Systematics of the gastrotrichs from the family Chaetonotidae (Gastrotricha, Chaetonotida) based on the integrative taxonomy approach

Małgorzata Kolicka

PhD thesis

Faculty of Biology
Adam Mickiewicz University, Poznań
2020, Poznań

The thesis has been prepared at the Department of Animal Taxonomy and Ecology
ZTiEZ 2020, Poznań

Supervisor: Prof. UAM dr hab. ✝ Ziemowit Olszanowski

Supervisor: Prof. UAM dr hab. Wojciech Magowski

Department of Animal Taxonomy and Ecology, Institute of Environmental Biology,

Faculty of Biology, Adam Mickiewicz University, Poznań; Poznań, Poland

TABLE OF CONTENTS

Abstract / Streszczenie 6

Introduction / Wprowadzenie 8

Doctoral dissertation 21

Bifidochaetus, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach 22

A new species of freshwater Chaetonotidae (Gastrotricha, Chaetonotida) from Obodska Cave (Montenegro) based on morphological and molecular characters 63

New Arctic Gastrotricha — Three *Chaetonotus* (Chaetonotidae) from Longyearbyen (Spitsbergen, Svalbard Archipelago) 95

Diversity of Gastrotricha on Spitsbergen (Svalbard Archipelago, Arctic) with a Description of Seven New Species 147

From saline to salty? *Heterolepidoderma sinus* (Chaetonotida, Chaetonotidae) from subsaline coal mine settling ponds 280

Gastrotricha – not only in sediments: new epiphytic species of Chaetonotida from the Jubilee Greenhouse of the Botanical Garden in Kraków 287

New *Chaetonotus* (*Wolterecka*) *semovitus* sp. nov. (Gastrotricha: Chaetonotida: Chaetonotidae) from a Palm House in Vienna (Austria) 389

ICZN Declaration 45: a remedy for the nomenclatural and typification dilemma regarding soft-bodied meiofaunal organisms? 420

Authorship statements of PhD candidate / Oświadczenia doktoranta o wkładzie w powstanie artykułów 433

Authorship statements of co-authors of the article / Oświadczenia współautorów o wkładzie w powstanie artykułów 450

Acknowledgments / Podziękowania 470

ABSTRACT

Considering the current state of knowledge about Gastrotricha, their unprecise phylogeny, the lack of knowledge about their morphology, and the evolution of their characteristics, the main aim of my work is to enhance our knowledge on gastrotrich evolution as well as to integrate them in a phylogenetic context with the construction of the natural systematics within Chaetonotidae. To achieve this goal, I studied chaetonotidian diversity from various geographical regions and habitat types based on morphological, morphometric and molecular data, with particular emphasis on evolutionary aspects of character diversification. I also focused on the new approaches to the taxonomy of gastrotrichs and their inclusion in the International Code of Zoological Nomenclature.

In the eight parts in this thesis, I describe one new genus namely *Bifidochaetus* gen. nov. and 16 species that are new to science i.e. *Bifidochaetus arcticus* sp. nov., *Chaetonotus* (*Chaetonotus*) *antrumus* sp. nov., *C.* (*C.*) *jaceki* sp. nov., *C.* (*C.*) *gelidus* sp. nov., *C.* (*C.*) *svalbardi* sp. nov., *C.* (*C.*) *bombardus* sp. nov., *C.* (*C.*) *subtilis* sp. nov., *C.* (*Hystricochaetonotus*) *borealis* sp. nov., *C.* (*H.*) *hornsundi* sp. nov., *C.* (*H.*) *persimilis* sp. nov., *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov., *C.* (*H.*) *horridus* sp. nov., *C.* (*H.*) *inaequabilis* sp. nov., *C.* (*Wolterecka*) *semovitus* sp. nov., *Lepidodermella polaris* sp. nov. and *Turbanella pilosum* sp. nov. I also show the presence of *C.* (*C.*) *paucisquamatus* Kisielewski, 1991 and *C.* (*Zonochaeta*) *cestacanthus* Balsamo, 1990 for the first time outside their *terra typica*. I also present pharynx-to-intestine length ratio (I), which enables objective determination of the degree of development of an individual. Even without other means of estimating age, this index can help identify whether the individual is a juvenile, subadult or adult. I also suggest the use of spine bifurcation ratio (B) parameters. The point at which the single spine divides into two separate spines is essential in distinguishing the two species now present within *Bifidochaetus*, gen. nov. Furthermore, I have noted a record abundance of Gastrotricha amounting to more than 1,000 specimens per 1 cm^3 of bottom sediment, in contrast to the maximum amount of 101 individuals that were recorded in the same volume of sediment from a temperate climate. Moreover, I focus on taxonomical studies of so-called soft-bodied meiofaunal organisms, in which deposition of physical type specimens is frequently either not possible or even not advisable at all because they are often lost before their formal designation as a name-bearing type. Me and co-authors postulate some arguments and recommendations concerning photomicrography-based taxonomy on these delicate organisms: high-definition light microscopy with high numerical aperture objectives and suitable contrast techniques such as DIC or phase contrast; extensive documentation in the form of a series of high-resolution image stacks and videos; deposition of digital image data in a scientific collection; multiple depositions of digital image data in other repositories as backup; assignment of accession numbers to those digital data; if possible, extraction of DNA from the documented type specimen(s); making DNA sequences or other molecular markers publicly available. All these observations have greatly contributed to better understanding of the diversity and evolution of the Chaetonotidae family; therefore, they represent a significant step towards the unavoidable redescription of the entire Chaetonotida order.

STRESZCZENIE

Mając na uwadze obecny stan wiedzy o Gastrotricha, nieprecyzyjną filogenezę, brak wiedzy o morfologii i ewolucji stanów cech, głównym celem mojej rozprawy jest poszerzenie obecnej wiedzy na temat ewolucji brzuchorzęsków oraz zintegrowanie jej w kontekście filogenetycznym z rekonstrukcją naturalnej systematyki w obrębie Chaetonotidae. Aby zrealizować to założenie, przeanalizowałam różnorodność gatunkową Chaetonotidae z różnych regionów geograficznych i typów siedlisk w oparciu o dane morfologiczne, morfometryczne i molekularne, ze szczególnym uwzględnieniem ewolucyjnych aspektów zmienności cech. Skoncentrowałam się również na nowych podejściach do opracowania najlepszej metody pracy nad taksonomią brzuchorzęsków i włączeniu jej do oficjalnej rekomendacji Międzynarodowego Kodeksu Nomenklatury Zoologicznej.

W ośmiu pracach składających się na tę dysertację opisałam jeden nowy rodzaj mianowicie *Bifidochaetus* gen. nov. oraz 16 gatunków nowych dla nauki którymi są *Bifidochaetus arcticus* sp. nov., *Chaetonotus* (*Chaetonotus*) *antrumus* sp. nov., *C.* (*C.*) *jaceki* sp. nov., *C.* (*C.*) *gelidus* sp. nov., *C.* (*C.*) *svalbardi* sp. nov., *C.* (*C.*) *bombardus* sp. nov., *C.* (*C.*) *subtilis* sp. nov., *C.* (*Hystricochaetonotus*) *borealis* sp. nov., *C.* (*H.*) *hornsundi* sp. nov., *C.* (*H.*) *persimilis* sp. nov., *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov., *C.* (*H.*) *horridus* sp. nov., *C.* (*H.*) *inaequabilis* sp. nov., *C.* (*Wolterecka*) *semovitus* sp. nov., *Lepidodermella polaris* sp. nov. oraz *Turbanella pilosum* sp. nov. Wykazałam także obecność *C.* (*C.*) *paucisquamatus* Kisielewski, 1991 i *C.* (*Zonochaeta*) *cestacanthus* Balsamo, 1990 dotychczas niestwierdzonych poza ich *terra typica*. Zaproponowałam także stosunek długości gardzieli do jelita (I), który umożliwia obiektywne wyznaczenie stopnia rozwoju osobnika. Ten wskaźnik może pomóc w określeniu czy dany okaz jest młodociany, prawie dorosły czy dorosły, nawet jeśli nie występują inne cechy pozwalające na oszacowanie wieku. Zasugerowałam również użycie współczynnika rozdwojenia szczeciny (B). Formuła ta określa punkt, w którym pojedyncza szczecina dzieli się na dwie osobne szczeciny i jest ona użyteczna przy rozróżnianiu dwóch gatunków obecnie należących do *Bifidochaetus* gen. *et* sp. nov. Ponadto odnotowałam rekordową liczebność brzuchorzęsków wynoszącą ponad tysiąc osobników na 1 cm^3 osadu dennego co stanowiło ponad dziesięciokrotność maksymalnie dotychczas zaobserwowanej liczby osobników w tej samej objętości osadów z obszaru klimatu umiarkowanego. Oprócz tego skupiłam się na badaniach taksonomicznych tak zwanej mejofauny o miękkim ciele, w obrębie których depozycja fizycznych okazów typowych jest często albo niemożliwa, albo wręcz niewskazana, ponieważ w zdecydowanej większości przypadków osobniki ulegają zniszczeniu jeszcze przed formalnym ustanowieniem ich jako holotypu. Wraz ze współautorami położyliśmy nacisk na niektóre argumenty i zalecenia dotyczące taksonomii wykorzystującej fotomikrografie tych delikatnych organizmów. Postulowaliśmy zastosowanie mikroskopii świetlnej o wysokiej rozdzielczości, z obiektywami o dużej aperturze numerycznej i odpowiednimi technikami kontrastu, takimi jak kontrast Nomarskiego lub kontrast fazowy, przygotowywanie obszernej dokumentacji składającej się z serii zdjęć i filmów w wysokiej rozdzielczości. Zwracaliśmy także uwagę na deponowanie fotomikrografii w kolekcjach naukowych, niezależne przechowywanie danych z cyfrowej dokumentacji osobników w różnych repozytoriach jako kopii zapasowej, przypisanie numerów dostępu do tych zbiorów fotomikrograficznych oraz, jeśli to możliwe, ekstrakcję DNA z udokumentowanego wcześniej fotomikrograficznie holotypu i publiczne udostępnienie pochodzących z niego sekwencji DNA lub innych markerów molekularnych. Poza tym przedyskutowaliśmy desygnowanie ilustracji jako reprezentacji typów nomenklaturowych w Międzynarodowym Kodeksie Nomenklatury dla glonów, grzybów i roślin, co stanowi praktykę stosowaną od pewnego czasu bez zauważalnych wad dotyczących jakości aktów taksonomicznych. Wszystkie te obserwacje w znacznym stopniu przyczyniły się do lepszego zrozumienia Chaetonotidae, jej różnorodności i ewolucji oraz poczyniły znaczący krok w kierunku nieuniknionej rewizji całego rzędu Chaetonotida.

INTRODUCTION

The majority of representatives of Kingdom Animalia are minute metazoans which play an important role in ecological networks, but regrettably their diversity is highly underestimated. One such example is phylum Gastrotricha, which represents small aquatic invertebrates ranging in size from 50 to 3500 μm (*e.g.*, Kisielewski 1997; Balsamo *et al.* 2014). Gastrotricha are regarded as a monophyletic group whose representatives inhabit all types of marine, brackish, freshwater, and semi-aquatic environments in natural, rural or urban habitats (see Balsamo *et al.* 2014 for references). They are ubiquitous even in unexpected types of habitats such as temporarily drying micro-reservoirs formed in the axils of bromeliads (Kisielewski 1991; Kolicka 2016), water reservoirs in caves (Renaund-Mornant 1986; Todaro *et al.* 2006a; Kolicka *et al.* 2017), and extreme ecosystems such as hot springs (Guerne 1888) or hydrothermal vent zones (Kieneke and Zekely 2007).

Currently, approximately 860 gastrotrich species are known (Todaro 2020), but this constitutes only a small fraction of their actual species richness. The estimated number of the entire marine species of Gastrotricha ranges from *ca*. 2,250 to 3,250, which shows that we currently know less than 20% of the diversity of these animals (Todaro in Appeltans *et al*. 2012). There are no such predictions concerning the probable number of taxa occurring in inland waters, but the degree of knowledge regarding these animals is most likely remarkably lower. Gastrotrichs are divided into two orders: Chaetonotida Remane, 1925 (*sensu* Rao *et* Clausen, 1970) and Macrodasyida Remane, 1925 (*sensu* Rao *et* Clausen, 1970). Representatives of Chaetonotida occur in fresh, brackish and marine waters, whereas species belonging to Macrodasyida are almost exclusively marine or brackish taxa.

Species belonging to Chaetonotida reach sizes from 50 to 780 μm; their body is usually bottle-shaped with a more or less remarkable neck narrowing, and the pharynx lumen has a "Y" cross section. The vast majority of representatives of this order have only distal adhesive tubes (usually one pair) set on the furca. In some taxa, these structures are partially or completely reduced. Macrodasyida comprises species ranging in size from 200 to 3500 μm, with an elongated "worm-like" body and, apart from several exceptions, with pharyngeal pores. Macrodasyida is characterised by the presence of many pairs of adhesive tubes (anterior, posterior, often also lateral, dorsolateral and dorsal) and an inverted "Y" cross section of the pharyngeal lumen. Current knowledge on Gastrotricha is fragmentary and constitutes a sort of *Tabula rasa* in studies on meiofaunal diversity. Next taxa are described as new species (*e.g.*, Kånneby *et al.* 2009; Kånneby 2011, 2013; Suzuki *et al.* 2013; Atherton 2014; Hochberg *et al.* 2014; Von Und zu Gilsa *et al.* 2014; Kånneby and Todaro 2017; Dal Zotto *et al*. 2018), genera (*e.g.*, Kisielewski 1987, 1991; Hummon *et al.* 1993; Todaro *et al.* 2005, 2015), and even as new families (*e.g.*, Kisielewski 1987; Todaro *et al.* 2006b, 2012, 2014). The main factor limiting the state of knowledge on gastrotrichs is the small number of specialists dealing with this group and the necessity of working only with living specimens (Kisielewski 1997; Balsamo *et al.* 2008, 2014). In the course of preserving gastrotrichs they lose almost all of their significant diagnostic characteristics,

therefore preserved material has very little taxonomic value and is usually not sufficient to be used for species determination (Kisielewski 1997).

The evolutionary history of Gastrotricha is fragmentary and constitutes a topic of debate. Despite rapid advances in phylogenetic analyses, it has been omitted due to its problematic nature (Laumer *et al*. 2015). Within gastrotrichs, the order Macrodasyida Remane, 1925 (*sensu* Rao *et* Clausen, 1970) is widely recognized as a more ancient group of gastrotrichs (Hochberg and Litvaitis 2000; Kieneke *et al*. 2008; Balsamo *et al*. 2014 for references), and only a single work postulates that Macrodasyida is a more advanced evolutionary taxon compared to the Chaetonotida baseline (see Wirz *et al.* 1999). Nevertheless, both of these hypotheses agree that Gastrotricha were originally saltwater psammon, and their expansion into habitats with lower salinity occurred later. Today, over 70% of species belonging to Chaetonotida occur in inland waters, and only seven species from two families of Macrodasyida (including four nominal ones, namely *Marinellina flagellata* Ruttner-Kolisko, 1955, *Redudasys fornerise* Kisielewski, 1987, *R. neotemperatus* Kånneby *et* Kirk, 2017, and *R. brasiliensis* Garraffoni, Araújo, Lourenço, Guidi *et* Balsamo, 2019) inhabit freshwater. According to traditional systematics (Remane 1936; d'Hond 1970; Hochberg and Litvaitis 2000; Zrzavý 2003), chaetonotid gastrotrichs are grouped in two suborders: Multitubulatina d'Hondt, 1971, which consists of one monogeneric marine family, Neodasyidae Remane, 1929, and the more species-rich suborder Paucitubulatina d'Hondt, 1971. Members of Paucitubulatina belong to seven families: the exclusively marine Muselliferidae Leasi *et* Todaro, 2008; Xenotrichulidae Remane, 1927a; the mostly freshwater Chaetonotidae Gosse, 1864 (*sensu* Leasi *et* Todaro, 2008); the exclusively freshwater Dasydytidae Daday, 1905; Dichaeturidae Remane, 1927b; Neogosseidae Remane, 1927b; and Proichthydiidae Remane, 1927b. The family Chaetonotidae, which contains 16 genera (*e.g.*, Balsamo *et al*. 2014; Todaro 2020), appears to be the most diverse and evolutionarily successful chaetonotid lineage. This family contains more species than all the remaining Chaetonotida families combined and includes freshwater, brackish, and marine species (Balsamo *et al*. 2014; Todaro 2020). Molecular phylogenetic analyses (Kånneby *et al*. 2012, 2013, 2015; Kånneby and Todaro 2015; Bekkouche and Worsaae 2016; Kolicka *et al*. 2016) have confirmed morphology-based hypotheses (Hochberg and Litvaitis 2000; Kieneke *et al*. 2008) that Chaetonotidae is paraphyletic with respect to Dasydytidae and Neogosseidae. Moreover, many types and subgenera within Chaetonotidae seem to be polyphyletic or paraphyletic groups that contain more closely unrelated taxa and which are grouped only on the basis of easily perceptible individual features.

The taxonomy of Gastrotricha is still based on a series of chosen morphological characteristics (Balsamo *et al.* 2008, 2014). Molecular data suggest that such an approach may be a misleading indicator of phylogenetic relations (*e.g.*, Paps and Riutort 2012; Kånneby *et al.* 2012, 2013, 2015; Kånneby and Todaro 2015; Kolicka *et al*. 2016). Also, the analysis of a wide range of morphological features indicates that species analyses that are based only on commonly accepted and easily noticeable characteristics (*e.g.*, the presence of scales and spines) may be misleading and may not reflect the relationships among taxa (Hochberg and Litvantis 2000; Kieneke *et al.* 2008). Among the eight families that belong to Chaetonotida, the family Chaetonotidae Gosse, 1864 (*sensu* Leasi and Todaro, 2008) is

the most species-rich, commonly distributed and best-studied; nonetheless, current knowledge on its phylogenesis is still very limited (Kånneby *et al.* 2012; 2013; 2015; Kånneby and Todaro 2015; Todaro *et al.* 2014). This family is divided into two subfamilies: Chaetonotinae Gosse, 1864 (*sensu* Kisielewski, 1991) and Undulinae Kisielewski, 1991. Chaetonotinae comprises 12 genera, which are traditionally established on the basis of morphological differences between the cuticular structures and their arrangements. Morphological analyses (Hochberg and Litvantis 2000; Kieneke *et al.* 2008) and molecular studies (*e.g.*, Paps and Riutort 2012; Kånneby *et al.* 2012, 2013; Bekkouche and Worsaae 2016) show that nearly all of the examined currently distinguished genera within Chaetonotinae (as well as the vast majority of subgenera within *Chaetonotus* Ehrenberg, 1830) are artificial taxa. The other subfamily, Undulinae, comprises a single monotypic genus that was previously known only from Brazil (Kisielewski 1991) and has not yet been subjected to molecular analyses. The polyphyletic nature of Chaetonotidae and the fact that the semi-planktonic family Dasydytidae and Neogosseidae are nested in Chaetonotidae (Kieneke *et al*. 2008; Kånneby *et al*. 2013; Todaro *et al*. 2013; Kånneby and Todaro 2015) indicates the necessity of a complete revision of Gastrotricha systematics. The considerable divergence between morphological and molecular data significantly increases the difficulty of interpretation of results and negatively affects their reliability. The discrepancy of signals from these two data sources makes it urgent to enrich the interpreted sets of features in such a way that the information obtained from them will fully and accurately reflect the course of evolutionary processes. Fiers and Kotwicki (2013) showed that the analysis of only chosen, easily noticeable morphological characters might be very misleading. These authors used morphology to prove that one taxon of Harpacticoida (Copepoda, Crustacea), which was previously regarded as a cosmopolitan species, actually comprises species that considerably differ in their appearance and geographical distribution. Also, Kånneby *et al*. (2012) observed morphological differences in gastrotrichs between two separate clades distinguished on the basis of molecular analyses and commonly regarded as the species *Lepidodermella squamata* (Dujardin, 1841); however, these authors explained the observed differences in the morphology of these two lines as developmental plasticity during the formation of cuticular structures (Kånneby *et al*. 2012). Noteworthy, earlier research suggested that the species *L. squamata* should be divided into separate species that differ morphologically in the shape and number of dorsal, dorsolateral, lateral, ventrolateral and ventral scales, as well as in the type, shape and number of ventral interciliary field scales, and the presence of spines on furcal appendages (Fregni *et al*. 1998). Similarly, using a confocal laser scanning microscope, Leasi and Todaro (2009) showed considerable differences in the structure of the muscular system in two geographically isolated groups that were classically regarded as the species *Xenotrichula intermedia* Remane, 1934. Their observations indicated that it is possible to distinguish species that were previously considered to be morphologically cryptic. It is very likely that the numerous taxa in Gastrotricha, which is now considered to be a cosmopolitan species, will appear to be a strictly specialised complex of cryptic species or an assemblage of species that are similar in morphology. It is also possible that the presently recognised complexes of cryptic species will appear to be an assemblage of taxa similar to each other in morphology which, however, will be distinguishable after detailed morphological analysis. Taking the above facts into

account and the constantly growing number of known species of gastrotrichs, it is crucial to combine morphological (qualitative and quantitative) and molecular analyses in studies instead of using only one of these methods.

A significant number of Gastrotricha descriptions come from the eighteenth, nineteenth and early twentieth centuries and contain only scant descriptions without many important taxonomical characteristics and detailed drawings. Due to their small size and delicate bodies, there is a lack of valid holotypes for subsequent comparisons because during preservation gastrotrichs are deformed and lose crucial taxonomical characteristics. However, despite the rapid development of available methods of taxonomy investigation, in gastrotrichs – as in many other organism groups – we are still struggling with the same fundamental question: What do these species look like? This issue, even if it is notching new and is commonly known, often remains widely undebated and unresolved. Reports of the occurrence of "old species" come from different parts of the world and from different authors, but this "species" seems to be truly cosmopolitan and globally distributed in a very wide range of ecosystem types. However, why do species with the same name habitually differ significantly according to various researchers, and which differences are clearly noticeable in emended descriptions and drawings? In gastrotrichs which require studying intravitally, different solutions to this problem have been tried and proposed in past decades but they are not widely used and recognized as part of standard methodology. Gastrotrichs are often omitted from ecological studies, although they are an important, universal, and common element of benthic communities. Due to their small body size and the difficult research methodology, many troubling evolutionary questions have not even been addressed.

WPROWADZENIE

Królestwo zwierząt reprezentowane jest w większości przez drobne wielokomórkowce odgrywające ważną rolę w sieciach ekologicznych. Niestety ich różnorodność pozostaje bardzo niedoszacowana. Jednym z przykładów jest typ brzuchorzęsków (Gastrotricha) reprezentująca małe bezkręgowce wodne mierzące od 50 do 3500 μm (np. Kisielewski 1997; Balsamo i in. 2014). Są one uważane za grupę monofiletyczną z przedstawicielami zamieszkującymi wszystkie typy środowisk morskich, słonawych, słodkowodnych oraz wilgotnych, zarówno naturalnych, jak i tych specyficznych dla terenów wiejskich lub miejskich (Balsamo i in. 2014 wraz z referencjami). Brzuchorzęski są wszechobecne nawet w nieoczekiwanych typach siedlisk, takich jak czasowo wysychające zbiorniki w kątach ulistnienia bromelii (Kisielewski 1991; Kolicka 2016), wody wewnątrz jaskiń (Renaund-Mornant 1986; Todaro i in. 2006a; Kolicka i in. 2017) oraz ekstremalne ekosystemy takie jak gorące źródła (Guerne 1888) czy ujścia kominów hydrotermalnych (Kieneke i Zekely 2007). Gatunki należące do Chaetonotida występują w wodach słodkich, słonawych i morskich, podczas gdy przedstawiciele rzędu Macrodasyida są niemal wyłącznie taksonami morskimi lub brakicznymi.

Obecnie znanych jest około 860 gatunków brzuchorzęsków (Todaro 2020), co stanowi niewielki ułamek ich rzeczywistego bogactwa taksonomicznego. Szacowana liczba wszystkich morskich gatunków Gastrotricha wynosi od ok. 2 250 do 3 250, co pokazuje, że aktualnie znamy mniej niż 20% różnorodności tych zwierząt (Todaro w: Appeltans i in. 2012). Jak dotąd nie powstały podobne prognozy dotyczące spodziewanej liczby taksonów występujących w wodach śródlądowych, niemniej poziom wiedzy dotyczącej słodkowodnych Gastrotricha jest prawdopodobnie jeszcze niższy. Gromada brzuchorzęsków podzielona jest na dwa rzędy: Chaetonotida Remane, 1925 (*sensu* Rao *et* Clausen, 1970) i Macrodasyida Remane, 1925 (*sensu* Rao *et* Clausen, 1970). Gatunki należące do Chaetonotida osiągają rozmiary od 50 do 780 μm, a ich ciało jest przeważnie butelkowate z mniej lub bardziej zauważalnym przewężeniem szyjnym. Natomiast światło ich gardzieli w przekroju poprzecznym ma kształt trójkątny, odpowiadający literze „Y". Zdecydowana większość przedstawicieli tego rzędu ma jedynie dystalne rurki lepowe (zazwyczaj jedną parę) znajdujące się na przydatkach widełek ogonowych. U niektórych taksonów struktury te zostały częściowo lub całkowicie zredukowane. Macrodasyida obejmuje gatunki mierzące od 200 do 3500 μm o wydłużonym, „robakowatym" ciele i, poza kilkoma wyjątkami, posiadające pory gardzielowe. Macrodasyida charakteryzują się obecnością wielu par rurek lepowych (przednich, tylnych, często również bocznych, grzbietowo-bocznych i grzbietowych), a światło ich gardzieli w przekroju poprzecznym ma kształt trójkątny, odpowiadający odwróconej literze „Y".

Dotychczasowa wiedza na temat Gastrotricha pozostaje fragmentaryczna i stanowi swoistą „białą kartę" w badaniach nad różnorodnością mejofauny. Kolejne taksony stale opisywane są jako nowe gatunki (np. Kånneby i in. 2009; Kånneby 2011, 2013; Suzuki i in. 2013; Atherton 2014; Hochberg i in. 2014; Von Und zu Gilsa i in. 2014; Kånneby i Todaro 2017; Dal Zotto i in. 2018), rodzaje (np. Kisielewski 1987, 1991; Hummon i in. 1993;

Todaro i in. 2005, 2015), a nawet jako nowe rodziny (np. Kisielewski 1987; Todaro i in. 2006b, 2012, 2014). Głównymi czynnikami ograniczającymi stan wiedzy o Gastrotricha jest niewielka liczba specjalistów zajmujących się tą grupą oraz konieczność pracy z wyłącznie żywymi okazami (Kisielewski 1997; Balsamo i in. 2008, 2014). Utrwalanie materiału skutkuje utratą niemal wszystkich znaczących cech diagnostycznych brzuchorzęsków. Z tego powodu zakonserwowane okazy mają bardzo ograniczoną wartość taksonomiczną i zwykle nie są wystarczające do oznaczenia ich do poziomu gatunku (Kisielewski 1997).

Znajomość historii ewolucyjnej Gastrotricha jest wciąż szczątkowa i stanowi temat do dyskusji. Pomimo szybkich postępów zachodzących w analizach filogenetycznych bywają one pomijane ze względu na ich problematyczny charakter (Laumer i in. 2015). W obrębie brzuchorzęsków rząd Macrodasyida Remane, 1925 (*sensu* Rao *et* Clausen, 1970) jest powszechnie uznawany za bardziej pierwotną grupę (Hochberg i Litvaitis 2000; Kieneke i in. 2008; Balsamo i in. 2014 w celach referencyjnych) w odniesieniu do linii Chaetonotida Jedynie pojedyncza praca postuluje uznanie Macrodasyida jako bardziej zaawansowanego ewolucyjnie taksonu (zobacz Wirz i in. 1999). Niemniej obie te hipotezy są zgodne co do faktu, że brzuchorzęski pierwotnie wchodziły w skład psammonu wód słonych i jedynie wtórnie dokonały ekspansji do siedlisk o niższym zasoleniu. Obecnie ponad 70% taksonów należących do Chaetonotida występuje w wodach śródlądowych, a tylko siedem gatunków z dwóch rodzajów należących do Macrodasyida (w tym cztery nominalne, a mianowicie *Marinellina flagellata* Ruttner-Kolisko, 1955, *Redudasys fornerise* Kisielewski, 1987, *R. neotemperatus* Kånneby *et* Kirk, 2017 i *R. brasiliensis* Garraffoni, Araújo, Lourenço, Guidi *et* Balsamo, 2019) zasiedla wody słodkie. Zgodnie z klasyczną systematyką (Remane 1936; d'Hond 1970; Hochberg i Litvaitis 2000; Zrzavý 2003), brzuchorzęski z rzędu Chaetonotida są zgrupowane w dwóch podrzędach: Multitubulatina d'Hondt, 1971, składającego się z jednej monotypowej rodziny morskiej - Neodasyidae Remane, 1929 oraz bogatszego w gatunki podrzędu Paucitubulatina d'Hondt, 1971. Przedstawiciele Paucitubulatina należą do siedmiu rodzin: wyłącznie morskich Muselliferidae Leasi *et* Todaro, 2008 i Xenotrichulidae Remane, 1927a, głównie słodkowodnej Chaetonotidae Gosse, 1864 (*sensu* Leasi *et* Todaro, 2008) oraz wyłącznie słodkowodnych Dasydytidae Daday, 1905, Dichaeturidae Remane, 1927b, Neogosseidae Remane, 1927b i Proichthydiidae Remane, 1927b. Rodzina Chaetonotidae wydaje się być najbardziej zróżnicowaną i rozpowszechnioną w różnych typach siedlisk linią Chaetonotida grupującą 16 rodzajów (np. Balsamo i in. 2014; Todaro 2020). Zawiera ona więcej gatunków niż wszystkie pozostałe rodziny należące do Chaetonotida łącznie i obejmuje gatunki słodkowodne, słonawowodne i morskie (Balsamo i in. 2014; Todaro 2020). Molekularne analizy filogenetyczne (Kånneby i in. 2012, 2013, 2015; Kånneby i Todaro 2015; Bekkouche i Worsaae 2016; Kolicka i in. 2016) potwierdziły hipotezy oparte na morfologii (Hochberg i Litvaitis 2000; Kieneke i in. 2008) postulujące, że Chaetonotidae jest parafiletyczna w odniesieniu do Dasydytidae i Neogosseidae. Ponadto wiele rodzajów i podrodzajów w obrębie Chaetonotidae wydaje się być grupami poli- lub parafiletycznymi, zawierającymi bliżej niepowiązane taksony pogrupowane wyłącznie na podstawie wybiórczych, łatwo dostrzegalnych cech.

Taksonomia brzuchorzęsków wciąż opiera się na szeregu wybranych cech morfologicznych (Balsamo i in. 2008, 2014, 2015). Dane molekularne sugerują, że takie podejście może być mylącym wskaźnikiem relacji filogenetycznych (np. Paps i Riutort 2012; Kånneby i in. 2012, 2013, 2015; Kånneby i Todaro 2015). Ponadto analiza szerokiego zakresu cech morfologicznych wskazuje, że opieranie się wyłącznie na powszechnie akceptowanych i łatwo zauważalnych strukturach (np. obecności tarczek i/lub szczecin) bywa zawodne i nie odzwierciedla poprawnie relacji między taksonami (Hochberg i Litvantis 2000; Kieneke i in. 2008). Spośród ośmiu rodzin należących do Chaetonotida, rodzina Chaetonotidae Gosse, 1864 (*sensu* Leasi *et* Todaro, 2008) jest najbogatsza gatunkowo, najszerzej rozpowszechniona oraz najdokładniej zbadana; niemniej jednak obecna wiedza na temat filogenezy tej rodziny jest nadal bardzo ograniczona (Kånneby i in. 2012; 2013; 2015; 2015; Kånneby i Todaro 2015; Todaro i in. 2014; Kolicka i in. 2016). Jest ona podzielona na dwie podrodziny: Chaetonotinae Gosse, 1864 (*sensu* Kisielewski, 1991) i Undulinae Kisielewski, 1991. Chaetonotinae składa się z 12 rodzajów, które zostały tradycyjnie ustanowione na podstawie różnic morfologicznych w strukturach kutykularnych oraz ich rozmieszczeniu. Analizy morfologiczne (Hochberg i Litvantis 2000; Kieneke i in. 2008) i badania molekularne (np. Paps i Riutort 2012; Kånneby i in. 2012, 2013; Bekkouche i Worsaae 2016) wskazują, że prawie wszystkie z badanych, obecnie wyróżnianych rodzajów w obrębie Chaetonotinae, a także znaczna większość podrodzajów w obrębie *Chaetonotus* Ehrenberg, 1830 to taksony sztuczne. Druga podrodzina, Undulinae, obejmuje jeden rodzaj monotypowy znany do tej pory wyłącznie z Brazylii (Kisielewski 1991) i do dziś nieuwzględniony w żadnej z analiz molekularnych. Polifiletyczny charakter Chaetonotidae oraz fakt, że semi-planktonowe rodziny Dasydytidae i Neogosseidae zawierają się w jej obrębie (Kieneke i in. 2008; Kånneby i in. 2013; Todaro i in. 2013; Kånneby i Todaro 2015) wskazują na konieczność całkowitej rewizji systematyki brzuchorzęsków. Duża rozbieżność między danymi morfologicznymi, a molekularnymi znacznie pogłębia trudności z interpretacją otrzymanych wyników i negatywnie wpływa na ich wiarygodność. Niezgodność sygnałów pochodzących z tych dwóch źródeł danych powoduje, że naglącą staje się potrzeba wzbogacenia interpretowanych zbiorów cech w taki sposób aby otrzymywany z nich obraz był pełny i odzwierciedlał prawdziwy przebieg procesów ewolucyjnych. Fiers i Kotwicki (2013) udowodnili, że analiza tylko wybranych, łatwo zauważalnych struktur morfologicznych może być bardzo myląca. Autorzy ci, wykorzystali badania morfologiczne, aby udowodnić, że takson należący do Harpacticoida (Copepoda, Crustacea), wcześniej uważany za jeden kosmopolityczny gatunek, w rzeczywistości obejmuje gatunki znacznie różniące się wyglądem i rozmieszczeniem geograficznym. Również wśród brzuchorzęsków Kånneby i in. (2012) zaobserwowali różnice morfologiczne między dwiema oddzielnymi grupami wyróżnionymi na podstawie analiz molekularnych i powszechnie uważanymi za gatunek *Lepidodermella squamata* (Dujardin, 1841). Zaobserwowane różnice w morfologii tych dwóch linii autorzy tłumaczyli plastycznością rozwojową powstałą podczas tworzenia się struktur oskórka (Kånneby i in. 2012). Już wcześniejsze badania sugerowały, że nominalny *L. squamata* należy podzielić na osobne gatunki różniące się od siebie morfologicznie kształtem i liczbą tarczek grzbietowych, grzbietowo-bocznych, bocznych, brzuszno-brzusznych i brzusznych, a także rodzajem, kształtem i liczbą tarczek międzyrzęskowego pólka brzusznego oraz obecnością

szczecin na przydatkach widełek ogonowych (Fregni i in. 1998). Podobnie Leasi i Todaro (2009), używając skanującego laserowego mikroskopu konfokalnego, wykazali znaczne zróżnicowanie struktury układu mięśniowego u dwóch izolowanych geograficznie grup, które klasycznie były uznawane za gatunek *Xenotrichula intermedia* Remane, 1934. Ich obserwacje wykazały, że jest możliwe rozróżnienie gatunków, które wcześniej uważano za morfologicznie kryptyczne. Jest bardzo prawdopodobne, że liczne taksony brzuchorzęsków, obecnie uważane za jeden kosmopolityczny gatunek, zostaną uznane jako ściśle wyspecjalizowany kompleks gatunków kryptycznych lub zbiór gatunków podobnych morfologicznie. Niewykluczone jest również, że obecnie rozpoznawane kompleksy gatunków kryptycznych okażą się zbiorem taksonów podobnych morfologicznie, jednak dających się odróżnić po szczegółowej analizie morfologicznej. Biorąc pod uwagę powyższe fakty i stale rosnącą liczbę znanych gatunków brzuchorzęsków, kluczowe jest połączenie analiz morfologicznych (jakościowych oraz ilościowych) i molekularnych w badaniach zamiast opierania się wyłącznie na jednej z tych metod.

Znaczna część opisów Gastrotricha powstała w XVIII, XIX oraz na początku XX wieku i często charakteryzuje się zwięzłością treści która nie uwzględnia wielu istotnych cech taksonomicznych oraz szczegółowych rysunków. Z powodu ich niewielkich rozmiarów oraz delikatnych ciał, brak odpowiednio zachowanych okazów typowych do późniejszych porównań ze względu na utratę kluczowych cech i zdeformowanie powstałe już podczas procesu konserwacji danego osobnika. Jednak pomimo szybkiego rozwoju dostępnych dla taksonomii metod badawczych, u brzuchorzęsków, podobnie jak u wielu innych grup organizmów, wciąż mamy do czynienia z tym samym podstawowym pytaniem: jak wyglądają te gatunki? Ten problem, choć nienowy i powszechnie znany, często pozostaje nierozważony i nierozwiązany. Doniesienia o występowaniu „starych gatunków" pochodzą z różnych części świata i są przedstawiane przez różnych autorów, dzięki czemu ten „gatunek" wydaje się być prawdziwie kosmopolitycznym, szeroko rozpowszechnionym na świecie i zasiedlającym rozległy zakres typów ekosystemów. Tylko dlaczego gatunki o tych samych nazwach, często znacznie się różnią w zależności od osoby je przedstawiającej czy też od miejsca z którego pochodzą? Jest to wyraźnie widoczne w dołączonych opisach uzupełniających i rysunkach. W typie brzuchorzęsków, które wymagają badań przyżyciowych, w ostatnich dziesięcioleciach wypróbowano i zaproponowano różne środki mające zaradzić powstawaniu takich wątpliwości, jednakże bez powszechnie obowiązujących wniosków. Ze względu na ich niewielki rozmiar ciała i trudną metodologię badań, wiele nurtujących pytań ewolucyjnych nawet nie zostało sformułowanych.

References / Literatura

Appeltans W., Ahyong S. T., Anderson G., Angel M.V., Artois T., Bailly N., Bamber R., Barber A., Bartsch I., Berta A., *et al*. 2012 The magnitude of global marine species diversity. *Curr. Biol.*, 22 (23): 2189-2202. http://dx.doi.org/10.1016/j.cub.2012.09.036.

Atherton S. 2014. *Urodasys poculostylis* sp. nov., a new stylet-bearing gastrotrich (Macrodasyida) from Capron Shoal, Florida. *Mar. Biol. Res.*, 10 (5): 530-536.

Balsamo M., Fregni E. 1995. Gastrotrichs from interstitial fresh water, with a description of four new species. *Hydrobiol*., 302: 163-175.

Balsamo M., d'Hondt J. –L., Kisielewski J., Pierboni L. 2008. Global diversity of gastrotrichs (Gastrotricha) in freshwaters. *Hydrobiol*., 595: 85-91. http://dx.doi.org/10.1007/s10750-007-9006-4

Balsamo M., Todaro M. A. 1987. *Aspidiophorus polystictos*, a new marine species (Gastrotricha, Chaetonotida) and its life cycle. *Boll. Zool.*, 54: 147-153.

Balsamo M. 1990. Gastrotrichs from Lakes Bolsena, Chiusi and Montepulciano (central Italy), with the description of four new species. *Boll. Zool.*, 57: 165-178.

Balsamo M., Grilli P., Guidi L., d'Hondt, J. –L. 2014. Gastrotricha: Biology, Ecology and Systematics: Families Dasydytidae, Dichaeturidae, Neogosseidae, Proichthydiidae. In: H. J. F. Dumont (Ed.) Identification Guides to the Plankton and Benthos of Inland Waters. 24 pp. 1-187.

Balsamo M., d'Hondt J. L., Kisielewski J., Todaro M. A., Tongiorgi P., Guidi L., De Jong Y. 2015. Fauna Europaea: Gastrotricha. *Biodivers. Data J.*, 3: (e5800). http://doi:10.3897/BDJ.3.e5800

Bekkouche N., Worsaae K. 2016. Neuromuscular study of early branching *Diuronotus aspetos* (Paucitubulatina) yields insights into the evolution of organs systems in Gastrotricha. *Zool. Lett.*, 2: 21. http://doi:10.1186/s40851-016-0054-3

Daday E. V. 1905. Untersuchungen uber die susswasser mikrofauna Paraguays. *Zool. Bulgaria*, 44: 72-86.

Dal Zotto M., Leasi F., Todaro M. A. 2018. A new species of Turbanellidae (Gastrotricha, Macrodasyida) from Jamaica, with a key to species of *Paraturbanella*. *Zookeys*, 734: 105-119. https://doi.org/10.3897/zookeys.734.23023

d'Hondt J. –L. 1971. Gastrotricha. *Oceanogr. Mar. Biol. Ann. Rev.*, 9: 141-192.

d'Hondt J. –L. 1970. Inventaire de la faune marine de Roscoff: Gastrotriches, Kinorhynques, Rotiferes, Tardigrades. Editions de la Station Biologique de Roscoff, Roscoff.

Dujardin F. 1841. Histoire Naturelle des Zoophytes, Infusoires. Librairie Encyclopedique de Roret, Paris, 684 & atlas 12.

Ehrenberg C. G. 1830. Organisation, Systematik, und geographisches Verhältnis der Infusionstierchen. F. Dümmler, Berlin, 1-108pp. http://dx.doi.org/10.5962/bhl.title.2077.

Fiers F., Kotwicki L. 2013. The multiple faces of *Nannopus palustris* auct. reconsidered: Amorphological approach (Copepoda: Harpacticoida: Nannopodidae). *Zool Anz.*, 253: 36-65.

Fregni E., Balsamo M., Tongiorgi P. 1998. Interstitial gastrotrichs from lotic Italian fresh waters. *Hydrobiol.*, 368: 175-187.

Garraffoni A. R. S., Araújo T. Q., Lourenço A. P., Guidi L., Balsamo M. 2016. A new genus and new species of freshwater Chaetonotidae (Gastrotricha: Chaetonotida) from Brazil with phylogenetic position inferred from nuclear and mitochondrial DNA sequences. *Syst. Biodivers.*, http://dx.doi.org/10.1080/14772000.2016.1214189

Garraffoni A. R. S., Araújo T. Q., Lourenço A. P., Guidi L., Balsamo M. 2019. Integrative taxonomy of a new *Redudasys* species (Gastrotricha: Macrodasyida) sheds light on the invasion of fresh water habitats by macrodasyids. *Sci. Rep.*, 9: 2067. https://doi.org/10.1038/s41598-018-38033-0

Gosse P. H. 1864. The natural history of the hairy–backed animalcules (Chaetonotidae). *Intel. Observ.*, 5: 387-406.

Guerne J. 1888. Campagnes scientifiques du Yacht monéasque l'Hirondelle, treoisiéme année 1887. Gauthier-Villars, Paris, 26: 72-78. https://doi.org/10.1080/00222938809460883

Hochberg R., Atherton S., Kieneke A. 2014. Marine Gastrotricha of Little Cayman Island with the description of one new species and an initial assessment of meiofaunal diversity. *Mar Biol.*, 44 (1): 89-113.

Hochberg R., Litvaitis M. K. 2000. Phylogeny of Gastrotricha: a morphology–based framework of gastrotricha relationships. *Biol Bull.*, 198: 299-305.

Hummon W. D, Todaro M. A., Tongiorgo P. 1993 Italian marine Gastrotricha: II. One new genus and ten new species of Macrodasyida. *Boll Zool.*, 60: 190-127.

Kånneby T. 2011. New species and new records of freshwater Chaetonotida (Gastrotricha) from Sweden. *Zootaxa*, 3115, 29-55.

Kånneby T., Todaro M. A., Jondelius U. 2012. A phylogenetic approach to species delimitation in freshwater Gastrotricha from Sweden. *Hydrobiol.*, 683: 185-202.

Kånneby T., Todaro M. A., Jondelius U. 2013. Phylogeny of Chaetonotidae and other Paucitubulatina (Gastrotricha: Chaetonotida) and colonization of aquatic ecosystems. *Zool. Scripta*, 42: 88-105. http://dx.doi.org/10.1111/j.1463-6409.2012.00558.x

Kånneby T., Wicksten M. K. 2014. First record of the enigmatic genus *Redudasys* Kisielewski, 1987 (Gastrotricha: Macrodasyida) from the Northern hemisphere. *Zoosystema*, 36 (4): 723-734. http://dx.doi.org/10.5252/z2014n4a2

Kånneby T. 2013. New species and records of freshwater *Chaetonotus* (Gastrotricha: Chaetonotidae) from Sweden. *Zootaxa*, 3701: 551-588.

Kånneby T., Kirk J. J. 2017. A new species of *Redudasys* (Gastrotricha: Macrodasyida: Redudasyidae) from the United States. *Proc. Biol. Soc. Wash.*, 130: 129-139.

Kånneby T., Todaro M. A., Jondelius U. 2012. A phylogenetic approach to species delimitation in freshwater Gastrotricha from Sweden. *Hydrobiol.*, 683: 185-202.

Kånneby T., Todaro M. A., Jondelius U. 2009. One new species and records of *Ichthydium* Ehrenberg, 1830 (Gastrotricha: Chaetonotida) from Sweden with a key to the genus. *Zootaxa*, 2278: 26-46.

Kånneby T., Todaro, M. A. 2015. The phylogenetic position of Neogosseidae (Gastrotricha: Chaetonotida) and the origin of planktonic Gastrotricha. *Org. Diver. Evol.*, 15: 459-469. https://doi.org/10.1007/s13127-015-0223-92

Kånneby T., Todaro M. A. 2017. A new species of *Aspidiophorus* (Gastrotricha: Chaetonotidae) from the Swedish west coast. *Zootaxa*, 4290 (2): 390-394. https://doi.org/10.11646/zootaxa.4290.2.11

Kieneke A., Zekely J. 2007. *Desmodasys abyssalis* sp. nov. – first record of a deep–sea gastrotrich from hydrothermal vents. *Mar. Biod. Rec.*, 1(e88), 1-7.

Kieneke A., Riemann O., Ahlrichs W. H. 2008. Novel implications for the basal internal relationschips of Gastrotricha revealed by an analysis of morphological characters. *Zool. Scr.*, 37: 429-460. http://dx.doi.org/10.1111/j.1463–6409.2008.00334.x

Kisielewski J. 1991. Inland-water Gastrotricha from Brazil. *Ann. Zool*., 43, 168 pp.

Kisielewski J. 1997. Brzuchorzeski (Gastrotricha). Fauna Słodkowodna Polski, Zeszyt 31. Wydawnictwo Uniwersytetu Łódzkiego, Łódź, 157 pp.

Kolicka M. 2016. Gastrotrichs in bromeliads – newly recorded *Chaetonotus* (*Hystricochaetonotus*) *furcatus* Kisielewski, 1991 (Chaetonotida) from the Łódź Palm House. *Zoosystema*, 38 (1): 141-155. http://dx.doi.org/10.5252/z2016n1a5

Kolicka M., Dabert M., Dabert J., Kånneby T., Kisielewski J. 2016. *Bifidochaetus*, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. *Invert. Syst.*, 30 (4): 398-419. http://dx.doi.org/10.1071/IS16001

Kolicka M., Gadawski P., Dabert M., 2017. A new species of freshwater Chaetonotidae (Gastrotricha, Chaetonotida) from Obodska Cave (Montenegro) based on morphological and molecular characters. *Eur. J. Taxon.*, 354, 1-30.

Kolicka M., Jankowska E., Kotwicki L. 2015. Baltic Sea Gastrotricha - one new species and one new record of Chaetonotida from Poland. *Zootaxa*, 4027: 487-508. http://dx.doi.org/10.11646/zootaxa.4027.4.2

Laumer C. E., Bekkouche N., Kerbl A., Goetz F., Neves R. C., Sørensen M. V., Kristensen R. M., Hejnol A., Dunn C. W., Giribet G., Worsaae K. 2015. Spiralian phylogeny informs the evolution of microscopic lineages. *Curr. Biol.*, 25 (15): 2000-2006. https://doi.org/10.1016/j.cub.2015.06.068

Leasi F., Todaro M. A. 2009. Meiofaunal cryptic species revealed by confocal microscopy: the case of *Xenotrichula intermedia* (Gastrotricha). *Mar Biol.*, 156: 1335-1346.

Leasi F., Todaro M. A. 2008. The muscular system of *Musellifer delamarei* (Renaud-Mornant, 1968) and other chaetonotidans with implications for the phylogeny and systematization of the Paucitubulatina (Gastrotricha). *Biol. J. Linn. Soc.*, 94: 379-398.

Levy D. P., Weiss M. J. 1980. Sperm in the life cycle of the freshwater gastrotrich, *Lepidodermella squamata*. *Am. Zool.*, 20: 749.

Mola P. 1932. Gastrotricha delle acque dolci italiane. *Int. Rev. Hydrobiol.*, 26: 397-423.

Paps J., Riutort M. 2012. Molecular phylogeny of the phylum Gastrotrich: New data brings together molecules and morphology. *Mol. Phylogenet. Evol.*, 63: 208-212.

Pfenninger M., Schwenk K. 2007. Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BMC Evol Biol.*, 7: 121.

Rao G. C., Clausen C. 1970. *Planodasys marginalis* gen. *et*. sp. nov. and Planodasyidae fam. nov. (Gastrotricha Macrodasyoidea). *Sarsia*, 42: 73-82.

Remane A. 1925. Organisation und systematische Stellung der aberranten Gastrotrichen. *Verh. Dtsch. Zool. Ges.*, 30: 121-128.

Remane A. 1929. Gastrotricha, In: W. Kükenthal & T. Krumback, eds. Handbuch der Zoologie, Band 2, Liefrung 6. Walter de Gruyter, Berlin.

Remane A. 1927a. *Xenotrichula velox* nov. gen. nov. spec., ein chaetonotoides Gastrotrich mit maennlichen Geschlectsorganen. *Zool. Anz.*, 71: 289-294.

Remane A., 1927b. Beiträge zur Systematik der Süßwassergastrotrichen. *Zool. Jahrb. (Syst. Oekol. Geogr. Tiere)*, 53: 269-320.

Remane A. 1934. Die Gastrotrichen des Küstengrundwassers von Schilksee. *Schr. Naturwiss. Ver. Schleswig-Holstein*, 20: 473-478.

Remane A. 1936. Gastrotricha, In: H. G. Bronns, ed. Klassen Ordnungen des Tierreichs, Band 4, Abteilung II, Buch I, Teil 2, Liefrungen 1-2. Akademie Verlagsgesellschaft, Berlin.

Renaud-Mornant J. 1986. Gastrotricha, In: L. Botosaneanu. Stygofauna Mundi: A faunistic, distributional, and ecological synthesis of the world fauna inhabiting subterranean waters (Including the Marine Interstitial). E.J. Brill, Leiden.

Ruttner-Kolisko A. 1955. *Rheomorpha neiswestnovae* und *Marinellina flagellata*, zwei phylogeneticsh interessante Wurmtypen aus dem Susswasserpsammon. *Österr. Zool. Z.*, 6: 55-69.

Schwank P. 1990. Gastrotricha. In: Schwoerbel J. & Zwick P. (eds), Süsswasserfauna von Mitteleuropa, Band 3.Gastrotricha und Nemertini. Gustav Fischer Verlag, Stuttgart, Jena, New York, 252 pp.

Suzuki T. G., Maeda M., Furuya H. 2013. Two new Japanese species of Gastrotricha (Chaetonotida, Chaetonotidae, Lepidodermella and Dichaeturidae, Dichaetura), with comments on the diversity of gastrotrichs in rice paddies. *Zootaxa*, 3691: 229-239.

Todaro M. A., Perissinotto R., Bownes S. J. 2013. Neogosseidae (Gastrotricha, Chaetonotida) from the iSimangaliso Wetland Park, KwaZulu-Natal, South Africa. *Zookeys*, 315: 77-94.

Todaro M. A, Leasi F., Bizzarri N., Tongiorgi P. 2006b Meiofauna densities and gastrotrich community composition in Mediterrarean sea cave. *Marine Biol*., 149: 1079-1091. http://dx.doi.org/10.1007/s00227-006-0299-z

Todaro M. A., Balsamo M., Kristensen R. M. 2005. A new genus of marine chaetonotids (Gastrotricha), with description of two new species from Greenland and Denmark. *J. Mar. Biol. Assoc UK.*, 25: 1391-1400.

Todaro M. A., Dal Zotto M., Leasi F. 2015. An integrated morphological and molecular approach to the description and systematisation of a novel genus and species of Macrodasyida (Gastrotricha). *PLoS ONE*, 10 (7): e0130278. https://doi.org/10.1371/journal.pone.0130278

Todaro M. A., Guidi L., Leasi F., Tongiorgi P. 2006b. Morphology of *Xenodasys* (Gastrotricha) the first species from Mediterranean Sea and estabilishment of *Chordodasiopsis* gen. nov. and Xenodasyidae fam. nov. *J. Mar. Biol. Assoc. UK.*, 86: 1005-1015. https://doi.org/10.1017/S0025315406013981

Todaro M. A., Leasi F., Bizzarri N., Tongiorgi, P. 2006a. Meiofauna densities and gastrotrich community composition in a Mediterranean sea cave. *Mar. Biol.*, 149: 1079-1091.

Todaro M. A., Leasi F., Hochberg R. 2014. A new species, genus and family of marine Gastrotricha from Jamaica, with a phylogenetic analysis of Macrodasyida based on molecular data. *Syst Biodivers*., 12 (4): 473-488.

Todaro M. A. 2020. Gastrotricha. World Register of Marine Species. Available from: http://www.marinespecies.org/ aphia.php?p=taxdetails&id=2078 (accessed 10 January 2020)

Von Und zu Gilsa A., Kieneke A., Hochberg R., Schmidt-Rhaesa A. 2014. Two new species of the genus Dactylopodola (Gastrotricha: Macrodasyida) from the Bahamas, with an updated key to the genus. *Cah. Biol. Mar.*, 55 (3): 333-345.

Wirz A., Pucciarelli S., Miceli C., Tongiorgi P., Balsamo M., 1999. Novelty in Phylogeny of Gastrotricha: Evidence from 18S rRNA Gene. *Mol. Phyl. Evol.*, 13 (2): 314-318.

Zrzavý J. 2003. Gastrotricha and metazoan phylogeny. *Zool. Scr.*, 32: 61-81. https://doi.org/10.1046/j.1463-6409.2003.00104.x

DOCTORAL DISSERTATION

List of original publications

1. **Kolicka M.**, Dabert M., Dabert J., Kånneby T. Kisielewski J. 2016. *Bifidochaetus*, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. *Invertebrate Systematics*, 30 (4): 398-419. https://doi.org/10.1071/IS16001
2. **Kolicka M.**, Dabert M., Gadawski P. 2017. A new species of freshwater Chaetonotidae (Gastrotricha, Chaetonotida) from Obodska Cave (Montenegro) based on morphological and molecular characters. *European Journal of Taxonomy*, 354: 1-30. https://doi.org/10.5852/ejt.2017.354
3. **Kolicka M.** 2017. New Arctic Gastrotricha — Three *Chaetonotus* (Chaetonotidae) from Longyearbyen (Spitsbergen, Svalbard Archipelago). *Annales Zoologici*, 67 (1): 131-180. https://doi.org/10.3161/00034541ANZ2017.67.1.015
4. **Kolicka M.**, Kotwicki L., Dabert M. 2018. Diversity of Gastrotricha on Spitsbergen (Svalbard Archipelago, Arctic) with a Description of Seven New Species. *Annales Zoologici*, 68 (4): 609-739. https://doi.org/10.3161/00034541ANZ2018.68.4.001
5. **Kolicka M.** 2019. From saline to salty? *Heterolepidoderma sinus* (Chaetonotida, Chaetonotidae) from subsaline coal mine settling ponds. *Zootaxa*, 4559 (3): 568-572. https://doi.org/10.11646/zootaxa.4559.3.7
6. **Kolicka M.** 2019. Gastrotricha – not only in sediments: new epiphytic species of Chaetonotida from the Jubilee Greenhouse of the Botanical Garden in Kraków. *European Journal of Taxonomy*, 511: 1-100. https://doi.org/10.5852/ejt.2019.511
7. **Kolicka M.** 2019. New *Chaetonotus* (*Wolterecka*) *semovitus* sp. nov. (Gastrotricha: Chaetonotida: Chaetonotidae) from a Palm House in Vienna (Austria). *Annales Zoologici*, 69 (2): 447-475. https://doi.org/10.3161/00034541ANZ2019.69.2.011
8. Garraffoni A. R. S., Kieneke A., **Kolicka M.**, Corgosinho P. H. C., Prado J., Nihei S. S., Freitas A. V. L. 2019. ICZN Declaration 45: a remedy for the nomenclatural and typification dilemma regarding soft-bodied meiofaunal organisms? *Marine Biodiversity*, https://doi.org/10.1007/s12526-019-00983-7

Poznań, 16.12.2019r.

Małgorzata Kolicka
Adam Mickiewicz University, Poznań
Faculty of Biology
Department of Animal Taxonomy and Ecology
Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Declaration

I declare that the publication: **Kolicka M.**, Dabert M., Dabert J., Kånneby T. Kisielewski J. 2016. *Bifidochaetus*, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. *Invertebrate Systematics*, 30 (4): 398-419, has been prepared as a part of my doctoral dissertation under the scientific supervision of Prof. UAM, dr hab. Ziemowit Olszanowski.

Yours faithfully,

Poznań, 16.12.2019r.

Małgorzata Kolicka
Uniwersytet im. Adama Mickiewicza, Poznań
Wydział Biologii
Zakład Taksonomii i Ekologii Zwierząt
ul. Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Oświadczenie

Oświadczam, że publikacja: **Kolicka M.**, Dabert M., Dabert J., Kånneby T. Kisielewski J. 2016. *Bifidochaetus*, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. *Invertebrate Systematics*, 30 (4): 398-419, została wykonana na poczet mojej rozprawy doktorskiej pod opieką promotora Prof. UAM dra hab. Ziemowita Olszanowskiego.

Z wyrazami szacunku,

CSIRO PUBLISHING

Invertebrate Systematics, 2016, **30**, 398–419
http://dx.doi.org/10.1071/IS16001

Bifidochaetus, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach

Małgorzata Kolicka[A,E], *Miroslawa Dabert*[B], *Jacek Dabert*[C], *Tobias Kånneby*[D] *and Jacek Kisielewski*[A]

[A]Department of Animal Taxonomy and Ecology, Institute of Environmental Biology, Faculty of Biology, Adam Mickiewicz University in Poznań, Umultowska 89, 61–614 Poznan, Poland.
[B]Molecular Biology Techniques Laboratory, Faculty of Biology, Adam Mickiewicz University in Poznań, Umultowska 89, 61–614 Poznań, Poland.
[C]Department of Animal Morphology, Institute of Environmental Biology, Faculty of Biology, Adam Mickiewicz University in Poznań, Umultowska 89, 61–614 Poznań, Poland.
[D]Department of Zoology, Swedish Museum of Natural History, PO Box 50007, SE–104 05 Stockholm, Sweden.
[E]Corresponding author. Email: kolicka@amu.edu.pl

Abstract. Gastrotricha is a cosmopolitan phylum of aquatic and semi-terrestrial invertebrates that comprises ~820 described species. To date, freshwater gastrotrichs have not been the subject of faunistic or taxonomic research in the polar regions. In this paper, we present the first species-level description of a freshwater gastrotrich from the Arctic (Svalbard Archipelago). Evidence from morphology, morphometry and molecular analyses reveals that the species represents a new genus in Chaetonotidae: *Bifidochaetus arcticus*, gen. *et* sp. nov. Taking into consideration many morphological similarities to *Chaetonotus (Primochaetus) veronicae* Kånneby, 2013 we propose to include *C. (P.) veronicae* in the newly established genus under the new combination *Bifidochaetus veronicae* (Kånneby, 2013), comb. nov. In the phylogenetic analysis based on nuclear 18S rRNA, 28S rRNA and mitochondrial cytochrome *c* oxidase subunit I sequence data, *B. arcticus*, gen. *et* sp. nov. is nested within the family Chaetonotidae, as the sister group to the genus *Lepidochaetus* Kisielewski, 1991. In this paper we also present new taxonomic characters useful for gastrotrich taxonomy: the pharynx-to-intestine length ratio (I) and the spine bifurcation ratio (B).

Received 1 January 2016, accepted 14 April 2016, published online 31 August 2016

Introduction

Gastrotricha is a group of microscopic acoelomate metazoans with a total body length of 50–3500 µm (Kisielewski 1997; Balsamo *et al*. 2014; Kieneke and Schmidt-Rhaesa 2015). The phylum is divided into two orders: Chaetonotida Remane, 1925 [Rao & Clausen, 1970] and Macrodasyida Remane, 1925 [Rao & Clausen, 1970]. Gastrotrichs can be found in virtually all aquatic environments, including semi-aquatic ecosystems (peatbogs, alder woods, riparian forests, etc.) in both natural and artificial habitats (Kolicka *et al*. 2013; Kolicka 2014). This includes microreservoirs that form between the leaves of Bromeliaceae species (Kisielewski 1991; Kolicka 2016), water in caves (e.g. Todaro *et al*. 2006), and even extreme ecosystems such as hot springs (Guerne 1888) and hydrothermal vent zones (Kieneke and Zekely 2007). Despite their abundance in various habitats, gastrotrichs have often been neglected in biodiversity surveys and ecological studies, or mentioned only as a group without identification to the species level (e.g. Kotwicki *et al*. 2005; Jankowska *et al*. 2014). The ~820 described species probably represent only a small fraction of their real taxonomic diversity (Balsamo *et al*. 2015; Todaro 2015).

Although marine gastrotrichs from the Arctic have been reported in several papers (Pfannkuche and Thiel 1987; Węsławski *et al*. 1997; Włodarska-Kowalczuk *et al*. 1999; Todaro *et al*. 2005, 2009; Urban-Malinga *et al*. 2005; Balsamo *et al*. 2010; Grzelak and Kotwicki 2012; Górska *et al*. 2014), none from the Svalbard Archipelago were identified to species level. Also, the sparse freshwater gastrotrich observations from the Svalbard area are limited only to the level of phylum or genus (Scourfield 1897; De Smet *et al*. 1987, 1988; De Smet 1993; Coulson *et al*. 2014). Furthermore, the observed genus *Chaetonotus* Ehrenberg, 1830, which is the most species-rich group within Chaetonotidae Gosse, 1864 (*sensu* Leasi & Todaro, 2008), is currently regarded as a polyphyletic taxon encompassing species from various evolutionary lineages (Balsamo *et al*. 2008, 2014; Kånneby *et al*. 2012, 2013).

© The authors 2016

Because the genus is virtually cosmopolitan and probably unnatural, reporting its presence without indicating the species is largely uninformative.

The taxonomy of Gastrotricha still relies primarily on morphological characters (Balsamo *et al*. 2008, 2014; Kånneby *et al*. 2012, 2013); however, molecular data suggest that such an approach may be a misleading indicator of phylogenetic relationships (e.g. Kånneby *et al*. 2012, 2013; Balsamo *et al*. 2014). Comprehensive phylogenetic analyses based on morphological characters also indicate that relying on generally accepted features such as the presence and shape of scales and spines can be misleading and not necessarily directly correlated with phylogenetic relationships (Hochberg and Litvaitis 2000; Balsamo *et al*. 2008; Kånneby *et al*. 2012, 2013). Even though knowledge regarding gastrotrichs continues to progress, there is still a lack of a unified set of key characters that should be taken into account when describing a new species. This problem arises from the necessity of working with live specimens, hence there is a short time before the animal deteriorates, and important diagnostic characters become increasingly more difficult to study. Another drawback is that none of the standard fixation methods have been completely successful. Even well-fixed material does not lead to high-quality permanent mounts (archival type specimens), so more appropriate documentation, especially for freshwater chaetonotids, is often recommended such as microphotographs, illustrations and detailed measurements (Kisielewski 1981).

The estimated number of marine gastrotrich species suggests that only ~20% of α diversity is currently known for these animals (Appeltans *et al*. 2012). Similar estimates are also available for the number of freshwater taxa in Europe (Balsamo *et al*. 2015), and they suggest that most freshwater gastrotrich species are so far undescribed. This is due to the low number of specialists working on this phylum, as reflected in part in the fact that new species are described on a regular basis. In contrast to other groups of small invertebrates, such as Tardigrada, which are relatively well-researched in the Arctic (for a reviews see Zawierucha *et al*. 2013, 2015), the diversity of gastrotrich species in the area of Spitsbergen as well as all the rest of Svalbard is still unknown.

In this study, we describe a new freshwater gastrotrich genus and species from the Arctic (the Svalbard Archipelago) based on morphology, morphometrics and genetic data. In light of our results, *Chaetonotus* (*Primochaetus*) *veronicae* Kånneby, 2013 is also included in the newly established genus. We also give a unified terminology for morphological characters in Gastrotricha. As a consequence of detailed morphological analysis, we introduce two new characters: the pharynx-to-intestine length ratio (I) and the spine bifurcation ratio (B).

Materials and methods

Study area

The study was conducted in Spitsbergen, the largest island of the Svalbard Archipelago. Svalbard is a high Arctic archipelago located between 74°–81°N and 10–35°E. It comprises five main islands (Spitsbergen, Nordaustlandet, Edgeøya, Barentsøya and Bjørnøya) with a total surface area of ~63 000 km^2, ~60% of which is permanently covered by snow and ice (Hagen *et al*. 2003). Geographically the islands are remote: they are located 700 km off the coast of northern Norway, which is the nearest mainland, and 600 km east of Greenland (Coulson 2007, 2013; Pilskog *et al*. 2014). The mean annual temperature in Spitsbergen is −6.7°C. The mean temperatures during the warmest month of the year, August, peak at +5.9°C, while in February, which is usually the coldest month, the average temperature reaches −14.6°C. The range of extreme temperatures listed for this island extends from −42.2°C to 17.0°C (Coulson 2013). The summer is short and lasts only four months, i.e. June through September, with a mean air temperature above 0°C. The ground may be snow-covered and the soil frozen for nine months during a year (Coulson *et al*. 1995; Norwegian Meteorological Institute 2014).

Sampling and documentation

The samples were collected on 7 and 8 August 2013 from temporary, small and shallow water reservoirs originating from melting snow or ice and surface runoff. These reservoirs were located in Longyearbyen, the capital city of the Svalbard Archipelago, at a distance of ~300–3000 m from the fjord coast (Fig. 1). Specimens of the new species were collected from the reservoir located ~500 m from the sea. Bottom sediments had organic and organic-clay characters and fine structure.

The top layer of bottom sediment together with ambient water from five reservoirs were collected by hand from shallow water zones into 100-cm^3 plastic containers. Approximately 10–20 cm^3 of the sediment and 80–90 cm^3 of ambient water were placed into each container. The collected samples were subsequently placed in an isothermal box and transported to the laboratory within 24 h. In the laboratory, samples were oxygenated and placed in a thermal chamber at a temperature of 10°C to create conditions similar to those at the sampling site. Within 10 days of collection, a total of 0.3 cm^3 of sediment from each sample was analysed. Specimens of Gastrotricha were extracted with a micropipette under an Olympus SZ51 stereomicroscope (Tokyo, Shinjuku, Japan). All specimens were observed, photographed and documented alive under an Olympus BX41 compound microscope (Tokyo, Shinjuku, Japan) equipped with phase contrast and an Array Artcam 300 digital camera (Tokyo, Shinjuku, Japan). Morphometric characters were measured in cellSens Entry 1.11 software (Olympus). All measurements are given in micrometres (µm) and all formulas are given as a percentage (%).

Morphological analyses

Measurements are provided for all sampled specimens of the new species and are given as ranges (Tables S1–S3). Because of the low taxonomic utility of fixed gastrotrichs, each specimen was photographed. The species description follows Hummon *et al*. (1992), where the position of certain morphological characters is given in units (U) proportional to total body length measured from the anterior end to the distal end of the furca. The present study uses a modified version of Kisielewski's (1991) convention to identify Gastrotricha by means of particular morphological measurements and indices

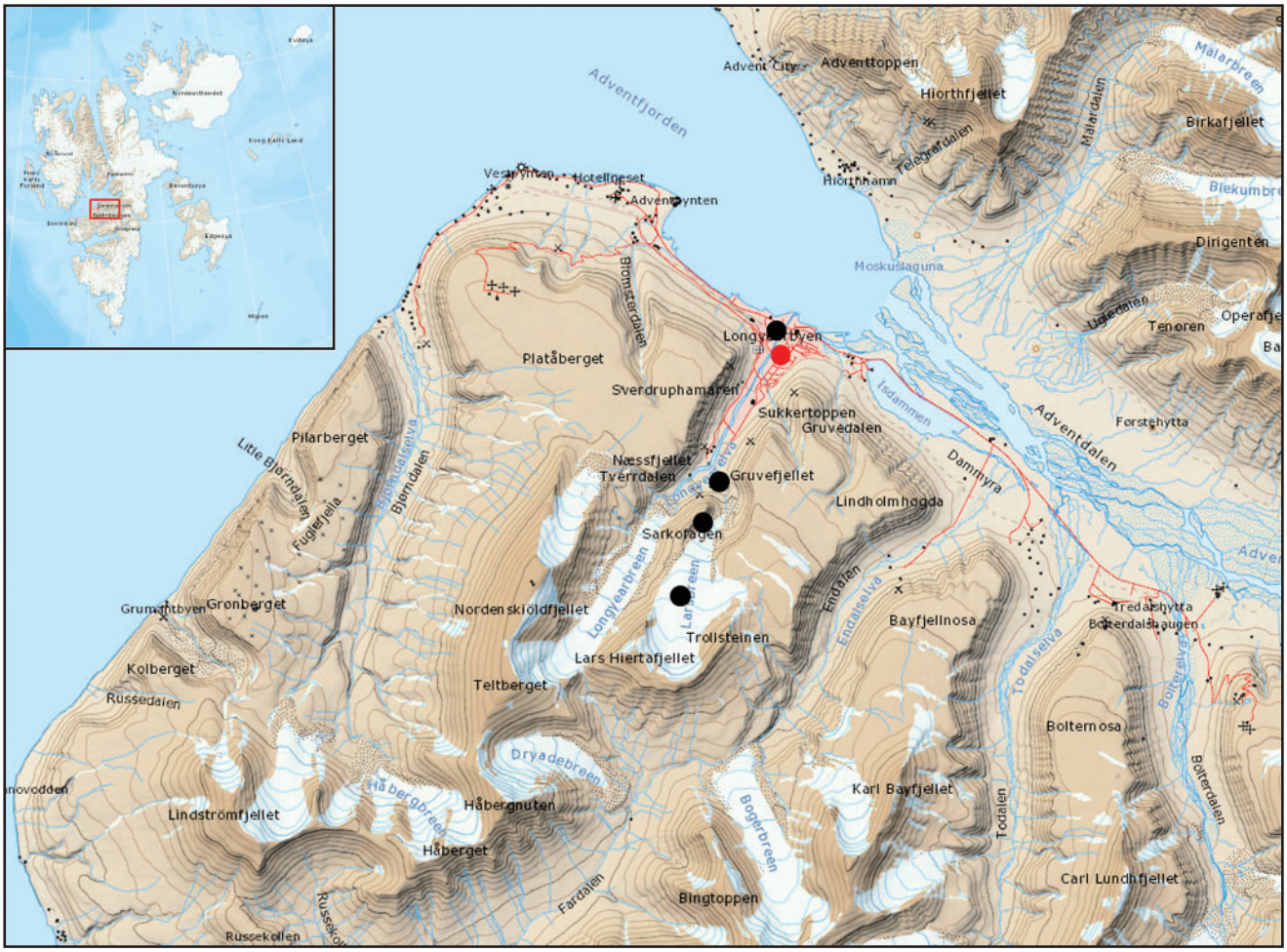


Fig. 1. Longyearbyen (Svalbard Archipelago). Study area with marked sampling places. Sites of reservoirs with gastrotrichs are marked in red is marked the site of reservoir with gastrotrichs.

(formulas and ratios). All morphological terms and measurable characters used in this study are listed in Figs S1–S3. This study also proposes a new index: the pharynx-to-intestine length ratio (I), which determines the age of a given specimen. The pharynx-to-intestine length ratio is the quotient of pharynx length and intestine length: $I = \frac{pharynx\ length}{intestine\ length} * 100\%$ (Fig. 2*A*). Juvenile and subadult specimens have a significantly higher I-ratio than adults. Furthermore, due to the presence of bifid spines in the taxon of interest, the spine bifurcation ratio (B) was introduced. The ratio is the quotient of the length of the bifid section of the spine and the length of the entire spine, expressed as a percentage: $B = \frac{length\ of\ the\ bifid\ section\ of\ the\ spine}{length\ of\ the\ entire\ spine} * 100\%$ (Fig. 2*B*).

Data accessibility

Microphotographs of the holotype and six paratypes (five adults and one subadult) were deposited in the Natural History Collections at Adam Mickiewicz University in Poznan under accession number NHC–GBA–5–1–20/h (holotype) and NHC–GBA–5–21–170/p (paratypes) and in the Morphbank under accession numbers 859852 (holotype); 859869, 859870, 859871, 859873, 859874, 859875 (paratypes).

Molecular data

Total genomic DNA was extracted from single specimens of the new species (six adults and one subadult; seven samples in total) using the DNeasy Blood and Tissue Kit (Qiagen GmbH, Hilden, Germany) as described by Dabert *et al.* (2008). A 670-bp fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was amplified with a 1 : 1 mixture of two forward primers, bcdF08 and bcdF09 (developed in this study), and reverse primer bcdR04 (Dabert *et al.* 2010). A complete sequence of 18S rRNA was amplified in two overlapping fragments using 18Sfw/rev960 and fw390/rev18S primer pairs (Dabert *et al.* 2010), respectively. A 2365-bp fragment of 28S rRNA was amplified using the primer pair U178Forward/2450R (Telford *et al.* 2003). Details of the primers used in this study are given in Table 1. Polymerase chain reactions were carried out in 10-μL reaction volumes containing 5 μL of the Type-it Microsatellite PCR Kit (Qiagen, Hilden, Germany), 0.5 μM of each primer and 4 μL of the DNA template using a thermocycling profile with one cycle of 5 min at 95°C followed by 35 steps each of 30 s at 95°C, 90 s at 50°C, 1 min at 72°C and with a final step of 5 min at 72°C for all reactions.

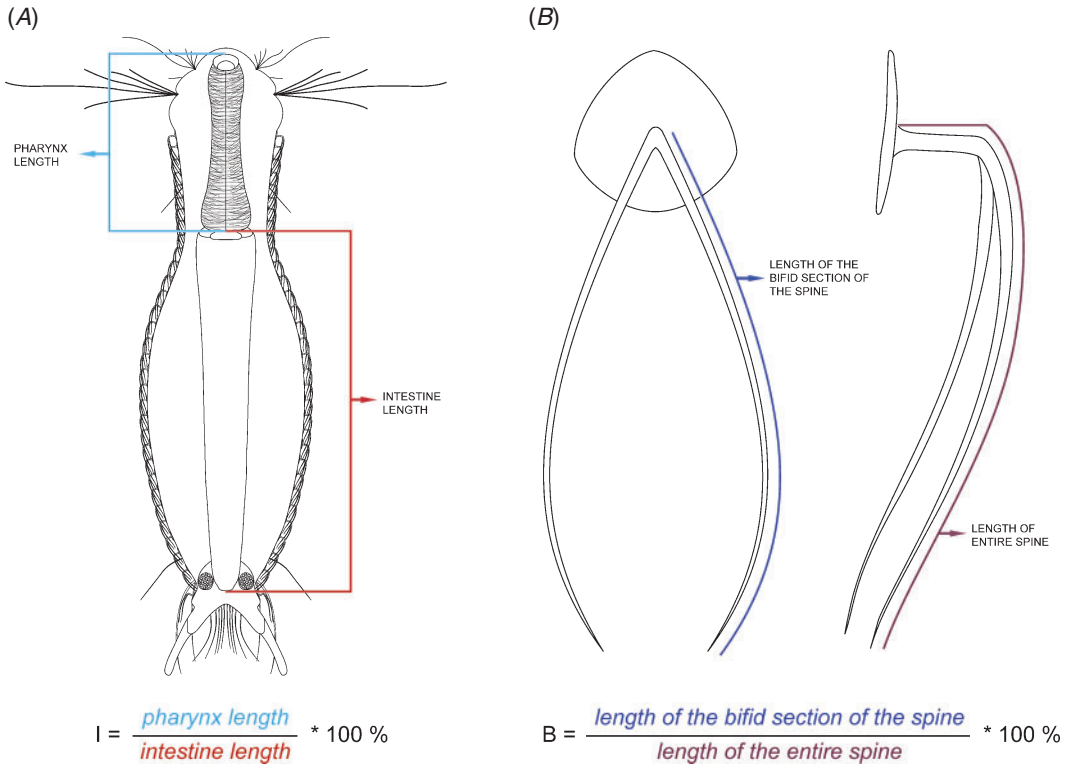


Fig. 2. Mode of measuring Chaetonotidae new indexes: (*A*) pharynx-to-intestine length ratio (I); (*B*) spine bifurcation ratio (B).

Table 1. Polymerase chain reaction primers used in this study
A and S refer to amplifying and sequencing, respectively

Primer name	Sequence (5′–3′)	Product	Use	Source
bcdF08	CGATGRTTTTTTTCHACWAACCAYAARGATATCGG	COI	A	This study
bcdF09	CGATGRTTTTTTTCHACWAACCAYAARGACATTGG	COI	A, S	This study
bcdR04	TATAAACYTCDGGATGNCCAAAAAA	COI	A, S	Dabert *et al.* 2010
18Sfw	CTTGTCTCAAAGATTAAGCCATGCA	18S rRNA	A, S	Dabert *et al.* 2010
rev480	GTTATTTTTCGTCACTACCT	18S rRNA	S	Dabert *et al.* 2010
fw390	AATCAGGGTTCGATTCCGGAGA	18S rRNA	A, S	Dabert *et al.* 2010
rev960	GACGGTCCAAGAATTTCAC	18S rRNA	A, S	Dabert *et al.* 2010
fw1230	TGAAACTTAAAGGAATTGACG	18S rRNA	S	Dabert *et al.* 2010
rev1460	CATCACAGACCTGTTATTGC	18S rRNA	S	Dabert *et al.* 2010
fw770	ACTTTGAAAAAATTAGAGTGC	18S rRNA	S	Dabert *et al.* 2010
rev18S	TGATCCTTCCGCAGGTTCACCT	18S rRNA	A, S	Dabert *et al.* 2010
U178Forward	GCACCCGCTGAAYTTAAG	28S rRNA	A, S	Dabert *et al.* 2010
1634LReverse	ATTCGGCAGGTGAGTTGTTACA	28S rRNA	S	Telford *et al.* 2003
1200F	CCCGAAAGATGGTGAACTATGC	28S rRNA	S	Telford *et al.* 2003
2450R	GCTTTGTTTTAATTAGACAGTCGGA	28S rRNA	A, S	Telford *et al.* 2003
300F	CAAGTACCGTGAGGGAAAGTTG	28S rRNA	S	Telford *et al.* 2003
300R	CAACTTTCCCTCACGGTACTTG	28S rRNA	S	Telford *et al.* 2003
1200R	GCATAGTTCACCATCTTTCGG	28S rRNA	S	Telford *et al.* 2003
UJR2176	CGGATCTAATTTGCCGACTTCCCTTA	28S rRNA	S	Telford *et al.* 2003
1600F	AGCAGGACGGTGGCCATGGAAG	28S rRNA	S	Telford *et al.* 2003

After amplification, the PCR products were diluted with 10 μL of MQ water; 5 μL of the diluted PCR reaction was analysed by electrophoresis on 1% agarose gel. Samples containing visible bands were purified with exonuclease I and Fast alkaline phosphatase (Fermentas, Waltham, MA) and sequenced using a BigDye Terminator v3.1 kit and an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, Waltham, MA), following the manufacturer's instructions. Individual sequence reads were

aligned and manually assembled into contigs in ChromasPro v. 1.32 (Technelysium, Brisbane, Australia) and GeneDoc v. 2.7.000 (Nicholas and Nicholas 1997). Genetic distance among the COI sequences was estimated using the Kimura 2-parameter model as implemented in MEGA 6 (Tamura *et al*. 2013).

Phylogenetic analyses

The nucleotide blast search of COI, 18S and 28S rRNA sequences of *Bifidochaetus arcticus*, gen. *et* sp. nov. suggested *Heterolepidoderma macrops* Kisielewski, 1981, *Ichthydium (Furficulichthys) skandicum* Kånneby, Todaro *et* Jondelius, 2009 and *Lepidochaetus zelinkai* (Grünspan, 1908) as the most similar taxa. Therefore, in our phylogenetic analyses we used representatives of the main clades reconstructed in the published molecular phylogeny of Chaetonotidae (Kånneby *et al*. 2013), including species that displayed some potential morphological affinities with the new species, as well as representatives of all species that clustered with *H. macrops*, *I. (F.) skandicum* and *L. zelinkai*. As the outgroup we used sequences of *Aspidiophorus polystictos* Balsamo *et* Todaro, 1987, which has been reconstructed as the sister group to all other species of Chaetonotidae by Kånneby *et al*. (2013). In total, 47 terminals were included in the analysis (Table 2).

Both rRNA markers were initially aligned by ClustalX 1.81 (Thompson *et al*. 1997) with default parameters; if necessary, the obtained alignment was subsequently adjusted manually. No hypervariable alignment positions in 28S rRNA were excluded from the dataset. The DNA substitution models for 18S and 28S rRNAs were tested with jModelltest 0.11 (Posada 2008) and MrModelltest (Nylander 2004) for maximum likelihood (ML) and Bayesian inference (BI), respectively. The GTR+I+G model was appropriate for both markers. For COI mtDNA sequences, the two-rate codon-based model was applied (Goldman and Yang 1994) with the invertebrate mtDNA genetic code. Tree inference was performed for the individual genes and for a concatenated dataset of the three genes using ML and BI with Markov Chain Monte Carlo. For the concatenated dataset, partitioned ML and BI analyses were performed with the appropriate substitution model for each partition. Maximum likelihood trees were searched using Garli v. 2.0 (Zwickl 2006) with 30 search replications. Bayesian inference analyses of four independent chains were run on a parallel version of MrBayes 3.2 (Ronquist *et al*. 2012) using the online Poznan Supercomputing Network Center at the Institute of Bioorganic Chemistry of the Polish Academy of Sciences, Poznan, Poland. Each run of the BI analyses was performed in 3–10 $\times$ 10^6 generations, and the trees were sampled every 1000th generation. The final 50% majority rule consensus tree was generated after discarding the 25% burn-in fraction of initial trees after assessing the chain convergence in Tracer v. 1. (Rambaut and Drummond 2007) judged by the average standard deviation of split frequencies dropping below 0.01. Support for the tree branches was calculated by the non-parametric bootstrap method (BS; Felsenstein 1985) with 100 replicates for ML analyses. Tree editing was performed using the programs FigTree 1.4.2 (Rambaut 2014) and TreeView 1.66 (Page 1996) and the tree editing tool of MEGA 6 (Tamura *et al*. 2013).

Results

In the recorded material, 308 specimens belonging to eight species were found, including 42 specimens of the new species. The remaining species found in the material belong to the subgenera *C. (Chaetonotus)* Ehrenberg, 1830 and *C. (Hystricochaetonotus)* Schwank, 1990. These species are also new to science and will be described in future publications.

Taxonomic account

Phylum **GASTROTRICHA** Mečnikow, 1865

Order **CHAETONOTIDA** Remane, 1925 [Rao & Clausen, 1970]

Suborder **PAUCITUBULATINA** d'Hondt, 1971

Family **CHAETONOTIDAE** Gosse, 1864 (*sensu* Leasi & Todaro, 2008)

Subfamily **CHAETONOTINAE** Gosse, 1864 (*sensu* Kisielewski, 1991)

Genus ***Bifidochaetus*** Kolicka *et* Kisielewski, gen. nov.

Type species: *Bifidochaetus arcticus* Kolicka *et* Kisielewski, gen. *et* sp. nov.

Terra typica: Spitsbergen, Svalbard Archipelago, Norway.

Locus typicus: Small water reservoir in Longyearbyen (78°13′14.28″N, 15°38′37.14″E).

http://zoobank.org/urn:lsid:zoobank.org:act:793CDB50-09EF-48B6-9ADA-41850438A9DB

Diagnosis

Total body length ranging from 107 to 154 µm. Dorsal, dorsolateral, lateral, ventrolateral and ventral sides of the body covered with thin, one-lobed scales without keels and notches. Scales located close to one another, without or with overlapping edges. Scales present on the inner part of the furca. Most body spines emerge vertically from the scales, strongly curved and divide into two spines of equal length. The pharyngeal section and a large part of the intestinal section of the ventral interciliary field are naked; scales present only in the posteriormost part of the field. The ventral interciliary field terminal scales large and with a keel. Head clearly five-lobed with a distinct cephalion, epipleuria and hypopleuria marked in the body outline. Two pairs of cephalic ciliary tufts. Posterior tufts with straighter and clearly thicker cilia than in the anterior tufts. Mouth ring located subterminally. Mouth ring internal reinforcements weakly separated, long and rod-like. Long cuticular bristles inside the mouth ring. Pharynx with weak anterior enlargement, then gradually widened towards the posterior end. Furcal base clearly marked, with external base of furcal appendages bearing hummocks with spined scales. Furca measures from 16.1 to 20.6 µm. Adhesive tubes thick, rigid and not tapered

Table 2. DNA sequences used in phylogenetic analysis

Species	GenBank acc. nos		
	18S	28S	COI
Arenotus strixinoi	JQ798537	JQ798608	JQ798677
Aspidiophorus ophiodermus	JN185463	JN185510	–
Aspidiophorus polystictos	JQ798597	JQ798664	JQ798726
Aspidiophorus tetrachaetus	JN185505	JN185540	JN185576
Chaetonotus (*Primochaetus*) *armatus*	JQ798594	–	JQ798723
Chaetonotus sp.	JQ798555	–	JQ798692
Chaetonotus (Primochaetus) acanthocephalus	JQ798569	–	JQ798701
Chaetonotus (Hystricochaetonotus) cf. *novenarius*	JQ798566	JQ798636	JQ798699
Chaetonotus (Chaetonotus) cf. *sphagnophilus*	JQ798604	JQ798671	JQ798733
Chaetonotus (Primochaetus) heideri 1	JQ798547	JQ798619	JQ798685
Chaetonotus (Primochaetus) heideri 2	JQ798590	JQ798657	JQ798720
Chaetonotus (Chaetonotus) maximus 1	JQ798577	–	JQ798709
Chaetonotus (Chaetonotus) maximus 2	JQ798574	JQ798646	JQ798706
Chaetonotus (Hystricochaetonotus) hystrix 1	JQ798557	JQ798627	–
Chaetonotus (Hystricochaetonotus) hystrix 2	JQ798603	JQ798670	JQ798732
Chaetonotus (Marinochaetus) mariae	JQ798558	JQ798628	–
Chaetonotus (Primochaetus) acanthodes 1	JQ798544	JQ798616	JQ798682
Chaetonotus (Primochaetus) acanthodes 2	JQ798552	JQ798624	–
Chaetonotus (Schizochaetonotus) dispar	JQ798561	JQ798631	JQ798696
Chaetonotus (Schizochaetonotus) neptuni 1	JQ798539	JQ798610	JQ798679
Chaetonotus (Schizochaetonotus) neptuni 2	JQ798595	JQ798662	JQ798724
Chaetonotus (Schizochaetonotus) schultzei	JQ798596	JQ798663	JQ798725
Chaetonotus (Wolterecka) uncinus	JQ798540	JQ798611	–
Chaetonotus (Zonochaeta) bisacer	JQ798565	JQ798635	–
Bifidochaetus arcticus, gen. *et* sp. nov.	KP713403	KP713404	KP713405
Halichaetonotus euromarinus	JQ798551	JQ798623	–
Halichaetonotus sp. 1	JQ798600	JQ798667	JQ798729
Halichaetonotus sp. 2	JQ798560	JQ798630	JQ798695
Halichaetonotus aculifer	JQ798550	JQ798622	JQ798688
Halichaetonotus paradoxus	JQ798599	JQ798666	JQ798728
Heterolepidoderma loricatum	JQ798541	JQ798612	–
Heterolepidoderma macrops	JN185469	JN185515	JN185548
Heterolepidoderma ocellatum	JN185475	JN185519	JN185554
Heterolepidoderma sp.	JQ798554	–	JQ798691
Ichthydium (Furficulichthys) skandicum	JQ798573	JQ798645	JQ798705
Lepidochaetus brasilense 1	JN185458	JN185507	JQ798680
Lepidochaetus brasilense 2	JN185495	JQ798658	JN185568
Lepidochaetus zelinkai	JN185486	JN185527	JN185564
Lepidodermella intermedia	JN185468	JN185514	JN185547
Lepidodermella minor minor	JN185474	–	JN185553
Polymerurus nodicaudus 1	JN185460	–	JN185542
Polymerurus nodicaudus 2	JN185502	JN185537	JN185573
Polymerurus nodicaudus 3	JN185465	JN185512	JQ798689
Polymerurus nodicaudus 4	JN185473	JQ798642	JN185552
Polymerurus rhomboides 1	JQ798584	–	JQ798715
Polymerurus rhomboides 2	JN185467	JN185513	JN185546
Polymerurus rhomboides 3	JN185493	JN185533	JN185567

towards the blunt slightly rounded distal ends; measure from 9.0 to 12.0 μm.

Taxonomic affinities

Bifidochaetus, gen. nov. shows a set of morphological features justifying its placement in the family Chaetonotidae: head separated from the trunk by a constricted neck; cephalic plates and ciliary tufts as well as dorsal sensory bristles present; furca with a pair of distal adhesive tubes present in the posterior end; body surface covered with cuticular scales and spines; ventral interciliary field with scales and a larger pair of ventral interciliary field terminal scales; ventral locomotory cilia in two separate longitiudinal bands. Moreover, molecular data also provide strong evidence for placement *Bifidochaetus*, gen. nov. within Chaetonotidae (see Figs 15, S4–S6).

The new genus presents a set of unique characters that separate it from all existing members of Chaetonotidae including: (1) thin rounded scales with distally bifurcating hair-like spines and (2) a bottle-shaped body with the

hummock at the external base of the furcal appendages. Despite these differences, the new taxon shares some similarities with the various subgenera of *Chaetonotus*. For example, the new taxon has rounded scales that lack a keel or posterior notch and also has spines that emerge from the centre or posterior scale region: similar features are present in *C. (Marinochaetus)* Kisielewski, 1997 and *C. (Primochaetus)* Kisielewski, 1997. The new taxon is also characterised by a spine type with strong diametrically opposed lateral branches similar to strong diametrically opposite lateral denticles on spines found in *C. (Schizochaetonotus)* Schwank, 1990. Lastly, the new taxon shares with *C. (Tristratachaetus)* Kolicka, Kisielewski, Nesteruk & Zawierucha, 2013 the presence of closely arranged scales and spines that emerge vertically from the centre of the scale and curve strongly beyond the short basal segment. Moreover, the new taxon has spines with a vertically emerging basal section that is reminiscent of the peduncle in *Aspidiophorus* Voigt, 1903.

Etymology

From the Latin '*bifidus*' (divided into two parts) and Greek 'khaite' (long hair, referring to the unique double spine).

Bifidochaetus arcticus Kolicka *et* Kisielewski, gen. *et* sp. nov.

(Figs 3–15; Tables S1–S3)

http://zoobank.org/urn:lsid:zoobank.org:act:5725037D-AF42-4E52-A8B5-7DEB4219F085

Material examined

One sample, 42 specimens (36 adults, five subadults, one juvenile), all photographed.

Diagnosis

Adult total body length ranging from 137 to 154 µm. Juvenile and subadult total body length ranging from 107 to 139 µm. Scales subtriangular with strongly rounded, convex edges, located close to one another but without overlapping edges (Figs 5, 11*A*, *C*). Scale size differentiated within the body and all scales well distinct. Scales distributed in 36–45 longitudinal alternating rows with 30–42 scales in the central row. Entire scale surface lies on the cuticle. Three pairs of parafurcal scales with bifid spines are present on the lateral sides of the furcal appendages. Scales present on the inner part of the furca. The ventral interciliary field terminal scales separated, very large, oval with narrowed anterior part and with keel (Figs 4*B*, 14*D*). Head clearly five-lobed with a distinct cephalion, epipleuria and hypopleuria well marked in the body outline (Fig. 7). Two pairs of cephalic ciliary tufts. Posterior tufts with straighter and clearly thicker cilia than in the anterior tuft (Fig. 3*A*). Pharynx length in adult specimens ranging from 35.8 to 43.1 µm, in juvenile and subadult specimens ranging from 34.2 to 43.6 µm. Pharynx with weak anterior enlargement, then gradually widened towards the posterior end (Figs 4*A*, 11*A*). Intestine straight with anterior section marked as a narrow bend. Three pairs of dorsal sensory bristles. Furcal base clearly marked, with external base of furcal appendages bearing hummocks with spined scales (Fig. 14*C*). Pear-shaped furcal indentation (Figs 3, 4, 7–9).

Description

This new species has a slender, bottle-shaped body with a clearly marked neck constriction and furcal base (Figs 3, 4, 7). The head is five-lobed and parabolic in shape. Cephalion and pleuria are separated. However, the presence of strongly curved spines around the cephalion, epipleuria and hypopleuria gives the impression that the plates are weakly marked and almost invisible in the body outline (Figs 3, 4, 7–9). The cephalion (U1–U4) is narrow and adheres to the head along the entire length. The epipleuria (U5–U8) and hypopleuria (U9–U13) are similar in size and separated by shallow notches. The epipleuria are more convex and more rounded than the hypopleuria (Fig. 6). The epipleuria and hypopleuria are mostly located on the lateral side of the head and only small parts of them are visible from the dorsal side (Fig. 12*A*, *B*). The hypostomium (U4–U5) is small, narrow and oval, and its lateral edges are stronger than anterior and posterior ones (Fig. 4*B*). Two pairs of cephalic ciliary tufts emerge from between the cephalic plates; the anterior tuft has five cilia and the posterior one has five to six cilia. The area they emerge from is clearly visible between the pleuria on the dorsolateral side (Fig. 3*B*). The anterior tuft consists of four short cilia of similar length and a fifth, much longer, posteriormost cilium. The posterior tuft has three to four cilia of similar length located in a row, followed by a much longer cilium and a cilium that is shorter than the preceding one but longer than the initial cilia. All cilia in the posterior tufts are straighter and clearly thicker than the cilia in the anterior tufts (0.81–1.37 µm vs 0.4–0.9 µm) (Figs 3, 4). The mouth ring is located subterminally on U2–U3 and has long, weakly marked cuticular reinforcements as well as long cuticular mouth bristles (Figs 4*B*, 12*B*). The pharynx (from U3 to U30) has a weak anterior dilatation, beyond which it dilates gradually towards the posterior end (Fig. 11*A*; Table S1). The pharynx is heavily muscularised, with muscles visible as horizontal bands (Figs 4*A*, 11*A*). It connects through the junction to the straight intestine, which extends from U30 to U86. The pharyngeal-intestinal junction is clearly marked, long and broad. The intestine has a distinct separate anterior section (from U30 to U31) that forms a narrow band located dorsally and partly laterally at the pharyngeal-intestinal junction (Figs 4*A*, 11*B*).

The head is wider than the neck. The neck tapers gradually from the head (from U13) to the beginning of the trunk (~U30) (Figs 3, 4, 7–9). The initial part of the trunk (from U30 to U35) is of the same width as the neck. The trunk dilates (initially rapidly, then gradually) from about U36 to about U61, where it is at its maximum width. Next it tapers (initially rapidly, then gradually) up to the furcal base (U87) (Fig. 7). From about U36 to about U86 the trunk is symmetrical, with a transverse axis of symmetry located at about U61, i.e. the shape of the trunk from the beginning of its wider part to its widest part is the same as from its widest part to the furcal base. The furcal base is clearly marked and narrow. On each furcal appendage, two clearly lateral indentations and one lateral hummock are present (Figs 3, 4, 7–9). The first indentation is located at U87 and is followed by a lateral hummock in the form of a thick fold of cuticle, and by a second indentation (U90). The furcal indentation (intrafurcal space) is pear-shaped. The furcal branches point slightly outwards at their distal ends and the

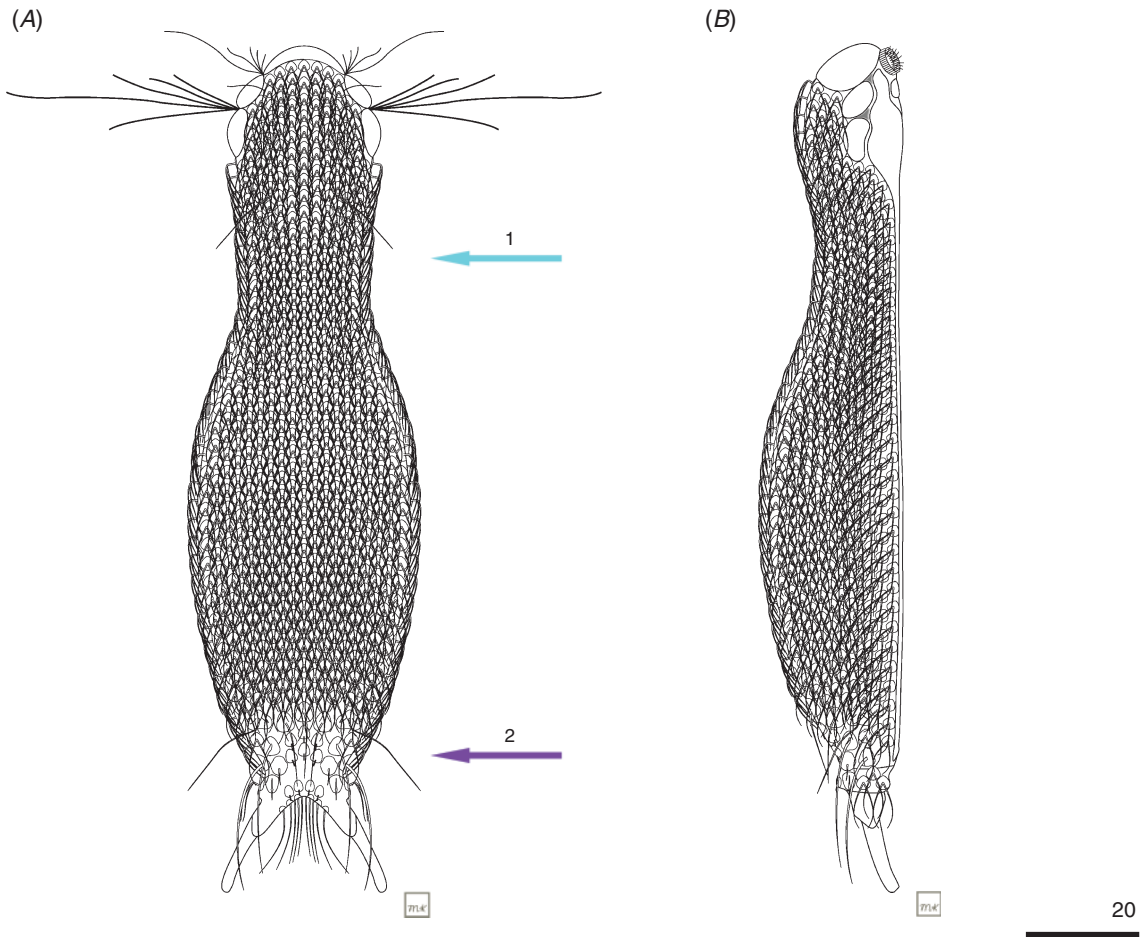


Fig. 3. *Bifidochaetus arcticus*, gen. *et* sp. nov. (*A*) Dorsal body view; (*B*) lateral body view. The arrows point to the neck narrowing (1) and furcal base (2).

distance between their tips is greater than the width of the furcal base (Figs 3, 4, 7–9). The adhesive tubes are thick (1.9–2.6 μm), rigid, slightly curved towards the centre of the indentation and have the same width along their entire length. The ends of the adhesive tubes are blunt with slightly rounded edges (Figs 3, 4, 7–9).

The entire body is covered with thin, one-lobed scales that adhere with their entire surface to the cuticle (Figs 3, 4, 7, 8). The scales are distributed in 36–45 longitudinal alternating rows with 30–42 scales in the central row (see Fig. S3). The scales are located close to one another. Their edges meet and are juxtaposed but never overlap (Figs 5, 12*A*, *C*). The longitudinal rows of scales begin on the head directly behind the cephalion, epipleuria and hypopleuria (Fig. 12*A*). The central longitudinal row of scales extends straight down the dorsal side, parallel to the longitudinal axis of the body. Scales in each longitudinal row located laterally to the central longitudinal row (on dorsal, dorsolateral, lateral and ventrolateral body surfaces) are slightly posterior to the scales in the central row (Figs 3, 12*A*, *C*). Each subsequent row counting from the central one has scales placed lower than the preceding longitudinal row. Such a distribution forms a system of scales that converge in the direction of the central longitudinal row. The scales that converge in the direction of the central longitudinal row on the head and the neck form rounded arcs, while on the trunk they form straight lines that are slanted at ~40° relative to the central row. The ventrolateral and ventral scales of the three subsequent longitudinal rows located closest to the ventral ciliary bands have tops of scales rotated by ~30° towards the bands (Figs 4*B*, 14*B*). The spines that emerge from these bands are adequately rotated. The scales of the dorsal, dorsolateral, lateral, ventrolateral and ventral sides of the body, except for those of the dorsal posterior trunk region, have no keel and no indentation of the posterior edge. They are subtriangular, with strongly rounded, convex edges (Fig. 5). The scales of the dorsal, dorsolateral, lateral, ventrolateral and ventral surfaces of the head, neck and trunk gradually decrease in size in the direction of the ventral ciliary bands (see Table S2). The scales in the ventral longitudinal row located closest to the ciliary band are distinctly smaller than the other scales (Figs 4*B*, 14*B*; Table S2). The dorsal, dorsolateral, lateral, ventrolateral and ventral spines emerge vertically from the exact centre of the scales (Fig. 5). After a short (0.6–3.0 μm), straight base segment that emerges vertically from the scale, the spine strongly curves (at an angle of ~90°) and bifid, branches off at the curvature into two separate, thick, strongly curved spines of equal length (5.0–10.8 μm) that taper towards their hair-like ends (Figs 5,

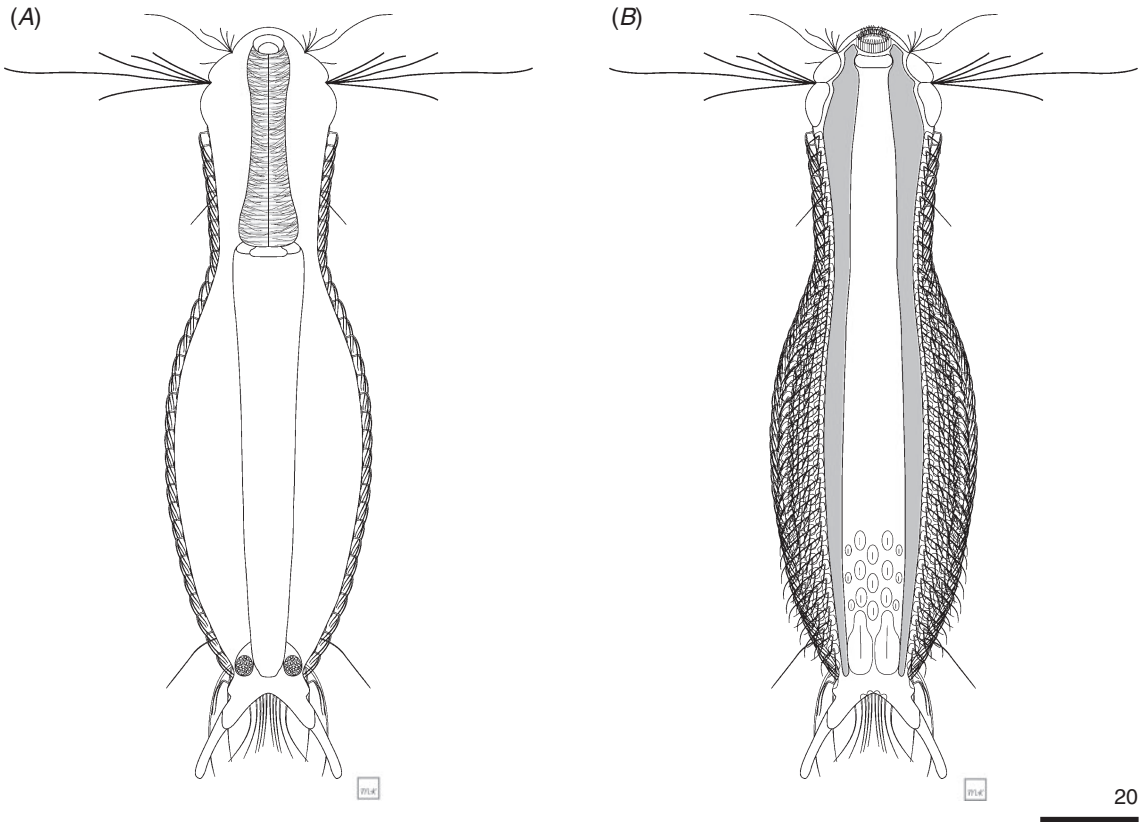


Fig. 4. *Bifidochaetus arcticus*, gen. *et* sp. nov. (*A*) Internal body view; (*B*) ventral body view. Light grey areas indicate the ciliary bands.

12*B*, *D*; Table S3). The longest spines are on the head (Figs 12*B*, 13*A*, *B*) and gradually shorten on the neck to become still shorter on the trunk (Figs 12*D*, 13*D*; Table S3). In addition to this the longest spines are dorsal and gradually shorten in the direction of ventral ciliary bands (Figs 12*B*, *D*, 13*A*, *B*, *D*, 14*B*; Table S3). The spines of the head and neck are more curved at the point of bifurcation than the trunk ones (Fig. 12*B*, *D*; Table S3).

An area with single-spined scales is present on the dorsal posterior trunk region (Figs 3*A*, 14*A*). The anteriormost scales in this area are located in the central longitudinal row and the two adjacent to it (U82–U84); they are shaped like elongated triangles with strongly curved, convex edges, do not have a keel and are greater in size than the surrounding scales (3.7–5.6 μm × 3.1–5.1 μm) (scales 1) (see Figs S1, 6, 14*A*). Their spines emerge from the centre of the scale, are long (12.2–17.8 μm), singular and hair-like. The scales located in two, or three in some specimens, subsequent transverse rows on the dorsal posterior trunk region (U85–U86) are smaller (1.6–4.0 μm × 1.3–3.7 μm), but similar to the previous scales (scales 2). Their spines emerge at the posterior edge of the scale, are short (1.7–4.1 μm), simple and straight, and have blunt ends. A pair of large (4.2–5.4 μm × 3.2–5.1 μm), hexagonal scales with rounded edges, a very weak keel and a short (2.3–4.5 μm), simple and straight spine with a blunt end is present on each dorsolateral side of the furcal base at U86 (scales 3). Two pairs of triangular scales with strongly rounded, convex edges are present on the furcal base. The anterior pair is located dorsolaterally at U85 (2.9–4.9 μm × 2.5–4.2 μm) (scales 4). Long (14.5–19.6 μm), single, curved spines that taper towards the end emerge from the centre of these scales. The posterior pair is located laterally at U86 (2.4–3.9 μm × 1.9–3.5 μm) (scales 5). Their spines constitute the posteriormost pair of the lateral spines, reaching up to about three-quarters of the length of the adhesive tubes (U98–U99) and are stouter and stronger than the other spines of the body (18.3–22.1 μm). A pair of large scales (4.4–7.0 μm × 3.8–5.5 μm) is located at the dorsal base of each furcal appendage at U87–U88 (scales 6). These two scales are oval, taper towards the posterior end and have a weak keel and a simple, straight spine (12.9–5.3 μm) with blunt ends. Four keel-less oval scales that have spines with blunt ends emerging from the posterior side of the scales are present in the centre of the dorsal side of the furcal appendages (1.4–3.9 μm × 1.1–3.0 μm) (scales 7). The spines of the central scales reach up to the furcal indentation (1.6–4.4 μm). Three pairs of parafurcal scales (2.4–3.9 μm × 2.4–4.0 μm; of the same type as on the trunk) with bifid spines (7.4–9.7 μm) are present laterally on the furcal appendages at U88 (scales 8) (Figs 3*A*, 14*A* and Fig. S1). The intrafurcal scales are almost circular and present on the inner side of the furcal appendages at U90 (1.1–2.0 μm × 1.2–2.6 μm) (scales 9). The edges of these scales are curved, thus making the entire scale adhere to the narrow surface of the inner furcal indentation (Figs 14*C*; S1). The inner scales of the furca are distributed in two rows with six

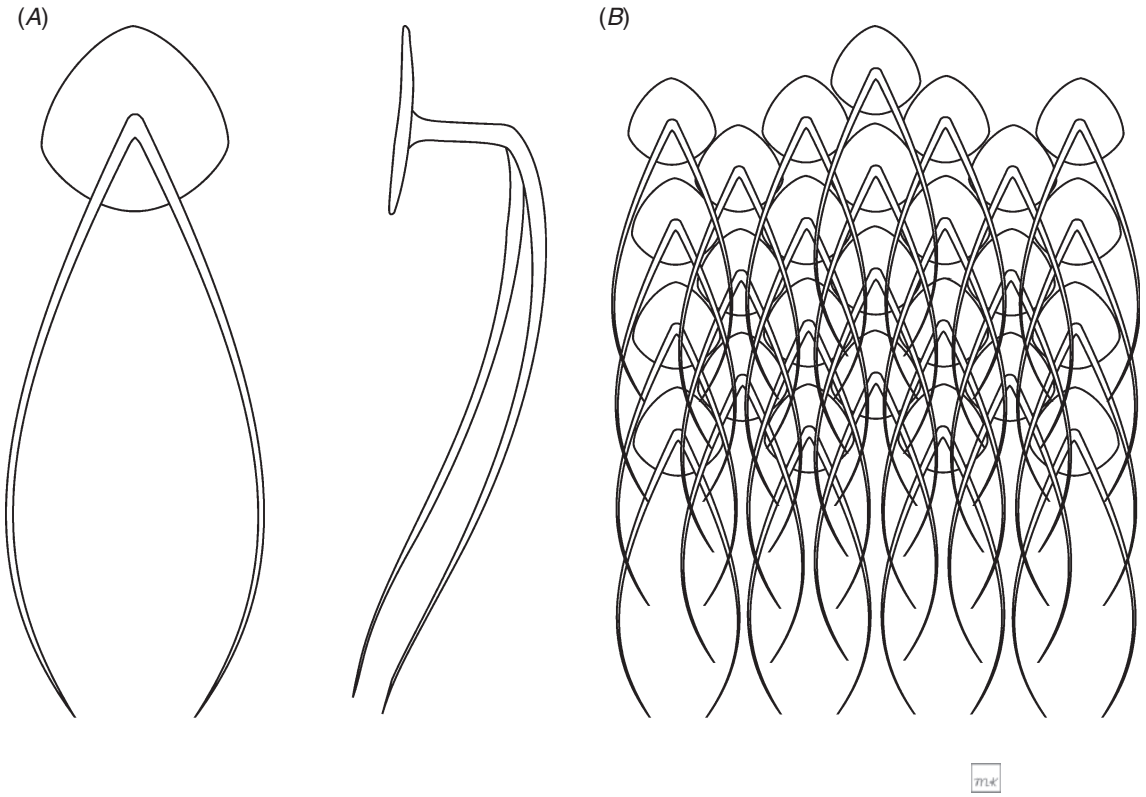


Fig. 5. *Bifidochaetus arcticus*, gen. *et* sp. nov. (*A*) Scales with bifid spines; (*B*) arrangements of scales.

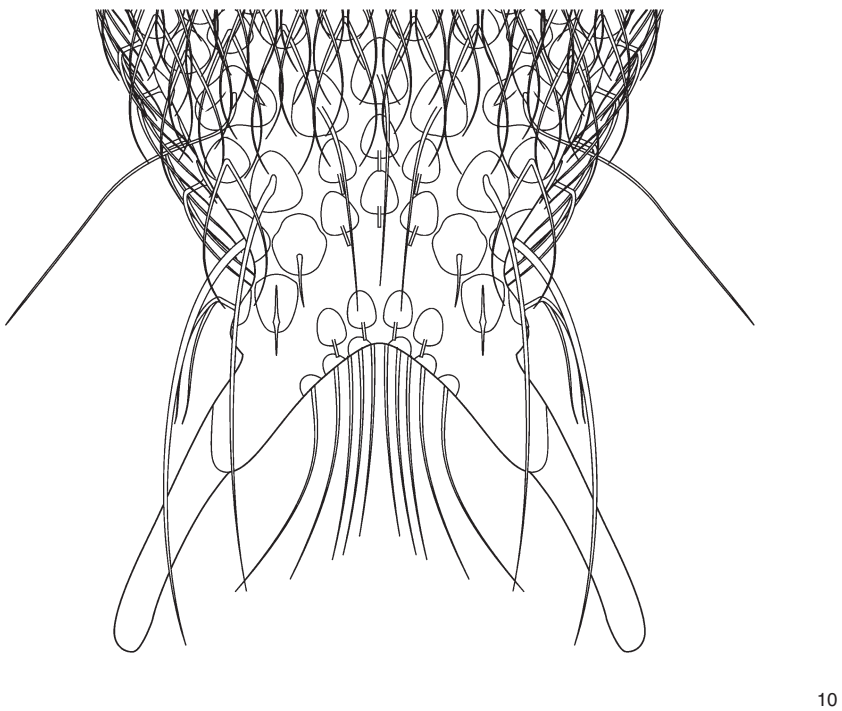


Fig. 6. *Bifidochaetus arcticus*, gen. *et* sp. nov. Arrangements of scales on dorsal posterior trunk region.

Fig. 7. *Bifidochaetus arcticus*, gen. *et* sp. nov. Habitus (phase contrast microphotographs).

scales in the row closer to the dorsal side and four scales in the row closer to the ventral side. A long (5.9–18.4 μm), strongly curved, thin spine emerges from all these scales that is visible inside the furcal indentation (Figs 3, 4, 7–9, 14*C*). Spines that emerge from the central scales of the inner part of the furca are less curved than the spines of the lateral pairs of scales (Figs 3, 4).

Longitudinal ventral ciliary bands begin at U2 and run up to U86 (Fig. 4*B*). The ciliary bands on the head lie directly next to the epipleuria and hypopleuria, enter the inside of the notch between them, and surround the lateral edges of the hypostomium. The ciliary bands are wider on the head and neck than on the trunk. The ventral interciliary field has no cuticular structures (Fig. 14*B*) except for the dorsal posterior trunk region (from U81 to U87) (Fig. 14*D*). Here they are oval, have a short, weakly marked keel and are distributed in three to four longitudinal rows with five scales in each row (Figs 4*B*, 8*C*, 9*C*, 14*B*, *D*). The longitudinal row of ventral field scales closest to the ventral ciliary bands has smaller scales than in the central rows (Fig. 14*B*; Table S2). The terminal scales of the ventral interciliary field are very large (9.3–15.0 μm × 4.6–7.9 μm), oval and have narrowed anterior parts as well as a weak keel (Figs 4*B*, 8, 9*C*, 14*D*).

The species has three pairs of dorsal sensory bristles (Fig. 3*A*). The first, anterior pair is located on the dorsal side of the head at U3, near the epipleuria. The bristles emerge from small, spherical papillae. The second pair of sensory bristles is located on the dorsal side of the neck and emerges from small, spherical papillae present between the third and fourth longitudinal rows of scales, counting from the central longitudinal row (U25). The third, posterior pair of sensory bristles is located dorsally at U81 before the area with the single-spined scales. These bristles emerge from scales shaped like an inverted heart with two keels and very weakly notched posterior edges.

The X-organ of this species (observed in six specimens) is located at U84–U86 and surrounds the terminal part of the intestine. It is bilobed, built from two extensions enveloped in a thin coat, and connected by a thinner band located behind the intestine at the ventral side. The extensions have a granular, grain-like structure. The thin coat and the cellular bridge connecting the extensions have a smooth and homogeneous structure (Fig. 10*D*). The sperm packets of this species take the form of long, coiled strings and are located at U65–U70 on the sides of the intestine (Fig. 10*C*).

Sequence diversity and phylogenetic relationships

Two different COI haplotypes were found among the six adult and one subadult specimens of *B. arcticus*, gen. *et* sp. nov. that were analysed; one of the haplotypes was shared by six individuals, and the second haplotype was found in one specimen with a developing egg. Although the mean genetic distance between the two haplotypes was relatively low and amounted to 0.13% (SD 0.08), one of the three nucleotide substitutions resulted in substitution of isoleucine by valine. No intraspecific sequence variation was found in the 18S and 28S rRNA sequences.

The total dataset for phylogenetic analysis comprised 4918 nucleotide positions (nps), including 657 nps for COI, 1741 nps for 18S rRNA and 2520 nps for 28S rRNA. Bayesian inference and ML phylogenetic analyses of the combined dataset provide strong support (PP = 1, BS = 84) for placement of *B. arcticus*, gen. *et* sp. nov. in the Chaetonotidae clade containing representatives of five genera: *Chaetonotus* Ehrenberg, 1830, *Aspidiophorus* Voigt, 1903, *Lepidodermella* Blake, 1933, *Ichthydium* Ehrenberg, 1830 and *Lepidochaetus* Kisielewski, 1991 (Fig. 15). The individual gene trees either strongly support this clade in 28S rRNA analysis (PP = 0.99, BS = 71 for ML) or do not conflict with it (18S rRNA, COI) (Figs S4–S6). In the BI tree for the combined dataset, *B. arcticus*, gen. *et* sp. nov. is resolved with strong support (PP = 1) as a sister group to the genus *Lepidochaetus*. This relationship is consistent, but varies in support across the datasets (COI, 28S rRNA and COI+18S+28S) and analysis methods (BI, ML), with one exception: in the ML and BI trees for 18S rRNA, *B. arcticus*, gen. *et* sp. nov. is resolved as basal to the clade grouping *Aspidiophorus tetrachaetus*, *Chaetonotus* (*Primochaetus*) *acanthocephalus*, *C.* (*P.*) *acanthodes*, *C.* (*P.*) *heideri* and *Lepidodermella*, but this relationship is unsupported supported (PP = 0.77, BS = 22).

Differential diagnosis

Among all the species known to date, *Bifidochaetus arcticus*, gen. *et.* sp. nov. is most similar to *Chaetonotus (Primochaetus) veronicae* Kånneby, 2013, reassigned to the genus

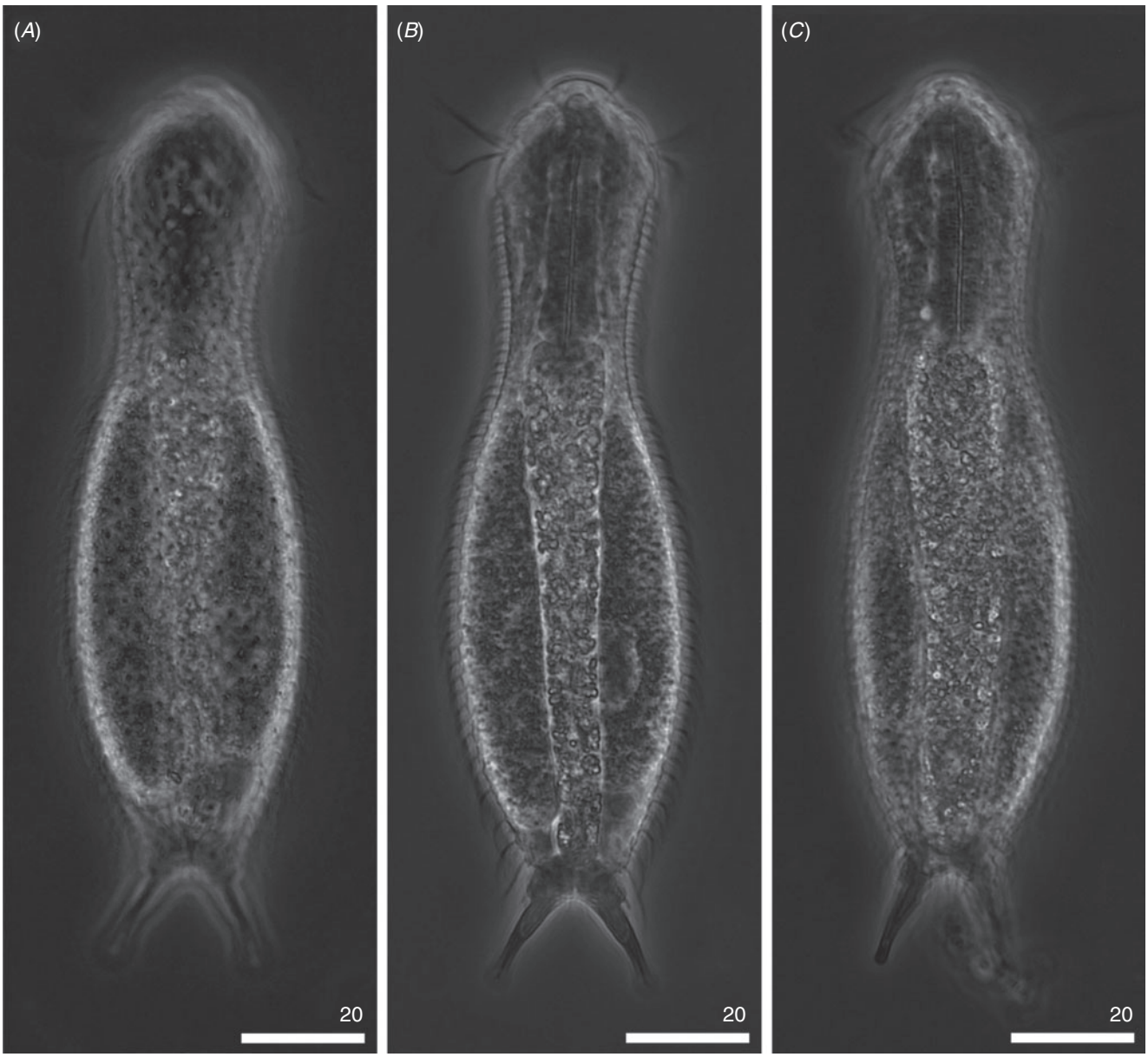


Fig. 8. *Bifidochaetus arcticus*, gen. *et* sp. nov. Holotype (phase contrast microphotographs): (*A*) dorsal body view; (*B*) internal body view; (*C*) ventral body view.

Bifidochaetus, gen. nov. in the present study (see below). These two species share the following characters: thin scales with no posterior notch that adhere to the cuticle with their entire surface, long bifid spines that curve strongly beyond a straight basal segment and taper towards their hair-like ends, spines present inside the inner furcal indentation (intrafurcal spines), the last pair of lateral trunk spines stronger than other spines of the body, hummocks on the lateral sides of furcal appendages, thick and rigid adhesive tubes, as well as muscular pharynx with marked anterior and no distinct posterior dilatation. However, *B. arcticus*, gen. *et* sp. nov. differs from *B. veronicae*, comb. nov. in terms of: body size (*B. veronicae*, comb. nov. is smaller and adults measure 123–135 μm); shape, size and arrangement of pleurae (in *B. veronicae*, comb. nov. they are small and weakly marked on head outline); thickness of cephalic cilia (*B. veronicae*, comb. nov. has cilia in both pairs of cephalic tufts of similar thickness); pharynx length (in *B. veronicae*, comb. nov. pharynx in adults measures 34–35 μm); number of scales (in *B. veronicae*, comb. nov. scales are distributed in 30–33 longitudinal alternating rows with 27–30 scales in central row); scale shape and type (*B. veronicae*, comb. nov. has round to suboval scales with overlapping edges, of which anterior parts are fused to the body surface); distinct scales in the furcal base (in *B. veronicae*, comb. nov. scales on the furcal base are more reduced than other scales of the dorsal body surface); presence

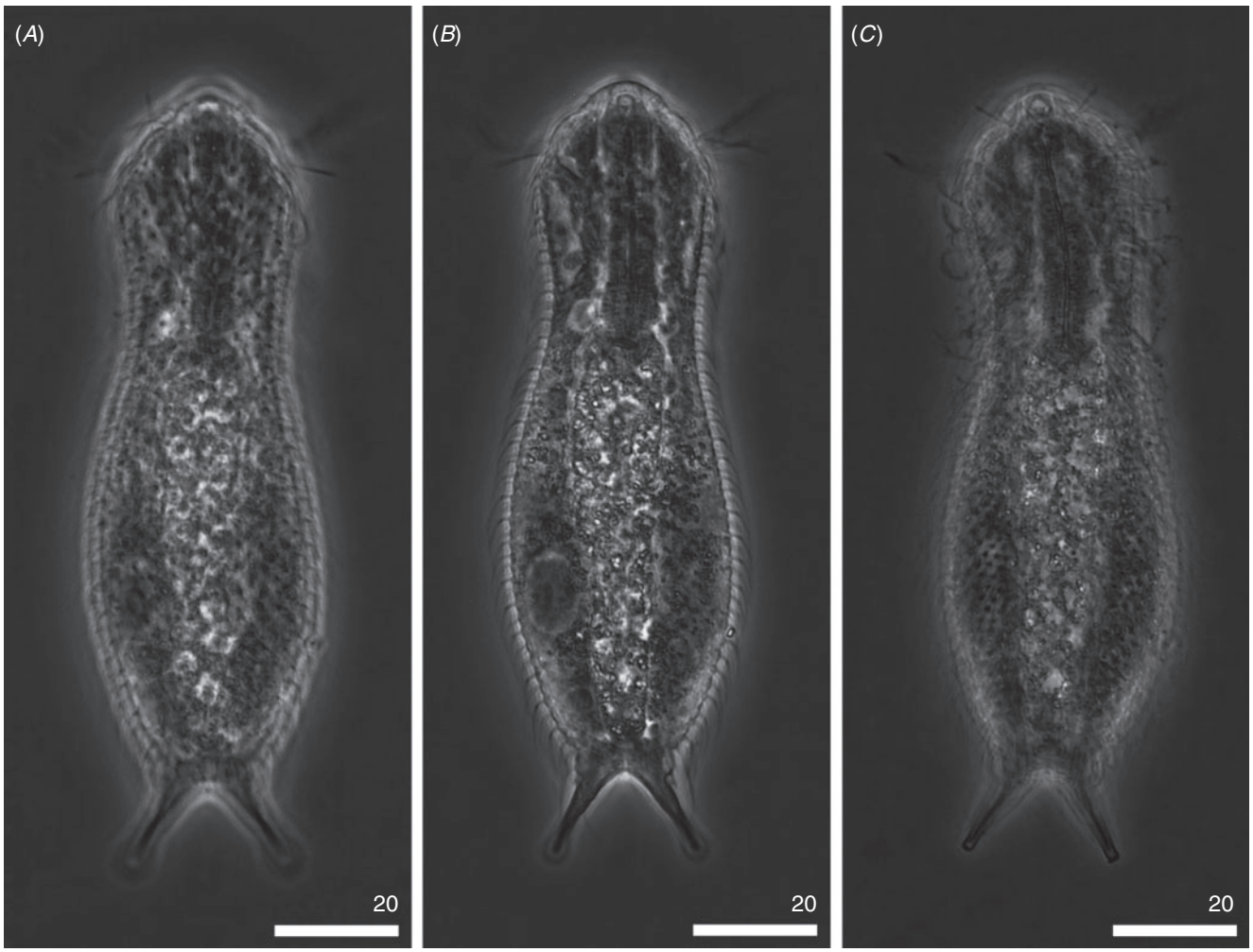


Fig. 9. *Bifidochaetus arcticus*, gen. *et* sp. nov. Subadult specimen (phase contrast microphotographs): (*A*) ventral body view; (*B*) internal body view; (*C*) ventral body view.

of scales with spines at the lateral side of furcal appendages (in *B. veronicae*, comb. nov. lateral scales are not present); location of intrafurcal scales with long, single spines (in *B. veronicae*, comb. nov. intrafurcal scales are located at ventral body side); the difference in scale size on the body (in *B. veronicae*, comb. nov. scales are a similar size all over the body, although slightly smaller anteriorly); the position where the spines emerge from scales (in *B. veronicae*, comb. nov. spines arise from the posterior part of each scale); length of basal (not bifid) part of spines (in *B. veronicae*, comb. nov. the basal part of spines is longer and is ~1/3 of the total spine length); thickness of bifid part of spines (in *B. veronicae*, comb. nov. the bifid part of spines is significantly thinner); degree of ventrolateral spine curvature (*B. veronicae*, comb. nov. has ventrolateral spines only slightly curved); number of pairs of dorsal sensory bristles (*B. veronicae*, comb. nov. has only one pair of dorsal sensory bristles, on the trunk); shape, type and size of ventral interciliary field terminal scale (*B. veronicae*, comb. nov. has keeled suboval scales strongly overlapping, which measure 6–8 μm × 4–5 μm); shape of furca (in *B. veronicae*, comb. nov. the furca is straight).

Remarks

In *B. arcticus*, gen. *et* sp. nov., three pairs of dorsal sensory bristles were observed. The feature is rarely listed among the freshwater members of the family Chaetonotidae and, so far, has been observed only in *Chaetonotus (Chaetonotus) brevispinosus* Zelinka, 1889 and *C. (C.) sanctipauli* Kisielewski, 1991. They are much more common in marine and brackish species, e.g. *Aspidiophorus lamellophorus* Balsamo, Hummon, Todaro *et* Tongiorgi, 1997 (see Kolicka *et al*. 2015). A larger number of dorsal sensory bristles (up to six pairs) is typically characteristic of marine genera, such as *Heteroxenotrichula* Wilke, 1954 and *Xenotrichula* Remane, 1927 (from Xenotrichulidae Remane, 1927).

The presence of intrafurcal scales similar to those observed in *B. arcticus*, gen. *et* sp. nov. is a very common character in representatives of the genus *Lepidodermella* Blake, 1933 and is also observed in some species from *Chaetonotus* Ehrenberg, 1830 – e.g. *Chaetonotus (Primochaetus) acanthocephalus* Valkanov, 1937, *C. (P.) brachyurus* Balsamo, 1980, *C. (Chaetonotus) heterospinosus* Balsamo, 1978, *C. (C.) pseudopolyspinosus*

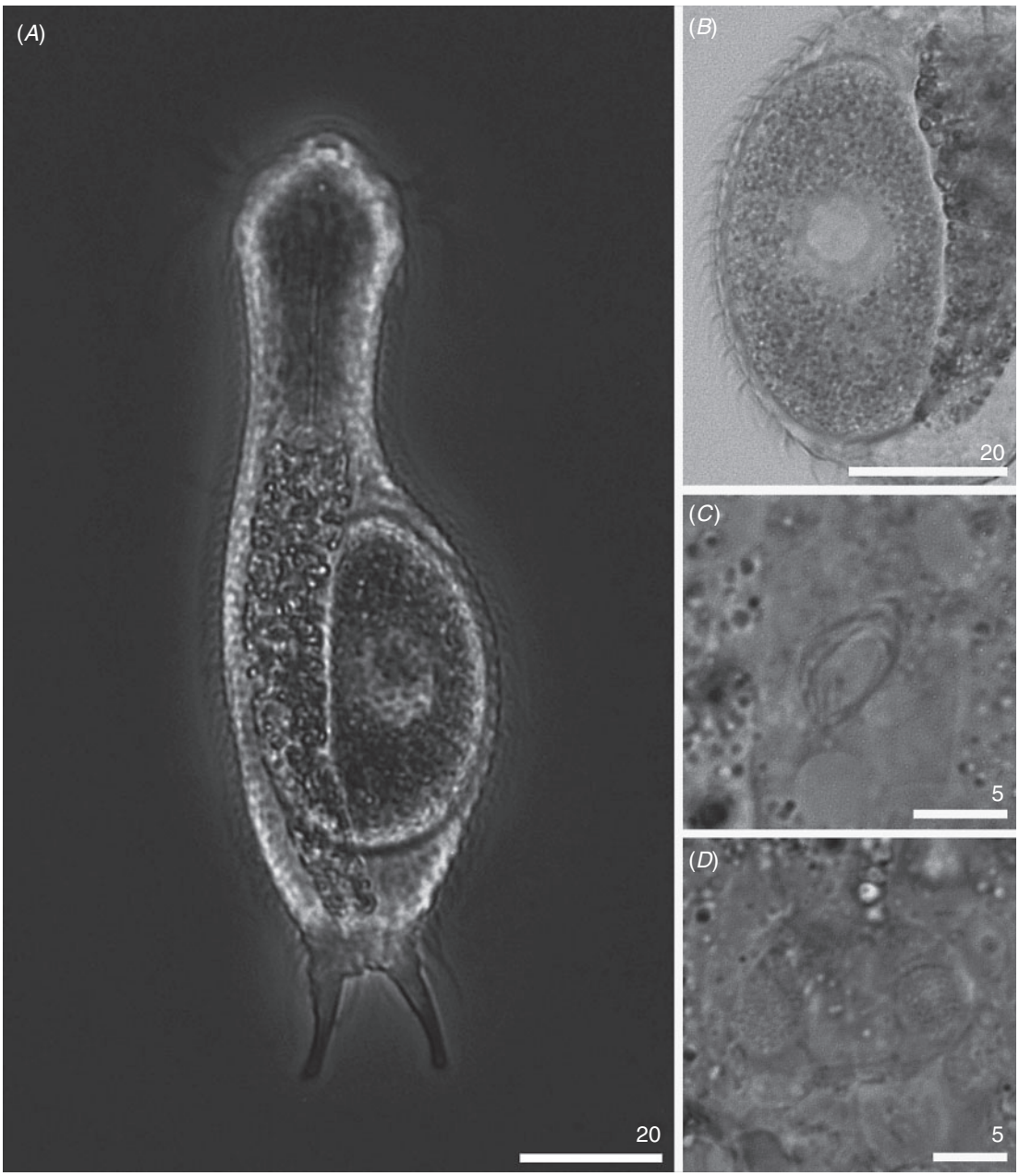


Fig. 10. *Bifidochaetus arcticus*, gen. *et* sp. nov. (phase contrast and brightfield microphotographs). (*A*) Specimen with mature egg; (*B*) mature egg; (*C*) a developing package of sperm; (*D*) X-organ.

Kisielewski, 1991 and *C. (C.) ventrochaetus* Kisielewski, 1991. However, other previously described species do not have intrafurcal scales with long, single, curved spines located internally in the furcal indentation. *Chaetonotus* representatives that have intrafurcal scales always have them located dorsally or ventrally, never internally. Among the observed specimens, one had a large developing egg. Furthermore, the X-organ was observed in six specimens and a maturing set of sperm cells was observed in two specimens. It should be noted that body size (length) is not an accurate indicator of age in Gastrotricha. In *B. arcticus*, gen. *et* sp. nov., the specimen with the developed egg and the specimen with the marked X-organ (whose presence may indicate a post-parthenogenetic life phase) both had body sizes similar to that of one of the subadult specimens. Clear differences between the adult and subadult or juvenile specimens were observed for head size (the head was disproportionately large in relation to the rest of the body in young specimens) (e.g. Remane 1936; Mock 1979; Balsamo and Todaro 1987; Hochberg 1998; Balsamo *et al*. 2014). Usually in juveniles the head is wider than the trunk and in adults the trunk is wider than the head (Balsamo *et al*. 2014). However, this rule does not apply to all species, e.g. *B. arcticus*, gen. *et* sp. nov. has a bottle-

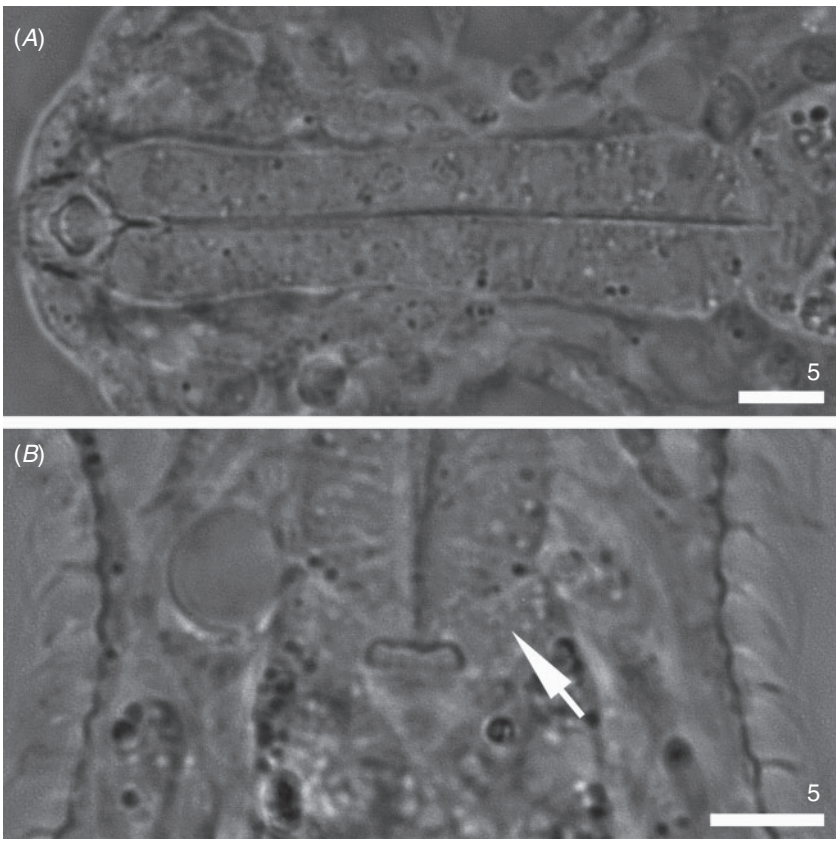


Fig. 11. *Bifidochaetus arcticus*, gen. *et* sp. nov. (brightfield microphotographs). (*A*) Pharynx; (*B*) pharyngeal-intestinal junction and anterior separate section of intestine. The arrow points to the anterior intestine section.

shaped body and its head is always narrower than the trunk, even in very young individuals. Therefore, we propose as a more adequate indicator the pharynx-to-intestine ratio (I), whose value in juvenile specimens is greater or equal to 55% (≥55% in juveniles or subadults and <55% in adults) and is not strongly associated with a species-specific body shape (Table S1).

Locality

Small water reservoirs in Longyearbyen (78°13′14.28″N, 15°38′37.14″E), Spitsbergen, Svalbard Archipelago, Norway.

Etymology

From the name of the geographic region and to commemorate the first freshwater species described from the area.

Bifidochaetus veronicae, comb. nov. (Kånneby, 2013)

Described as: *Chaetonotus* (*Primochaetus*) *veronicae* Kånneby, 2013.
Emended description: see Kånneby 2015.

Diagnosis

Adult total body length ranging from 123 to 135 μm. Scales of similar size although slightly smaller anteriorly. Scales distributed in 30–33 longitudinal alternating rows with 27–30 scales in central row. Anterior part of scales fused with body surface, some scales reduced. Scales round to suboval with overlapping edges. Parafurcal scales on lateral sides of furcal appendages absent. Scales present on inner part of furca. Ventral interciliary field terminal scales large, oval with keel and with overlapping edges. Head five-lobed with a small cephalion, epipleuria and hypopleuria weakly marked in body outline. Two pairs of cephalic ciliary tufts; their cilia of similar thickness. Pharynx length of adult specimens ranging from 34 to 35 μm. Pharynx with weak anterior enlargement, then gradually widening towards the posterior end. Intestine straight without differentiated anterior section. One pair of dorsal sensory bristles, only on trunk. Furcal base clearly marked, with external base of furcal appendages bearing hummocks. Furca straight.

Remarks

The reason for transferring this species to the genus *Bifidochaetus*, gen. nov. is the presence of bifid spines as opposed to the single spines of the genus *Chaetonotus*. In the original description and drawing (see Kånneby 2013: fig. 23), as well as in selected holotype microphotographs (see Kånneby 2013: fig. 22), only simple non-bifid spines were present and an incorrect impression is given with respect to whether this species has bifid spines arising from dorsal, dorsolateral, lateral,

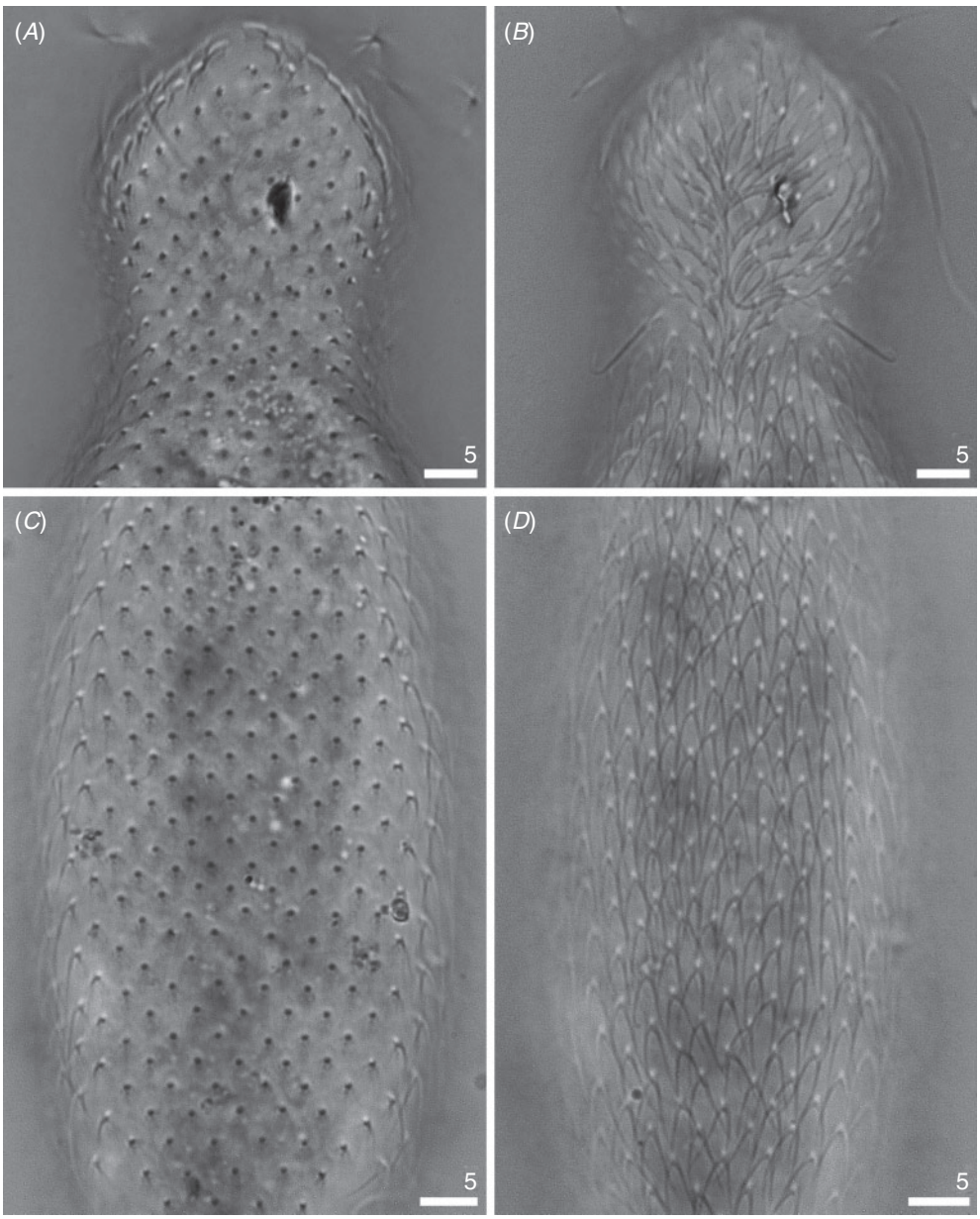


Fig. 12. *Bifidochaetus arcticus*, gen. *et* sp. nov. (brightfield microphotographs). Dorsal view of scales: (*A*) on the head and neck; (*C*) on the trunk. Dorsal view of spines: (*B*) on the head and neck; (*D*) on the trunk.

ventrolateral and ventral scales (see Kånneby 2015). The following features justify the inclusion of this species in the newly established genus: the presence of bifid spines and a bottle-shaped body with a hummock at the external base of the furcal appendages; thin, one-lobed scales; thick, rigid adhesive tubes; mouth ring with weakly marked cuticular reinforcements and long cuticular mouth bristles; heavily muscularised pharynx with weak anterior dilatation.

Discussion

Our phylogenetic analyses of DNA sequences indicate a close relationship between *Bifidochaetus*, gen. nov. and *Lepidochaetus* Kisielewski, 1991 (Figs 15, S4–S6). Morphological features suggesting this relationship are as follows: most of body covered in scales with rounded edges, without posterior notches or keel (except for scales with posterior sensory bristles and a single pair of scales at the posterior part of the trunk in *Bifidochaetus*, gen. nov.); presence of longer spines of different type on the furcal base than the other spines on the dorsal, dorsolateral, lateral and ventral sides of the posterior part of trunk; short cuticular reinforcements of the mouth ring with long internal cuticular hairs; ventral locomotor cilia bands wider in the head region; and thick, rigid adhesive tubes. However, the two genera differ significantly in other essential characters, such as: body habitus (*Lepidochaetus* has a large, stocky body with a

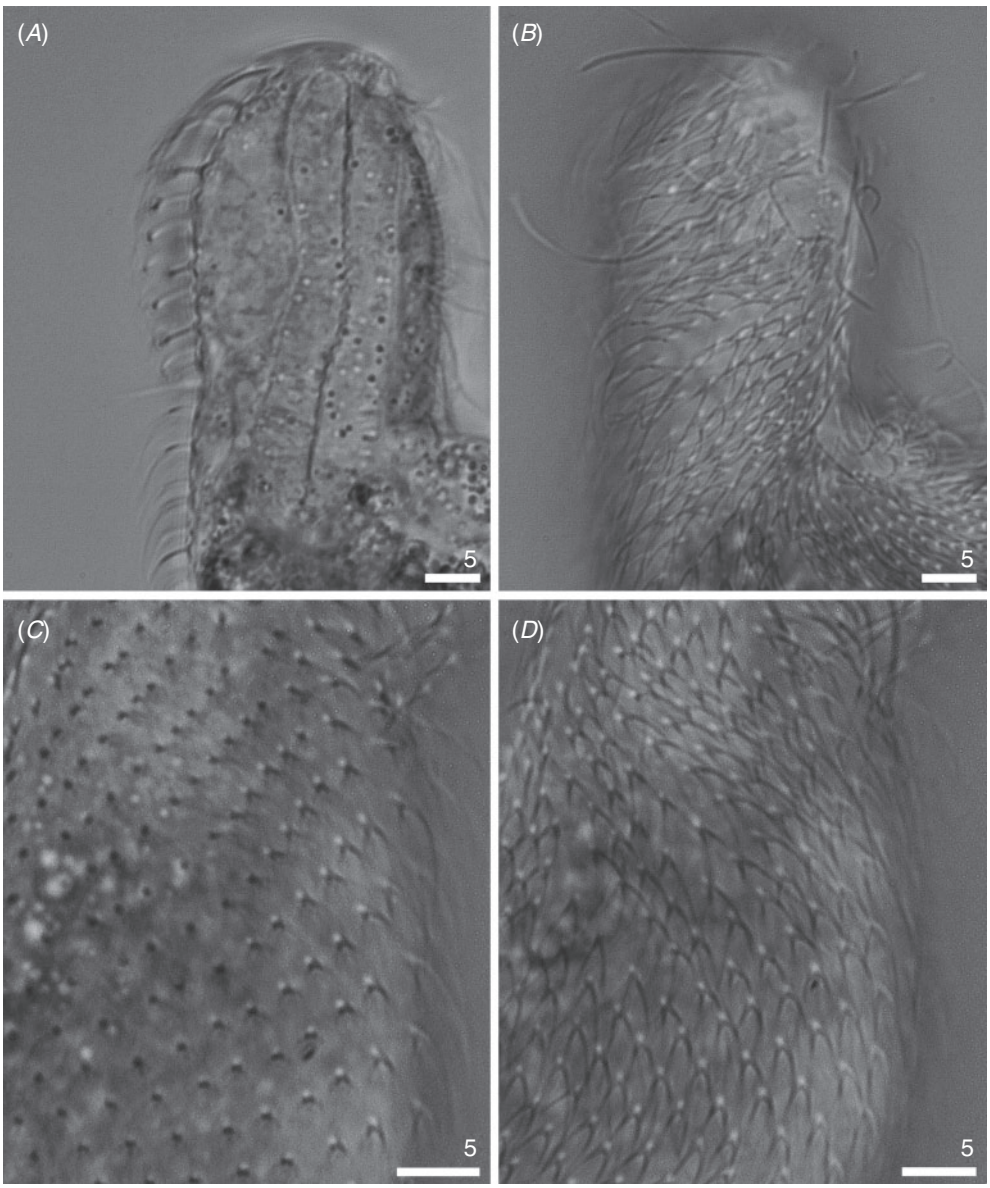


Fig. 13. *Bifidochaetus arcticus*, gen. *et* sp. nov. (brightfield microphotographs). (*A*) Internal view of head and neck. Lateral view: (*B*) of head and neck; (*C*) of scales on trunk; (*D*) of spines on trunk.

weak neck constriction and not a clearly marked furcal base); scale type (*Lepidochaetus* exhibits extraverted scales, i.e. with double anterior edges, connected to the body surface by their anterior part only); scale type and size variation (all scales in *Lepidochaetus* are of similar size and shape); type of spines (*Lepidochaetus* possesses dentate posterior spines); as well as the type of furcal base and shape of adhesive tubes (*Lepidochaetus* has an indistinct furcal base, the furcal indentations are narrow and the adhesive tubes are straight). Foregoing similarities and differences in morphology between these two genera and the clear separation of the clades in our phylogenetic trees indicate the high evolutionary distinctiveness of *Bifidochaetus–Lepidochaetus*.

The results of our molecular phylogenetic analyses are mostly consistent with the previous molecular phylogeny of Chaetonotidae (Kånneby *et al*. 2013), except for relationships among species of the most distal clade of Chaetonotidae (see Kånneby *et al*. 2013: fig. 4). In the earlier reconstruction (Kånneby *et al*. 2013), the *Lepidochaetus* clade was sister group to a clade grouping species of *Chaetonotus (Primochaetus)*, *Aspidophorus*, *Lepidodermella* and *Ichthydium*. In our tree, the genus *Lepidochaetus* forms a clade with *Bifidochaetus arcticus*, gen. *et* sp. nov.; this monophyletic group is the sister clade to the *Ichthydium–Aspidiophorus tetrachaetus–Lepidodermella* group. Although *Bifidochaetus*, gen. nov. shares several morphological characters with other groups within

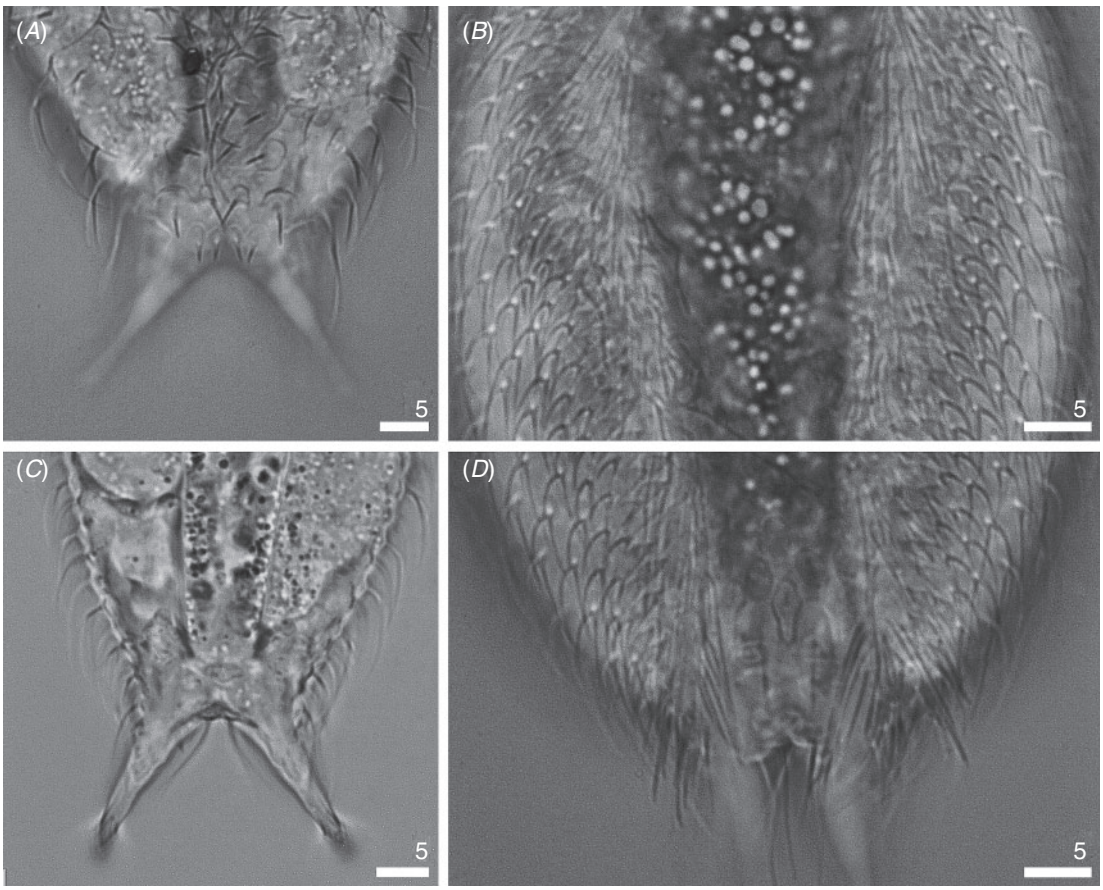


Fig. 14. *Bifidochaetus arcticus*, gen. nov. *et* sp. nov. (brightfield microphotographs). (*A*) Dorsal scales on posterior trunk region; (*B*) ventral view of trunk; (*C*) furca and adhesive tubes; (*D*) ventral view of posterior trunk region with visible interciliary field scales and posteriormost interciliary field scales.

Chaetonotidae, especially with certain subgenera of *Chaetonotus* (see 'Taxonomic affinities' for the genus), these characters may reflect homoplasy in the perspective of the phylogenetic relationships at genus level inferred from molecular data (see Kånneby *et al*. 2013). Further sampling for molecular studies including detailed morphological and morphometric analyses would be of great value in solving the taxonomy of Chaetonotidae, where most current genera are indeed polyphyletic. Further research could result in significant changes to the systematics of this group.

From a morphological point of view, a hypothesised evolutionary scenario could see the bifid spines evolve through the formation of a thin lamella partially and subsequently entirely linking the bifid spine sections, thus forming a pedunculate scale similar to those present in *Aspidiophorus* Voight, 1903. A similar evolutionary scenario could also be the case for ancestors of *Chaetonotus (Schizochaetonotus)* Schwank, 1990. On the other hand, the opposite is also possible, i.e. a reduction of the pedunculate secondary scales in *Aspidiophorus* to a bifid or dentate spine as in *Bifidochaetus*, gen. nov. and *C. (Schizochaetonotus)*, respectively.

In many representatives of chaetonotid genera, characters considered to be distinguishing at the genus level are ambiguous and can appear in varying proportions in particular species (e.g. scales, spines or lamellae). Many species display characters that are present in several different genera or characteristics considered to be representative of another genus. Species with non-specific features for their genus are highly common within Chaetonotidae. According to Kånneby *et al*. (2013), while differences in cuticular coverage constitute a very accurate indicator of specific level, they have no diagnostic validity for genera. In case of present discrepancies between morphological and molecular data, future research will have to analyse both detailed morphological and molecular data and carefully examine their consistency. Fiers and Kotwicki (2013) demonstrate that analysing only selected, easily discernible morphological characters can be misleading. The two authors used a morphological analysis to prove that a taxon of cosmopolitan Harpacticoida (Crustacea, Copepoda), considered to be one species, constitutes in fact four species with significant differences in external morphology and geographical distribution. In Gastrotricha, Kånneby *et al*. (2012) drew attention to the morphological differences between two separate clades that were currently considered species, *Lepidodermella squamata* (Dujardin, 1841), obtained based on molecular analyses. These authors, however, find that morphological differences between the clades could be explained by developmental flexibility during cuticle formation, like that observed by Amato and Weiss

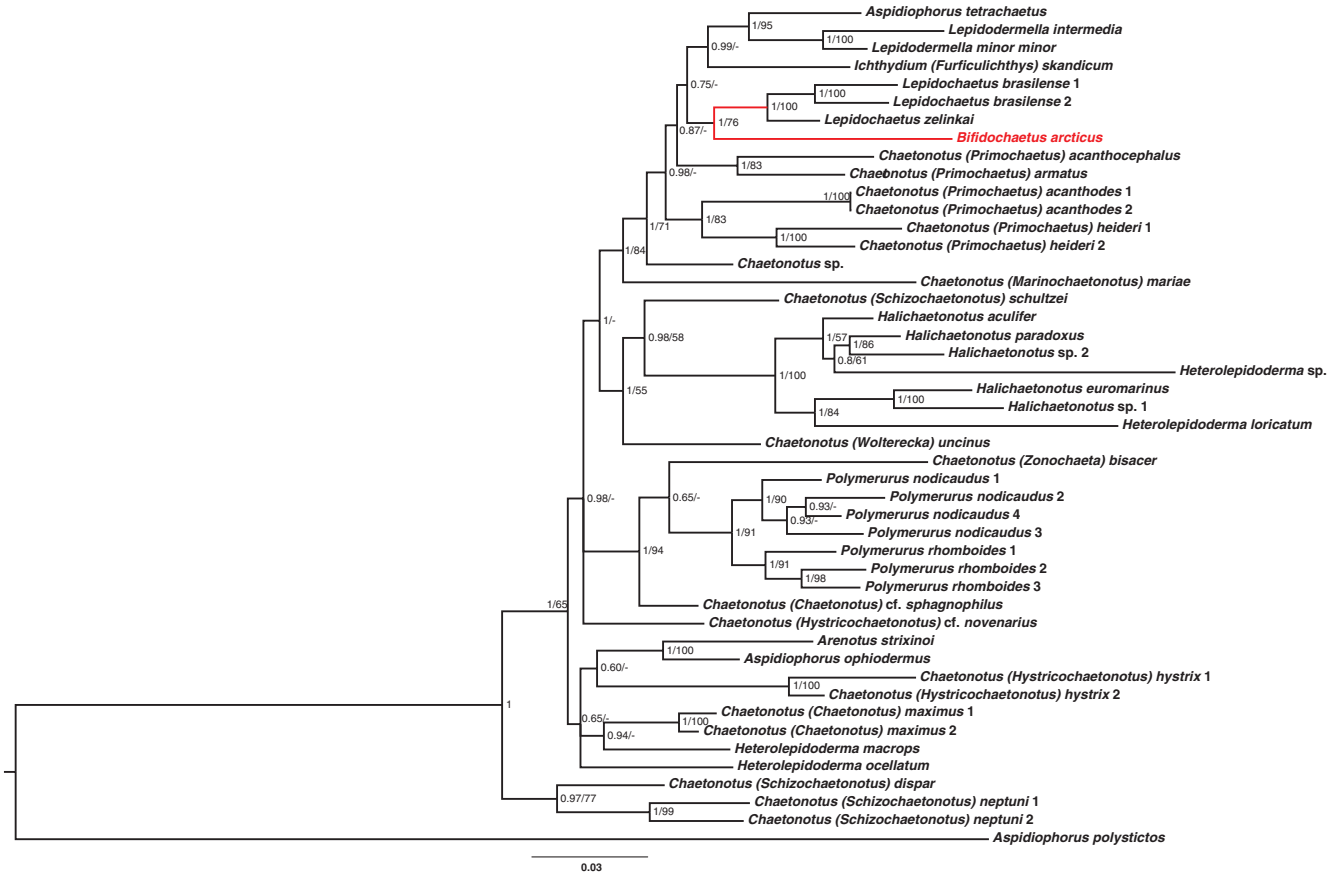


Fig. 15. Phylogenetic relationships of Chaetonotidae inferred from Bayesian and maximum likelihood analyses of 18S rRNA, 28S rRNA and COI sequence data. Values near branches show Bayesian posterior probability and bootstrap support above 0.5 and 50, respectively.

(1982) in research on clonal individuals of this species, but also speculate that cryptic speciation may be taking place (Kånneby *et al*. 2012). Also, earlier studies (e.g. Fregni *et al*. 1998) suggest that the species *L. squamata* should be divided into distinct species based on differences in shape and number of dorsal, dorsolateral, lateral, ventrolateral and ventral scales; type, shape and number of ventral interciliary field terminal scales and the presence or absence of parafurcal spines. Likewise, Leasi and Todaro (2009), using confocal laser scanning microscopy, found significant differences in the construction of the muscular system in two geographically separate groups currently known as species *Xenotrichula intermedia* Remane, 1934. Their observation indicates that morphological differences of species regarded as cryptic are possible. Within Gastrotricha it is highly plausible that numerous taxa currently considered to be cryptic species will prove to be a complex of species that are similar yet differentiable in terms of appearance after thorough morphological analysis.

During gastrotrich postembryonic development, the number and size of cuticular structures as well as the degree of their separation from the cuticle can change (Amato and Weiss 1982). Moreover, in juvenile and subadult specimens the spines can be thinner and do not possess marked lateral denticles even if this structure exists in adult individuals (Kisielewski 1997). In our study, we proposed a new parameter: the pharynx-to-intestine length ratio (I), which enables an objective determination of the degree of development of an individual. This index can help identify whether the individual is a juvenile, subadult or adult even without other means to estimate age (such as a developing egg or visible X-organ). Gauging age is important to avoid errors in species identification and description of morphological characters. Also, to improve the quality of morphological differentiation, we suggest the use of spine bifurcation ratio (B) parameters. The point at which the single spine divides into two separate spines is important in distinguishing the two species now present within *Bifidochaetus*, gen. nov., and may be useful if additional species are described in the genus.

It should be emphasised that some characters may have appeared independently during the evolution of Gastrotricha, such as the presence of pedunculated scales in *Aspidiophorus* Voigt, 1903, some representatives of *Polymerurus* Remane, 1927, *Lepidodermella* Blacke, 1933, as well as the marine *Xenotrichula* Remane, 1927 and *Draculiciteria* Hummon, 1974.

The taxon discovered in our research is characterised by a unique composition of morphological features. The combination of these characters did not allow us to assign the taxon to any of the currently known species or genera. Molecular examination of the taxon also indicated its distinctness from the other included Chaetonotidae. Therefore, in this paper we included

a description of a new species and new combination of a previously described species for which we believe a separate genus should be defined.

Conclusion

In this paper we propose the new genus *Bifidochaetus*, gen. nov. within the family Chaetonotidae (Gastrotricha) based on morphological characters and its placement in a molecular phylogenetic analysis. *Bifidochaetus arcticus*, gen. *et* sp. nov. is the first species of freshwater gastrotrich determined to species level from the Arctic. Moreover, the redescription of *Chaetonotus (Primochaetus) veronicae* Kånneby, 2013 (Kånneby 2015) brings characters into light that place it in our newly proposed genus, hence it is reclassified as *B. veronicae*, comb. nov. (Kånneby 2013). Detailed morphological comparisons of these taxa indicate they are two separate species. Moreover, we propose the use of two new parameters: the pharynx-to-intestine length ratio (I) and the spine bifurcation ratio (B).

Acknowledgements

We thank Krzysztof Zawierucha for collecting the material and for his helpful advice during manuscript preparation; Ziemowit Olszanowski, M. Antonio Todaro and two anonymous reviewers for their kindly advice and insightful and helpful comments on earlier versions of the manuscript. Special thanks to Małgorzata Kuźnik for preparing the drawings of the new species and for all the figures used in this paper. Last, but not least, the authors are grateful to Łukasz Kaczmarek and Wojciech Magowski for their help during the research. All samples were collected in public spaces. Collection and transportation of material were made possible under the authorisation number RIS–ID 6390 as part of the research project 'Influence of islands size on invertebrate diversity in Svalbard Archipelago'. This permit was given by the Governor of Svalbard in 2013. Further work with Gastrotricha did not require any additional permits. The study was founded by Polish MSHE grants DI 2011 035241 (sampling) and DI 2014 013844 (research).

References

Amato, A. J., and Weiss, M. J. (1982). Developmental flexibility in the cuticular pattern of a cell-constant organism, *Lepidodermella squammata* (Gastrotricha). *Transactions of the American Microscopical Society* **101**, 229–240. doi:10.2307/3225811

Appeltans, W., Ahyong, S. T., Anderson, G., Angel, M. V., Artois, T., Bailly, N., Bamber, R., Barber, A., Bartsch, I., Berta, A., Błażewicz-Paszkowycz, M., Bock, P., Boxshall, G., Boyko, C. B., Brandão, S. N., Bray, R. A., Bruce, N. L., Cairns, S. D., Chan, T. Y., Cheng, L., Collins, A. G., Cribb, T., Curini-Galletti, M., Dahdouh-Guebas, F., Davie, P. J., Dawson, M. N., De Clerck, O., Decock, W., De Grave, S., de Voogd, N. J., Domning, D. P., Emig, C. C., Erséus, C., Eschmeyer, W., Fauchald, K., Fautin, D. G., Feist, S. W., Fransen, C. H., Furuya, H., Garcia-Alvarez, O., Gerken, S., Gibson, D., Gittenberger, A., Gofas, S., Gómez-Daglio, L., Gordon, D. P., Guiry, M. D., Hernandez, F., Hoeksema, B. W., Hopcroft, R. R., Jaume, D., Kirk, P., Koedam, N., Koenemann, S., Kolb, J. B., Kristensen, R. M., Kroh, A., Lambert, G., Lazarus, D. B., Lemaitre, R., Longshaw, M., Lowry, J., Macpherson, E., Madin, L. P., Mah, C., Mapstone, G., McLaughlin, P. A., Mees, J., Meland, K., Messing, C. G., Mills, C. E., Molodtsova, T. N., Mooi, R., Neuhaus, B., Ng, P. K., Nielsen, C., Norenburg, J., Opresko, D. M., Osawa, M., Paulay, G., Perrin, W., Pilger, J. F., Poore, G. C., Pugh, P., Read, G. B., Reimer, J. D., Rius, M., Rocha, R. M., Saiz-Salinas, J. I., Scarabino, V., Schierwater, B., Schmidt-Rhaesa, A., Schnabel, K. E., Schotte, M., Schuchert, P., Schwabe, E., Segers, H., Self-Sullivan, C., Shenkar, N., Siegel, V., Sterrer, W., Stöhr, S., Swalla, B., Tasker, M. L., Thuesen, E. V., Timm, T., Todaro, M. A., Turon, X., Tyler, S., Uetz, P., van der Land, J., Vanhoorne, B., van Ofwegen, L. P., van Soest, R. W., Vanaverbeke, J., Walker-Smith, G., Walter, T. C., Warren, A., Williams, G. C., Wilson, S. P., and Costello, M. J. (2012). The magnitude of global marine species diversity. *Current Biology* **22**(23), 2189–2202. doi:10.1016/j.cub.2012.09.036

Balsamo, M., and Todaro, M. A. (1987). *Aspidiophorus polystictos*, a new marine species (Gastrotricha, Chaetonotida) and its life cycle. *Bollettino di Zoologia* **54**, 147–153. doi:10.1080/11250008709355574

Balsamo, M., d'Hondt, J.-L., Kisielewski, J., and Pierboni, L. (2008). Global diversity of gastrotrichs (Gastrotricha) in freshwaters. *Hydrobiologia* **595**, 85–91. doi:10.1007/s10750-007-9006-4

Balsamo, M., Guidi, L., Ferraguti, M., Pierboni, L., and Kristensen, R. M. (2010). *Diuronotus aspetos* (Gastrotricha): new morphological data and description of the spermatozoon. *Helgoland Marine Research* **64**, 27–34. doi:10.1007/s10152-009-0163-x

Balsamo, M., Grilli, P., Guidi, L., and d'Hondt, J.-L. (2014). Gastrotricha: biology, ecology and systematics: families Dasydytidae, Dichaeturidae, Neogosseidae, Proichthydiidae. In 'Identification Guides to the Plankton and Benthos of Inland Waters. 24'. (Ed. H. J. F. Dumont.) pp. 1–187. (Backhuys Publishers, Margraf Publishers GmbH: Weikeirsheim, Germany.)

Balsamo, M., d'Hondt, J.-L., Kisielewski, J., Todaro, M. A., Tongiorgi, P., Guidi, L., Grilli, P., and de Jong, Y. (2015). Fauna Europea: Gastrotricha. *Biodiversity Data Journal* **3**, e5800. doi:10.3897/BDJ.3.e5800

Coulson, S. J. (2007). The terrestrial and freshwater invertebrate fauna of the High Arctic archipelago of Svalbard. *Zootaxa* **1448**, 41–58.

Coulson, S. J. (2013). The terrestrial invertebrate fauna of the Svalbard archipelago in a changing world: history of research and challenges. *Canadian Entomologist* **145**, 131–146. doi:10.4039/tce.2012.110

Coulson, S. J., Hodkinson, I. D., Strathdee, A. T., Block, W., Webb, N. R., Bale, J. S., and Worland, M. R. (1995). Thermal environments of Arctic soil organisms during winter. *Arctic and Alpine Research* **27**, 364–371. doi:10.2307/1552029

Coulson, S. J., Convey, P., Aakra, K., Aarvik, L., Avila-Jimenez, M. L., Babenko, A., Biersma, E. M., Bostrom, S., Brittain, J. E., Carlsson, A. M., Christoffersen, K., De Smet, W. H., Ekremj, T., Fjellberg, A., Fureder, L., Gustafssonm, D., Gwiazdowicz, D. J., Hansen, L. O., Holmstrup, M., Hulle, M., Kaczmarek, Ł., Kolicka, M., Kuklin, V., Lakka, H. K., Lebedeva, N., Makarova, O., Maraldo, K., Melekhina, E., Odegaard, F., Pilskog, H. E., Simon, J. C., Sohlenius, B., Solhoy, T., Soli, G., Sture, E., Tanasevitch, A., Taskaeva, A., Velle, G., Zawierucha, K., and Zmudczyńska-Skarbek, K. (2014). The terrestrial and freshwater invertebrate biodiversity of the archipelagoes of the Barents Sea; Svalbard, Franz Josef Land and Novaya Zemlya. *Soil Biology & Biochemistry* **68**, 440–470. doi:10.1016/j.soilbio.2013.10.006

Dabert, J., Ehrnsberger, R., and Dabert, M. (2008). *Glaucalges tytonis* sp. nov. (Analgoidea: Xolalgidae) from the barn owl *Tyto alba* (Strigiformes: Tytonidae): compiling morphology with DNA barcode data for taxa descriptions in mites (Acari). *Zootaxa* **1719**, 41–52.

Dabert, M., Witalinski, W., Kaźmierski, A., Olszanowski, Z., and Dabert, J. (2010). Molecular phylogeny of acariform mites (Acari, Arachnida): strong conflict between phylogenetic signal and long branch attraction artifacts. *Molecular Phylogenetics and Evolution* **56**, 222–241. doi:10.1016/j.ympev.2009.12.020

De Smet, W. H. (1993). Report on rotifers from Barentsøya, Svalbard (78°30′N). *Fauna Norvegica Serie A* **14**, 1–26.

De Smet, W. H., Van Rompu, E. A., and Beyens, L. (1987). Rotifera, Gastrotricha en Tardigrada uit Shetland, de Faroër en Spitsbergen (Rotifera, Gastrotricha et Tardigrada provenant du Shetland, les îles Féroé et du Spitsberg). *Natuurwetenschappelijk Tijdschrift* **69**, 81–102.

De Smet, W. H., Van Rompu, E. A., and Beyens, L. (1988). Contribution to the rotifers and aquatic Tardigrada of Edgeøya (Svalbard). *Fauna Norvegica Serie A* **9**, 19–30.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791. doi:10.2307/2408678

Fiers, F., and Kotwicki, L. (2013). The multiple faces of *Nannopus palustris* auct. reconsidered: a morphological approach (Copepoda: Harpacticoida: Nannopodidae). *Zoologischer Anzeiger* **253**, 36–65. doi:10.1016/j.jcz.2013.08.001

Fregni, E., Balsamo, M., and Tongiorgi, P. (1998). Interstitial gastrotrichs from lotic Italian fresh waters. *Hydrobiologia* **368**, 175–187. doi:10.1023/A:1003250217483

Goldman, N., and Yang, Z. (1994). A codon-based model of nucleotide substitution for protein-coding DNA sequences. *Molecular Biology and Evolution* **11**, 725–736.

Górska, B., Grzelak, K., Kotwicki, L., Hasemann, C., Schew, I., Soltwed, T., and Włodarska-Kowalczuk, M. (2014). Bathymetric variations in vertical distribuction patterns of meiofauna in the surface sediments of the deep Arctic ocean. *Deep-sea Research. Part I, Oceanographic Research Papers* **91**, 36–49. doi:10.1016/j.dsr.2014.05.010

Grzelak, K., and Kotwicki, L. (2012). Meiofaunal distribution in Horsund fjord, Spitsbergen. *Polar Biology* **35**, 269–280. doi:10.1007/s00300-011-1071-5

Guerne, J. (1888). Campagnes scientifiques du Yacht monégasque l'Hirondelle, treoisième année 1887. [Scientific surveys of the Monaco Yacht Hirondelle, third year 1887]. Paris *Gauthier-Villars* **10** (26), 72–78.

Hagen, J. O., Kohler, J., Melvold, K., and Winther, J. G. (2003). Glaciers in Svalbard: mass balance, runoff and freshwater flux. *Polar Research* **22**, 145–159. doi:10.1111/j.1751-8369.2003.tb00104.x

Hochberg, R. (1998). Postembryonic growth and morphological variability in *Turbanella mustela* (Gastrotricha, Macrodasyida). *Journal of Morphology* **237**, 117–126. doi:10.1002/(SICI)1097-4687(199808)237:2<117::AID-JMOR3>3.0.CO;2-Z

Hochberg, R., and Litvaitis, M. K. (2000). Phylogeny of Gastrotricha: a morphology-based framework of Gastrotricha relationships. *The Biological Bulletin* **198**, 299–305. doi:10.2307/1542532

Hummon, W. D., Balsamo, M., and Todaro, M. A. (1992). Italian marine Gastrotricha: I. Six new and one redescribed species of Chaetonotida. *Bollettino di Zoologia* **59**, 499–516. doi:10.1080/11250009209386711

Jankowska, E., Włodarska-Kowalczuk, M., Kotwicki, L., Balazy, P., and Kukliński, K. (2014). Seasonality in the vegetation cover and its effects on sediment characteristics and meiofauna in the Baltic seagrass meadows. *Estuarine, Coastal and Shelf Science* **139**, 159–170. doi:10.1016/j.ecss.2014.01.003

Kånneby, T. (2013). New species and records of freshwater *Chaetonotus* (Gastrotricha: Chaetonotidae) from Sweden. *Zootaxa* **3701**, 551–588. doi:10.11646/zootaxa.3701.5.3

Kånneby, T. (2015). A redescription of *Chaetonotus* (*Primochaetus*) *veronicae* Kånneby, 2013 (Gastrotricha: Chaetonotidae). *Zootaxa* **4027** (3), 442–446. doi:10.11646/zootaxa.4027.3.8

Kånneby, T., Todaro, M. A., and Jondelius, U. (2012). A phylogenetic approach to species delimitation in freshwater Gastrotricha from Sweden. *Hydrobiologia* **683**, 185–202. doi:10.1007/s10750-011-0956-1

Kånneby, T., Todaro, M. A., and Jondelius, U. (2013). Phylogeny of Chaetonotidae and other Paucitubulatina (Gastrotricha: Chaetonotida) and colonization of aquatic ecosystems. *Zoologica Scripta* **42**, 88–105. doi:10.1111/j.1463-6409.2012.00558.x

Kieneke, A., and Schmidt-Rhaesa, A. (2015). Gastrotricha. In 'Handbook of Zoology. Vol. 3 Gastrotricha and Gnathifera'. (Ed. A. Schmidt-Rhaesa.) pp. 1–134. (De Gruyter: Berlin, Boston.)

Kieneke, A., and Zekely, J. (2007). *Desmodasys abyssalis* sp. nov. – first record of a deep-sea gastrotrich from hydrothermal vents. *Marine Biodiversity Records* **1**(88), 1–7.

Kisielewski, J. (1981). 'Gastrotricha from Raised and Transitional Peat Bogs in Poland. Monografie Fauny Polski, 11.' (Polska Akademia Nauk: Kraków, Poland.)

Kisielewski, J. (1991). Inland-water Gastrotricha from Brazil. *Annales Zoologici* **43**, 1–168.

Kisielewski, J. (1997). 'Brzuchorzeski (Gastrotricha). Fauna Słodkowodna Polski, Zeszyt 31.' (Wydawnictwo Uniwersytetu Łódzkiego: Łódź, Poland.)

Kolicka, M. (2014). Gastrotricha from Copenhagen Palm House – contribution to the knowledge of *Lepidodermella intermedia* Kånneby, Todaro & Jondelius, 2012 (Chaetonotida, Gastrotricha). *Zoosystema* **36**(4), 713–722. doi:10.5252/z2014n4a1

Kolicka, M. (2016). Gastrotrichs in bromeliads – newly recorded *Chaetonotus* (*Hystricochaetonotus*) *furcatus* Kisielewski, 1991 (Chaetonotida) from the Łódź Palm House. *Zoosystema* **38**, 141–155. doi:10.5252/z2016n1a5

Kolicka, M., Kisielewski, J., Nesteruk, T., and Zawierucha, K. (2013). Gastrotricha from the Poznań Palm House – one new subgenus and three new species of freshwater Chaetonotida (Gastrotricha). *Zootaxa* **3717**(2), 231–279. doi:10.11646/zootaxa.3717.2.7

Kolicka, M., Jankowska, E., and Kotwicki, L. (2015). Baltic Sea Gastrotricha – one new species and one new record of Chaetonotida from Poland. *Zootaxa* **4027**(4), 487–508. doi:10.11646/zootaxa.4027.4.2

Kotwicki, L., De Troch, M., Urban-Malinga, B., Gheskiere, T., and Węsławski, J. M. (2005). Horizontal and vertical distribution of meiofauna on sandy beaches of the North Sea (The Netherlands, Belgium, France). *Helgoland Marine Research* **59**, 255–264. doi:10.1007/s10152-005-0001-8

Leasi, F., and Todaro, M. A. (2008). The muscular system of *Musellifer delamarei* (Reanud-Mornant, 1968) and other chaetonotidans with implication for the phylogeny and systematisation of the Paucitubulatina (Gastrotricha). *Biological Journal of the Linnean Society. Linnean Society of London* **94**, 379–398. doi:10.1111/j.1095-8312.2008.00974.x

Leasi, F., and Todaro, M. A. (2009). Meiofaunal cryptic species revealed by confocal microscopy: the case of *Xenotrichula intermedia* (Gastrotricha). *Marine Biology* **156**, 1335–1346. doi:10.1007/s00227-009-1175-4

Mock, H. (1979). Chaetonotidea (Gastrotricha) from the North Sea Island of Sylt. *Mikrofauna Meeresbodens* **78**, 1–107.

Nicholas, K. B., and Nicholas, H. B. (1997). GeneDoc: a tool for editing and annotating multiple sequence alignments. Pittsburgh Supercomputing Center's National Resource for Biomedical Supercomputing. Available at http://www.nrbsc.org/downloads/ [Accessed 10 January 2014]

Norwegian Meteorological Institute (2014). Available at http://retro.met.no/observasjoner/svalbard/index.html [Accessed 12 July 2014]

Nylander, J. A. A. (2004). 'MrModeltest v2. Program distributed by the author.' (Evolutionary Biology Centre, Uppsala University: Uppsala, Sweden.)

Page, R. D. M. (1996). TREEVIEW: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* **12**, 357–358.

Pfannkuche, O., and Thiel, H. (1987). Meiobenthic stocks and benthic activity on the NE-Svalbard Shelf and in the Nansen Basin. *Polar Biology* **7**, 253–266. doi:10.1007/BF00443943

Pilskog, H. E., Solhøy, T., Gwiazdowicz, D. J., Grytnes, J.-A., and Coulson, S. J. (2014). Invertebrate communities inhabiting nests of migrating passerine, wild fowl and sea birds breeding in the High Arctic, Svalbard. *Polar Biology* **37**, 981–998. doi:10.1007/s00300-014-1495-9

Posada, D. (2008). jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* **25**, 1253–1256. doi:10.1093/molbev/msn083

Rao, G. C., and Clausen, C. (1970). *Planodasys marginalis* gen. et. sp. nov. and Planodasyidae fam. nov. (Gastrotricha Macrodasyoidea). *Sarsia* **42**, 73–82.

Rambaut, A. (2014). FigTree v. 1.4.2. Available at http://tree.bio.ed.ac.uk/software/figtree/ [Accessed 10 January 2014]

Rambaut, A., and Drummond, A. J. (2007). Tracer v1.4. Available at http://beast.bio.ed.ac.uk/Tracer [Accessed 12 July 2014]

Remane, A. (1936). Gastrotricha. In 'Klassen Ordnungen des Tierreichs, Band 4, Abteilung II, Buch I, Teil 2, Liefrungen 1–2'. (Ed. H. G. Bronns.) pp. 1–385. (Akademie Verlagsgesellschaft: Berlin, Germany.)

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., and Huelsenbeck, J. P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**, 539–542. doi:10.1093/sysbio/sys029

Scourfield, D. J. (1897). Contribution to the non-marine fauna of Spitsbergen. Part I. Preliminary notes, and reports on the Rhizopoda, Tardigrada, Entomostraca, *etc. Catalogue of the Birds in the British Museum* **20**, 784–792.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30**(12), 2725–2729. doi:10.1093/molbev/mst197

Telford, M. J., Lockyer, A. E., Cartwright-Finch, C., and Littlewood, D. T. J. (2003). Combined large and small subunit ribosomal RNA phylogenies support a basal position of the acoelomorph flatworms. *Proceedings. Biological Sciences* **270**(1519), 1077–1083. doi:10.1098/rspb.2003.2342

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25**, 4876–4882. doi:10.1093/nar/25.24.4876

Todaro, M. A. (2015). Gastrotricha. World Register of Marine Species. Available at http://www.marinespecies.org/aphia.php?p=taxdetails&id=2078 [Verified 1 September 2015].

Todaro, M. A., Balsamo, M., and Kristensen, R. (2005). A new genus of marine chaetonotids (Gastrotricha), with a description of two new species from Greenland and Denmark. *Journal of the Marine Biological Association of the United Kingdom* **85**, 1391–1400. doi:10.1017/S0025315405012579

Todaro, M. A., Leasi, F., Bizzarri, N., and Tongiorgi, P. (2006). Meiofauna densities and gastrotrich community composition in a Mediterranean sea cave. *Marine Biology* **149**, 1079–1091. doi:10.1007/s00227-006-0299-z

Todaro, M. A., Dal Zotto, M., Maiorova, A. S., and Adrianov, A. V. (2009). A new species of *Aspidiophorus* (Gastrotricha, Chaetonotida) from the Russian Far East with a key to marine species of the genus. *Marine Biology Research* **5**, 297–303. doi:10.1080/17451000802419430

Urban-Malinga, B., Wiktor, J., Jabłońska, A., and Moens, T. (2005). Intertidal meiofauna of high-latitude glacial Arctic fjord (Kongsfjorden, Svalbard) with emphasis on the structure of free-living Nematoda communities. *Polar Biology* **28**, 940–950. doi:10.1007/s00300-005-0022-4

Węsławski, J. M., Zajączkowski, M., Wiktor, J., and Szymelfening, M. (1997). Intertidal zone of Svalbard. 3. Littoral of subartic, oceanic island: Bjornoya. *Polar Biology* **18**, 45–52.

Włodarska-Kowalczuk, M., Szymfeling, M., and Kotwicki, L. (1999). Macro- and meiobenthic fauna of Yaldiabucta glacial bay (Isfjorden, Spitsbergen). *Polish Polar Research* **4**, 367–386.

Zawierucha, K., Coulson, S. J., Michalczyk, Ł., and Kaczmarek, Ł. (2013). Current knowledge of the Tardigrada of Svalbard with the first records of water bears from Nordaustlandet (High Arctic). *Polar Research* **32**, 20886. doi:10.3402/polar.v32i0.20886

Zawierucha, K., Kolicka, M., Takeuchi, N., and Kaczmarek, Ł. (2015). Which animals can live in cryoconite holes of glaciers? A faunal review. *Journal of Zoology* **295**, 159–169. doi:10.1111/jzo.12195

Zwickl, D. J. (2006). 'Genetic algorithm approaches for the phylogenetic analysis of large biological sequence data sets under the maximum likelihood criterion.' PhD Thesis. (The University of Texas, Austin, TX.)

Handling editor: Gonzalo Giribet

Publisher: CSIRO; Journal: IS:Invertebrate Systematics
Article Type: research-article; Volume: ; Issue: ; Article ID: IS16001
DOI: 10.1071/IS16001; TOC Head:

Bifidochaetus, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach

Małgorzata Kolicka[A,E], *Miroslawa Dabert*[B], *Jacek Dabert*[C], *Tobias Kånneby*[D] *and Jacek Kisielewski*[A]

[A]Department of Animal Taxonomy and Ecology, Institute of Environmental Biology, Adam Mickiewicz University in Poznań, Umultowska 89, 61–614 Poznan, Poland.

[B]Molecular Biology Techniques Laboratory, Faculty of Biology, Adam Mickiewicz University in Poznań, Umultowska 89, 61–614 Poznan, Poland.

[C]Department of Animal Morphology, Institute of Environmental Biology, Adam Mickiewicz University in Poznań, Umultowska 89, 61–614 Poznan, Poland.

[D]Department of Zoology, Swedish Museum of Natural History, PO Box 50007, SE–104 05 Stockholm, Sweden.

[E]Corresponding author. Email: kolicka@amu.edu.pl

Supplementary data

Fig. S1. Morphological characters in *Bifidochaetus arcticus*, gen. *et* sp. nov. (*A*) Dorsal body view; (*B*) internal body view; (*C*) ventral body view.

Fig. S2. Division of the scales section in *Bifidochaetus arcticus*, gen. *et* sp. nov. (*A*) Dorsal body view; (*B*) lateral body view.

Fig. S3. Method of counting scales in Chaetonotida: dorsal, lateral and ventral view. The vertical line indicates the number of scales in a single longitudinal row; the horizontal, alternating line indicates the number of scales in a single longitudinal row.

Fig. S4. Phylogenetic relationships of Chaetonotidae inferred from Bayesian and maximum likelihood analyses of 18S rRNA sequence data. Values near branches show Bayesian posterior probability and bootstrap support above 0.5 and 50, respectively.

Fig. S5. Phylogenetic relationships of Chaetonotidae inferred from Bayesian and maximum likelihood analyses of 28S rRNA sequence data. Values near branches show Bayesian posterior probability and bootstrap support above 0.5 and 50, respectively.

Fig. S6. Phylogenetic relationships of Chaetonotidae inferred from Bayesian and maximum likelihood analyses of COI sequence data. Values near branches show Bayesian posterior probability and bootstrap support above 0.5 and 50, respectively.

Table S1. Morphometric of major parameters for *Bifidochaetus arcticus*, gen. *et* sp. nov.

N, number of specimens or structures analysed; Range, the smallest and largest structures found among all specimens measured; s.d., standard deviation; all measurements are given in micrometres (μm); all indicators are given as a percentage (%) and italicised

Publisher: CSIRO; Journal: IS:Invertebrate Systematics
Article Type: research-article; Volume: ; Issue: ; Article ID: IS16001
DOI: 10.1071/IS16001; TOC Head:

Characters	N	Holotype	Range of adult paratypes	s.d.	N	Range of juvenile and subadult paratypes	s.d.
Body length	36	150.50	137.34–153.60	4.608	6	107.24–139.11	11.236
Pharynx length	28	39.48	35.81–43.07	1.653	5	34.18–43.55	3.522
Width of anterior pharynx thickening (a)	25	9.19	7.80–10.57	0.642	4	7.41–10.00	1.206
Width of pharynx narrowing that follows anterior thickening (n)	25	7.61	5.65–8.56	0.672	4	6.35–9.21	1.237
Width of pharynx at its middle length (m)	25	7.81	7.13–8.95	0.273	4	6.61–9.84	1.346
Width of posterior pharynx thickening (p)	26	10.13	10.02–11.43	0.344	4	9.45–12.34	1.306
Width of intestine junction	26	6.98	5.14–7.75	0.582	4	4.78–6.30	1.306
Length of intestine enzymatic section	26	2.75	1.97–3.80	0.455	4	2.26–2.71	0.227
Intestine length	26	86.82	73.61–93.87	5.158	5	48.31–65.28	6.918
Length of cephalic cilia (anterior tuft)	30	6.48–20.27	(4.65–7.14)–(14.94–31.54)	0.656; 3.417	5	(5.91–7.10)–(17.90–20.14)	0.482; 0.968
Length of cephalic cilia (posterior tuft)	30	10.05–27.03	(6.72–13.60)–(20.66–38.00)	1.591; 4.012	5	(7.44–10.36)–(24.78–29.30)	1.193; 2.095
Width of cephalic cilia (anterior tuft)	29	0.49–0.69	(0.39–0.56)–(0.43–0.86)	0.047; 0.087	5	(0.43–0.69)–(0.51–0.72)	0.106; 0.081
Width of cephalic cilia (posterior tuft)	29	0.90–0.94	(0.81–1.00)–(0.86–1.17)	0.053; 0.073	5	(0.82–1.09)–(0.94–1.37)	0.105; 0.183
Hypostomium width	28	7.21	2.09–8.09	2.048	4	6.09–7.36	0.560
Hypostomium length	28	2.26	2.03–7.60	1.984	4	2.13–2.79	0.298
Cephalion length	31	9.22	8.14–10.46	0.511	6	7.53–9.81	0.804
Cephalion width	31	9.49	9.11–11.38	0.589	6	8.65–10.60	0.755
Diameter of mouth ring	30	6.04	5.17–6.14	0.296	5	4.40–5.83	0.524
Furca length	30	18.59	17.39–20.57	0.743	5	16.06–18.66	1.339
Length of adhesive tube	30	10.24	9.38–11.64	0.641	5	9.03–11.98	1.046
Width of adhesive tube	26	2.21	1.94–2.60	0.116	4	2.04–2.34	0.116
Head dorsal sensory bristles length	29	*10.29*	*7.75–16.61*	1.640	6	*9.27–12.25*	1.265
Neck dorsal sensory bristles length	28	*13.94*	*10.35–16.99*	1.406	6	*11.33–13.27*	0.715
Posterior dorsal sensory bristles length	28	*14.97*	*12.46–21.75*	1.826	6	*12.48–16.03*	1.370
Length of matured egg	1	–	*48.65*	–	–	–	–
Width of matured egg	1	–	*34.34*	–	–	–	–
Length of X-organ	6	–	*9.22–11.33*	0.905	–	–	–
Width of X-organ	6	–	*13.06–18.84*	1.952	–	–	–
Number of cephalic cilia in anterior tuft	36	*5*	*5*	0	6	*5*	0
Number of cephalic cilia in posterior tuft	36	*5*	*5–6*	0.167	6	*5–6*	0.408
Number of separated cephalic tufts	36	*4*	*4*	0	6	*4*	0
Number of scales in	36	*40*	*30–42*	2.490	6	*31–40*	3.312

Publisher: CSIRO; Journal: IS:Invertebrate Systematics
Article Type: research-article; Volume: ; Issue: ; Article ID: IS16001
DOI: 10.1071/IS16001; TOC Head:

Characters	N	Holotype	Range of adult paratypes	s.d.	N	Range of juvenile and subadult paratypes	s.d.
single longitudinal row							
Total number of longitudinal alternating rows of scales	36	*41*	*36–45*	2.234	6	*33–43*	3.445
Number of scales in single longitudinal row on ventral interciliary field	36	*3*	*3–7*	1.069	6	*3–6*	1.225
Total number of longitudinal alternating rows of scales	36	*5*	*5–7*	0.701	6	*5*	0
Pharynx formula a	25	*23.278*	*18.470–26.073*	1.701	4	*20.158–29.257*	3.925
Pharynx formula n	25	*19.276*	*13.379–21.209*	1.750	4	*17.274–26.946*	4.317
Pharynx formula m	25	*19.782*	*16.889–23.105*	1.342	4	*17.982–28.789*	4.780
Pharynx formula p	25	*25.659*	*24.338–29.158*	1.193	4	*25.097–36.103*	5.339
Pharynx–intenstine ratio I	28	*45.473*	*42.239–54.508*	2.670	5	*56.254–76.092*	7.426
Ratio of scale distribution	36	*102.50*	*102.50–123.33*	4.767	6	*102.50–113.16*	4.076

Table S2. Morphometric of scale parameters for *Bifidochaetus arcticus*, gen. *et* sp. nov.

N, number of specimens or structures analysed; Range, the smallest and largest structures found among all specimens measured; s.d., standard deviation; all measurements are given in micrometres (μm)

Characters	N	Holotype	Range of adult paratypes	s.d.	N	Range of juvenile and subadult paratypes	s.d.
Length of head dorsal scales	26	1.36–3.88	(1.28–1.87)–(2.57–4.38)	0.131; 0.370	4	(1.18–1.58)–(3.03–3.88)	0.194; 0.355
Width of head dorsal scales	26	1.43–4.11	(1.32–1.93)–(2.62–4.42)	0.135; 0.368	4	(1.23–1.64)–(3.09–3.95)	0.196; 0.358
Length of head dorsolateral scales	26	1.30–2.75	(1.17–1.88)–(2.52–3.92)	0.180; 0.366	4	(1.09–1.86)–(2.32–3.12)	0.339; 0.340
Width of head dorsolateral scales	26	1.37–2.82	(1.21–2.24)–(2.59–3.95)	0.223; 0.349	4	(1.13–1.87)–(2.39–3.17)	0.327; 0.334
Length of head lateral scales	26	1.23–2.73	(1.11–1.69)–(2.40–3.42)	0.150; 0.278	4	(1.02–1.52)–(2.17–3.02)	0.256; 0.396
Width of head lateral scales	26	1.29–2.80	(1.17–2.47)–(2.43–3.52)	0.260; 0.265	4	(1.10–1.58)–(2.22–3.07)	0.239; 0.394
Length of head ventral scales	26	0.80–1.65	(0.53–1.11)–(1.39–2.25)	0.132; 0.214	4	(0.61–0.90)–(1.30–1.67)	0.134; 0.158
Width of head ventral scales	26	0.86–1.78	(0.59–1.18)–(1.50–2.28)	0.135; 0.201	4	(0.64–0.94)–(1.33–1.72)	0.138; 0.169
Length of neck dorsal scales	26	2.33–2.93	(1.74–2.70)–(2.84–3.93)	0.202; 0.264	4	(1.56–2.09)–(2.41–3.32)	0.250; 0.389
Width of neck dorsal scales	26	2.37–2.96	(1.76–2.73)–(2.87–3.99)	0.204; 0.274	4	(1.61–2.11)–(2.44–3.37)	0.241; 0.400
Length of neck dorsolateral scales	26	2.11–2.63	(1.38–2.47)–(2.62–3.55)	0.219; 0.234	4	(1.33–2.00)–(2.20–2.84)	0.280; 0.303
Width of neck dorsolateral scales	26	2.13–2.66	(1.44–2.52)–(2.66–3.58)	0.216; 0.235	4	(1.37–2.06)–(2.24–2.88)	0.285; 0.297
Length of neck	26	2.09–2.56	(1.23–2.39)–	0.301; 0.201	4	(1.21–1.69)–	0.203; 0.278

Publisher: CSIRO; Journal: IS:Invertebrate Systematics
Article Type: research-article; Volume: ; Issue: ; Article ID: IS16001
DOI: 10.1071/IS16001; TOC Head:

Characters	N	Holotype	Range of adult paratypes	s.d.	N	Range of juvenile and subadult paratypes	s.d.
lateral scales			(2.42–3.41)			(2.09–2.76)	
Width of neck lateral scales	26	2.15–2.60	(1.26–2.42)–(2.49–3.49)	0.230; 0.202	4	(1.25–1.71)–(2.14–2.80)	0.195; 0.272
Length of neck ventrolateral scales	26	1.35–2.03	(1.07–2.19)–(1.89–2.83)	0.259; 0.320	4	(1.01–1.67)–(1.84–2.42)	0.290; 0.269
Width of neck ventrolateral scales	26	1.39–2.09	(1.10–2.21)–(1.93–2.85)	0.265; 0.320	4	(1.05–1.73)–(1.89–2.51)	0.297; 0.280
Length of neck ventral scales	26	0.87–1.60	(0.68–1.31)–(1.34–2.32)	0.147; 0.248	4	(0.67–0.93)–(1.64–1.88)	0.114; 0.106
Width of neck ventral scales	26	0.90–1.63	(0.74–1.33)–(1.38–2.35)	0.148; 0.248	4	(0.71–1.00)–(1.68–1.93)	0.121; 0.110
Length of trunk dorsal scales	26	2.42–4.07	(2.12–3.06)–(3.20–4.56)	0.213; 0.361	4	(2.04–2.53)–(2.94–3.85)	0.228; 0.386
Width of trunk dorsal scales	26	2.47–4.11	(2.13–3.13)–(3.26–4.62)	0.218; 0.357	4	(2.08–2.56)–(3.03–3.89)	0.226; 0.362
Length of trunk dorsolateral scales	26	2.33–4.01	(2.06–2.68)–(3.00–4.14)	0.182; 0.335	4	(1.80–2.29)–(2.82–3.12)	0.216; 0.137
Width of trunk dorsolateral scales	26	2.38–4.06	(2.09–2.83)–(3.04–4.19)	0.201; 0.326	4	(1.83–2.33)–(2.85–3.16)	0.224; 0.154
Length of trunk lateral scales	26	2.26–3.38	(1.90–2.53)–(2.88–3.93)	0.190; 0.302	4	(1.82–2.18)–(2.45–3.02)	0.148; 0.261
Width of trunk lateral scales	26	2.33–3.40	(1.96–2.66)–(2.91–3.95)	0.196; 0.300	4	(1.86–2.21)–(2.53–3.07)	0.144; 0.266
Length of trunk ventrolateral scales	26	1.46–2.50	(1.26–2.27)–(2.50–3.96)	0.306; 0.305	4	(1.34–1.71)–(2.21–2.83)	0.170; 0.261
Width of trunk ventrolateral scales	26	1.54–2.56	(1.28–2.29)–(2.54–3.27)	0.303; 0.203	4	(1.38–1.75)–(2.23–2.87)	0.165; 0.268
Length of trunk ventral scales	26	1.10–2.02	(0.88–1.76)–(1.72–2.62)	0.186; 0.187	4	(1.04–1.10)–(1.81–2.24)	0.026; 0.176
Width of trunk ventral scales	26	1.13–2.07	(0.91–1.79)–(1.74–2.67)	0.182; 0.118	4	(1.09–1.19)–(1.87–2.26)	0.046; 0.161
Length of scales 1	27	4.32	3.69–5.61	0.510	4	3.53–5.09	0.642
Width of scales 1	27	3.37	3.13–5.10	0.592	4	3.03–4.78	
Length of scales 2	28	2.57–3.00	(2.09–3.34)–(2.82–3.98)	0.338; 0.357	4	(1.56–2.67)–(2.48–3.33)	0.519; 0.354
Width of scales 2	28	2.23–2.70	(1.34–3.11)–(1.88–3.66)	0.399; 0.357	4	(1.47–2.26)–(2.01–3.45)	0.327; 0.624
Length of scales 3	26	4.94	4.18–5.37	0.356	4	4.22–4.49	0.131
Width of scales 3	26	4.19	3.24–5.13	0.375	4	3.72–4.30	0.268
Length of scales 4	26	4.24	2.87–4.90	0.504	4	3.24–3.97	0.337
Width of scales 4	26	3.83	2.53–4.18	0.462	4	2.83–3.55	0.314
Length of posteriormost pair of lateral scales (scales 5)	24	3.49	2.35–3.88	0.388	4	2.50–3.77	0.519
Width of posteriormost pair of lateral scales (scales 5)	24	2.48	1.99–3.54	0.419	4	1.94–2.84	0.405
Length of scales 6	26	5.72	4.36–6.95	0.488	4	4.72–5.08	0.151
Width of scales 6	23	4.47	3.79–5.52	0.438	4	3.22–4.72	0.438
Length of scales 7	25	2.58–2.63	(1.36–3.08)–(2.28–3.82)	0.367; 0.436	4	(1.59–2.24)–(1.76–2.78)	0.297; 0.525
Width of scales 7	25	2.17–2.33	(1.26–2.71)–(1.58–3.01)	0.348; 0.353	4	(1.13–1.93)–(1.43–2.40)	0.363; 0.512
Length of scales 8	28	3.38	2.44–3.88	0.343	4	2.36–3.55	0.537
Width of scales 8	28	3.42	2.51–3.95	0.341	4	2.41–3.63	0.550

Publisher: CSIRO; Journal: IS:Invertebrate Systematics
Article Type: research-article; Volume: ; Issue: ; Article ID: IS16001
DOI: 10.1071/IS16001; TOC Head:

Characters	N	Holotype	Range of adult paratypes	s.d.	N	Range of juvenile and subadult paratypes	s.d.
Length of intrafurcal scales (scales 9)	26	1.30–1.81	(1.07–1.64)–(1.38–2.03)	0.151; 0.204	4	(1.15–1.76)–(1.30–2.04)	0.285; 0.339
Width of intrafurcal scales (scales 9)	26	1.52–2.12	(1.19–1.79)–(1.43–2.64)	0.159; 0.255	4	(1.21–1.67)–(1.35–2.11)	0.220; 0.345
Length of ventral interciliary field scales	29	1.74–3.20	(1.28–2.62)–(2.26–4.54)	0.354; 0.527	4	(1.22–1.89)–(2.10–3.59)	0.275; 0.666
Width of ventral interciliary field scales	29	1.36–1.93	(0.71–1.67)–(1.32–2.66)	0.243; 0.296	4	(0.82–1.26)–(1.30–2.29)	0.220; 0.479
Length of ventral interciliary field terminal scales	29	11.28	9.35–14.95	1.106	4	9.32–10.99	0.791
Width of ventral interciliary field terminal scales	29	6.14	4.59–7.94	0.636	4	4.91–5.62	0.354

Table S3. Morphometric of spine parameters for *Bifidochaetus arcticus*, gen. *et* sp. nov.

N, number of specimens or structures analysed; Range, the smallest and largest structures found among all specimens measured; s.d., standard deviation; all measurements are given in micrometres (μm); all indicators are given as a percentage (%) and italicised

Characters	N	Holotype	Range of adult paratypes	s.d.	N	Range of juvenile and subadult paratypes	s.d.
Length of head dorsal spines (1.1)	26	6.74–13.10	(5.61–9.21)–(11.94–14.47)	0.905; 0.663	4	(5.62–6.61)–(10.92–11.63)	0.421; 0.309
Length of head dorsal not divided spines segment	26	0.91–2.32	(0.76–1.77)–(1.72–2.95)	0.200; 0.254	4	(0.64–0.80)–(1.93–2.17)	0.076; 0.107
Length of head dorsal bifurcation spines segment	26	5.79–10.81	(4.59–7.28)–(9.76–12.82)	0.444; 0.595	4	(4.95–5.79)–(8.97–9.72)	0.363; 0.351
Length of head dorsolateral spines (1.2)	26	6.76–11.63	(6.07–8.41)–(10.30–12.42)	0.471; 0.647	4	(5.65–6.63)–(9.34–10.61)	0.493; 0.582
Length of head dorsolateral not divided spines segment	26	0.95–2.02	(0.78–1.62)–(1.59–2.43)	0.182; 0.178	4	(0.66–0.82)–(1.69–1.92)	0.076; 0.100
Length of head dorsolateral bifurcation spines segment	26	5.78–9.64	(5.25–6.94)–(8.56–10.81)	0.337; 0.582	4	(4.98–5.79)–(7.69–8.78)	0.350; 0.510
Length of head lateral spines (1.3)	26	7.66–10.78	(6.83–8.39)–(9.54–11.94)	0.425; 0.571	4	(6.91–7.24)–(8.94–10.32)	0.146; 0.667
Length of head lateral not divided spines segment	26	1.10–1.87	(0.74–1.38)–(1.45–2.11)	0.137; 0.162	4	(0.88–1.01)–(1.23–1.78)	0.060; 0.238
Length of head lateral bifurcation spines segment	26	6.53–8.94	(5.76–6.95)–(8.03–9.96)	0.326; 0.472	4	(5.99–6.21)–(7.67–8.76)	0.093; 0.558
Length of head ventral spines (1.4)	26	6.87–9.31	(5.20–7.54)–(8.01–10.71)	0.560; 0.668	4	(6.02–6.73)–(7.68–9.17)	0.315; 0.749
Length of head	26	1.01–	(0.56–1.13)–	0.173; 0.175	4	(0.80–0.91)–(1.12–	0.050; 0.198

Publisher: CSIRO; Journal: IS:Invertebrate Systematics
Article Type: research-article; Volume: ; Issue: ; Article ID: IS16001
DOI: 10.1071/IS16001; TOC Head:

Characters	N	Holotype	Range of adult paratypes	s.d.	N	Range of juvenile and subadult paratypes	s.d.
ventral not divided spines segment		1.31	(1.25–1.96)			1.58)	
Length of head ventral bifurcation spines segment	26	5.77–8.09	(4.39–6.41)–(6.58–9.30)	0.475; 0.655	4	(5.19–5.86)–(6.58–7.95)	0.298; 0.657
Length of neck dorsal spines (2.1)	26	6.45–10.46	(4.85–8.44)–(9.21–11.69)	0.570; 0.612	4	(5.33–6.26)–(8.56–10.21)	0.480; 0.744
Length of neck dorsal not divided spines segment	26	1.06–1.75	(0.64–1.79)–(1.35–2.51)	0.225; 0.226	4	(0.57–1.01)–(1.26–1.58)	0.200; 0.137
Length of neck dorsal bifurcation spines segment	26	5.36–8.77	(3.83–6.47)–(7.60–10.04)	0.431; 0.522	4	(4.74–5.32)–(7.31–8.68)	0.300; 0.655
Length of neck dorsolateral spines (2.2)	26	6.32–8.79	(4.81–7.24)–(7.96–10.65)	0.479; 0.664	4	(5.30–6.22)–(7.13–8.13)	0.467; 0.487
Length of neck dorsolateral not divided spines segment	26	1.03–1.61	(0.59–1.29)–(1.18–2.09)	0.178; 0.210	4	(0.52–0.96)–(0.86–1.46)	0.198; 0.270
Length of neck dorsolateral bifurcation spines segment	26	5.24–7.18	(4.13–6.14)–(6.52–8.91)	0.401; 0.563	4	(4.75–5.26)–(6.06–6.96)	0.283; 0.390
Length of neck lateral spines (2.3)	25	6.24–8.07	(4.79–7.15)–(7.73–10.06)	0.462; 0.696	4	(5.21–6.18)–(5.97–7.76)	0.476; 0.449
Length of neck lateral not divided spines segment	25	0.90–1.53	(0.56–1.17)–(1.16–1.96)	0.171; 0.192	4	(0.50–0.84)–(0.80–1.41)	0.157; 0.279
Length of neck lateral bifurcation spines segment	25	5.31–6.56	(4.24–5.84)–(6.48–8.51)	0.366; 0.607	4	(4.64–5.33)–(5.84–6.72)	0.328; 0.363
Length of neck ventrolateral spines (2.4)	25	5.30–7.88	(4.52–7.01)–(7.09–9.15)	0.563; 0.504	4	(4.36–5.17)–(6.54–7.21)	0.429; 0.295
Length of neck ventrolateral not divided spines segment	25	0.71–1.13	(0.47–1.15)–(1.03–1.82)	0.162; 0.197	4	(0.48–0.63)–(0.76–1.05)	0.076; 0.135
Length of neck ventrolateral bifurcation spines segment	25	4.67–6.78	(3.94–5.48)–(6.08–7.42)	0.433; 0.372	4	(3.84–4.52)–(5.69–6.34)	0.350; 0.277
Length of neck ventral spines (2.5)	25	4.63–7.41	(3.83–5.10)–(6.04–7.97)	0.381; 0.567	4	(3.95–4.51)–(6.12–6.96)	0.236; 0.354
Length of neck ventral not divided spines segment	25	0.69–0.98	(0.36–0.93)–(0.74–1.53)	0.138; 0.182	4	(0.42–0.58)–(0.71–0.98)	0.076; 0.123
Length of neck ventral bifurcation spines segment	25	3.86–6.45	(3.37–4.35)–(5.26–6.84)	0.282; 0.480	4	(3.51–3.90)–(5.36–6.01)	0.168; 0.303
Length of trunk dorsal spines (3.1)	26	6.36–8.59	(5.18–6.79)–(7.93–10.04)	0.352; 0.554	4	(5.27–6.10)–(6.03–8.32)	0.441; 0.968
Length of trunk dorsal not divided spines segment	26	1.10–1.51	(0.57–1.25)–(1.03–1.77)	0.189; 0.179	4	(0.55–1.04)–(0.91–1.45)	0.224; 0.251
Length of trunk dorsal bifurcation	26	5.26–7.11	(4.22–5.58)–(6.37–8.43)	0.293; 0.474	4	(4.68–5.29)–(6.67–6.92)	0.289; 0.103

Publisher: CSIRO; Journal: IS:Invertebrate Systematics
Article Type: research-article; Volume: ; Issue: ; Article ID: IS16001
DOI: 10.1071/IS16001; TOC Head:

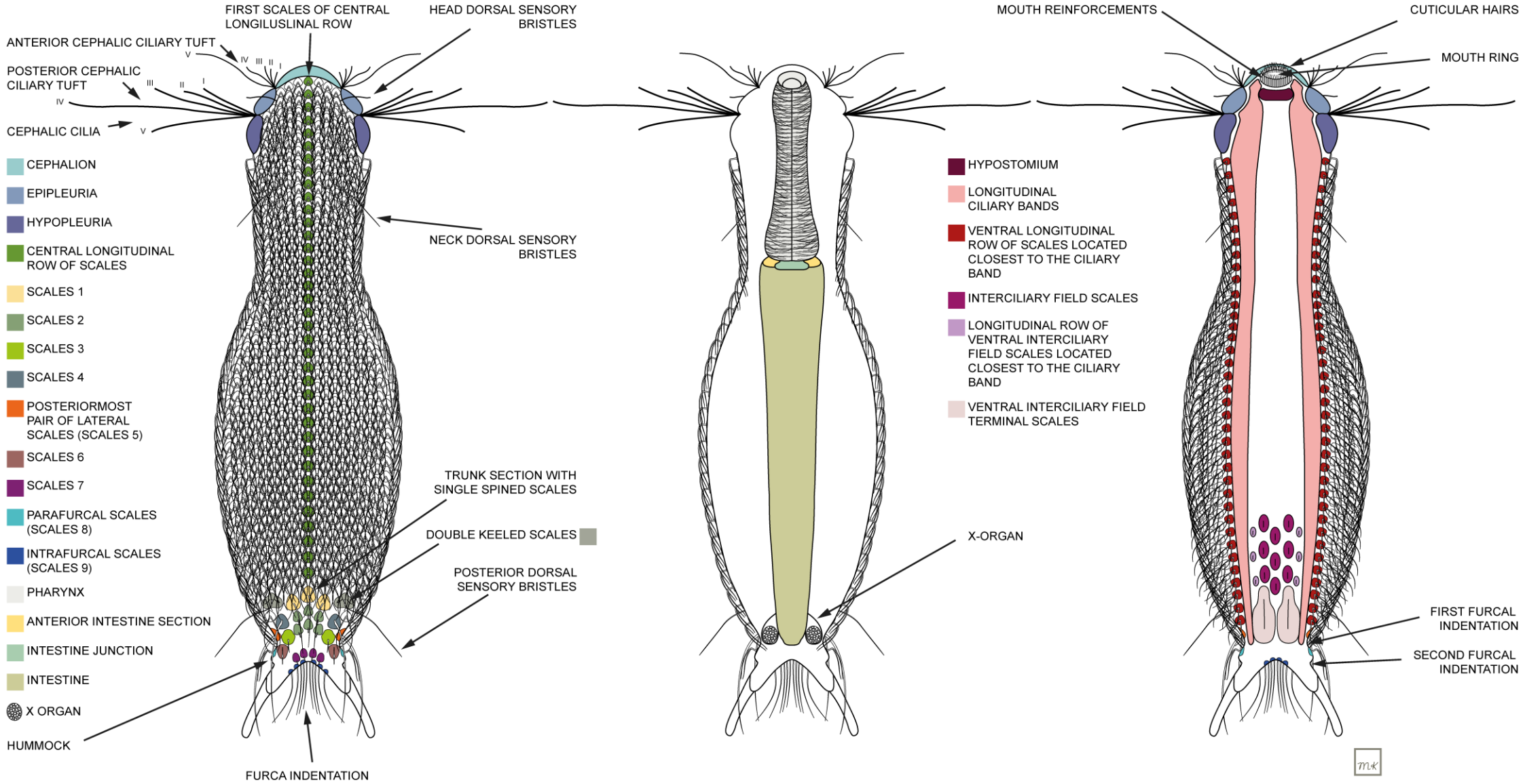
Characters	N	Holotype	Range of adult paratypes	s.d.	N	Range of juvenile and subadult paratypes	s.d.
spines segment							
Length of trunk dorsolateral spines (3.2)	26	6.32–8.56	(5.10–6.75)–(7.29–10.01)	0.371; 0.627	4	(5.22–6.07)–(7.55–8.29)	0.380; 0.341
Length of trunk dorsolateral not divided spines segment	26	0.93–1.49	(0.51–1.21)–(0.98–1.75)	0.195; 0.183	4	(0.52–1.00)–(0.87–1.42)	0.218; 0.253
Length of trunk dorsolateral bifurcation spines segment	26	5.38–7.09	(4.53–5.56)–(6.29–8.90)	0.292; 0.553	4	(4.66–5.04)–(6.72–6.92)	0.163; 0.095
Length of trunk lateral spines (3.3)	26	6.29–8.53	(5.04–6.72)–(7.65–9.95)	0.418; 0.562	4	(5.19–6.04)–(7.51–8.25)	0.378; 0.339
Length of trunk lateral not divided spines segment	26	0.88–1.39	(0.43–1.18)–(0.96–1.73)	0.197; 0.187	4	(0.50–0.98)–(0.85–1.39)	0.222; 0.249
Length of trunk lateral bifurcation spines segment	26	5.40–7.16	(4.49–5.76)–(6.39–8.42)	0.353; 0.444	4	(4.67–5.04)–(6.70–6.89)	0.162; 0.087
Length of trunk ventrolateral spines (3.4)	26	4.36–7.73	(3.79–5.50)–(6.71–8.51)	0.557; 0.491	4	(3.39–4.17)–(6.80–7.51)	0.396; 0.311
Length of trunk ventrolateral not divided spines segment	26	0.62–1.11	(0.42–1.04)–(0.77–1.65)	0.170; 0.187	4	(0.41–0.56)–(0.68–1.02)	0.070; 0.150
Length of trunk ventrolateral bifurcation spines segment	26	3.66–6.64	(3.32–4.79)–(5.83–7.09)	0.442; 0.360	4	(2.97–3.60)–(6.14–6.52)	0.326; 0.174
Length of trunk ventral spines (3.5)	26	3.30–5.58	(2.65–4.10)–(4.97–7.38)	0.349; 0.565	4	(2.39–3.09)–(4.66–5.35)	0.328; 0.300
Length of trunk ventral not divided spines segment	26	0.53–0.89	(0.27–0.67)–(0.53–1.22)	0.116; 0.166	4	(0.33–0.47)–(0.64–0.78)	0.059; 0.059
Length of trunk ventral bifurcation spines segment	26	2.71–4.72	(2.37–3.57)–(4.19–6.38)	0.271; 0.476	4	(2.06–2.67)–(4.05–4.70)	0.281; 0.270
Length of spines scales 1	29	13.79	12.20–17.82	1.214	5	13.11–13.41	0.132
Length of spines scales 2	28	2.94–3.02	(1.72–3.85)–(2.20–4.08)	0.543; 0.508	4	(1.71–2.40)–(2.52–3.03)	0.289; 0.254
Length of spines scales 3	27	2.49	2.33–4.51	0.674	4	2.30–3.18	0.363
Length of spines scales 4	30	18.14	14.52–19.64	1.031	4	16.78–17.89	0.409
Length of posteriormost pair of lateral spines (scales 5)	31	20.19	18.33–22.08	0.891	4	18.03–19.98	1.180
Length of spines scales 6	23	3.27	2.86–5.25	0.605	4	2.35–4.12	0.737
Length of spines scales 7	24	2.42–2.53	(1.60–3.61)–(2.27–4.44)	0.520; 0.570	4	(1.47–2.01)–(1.55–2.74)	0.236; 0.495
Length of spines scales 8 (4)	28	9.33	7.38–9.70	0.705	4	5.57–6.73	0.544
Length of not	28	1.62	1.21–1.88	0.182	4	1.02–1.36	0.152

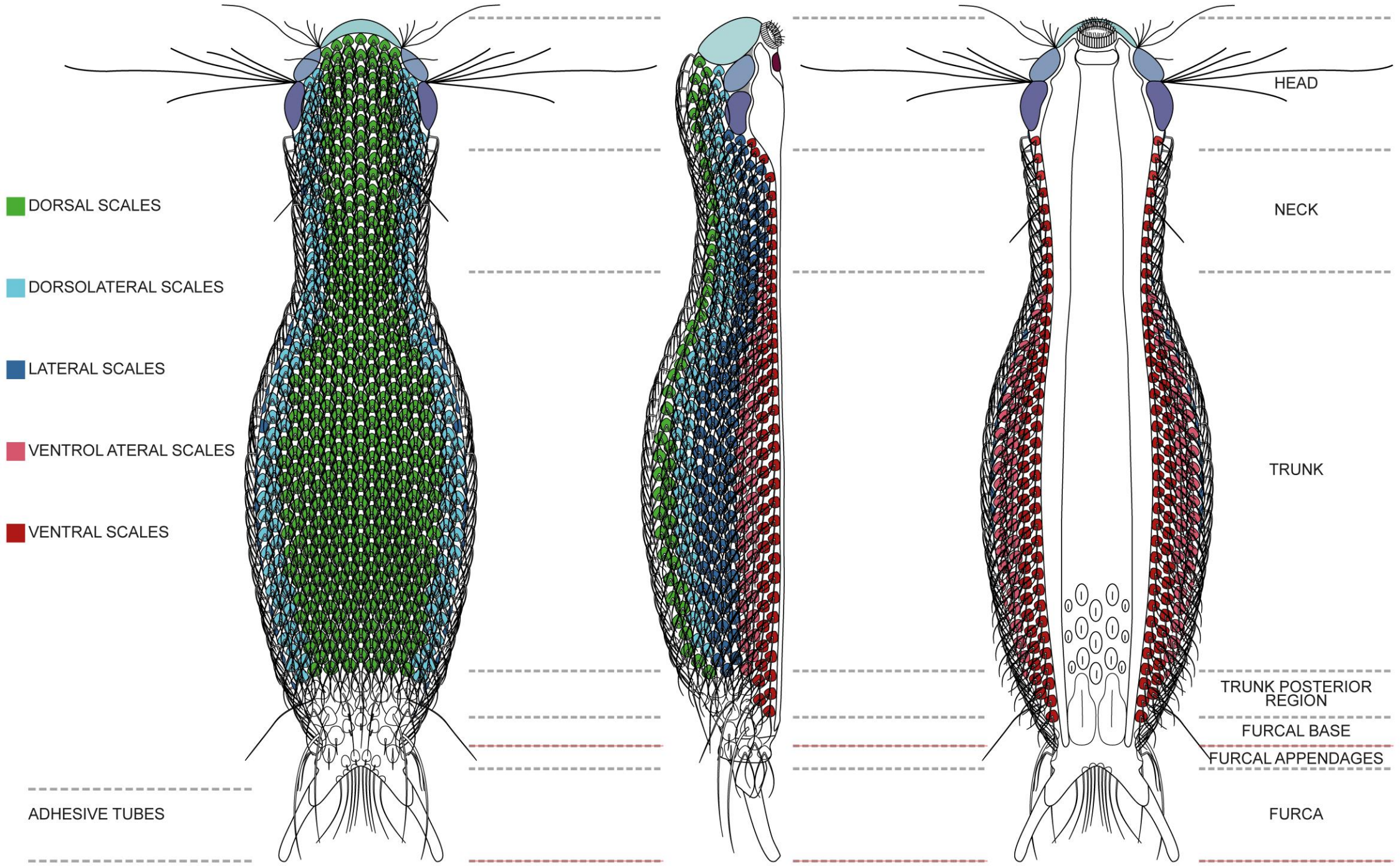
Publisher: CSIRO; Journal: IS:Invertebrate Systematics
Article Type: research-article; Volume: ; Issue: ; Article ID: IS16001
DOI: 10.1071/IS16001; TOC Head:

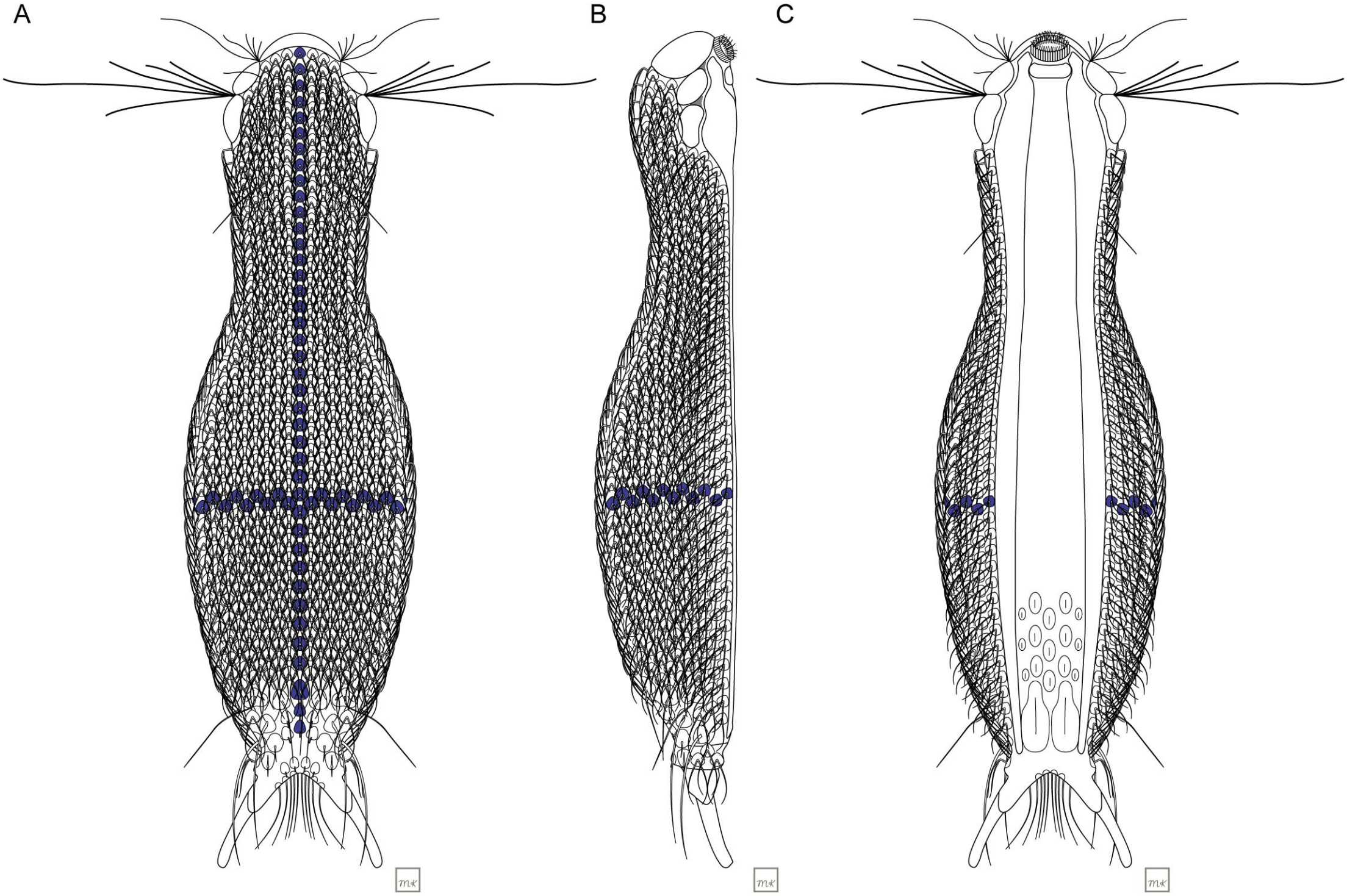
Characters	N	Holotype	Range of adult paratypes	s.d.	N	Range of juvenile and subadult paratypes	s.d.
divided scales 8 spines segment							
Length of bifurcation scales 8 spines segment	28	7.71	6.03–7.92	0.593	4	4.36–5.65	0.593
Length of spines intrafurcal scales (scales 9)	32	7.33–15.09	(5.88–11.88)–(12.87–18.43)	1.147; 1.357	5	(6.23–9.65)–(12.18–14.34)	1.280; 0.817
Ratio of bifurcation dorsal head spines (B 1.1)	26	*82.519–85.905*	(*79.045–84.452*)–(*83.518–88.752*)	1.3015; 1.0264	4	(*80.927–83.577*)–(*86.678–88.078*)	1.1221; 0.6667
Ratio of bifurcation dorsolateral head spines (B 1.2)	26	*82.889–85.503*	(*77.230–85.806*)–(*81.751–89.635*)	1.4466; 1.4716	4	(*82.281–83.619*)–(*86.517–88.142*)	0.6296; 0.7295
Ratio of bifurcation lateral head spines (B 1.3)	26	*82.931–85.248*	(*75.804–85.814*)–(*83.037–88.366*)	1.8567; 1.0449	4	(*82.829–86.577*)–(*85.773–86.819*)	1.9630; 0.4656
Ratio of bifurcation ventral head spines (B 1.4)	26	*83.988–86.896*	(*71.849–87.132*)–(*78.333–90.020*)	2.9622; 2.1793	4	(*82.260–87.073*)–(*85.016–86.696*)	2.0395; 0.7220
Ratio of bifurcation dorsal neck spines (B 2.1)	26	*83.101–83.843*	(*76.659–86.211*)–(*79.321–89.448*)	1.9780; 2.1853	4	(*83.546–86.181*)–(*85.015–88.931*)	1.2062; 1.7381
Ratio of bifurcation dorsolateral neck spines (B 2.2)	26	*81.684–82.911*	(*79.686–85.804*)–(*82.840–90.180*)	1.6094; 2.1460	4	(*82.288–88.550*)–(*84.084–89.811*)	2.9583; 2.4951
Ratio of bifurcation lateral neck spines (B 2.3)	25	*81.289–85.096*	(*78.364–86.805*)–(*81.678–90.429*)	1.8878; 2.4221	4	(*82.216–89.125*)–(*86.246–90.341*)	3.2658; 1.8084
Ratio of bifurcation ventrolateral neck spines (B 2.4)	25	*86.041–88.113*	(*78.174–89.677*)–(*79.016–90.347*)	2.1556; 2.4542	4	(*85.714–88.864*)–(*87.377–89.548*)	1.3657; 1.1110
Ratio of bifurcation ventral neck spines (B 2.5)	25	*83.369–87.045*	(*80.162–88.079*)–(*83.231–89.912*)	1.8664; 1.8617	4	(*85.748–88.861*)–(*86.475–89.571*)	1.4767; 1.5351
Ratio of bifurcation dorsal trunk spines (B 3.1)	26	*82.704–82.771*	(*80.328–87.952*)–(*81.592–89.583*)	1.6751; 2.4085	4	(*82.787–88.613*)–(*83.173–88.805*)	2.7085; 2.6052
Ratio of bifurcation dorsolateral trunktrunk (B 3.2)	26	*82.827–85.127*	(*77.250–88.655*)–(*82.063–90.193*)	2.0572; 2.6047	4	(*83.031–89.007*)–(*83.474–89.286*)	2.7817; 2.7492
Ratio of bifurcation lateral neck spines (B 3.3)	26	*83.939–85.851*	(*78.397–88.509*)–(*83.470–90.300*)	1.9588; 2.2654	4	(*83.444–89.214*)–(*83.515–89.981*)	2.6328–2.9694
Ratio of bifurcation	26	*83.945–85.899*	(*76.364–89.078*)–	2.6229; 1.6822	4	(*86.331–87.611*)–(*86.818–90.294*)	0.5929; 1.5558

Publisher: CSIRO; Journal: IS:Invertebrate Systematics
Article Type: research-article; Volume: ; Issue: ; Article ID: IS16001
DOI: 10.1071/IS16001; TOC Head:

Characters	N	Holotype	Range of adult paratypes	s.d.	N	Range of juvenile and subadult paratypes	s.d.
ventrolateral trunk spines (B 3.4)			(*83.293–89.859*)				
Ratio of bifurcation ventral trunk spines (B 3.5)	26	*82.121–84.588*	(*77.364–90.092*)–(*81.472–90.323*)	2.5174; 2.2267	4	(*84.790–86.680*)–(*85.632–87.850*)	0.8034; 0.9374
Ratio of bifurcation parafulcal spines (scales 8) (B 4)	28	*82.637*	*76.412–87.438*	1.9439	4	*77.741–84.708*	3.167


FIRST SCALES OF CENTRAL LONGILUSLINAL ROW
HEAD DORSAL SENSORY BRISTLES
ANTERIOR CEPHALIC CILIARY TUFT
POSTERIOR CEPHALIC CILIARY TUFT
CEPHALIC CILIA
V
IV
III
II
I
CEPHALION
EPIPLEURIA
HYPOPLEURIA
CENTRAL LONGITUDINAL ROW OF SCALES
SCALES 1
SCALES 2
SCALES 3
SCALES 4
POSTERIORMOST PAIR OF LATERAL SCALES (SCALES 5)
SCALES 6
SCALES 7
PARAFURCAL SCALES (SCALES 8)
INTRAFURCAL SCALES (SCALES 9)
PHARYNX
ANTERIOR INTESTINE SECTION
INTESTINE JUNCTION
INTESTINE
X ORGAN
HUMMOCK
FURCA INDENTATION
NECK DORSAL SENSORY BRISTLES
TRUNK SECTION WITH SINGLE SPINED SCALES
DOUBLE KEELED SCALES
POSTERIOR DORSAL SENSORY BRISTLES
HYPOSTOMIUM
LONGITUDINAL CILIARY BANDS
VENTRAL LONGITUDINAL ROW OF SCALES LOCATED CLOSEST TO THE CILIARY BAND
INTERCILIARY FIELD SCALES
LONGITUDINAL ROW OF VENTRAL INTERCILIARY FIELD SCALES LOCATED CLOSEST TO THE CILIARY BAND
VENTRAL INTERCILIARY FIELD TERMINAL SCALES
X-ORGAN
MOUTH REINFORCEMENTS
CUTICULAR HAIRS
MOUTH RING
FIRST FURCAL INDENTATION
SECOND FURCAL INDENTATION
MK


DORSAL SCALES
DORSOLATERAL SCALES
LATERAL SCALES
VENTROL ATERAL SCALES
VENTRAL SCALES
ADHESIVE TUBES
HEAD
NECK
TRUNK
TRUNK POSTERIOR REGION
FURCAL BASE
FURCAL APPENDAGES
FURCA


A
B
C

Poznań, 16.12.2019r.

Małgorzata Kolicka
Adam Mickiewicz University, Poznań
Faculty of Biology
Department of Animal Taxonomy and Ecology
Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Declaration

I declare that the publication: **Kolicka M.**, Dabert M., Gadawski P. 2017. A new species of freshwater Chaetonotidae (Gastrotricha, Chaetonotida) from Obodska Cave (Montenegro) based on morphological and molecular characters. *European Journal of Taxonomy*, 354: 1-30, has been prepared as a part of my doctoral dissertation under the scientific supervision of Prof. UAM, dr hab. Ziemowit Olszanowski.

Yours faithfully,

Poznań, 16.12.2019r.

Małgorzata Kolicka
Uniwersytet im. Adama Mickiewicza, Poznań
Wydział Biologii
Zakład Taksonomii i Ekologii Zwierząt
ul. Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Oświadczenie

Oświadczam, że publikacja: **Kolicka M.**, Dabert M., Gadawski P. 2017. A new species of freshwater Chaetonotidae (Gastrotricha, Chaetonotida) from Obodska Cave (Montenegro) based on morphological and molecular characters. *European Journal of Taxonomy*, 354: 1-30, została wykonana na poczet mojej rozprawy doktorskiej pod opieką promotora Prof. UAM dra hab. Ziemowita Olszanowskiego.

Z wyrazami szacunku,

European Journal of Taxonomy 354: 1–30
https://doi.org/10.5852/ejt.2017.354
CC BY
ISSN 2118-9773
www.europeanjournaloftaxonomy.eu
2017 · Kolicka M. *et al.*

This work is licensed under a Creative Commons Attribution 3.0 License.

Research article

urn:lsid:zoobank.org:pub:51C2BE54-B99B-4464-8FC1-28A5CC6B9586

A new species of freshwater Chaetonotidae (Gastrotricha, Chaetonotida) from Obodska Cave (Montenegro) based on morphological and molecular characters

Małgorzata KOLICKA[1,*], Piotr GADAWSKI[2] & Miroslawa DABERT[3]

[1]Department of Animal Taxonomy and Ecology, Institute of Environmental Biology, Adam Mickiewicz University Poznan, Umultowska 89, 61–614 Poznan, Poland.
[2]Department of Invertebrate Zoology and Hydrobiology, University of Łódź, Banacha 12/16, 90–237 Łódź, Poland.
[3]Molecular Biology Techniques Laboratory, Faculty of Biology, Adam Mickiewicz University Poznan, Umultowska 89, 61–614 Poznan, Poland.

[*]Corresponding author: kolicka@amu.edu.pl
[2]E-mail: gadawski@biol.uni.lodz.pl
[3]E-mail: mirkad@amu.edu.pl

[1]urn:lsid:zoobank.org:author:550BCAA1-FB2B-47CC-A657-0340113C2D83
[2]urn:lsid:zoobank.org:author:BCA3F37A-28BD-484C-A3B3-C2169D695A82
[3]urn:lsid:zoobank.org:author:8F04FE81-3BC7-44C5-AFAB-6236607130F9

Abstract. Gastrotricha is a cosmopolitan phylum of aquatic and semi-aquatic invertebrates that comprises about 820 described species. Current knowledge regarding freshwater gastrotrichs inhabiting caves is extremely poor and there are no extant data regarding Gastrotricha from Montenegro. We describe a new species from Obodska Cave, which is also the first record of a gastrotrich from this region. Due to its unusual habitat and morphological characteristics, this species may be important when considering the evolution and dispersion routes of Chaetonotidae Gosse, 1864 (*sensu* Leasi & Todaro 2008). We provide morphometric, molecular and phylogenetic data for the new species, together with photomicrographs and drawings.

Keywords. Balkan Peninsula, cave fauna, *Chaetonotus (Chaetonotus) antrumus* sp. nov., DNA barcode, Gastrotricha.

Kolicka M., Gadawski P. & Dabert M. 2017. A new species of freshwater Chaetonotidae (Gastrotricha, Chaetonotida) from Obodska Cave (Montenegro) based on morphological and molecular characters. *European Journal of Taxonomy* 354: 1–30. https://doi.org/10.5852/ejt.2017.354

Introduction

The eumetazoan meiofauna is considered a significant component of both rocky and soft bottoms of various natural aquatic ecosystems (Giere 2009). The meiofauna is an important source of food for macrofauna, small fish, juveniles of large fish and other epibenthic predators (Danovaro *et al.* 2007).

While trophic connections within meiofaunal assemblages are not particularly well studied, there is evidence to suggest that changes in community structure may affect higher and lower trophic levels (McCall & Fleeger 1995; Danovaro *et al.* 2007; Giere 2009). Moreover, meiofauna feeds on detritus, prokaryotes and microscopic eukaryotes and, therefore, plays essential roles in modulation of nutrient cycling processes, secondary production, sediment transport and detritus remineralization (Nozais *et al.* 2005; Danovaro *et al.* 2008). Due to their life cycle characteristics (small size, high turnover and lack of pelagic larval dispersal), these organisms are highly sensitive to environmental disturbance and respond rapidly to changes in food availability (Danovaro 1996; Fraschetti *et al.* 2006).

Within the meiofauna, gastrotrichs are microscopic free-living acoelomate eumetazoans with a total body length from 50 to 3500 µm (e.g., Kisielewski 1997; Balsamo *et al.* 2014; Kieneke & Schmidt-Rhaesa 2015). They inhabit all types of aquatic (fresh, brackish and marine waters) and semi-terrestrial ecosystems (e.g., peat bogs, sedge swamps and alder forests) throughout the world (Kisielewski 1981, 1997; Balsamo *et al.* 2008, 2014; Kieneke & Schmidt-Rhaesa 2015). Currently, there are about 820 known species of Gastrotricha belonging to two orders: Chaetonotida Remane, 1925 [Rao & Clausen, 1970] and Macrodasyida Remane, 1925 [Rao & Clausen, 1970] (Balsamo *et al.* 2009; Hummon & Todaro 2010; Kieneke & Schmidt-Rhaesa 2015; Todaro 2016). Gastrotrichs are known from across the globe, but not all regions have been studied equally. Europe is the most thoroughly studied continent with respect to the gastrotrich fauna, with ca 225 freshwater and ca 150 marine species described (Balsamo *et al.* 2015; Todaro 2016). However, some countries have been studied relatively well, while others are still blank areas on a map of gastrotrich research; for example, Poland is a country with one of the longest histories of detailed studies on gastrotrichs with 100 freshwater (Kisielewski 1997; Kolicka *et al.* 2013; Kolicka 2016) and 31 marine species currently known (Kisielewski 1997; Kolicka *et al.* 2014, 2015). At the same time there are no data available on the freshwater gastrotrich fauna of countries like Portugal, The Netherlands, Albania, Serbia and Montenegro (Balsamo *et al.* 2008, 2014, 2015). The number of species recorded on other continents, including the tropical regions, is even lower, especially when compared with the potential species richness of those areas: there are ca 75 freshwater species of Gastrotricha known from North America, ca 95 from South America, ca 65 from Asia and 10 species from Africa and Australia (Balsamo *et al.* 2008, 2014). Moreover, there are no data on the occurrence of Antarctic freshwater Gastrotricha (Balsamo *et al.* 2008, 2014; Todaro 2016).

Until now, there has been research on neither the freshwater nor the marine gastrotrich fauna in Montenegro or even in all of the Balkan Peninsula region (except Romania), despite the fact that both the high average annual temperature and the diversity of aquatic habitats in this area are favourable to the presence of diverse and abundant gastrotrich communities. During the past five decades, the biota inhabiting dark caves has attracted the attention of many biologists. Cave waters host a great variety of species associated only with this type of habitat, and are often characterized by very restricted geographic distributions (Jones *et al.* 2003). However, studies of the meiofauna of these peculiar biotopes are still very scarce. So far, Gastrotricha associated with inland caves has only been reported once (Vandel 1964), but not identified to the species level. The only comprehensive study on the gastrotrich fauna in a cave habitat was carried out in a sea cave, 'Grotta Piccola del Ciolo', by Todaro *et al.* (2006), who revealed that cave ecosystems can be hotspots of biodiversity and endemism for marine Gastrotricha. It is possible that further research of inland caves might result in similar conclusions regarding freshwater gastrotrichs. The new species described in this study will turn out to be crucial in considering representatives of Chaetonotida as typical cavernicolous organisms (Vandel 1964). Such recognition is highly important for further studies of the adaptation, evolution and dispersal routes of freshwater Gastrotricha.

Material and methods

Study area

This study was performed in the Obodska Cave (42°21.118′ N, 19°0.304′ E), which is located west of the Rijeka Crnojevica, in the cadastral municipality of Ljubotinj II, Montenegro (Fig. 1). The area is not cultivated and most of the natural vegetation is still intact. The landscape is mostly covered with deciduous forest. The climate is classified as humid subtropical (no dry season, hot summer), with a temperate warm and wet forest biozone (Bonada *et al.* 2008). The area is high in leptosol (lp), a weakly developed shallow soil. Cetinje field and its surroundings are inclined to the southeast toward Skadar Lake, which causes gravity flows of groundwater in that direction (Bonada *et al.* 2008). There are numerous caves and cavities in this region, indicating the degree and depth of karstification. Cavities are vertical or horizontal with an opening on the surface. In most cases they are located in areas of vertical cracks or fracture systems, where extended karst processes occur on the tectonic lines and on the contact zone between limestone and dolomite (Martinović 1964; Lješević 1968; Doderović *et al.* 2013).

Obodska Cave is a deep cave with the spring-fed Crnojevica River flowing through it (Figs 2–3). The major part of the cave is formed beneath Pecki Hill. The Crnojevica River flows into the cave through

Fig. 1. Map of Montenegro with the location of Obodska Cave.

a trench that plunges beneath the surface at the foot of Pecki Hill. Obodska Cave was created from stratified limestone, where edges of layers create horns visible on the vault. The sides and bottom of the upper channels are polished, in places, with narrow shelves on the horns of the layers. The cave is situated on three to five morphological levels (Palmer 1991). The total length of the cave is more than 350 meters and comprises three compartments connected by two siphons. The cave was formed through erosion by an underground river flowing along the initial chasm. The river flowed fast through stones and gravel in the whole cave and left holes in the solid rock (Martel 1893). Water in the cave leads to a humid microclimate (Obodska Cave has a precipitation/potential evapotranspiration index higher than 0.65) (Martel 1893; Lješević 1968; Mihavc 1983). Obod spring is characterized by a very variable flow that ranges from a minimum of 0.24 m^3/s to a maximum of 46 m^3/s. Typically, the minimum water level occurs in November or December, and the maximum occurs in March or April, or rarely in February. The first post-summer minimum peak in flow is caused by minimal autumn rainfall and high temperatures, while the maximum peak in flow is due to rain with snow and low temperatures. The low precipitation in Cetinje field and its adjacent surroundings results from a lack of water streams on land surface (Martinović 1964; Lješević 1969; Radulović & Radulović 2004).

The entrance to the cave is situated at 375 m above sea level, and the lowest point of the cave reaches an elevation of 244 m (192 m below the cave entrance). The main channel of the cave is divided by two siphons and creates the upper and lower channel. The lower channel is only partially passable (Mihavc 1983).

Obodska Cave was created in several morphological and hydrological stages. The main upper channel with a constant water flow was created during the first stage, when narrow pits were created at the bottom of the channel. The extension of the pits gradually allowed an increase of the amount of water that plunged through them and formed the second channel. This bifurcation of the channel has migrated during the second stage of development from a point close to the cave entrance to a point 45 meters from the entrance to the cave, where it is currently located. The third stage was characterized by the further scouring of the cave by the water flow, so that the upper part of the channel became dry. It is impossible to observe the bifurcation, because the lower water plunges through sinkholes in the bottom

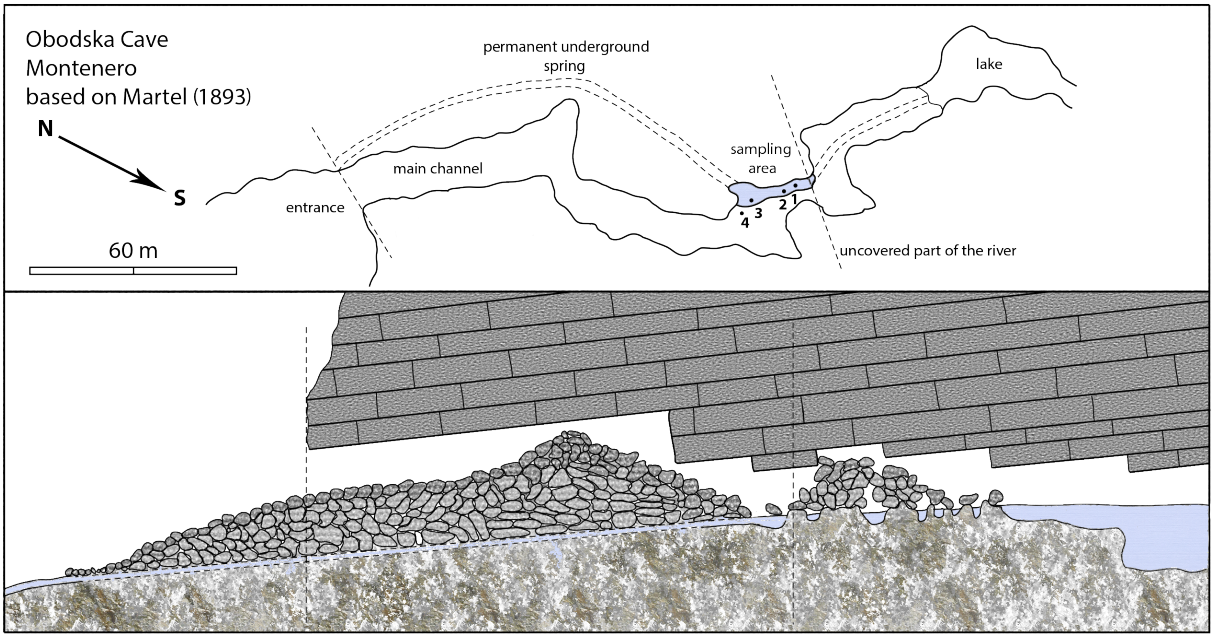


Fig. 2. Scheme of Obodska Cave. 1, 2, 3, 4 = sampling localities.

and flows through cave channels underground. All this shows that there is another, lower cave channel, which today contains warm water. The genesis of this cave and its water flow reflect the typical lowering of the underground water flow in karst. Finally, it should be noted that the regime of the Crnojevic springs, which emerge from the cave, is very uneven. The flow of water fluctuates from 0.03 to 2 m^3 per year (Dinić 1965). A few endemic species of invertebrates are known from this cave, e.g., Amphipoda: *Metohija carinata* Absolon, 1927; Gastropoda: *Plagigeyeria montenigrina* Bole, 1961 and Coleoptera: *Adriaphaenops stirni* Pretner, 1959. They occur only there or in a few other caves on the Adriatic coast (Pretner 1972; Pešić 2010; Hou & Sket 2015).

Sampling and documentation

One sample of approximately 50–70 cm^3 from the top layer of the bottom sediment, together with approximately 100–120 cm^3 of ambient water, was collected from each of 4 sites within one locality on 29 July 2015. Each sample was placed in a 200 cm^3 plastic container. The sampling area was situated at the end of the middle compartment approximately 150 meters from the entrance to the cave. Samples were collected in an area not covered by stones and gravel close to the second siphon, connecting the second and third compartments. Four samples were collected from different parts of the river channel: (1) in the main channel at a depth of 0.4 m, (2) from the main channel at a depth of 0.25 m, (3) from shallows at a depth of 0.1 m, and (4) from pockets among stones and gravel. The last sample (4) was collected from a place where water filled the space between stones and boulders which had fallen from the roof of the cave. The sampling locations were separated by no less than 2 m (Fig. 2).

Water parameters, such as temperature, pH, electrolytic conductivity and dissolved oxygen content, were measured in the field with an Elmetron CX-401 multiparametric sampling probe; BOD5 by Winkler's method, and NH_4, NO_3, PO_3 with a Slandi LF205 photometer. The collected samples were subsequently placed in an insulated box and transported to the laboratory, where the samples were oxygenated and held at 12°C in order to create conditions similar to those at the sampling site. Within 10 days after collection, a total of 2 cm^3 of sediment from each sample was searched for gastrotrichs. Specimens were extracted with a micropipette under an Olympus SZ51 stereo microscope. All specimens were observed, photographed and documented alive with an Olympus BX53 microscope equipped with phase contrast and an Array Artcam 500 digital camera or a Leica DM 5500 B microscope equipped with

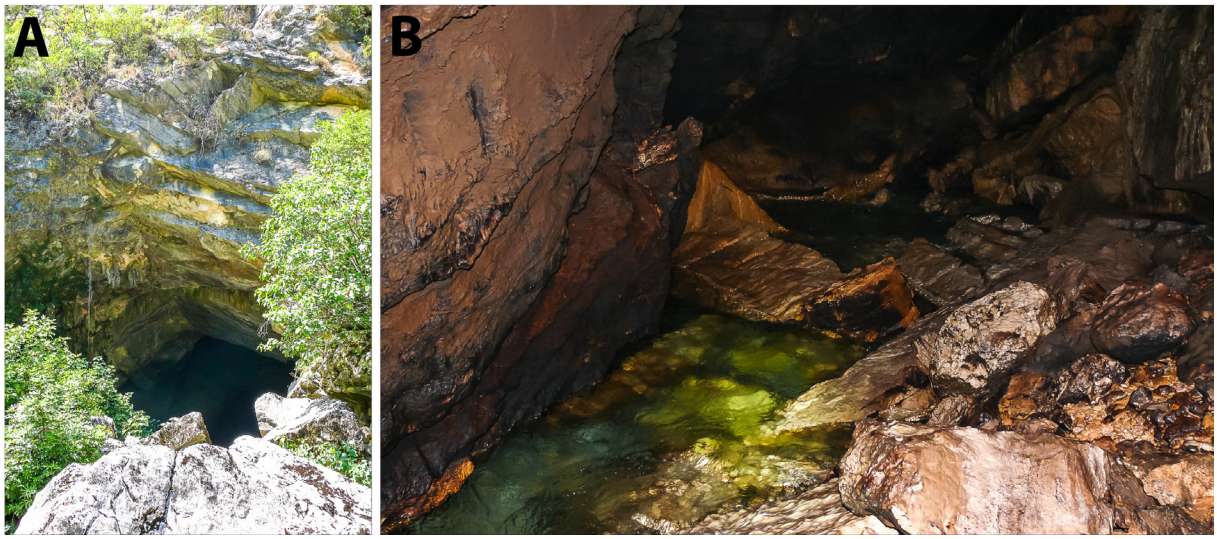


Fig. 3. Photographs of Obodska Cave. **A**. Entrance to the Cave. **B**. Sampling area inside the Cave. (Photographs by Przemysław Śmietana.)

differential interference contrast (DIC) and a Leica DFC 450 digital camera. Morphometric characters were measured in cellSens Entry 1.11 software (Olympus). All measurements are given in micrometers (μm) and all formulas used are given as a percentage (%).

Granulometric analyses

To determine the grain-size properties, the granulometric protocol described by Buchanan (1984) was applied. The grain-size statistics were calculated through Gradistat software using a logarithmic method of moments (Blott & Pye 2001) and sediments were classified according to the Folk and Ward system (Folk & Ward 1957). The organic matter content of the same sediment samples was determined using the combustion method. The main results of the granulometric analyses are provided in Table 1.

Morphological analyses

A morphological feature was measured only if its orientation was conducive to accurate measurement. Ranges of measurements are presented. Each of the specimens was documented by means of a set of photomicrographs. The species description follows the convention of Hummon *et al.* (1992), in which the longitudinal distance of a morphological character from the anterior end is expressed as percentage units (U) of the animal's total length (i.e., the distance of the character from the anterior end, divided by the total body length, and multiplied by 100). The identification of gastrotrichs, their morphological study and terms follows Kisielewski (1981, 1991, 1997) and Kolicka *et al.* (2016). The terms describing the shape of furcal branches and furcal indentation follows Roszczak (1969). In this paper, the following formula describing the distribution of scales was used:

$$\text{Ratio of scales distribution} = \frac{\text{total number of longitudinal alternating rows of scales}}{\text{number of scales in central longitudinal row}} \times 100\%$$

as well as the pharynx formulae according to Kisielewski (1991):

$$a = \frac{\text{width of anterior thickening}}{\text{pharynx length}} \times 100\% \qquad n = \frac{\text{width of pharynx narrowing}}{\text{pharynx length}} \times 100\%$$

$$m = \frac{\text{width of middle pharynx}}{\text{pharynx length}} \times 100\% \qquad p = \frac{\text{width of anterior thickening}}{\text{pharynx length}} \times 100\%$$

Abbreviations used: D = dorsal; DL = dorsolateral; L = lateral; LV = ventrolateral; V= ventral.

The new species proposed in this paper is described by the first author only. This solution is consistent with chapter 11, article 50 of the International Code of Zoological Nomenclature (The International Commission on Zoological Nomenclature 1999).

Molecular analyses

Total genomic DNA was extracted from three single specimens of the new species using the DNeasy Blood and Tissue Kit (Qiagen GmbH, Hilden, Germany) as described by Dabert *et al.* (2008). A 628 bp fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was amplified with a 1:1 mixture of two forward primers, bcdF08 and bcdF09 (Kolicka *et al.* 2016), and reverse primer bcdR04 (Dabert *et al.* 2010). A fragment coding for both internal transcribed spacers (ITS1-5.8S rDNA-ITS2) was amplified with ITS1_18S and ITS2_28S primers (Navajas *et al.* 1998). A complete sequence of 18S rRNA was amplified in two overlapping fragments using 18Sfw/rev960 and fw390/rev18S primer pairs (Dabert *et al.* 2010), respectively. A 2381 bp fragment of 28S rRNA was amplified using the primer pair 28SF0001/28SR2850 (Dabert *et al.* 2016). For primer details see Table 2. PCRs were carried out in 10 μl reaction volumes containing 5 μl of the Type-it Microsatellite PCR Kit (Qiagen), 0.5 μM of each primer and 4 μl of the DNA template using a thermocycling profile with one cycle

Table 1. Main results of sediment sample granulometric analyses. All measurements are given in micrometres (μm); indicators are given as a percentage (%) and italicized.

Sites		**1**	**2**	**3**	**4**
Mean		Coarse sand	Very coarse sand	Medium sand	Very coarse sand
	(M_G)	859.5	1235.8	384.2	1318.4
Sorting		Moderately sorted	Well sorted	Poorly sorted	Very well sorted
	(σ_G)	1.748	1.277	2.190	1.071
Skewness		Symmetrical	Very fine skewed	Fine skewed	Very coarsely skewed
		-0.016	-2.521	-0.104	1.076
Kurtosis		Mesokurtic	Very fine skewed	Leptokurtic	Very platykurtic
		1.080	1.069	1.131	-0.596
Organic matter		*6.61*	*2.64*	*4.09*	*2.63*

of 5 min at 95°C followed by 40 steps of 30 s at 95°C, 90 s at 50°C, 1 min at 72°C, and with a final step of 5 min at 72°C for all reactions. After amplification, the PCR products were diluted with 10 μl of MQ water; 5 μl of the diluted PCR reaction was analysed by electrophoresis on 1% agarose gel. Samples containing visible bands were purified with exonuclease I and Fast alkaline phosphatase (Fermentas) and sequenced using the BigDye Terminator v3.1 kit and the ABI Prism 3130xl Genetic Analyzer (Applied Biosystems), following the manufacturer's instructions. Individual sequence reads were aligned and manually assembled into contigs in ChromasPro v. 1.32 (Technelysium) and GeneDoc v. 2.7.000 (Nicholas & Nicholas 1997). Genetic distance among the COI sequences was estimated using the Kimura 2-parameter model as implemented in MEGA 7 (Kimura 1980; Tamura *et al.* 2013).

Phylogenetic analyses

The nucleotide blast search of COI, 18S and 28S rRNA sequences of *Chaetonotus (Chaetonotus) antrumus* sp. nov. suggested *Chaetonotus* sp. 1 (in Kånneby, Todaro & Jondelius 2013), *Chaetonotus (Chaetonotus)* cf. *sphagnophilus* Kisielewski, 1981 and *Chaetonotus (Chaetonotus)* cf. *laroides* Marcolango, 1910 as the most similar taxa. Therefore, in our phylogenetic analyses we used representatives of the main clades reconstructed in the published molecular phylogeny of Chaetonotidae (Kånneby *et al.* 2013; Kolicka *et al.* 2016). As the outgroup we used sequences of *Aspidiophorus polystictos* Balsamo & Todaro, 1987, which has been reconstructed as the sister group to all other species of Chaetonotidae by Kånneby *et al.* (2013). In total, our data set consisted of 4881 nucleotide positions for 55 terminals (Table 3) and involved COI+18S+28S markers. ITS sequences were excluded from the data set because of the lack of sequence data for this marker for most species included in the phylogenetic analysis.

Choice of an appropriate model of DNA sequence evolution for 18S and 28S rDNA was made using jModeltest 0.11 (Posada 2008); the GTR + I + G model was appropriate for both markers. For COI DNA sequences the two-rate codon-based model was applied (Goldman & Yang 1994) with invertebrate mtDNA genetic code. Tree inference was performed using Bayesian Inference with Markov Chain Monte Carlo (BI), with the appropriate substitution model for each partition. Four independent chains were run on a parallel version of MrBayes 3.2 (Ronquist *et al.* 2012). Each run of the BI analyses was performed in 3–10 × 10^6 generations, and the trees were sampled every 1000th generation. The final 50% majority rule consensus tree was generated after discarding the 25% burn-in fraction of initial trees after assessing the chain convergence in Tracer v.1. (Rambaut & Drummond 2007) judged by the average standard deviation of split frequencies dropping below 0.01. Tree editing was performed using FigTree 1.4.2 (Rambaut 2014).

Table 2. PCR primers used in this study; A and S refer to amplifying and sequencing, respectively.

Primer name	Sequence (5'–3')	Product	Use	Source
bcdF08	CGATGRTTTTTTTCHACWAACCAYAARGATATCGG	COI	A	Kolicka *et al.* 2016
bcdF09	CGATGRTTTTTTTCHACWAACCAYAARGACATTGG	COI	A, S	Kolicka *et al.* 2016
bcdR04	TATAAACYTCDGGATGNCCAAAAAA	COI	A, S	Dabert *et al.* 2008
ITS1_18S	AGAGGAAGTAAAAGTCGTAACAAG	ITS	A, S	Navajas *et al.* 1998
ITS2_28S	ATATGCTTAAATTCAGGGGG	ITS	A, S	Navajas *et al.* 1998
18Sfw	CTTGTCTCAAAGATTAAGCCATGCA	18S rDNA	A, S	Dabert *et al.* 2010
rev480	GTTATTTTTCGTCACTACCT	18S rDNA	S	Dabert *et al.* 2010
fw390	AATCAGGGTTCGATTCCGGAGA	18S rDNA	A, S	Dabert *et al.* 2010
rev960	GACGGTCCAAGAATTTCAC	18S rDNA	A, S	Dabert *et al.* 2010
for1300	TGCATGGCCGTTCTTAGTTG	18S rDNA	S	Dabert *et al.* 2010
rev1460	CATCACAGACCTGTTATTGC	18S rDNA	S	Dabert *et al.* 2010
rev18S	TGATCCTTCCGCAGGTTCACCT	18S rDNA	A, S	Dabert *et al.* 2010
28SF0001	ACCCVCYNAATTTAAGCATAT	28S rDNA	A	Dabert *et al.* 2016
1634LReverse	ATTCGGCAGGTGAGTTGTTACA	28S rDNA	S	Telford *et al.* 2003
1200F	CCCGAAAGATGGTGAACTATGC	28S rDNA	S	Telford *et al.* 2003
2450R	GCTTTGTTTTAATTAGACAGTCGGA	28S rDNA	A, S	Telford *et al.* 2003
28SR2850	GTGGTTTCGCTAGATAGTAGATA	28S rDNA	A, S	Dabert *et al.* 2016
300F	CAAGTACCGTGAGGGAAAGTTG	28S rDNA	S	Telford *et al.* 2003
300R	CAACTTTCCCTCACGGTACTTG	28S rDNA	S	Telford *et al.* 2003
1200R	GCATAGTTCACCATCTTTCGG	28S rDNA	S	Telford *et al.* 2003
UJR2176	CGGATCTAATTTGCCGACTTCCCTTA	28S rDNA	S	Telford *et al.* 2003
1600F	AGCAGGACGGTGGCCATGGAAG	28S rDNA	S	Telford *et al.* 2003

Results

Gastrotrichs, belonging to only one species, were present in two of the four sampled sites: Site 1 (9 specimens) and Site 3 (27 specimens) in Fig. 2.

The physicochemical parameters of the water on the investigated cave river did not vary between sampling sites and were the following: temperature: 12°C; conductivity: 3.18 µS/cm; dissolved oxygen: 10.66 mg/dm^3; biochemical oxygen demand over 5 days (BOD_5): 1.98; NO_3: <0.10 mg/dm^3; NH_4 concentration: 0.207 mg/dm^3; PO_3 concentration: 0.170 mg/dm^3. The granulometry varied among sites (Table 1). Sites 1 and 3 had a high organic content with moderately to poorly sorted fine sediments, while Sites 2 and 4 had lower organic content and more coarse sands. In addition to Gastrotricha in the examined material, we found protozoa (mainly Ciliata), nematodes and rotifers (Bdelloidea as well as Monogononta).

Table 3. DNA sequences of the gastrotrichs species used in phylogenetic analysis.

Species	GenBank acc. nos.		
	18S	**28S**	**COI**
Arenotus strixinoi	JQ798537	JQ798608	JQ798677
Aspidiophorus ophiodermus	JN185463	JN185510	–
Aspidiophorus polystictos	JQ798597	JQ798664	JQ798726
Aspidiophorus tetrachaetus	JN185505	JN185540	JN185576
Bifidochaetus arcticus	KP713403	KP713404	KP713405
Chaetonotus sp. Kånneby, Todaro & Jondelius, 2013	JQ798555	–	JQ798692
Chaetonotus sp. 1 Kånneby, Todaro & Jondelius, 2013	JQ798601	JQ798668	JQ798730
Chaetonotus (Chaetonotus) antrumus Kolicka sp. nov.	KX538804	KU705232	KU705230
Chaetonotus (Chaetonotus) daphnes	JQ798549	JQ798617	JQ798687
Chaetonotus (Chaetonotus) cf. *sphagnophilus*	JQ798604	JQ798671	JQ798733
Chaetonotus (Chaetonotus) cf. *laroides*	JQ798602	JQ798669	JQ798731
Chaetonotus (Chaetonotus) microchaetus	JQ798575	JQ798618	JQ798684
Chaetonotus (Chaetonotus) cf. *similis*	JQ798592	JQ798660	JQ798722
Chaetonotus (Chaetonotus) similis	JQ798578	JQ798648	JQ798710
Chaetonotus (Chaetonotus) polyspinosus	JQ798563	JQ798654	JQ798717
Chaetonotus (Chaetonotus) maximus 1	JQ798577	–	JQ798709
Chaetonotus (Chaetonotus) maximus 2	JQ798574	JQ798646	JQ798706
Chaetonotus (Primochaetus) armatus	JQ798594	–	JQ798723
Chaetonotus (Primochaetus) acanthocephalus	JQ798569	–	JQ798701
Chaetonotus (Primochaetus) acanthodes 1	JQ798544	JQ798616	JQ798682
Chaetonotus (Primochaetus) acanthodes 2	JQ798552	JQ798624	–
Chaetonotus (Primochaetus) heideri 1	JQ798547	JQ798619	JQ798685
Chaetonotus (Primochaetus) heideri 2	JQ798590	JQ798657	JQ798720
Chaetonotus (Hystricochaetonotus) hystrix 1	JQ798557	JQ798627	–
Chaetonotus (Hystricochaetonotus) hystrix 2	JQ798603	JQ798670	JQ798732
Chaetonotus (Hystricochaetonotus) cf. *novenarius*	JQ798566	JQ798636	JQ798699
Chaetonotus (Marinochaetus) mariae	JQ798558	JQ798628	–
Chaetonotus (Schizochaetonotus) dispar	JQ798561	JQ798631	JQ798696
Chaetonotus (Schizochaetonotus) neptuni 1	JQ798539	JQ798610	JQ798679
Chaetonotus (Schizochaetonotus) neptuni 2	JQ798595	JQ798662	JQ798724
Chaetonotus (Schizochaetonotus) schultzei	JQ798596	JQ798663	JQ798725
Chaetonotus (Wolterecka) uncinus	JQ798540	JQ798611	–
Chaetonotus (Zonochaeta) bisacer	JQ798565	JQ798635	–
Halichaetonotus euromarinus	JQ798551	JQ798623	–
Halichaetonotus sp. 1 Kånneby, Todaro & Jondelius, 2013	JQ798600	JQ798667	JQ798729
Halichaetonotus sp. 2 Kånneby, Todaro & Jondelius, 2013	JQ798560	JQ798630	JQ798695
Halichaetonotus aculifer	JQ798550	JQ798622	JQ798688
Halichaetonotus paradoxus	JQ798599	JQ798666	JQ798728
Heterolepidoderma loricatum	JQ798541	JQ798612	–
Heterolepidoderma macrops	JN185469	JN185515	JN185548
Heterolepidoderma ocellatum	JN185475	JN185519	JN185554
Heterolepidoderma sp.	JQ798554	–	JQ798691
Ichthydium (Furficulichthys) skandicum	JQ798573	JQ798645	JQ798705
Lepidochaetus brasilense 1	JN185458	JN185507	JQ798680
Lepidochaetus brasilense 2	JN185495	JQ798658	JN185568
Lepidochaetus zelinkai	JN185486	JN185527	JN185564
Lepidodermella intermedia	JN185468	JN185514	JN185547
Lepidodermella minor minor	JN185474	–	JN185553
Polymerurus nodicaudus 1	JN185460	–	JN185542
Polymerurus nodicaudus 2	JN185502	JN185537	JN185573
Polymerurus nodicaudus 3	JN185465	JN185512	JQ798689
Polymerurus nodicaudus 4	JN185473	JQ798642	JN185552
Polymerurus rhomboides 1	JQ798584	–	JQ798715
Polymerurus rhomboides 2	JN185467	JN185513	JN185546
Polymerurus rhomboides 3	JN185493	JN185533	JN185567

Taxonomic account

Phylum Gastrotricha Mečnikow, 1865
Order Chaetonotida Remane, 1925 [Rao & Clausen 1970]
Suborder Paucitubulatina d'Hondt, 1971
Family Chaetonotidae Gosse, 1864 (*sensu* Leasi & Todaro 2008)
Subfamily Chaetonotinae Gosse, 1864 (*sensu* Kisielewski 1991)

Genus ***Chaetonotus*** Ehrenberg, 1830

Type species

Chaetonotus larus (Müller, 1773).

Type area

Denmark.

Subgenus ***Chaetonotus*** *sensu stricto* Ehrenberg, 1830

Type species

Chaetonotus larus (Müller, 1773).

Type area

Denmark.

Chaetonotus (Chaetonotus) antrumus Kolicka sp. nov.
urn:lsid:zoobank.org:act:BF874601-C073-465F-B50B-48D73DB5A4C5
Figs 4–11; Table 4; Appendix

Diagnosis

Slender body, length from 91.2 to 129.7 µm. Head five-lobed, cephalion and pleuria weakly marked in the head outline. Hypostomium small and rhomboidal. Ocellar granules absent. Scales small, three-lobed and with strong keels. One pair of one-lobed, keeled scales on the dorsal side of the posterior part of the trunk, and on the dorsal and dorsolateral sides of the furcal appendages. Two pairs of three-lobed, spined scales present on the ventral side of the furcal appendages. Scales distributed in 29–35 total longitudinal rows (11–13D+6–8DL+6L+4–6LV+2V) with 23–27 scales in the central row, and differing morphologically in the areas of the head, neck and trunk, respectively. Spines thick, simple, with blunt ends. Spine lengths strongly vary: spines of the head are longer than those of the neck; spines of the neck are short, but become progressively longer to the widest body region, after which they gradually shorten towards the furcal base. The spines gradually increase in length from lateral to ventral body side towards the ciliary bands. Ventral scale spines longer than the others and hair-like. Last pair of parafurcal spines longer and stronger. Ventral interciliary field naked, except for the posterior trunk region. A pair of ventral terminal scales long, oval with shallow posterior notches, keeled and spineless. Pharynx narrow with two weakly marked dilatations. Straight intestine with a distinct, short anterior section appearing as a narrow band.

Etymology

From Latin '*antrum*', cave, referring to the habitat where the species was found.

Material examined

Holotype

MONTENEGRO: adult, Crnojevica River flowing in Obodska Cave, 42°21.118′ N, 19°0.304′ E, sample 3, sampling site 3, 29 Jul. 2015, Piotr Gadawski leg., determined by Małgorzata Kolicka (photomicrograph in the Natural History Collections, Adam Mickiewicz University, Poznan: NHC-GCCA-12-1-20/h).

Paratypes

MONTENEGRO: 31 adults, 2 subadults, 2 juveniles, Crnojevica River flowing in Obodska Cave, 42°21.118′ N, 19°0.304′ E, samples 1 and 3, sampling sites 1 and 3, 29 Jul. 2015, Piotr Gadawski leg., determined by Małgorzata Kolicka (Natural History Collections, Adam Mickiewicz University, Poznan under accesing number: NHC-GCCA-12-21-50).

Description

This new species has a slender body. Its head is wider than the neck and separated from the trunk by a distinct neck constriction. The neck extends into the trunk, which gradually widens towards its widest region beyond its midpoint (ca U61), after which it gradually tapers towards a distinct furcal base at U84. The branches of the furca are set wide apart. The furcal indentation is V-shaped, and the adhesive tubes diverge posteriorly. They measure 10.1–11.3 μm, are straight and thin and do not taper towards their blunt ends (Figs 4–6).

The head is five-lobed and semi-circular. All plates are visible in the dorsal head outline. The cephalion (U1–U5) adheres to the head along its entire length, is narrow and widens at the dorsal edge. The epipleuria (U4–U6) are small and slightly convex. The hypopleuria (U7–U13) are more than twice as large as the epipleuria. The hypopleuria are not visible from the dorsal side; only their outline is marked in body shape (Figs 4A, 7). The hypostomium (U5–U8) is short and rhomboidal with slightly rounded edges and a strong anterior edge (Fig. 4C). Two pairs of cephalic ciliary tufts are present. The anterior tufts have four cilia each that emerge from between the cephalion and epipleuria and are arranged in lines around the lateral edges of the cephalion. The beginning of these lines (the first two cilia) is clearly visible on the dorsal side. The anteriormost cilium in both anterior tufts is fairly short (the shortest). The second cilium is longer than the first. The third cilium is very long and is the longest of all cilia in the tuft. The posteriormost cilium is shorter than the third and similar in length to the second cilium. The posterior tufts have five cilia each and emerge ventrally at the anterior edge of the hypopleuria. The length of the cephalic cilia in the posterior tufts increases from the anteriormost to the fourth cilium. The posteriormost cilium is similar in length to the first (see Appendix). Ocellar granules are not present. The mouth ring is narrow, located sub-terminally at U2–U3 and has weakly marked, granular reinforcements. Short inner hairs are present inside the mouth ring and suboral hairs are located around it.

The body is covered by small, three-lobed scales and single one-lobed scales that adhere over their entire surface to the cuticle (Figs 5–9). Scales are distributed in 29–35 total longitudinal and alternating rows (11–13D+6–8DL+6L+4–6LV+2V) with 23–27 scales in the dorsal central row. Each scale has a strong keel and triangular with a deeply-notched posterior edge. On the head, neck and anterior and middle parts of the trunk, the longitudinal rows of scales run parallel to each other, while in the posterior part of the trunk and on the furcal base the scales gradually converge towards the central longitudinal row (Figs 4, 7–9). The scales on the head, neck and trunk are arranged close to one another, but their edges do not adhere or overlap. The longitudinal rows of scales begin on the head beyond the posterior edges of the cephalion, epipleuria and hypopleuria. The scales show a strong morphological diversity in postero-lateral lobe distinctness, edge roundness and size throughout the different surfaces of the head, neck and trunk regions. On the head, there are deeply-notched, rounded, wide triangles with rounded postero-lateral lobes that are weakly differentiated from the central lobe. On the neck, scales are narrower, and

their postero-lateral lobes show a weaker separation from the central lobe (see Appendix; Figs 4A, 5). On the trunk, scales are shaped like narrow, deeply-notched triangles with their postero-lateral lobes clearly differentiated from the central lobe and with edges that are less rounded (Figs 4–5, 8A, C, E, 9A). The size of the scales decreases rapidly at the beginning of the neck, after which it gradually increases towards the beginning of the trunk (head: 1.6–3.8 μm length × 1.8–4.3 μm width vs neck: 1.3–3.3 μm length × 1.4–3.9 μm width vs trunk: 2.5–5.6 μm length × 1.6–4.3 μm width). The dorsal head and neck scales differ most from one another (Figs 4A, 5, 7A), whereas in the other areas of the head and neck, the differences in shape and size between the scales are gradual. The size of the scales of the trunk increases from the anterior end towards the widest trunk region, after which it decreases towards the furcal base (see Appendix). On the posterior part of the trunk, scales are clearly smaller than those at the widest point of the body. A fine (1.9–2.7 μm length × 1.3–1.8 μm width) one-lobed, keeled and spineless scale is located at U78 on the dorsal part of the trunk, anterior to each scale bearing a sensory bristle (Figs 4A, 8C) and is shaped like a strongly-rounded triangle. Medially, on the dorsal side of the furcal appendages (U86–U88), three three-lobed scales are present that are slightly narrower than the other scales of the trunk (see Appendix; Figs 4A, 8G). Lateral to these scales, on the furcal appendages (U85–U88), there are two pairs of elongated one-lobed scales shaped like narrow ovals with a weakly-notched posterior edge. The anterior one of each pair of scales is located dorsally, has a strong keel and a long, straight spine, whereas the posterior one is located dorsolaterally and has a long keel, but no spine. The lateral edges of these scales are partially overlapping. On the lateral side of the furcal appendages (U86–U90), two pairs of three-lobed scales, of the same type as the scales of the trunk, with spines are present (Figs 6, 9A). The dorsal, dorsolateral, lateral, ventrolateral and ventral scales do not strongly vary in size, except that the scales in the area of the neck and in the longitudinal rows next to the ciliary bands are considerably smaller than the others. The ventral scales of the longitudinal rows located closest to the ventral ciliary bands are about half the size of the scales in the other rows (head: 1.6–2.9 μm length × 1.8–2.3 μm width; neck: 1.3–1.8 μm length × 1.4–2.0 μm width; trunk: 2.5–3.0 μm length × 1.6–2.0 μm width) and have their central lobe rotated about 20° towards the bands (see Appendix; Figs 4C, 8C, 10C).

The spines arising from the posterior scales region are thick and straight, taper very slightly towards their blunt ends and have no lateral denticles (Figs 4, 7B, D, 8B, D, F, 9B, 10E). The spines that adhere directly to the cephalion and pleuria are the shortest of the head spines (Fig. 7B). The spines on the head increase in length (1.1–3.1 μm), whereas those on the neck decrease rapidly in length. The spines on the neck are much shorter than those in the head area; the spines are merely vestigial halfway down the neck, after which they gradually lengthen towards the trunk (0.5–2.7 μm) (Figs 4, 7B). The spines on the trunk gradually and slightly lengthen from the beginning of the trunk (ca U30) up to the widest body region (ca U61), after which they gradually shorten towards the furcal base at U84 (1.3–4.1 μm) (Figs 4, 8B, D, F). The pair of posteriormost lateral trunk spines is slightly longer and thicker than the surrounding spines (3.4–5.9 μm). Parafurcal spines emerging from two lateral scales per side on the furcal appendages are slightly thicker and longer than the other spines of the body, the posteriormost pair is longer, thicker and stronger than those of the preceding pair (see Appendix; Fig. 4). These spines taper slightly towards their blunt distal ends. The dorsal and dorsolateral spines do not vary substantially in length (Table 4). The spines lengthen gradually and slightly from the lateral side towards the ciliary bands (Fig. 9B). The spines arising from the ventral longitudinal row of scales located closest to the ciliary bands are much longer than those of the body, curved, and hair-like along their entire length (head: 5.1–8.6 μm; neck: 5.0–9.0 μm; trunk: 7.1–14.0 μm).

This species has three pairs of dorsal sensory bristles (Fig. 4). The first pair is located on the head, directly posterior to the cephalion, near the lateral edges of the epipleuria (U5), and each bristle emerges from a small, round papilla. The second pair is located on the neck (U25) and each bristle emerges from a small, rounded papilla. The third, posterior pair, which emerges from double-keeled scales located in

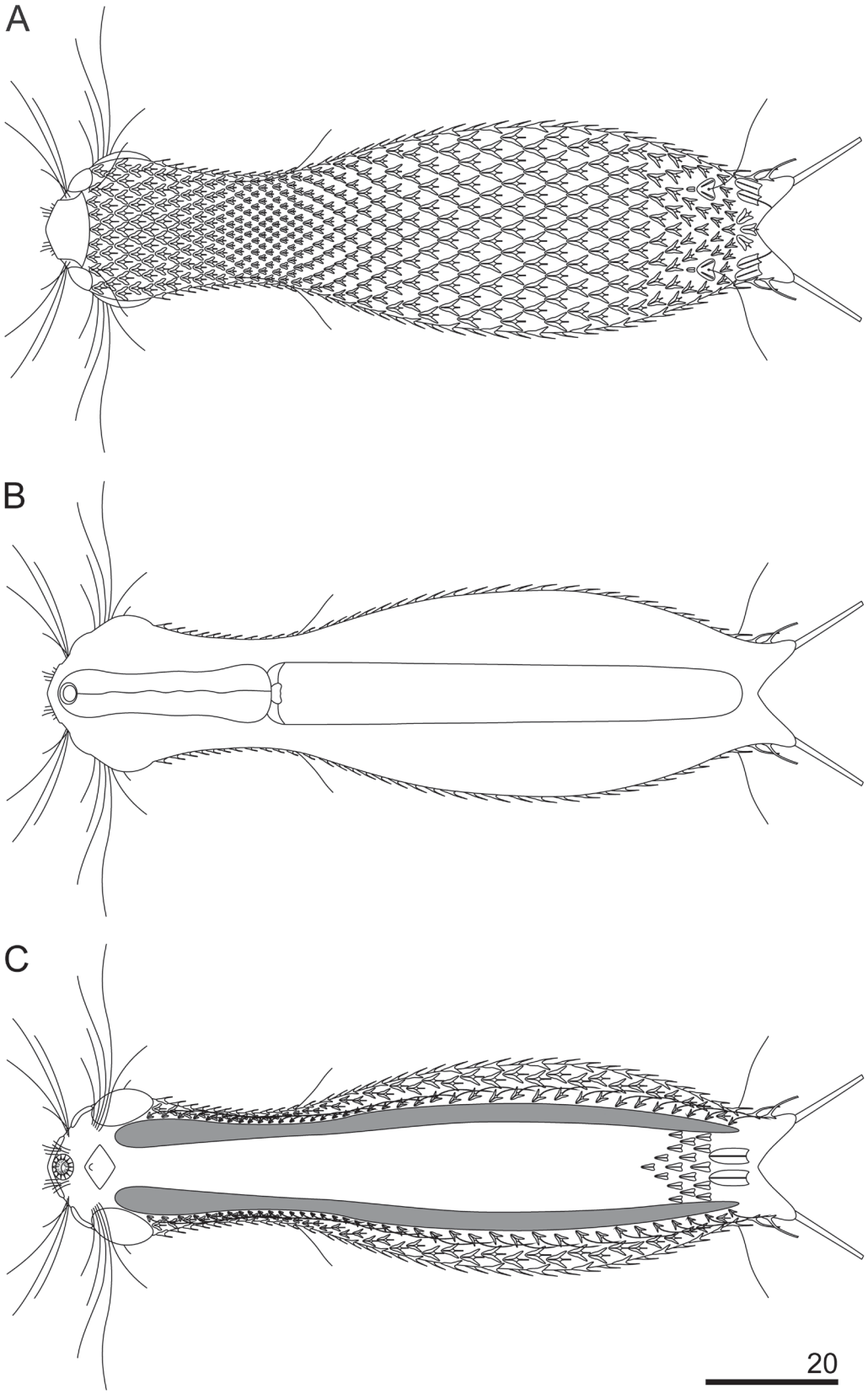


Fig. 4. *Chaetonotus (Chaetonotus) antrumus* Kolicka sp. nov., schematic drawings. **A**. Dorsal body view. **B**. Internal body view. **C**. Ventral body view.

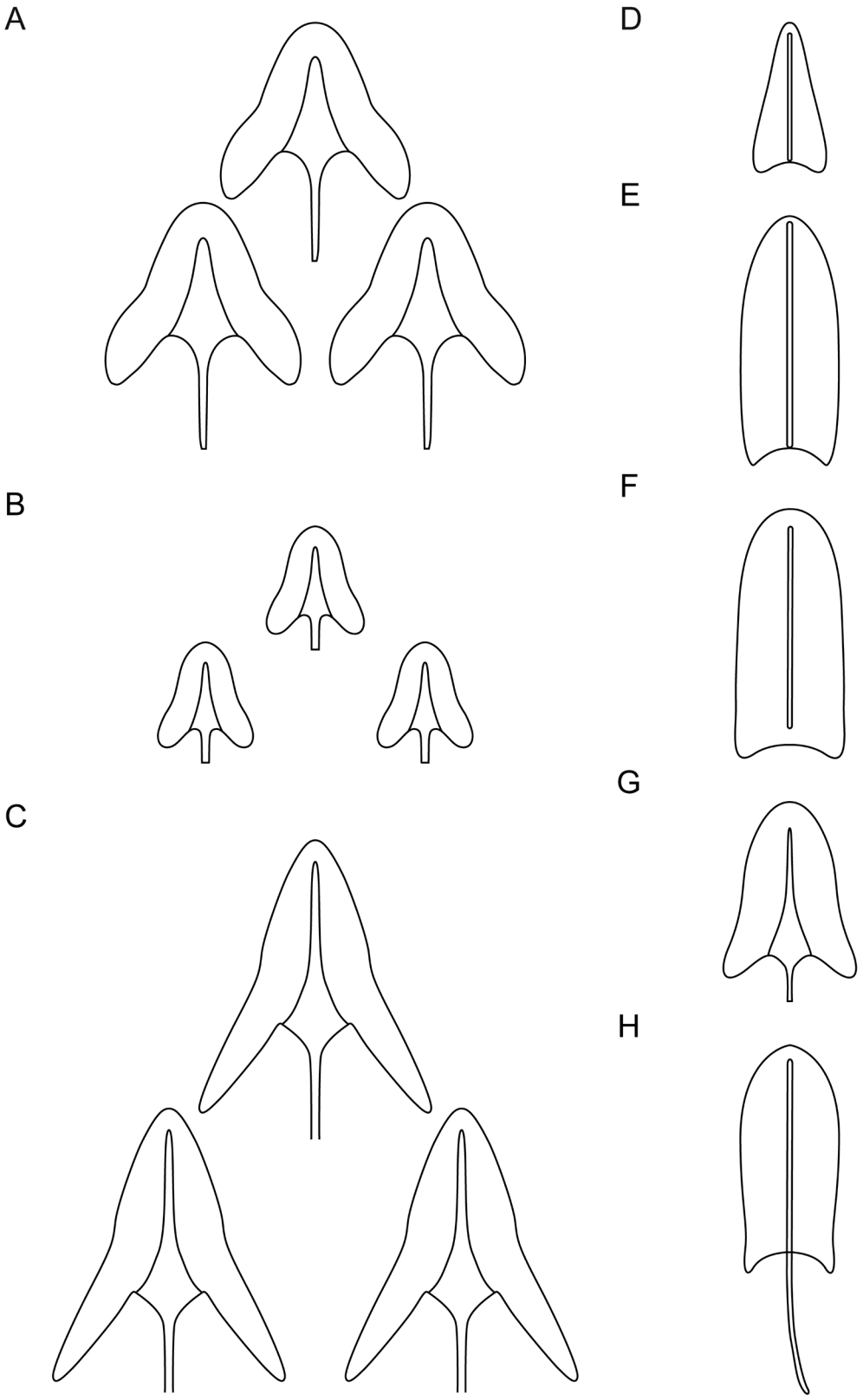


Fig. 5. *Chaetonotus (Chaetonotus) antrumus* Kolicka sp. nov., schematic drawings of the scales. **A**. Head region scales. **B**. Neck region scales. **C**. Trunk region scales. **D**. Ventral interciliary field scales. **E**. Posteriormost ventral field scales. **F**, **H**. Furcal appendages dorsolateral one–lobed scales. **G**. Furcal appendages dorsal three-lobed scales.

the posterior part of the trunk (U79–U80), is shaped like deeply-notched triangles with unconnected keels (Figs 4A, 5).

On the ventral side, the longitudinal ciliary bands begin at U8 and run back to U84 (Fig. 4C). They are wider in the area of the head than in the other parts of the body. Most of the ventral interciliary field is naked: fine, keeled, spined scales are present only in the posterior part of the trunk (from ca U78 to U82) (Figs 4C, 8H). Their differentiation increases towards the posterior body region: the anterior scales are weakly delineated and partially submerged in the cuticle. These scales are shaped like narrow triangles with a very weakly notched posterior edge. The scales of the ventral interciliary field increase in size towards the posterior end of the body (1.4–3.7 μm length × 1.1–1.9 μm width). The terminal scales are located at U82–U85 and are shaped like long, narrow ovals with a very weakly notched posterior edge and have a long keel running along their entire length, but are spineless (Figs 4C, 5E).

The pharynx (U2–U28) is relatively narrow and has weak anterior and posterior dilatations, with the posterior dilatation wider than the anterior one (Figs 4B, 7C; Appendix). The pharynx connects through the small and narrow pharyngeal intestinal junction (U30) to a straight intestine (running from U29 to U86). The intestine has a distinct, short (U29–U31) anterior section marked as a narrow band (Fig. 4B).

Remarks

Chaetonotus (Chaetonotus) antrumus sp. nov. is an interstitial species which was recorded in the lotic system in a cave. The gastrotrich fauna in interstitial freshwater habitats is relatively rich, but composed

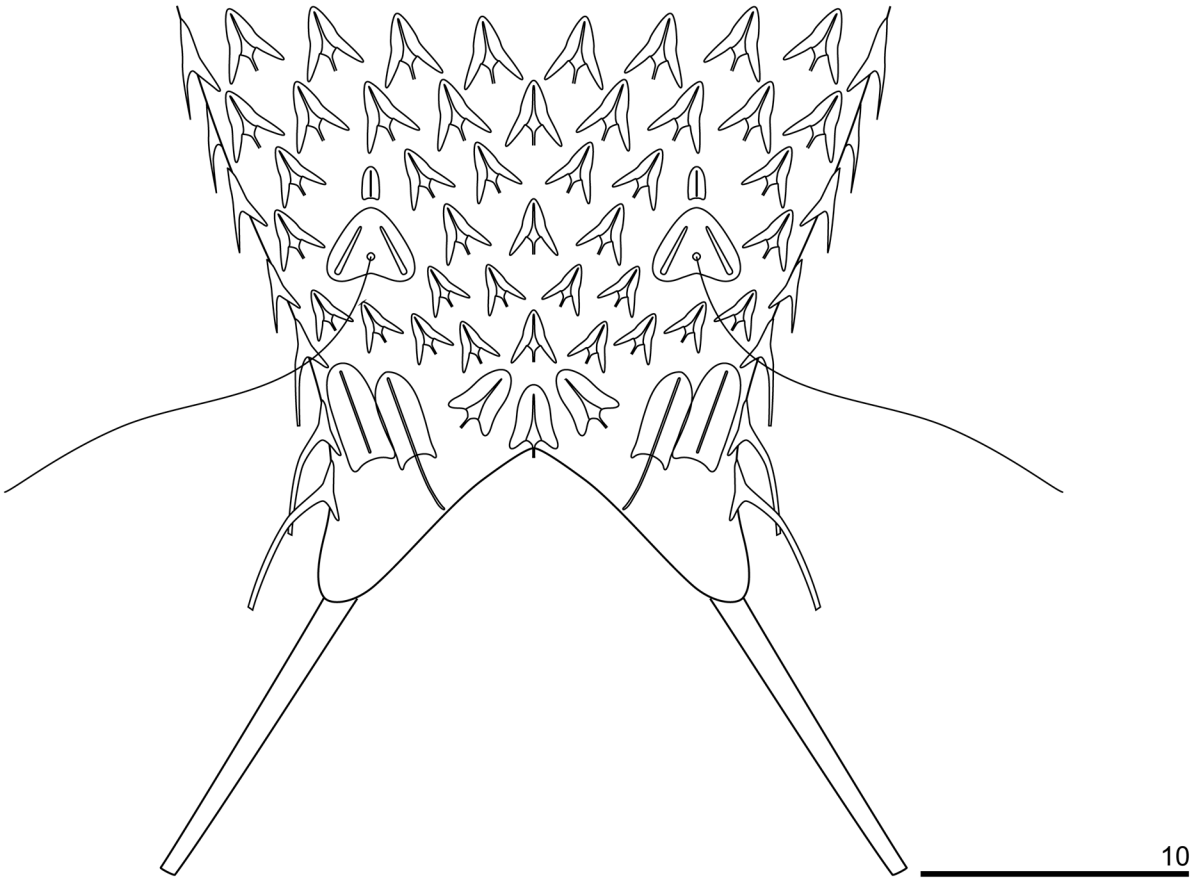


Fig. 6. *Chaetonotus (Chaetonotus) antrumus* Kolicka sp. nov., schematic drawings of the posterior trunk region, furcal base and furcal appendages.

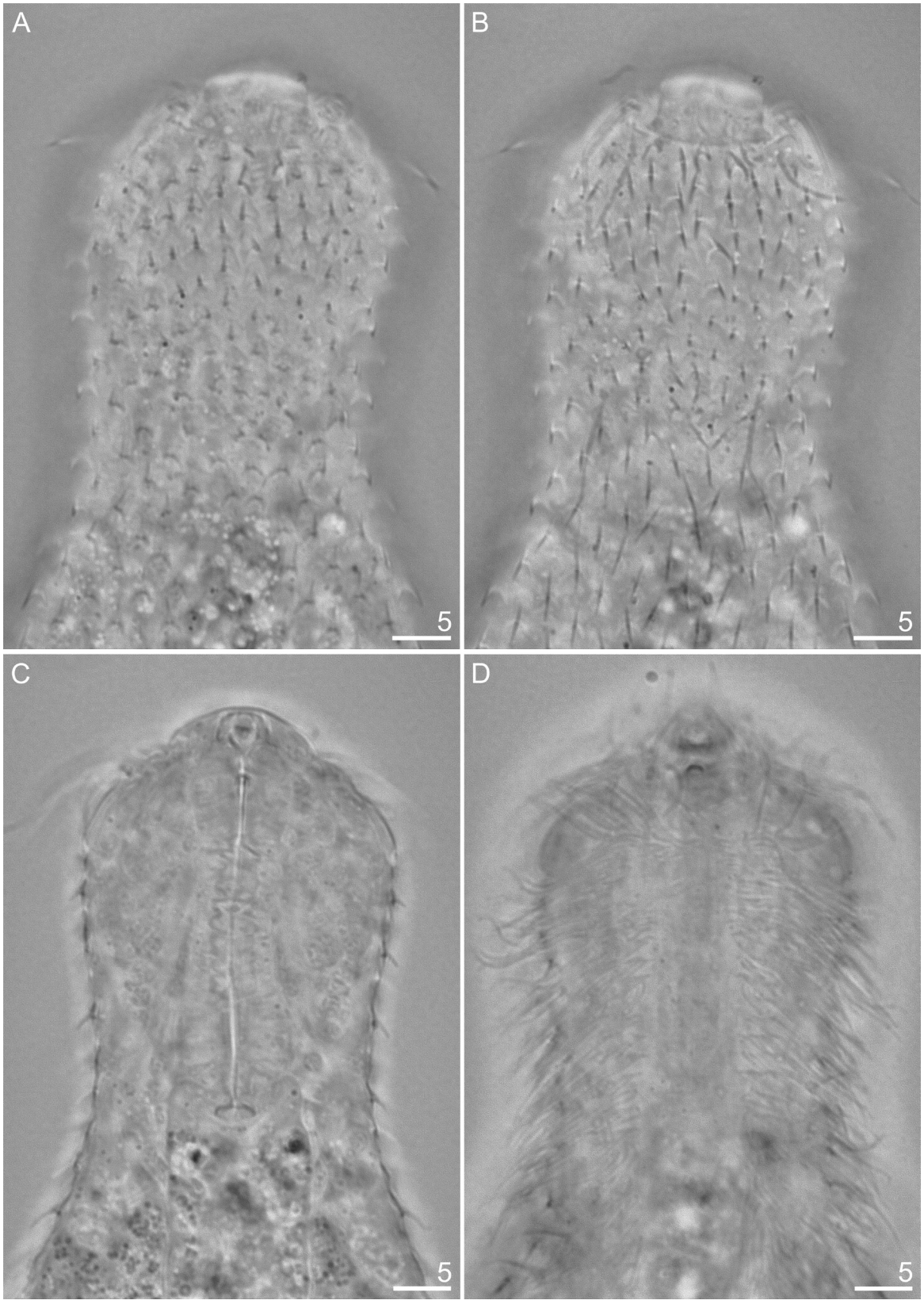


Fig. 7. *Chaetonotus (Chaetonotus) antrumus* Kolicka sp. nov. **A**. Dorsal view of scales on head and neck. **B**. Dorsal view of spines on head and neck. **C**. Internal view of head and neck. **D**. Head and neck, ventral view. (Bright field microphotographs.)

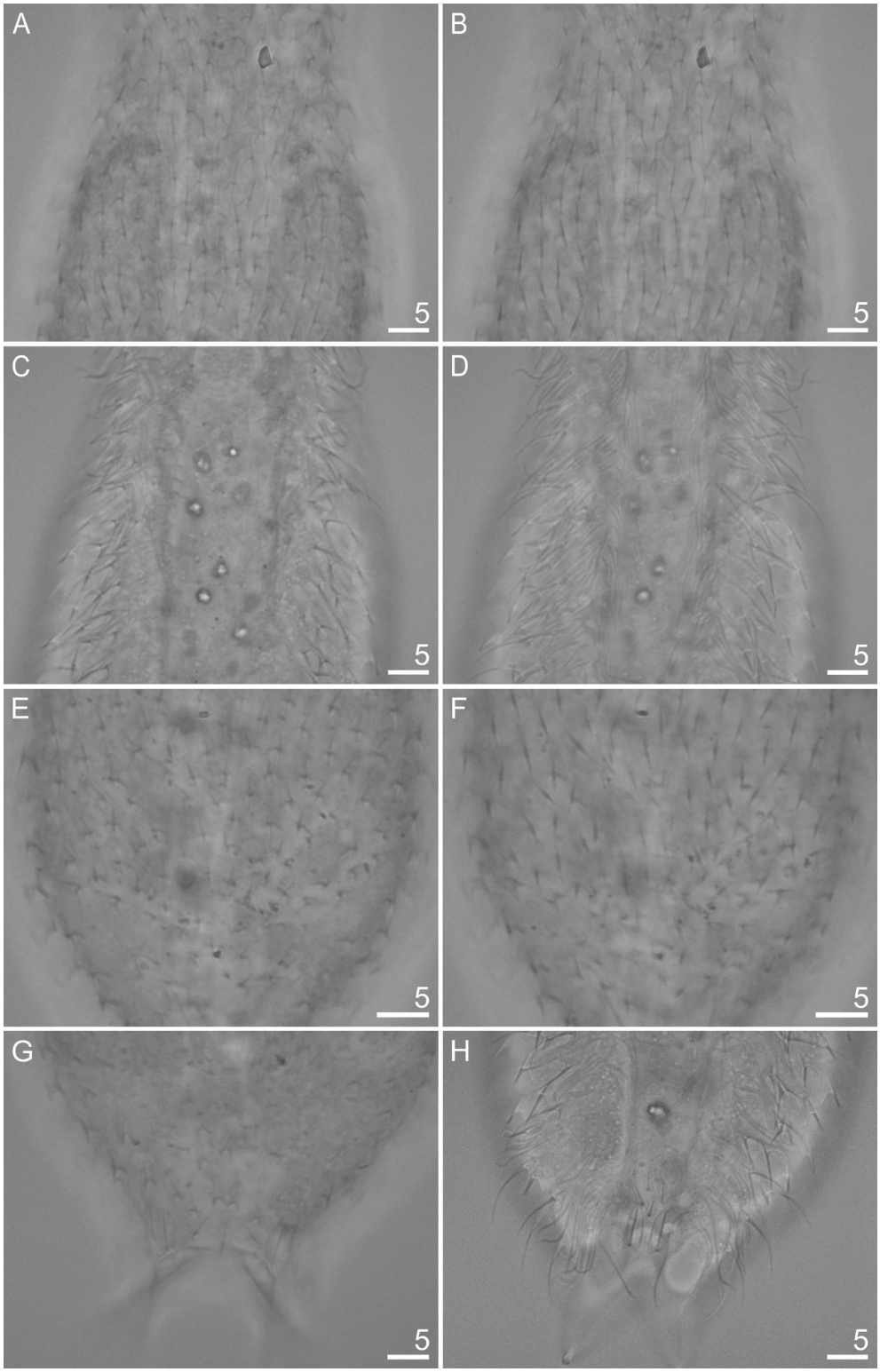


Fig. 8. *Chaetonotus (Chaetonotus) antrumus* Kolicka sp. nov., bright field microphotographs. **A**. Dorsal view of scales on trunk region. **B**. Dorsal view of spines on trunk region. **C**. Ventral view of scales on trunk region. **D**. Ventral view of spines on trunk region. **E**. Dorsal view of scales on posterior trunk region. **F**. Dorsal view of spines on posterior trunk region. **G**. Dorsal view of scales on furcal base and furcal appendages. **H**. Ventral view of posterior trunk region with visible interciliary field scales and posteriormost interciliary field scales.

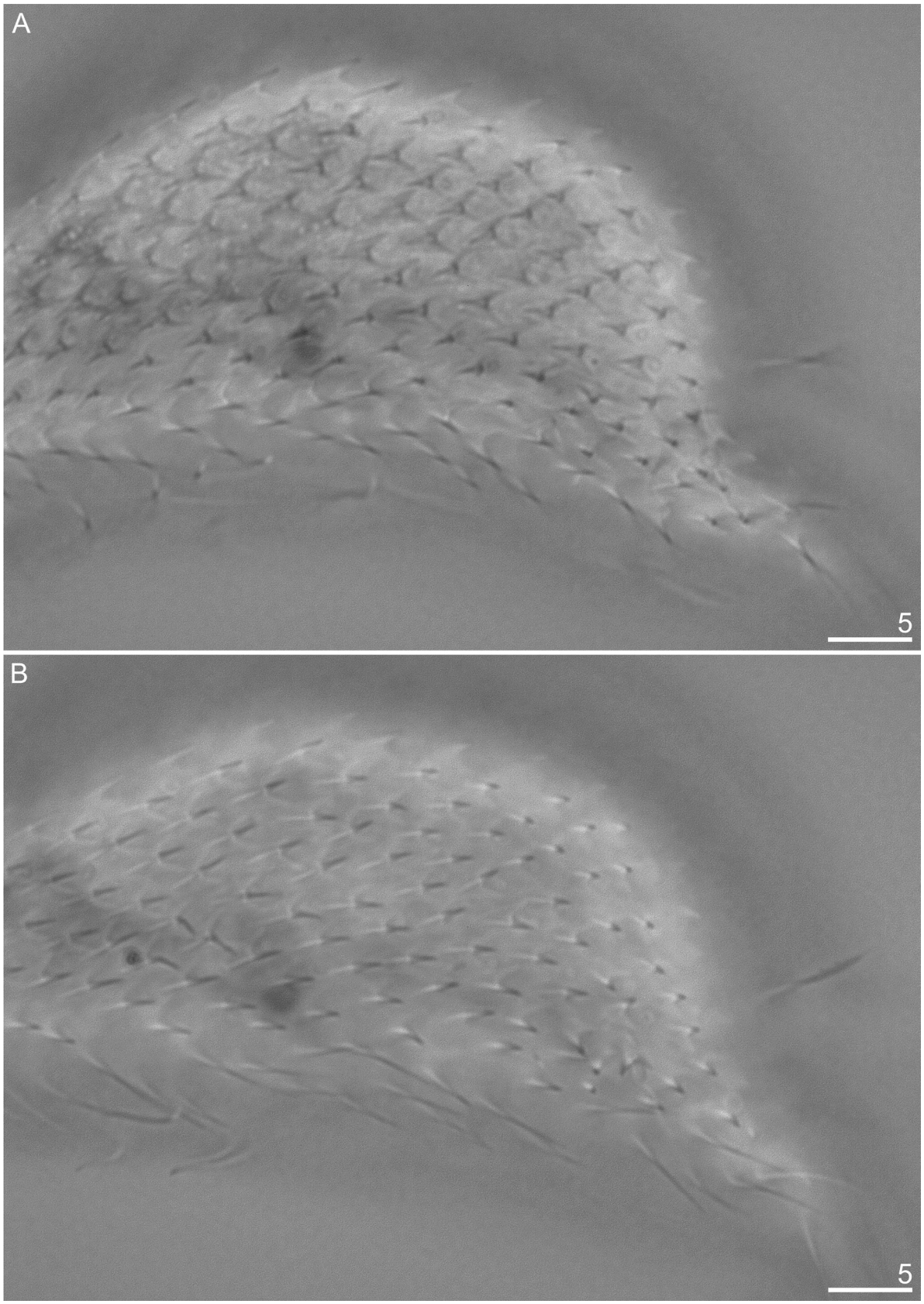


Fig. 9. *Chaetonotus (Chaetonotus) antrumus* Kolicka sp. nov., bright field microphotographs. **A**. Lateral view of scales on trunk. **B**. Lateral view of spines on trunk.

Table 4. Condensed comparison of the most important differential characters between species most similar to *Chaetonotus (Chaetonotus) antrumus* Kolicka sp. nov. Measurements are given in micrometers (μm).

Character	***C. (C.) antrumus*** Kolicka sp. nov.	***C. (C.) naiadis*** Balsamo & Todaro, 1995	***C. (C.) daphnes*** Balsamo & Todaro, 1995
Body length	119.1–129.7	89.0–140.0	141.0–173.0
Cephalic pleuria	Cephalion narrow, extended dorsally; epipleuria small visible from the dorsal body side; hypopleuria large not visible from dorsal body side. Hypostomium present	Cephalion wide; epipleuria large; hypopleuria small; clearly visible from the dorsal body side. Hypostomium present	Cephalion wide; epipleuria large, clearly visible on the dorsal body side; hypopleuria smallpresent only on the ventral body side. Hypostomium absent
Place of arising cephalic cilia	Anterior tufts: in lines between cephalion and epipleuria; posterior tufts: at the anterior edge of the hypopleuria on the ventral side	Anterior and posterior tufts: lateral	Anterior tufts: dorsally at the head top; posterior: dorsally
Pharynx length	31.9–34.9	26.0–33.0	36.0–44.0
Anterior intestinal section morphologically different	Present	Absent	Absent
Furca length	19.8–23.2	12.0–17.0	14.0–20.0
Adhesive tube length	10.1–11.3	9.0	7.0–11.0
Type of furca indentation	V-shaped	V-shaped	Helmet-shaped
Number of scales in single longitudinal row	24–27	20	25–33
Total number of longitudinal alternating rows of scales	29–35	23–31	45–53
Type of scales	Almost all three-lobed with strong keels and straight spines; shape strongly differentiated within body regions	One-lobed with strong keels and straight spines; shape undifferentiated within body regions	One-lobed with strong keels and straight spines; shape undifferentiated within body regions
Size of scales	Small; size strongly differentiated within body regions	Small; size slightly increasing posteriorly	Small; similar size all over the body
Presence of scales with two keels and two spines on the furcal appendages	Absent	One pair of scales with two keels and two spines present dorsolateraly	Absent
Scale coverage of the ventral interciliary field	Only the posterior end of the trunk covered by scales	Only the posterior end of the trunk covered by scales	Entire ventral interciliary field covered by scales
Type of terminal scales of the ventral interciliary field	Oval with shallow posterior notches, keeled, spineless	Oval without posterior notches, keeled, spined	Oval without posterior notches, keeled, spineless
Variety of spines length	Strongly differentiated length of spines on the head, neck (short, vestigial on dorsal area) and trunk; length of the spines gradually lengthen from the lateral to the ventral sides of the body; much longer spines on the ventral body sides	Similar length of spines on the dorsal, dorsolateral, lateral sides of the head, neck and trunk; much longer spines on the ventrolateral body sides	All spines similar length
Type of posteriormost pair of the lateral trunk spines	Slightly longer and thicker than surrounding spines	Same as other spines	Same as other spines
Type of last pair of the parafurcal spines	Longer, thicker and stronger than other spines	Same as other spines	Same as other spines
Number of dorsal sensory bristles	3 pairs	2 pairs	2 pairs

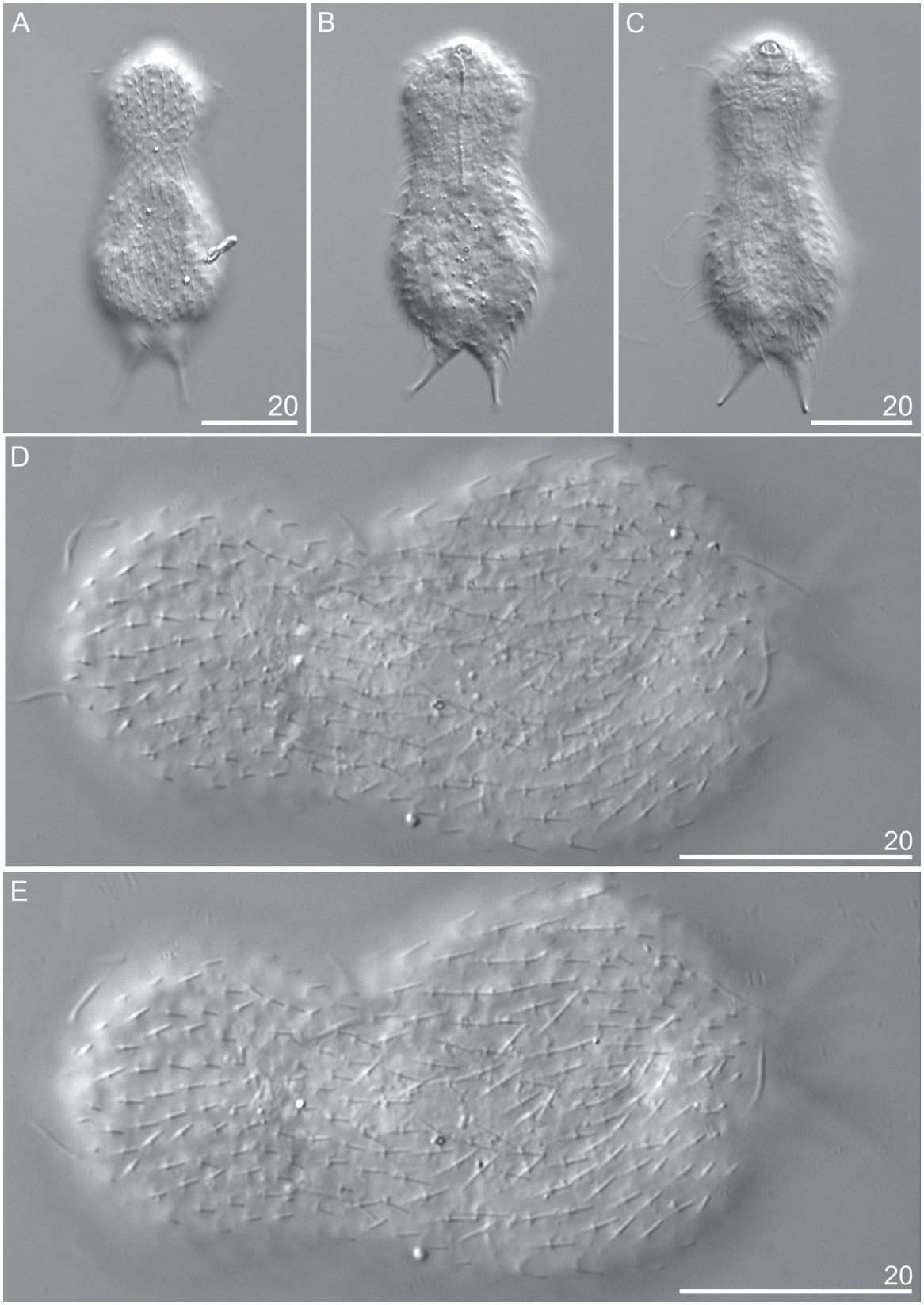


Fig. 10. *Chaetonotus (Chaetonotus) antrumus* Kolicka sp. nov., juvenile specimen, differential interference contrast microphotographs. **A**. Body, dorsal view. **B**. Body, internal view. **C**. Ventral body view. **D**. Dorsal view of scales. **E**. Dorsal view of spines.

of fewer species than in epibenthic or periphytic habitats (e.g., Balsamo *et al.* 2015). Interstitial communities are composed of not only taxa specific to them, but also of eurytopic species. Out of ca 40 species found in sandy biotopes, fewer than 10 seem to constitute exclusively interstitial taxa (Balsamo *et al.* 2015). All of the species share certain morphological traits, e.g., a small body size, a poorly ornamented cuticular covering, a well developed locomotory ciliature and adhesive organs (Balsamo & Fregni 1995; Balsamo *et al.* 2015). Entire sets of these characteristics also occur in *C. (C.) antrumus* sp. nov.

Usually, one or two pairs of sensory bristles have been described in Chaetonotidae, but in *C. (C.) antrumus* sp. nov., three pairs of dorsal sensory bristles are present. The presence of dorsal sensory bristles in the area of the head has previously been noted in only two species of *Chaetonotus*: *C. (C.) brevispinosus* Zelinka, 1889 and *C. (C.) sanctipauli* Kisielewski, 1991. Perhaps three pairs of dorsal sensory bristles occur more frequently in Chaetonotidae; the location of the first pair of dorsal sensory bristles on the head near the cephalion and the cephalic cilia, however, may have led to these sensory bristles being

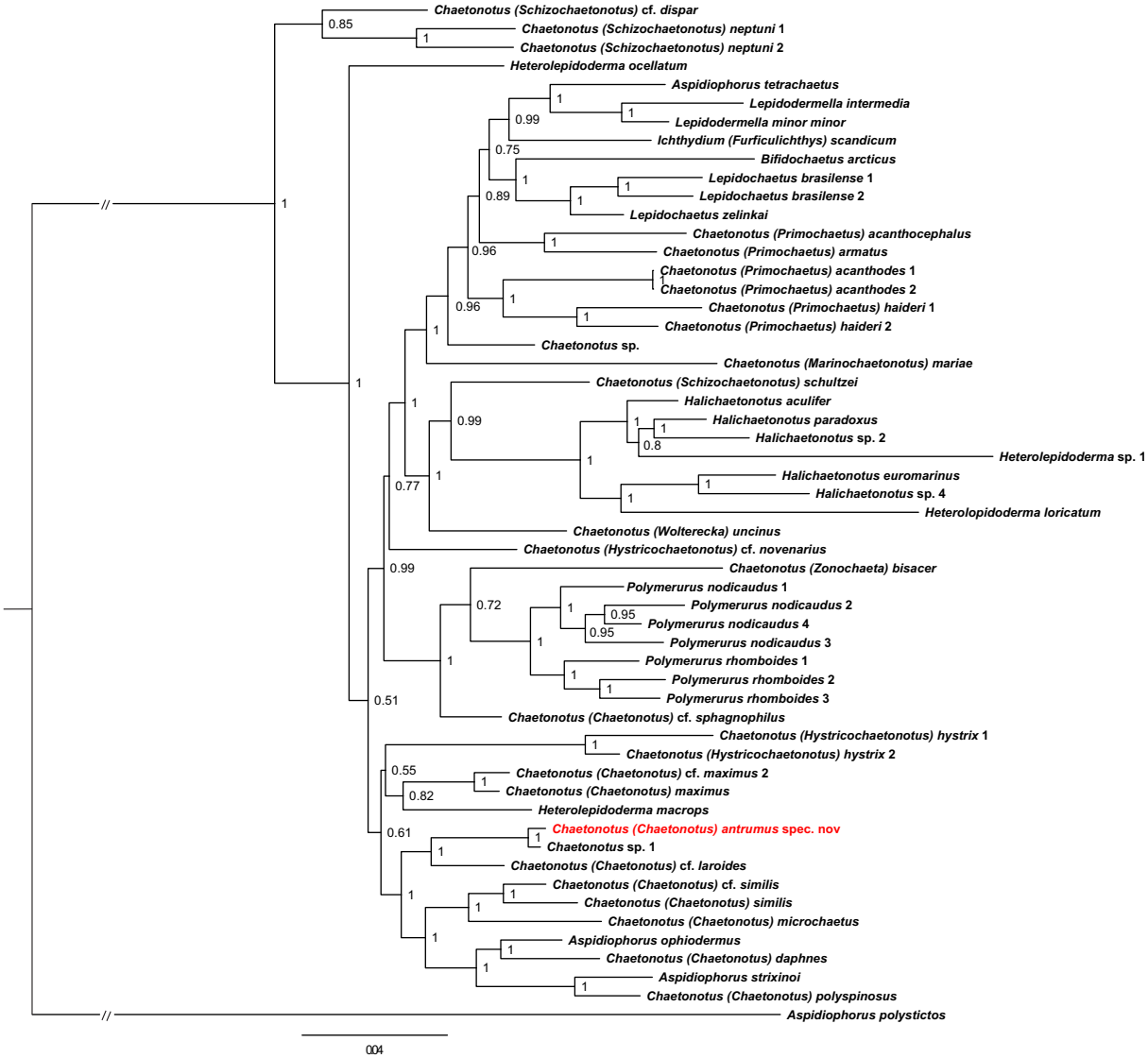


Fig. 11. Phylogenetic relationships of Chaetonotidae inferred from the Bayesian analysis of 18S rRNA, 28S rRNA and COI sequence data (for details on the species names see Table 3).

obscured by the cilia or erroneously interpreted as one of the cilia. Thus, the presence of dorsal sensory bristles on the head cannot be considered as a good diagnostic characters at the species level.

The presence of a developing egg was observed in 21 out of 32 adult specimens of *C. (C.) antrumus* sp. nov. However, sperm and an X-organ were not observed.

Chaetonotus (Chaetonotus) antrumus sp. nov. showed very quick locomotion under microscopic observation. The species often remained in motion even after its integument had burst from compression under a cover glass, thus producing a visible deformation.

Sequence diversity and phylogenetic relationships

The COI alignment for the distance calculations comprised 628 bp of unambiguous sequence data for three specimens of *Chaetonotus (Chaetonotus) antrumus* sp. nov. We found two haplotypes that differed in eight nucleotide positions (K2P = 0.013; SD = 0.005). All substitutions were located at synonymous sites, i.e., both haplotypes coded for the same amino acid sequence. The nuclear data, including 4809-bp of the DNA region coding for 18S rRNA, ITS1, 5.8S, ITS2, and 28S rRNA, showed no intraspecific variation. All sequences are deposited in GenBank under accession numbers KU705230, KU705231 (COI); KX538804 (18S); KU705232 (28S); KU705233 (ITS).

A phylogenetic analysis (Fig. 11) recovered, with maximum support, *C. (C.) antrumus* sp. nov. as closely related to the undetermined *Chaetonotus* sp. 1 TK-2012 (Kånneby *et al.* 2013); both grouped with *C. (C.)* cf. *laroides.* This clade was in a sister relation to a clade consisting of some representatives of *Chaetonotus (Chaetonotus)* Ehrenberg, 1830 and *Aspidiophorus* (Voigt, 1903) and including *Chatonotus (Chaetonotus) daphnes* Balsamo & Todaro, 1995, which is morphologically most similar to the new species.

Differential diagnosis

Even if the new species was recorded from a sandy habitat, it is not similar to any other species considered as exclusively interstitial taxa. *Chaetonotus* Ehrenberg, 1830 is a polyphyletic genus, containing varied species. Of all the 165 currently known nominal freshwater representatives of this genus, *C.(C.) antrumus* sp. nov. is morphologically closest to *C. (C.) naiadis* Balsamo & Todaro, 1995 and *C. (C.) daphnes* Balsamo & Todaro, 1995, both of which were originally reported from Italian mountain pools. These two species were selected from all the *Chaetonotus* representatives for the comparison of the new species due to a similarity in terms of (1) the alignment of scales whose edges are juxtaposed; the presence of a different type of scales on the dorsal and dorsolateral sides of the furcal base; (2) the type of scales on the ventral interciliary field; (3) the number of terminal scales of the ventral interciliary field; (4) two longitudinal bands of ventral locomotor cilia wider on the head region. Despite the fact that from all of the hitherto known species, *C. (C.) naiadis* and *C. (C.) daphnes* have the highest number of common features with the newly described species, they are significantly different from *C. (C.) antrumus* sp. nov., most strikingly in the scale type and shape. All the differences between the new species and the most morphologically similar taxa have been summarized in Table 4.

Discussion

The presence of *Chaetonotus (Chaetonotus) antrumus* sp. nov. in only two of four sampling sites may reflect the habitat selectivity by the species. The species was observed only in the samples with a high organic matter content, a minimum diameter spectra of the sand grain, and moderately to poorly sorted sediments. This result confirms previous observations regarding the habitat preferences of many freshwater gastrotrichs, which appear to prefer finer sediments with high organic matter content (e.g., Kisielewski 1997; Balsamo & Todaro 2002; Balsamo *et al.* 2014). However, the occurrence and

composition of the Gastrotricha fauna inhabiting caves may depend not only on the physicochemical habitat properties (temperature, sunlight, granulometry of the sediments, the water flow rate) (e.g., Balsamo *et al.* 2014), but also on the possibility of colonization and food availability. Gastrotrichs, as other meiobenthic invertebrates, have limited locomotory abilities. Dispersal in freshwater gastrotrichs is probably limited to migration through aquatic sediments and dispersal of resistant eggs (see below).

The result of our molecular phylogenetic analysis is for the greater part congruent with the previous molecular phylogenies of Chaetonotidae (Kånneby *et al.* 2013; Kolicka *et al.* 2016) and gave further evidence to the high degree of polyphyletism of chaetonotid genera. Freshwater species of the order Chaetonotida, which may produce opsiblastic, resting eggs, are known for passive migration with water currents, watercourses, surface run-offs, wind and being carried by more mobile animals, e.g., birds, bats, amphibians, insects or annelids (e.g., Gerlach 1977; Kolicka *et al.* 2014). One of those vectors was most probably responsible for the transport of gastrotrichs into Obodska Cave. Because of their wide range of ecological tolerance and parthenogenetic reproduction, Gastrotricha may have persisted there and developed a population. When analyzing the fauna of cave ecosystems, it must be remembered that those habitats are not influenced by sunlight. Thus, there are no photoautotrophs and the ecosystem is based mainly on the influx of organic matter from outside the cave. Most of the currently known species of Gastrotricha feed on bacteria, detritus, and small algae (e.g., Bennett 1979; Balsamo & Todaro 2002; Todaro & Hummon 2008). The lack of unicellular autotrophs may not be a limiting factor for the majority of Gastrotricha species that feed mainly on bacteria (e.g., Balsamo *et al.* 2014).

Further studies will show whether *Chaetonotus (Chaetonotus) antrumus* sp. nov. is endemic to Obodska Cave, or more widely distributed and not associated with only one specific type of environment. The application of methods typical for integrative taxonomy (i.e., combined morphological, morphometric and molecular analyses), will allow verification of future reports regarding the presence of this species and accurate determination of its biogeography.

Acknowledgements

We would like to thank James Kirk for his invaluable comments on the draft version of the manuscript. The authors wish to thank two anonymous reviewers for their helpful comments. Special thanks to Małgorzata Kuźnik for figures of the new species used in this paper. Last but not least, we are grateful to Lech Kotwicki for help with the granulometric analysis, to Krzysztof Pabis for his helpful advise to this paper, and to Katarzyna Gadawska, Aleksandra Bańkowska, Magdalena Kłosowska, Grzegorz Michoński and Przemysław Śmietana for their help during the research expedition.

References

Balsamo M. & Fregni E. 1995. Gastrotrichs from interstitial fresh water, with a description of four new species. *Hydrobiologia* 302: 163–175. https://doi.org/10.1007/BF00027040

Balsamo M. & Todaro M.A. 2002. Gastrotricha. *In*: Rundle S.D., Robertson A.L. & Schmid-Araya J.M. (eds) *Freshwater Meiofauna: Biology and Ecology*: 45–61. Backhuys Publishers, Leiden.

Balsamo M., Grilli P., Guidi L. & d'Hondt J.-L. 2014. Gastrotricha: biology, ecology and systematics: families Dasydytidae, Dichaeturidae, Neogosseidae, Proichthydiidae. *In*: Dumont H.J.F. (ed.) *Identification Guides to the Plankton and Benthos of Inland Waters* 24. Backhuys Publishers and Margraf Publishers, Weikersheim.

Balsamo M., d'Hondt J.-L., Kisielewski J. & Pierboni L. 2008. Global diversity of gastrotrichs (Gastrotricha) in fresh waters. *Hydrobiologia* 595: 85–91. https://doi.org/10.1007/978-1-4020-8259-7_10

Balsamo M., d'Hondt J.-L., Kisielewski J., Todaro M.A., Tongiorgi P., Guidi L., Grilli P. & de Jong Y. 2015. Fauna Europea: Gastrotricha. *Biodiversity Data Journal* 3: e5800. https://doi.org/10.3897/BDJ.3.e5800

Balsamo M., d'Hondt J.-L., Pierboni L. & Grilli P. 2009. Taxonomic and nomenclatural notes on freshwater Gastrotricha. *Zootaxa* 2158: 1–19.

Bennett L.W. 1979. Experimental analysis of the trophic ecology of *Lepidodermella squamata* (Gastrotricha: Chaetonotida) in mixed culture. *Transactions of the American Microscopy Society* 98: 254–260.

Blott S. & Pye K. 2001. Gradistat: a grain size distribution and statistics package for the analysis of unconsolidated sediments. *Earth Surface Processes and Landforms* 26: 1237–1248. https://doi.org/10.1002/esp.261

Bonada N., Rieradevall M., Dallas H., Davis J., Day J., Figueroa R., Resh V.H. & Prat N. 2008. Multi-scale assessment of macroinvertebrate richness and composition in Mediterranean-climate rivers. *Freshwater Biology* 53: 772–788. https://doi.org/10.1111/j.1365-2427.2007.01940.x

Buchanan J.B. 1984. Sediment analysis. *In*: Holme N.A. & McIntyre A.D. (eds) *Methods for the Study of Marine Benthos*: 41–65. Blackwell Scientific Publications, Boston, MA.

Dabert J., Ehrnsberger R. & Dabert M. 2008. *Glaucalges tytonis* sp. nov. (Analgoidea: Xolalgidae) from the barn owl *Tyto alba* (Strigiformes: Tytonidae): compiling morphology with DNA barcode data for taxa descriptions in mites (Acari). *Zootaxa* 1719: 41–52.

Dabert M., Proctor H.C. & Dabert J. 2016. Higher-level molecular phylogeny of the water mites (Acariformes: Prostigmata: Parasitengonina: Hydrachnidiae). *Molecular Phylogenetics and Evolution* 101: 75–90. https://doi.org/10.1016/j.ympev.2016.05.004

Dabert M., Witalinski W., Kaźmierski A., Olszanowski Z. & Dabert J. 2010. Molecular phylogeny of acariform mites (Acari, Arachnida): strong conflict between phylogenetic signal and long branch attraction artifacts. *Molecular Phylogenetics and Evolution* 56: 222–241. https://doi.org/10.1016/j.ympev.2009.12.020

Danovaro R. 1996. Detritus-bacteria-meiofauna interactions in a seagrass bed (*Posidonia oceanica*) of the NW Mediterranean. *Marine Biology* 127: 1–13. https://doi.org/10.1007/BF00993638

Danovaro R., Corinaldesi C., Filippini M., Fischer U.R., Gessner M.O., Jacquet S., Magagnini M. & Velimirov B. 2008. Viriobenthos in freshwater and marine sediments: a review. *Freshwater Biology* 53: 1186–1213. https://doi.org/10.1111/j.1365-2427.2008.01961.x

Danovaro R., Luna G.M., Dell' Anno A. & Pietrangeli B. 2007. Measuring species richness based on microbial community fingerprints: the emperor has no clothes. *Applied and Environmental Microbiology* 73: 2399–2401. https://doi.org/10.1128/AEM.02383-06

d'Hondt J.-L. 1971. Gastrotricha. *In*: Barnes H. (ed.) *Oceanography and Marine Biology: an Annual Review* 9: 141–192. George Allen and Unwin Ltd, London.

Dinić J.B. 1965. *Crnojevićka Pecina, in Nase Jame.* Bohinec V. & Savnik R. (eds), Ljubljana.

Doderović M., Bulić Z. & Mijanović D. 2013. *Turistička valorizacija speološkog potencijala cetinjske subregije tourist valorization of caving in Cetinje.* Človjek I krś 2014, Sarajevo.

Folk R.L. & Ward W.C. 1957. Brazos River bar: a study in the significance of grain size parameters. *Journal of Sedimentary Research* 27: 3–26.
https://doi.org/10.1306/74D70646-2B21-11D7-8648000102C1865D

Fraschetti S., Gambi C., Giangrande A., Musco L., Terlizzi A. & Danovaro R. 2006. Structural and functional response of meiofauna rocky assemblages exposed to sewage pollution. *Marine Pollution Bulletin* 52: 540–548. https://doi.org/10.1016/j.marpolbul.2005.10.001

Gerlach S. 1977. Means of meiofauna dispersal. *Mikrofauna Meeresboden* 61: 89–103.

Giere O. 2009. *Meiobenthology. The Microscopic Fauna in Aquatic Sediments*. Springer Verlag, Berlin.

Goldman N. & Yang Z. 1994. A codon-based model of nucleotide substitution for protein-coding DNA sequences. *Molecular Biology and Evolution* 11: 725–736.

Hou Z. & Sket B. 2015. A review of Gammaridae (Crustacea: Amphipoda): the family extent, its evolutionary history, and taxonomic redefinition of genera. *Zoological Journal of the Linnean Society* 176 (2): 323–348. https://doi.org/10.1111/zoj.12318

Hummon W.D., Balsamo M. & Todaro M.A. 1992. Italian marine Gastrotricha: I. Six new and one redescribed species of Chaetonotida. *Bollettino di Zoologia* 59: 499–516. https://doi.org/10.1080/11250009209386711

Hummon W.D. & Todaro M.A. 2010. Analytic taxonomy and notes on marine, brackish-water and estuarine Gastrotricha. *Zootaxa* 2392: 1–32.

International Commission on Zoological Nomenclature 1999. *International Code of Zoological Nomenclature, Fourth Edition*. The International Trust for Zoological Nomenclature, The Natural History Museum, London.

Jones W.K., Hobbs H.H., Wicks C.M., Currie R.R., Hose L.D., Kerbo R.C., Goodbar J.R. & Trout J. 2003. *Recommendations and Guidelines for Managing Caves on Protected Lands*. Karst Waters Institute Special Publication, Charles Town, West Virginia.

Kånneby T., Todaro M.A. & Jondelius U. 2013. Phylogeny of Chaetonotidae and other Paucitubulatina (Gastrotricha: Chaetonotida) and colonization of aquatic ecosystems. *Zoologica Scripta* 42: 88–105. https://doi.org/10.1111/j.1463-6409.2012.00558.x

Kieneke A. & Schmidt-Rhaesa A. 2015. Gastrotricha. *In*: Schmidt-Rhaesa A. (ed.) *Handbook of Zoology. Gastrotricha and Gnathifera. Vol. 3*. De Gruyter, Berlin/Boston.

Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111–120. https://doi.org/10.1007/BF01731581

Kisielewski J. 1981. *Gastrotricha from Raised and Transitional Peat Bogs in Poland*. Monografie Fauny Polski, 11. Polska Akademia Nauk, Kraków.

Kisielewski J. 1991. Inland-water Gastrotricha from Brazil. *Annales Zoologici* 43: 1–168.

Kisielewski J. 1997. *Brzuchorzeski (Gastrotricha). Fauna Słodkowodna Polski*. Zeszyt 31, Wydawnictwo Uniwersytetu Łódzkiego, Łódź.

Kolicka M. 2016. Gastrotrichs in bromeliads – newly recorded *Chaetonotus (Hystricochaetonotus) furcatus* Kisielewski, 1991 (Chaetonotida) from the Łódź Palm House. *Zoosystema* 38 (1): 141–155. https://doi.org/10.5252/z2016n1a5

Kolicka M., Dabert M., Dabert J., Kånneby T. & Kisielewski J. 2016. *Bifidochaetus*: a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by the integrative taxonomy approach. *Invertebrate Systematics* 30: 398–419. https://doi.org/10.1071/IS16001

Kolicka M., Jankowska E. & Kotwicki L. 2015. Baltic Sea Gastrotricha – one new species and one new record of Chaetonotida from Poland. *Zootaxa* 4027: 487–508. https://doi.org/10.11646/zootaxa.4027.4.2

Kolicka M., Kisielewski J., Kotwicki L., Zawierucha K. & Grzelak K. 2014. Checklist of Gastrotricha of the Polish Baltic Sea with the first reports of *Heterolepidoderma joermungandri* Kånneby, 2011, and *Turbanella hyalina* Schultze, 1853. *Zootaxa* 3869 (2): 101–130. https://doi.org/10.11646/zootaxa.3869.2.1

Kolicka M., Kisielewski J., Nesteruk T. & Zawierucha K. 2013. Gastrotricha from the Poznań Palm House – one new subgenus and three new species of freshwater Chaetonotida (Gastrotricha). *Zootaxa* 3717 (2): 231–279. https://doi.org/10.11646/zootaxa.3717.2.7

Leasi F. & Todaro M.A. 2008. The muscular system of *Musellifer delamarei* (Renaud-Mornant, 1968) and other chaetonotidans with implications for the phylogeny and systematization of the Paucitubulatina (Gastrotricha). *Biological Journal of the Linnean Society* 94: 379–398. https://doi.org/10.1111/j.1095-8312.2008.00974.x

Lješević A.M. 1968. *Izveštaj o speleološkim istraživanjima Lipske pećine, (elaborat) u okviru projekta "Hidrogeologija sliva Skadarskog jezera, Zavod za geološka istraživanja CG"*. Titograd.

Lješević A.M. 1969. *Izveštaj o speleološkim istraživanjima Cetinjske pećine, Zavod za geološka istraživanja CG*. Titograd.

Martel E.A. 1893. *Les abîmes: les eaux souterraines, les cavernes, les sources, la speleology: explorations souterraines effectuées de 1888 à 1893 en France, Belgique, Autriche et Grèce*. Charles Delagrave, Paris.

Martinović D. 1964. Cetinjsko polje (fizičko - geografska ispitivanja). *Glasnik Srpskog Geografskog Društva* 44: 27–37.

McCall J.N. & Fleeger J.W. 1995. Predation by juvenile fish on hyperbenthic meiofauna, a review with data on post-larval *Lelostomus xanthurus*. *Vie et Milieu* 45: 61–73.

Mihavc A. 1983. Lipska in Začirska pečina, dve veliki Črnogorski jami. Published by Jamarska Zveza Slovenje, Speleological Associaton of Slovenia. *Nase Jame* 25: 37–39.

Navajas M., Lagnel J., Gutierrez J. & Boursot P. 1998. Species-wide homogeneity of nuclear ribosomal ITS2 sequences in the spider mite *Tetranychus urticae* contrasts with extensive mitochondrial COI polymorphism. *Heredity* 80: 742–752.

Nicholas K.B. & Nicholas H.B. 1997. *GeneDoc: a Tool for Editing and Annotating Multiple Sequence Alignments*. Distributed by the author.

Nozais C., Perissinotto R. & Tita G. 2005. Seasonal dynamics of meiofauna in a South African temporarily open/closed estuary (Mdloti Estuary, Indian Ocean). *Estuarine Coastal and Shelf* 62: 325–338. https://doi.org/10.1016/j.ecss.2004.09.020

Palmer A.N. 1991. Origin and morphology of limestone caves. Vol. 103. *Bulletin of the Geological Society of America* 103 (1): 1–21.

Pešić V. 2010. *Plagigeyeria montenigrina*. The IUCN Red List of Threatened Species 2010: e.T155489A4786103. Available from http://www.iucnredlist.org/ [accessed 8 Sep. 2017].

Posada D. 2008. jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253–1256. https://doi.org/10.1093/molbev/msn083

Pretner E. 1972. La biospeleologia in Jugoslavia nel dopoguerra. *Italian Journal of Zoology* 39 (4): 491–502. https://doi.org/10.1080/11250007209431403

Radulović M. & Radulović V. 2004. *Hydrogeological Map of Montenegro* 1:200,000. Geological Survey of Montenegro, Podgorica.

Rambaut A. 2014. FigTree v. 1.4.2. Available from http://tree.bio.ed.ac.uk/software/figtree/ [accessed 12 Oct. 2016].

Rambaut A. & Drummond A.J. 2007. Tracer v1.4. Distributed by the author.

Rao G.C. & Clausen C. 1970. *Planodasys marginalis* gen. et. sp. nov. and Planodasyidae fam. nov. (Gastrotricha Macrodasyoidea). *Sarsia* 42: 73–82. https://doi.org/10.1080/00364827.1970.10411164

Ronquist F., Teslenko M., van der Mark P., Ayres D.L., Darling A., Höhna S., Larget B., Liu L., Suchard M.A. & Huelsenbeck J.P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Roszczak R. 1969. Brzuchorzęski (Gastrotricha) środkowej Wielkopolski. Poznańskie Towarzystwo Przyjaciół Nauk. Wydział matematyczno-przyrodniczy. *Prace Komisji Biologicznej* 32 (6): 1–92.

Tamura K., Stecher G., Peterson D., Filipski A. & Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30 (12): 2725–2729. https://doi.org/10.1093/molbev/mst197

Telford M.J., Lockyer A.E., Cartwright-Finch C. & Littlewood D.T.J. 2003. Combined large and small subunit ribosomal RNA phylogenies support a basal position of the acoelomorph flatworms. *Proceedings of the Royal Society B, Biological Sciences* 1519 (270): 1077–1083. https://doi.org/10.1098/rspb.2003.2342

Todaro M.A. 2016. Gastrotricha [online]. Available from World Register of Marine Species at http://www.marinespecies.org/aphia.php?p=taxdetailsetid=2078 [accessed 7 May 2016].

Todaro M.A. & Hummon W.D. 2008. An overview and a dichotomous key to genera of the phylum Gastrotricha. *Meiofauna Marina* 16: 3–20.

Todaro M.A., Leasi F., Bizzarri N. & Tongiorgi P. 2006. Meiofauna densities and gastrotrich community composition in a Mediterranean sea cave. *Marine Biology* 149: 1079–1091. https://doi.org/10.1007/s00227-006-0299-z

Vandel A. 1964. *Biospeleology. The Biology of Cavernicolous Animals*. Pergamon Press, Oxford.

Manuscript received: 13 July 2016
Manuscript accepted: 19 January 2017
Published on: 28 September 2017
Topic editor: Rudy Jocqué
Desk editor: Kristiaan Hoedemakers

Printed versions of all papers are also deposited in the libraries of the institutes that are members of the *EJT* consortium: Muséum national d'Histoire naturelle, Paris, France; Botanic Garden Meise, Belgium; Royal Museum for Central Africa, Tervuren, Belgium; Natural History Museum, London, United Kingdom; Royal Belgian Institute of Natural Sciences, Brussels, Belgium; Natural History Museum of Denmark, Copenhagen, Denmark; Naturalis Biodiversity Center, Leiden, the Netherlands; Museo Nacional de Ciencias Naturales-CSIC, Madrid, Spain; Real Jardín Botánico de Madrid CSIC, Spain.

Appendix

Morphometric parameters of *Chaetonotus* (*Chaetonotus*) *antrumus* Kolicka sp. nov. Abbreviations: N = number of specimens or structures analysed; Range = the smallest and the largest structure measurement found among all specimens measured; SD = standard deviation. All measurements are given in micrometers (μm); all indicators are given as a percentage (%) and italicized.

Characters	N	Holotype	Range of adult paratypes	SD	N	Range of juvenile and subadult paratypes	SD
Body length	11	123.08	119.12–129.72	2.941	2	91.19–118.46	19.283
Pharynx length	11	32.79	31.85–34.85	0.966	2	30.47–31.05	0.410
Width of anterior pharynx thickening (a)	11	8.54	8.32–10.14	0.522	2	8.17–8.52	0.247
Width of pharynx narrowing (n)	11	5.81	5.06–6.22	0.367	2	5.25–5.31	0.042
Width of middle pharynx (m)	11	7.04	6.51–7.46	0.281	2	6.76–6.78	0.014
Width of posterior pharynx thickening (p)	11	9.64	9.16–11.28	0.590	2	8.84–9.05	0.148
Length of cephalic cilia (anterior tuft)	10	6.27–16.04	(5.46–6.86)–(14.54–7.21)	0.421; 0.873	2	(5.43–5.77)–(15.94–6.01)	0.247; 0.049
Length of cephalic cilia (posterior tuft)	10	8.74–18.35	(7.94–9.07)–(18.16–0.74)	0.368; 0.822	2	(8.26–8.28)–(17.82–7.96)	0.014; 0.099
Hypostomium length	10	5.29	4.56–6.08	0.438	2	4.19–5.14	0.672
Hypostomium width	10	6.58	5.73–6.97	0.395	2	5.26–6.09	0.587
Cephalion length	10	9.29	8.92–10.71	0.632	2	8.86–9.02	0.113
Cephalion width	10	8.82	8.33–9.41	0.291	2	7.81–8.40	0.417
Cephalion maximum width	10	11.37	11.14–12.18	0.375	2	10.94–11.34	0.283
Diameter of mouth ring	10	4.67	4.24–4.84	0.174	2	4.37–4.46	0.064
Furca length	10	21.71	19.77–23.24	0.868	2	20.82–21.39	0.403
Length of adhesive tube	10	10.39	10.08–11.26	0.361	2	10.02–10.07	0.035
Width of adhesive tube	10	1.29	1.26–1.34	0.029	2	1.23–1.27	0.028
Length of head scales	10	1.91–3.50	(1.57–2.19)–(3.34–3.82)	0.219; 0.182	2	(1.49–1.56)–(3.28–3.32)	0.049; 0.028
Width of head scales	10	2.04–4.14	(1.78–2.32)–(3.56–4.26)	0.207; 0.214	2	(1.62–1.69)–(3.59–3.94)	0.049; 0.247
Length of neck scales	10	1.39–3.14	(1.27–1.82)–(2.93–3.29)	0.173; 0.130	2	(1.24–1.37)–(2.89–3.06)	0.092; 0.120
Width of neck scales	10	1.66–3.05	(1.42–2.04)–(2.76–3.94)	0.195; 0.345	2	(1.47–1.57)–(2.76–2.84)	0.071; 0.057
Length of trunk scales	10	2.76–4.52	(2.47–3.02)–(4.12–5.61)	0.185; 0.453	2	(2.38–2.61)–(4.10–4.23)	0.163; 0.092

Characters	N	Holotype	Range of adult paratypes	SD	N	Range of juvenile and subadult paratypes	SD
Width of trunk scales	10	1.84–3.43	(1.64–2.04)–(3.10–4.27)	0.153; 0.347	2	(1.59–1.70)–(2.92–3.08)	0.078; 0.113
Length of small one-lobed scales	10	2.12	1.88–2.66	0.267	2	1.62–2.88	0.891
Width of small one-lobed scales	10	1.51	1.32–1.78	0.150	2	1.08–1.56	0.339
Length of furcal appendages one-lobed scales	10	4.55–4.72	(4.02–5.23)–(4.32–5.72)	0.395; 0.461	2	(3.83–4.06)–(4.09–4.27)	0.163; 0.127
Width of furcal appendages one-lobed scales	10	2.21–2.42	(2.02–2.71)–(2.08–3.17)	0.208; 0.308	2	(1.77–1.94)–(1.92–2.03)	0.120; 0.078
Length of both pairs of parafurcal scales	10	2.76–2.81	(2.04–3.38)–(2.53–3.60)	0.299; 0.324	2	(2.41–2.54)–(2.54–2.73)	0.092; 0.134
Width of parafurcal scales	10	2.27–2.34	(2.11–2.82)–(2.21–3.22)	0.239; 0.314	2	(1.87–2.04)–(2.04–2.16)	0.120; 0.085
Length of head spines	10	1.14–2.06	(1.05–1.48)–(1.83–3.09)	0.164; 0.365	2	(1.01–1.09)–(1.97–2.03)	0.057; 0.042
Length of head hair-like ventral spines	10	5.51–6.88	(5.09–6.38)–(6.38–8.55)	0.437; 0.716	2	(4.94–5.18)–(5.67–6.33)	0.170; 0.467
Length of neck spines	10	0.61–1.72	(0.47–1.26)–(1.53–2.67)	0.220; 0.329	2	(0.51–0.53)–(1.63–1.76)	0.014; 0.092
Length of neck hairlike ventral spines	10	5.36–7.24	(4.98–6.25)–(6.89–9.02)	0.430; 0.683	2	(4.57–4.97)–(6.12–6.86)	0.283; 0.523
Length of trunk spines	10	1.50–2.97	(1.33–1.94)–(2.52–4.12)	0.176; 0.448	2	(1.29–1.39)–(2.72–2.74)	0.071; 0.014
Length of trunk hair-like ventral spines	10	7.31–12.09	(7.08–9.16)–(10.49–13.96)	0.664; 1.021	2	(6.24–6.97)–(10.49–10.52)	0.516; 0.021
Length of posteriormost trunk lateral spines	10	3.77	3.39–5.93	0.721	2	3.52–3.61	0.064
Length of furcal appendages dorsal spines	10	2.64	2.42–4.08	0.530	2	2.23–2.33	0.071
Length of parafurcal spines	10	6.02	5.53–7.67	0.639	2	5.47–5.61	0.099
Length of ventral interciliary field scales	11	1.93–3.44	(1.44–1.93)–(3.08–3.72)	0.202; 0.230	2	(1.52–1.73)–(3.10–3.19)	0.148; 0.064
Width of ventral interciliary field scales	11	1.21–1.68	(1.07–1.29)–(1.56–1.88)	0.059; 0.111	2	(1.06–1.12)–(1.59–1.62)	0.042; 0.021
Length of posteriormost ventral interciliary field scales	11	5.91	5.37–6.12	0.231	2	5.46–5.62	0.113
Width of posteriormost ventral interciliary field scales	11	2.37	2.22–3.24	0.279	2	2.19–2.24	0.035
Head dorsal sensory bristles length	11	8.41	8.19–10.13	0.569	2	8.28–8.47	0.134

Characters	N	Holotype	Range of adult paratypes	SD	N	Range of juvenile and subadult paratypes	SD
Neck dorsal sensory bristles length	11	14.77	13.28–15.68	0.796	2	13.28–13.94	0.467
Posterior dorsal sensory bristles length	11	11.72	10.13–12.67	0.755	2	10.01–11.08	0.757
Length of mature egg	1	–	40.96	–	–	–	–
Width of mature egg	1		26.02		–	–	–
Number of cephalic cilia in anterior tuft	31	4	4	0	5	4	0
Number of cephalic cilia in posterior tuft	31	5	5	0	5	5	0
Number of scales in central longitudinal row	31	25	24–27	1.104	5	23–26	2.121
Total number of longitudinal alternating rows of scales	31	31	29–35	1.748	5	29–31	1.414
Number of scales in single longitudinal row on ventral interciliary field	31	3	3–4	0.467	5	3	0
Total number of longitudinal alternating rows of scales	31	9	7–9	0.809	5	9	0
Pharynx formula a	11	*26.045*	*24.317–29.096*	1.406	2	*26.312–27.962*	1.167
Pharynx formula n	11	*17.719*	*15.852–19.036*	1.028	2	*16.908–17.427*	0.367
Pharynx formula m	11	*21.47*	*20.331–22.732*	0.800	2	*21.771–22.251*	0.339
Pharynx formula p	11	*29.399*	*27.607–32.367*	1.560	2	*29.012–29.147*	0.095
Ratio of scale distribution	31	*124*	*111.5–129.6*	4.912	5	*119.2–126.1*	4.879

Poznań, 16.12.2019r.

Małgorzata Kolicka
Adam Mickiewicz University, Poznań
Faculty of Biology
Department of Animal Taxonomy and Ecology
Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Declaration

I declare that the publication: **Kolicka M**. 2017. New Arctic Gastrotricha – Three *Chaetonotus* (Chaetonotidae) from Longyearbyen (Spitsbergen, Svalbard Archipelago). *Annales Zoologici*, 67 (1): 131-180, has been prepared as a part of my doctoral dissertation under the scientific supervision of Prof. UAM, dr hab. Ziemowit Olszanowski.

Yours faithfully,

Poznań, 16.12.2019r.

Małgorzata Kolicka
Uniwersytet im. Adama Mickiewicza, Poznań
Wydział Biologii
Zakład Taksonomii i Ekologii Zwierząt
ul. Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Oświadczenie

Oświadczam, że publikacja: **Kolicka M**. 2017. New Arctic Gastrotricha – Three *Chaetonotus* (Chaetonotidae) from Longyearbyen (Spitsbergen, Svalbard Archipelago). *Annales Zoologici*, 67 (1): 131-180, została wykonana na poczet mojej rozprawy doktorskiej pod opieką promotora Prof. UAM dra hab. Ziemowita Olszanowskiego.

Z wyrazami szacunku,

ANNALES ZOOLOGICI (Warszawa), 2017, 67(1): 131-180

NEW ARCTIC GASTROTRICHA – THREE *CHAETONOTUS* (CHAETONOTIDAE) FROM LONGYEARBYEN (SPITSBERGEN, SVALBARD ARCHIPELAGO)

MAŁGORZATA KOLICKA

Department of Animal Taxonomy and Ecology, Institute of Environmental Biology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland; e-mail: kolicka@amu.edu.pl

Abstract.— Gastrotricha is a cosmopolitan phylum of aquatic and semi-terrestrial invertebrates comprising over 820 described species. Virtually no taxonomic or faunistic data exist on freshwater Gastrotricha of the Arctic. So far, 10 studies have recorded Gastrotricha from the entire area of the Svalbard Archipelago; only in two were gastrotrichs determined to the genus and species levels. The remaining studies only reported the presence of Gastrotricha as a phylum. In this article, I present the results of research on freshwater Gastrotricha from the area of fjord Isfjorden (Spitsbergen, Arctic). The collected material contained representatives of 8 species, 2 subgenera of *Chaetonotus* namely *Chaetonotus* (*Chaetonotus*) Erhenberg, 1830 and *Chaetonotus* (*Hystricochaetonotus*) Schwank, 1990, two genera namely *Chaetonotus* Erhenberg, 1830 and *Bifidochaetus* Kolicka *et* Kisielewski, 2016, both belonging to the family Chaetonotidae Gosse, 1864 (sensu Leasi and Todaro, 2008). I describe 3 new species from the Chaetonotidae: *Chaetonotus* (*Chaetonotus*) *jaceki* **sp. nov.**, *C.* (*C.*) *gelidus* **sp. nov.**, and *C.* (*C.*) *svalbardi* **sp. nov.**, together with data on the range, frequency and abundance of all noted species. Furthermore, a record abundance of Gastrotricha was noted, amounting to more than one thousand specimens per 1 cm^3 of bottom sediment.

Key words.— Chaetonotida, freshwater, gastrotrichs, taxonomy, zoogeography.

INTRODUCTION

Gastrotrichs are a group of common and widespread microscopic animals ranging in size from 50 to 3500 μm. They can live in any aquatic and wet terrestrial habitat worldwide. To date, *ca.* 820 nominal species of Gastrotricha are divided into two orders, i.e. Chaetonotida Remane, 1925 [Rao and Clausen, 1970] and Macrodasyida Remane, 1925 [Rao and Clausen, 1970] (Kisielewski 1997, Balsamo *et al*. 2014, Todaro 2016). They are a common component of meiofauna communities and constitute a constant and significant component of the benthic psammon and epiphytic ecosystems (Balsamo and Todaro 2002, Nesteruk 2004, Balsamo *et al*. 2008, 2014). To date, the highest noted abundance of freshwater gastrotrichs in the literature was recorded in sediments from a small hypertrophic pond in the Białowieża Glade (Poland), reaching 101 individuals per 1 cm^3 of bottom sediments (Kisielewska and Kisielewski 1986). Despite the fact that gastrotrichs are ubiquitous and numerous, the state of knowledge on their biodiversity and biogeography is very heterogeneous. The main factors accounting for our poor knowledge on these animals are methodological problems (*e.g.*, collecting, extracting, preserving and determining) and the small number of taxonomic specialists for this group (Balsamo *et al*. 2008, 2014, Garraffoni *et al*. 2016). Moreover, many descriptions appear to be incomplete or insufficient in the light of new knowledge about the detailed species morphology

PL ISSN 0003-4541 © Museum and Institute of Zoology PAS
doi: 10.3161/00034541ANZ2017.67.1.015

and cryptic speciation. These taxonomical problems are the main reasons why the ecology of these invertebrates is virtually unknown in relation to the current state of general zoological knowledge (Kisielewski 1981).

Because current knowledge on Gastrotricha is far from complete, new species are described regularly (*e.g.* Kånneby *et al*. 2009, Kånneby 2011, 2013, Suzuki *et al*. 2013, Atherton 2014, Hochberg *et al*. 2014, Araujo *et al*. 2015, Garraffoni and Melchior 2015), as new genera (*e.g.*, Kisielewski 1987, 1991, Hummon *et al*. 1993, Todaro *et al*. 2005, 2014, 2015, Kolicka *et al*. 2016, Garraffoni *et al*. 2016), and even new families are a common occurrence (*e.g.* Kisielewski 1987, Todaro *et al*. 2006, 2014). Although gastrotrichs prevail in many types of environments in different word regions, they are still unsatisfactorily known or even unknown in other geographic areas like Portugal, The Netherlands, Albania or Serbia(Balsamo *et al*. 2008, 2014, 2015).

To date, more than 1,000 identified invertebrate species are known from the Svalbard Archipelago (Coulson *et al*. 2014), yet taxonomic and faunistic knowledge on Gastrotricha is very limited. Most of the previous records from this region concern marine habitats and note the presence of gastrotrichs only at phylum level without further determination (Pfannkuche and Thiel 1987, Węsławski *et al*. 1997, Włodarska-Kowalczuk *et al*. 1999, Urban-Malinga *et al*. 2005, Grzelak and Kotwicki 2012, Górska *et al*. 2014). Gastrotrichs from Svalbard freshwater environments were mentioned only four times – in three papers only as a phylum, Gastrotricha, and a genus, *Chaetonotus* Ehrenberg, 1830, and in one work as an identified species – *Bifidochaetus arcticus* Kolicka *et* Kisielewski, 2016 (Scourfield 1897, De Smet *et al*. 1988, De Smet 1993, Coulson *et al*. 2014, Kolicka *et al*. 2016). In this work I present the first comprehensive data on freshwater gastrotrichs recorded from the Arctic region with a description of 3 new species of *Chaetonotus* and note their unprecedented abundances.

MATERIAL AND METHODS

Study site

The material for the research was collected on Spitsbergen, which is the largest island of the Svalbard Archipelago. Spitsbergen has an area of 37.673 km^2 and is surrounded by the Arctic Ocean, Norwegian Sea and Greenland Sea. Two currents flow around the island: the warm West Spitsbergen Current, which constitutes the northern extension of the Norway Current (an extension of the Gulf Stream) and the cold East Spitsbergen Current, originating in the Barents Sea. The currents flowing around Spitsbergen cause strong thermal differentiation between the regions of the island. The conditions in the northern part are harsh and extend over to the eastern coast (affected by the East Spitsbergen Current). On the other hand, the climatic and environmental conditions on the western coast are milder thanks to the warm West Spitsbergen Current, which considerably increases the temperature in the area (Głowacki 2007). Finally, the central part of the island is close to continental in terms of climate (Stanowski *et al*. 2013). Most of Spitsbergen area is covered by a long-term permafrost that thaws only down to 2 m deep in the summer (Giżejewski and Karczewski 2013). The vegetation period is very short, lasting usually from July to September during the polar summer. Most of the precipitation on the island is solid. For about eight months out of the year the ground is covered by snow, and the average monthly temperature does not exceed 0°C (Marsz *et al*. 2013, Migała and Wawrzyniak 2013).

Longyearbyen is the capital of Svalbard and with a population of *ca.* 1800 people, is the largest settlement in the northern Arctic. A fishing village was established in the area in the city in the 16th century. Today, the area of Longyearbyen includes an airport and bituminous coal mines. Due to its long settlement history, economic activities and intense tourism, the city and its vicinity have been exposed to strong human pressure as compared to the remaining part of the Svalbard Archipelago. All samples were collected either within or in the vicinity of the city of Longyearbyen (Fig. 1, Table 1). The average yearly temperature in the region is -5.1°C, with the lowest average monthly temperature (-13.5°C) occurring in February and the highest (6.4°C) occurring in July. The mean sum of precipitation in the region is 183.5 mm (Marsz *et al*. 2013).

The samples were collected from temporary, small and shallow water reservoirs originating from melting snow or ice and surface runoff on 7 and 8 August 2013. These reservoirs were at a distance of approximately 300–3000 metres from the Isfjorden coast (Fig. 1).

Methods

The gastrotrichs were collected from a reservoir located approximately 500 metres from the sea. The bottom sediments had organic and organic-clay characters and a fine texture. The top layer of the bottom sediments together with ambient water from five reservoirs were collected by hand from shallow water zones into 100 cm^3 plastic containers. Approximately 10–20 cm^3 of the sediment and 80–90 cm^3 of ambient water were placed into each container. The collected samples were subsequently placed in an isothermal box and transported to the laboratory within 24 hours. In the laboratory the samples were oxygenated and placed in a thermal chamber at a temperature of 10°C in order to

Figure 1. Longyearbyen (Svalbard Archipelago) – Study area with marked sampling places.

create conditions similar to those at the sampling site. Within 10 days of collection, a total of 0.3 cm^3 of sediment from each sample was analysed. Specimens of Gastrotricha were extracted with a micropipette under an Olympus SZ51 stereoscopic microscope. All specimens were observed, photographed and documented alive under an Olympus BX41 microscope equipped with phase contrast and an Array Artcam 300 digital camera. Morphometric characters were measured in cellSens Entry 1.11 software (Olympus). All measurements are given in micrometres (μm) and all used formulas are given as a percentage (%).

Detailed measurements are provided and expressed as ranges for all new species reported. Because of their very limited utility specimens preserved as permanent slides for taxonomic purposes, all animals were documented as a sets of photomicrographs. Such solution is allowed by The International Code of Zoological Nomenclature, namely in the article 73.1.4 (The International Commission on Zoological

Table 1. Sampling sites.

Site	GPS coordinates	Altitude above sea level (m)	Collecting data
1	N78° 13.392' E15° 38.759'	1	07.08.2013
2	N78° 13.238' E15° 38.619'	15	07.08.2013
3	N78° 10.942' E15° 32.629'	519	08.08.2013
4	N78° 11.771' E15° 34.096'	151	08.08.2013
5	N78° 12.072' E15° 35.523'	-70	08.08.2013

Nomenclature 1999). The description of new species in this paper follows the convention of Hummon *et al*. (1992), in which the positions of certain morphological characters are provided in percentage units (U) of the total body length measured from the anterior to the posterior (distal end of the furca). The identification of gastrotrichs and their morphological study follows Kisielewski (1991, 1997) and Kolicka *et al*. (2016). The terms describing the shape of furcal branches and furcal indentation follows Roszczak (1969). In this paper, the following formulae describing the distribution of scales were used, namely:

$$\textit{Ratio of scales distribution} = \frac{\text{total number of longitudinal alternating rows of scales}}{\text{number of scales in central longitudinal row}} \times 100\%$$

and the pharynx shape, namely: pharynx formula

$$a = \frac{\text{width of anterior thickenning}}{\text{pharynx length}} \times 100\%$$

and pharynx formula

$$n = \frac{\text{width of pharynx narrowing}}{\text{pharynx length}} \times 100\%$$

$$m = \frac{\text{width of middle pharynx}}{\text{pharynx length}} \times 100\%$$

$$p = \frac{\text{width of anterior thickenning}}{\text{pharynx length}} \times 100\%$$

according to Kisielewski (1991).

The pharynx-intestine ratio

$$I = \frac{\text{pharynx length}}{\text{intestine length}} \times 100\%$$

was used according to Kolicka *et al*. 2016.

Results

The analysed material contained 308 specimens belonging to 8 species. A representative of a new genus, *Bifidochaetus arcticus* Kolicka *et* Kisielewski, 2016, found in this material was described previously in a separate article (Kolicka *et al*. 2016). The other found species, namely *Chaetonotus* (*Chaetonotus*) sp. 1, *Chaetonotus* (*Chaetonotus*) sp. 2, *Chaetonotus* (*Chaetonotus*) sp. 3 and *Chaetonotus* (*Hystricochaetonotus*) sp. 4, are most probably new to science but cannot be described due to insufficient material.

TAXONOMIC ACCOUNTS

Phylum **Gastrotricha** Mečnikow, 1865

Order **Chaetonotida** Remane, 1925 [Rao and Clausen, 1970]

Suborder **Paucitubulatina** d'Hondt, 1971

Family **Chaetonotidae** Gosse, 1864 (*sensu* Leasi and Todaro, 2008)

Subfamily **Chaetonotinae** Gosse, 1864 (*sensu* Kisielewski, 1991)

Genus ***Chaetonotus*** Ehrenberg, 1830

Typus generis. *Chaetonotus larus* (Müller, 1773).
Terra typical. Denmark.

Subgenus ***Chaetonotus*** *sensu stricto* Ehrenberg, 1830

Type species. *Chaetonotus larus* (Müller, 1773).
Terra typica. Denmark.

Chaetonotus* (*Chaetonotus*) *jaceki sp. nov.
(Figs 2–12, Tables 2–3 and A1)

Location. Spitsbergen, Svalbard Archipelago (Norway), Site 2.

Material. One sample, 116 specimens (81 adults, 18 subadults, 17 juveniles), all photographed. The holotype was established and their detail photomicrographs documentation are deposited in the Natural History Collections at Adam Mickiewicz University in Poznań under access number NHC–GCCJ–7–1–20/h (The International Commission on Zoological Nomenclature 1999)). The photomicrographs documentation for paratypes are also available in the Natural History Collections at Adam Mickiewicz University in Poznań under access number NHC–GCCJ–7–21–150/p and in the author's collection.

Etymology. Named to honour Professor Jacek Kisielewski, a prominent Polish gastrotrichologist, eminent taxonomist and wonderful person with a great passion for Gastrotricha.

Diagnosis. Body length from 81.4 to 131.1 m. Slender body. Head five-lobed, cephalion and pleuria marked in the head outline. Hypostomium small, rhomboidal. Thick cephalic cilia. No ocellar granules. Scales small and three-lobed with a strong keel. Single one-lobed scales with a keel present on the dorsal side of the posterior trunk region. Scales of the head, neck and trunk differ morphologically. Scales distributed in

33–47 total longitudinal rows (9–13D+4–8DL+6-8L+10-12LV+4-6V) of 26–44 scales in central row. Simple spines thick, with blunt end. Spine lengths vary throughout the body: head spines longer than those of neck; spines on the trunk the widest body region the longest. The length of the spines slightly and gradually increases from the dorsal side to the ventral side, towards the ciliary bands. The pair of posteriormost lateral trunk spines and the two pairs of the parafurcal spines longer and stronger than the surrounding spines. Ventral scales spines thin and hair-like. Ventral interciliary field naked except for the posterior trunk region, where small scales and a pair of terminal scales of the ventral interciliary field present. Ventral interciliary field scales shaped like narrow triangles, with a shallow, sharp notch in the posterior edge; a long keel and spineless. Pharynx with marked anterior and posterior dilatations with single, narrow, curved cuticular rod inside the anterior dilatation. Straight intestine with a marked distinct anterior section.

Description. *Chaetonotus* (*Chaetonotus*) *jaceki* sp. nov. has a slender body (Fig. 2). The head is wider than the neck and separated from the trunk by a clear neck narrowing. The neck gradually expands towards to the trunk, which gradually widens towards to the widest region located about halfway along its length (U59), after which it gradually narrows up to a narrow furcal base at U82 (Figs 2, 5–6). The furca branches are narrowly spaced. The furcal indentation is V-shaped, and the ends of the adhesive tubes point outwards. The adhesive tubes are straight, narrow and short in relation to body length, and slightly taper towards their blunt ends.

The head is short, five-lobed and shaped like a rounded triangle. All plates are clearly marked in the head outline (Fig. 2C). The cephalion (U1–U5) adheres along its entire length to the head and rapidly widens at the dorsal edge. The epipleuria (U4–U6) are small and strongly convex. The hypopleuria (U6–U13) are large, about three times larger than the epipleuria, and convex. The epipleuria are located dorsolaterally, whereas the hypopleuria are located more ventrolaterally, with only a small part of them visible on the dorsal side (Figs 2, 5–6). The hypostomium (U6–U9) is short and rhomboidal in shape with slightly rounded edges and a strong anterior edge (see Table A1). Two pairs of cephalic ciliary tufts are present. Each anterior tuft has 5–6 cilia that emerge from between the cephalion and the epipleuria. The cilia of the anterior tufts emerge in lines around the lateral edges of the cephalion; the beginning of these lines (the two first cilia) is clearly visible on the dorsal side. The anteriormost cilium in both anterior tufts is relatively short. The second cilium is very long, the longest of all the cilia in the tuft, and orientated towards the dorsal side of the head. The next cilium or the next two cilia are shorter than the second cilium but longer than the first one. The subsequent cilium is shorter and orientated laterally. The posteriormost cilium in both anterior tufts is shorter than second one but longer than the rest cilia and orientated ventrolaterally. Each posterior tuft has 5–7 cilia and emerges from between the epipleuria and the hypopleuria. The posterior tufts are located ventrolaterally, beneath the posterior edges of the epipleuria. The first cilium in both posterior tufts is the shortest. The next cilium is slightly longer than the first one.

Table 2. Main morphometric of *Chaetonotus* (*Chaetonotus*) *jaceki* sp. nov; N – number of specimens or structures analyzed, Range – the smallest and the largest structure found among all specimens measured. All measurements are given in micrometers (μm).

Characters	N	Holotype	Range of adult paratypes
Body length	79	121.55	110.01–131.12
Furca length	22	13.38–19.68	13.38–19.68
Adhesive tube length	22	8.63	8.19–9.73
Number of scales in central longitudinal row	81	30	28–44
Total number of longitudinal alternating rows of scales	81	35	33–47
Head scales length x width	22	0.83–3.94 x 0.62–4.21	0.62–4.21 x 0.49–4.19
Neck scales length x width	22	1.41–4.42 x 1.24–3.08	1.10–5.07 x 0.87–4.01
Trunk scales length x width	22	2.21–5.10 x 1.87–4.33	2.54–5.91 x 1.78–5.05
Head spines length	15	1.81–8.61	1.26–9.95
Neck spines length	15	2.19–7.24	1.40–8.89
Trunk spines length	15	2.26–11.88	1.52–13.10
Mouth ring diameter	15	5.23	4.73–5.37
Pharynx length	65	30.52	26.51–36.13

Table 3. Condensed comparison of the most important differential characters between the most similar species to *Chaetonotus* (*Chaetonotus*) *jaceki* sp. nov. Measurements are given in micrometres (μm). NA – data not available, – – not applicable.

Character	*C.* (*C.*) *jaceki* sp. nov.	*C.* (*C.*) *naiadis*	*C.* (*C.*) *sphagnophilus*
Body length	110.0–131.1	89–140	125–148
Cephalic pleuria	Cephalion narrow, rapidly widening at the dorsal edge; epipleuria small, dorsolateral; hypopleuria large, located more ventrolaterally. Hypostomium short, rhomboidal	Cephalion wide; epipleuria large; hypopleuria small; clearly visible from the dorsal body side. Hypostomium small, subrectangular	Cephalion narrow, epipleuria hypopleuria similar in size. clearly visible from the dorsal body side. Hypostomium present
Place of emerging cephalic cilia	Anterior tufts: in lines arround lateral cephalion edges; posterior tufts: ventrolllateraly, beyound the posterior edges of epipleuria	Anterior and posterior tufts: laterally	Anterior and posterior tufts: laterally
Pharynx length	26.5–35.1	26–33	32–42
Pharynx shape	Pharynx with anterior and wider posterior dilatation	Pharynx widens towards its end, without clear dilatation	Pharynx with anterior and wider posterior dilatation
Presence of cuticular reinforcements in the pharynx	Present (single, narrow, curved cuticular rod)	Absent	Absent
Anterior intestine section differ in form of morphology	Present	Absent	Absent
Furca length	13.4–19.7	12–17	15–21
Adhesive tube length	8.2–9.7	9	8.5–10.5
Type of furca indentation	V-shaped	V-shaped	V-shaped
Number of scales in single longitudinal row	26–44	20	21–26
Total number of longitudinal alternating rows of scales	33–47	23–31	12–19
Type of scales	Scales small; almost all three-lobed with strong keels and straight, thick spines; shape strongly differentiated within different body regions	Scales small; one-lobed with strong keels and straight spines; shape undifferentiated within body regions	Scales small; three-lobed with thin postero-lateral lobes, with keels and slightly bent spines; shape undifferentiated within body regions
Scales size variation	Scales size decrease on the neck area and then increase towards to the widest trunk region; the size of the ventrolateral and ventral scales gradually decrease towards the ciliary bands	Similar size all over the body	Size scales slightly decrease from head to the posterior trunk part
Scales size	Head: 0.62–4.21 x 0.49–3.84 Neck: 1.10–5.07 x 0.87–4.01 Trunk: 1.74–5.91 x 1.39–5.05	Head: 2.5–2.8 x 2.3–2.5 Neck: NA Trunk: 3.1–4.2 x 1.5–2.3	Head: NA Neck: 3.0–5.5 x NA Trunk:5.5–7.5 x NA
Alignment of scales	Edges juxtaposed but do not overlap	Edges juxtaposed but do not overlap	Located far from each other

Table 3. Continued.

Character	*C.* (*C.*) *jaceki* sp. nov.	*C.* (*C.*) *naiadis*	*C.* (*C.*) *sphagnophilus*
Presence of a different type scales on the dorsal and dorsolateral side of the furcal base	Present	Present	Absent
Presence of scales with two keels and two spines on the furcal appendages	Absent	Present	Absent
Scales coverage of the ventral interciliary field	Only the posterior part of the trunk covered	Only the posterior end of the trunk covered	Covered from the beginning of the intestinal section to furcal base
Type of scales of the ventral interciliary field	Narrow triangle with shallow posterior notches scales with keels and spineless	Oval scales with keels and spineless	Only keels visible
Number of terminal scales of the ventral interciliary field	1 pair	1 pair	1 pair
Type of terminal scales of the ventral interciliary field	Narrow triangles with shallow posterior notches, keeled, spineless	Oval without posterior notches, keeled, spined	NA
Spines length variation	Spines of head longer than those of neck; spines of neck getting progressively longer towards the beginning of the trunk region; spines of the trunk getting progressively longer towards the widest body region, after which they slightly and gradually shorten towards the furcal base; length of the spines slightly and gradually increases from the dorsal side to the ventral side, towards the ciliary bands; ventral spines long	Similar length of spines on the dorsal, dorsolateral, lateral and ventrolateral sides of the head, neck and trunk; longer spines on the ventral body side	Spines lengthen from the head to the posterior trunk region; similar length of dorsal, dorsolateral, lateral, ventrolateral and ventral spines
Spines length	Head: 1.26–9.95 Neck: 1.40–8.89 Trunk: 1.52–13.10	1–2	Head: NA Neck: 3.5–8.5 Trunk: 8–13
Presence of hair-like spines on the ventral body side	Present	Absent	Absent
Type of posteriormost pair of the trunk lateral spines	Longer and slightly thicker than other trunk spines	The same as other spines	Similar or slightly shorter than other trunk spines
Type of last pair of the furcal appendages lateral spines	Longer, thicker and stronger than other spines	The same as other spines	–
Ventral locomotor cilia	Two longitudinal bands wider on the head region	Two longitudinal bands wider on the head region	NA
Number of dorsal sensory bristles	3 pairs	2 pairs	1–2 pairs

The third cilium in both tufts is long, the longest of all the cilia in the posterior tufts. The next 1–3 cilia are slightly shorter than the third cilium. The last cilium is shorter than the preceding cilia. All cephalic cilia are thick. Ocellar granules are not present. The mouth ring is located subterminally on U2–U5 and has granular reinforcements. Short cuticular inner hairs are present inside the mouth ring, and suboral bristles are present around it.

The entire body, except for the ventral interciliary field, is covered with small, three-lobed scales and one pair of one-lobed scales that adhere with their entire surface to the cuticle (Figs 2, 3–4, 6–7, 9A, 11A, B, C). The scales have a strong keel and are shaped like triangles with a deep notch in the posterior edge. The scales are distributed in 33–47 longitudinal alternating rows (9–13D+4–8DL+6-8L+10-12LV+4-6V) with 26–44 scales in the central row. On the head, neck and anterior and middle parts of the trunk, longitudinal rows of scales run parallel to one another, whereas on the posterior part of the trunk and on the furcal base the scales gradually converge towards the central longitudinal row (Fig. 2). The scales are located close to one another but do not overlap. The longitudinal rows of scales begin on the head just posteriorly to the posterior edge of the cephalion, epipleuria and hypopleuria The scales show strong morphological diversity throughout the different body areas in the distinction of postero-lateral lobes, the length of the central lobe, the roundness of the edges, and the size of the scale (Figs 3–4). The anteriormost scales of the head are much smaller than the other scales of the body (Figs 2, 4A, 9A). The head scales have weakly marked, narrow postero-lateral lobes that transition directly from the central lobe (Figs 2, 3A, B, F, G, K, L, R, S). On the neck, towards the trunk, the postero-lateral lobes of the scales become consistently and clearly distinct (Figs 2, 3C, H, M, T). The postero-lateral lobes of the scales of the trunk are already clearly distinct from the central lobe (Figs 2, 3D, E, I, J, N, P, U, W). The scales of the head have short central lobes and rounded edges. On the neck and towards the central part of the trunk the central lobes of the scales gradually lengthen, and their rounded edges gradually transition into sharp edges (Figs 2–3). Furthermore, the edges of the dorsolateral, lateral, ventrolateral and ventral scales gradually become more rounded towards the ventral scales as compared to the edges of the dorsal scales (Figs 2–3).

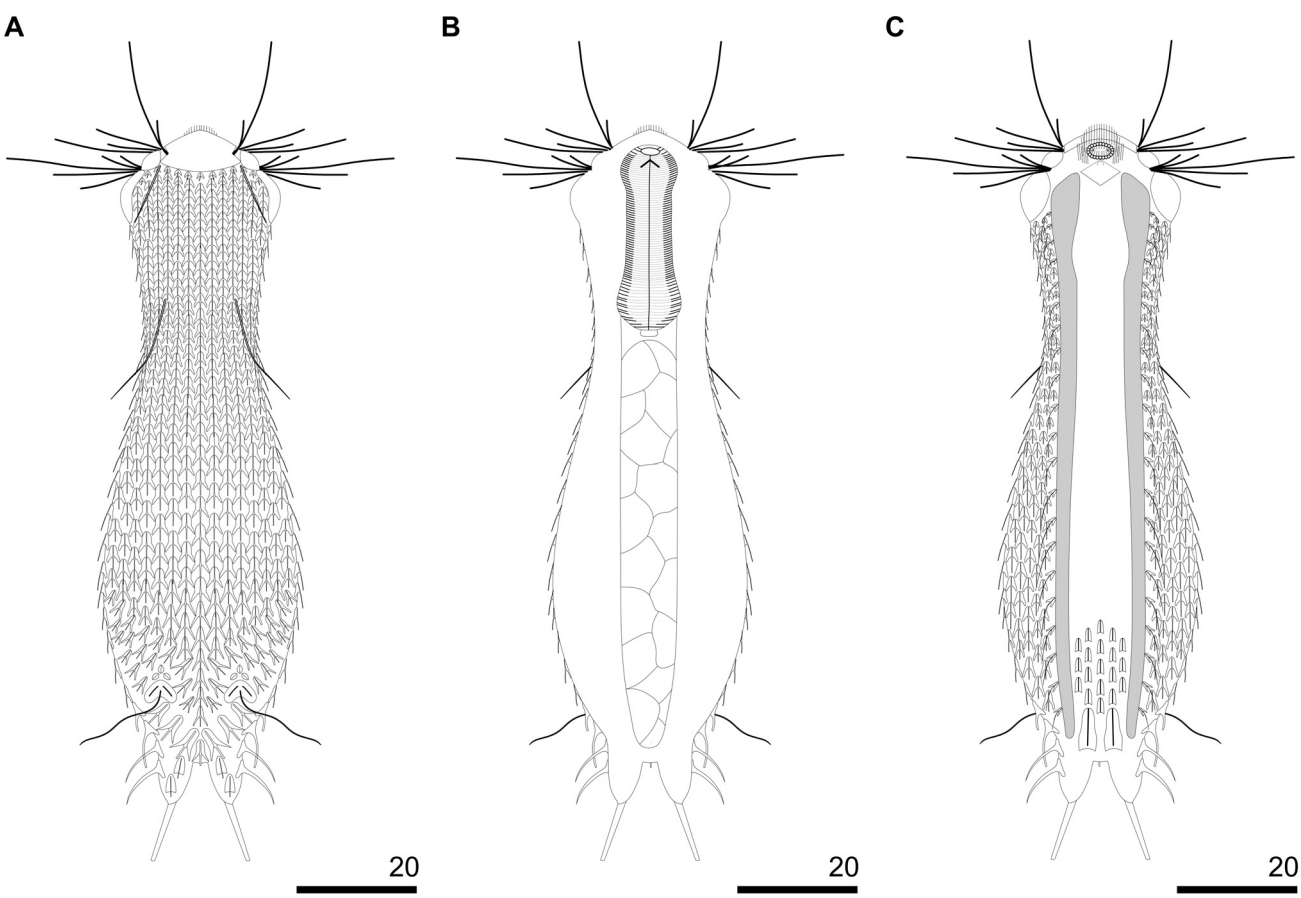


Figure 2. *Chaetonotus* (*Chaetonotus*) *jaceki* sp. nov. Schematic drawings. A – Dorsal body view. B – View of internal morphology. C – Ventral body view. Light grey areas indicate the areas of the insertion of ciliary bands.

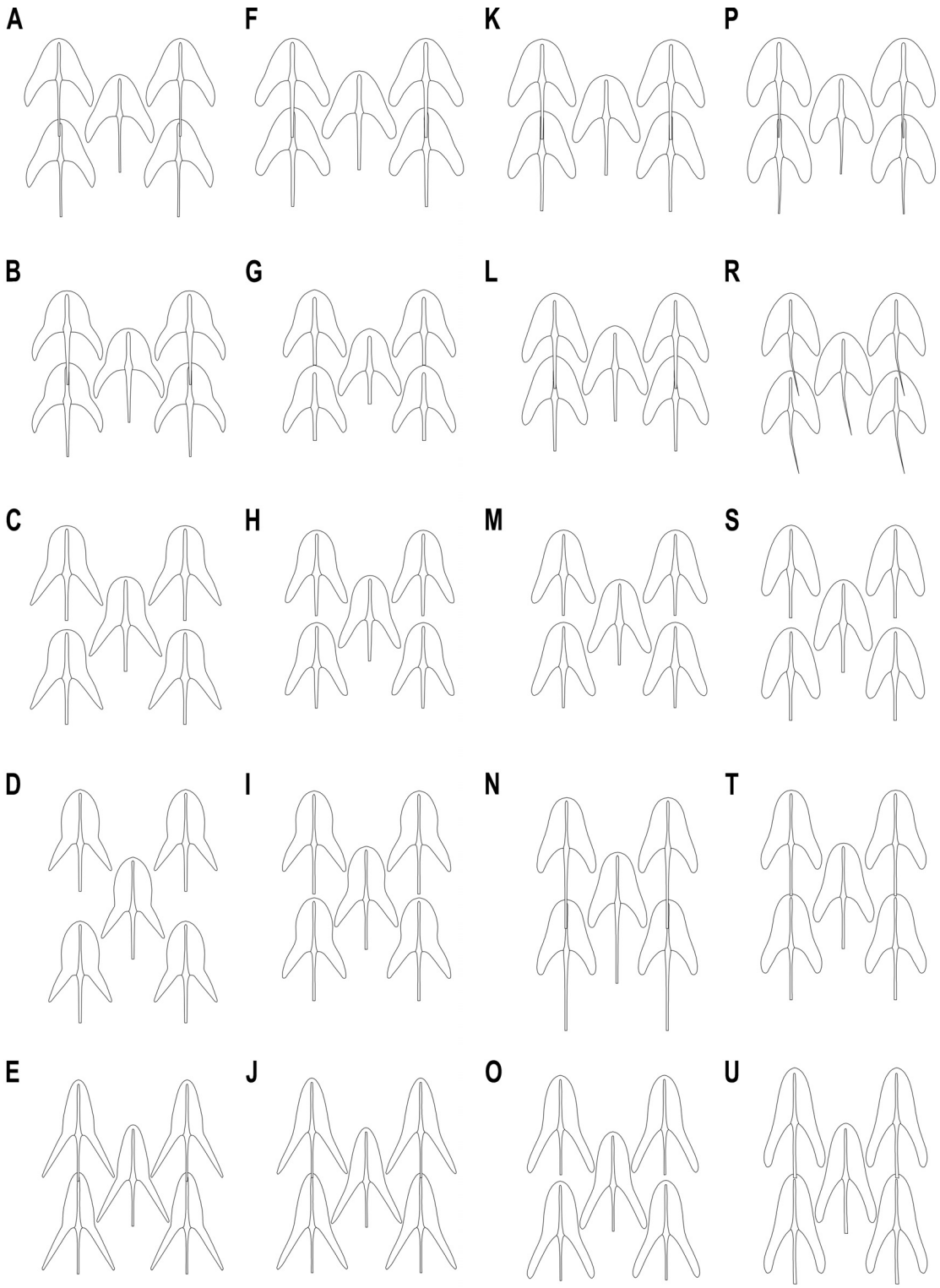


Figure 3. *Chaetonotus* (*Chaetonotus*) *jaceki* sp. nov. Schematic scales drawings. A –Head anterior region dorsal scales. B – Head posterior region dorsal scales. C – Neck dorsal scales. D – Trunk anterior region dorsal scales. E – Trunk posterior region dorsal scales. F – Head anterior region dorsolateral scales. G – Head posterior region dorolateral scales. H – Neck dorsolateral scales. I – Trunk anterior region dorsolateral scales. J – Trunk posterior region dorsolateral scales. K – Head anterior region lateral and ventrolateral scales. L – Head posterior region lateral and ventrolateral scales. M – Neck lateral and ventrolateral scales. N – Trunk anterior region lateral and ventrolateral scales. O – Trunk posterior region lateral and ventrolateral scales. P – Head anterior region ventral scales. R – Head posterior region ventral scales. S – Neck ventral scales. T – Ventral anterior region trunk scales. U – Trunk posterior region ventral scales.

The size of the ventrolateral and ventral scales gradually decreases towards the ciliary bands (see Tables 2 and A1). The scales in the ventral longitudinal row located closest to the ciliary bands are much smaller than the scales of the other rows, and their central lobe is orientated towards the bands at an angle of *ca*. 45° (Figs 2, 6–8). Three spineless, small, one-lobed scales with 43a keel are present on the dorsal side of the trunk, anterior to each double keeled scale bearing the posterior sensory bristle (at U74–U76) (Figs 2, 4D). These scales are fusiform and have rounded edges. One central and three pairs of three-lobed scales slightly narrower than the other trunk scales are present on the dorsal and dorsolateral sides of the furcal base (U79–U85) (Figs 2, 4B, 10). Two pairs of elongated scales shaped like narrow triangles with weakly marked postero-lateral lobes are present on the furcal appendages (on U86–U89) (Figs 2, 4E). The first pair of these scales is located dorsally, and the second pair is located dorsolaterally. Two pairs of three-lobed scales of the same type as the scales of the trunk are present on each lateral side of the furcal appendages (at U85–U90).

Spines emerge from the posterior part of the scales and are thick, straight and taper very slightly towards their blunt edges. The spines of the scales that adhere directly to the cephalion and pleuria are short, the shortest on the head (Figs 2–8; Tables 2 and A1). The length of the spines gradually increase from the anteriormost to central head region. On the posterior head region the length of the spines gradually decrease towards to the neck. The spines of the neck are slightly shorter than the posterior head spines and gradually lengthen towards the trunk (Figs 2–9; Tables 2 and A1). The spines of the trunk slightly and gradually lengthen from the beginning of the trunk up to the widest point of the body (*ca*. U59), after which they slightly shorten towards the furcal base (U82) (Figs 2–8, 10A, B, C, 11A, B, C; Tables 2 and A1). The lateral, ventrolateral and ventral spines are slightly longer than the dorsal and dorsolateral spines, and their length slowly and gradually increases towards the ciliary bands (Tables 2 and A1). The spines of the scales located in the three longitudinal rows located closest to the ventral ciliary bands are long, curved, thin and hair-like (Figs 2, 9C, D). The posteriormost pair of lateral trunk spines is longer and slightly thicker than the surrounding spines (Figs 2–8). Long, thick parafurcal spines that are longer and stronger than the other spines of the body emerge from two pairs of lateral scales of the furcal appendages. These parafurcal spines slightly taper towards their blunt edges. The spines of the posteriormost pair of scales of the furcal appendages are longer, thicker and stronger than the spines of the preceding pair (Fig. 2; Tables 2 and A1).

The species has three pairs of dorsal sensory bristles. The first pair is located on the head, directly

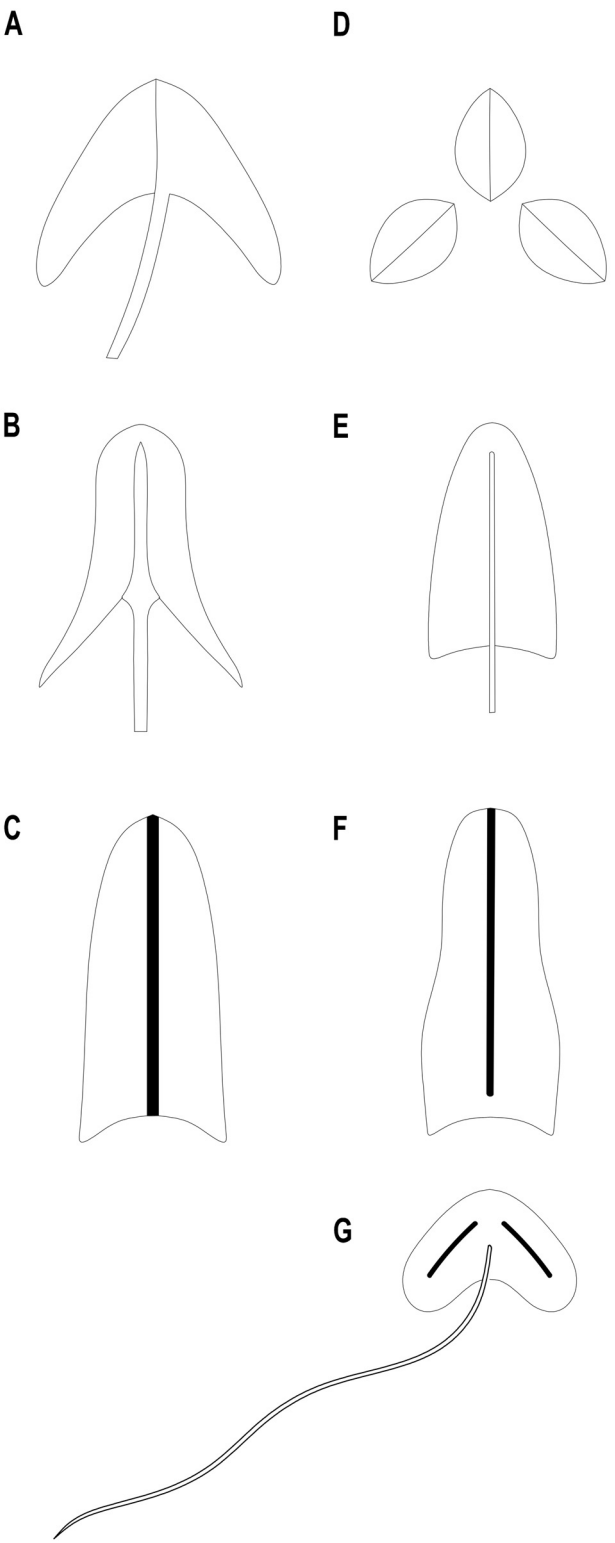


Figure 4. *Chaetonotus* (*Chaetonotus*) *jaceki* sp. nov. Schematic scales drawings. A – Head anteriormost dorsal scale. B – Furcal base dorsal and dorsolateral scale. C– Ventral interciliary field scale. D – Small one–lobed dorsal scales. E – Furcal appendages dorsal and dorsolateral scale. F – Ventral interciliary field terminal scale. G – Double-keeled scale with trunk dorsal sensory bristle.

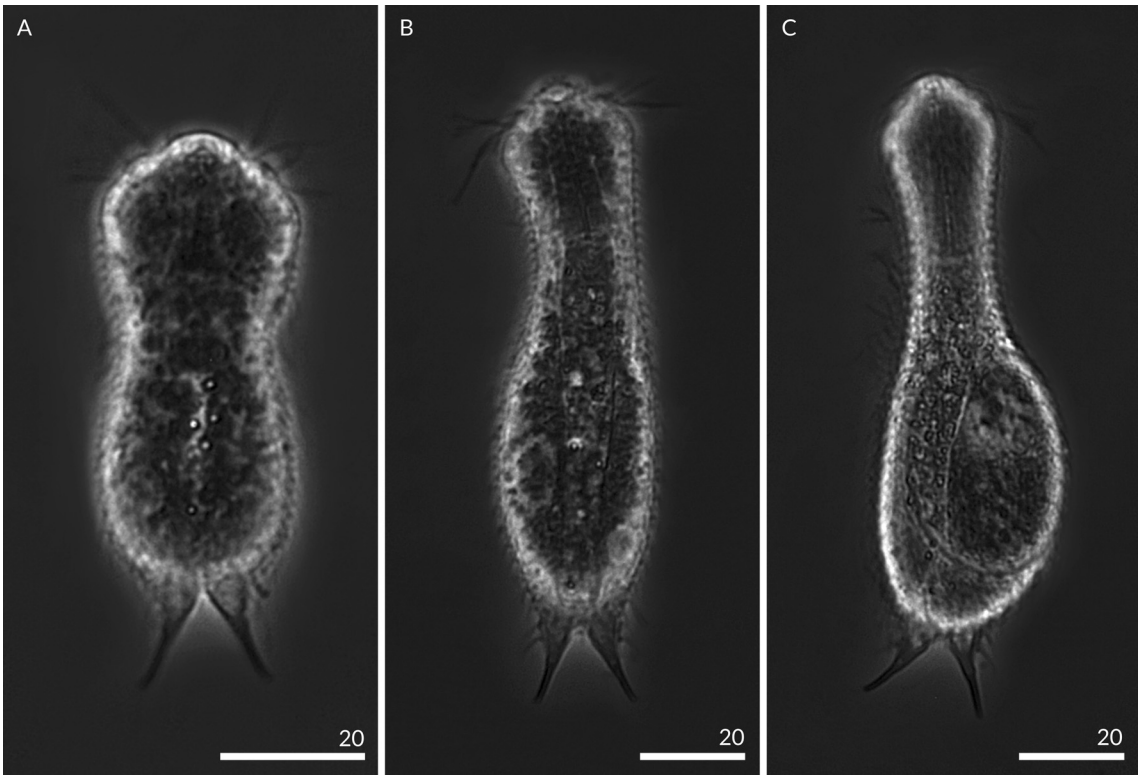


Figure 5. *Chaetonotus* (*Chaetonotus*) *jaceki* sp. nov. A – Juvenile specimen. Habitus. B – Adult specimen. Habitus. C – Specimen with mature egg. Habitus. (Phase contrast photomicrographs).

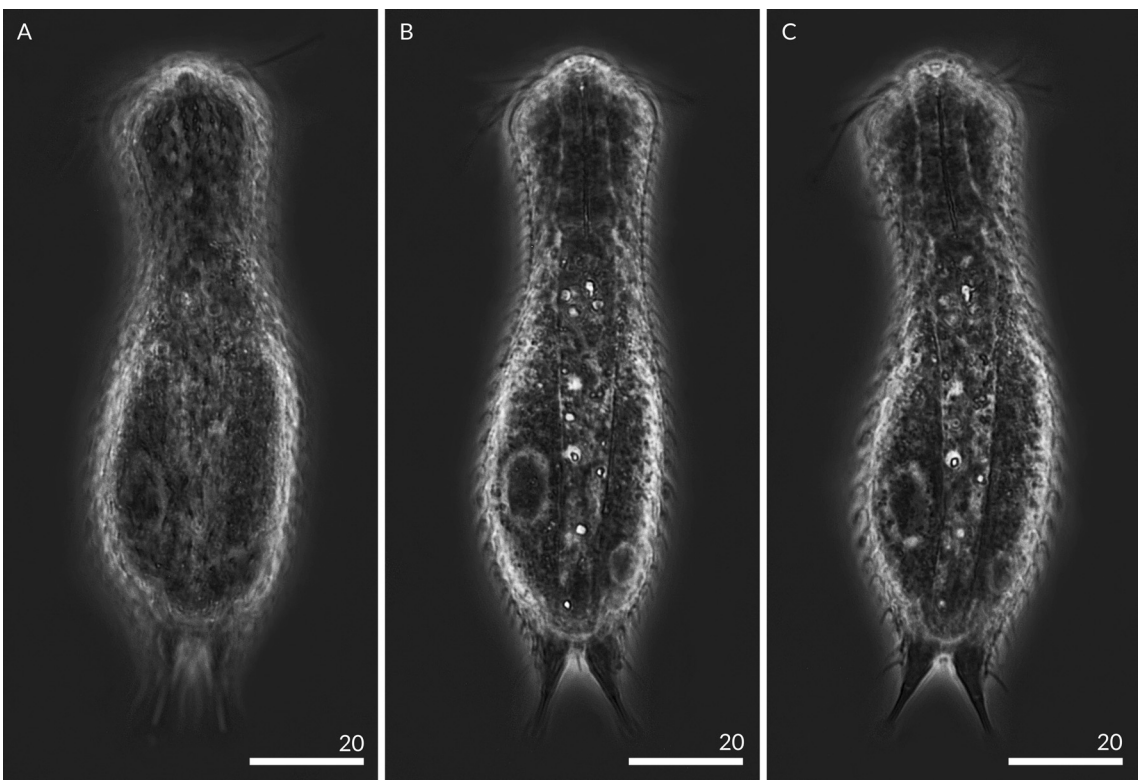


Figure 6. *Chaetonotus* (*Chaetonotus*) *jaceki* sp. nov. Adult specimen. A – Dorsal body view. B – View of internal morphology. C – Ventral body view. (Phase contrast photomicrographs).

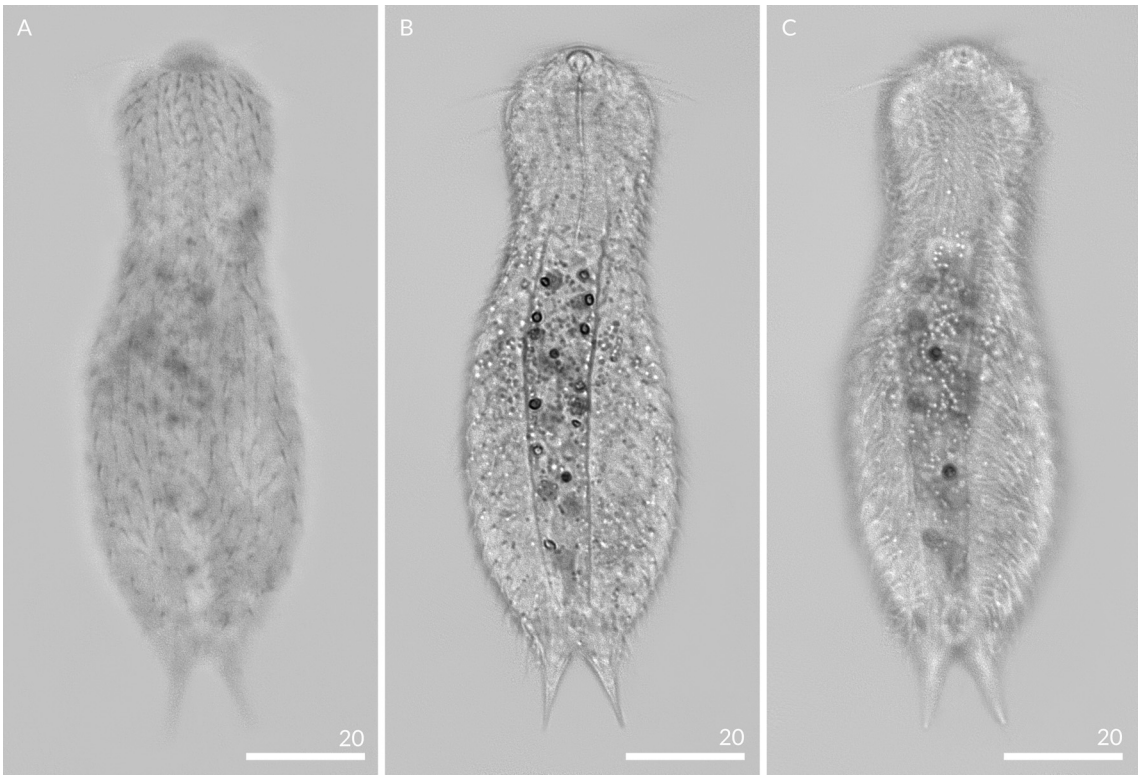


Figure 7. *Chaetonotus* (*Chaetonotus*) *jaceki* sp. nov. Adult specimen. A – Dorsal body view. B – View of internal morphology. C – Ventral body view. (Brightfield photomicrographs).

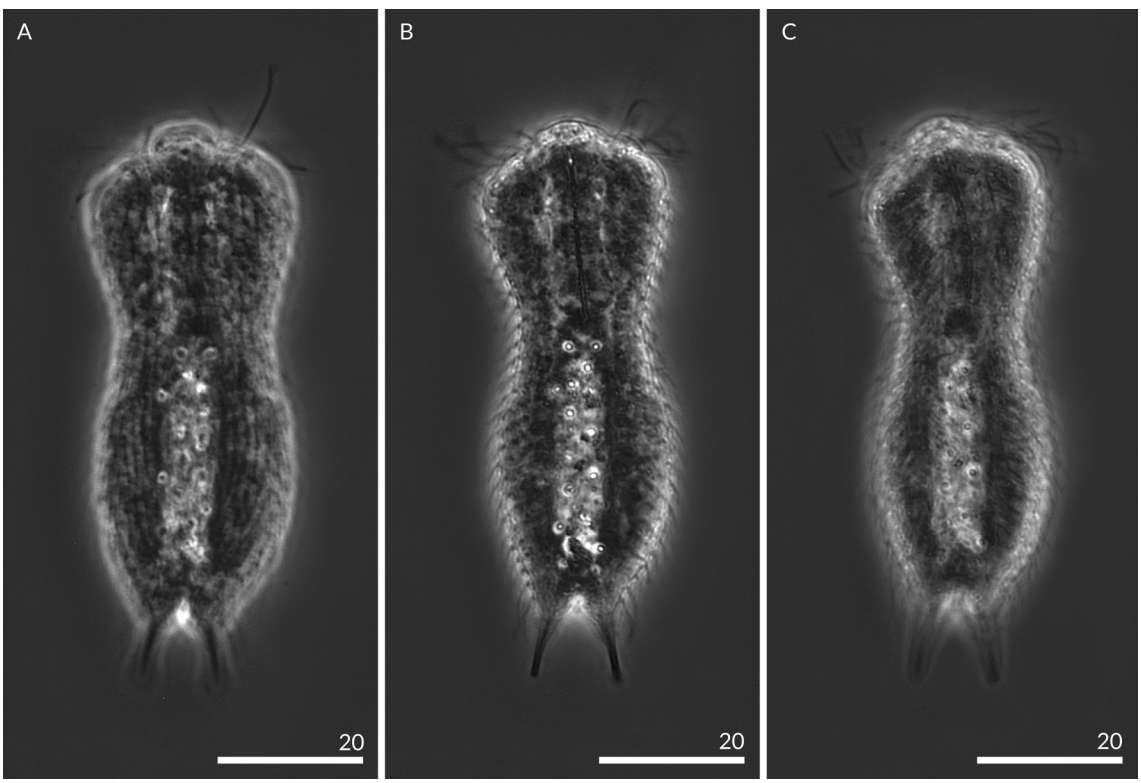


Figure 8. *Chaetonotus* (*Chaetonotus*) *jaceki* sp. nov. Juvenile specimen. A – Dorsal body view. B – View of internal morphology. C – Ventral body view. (Phase contrast photomicrographs).

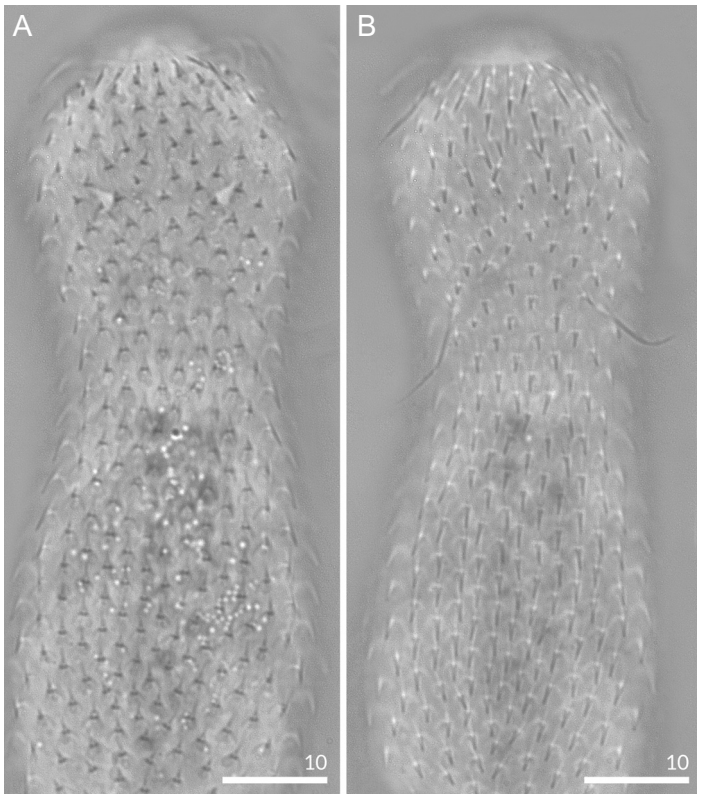


Figure 9. *Chaetonotus* (*Chaetonotus*) *jaceki* sp. nov. Subadult specimen. A – Dorsal view of scales. B – Dorsal view of spines. (Brightfield photomicrographs).

Figure 10. *Chaetonotus* (*Chaetonotus*) *jaceki* sp. nov. Adult specimen. A – Dorsal spines D – Ventral spines. (Brightfield photomicrographs).

beyond the epipleuria (at U4) and emerges from small, spherical papillae. The second pair of sensory bristles is located on the neck (U24) and emerges from small, spherical papillae. The third, posterior pair of sensory bristles emerges from the scales with two keels, located in the posterior part of the trunk (U76–U78). These scales are shaped like wide, deeply notched triangles (Figs 2, 4G, 9B).

On the ventral side, the longitudinal ciliary bands begin on U7 and extend to U82. The bands are much wider on the head than on the other parts of the body (Figs 2C, 6C, 7C). Most of the ventral interciliary field is naked. Small spineless scales with a keel are present only in the posterior part of the trunk (from U71 to U79) (Figs 2, 4C). Their distinction increases towards the posterior part of the body. The first scales are weakly marked and partially submerged into the cuticle. These scales are shaped like narrow triangles with a very shallow notch in the posterior edge. The terminal scales of the ventral interciliary field are located on U79–U83 and shaped like narrow triangles with a very shallow notch in the posterior edge. They have a long keel running along their entire length and no spines (Figs 2, 4F).

The pharynx (from U3 to U27) has anterior and posterior dilatations, with the posterior dilatation being wider than the anterior one. A weak reinforcement composed of a single, thin, strongly curved cuticular rod is present inside the anterior dilatation. The pharynx connects through the pharyngeal intestinal junction (U28) to a straight intestine (running from U28 to U82). The pharyngeal intestinal junction is short and narrow. The intestine has a distinct anterior section that differs in structure from the remaining part of the intestine. Anterior differ section is laterally long and in central part short (Figs 2B, 5) (from U28 to U32). Posteriorly to this section, the intestine is composed of large, polygonal cells clearly visible under a light optical microscope (Figs 2B, 6B, 7B). The X-organ of this species (observed in one specimen) is located at U82–U83 and surrounds the terminal part of the intestine. It is bilobed, built from two extensions enveloped in a thin coat and connected by a thinner band located behind the intestine at the ventral side. The extensions have a granular, grain-like structure. The thin coat and the cellular bridge connecting the extensions have a smooth and homogeneous structure. No sperm packets were observed in any of the specimens.

Remarks. Out of 81 reported adults, 13 individuals had a large developing egg (10 matured eggs had formed a chorion). The maximum number of oocytes

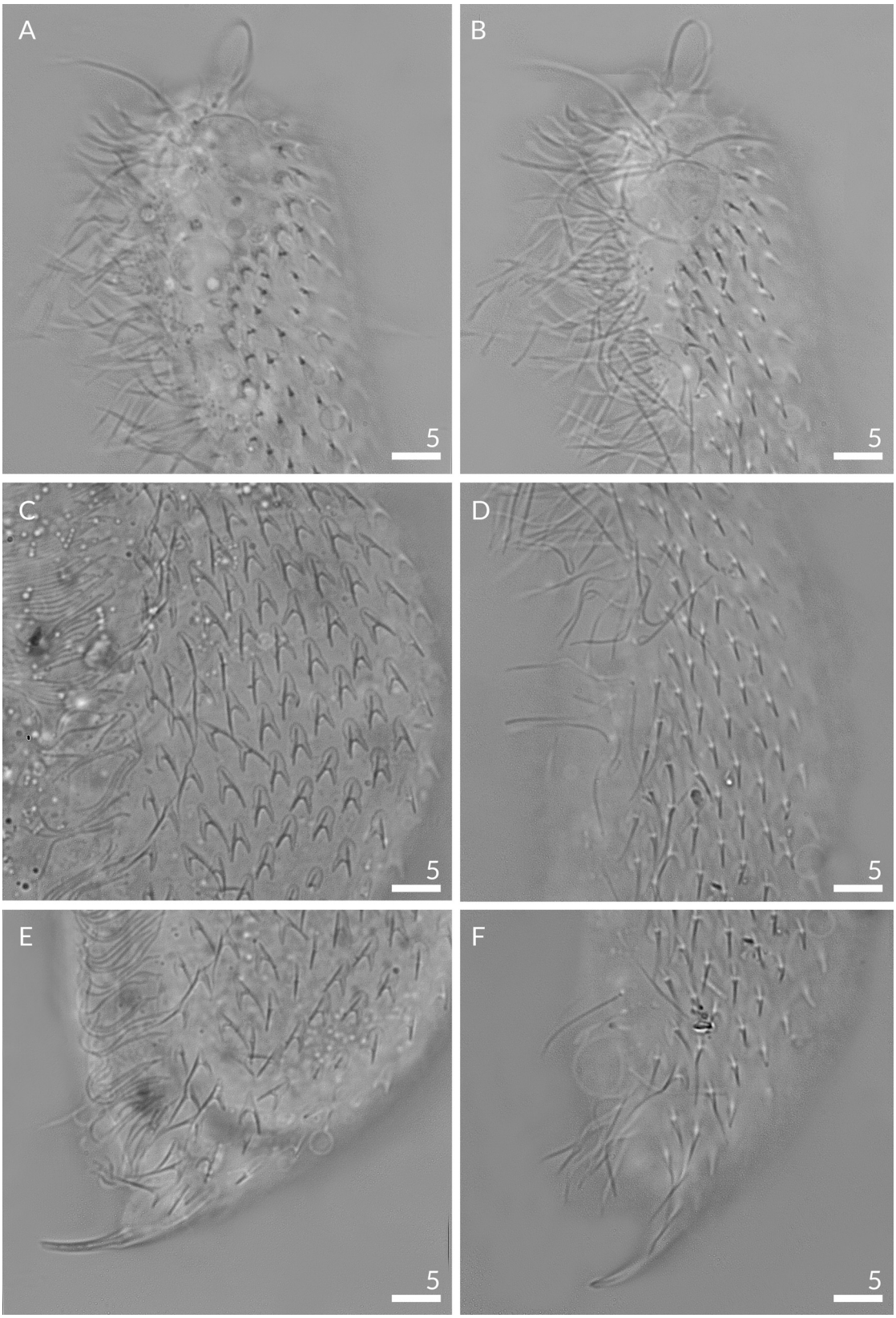


Figure 11. *Chaetonotus* (*Chaetonotus*) *jaceki* sp. nov. Adult specimen. A – Lateral view of scales in head region. B – Lateral view of scales in trunk region. C – Lateral view of scales in posterior trunk region, on furcal base and furcal appendages. D – Lateral spines in head region. E – Lateral spines in trunk region. F – Lateral spines in posterior trunk region, on furcal base and furcal appendages. (Brightfield photomicrographs).

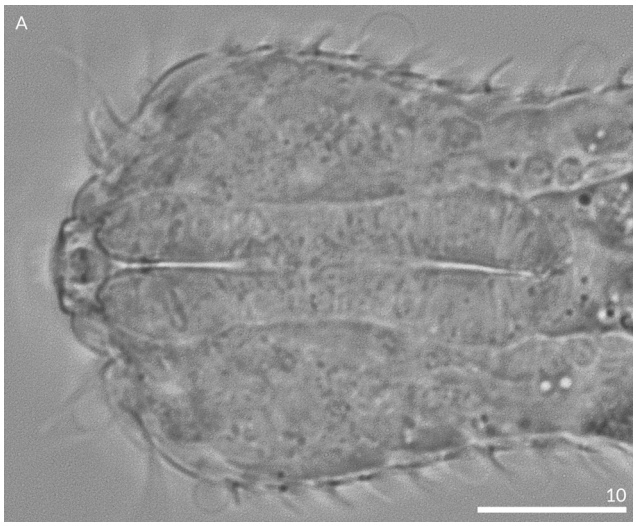


Figure 12. *Chaetonotus* (*Chaetonotus*) *jaceki* sp. nov. Detail of the pharynx. (Brightfield photomicrographs).

present simultaneously is from 1 to 2 per intestine side. Two specimens have two large and similar-sized eggs.

Three pairs of dorsal sensory bristles were noted in *Chaetonotus* (*Chaetonotus*) *jaceki* sp. nov. To date, the presence of dorsal sensory bristles on the head has been observed in 5 species from the genus *Chaetonotus*, i.e. *Chaetonotus* (*Chaetonotus*) *antrumus* Kolicka, 2016, *C.* (*C.*) *brevispinosus* Zelinka, 1889, *C.* (*C.*) *sanctipauli* Kisielewski, 1991, and, as described below, *C.* (*C.*) *gelidus* sp. nov., *C.* (*C.*) *svalbardi* sp. nov., and from another genus in *Bifidochaetus arcticus* Kolicka *et* Kisielewski, 2016. It is also possible that the presence of three pairs of dorsal sensory bristles is a more common occurrence among the Chaetonotidae, but due to the location of the first pair near the cephalic cilia the bristles of this pair may be wrongly interpreted as other cephalic cilia.

Inside the bodies of 46 out of 116 specimens were traces of an infestation, probably by a parasite. The issue of undetermined changes inside the body concerned mainly adult individuals, and the formations were non-aggregated and in diffuse form (see also Kisielewska *et al*. 2015, Kolicka 2016).

Differential diagnosis. *Chaetonotus* Ehrenberg, 1930 is a polyphyletic genus and contains varied species. Of all the currently known 165 nominal freshwater representatives of this genus, *C.* (*C.*) *jaceki* sp. nov. morphologically most closely resembles *C.* (*C.*) *naiadis* Balsamo *et* Todaro, 1995, originally described from Italian mountain pools and *C.* (*C.*) *sphagnophilus* Kisielewski, 1981 which was originally reported from Polish peat bogs. These two species were selected from all the *Chaetonotus* representatives for the comparison of new species due to a similarity in terms of: body shape and length, pharynx length, furca length, adhesive tube length, type of furca indentation and number of terminal scales of ventral interciliary field. Despite the fact that from all of the hitherto known species *C.* (*C.*) *naiadis* and *C.* (*C.*) *sphagnophilus* have the highest number of common features with the *C.* (*C.*) *jaceki* sp. nov. they are significantly different from new species – most strikingly by scale type and shape as well as by spines type and length. All the differences between the new species and the most morphologically similar taxa have been summarised in Table 3.

Chaetonotus* (*Chaetonotus*) *gelidus sp. nov.
(Figs 13–24; Tables 4–5 and A2)

Location. Spitsbergen, Svalbard Archipelago (Norway), Site 2

Material. One sample, 140 specimens (115 adults, 9 subadults, 16 juveniles), all photographed. The holotype was established and their detail photomicrographs documentation are deposited in the Natural History Collections at Adam Mickiewicz University in Poznań under access number NHC–GCCG–8–1–20/h (The International Commission on Zoological Nomenclature 1999)). The photomicrographs documentation for paratypes are also available in the Natural History Collections at Adam Mickiewicz University in Poznań under access number NHC–GCCG–8–21–150/p and in the author's collection.

Etymology. From Latin "*gelidus*" – cold and freezing, referring to the climate of the Arctic where it was found.

Diagnosis. Body length from 75.2 to 115.2 μm. Slender body. Head five-lobed, cephalion and pleuria weakly marked in the head outline. Hypostomium small, narrow, rhomboidal. No ocellar granules. Scales semi-three-lobed and one-lobed with a strong keel and spine. Scales of the head, neck and trunk differ morphologically. Scales located in 31–37 total longitudinal rows (9–13D+6DL+6L+6-8LV+4V) of 23–28 scales in central row. Spines thick, curved and tapering towards their ends, without lateral denticle. Spines of the head longer and more curved than those of the neck, neck spines gradually turn into shorter spines of the trunk. Spines of the ventral longitudinal row of scales located closest to the ciliary band thin and hair-like. Pharyngeal region of the ventral interciliary field naked. Intestinal region covered with one-lobed, narrow scales with a keel. A pair of terminal scales of the ventral interciliary field narrow, long, strongly rounded triangle in shape, with a long and without spines. Pharynx with anterior and posterior dilatations with a single, narrow, curved cuticular rod weak inside the anterior dilatation. Straight intestine with a distinct marked anterior section.

Description. *Chaetonotus* (*Chaetonotus*) *gelidus* sp. nov. has a slender body. Its head is wider than the neck and separated from the trunk with a clear neck narrowing, the body then gradually widens up to about a half-way region along the trunk length (U59), after which the trunk gradually narrows up to the furcal base on U85. The furca branches are narrowly spaced. The furcal indentation is helmet like shaped and the ends of the adhesive tubes point outwards. The adhesive tubes are straight and short in relation to body length (Table 4). They are thin, equally thick along their entire length and have blunt edges (Fig. 13).

The head is round and five-lobed. All lobes are distinct but weakly marked in the head outline. The cephalion (U1–U6) adheres along its entire length to the head, is narrow and widens at the posterior edge (Fig. 13A). The epipleuria (U4–U8) and hypopleuria (U8–U14) are slightly convex. The hypopleuria are larger than the epipleuria by about one-third (Figs 13, 16). The epipleuria are located dorsolaterally, whereas the hypopleuria are located more ventrolaterally, with only a small part of them visible on the dorsal side. The hypostomium (U6–U8) is small and rhomboidal in shape with a strongly reinforced anterior edge (Figs 13, 16C, 17C, 18C, 19C; see Table A2). Two pairs of cephalic ciliary tufts are present. Each anterior tuft has 7–8 cilia and emerges at the lateral edges of the cephalion. The point from where the tufts emerge is clearly visible on the dorsolateral (5 cilia) and ventrolateral sides (2–3 cilia). The anteriormost cilium in both anterior tufts is very long, the longest in the tuft. The next two or three cilia are short and have a similar length. The subsequent cilium in each tuft is longer than the preceding ones but shorter than the first cilium. Furthermore, it is much thicker than the other cilia in the tuft. The next two cilia have a similar length and are shorter than the preceding thick cilium. The posteriormost cilium is slightly shorter than the cilia directly before it. Each posterior tuft has 7–8 cilia and emerges from between the epipleuria and hypopleuria. The tufts are located on the ventral side, beyond the posterior edges of the epipleuria. The length of the cephalic cilia in the posterior tufts increases from the anteriormost cilium to the posteriormost one (Table A2). In addition, the fourth cilium in both posterior tufts is much thicker than the other cilia in the tuft (Fig. 13). Ocellar granules are not present. The mouth ring is small, located subterminally on U2–U5 and has reinforcements typical for the genus *Chaetonotus*. Short cuticular inner hairs are present inside the mouth ring, and suboral bristles are present around it. The entire body is covered with semithree-lobed scales and two pairs of one-lobed scales adhering to the cuticle (Figs 13, 14, 20). The scales of the dorsal, dorsolateral, lateral, ventrolateral and ventral sides are shaped like triangles with a deeply notched posterior edge. Each of these scales has a strong, long keel running along its entire length. The scales form 31–37 total longitudinal alternating rows (9–13D+6DL+6L+6-8LV+4V) of 23–28 scales in central row. The scales on the head slightly converge towards the central longitudinal row. The longitudinal rows on the neck and the anterior and central region of the trunk run parallel to one another, and the scales on the posterior part of the trunk and on the furcal base also slightly and gradually converge towards the central longitudinal row. The scales are located close to one another but do not adhere, and their edges do not overlap. The longitudinal

Table 4. Main morphometric of *Chaetonotus* (*Chaetonotus*) *gelidus* sp. nov; N – number of specimens or structures analyzed, Range – the smallest and the largest structure found among all specimens measured. All measurements are given in micrometers (μm).

Characters	N	Holotype	Range of adult paratypes
Body length	112	106.91	80.29–115.16
Furca length	21	18.80	16.82–19.05
Adhesive tube length	21	7.58	6.91–8.13
Number of scales in central longitudinal row	115	26	23–28
Total number of longitudinal alternating rows of scales	115	35	31–37
Head scales length x width	21	1.10–3.53 x 1.39–3.09	0.88–4.26 x 1.11–3.76
Neck scales length x width	21	1.33–4.31 x 1.06–2.75	1.10–4.96 x 0.86–3.08
Trunk scales length x width	21	1.48–5.51 x 1.17–3.22	1.28–6.22 x 1.05–3.86
Head spines length	21	2.40–5.28	1.69–6.37
Neck spines length	21	2.13–5.14	1.22–6.23
Trunk spines length	21	1.64–5.10	1.09–6.12
Mouth ring diameter	21	4.37	4.16–5.03
Pharynx length	92	26.98	21.65–32.99

Table 5. Condensed comparison of the most important differential characters between the most similar species to *Chaetonotus* (*Chaetonotus*) *gelidus* sp. nov. Measurements are given in micrometres (μm). NA – data not available.

Character	*C.* (*C.*) *gelidus* sp. nov.	*C.* (*C.*) *naiadis*	*C.* (*C.*) *oculifer*	*C.* (*C.*) *jaceki* sp. nov.
Body length	80.3–115.2	89–140	97–137	110.0–131.1
Cephalic pleuria	Cephalion narrow, rapidly widening at the dorsal edge; epipleuria and hypopleuria large; hypopleuria larger than epipleuri; epipleuria located dorsolaterally; hypopleuria located more ventrolaterally. Hypostomium small, rhomboidal	Cephalion wide; epipleuria large; hypopleuria small; clearly visible from the dorsal body side. Hypostomium small, subrectangular	Cephalion narrow, epipleuria hypopleuria similar in size. clearly visible from the dorsal body side. Hypostomium present	Cephalion narrow, rapidly widens at the dorsal edge; epipleuria small located dorsolaterally; hypopleuria large, located more ventrolaterally. Hypostomium short, rhomboidal
Place of emerging cephalic cilia	Anterior tufts: at the lateral edges of the cephalion; posterior tufts: on the ventral side, beyond the posterior edges of the epipleuria	Anterior and posterior tufts: laterally	Anterior and posterior tufts: laterally	Anterior tufts: in lines around lateral cephalion edges; posterior tufts: ventrolllateraly, beyond the posterior edges of epipleuria
Presence of ocellar granules	Absent	Absent	Present	Absent
Pharynx length	21.7–33.0	26–33	27–38	26.5–35.1
Pharynx shape	Pharynx with anterior and wider posterior dilatation	Pharynx widens towards its end, without clear dilatation	Pharynx with anterior and wider posterior dilatation	Pharynx with anterior and wider posterior dilatation
Presence of cuticular reinforcements in the pharynx	Present (single, narrow, curved cuticular rod)	Absent	Absent	Present (single, narrow, curved cuticular rod)
Anterior intestine section differ in form of morphology	Present	Absent	Absent	Present
Furca length	16.8–19.1	12–17	12–18	13.4–19.7
Adhesive tube length	6.9–8.1	9	6.5–9.0	8.2–9.7
Type of furca indentation	Helmet like shaped	V-shaped	V-shaped	V-shaped
Number of scales in single longitudinal row	23–28	20	20–24	26–44
Total number of longitudinal alternating rows of scales	31–37	23–31	17–26	33–47
Type of scales	Scales small; semithree-lobed and two pairs one-lobed scales with strong keels and spines; shape differentiated within different body regions	Scales small; one-lobed with strong keels and straight spines; shape undifferentiated within body regions	Scales small, three-lobed with bad-pronounced lobes, keeled and spined; shape weak differentiated within body regions	Scales small; almost all three-lobed with strong keels and straight, thick spines; shape strongly differentiated within different body regions

Table 5. Condensed.

Character	*C.* (*C.*) *gelidus* sp. nov.	*C.* (*C.*) *naiadis*	*C.* (*C.*) *oculifer*	*C.* (*C.*) *jaceki* sp. nov.
Scales size variation	Size and length of the scales relative to their width gradually increase from the posterior part of the head to the widest point of the trunk; scales in three longitudinal rows located at ventral body surface gradually decrease in size towards the ciliary band	Similar size all over the body	Similar size all over the body	Scales size decrease on the neck area and then increase towards to the widest trunk region; the size of the ventrolateral and ventral scales gradually decrease towards the ciliary bands
Scales size	Head: 0.88–4.26 x 1.11–3.76 Neck: 1.10–4.96 x 0.86–3.08 Trunk: 1.28–6.22 x 1.05–3.86	Head: 2.5–2.8 x 2.3–2.5 Neck: NA Trunk: 3.1–4.2 x 1.5–2.3	Head: NA Neck: 2.5–3.5 x NA Trunk: 2.5–5.5 x NA	Head: 0.62–4.21 x 0.49–3.84 Neck: 1.10–5.07 x 0.87–4.01 Trunk: 1.74–5.91 x 1.39–5.05
Alignment of scales	Edges juxtaposed but do not overlap	Edges juxtaposed but do not overlap	Edges juxtaposed but do not overlap	Edges juxtaposed but do not overlap
Presence of a different type scales on the dorsal and dorso-lateral side of the furcal base	Present	Present	Present	Present
Presence of scales with two keels on the furcal appendages	Present	Present	Absent	Absent
Scales coverage of the ventral interciliary field	Covered from the pharyngeal region from the posterior dilatation of the pharynx to the furcal base	Only the posterior end of the trunk covered	Covered from the pharyngeal region from the posterior dilatation of the pharynx to the furcal base	Only the posterior part of the trunk covered
Type of scales of the ventral interciliary field	One-lobed scales shaped like rectangles with rounded edges without a posterior notches, with long keel and vestigial spines	Oval scales with keels and spineless	Keels/ weakly marked scales	Narrow triangle with shallow posterior notches scales with keels and spineless
Number of terminal scales of the ventral interciliary field	1 pair	1 pair	1 pair	1 pair

Table 5. Condensed.

Character	*C.* (*C.*) *gelidus* sp. nov.	*C.* (*C.*) *naiadis*	*C.* (*C.*) *oculifer*	*C.* (*C.*) *jaceki* sp. nov.
Type of terminal scales of the ventral interciliary field	Narrow triangles without posterior notches, keeled with vestigial spines	Oval without posterior notches, keeled, spined	Kelled, spineless	Narrow triangles with shallow posterior notches, keeled, spineless
Spines length variation	Spines of the head longer and more curved than those of theneck. Spines of the neck gradually turn into shorter spines of the trunk; length of the spines slightly and gradually increases from the dorsal sideto the ventral side, towards theciliary bands; ventral spines long	Similar length of spines on the dorsal, dorsolateral, lateral and ventrolateral sides of the head, neck and trunk; longer spines on the ventral body side	Spines lengthen from the head to the posterior trunk region; similar length of dorsal, dorsolateral, lateral, ventrolateral and ventral spines	Spines of head longer than those of neck; spines of neck getting progressively longer towards the beginning of the trunk region; spines of the trunk getting progressively longer towards the widest body region, after which they slightly and gradually shorten towards the furcal base; length of the spines slightly and gradually increases from the dorsal side to the ventral side, towards the ciliary bands; ventral spines long
Spines length	Head: 1.69–6.37 Neck: 1.22–6.23 Trunk: 1.09–6.12	1–2	Head: NA Neck: 2–6 Trunk: 4–8	Head: 1.26–9.95 Neck: 1.40–8.89 Trunk: 1.52–13.10
Presence of hair-like spines on the ventral body side	Present	Absent	Absent	Present
Type of posteriormost pair of the trunk lateral spines	Slightly longer than the other lateral trunk spines, not thicker or more curved	The same as other spines Slightly shorter than on mid-trunk spines		Longer and slightly thicker than other trunk spines
Type of last pair of the furcal appendages lateral spine	Slightly longer than the trunk lateral spines, not thicker or more curved	The same as other spines	NA	Longer, thicker and stronger than other spines
Ventral locomotor cilia	Two longitudinal bands wider on the head and neck region	Two longitudinal bands wider on the head region	NA	Two longitudinal bands wider on the head region
Number of dorsal sensory bristles	3 pairs	2 pairs	0–2 pairs	3 pairs

rows of scales begin on the head directly posteriorly to cephalion, epipleuria and hypopleuria. The dorsal scales of the head located directly posteriorly to the pleuria are clearly smaller than the other scales (Figs 13, 20A; Table A2). The edges of the scales of the head and neck are more rounded than those of the scales of the trunk (Figs 13, 14). The size and length of the scales relative to their width gradually increase from the posterior part of the head to the widest point of the trunk (*ca*. U59) (Figs 13, 14, 15–20; Table 5). The scales arranged in three longitudinal rows located at ventral body surface gradually decrease in size towards the ciliary band (Figs 13C, 15C, 16C, 17C, 19C, 20C, D; Tables 4 and A2). The anterior edges of the scales in the ventral longitudinal row located closest to the ciliary band are orientated towards the band at an angle of *ca*. 10° (Figs 13C; 20C, D). One-lobed scales that are smaller and have more rounded edges than the surrounding scales are located posteriorly to the double-keeled scales with a sensory bristle, inside their posterior notch (U81) (Figs 13A, 14E, 20A). Three pairs of scales slightly narrower than the other scales are present on the dorsal side of the furcal base (U82–U85) (Figs 13A, 14K, 20A). The trunk lateral posteriormost pair of scales is located on the furcal base at U84. These scales are of the same type as the other trunk scales. A pair of large,one-lobed, rectangular scales with slightly rounded edges is located on the dorsolateral side of the furcal appendages (U85–U88) and have two parallel keels running along their entire length (Figs 13A, 14D, 20A). Three pairs of scales of the same type as the trunk scales are aligned on the lateral side of the furcal appendages (U85–U89) (Figs 13, 20; Table A2).

Spines emerge from the posterior part of the scales, are thick, without lateral denticles, curved and taper towards the ends. The spines of the head are longer and more curved than those of the neck (Figs 13, 21; Tables 4 and A2). The spines of the neck gradually taper and gradually become shorter on the trunk (Figs 13, 21; Tables 4 and A2). The dorsolateral, lateral, ventrolateral and ventral spines are slightly longer than the dorsal spines, and their length gradually increases towards the ciliary bands (Figs 13, 15–19, 21; Tables 4 and A2). The spines emerging from the scales arranged in a ventral longitudinal row located closest to the ciliary band are thin, long and hair-like (Figs 13C, 21C, D). The posteriormost three pairs of lateral spines are slightly longer than the other lateral trunk spines but

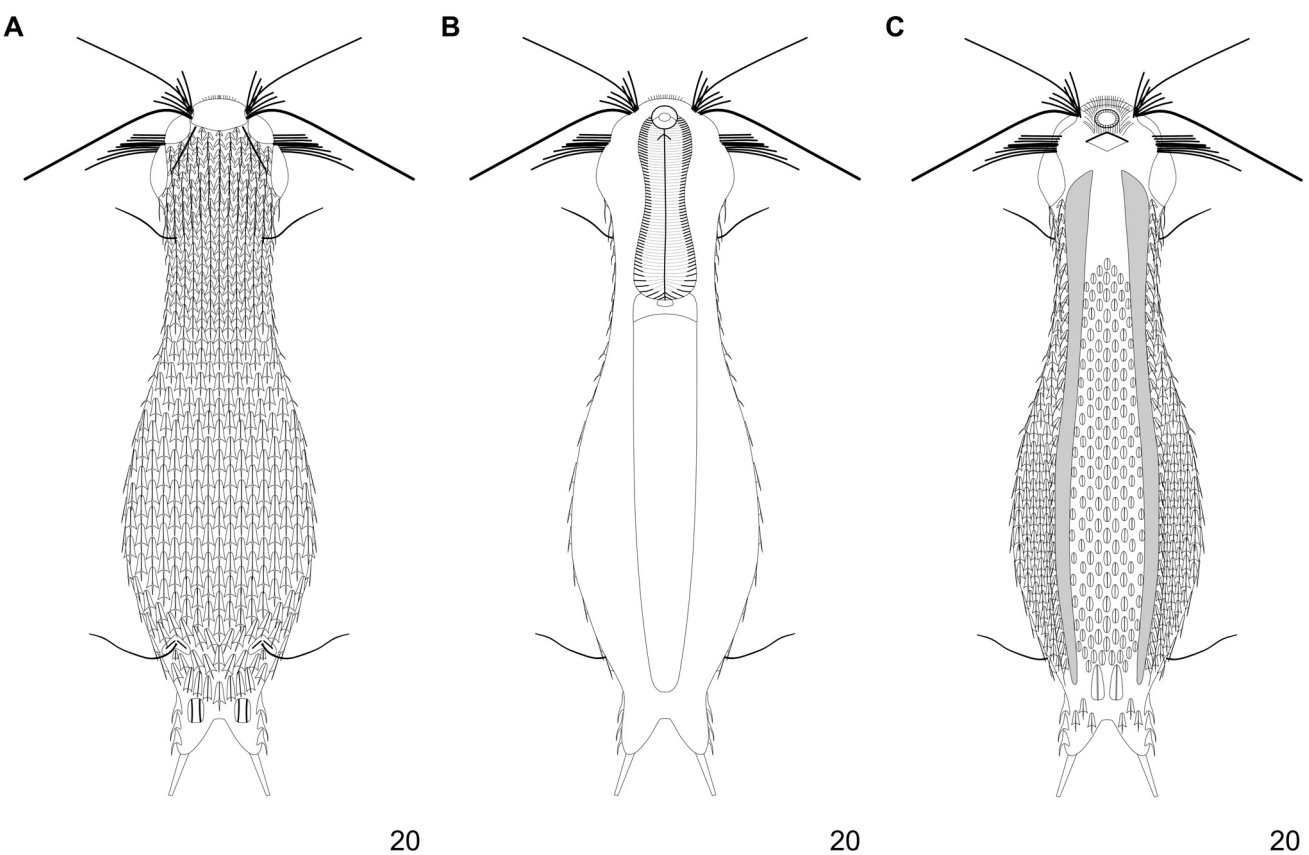


Figure 13. *Chaetonotus* (*Chaetonotus*) *gelidus* sp. nov. Schematic drawings. A – Dorsal body view. B – View of internal morphology. C – Ventral body view. Light grey areas indicate the ciliary bands.

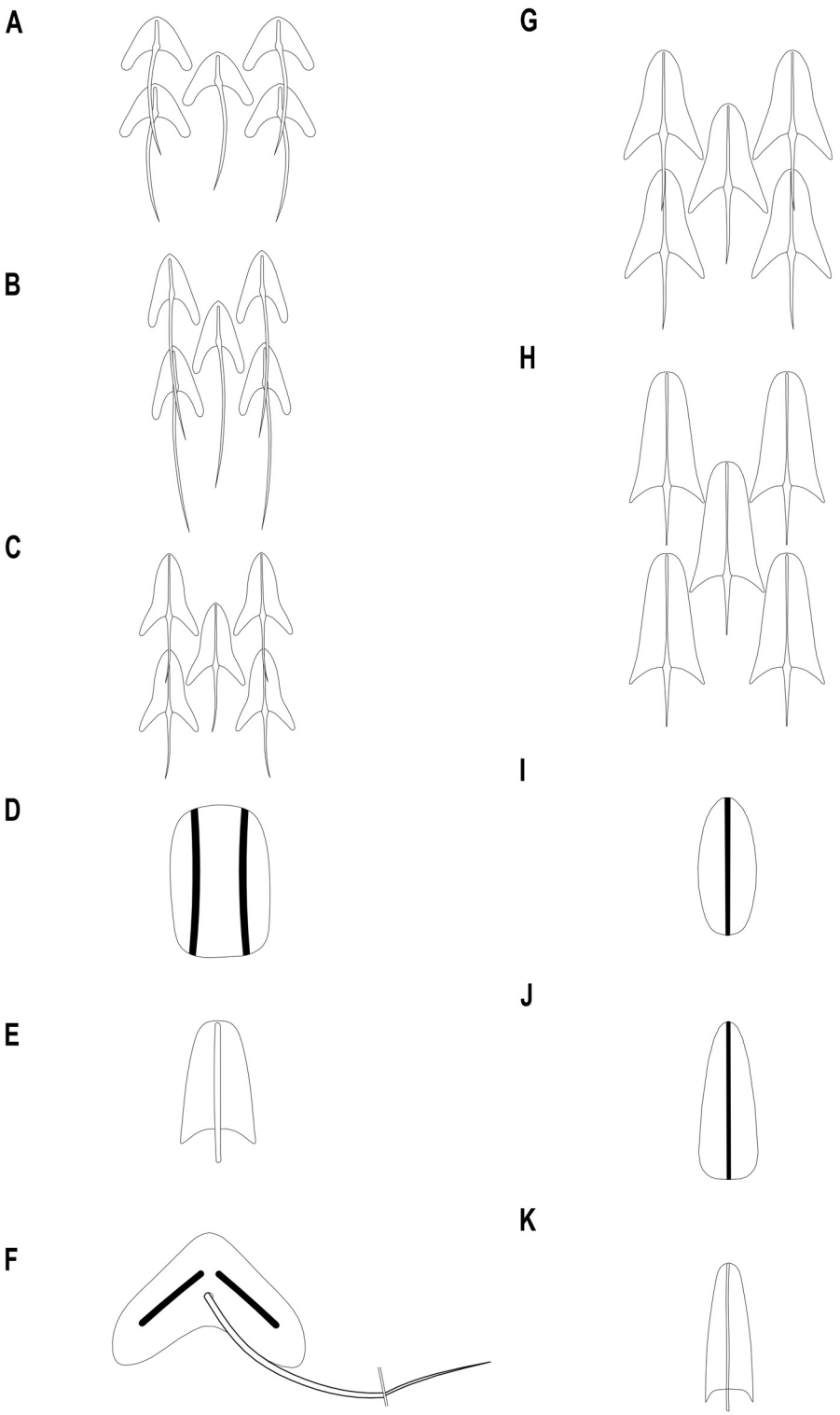


Figure 14. *Chaetonotus* (*Chaetonotus*) *gelidus* sp. nov. Schematic scales drawings. A – Head anterior region scale. B – Head posterior region scale. C – Neck scale. D – Furcal appendages dorsal double-keeled scale. E – Small one-lobed dorsal scale. F – Double-keeled scale with dorsal sensory bristle. G – Trunk anterior region scale. H – Trunk posterior region scale. I – Ventral interciliary field scale. J – Ventral interciliary field terminal scale. K – Furcal base dorsal scale.

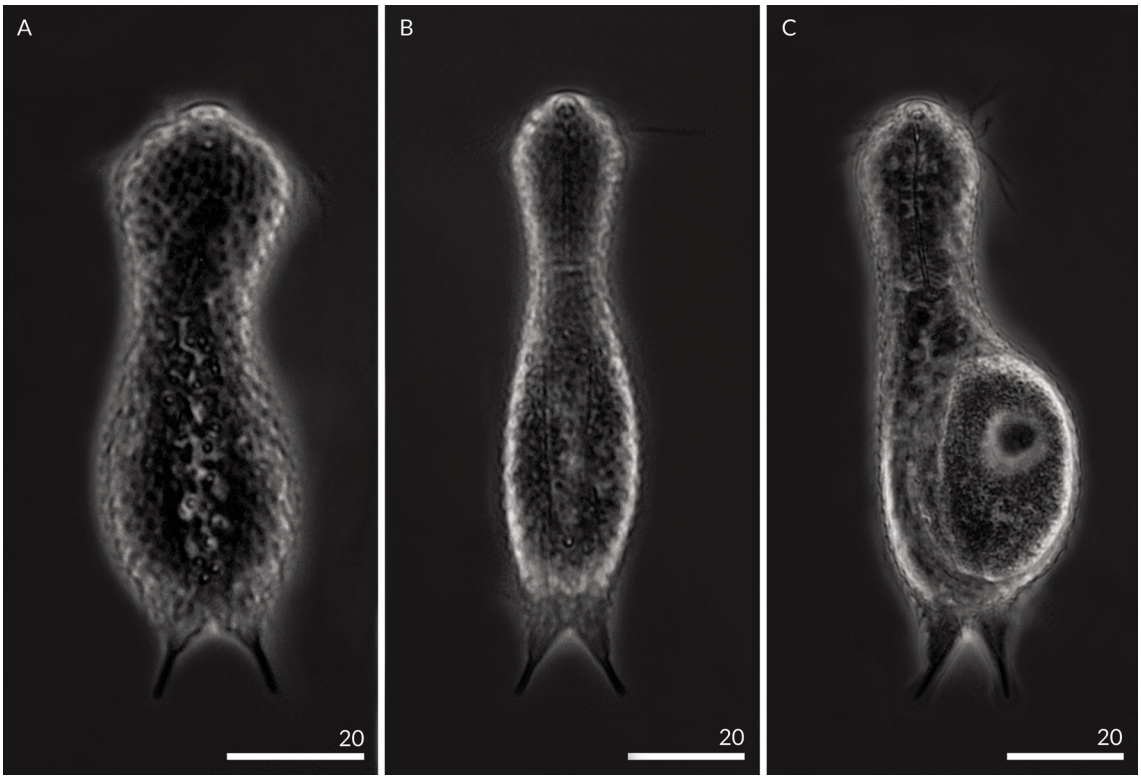


Figure 15. *Chaetonotus* (*Chaetonotus*) *gelidus* sp. nov. A – Juvenile specimen. Habitus. B – Adult specimen. Habitus. C – Specimen with mature egg. Habitus. (Phase contrast photomicrographs).

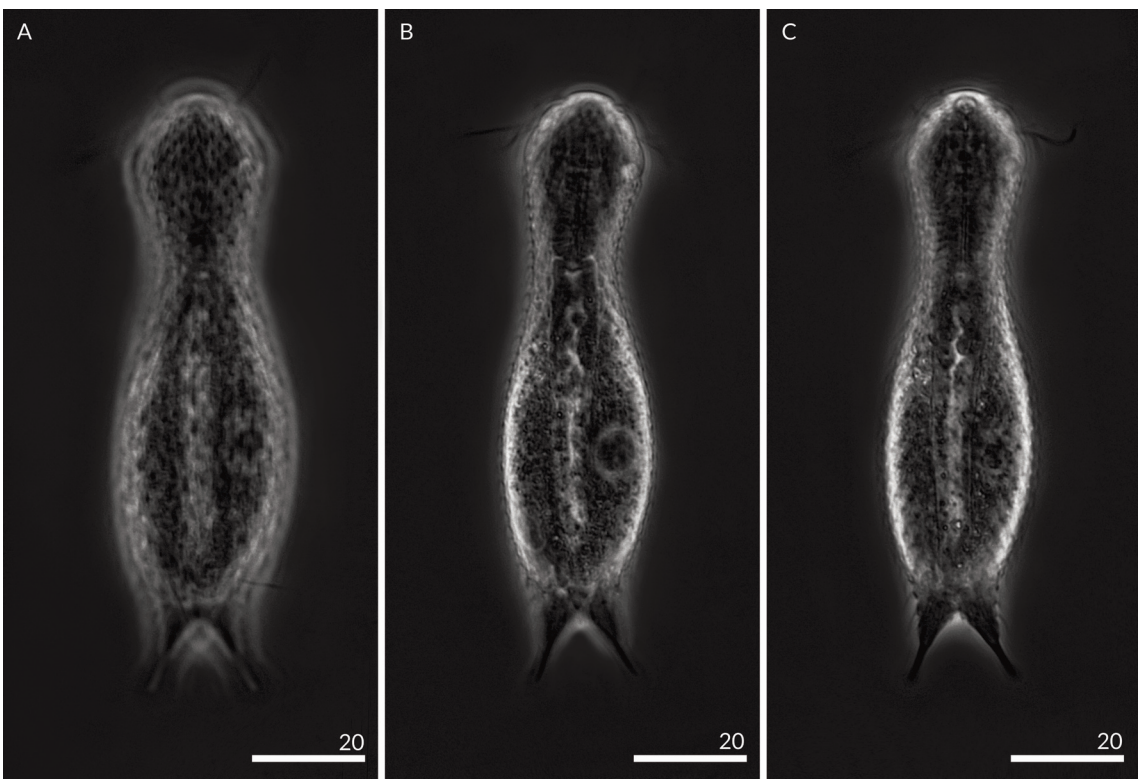


Figure 16. *Chaetonotus* (*Chaetonotus*) *gelidus* sp. nov. Adult specimen. A – Dorsal body view. B – View of internal morphology. C – Ventral body view. (Phase contrast photomicrographs).

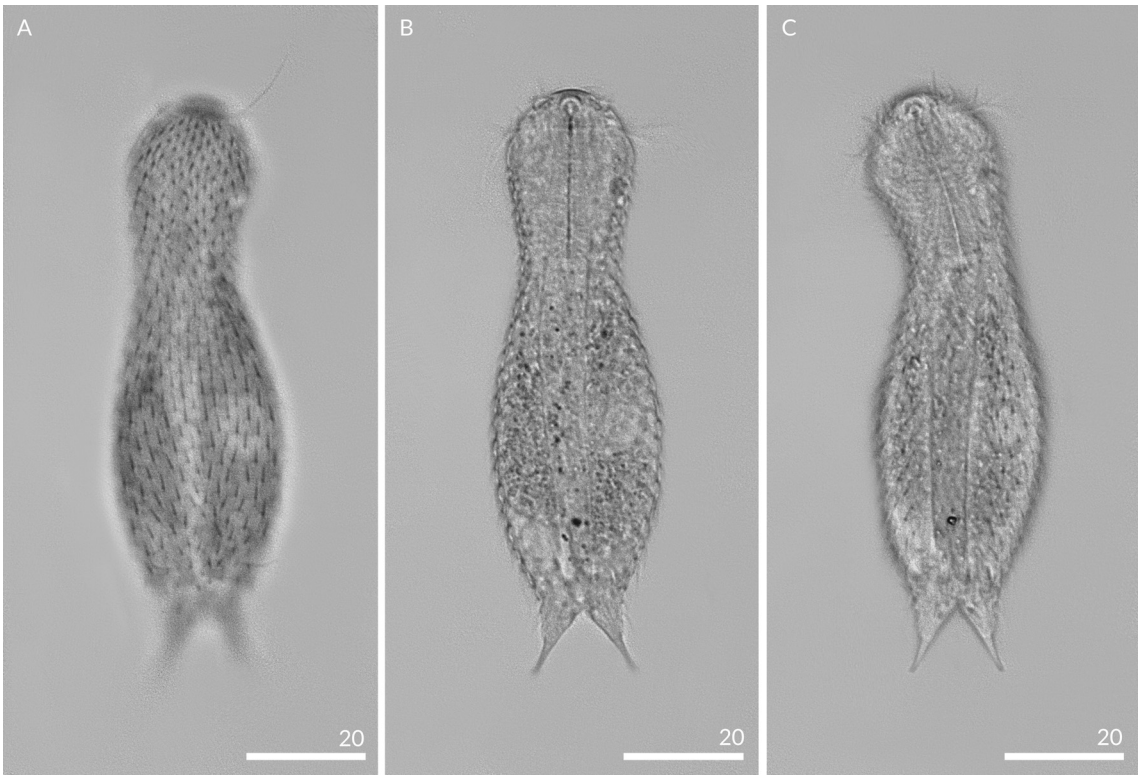


Figure 17. *Chaetonotus* (*Chaetonotus*) *gelidus* sp. nov. Adult specimen. A – Dorsal body view. B – View of internal morphology. C – Ventral body view. (Brightfield photomicrographs).

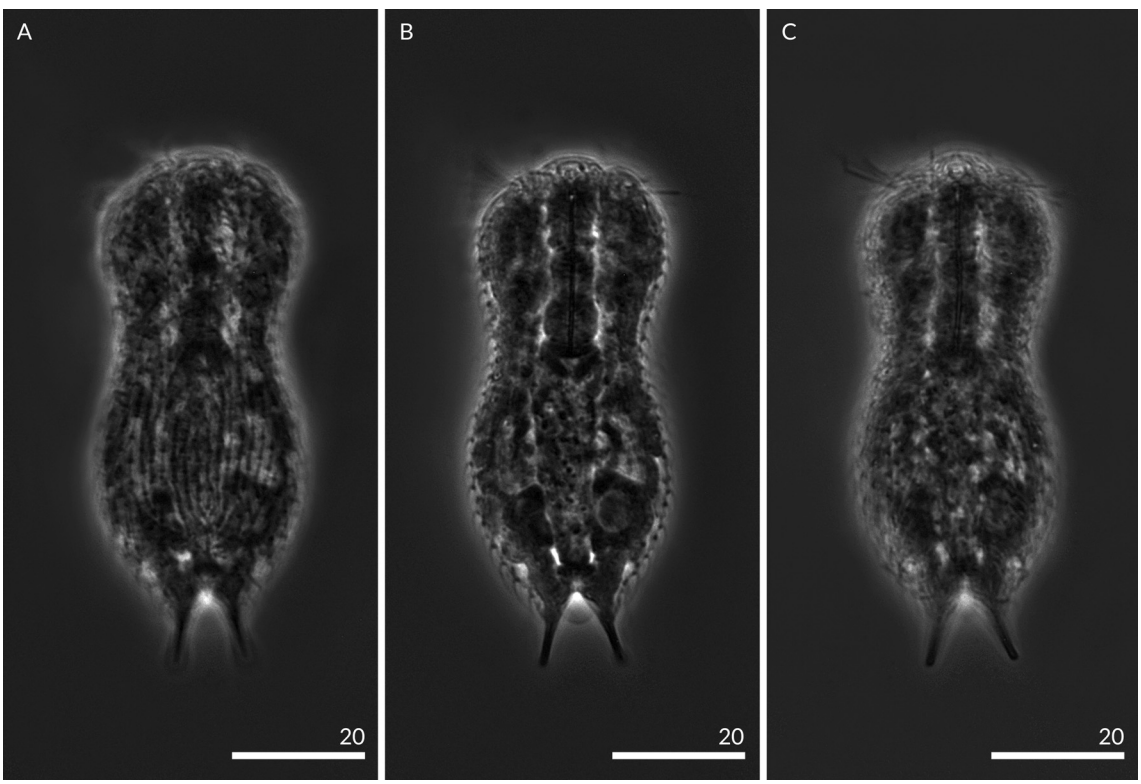


Figure 18. *Chaetonotus* (*Chaetonotus*) *gelidus* sp. nov. Juvenile specimen. A – Dorsal body view. B – View of internal morphology. C – Ventral body view. (Phase contrast photomicrographs).

Figure 19. *Chaetonotus* (*Chaetonotus*) *gelidus* sp. nov. Adult with matured egg. A – Dorsal body view. B – View of internal morphology. C – Ventral body view. (Phase contrast photomicrographs).

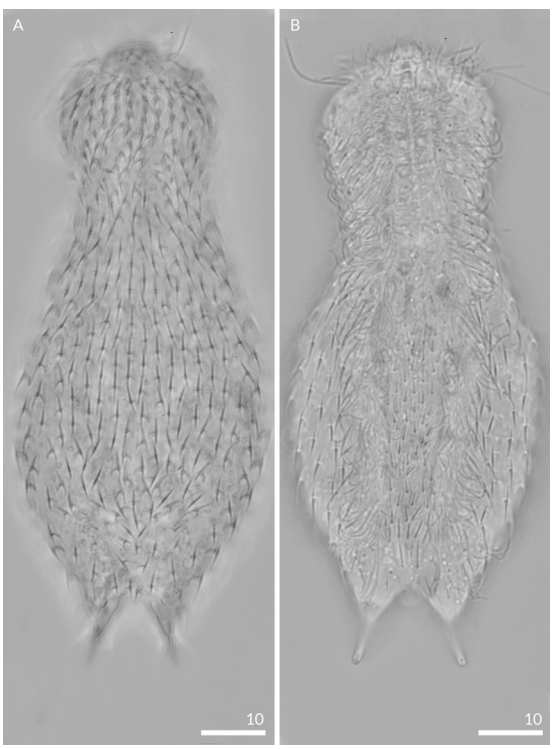


Figure 20. *Chaetonotus* (*Chaetonotus*) *gelidus* sp. nov. Adult specimen. A – Dorsal view of scales. B – Ventral view of scales. (Brightfield photomicrographs).

Figure 21. *Chaetonotus* (*Chaetonotus*) *gelidus* sp. nov. Adult specimen. A, B – Dorsal spines. C, D – Ventral spines. (Brightfield photomicrographs).

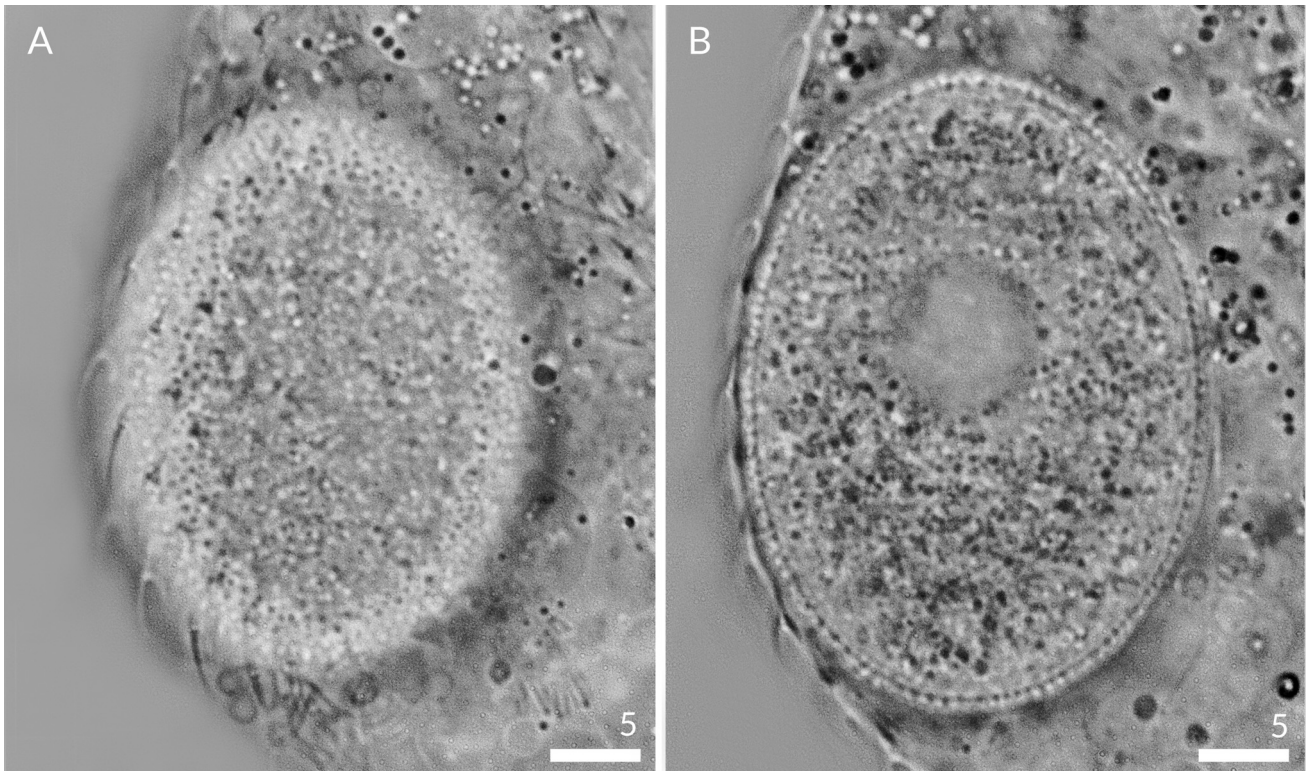


Figure 22. *Chaetonotus* (*Chaetonotus*) *gelidus* sp. nov. A, B – Matured egg with a chorion. (Brightfield photomicrographs).

similar in thickness and curvature (Fig. 13; Table 2A). Parafurcal scales have spines that are slightly longer than the trunk lateral spines. The furcal appendages posteriormost pair of spines is neither longer nor stronger than the anterior pairs (Fig. 13; Table 2A).

The species has three pairs of dorsal sensory bristles. The first pair is located on the head, directly posterior to the cephalion, at the lateral edges of the epipleuria (U6) and emerges from small, spherical papillae. The second pair of dorsal sensory bristles emerges from small, spherical papillae and is located in the posterior part of the neck (U19). The posterior pair of dorsal sensory bristles emerges from large scales with two long keels located in the posterior part of the trunk (U78–U80). These scales are shaped like wide triangles with rounded edges and a deep posterior notch (Figs 13, 14F, 20A).

On the ventral body side, the longitudinal ciliary bands begin at U11 and extend to U84. The ciliary bands on the head and neck area are wider than in the trunk area. The pharyngeal area of the ventral interciliary field up to the posterior dilatation of the pharynx is naked. One-lobed scales shaped like rectangles with rounded edges and without a posterior notch that have a strong, long keel extending into vestigial spines are present from the posterior pharyngeal region (from *ca*. U21) to the posterior end of the ventral interciliary field (Figs 13C, 14I, 16C, 17C, 18C, 20C, D). These scales form 5–9 transverse rows and are located far from one another. Their length gradually increases posteriorly (Table 2A). The most lateral longitudinal rows of the scales of the ventral interciliary field have scales that

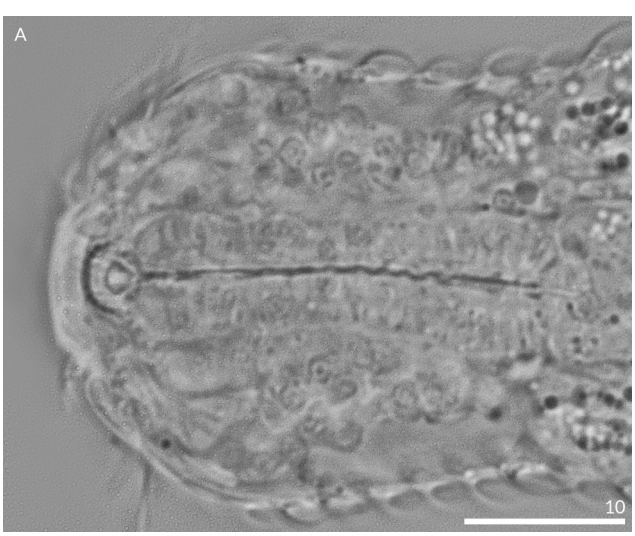


Figure 23. *Chaetonotus* (*Chaetonotus*) *gelidus* sp. nov. Detail of the pharynx. (Brightfield photomicrographs).

are smaller than those central rows (Figs 13C, 16C, 17C, 18C, 19C 20C, D). A pair of terminal scales of the ventral interciliary field is located at U83–U86. They are long, shaped like triangles with rounded edges and their posterior edge has no notch. They have a keel that runs along their entire length and extends into a vestigial spine (Figs 13C, 14I, 16C, 17C, 18C, 19C, 20C, D).

The pharynx (from U4 to U29) has one anterior and one posterior dilatations, with the posterior dilatation wider than the anterior one. A weak reinforcement composed of a single, thin, strongly curved cuticular rod is present inside the anterior dilatation. The pharynx connects through the pharyngeal intestinal junction (U30) to a straight intestine (running from U28 to U86). The pharyngeal intestinal junction is short and narrow. The intestine has a distinct anterior section differ morphologically from remaining intestine part marked as a bend (from U28 to U32) (Figs 13, 15B, 16B, 17B). The X-organ of this species (observed in two specimens) is located at U84–U85, near the end of the intestine. It is bilobed, built from two extensions enveloped in a thin coat and connected by a thinner band located behind the intestine at the ventral side. The extensions have a granular, grain-like structure. The thin coat and the cellular bridge connecting the extensions have a smooth and homogeneous structure. No sperm packets were observed in any of the specimens.

Remarks. Out of 115 reported adults, 20 individuals had a large developing egg (6 matured eggs had formed a chorion) (Fig. 22). The maximum number of oocytes simultaneously present per intestine side is from 1 to 3, but most often 2 per side.

Three pairs of dorsal sensory bristles were noted in *Chaetonotus* (*Chaetonotus*) *gelidus* sp. nov. The presence of dorsal sensory bristles on the head has been observed in five species from the genus *Chaetonotus*. See the 'Remarks' section for the species *C.* (*C.*) *jaceki* sp. nov.

Inside the bodies of 31 out of 140 specimens were traces of an infestation, probably by a parasite. The issue of undetermined changes inside the body concerned mainly adult individuals, and the formations were non-aggregated and in diffuse form (see also Kisielewska *et al*. 2015; Kolicka 2016).

Differential diagnosis. From all currently known Chaetonotida species, *C.* (*C.*) *gelidus* sp. nov. most closely resembles *C.* (*C.*) *naiadis* Balsamo *et* Todaro, 1995 originally reported from Italian mountain pools, *Chaetonotus* (*Chaetonotus*) *oculifer* Kisielewski, 1981 described from Polish peat bogs and *Chaetonotus* (*Chaetonotus*) *jaceki* sp. nov. which comes from the same sample and is described above. This species were selected for the comparison with new species due to a similarity in terms of: body length, furca length, similar range of adhesive tube length, number of scales in single longitudinal row, small scales size, alignment of scales, presence of a different type scales on the dorsal and dorsolateral side of the furcal base, number of terminal scales of the ventral interciliary field. Despite the fact that from all of the hitherto known taxa, this listed above have the highest number of common features with the *C.* (*C.*) *gelidus* sp. nov., they are significantly different mostly by scale type and shape. Comparition between the new species and the most morphologically similar taxa have been summarised in Table 5.

Chaetonotus (*Chaetonotus*) *svalbardi* sp. nov.
(Figs 24–31, Tables 6–7 and A3)

Location. Spitsbergen, Svalbard Archipelago (Norway), Site 2.

Material. One sample, 5 specimens (all adults), all photographed. The holotype was established and their detail photomicrographs documentation are deposited in the Natural History Collections at Adam Mickiewicz University in Poznań under access number NHC–GCCS–9–1–20/h (The International Commission on Zoological Nomenclature 1999). The photomicrographs documentation for paratypes are also available in the Natural History Collections at Adam Mickiewicz University in Poznań under access number NHC–GCCS–9–21–100/p and in the author's collection.

Etymology. From the geographical name of the Svalbard Archipelago.

Diagnosis. Body length from 109.1 to 120.9 m. Stocky body. Head, five-lobed, cephalion and pleuria weakly marked in the head outline. Hypostomium small and kidney-shaped. Thick cephalic cilia. No ocellar granules. Scales three-lobed and have a strong keel, with few one-lobed scales with a keel. Scales of the head, neck and trunk show morphological diversity and form 25–27 total longitudinal rows (9–11D+4DL+4L+4LV+4V) of 21–23 scales in central row. Spines thick, without lateral denticle, slightly curved on the dorsal body side and straight on the dorsolateral, lateral and ventral sides. All spines have blunt edges. Spines of the ventral scales longer and hair-like. Ventral interciliary field naked on the pharynx section and covered with small, one-lobed scales with a keel on the intestine section, clearly visible only in the posterior trunk region. Two pairs of ventral interciliary field terminal scales. The anterior pair narrow rectangles with a rounded anterior edge in shape, with a long, clear keel and straight spines. The posterior pair one-lobed, narrow triangles in shape, with a keel and short spines. Pharynx with anterior and posterior dilatations and two thin connected cuticular rods inside the anterior dilatation. Straight intestine with a distinct anterior section marked as a short bend.

Table 6. Main morphometric of *Chaetonotus* (*Chaetonotus*) *svalbardi* sp. nov; N – number of specimens or structures analyzed, Range – the smallest and the largest structure found among all specimens measured. All measurements are given in micrometers (μm).

Characters	N	Holotype	Range of adult paratypes
Body length	5	119.59	109.12–120.87
Furca length	5	19.49	18.85–19.62
Adhesive tube length	5	11.43	10.39–11.51
Number of scales in central longitudinal row	5	21	21–23
Total number of longitudinal alternating rows of scales	5	25	25–27
Head scales length x width	5	1.03–3.39 x 0.85–4.31	0.96–3.91 x 0.81–4.98
Neck scales length x width	5	1.41–4.94 x 1.16–4.89	1.37–5.19 x 1.14–4.97
Trunk scales length x width	5	1.90–8.03 x 1.46–6.90	1.83–8.28 x 1.38–7.12
Head spines length	5	1.81–7.77	1.79–7.83
Neck spines length	5	3.13–8.85	3.10–8.87
Trunk spines length	5	3.57–12.44	3.57–12.89
Mouth ring diameter	5	4.46	4.44–4.92
Pharynx length	5	31.02	29.55–32.06

Description. *Chaetonotus* (*Chaetonotus*) *svalbardi* sp. nov. has a stocky body. Its head is wider than the neck. The neck gradually narrows from the head to the trunk, forming a neck narrowing. The trunk gradually widens up to its widest region on *ca*. U62, after which it gradually tapers up to the furcal base (U85) that is clearly marked. The furca has a V-shaped notch, and the ends of its branches point outwards.

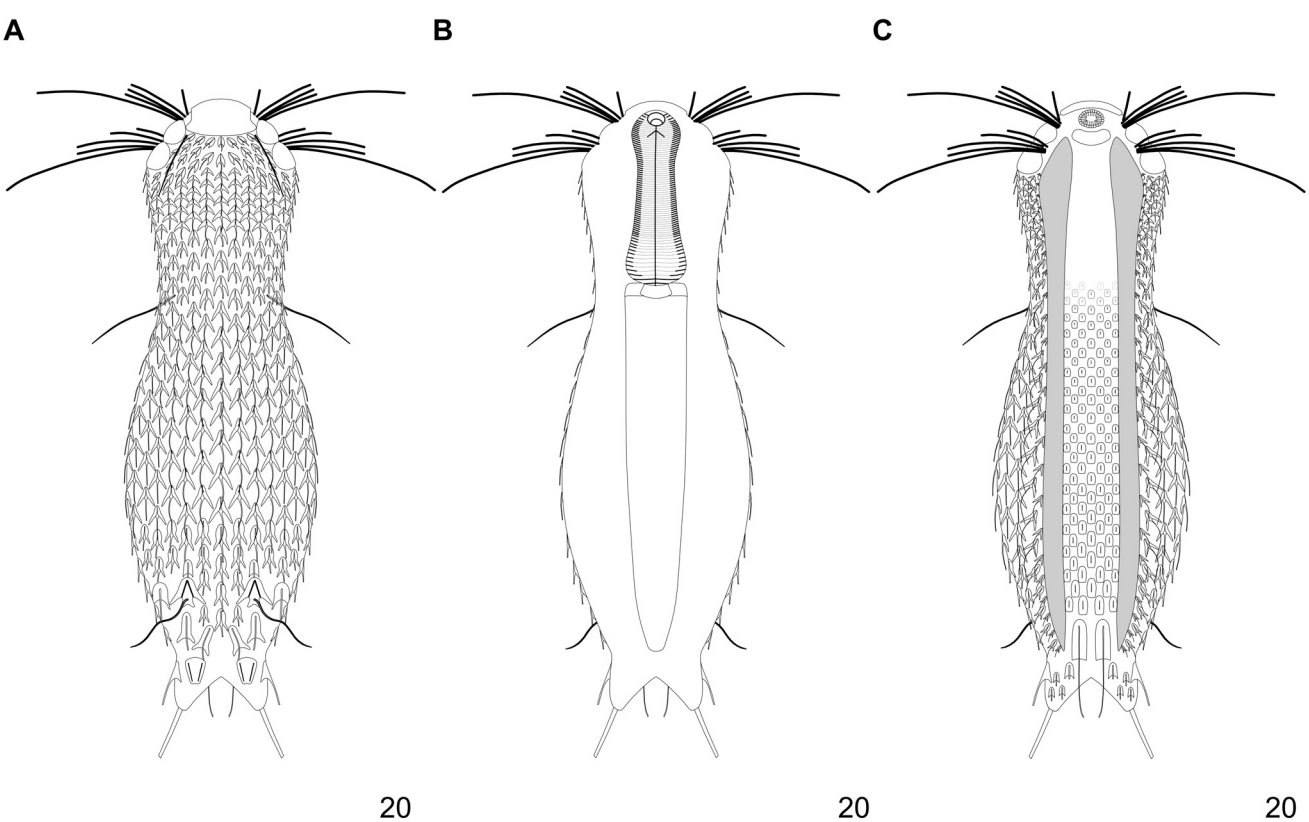


Figure 24. *Chaetonotus* (*Chaetonotus*) *svalbardi* sp. nov. Schematic drawings. A – Dorsal body view. B – View of internal morphology. C – Ventral body view. Light grey areas indicate the ciliary bands.

Table 7. Condensed comparison of the most important differential characters between the most similar species to *Chaetonotus* (*Chaetonotus*) *svalbardi* sp. nov. Measurements are given in micrometres (μm). NA – data not available; – – not applicable.

Character	*C.* (*C.*) *svalbardi* sp. nov.	*C.* (*C.*) *fluviatilis*	*C.* (*H.*) *italicus*
Body length	109.1–120.9	107–140	91–110
Cephalic pleuria	Cephalion narrow; epipleuria nad hypopleuria small, simillar size; visible from the dorsal body side. Hypostomium small, kidney sheyped	Cephalion narrow, widens at the dorsal edge; epipleuria small; hypopleuria large; clearly visible from the dorsal body side. Hypostomium absent	Cephalion narrow; epipleuria large; hypopleuria small; located alterally. Hypostomium small, trapezoidal
Place of emerging cephalic cilia	Anterior tufts: ventrally, between cephalion and epipleuria; posterior tufts: ventrally, in notches between epipleuria and hypopleuria	Anterior tufts: Lateralo-terminal, betwen cephalion and epipleuria; posterior tufts: laterally, between epipleuria and hypopleuria	Anterior tufts: Lateralo-terminal, betwen cephalion and epipleuria; posterior tufts: laterally, between epipleuria and hypopleuria
Pharynx length	29.6–32.1	30.5–34.5	27–29
Pharynx shape	Pharynx with anterior and wider posterior dilatation	Pharynx with anterior and wider posterior dilatation	Pharynx cylindrical
Presence of cuticular reinforcements in the pharynx	Present (two slight, thin and straight linked cuticular rods)	Absent	Absent
Anterior intestine section differ in form of morphology	Present	Absent	Absent
Furca length	18.9–19.6	21–23	11–17
Adhesive tube length	10.4–11.5	10–14	NA
Type of furca indentation	V-shaped	Parablic in shape	Eliptical in shape
Number of scales in single longitudinal row	21–23	9	13–14
Total number of longitudinal alternating rows of scales	25–27	13–15	13
Type of scales	Almost all tree-lobed with strong keels and bent spines; shape strongly differentiated within body regions	Almost all tree-lobed with strong keels and straight spines; shape strongly differentiated within body regions	Almost all tree-lobed with strong keels and slightly bent spines; shape strongly differentiated within body regions
Scales size variation	Strongly differentiated within body regions	Differentiated within body regions	Differentiated within body regions
Scales size	Head: 0.96–3.91 x 0.81–4.98 Neck: 1.37–5.19 x 1.14–4.64 Trunk: 1.38–8.28 x 1.83–7.12	Head: NA Neck: 4.0–4.5 x 2.5–3.0 Trunk: 5.0–8.5 x 3.0–6.0	Head: 2.0–3.1 x 2.0–3.1 Neck: NA Trunk: 6.2–7.0 x 4.6–5.0

Table 7. Continued.

Character	*C.* (*C.*) *svalbardi* sp. nov.	*C.* (*C.*) *fluviatilis*	*C.* (*H.*) *italicus*
Alignment of scales	Located far from one another, edges do not overlap	Located far from one another, edges do not overlap	Located far from one another, edges do not overlap
Presence of a different type scales on the dorsal and dorsolateral side of the furcal base	Present	Present	Present
Presence of scales with two keels on the furcal appendages	Present	Absent	Present
Scales coverage of the ventral interciliary field	Covered from the beginning of the intestinal section to furcal base; scales clearly visible only on the posterior trunk region	Ventral interciliary field naked	Entire ventral interciliary field covered by scales; all scales clearly visible
Type of scales of the ventral interciliary field	Small, one-lobed rectangles scales with rounded anterior edge, weakly keeled and spineless	–	Rounded, pleat like scales
Number of terminal scales of the ventral interciliary field	2 pairs	2 pairs	1 pair
Type of terminal scales of the ventral interciliary field	Anterior pair: Long, narrow rectangles with rounded anterior edges and very shallow posterior notches, keeled and spined Posterior pair: Narrow triangles with shallow posterior notches, keeled and spined (short thin spines)	Anterior pair: Narrow, rounded triangles, keeled and spined Posterior pair: Narrow triangles with shallow posterior notches, keeled and spined	Elongated, rectangular with rounded edges, keeled and spinless
Spines length variation	Spines length gradually increases from the beginning of the head towards to about three-fourths of the body length; spines on dorsal side lengthen more rapidly and are longer than the dorsolateral, lateral and ventrolateral spines; ventral spines much longer than the other spines	Spines lengthen from the head to the posterior trunk region; spines of the three dorsal central rows longer than other spines	Spines length gradually increases from the beginning of the head towards to the widest body region; length of the spines decreases from the dorsal side to the ventral side, towards the ciliary bands
Spines length	Head: 1.8–7.8 Neck: 3.1–8.9 Trunk: 3.6–12.9	Head: NA Neck: 3.0–7.5 Trunk: 5.0–15.5	Head: 3.2–3.6 Neck: 4.5 Trunk: 6.5–9.7

Table 7. Continued.

Character	*C.* (*C.*) *svalbardi* sp. nov.	*C.* (*C.*) *fluviatilis*	*C.* (*H.*) *italicus*
Presence of lateral denticle on the spines	Absent	Absent	Absent
Presence of hair-like spines on the ventral body side	Present	Absent	Absent
Type of posteriormost pair of the trunk lateral spines	Similar to other posterior trunk spines, nor longer or thicker	Markedly shorter than other trunk spines, nor thicker	Similar to other posterior trunk spines, nor longer or thicker
Type of last pair of the furcal appendages lateral spines	Similar to the posterior trunk spines, nor longer or thicker	Markedly shorter than trunk spines, nor longer or thicker	–
Ventral locomotor cilia	Two longitudinal bands wider on the head region	Two longitudinal bands slightly wider on the head region	Two longitudinal bands merged on the head
Number of dorsal sensory bristles	3 pairs	1 pair	2 pairs

The adhesive tubes are straight, relatively thin, equally thick along their entire length and do not taper towards their ends. The ends of the adhesive tubes are blunt (Figs 24, 26–27).

The head is five-lobed and semicircular. All lobes are distinct but weakly marked in the head outline. The cephalion (U1–U5) is narrow and adheres along its entire length to the head. The epipleuria (U4–U7) and hypopleuria (U7–U10) are small and of a similar size. The epipleuria are more convex and more prominently domed than the hypopleuria. The hypostomium (U6–U7) is small and kidney-shaped and has weak reinforcements at the anterior edge (Figs 24C, 26C, 27C; see Table A3). Two pairs of cephalic ciliary tufts are present, between the cephalion and the epipleuria and between the epipleuria and the hypopleuria, respectively. The point from which these tufts emerge is clearly marked on the ventral side, beyond the edges of the pleuria, in the notch between them. Each anterior tuft has five cilia. The anteriormost cilium in each tuft is very short, the next three cilia are longer and have a similar length, and the posteriormost cilium is much longer than the other ones. The posterior tufts have five cilia each and length of the cilia increases from the anteriormost cilium to the posteriormost one. All cephalic cilia are thick. Ocellar granules are not present. The mouth ring is small (see Table 6), located sub-terminally on U3–U5 and has cuticular reinforcements typical for the genus *Chaetonotus*. Short cuticular inner hairs are present inside the mouth ring, and suboral bristles are present around it.

The entire body, except for the ventral interciliary field, is covered with three-lobed scales and individual one-lobed scales that adhere with their entire surface to the cuticle (Figs 24–26). The scales are distributed in 25–27 longitudinal alternating rows (9–11D+4DL +4L+4LV+4V) of 21–23 scales each. The scales are located far from one another and their edges do not overlap. The longitudinal rows of scales begin on the head posteriorly to at the cephalion, epipleuria and hypopleuria. The scales on the dorsal, dorsolateral, lateral, ventrolateral and ventral sides of the head and neck lengthen from the beginning of the head towards the neck and have a strong keel and narrow postero-lateral lobes (Figs 24–25, 28A; Table 7). The alignment of the postero-lateral lobes relative to the central lobe of a scale changes from the beginning of the head towards the neck. In the two anteriormost transverse rows of scales (from U6 to U7) the scales are narrow and their postero-lateral lobes are located close to one another (Figs 24A, 25A, B, 28A). From the line formed by the hypopleuria to the area before the posterior dilatation (from U7 to U21) the scales gradually widen (their width exceeds their length) (Figs 24A, 25C, 28A). The postero-lateral lobes of scales gradually move

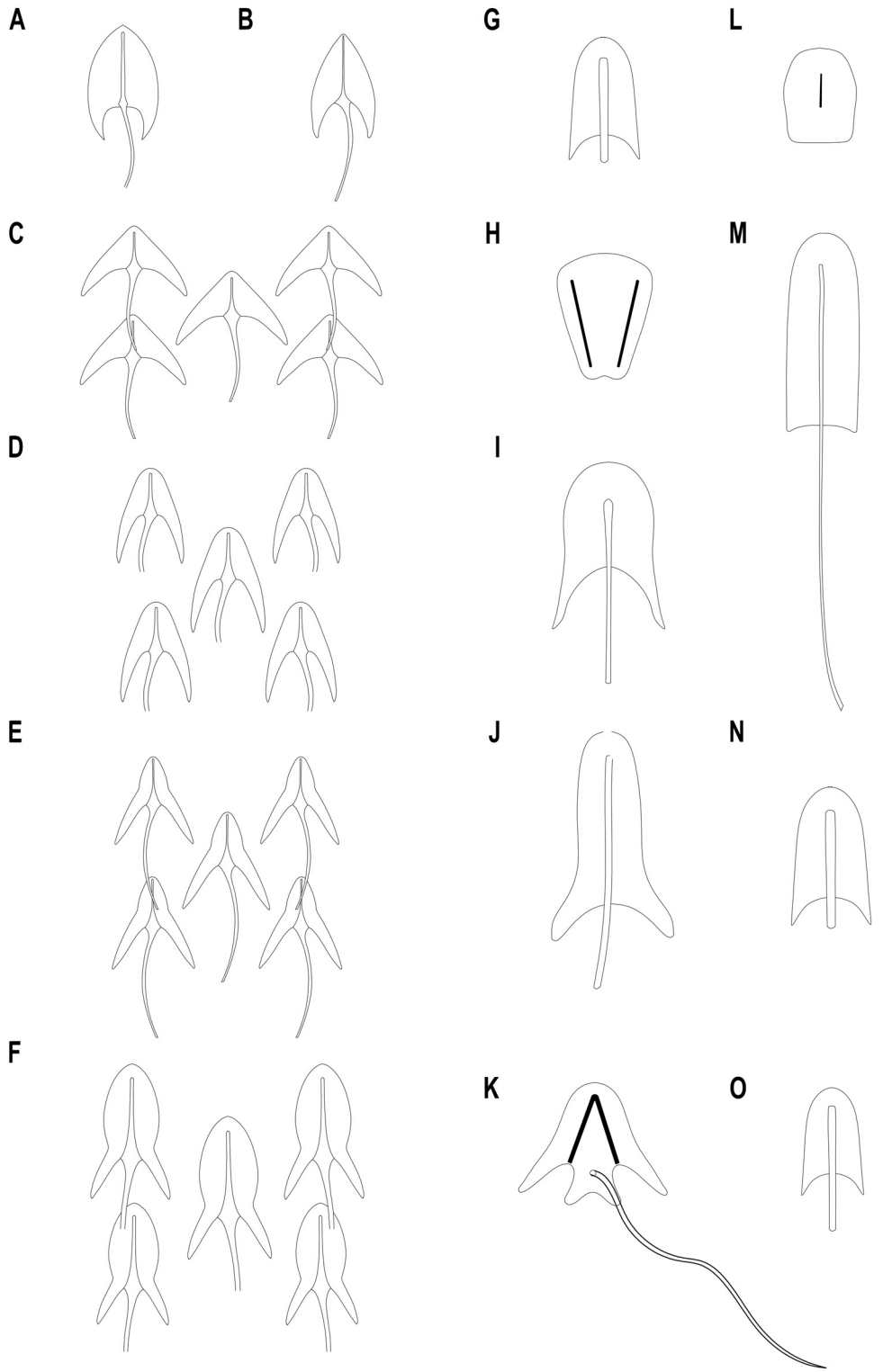


Figure 25. *Chaetonotus* (*Chaetonotus*) *svalbardi* sp. nov. Schematic scales drawings. A – Head anteriormost scale. B – Head anterior region scale. C – Head posterior region scale. D – Neck scale. E – Trunk anterior region scale. F – Trunk posterior region scale. G – Small one-lobed dorsal scale. H – Furcal appendages dorsal double-keeled scale. I – Trunk dorsolateral one-lobed scale. J – Furcal base dorsal scale. K – Double-keeled scale with trunk dorsal sensory bristle. L – Ventral interciliary field scale. M – Ve ntral interciliary field first pair terminal scale. N – Ventral interciliary field second pair terminal scale.

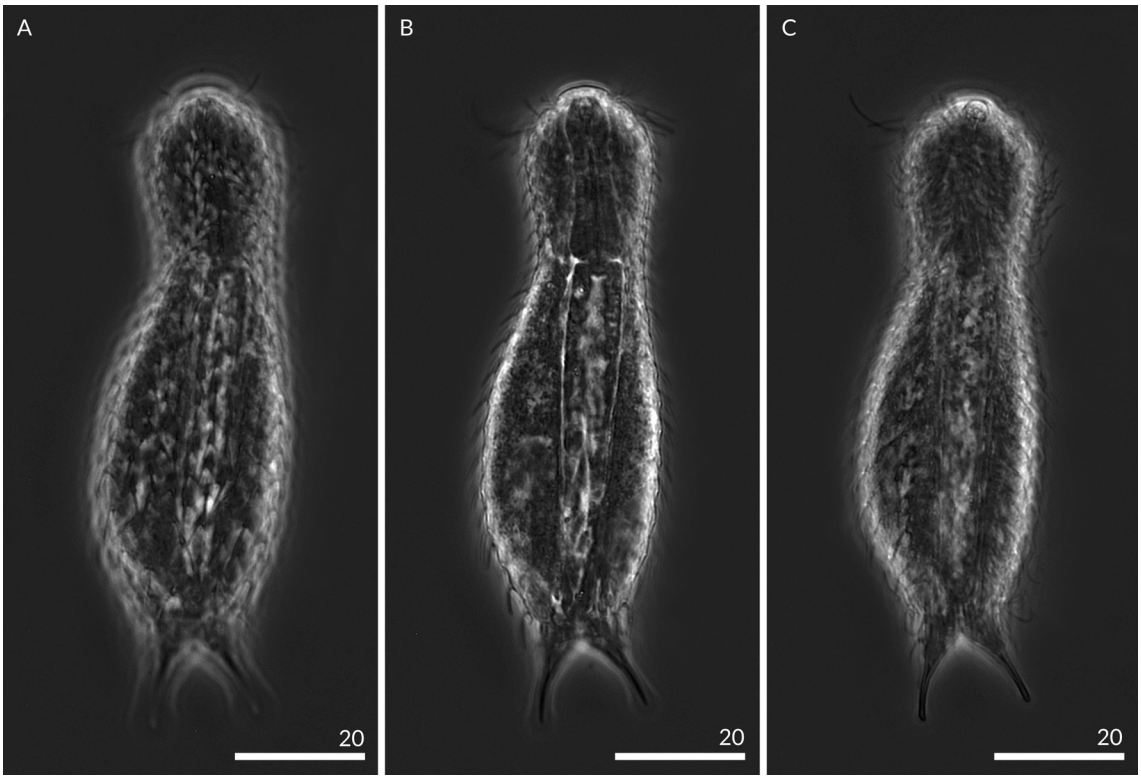


Figure 26. *Chaetonotus* (*Chaetonotus*) *svalbardi* sp. nov. Adult specimen. A – Dorsal body view. B – View of internal morphology. C – Ventral body view. (Phase contrast photomicrographs).

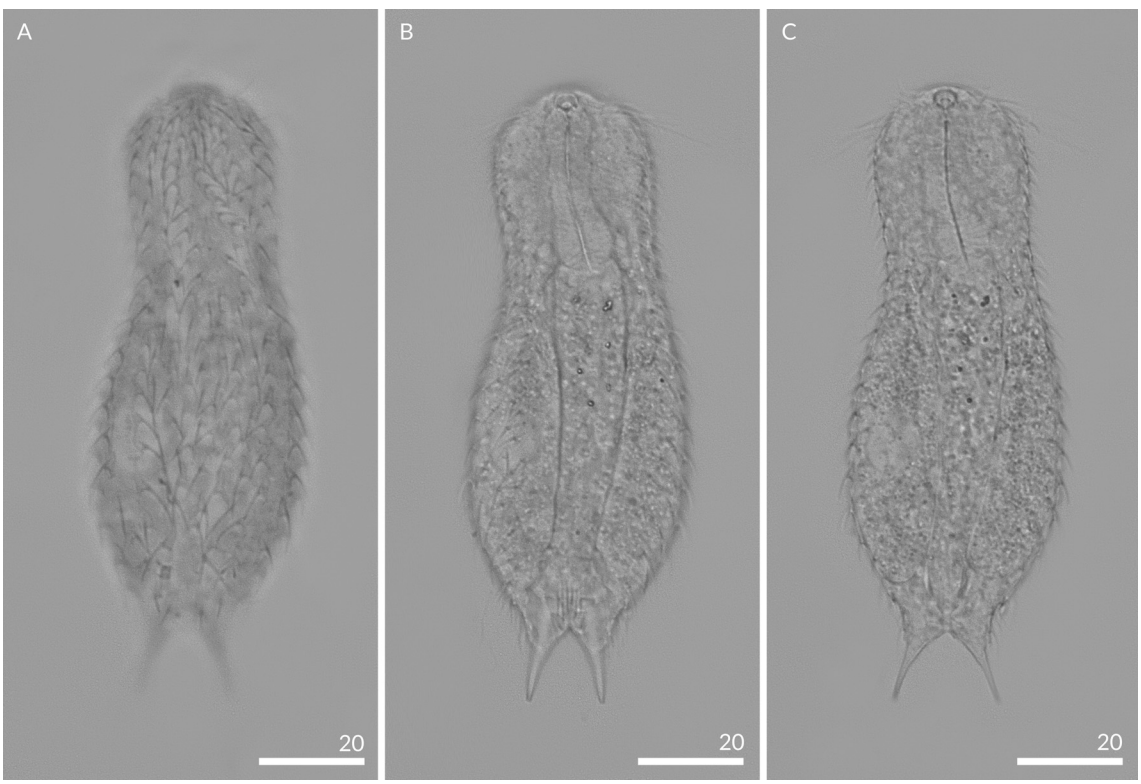


Figure 27. *Chaetonotus* (*Chaetonotus*) *svalbardi* sp. nov. Adult specimen. A – Dorsal body view. B – View of internal morphology. C – Ventral body view. (Brightfield photomicrographs).

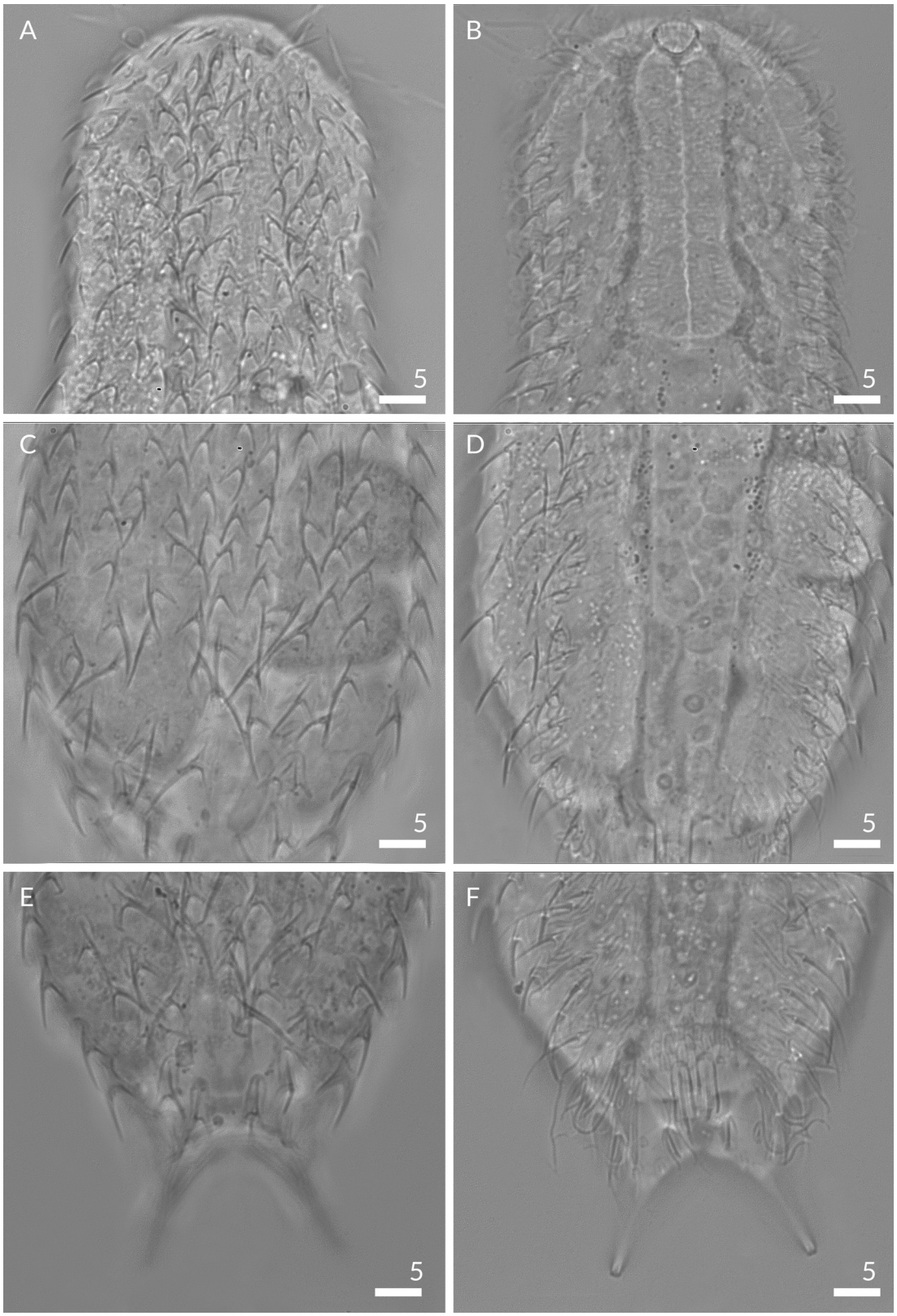


Figure 28. *Chaetonotus* (*Chaetonotus*) *svalbardi* sp. nov. Adult specimen. A – Dorsal view of scales in head region. B – Dorsal view of scales in trunk region. C – Dorsal view of scales in posterior trunk region, on furcal base and furcal appendages. D – Ventral view of scales in head region. E – Ventral view of scales in trunk region. F – Ventral view of scales in posterior trunk region, on furcal base and furcal appendages. (Brightfield photomicrographs).

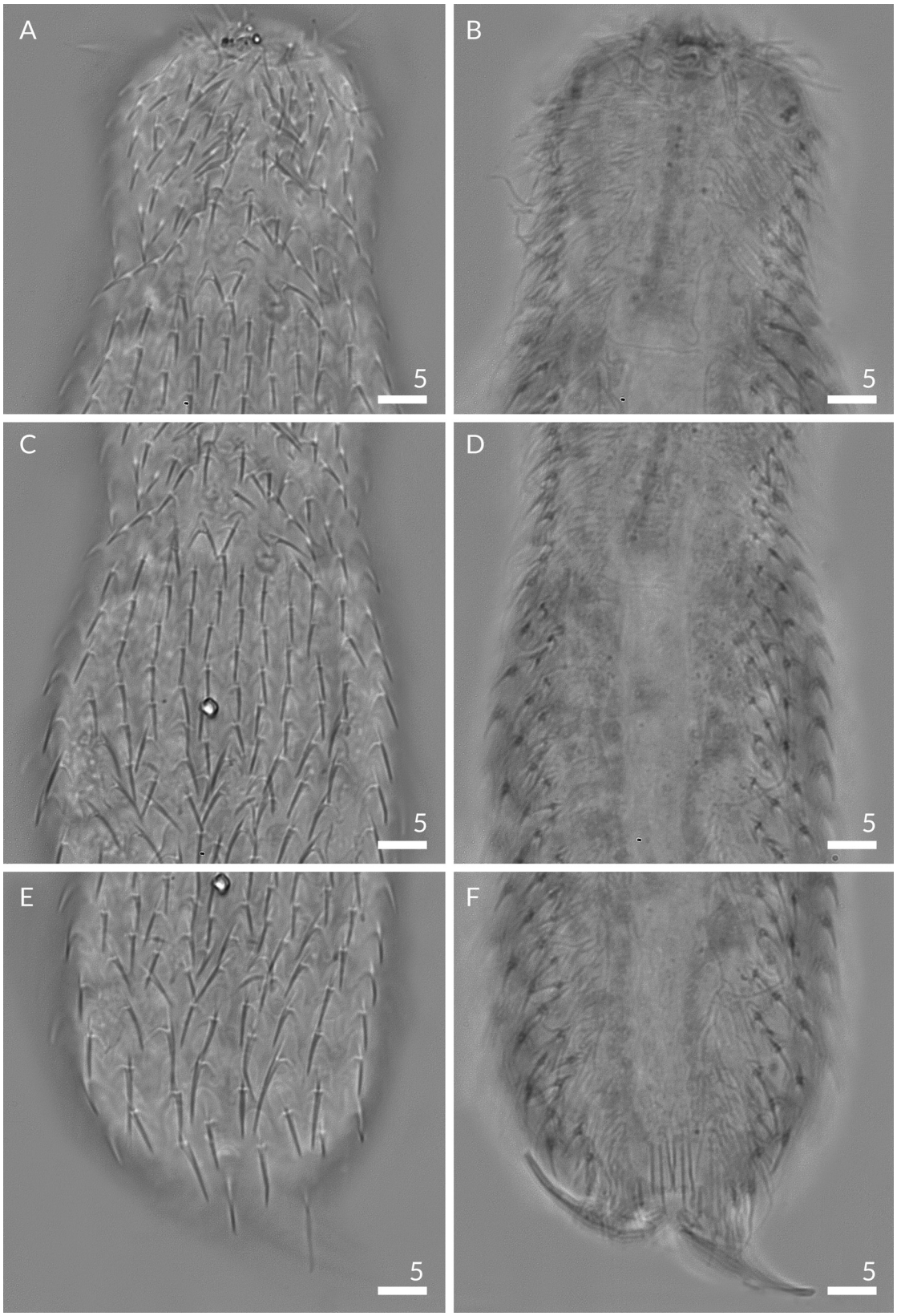


Figure 29. *Chaetonotus* (*Chaetonotus*) *svalbardi* sp. nov. Adult specimen. A – Dorsal spines in head region. B – Dorsal spines in trunk region. C – Dorsal spines in posterior trunk region, on furcal base and furcal appendages. D – Ventral spines in head region. E – Ventral spines in trunk region. F – Ventral spines in posterior trunk region, on furcal base and furcal appendages. (Brightfield photomicrographs).

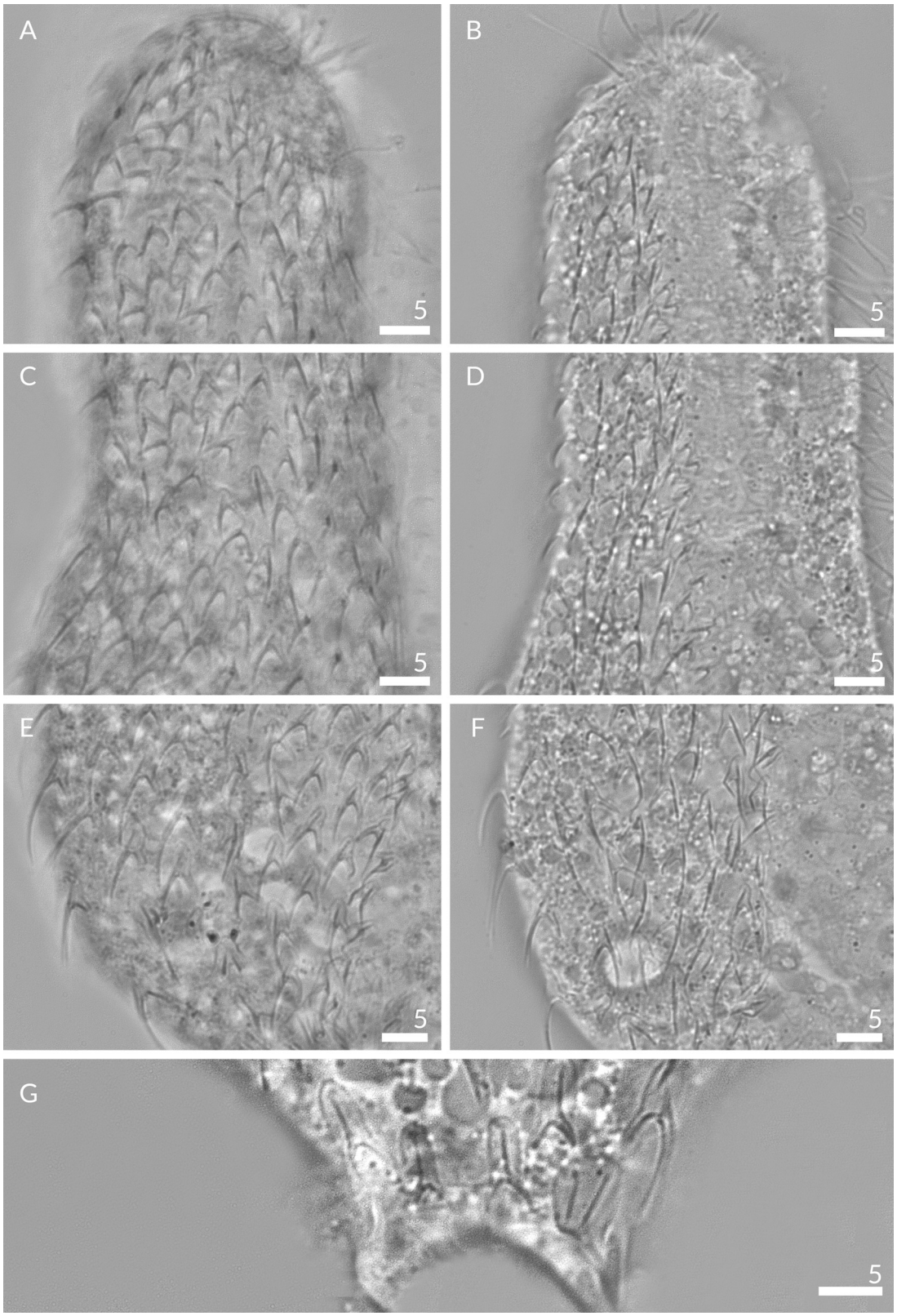


Figure 30. *Chaetonotus* (*Chaetonotus*) *svalbardi* sp. nov. Adult specimen. A – Lateral view of scales in head region. B – Lateral view of scales in trunk region. C – Lateral view of scales in posterior trunk region, on furcal base and furcal appendages. D – Dorsal view of scales in posterior trunk region, on furcal base and furcal appendages. E – Lateral spines in head region. F – Lateral spines in trunk region. G – Lateral spines in posterior trunk region, on furcal base and furcal appendages. (Brightfield photomicrographs).

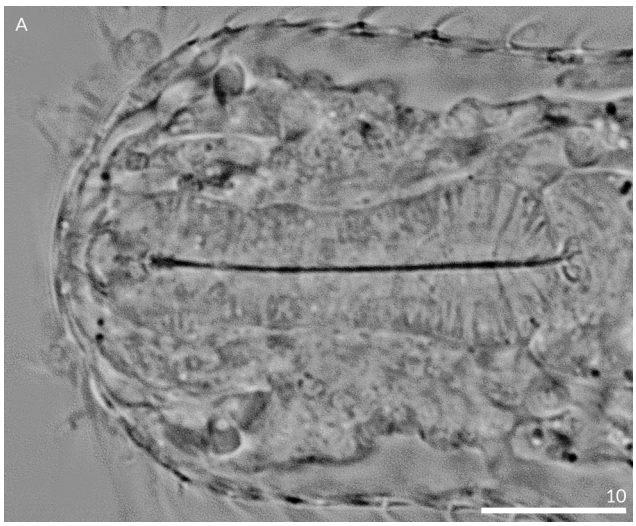


Figure 31. *Chaetonotus* (*Chaetonotus*) *svalbardi* sp. nov. Detail of the pharynx. (Brightfield photomicrographs).

lateral, and thus the distance between them increases. From the area of the posterior pharynx dilatation (from U21) the width of the scales gradually decreases and the postero-lateral lobes align gradually downwards relative to the central lobe (Figs 24A, 25D, 28A, B, C). The scales of the trunk have a strong keel. From the beginning of the trunk (U29) to about three-fourths of the body length (U75), the size of the scales increases and length of central lobes gradually increase from the dorsal to the ventral body sides (Figs 24A, 25E, 28A, B, C; Tables 6 and A3). From about two-thirds of the trunk length (U66) the shape of the scales changes, i.e. a central lobes become larger than postero-lateral lobes and have more rounded anterior edges (Figs 24A, 25F, 28). A pair of small, one-lobed scales with strongly rounded anterior edges, a keel and short, straight spines is present dorsally on U77, directly above the scales with the sensory bristles (Figs 24A, 25G, 28C). A pair of one-lobed scales with a strongly rounded anterior edge and a deep notch in the posterior edge is present dorsolaterally in longitudinal transverse rows next to the scales with the sensory bristles (U78–U80) (Figs 24A, 25I, 28C). Two pairs of narrow scales with a significantly elongated central lobe and short, narrow postero-lateral lobes are present on the dorsal side of the furcal base (Figs 24, 25J). A pair of large, spineless double-keeled scales shaped like ovals that taper towards the end is present dorsolaterally on the furcal appendages (U86–U89) (Figs 24A, 25H, 29D). A pair of scales with a keel and spines of the same type as the r trunk scales is present on the lateral side of the furcal appendages (parafurcal scales) (*ca*. U87–U89). On the ventral side of the head, neck and trunk the scales in a longitudinal row located closest to the ciliary band are about half as large as the surrounding scales (Figs 24C, 26C, 27C, 28D, E, F; Tables 6 and A3). The central lobes of the scales in the two longitudinal rows closest to the ciliary bands are orientated towards the band at an angle of *ca*. 30°. The spines emerge from the posterior part of the scales, are thick, have blunt ends and do not have a lateral denticle (Figs 24, 26–27, 29). The length of the spines gradually increases from the beginning of the head to about three-fourths of the body length (*ca*. U75) (Tables 6 and A3). The dorsal spines lengthen more rapidly and are longer than the dorsolateral, lateral and ventrolateral spines (Figs 24, 26–27, 29). The ventral spines are much longer than the other spines (Figs 24C, 26C, 27C, 29D, E, F). The dorsal spines of the head, neck and trunk are slightly curved and taper slightly towards their ends (Figs 24–25), contrary to the dorsolateral, lateral and ventrolateral spines of the head, neck and trunk that are straight and do not taper towards their ends (Figs 24–25). Ventral sides spines are thin, hair-like and equally thick along their entire length (Figs 24C, 26C, 27C, 29D, E, F).The posteriormost pair of lateral trunk spines is the same type and has a similar length than the spines on the posterior trunk region. The pair of parafurcal spines is longer and thicker than posterior trunk region spines, is straight and has a blunt ends.

The species has three pairs of dorsal sensory bristles. The first pair is located on the head between the cephalion and the epipleuria (U5) and emerges from small, spherical papillae. The second pair of dorsal sensory bristles emerges from small, spherical papillae and is located in the posterior part of the neck (U27). The third pair of dorsal sensory bristles emerges from large, three-lobed scales with two strong keels that are shaped like triangles with thin (narrow) postero-lateral lobes. These scales have a double posterior notch and are located on U78–U80 (Figs 24A, 25K, 28C).

On the ventral side, the longitudinal ciliary bands begin on U6 and run up to U84. These bands are wider on the head than on the rest of the body. The pharyngeal area of the ventral interciliary field is naked. Small, one-lobed scales shaped like squares with a rounded anterior edge with a weak keel are present in the intestinal area of the ventral interciliary field (Figs 24C, 25L, 26C, 27C, 28D, E, F). These scales form 7 longitudinal rows. The scales in the anterior section of the intestinal area of the ventral interciliary field (*ca*. U29) are nearly invisible and rudimentary, and appear to be submerged in the cuticle, wherease in the posterior part of the body they become more visible and separate. However, they are clearly visible only in the posterior part of the body (from *ca*. U75) (Fig. 30F). Two pairs of terminal scales of the ventral interciliary field are present. The first pair is located on U82–U86 and

has a long, clear keel and a straight spine (Figs 24C, 25M). These scales are shaped like long, narrow rectangles with a rounded anterior edge and a very shallow notch at the posterior edge. The second pair is located on the furcal appendages (U86–U87); these scales are one-lobed and shaped like narrow triangles with a shallow posterior notch, have a keel and short, thin spines (Figs 24C, 25N). Three pairs of small, one-lobed scales shaped like narrow triangles with a shallow notch in the posterior edge, a weak keel and a short, vestigial spine are located on each furcal appendage (from U88 to U91), on the ventrolateral and ventral sides (Figs 24C, 25O, 26C, 27C, 28D, E, F).

The pharynx (from U3 to U28) has clearly marked anterior and posterior dilatations, with the posterior dilatation wider than the anterior one. A weak reinforcement composed of two thin, straight connected cuticular rods is present inside the anterior pharynx dilatation on *ca*. U6. The pharynx connects through a wide pharyngeal intestinal junction (U29) to a straight intestine (running from U29 to U84). The intestine has a short differ morphologically from remaining intestine part marked as a bend (on U29) (Figs 24B, 26B, 27B). A large developing egg, X-organ and sperm packets were not observed.

Remarks. In *Chaetonotus* (*Chaetonotus*) *svalbardi* sp. nov. specimens the maximum number of oocytes simultaneously present is from 1 to 2 per intestine side.

Three pairs of dorsal sensory bristles were noted in *Chaetonotus* (*Chaetonotus*) *svalbardi* sp. nov. The presence of dorsal sensory bristles on the head has been observed in five species from the genus *Chaetonotus*. See the 'Remarks' section for the species *C.* (*C.*) *jaceki* sp. nov.

Differential diagnosis. From all currently known Chaetonotida species, *Chaetonotus* (*Chaetonotus*) *svalbardi* sp. nov. most closely resembles *C.* (*C.*) *fluviatilis* Balsamo *et* Kisielewski, 1986 and *C.* (*Hystricochaetonotus*) *italicus* Balsamo *et* Todaro, 1995, both originally described from Italy. This species were selected for the comparison with new species due to a similarity in terms of: body shape and length, type of cephalion, similar range of pharynx length, strongly differentiated of scales shape within body regions, scales size variation within body regions, similar range of scales size, alignment of scales, presence of a different type scales on the dorsal and dorsolateral side of the furcal base. Despite the fact that from all of the hitherto known taxa, this listed above have the highest number of common features with the *C.* (*C.*) *svalbardi* sp. nov., they are significantly different mostly by scale shape, the number of scales in single longitudinal row and in total number of longitudinal alternating rows of scales as well as similar range of spines length. Comparition between the new species and the most morphologically similar taxa have been summarised in Table 7.

Discussion

The life of plants and animals throughout the Arctic is significantly limited in both time and space (Remmert 1980). There are two possible reasons for the poor diversity of species in Arctic habitats. First, the values of environmental factors exceed the tolerance of individual species during periods of disadvantageous conditions for living organisms and undergo violent changes during periods that benefit life. Second, Arctic areas are 'young', i.e. glaciation ended relatively recently, and Spitsbergen is located far from the continents (Pirożnikow 2013). Consequently, is should come as no surprise that while only a small number of species were found, a vast majority of them had very large populations. The number of species observed in the astatic reservoir in the area of Isfjorden may have been limited by both the dispersal pathways along which the species may have reached their habitats and the ecological tolerance of these species. In turn, the fact that a record specimen abundance was noted in an area where the active life of minute invertebrates in small freshwater habitats is possible for only four months of the year may be explained by the consecutive hatching of numerous dormant eggs deposited in the reservoir sediments or transported there with surface runoffs as well as by the wind (*e.g.* Kaya *et al.* 2010) and/or very rapid reproduction and development of the species as a response to unstable environmental conditions.

A vast majority of animals inhabiting the Svalbard Archipelago are invertebrates (Coulson *et al*. 2014). Coulson (2007) suggests that the fauna currently present on Spitsbergen recolonised the island in the Holocene after the last glaciation ended, i.e. within 10 000 years. Coulson (2007) states that only a small percentage of species may have survived in the area. It is possible that the Gastrotricha inhabiting the Arctic underwent speciation and thus have a single, close common ancestor which survived the glaciation or occupied the Svalbard Archipelago during a warm period (Kolicka *et al*. 2016). These speculations about a common ancestry of the newly described Arctic species are substantiated by a large number of morphologically very similar traits in these species. The traits that rarely appear in other freshwater taxa but are present in *Chaetonotus* (*Chaetonotus*) *jaceki* sp. nov., *C.* (*C.*) *gelidus* sp. nov. and *C.* (*C.*) *svalbardi* sp. nov. are: displacement of the point from which the cephalic cilia emerge towards the ventral side, a weak cuticular reinforcement in the anterior pharynx dilatation, the intestine anterior

section differ from remaining part, the presence of three pairs of dorsal sensory bristles, and the presence of one-lobed scales that differ from the other scales of the body near the base scale of the sensory bristles on the posterior part of the trunk. All of these morphological characteristics may also stem from evolutionary parallelism or convergence and could be the result of environmental selection pressure. The use of molecular analyses with respect to these taxa in the future may help resolve these doubts.

Taking into account the young age (on the evolutionary timescale) of the habitats, it is possible that the processes of speciation or even emergence of individual genera of gastrotrichs in the Arctic are still open to observation. In contrast to other groups of small invertebrates, *e.g.* tardigrades (Zawierucha 2013, Zawierucha *et al*. 2013, 2015, 2015), Arctic gastrotrichs have so far remained virtually unknown. Thus it is very likely that further research in the area will bring many more taxa that are new to science. It is also possible that investigating the diversity of gastrotrichs in young Arctic habitats will help broaden our knowledge on both diversity and speciation within the entire group of Chaetonotidae as well as provide information on the ecological tolerance and both adaptive and dispersive capacity of these poorly researched invertebrates.

Acknowledgments

I thank Dr. Krzysztof Zawierucha for collecting the material and Prof. Ziemowit Olszanowski as well as Prof. André R. S. Garraffoni and Dr. Thiago Araujo for his kindly and helpfully advice to earlier versions of the manuscript. Special thanks to Alicja Kubicka for preparing the drawings of the new species and for all the figures used in this paper. Last but not least, the authors are grateful to Łukasz Kaczmarek and Wojciech Magowski for their help during the research. The study was founded by Polish MSHE grants DI 2011 035241 (sampling) and DI 2014 013844 (research). Author is a beneficiary of the Adam Mickiewicz Foundation scholarship scheme.

References

Araujo, T., Atherton, S. and R. Hochberg. 2015. First record and a description of a new species of *Oregodasys* (Gastrotricha: Macrodasyida: Thaumastodermatidae) from Tobago. Proceedings of the Biological Society of Washington, 128(3): 176–185.

Atherton, S. 2014. *Urodasys poculostylis* sp. nov., a new stylet-bearing gastrotrich (Macrodasyida) from Capron Shoal, Florida. Marine Biology Research, 10(5): 530–536.

Balsamo, M., d'Hondt, J.-L., Kisielewski, J. and L.Pierboni. 2008. Global diversity of gastrotrichs (Gastrotricha) in freshwaters. Hydrobiologia, 595: 85–91.

Balsamo, M., Grilli, P., Guidi, L. and J.-L. d'Hondt. 2014. Gastrotricha: biology, ecology and systematics: families Dasydytidae, Dichaeturidae, Neogosseidae, Proichthydiidae. *In*: 'Identification Guides to the Plankton and Benthos of Inland Waters. 24'. (Ed. H. J. F. Dumont.) pp. 1–187. (Backhuys Publishers, Margraf Publishers GmbH: Weikeirsheim, Germany.).

Balsamo, M., d'Hondt, J.-L., Kisielewski, J., Todaro, M. A., Tongiorgi, P., Gudi, L., Grilli, P. and Y. de Jong. 2015. Fauna Europea: Gastrotricha. Biodiversity Data Journal, 3: e5800.

Balsamo, M. and J. Kisielewski. 1986. *Chaetonotus fluviatilis*, a new freshwater gastrotrich. Bolletino di Zoologia, 53, 111–114. http://dx.doi.org/10.1080/11250008609355491.

Balsamo, M. and M. A. Todaro. 1995. Gastrotrichi del Trentino: le Viotte del Monte Bondone (Trento). Studi Trentini di Science Naturali – Acta Biologica, 70: 9–22.

Balsamo, M. and M. A. Todaro. 2002. Gastrotricha. *In*: Rundle, S. D., Robertson, A. L & Schmid-Araya, J. M. (Eds.), Freshwater Meiofauna: Biology and Ecology. Backhuys Publishers, Leiden, 45–61.

Coulson, S. J. 2007. The terrestrial and freshwater invertebrate fauna of the High Arctic archipelago of Svalbard. Zootaxa, 1448: 41–58.

Coulson, S. J., Convey, P., Aakra, K., Aarvik, L., Avila-Jimenez, M. L., Babenko, A., Biersma, E. M., Bostrom, S., Brittain, J. E., Carlsson, A. M., Christoffersen, K., De Smet, W. H., Ekremj, T., Fjellberg, A., Fureder, L., Gustafssonm, D., Gwiazdowicz, D. J., Hansen, L. O., Holmstrup, M., Hulle, M., Kaczmarek, Ł., Kolicka, M., Kuklin, V., Lakka, H. K., Lebedeva, N., Makarova, O., Maraldo, K., Melekhina, E., Odegaard, F., Pilskog, H. E., Simon, J. C., Sohlenius, B., Solhoy, T., Soli, G., Sture, E., Tanasevitch, A., Taskaeva, A., Velle, G., Zawierucha, K. and K. Zmudczyńska-Skarbek. 2014. The terrestrial and freshwater invertebrate biodiversity of the archipelagoes of the Barents Sea; Svalbard, Franz Josef Land and Novaya Zemlya. Soil Biology and Biochemistry, 68: 440–470.

De Smet, W. H. 1993. Report on rotifers from Barentsøya, Svalbard (78°30'N). Fauna Norvegica Serie A, 14: 1–26.

De Smet, W. H., Van Rompu, E. A. and L. Beyens. 1988. Contribution to the rotifers and aquatic Tardigrada of Edgeøya (Svalbard). Fauna Norvegica Serie A, 9: 19–30.

Ehrenberg, C. G. 1830. Organisation, Systematik, und geographisches Verhältnis der Infusionstierchen. F. Dümmler, Berlin, 108 pp. http://dx.doi.org/10.5962/bhl.title.2077

Garraffoni, A. R. S., Araújo, T. Q., Laurenço, A. P., Guidi, L. and M. Balsamo. 2016. A new genus and new species of freshwater Chaetonotidae (Gastrotricha: Chaetonotida) from Brazil with phylogenetic position inferred from nuclear and mitochondrial DNA sequences. Systematic and Biodiversity, 15(1): 49-62. DOI: 10.1080/14772000.2016.1214189

Garraffoni, A. R. S., Alcântara F. C. and H. H. Checon. 2016. Evaluating the anesthetization and fixation efficacy of "soft" and "hard" freshwater benthic meiofauna: what is the best method for specimen preservation? Limnology, DOI: 10.1007/s10201-016-0502-y.

Garraffoni, A. R. S. and M. P. Melchiorm. 2015. New species and new records of freshwater Heterolepidoderma (Gastrotricha: Chaetonotidae) from Brazil with an identification key to the genus. Zootaxa, 4057(4): 551. DOI: 10.11646/zootaxa.4057.4.5

Giżejewski, J. and A. Karczewski. 2013. Landforms. *In*: Zwoliński, Z., Kostrzewski, A., Puliny, M. (Eds.) Acient and modern geoecosystems of Spitsbergen. Polish geomorphological research. Bogucki Wydawnictwo Naukowe, Poznań, 65–69.

Głowacki, P. 2007. [Rola procesów fizyczno-chemicznych w kształtowaniu struktury wewnętrznej i obiegu masy lodowców Spitsbergenu]. [Book in Polish]. Publications of the Institute of Geophysics Polish Academy of Sciences, Warszawa, vol. M-30, no. 400.

Górska, B., Grzelak, K., Kotwicki L., Hasemann, C., Schew, I., Soltwed, T. and M. Włodarska-Kowalczuk. 2014. Bathymetric variations in vertical distribuction patterns of meiofauna in the surface sediments of the deep Arctic ocean. Deep-Sea Research Part I: Oceanographic Research Papers, 91: 36–49.

Gosse, P. H. 1864. The natural history of the hairy-backed animalcules (Chaetonotidae). The Intellectual Observer, 5: 387–406.

Grzelak, K. and L. Kotwicki. 2012. Meiofaunal distribution in Horsund fjord, Spitsbergen. Polar Biology, 35: 269–286.

Hochberg, R., Atherton, S., Kieneke, A. 2014. Marine Gastrotricha of Little Cayman Island with the description of one new species and an initial assessment of meiofaunal diversity. Marine Biodiversity, 44: 89–113.

d'Hondt, J.-L. 1971. Gastrotricha. *In*: Barnes, H. (Ed.), Oceanography and marine biology: an annual review, 9, pp. 141–192. [George Allen and Unwin Ltd.]

Hummon, W. D., Balsamo, M. and M. A. Todaro. 1992. Italian marine Gastrotricha: I. Six new and one redescribed species of Chaetonotida. Bollettino di Zoologia, 59: 499–516.

Hummon, W. D., Todaro, M. A. and P. Tongiorgi. 1993. Italian Marine Gastrotricha: II. One new genus and ten new species of Macrodasyida. Bollettino di Zoologia, 60: 109–127.

Kånneby, T. 2011. New species and new records of freshwater Chaetonotida (Gastrotricha) from Sweden. Zootaxa, 3115: 29–55.

Kånneby, T. 2013. New species and records of freshwater *Chaetonotus* (Gastrotricha: Chaetonotidae) from Sweden. Zootaxa, 3701: 551–588.

Kånneby, T., Todaro, M. A., U. Jondelius. 2009. One new species and records of Ichthydium Ehrenberg, 1830 (Gastrotricha: Chaetonotida) from Sweden with a key to the genus. Zootaxa, 2278: 26–46.

Kånneby, T., Todaro, M. A. and U. Jondelius. 2012. A phylogenetic approach to species delimitation in freshwater Gastrotricha from Sweden. Hydrobiologia, 683: 185–202.

Kånneby, T., Todaro, M. A. and U. Jondelius. 2013. Phylogeny of Chaetonotidae and other Paucitubulatina (Gastrotricha: Chaetonotida) and colonization of aquatic ecosystems. Zoologica Scripta, 42: 88–105.

Kisielewska, G. and J. Kisielewski. 1986. Freshwater Gastrotricha of Poland. III. Gastrotricha from the Białowieża Forest and the Białowieża Glade. Fragmenta Faunistica, 30: 195–215.

Kisielewska, G., Kolicka, M. and K. Zawierucha. 2015. Prey or parasite? The first observations of live Euglenida in the intestine of Gastrotricha. European Journal of Protistology, 51: 138–141.

Kisielewski, J. 1981. Gastrotricha from raised and transitional peat bogs in Poland. Monografie Fauny Polski, 11. Polska Akademia Nauk, Kraków, 143 pp.

Kisielewski, J. 1987. New records of marine Gastrotricha from the French coasts of Manche and Atlantic I. Macrodasyida, with descriptions of seven new species. Bulletin du Museum national d'histoire naturelle, 9: 837–877.

Kisielewski, J. 1991. Inland-water Gastrotricha from Brazil. Annales Zoologici, Warszawa, 43: 1–168.

Kisielewski, J. 1997. Brzuchorzeski (Gastrotricha). Fauna Słodkowodna Polski, Zeszyt 31. Wydawnictwo Uniwersytetu Łódzkiego, Łódź, 157 pp.

Kolicka, M. 2016. Gastrotrichs in bromeliads – newly recorded *Chaetonotus* (*Hystricochaetonotus*) *furcatus* Kisielewski, 1991 (Chaetonotida) from the Łódź Palm House. Zoosystema, 38(1): 141–155. http://dx.doi.org/10.5252/z2016n1a5.

Kolicka, M., Gadawski, P., Dabert, M. 2016. A new species of freshwater Chaetonotidae (Gastrotricha, Chaetonotida) from Obodska Cave (Montenegro) based on morphological and molecular characters. European Journal of Taxonomy (in press).

Kolicka, M., Dabert, M., Dabert, J., Kånneby, T., Kisielewski, J. 2016. *Bifidochaetus*, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. Invertebrate Systematics, 30: 398–419. http://dx.doi.org/10.1071/IS16001.

Leasi, F. and M. A. Todaro. 2008. The muscular system of Musellifer delamarei (Renaud-Mornant, 1968) and other chaetonotidans with implications for the phylogeny and systematization of the Paucitubulatina (Gastrotricha). Biological Journal of the Linnean Society, 94, 379–398. http://dx.doi.org/10.1111/j.1095–8312.2008.00974.x

Marsz, A. A., Niedźwiedź, T. and A. Styszyńska. 2013. Modern climate changes on Spitsbergen as a basis for determining landscape metamorphosis *In*: Zwoliński, Z., Kostrzewski, A., Puliny, M. (Eds.) Acient and modern geoecosystems of Spitsbergen. Polish geomorphological research. Bogucki Wydawnictwo Naukowe, Poznań, 391–413.

Mečnikow, E. 1865. Über einige wenig bekannte niedere Thierformen. Zeitschrift für Wissenschaftliche Zoologie, 15: 450–463.

Migała, K. and T. Wawrzyniak. 2013. Climate. *In*: Zwoliński, Z., Kostrzewski, A., Puliny, M. (Eds.) Acient and modern geoecosystems of Spitsbergen. Polish geomorphological research. Bogucki Wydawnictwo Naukowe, Poznań, 71–76.

Müller, O. F. 1773. Vermium terrestrium et fluviatilium saeu animalium infosoriorum, helminthicorum et testaceorum non marinorum, succincta historia. Heineck et Faber, Havniae et Lipsiae, 135 pp. http://dx.doi.org/10.5962/bhl.title.12733

Nesteruk, T. 2004. Benthic and epiphytic fauna of Gastrotricha in littoral of mesotrophic lake in Łęczna–Włodawa Lakeland, Poland. Fragmenta Faunistica, 47(1): 1–6.

Pfannkuche, O. and H. Thiel. 1987. Meiobenthic Stocks and Benthic Activity on the NE-Svalbard Shelf and in the Nansen Basin. Polar Biology, 7: 253–266.

Pirożnikow, E. 2013. Flora. *In*: Zwoliński, Z., Kostrzewski, A., Puliny, M. (Eds.) Acient and modern geoecosystems of Spitsbergen. Polish geomorphological research. Bogucki Wydawnictwo Naukowe, Poznań, 79–81.

Rao, G. C. and C. Clausen. 1970. *Planodasys marginalis* gen. et. sp. nov. and Planodasyidae fam. nov. (Gastrotricha Macrodasyoidea). Sarsia, 42: 73–82.

Remane, A. 1925. Organisation und systematische Stellung der aberranten Gastrotrichen. Verhandlungen der deutschen zoologischen. Gesellschaft, 30: 121–128.

Remane, A. 1927. Beiträge zur Systematik der Süsswassergastrotrichen. Zoologische Jahrbücher Abteilung für Systematik. Oekologie und Geographie der Tiere, 53: 269–320.

Remmert, H. 1980. Arctic animal ecology. Springer Verlag, Berlin, Heidelberg, New York, 280 pp.

Scourfield, D. J. 1897. Contribution to the Non-Marine Fauna of Spitsbergen. Part I. Preliminary Notes, and Reports on the Rhizopoda, Tardigrada, Entomostraca, etc. Catalogue of Birds in the British Museum, 20, 784–792.

Suzuki, T. G., Maeda, M. and H. Furuya. 2013. Two new Japanese species of Gastrotricha (Chaetonotida, Chaetonotidae, Lepidodermella and Dichaeturidae, Dichaetura), with comments on the diversity of gastrotrichs in rice paddies. Zootaxa, 3691: 229–239.

The International Commission on Zoological Nomenclature. 1999. International Code of Zoological Nomenclature, Fourth Edition. The International Trust for Zoological Nomenclature, The Natural History Museum, Cromwell Road, London. Kaya, M., De Smet, W. H. and D. Fontaneto. 2010. Survey of moss-dwelling bdelloid rotifers from middle Arctic Spitsbergen (Svalbard). Polar Biology, 33: 833–842.

Todaro, M. A. 2016. Gastrotricha. Accessed through: World Register of Marine Species at http://www.marinespecies.org/aphia.php?p=taxdetailsetid=2078 (accessed 10 December 2016)

Todaro, M. A., Balsamo, M. and R. M. Kristensen. 2005. A new genus of marine chaetonotids (Gastrotricha), with a description of two new species from Greenland and Denmark. Journal of the Marine Biological Association of the United Kingdom, 85: 1391–1400.

Todaro, M. A., Dal Zotto, M. and F. Leasi. 2015. An Integrated Morphological and Molecular Approach to the Description and Systematisation of a Novel Genus and Species of Macrodasyida (Gastrotricha). PLoS ONE 10(7): e0130278. doi:10.1371/journal.pone.0130278

Todaro, M. A., Guidi, L., Leasi, F. and P. Tongiorgi. 2006. Morphology of *Xenodasys* (Gastrotricha): the first species from the Mediterranean Sea and the establishment of Chordodasiopsis gen. nov. and Xenodasyidae fam. nov. Journal of Marine Association of United Kingdom, 86: 1005–1015.

Todaro, M. A., Leasi, F. and R. Hochberg. 2014. A new species, genus and family of marine Gastrotricha from Jamaica, with a phylogenetic analysis of Macrodasyida based on molecular data. Systematic and Biodiversity, 12(4): 473–488.

Urban-Malinga, B., Wiktor, J., Jabłońska, A. and T. Moens. 2005. Intertidial meiofauna of high-latitude glacial Arctic fjord (Kongsfjorden, Svalbard) with emphasis on the structure of free-living Nematoda communities. Polar Biology, 28: 940–950.

Węsławski, J. M, Zajączkowski, M., Wiktor, J. and M. Szymelfening. 1997. Intertidal Zone of Svalbard. 3. Littoral of subartic, oceanic island: Bjornoya. Polar Biology, 18, 45–52.

Włodarska-Kowalczuk, M., Szymfeling, M. and L. Kotwicki. 1999. Macro- and meiobenthic fauna of Yaldiabucta glacial bay (Isfjorden, Spitsbergen). Polish Polar Research, 4: 367–386.

Zawierucha, K., Coulson, S. J, Michalczyk, Ł. and Ł. Kaczmarek. 2013. Current knowledge of the Tardigrada of Svalbard with the first records of water bears from Nordaustlandet (High Arctic). Polar Research 32, 20886, http://dx.doi.org/10.3402/polar.v32i0.20886.

Zawierucha, K. 2013. Tardigrada from Arctic tundra (Spitsbergen) with description of *Isohypsibius karenae* sp. n. (Isohypsibiidae). Polish Polar Research, 34 (4): 383–396. DOI: 10.2478/popore–2013–0016.

Zawierucha, K., Zmudczyńska-Skarbek, K., Kaczmarek, Ł. and K. Wojczulanis-Jakubas. 2015. The influence of a seabird colony on abundance and species composition of water bears (Tardigrada) in Hornsund (Spitsbergen, Arctic). Polar Biology, http://dx.doi.org/10.1007/s00300–015–1827–4.

Zelinka, C. 1889. Die Gastrotrichen. Eine monographische Darstellung ihrer Anatomie, Biologie und Systematik. Zeitschrift für Wissenschaftliche Zoologie, 49, 209–384.

Received: December 11, 2016
Accepted: February 15, 2017

Table A1. Morphometric of *Chaetonotus* (*Chaetonotus*) *jaceki* sp. nov.; N – number of specimens or structures analysed, Range – the smallest and the largest structure found among all specimens measured, SD – standard deviation. All measurements are given in micrometres (μm); all indicators are given as a percentage (%) and italicized.

Characters	N	Holotype	Range of adult paratypes	SD	N	Range of juvenile and subadult paratypes	SD
Body length	79	121.55	110.01–131.12	5.451	35	81.43–113.94	9.819
Cephalion length	22	10.16	9.89–11.38	0.422	20	9.37–10.22	0.243
Cephalion width	22	9.94	9.20–10.82	0.319	20	8.92–10.32	0.352
Cephalion maximum width	22	12.55	12.09–13.67	0.451	20	1.05–12.95	0.457
Hypostomium length	22	5.04	3.85–5.57	0.427	20	3.74–5.64	0.482
Hypostomium width	22	7.23	6.23–8.10	0.474	20	6.34–7.73	0.440
Length of cephalic cilia (anterior tuft)	22	5.40–22.12	(4.51–6.19)–(19.49–22.88)	0.412; 0.904	20	(4.08–5.48)–(15.76–22.11)	0.412; 1.350
Length of cephalic cilia (posterior tuft)	22	10.51–25.46	(9.33–11.75)–(21.52–26.64)	0.444–1.237	20	(8.06–10.78)–(17.42–24.53)	0.800; 1.738
Width of cephalic cilia (anterior tuft)	22	0.84–0.87	(0.77–0.9)–(0.80–0.94)	0.035–0.037	20	(0.73–0.90)–(0.81–0.94)	0.053; 0.050
Width of cephalic cilia (posterior tuft)	22	0.86–0.90	(0.81–0.94)–(0.84–0.97)	0.037–0.036	20	(0.74–0.91)–(0.81–0.98)	0.051; 0.055
Mouth ring diameter	22	5.23	4.73–5.37	0.149	20	4.18–5.22	0.367
Furca length	22	18.87	13.38–19.68	0.317	20	16.83–18.94	0.679
Adhesive tube length	22	8.63	8.19–9.73	0.370	20	7.34–8.81	0.404
Adhesive tube width	22	1.48	1.43–1.55	0.033	20	1.30–1.52	0.056
Number of scales in central longitudinal row	81	30	28–44	2.778	35	26–42	3.399
Total number of longitudinal alternating rows of scales	81	35	33–47	2.480	35	33–47	3.100
Length of head dorsal scales	22	1.24–3.94	(1.16–1.83)–(3.17–4.21)	0.186; 0.268	20	(0.83–1.31)–(2.94–3.89)	0.105; 0.265
Width of head dorsal scales	22	1.17–3.12	(1.10–1.59)–(2.72–3.84)	0.132; 0.294	20	(0.70–1.22)–(2.32–3.10)	0.147; 0.211
Length of head dorsolateral scales	22	1.81–3.85	(1.63–2.19)–(3.02–4.19)	0.142; 0.267	20	(1.17–1.83)–(2.89–3.85)	0.170; 0.268
Width of head dorsolateral scales	22	1.23–3.10	(1.19–1.62)–(2.69–3.79)	0.123; 0.289	20	(0.91–1.39)–(2.27–3.15)	0.104; 0.220
Length of head lateral scales	22	1.83–3.82	(1.63–2.22)–(3.01–4.17)	0.146; 0.226	20	(1.20–1.87)–(2.83–3.81)	0.172; 0.272
Width of head lateral scales	22	1.25–3.06	(1.21–1.65)–(2.66–3.76)	0.125; 0.292	20	(0.93–1.41)–(2.32–3.11)	0.106; 0.215
Length of head ventrolateral scales	22	1.76–3.68	(1.52–2.13)–(2.63–4.01)	0.147; 0.365	20	(1.11–1.81)–(2.59–3.69)	0.173; 0.273
Width of head ventrolateral scales	22	1.20–2.83	(1.06–1.60)–(2.41–3.49)	0.122; 0.278	20	(0.78–1.34)–(2.02–2.87)	0.122; 0.211
Length of head ventral scales	22	0.83–2.73	(0.62–1.14)–(1.91–3.14)	0.139; 0.351	20	(0.53–0.91)–(1.61–2.58)	0.077; 0.272
Width of head ventral scales	22	0.68–2.11	(0.49–0.96)–(1.38–2.71)	0.139; 0.303	20	(0.45–0.77)–(1.26–2.06)	0.074; 0.237
Length of neck dorsal scales	22	3.01–4.42	(2.60–3.61)–(4.03–5.07)	0.243; 0.293	20	(2.03–2.91)–(3.21–4.23)	0.245; 0.301
Width of neck dorsal scales	22	2.22–3.08	(1.83–2.97)–(2.67–4.01)	0.267; 0.346	20	(1.44–2.16)–(2.18–2.95)	0.191; 0.226
Length of neck dorsolateral scales	22	2.96–4.38	(2.55–3.58)–(3.71–5.01)	0.243; 0.329	20	(1.96–2.89)–(3.17–4.18)	0.252; 0.303
Width of neck dorsolateral scales	22	2.20–3.05	(1.81–2.95)–(2.65–3.96)	0.267; 0.339	20	(1.39–2.13)–(2.13–2.90)	0.191; 0.230
Length of neck lateral scales	22	2.93–4.34	(2.51–3.52)–(3.67–4.96)	0.506; 0.333	20	(1.89–2.86)–(3.13–4.14)	0.254; 0.301
Width of neck lateral scales	22	2.17–3.03	(1.77–2.90)–(2.62–3.92)	0.266; 0.337	20	(1.33–2.09)–(2.11–2.86)	0.192; 0.227
Length of neck ventrolateral scales	22	2.46–3.85	(2.07–3.02)–(3.07–4.39)	0.246–0.317	20	(1.49–2.40)–(2.09–3.66)	0.226; 0.384

Table A1. Continued.

Characters	N	Holotype	Range of adult paratypes	SD	N	Range of juvenile and subadult paratypes	SD
Width of neck ventrolateral scales	22	1.82–2.39	(1.43–2.36)–(2.12–3.32)	0.259; 0.312	20	(1.02–1.78)–(1.59–2.66)	0.187; 0.240
Length of neck ventral scales	22	1.41–2.86	(1.10–1.69)–(2.14–3.48)	0.175; 0.331	20	(0.82–1.38)–(1.62–2.70)	0.159; 0.327
Width of neck ventral scales	22	1.24–2.18	(0.87–1.63)–(1.71–2.94)	0.170; 0.299	20	(0.71–1.20)–(1.22–2.37)	0.139; 0.295
Length of trunk dorsal scales	22	3.62–5.10	(3.14–4.06)–(4.60–5.91)	0.284; 0.332	20	(2.57–3.47)–(3.98–4.91)	0.239; 0.295
Width of trunk dorsal scales	22	2.53–4.33	(2.12–3.08)–(3.29–5.05)	0.263; 0.399	20	(1.77–2.41)–(3.11–4.13)	0.191; 0.304
Length of trunk dorsolateral scales	22	3.59–5.02	(3.08–4.02)–(4.58–5.82)	0.287; 0.310	20	(2.53–3.42)–(3.92–4.84)	0.241; 0.302
Width of trunk dorsolateral scales	22	2.51–4.19	(2.09–3.06)–(3.09–4.82)	0.264; 0.379	20	(1.74–2.37)–(3.02–4.03)	0.192; 0.311
Length of trunk lateral scales	22	3.56–4.97	(3.03–3.97)–(4.55–5.76)	0.287; 0.309	20	(2.48–3.38)–(3.86–4.79)	0.246; 0.310
Width of trunk lateral scales	22	2.47–4.14	(2.02–3.03)–(3.03–4.79)	0.265; 0.389	20	(1.70–2.33)–(2.97–3.97)	0.193; 0.313
Length of trunk ventrolateral scales	22	3.06–4.91	(2.54–3.48)–(4.45–5.69)	0.271; 0.306	20	(2.01–2.94)–(3.42–4.72)	0.258; 0.368
Width of trunk ventrolateral scales	22	2.15–4.10	(1.78–2.42)–(2.99–4.75)	0.213; 0.405	20	(1.44–2.08)–(2.88–3.95)	0.186; 0.312
Length of trunk ventral scales	22	2.21–3.36	(1.74–2.56)–(3.02–4.34)	0.239; 0.381	20	(1.18–2.10)–(2.42–3.34)	0.275; 0.253
Width of trunk ventral scales	22	1.87–2.88	(1.39–2.09)–(2.06–4.04)	0.199; 0.456	20	(0.94–1.77)–(1.99–2.85)	0.242; 0.235
Length of small one–lobed scales	22	1.91–2.48	(1.24–2.44)–(1.96–3.13)	0.362; 0.368	20	(0.91–2.00)–(1.44–2.79)	0.253; 0.357
Width of small one–lobed scales	22	1.28–1.97	(0.96–1.95)–(1.37–2.42)	0.289; 0.295	20	(0.49–1.23)–(0.81–1.96)	0.162; 0.252
Length of furcal appendages dorsal scales	22	5.11–5.23	(3.43–5.59)–(3.94–5.92)	0.540; 0.484	20	(2.63–5.07)–(3.08–5.29)	0.598; 0.598
Width of furcal appendages dorsal scales	22	2.26–2.39	(1.59–2.74)–(1.66–2.83)	0.307; 0.316	20	(0.97–2.20)–(1.08–2.64)	0.286; 0.355
Length of parafurcal scales	22	3.02–3.17	(2.12–3.82)–(2.29–3.94)	0.489; 0.455	20	(1.62–3.53)–(1.89–3.76)	0.505; 0.529
Width of parafurcal scales	22	2.43–2.62	(1.53–3.31)–(1.66–3.49)	0.444; 0.427	20	(1.30–2.86)–(1.52–2.98)	0.424; 0.415
Length of head dorsal spines	22	1.81–3.23	(1.26–2.35)–(2.76–4.01)	0.274; 0.306	20	(1.08–1.86)–(2.24–3.12)	0.225; 0.245
Length of head dorsolateral spines	22	2.04–3.36	(1.35–2.49)–(2.92–4.12)	0.272; 0.292	20	(1.19–2.09)–(2.31–3.24)	0.264; 0.251
Length of head lateral spines	22	2.41–3.49	(1.68–2.74)–(3.04–4.32)	0.276; 0.315	20	(1.34–2.43)–(2.42–3.33)	0.308; 0.263
Length of head ventrolateral spines	22	2.58–3.62	(1.84–2.89)–(3.18–4.56)	0.270; 0.322	20	(1.41–2.57)–(2.51–3.46)	0.319; 0.275
Length of head ventral spines	22	5.96–8.61	(4.66–.686)–(7.81–9.95)	0.638; 0.667	20	(3.96–5.61)–(6.21–9.04)	0.508; 0.819
Length of neck dorsal spines	22	2.19–2.96	(1.40–2.42)–(2.57–3.36)	0.261; 0.231	20	(1.20–2.06)–(2.12–2.87)	0.245; 0.200
Length of neck dorsolateral spines	22	2.30–3.18	(1.00–2.56)–(2.70–3.52)	0.403; 0.230	20	(1.26–2.14)–(2.26–3.01)	0.267; 0.200
Length of neck lateral spines	22	2.37–3.26	(1.63–2.62)–(2.79–3.67)	0.247; 0.232	20	(1.31–2.20)–(2.34–3.12)	0.269; 0.213
Length of neck ventrolateral spines	22	2.49–3.40	(1.76–2.78)–(2.94–3.75)	0.256; 0.232	20	(1.39–2.31)–(2.42–3.26)	0.275; 0.220
Length of neck ventral spines	22	5.72–7.24	(4.22–6.41)–(6.89–8.89)	0.631; 0.604	20	(3.49–5.09)–(5.59–8.43)	0.418; 0.719
Length of trunk dorsal spines	22	2.26–3.32	(1.52–2.63)–(2.95–4.28)	0.252; 0.349	20	(1.28–2.18)–(2.51–3.22)	0.246; 0.205
Length of trunk dorsolateral spines	22	2.39–3.47	(1.66–2.87)–(3.08–4.35)	0.262; 0.344	20	(1.40–2.24)–(2.62–3.37)	0.241; 0.213
Length of trunk lateral spines	22	2.51–3.65	(1.74–2.94)–(3.27–4.51)	0.267; 0.333	20	(1.48–2.37)–(2.71–3.48)	0.251; 0.226
Length of trunk ventrolateral spines	22	2.62–3.96	(1.91–3.06)–(3.34–4.74)	0.263; 0.356	20	(1.53–2.43)–(2.82–3.63)	0.246; 0.217
Length of trunk ventral spines	22	6.29–11.88	(5.15–7.19)–(8.54–13.10)	0.588; 1.297	20	(4.19–6.16)–(7.38–11.91)	0.536; 1.201
Length of furcal appendages dorsal spines	22	2.96–3.23	(2.11–3.19)–(2.33–3.75)	0.346–0.437	20	(1.69–2.86)–(2.19–3.44)	0.325; 0.342

Table A1. Continued.

Characters	N	Holotype	Range of adult paratypes	SD	N	Range of juvenile and subadult paratypes	SD
Length of trunk posteriormost pair of lateral spines	22	6.36	4.33–7.18	0.855	20	4.03–6.59	0.803
Length of posteriormost parafurcal spines	22	7.1	5.54–7.59	0.622	20	5.16–7.34	0.618
Number of scales in single longitudinal row on ventral interciliary field	81	5	3–8	0.758	35	3–7	0.810
Total number of longitudinal alternating rows of scales on ventral interciliary field	81	5	5–7	0.782	35	5–7	0.646
Length of ventral interciliary field scales	22	2.46–2.82	(1.67–2.61)–(2.32–3.70)	0.264; 0.371	20	(0.81–2.60)–(1.59–3.11)	0.420; 0.435
Width of ventral interciliary field scales	22	1.46–1.69	(0.90–1.68)–(1.25–2.34)	0.185; 0.319	20	(0.46–1.72)–(0.90–1.85)	0.266; 0.232
Length of posteriormost ventral interciliary field scales	22	6.37	4.91–8.91	0.821	20	3.69–5.86	0.554
Width of posteriormost ventral interciliary field scales	22	2.62	2.26–2.90	0.211	20	1.63–2.83	0.315
Head dorsal sensory bristles length	22	10.21	8.36–13.38	1.158	20	8.07–10.45	0.819
Neck dorsal sensory bristles length	22	13.78	10.59–15.96	1.51	20	10.20–13.41	0.867
Posterior dorsal sensory bristles length	22	11.21	8.79–12.31	0.877	20	8.67–11.25	0.837
Number of cephalic cilia in anterior tuft	81	5	5–6	0.190	35	5–6	0.236
Number of cephalic cilia in posterior tuft	81	5	5–7	0.289	35	5–6	0.168
Pharynx length	65	30.52	26.48–35.10	1.801	29	26.51–36.13	2.222
Width of anterior pharynx thickening (a)	22	10.17	9.26–10.87	0.383	20	7.37–10.97	0.775
Width of pharynx narrowing (n)	22	6.93	6.46–7.47	0.224	20	5.17–7.73	0.528
Width of middle pharynx (m)	22	7.49	7.14–8.39	0.349	20	6.19–8.76	0.561
Width of posterior pharynx thickening (p)	22	11.36	10.16–11.78	0.412	20	8.06–11.70	0.779
Width of pharyngeal–intestinal junction	22	1.62	1.61–1.95	0.099	20	1.37–2.02	0.14
Length of intestine anterior differ section – in it central part	22	2.67	1.94–4.46	0.530	20	2.05–3.18	0.309
Length of intestine anterior differ section – in it lateral part	22	7.30	6.51–8.90	0.540	20	5.56–7.90	0.697
Pharynx formula a	22	*33.322*	*29.086–35.808*	2.0189	20	*27.801–36.662*	2.6467
Pharynx formula n	22	*22.706*	*19.84–24.522*	1.2052	20	*18.990–26.775*	2.0475
Pharynx formula m	22	*24.541*	*24.937–28.552*	1.7202	20	*22.407–28.131*	1.9525
Pharynx formula p	22	*37.221*	*32.501–40.232*	2.0758	20	*30.404–39.371*	2.5046
Pharynx–intestine ratio I	65	*44.419*	*43.250–54.998*	3.2250	29	*55.383–89.096*	9.2504
Ratio of scale distribution	81	*116.67*	*94.74–120*	4.7292	35	*102.630–138.460*	7.2420
Length of matured egg	13	–	*35.62–56.95*	5.169	–	–	–
Width of matured egg	13	–	*28.26–38.84*	3.043	–	–	–

Tabela A2. Morphometric of *Chaetonotus* (*Chaetonotus*) *gelidus* sp. nov; N – number of specimens or structures analysed, Range – the smallest and the largest structure found among all specimens measured, SD – standard deviation. All measurements are given in micrometres (μm); all indicators are given as a percentage (%) and italicized.

Characters	N	Holotype	Range of adult paratypes	SD	N	Range of juvenile and subadult paratypes	SD
Body length	112	106.91	80.29–115.16	5.207	25	75.15–103.27	6.281
Cephalion length	21	9.62	8.92–10.19	0.380	20	8.13–9.96	0.468
Cephalion width	21	8.22	8.22–9.52	0.349	20	7.66–9.36	0.429
Cephalion maximum width	21	12.02	11.15–12.97	0.517	20	10.38–12.58	0.627
Hypostomium length	21	4.96	3.69–4.96	0.301	20	3.09–5.47	0.474
Hypostomium width	21	7.97	6.05–9.72	0.866	20	5.05–8.16	0.793
Length of cephalic cilia (anterior tuft)	21	6.12–25.24	(5.28–7.09)–(20.55–26.71)	0.399; 1.445	20	(4.79–6.57)–(22.26–25.46)	0.420; 0.979
Length of cephalic cilia (posterior tuft)	21	6.35–15.99	(6.04–7.33)–(12.42–16.74)	0.33; 1.031	20	(5.71–7.24)–(13.89–15.98)	0.406; 0.534
Width of cephalic cilia (anterior tuft)	21	0.51–1.05	(0.47–0.59)–(0.97–1.23)	0.032; 0.60	20	(0.47–0.54)–(0.97–1.14)	0.021; 0.035
Width of cephalic cilia (posterior tuft)	21	0.52–0.97	(0.52–0.61)–(0.89–1.16)	0.031–0.055	20	(0.47–0.58)–(0.90–0.98)	0.027; 0.026
Mouth ring diameter	21	4.37	4.16–5.03	0.212	20	3.81–4.74	0.225
Furca length	21	18.80	16.82–19.05	0.603	20	3.81–4.74	0.225
Adhesive tube length	21	7.58	6.91–8.13	0.318	20	6.69–7.96	0.349
Adhesive tube width	21	1.52	1.49–1.62	0.037	20	1.28–1.560	0.060
Number of scales in central longitudinal row	115	26	23–28	1.436	25	23–28	1.500
Total number of longitudinal alternating rows of scales	115	35	31–37	1.743	25	31–37	1.665
Length of head dorsal scales	21	1.40–3.53	(1.19–2.01)–(3.04–4.26)	0.197; 0.304	20	(0.79–1.37)–(2.26–3.46)	0.140–0.319
Width of head dorsal scales	21	2.02–3.09	(1.62–2.63)–(2.67–3.76)	0.227; 0.288	20	(1.14–1.99)–(1.99–3.37)	0.206; 0.317
Length of head dorsolateral scales	21	1.28–3.06	(1.05–1.84)–(2.52–3.72)	0.181; 0.294	20	(0.72–1.26)–(1.97–3.01)	0.130; 0.276
Width of head dorsolateral scales	21	1.94–2.84	(1.48–2.52)–(2.33–3.49)	0.227; 0.289	20	(1.09–1.91)–(1.83–2.79)	0.199; 0.256
Length of head lateral scales	21	1.72–2.92	(1.38–2.34)–(2.39–3.61)	0.218; 0.292	20	(0.94–1.70)–(1.89–2.88)	0.180; 0.261
Width of head lateral scales	21	2.24–2.79	(1.82–2.87)–(2.28–3.43)	0.257; 0.283	20	(1.26–2.21)–(1.80–2.74)	0.234; 0.248
Length of head ventrolateral scales	21	1.56–2.81	(1.23–2.21)–(2.29–3.50)	0.219; 0.283	20	(0.88–1.55)–(1.81–2.77)	0.171; 0.254
Width of head ventrolateral scales	21	2.01–2.68	(1.59–2.64)–(2.17–3.31)	0.249; 0.278	20	(1.13–1.99)1.72–2.63)	0.220; 0.238
Length of head ventral scales	21	1.10–1.93	(0.88–1.59)–(1.44–2.42)	0.170; 0.232	20	(0.68–1.09)–(1.30–1.90)	0.107; 0.164
Width of head ventral scales	21	1.39–1.78	(1.11–1.93)–(1.33–2.29)	0.202; 0.224	20	(0.86–1.38)–(1.20–1.76)	0.135; 0.152
Length of neck dorsal scales	21	2.92–4.31	(2.34–3.52)–(3.84–4.96)	0.310; 0.270	20	(1.64–2.85)–(2.76–4.22)	0.302; 0.396
Width of neck dorsal scales	21	1.97–2.75	(1.55–2.59)–(2.36–3.08)	0.247; 0.194	20	(1.11–1.93)–(1.70–2.69)	0.202; 0.250
Length of neck dorsolateral scales	21	2.63–4.01	(2.06–3.30)–(3.61–4.52)	0.312; 0.236	20	(1.48–2.57)–(2.58–3.94)	0.270; 0.369
Width of neck dorsolateral scales	21	1.74–2.52	(1.36–2.41)–(2.22–2.88)	0.250; 0.180	20	(0.98–1.70)–(1.62–2.48)	0.175; 0.230
Length of neck lateral scales	21	2.41–3.88	(1.87–3.08)–(3.49–4.37)	0.296; 0.230	20	(1.36–2.36)–(2.50–3.83)	0.247; 0.362
Width of neck lateral scales	21	1.62–2.43	(1.23–2.29)–(2.08–2.76)	0.244; 0.179	20	(0.91–1.58)–(1.56–2.39)	0.163; 0.223

Table A2. Continued.

Characters	N	Holotype	Range of adult paratypes	SD	N	Range of juvenile and subadult paratypes	SD
Length of neck ventrolateral scales	21	2.35–3.72	(1.79–3.01)–(2.57–4.21)	0.292; 0.327	20	(1.32–2.29)–(2.40–3.68)	0.242; 0.353
Width of neck ventrolateral scales	21	1.57–2.36	(1.19–2.22)–(1.99–2.68)	0.235; 0.182	20	(0.88–1.53)–(1.52–2.37)	0.161; 0.252
Length of neck ventral scales	21	1.33–2.21	(1.10–1.83)–(1.81–2.86)	0.182; 0.246	20	(0.82–1.29)–(1.52–2.19)	0.122; 0.185
Width of neck ventral scales	21	1.06–1.62	(0.86–1.54)–(1.34–2.25)	0.168; 0.214	20	(0.67–1.25)–(1.12–1.61)	0.154; 0.135
Length of trunk dorsal scales	21	3.48–5.51	(2.97–3.73)–(4.58–6.22)	0.249; 0.426	20	(2.24–3.42)–(3.53–5.41)	0.324; 0.510
Width of trunk dorsal scales	21	2.21–3.22	(1.83–2.42)–(2.59–3.86)	0.196; 0.314	20	(1.43–2.18)–(2.06–3.16)	0.205; 0.296
Length of trunk dorsolateral scales	21	3.23–4.78	(2.78–3.56)–(3.83–5.43)	0.246–0.408	20	(2.07–3.17)–(3.07–4.70)	0.301; 0.442
Width of trunk dorsolateral scales	21	2.03–2.76	(1.61–2.37)–(2.21–3.46)	0.207; 0.311	20	(1.30–1.99)–(1.77–2.76)	0.188; 0.266
Length of trunk lateral scales	21	3.02–4.61	(2.61–3.34)–(3.64–5.32)	0.243; 0.407	20	(1.94–2.96)–(2.95–4.52)	0.281; 0.428
Width of trunk lateral scales	21	1.84–2.52	(1.43–2.13)–(1.98–3.19)	0.193; 0.299	20	(1.18–1.80)–(1.61–2.65)	0.170; 0.263
Length of trunk ventrolateral scales	21	2.86–4.42	(2.32–3.12)–(3.61–5.04)	0.244; 0.385	20	(1.83–2.79)–(2.84–4.35)	0.267; 0.412
Width of trunk ventrolateral scales	21	1.79–2.43	(1.28–2.07)–(1.85–3.06)	0.196; 0.283	20	(1.14–1.74)–(1.56–2.39)	0.164; 0.225
Length of trunk ventral scales	21	1.48–2.77	(1.28–2.02)–(2.25–3.82)	0.193; 0.380	20	(0.95–1.45)–(1.78–2.72)	0.135; 0.252
Width of trunk ventral scales	21	1.17–1.90	(1.05–1.64)–(1.59–2.93)	0.167; 0.300	20	(0.74–1.26)–(1.22–1.90)	0.122; 0.191
Length of small one-lobed scales	21	2.28	1.63–3.28	0.455	20	1.07–2.44	0.35
Width of small one-lobed scales	21	1.61	1.06–2.66	0.459	20	0.62–1.56	0.255
Length of furcal base scales	21	3.49–4.46	(2.98–4.89)–(3.73–5.84)	0.578; 0.544	20	(1.86–3.72)–(2.23–4.39)	0.475; 0.596
Width of furcal base scales	21	1.84–2.37	(1.16–2.82)–(1.60–3.18)	0.432; 0.414	20	(0.96–2.28)–(1.13–2.46)	0.306; 0.311
Length of furcal appendages double--keeled scales	21	4.08	3.80–6.11	0.646	20	2.39–4.42	0.448
Width of furcal appendages double--keeled scales	21	3.42	2.89–4.68	0.494	20	1.77–3.62	0.412
Length of parafurcal scales	21	2.77–2.90	(2.28–3.82)–(2.68–4.15)	0.337; 0.411	20	(1.71–2.84)–(2.07–2.92)	0.280; 0.226
Width of parafurcal scales	21	1.86–1.94	(1.63–2.62)–(1.77–3.34)	0.276; 0.429	20	(1.31–2.02)–(1.41–2.17)	0.218; 0.220
Length of head dorsal spines	21	2.40–3.89	(1.69–2.78)–(3.02–4.48)	0.288; 0.417	20	(1.26–2.22)–(2.54–3.43)	0.267; 0.271
Length of head dorsolateral spines	21	2.45–3.92	(1.73–2.82)–(3.04–4.53)	0.293; 0.421	20	(1.29–2.26)–(2.56–3.45)	0.271; 0.270
Length of head lateral spines	21	2.53–4.04	(1.81–2.88)–(3.16–4.62)	0.295; 0.418	20	(1.33–2.33)–(2.64–3.57)	0.275; 0.272
Length of head ventrolateral spines	21	2.57–4.10	(1.87–2.91)–(2.76–4.69)	0.296; 0.492	20	(1.36–2.37)–(2.69–3.63)	0.272; 0.276
Length of head ventral spines	21	2.96–5.28	(2.44–3.29)–(4.55–6.37)	0.242; 0.493	20	(1.91–2.78)–(4.12–5.09)	0.226; 0.272
Length of neck dorsal spines	21	2.13–2.39	(1.22–2.46)–(1.67–2.73)	0.347; 0.283	20	(1.03–1.84)–(1.23–2.18)	0.236; 0.268
Length of neck dorsolateral spines	21	2.17–2.44	(1.29–2.51)–(1.71–2.78)	0.346; 0.291	20	(1.07–1.87)–(1.27–2.21)	0.235; 0.269
Length of neck lateral spines	21	2.28–2.56	(1.38–2.60)–(1.85–2.91)	0.351; 0.293	20	(1.14–1.96)–(1.34–2.32)	0.235; 0.281
Length of neck ventrolateral spines	21	2.32–2.63	(1.43–2.65)–(1.93–2.99)	0.354; 0.289	20	(1.18–1.99)–(1.41–2.41)	0.233; 0.272
Length of neck ventral spines	21	2.70–5.14	(2.02–3.08)–(4.39–6.23)	0.302; 0.490	20	(1.68–2.43)–(4.04–4.93)	0.201; 0.271

Table A2. Continued.

Characters	N	Holotype	Range of adult paratypes	SD	N	Range of juvenile and subadult paratypes	SD
Length of trunk dorsal spines	21	1.64–2.36	(1.09–2.07)–(1.63–2.68)	0.249; 0.285	20	(0.64–1.43)–(1.22–2.15)	0.180; 0.278
Length of trunk dorsolateral spines	21	1.68–2.42	(1.14–2.12)–(1.70–2.74)	0.250; 0.289	20	(0.66–1.48)–(1.26–2.19)	0.185; 0.280
Length of trunk lateral spines	21	1.77–2.52	(1.25–2.23)–(1.82–2.86)	0.254; 0.294	20	(0.75–1.56)–(1.32–2.30)	0.183; 0.287
Length of trunk ventrolateral spines	21	1.82–4.18	(1.34–2.29)–(3.11–5.03)	0.253; 0.479	20	(0.82–1.61)–(2.92–3.88)	0.179; 0.275
Length of trunk ventral spines	21	2.61–5.10	(1.96–3.01)–(4.34–6.12)	0.293; 0.481	20	(1.66–2.37)–(2.24–4.88)	0.187; 0.574
Length of small one-lobed scales spines	21	0.82	0.54–1.22	0.224	20	0.37–0.74	0.113
Length of furcal appendages dorsal spines	21	1.49–1.66	(1.20–1.94)–(1.36–2.09)	0.221; 0.185	20	(1.01–1.49)–(1.15–1.69)	0.123; 0.128
Length of trunk posteriormost pair of lateral spines	21	4.06	2.74–4.67	0.543	20	2.71–3.93	0.404
Length of posteriormost parafurcal spines	21	3.92	2.61–4.53	0.556	20	2.54–3.74	0.386
Head dorsal sensory bristles length	21	8.97	7.36–9.74	0.593	20	6.57–10.40	0.914
Neck dorsal sensory bristles length	21	12.41	10.48–14.04	0.961	20	10.06–13.54	0.927
Posterior dorsal sensory bristles length	21	9.87	8.43–11.03	0.634	20	6.54–11.75	1.162
Number of scales in single longitudinal row on ventral interciliary field	115	21	19–24	1.445	25	17–21	1.114
Total number of longitudinal alternating rows of scales on ventral interciliary field	115	8	5–9	0.468	25	7–8	0.200
Length of ventral interciliary field scales	21	1.41–4.43	(1.06–1.69)–(3.39–4.66)	0.129; 0.362	20	(0.61–1.28)–(2.26–3.72)	0.211; 0.325
Width of ventral interciliary field scales	21	0.90–1.36	(0.60–1.69)–(1.10–2.38)	0.209; 0.278	20	(0.34–0.73)–(0.73–1.28)	0.122; 0.156
Length of posteriormost ventral interciliary field scales	21	5.21	4.82–6.77	0.399	20	3.60–6.06	0.526
Width of posteriormost ventral interciliary field scales	21	2.19	1.81–3.36	0.305	20	1.07–2.24	0.259
Number of cephalic cilia in anterior tuft	115	8	7–8	0.131	25	7–8	0.200
Number of cephalic cilia in posterior tuft	115	8	7–8	0.161	25	8	0
Pharynx length	92	26.98	21.65–32.99	1.469	23	25.05–29.20	1.014
Width of anterior pharynx thickening (a)	21	8.14	7.70–8.76	0.236	20	7.42–8.48	0.281
Width of pharynx narrowing (n)	21	5.28	5.28–6.47	0.292	20	4.95–6.70	0.46
Width of middle pharynx (m)	21	5.54	5.54–7.09	0.333	20	5.26–7.05	0.435
Width of posterior pharynx thickening (p)	21	8.47	8.47–9.11	0.169	20	7.76–8.96	0.341
Width of pharyngeal-intestinal junction	21	2.26	2.02–3.25	0.269	20	1.84–2.75	0.228

Table A2. Continued.

Characters	N	Holotype	Range of adult paratypes	SD	N	Range of juvenile and subadult paratypes	SD
Length of intestine anterior differ section – in it central part	21	2.18	1.82–2.81	0.233	20	1.68–2.33	0.155
Length of intestine anterior differ section – in it lateral part	21	4.76	2.85–4.87	0.472	20	2.26–4.14	0.498
Pharynx formula a	21	*30.17*	*27.753–32.076*	1.2000	20	*27.386–33.812*	1.8114
Pharynx formula n	21	*19.57*	*19.166–23.173*	1.1154	20	*18.092–25.230*	2.005
Pharynx formula m	21	*20.534*	*20.534–25.394*	1.2077	20	*19.191–26.188*	1.8737
Pharynx formula p	21	*31.394*	*29.799–33.908*	1.0723	20	*28.450–35.409*	1.8700
Pharynx–intestine ratio I	92	*45.072*	*37.876–53.935*	2.8975	23	*49.845–83.020*	8.7903
Ratio of scale distribution	115	*134.62*	*110.71–154.17*	7.724	25	*110.71–154.16*	9.791
Length of matured egg	21	–	34.5–48.08	2.936	–	–	–
Width of matured egg	21	–	20.95–34.69	3.175	–	–	–
Length of organ X	21	–	5.52–6.09	0.403	–	–	–
Width of organ X	21	–	*1*1.74–12.44	0.495	–	–	–

Table A3. Morphometric of *Chaetonotus* (*Chaetonotus*) *svalbardi* sp. nov; N – number of specimens or structures analysed, Range – the smallest and the largest structure found among all specimens measured, SD – standard deviation. All measurements are given in micrometres (μm); all indicators are given as a percentage (%) and italicized.

Characters	N	Holotype	Range of adult paratypes	SD
Body length	5	119.59	109.12–120.87	4.925
Cephalion length	5	8.49	8.09–8.55	0.177
Cephalion width	5	10.23	10.17–10.59	0.184
Cephalion maximum width	5	13.1	12.82–13.13	0.122
Hypostomium length	5	6.17	6.07–7.20	0.491
Hypostomium width	5	2.95	2.79–3.15	0.136
Length of cephalic cilia (anterior tuft)	5	5.33–16.67	(4.72–5.89)–(16.62–18.04)	0.416; 0.574
Length of cephalic cilia (posterior tuft)	5	9.32–22.46	(9.06–10.80)–(19.54–22.46)	0.806; 1.177
Width of cephalic cilia (anterior tuft)	5	0.65–0.74	(0.61–0.72)–(0.73–0.76)	0.040; 0.130
Width of cephalic cilia (posterior tuft)	5	0.71–0.80	(0.69–0.76)–(0.80–0.89)	0.028; 0.035
Mouth ring diameter	5	4.46	4.44–4.92	0.220
Furca length	5	19.49	18.85–19.62	0.311
Adhesive tube length	5	11.43	10.39–11.51	0.448
Adhesive tube width	5	1.49	1.43–1.49	0.029
Number of scales in central longitudinal row	5	21	21–23	0.894
Total number of longitudinal alternating rows of scales	5	25	25–27	1.095
Length of head dorsal scales	5	2.37–3.39	(2.30–2.40)–(3.39–3.91)	0.038; 0.188
Width of head dorsal scales	5	1.49–4.31	(1.41–1.71)–(4.31–4.98)	0.121; 0.293
Length of head dorsolateral scales	5	2.21–3.32	(2.17–2.32)–(3.32–3.87)	0.062; 0.203
Width of head dorsolateral scales	5	1.61–4.16	(1.51–1.64)–(4.16–4.56)	0.056; 0.156
Length of head lateral scales	5	2.17–3.28	(2.10–2.28)–(3.28–3.82)	0.068; 0.205
Width of head lateral scales	5	1.77–4.11	(1.60–1.79)–(4.11–4.42)	0.089; 0.123
Length of head ventrolateral scales	5	2.14–3.25	(2.06–2.19)–(3.25–3.77)	0.053; 0.197
Width of head ventrolateral scales	5	1.80–4.08	(1.68–1.84)–(4.08–4.38)	0.069; 0.118
Length of head ventral scales	5	1.03–2.87	(0.96–1.19)–(2.41–3.65)	0.091; 0.448
Width of head ventral scales	5	0.85–3.42	(0.81–0.98)–(2.39–4.04)	0.077; 0.611
Length of neck dorsal scales	5	3.04–4.94	(3.01–3.39)–(4.88–5.19)	0.178; 0.122
Width of neck dorsal scales	5	3.11–4.35	(3.07–3.62)–(4.35–4.64)	0.281; 0.122
Length of neck dorsolateral scales	5	2.98–4.89	(2.98–3.47)–(4.81–4.97)	0.220; 0.071
Width of neck dorsolateral scales	5	2.87–4.16	(2.87–3.57)–(4.16–4.60)	0.281; 0.185
Length of neck lateral scales	5	2.96–4.64	(2.81–3.33)–(4.64–4.93)	0.223; 0.108
Width of neck lateral scales	5	2.83–4.12	(2.77–3.52)–(4.12–4.54)	0.306; 0.179
Length of neck ventrolateral scales	5	2.76–4.57	(2.74–3.24)–(4.57–4.79)	0.251; 0.078
Width of neck ventrolateral scales	5	2.81–4.07	(2.79–3.48)–(4.07–4.44)	0.302; 0.147
Length of neck ventral scales	5	1.41–3.59	(1.37–1.93)–(3.05–3.67)	0.223; 0.265
Width of neck ventral scales	5	1.16–3.06	(1.14–1.78)–(2.89–3.18)	0.268; 0.125
Length of trunk dorsal scales	5	3.79–8.03	(3.50–4.04)–(7.74–8.28)	0.213; 0.201
Width of trunk dorsal scales	5	3.31–6.90	(3.17–3.66)–(6.45–7.12)	0.210; 0.243
Length of trunk dorsolateral scales	5	3.72–6.50	(3.48–4.00)–(6.23–7.08)	0.205; 0.322
Width of trunk dorsolateral scales	5	3.27–6.19	(3.12–3.61)–(4.97–6.45)	0.206; 0.584
Length of trunk lateral scales	5	3.80–6.45	(3.52–3.94)–(6.18–6.76)	0.163; 0.242
Width of trunk lateral scales	5	3.24–6.04	(3.19–3.58)–(4.95–6.12)	0.184; 0.496
Length of trunk ventrolateral scales	5	3.76–5.54	(3.38–3.92)–(5.53–5.90)	0.208; 0.154
Width of trunk ventrolateral scales	5	3.18–4.47	(3.10–3.55)–(4.47–4.86)	0.205; 0.164

Table A3. Continued.

Characters	N	Holotype	Range of adult paratypes	SD
Length of trunk dorsolateral scales	5	3.72–6.50	(3.48–4.00)–(6.23–7.08)	0.205; 0.322
Width of trunk dorsolateral scales	5	3.27–6.19	(3.12–3.61)–(4.97–6.45)	0.206; 0.584
Length of trunk lateral scales	5	3.80–6.45	(3.52–3.94)–(6.18–6.76)	0.163; 0.242
Width of trunk lateral scales	5	3.24–6.04	(3.19–3.58)–(4.95–6.12)	0.184; 0.496
Length of trunk ventrolateral scales	5	3.76–5.54	(3.38–3.92)–(5.53–5.90)	0.208; 0.154
Width of trunk ventrolateral scales	5	3.18–4.47	(3.10–3.55)–(4.47–4.86)	0.205; 0.164
Length of trunk ventral scales	5	1.90–5.18	(1.83–2.31)–(4.17–5.21)	0.186; 0.436
Width of trunk ventral scales	5	1.46–4.15	(1.38–1.69)–(3.60–4.19)	0.120; 0.283
Length of small one-lobed scales	5	4.06	3.32–4.06	0.310
Width of small one-lobed scales	5	2.12	2.10–2.87	0.322
Length of furcal base scales	5	6.21–7.51	(6.4–6.65)–(6.50–7.70)	0.295; 0.539
Width of furcal base scales	5	3.66–4.03	(3.28–3.77)–(3.65–4.14)	0.211; 0.221
Length of furcal appendages double-eeled scales	5	5.74	5.33–5.83	0.237
Width of furcal appendages double-keeled scales	5	4.27	4.27–4.65	0.201
Length of parafurcal scales	5	2.94	2.74–3.29	0.257
Width of furcal parafurcal scales	5	2.02	1.86–2.37	0.237
Length of furcal appendages ventrolateral and ventral scales	4	2.33–2.96	(2.13–2.35)–(2.49–2.96)	0.104; 0.203
Width of furcal appendages ventrolateral and ventral scales	4	1.36–1.56	(1.29–1.42)–(1.52–1.63)	0.054; 0.045
Length of head dorsal spines	5	1.99–3.98	(1.94–2.10)–(3.91–4.23)	0.063; 0119
Length of head dorsolateral spines	5	1.95–3.96	(1.90–2.06)–(3.844.11)	0.059; 0.103
Length of head lateral spines	5	1.89–3.49	(1.84–1.97)–(3.43–4.05)	0.052; 0.248
Length of head ventrolateral spines	5	1.81–3.41	(1.79–1.90)–(3.39–3.72)	0.047; 0.142
Length of head ventral spines	5	2.52–7.77	(2.46–3.14)–(7.54–7.83)	0.281; 0.119
Length of neck dorsal spines	5	4.07–5.13	(3.54–4.27)–(5.02–5.49)	0.279; 0.177
Length of neck dorsolateral spines	5	3.73–4.90	(3.51–4.19)–(4.88–5.12)	0.264; 0.096
Length of neck lateral spines	5	3.46–4.67	(3.38–3.84)–(4.62–4.79)	0.176; 0.065
Length of neck ventrolateral spines	5	3.13–4.53	(3.10–3.54)–(4.34–4.60)	0.176; 0.102
Length of neck ventral spines	5	7.80–8.85	(6.09–7.99)–(7.50–8.87)	0.770; 0.575
Length of trunk dorsal spines	5	4.32–9.38	(3.99–4.46)–(9.34–10.40)	0.173; 0.463
Length of trunk dorsolateral spines	5	3.80–8.81	(3.80–4.35)–(8.39–8.87)	0.221; 0.215
Length of trunk lateral spines	5	3.68–7.04	(3.68–4.27)–(6.92–7.38)	0.239; 0.207
Length of trunk ventrolateral spines	5	3.57–5.72	(3.57–4.07)–(5.51–5.86)	0.199; 0.163
Length of trunk ventral spines	5	7.66–12.44	(6.64–8.26)–(11.42–12.89)	0.858; 0.568
Length of small one-lobed scales spines	5	1.09	1.02–1.34	0.127
Length of furcal appendages dorsal spines	5	2.47–2.56	(1.64–2.47)–(1.88–2.79)	0.323; 0.336
Length of parafurcal spines	5	4.82	4.50–5.01	0.185
Length of trunk posteriormost pair of lateral spines	5	6.97	6.38–6.97	0.243
Length of furcal appendages ventrolateral and ventral spines	4	0.40–0.43	(0.36–0.47)–(0.43–0.55)	0.457; 0.051
Head dorsal sensory bristles length	5	8.62	8.19–9.06	0.333
Neck dorsal sensory bristles length	5	9.72	9.72–9.92	0.098
Posterior dorsal sensory bristles length	5	11.73	10.14–11.94	0.715

Table A3. Continued.

Characters	N	Holotype	Range of adult paratypes	SD
Number of scales in single longitudinal row on ventral interciliary field	5	16	15–16	0.447
Total number of longitudinal alternating rows of scales on ventral interciliary field	5	7	7–8	0.447
Length of ventral interciliary field scales	5	2.12–3.2	(1.85–2.14)–(2.70–3.95)	0.138; 0.490
Width of ventral interciliary field scales	5	1.32–1.97	(1.02–1.36)–(1.40–2.02)	0.161; 0.255
Length of ventral interciliary field first pair of terminal scales	5	7.86	7.12–8.07	0.367
Width of ventral interciliary field first pair of terminal scales	5	2.92	2.44–2.97	0.208
Length of ventral interciliary field second pair of terminal	5	3.76	3.59–3.83	0.092
Width of ventral interciliary field second pair of terminal scales	5	2.24	2.07–2.24	0.072
Length of spines first pair of ventral interciliary field terminal scales	5	7.16	7.01–7.24	0.095
Length of spines second pair of ventral interciliary field terminal scales	5	0.87	0.80–0.87	0.027
Number of cephalic cilia in anterior tuft	5	5	5	0
Number of cephalic cilia in posterior tuft	5	5	5	0
Pharynx length	5	31.02	29.55–32.06	0.993
Width of anterior pharynx thickening (a)	5	9.22	8.46–9.55	0.401
Width of pharynx narrowing (n)	5	6.2	6.06–6.73	0.270
Width of middle pharynx (m)	5	7.35	6.98–7.35	0.148
Width of posterior pharynx thickening (p)	5	11.15	9.87–11.15	0.537
Width of pharyngeal-intestinal junction	5	3.63	3.57–3.78	0.077
Length of intestine anterior differ section	5	2.52	2.48–2.76	0.119
Intestine length	5	69.31	57.08–69.31	4.800
Pharynx formula a	5	*29.723*	*27.838–31.066*	1.1704
Pharynx formula n	5	*19.987*	*19.941–20.992*	0.4879
Pharynx formula m	5	*23.694*	*22.493–23.694*	0.5480
Pharynx formula p	5	*35.945*	*31.224–35.945*	1.8383
Pharynx-intestine ratio I	5	*44.755*	*43.424–53.241*	3.8362
Ratio of scale distribution	5	*119.05*	*113.64–128.57*	5.5103
Length of matured egg	2	–	49.06–50.08	0.721
Width of matured egg	2	–	25.36–27.97	1.846

Poznań, 16.12.2019r.

Małgorzata Kolicka
Adam Mickiewicz University, Poznań
Faculty of Biology
Department of Animal Taxonomy and Ecology
Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Declaration

I declare that the publication: **Kolicka M.**, Kotwicki L., Dabert M. 2018. Diversity of Gastrotricha on Spitsbergen (Svalbard Archipelago, Arctic) with a Description of Seven New Species. *Annales Zoologici*, 68 (4): 609-739, has been prepared as a part of my doctoral dissertation under the scientific supervision of Prof. UAM, dr hab. Ziemowit Olszanowski.

Yours faithfully,

Poznań, 16.12.2019r.

Małgorzata Kolicka
Uniwersytet im. Adama Mickiewicza, Poznań
Wydział Biologii
Zakład Taksonomii i Ekologii Zwierząt
ul. Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Oświadczenie

Oświadczam, że publikacja: **Kolicka M.**, Kotwicki L., Dabert M. 2018. Diversity of Gastrotricha on Spitsbergen (Svalbard Archipelago, Arctic) with a Description of Seven New Species. *Annales Zoologici*, 68 (4): 609-739, została wykonana na poczet mojej rozprawy doktorskiej pod opieką promotora Prof. UAM dra hab. Ziemowita Olszanowskiego.

Z wyrazami szacunku,

ANNALES ZOOLOGICI (Warszawa), 2018, 68(4): 609-739

DIVERSITY OF GASTROTRICHA ON SPITSBERGEN (SVALBARD ARCHIPELAGO, ARCTIC) WITH A DESCRIPTION OF SEVEN NEW SPECIES

MAŁGORZATA KOLICKA[1*], LECH KOTWICKI[2], and MIROSLAWA DABERT[3]

[1]*Department of Animal Taxonomy and Ecology, Institute of Environmental Biology, Adam Mickiewicz University, Poznan, Umultowska 89, 61-614 Poznań, Poland; e-mail: kolicka@amu.edu.pl*
[2]*Department of Marine Ecology, Institute of Oceanology, Polish Academy of Sciences, Powstańców Warszawy 55, 81-712 Sopot, Poland; e-mail: lechk@iopan.gda.pl*
[3]*Molecular Biology Techniques Laboratory, Faculty of Biology, Adam Mickiewicz University, Poznan, Umultowska 89, 61-614 Poznań, Poland; e-mail: mirkad@amu.edu.pl*
[*]*Corresponding author*

Abstract.— Gastrotrich biodiversity in the Arctic region is virtually unknown. Here we present, for the first time, a Gastrotricha investigation from both freshwater and marine habitats in the Hornsund fjord area (Spitsbergen, Arctic). Our research was conducted on the transect at various distances from the foreland of the Hans glacier (Spitsbergen, Arctic). The collected material contained representatives of 15 species belonging to 6 genera, 3 families and 2 orders. From all of the recorded taxa, 6 freshwater and 1 marine species proved to be new species and are described in this paper as *Chaetonotus* (*Chaetonotus*) *bombardus* **sp. nov.**, *C.* (*C.*) *subtilis* **sp. nov.**, *C.* (*Hystricochaetonotus*) *borealis* **sp. nov.**, *C.* (*H.*) *hornsundi* **sp. nov.**, *C.* (*H.*) *persimilis* **sp. nov.**, *Lepidodermella polaris* **sp. nov.** and *Turbanella pilosum* **sp. nov.** Moreover, 3 other species described from Isfjorden area, another Spitsbergen locality, namely *C.* (*C.*) *gelidus*, *C.* (*C.*) *jaceki* and *C.* (*C.*) *svalbardi*, were found during our research, which may suggest a high specificity of Arctic Gastrotricha fauna. In this work we provide both morphological and morphometric data and we present the phylogenetic position of the newly described and newly found species based on nuclear 18S rRNA, 28S rRNA and mitochondrial cytochrome c oxidase subunit I sequence data.

Key words.— Biodiversity, Chaetonotida, Macrodasyida, molecular phylogeny, new species, DNA-barcode, Svalbard, Arctic

INTRODUCTION

Gastrotricha comprise microscopic acoelomate metazoans ranging in size from 50 μm to 3500 μm that inhabit all types of aquatic as well as semi-aquatic ecosystems throughout the world (*e.g.* Hochberg *et* Litvaitis 2000, Kisielewski 1997, Todaro *et al.* 2006, Balsamo *et al.* 2014, 2015). Gastrotrichs comprise more than 820 nominal species (Todaro 2018) and are considered a monophyletic phylum that is classically divided into two orders: Chaetonotida Remane, 1925 [Rao & Clausen, 1970] and Macrodasyida Remane, 1925 [Rao & Clausen, 1970].

PL ISSN 0003-4541 © Museum and Institute of Zoology PAS
doi: 10.3161/00034541ANZ2018.68.4.001

The order Chaetonotida includes 8 families, 32 genera, and contains approximately 470 nominal species, of which *ca*. 70% are freshwater and the remaining are marine or brackish taxa (Todaro *et al*. 2009, Todaro *et* Hummon 2008, Balsamo *et al*. 2013, Garraffoni *et al*. 2016, Kolicka *et al*. 2016, Todaro 2018). Chaetonotidans have a total body length of 50–780 μm, are usually tenpin-shaped and have a mostly furcated caudal end. The furca generally bears only one pair or, very rarely, two pairs of distal adhesive tubes or the adhesive tubes are reduced. Certain semi-planktonic families, viz. Dasydytidae Daday, 1905 and Neogosseidae Remane, 1927a, lack the distal adhesive tubes altogether and have a rounded caudal end (Kisielewski 1997, Todaro *et al*. 2013, Kånneby *et* Todaro 2015). According to the current classification, the order Chaetonotida is divided into two suborders, viz. Multitubulatina d'Hondt, 1971, comprising the single genus *Neodasys* Remane, 1927b, and Paucitubulatina d'Hondt, 1971, grouping all of the remaning Chaetonotida. Representatives of the genus *Neodasys* have many pairs of adhesive tubes and a strap-shaped body in comparison to other chaetonotidians and are hypothesised as the earliest branching lineage and sister group of the suborder Paucitubulatina (*e.g*. Kieneke *et al*. 2008).

Macrodasyidans include 8 families, 33 genera and contain approximately 350 marine and brackish species, with the exception of 6 freshwater taxa from two genera (Hummon *et* Todaro 2010, Kånneby *et* Wicksten 2014, Todaro *et al*. 2014, 2015, Kånneby *et* Kirk 2017, Todaro 2018). Representatives of this order have a strap-shaped body, total length of 200–3500 μm, pharyngeal pores and bear anterior, posterior, also often dorsal, dorsolateral, lateral, ventrolateral and ventral adhesive tubes (Kisielewski 1997, Todaro *et* Hummon 2008, Kieneke *et* Schmidt-Rhaesa 2015).

To the present day marine Gastrotricha from the Arctic region, which usually have been determined only to the phylum level, have, been mentioned in several scientific papers (*e.g*. Pflannkuche *et* Thiel 1987, Węsławski *et al*. 1997, Włodarska-Kowalczuk *et al*. 1999, Todaro *et al*. 2005, 2009, Urban-Malinga *et al*. 2004, 2005, Balsamo *et al*. 2010, Grzelak *et* Kotwicki 2012). Similarly, most reports on freshwater gastrotrich taxa from the Arctic region provide identifications only to the phylum (Scourfield 1897, De Smet 1993) or genus level (De Smet *et al*. 1987, Coulson *et al*. 2014). Determinations to species level has been reported in only two recent works: Kolicka *et al*. (2016) conducted an integrated morphological and molecular analyses and described one new species and genus, and Kolicka (2017) conducted a morphological study and reported 8 taxa from the genus *Chaetonotus* Ehrenberg, 1830, including three new species.

This paper presents a comprehensive research approach to both freshwater and marine gastrotrichs from the Hans glacier foreground in Hornsund fjord, with a description of 7 new species, 5 of the genus *Chaetonotus* Ehrenberg, 1830, 1 of the genus *Lepidodermella* Blake, 1933 and 1 of the genus *Turbanella* Schultze, 1853. Of all these new species, and for the newly recorded *Chaetonotus* (*Chaetonotus*) *jaceki* Kolicka, 2017 and *C.* (*C.*) *gelidus* Kolicka, 2017, we provide hypotheses regarding their relationships with other gastrotrichs based on morphological and molecular data.

MATERIAL AND METHODS

Study area

The material for the research was collected on Spitsbergen, which is the largest island of the Svalbard Archipelago. Spitsbergen has an area of 37,673 km^2 and is surrounded by the Arctic Ocean, Norwegian Sea and Greenland Sea. The Hornsund fjord is the most southern glacial fjord on the west coast of Spitsbergen. The entire area of Hornsund is protected, as South Spitsbergen National Park, and is a flagship destination for research on biodiversity (Giżejewski *et al*. 2013). Approximately 70% of the Hornsund catchment area is covered by glaciers, of which thirteen directly enter the fjord (Błaszczyk *et al*. 2013). The total outflow of the Hornsund melting ice cap is estimated at 0.79 km^3 per year (Beszczynska-Moller *et al*. 1997). Currently, the volume of freshwater run-off might be even higher due to rising temperatures and the continuous retreat of glaciers (Jania 1988, Palli *et al*. 2003, Grzelak *et* Kotwicki 2012), hence the Hornsund region may be more vulnerable to climate change than the rest of the archipelago.

The average annual air temperature in Hornsund in the period 1979–2012 was 3.8°C, with July as the warmest (mean +4.4°C) and February and March (mean -10.2°C) as the coldest months. The maximum temperature was recorded in July 2005 at 13.5°C and the lowest, in January of 1981, reached -35.9°C (Migała *et* Wawrzyniak 2013).

Throughout the Hornsund area, snow remains on the tundra for 8 months a year, while permafrost is present even at a depth of several hundred metres (Humlum *et al*. 2003). The frozen surface thaws in the summer seasons to a depth of no more than 2 m, forming an active layer in which various biogeochemical processes occur (Giżejewski *et* Karczewski 2013). A permanent winter snow cover usually appears in October, sometimes earlier. In the first part of winter the snow cover is highly variable, with a tendency to shrink or transform into firn or ice due to thawing and rain. The thickness of snow sheet in the coastal area is very diverse and varies due to wind-blown drifting from

a depth of several metres on the see side of rocks, morphological edges and concave forms to bare areas at sites exposed to wind. In flat regions, the average snow cover depth is 30–60 cm, with an average density of 0.3 to 0.4 g/cm^3. Seasonal disappearance of snow usually falls at the end of May or the beginning of June. In selected places, such as gullies and the upper parts of mountains, snow remains until the end of July, or sometimes for years (Migała *et* Wawrzyniak 2013).

The hydrographical regime is one of the key factors driving the biodiversity and ecological functioning of the Hornsund fjord, and hydrodynamic complexity shapes the temporal and spatial variations of its physical and biological components (Gilbert 2000).

The Hornsund fjord is significantly affected by cold Arctic waters carried by the Sørkapp Current from the Barents Sea in the eastern part of the archipelago (temperature range from -1.5 to 1.0°C and salinity from 34.3 to 34.8 Practical Salinity Units); therefore the resident biota may exhibit typical low-temperature Arctic features (Węsławski *et al*. 2012).

Sampling and documentation

Field works was carried out from 26 July to 8 August 2014. Samples were collected from 20 stations: 14 freshwater and 6 marine (Fig. 1, Table 1).

The freshwater samples were collected from temporary, small and shallow water reservoirs located on the tundra (samples 1–13) and Revvatnet Lake (sample 14) (Figs 1–2). The marine samples were taken on the northern coast of the Hornsund fjord from the shallow sublitoral zone during low tide (Figs 1, 3).

All samples were collected manually with a Plexiglass tube, with marked centymeter scales and a cross section area of 10 cm^2, in such a manner that a 5 cm upper layer of sediment and approximately 5 cm of ambient water were placed in a 200 cm^3 plastic container. The additional water above the sediment was carefully cast by gentle tilting of the tube, in such manner that remains only 5 cm high layer above the bottom substrat. The collected samples were subsequently placed in an isothermal box to maintain the ambient

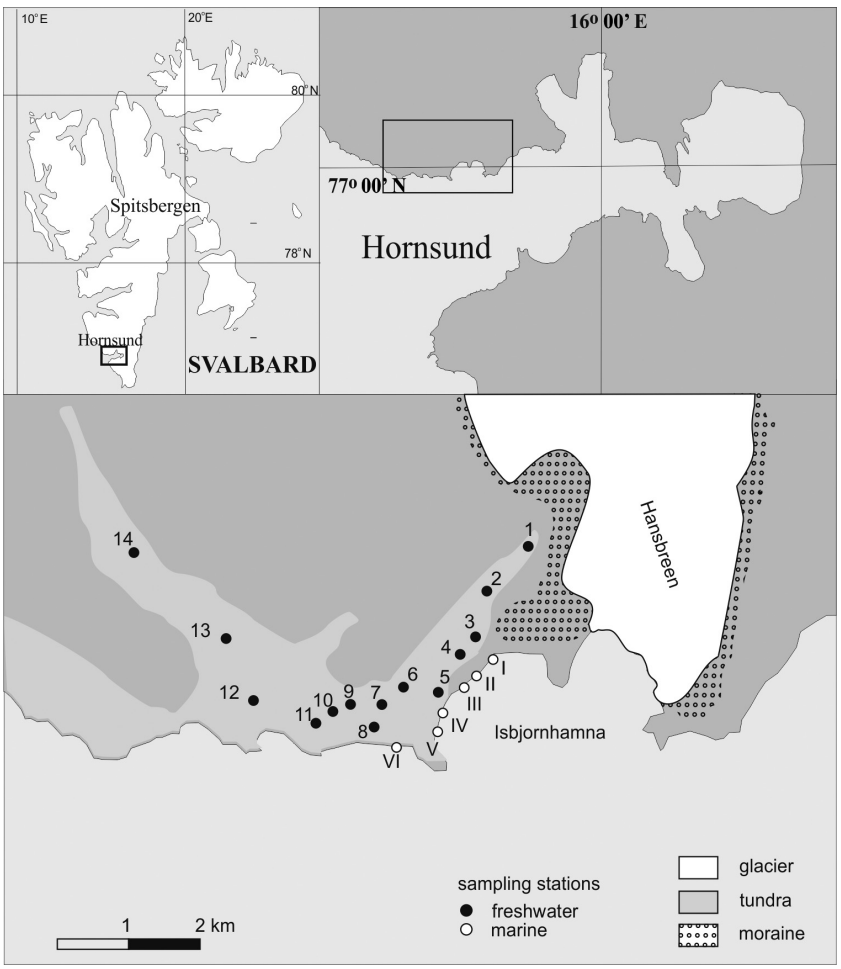


Figure 1. Study area with marked sampling sites (1–14 freshwater; I–VI marine).

temperature and immediately transported to the laboratory at the Stanisław Siedlecki Polish Polar Station in Hornsund. In the laboratory the samples were oxygenated and placed in a thermal chamber at 5°C to create conditions similar to those at the sampling site. The material was examined at the earliest possible time, starting 0.5–6 hours after sampling and finishing within 10 days after collecting the material. The samples examined time were extended in comparison to the literature recommendations (*e.g.* Kisielewski 1981,

Figure 2. Various freshwater sampling stations.

1991) in order to analyse as many specimens as possible and to provide the fullest list of species present at each location. That long analyses time allow including also individuals heated from eggs deposited into sediments samples and observe the rearrangements of the fauna composition, what imitated the conditions and processes occurring in the natural environment. A total of 0.1 cm^3 of sediment from each freshwater sample and the entire contents of the marine samples were analysed under a Nikon SMZ1500 stereomicroscope.

Figure 3. Various marine sampling stations.

The freshwater sediment was scanned for gastrotrichs with using a Nikon SMZ1500 stereomicroscope and all specimens were extracted with a micropipette and studied alive (Kisielewski 1997, Balsamo *et al.* 2014). The marine specimens were extracted using the narcotisation-decantation technique (Todaro *et* Hummon 2008). All specimens were observed, photographed and documented alive under a Nikon Eclipse E600 compound microscope equipped with differential interference contrast (DIC) and an AxioCam ERc5s digital camera. Morphometric characters were measured in cellSens Entry 1.12 software (Olympus). All measurements are given in micrometres (μm) and all formulas used here are given as a percentage (%).

Granulometric analyses

The marine sediment samples were analysed for their grain-size properties. To determine the sediment characteristic (mean, sorting, skewness and kurtosis), the granulometric protocol as described by Buchanan (1984) was applied. The grain-size statistics were calculated through Gradistat software using a logarithmic method of moments (Blott *et* Pye 2001). Sediments were classified according to the Folk and Ward method (Folk *et* Ward 1957). The organic matter content of the same sediment samples was determined using the combustion method (LOI – Loss On Ignition).

Morphological analyses

A morphological character was measured only if the orientation of that feature was conducive to accurate measurement. The ranges of measurements are presented below in Tables 9–10, 12–13, 15–120, 22–23, 25. Because of the low taxonomic usefulness of preserved gastrotrichs, each specimen was documented by means of a set of photomicrographs and each holotype was additionally documented by detailed schematic. Such documentation of types is allowed by The International Code of Zoological Nomenclature, namely in article 73.1.4 (The International Commission on Zoological Nomenclature 1999), even if the specimen documented no longer exists. The taxon descriptions follow the convention of Hummon *et al.* (1992), in which the longitudinal distance of a morphological character from the anterior end is expressed as percentage units (U) of the animal's total length (i.e. the distance of the character from the anterior end divided by the total body length and multiplied by 100) and, for the Macrodasyida representatives, detailing was used as proposed by Kieneke and Nikoukar (2017). In this work, in new species descriptions, measurements of width of different body sections were omitted because they would be strongly influenced by the pressure of the coverglass on the live specimens being studied. The identification of gastrotrichs, their morphological study and terms follow Kisielewski (1981, 1991, 1997) and Kolicka *et al.* (2016). The terms describing the shape of the furcal branches and furcal indentation follow Roszczak (1969). In this paper, the following formulae describing the distribution of scales were used, namely:

$$\textit{Ratio of scales distribution} = \frac{\text{total number of longitudinal alternating rows of scales}}{\text{number of scales in central longitudinal row}} \times 100$$

and the pharynx shape, namely: pharynx formula

$$\alpha = \frac{\text{width of anterior thickening}}{\text{pharynx length}} \times 100\%$$

$$n = \frac{\text{width of pharynx narrowing}}{\text{pharynx length}} \times 100\%$$

$$m = \frac{\text{width of middle pharynx}}{\text{pharynx length}} \times 100\%$$

$$p = \frac{\text{width of posterior thickening}}{\text{pharynx length}} \times 100\%$$

according to Kisielewski (1991), and the pharynx-to-intestine length ratio:

$$I = \frac{\text{pharynx length}}{\text{intestine length}} \times 100\%$$

according to Kolicka *et al.* (2016). In this work, the following abbreviations were applied: D – dorsal, DL – dorsolateral, L – lateral, LV – ventrolateral, V – ventral *sensu* Kolicka *et al.* (2016, see S1 and S2).

The new species proposed in this paper are described by the first author only. This solution is consistent with Chapter 11, Article 50 of the International Code of Zoological Nomenclature (The International Commission on Zoological Nomenclature 1999).

Molecular data

We generated new sequence data from 27 individuals representing all new species and *Chaetonotus* (*Chaetonotus*) *gelidus* Kolicka, 2017 and *C.* (*C.*) *jaceki* Kolicka, 2017. Total genomic DNA was extracted from single specimens using the DNeasy Blood and Tissue Kit (Qiagen GmbH, Hilden, Germany) as described by Dabert *et al.* (2008). The mitochondrial cytochrome *c* oxidase subunit I (COI) gene fragments were amplified with two pairs of primers: a 1:1 mixture of two forward primers, bcdF08 and bcdF09 (Kolicka *et al.* 2016) with reverse primer bcdR04 (Dabert *et al.* 2010), and forward primer LCO1490 (Folmer *et al.* 1994) with reverse primer HCO2198 (Folmer *et al.* 1994). The fragment coding for internal transcribed spacers (ITS1–5.8S rRNA–ITS2) was amplified with ITS1_18S and ITS2_28S primers (Navajas *et al.* 1998). Complete sequences of 18S rRNA were amplified in two

Table 1. Geografical position of freshwater and marine sampling stations.

Freshwater		Marine	
Station	Localization	Station	Localization
1	N77° 00.751' E15° 34.975'	I	N77° 00.280' E15° 33.920'
2	N77° 00.532' E15° 33.914'		
3	N77° 00.285' E15° 33.906'	II	N77° 00.253' E15° 33.580'
4	N77° 00.282' E15° 33.911'		
5	N77° 00.386' E15° 33.136'	III	N77° 00.037' E15° 32.918'
6	N77° 00.062' E15° 31.977'		
7	N76° 59.980' E15° 31.667'	IV	N77° 00.100' E15° 32.670'
8	N77° 00.021' E15° 31.697'		
9	N77° 00.052' E15° 30.813'	V	N77° 00.085' E15° 32.411'
10	N77° 00.004' E15° 30.486'		
11	N76° 59.964' E15° 30.055'	VI	N76° 59.908' E15° 31.557'
12	N77° 00.062' E15° 28.503'		
13	N77° 00.351' E15° 27.630'		
14	N77° 01.099' E15° 23.759'		

Table 2. PCR primers used in this study; A and S refer to amplifying and sequencing, respectively.

Primer name	Sequence (5'–3')	Product	Use	Source
bcdF08	CGATGRTTTTTTTCHACWAACCAYAARGATATCGG	COI	A, S	Kolicka *et al*. 2016
bcdF09	CGATGRTTTTTTTCHACWAACCAYAARGACATTGG	COI	A, S	Kolicka *et al*. 2016
LCO1490	GGTCAACAAATCATAAAGATATTGG	COI	A, S	Folmer *et al*. 1994
bcdR04	TATAAACYTCDGGATGNCCAAAAAA	COI	A, S	Dabert *et al*. 2008
HCO 2198	TAAACTTCAGGGTGACCAAAAAATCA	COI	A, S	Folmer *et al*. 1994
ITS1_18S	AGAGGAAGTAAAAGTCGTAACAAG	ITS	A, S	Navajas *et al*. 1998
ITS2_28S	ATATGCTTAAATTCAGGGGG	ITS	A, S	Navajas *et al*. 1998
18Sfw	CTTGTCTCAAAGATTAAGCCATGCA	18S rDNA	A, S	Dabert *et al*. 2010
rev480	GTTATTTTTCGTCACTACCT	18S rDNA	S	Dabert *et al*. 2010
fw390	AATCAGGGTTCGATTCCGGAGA	18S rDNA	A, S	Dabert *et al*. 2010
rev960	GACGGTCCAAGAATTTCAC	18S rDNA	A, S	Dabert *et al*. 2010
for1300	TGCATGGCCGTTCTTAGTTG	18S rDNA	S	Dabert *et al*. 2010
rev1460	CATCACAGACCTGTTATTGC	18S rDNA	S	Dabert *et al*. 2010
rev18S	TGATCCTTCCGCAGGTTCACCT	18S rDNA	A, S	Dabert *et al*. 2010
F0001	ACCCVCYNAATTTAAGCATAT	28S rDNA	A	Dabert *et al*. 2016
300R	CAACTTTCCCTCACGGTACTTG	28S rDNA	S	Telford *et al*. 2003
F440	ACAAGTACCGTGAGGGAAAGTTG	28S rDNA	S	Sonnenberg *et al*. 2007
1200R	GCATAGTTCACCATCTTTCGG	28S rDNA	S	Telford *et al*. 2003
R1634	ATTCGGCAGGTGAGTTGTTACA	28S rDNA	A, S	Telford *et al*. 2003
1200F	CCCGAAAGATGGTGAACTATGC	28S rDNA	A, S	Telford *et al*. 2003
1600F	AGCAGGACGGTGGCCATGGAAG	28S rDNA	S	Telford *et al*. 2003
F1800	AGAAGGGTTCCATGTGAAC	28S rDNA	A, S	Dabert *et al*. 2016
R2450	GCTTTGTTTTAATTAGACAGTCGGA	28S rDNA	A, S	Telford *et al*. 2003
R2850	GTGGTTTCGCTAGATAGTAGATA	28S rDNA	S	Dabert *et al*. 2016
F2120	GCCAGGCCGTACCCATATCC	28S rDNA	A, S	Kolicka *et al*. This study
F2960	CTTTTATCGTTTCTTTACTTATTCAG	28S rDNA	S	Kolicka *et al*. This study
F3080	TCTGTCAAATGGTAACGCAGGT	28S rDNA	S	Kolicka *et al*. This study
R3530	CTAACCTGTCTCACGACGGTCT	28S rDNA	A, S	Dabert *et al*. 2016
R3950	TGCTCTACTAAGTACAACACC	28S rDNA	A, S	Kolicka *et al*. This study
R3980	CAGTGTGGTGGCTGCTCTACT	28S rDNA	A, S	Kolicka *et al*. This study

Table 3. List of sequences from new and newly recorded species.

Species	Specimen ID	GenBank acc. nos.			
		COI	ITS	18S	28S
Chaetonotus (*Chaetonotus*) *bombardus* sp. nov.	AMU217.5	MF374689	MF325869		MF325907
	AMU217.7	MF374690	MF325870		MF325908
	AMU217.14	MF374691	MF325871		MF325909
	AMU217.56	MF374692	MF325872		MF325910
	AMU217.62	MF374693	MF325873	MF325912	MF325906
Chaetonotus (*Chaetonotus*) *gelidus*	AMU234.10	MF374695	MF325875	MF325914	MF325922
Chaetonotus (*Chaetonotus*) *jaceki*	AMU217.8	MF417812	MF325878		MF325924
	AMU217.11	MF417813	MF325879		MF325925
	AMU217.59	MF417814	MF325880		MF325926
	AMU217.60	MF417815	MF325881		MF325927
	AMU217.63	MF417816	MF325882	MF325916	MF325923
	AMU217.67	MF417817	MF325883		MF325928
	AMU241.25	MF417818	MF325884		MF325929
	AMU241.34	MF417819	MF325885		MF325930
Chaetonotus (*Chaetonotus*) *subtilis* sp. nov.	AMU241.26	MF374699	MF325887		MF325897
	AMU237.42	MF374700	MF325888		MF325896
	AMU237.45	MF374701	MF325889	MF325918	MF325895
Chaetonotus (*Hystricochaetonotus*) *borealis* sp. nov.	AMU237.46	MF374694	MF325868	MF325913	MF325911
Chaetonotus (*Hystricochaetonotus*) *hornsundi* sp. nov.	AMU240.4	MF374696	MF325874	MF325915	MF325921
Chaetonotus (*Hystricochaetonotus*) *persimilis* sp. nov.	AMU241.21	MF374697	MF325876		MF325899
	AMU241.38	MF374698	MF325877	MF325917	MF325898
Lepidodermella polaris sp. nov.	AMU234.16	MF374702	MF325886	MF325919	MF325900
Turbanella pilosum sp. nov.	AMU2.2	MF374703	MF325890		MF325902
	AMU2.3	MF374704	MF325891	MF325920	MF325901
	AMU2.5	MF374705	MF325892		MF325903
	AMU2.16	MF374706	MF325893		MF325904
	AMU2.21	MF374707	MF325894		MF325905

Table 4. DNA sequences of the Chaetonotida gastrotrichs species used in phylogenetic analysis. NA – not available.

Family/Species	GenBank acc. nos.		
	COI	18S	28S
Chaetonotidae			
Arenotus strixinoi Kisielewski, 1987	JQ798677	JQ798537	JQ798608
Aspidiophorus ophiodermus Balsamo, 1983	NA	JN185463	JN185510
Aspidiophorus paramediterraneus Hummon, 1974	JQ798678	JQ798538	JQ798609
Aspidiophorus polystictos Balsamo *et* Todaro, 1987	JQ798726	JQ798597	JQ798677
Aspidiophorus tetrachaetu Kisielewski, 1986	JN185576	JN185505	JN185540
Bifidochaetus arcticus Kolicka *et* Kisielewski, 2016	KP713405	KP713403	KP713404
Cephalionotus kisielewskii Garraffoni, Araújo, Lourenço, Guidi *et* Balsamo, 2016	KX159487	KX159486	NA
Chaetonotus (*Chaetonotus*) *antrumus* Kolicka, 2017	KU705230	KX538804	KU705232
Chaetonotus (*Chaetonotus*) *bombardus* spec. nov. Kolicka, 2018	MF374693	MF325912	MF325906
Chaetonotus (*Chaetonotus*) cf. *oculifer* Kisielewski, 1981	JQ798708	JQ798581	JQ798647
Chaetonotus (*Chaetonotus*) cf. *sphagnophilus* Kisielewski, 1981	JQ798733	JQ798604	JQ798671
Chaetonotus (*Chaetonotus*) *daphnes* Balsamo *et* Todaro, 1995	JQ798687	JQ798549	JQ798617
Chaetonotus (*Chaetonotus*) *gelidus* Kolicka, 2017	MF374695	MF325914	MF325922
Chaetonotus (*Chaetonotus*) *jaceki* Kolicka, 2017	MF417816	MF325916	MF325923
Chaetonotus (*Chaetonotus*) *laroides* Marcolongo, 1910	JQ798731	JQ798602	JQ798669
Chaetonotus (*Chaetonotus*) *maximus* 1 Ehrenberg, 1838	JQ798709	JQ798577	NA

Table 4. Continued.

Family/Species	GenBank acc. nos.		
	COI	18S	28S
Chaetonotus (*Chaetonotus*) *maximus* 2 Ehrenberg, 1938	JQ798706	JQ798574	JQ798646
Chaetonotus (*Chaetonotus*) *microchaetus* Preobrajenskaja, 1926	JQ798684	JQ798575	JQ798618
Chaetonotus (*Chaetonotus*) *polyspinosus* Greuter, 1917	JQ798717	JQ798563	JQ798654
Chaetonotus (*Chaetonotus*) *similis* Zelinka, 1889	JQ798710	JQ798578	JQ798648
Chaetonotus (*Chaetonotus*) cf. *similis* Zelinka, 1889	JQ798722	JQ798592	JQ798660
Chaetonotus (*Chaetonotus*) sp. 1 Kånneby, Todaro *et* Jondelis, 2013	JQ798730	JQ798601	JQ798668
Chaetonotus (*Chaetonotus*) sp. 2 Kånneby, Todaro *et* Jondelis, 2013	JQ798692	JQ798555	NA
Chaetonotus (*Chaetonotus*) *subtilis* sp. nov. Kolicka, 2018	MF374701	MF325918	MF325895
Chaetonotus (*Hystricochaetonotus*) *aemilianus* Balsamo, 1978	JQ798693	JQ798556	JQ798626
Chaetonotus (*Hystricochaetonotus*) *borealis* sp. nov. Kolicka, 2018	MF374694	MF325913	MF325911
Chaetonotus (*Hystricochaetonotus*) cf. *novenarius* Greuter, 1917	JQ798699	JQ798566	JQ798636
Chaetonotus (*Hystricochaetonotus*) *hornsundi* sp. nov. Kolicka, 2018	MF374696	MF325915	MF325921
Chaetonotus (*Hystricochaetonotus*) *hystrix* 1 Mečnikow, 1865	NA	JQ798557	JQ798627
Chaetonotus (*Hystricochaetonotus*) *hystrix* 2 Mečnikow, 1865	JQ798732	JQ798603	JQ798670
Chaetonotus (*Hystricochaetonotus*) *persimilis* sp. nov. Kolicka, 2018	MF374698	MF325917	MF325898
Chaetonotus (*Marinochaetus*) *mariae* Todaro, 1992	NA	JQ798558	JQ798628
Chaetonotus (*Primochaetus*) *acanthocephalus* Valkanov, 1937	JQ798701	JQ798569	NA
Chaetonotus (*Primochaetus*) *acanthodes* 1 Stokes, 1887	JQ798682	JQ798544	JQ798616
Chaetonotus (*Primochaetus*) *acanthodes* 2 Stokes, 1887	NA	JQ798552	JQ798624
Chaetonotus (*Primochaetus*) *armatus* Kisielewski, 1981	JQ798723	JQ798594	NA
Chaetonotus (*Primochaetus*) *heideri* 1 Brehm, 1917	JQ798685	JQ798547	JQ798619
Chaetonotus (*Primochaetus*) *heideri* 2 Brehm, 1917	JQ798720	JQ798590	JQ798657
Chaetonotus (*Primochaetus*) *heteracanthus* Remane, 1927a	JQ798681	JQ798615	JQ798681
Chaetonotus (*Schizochaetonotus*) *dispar* Wilke, 1954	JQ798696	JQ798561	JQ798631
Chaetonotus (*Schizochaetonotus*) *neptuni* 1 Wilke, 1954	JQ798679	JQ798539	JQ798610
Chaetonotus (*Schizochaetonotus*) *neptuni* 2 Wilke, 1954	JQ798724	JQ798595	JQ798662
Chaetonotus (*Schizochaetonotus*) *schultzei* Mečnikow, 1865	JQ798725	JQ798596	JQ798663
Chaetonotus (*Wolterecka*) *uncinus* Voigt, 1902	NA	JQ798540	JQ798611
Chaetonotus (*Zonochaeta*) *bisacer* Greuter, 1917	NA	JQ798565	JQ798635
Halichaetonotus paradoxus (Remane, 1927a)	JQ798728	JQ798599	JQ798666
Halichaetonotus euromarinus Hummon *et* Todaro, 2010	NA	JQ798551	JQ798623
Halichaetonotus sp. 2 Kånneby, Todaro *et* Jondelis, 2013	JQ798695	JQ798560	JQ798630
Halichaetonotus sp. 4 Kånneby, Todaro *et* Jondelis, 2013	JQ798729	JQ798600	JQ798667
Halichaetonotus aculifer (Gerlach, 1953)	JQ798688	JQ798550	JQ798622
Heterolepidoderma acidophilum Kånneby, Todaro *et* Jondelis, 2012	JN185556	JN185462	JN185521
Heterolepidoderma macrops Kisielewski, 1981	JN185548	JN185469	JN185515
Heterolepidoderma ocellatum (Mečnikow, 1865)	JN185554	JN185475	JN185519
Heterolepidoderma loricatum Schrom, 1972	NA	JQ798541	JQ798612
Heterolepidoderma sp.1 Kånneby, Todaro *et* Jondelis, 2013	JQ798691	JQ798554	NA
Heterolepidoderma sp. 2 Kånneby, Todaro *et* Jondelis, 2013	JN185563	JN185485	JQ798644
Ichthydium skandicum Kånneby, Todaro *et* Jondelis, 2009	JQ798705	JQ798573	JQ798645
Lepidochaetus brasilense 1 Kisielewski, 1991	JQ798680	JN185458	JN185507
Lepidochaetus brasilense 2 Kisielewski, 1991	JN185568	JN185495	JQ798658
Lepidochaetus zelinkai (Grünspan, 1908)	JN185564	JN185486	JN185527
Lepidodermella intermedia Kånneby, Todaro *et* Jondelis, 2012	JN185547	JN185468	JN185514
Lepidodermella minor minor (Remane, 1936)	JN185553	JN185474	NA
Lepidodermella polaris sp. nov. Kolicka, 2018	MF374702	MF325919	MF325900
Lepidodermella squamata 1 (Dujardin, 1841)	KP965862	KC193102	KC193103
Lepidodermella squamata 2 (Dujardin, 1841)	JN185557	JN185478	JN185522
Polymerurus nodicaudus 1 (Voigt, 1901)	JN185542	JN185460	NA
Polymerurus nodicaudus 2 (Voigt, 1901)	JN185573	JN185502	JN185537
Polymerurus nodicaudus 3 (Voigt, 1901)	JQ798689	JN185465	JN185512

Table 4. Continued.

Family/Species	GenBank acc. nos.		
	COI	18S	28S
Polymerurus nodicaudus 4 (Voigt, 1901)	JN185552	JN185473	JQ798642
Polymerurus rhomboides 1 (Stokes, 1887)	JQ798715	JQ798584	NA
Polymerurus rhomboides 2 (Stokes, 1887)	JN185546	JN185467	JN185513
Polymerurus rhomboides 3 (Stokes, 1887)	JN185567	JN185493	JN185533
Dasydytidae			
Dasydytes (*Prodasydytes*) *elongatus* Kisielewski, 1991	JQ798700	JQ798568	JQ798638
Dasydytes (*Prodasydytes*) *papaveroi* Kisielewski, 1991	JQ798703	JQ798564	JQ798634
Haltidytes squamosus Kisielewski, 1991	NA	JQ798567	JQ798637
Ornamentula paraensis Kisielewski, 1991	JQ798697	JQ798562	JQ798632
Stylochaeta fusiformis (Spencer, 1890)	JN185550	JN185471	JN185517
Stylochaeta scirtetica Hlava, 1904	JN185566	JN185491	JN185532
Gnathostomulidae			
Gnathostomula paradoxa Ax, 1956	DQ079967	DQ079925	DQ079950
Haplognathia ruberrima (Sterrer, 1966)	DQ079968	DQ079930	DQ079954
Muselliferidae			
Musellifer delamarei (Renaud-Mornant, 1968)	NA	AM231775	NA
Musellifer reichardti Kånneby, Atherton *et* Hochberg, 2014	NA	KF578503	NA
Diuronotus aspetos Todaro, Balsamo *et* Kristensen, 2005	KX531007	KX531005	KX531006
Neodasyidae			
Neodasys chaetonotoideus Remane, 1927b	JQ798675	JQ798535	NA
Neodasys cirritus Evans, 1992	JQ798676	JQ798536	NA
Neogosseidae			
Kijanebalola devestiva Todaro, Perissinotto *et* Bownes, 2013	KR822120	KR822112	KR822117
Kijanebalola devestiva 2 Todaro, Perissinotto *et* Bownes, 2013	NA	KR822113	KR822118
Neogossea antennigera 1 (Gosse, 1851)	NA	KR822110	KR822116
Neogossea antennigera 2 (Gosse, 1851)	NA	KR822111	KR822115
Xenotrichulidae			
Draculiciteria tesselata (Renaud-Mornant, 1968)	JN185549	JN185470	JN185516
Heteroxenotrichula squamosa Wilke, 1954	NA	JQ798542	JQ798613
Xenotrichula intermedia Remane, 1934	JF432048	JF357664	JF357712
Xenotrichula punctata Wilke, 1954	NA	JN185464	JN185511
Xenotrichula velox Remane, 1927b	NA	JN185499	JQ798652

Table 5. DNA sequences of the Macrodasyida gastrotrichs species used in phylogenetic analysis.

Family/Species	GenBank acc. nos.		
	COI	18S	28S
Planodasyidae			
Megadasys minor Kisielewski, 1987	NA	AY228131	NA
Megadasys sp. Todaro, Kånneby, Dal Zotto *et* Jondelius, 2011	NA	JF357655	JF357703
Turbanellidae			
Paraturbanella dohrni 2 Remane, 1927b	NA	JF357659	JF357707
Paraturbanella pallida Luporini, Magagnini *et* Tongiorgi, 1973	JF432045	JF357660	JF357708
Paraturbanella teissieri Swedmark, 1954	NA	JF357661	JF357709
Turbanella bocqueti Kaplan, 1958 *sensu* Boaden, 1974	JF432046	JF357662	JF357710
Turbanella cornuta Remane, 1925	NA	AF157007	NA
Turbanella cornuta 2 Remane, 1925	NA	JF357663	NA
Turbanella cornuta 3 Remane, 1925	JF432047	JF357666	JF357711
Turbanella cornuta 4 Remane, 1925	JN099907	JN099882	NA
Turbanella lutheri 1 Remane, 1952	NA	AY963693	NA
Turbanella lutheri 2 Remane, 1952	JF432051	JF357669	NA
Turbanella mustela Wieser, 1957	JN086974	JN688297	NA
Turbanella pilosum sp. nov. Kolicka, 2018	MF374704	MF325920	MF325901
Turbanella sp. Paps *et* Riutort, 2012	NA	JF970238	NA

Table 6. Total number of particular freshwater gastrotrichs species in analysed samples at investigated stations.

CHAETONOTIDA	Station													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	Number of individuals per sample													
Chaetonotus (*Chaetonotus*) *bombardus* sp. nov.	–	36	–	–	–	–	–	–	–	–	–	1	–	–
Chaetonotus (*Chaetonotus*) *gelidus*	–	4	–	2	–		–	–	–	1	–	7	–	–
Chaetonotus (*Chaetonotus*) *jaceki*	–	38	2	3	–	10	–	–	–	1	–	–	–	–
Chaetonotus (*Chaetonotus*) *subtilis* sp. nov.	–	1	21	–	–	9	–	–	–	–	–	–	–	–
Chaetonotus (*Chaetonotus*) *svalbardi*	–	2	–	–	–	–	–	–	–	–	–	–	–	–
Chaetonotus (*Hystricochaetonotus*) *borealis* sp. nov	–	–	24	–	–	1	–	–	1	–	–	1	–	–
Chaetonotus (*Hystricochaetonotus*) *hornsundi* sp. nov.	–	–	11	4	–	5	–	–	–	–	5	4	–	–
Chaetonotus (*Hystricochaetonotus*) *persimilis* sp. nov.	–	–	6	2	–	13	–	–	–	–	–	1	–	–
Lepidodermella polaris sp. nov.	–	–	–	–	–	2	–	–	1	–	–	4	–	–
Chaetonotus (*Chaetonotus*) sp. 1	–	–	–	–	–	–	–	–	–	–	–	2	–	–
Ichthydium sp. 2	–	–	–	–	–	1	–	–	–	–	–	1	–	–
Heterolepidoderma sp. 3	–	–	–	–	–	2	–	–	–	–	–	–	–	–
Lepidodermella sp. 4	–	–	–	–	–	–	–	–	–	–	–	1	–	–
Number of species	–	5	5	4	–	8	–	–	2	2	1	9	–	–
Number of specimens	–	81	64	11	–	43	–	–	2	2	5	22	–	–
Abundance [ind. per cm^3]	–	810	640	110	–	430	–	–	20	20	50	220	–	–

Table 7. Total number of particular marine gastrotrichs species in analysed samples at investigated stations.

MACRODASYIDA	Station					
	I	II	III	IV	V	VI
	Number of individuals per sample					
Turbanella pilosum sp. nov.	–	7	73	–	–	–
Mesodasys sp. 4	–	–	–	–	1	–
Number of species	–	1	1	–	1	–
Number of specimens	–	7	73	–	1	–

Table 8. Sediment characteristics at marine sampling sites.

Sites	I	II	III	IV	V	VI
Mean [μm] (M_G)	1458.4	1366.7	1407.4	1008.4	1503.1	1421.2
Sorting (σ_G)	0.814	1.078	0.970	1.598	0.829	0.806
Skewness	0.161	0.381	-11.371	-0.663	0.642	0.000
Kurtosis	0.558	-0.769	-0.068	0.565	0.323	0.738
Organic matter content [%]	*1.67*	*2.37*	*0.11*	*4.48*	*3.67*	*3.93*

overlapping fragments using 18Sfw/rev960 and fw390/rev18S primer pairs (Dabert *et al*. 2010), respectively. A fragment of 28S rRNA gene was amplified for one representative of all new and newly recorded species using the forward primer 28SF0001 (Dabert *et al*. 2016) with a 1:1 mixture of two reverse primers, R3950 and R3980 (this study). For all of the remaining specimens, the D1–D3 region of 28S rRNA gene was amplified with 28SF0001 (Dabert *et al*. 2016) and 1634LReverse (Telford *et al*. 2003) primers. PCRs were conducted in 10 μL reaction volumes containing 5 μL of the Type-it Microsatellite PCR Kit (Qiagen, Hilden, Germany), 0.5 μM of each primer and 4 μL of the DNA template using a thermocycling profile with one cycle of 5 min at 95°C followed by 35 steps of 30 s each at 95°C, 90 s at 50°C, 1–3 min at 72°C, and with a final step of 5 min at 72°C for all DNA fragments. For all primers details see Table 2. After amplification, the PCR products were diluted with 10 μL of MQ water; 5 μL of the diluted PCR reaction was analysed by electrophoresis on 1% agarose gel. Samples containing visible bands were purified with exonuclease I and Fast alkaline phosphatase (Thermo Fisher Scientific Inc.) and sequenced using the BigDye Terminator v3.1 kit and the ABI Prism 3130xl Genetic Analyzer (Applied Biosystems), following the manufacturer's instructions. Sequence chromatograms were checked for accuracy and sequences were aligned using Geneious 10.2.3 (Biomatters Ltd.). Genetic distances among the COI sequences were estimated using the Kimura 2-parameter model as implemented in MEGA 7 (Tamura *et al*. 2013). All new sequences used in this work have been deposited in GenBank under accession nos. MF325868-930, MF374689-707, MF41782-819 (see Table 3 for a full list of the deposited sequences).

Phylogenetic analyses

For the Chaetonotidae phylogenetic reconstruction, 93 species from six Chaetonotida families and two Gnathostomulida outgroup taxa were used (Table 4). For the Turbanellidae phylogenetic analyses, 16 species were applied (Table 5). Due to the high heterogeneity of available Macrodasyida sequences, we restricted our phylogenetic analysis to the family Turbanellidae and its sister group and the outgroup according to Todaro *et al*. (2015). This approach enabled us to obtain stronger support for the new species relationships within Turbanellidae. Sequences were aligned using the L–INS–i algorithm in MAFFT v7.308 (Katoh *et* Standley 2013) as implemented in Geneious 10.2.3 (Biomatters Ltd.). The best fit model of DNA evolution for each partition was chosen by PartitionFinder2 (Lanfear *et al*. 2012). Phylogenetic trees were reconstructed using Maximum Likelihood (ML) in Garli v.2.0 (Zwickl 2006) and Bayesian inference (BI) in MrBayes 3.2 (Ronquist *et al*. 2012). Each BI run of four independent chains was performed in 15×10^6 generations, and the trees were sampled every 1000 generations. The final consensus tree was generated after discarding the burn-in fraction of 0.25% of initial trees; however, the average standard deviation of split frequencies never dropped below 0.025. Support for the ML tree branches was calculated by the nonparametric bootstrap method with 500 replicates in Grali (Stamatakis 2006) and by analysing the consensus tree generated from 1000 ML trees reconstructed from the original dataset. We used the latter approach because the bootstrapping methods mostly failed due to very short branches in the ingroup taxa. The trees were edited in FigTree 1.4.2 (Rambaut 2014) and further in Corel Draw X5.

RESULTS

Taxonomic analysis revealed the presence of Gastrotricha representatives from two orders: Chaetonotida Remane, 1925 [Rao & Clausen, 1970] (in the freshwater samples) and Macrodasyida Remane, 1925 [Rao & Clausen, 1970] (in the marine samples).

In freshwater habitats, Gastrotricha were found in 8 stations with a density ranging from 20 to 850 individuals per 1 cm^3. The number of species in the sampling stations also varied over a wide range, from 1 to 9 (Table 6). The freshwater samples contained, in total, 231 specimens belonging to 1 family, 4 genera, 2 subgenera and 13 species. Most of the freshwater species are new to science; 6 of them are described in this paper, namely: *Chaetonotus* (*Chaetonotus*) *bombardus* sp. nov., *C.* (*C.*) *subtilis* sp. nov., *C.* (*Hystricochaetonotus*) *borealis* sp. nov., *C.* (*H.*) *hornsundi* sp. nov., *C.* (*H.*) *persimilis* sp. nov. and *Lepidodermella polaris* sp. nov.; the other 4 species: *C.* (*C.*) sp. 1, *Ichthydium* sp. 2, *Heterolepidoderma* sp. 3 and *Lepidodermella* sp. 4 are most probably new to science but could not be described due to insufficient material (only one or two specimens of each taxon). The remaining 3 species, namely *C.* (*C.*) *gelidus* Kolicka, 2017, *C.*(*C.*) *jaceki* Kolicka, 2017 and *C.* (*C.*) *svalbardi* Kolicka, 2017 belong to known taxa described from Isfjorden fjord area, another Arctic localities (Kolicka 2017). Inside the bodies of 171 out of 231 specimens, belonging to all of the recorded species at all stages of development, there was evidence for the presence of endosymbionts. Five of the specimens had isolated circular cysts within the body; the remaining individuals had either non-aggregated or diffuse formations (see Kisielewska *et al*. 2015, Kolicka 2016).

Marine gastrotrichs were collected from 6 stations along the Hornsund fjord coast in the tidal zone. Marine Gastrotricha species were noted at only

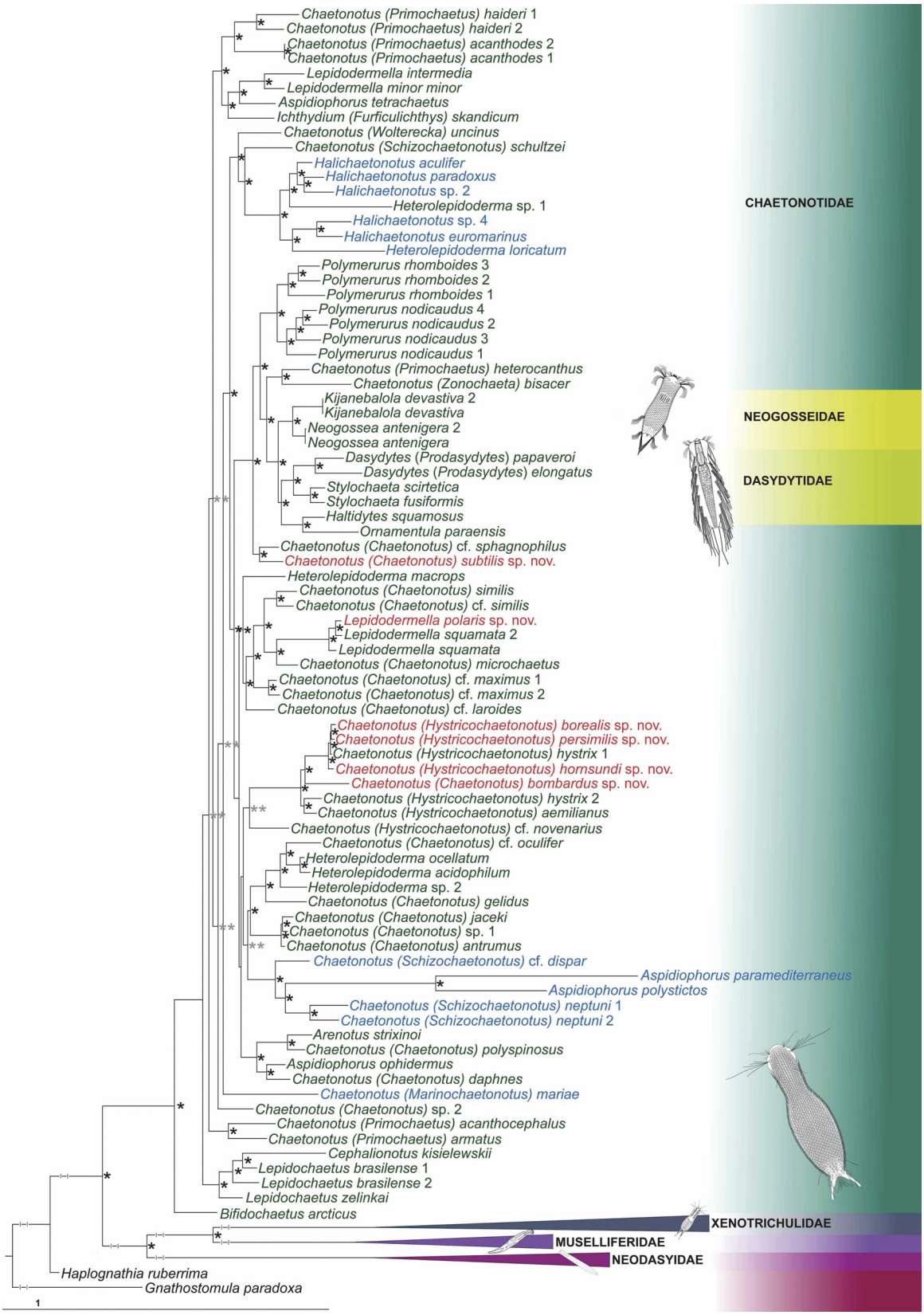


Figure 4. Phylogenetic relationships of Chaetonotida inferred from ML and BI analyses of concatenate 18S rRNA, 28S rRNA, and COI sequence data. Marks near branches show bootstrap support values above (* = 50 ≥ 70; ** > 70). The new species are indicated by red font; freshwater species marked by green font, marine by blue font. Drawings after MK and Jacek Kisielewski with permission.

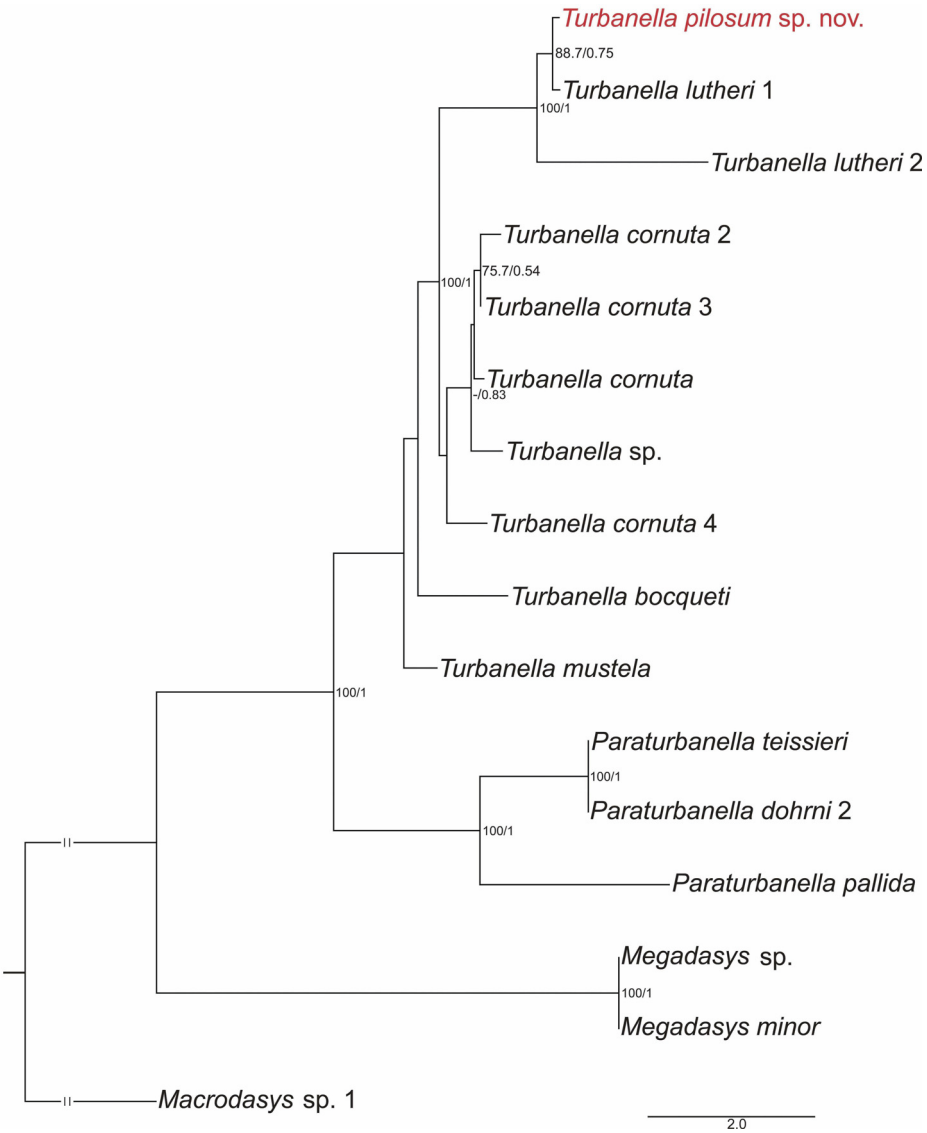


Figure 5. Phylogenetic relationships of Turbanellidae inferred from ML and BI analyses of concatenate 18S rRNA, 28S rRNA, and COI sequence data. Values near branches show bootstrap support and Bayesian posterior probability, respectively. The new species is indicated by red font.

3 sampling points: 1 individual from station V, 7 individuals from station II, and 73 individuals from station III (Table 7). In total, 81 specimens of Macrodasyida belonged to 2 families, 2 genera and 2 species. The two species are new to science: *Turbanella pilosum* sp. nov. is described in this paper and the second marine taxon, viz. *Mesodasys* sp. 4, could not be described due to insufficient material (only one specimen).

All of the freshwater samples included sediment with organic or organic-clay characters and a fine structure. The analysed marine samples included very coarse sandy sediment, with mean size ranging from 1.0 mm to 1.5 mm, and with the silt and clay content generally comprising less than 1% of the sediment volume (Table 8). All of the sediment samples were composed of very well-sorted sand, with the exception of samples from station IV whose sediments were composed of moderately well-sorted sand. The grain size distribution was mostly fine or coarse skewed and very platykurtic, with one exception of symmetrical and platykurtic sediments observed at station VI. The sediment characteristics did not vary distinctly among the sites; the only factor that clearly differed was the organic matter content, which ranged from 0.11% (station III) to 4.48% (station IV) of dry sediment.

Although our phylogenetic reconstruction of Chaetonotida inferred from ML and BI analyses in most parts is consistent with the previously published

(*e.g.* Kånneby *et al.* 2012, 2013, Kånneby *et* Todaro 2015), some of the inferred relationships vary due to the basal position of *Bifidochaetus arcticus* and *Lepidochaetus-Cephalionotus* clade (Fig. 4). Very short branches in this part of the tree may be responsible for low support values. However, all clades clustering the new species are well supported; therefore the results of the phylogenetic analysis concerning the new taxa are reliable. In our Turbanellidae phylogenetic reconstruction the family forms a monophyletic clade (Fig. 5). This clade containing two monophyletic groups, one containing only the representatives of the *Turbanella* genus and the second grouping *Paraturbanella* species. For details concerning phylogenetic position of the new species see 'Remarks' sections.

Taxonomic accounts

Phylum **Gastrotricha** Mečnikow, 1865
Order **Chaetonotida** Remane, 1925 [Rao & Clausen, 1970]
Suborder **Paucitubulatina** d'Hondt, 1971
Family **Chaetonotidae** Gosse, 1864 (*sensu* Leasi & Todaro, 2008)
Subfamily **Chaetonotinae** Gosse, 1864 (*sensu* Kisielewski, 1991)

Genus ***Chaetonotus*** Ehrenberg, 1830

Typus generis. *Chaetonotus larus* (Müller, 1773).
Terra typica. Denmark.

A polyphyletic taxon with only one apparently monophyletic subgenus (*Chaetonotus* (*Zonochaeta*) Remane, 1927a); encompasses 169 nominal freshwater species and 44 nominal marine species (Kånneby *et al.* 2012, 2013, Todaro 2018). Present in benthic, interstitial, periphytic and rarely semi-planktonic habitats (Kisielewski 1997).

Subgenus ***Chaetonotus*** *sensu stricto* Ehrenberg, 1830

Type species. *Chaetonotus larus* (Müller, 1773).
Terra typica. Denmark.

Clearly a polyphyletic subgenus in terms of molecular data (Kieneke *et al.* 2008, Kånneby *et al.* 2012, 2013); encompasses 82 nominal freshwater and 4 nominal marine species (Kieneke *et* Schmidt-Rhaesa 2015, Todaro 2018). Present in benthic, interstitial, periphytic and rarely also in semi-planktonic habitats (Kisielewski 1997).

Chaetonotus (***Chaetonotus***) ***bombardus*** sp. nov.
(Figs 6–14; Tables 9–11)

Localities: 2, 12.

Material. Three samples, in total 37 specimens (29 adults, 2 subadults, 6 juveniles), all photographed. The photomicrographs are available in the Natural History Collections at Adam Mickiewicz University in Poznań under accession numbers NHC–GCCB–13–1–10/h (holotype) and NHC–GCCB–13–11–50/p (paratypes) and in the author's collection.

Etymology. The term 'bombardus' is a Latinised species name referring to the pontoon named "Bombard" which with great sacrifice took a stereoscopic microscope to the Polish Polar Station during this research study.

Diagnosis. Slender body, measuring from 62.5 to 126.3 μm. Head five-lobed, cephalion and epipleurae virtually invisible in the head outline. Hypostomium wide and ship-shaped. Ocellar granules absent. Scales, with few exceptions, three-lobed with straight keel and strong spine. Two pairs of one-lobed, elongated, keeled and spined scale on the dorsal and dorsolateral furcal base surfaces. Scales distributed in 29–31 total longitudinal rows (9–11D+4DL+6L+6LV+4V) with 21–25 scales in the central row and differing morphologically in the areas of the head, neck and trunk. Spines strongly bent basally and subsequently straight, slightly tapering to the blunt ends. Spines thick without lateral denticle. Lengths of spines gradually increase slightly from head towards the widest trunk region, after which they gradually decrease slightly to the furcal base; one pair of parafurcal spines significantly stronger and longer than other spines. Ventral interciliary field from anterior pharynx dilatation region covered by tear-shaped scales with keele and rudimentary to short and straight spine. Three pairs of ventral interciliary field terminal scales. Pharynx narrow with pronounced anterior and posterior dilatations. A single, narrow, curved cuticular rod inside the anterior pharynx dilatation. Intestine straight with anterior section differing in form and morphology.

Description. *Chaetonotus* (*Chaetonotus*) *bombardus* sp. nov. has a slender body. Its head is wider than the neck and the neck constriction is clearly marked. The neck gradually tapers from the head (from U20) to the beginning of the trunk (*ca.* U30) (Figs 6, 8–10). The trunk has a width similar to the head and its widest part is slightly wider than the head. The trunk gradually dilates from *ca.* U30 to *ca.* U61, where it is at its maximum width, and then it gradually tapers down to the furcal base (from U83) (Figs 6, 8–10). The furcal base is clearly demarcated and narrow. The furcal indentation (intrafurcal space) is parabolic in shape. The branches of the furca are set close together and point slightly outwards (Figs 6, 8–10). The furca and adhesive tubes are relatively short in comparison to the body length (Table 9). The adhesive tubes are thin, slightly curved towards the centre of the indentation (very slightly thong-shaped) and have the same

width along their entire length. The adhesive tubes' ends are blunt with slightly rounded edges (Figs 6, 8–10).

The head is five-lobed and semicircular in shape. The cephalion (U1–U5) is short, narrow and with a slightly lateral notch near the dorsal edge. It is very weakly demarcated in the body outline but clearly visible from the dorsal side. It adheres to the head along the entire length. The epipleurae (U5–U7) and hypopleurae (U7–U14) are flat. The epipleurae are small and the hypopleurae are over double the size of the epipleurae (Fig. 6). The epipleurae adhere to the hypopleurae and there are no notches between them. The pleurae are not visible from the dorsal side; only their outline is demarcated, their greatest portion is located on the ventrolateral and ventral sides. The hypostomium (U5–U8) is wide and ship-shaped with a deep, semicircular anterior notch. Its anterolateral edges are reinforced (Figs 6C, 9C). Two pairs of cephalic ciliary tufts are present. The anterior tufts emerge in lines between the lateral cephalion edges and the anterior edges of the epipleurae. The anterior tufts consist of 5 cilia. The anteriormost cilium in both anterior tufts is the shortest. The second cilium is much longer, and the longest in the tuft. The third cilium is shorter than the second one. Starting from the third to fifth, the cilia gradually increase in length. The posterior tufts have 5 cilia each and emerge in ventral notches between the epipleurae and hypopleurae on the ventral side. The anteriormost and third cilia in both tufts are short and similar in length. The second and fifth cilia are similar in length and longer than the first and third cilia. The fourth cilium is very long and the longest of all the cilia in both pairs of cephalic tufts. All of the cilia are thick. Ocellar granules absent. The mouth ring is subterminal at U3–U5 and has granular cuticular reinforcements. Short cuticular inner hairs are present inside it (Figs 6C, 9C). Suboral bristles are located in two tufts around the mouth ring's lateral sides (Figs 6C, 9C).

With the exception of the three pairs of one-lobed scales on the dorsal posterior trunk region and two pairs of one-lobed scales on the furcal base and furcal appendages and the ventral interciliary field scales, the entire body is covered with three-lobed scales with a strong keel running from halfway down the central lobe length and with a strong spine. The scales are shaped as wide triangles with a wide, strongly rounded central lobe and long, narrow posterolateral lobes, and very deep, parabolic shaped posterior notches. The scales are distributed in 29–31 longitudinal alternating rows (9–11D+4DL+6L+6LV+4V) with 21–25 scales in the central row. The central longitudinal row of scales runs straight throughout the dorsal surface, parallel to the longitudinal axis of the body. The rest of the longitudinal rows of scales also run straight and are arranged almost parallel from the top of the head to the widest body region. Posterior to the widest body region, the longitudinal rows of scales form gradually weakly bent arches that slightly converge towards the central row of scales. The scales are located close to another and adjacent scales have overlapping posterolateral lobes (Figs 6–10). The head scales possess weakly separated posterolateral lobes from the central lobe (scales 1; Fig. 7A). On the neck, towards the trunk, there is a gradual separation of the posterolateral scale lobes (scales 2; Fig. 7B). On the trunk scales the posterolateral lobes are clearly separated from the central lobe (scales 3; Fig. 7C). In addition, the length of the central scale lobe increases, relative to the posterolateral length, from the top of the head to the widest trunk region, then it subsequently decreases in length towards the furcal base (scales 4; Fig. 7D). Three small, one-lobed scales with straight keels and rudimentary spines are on the dorsal surface in the posterior trunk region, anterior to each double-keeled scale bearing a dorsal sensory bristle (at U77–U79) (scales 5; Fig. 7E). Posterior to the double-keeled scale with dorsal sensory bristles (scale 6), two pairs of three-lobed, narrower and elongated scales with long straight keels and spines are present at the dorsal and dorsolateral surfaces of the furcal base and furcal appendages. The anterior of these pairs is located dorsolaterally at U83–U87 and each scale is shaped as a narrow and elongated triangle with posterolateral lobes that extend only slightly outwards, with a straight keel and a shallow posterior notch (scales 7; Fig. 7G). The posterior pair is located dorsally at U86–U90, and each scale is shorter and wider with clearly separated posterolateral lobes, with a keel and a straight, strong spine (scales 8; Fig. 7H). These scales are oriented diagonally to the body axis, with posterior scales edges directed to the central body region (Fig. 6A). Two pairs of one-lobed, narrow scales are located dorsolaterally on the furcal appendages, at U90–U91. These scales are rectangular with rounded corners, have straight keels, and lack posterior notches and spines (scales 9; Fig. 7I). The scales are arranged very close to another and meet at the lateral edges (Fig. 6A). One pair of scales with parafurcal spines is located at U89–U91, is smaller and similar in shape to those from the posterior trunk region (scales 10; Fig. 7J).

The size of the scales gradually increases from the head towards the widest body region, then their size gradually decreases to the furcal base. In addition, the size of the scales gradually decreases from the dorsal body surface, throughout the dorsolateral, lateral, ventrolateral and ventral surface of the head, neck and trunk towards the ventral ciliary bands (Table 10). The scales in the ventral longitudinal row located closest to the ciliary band are distinctly smaller than the other

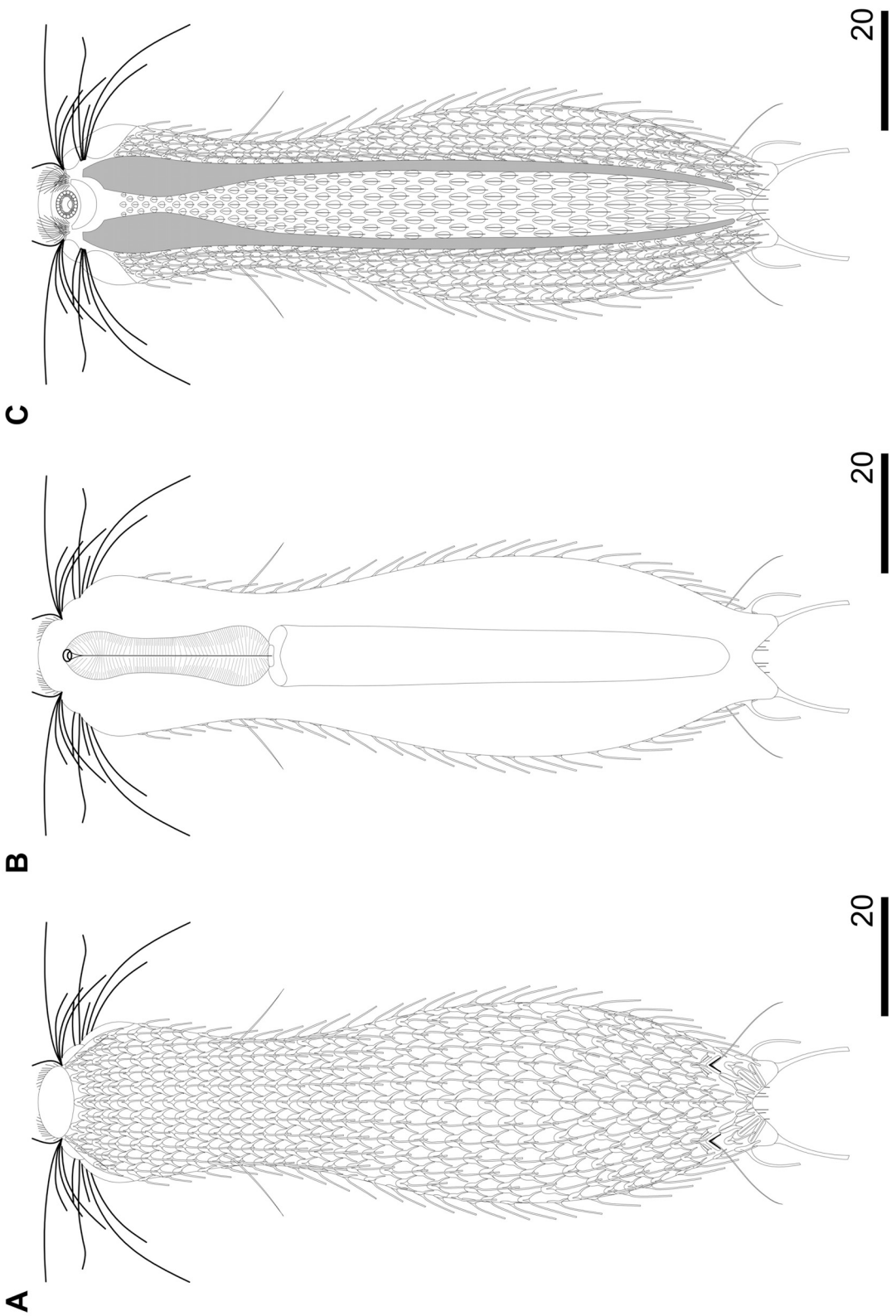


Figure 6. *Chaetonotus* (*Chaetonotus*) *bombardus* sp. nov. Schematic drawings. A – Dorsal body view. B – View of internal morphology. C – Ventral body view. Light grey areas indicate the areas of the insertion of ciliary bands.

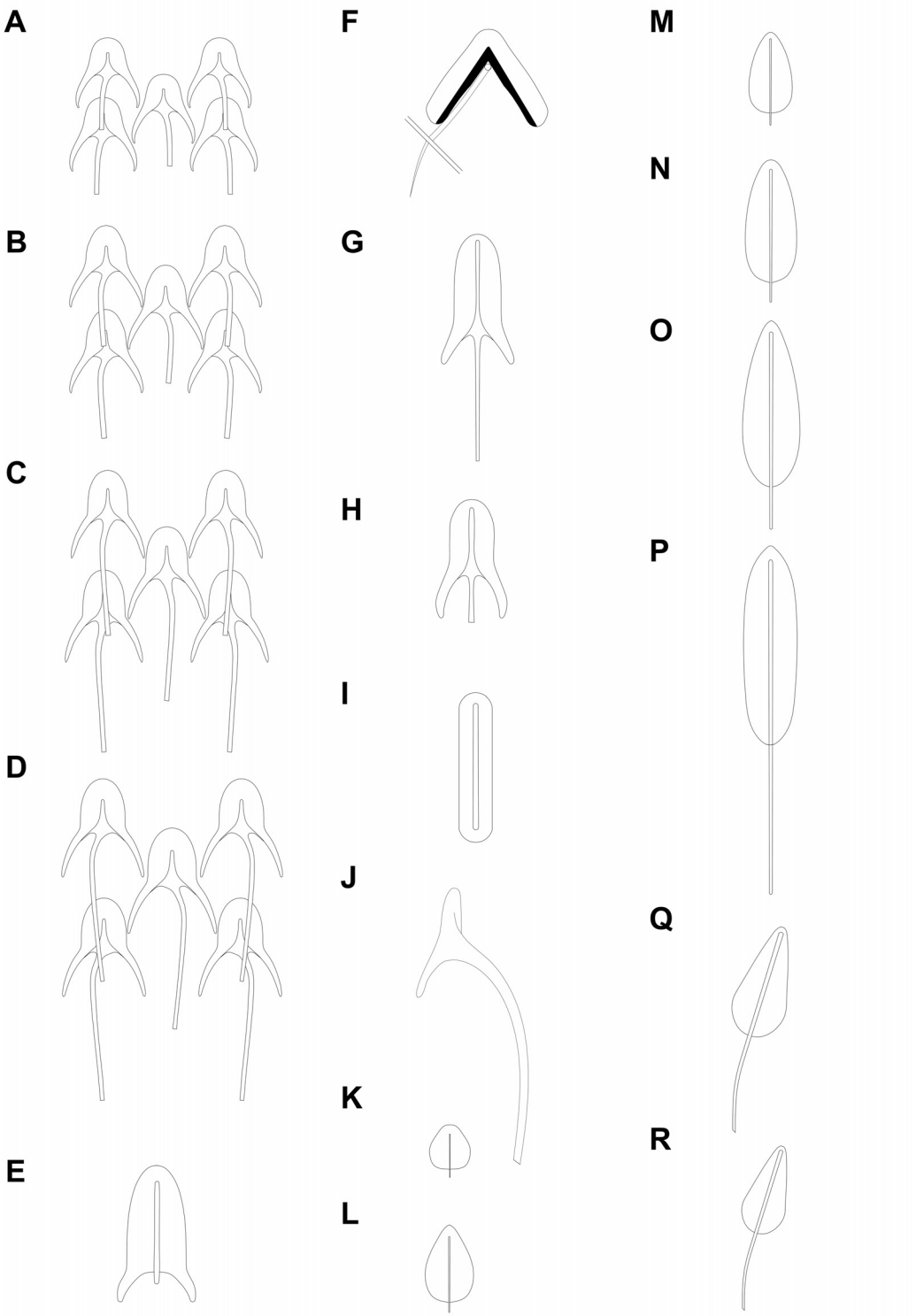


Figure 7. *Chaetonotus* (*Chaetonotus*) *bombardus* sp. nov. Schematic scales drawings. A – Head scales (scales 1). B – Neck scales (scales 2). C – Trunk anterior scales (scales 3). D – Trunk central and posterior scales (scales 4). E – One-lobed scale with straight keels and rudimentary spines anterior to each double-keeled scale with dorsal sensory bristle (scale 5). F – Double-keeled scale with trunk dorsal sensory bristle (scale 6). G – Furcal base dorsal scale (scale 7). H – Furcal appendages dorsal scale (scale 8). I – Furcal appendages dorsal elongated, spinless scale (scale 9). J – Scales with parafurcal spines (scale 10). K–O – Ventral interciliary field scale (scales 11–13). P – First pair of ventral interciliary field terminal scale (scale 14). Q – Second pair of ventral interciliary field terminal scale (scale 15). R – Third pair of ventral interciliary field terminal scale (scale 16).

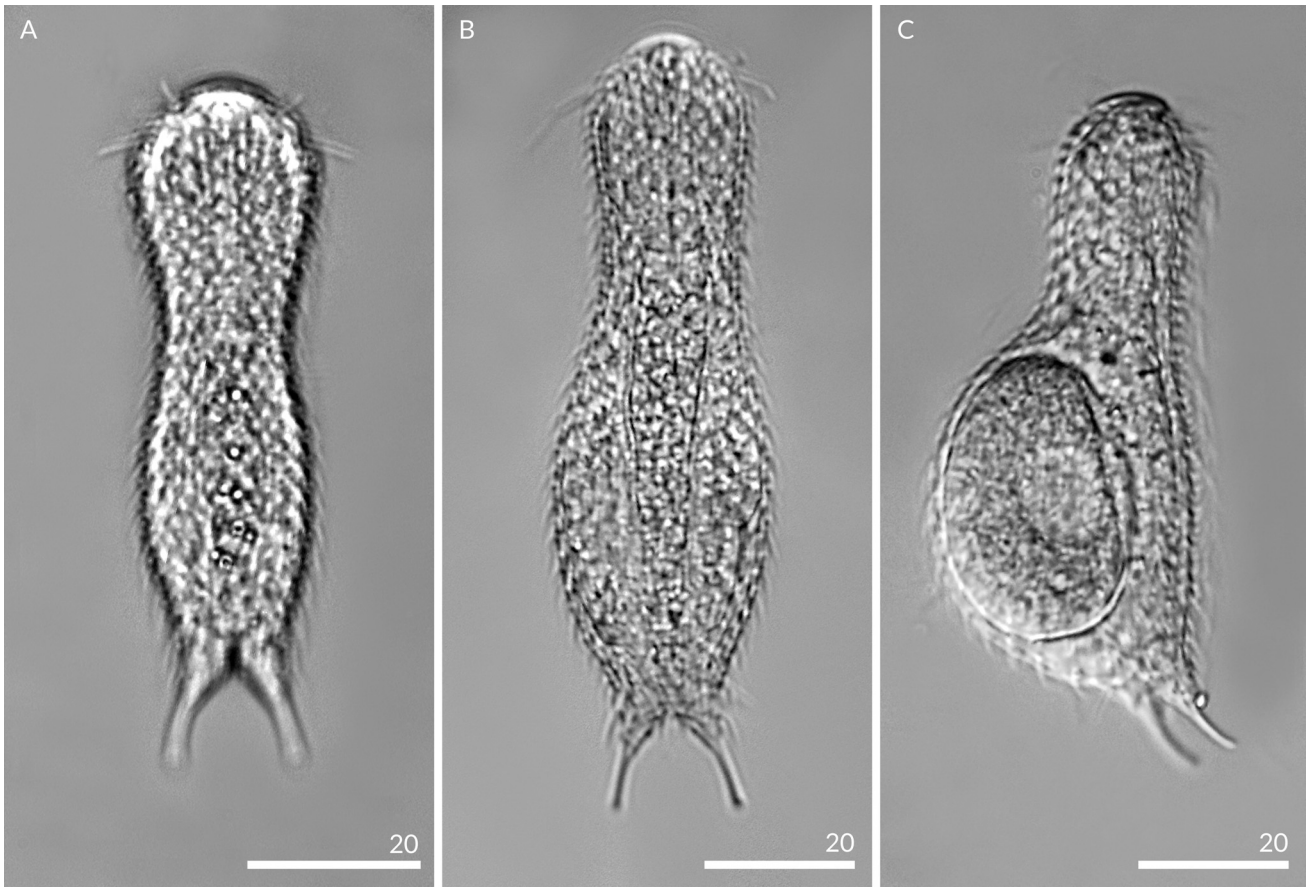


Figure 8. *Chaetonotus* (*Chaetonotus*) *bombardus* sp. nov. A – Juvenile specimen. Habitus. B – Adult specimen. Habitus. C – Specimen with mature egg. Habitus.

scales and their anterior edge is oriented towards the bands at an angle of *ca.* 10° (Figs 6C, 8C, 9C).

The spines emerging from the posterior scales region are thick, basally strongly curved and then subsequently straight. The spines taper gradually towards their blunt ends and have no lateral denticles. The length of the spines gradually increases from the head towards the widest body region, then decreases gradually to the furcal base. The posteriormost lateral trunk spines do not vary in length from the other spines on the lateral surfaces of the posterior trunk region. Moreover, the spines gradually increase from the dorsal body surfaces, throughout the dorsolateral, lateral, ventrolateral and ventral body surfaces towards the ciliary bands (Table 10). Spines emerging from the elongated scales on the furcal base and furcal appendages are thick, straight and do not taper to their ends. The parafurcal spines (U89–U91) are strongly curved, taper towards their blunt ends, and are thicker and much longer than the other spines of the body (Table 10).

The species has three pairs of dorsal sensory bristles (Figs 6, 8–10). The first, anterior pair is located on the dorsal surface of the head at U5, between the lateral cephalion edges and the epipleurae. The bristles emerge from small, spherical papillae. The second pair of sensory bristles is located on the dorsolateral surface of the neck at U26 and emerges from small, spherical papillae. The third, posterior pair of sensory bristles is located dorsally at U80–U82 on the posterior trunk region. These bristles emerge from the triangle-shaped scales with two strong keels connected in the scales' centre and with very deeply notched posterior edges (Figs 6A, 7F).

On the ventral surface, the longitudinal ciliary bands begin at U7 and run back to U86 (Figs 6C, 9C, 10C). The ciliary bands on the head are arranged close to the hypopleurae and lateral edges of the hypostomium. The ciliary bands are wider on the head and neck region than in the other parts of the body, where they are narrow. The ventral interciliary band in the intestine region runs almost parallel to one another. The ventral interciliary field is covered with small, one-lobed scales from the end of the pharynx posterior dilatation to the furcal appendages (Figs 6C, 9C, 10C).

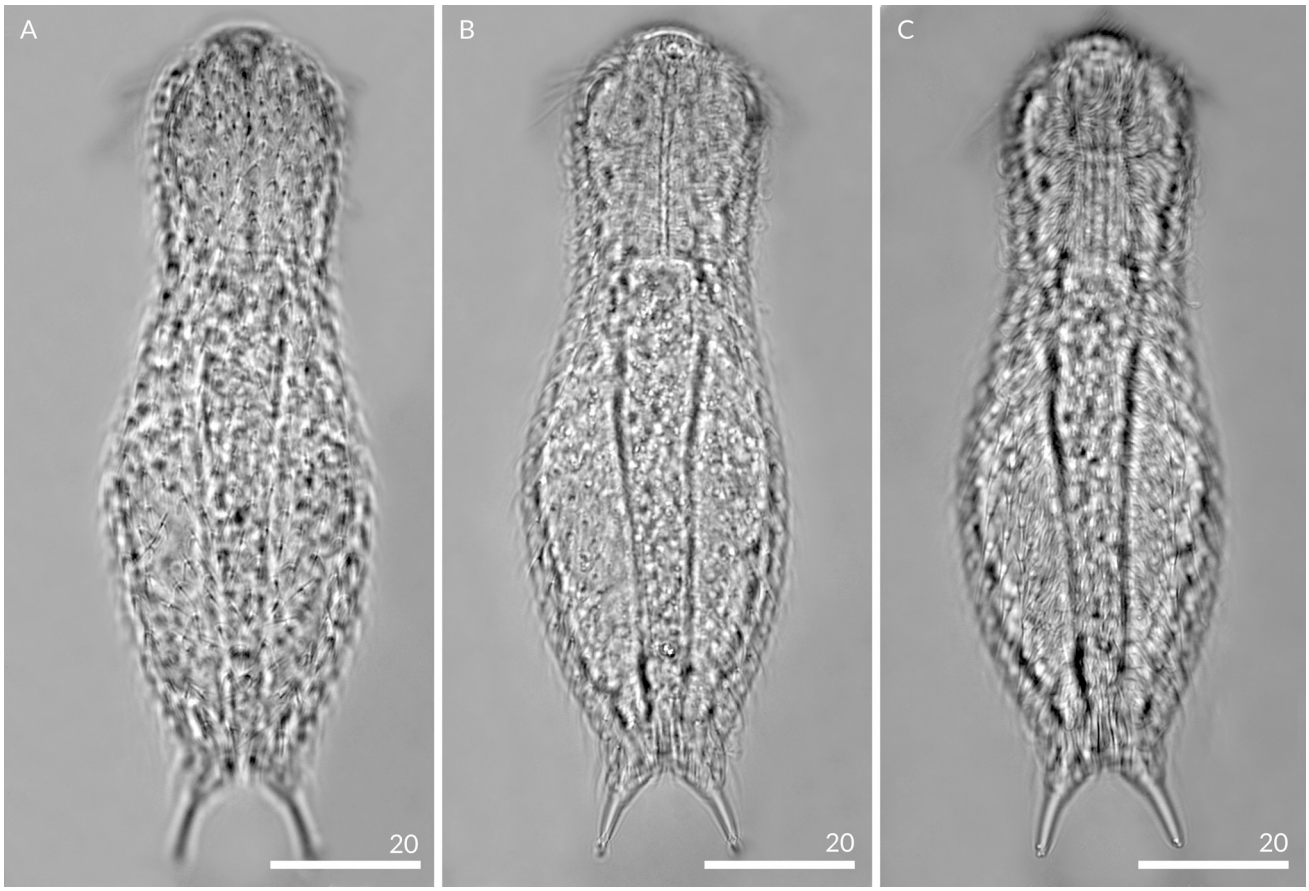


Figure 9. *Chaetonotus* (*Chaetonotus*) *bombardus* sp. nov. Adult specimen. A – Dorsal body view. B – View of internal morphology. C – Ventral body view.

The scales are tear-shaped with a straight keel and a rudimentary to short and straight spine (scales 11–13; Fig. 7M, N, O). The size and number of the scales and the length of their spines increase from anterior to posterior (see Table 10). These scales are distributed in 9 longitudinal rows with 21–25 scales in the central row. Their separation from cuticle increases towards the posterior part of the body, where they are completely distinguished. Three pairs of ventral interciliary field terminal scales are present. The first pair is large, located at U84–U89, the second at U87–U90 and the third at U89–U91. The scales in these three pairs are triangular with rounded edges and without any posterior notches. They have straight keels and straight spines that extend to the furcal indentation (scales 14–16; Figs 6C, 7P, 7Q, 7R, 9C).

The pharynx (from U4 to U29) is narrow and has clearly marked anterior and posterior dilatations. In the anterior dilatation, weak cuticular reinforcements are located in the form of a single slightly curved rod (at U6). The posterior dilatation is greater than the anterior (Figs 6B, 9B, 10B, 13). The pharynx is connected through the pharyngeal pharyngeal-intestinal junction to the straight intestine (running from U30 to U86). The pharyngeal-intestinal junction is clearly demarcated, short and wide (U30). The intestine has a short separate anterior section running from U30 to U32 (Figs 6B, 9B, 10B).

The X-organ of this species (observed in one specimen) is located at U81–U82 near the terminal part of the intestine. It is bilobed, built from two extensions enveloped in a thin coat and connected by a thinner band located behind the intestine at the ventral side (Fig. 14B). The extensions have a granular appearance. The thin coat and the cellular bridge connecting the extensions have a smooth and homogeneous structure. The sperm packets of this species are circular in shape and contain spermatozoids (4 per packet) in the form of a short rod (Fig. 14A). They are located at U66–U67 on both sides, juxtaposed to the intestine.

Remarks. Previously, two specimens belonging to *C.* (*C.*) *bombardus* sp. nov. were presented as *Chaetonotus* (*Chaetonotus*) sp. 1 in the Longyerbyen region (Isfjorden, Spitsbergen) (Kolicka 2017).

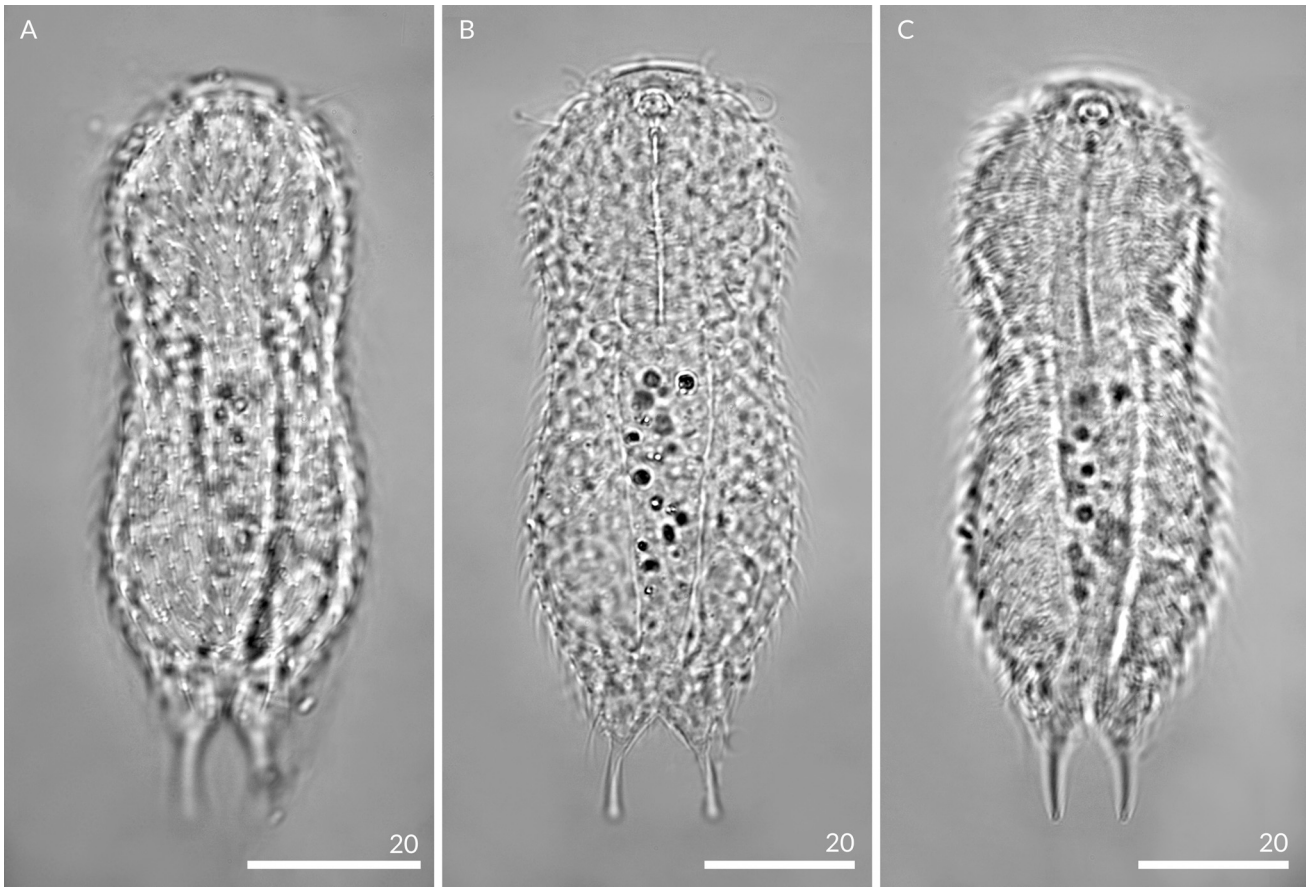


Figure 10. *Chaetonotus* (*Chaetonotus*) *bombardus* sp. nov. Juvenile specimen. A – Dorsal body view. B – View of internal morphology. C – Ventral body view.

The total body length of the juvenile and subadult specimens of *C.* (*C.*) *bombardus* sp. nov. varies from 62.5 to 104.7 μm; the length of the pharynx is between 24.2 and 31.1 μm; of the intestine from 24.8 to 51.9 μm; and there are 27–31 total longitudinal rows of scales (7–11D+4DL+6L+6LV+4V) with 21–23 scales in the central row.

Phylogenetic analysis showed *Chaetonotus* (*Chaetonotus*) *bombardus* sp. nov. to be a sister taxon to the clade containing *C.* (*H.*) *boralis* sp. nov., *C.* (*H.*) *hornsundi* sp. nov. and *C.* (*H.*) *persimilis* sp. nov. together with *C.* (*H.*) *hystrix* 1. These four new species fall within the maximally supported clade that including all new described in this paper representatives of the subgenus *Hystricochaetonotus*, although the main morphological features of *C.* (*C.*) *bombardus*, such as the lack of a lateral denticle on the spines, strongly suggest their affinity to subgenus *Chaetonotus* (see the description above) (Fig. 4). Subgenus *Chaetonotus* is currently very broad and includes strongly diverse species, while the range of subgenus *Hystricochaetonotus* is narrower and more precise membership criteria have been defined. Perhaps adding further species to the phylogenetic tree will help either resolve or revise this species affiliation.

Of the 29 reported adults, 7 individuals had a large developing egg, 1 had two eggs that were large and similar in size, and 1 had an X-organ and two packages of sperm.

Differential diagnosis. *Chaetonotus* Ehrenberg, 1830 is a polyphyletic genus and contains very varied species. Of all the currently known 169 nominal freshwater representatives of this genus, *Chaetonotus* (*Chaetonotus*) *bombardus* sp. nov. morphologically most closely resembles *C.* (*C.*) *gelidus* Kolicka, 2017, *C.* (*C.*) *svalbardi* Kolicka, 2017 and *C.* (*C.*) *disjunctus* Greuter, 1917. *C.* (*C.*) *gelidus* was selected for comparison with the new species due to its similarity in terms of: range of body length; place of emerging cephalic cilia; range of pharynx length; pharynx shape; presence of cuticular reinforcements in the pharynx; anterior intestine section differing in form of morphology; range of furca length; range of number of scales in the central longitudinal row; strongly differentiated scales' shape

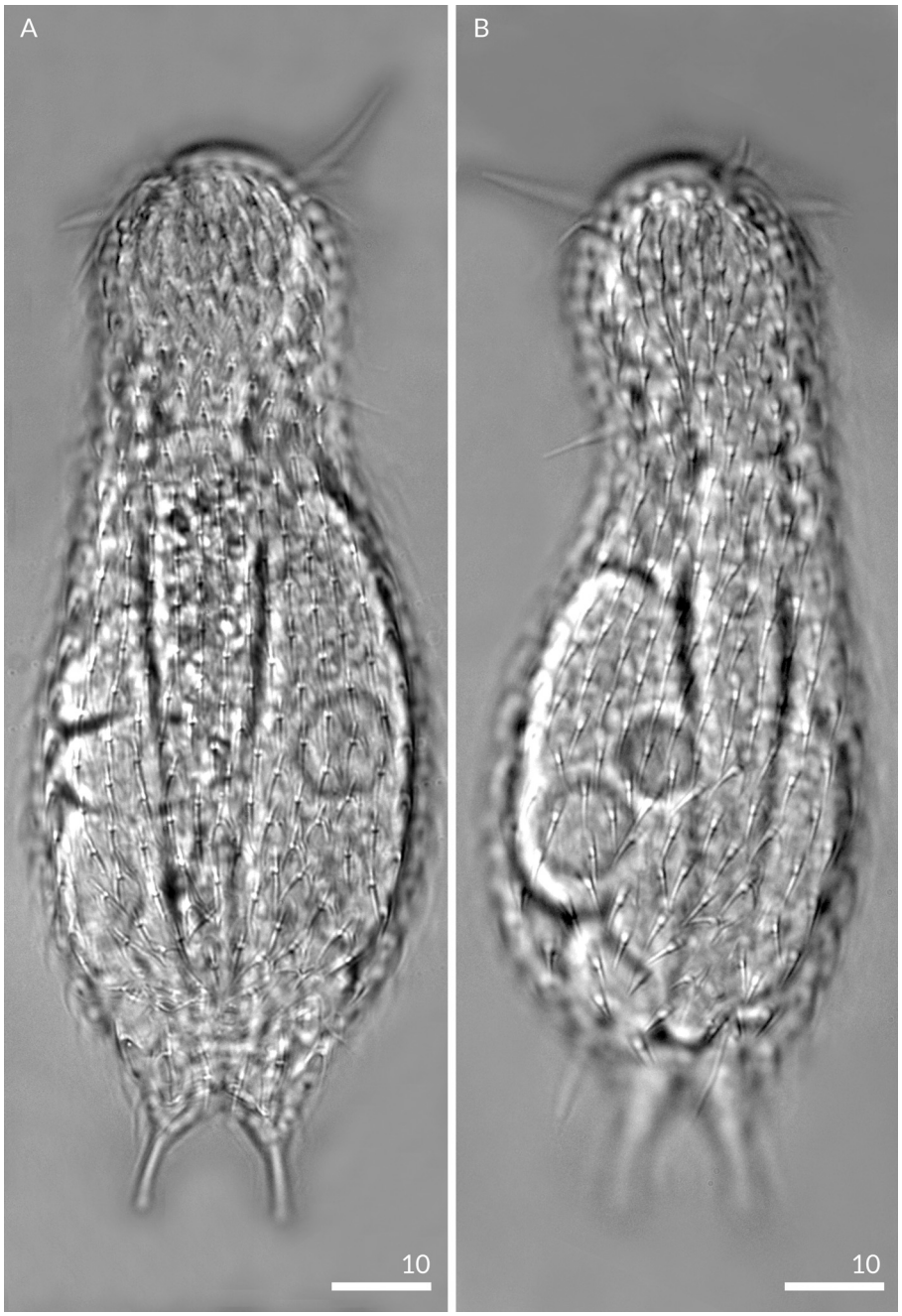


Figure 11. *Chaetonotus* (*Chaetonotus*) *bombardus* sp. nov. Adult specimen. A – Dorsal view of scales. B – Dorsal view of spines.

within the body regions; similar range of scale length; presence of scales of a different type on the dorsal and dorsolateral surfaces of the furcal base; ventral interciliary field covered by scales from the posterior dilatation of the pharynx to the furcal base; ventral ciliary bands wider on the head and neck region; and the number of dorsal sensory bristles. *C.* (*C.*) *svalbardi* shares with *C.* (*C.*) *bombardus* sp. nov. the following: range of body length; places of emerging posterior tufts of the cephalic cilia; range of pharynx length; pharynx shape; presence of cuticular reinforcements in the pharynx; anterior intestine section differing in form of morphology; range of furca length; range of adhesive tube length; range of number of scales in the central longitudinal row; type of scales; scale size variation; range of scale size; presence of scales of a different type on the dorsal and dorsolateral surfaces of the furcal base; range of spine length; type of posteriormost

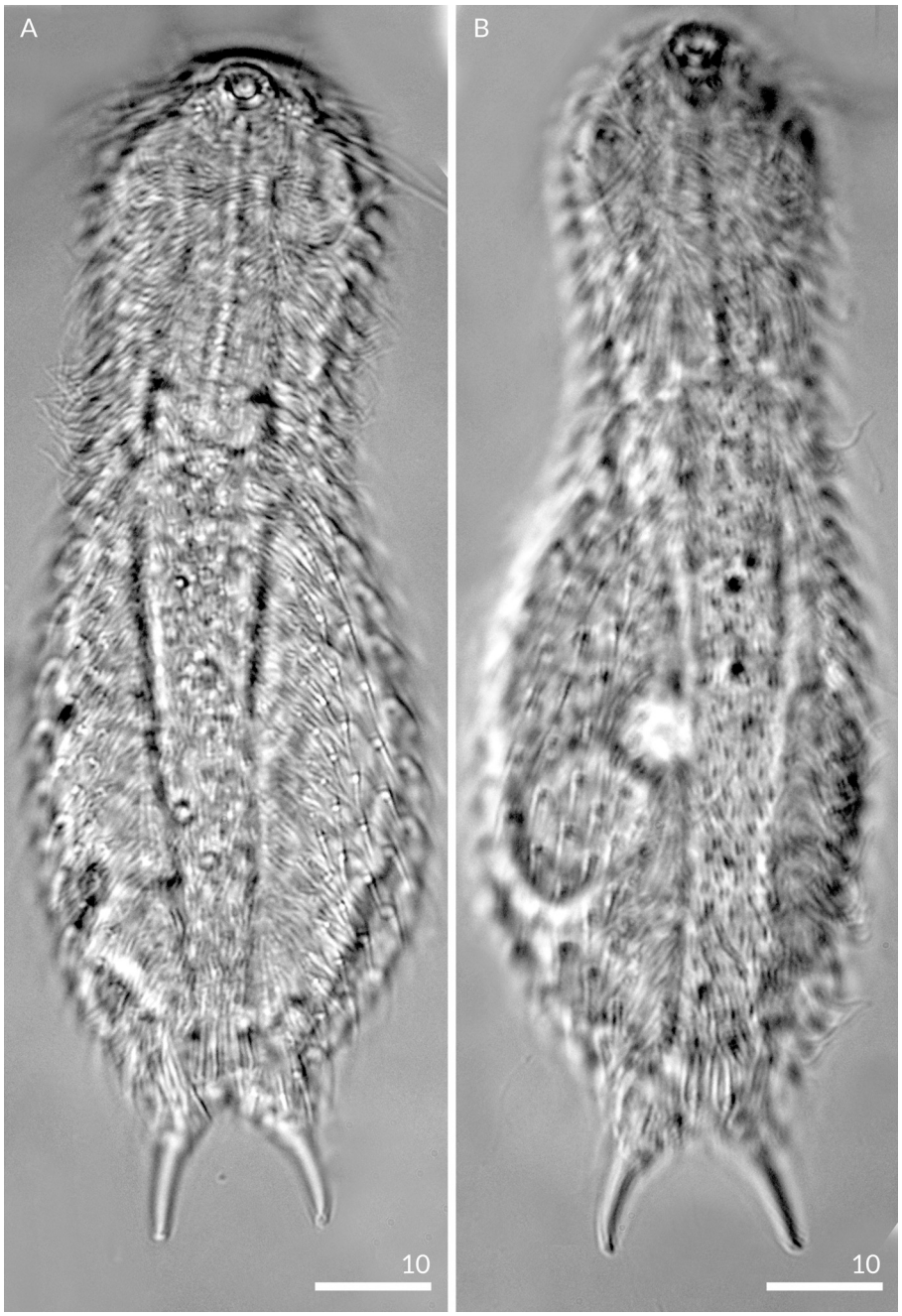


Figure 12. *Chaetonotus* (*Chaetonotus*) *bombardus* sp. nov. Adult specimen. A – Ventral view of scales. B – Ventral view of spines.

pair of trunk lateral spines; ventral ciliary bands wider on the head and neck region; and number of dorsal sensory bristles. *C.* (*C.*) *disjunctus* resembles the *C.* (*C.*) *bombardus* sp. nov. in terms of: range of furca length; type of furcal indentation; range of number of scales in the central longitudinal row; range of scale length; presence of a different type of scales on the dorsal and dorsolateral surfaces of the furcal base and absence of hair-like spines on the ventral body surface. Despite the fact that of all the hitherto known taxa, *C.* (*C.*) *gelidus, C.* (*C.*) *svalbardi* and *C.* (*C.*) *disjunctus* have the highest number of common features with the *C.* (*C.*) *bombardus* sp. nov., they are significantly different from them – most strikingly by scale type and shape as well as by variation in the length of the spines. The comparison between the new species and the most morphologically similar taxa is summarised in Table 11.

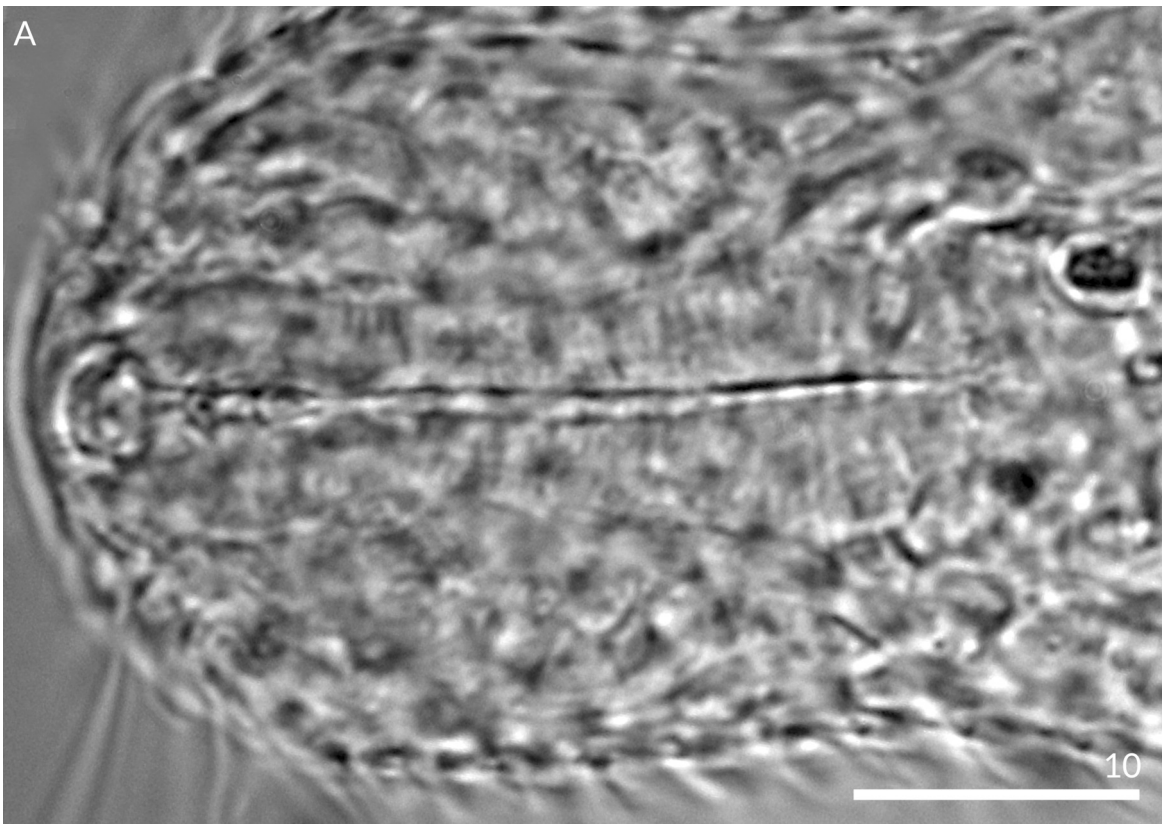


Figure 13. *Chaetonotus* (*Chaetonotus*) *bombardus* sp. nov. Adult specimen. Detail of the pharynx.

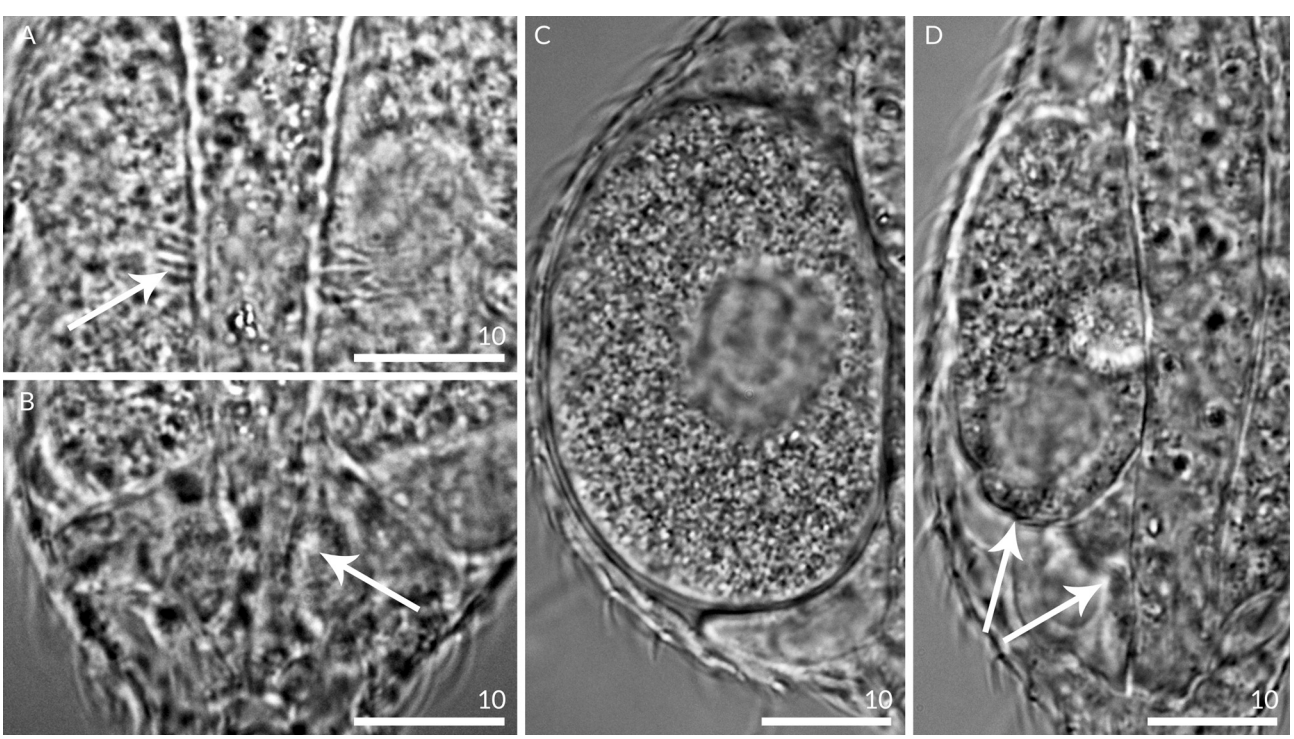


Figure 14. *Chaetonotus* (*Chaetonotus*) *bombardus* sp. nov. Detail of the internal morfology. A – Sperm packet. B – X-organ. C – Matured egg. D – Two maturing eggs.

Table 9. Main morphometric characters of *Chaetonotus* (*Chaetonotus*) *bombardus* sp. nov; N – number of specimens or structures analysed, Range – the smallest and the largest structure found among all specimens measured. All measurements are given in micrometers (μm).

Characters	N	Holotype	Range of adult paratypes
Body length	29	123.12	107.07–126.30
Furca length	15	21.36	18.18–22.23
Adhesive tube length	15	11.33	8.92–11.58
Number of scales in central longitudinal row	29	25	21–25
Total number of longitudinal alternating rows of scales	29	29	29–31
Head scales length x width	15	1.42–4.39 x 1.41–4.16	1.06–4.76 x 1.03–4.51
Neck scales length x width	15	2.27–5.25 x 2.15–4.32	1.78–5.68 x 1.69–4.67
Trunk scales length x width	15	2.78–6.19 x 2.64–5.98	2.19–6.73 x 2.07–6.50
Head spines length	15	2.97–5.29	2.31–6.18
Neck spines length	15	4.55–6.61	3.87–7.60
Trunk spines length	15	5.77–8.16	4.90–9.37
Mouth ring diameter	15	4.38	4.03–4.95
Pharynx length	29	31.26	28.72–34.96

Table 10. Detailed morphometric characters of *Chaetonotus* (*Chaetonotus*) *bombardus* sp. nov; N – number of specimens or structures analysed, Range – the smallest and the largest structure found among all specimens measured, SD – standard deviation. All measurements are given in micrometers (μm); all indicators are given as a percentage (%) and italicized.

Characters	N	Holotype	Range of adult paratypes	SD
Cephalion length	15	10.34	9.64–11.02	0.325
Cephalion width	15	13.18	12.08–13.88	0.396
Hypostomium length	15	3.41	3.41–5.15	0.464
Hypostomium width	15	6.47	6.14–7.50	0.397
Length of cephalic cilia (anterior tuft)	15	7.66–16.21	(6.56–8.28)–(14.27–17.81)	0.507; 0.982
Length of cephalic cilia (posterior tuft)	15	10.69–17.87	(8.95–11.15)–(14.68–18.28)	0.528; 0.869
Width of cephalic cilia (anterior tuft)	15	0.98–1.02	(0.88–1.02)–(0.92–1.04)	0.0403; 0.034
Width of cephalic cilia (posterior tuft)	15	1.04–1.09	(0.91–1.05)–(1.01–1.10)	0.035; 0.029
Width of adhesive tube	15	1.64	1.48–1.67	0.053
Ratio of scale distribution	29	116	*116.000–138.100*	6.534
Length of head dorsal scales (scales 1)	15	2.71–4.39	(1.99–2.94)–(3.43–4.76)	0.272; 0.354
Width of head dorsal scales (scales 1)	15	2.68–4.16	(1.97–2.91)–(3.25–4.51)	0.269; 0.329
Length of head dorsolateral scales (scales 1)	15	2.57–4.27	(1.89–2.79)–(3.34–4.63)	0.253; 0.346
Width of head dorsolateral scales (scales 1)	15	2.54–4.05	(1.87–2.76)–93.16–4.39)	0.253; 0.320
Length of head lateral scales (scales 1)	15	2.44–4.12	(1.79–2.65)–(3.22–4.47)	0.243; 0.340
Width of head lateral scales (scales 1)	15	2.40–3.90	(1.76–2.61)–(3.05–4.23)	0.238; 0.308
Length of head ventrolateral scales (scales 1)	15	2.16–3.72	(1.59–2.35)–(2.91–4.04)	0.214; 0.319
Width of head ventrolateral scales (scales 1)	15	2.13–3.53	(1.57–2.32)–(2.75–3.83)	0.213; 0.280
Length of head ventral scales (scales 1)	15	1.42–2.69	(1.06–1.54)–(2.10–2.92)	0.137; 0.224
Width of head ventral scales (scales 1)	15	1.41–2.55	(1.03–1.52)–(1.99–2.77)	0.138; 0.203
Length of neck dorsal scales (scales 2)	15	4.22–5.25	(3.31–4.57)–(4.12–5.68)	0.0338; 0.433
Width of neck dorsal scales (scales 2)	15	3.96–4.32	(3.10–4.28)–(3.38–4.67)	0.311; 0.332
Length of neck dorsolateral scales (scales 2)	15	4.06–5.12	(3.18–4.39)–(4.02–5.54)	0.325; 0.409
Width of neck dorsolateral scales (scales 2)	15	3.81–4.21	(2.97–4.12)–(3.30–4.56)	0.302; 0.326
Length of neck lateral scales (scales 2)	15	3.91–5.01	(3.06–4.23)–(3.93–5.42)	0.312; 0.414
Width of neck lateral scales (scales 2)	15	3.67–4.12	(2.86–3.97)–(3.22–4.46)	0.289; 0.317
Length of neck ventrolateral scales (scales 2)	15	3.56–4.84	(2.79–3.85)–(3.80–5.25)	0.283; 0.402
Width of neck ventrolateral scales (scales 2)	15	3.34–3.97	(2.62–3.61)–(3.12–4.31)	0.260; 0.283
Length of neck ventral scales (scales 2)	15	2.27–4.29	(1.78–2.45)–(3.38–4.65)	0.181; 0.337
Width of neck ventral scales (scales 2)	15	2.15–3.53	(1.69–2.32)–(2.78–3.83)	0.174; 0.278

Table 10. Continued.

Characters	N	Holotype	Range of adult paratypes	SD
Length of trunk dorsal scales (scales 3 and 4)	15	5.12–6.19	(4.02–5.54)–(5.19–6.73)	0.407; 0.459
Width of trunk dorsal scales (scales 3 and 4)	15	4.29–5.98	(3.37–4.64)–(5.01–6.50)	0.325; 0.393
Length of trunk dorsolateral scales (scales 3 and 4)	15	4.94–6.01	(3.39–5.35)–(5.05–6.53)	0.392; 0.460
Width of trunk dorsolateral scales (scales 3 and 4)	15	4.14–5.77	(3.26–4.48)–(3.89–6.27)	0.312; 0.535
Length of trunk lateral scales (scales 3 and 4)	15	4.80–5.86	(3.78–5.20)–(3.95–6.37)	0.381; 0.609
Width of trunk lateral scales (scales 3 and 4)	15	4.02–5.63	(3.17–4.36)–(3.79–6.11)	0.303; 0.524
Length of trunk ventrolateral scales (scales 3 and 4)	15	4.62–5.69	(3.64–5.00)–(3.84–6.19)	0.366; 0.571
Width of trunk ventrolateral scales (scales 3 and 4)	15	3.87–5.44	(3.05–4.19)–93.67–5.91)	0.292; 0.506
Length of trunk ventral scales (scales 3 and 4)	15	2.78–5.47	(2.19–3.01)–(3.69–5.95)	0.221; 0.538
Width of trunk ventral scales (scales 3 and 4)	15	2.64–5.22	(2.07–2.86)–(3.52–5.67)	0.220; 0.475
Length of small one-lobed scales on posterior trunk region (scales 5)	15	2.23–2.37	(1.71–2.89)–(1.86–3.44)	0.356; 0.442
Width of small one-lobed scales on posterior trunk region (scales 5)	15	1.73–1.92	(1.23–2.31)–(1.31–2.93)	0.318; 0.436
Length of dorsal pair of scales on the furcal base (scales 7)	15	6.71	4.96–7.03	0.592
Width of dorsal pair of scales on the furcal base (scales 7)	15	3.97	2.94–4.29	0.378
Length of dorsal pair of scales on the furcal appendages (scales 8)	15	7.51	5.76–8.56	0.689
Length of dorsal pair of scales on the furcal appendages (scales 8)	15	4.42	3.41–5.04	0.398
Length of one-lobed scales on furcal appendages (scales 9)	15	4.78–4.85	(3.69–5.52)–(3.93–5.82)	0.470; 0.466
Width of one-lobed scales on furcal appendages (scales 9)	15	1.23–1.28	(0.94–1.59)–(1.02–1.65)	0.145; 0.134
Length of lateral scales with parafurcal spines (scales 10)	15	5.59	4.72–6.04	0.339
Width of lateral scales with parafurcal spines (scales 10)	15	4.92	4.15–5.32	0.304
Length of head dorsal spines (scales 1)	15	2.97–4.38	(2.31–3.46)–(3.73–5.11)	0.284; 0.346
Length of head dorsolateral spines (scales 1)	15	3.27–4.61	(2.59–3.80)–(3.92–5.38)	0.304; 0.366
Length of head lateral spines (scales 1)	15	3.44–4.89	(2.73–4.00)–(4.17–5.71)	0.323; 0.398
Length of head ventrolateral spines (scales 1)	15	3.62–5.04	(2.89–4.21)–(4.31–5.89)	0.342; 0.410
Length of head ventral spines (scales 1)	15	3.97–5.29	(3.19–4.62)–(4.52–6.18)	0.356; 0.429
Length of neck dorsal spines (scales 2)	15	4.55–5.59	(3.87–5.26)–(4.75–6.44)	0.342; 0.413
Length of neck dorsolateral spines (scales 2)	15	4.79–5.83	(4.07–5.54)–(4.95–6.72)	0.362; 0.433
Length of neck lateral spines (scales 2)	15	4.94–6.05	(4.21–5.71)–(5.15–6.97)	0.371; 0.446
Length of neck ventrolateral spines (scales 2)	15	5.32–6.26	(4.53–6.14)–(5.32–7.21)	0.396; 0.463
Length of neck ventral spines (scales 2)	15	5.68–6.61	(4.83–6.56)–(5.62–7.60)	0.424; 0.482
Length of trunk dorsal spines (scales 3 and 4)	15	5.77–7.03	(4.90–6.63)–(5.99–8.08)	0.424; 0.482
Length of trunk dorsolateral spines (scales 3 and 4)	15	5.94–7.26	(5.04–6.82)–(6.18–8.34)	0.436; 0.499
Length of trunk lateral spines (scales 3 and 4)	15	6.19–7.52	(5.25–7.11)–(6.41–8.64)	0.456; 0.520
Length of trunk ventrolateral spines (scales 3 and 4)	15	6.42–7.81	(5.44–7.37)–(6.67–8.97)	0.471; 0.538
Length of trunk ventral spines (scales 3 and 4)	15	6.76–8.16	(5.73–7.73)–(6.97–9.37)	0.492; 0.558
Length of small one-lobed scales on posterior trunk region spines (scales 5)	15	0.37–0.45	(0.23–0.43)–(0.36–0.59)	0.069–0.075
Length of dorsal pair of scales on the furcal base spines (scales 6)	15	4.76	3.43–5.08	0.494
Length of dorsal pair of scales on the furcal appendages spines (scales 7)	15	5.96	4.92–6.07	0.448
Length of trunk posteriormost pair of lateral spines (scales 4)	15	6.56	5.51–7.32	0.444

Table 10. Continued.

Characters	N	Holotype	Range of adult paratypes	SD
Length of parafurcal lateral spines (scales 10)	15	9.54	8.22–12.19	1.045
Head dorsal sensory bristles length	15	9.36	7.09–11.04	1.062
Neck dorsal sensory bristles length	15	10.63	9.29–12.34	0.807
Posterior dorsal sensory bristles length	15	11.52	10.29–13.18	0.740
Number of scales in central longitudinal row on ventral interciliary field	29	25	21–25	1.265
Total number of longitudinal alternating rows of scales on ventral interciliary field	29	9	9	0
Length of ventral interciliary field scales (scales 11–13)	15	0.87–3.06	(0.68–0.87)–(2.62–3.16)	0.057; 0.146
Width of ventral interciliary field scales (scales 11–13)	15	0.68–1.12	(0.42–0.72)–(0.98–1.20)	0.074; 0.065
Length of terminal ventral interciliary field scales I pair (scales 14)	15	7.67	6.38–8.03	0.438
Width of terminal ventral interciliary field scales I pair (scales 14)	15	3.23	2.37–3.36	0.249
Length of terminal ventral interciliary field scales II pair (scales 15)	15	3.28	2.44–3.51	0.334
Width of terminal ventral interciliary field scales II pair (scales 15)	15	1.61	1.23–1.91	0.208
Length of terminal ventral interciliary field scales III pair (scales 16)	15	3.11	2.01–3.21	0.403
Width of terminal ventral interciliary field scales III pair (scales 16)	15	1.43	1.08–1.74	0.180
Length of ventral interciliary field scales spines (scales 11–13)	15	0.10–1.61	(0.06–0.13)–(1.29–1.77)	0.027; 0.145
Length of terminal ventral interciliary field scales I pair spines (scales 14)	15	5.28	4.74–6.01	0.400
Length of terminal ventral interciliary field scales II pair spines (scales 15)	15	3.69	3.05–4.09	0.323
Length of terminal ventral interciliary field scales III pair spines (scales 16)	15	2.66	2.08–3.39	0.414
Width of anterior pharynx thickening (a)	15	8.42	7.37–9.31	0.635
Width of pharynx narrowing (n)	15	6.07	5.39–6.92	0.481
Width of middle pharynx (m)	15	7.09	6.31–7.83	0.473
Width of posterior pharynx thickening (p)	15	9.67	8.72–10.77	0.508
Width of pharyngeal-intestinal junction	15	4.06	3.42–4.32	0.309
Length of intestine anterior differ section – in it central part	15	2.82	2.15–3.29	0.338
Length of intestine anterior differ section – in it lateral part	15	3.45	2.56–4.42	0.566
Intestine length	29	66.29	54.64–66.95	3.270
Pharynx formula a	29	26.935	*24.518–31.823*	2.094
Pharynx formula n	29	19.418	*17.265–22.952*	1.631
Pharynx formula m	29	22.681	*20.948–25.970*	1.361
Pharynx formula p	29	30.934	*30.059–34.912*	1.551
Pharynx-intestine ratio I	29	47.156	*46.207–53.839*	2.102
Length of matured egg	1	–	44.26	0
Width of matured egg	1	–	29.27	0

Table 11. Condensed comparison of the most important differential characters between species most similar to *Chaetonotus* (*Chaetonotus*) *bombarus* sp. nov. Measurements are given in micrometres (μm). NA – data not available; – – not applicable.

Character	*C.* (*C.*) *bombardus* sp. nov.	*C.* (*C.*) *gelidus*	*C.* (*C.*) *svalbardi*	*C.* (*C.*) *disiunctus*
Body length	107.1–126.3	80.3–115.2	109.1–120.9	150.0–256.0
Cephalic pleurae	Cephalion narrow; epipleurae flat and small; hypopleurae flat and large, two times larger than epipleurae; epipleurae and hypopleurae located on the lateral, ventrolateral and ventral sides in such a manner that their greatest portion is located on the ventrolateral and ventral sides. Pleurae not visible from the dorsal side; only their outline is demarcated. Epipleurae adhere to hypopleurae and there are no notches between them. Hypostomium wide, ship-shaped with reinforced anterior edge	Cephalion narrow, widens at the dorsal edge; epipleurae and hypopleurae large; hypopleurae larger than epipleurae; epipleurae located dorsolaterally; hypopleurae located more ventrolaterally. Hypostomium small, rhomboidal	Cephalion narrow; epipleurae and hypopleurae small, similar size; visible from the dorsal body side. Hypostomium small, kidney-shaped	Cephalion narrow; epipleurae small; hypopleurae large, two times larger than epipleurae; epipleurae and hypopleurae located mostly laterally. Hypostomium present
Place of emerging cephalic cilia	Anterior tufts: in the line between lateral cephalion edges and anterior edges of epipleurae; posterior tufts: on ventral side in notches between epipleurae and hypopleurae	Anterior tufts: at the lateral edges of the cephalion; posterior tufts: on the ventral side, beneath the posterior edges of the epipleurae	Anterior tufts: ventrally, between cephalion and epipleurae; posterior tufts: ventrally, in notches between epipleurae and hypopleurae	Anterior tufts: dorsoterminal between cephalion and epipleurae; posterior tufts: laterally, between epipleurae and hypopleurae
Pharynx length	28.7–35.0	21.7–33.0	29.6–32.1	36.0–53.0
Pharynx shape	Pharynx with anterior and wider posterior dilatation	Pharynx with anterior and wider posterior dilatation	Pharynx with anterior and wider posterior dilatation	Pharynx with clearly marked anterior and wider, slightly marked posterior dilatation
Presence of cuticular reinforcements in the pharynx	Present (single, narrow, curved cuticular rod)	Present (single, narrow, curved cuticular rod)	Present (two slight, thin and straight linked cuticular rods)	Absent
Anterior intestine section differing in form of morphology	Present	Present	Present	Absent
Furca length	18.2–22.2	16.8–19.1	18.9–19.6	17.0–26.0
Adhesive tube length	9.9–11.6	6.9–8.1	10.4–11.5	NA
Type of furcal indentation	Parabolic in shape	Helmet-like shaped	V-shaped	Parabolic in shape

Table 11. Continued.

Character	*C.* (*C.*) *bombardus* sp. nov.	*C.* (*C.*) *gelidus*	*C.* (*C.*) *svalbardi*	*C.* (*C.*) *disiunctus*
Number of scales in central longitudinal row	21–25	23–28	21–23	20–27
Total number of longitudinal alternating rows of scales	29–31	31–37	25–27	17–19
Type of scales	Almost all three-lobed with strong keels and bent spines; shape strongly differentiated within body regions	Scales small; one-lobed with strong keels and spines; shape differentiated within different body regions	Almost all three-lobed with strong keels and bent spines; shape strongly differentiated within body regions	One-lobed without keels and with spines; shape undifferentiated within different body regions
Scale size variation	Size gradually increases from the head towards the widest body region and then size gradually decreases towards the furcal base; scale size gradually decreases gradually from the dorsal body side, through to the dorsolateral, lateral, ventrolateral and ventral surface of the head, neck and trunk towards the direction of the ventral ciliary bands	Size and length of the scales relative to their width gradually increases from the posterior part of the head to the widest point of the trunk; scales in three longitudinal rows located on ventral body side gradually decrease in size towards the ciliary bands	Strongly differentiated within body regions	Similar size all over the body
Scale size	Head: 1.1–4.8 x 1.0–4.5 Neck: 1.8–5.7 x 1.7–4.7 Trunk: 2.2–6.3 x 2.1–6.5	Head: 0.9–4.3 x 1.1–3.8 Neck: 1.1–5.0 x 0.9–3.1 Trunk: 1.3–6.2 x 1.1–3.9	Head: 1.0–3.9 x 0.8–5.0 Neck: 1.4–5.2 x 1.1–4.6 Trunk: 1.4–8.3 x 1.8–7.1	Head: NA Neck: 4.0–5.0 x NA Trunk: 5.0–8.0 x NA
Alignment of scales	Located close to another and with overlapping posterolateral lobes	Edges juxtaposed but do not overlap	Located far from another, edges do not overlap	Located far from another, edges do not overlap
Presence of a different type of scales on the dorsal and dorsolateral side of the furcal base	Present	Present	Present	Present
Scale coverage of the ventral interciliary field	Covered from the pharyngeal region from the posterior dilatation of the pharynx to the furcal base	Covered from the pharyngeal region from the posterior dilatation of the pharynx to the furcal base	Covered from the beginning of the intestinal section to the furcal base; scales clearly visible only on the posterior trunk region	NA
Type of scales of the ventral interciliary field	Small, one-lobed, tear-shaped scales, with long keel and vestigial spines	Small, one-lobed scales shaped like rectangles with rounded edges without posterior notches, with long keel and vestigial spines	Small, one-lobed rectangle scales with rounded anterior edge, weakly keeled and spineless	NA

Table 11. Continued.

Character	*C.* (*C.*) *bombardus* sp. nov.	*C.* (*C.*) *gelidus*	*C.* (*C.*) *svalbardi*	*C.* (*C.*) *disiunctus*
Number of terminal scales of the ventral interciliary field	3 pairs	1 pair	2 pairs	NA
Type of terminal scales of the ventral interciliary field	All pairs: shaped like a triangle with rounded edges, keeled and spined	Narrow triangles without posterior notches, keeled with vestigial spines	Anterior pair: Long, narrow rectangles with rounded anterior edges and very shallow posterior notches, keeled and spined Posterior pair: Narrow triangles with shallow posterior notches, keeled and spined (short thin spines)	NA
Spine length variation	Spine length gradually increases gradually from the head towards the widest body region and then their length gradually decreases towards the furcal base; spines lengthen from dorsal body surface, on the dorsolateral, lateral, ventrolateral and ventral body sides towards the ciliary bands	Spines of the head longer and more curved than those of the neck. Spines of the neck gradually turn into shorter spines of the trunk; length of the spines slightly and gradually increases from the dorsal side to the ventral side, towards the ciliary bands; ventral spines long	Spine length gradually increases from the beginning of the head towards about three-fourths of the body length; spines on dorsal side lengthen more rapidly and are longer than the dorsolateral, lateral and ventrolateral spines; ventral spines much longer than the other spines	All spines of similar length, only posteriormost pair of trunk lateral spines are longer
Spine length	Head: 2.3–6.2 Neck: 3.9–7.6 Trunk: 4.9–9.4	Head: 1.7–6.4 Neck: 1.2–6.2 Trunk: 1.1–6.1	Head: 1.8–7.8 Neck: 3.1–8.9 Trunk: 3.6–12.9	Head: NA Neck: 8.0–13.0 Trunk: 8.0–14.0
Presence of hair-like spines on the ventral body side	Absent	Present	Present	Absent
Type of posteriormost pair of trunk lateral spines	Similar to other posterior trunk spines, not longer or thicker	Slightly longer than the other lateral trunk spines, not thicker or more curved	Similar to other posterior trunk spines, not longer or thicker	Longer and stronger than other spines
Type of furcal appendage lateral spines	Longer, stronger and more curved than other spines	Slightly longer than the trunk lateral spines, not thicker or more curved	Similar to the posterior trunk spines, not longer or thicker	–
Ventral locomotor cilia	Two longitudinal bands wider on the head and neck region	Two longitudinal bands wider on the head and neck region	Two longitudinal bands wider on the head region	Two longitudinal bands
Number of dorsal sensory bristles	3 pairs	3 pairs	3 pairs	2 pairs

Chaetonotus* (*Chaetonotus*) *jaceki Kolicka, 2017

Localities: 2, 3, 4, 6, 10.

Material. Five samples, 54 specimens (43 adults, 6 subadults and 5 juveniles), all photographed. The photomicrographs are available in the author's collection.

Remarks. *Chaetonotus* (*Chaetonotus*) *jaceki* Kolicka, 2017 was the most abundant and common species in the Hornsund freshwater samples. The total body length of adult specimens of *C.* (*C.*) *jaceki* varied from 116.3 to 135.1 μm; length of pharynx was between 27.6 and 35.4 μm; of intestine from 58.9 to 76.5 μm; there were 31–39 total longitudinal rows of scales (11–17D+4–6DL+4L+8LV+4V) with 28–37 scales in the central row. Total body length of juvenile and subadult specimens of *C.* (*C.*) *jaceki* varied from 95.7 to 116.3 μm; length of pharynx was between 28.2 and 34.2 μm; of intestine from 39.5 to 57.9 μm; there were 29–39 total longitudinal rows of scales (11–15D+4–6DL+4L+6LV+4V) with 25–35 scales in the central row. The found specimens corresponded very well with the morphological and morphometric features of the original description (Kolicka 2017).

In our phylogenetic analyses, *C.* (*C.*) *jaceki* forms a clade with *C.* (*C.*) *antrumus* and undescribed *Chaetonotus* (*Chaetonotus*) sp. 1 Kånneby, Todaro *et* Jondelis, 2013. This clade is a sister group to the clade containing *Heterolepidoderma ocellatum*, *H. acidophilum*, *Heterolepidoderma* sp. 2 Kånneby, Todaro *et* Jondelis, 2013, *C* (*C.*) cf. *oculifer* as well as the newly sequenced *C.* (*C.*) *gelidus* (Fig. 4). Phylogenetic position of *C.* (*C.*) *jaceki* indicated by molecular data is consistent with its morphological features (Kolicka 2017).

Of the 43 reported adults, 7 individuals had a large, developing egg, 1 had two eggs that were large and similar in size; and 3 had an X-organ and two packages of sperm. None of the specimens had two large developing eggs in the original description (Kolicka 2017).

Distribution. *Chaetonotus* (*Chaetonotus*) *jaceki* Kolicka, 2017 was previously known from only one locality on Spitsbergen (Svalbard) in the Longyearbyen region (Kolicka 2017).

Chaetonotus* (*Chaetonotus*) *gelidus Kolicka, 2017

Localities: 2, 4, 10, 12.

Material. Four samples, 14 specimens (13 adults and 1 juvenile), all photographed. The photomicrographs are available in the author's collection.

Remarks. Total body length of adult specimens of *Chaetonotus* (*Chaetonotus*) *gelidus* varied from 105.1 to 114.8 μm; length of pharynx was between 26.2 and 31.9 μm; of intestine from 55.4 to 64.8 μm; there were 31–37 total longitudinal rows of scales (11–15D+4–6DL+6L+6LV+4V) with 23–29 scales in the central row. The only reported juvenile *C.* (*C.*) *gelidus* was 98.1 μm in total body length; the pharynx was 26.5 μm in length; the intestine was 49.8 μm. It has 31 rows of scales in total with 25 scales in the central row. The specimens found during this research correspond very well with the morphological and morphometric features from the original description (Kolicka 2017).

In the phylogenetic tree (Fig. 4), *C.* (*C.*) *gelidus* is a sister group to the clade containing *Heterolepidoderma* sp. 2, *C.* (*C.*) *oculifer*, *H. ocellatum* and *H. acidophilum*. This whole branch forms a sister group to the clade with *C.* (*C.*) *jaceki*, *Chaetonotus* (*C.*) sp. 1 Kånneby, Todaro *et* Jondelis, 2013 and *C.* (*C.*) *antrumus*.

Of the 13 reported adults, 3 individuals had a large, developing egg.

Distribution. *Chaetonotus* (*Chaetonotus*) *gelidus* Kolicka, 2017 was previously known only from one locality on Spitsbergen (Svalbard) in the Longyearbyen region (Kolicka 2017).

Chaetonotus* (*Chaetonotus*) *subtilis sp. nov.
(Figs 15–22; Table 12–14)

Localities: 2, 3, 6.

Material. Three samples, 31 specimens (26 adults, 3 subadults and 2 juveniles) in total, all photographed. The photomicrographs are available in the Natural History Collections at Adam Mickiewicz University in Poznań under accession numbers NHC–GCCS–14–1–10/h (holotype) and NHC–GCCS–14–11–50/p (paratypes) and in the author's collection.

Etymology. From the Latin *subtile*, "subtle", referring to the long, slender body shape and numerous, small and delicate scales.

Diagnosis. Slender, elongated body measuring from 121.6 to 172.1 μm. Head five-lobed, cephalion clearly demarcated, epipleurae virtually invisible in head outline. Hypostomium wide and short, rhomboidal in shape. Ocellar granules absent. Scales small, one-lobed and sub-three-lobed, deeply posteriorily notched with a keel and straight spine. On furcal base and furcal appendages, numerous smaller and narrower scales; dorsally on furcal appendages four pairs of large, elongated one-lobed scales with thick, straight spine. Scales distributed in 33–41 total longitudinal rows (13–15D+4–6DL+4–6L+8–10LV+4V) with 31–39 scales in the central row and differing morphologically in the areas of head, neck and trunk. Scales located close to another and meeting at the posterolateral parts. Body spines straight and thin, tapering towards end, without lateral denticle. Spines on ventral body surface hair-like along their entire length. Length of

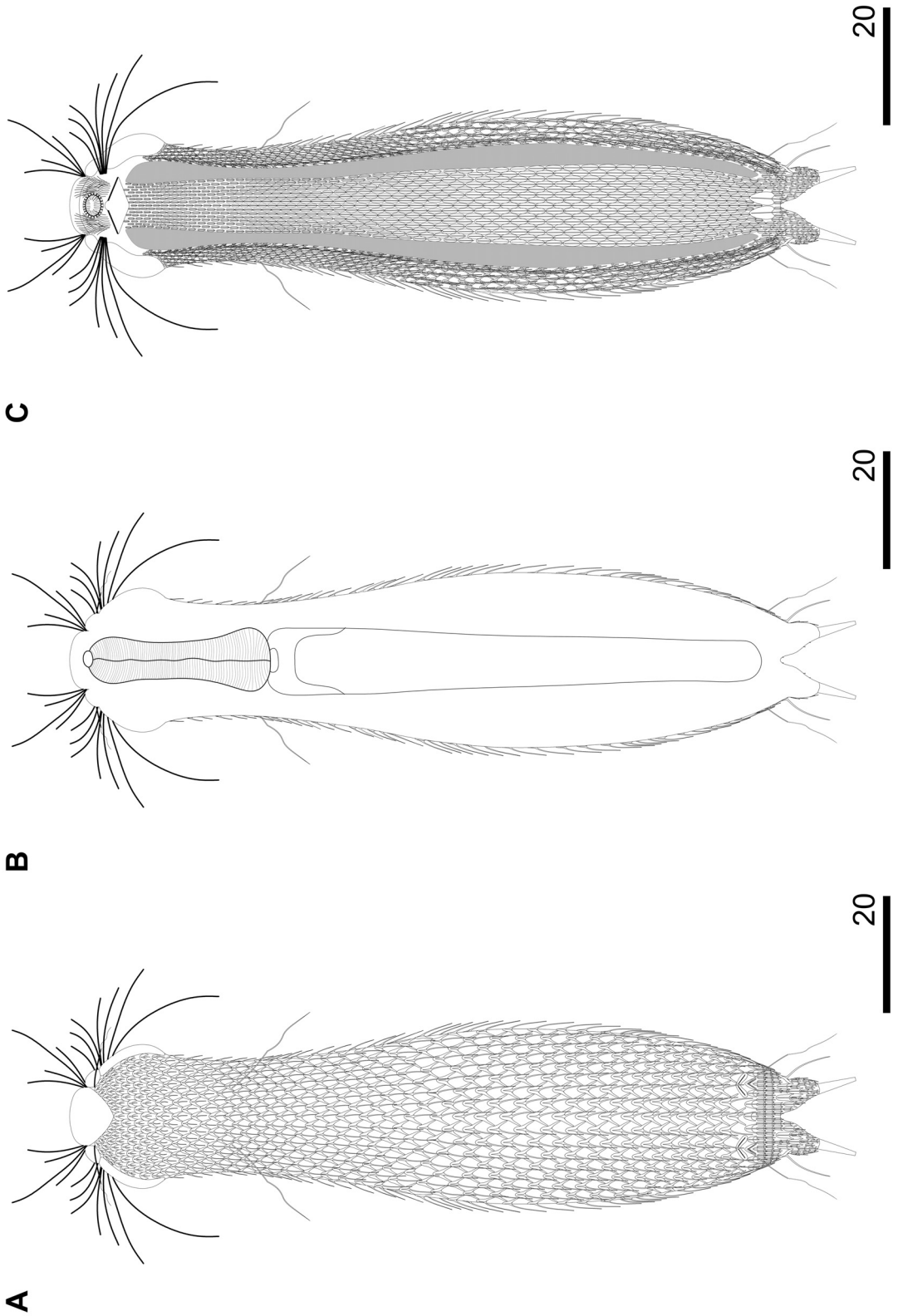


Figure 15. *Chaetonotus* (*Chaetonotus*) *subtilis* sp. nov. Schematic drawings. A – Dorsal body view. B – View of internal morphology. C – Ventral body view. Light grey areas indicate the areas of the insertion of ciliary bands.

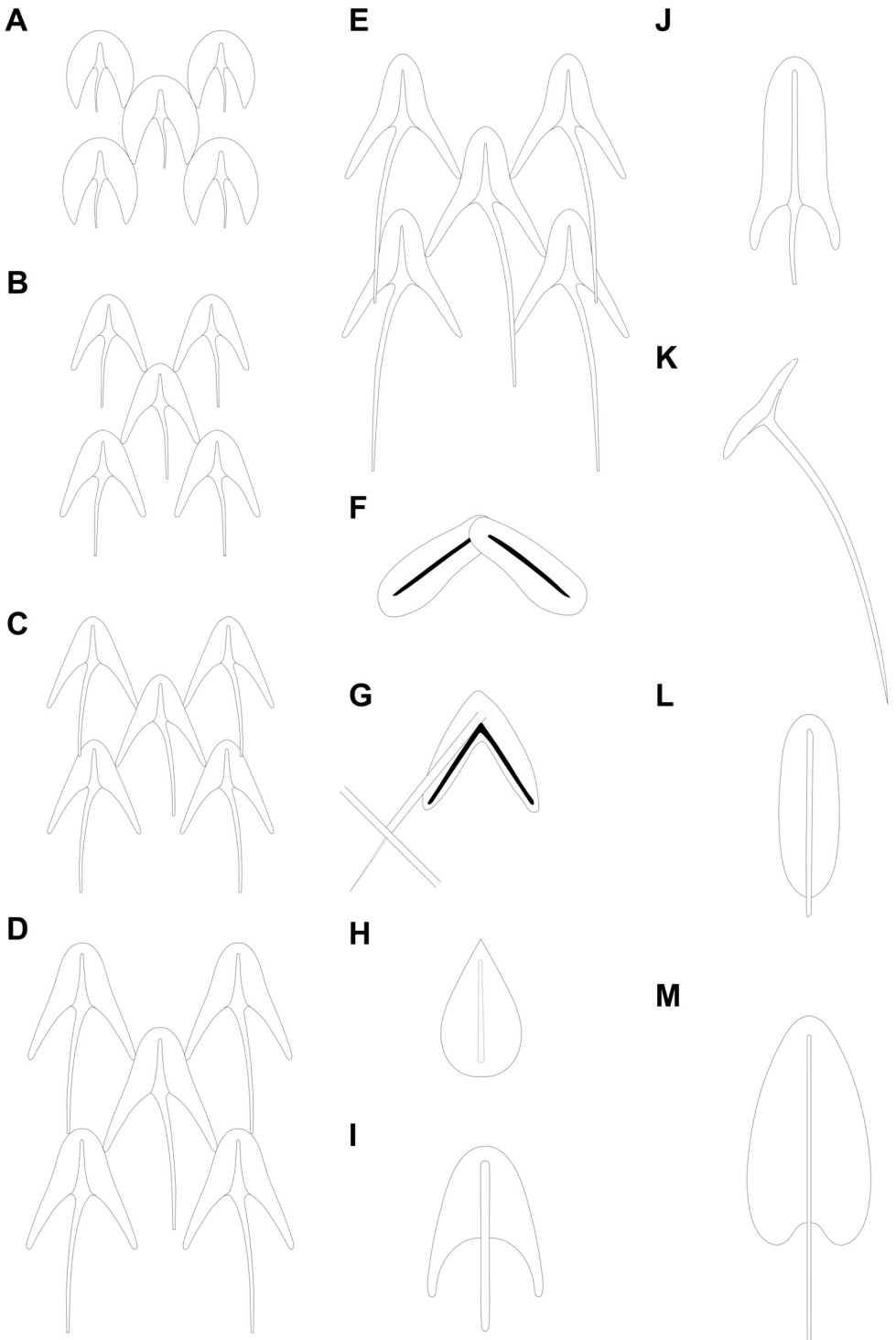


Figure 16. *Chaetonotus* (*Chaetonotus*) *subtilis* sp. nov. Schematic scales drawings. A – Head scales (scales 1). B – Anterior neck scales (scales 2). C – Posterior neck scales (scales 3). D – Anterior trunk scales (scales 4). E – Trunk central and posterior scales (scales 5). F – Pair of one-lobed, elongated scale with straight keels anterior to each double-keeled scale with dorsal sensory bristle (scales 6). G – Double-keeled scale with trunk dorsal sensory bristle (scales 7). H – Small, tear-shaped scale located inside the posterior notch of double-keeled scale with dorsal sensory bristle (scales 8). I – Furcal base scale (scales 9). J – Furcal base and furcal appendages scale (scales 10). K – Scales with parafurcal spines (scales 11). L – Ventral interciliary field scales (scales 12). M – Pair of ventral interciliary field terminal scale (scales 13).

spines gradually and slightly increases from head towards widest trunk region, after which it gradually and slightly shortens to furcal base; only one pair of parafurcal spines significantly longer. Entire ventral interciliary field covered with numerous, fine scales. One pair of ventral interciliary field terminal scales. Pharynx narrow with pronounced anterior and posterior dilatations. Single, narrow, curved cuticular rod inside the anterior pharynx dilatation. Intestine straight with anterior section differing in form and morphology.

Description. *Chaetonotus* (*Chaetonotus*) *subtilis* sp. nov. has a slender, elongated body (Figs 15, 17–18). Its head is only slightly wider than the neck, and neck constriction is weakly marked. The neck gradually tapers from the head (from U16) to the beginning of the trunk (*ca*. U25) (Figs 115, 17–18). The trunk has a similar width as the head and its widest part is only slightly wider. The trunk gradually dilates from *ca*. U26 to *ca*. U60, where it is at its maximum width. It then gradually tapers towards the narrow furcal base (from U90) (Figs 15, 17). The furcal base is clearly demarcated and narrow. The furcal indentation (intrafurcal space) is helmet-like shaped. The furcal branches are set narrowly apart and point slightly outwards (Figs 15, 17). The furca and adhesive tubes are short in comparison to the body length (Table 12). The adhesive tubes are straight, thick and gradually taper slightly towards their ends. The adhesive tube ends are blunt with slightly rounded edges (Figs 15, 17).

The head is five-lobed, short and semicircular. The cephalion (U1–U5) is long and narrow, it is visible on the dorsal head surface as an inverted, rounded triangle. It is clearly demarcated in the body outline and adheres to the head along the entire length. The epipleurae (U3–U4) are small and convex, whereas the hypopleurae (U4–U12) are very large (Figs 15, 17). The hypopleurae are not visible from the dorsal side; only their outline is demarcated in body shape. The hypostomium (U5–U7) is wide, short and rhomboid, and its anterior edge is reinforced (Figs 15C, 17C). Two pairs of cephalic ciliary tufts are present. The anterior tufts emerge in lines between the lateral cephalion edge and the anterior edge of the epipleurae and consist of 4 cilia. The anteriormost cilium in both anterior tufts is long. The second cilium is slightly shorter than the

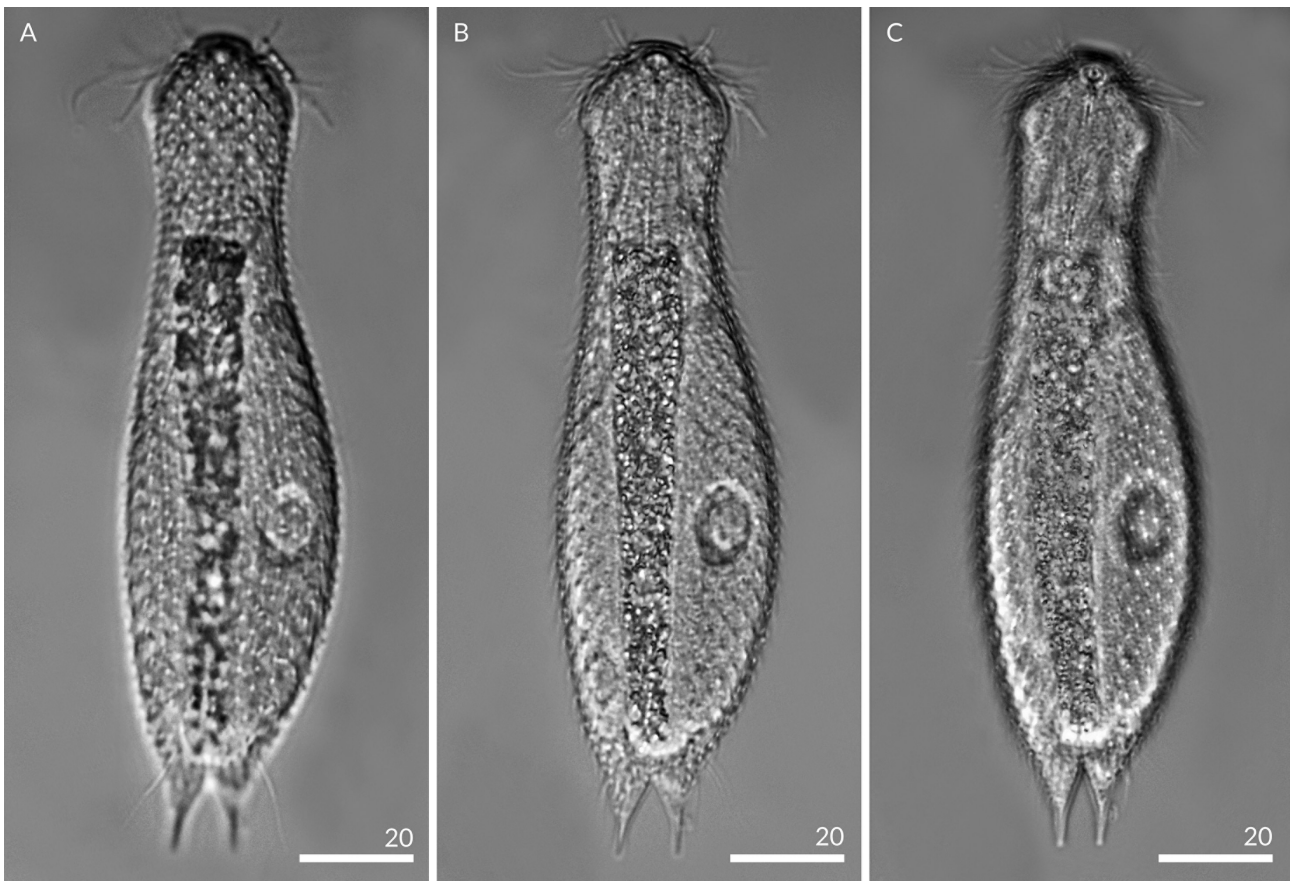


Figure 17. *Chaetonotus* (*Chaetonotus*) *subtilis* sp. nov. Adult specimen. A – Dorsal body view. B – View of internal morphology. C – Ventral body view.

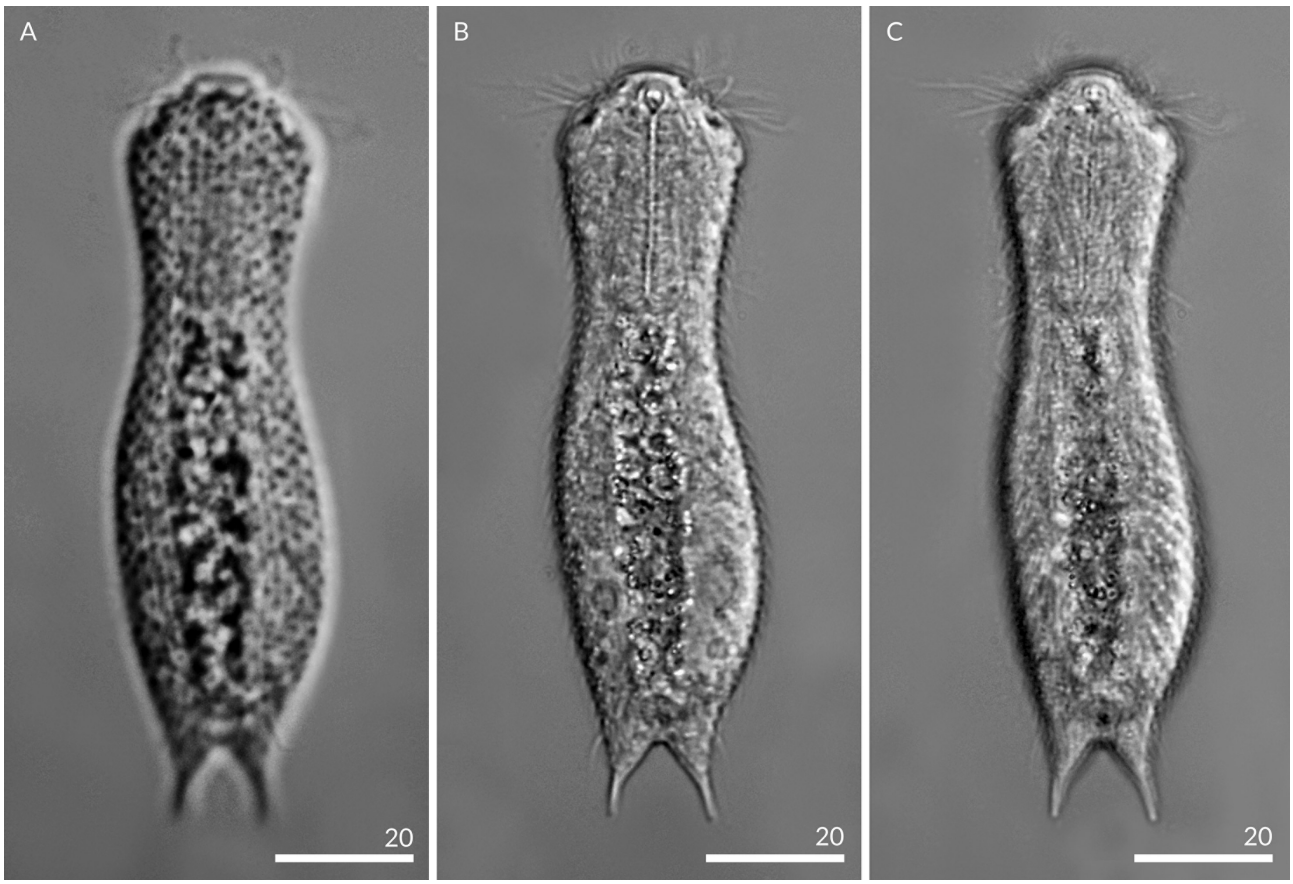


Figure 18. *Chaetonotus* (*Chaetonotus*) *subtilis* sp. nov. Juvenile specimen. A – Dorsal body view. B – View of internal morphology. C – Ventral body view.

first. The third cilium is the longest of all the cilia in the tuft. The fourth (posteriormost) cilium is similar in length to the second cilium. The posterior tufts have 7 cilia each and emerge ventrally at the anterolateral edges of the hypopleurae. The length of the cephalic cilia in the posterior tufts increases from the anteriormost to the posteriormost, long cilium. Cilia in both tufts are thick. Ocellar granules are absent. The mouth ring is located subterminally at U3–U4, is narrow and has granular cuticular reinforcements as well as long cuticular inner hairs (Figs 15, 22A). The suboral bristles are located in two tufts around the mouth ring's lateral sides.

The entire body is covered with small, one-lobed and sub-three-lobed delicate scales with straight keels and spines. The scales are distributed in 33–41 total longitudinal alternating rows (13–15D+4–6DL+4–6L+ +8–10LV+4V), with 31–39 scales in the central row. The longitudinal rows of scales run straight and are arranged almost parallel from the anteriormost head region to the furcal base. The scales are located close to another; their posterolateral parts adhere to adjacent scales. The edges of most of the scales meet and are juxtaposed but never overlap, as only two pairs of scales of differentiated shape are located on the dorsal surface of the posterior trunk region and have overlapping antero-lateral edges (Figs 15, 17, 20). The head scales are one-lobed, strongly rounded with very deep posterior notches (scales 1; Fig. 16A). On the neck towards the trunk the scales become more trilangular in shape with deep parabolic posterior notches (scales 2 and 3; Figs 16B, 16C). On the trunk the scales are shaped like triangles with rounded edges and with very deep, posterior notches that are parabolic in shape (scales 4; Fig. 16D). The trunk scales, from the anterior to the widest body region, start to show weak separation of their posterior parts into narrow posteriolateral lobes and are sub-three-lobed (scales 5; Fig. 16E). From the anterior to the widest body region the length of the central lobe of the trunk scales gradually increases slightly, relative to the posterolateral length. On the dorsal surface in the posterior trunk region, two pairs of oval, elongated scales are present with a keel and without a spine (scales 6; Fig. 16F). These scales are located at U87, anteriorly to the double-keeled scales with the trunk dorsal sensory bristles. The

Figure 19. *Chaetonotus* (*Chaetonotus*) *subtilis* sp. nov. Specimen with mature egg. A – Dorsal body view. B – View of internal morphology. C – Ventral body view.

antero-lateral edges of these scales within the pair overlap another. At U89, inside the deep posterior notches of the pair of double-keeled scales bearing the trunk dorsal sensory bristles, a single, small, tear-shaped scale is located with a straight keel and without spines (scales 8; Fig. 16H). Three types of scales are located on the furcal base and furcal appendages. Numerous, fine, small scales are present on the dorsal, dorsolateral, lateral, ventrolateral and ventral surfaces of the furcal base and on the internal, dorsal, dorsolateral, lateral, ventrolateral and ventral surfaces of the furcal appendages. These scales have straight keels, shaped like triangles with deep, semicircular posterior notches and are smaller and narrower than the other body scales (scales 9; Fig. 16I), and on the ventral surfaces they surround the terminal pair of ventral interciliary field scales and the posterior end of the longitudinal ciliary bands. Dorsally and dorsolaterally on the furcal base and furcal appendages, four pairs of large, one-lobed, elongated scales with a straight keel and straight, thick spines are present (scales 10; Fig. 16J). The first pair of scales located laterally on the furcal appendages at U92 (scales with parafurcal spines) is different than the surrounding scales. This pair is larger and similar in shape to those of the head, neck and trunk region (scales 11; Fig. 16K).

The size of the scales increases very slightly and gradually from the head towards the widest body region, and then decreases slightly and gradually towards the furcal base. Moreover, the scales of the dorsal, dorsolateral, lateral, ventrolateral and ventral surface of the head, neck and trunk decrease in size slightly and gradually towards the ventral ciliary bands (Table 13). The scales in the ventral longitudinal row located closest to the ciliary band are distinctly smaller than the other scales and their anterior edge is oriented towards the bands at an angle of *ca.* 20° (Figs 15, 17, 20).

The spines emerging from the posterior region of scales and are thin and straight, except for four pairs of spines arising from elongated scales located dorsally and dorsolaterally on the furcal base and furcal appendages. These spines are thick and rigid. All spines gradually taper towards their blunt ends and

have no lateral denticles. The length of the spines increases very slightly and gradually from the head towards the widest body region; then their length decreases slightly and gradually towards the furcal base. The posteriormost lateral trunk spines do not vary in length from the other lateral spines in the posterior trunk region. Moreover, the length of the spines increases slightly and gradually from the dorsal surface, through the dorsolateral, lateral, ventrolateral and ventral surfaces towards the ciliary bands (Table 13). On the ventral surface, spines emerging from the scales arranged in two longitudinal rows located closest to the longitudinal bands are long, thin and hair-like along their entire length of those rows. Spines emerging from the furcal base and furcal appendages are straight, thinner and shorter than the other spines. Their length decreases slightly and gradually from the dorsal furcal base and furcal appendages' surface along the dorsolateral, lateral and ventrolateral sides towards the ventral surface. Only parafurcal spines,

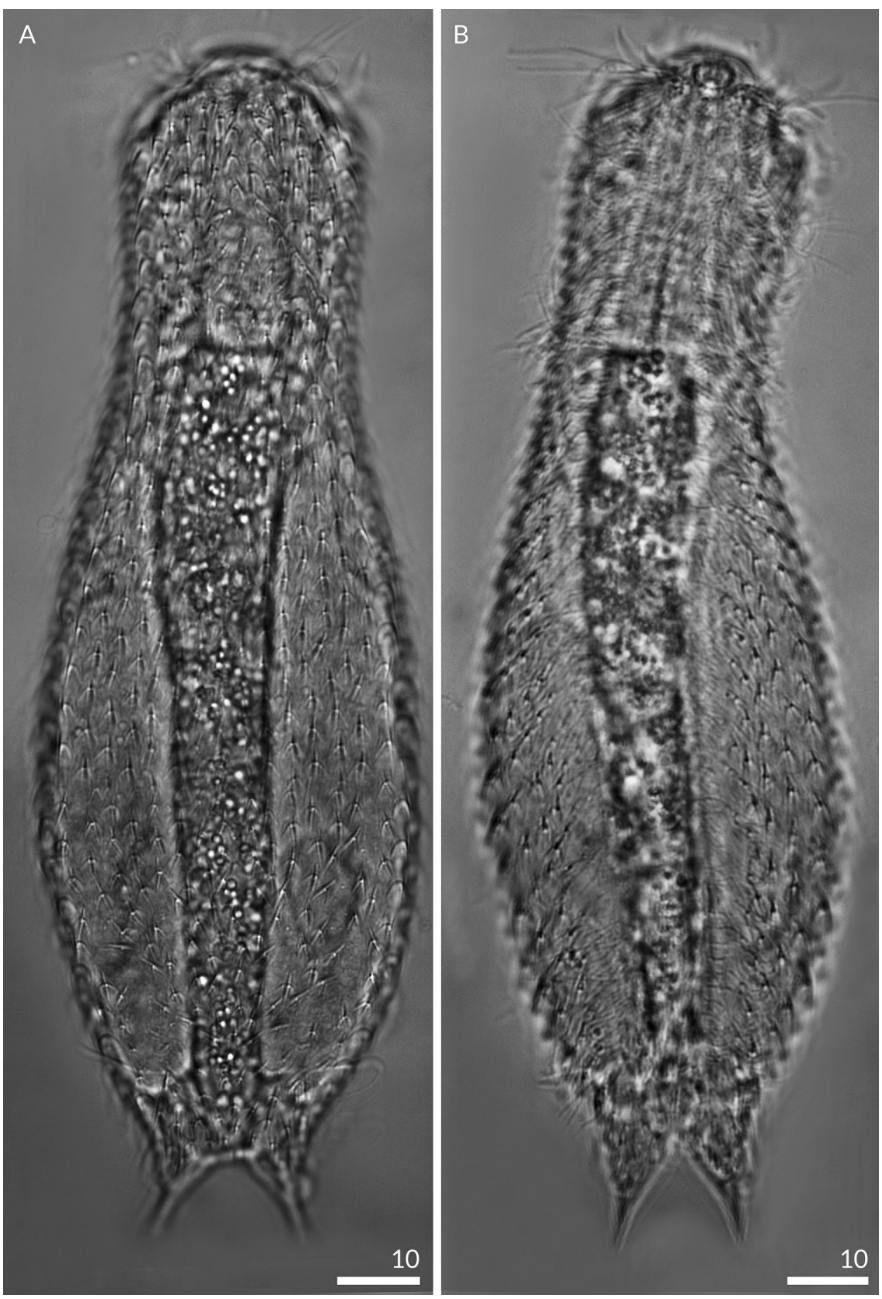


Figure 20. *Chaetonotus* (*Chaetonotus*) *subtilis* sp. nov. Adult specimen. A – Dorsal view of scales. B – Ventral view of scales.

emerging from the first pairs of lateral scales of the furcal appendages (U92), are thicker and much longer than the other spines of the body. These spines are bent and taper slightly towards their ends.

The species has three pairs of dorsal sensory bristles which have a dual structure (Figs 15A, 17A, 20A). The basal segment of each dorsal sensory bristle is significantly thicker and straighter than the remainder of the bristle, which is very thin and flexible. The anterior pair is located on the dorsal surface of the head at U3, between the lateral cephalion edges and the epipleurae, and emerge from small, spherical papillae. The second pair of sensory bristles is located on the dorsal surface of the neck at U23 and each emerges from a small, spherical papilla. The third, posterior pair of sensory bristles is located dorsally at U88–U89 on the posterior trunk region, and each emerges from a trianglar scale with two strong keels which converge and connect in the scales' centre. These double-keeled scales have very deeply notched posterior edges (Fig. 16G).

On the ventral surface, the longitudinal ciliary bands begin at U5 and run back to U83 (Figs 15C, 17C, 20C). The ciliary bands on the head are arranged close to the hypopleurae and lateral edges of the hypostomium. The ciliary bands on the head are slightly wider than in other parts of the body. The entire ventral interciliary field is covered with very fine scales that sit close to another but whose edges do not overlap (scales 12; Figs 15C, 17C, 20C). The scales are narrow, oval in shape, with straight keels and short, thin spines (Fig. 16L). They are distributed in 15–19 total longitudinal rows with 49–57 scales in the central row (Figs 15C, 17C, 20C). The scales gradually become larger, from the anterior to the posterior end of the interciliary field. Thescales on the anterior ventral interciliary field are weakly distinguishable from the cuticle anteriorly, but become progressively more distinguishable posteriorly. The ventral interciliary field terminal scales (U88–U90) are small, oval, and have posterior notches, straight keels, and short, thin spines (scales 13; Figs 15C, 16M, 17C, 20C).

The pharynx (from U4 to U25) is narrow and has marked anterior and stronger posterior dilatations (Fig. 22A). In the anterior dilatation, weak cuticular reinforcements are located in the form of a single slightly curved rod (at U6–U7). The pharynx is

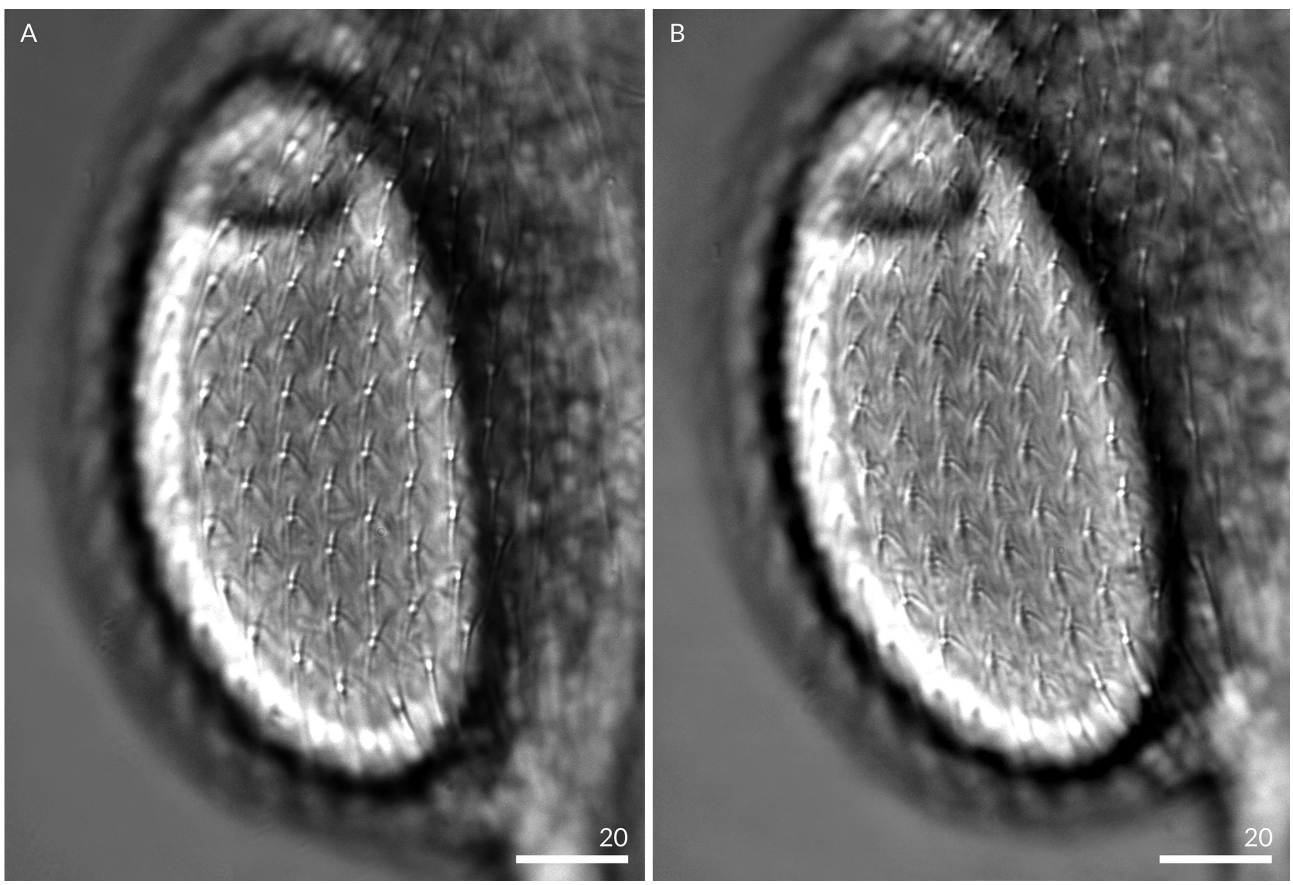


Figure 21. *Chaetonotus* (*Chaetonotus*) *subtilis* sp. nov. Adult specimen. A – Lateral view of trunk spines. B – Lateral view of trunk scales.

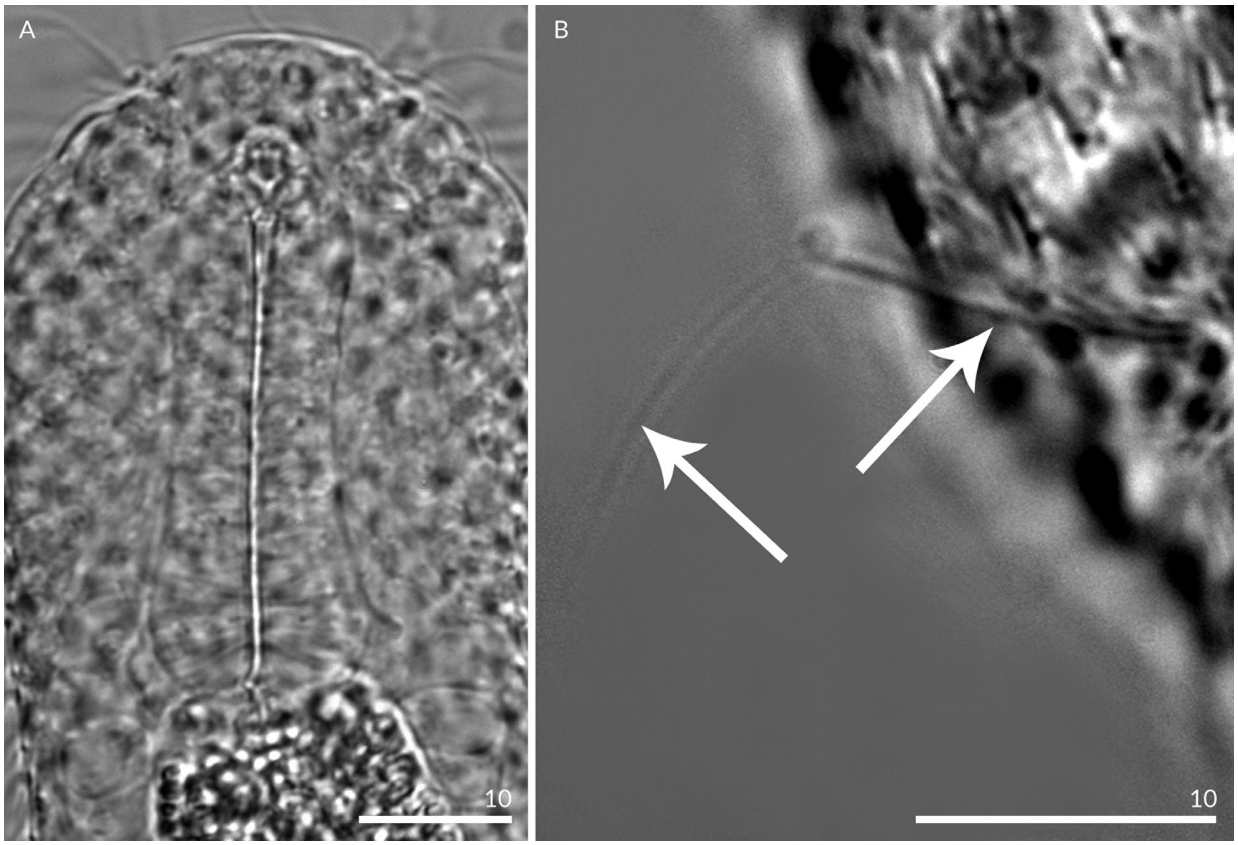


Figure 22. *Chaetonotus* (*Chaetonotus*) *subtilis* spec nov. Adult specimen. A – Detail of the pharynx. B – Trunk dorsal sensory bristle.

connected through the pharyngeal-intestinal junction to the straight intestine (running from U26 to U88). The pharyngeal-intestinal junction is clearly demarcated, short and narrow (U26). The intestine has a distinct, long, separate anterior section running from U26 to U33 (Figs 15B, 17B).

Remarks. Because of a short pharynx in relation to the length of the body and intestine, the pharynx-intestine ratio (I) has lower values in this species. The juvenile and subadult specimens have a pharynx-intestine ratio (I) that is higher than 45% but not higher than 55%.

The total body length of juvenile and subadult specimens of *Chaetonotus* (*Chaetonotus*) *subtilis* sp. nov. varied from 121.6 to 144.8 μm; the length of the pharynx was between 34.6 and 36.7 μm; of the intestine from 57.4 to 86.8 μm; and there were 35–39 total longitudinal rows of scales (13–15D+4–6DL+6L+8LV+4V) with 33–37 scales in the central row. Of the 26 adults examined, 6 individuals had a large, developing egg.

The *C.*(*C.*) *subtilis* sp. nov. and the morphologicaly similar *C.* (*C.*) cf. *sphagnophilus* form a clade which is a sister group to the paraphyletic group containing *C.* (*Zonochaeta*) *bisacer* and *C.* (*Primochaetus*) *heterocanthus*, all representatives of the genus *Polymerurus* as well as the families Neogosseidae and Dasydytidae (Fig. 4).

Differential diagnosis. *Chaetonotus* (*Chaetonotus*) *subtilis* sp. nov. morphologically more closely resembles *C.* (*C.*) *greuteri* Remane, 1927a, *C.* (*C.*) *multispinosus* Grünspan, 1908 and *C.* (*C.*) *pawlowskii* Kisielewski, 1984. *C.* (*C.*) *poznaniensis* Kisielewski, 1981. *C.* (*C.*) *greuteri* was selected for a comparison with the new species due to similarity in terms of: range of body length; head and body shape; size of cephalic pleurae; range of pharynx length; pharynx shape; range of the total number of longitudinal alternating rows of scales; type of scales; and type of posteriormost pair of trunk lateral spines. *C.* (*C.*) *multispinosus* shares with *C.* (*C.*) *subtilis* sp. nov. the following: range of body length; range of pharynx length; range of number of scales in the central longitudinal row; type of scale alignment; type of scales and scale coverage on the ventral interciliary field; and type of posteriormost pair of trunk lateral spines. *C.* (*C.*) *pawlowski* resembles the newly described taxon in terms of: range of body length; head and body shape; size of cephalic pleurae; range of pharynx

Table 12. Main morphometric characters of *Chaetonotus* (*Chaetonotus*) *subtilis* sp. nov.; N – number of specimens or structures analysed, Range – the smallest and the largest structure found among all specimens measured. All measurements are given in micrometers (μm).

Characters	N	Holotype	Range of adult paratypes
Body length	26	156.69	139.16–172.11
Furca length	15	17.32	17.09–22.91
Adhesive tube length	15	8.44	8.12–10.09
Number of scales in central longitudinal row	26	33	31–39
Total number of longitudinal alternating rows of scales	26	35	33–41
Head scales length x width	15	1.22–3.92 x 1.19–2.87	1.04–4.12 x 1.01–4.08
Neck scales length x width	15	1.78–4.56 x 1.70–4.48	1.58–4.88 x 1.45–4.78
Trunk scales length x width	15	2.10–5.06 x 1.92–4.81	1.84–6.21 x 1.69–5.87
Head spines length	15	2.93–5.10	2.36–6.70
Neck spines length	15	4.41–5.54	3.82–7.47
Trunk spines length	15	4.77–6.21	4.16–8.78
Mouth ring diameter	15	4.56	4.19–6.34
Pharynx length	26	34.5	32.14–39.09

Table 13. Detailed morphometric characters of *Chaetonotus* (*Chaetonotus*) *subtilis* sp. nov; N – number of specimens or structures analysed, Range – the smallest and the largest structure found among all specimens measured, SD – standard deviation. All measurements are given in micrometers (μm); all indicators are given as a percentage (%) and italicized.

Characters	N	Holotype	Range of adult paratypes	SD
Cephalion length	15	14.51	14.08–16.19	0.557
Cephalion width	15	11.42	11.10–15.02	0.929
Hypostomium length	15	5.06	3.98–6.01	0.520
Hypostomium width	15	10.2	9.08–11.47	0.703
Length of cephalic cilia (anterior tuft)	15	10.38–23.02	(8.50–11.60)–(20.96–26.10)	0.741; 1.507
Length of cephalic cilia (posterior tuft)	15	11.07–26.53	(10.67–12.34)–(24.73–31.91)	0.495; 1.841
Width of cephalic cilia (anterior tuft)	15	1.03–1.05	(1.00–1.06)–(1.04–1.11)	0.020; 0.021
Width of cephalic cilia (posterior tuft)	15	1.07–1.10	(1.04–1.13)–(1.07–1.16)	0.026; 0.027
Width of adhesive tube	15	1.97	1.91–2.08	0.057
Ratio of scale distribution	26	106.061	*100.000–118.182*	3.697
Length of head dorsal scales (scales 1)	15	2.87–3.92	(2.27–3.17)–(3.19–4.12)	0.284; 0.306
Width of head dorsal scales (scales 1)	15	2.77–3.89	(2.18–3.10)–(3.15–4.08)	0.291; 0.307
Length of head dorsolateral scales (scales 1)	15	2.80–3.78	(2.22–3.09)–(3.10–4.02)	0.276; 0.303
Width of head dorsolateral scales (scales 1)	15	2.70–3.75	(2.14–2.99)–(3.04–3.98)	0.276; 0.314
Length of head lateral scales (scales 1)	15	2.74–3.71	(2.19–3.01)–(3.05–3.96)	0.266; 0.299
Width of head lateral scales (scales 1)	15	2.66–3.67	(2.10–2.91)–(2.98–3.91)	0.264; 0.307
Length of head ventrolateral scales (scales 1)	15	2.64–3.57	(2.12–2.89)–(2.96–3.89)	0.254; 0.295
Width of head ventrolateral scales (scales 1)	15	2.56–3.51	(2.04–2.82)–(2.87–3.81)	0.256; 0.304
Length of head ventral scales (scales 1)	15	1.22–2.96	(1.04–1.41)–(2.23–3.28)	0.112; 0.330
Width of head ventral scales (scales 1)	15	1.19–2.87	(1.01–1.37)–(1.79–3.18)	0.109; 0.384
Length of neck dorsal scales (scales 2 and 3)	15	4.01–4.56	(3.26–4.23)–(3.79–4.88)	0.315; 0.359
Width of neck dorsal scales (scales 2 and 3)	15	3.94–4.48	(3.22–4.19)–(3.63–4.78)	0.313; 0.390
Length of neck dorsolateral scales (scales 2 and 3)	15	3.93–4.49	(3.21–4.14)–(3.73–4.81)	0.307; 0.353
Width of neck dorsolateral scales (scales 2 and 3)	15	3.85–4.40	(3.15–4.31)–(3.57–4.72)	0.340; 0.390
Length of neck lateral scales (scales 2 and 3)	15	3.87–4.41	(3.17–4.09)–(3.68–4.72)	0.307; 0.350
Width of neck lateral scales (scales 2 and 3)	15	3.78–4.32	(3.09–4.00)–(3.50–4.63)	0.299; 0.390
Length of neck ventrolateral scales (scales 2 and 3)	15	3.71–4.32	(3.07–3.91)–(3.63–4.62)	0.293; 0.337
Width of neck ventrolateral scales (scales 2 and 3)	15	3.60–4.24	(2.94–3.80)–(3.42–4.52)	0.290; 0.378

Table 13. Continued.

Characters	N	Holotype	Range of adult paratypes	SD
Length of neck ventral scales (scales 2 and 3)	15	1.78–3.22	(1.58–1.90)–(2.68–3.60)	0.111; 0.296
Width of neck ventral scales (scales 2 and 3)	15	1.70–3.14	(1.45–1.81)–(254–3.50)	0.121; 0.325
Length of trunk dorsal scales (scales 4 and 5)	15	4.64–5.06	(3.57–4.97)–(4.37–6.21)	0.422; 0.543
Width of trunk dorsal scales (scales 4 and 5)	15	4.47–4.81	(3.40–4.78)–(4.16–5.87)	0.432; 0.558
Length of trunk dorsolateral scales (scales 4 and 5)	15	4.52–4.91	(3.47–4.84)–(4.30–6.06)	0.424; 0.530
Width of trunk dorsolateral scales (scales 4 and 5)	15	4.35–4.66	(3.30–4.65)–(4.03–5.74)	0.426; 0.535
Length of trunk lateral scales (scales 4 and 5)	15	4.45–4.83	(3.42–4.76)–(4.25–5.98)	0.407; 0.521
Width of trunk lateral scales (scales 4 and 5)	15	4.26–4.61	(3.23–4.58)–(3.95–5.69)	0.422; 0.540
Length of trunk ventrolateral scales (scales 4 and 5)	15	4.36–4.72	(3.34–4.66)–(4.19–5.87)	0.400; 0.511
Width of trunk ventrolateral scales (scales 4 and 5)	15	4.18–4.47	(3.17–4.49)–(3.84–5.54)	0.415; 0.503
Length of trunk ventral scales (scales 4 and 5)	15	2.10–4.19	(1.84–2.27)–(3.70–5.36)	0.158; 0.467
Width of trunk ventral scales (scales 4 and 5)	15	1.92–3.96	(1.69–2.08)–(3.49–5.06)	0.144; 0.397
Length of elongated overlapping scales (scales 6)	15	3.84	2.97–4.21	0.401
Width of elongated overlapping scales (scales 6)	15	1.51	1.30–1.88	0.190
Length of small one-lobed scales (scales 8)	15	1.47	1.17–2.14	0.277
Width of small one-lobed scales (scales 8)	15	0.96	0.82–1.42	0.188
Length of small furcal base and furcal appendages dorsal scales (scales 9 and 10)	15	2.14–2.59	(1.78–3.77)–(2.14–4.26)	0.580; 0.647
Width of small furcal base and furcal appendages dorsal scales (scales 9 and 10)	15	1.37–1.56	(1.16–3.14)–(1.32–3.42)	0.575; 0.658
Length of small furcal base and furcal appendages dorsolateral scales (scales 9 and 10)	15	2.10–2.55	(1.74–3.71)–(2.10–4.19)	0.578; 0.641
Width of small furcal base and furcal appendages dorsolateral scales (scales 9 and 10)	15	1.32–1.51	(1.09–3.07)–(1.26–3.34)	0.566; 0.633
Length of small furcal base and furcal appendages lateral scales (scales 9 and 10)	15	2.04–2.49	(1.68–3.63)–(2.04–4.10)	0.569; 0.629
Width of small furcal base and furcal appendages lateral scales (scales 9 and 10)	15	1.29–1.47	(1.07–2.97)–(1.21–3.27)	0.549; 0.631
Length of small furcal base and furcal appendages ventrolateral scales (scales 9 and 10)	15	1.97–2.41	(1.61–3.52)–(1.96–3.97)	0.558; 0.616
Width of small furcal base and furcal appendages ventrolateral scales (scales 9 and 10)	15	1.25–1.43	(1.04–2.91)–(1.18–3.01)	0.541; 0.473
Length of small furcal base and furcal appendages ventral scales (scales 9 and 10)	15	1.73–2.27	(1.39–3.24)–(1.83–3.80)	0.541; 0.480
Width of small furcal base and furcal appendages ventral scales (scales 9 and 10)	15	1.17–1.38	(0.96–2.82)–(1.11–3.12)	0.501; 0.620
Length of large, elongated furcal base and furcal appendages scales (scales 10)	15	5.28–5.73	(4.27–7.21)–(4.90–8.17)	0.917; 0.949
Width of large, elongated furcal base and furcal appendages scales (scales 10)	15	2.00–2.18	(1.71–3.01)–(1.79–3.26)	0.378; 0.406
Length of lateral scales with long parafurcal spines (scales 11)	15	4.11	3.73–6.21	0.721
Width of lateral scales with long parafurcal spines (scales 11)	15	3.94	3.22–5.89	0.677
Length of head dorsal spines (scales 1)	15	2.93–4.37	(2.36–3.73)–3.77–5.66)	0.378; 0.536
Length of head dorsolateral spines (scales 1)	15	2.98–4.42	(2.40–3.81)–(3.81–5.73)	0.386; 0.542
Length of head lateral spines (scales 1)	15	3.06–4.51	(2.47–3.94)–(3.88–5.86)	0.401; 0.549
Length of head ventrolateral spines (scales 1)	15	3.12–4.58	(2.55–4.02)–(3.94–5.97)	0.402; 0.566
Length of head ventral spines (scales 1)	15	4.07–5.10	(3.23–5.28)–(4.22–6.70)	0.507; 0.661
Length of neck dorsal spines (scales 2 and 3)	15	4.41–4.72	(3.82–5.71)–(4.13–6.37)	0.548; 0.621
Length of neck dorsolateral spines (scales 2 and 3)	15	4.47–4.79	(3.87–5.79)–(4.19–6.47)	0.550; 0.629
Length of neck lateral spines (scales 2 and 3)	15	4.52–4.85	(3.93–5.86)–(4.25–6.55)	0.554; 0.633
Length of neck ventrolateral spines (scales 2 and 3)	15	4.60–4.91	(4.01–5.96)–(4.06–6.63)	0.563; 0.685

Table 13. Continued.

Characters	N	Holotype	Range of adult paratypes	SD
Length of neck ventral spines (scales 2 and 3)	15	4.66–5.54	(4.09–6.06)–(4.67–7.47)	0.573; 0.761
Length of trunk dorsal spines (scales 4 and 5)	15	4.77–5.68	(4.16–6.44)–(5.28–8.04)	0.626; 0.855
Length of trunk dorsolateral spines (scales 4 and 5)	15	4.83–5.77	(4.21–6.53)–(5.37–8.17)	0.641; 0.876
Length of trunk lateral spines (scales 4 and 5)	15	4.89–5.84	(4.26–6.61)–(5.47–8.28)	0.647; 0.884
Length of trunk ventrolateral spines (scales 4 and 5)	15	4.97–5.92	(4.35–6.72)–(5.56–8.38)	0.656; 0.902
Length of trunk ventral spines (scales 4 and 5)	15	5.03–6.21	(4.42–6.81)–(5.85–8.78)	0.660; 0.948
Length of large, elongated furcal base and furcal appendages scales spines (scales 6)	15	4.07–4.39	(3.59–7.93)–(3.93–9.34)	1.322; 1.614
Length of furcal base and furcal appendages dorsal spines (scales 9 and 10)	15	2.51–3.37	(2.03–3.18)–(2.97–5.42)	0.293; 0.664
Length of furcal base and furcal appendages dorsolateral spines (scales 9 and 10)	15	2.47–3.34	(1.99–3.14)–(2.94–5.38)	0.292; 0.658
Length of furcal base and furcal appendages lateral spines (scales 9 and 10)	15	2.42–3.30	(1.94–3.08)–(2.90–5.30)	0.290; 0.644
Length of furcal base and furcal appendages ventrolateral spines (scales 9 and 10)	15	2.37–3.25	(1.88–3.01)–(2.85–5.17)	0.295; 0.623
Length of furcal base and furcal appendages ventral spines (scales 9 and 10)	15	2.29–3.18	(1.80–2.91)–(2.79–5.06)	0.281; 0.612
Length of trunk posteriormost pair of lateral spines (scales 5)	15	5.07	4.84–6.97	0.656
Length of parafurcal long lateral spines (scales 11)	15	11.94	10.20–13.51	1.015
Head dorsal sensory bristles length	15	13.43	13.06–15.16	0.636
Neck dorsal sensory bristles length	15	16.96	15.63–20.68	1.326
Posterior dorsal sensory bristles length	15	21.83	20.69–31.43	3.157
Number of scales in central longitudinal row on ventral interciliary field	26	53	49–57	1.865
Total number of longitudinal alternating rows of scales on ventral interciliary field	26	17	15–19	1.208
Length of ventral interciliary field scales (scales 12)	15	1.46–3.61	(1.14–1.59)–93.23–4.54)	0.131; 0.450
Width of ventral interciliary field scales (scales 12)	15	0.73–1.29	(0.50–0.82)–(1.11–1.79)	0.099; 0.227
Length of terminal ventral interciliary field scales (scales 13)	15	4.18	4.01–6.88	0.819
Width of terminal ventral interciliary field scales (scales 13)	15	2.15	1.91–3.27	0.337
Length of ventral interciliary field scales spines (scales 12)	15	0.14–1.67	(0.10–0.23)–(1.23–1.77)	0.040; 0.142
Length of terminal ventral interciliary field scales spines (scales 13)	15	3.61	3.06–4.19	0.335
Width of anterior pharynx thickening (a)	15	9.06	8.87–11.47	0.926
Width of pharynx narrowing (n)	15	6.62	6.22–8.20	1.001
Width of middle pharynx (m)	15	8.09	7.96–9.87	0.704
Width of posterior pharynx thickening (p)	15	11.48	10.83–12.87	0.627
Width of pharyngeal-intestinal junction	15	3.31	3.11–3.81	0.353
Length of intestine anterior differ section – in it central part	15	9.58	8.52–12.13	1.615
Length of intestine anterior differ section – in it lateral part	15	19.97	16.28–25.09	2.628
Intestine length	26	93.59	75.02–112.27	7.679
Pharynx formula a	15	*26.261*	*25.083–31.932*	1.795
Pharynx formula n	15	*19.188*	*17.906–25.249*	2.096
Pharynx formula m	15	*23.449*	*22.803–26.531*	1.241
Pharynx formula p	15	*33.275*	*32.259–36.077*	1.117
Pharynx-intestine ratio I	26	*36.863*	*33.872–47.521*	2.903
Length of matured egg	4	–	55.01–64.20	3.804
Width of matured egg	4	–	31.12–40.13	3.862

Table 14. Condensed comparison of the most important differential characters between species most similar to *Chaetonotus* (*Chaetonotus*) subtilis sp. nov. Measurements are given in micrometres (μm). NA – data not available; – – not applicable.

Character	*C.* (*C.*) *subtilis* sp. nov.	*C.* (*C.*) *greuteri*	*C.* (*C.*) *multispinosus*	*C.* (*C.*) *pawlowski*	*C.* (*C.*) *poznaniensis*	*C.* (*C.*) *daphnes*
Body length	139.2–172.1	150.0–210.0	85.0–150.0	167.0–172.0	125.0–127.0	141.0–173.0
Cephalic pleurae	Cephalion long and narrow; epipleurae small and convex; hypopleurae very large. Epipleurae located on dorsal, dorsolateral, lateral and ventrolateral head sides; hypopleurae located on lateral, ventrolateral and ventral sides, they are not visible from the dorsal side, only their outline is demarcated. Hypostomium wide, short, rhomboidal with reinforced anterior edge	Cephalion long and wide; epipleurae small; hypopleurae very large. Epipleurae located dorsally; hypopleurae located on lateral, ventrolateral and ventral sides; hypopleurae located on lateral, ventrolateral and ventral sides. Hypostomium present	Cephalion narrow; epipleurae and hypopleurae similar in size, located mostly laterally but clearly visible on the dorsal body side. Hypostomium present	Cephalion narrow; epipleurae small and convex; hypopleurae very large. Epipleuria and hypopleurae located mostly laterally but clearly visible on the dorsal body side. Hypostomium large, very strong with strong transverse furrow	Cephalion narrow; epipleurae small and convex; hypopleurae very large. Epipleurae and hypopleurae located mostly laterally but clearly visible on the dorsal body side. Hypostomium present	Cephalion long and wide; epipleurae large located mostly laterally but clearly visible on the dorsal body side; hypopleurae small, present only on the ventral body side. Hypostomium absent
Place of emerging cephalic cilia	Anterior tufts: in lines between lateral cephalion edge and anterior edge of epipleurae; posterior tufts: on the ventral side, near the anterolateral edges of the hypopleurae	Only on pair of tufts: ventrolaterally, between cephalion and hypopleurae	Anterior tufts: dorsoterminal between cephalion and epipleuria; posterior tufts: laterally, between epipleurae and hypopleurae	Anterior tufts: dorsoterminal between cephalion and epipleurae; posterior tufts: laterally, between epipleurae and hypopleurae	Anterior tufts: dorsoterminal between cephalion and epipleurae; posterior tufts: laterally, between epipleurae and hypopleurae	Anterior tufts: dorsoterminal between cephalion and epipleurae; posterior tufts: on dorsal body sides
Pharynx length	32.1–39.1	36.0–50.0	37.0–39.0	37.0–47.0	29.0–31.0	36.0–44.0
Pharynx shape	Anterior and wider posterior dilatation	Anterior and wider posterior dilatation	Cylindrical, widening towards the posterior end	With anterior and posterior very strong, bulb-like dilatation; posterior dilatation wider than the anterior one	Distinct anterior and wider posterior dilatation	Anterior and wider posterior dilatation

Table 14. Continued.

Character	*C.* (*C.*) *subtilis* sp. nov.	*C.* (*C.*) *greuteri*	*C.* (*C.*) *multispinosus*	*C.* (*C.*) *pawlowski*	*C.* (*C.*) *poznaniensis*	*C.* (*C.*) *daphnes*
Presence of cuticular reinforcements in the pharynx	Present (weak, single cuticular curved rod)	Absent	Absent	Present (a complex system of strong reinforcements composed of rods and pellets)	Absent	Absent
Anterior intestine section differing in form of morphology	Present	Absent	Absent	Absent	Absent	Absent
Furca length	17.1–22.9	21.0–34.0	14.0–16.0	NA	18.0	14.0–20.0
Adhesive tube length	8.1–10.1	NA	NA	10.0–12.0	10.0	7.0–11.0
Type of furcal indentation	Helmet-like shape	V-shaped	V-shaped	Elliptical in shape	V-shaped	Helmet-like shaped
Number of scales in central longitudinal row	33–41	29	38–40	22–25	20–25	25–33
Total number of longitudinal alternating rows of scales	31–39	31–32	25–29	23–30	24–25	45–53
Type of scales	One-lobed and sub-three-lobed, fine, small, with straight keels and spines. Except for the single scales on posterior trunk region and smaller scales on the furcal base and furcal appendages, shape undifferentiated within body regions	One-lobed, small, with weak keels and with spines	One-lobed, fine, small, without keels and with spines	One-lobed, small, with weak keels and with spines	One-lobed, small, without keels and with spines	One-lobed with strong keels and straight spines; shape undifferentiated within body regions
Scale size variation	Size increases very slightly and gradually from the head towards the widest body region and subsequently their size decreases slightly	Similar size all over the body	Similar size all over the body	Size similar on the head, neck and trunk; on the furcal base and furcal appendages scales smaller	Similar size all over the body	Similar size all over the body

Table 14. Continued.

Character	*C.* (*C.*) *subtilis* sp. nov.	*C.* (*C.*) *greuteri*	*C.* (*C.*) *multispinosus*	*C.* (*C.*) *pawlowski*	*C.* (*C.*) *poznaniensis*	*C.* (*C.*) *daphnes*
	and gradually towards the furcal base; on the furcal base and furcal appendages scales much smaller; scales of the dorsal, dorsolateral, lateral, ventrolateral and ventral surface of the head, neck and trunk slightly, insufficiently and gradually decrease in size towards the ventral ciliary bands					
Scale size	Head: 1.0–4.1 x 1.0–4.1 Neck: 1.6–4.9 x 1.5–4.8 Trunk: 1.8–6.2 x 1.7–5.9	NA	NA	Head: NA Neck: 4.0 x NA Trunk: 6.0 x NA	4.0 x NA	2.5–3.4 x 0.6–2.0
Alignment of scales	Located close to another; their posterolateral parts adhere to adjacent scales. Edges of most of scales meet and are juxtaposed but never overlap. Only two pairs of dorsal scales of different shape, located on the posterior trunk region have overlapping anterolateral edges	Located far from another	Located close to another; their posterolateral parts overlap adjacent scales	Located close to another but do not overlap	Located close to another but do not overlap	Edges juxtaposed but do not overlap
Presence of a different type of scales on the dorsal and dorsolateral side of the furcal base	Present	Absent	Absent	Absent	Absent	Present

Table 14. Continued.

Character	*C.* (*C.*) *subtilis* sp. nov.	*C.* (*C.*) *greuteri*	*C.* (*C.*) *multispinosus*	*C.* (*C.*) *pawlowski*	*C.* (*C.*) *poznaniensis*	*C.* (*C.*) *daphnes*
Scale coverage of the ventral interciliary field	Entire ventral interciliary field covered with scales	Entire ventral interciliary field covered by keels	Entire ventral interciliary field covered with scales	NA	Ventral interciliary field naked	Entire ventral interciliary field covered with scales
Type of scales of the ventral interciliary field	Scales fine, narrow, oval in shape, keeled and with rudimentary spines	Only keels visible	Scales small, round, keeled and/or spined	NA	–	Scales small, oval without posterior notches, keeled, spineless
Number of terminal scales of the ventral interciliary field	1 pair	NA	2 pairs	NA	2 pairs	1 pair
Type of terminal scales of the ventral interciliary field	Small, oval, without posterior notches, keeled and spined	NA	Anterior and posterior pair elongated, keeled and spineless	NA	Anterior and posterior pair extremely narrow, keeled and with rudimentary spines	Oval without posterior notches, keeled, spineless
Spine length variation	Spine length gradually increases slightly from the head towards the widest body region, after which they gradually, slightly shorten towards the furcal base; spine length gradually increases slightly from dorsal body side, on the dorsolateral, lateral, ventrolateral and ventral body sides towards the ciliary bands	Spine length gradually increases slightly from the head towards the body end; lateral spines longer than dorsal spines	All spines similar in length	Head, neck and trunk spines similar in length; furcal base and furcal appendage spines shorter	Head, neck and trunk spines similar in length; furcal base and furcal appendage spines slightly longer	All spines similar in length
Spine length	Head: 2.4–6.7 Neck: 3.8–7.5 Trunk: 4.2–8.3	Head: NA Neck: NA Trunk: 11.0–17.0	Very short	Head: NA Neck: 3.0–6.0 Trunk: 3.0–5.0 (13.0 in “long spines” form)	1.0	1.7–4.3
Presence of hair-like spines on the ventral body side	Present	Absent	Absent	Absent	Absent	Absent

Table 14. Continued.

Character	*C.* (*C.*) *subtilis* sp. nov.	*C.* (*C.*) *greuteri*	*C.* (*C.*) *multispinosus*	*C.* (*C.*) *pawlowski*	*C.* (*C.*) *poznaniensis*	*C.* (*C.*) *daphnes*
Type of posteriormost pair of trunk lateral spines	Do not vary from other posterior trunk spines, not longer or thicker	The same as other lateral spines	The same as other spines	Shorter than the other trunk spines	The same as other spines	The same as other spines
Type of furcal appendage lateral spines	First pair of parafurcal spines on lateral sides of furcal appendages much thicker and longer than the other body spines; other lateral spines short and thin	Longer than the other spines	Furcal appendages without spines	Short and thin	Slightly longer than the other spines	The same as other spines
Ventral locomotor cilia	Two longitudinal bands wider on the head region	Two longitudinal bands	Two longitudinal bands	Two longitudinal bands	Two longitudinal bands	Two longitudinal bands wider on the head region
Number of dorsal sensory bristles	3 pairs	NA	2 pairs	NA	NA	2 pairs

length; presence of cuticular reinforcements in the pharynx; type of scales; range of scale size; type of scale alignment; range of spine length. *C.* (*C.*) *poznaniensis* was selected for a comparison with the new species due to similarity in terms of: size of cephalic pleurae; body shape; range of furca length; and range of adhesive tube length. *C.* (*C.*) *daphnes* Balsamo et Todaro, 1995 shares with *C.* (*C.*) *subtilis* sp. nov. the following: range of body length; head and body shape; range of pharynx length; pharynx shape; range of furca length; range of adhesive tube length; type of furcal indentation; type of scales; presence of a different type of scales on the dorsal and dorsolateral surfaces of the furcal base; scale coverage of the ventral interciliary field; number of ventral terminal scales; type of posteriormost pair of trunk lateral spines; and ventral ciliary bands wider on the head region. Although of all the hitherto known taxa those listed above have the highest number of common features with *C.* (*C.*) *subtilis* sp. nov., they are significantly different, mostly in scale type and shape as well as in variation of length of spines. The comparison between the new species and the more morphologically similar taxa is summarised in Table 14.

Chaetonotus* (*Chaetonotus*) *svalbardi Kolicka, 2017

Localities: 2.

Material. One sample, 2 specimens (both adults), all photographed. The micro-photographs are available in the author's collection.

Remarks. The total body length of adult specimens of *Chaetonotus* (*Chaetonotus*) *svalbardi* varied from 119.2 to 121.6 μm; the length of the pharynx was between 30.0 and 32.7 μm; of the intestine from 63.3 to 64.3 μm; there were 23–25 total longitudinal rows of scales (7–9D+4DL+4L+4LV+4V) with 19–21 scales in the central row. The specimen corresponds very well with the morphological and morphometric features from the original description (Kolicka 2017).

Distribution. *Chaetonotus* (*Chaetonotus*) *svalbardi* Kolicka, 2016 was previously known only from one locality on Spitsbergen (Svalbard) in the Longyearbyen region (Kolicka 2017).

Subgenus ***Hystricochaetonotus*** Schwank, 1990

Type species. *Chaetonotus hystrix* Mečnikow, 1865.
Terra typica. Russia.

A polyphyletic subgenus according to molecular data (Kånneby *et al*. 2013); it encompasses 28 nominal freshwater and 10 nominal marine species (Todaro 2018). It is present in benthic and periphytic habitats (Kisielewski 1997).

Chaetonotus* (*Hystricochaetonotus*) *borealis sp. nov.
(Figs 23–31; Tables 15–16, 21)

Localities: 3, 6, 9, 12.

Material. Four samples, in total 27 specimens (22 adults, 2 subadults and 3 juveniles), all photographed. The photomicrographs are available in the Natural History Collections at Adam Mickiewicz University in Poznań under accession numbers NHC–GCHB–15–1––10/h (holotype) and NHC–GCHB–15–11–50/p (paratypes) and in the author's collection.

Etymology. From the Latin *borealis*, referring to the habitat in which it was found.

Diagnosis. Stocky body measuring from 98.4 to 124.4 μm. Head five-lobed, cephalion and pleurae well demarcated in the head outline. Hypostomium wide and short, pentagonal in shape. Ocellar granules absent. In mouth ring, two weak cuticular teeth arising from anterior pharynx region. Scales three-lobed with strong keels and spine. One pair of three-lobed, keeled and spineless scales located on dorsal surface of trunk posterior region. Four pairs of three-lobed, keeled scales with a short spine on dorsal surfaces of furcal base and furcal appendages. One pair of double-keeled oval scales on dorsolateral surface of furcal appendages. Scales distributed in 15–17 total longitudinal rows (5–7D+2DL+2L+4LV+2V) with 12–14 scales in the central row. Scales in areas of head, neck and trunk strongly differing morphologically. Spines on dorsal, dorsolateral, lateral and ventrolateral surfacesstrong and curved, tapering towards end, with a lateral denticle. Spines on ventral body surface thin and hair-like with narrow, delicate *lamellae* along their entire length, without a lateral denticle. Length of spines gradually increases from head towards furcal base and decreases from dorsal, dorsolateral, lateral, ventrolateral surfaces towards ventral body side. Posteriormost trunk lateral spines are the longest, strongest and most curved. Furcal appendages with one pair of scales with a short, strong parafurcal spine with a lateral denticle. Inside the furcal indentation, three pairs of small intrafurcal scales with keels and rudimetary spines. Ventral interciliary field from end of anterior pharynx dilatation to posterior body end covered with strongly differentiated and overlapping scales. Three pairs of ventral interciliary field terminal scales. Pharynx with pronounced anterior and posterior dilatations. A single, narrow, curved cuticular rod inside the anterior pharynx dilatation. Intestine straight with anterior section as a short band differing in form of morphology.

Description. *Chaetonotus* (*Hystricochaetonotus*) *borealis* sp. nov. has a stocky body (Figs 23, 26–27). Its head is wide and the neck is only slightly narrower than the head. The neck constriction is present but not strongly marked. The neck gradually tapers

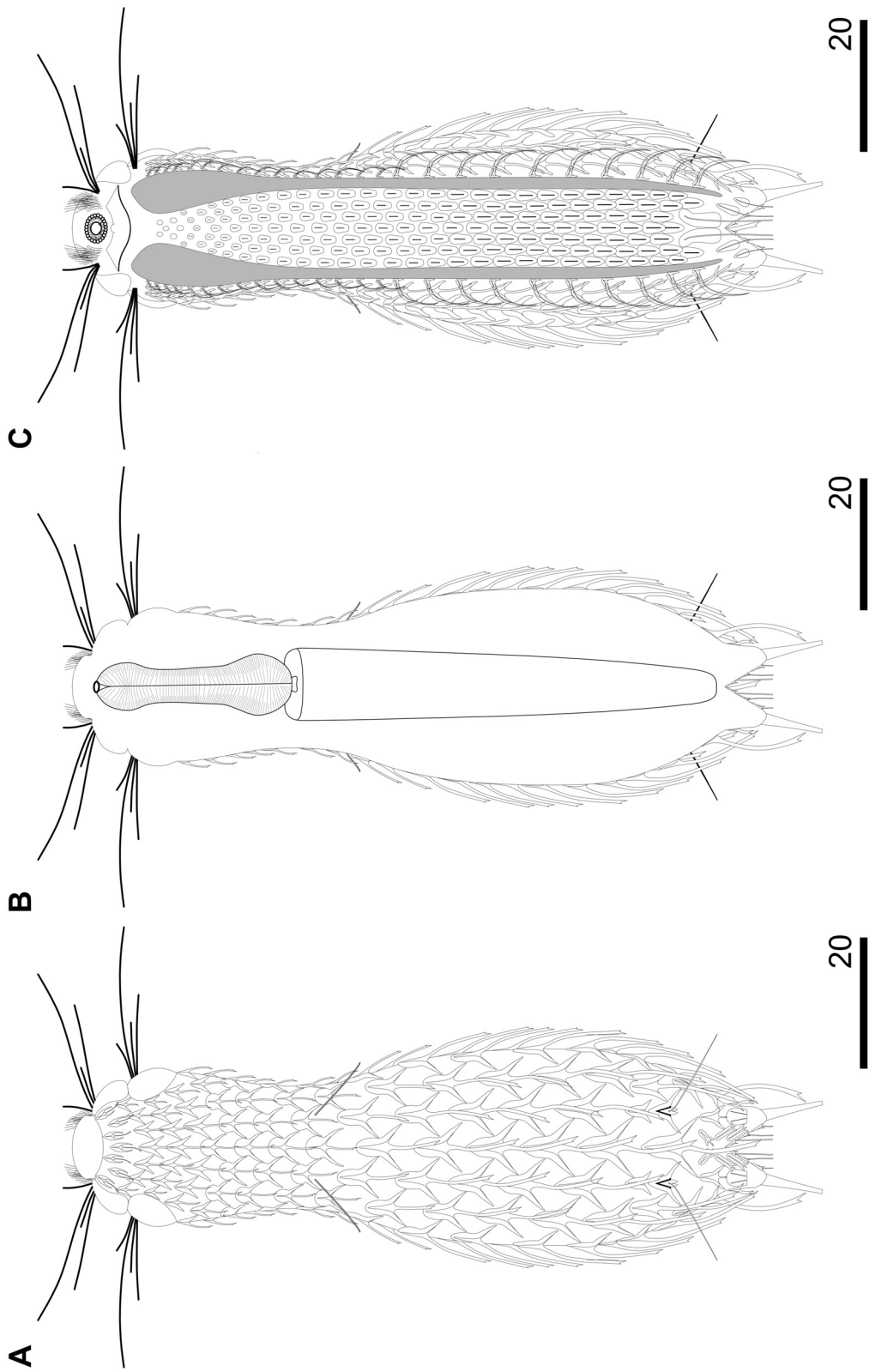


Figure 23. *Chaetonotus* (*Hystricochaetonotus*) *borealis* sp. nov. Schematic drawings. A – Dorsal body view. B – View of internal morphology. C – Ventral body view. Light grey areas indicate the areas of the insertion of ciliary bands.

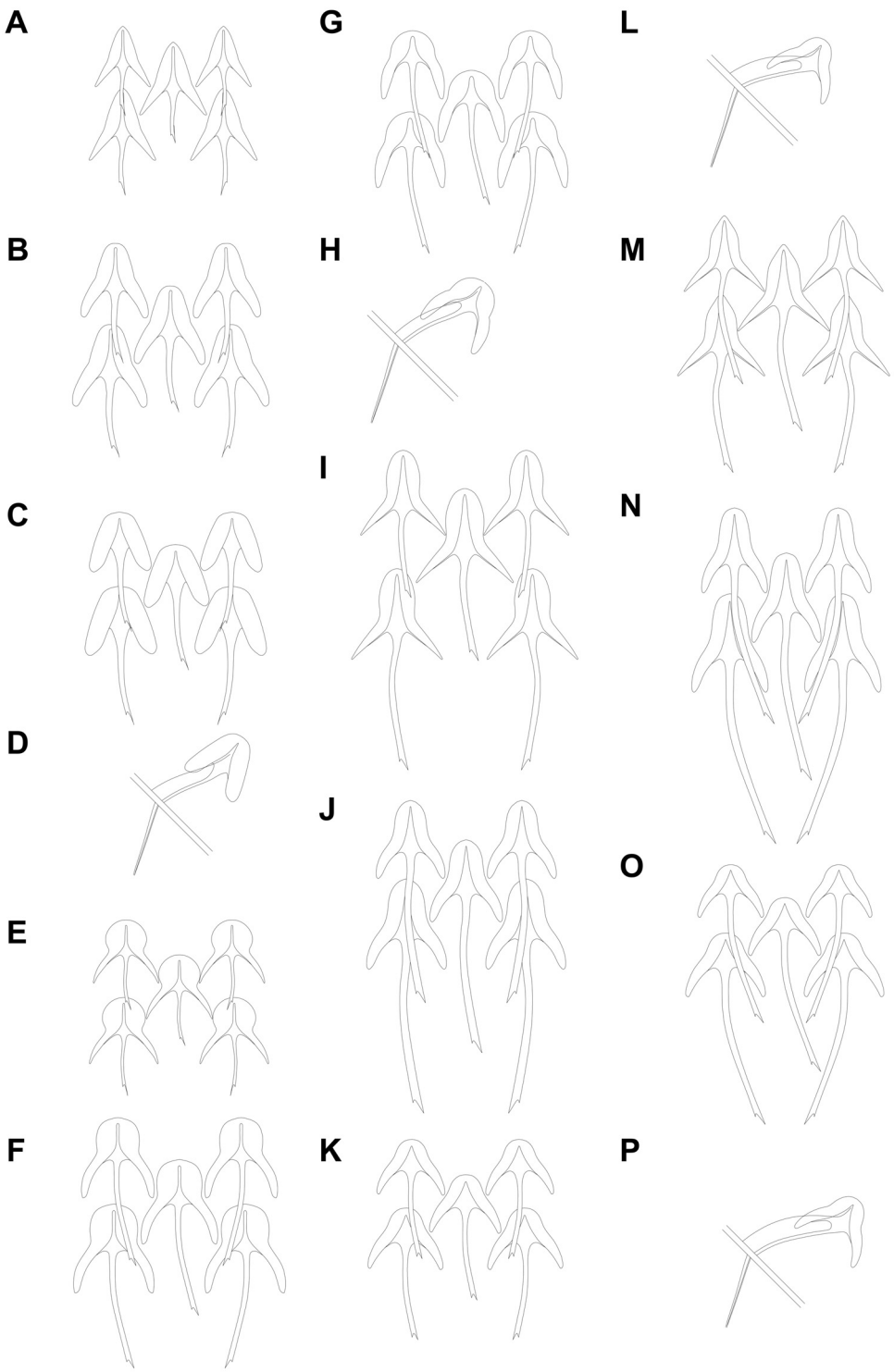


Figure 24. *Chaetonotus* (*Hystricochaetonotus*) *borealis* sp. nov. Schematic scales drawings. A – Head dorsal scales (scales 2). B – Head dorsolateral and lateral scales (scales 3). C – Head ventrolateral scales (scales 4). D – Head ventral scales (scales 5). E – Neck dorsal scales (scales 6). F – Neck dorsolateral and lateral scales (scales 7). G – Neck ventrolateral scales (scales 8). H – Neck ventral scales (scales 9). I – Trunk anterior region dorsal scales (scales 10). J – Trunk anterior region dorsolateral and lateral scales (scales 11). K – Trunk anterior region ventrolateral scales (scales 12). L – Trunk anterior region ventral scales (scales 13). M – Trunk posterior region dorsal scales (scales 14). N – Trunk posterior region dorsolateral and lateral scales (scales 15). O – Trunk posterior region ventrolateral scales (scales 16). P – Trunk posterior region ventral scales (scales 17).

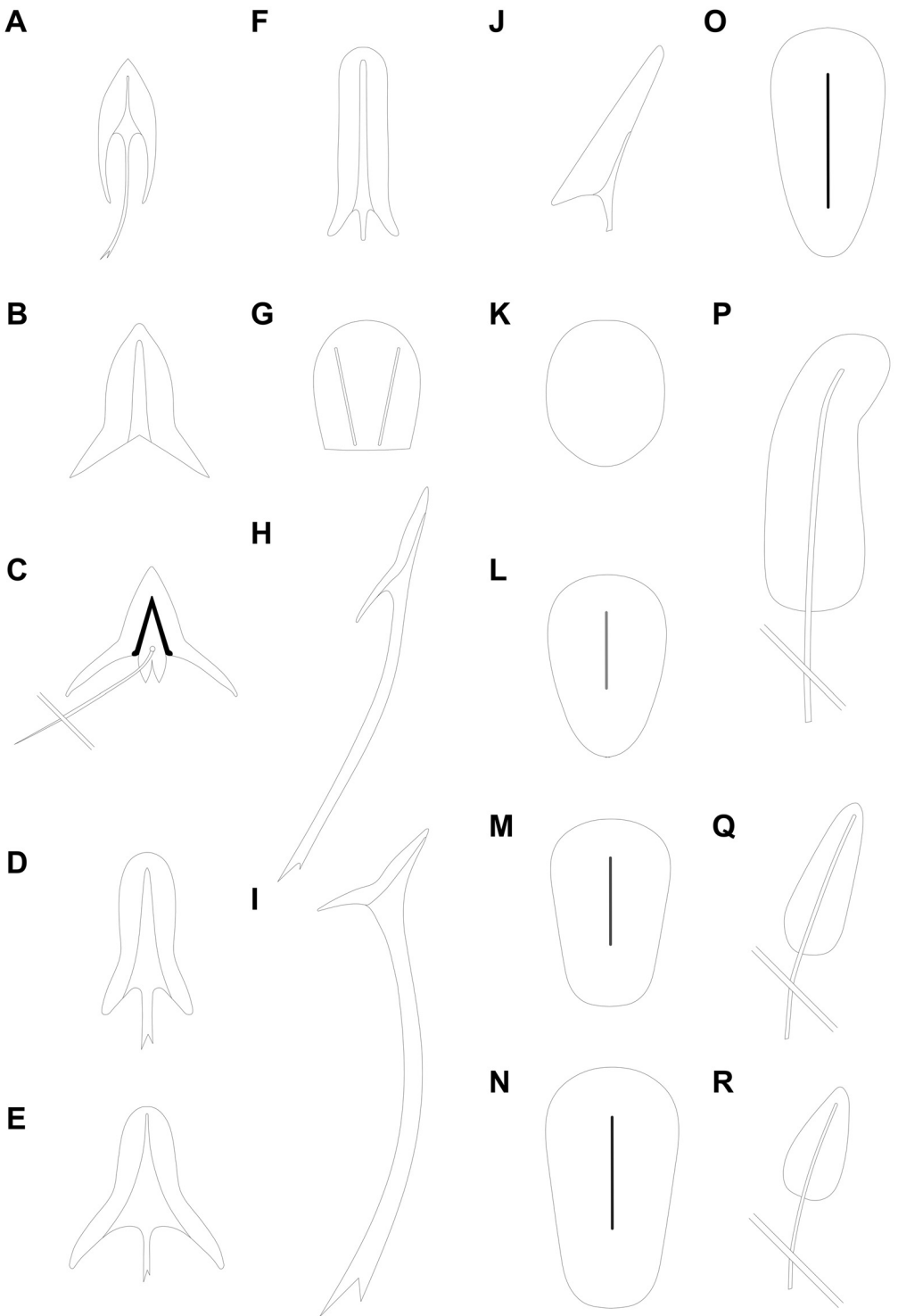


Figure 25. *Chaetonotus* (*Hystricochaetonotus*) *borealis* sp. nov. Schematic scales drawings. A – Head anteriormost dorsal scale (scales 1). B – Spineless scale anterior to each double-keeled scale with dorsal sensory bristle (scales 18). C – Double-keeled scale with trunk dorsal sensory bristle (scales 19). D – Small furcal appendages dorsal scales . E–F – Furcal appendages dorsal scale (scales 20 and 21). G – Furcal appendages double-keeled scale (scales 23). H – Second to the posteriormost trunk lateral scales (scales 24). I – Posteriormost trunk lateral scales (scales 25). J – Intrafurcal scale (scales 26). K–O – Ventral interciliary field scale (scales 27–31). P – First pair of ventral interciliary field terminal scale (scales 32). Q – Second pair of ventral interciliary field terminal scale (scales 33). R – Third pair of ventral interciliary field terminal scale (scales 34).

from the head (from U21) to the beginning of the trunk (*ca*. U33) (Figs 23, 26–27). The trunk is wider than the head and gradually dilates from *ca*. U34 to *ca*. U61, where it is at its maximum width, and subsequently gradually tapers to the furcal base (from U83), which is clearly demarcated and narrow (Figs 23, 26–27). The furcal indentation (intrafurcal space) is V-shaped. The furcal branches are set close together and point slightly outwards (Figs 23, 26–27). The furcal appendages and the adhesive tubes are relatively short in comparison to body length. The adhesive tubes are straight and taper towards their blunt ends (Figs 23, 26).

The head is five-lobed and quarter-circle in shape. The cephalion (U1–U5) is short, narrow, strongly convex and clearly demarcated in the body outline. It adheres to the head along its entire length and rapidly widens at the dorsal edge (Figs 23A, 27A). The epipleurae (U3–U8) and hypopleurae (U8–U14) are large, convex and similar in size (Figs 23A, 27A). The lateral edges of the cephalion and epipleurae do not meet and there is a prominent space between them which is largest on the ventral side. Deep notches are present between the cephalion, epipleurae and hypopleurae. The hypostomium (U5–U8) is wide and short, and pentagonal in shape (Figs 23C, 27C, 30C). It has a granular protuberance in the centre of the anterior edge and a reinforced posterior edge. Two pairs of cephalic ciliary tufts are present. The anterior tufts (at U4) emerge in the area between the cephalion and epipleurae edges on the ventral head side. The anterior tufts consist of 4 cilia. The anteriormost and third cilium in both of the anterior tufts are short. The second cilium is the longest in the tuft. The posteriormost cilium is shorter than the second one and longer than the first and third cilium. The posterior tufts (at U9) have 4 cilia each and emerge in ventral notches between the epipleurae and hypopleurae on the ventral head side. The anteriormost cilium in both tufts is the shortest. The second cilium is very long and the longest of all the cilia in both pairs of cephalic tufts. The third is shorter than the second one and longer than the first. The posteriormost one is longer than the third. Cilia in both tufts are thick and almost straight. Ocellar granules are absent. The mouth ring is located subterminally at U3–U5 and

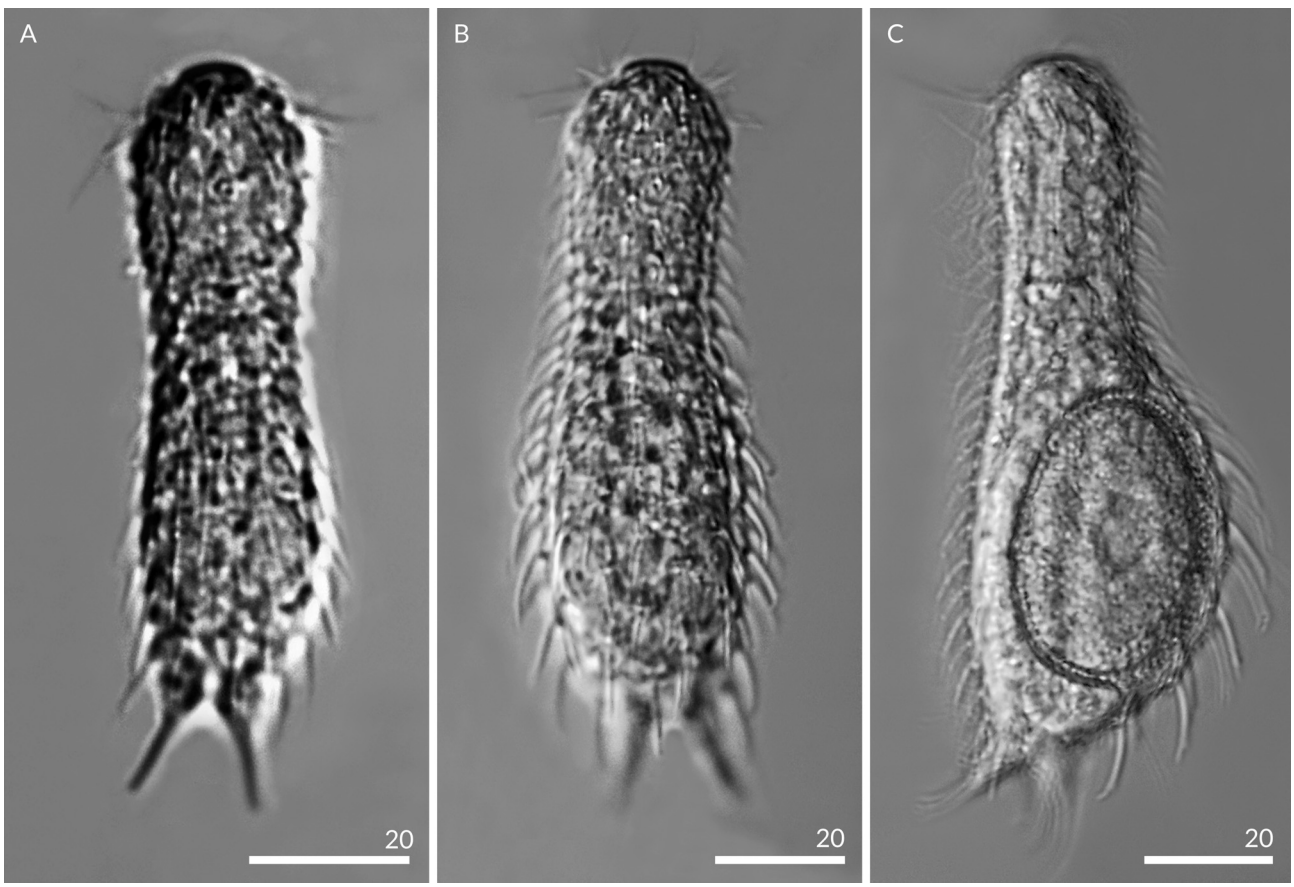


Figure 26. *Chaetonotus* (*Hystricochaetonotus*) *borealis* sp. nov. A – Juvenile specimen. Habitus. B – Adult specimen. Habitus. C – Specimen with mature egg. Habitus.

has strong, granular cuticular reinforcements and short inner hairs. Inside the mouth ring, two small and weak cuticular teeth arising from the anterior pharynx region are present. Suboral bristles are located in two tufts around the mouth ring's lateral sides.

The entire body is covered with three-lobed scales that adhere over their entire surfaces to the cuticle. Each triangular scale has a strong, high, triangular keel and a deeply notched posterior edge. The scales are distributed in 15–17 total longitudinal alternating rows (5–7D+2DL+2L+4LV+2V) with 12–14 scales in the central row. The longitudinal rows of scales run almost parallel to each other. The scales on the head, neck and trunk do not meet and their edges do not overlap. The edges of the scales strongly overlap only on the furcal base and furcal appendages. The longitudinal rows of scales begin on the anteriormost head area. The scales show strong morphological diversity among the various parts of the body: the distinction of the lateral lobes, the length of the central lobe, the roundness of the edges, and scale size (Fig. 23). The anteriormost scales of the head that adhere directly to the posterior edge of the cephalion and epipleurae are very narrow, with long, posterolateral lobes directed downwards (scales 1; Figs 23A, 25A). The remaining scales of the head are sharp and triangular and have weakly demarcated, wide lateral lobes that transition directly from the central lobe (scales 2; Figs 23A, 24A). The neck scales have a shorter, rounded, semicircular central lobe and long and narrow posterolateral lobes that are distinctly separated from the central lobe (scales 6; Figs 23A, 24E). The central lobes of the scales gradually lengthen on the neck towards the anterior trunk region and their rounded edges gradually transition into sharp edges (scales 10 and 14; Figs 23A, 24I, 24M). Scales on the trunk are triangularwith a long central lobe. The posterolateral lobes are clearly separated from the central lobe and directed laterally. One pair of scales different in shape is located dorsolaterally on the trunk, lateral and slightly anterior to the scales with dorsal sensory bristles (*ca.* at U77–80) (Fig. 23A). These scales have more rounded central lobes and shorter posterolateral lobes, but the keels and spines are similar to those of neighbouring scales

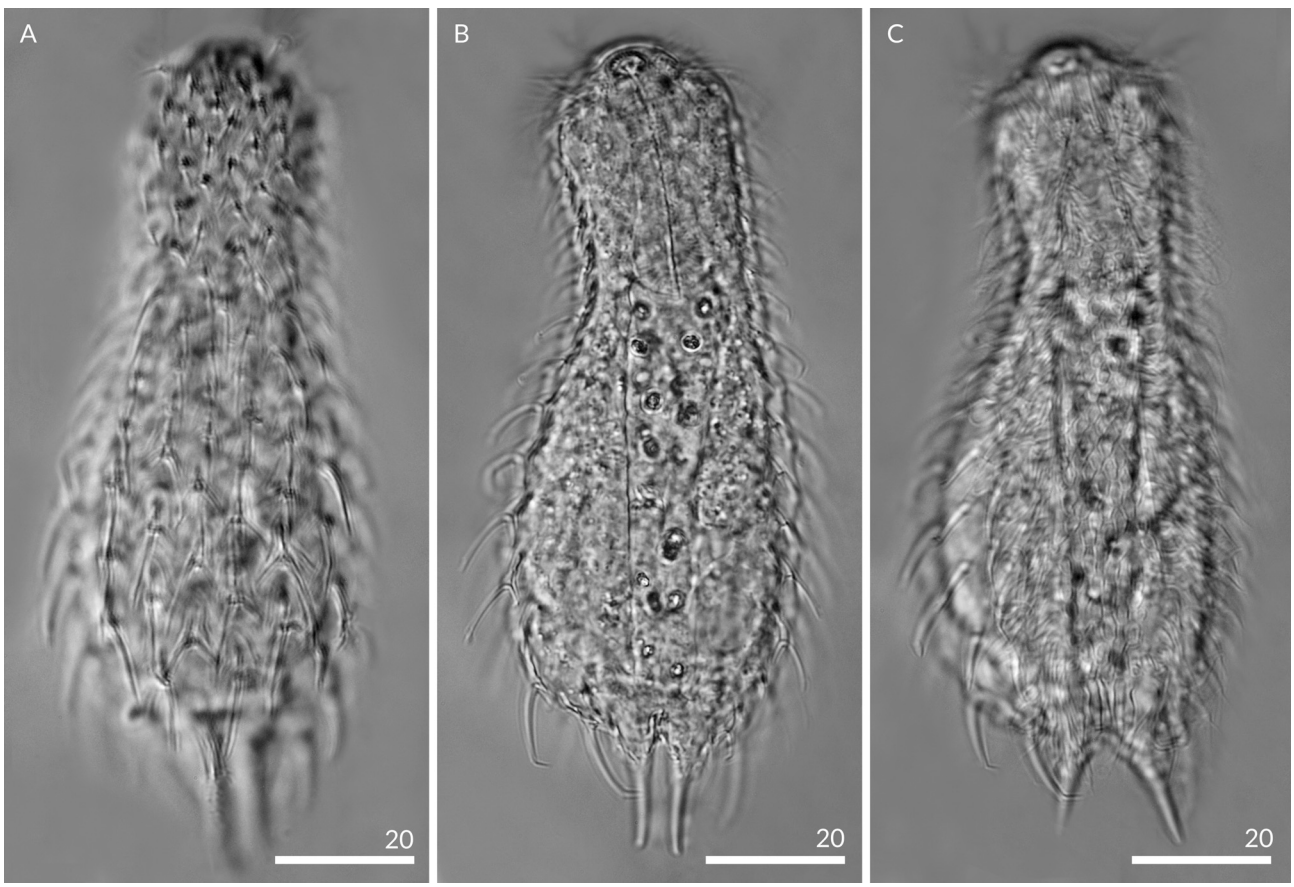


Figure 27. *Chaetonotus* (*Hystricochaetonotus*) *borealis* sp. nov. Adult specimen. A – Dorsal body view. B – View of internal morphology. C – Ventral body view.

(Figs 23A, 27A). The edges of the dorsolateral, lateral, ventrolateral and ventral scales gradually become more rounded towards the ciliary bands as compared to the edges of the dorsal scales (scales from 2 to 5; from 6 to 9; from 10 to 17; Figs 23–25). The size of the scales gradually decreases from the dorsal, dorsolateral, lateral and ventrolateral surfaces towards the ventral ciliary bands, but the posteriormost pair of trunk scales is slightly smaller than the other lateral scales of the posterior trunk region. The scales in the ventral longitudinal row located closest to the ciliary bands are much smaller than the scales of the other rows (Figs 23C, 27C). In the trunk region, the width of the ventral scales decreases more slowly than their length, and thus they are shorter relative to their width (scales 13 and 17; Figs 24L, 24P). The central lobes of the ventral scales are oriented towards the ventral locomotory bands at an angle of *ca*. 45° (Figs 23C, 27C). Four pairs of three-lobed scales of differentiated shape are present on the dorsal surface of the furcal base and the dorsal and dorsolateral sides of the furcal appendages (Figs 23A, 27A, 29A, 29C). The first pair located on the dorsal surface of the furcal base (at U84–U86), is elongated with a wide central lobe and short posterolateral lobes. This pair has a strong triangular keel and short spines with lateral denticles (scales 20; Fig. 25D). The second pair, is located dorsally at U86–U89 on the furcal appendages. The scales are shaped like wide triangles with the posterolateral lobes set wide apart, a strong triangular keel and short spines with lateral denticles (scales 21; Fig. 25E). The third pair is located on the dorsal surface of the furcal base (at U85–U87). The fourth pair is located on the furcal appendages near their internal edges and parallel to the third pair (at U87–U90) (scales 22; Fig. 25F). The third and fourth pairs are narrow, elongated, have only very short and narrow posterolateral lobes, have long, straight keels and very short spines without lateral denticles (Fig. 23A). Dorsolaterally and laterally at U89–U92, the furcal appendages of one pair of scales with two keels are present. These scales are oval with a straight posterior edge and have no posterior notches or spines. Their keels are situated in a V-shape but are not connected (scales 23; Figs 23A, 25G, 27A). The edges of these

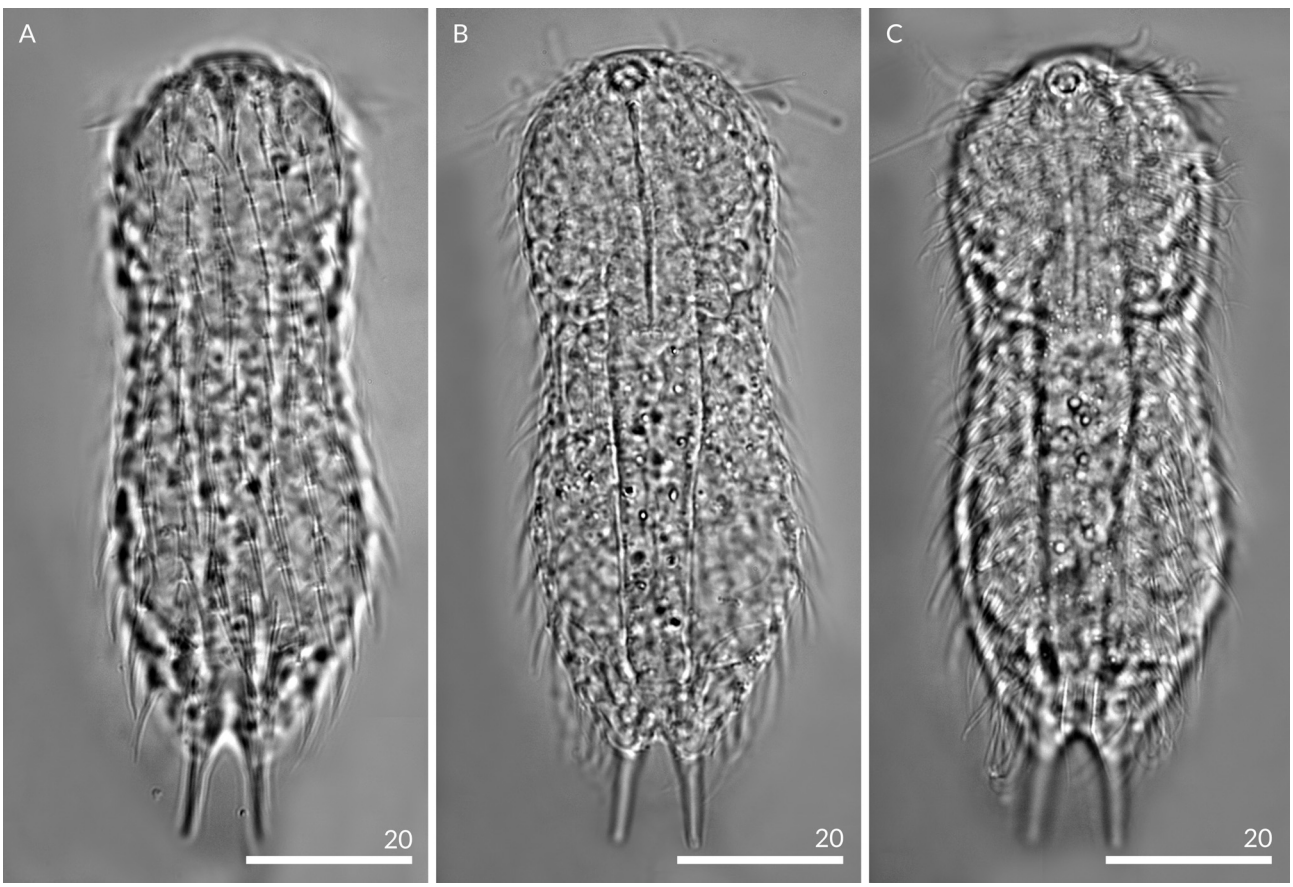


Figure 28. *Chaetonotus* (*Hystricochaetonotus*) *borealis* sp. nov. Juvenile specimen. A – Dorsal body view. B – View of internal morphology. C – Ventral body view.

scales overlap the neighbouring dorsolateral and lateral scales. One pair of scales with parafurcal spines of the same type as the trunk lateral scales is located on the lateral, external surface of the furcal appendages (at U89–U90). Three pairs of intrafurcal scales are present on the inner surface of the furcal appendages at *ca*. U88–U92 (scales 26; Figs 23, 25J). These scales are small, three-lobed, almost triangular in shape, and have keels and short, simple, rudimentary spines. The edges of these scales are curved, thus making the entire scale adhere to the narrow, medial surface of the furcal indentation (scales 26; Figs 23, 25J, 27).

Spines emerge from the posterior part of the scales. On the dorsal, dorsolateral, lateral and ventrolateral body surfaces, the spines are strong, curved, have a lateral denticle near their end and gradually taper

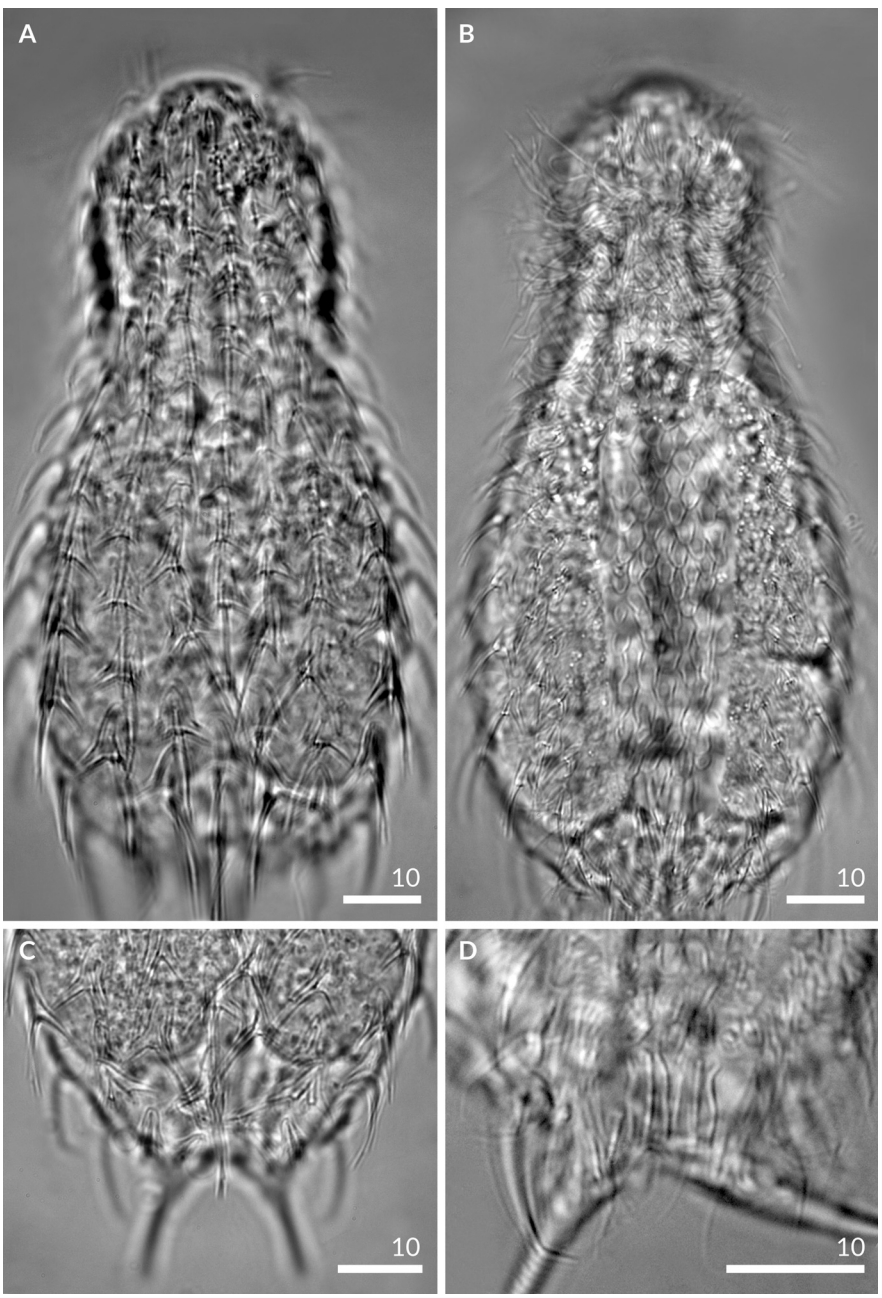


Figure 29. *Chaetonotus* (*Hystricochaetonotus*) *borealis* sp. nov. Adult specimen. A – Dorsal view of scales and spines. B – Ventral view of scales and spines. C – Posterior trunk region and furcal base dorsal view of scales and spines. D – Posterior trunk region and furcal base dorsal view of scales and spines.

towards their pointed ends. The length and thickness of the spines gradually increase from the top of the head towards the anterior one-third of the trunk, on the posterior two-thirds of the trunk the spines are conspicusly longer and their length gradually increases towardthe furcal base (Figs 23, 26–29). Also, the lateral denticles on the spines become more pronounced from the head towards the furcal base. All of the dorsolateral, lateral and ventrolateral spines, except for the posteriormost lateral pair, are shorter than the dorsal spines and their length gradually decreases towards the ventrolateral body surfaces. The posteriormost pair of lateral trunk spines are longer, stronger and more curved than the other body spines. The pair of parafurcal spines are strong and curved, but shorter than the trunk spines. On the ventral body surface, spines emerging from scales arranged in two longitudinal rows located closest to the longitudinal bands are long, thin, hair-like and have narrow, delicate *lamellae* along their entire length. The *lamellae* are the widest near the spine base and taper towards the end of the spines. These spines do not have a lateral denticle (Figs 23, 24D, 24H, 24L, 27C, 28C, 29B, 29D).

The species has three pairs of dorsal sensory bristles (Fig. 23). The anterior pair is located on the dorsal surface of the head at U3, between the lateral cephalion edges and the epipleurae. The bristles emerge from small, spherical papillae. The second pair of sensory bristles is located on the dorsal surface of the neck at U31 and emerges from small, spherical papillae. The third, posterior pair of sensory bristles is located dorsally on the posterior trunk region. The third pair of dorsal sensory bristles emerges from the three-lobed scales with two keels located at U79–U82. These scales have keels that are strongly connected in the centre of the scales. These scales are shaped like triangles with long and narrow posterolateral lobes set very wide apart (scales 19; Fig. 25C).

On the ventral surface, the longitudinal ciliary bands begin at U9 and run back to U87 (Figs 23C, 27C, 28C, 29B, 29D). The ciliary bands are wider on the head than on the other parts of the body. The ventral interciliary field is covered with scales from the end of the

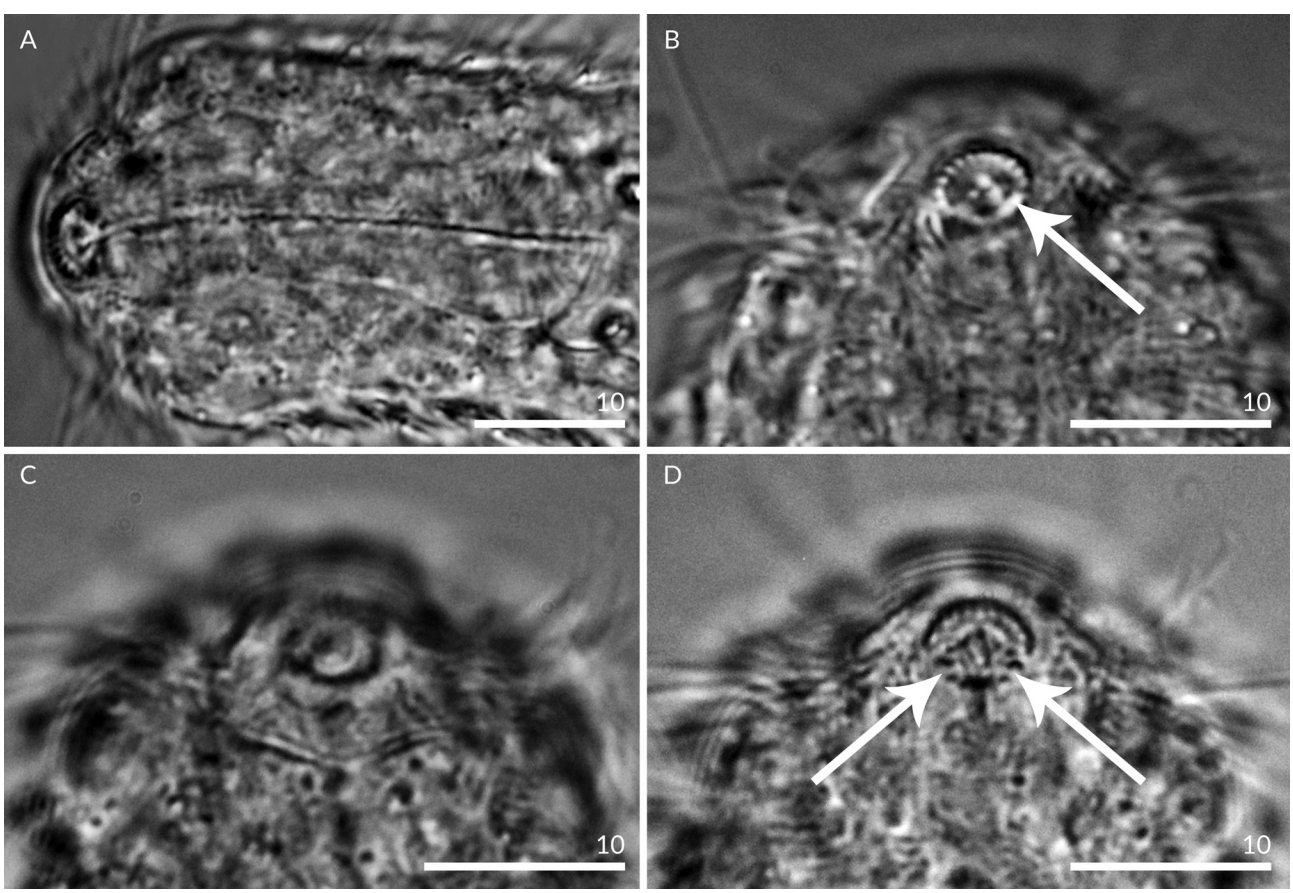


Figure 30. *Chaetonotus* (*Hystricochaetonotus*) *borealis* spec nov. Adult specimen. A – Detail of the pharynx. B, D – Detail of mouth ring with cuticular denticles. C – Detail of hypostomium.

anterior pharynx dilatation (Figs 23C, 27C, 28C, 29B, 29D). Scales on the ventral interciliary field are one-lobed and strongly differentiated in shape and size. They are distributed in 9–11 total longitudinal rows with 20–27 scales in the central row (scales 27–31; Figs 23C, 25K–O, 27C, 28C, 29B, 29D). On the anterior body part the scales are round and located far from each other (scales 27; Fig. 25K). Towards the posterior body region the scales become gradually elongated, larger and shaped like ovals tapering towards their posterior edges (scales 27 and 28; Figs 25K–L). Towards the trunk posterior region, keels on the scales become more distinct and the distance between the scales decreases, so that the scales' edges start adjacent to another and subsequently their edges initially are to overlap each other (Figs 23C, 27C, 28C, 29B, 29D). On the posterior trunk region, the ventral interciliary field scales overlap(scales 29–31; Figs 25M–O). Three pairs of ventral interciliary field terminal scales are present. The first pair is large, elongated and asymmetrical and without the posterior notch (at U81–U87) (scales 32; Figs 23C, 25P, 27C, 29D). These scales have an oval shape with the anterior edges directed aside, whereas the remaining parts of scales are straight in relation to the longitudinal body axis. They have a long keel and a long, thin, straight spine extending towards the internal furcal indentation. The second and third pairs are located at the furcal appendages (at U86–U89 and U89–U90, respectively). These pairs are triangular, with strong, rounded edges, and without posterior notches. They have a long, straight keel and a long, thin, straight spine extending towards the internal furcal indentation (scales 33 and 34; Figs 23C, 25Q–R, 27C, 29D).

The pharynx (from U4 to U30) has clearly marked anterior and stronger posterior dilatations. Weak cuticular reinforcements in the form of a single strongly curved rod are located in the anterior dilatation (at U6–U7). The pharynx is connected through the pharyngeal-intestinal junction to the straight intestine (running from U30 to U86). The pharyngeal-intestinal junction is clearly demarcated, short and narrow (U30). The intestine has a short separate anterior section running from U30 to U31 (Figs 23B, 27B, 28B). The X-organ of this species (observed in one specimen) is located at U84–U86 on both sides of the terminal part

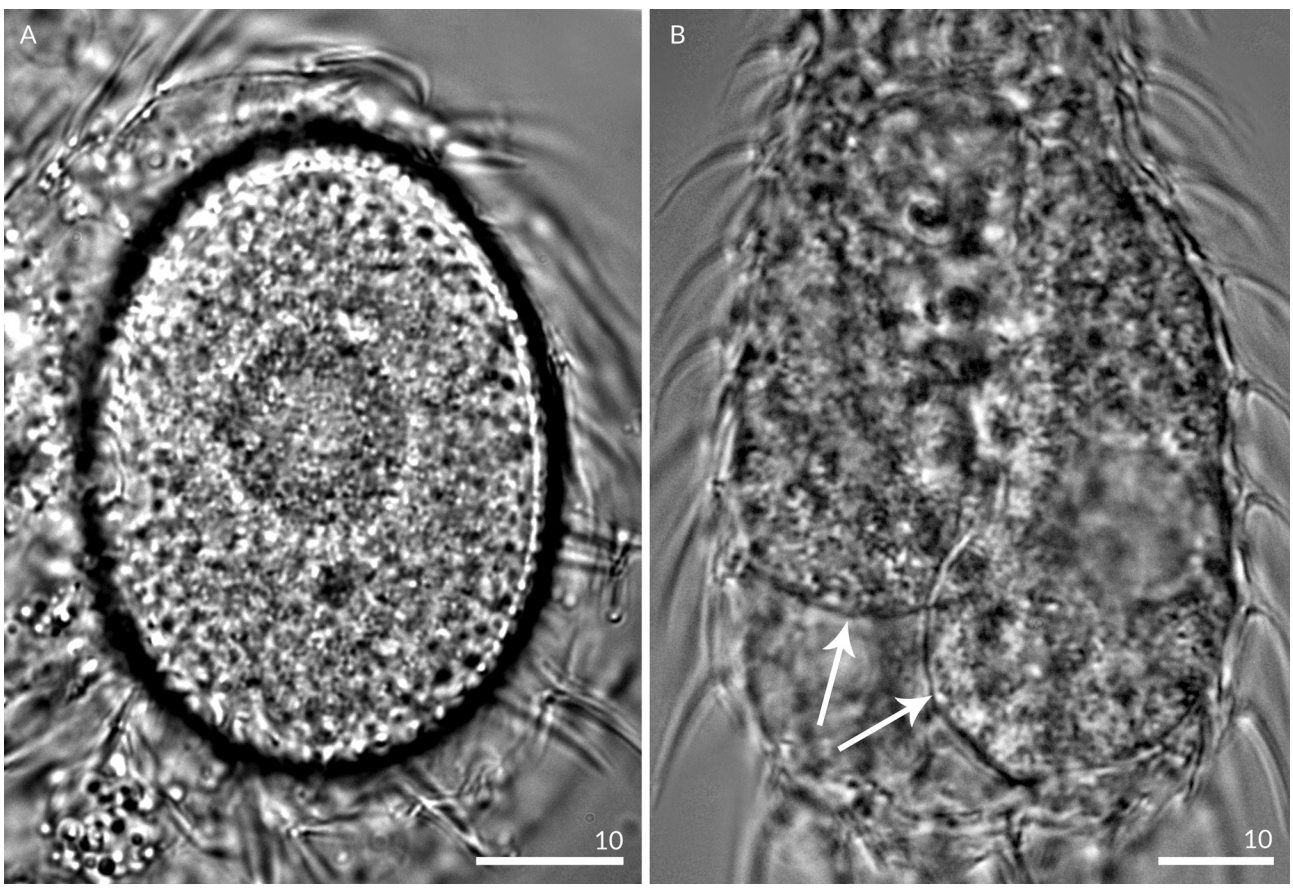


Figure 31. *Chaetonotus* (*Hystricochaetonotus*) *borealis* sp. nov. Detail of the internal morfology. A – Matured egg. B – Two maturing eggs.

Table 15. Main morphometric characters of *Chaetonotus* (*Hystricochaetonotus*) *borealis* sp. nov; N – number of specimens or structures analysed, Range – the smallest and the largest structure found among all specimens measured. All measurements are given in micrometers (μm); all indicators are given as a percentage (%) and italicized.

Characters	N	Holotype	Range of adult paratypes
Body length	23	115.52	115.25–127.40
Furca length	15	20.86	20.86–22.14
Adhesive tube length	15	10.64	10.64–12.06
Number of scales in central longitudinal row	15	13	12–14
Total number of longitudinal alternating rows of scales	15	15	15–17
Head scales length x width	15	1.93–5.76 x 1.87–5.58	1.87–7.08 x 1.81–6.85
Neck scales length x width	15	3.09–7.09 x 3.07–6.83	2.75–8.63 x 2.73–8.30
Trunk scales length x width	15	3.92–10.84 x 4.16–9.77	3.51–13.10 x 3.72–11.80
Head spines length	15	2.92–8.34	2.86–11.20
Localization of lateral denticle on head spines	15	*13.014–15.781*	*11.963–18.557*
Neck spines length	15	6.36–11.24	5.95–13.99
Localization of lateral denticle on neck spines	15	*16.593–18.163*	*16.006–18.886*
Trunk spines length	15	9.58–16.93	7.87–20.21
Localization of lateral denticle on trunk spines	15	*18.789–23.690*	*18.043–25.824*
Length of posteriormost trunk lateral spines	15	15.34	15.20–19.60
Localization of lateral denticle on posteriormost trunk lateral spines	15	*23.729*	*22.995–23.795*
Length of posteriormost parafurcal lateral spines	15	4.24	3.94–5.25
Localization of lateral denticle on posteriormost parafurcal lateral spines	15	*14.623*	*13.429–15.741*
Mouth ring diameter	15	4.63	4.41–4.92
Pharynx length	23	32.49	31.05–34.78

Table 16. Detailed morphometric characters of *Chaetonotus* (*Hystricochaetonotus*) *borealis* sp. nov; N – number of specimens or structures analysed, Range – the smallest and the largest structure found among all specimens measured, SD – standard deviation. All measurements are given in micrometers (μm); all indicators are given as a percentage (%) and italicized.

Characters	N	Holotype	Range of adult paratypes	SD
Cephalion length	15	12.10	11.97–13.07	0.404
Cephalion width	15	10.16	10.08–11.71	0.487
Hypostomium length	15	5.59	5.21–6.03	0.202
Hypostomium width	15	13.57	11.60–14.17	0.601
Length of cephalic cilia (anterior tuft)	15	7.02–16.93	(6.57–7.52)–(16.89–24.16)	0.259; 1.858
Length of cephalic cilia (posterior tuft)	15	8.34–18.52	(8.21–9.07)–(18.39–29.70)	0.255; 2.795
Width of cephalic cilia (anterior tuft)	15	1.02–1.07	(1.01–1.05)–(1.06–1.10)	0.014; 0.011
Width of cephalic cilia (posterior tuft)	15	1.05–1.10	(1.04–1.08)–(1.08–1.14)	0.012; 0.015
Width of adhesive tube	15	1.85	1.85–1.94	0.029
Ratio of scale distribution	23	*115.385*	*115.385–125.000*	3.509
Length of head dorsal scales (scales 1 and 2)	15	4.07–5.76	(3.93–5.06)–(5.53–7.08)	0.342; 0.410
Width of head dorsal scales (scales 1 and 2)	15	1.96–5.58	(1.91–2.43)–(5.36–6.85)	0.157; 0.396
Length of head dorsolateral scales (scales 3)	15	3.84–5.53	(3.71–4.77)–(5.31–6.80)	0.322; 0.390
Width of head dorsolateral scales (scales 3)	15	2.93–5.31	(2.83–3.64)–(5.10–6.53)	0.247; 0.370
Length of head lateral scales (scales 3)	15	3.69–5.38	(3.57–4.58)–(5.17–6.62)	0.307; 0.378
Width of head lateral scales (scales 3)	15	2.80–5.12	(2.71–3.47)–(4.92–6.30)	0.235; 0.345
Length of head ventrolateral scales (scales 4)	15	3.44–5.07	(3.33–4.27)–(4.87–6.24)	0.284; 0.356
Width of head ventrolateral scales (scales 4)	15	2.59–4.75	(2.51–3.21)–(4.56–5.83)	0.213; 0.333
Length of head ventral scales (scales 5)	15	1.93–2.96	(1.87–2.37)–(2.86–3.64)	0.149; 0.220
Width of head ventral scales (scales 5)	15	1.87–2.74	(1.81–2.28)–(2.65–3.37)	0.144; 0.203
Length of neck dorsal scales (scales 6)	15	6.13–7.09	(5.89–7.47)–(6.81–8.63)	0.427; 0.492
Width of neck dorsal scales (scales 6)	15	6.20–6.83	(5.96–7.55)–(6.56–8.30)	0.433; 0.480
Length of neck dorsolateral scales (scales 7)	15	5.82–6.88	(5.60–7.09)–(6.61–8.37)	0.402; 0.479
Width of neck dorsolateral scales (scales 7)	15	5.86–6.54	(5.64–7.14)–(6.28–7.95)	0.407; 0.456
Length of neck lateral scales (scales 7)	15	5.67–6.67	(5.47–6.91)–(6.41–8.11)	0.392; 0.465
Width of neck lateral scales (scales 7)	15	5.69–6.31	(5.49–6.93)–(6.06–7.67)	0.395; 0.444
Length of neck ventrolateral scales (scales 8)	15	5.49–6.38	(5.31–6.69)–(6.13–7.76)	0.378; 0.445
Width of neck ventrolateral scales (scales 8)	15	5.48–6.00	(5.30–6.68)–(5.76–7.30)	0.376; 0.417

Table 16. Continued.

Characters	N	Holotype	Range of adult paratypes	SD
Length of neck ventral scales (scales 9)	15	3.09–3.76	(2.75–3.0)–(3.38–4.61)	0.275; 0.320
Width of neck ventral scales (scales 9)	15	3.07–3.53	(2.73–3.77)–(3.17–4.32)	0.261; 0.307
Length of trunk dorsal scales (scales 10 and 14)	15	7.42–10.84	(7.13–8.98)–(10.42–13.10)	0.515; 0.740
Width of trunk dorsal scales (scales 10 and 14)	15	7.03–9.77	(6.76–8.51)–(9.38–11.80)	0.486; 0.667
Length of trunk dorsolateral scales (scales 11 and 15)	15	7.21–9.97	(6.93–8.73)–(9.56–12.05)	0.500; 0.681
Width of trunk dorsolateral scales (scales 11 and 15)	15	6.79–8.92	(6.53–8.22)–(8.55–10.77)	0.472; 0.607
Length of trunk lateral scales (scales 11 and 15)	15	7.01–9.68	(6.74–8.49)–(9.28–11.69)	0.489; 0.661
Width of trunk lateral scales (scales 11 and 15)	15	6.57–8.63	(6.31–7.95)–(8.26–10.42)	0.460; 0.589
Length of trunk ventrolateral scales (scales 12 and 16)	15	6.54–8.97	(6.29–7.92)–(8.60–10.83)	0.454; 0.580
Width of trunk ventrolateral scales (scales 12 and 16)	15	6.08–7.96	(5.85–7.35)–(7.62–9.60)	0.422; 0.518
Length of trunk ventral scales (scales 13 and 17)	15	3.92–4.23	(3.51–4.81)–(3.79–6.36)	0.310; 0.773
Width of trunk ventral scales (scales 13 and 17)	15	4.16–5.37	(3.72–5.09)–(4.78–7.28)	0.336; 0.627
Length of posterior trunk spineless scales (scales 18)	15	7.29	6.92–9.81	0.854
Width of posterior trunk spineless scales (scales 18)	15	6.30	5.69–8.47	0.746
Length of trunk posteriormost pair of lateral scales (scales 25)	15	7.24	7.03–10.38	1.122
Width of trunk posteriormost pair of lateral scales (scales 25)	15	5.82	5.65–8.30	0.897
Length of posteriormost lateral scales with parafurcal spines	15	4.28	4.03–5.58	0.447
Width of posteriormost lateral scales with parafurcal spines	15	3.97	3.61–5.19	0.427
Length of the first pair of different shape furcal base dorsal scales (scales 20)	15	7.06	5.03–8.13	0.852
Width of the first pair of different shape furcal base dorsal scales (scales 20)	15	4.22	2.91–4.86	0.490
Length of the second pair of different shape furcal appendages dorsal scales (scales 21)	15	6.44	5.76–7.98	0.719
Width of the second pair of different shape furcal appendages dorsal scales (scales 21)	15	6.20	5.54–7.68	0.678
Length of the third and four pair of different shape furcal base and appendages dorsal scales (scales 22)	15	8.50–8.76	(6.41–9.13)–(6.98–9.79)	0.834; 0.888
Width of the third and four pair of different shape furcal base and appendages dorsal scales (scales 22)	15	3.51–3.78	(2.77–4.19)–(3.03–4.37)	0.384; 0.399
Length of double-keeled scales on the furcal appendages (scales 23)	15	6.51	5.44–8.06	0.735
Width of double-keeled scales on the furcal appendages (scales 23)	15	5.25	4.28–6.49	0.629
Length of intrafurcal scales (scales 26)	15	1.89–2.16	(1.68–2.18)–(1.93–2.63)	0.117; 0.185
Width of intrafurcal scales (scales 26)	15	1.47–1.64	(1.11–1.77)–(1.26–1.99)	0.154; 0.187
Length of head dorsal spines (scales 1 and 2)	15	4.03–7.06	(3.95–5.09)–(6.56–8.83)	0.399; 0.754
Head dorsal spines length between lateral denticle and spine top (scales 1 and 2)	15	0.53–1.11	(0.51–0.82)–(1.05–1.04)	0.074; 0.117
Length of head dorsolateral spines (scales 3)	15	3.57–6.83	(3.50–4.51)–(6.34–8.54)	0.354; 0.732
Head dorsolateral spines length between lateral denticle and spine top (scales 3)	15	0.47–1.07	(0.46–0.72)–(1.01–1.34)	0.064; 0.113
Length of head lateral spines (scales 3)	15	3.39–6.59	(3.32–4.28)–(6.47–8.24)	0.338; 0.646
Head lateral spines length between lateral denticle and spine top (scales 3)	15	0.45–1.04	(0.43–0.68)–(0.98–1.30)	0.060; 0.111
Length of head ventrolateral spines (scales 4)	15	2.92–6.22	(2.86–3.68)–(6.12–7.78)	0.290; 0.608
Head ventrolateral spines length between lateral denticle and spine top (scales 4)	15	0.38–0.98	(0.37–0.56)–(0.93–1.22)	0.049; 0.099
Length of head ventral spines (scales 5)	15	7.18–8.34	(7.01–8.96)–(8.14–11.20)	0.648; 0.947
Lamellae width on the ventral head spines (scales 5)	15	0.73–0.96	(0.55–0.92)–(0.91–1.08)	0.097; 0.057
Length of neck dorsal spines (scales 6)	15	7.23–9.91	(6.73–9.04)–(8.26–12.39)	0.765; 1.158
Neck dorsal spines length between lateral denticle and spine top (scales 6)	15	1.21–1.80	(1.13–1.51)–(1.49–2.34)	0.126; 0.220
Length of neck dorsolateral spines (scales 7)	15	6.99–9.72	(6.52–8.74)–(8.10–12.15)	0.739; 1.138
Neck dorsolateral spines length between lateral denticle and spine top (scales 7)	15	1.16–1.76	(1.05–1.45)–(1.46–2.19)	0.125; 0.205
Length of neck lateral spines (scales 7)	15	6.75–9.58	(6.31–8.44)–(7.98–11.98)	0.713; 1.123

Table 16. Continued.

Characters	N	Holotype	Range of adult paratypes	SD
Neck lateral spines length between lateral denticle and spine top (scales 7)	15	1.12–1.74	(1.01–1.40)–(1.44–2.17)	0.119; 0.203
Length of neck ventrolateral spines (scales 8)	15	6.36–9.36	(5.95–7.95)–(7.79–11.69)	0.671; 1.094
Neck ventrolateral spines length between lateral denticle and spine top (scales 8)	15	1.06–1.69	(0.96–1.32)–(1.39–2.11)	0.112; 0.196
Length of neck ventral spines (scales 9)	15	8.48–11.24	(8.28–11.32)–(10.94–13.99)	0.947; 1.015
Lamellae width on the ventral neck spines (scales 9)	15	0.98–1.19	(0.92–1.10)–(1.10–1.27)	0.059; 0.063
Length of trunk dorsal spines (scales 10 and 14)	15	10.27–14.31	(8.49–12.79)–(13.29–17.64)	1.192; 1.251
Trunk dorsal spines length between lateral denticle and spine top (scales 10 and 14)	15	1.94–3.39	(1.54–2.42)–(3.07–4.21)	0.232; 0.336
Length of trunk dorsolateral spines (scales 11 and 15)	15	10.08–13.22	(8.33–12.55)–(10.92–16.30)	1.175; 1.358
Trunk dorsolateral spines length between lateral denticle and spine top (scales 11 and 15)	15	1.90–3.13	(1.51–2.36)–(3.05–3.90)	0.228; 0.273
Length of trunk lateral spines (scales 11 and 15)	15	9.77–12.63	(8.07–12.16)–(12.41–15.57)	1.138; 0.979
Trunk lateral spines length between lateral denticle and spine top (scales 11 and 15)	15	1.84–2.99	(1.46–2.28)–(2.92–3.72)	0.219; 0.263
Length of trunk ventrolateral spines (scales 12 and 16)	15	9.58–11.19	(7.87–11.91)–(11.01–13.76)	1.119; 0.869
Trunk ventrolateral spines length between lateral denticle and spine top (scales 12 and 16)	15	1.80–2.65	(1.42–2.23)–(1.62–3.24)	0.213; 0.391
Length of trunk ventral spines (scales 13 and 17)	15	11.67–16.93	(9.61–14.50)–(12.36–20.21)	1.307; 2.109
Lamellae width on the ventral trunk spines (scales 13 and 17)	15	1.21–1.47	(1.12–1.29)–(1.21–1.62)	0.063; 0.108
Length of trunk posteriormost pair of lateral spines (scales 25)	15	15.34	15.20–19.60	1.478
Trunk posteriormost pair of lateral spines length between lateral denticle and spine base (scales 25)	15	3.64	3.57–4.65	0.355
Length of posteriormost parafurcal lateral spines	15	4.24	3.94–5.25	0.431
Posteriormost parafurcal lateral spines length between lateral denticle and spine base	15	0.62	0.56–0.78	0.073
First pair of different shape furcal base dorsal scales spines (scales 20)	15	3.52	3.19–4.14	0.237
Length between lateral denticle and top on spines first pair of different shape furcal top dorsal scales spines (scales 20)	15	0.56	0.51–0.84	0.084
Second pair of different shape furcal appendages dorsal scales spines (scales 21)	15	1.97	1.84–3.38	0.422
Length between lateral denticle and top on spines second pair of different shape furcal top dorsal scales spines (scales 21)	15	0.23	0.19–0.53	0.094
Third and four pair of different shape furcal base and appendages dorsal scales spines (scales 22)	15	1.41–1.53	(1.29–2.05)–(1.44–2.26)	0.223; 0.217
Length of intrafurcal scales spines (scales 26)	15	0.77–0.92	(0.71–0.99)–(0.84–1.17)	0.085; 0.102
Length ratio of posteriormost trunk lateral spines	15	13.279	12.818–16.186	0.975
Length ratio of posteriormost parafurcal lateral spines	15	3.670	3.207–4.336	0.312
Localization of lateral denticle on dorsal head spines (scales 1 and 2)	15	*13.151–15.722*	(*12.528–16.006*)–(*15.638–16.368*)	0.966; 0.179
Localization of lateral denticle on dorsolateral head spines (scales 3)	15	*13.165–15.666*	(*12.626–15.931*)–(*15.481–18.557*)	0.915; 0.756
Localization of lateral denticle on lateral head spines (scales 3)	15	*13.274–15.781*	*12.687–16.013*)–((*15.462–18.478*)	0.926; 0.715
Localization of lateral denticle on ventrolateral head spines (scales 4)	15	*13.014–15.756*	(*11.963–16.09*)–(*15.426–17.666*)	
Localization of lateral denticle on dorsal neck spines (scales 6)	15	*16.736–18.163*	(*16.591–17.125*)–(*17.692–18.886*)	0.120; 0.226
Localization of lateral denticle on dorsolateral neck spines (scales 7)	15	*16.595–18.107*	(*16.104–16.787*)–(*17.973–18.134*)	0.144; 0.044
Localization of lateral denticle on lateral neck spines (scales 7)	15	*16.593–18.163*	(*16.006–16.792*)–(*17.898–18.191*)	0.180; 0.078
Localization of lateral denticle on ventrolateral head spines (scales 8)	15	*16.667–18.056*	(*16.162–16.720*)–(*17.699–18.083*)	0.145; 0.107

Table 16. Continued.

Characters	N	Holotype	Range of adult paratypes	SD
Localization of lateral denticle on dorsal trunk spines (scales 10 and 14)	15	*18.890–23.690*	(*18.137–18.966*)–(*22.885–25.089*)	0.277; 0.460
Localization of lateral denticle on dorsolateral trunk spines (scales 11 and 15)	15	*18.849–23.676*	(*18.091–18.862*)–(*22.898–25.824*)	0.251; 0.700
Localization of lateral denticle on lateral trunk spines (scales 11 and 15)	15	*18.833–23.674*	(18.092–18.874)–(*22.956–25.816*)	0.240; 0.698
Localization of lateral denticle on ventrolateral trunk spines (scales 12 and 16)	15	*18.789–23.682*	(*18.043–19.002*)–(*22.893–24.036*)	0.251; 0.274
Localization of lateral denticle on posteriormost trunk lateral spines (scales 25)	15	*23.729*	*22.995–23.795*	0.196
Localization of lateral denticle on first pair of different shape furcal top dorsal scales spines (scales 20)	15	*15.909*	*15.245–22.581*	2.017
Localization of lateral denticle on second pair of different shape furcal appendages dorsal scales spines (scales 21)	15	*11.675*	*10.326–19.134*	2.585
Localization of lateral denticle on posteriormost parafurcal lateral spines	15	*14.623*	*13.429–15.741*	0.495
Head dorsal sensory bristles length	15	12.93	12.45–14.98	0.638
Neck dorsal sensory bristles length	15	13.38	13.11–15.33	0.624
Posterior dorsal sensory bristles length	15	17.02	16.92–19.71	0.880
Number of scales in central longitudinal row on ventral interciliary field	23	25	20–25	1.260
Total number of longitudinal alternating rows of scales on ventral interciliary field	23	9	9–11	0.703
Length of ventral interciliary field scales (scales 27–31)	15	0.87–4.58	(0.84–1.18)–(3.90–4.73)	0.102; 0.276
Width of ventral interciliary field scales (scales 27–31)	15	0.91–2.63	(0.90–1.20)–(2.34–3.62)	0.095; 0.306
Length of terminal ventral interciliary field scales I pair (scales 32)	15	9.58	9.24–10.73	0.4613469
Width of terminal ventral interciliary field scales I pair (scales 32)	15	3.22	3.17–4.07	0.310
Length of terminal ventral interciliary field scales II pair (scales 33)	15	5.21	5.09–5.84	0.206
Width of terminal ventral interciliary field scales II pair (scales 33)	15	2.11	1.98–2.54	0.160
Length of terminal ventral interciliary field scales III pair (scales 34)	15	4.02	3.94–4.48	0.174
Width of terminal ventral interciliary field scales III pair (scales 34)	15	1.83	1.78–2.19	0.113
Length of terminal ventral interciliary field scales I pair spines (scales 32)	15	11.72	10.24–12.28	0.528
Length of terminal ventral interciliary field scales II pair spines (scales 33)	15	9.67	8.26–10.15	0.568
Length of terminal ventral interciliary field scales III pair spines (scales 34)	15	6.71	6.27–7.19	0.245
Width of anterior pharynx thickening (a)	15	9.68	9.41–11.69	0.622
Width of pharynx narrowing (n)	15	7.08	6.31–8.55	0.531
Width of middle pharynx (m)	15	7.74	6.83–9.22	0.584
Width of posterior pharynx thickening (p)	15	11.97	11.43–13.43	0.499
Width of pharyngeal–intestinal junction	15	3.01	3.01–3.32	0.078
Length of intestine anterior differ section	15	2.78	2.43–3.31	0.237
Intestine length	23	59.25	58.9–67.76	2.445
Pharynx formula a	15	*29.794*	*28.522–35.532*	1.818
Pharynx formula n	15	*21.791*	*19.427–25.988*	1.585
Pharynx formula m	15	*23.823*	*21.028–28.024*	1.773
Pharynx formula p	15	*36.842*	*35.049–40.821*	1.546
Pharynx-intestine ratio I	23	*54.835*	*49.049–57.046*	1.942
Length of matured egg	3	–	46.41–54.33	4.541
Width of matured egg	3	–	32.18–38.16	2.998
Length of X-organ	1	–	12.55	–
Width of X-organ	1	–	7.47	–

of the intestine. It is bilobed, built from two extensions enveloped in a thin coat and connected by a thin band located behind the intestine at the ventral side. The extensions have a granularappearance. The thin coat and the cellular bridge connecting the extensions have a smooth and homogeneous structure.

Remarks. The total body length of juvenile and subadult specimens of *C.* (*H.*) *borealis* sp. nov. varied from 98.4 to 109.3 μm; the length of the pharynx was between 31.0 to 33.7 μm; of the intestine from 44.6 to 51.6 μm. There were 15 total longitudinal rows of scales (5D+2DL+2L+4LV+2V) with 12–14 scales in the central row. Of the 22 adults, 8 individuals had a large, developing egg (Fig. 31A), 2 had two eggs that were large and similar in size (Fig. 31B), 1 had the X-organ and a developing egg, and 1 had spherical formations inside the integuments (probably some kind of microsymbiont structures).

All three of the new representatives of the *Hystricochaetonotus* subgenus listed in this paper are morphologically similar (see the 'Differential diagnosis' section below); therefore, their grouping into one clade is not surprising. *C.* (*H.*) *hystrix* 1 is also included in this species group. On this branch, *C.* (*C.*) *bombardus* sp. nov. forms the sister group, which together with *C.* (*H.*) *borealis* sp. nov., *C.* (*H.*) *hornsundi* sp. nov., *C.* (*H.*) *hystrix* 1 and *C.* (*H.*) *persimilis* sp. nov. remain a sister clade to *C.* (*H.*) *hystrix* 2 and *C.* (*H.*) *aemilanus*.

Differential diagnosis. The newly described species belongs to the subgenus *Hystricochaetonotus* within the genus *Chaetonotus*. Three similar species described in the 19th century belong to this subgenus, viz. *C.* (*H.*) *hystrix* Mečnikow, 1865, *C.* (*H.*) *macrochaetus* Zelinka, 1889 and *C.* (*H.*) *perse*t*osus* Zelinka, 1889. These taxa were recorded in many different regions of the world and were presented differently by various researchers (see *e.g.* Zelinka 1889, Daday 1905, Konsuloff 1913, Roszczak 1969, Rudescu 1967, Kisielewski 1981, 1991, 1997, Balsamo 1983, Schwank 1990, Kånneby 2013). Thus a different description/specification or a taxonomic classification of theoretically the same species may suggest that we are dealing with a very large range of plasticity of these taxa or with a larger number of undiagnosed, separate species. In order to avoid any doubts as to a differential diagnosis with the new Arctic species, only original descriptions of these species and two of the most detailed papers with morphological data were used (Balsamo 1983, Kisielewski 1997).

Within the subgenus *Hystricochaetonotus*, *Chaetonotus* (*Hystricochaetonotus*) *borealis* sp. nov. most closely resembles two new species from the Hornsund fjord area as described below, namely *C.* (*H.*) *hornsundi* sp. nov. and *C.* (*H.*) *persimilis* sp. nov., as well as *C.* (*H.*) *hystrix* Mečnikow, 1865, *C.* (*H.*) *euhystrix* Schwank, 1990, *C.* (*H.*) *macrochaetus* Zelinka, 1889, *C.* (*H.*) *persetosus* Zelinka, 1889 and *Chaetonotus* (*Chaetonotus*) *benacensis* Balsamo *et* Fregni, 1995. All of these species were chosen for comparison because they possessed similar features such as: range of body length; body shape; range of scale number in central longitudinal alternating rows; range of total number of longitudinal alternating rows of scales; type of scales; range of scale size; and presence of different types of scales on the dorsal and dorsolateral surfaces of the furcal base. Despite these common features, all of the above species differ significantly and a differential diagnosis for all new *Hystricochaetonotus* representatives is given in Table 21.

Chaetonotus (*Hystricochaetonotus*) *hornsundi* sp. nov.
(Figs 32–42; Tables 17–18, 21)

Localities: 3, 4, 6, 11, 12.

Material. Five samples, in total 29 specimens (23 adults, 1 subadult and 5 juveniles), all photographed. The photomicrographs are available in the Natural History Collections at Adam Mickiewicz University in Poznań under accession numbers NHC–GCHH–16–1–10/h (holotype) and NHC–GCHH–16–11–50/p (paratypes) and in the author's collection.

Etymology. From the geographical name of Hornsund fjord where the species was found.

Diagnosis. Mid-slender body, measuring from 84.2 to 138.2 μm. Head five-lobed, cephalion narrow, epipleurae small, hypopleurae large. All cephalic plates well demarcated in dorsal head outline. Hypostomium three-pieced, consisting of a central rectangular narrow plate and a pair of additional tear-shaped plates. Ocellar granules absent. In mouth ring, two weak cuticular teeth arising from anterior pharynx region. Scales, except for one pair on furcal appendages as well as those on ventral interciliary field, three-lobed with strong keel and, except for one pair, spined. Edges of scales overlap: posterolateral lobes of one scale overlap posterolateral lobe of neighbouring scales to the left and right. One pair of three-lobed, keeled and spineless scales located on dorsal surface of the posterior trunk region. One pair of small, rounded, three-lobed scales with posterior edges rotate toward each other located dorsaly on furcal base. Two pairs of three-lobed, keeled and spined elongated scales on dorsal surface of furcal base and furcal appendages. One pair of one-lobed, keeled and spineless long and narrow scales on dorsal surface of furcal appendages. One pair of double-keeled hexagonal scales on dorsolateral surface of furcal appendages. Scales distributed in 15–17 total longitudinal rows (5–7D+2DL+2L+4LV+2V) with 13–15 scales in the central row. Scales have overlapping edges and differ morphologically in areas of

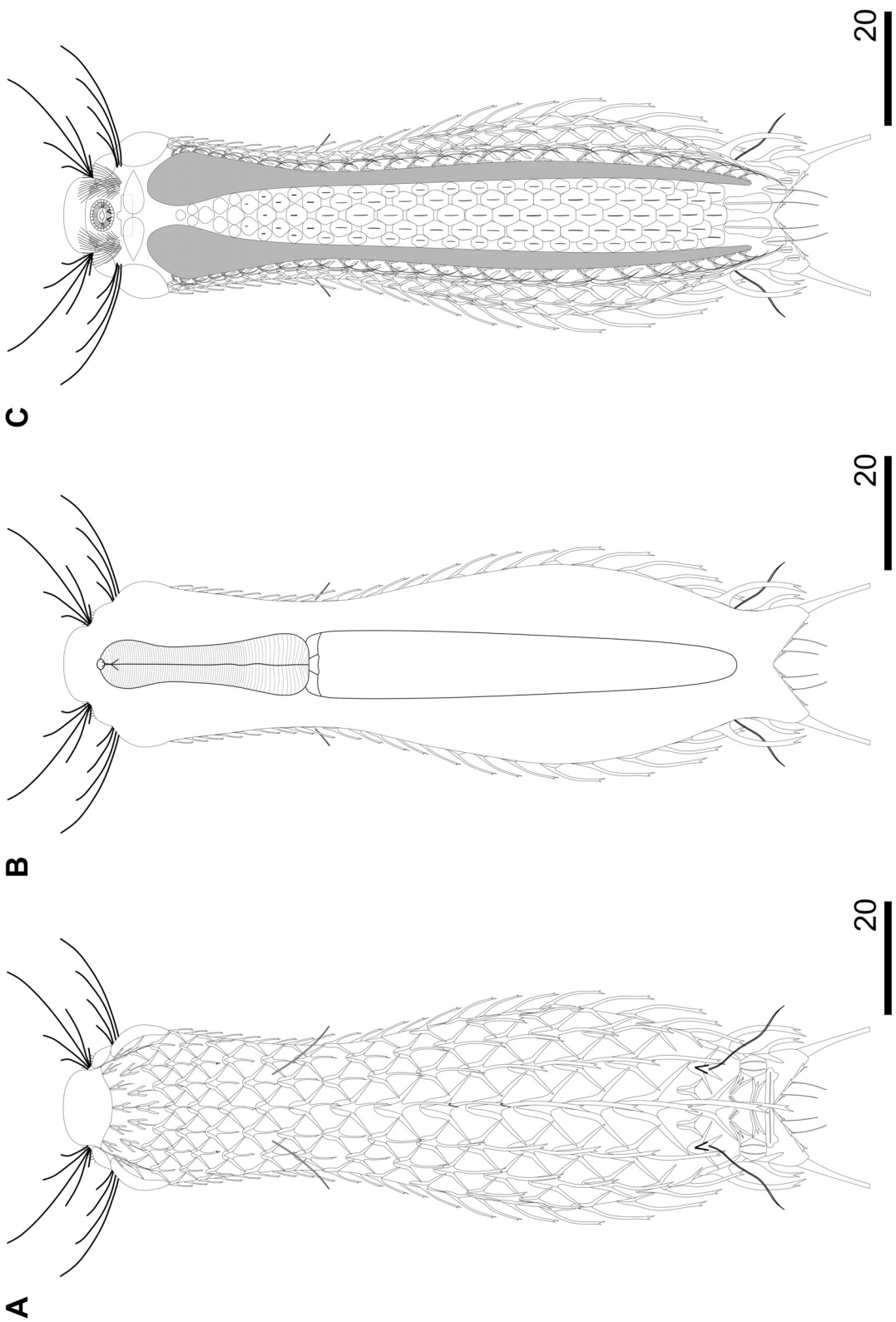


Figure 32. *Chaetonotus* (*Hystricochaetonotus*) *hornsundi* sp. nov. Schematic drawings. A – Dorsal body view. B – View of internal morphology. C – Ventral body view. Light grey areas indicate the areas of the insertion of ciliary bands.

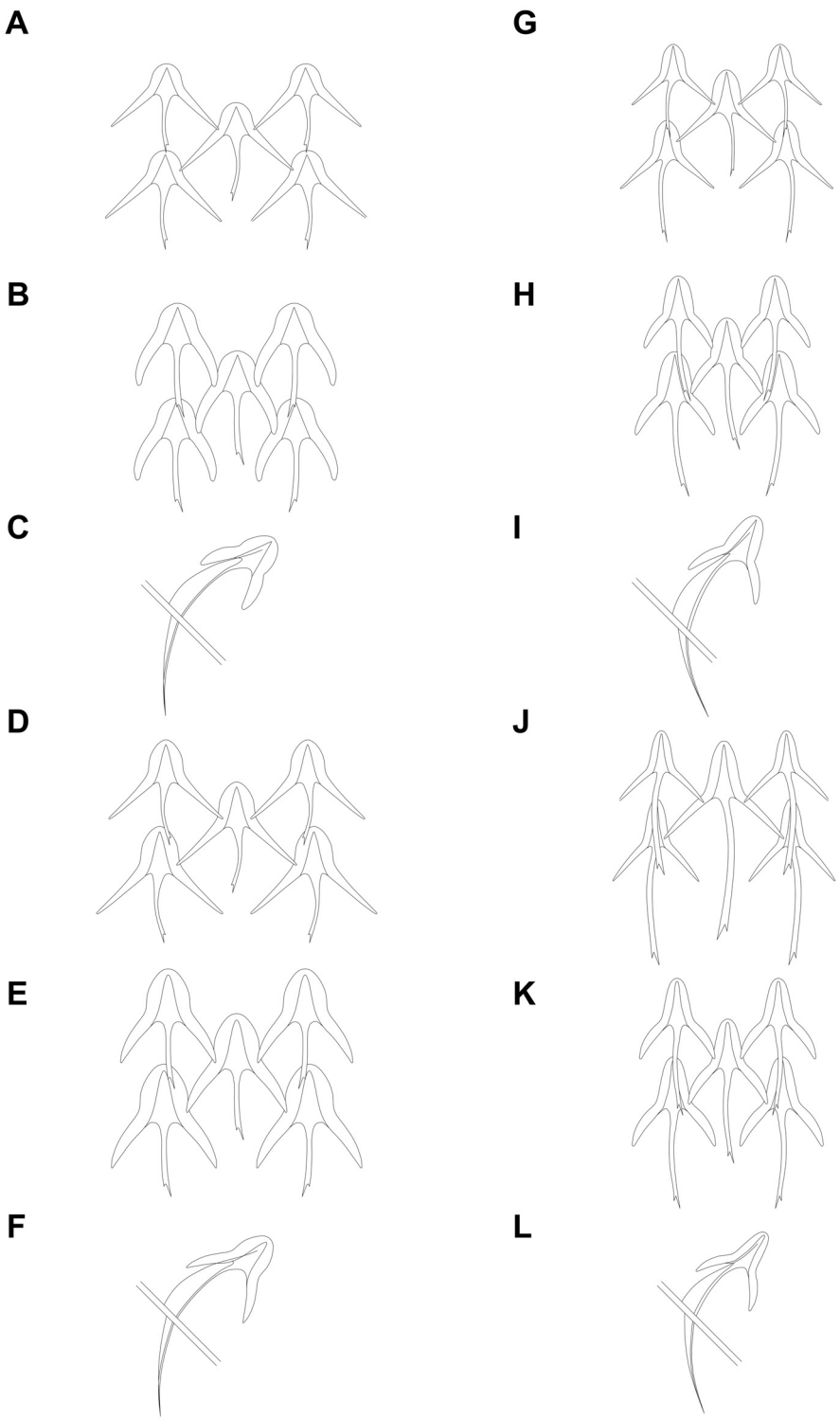


Figure 33. *Chaetonotus* (*Hystricochaetonotus*) *hornsundi* sp. nov. Schematic scales drawings. A – Head anterior region dorsal, dorsolateral and lateral scales (scales 2). B – Head anterior region ventrolateral scales (scales 3). C – Head anterior region ventral scales (scales 4). D – Head posterior region dorsal, dorsolateral and lateral scales (scales 5). E – Head posterior region ventrolateral scales (scales 6). F – Head posterior region ventral scales (scales 7). G – Neck dorsal, dorsolateral and lateral scales (scales 8). H – Neck ventrolateral scales (scales 9). I – Neck ventral scales (scales 10). J – Trunk dorsal, dorsolateral and lateral scales (scales 11). K – Trunk ventrolateral scales (scales 12). L – Trunk ventral scales (scales 13).

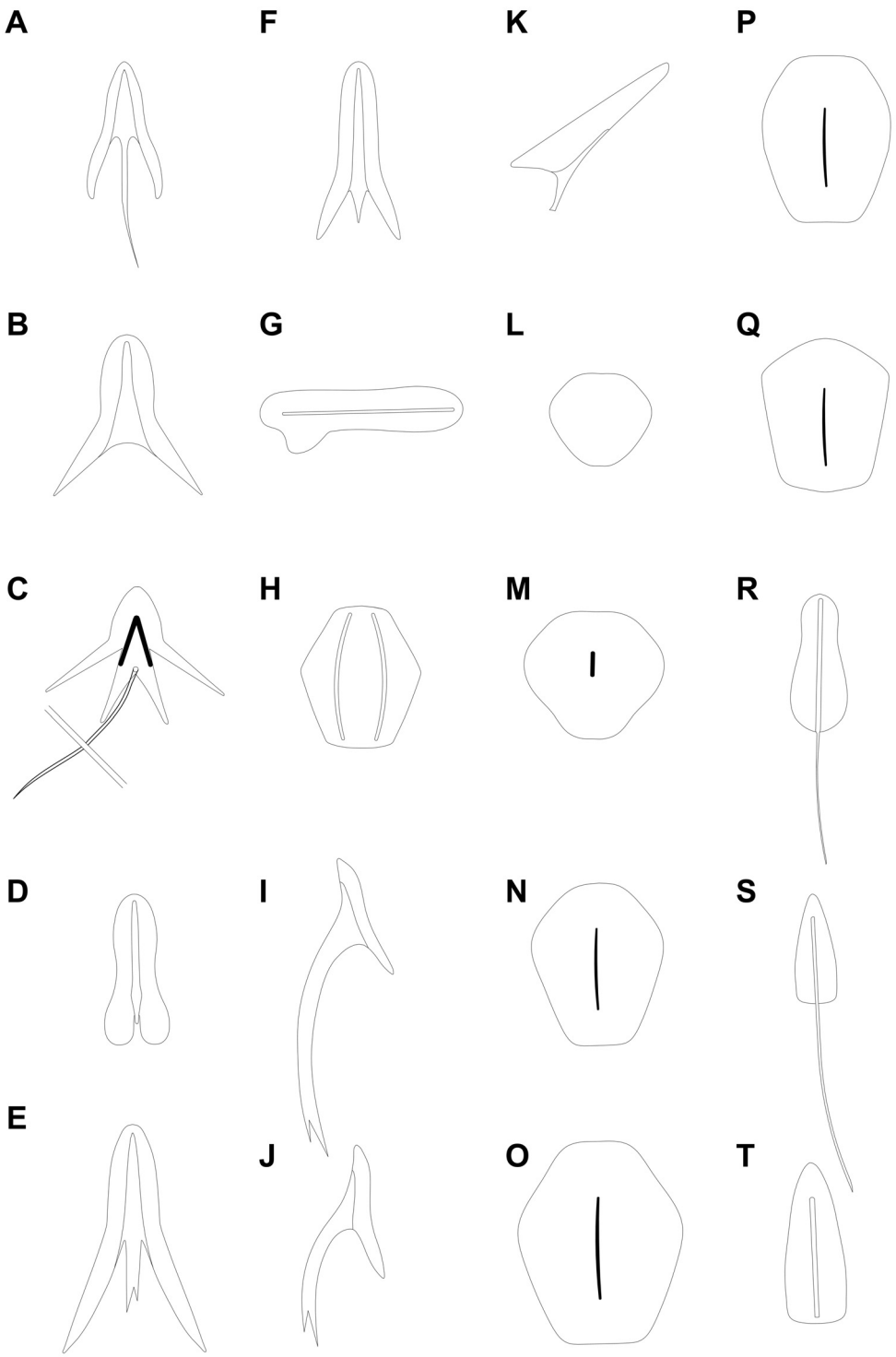


Figure 34. *Chaetonotus* (*Hystricochaetonotus*) *hornsundi* sp. nov. Schematic scales drawings. A – Head anteriormost dorsal scale (scale 1). B – Spineless scale anterior to each double-keeled scale with dorsal sensory bristle (scale 14). C– Double-keeled scale with trunk dorsal sensory bristle (scale 15). D – Small furcal base dorsal scales (scale 16). E – Furcal base dorsal and furcal appendages dorsal scale (scale 17). F – Furcal appendages dorsal scale (scale 18). G – Furcal appendages one-lobed, spineless, elongated scale (scale 19). H – Furcal appendages double-keeled scale (scale 20). I – Posteriormost trunk lateral scales (scales 21). J – Scale with parafurcal spine (scale 22). K – Intrafurcal scale (scale 23). L–Q – Ventral interciliary field scale (scales 24–29). R – First pair of ventral interciliary field terminal scale (scales 30). S – Second pair of ventral interciliary field terminal scale (scales 31). T – Third and foure pairs of ventral interciliary field terminal scale (scales 32).

head, neck and trunk. Spines on dorsal, dorsolateral, lateral and ventrolateral body surfaces strong and slightly curved, tapering towards end, with a lateral denticle. Spines on ventral body surfaces thin and hair-like with narrow, delicate *lamellae* along their entire length, without a lateral denticle. Length of spines gradually increases from head towards furcal base and decreases from dorsal towards ventral. Posteriormost trunk lateral scales are the longest, strongest and most curved. Furcal appendages with one pair of scales with short, strong parafurcal spine with a lateral denticle. Inside the furcal indentation, three pairs of small intrafurcal scales with keels and rudimetary spines. Ventral interciliary field beneath the end of anterior pharynx dilatation to posterior body end covered with large, plate-like, strongly differentiated scales; scales overlap on trunk region. Four pairs of ventral interciliary field terminal scales. Pharynx with pronounced anterior and posterior dilatations. Two thin cuticular rods connected together in the centre inside anterior pharynx dilatation. Intestine straight with anterior section as a short band differing in form of morphology.

Description. *Chaetonotus* (*Hystricochaetonotus*) *hornsundi* sp. nov. has a mid-slender body (Figs 32, 35–38). The head is wide but only slightly wider than the neck. The neck constriction is present but not strongly marked. The neck gradually tapers from the head (from U22) to the beginning of the trunk (*ca*. U32) (Figs 32, 35–38). The trunk is wider than the head and gradually dilates from *ca*. U33 to *ca*. U61, where it is at its maximum width and subsequently gradually tapers to the furcal base (from U81) (Figs 32, 35–38). The furcal base is clearly demarcated and is as wide as the head. The furcal indentation (intrafurcal space) is V-shaped. The furcal branches are set wide apart and point outwards (Figs 32, 35–38). The adhesive tubes are straight and taper towards their blunt ends (Figs 32, 35–38).

The head is five-lobed, short and quarter-circle in shape. The cephalion (U1–U6) is narrow, placed forward, flat-ended and clearly demarcated in the body outline. It adheres to the head along its entire length and rapidly widens at the dorsal edge (Figs 32, 35–38). The epipleurae (U4–U6) are small, *ca*. one-third the

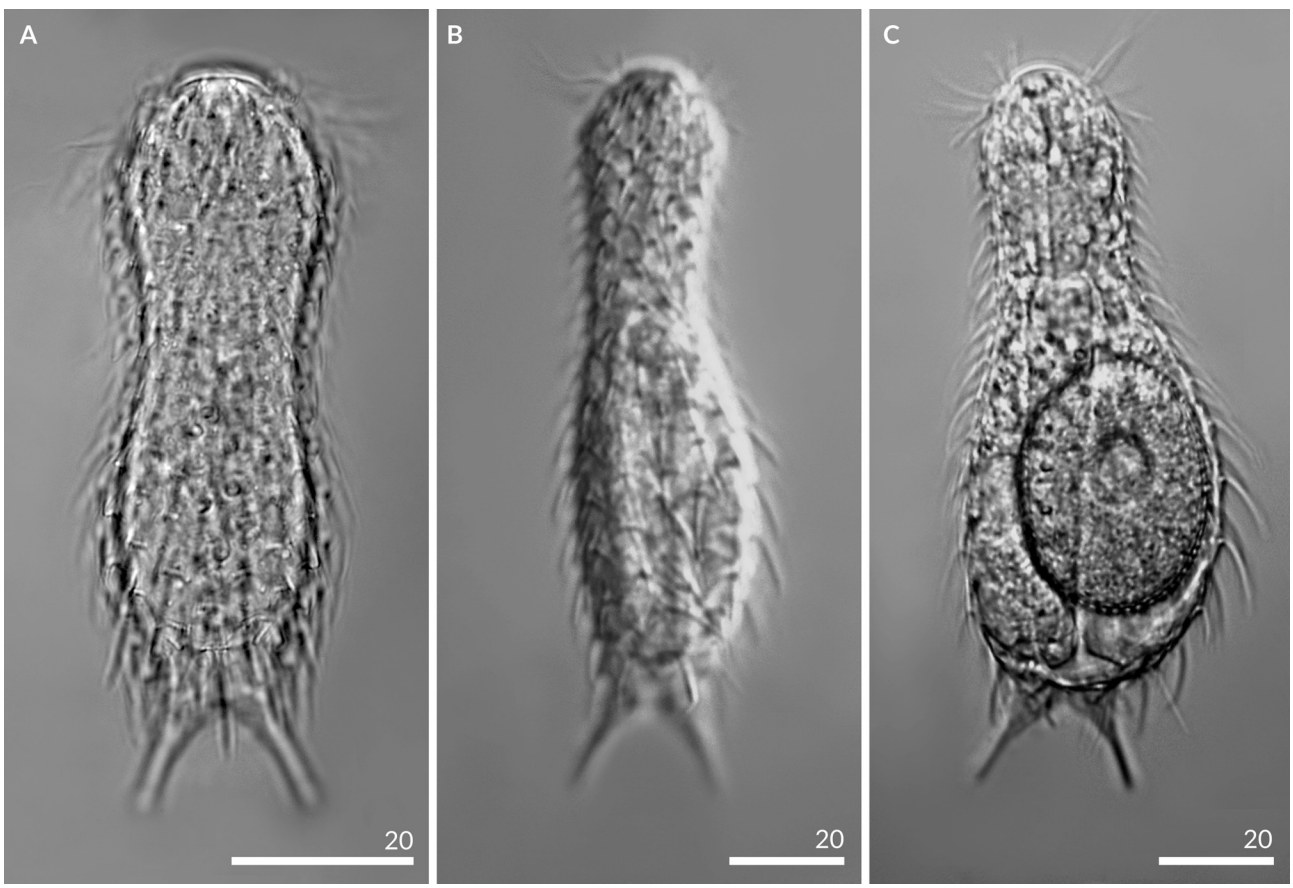


Figure 35. *Chaetonotus* (*Hystricochaetonotus*) *hornsundi* sp. nov. A – Juvenile specimen. Habitus. B – Adult specimen. Habitus. C – Specimen with mature egg. Habitus.

size of the hypopleurae, which are large (U7–U13) (Figs 32, 35–38). On the dorsal head surface, the lateral edges of the cephalion and epipleurar do not meet, and there is a prominent space between them. Deep notches are present between the cephalion and epipleurae, and shallow notches are present between the epipleurae and hypopleurae. The hypostomium (U7–U10) is composed of three plates. The anterior plate is located centrally beneath the mouth ring and are rectangle, short, with a granular protuberance present in the centre of the anterior edge. Slightly beneath the anterior edges of the central plate, two transverse tear-shaped plates are situated. Each of these plates is larger than the central hypostomium plates and strongly but partially overlaps it (Figs 31C, 32B, 41C). Two pairs of cephalic ciliary tufts are present. The anterior tufts emerge dorsolaterally between the lateral cephalion edge and the anterior epipleurae edge. The anterior tufts (at U4) consist of 5 cilia. The anteriormost cilium in both anterior tufts is short. The second and fourth cilia are of similar lengths and are longer than the first cilium. The third cilium is the longest in the tuft. The posteriormost cilium is very short and is the shortest in the tuft. The posterior tufts (at U7) have 4 cilia each and emerge in ventral notches between the epipleurae and hypopleurae. The length of the cilia in the posterior tuft gradually increases from the anteriormost to the posteriormost cilium. The fourth, longest cilium in this tuft is shorter than the longest cilim in the anterior tuft. All cilia in both tufts are thick and almost straight. Ocellar granules are absent. The mouth ring is located subterminally at U4–U6 and has strong, granular cuticular reinforcements and short inner hairs. Inside the mouth ring, two small and weak cuticular teeth arising from the anterior pharynx region are present (Figs 32C, 41B, 41D). Suboral bristles are located in two tufts slightly beneath the lateral edges of the mouth ring.

The entire body, except for the one pair of scales on the dorsal surfaces of the furcal appendages, is covered with three-lobed scales that adhere to the cuticle along their entire surface. The scales have a strong, high, triangular keel and are triangular with a deeply notched posterior edge. The scales are

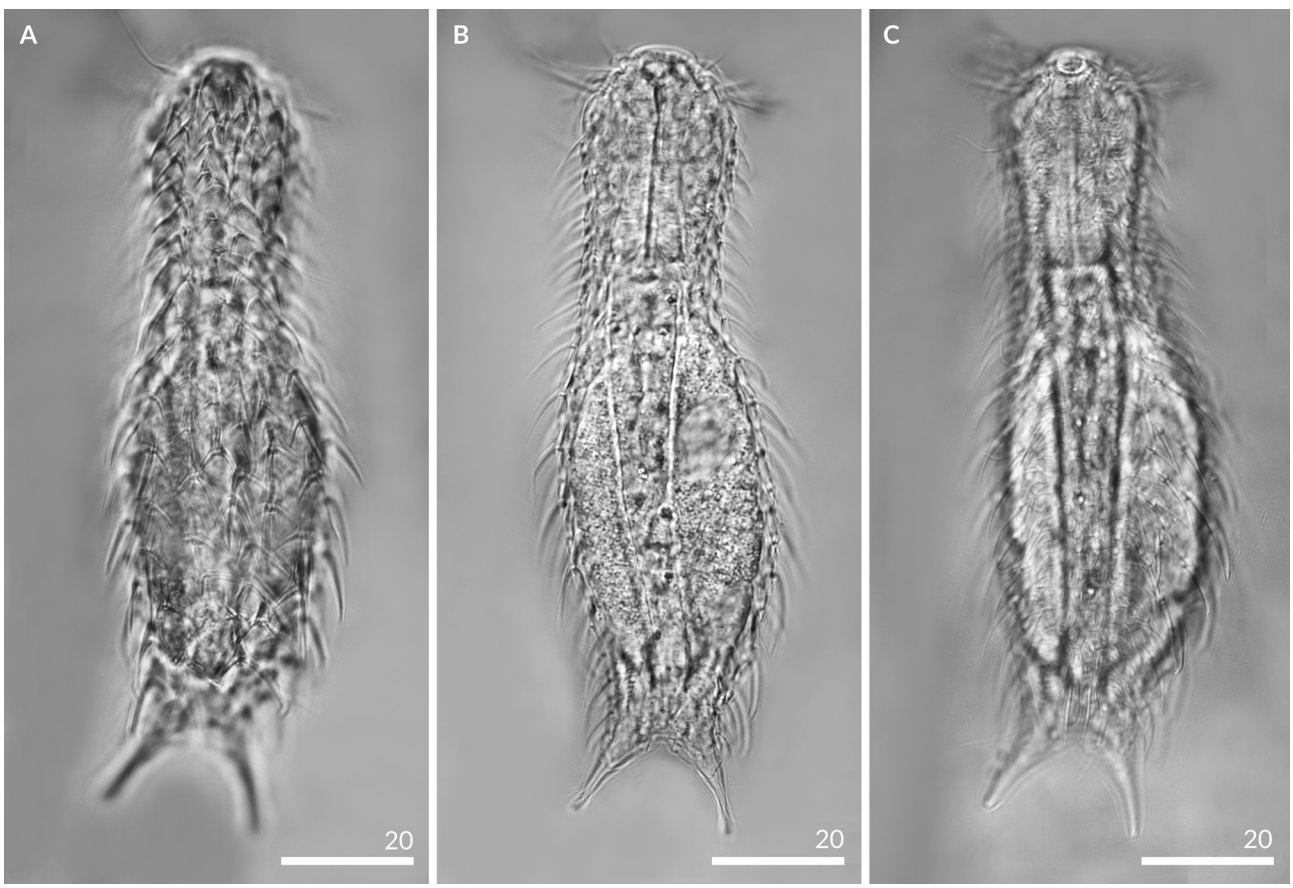


Figure 36. *Chaetonotus* (*Hystricochaetonotus*) *hornsundi* sp. nov. Adult specimen. A – Dorsal body view. B – View of internal morphology. C – Ventral body view.

distributed in 15–17 total longitudinal alternating rows (5–7D+2DL+2L+4LV+2V) with 13–15 scales in the central row. The longitudinal rows of scales from the head to the trunk posterior region run parallel to another (Fig. 32). The scales are located far from another but their posterolateral lobes slightly overlap adjacent scales. The longitudinal rows of scales begin on the head directly beneath the posterior edge of the cephalion, epipleurae and hypopleurae. The scales show strong morphological diversity between various parts of the body (Figs 32–33). The length of the central lobe, the roundness of the edges and the size of the scales differ. The scales of the head, located in the two anteriormost transverse rows situated near the posterior edge of the cephalion and pleurae, are narrow with long and narrow posterolateral lobes (scales 1; Figs 32A, 34A). The remaining scales of the head are wide and shaped like triangles. The central lobe of these scales is rounded, the posterolateral lobes are very narrow, sharp in shape, widely set apart and strongly directed diagonally (scales 2; Figs 32A, 33A). From the head to the posterior trunk region, the central lobe of the scales gradually lengthens and the size of the scales gradually increases (scales 2–13; Figs 32–33). Furthermore, the edges of the ventrolateral and ventral scales become gradually more rounded towards the ciliary bands (scales from 2 to 7, from 8 to 10, from 10 to 13; Figs 32-33). The posterolateral lobes are slightly shorter and wider as compared to the dorsal, dorsolateral and lateral scales. The size of the scales slightly and gradually decreases from the dorsal body sides towards the ciliary bands. Scales arranged in the ventral longitudinal row located closest to the ciliary bands are small, much smaller than the scales of the other rows (see Table 18), and their central lobe is oriented towards the bands at an angle of *ca*. 45° (Fig. 32C). The scales arranged in the second ventral longitudinal row from the ciliary bands have their central lobe oriented towards the bands at an angle of *ca*. 20°. One pair of three-lobed scales of a different type is located on the dorsal surface of the posterior trunk region (at U78–U79), slightly anteriorly to the double-keeled scales with dorsal sensory bristles. These scales have wider and shorter posterolateral lobes,

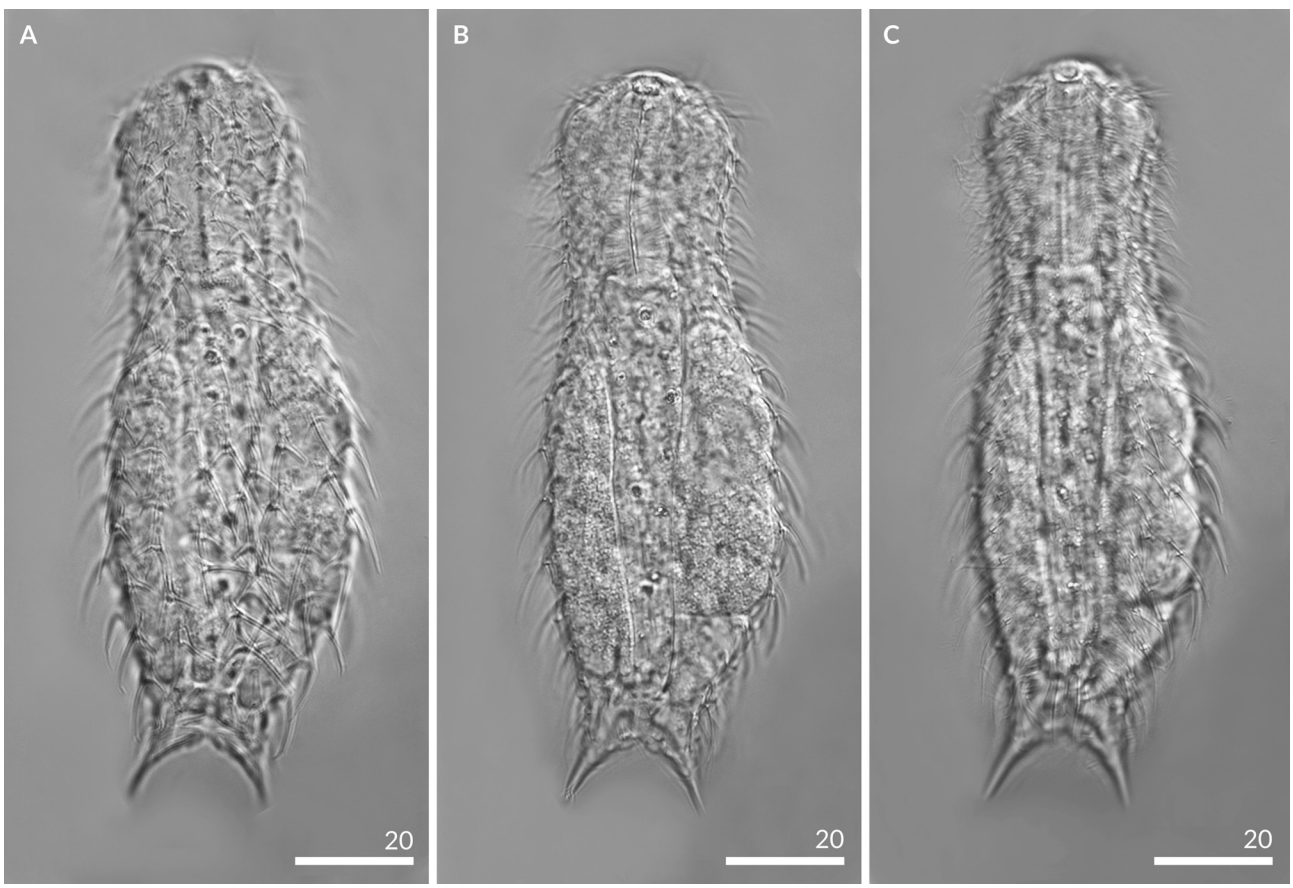


Figure 37. *Chaetonotus* (*Hystricochaetonotus*) *hornsundi* sp. nov. Juvenile specimen. A – Dorsal body view. B – View of internal morphology. C – Ventral body view.

a strong triangular keel lack a spine (scales 14; Figs 32A, 34B). One pair of small three-lobed scales shaped like triangles with all lobes strongly rounded and with a narrow, deep posterior notch is located diagonally on the dorsal surface of the furcal base (at U82–U83) (scales 16; Figs 32A, 34D). These scales have a long, thin keel and a short, simple spine. Two pairs of three-lobed, elongated and narrow scales are located on the dorsal surface of the furcal base and furcal appendages (at U82–U87). The anterior pair of these scales has long posterolateral lobes, a strong triangular keel and a short, strong spine with a lateral denticle (scales 17; Figs 32A, 34E). The posterior pair of these scales has an elongated central lobe and short posterio-lateral lobes, a straight keel and short, simple spines without a lateral denticle (scales 18; Figs 32A, 34F). These scales are diagonally arranged and directed towards the central body axis (Figs 32, 38, 39C). One pair of one-lobed, narrow scales is located on the dorsal surface of the furcal appendages. These scales are shaped like strongly elongated ovals with a slight protrusion at the posterior scales part, they have long keels running along their length and do not have a spine (scales 19; Figs 32A, 34G). They are located at U88, straight, arranged orthogonally to the central body axis. One pair of scales with two keels is located dorsolaterally at the furcal appendages (at U85–U88). These scales are hexagonal in shape and do not have any posterior notches or spines. Their keels are long and slightly bent (scales 20; Figs 32A, 34H, 39C). The edges of these scales overlap the neighbouring dorsolateral and lateral scales. One pair of scales with parafurcal spines of the same type as the trunk lateral scales is located at U85–U89. Three pairs of intrafurcal scales are present on the inner surface of the furcal appendages at *ca*. U89–U92 (scales 23; Figs 32, 34K). These scales are small, three-lobed, almost triangular, and have keels and short, rudimentary simple spines. The edges of these scales are curved, thus making the entire scale adhere to the narrow surface of the inner furcal indentation (scales 23; Figs 32, 34K).

Spines emerge from the posterior part of the scales. On the dorsal, dorsolateral, lateral and ventrolateral body surfaces the spines are strong, curved, have

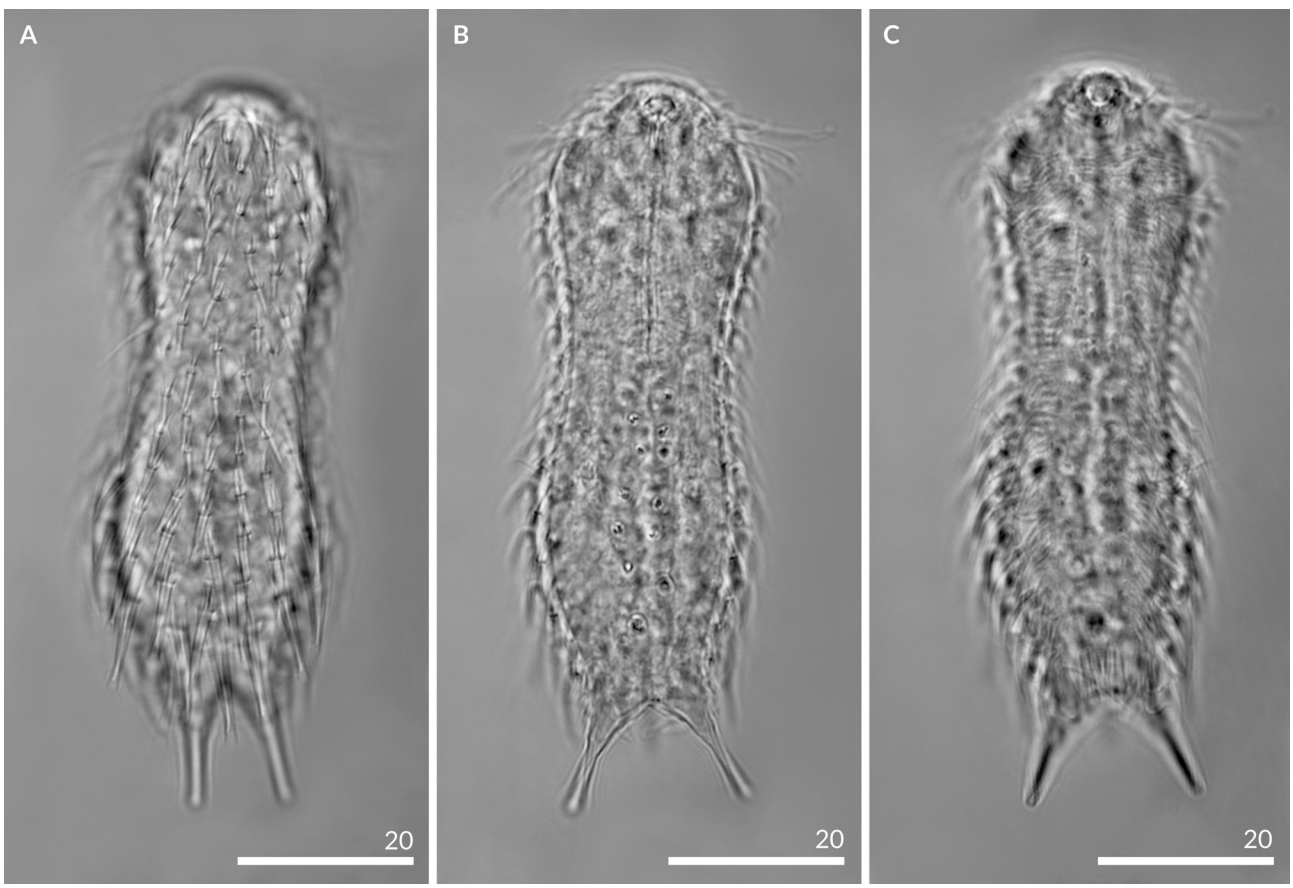


Figure 38. *Chaetonotus* (*Hystricochaetonotus*) *hornsundi* sp. nov. Specimen with maturing egg. A – Dorsal body view. B – View of internal morphology. C – Ventral body view.

a lateral denticle near their ends and gradually taper towards their pointed ends. The length and thickness of the spines gradually increase from the top of the head towards the anterior one-third of the trunk, on the posterior two-thirds of the trunk the spines are conspicuously longer and their length gradually increases to the furcal base (Figs 32, 35–40). The lateral denticles on the spines become more pronounced from the head towards the furcal base. All of the dorsolateral, lateral, ventrolateral and ventral spines, except for the posteriormost lateral pair, are shorter than the dorsal spines and their length gradually decreases towards the ventrolateral body surface. The posteriormost pair of lateral spines on the trunk are longer, stronger and more curved than the other body spines. The pair of parafurcal spines is strong and curved but shorter than

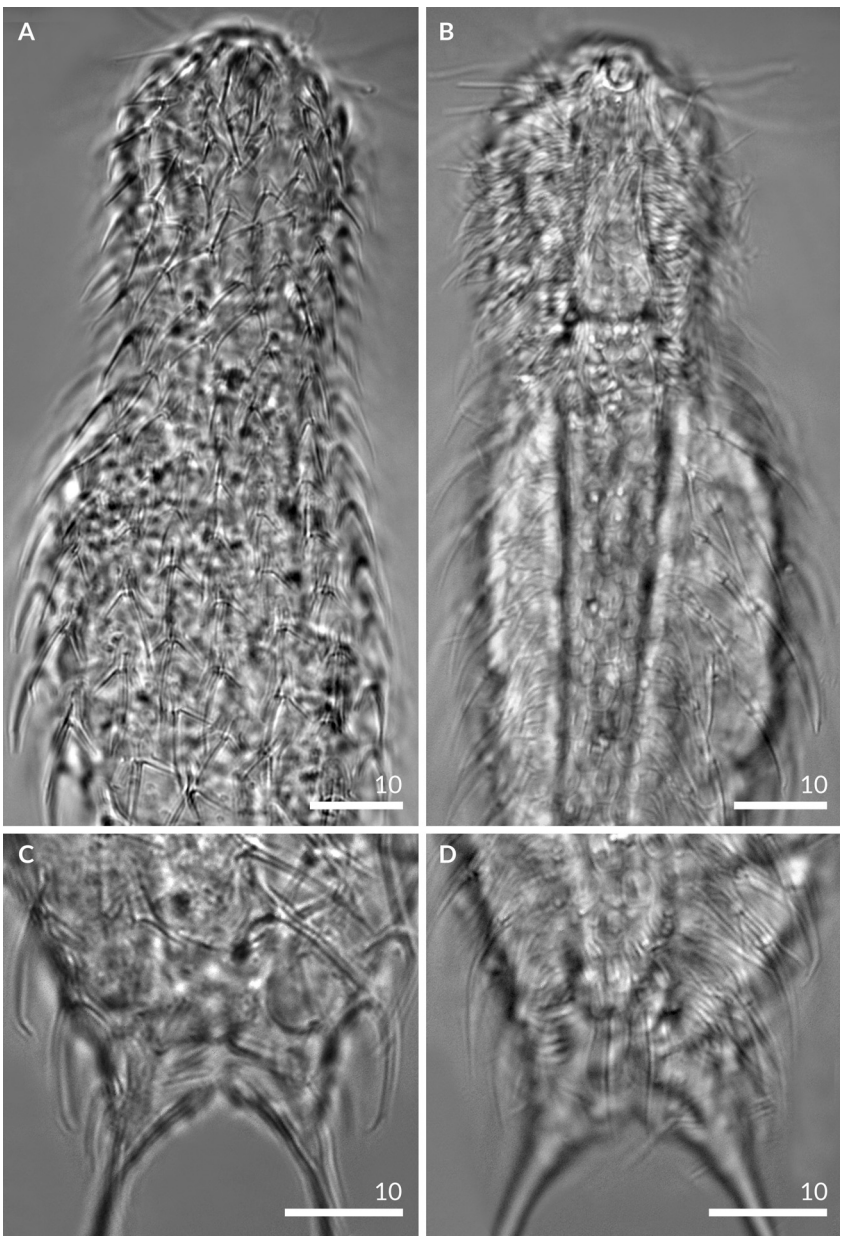


Figure 39. *Chaetonotus* (*Hystricochaetonotus*) *hornsundi* sp. nov. Adult specimen. A – Dorsal view of scales and spines. B – Ventral view of scales and spines. C – Posterior trunk region and furcal base dorsal view of scales and spines. D – Posterior trunk region and furcal base dorsal view of scales and spines.

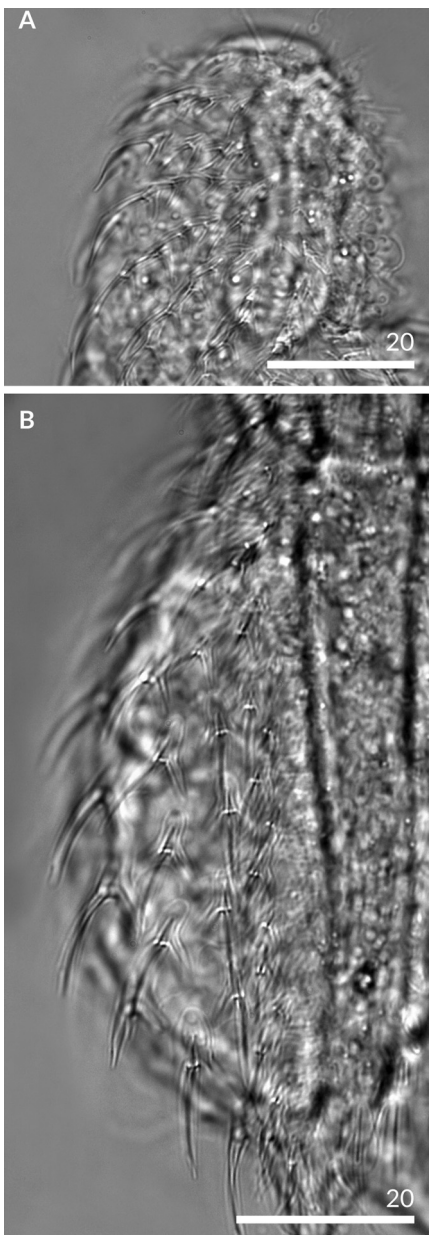


Figure 40. *Chaetonotus* (*Hystricochaetonotus*) *hornsundi* spec nov. Adult specimen. A – Lateral, ventrolateral and ventral view of head scales and spines. B – Lateral, ventrolateral and ventral view of neck and trunk scales and spines.

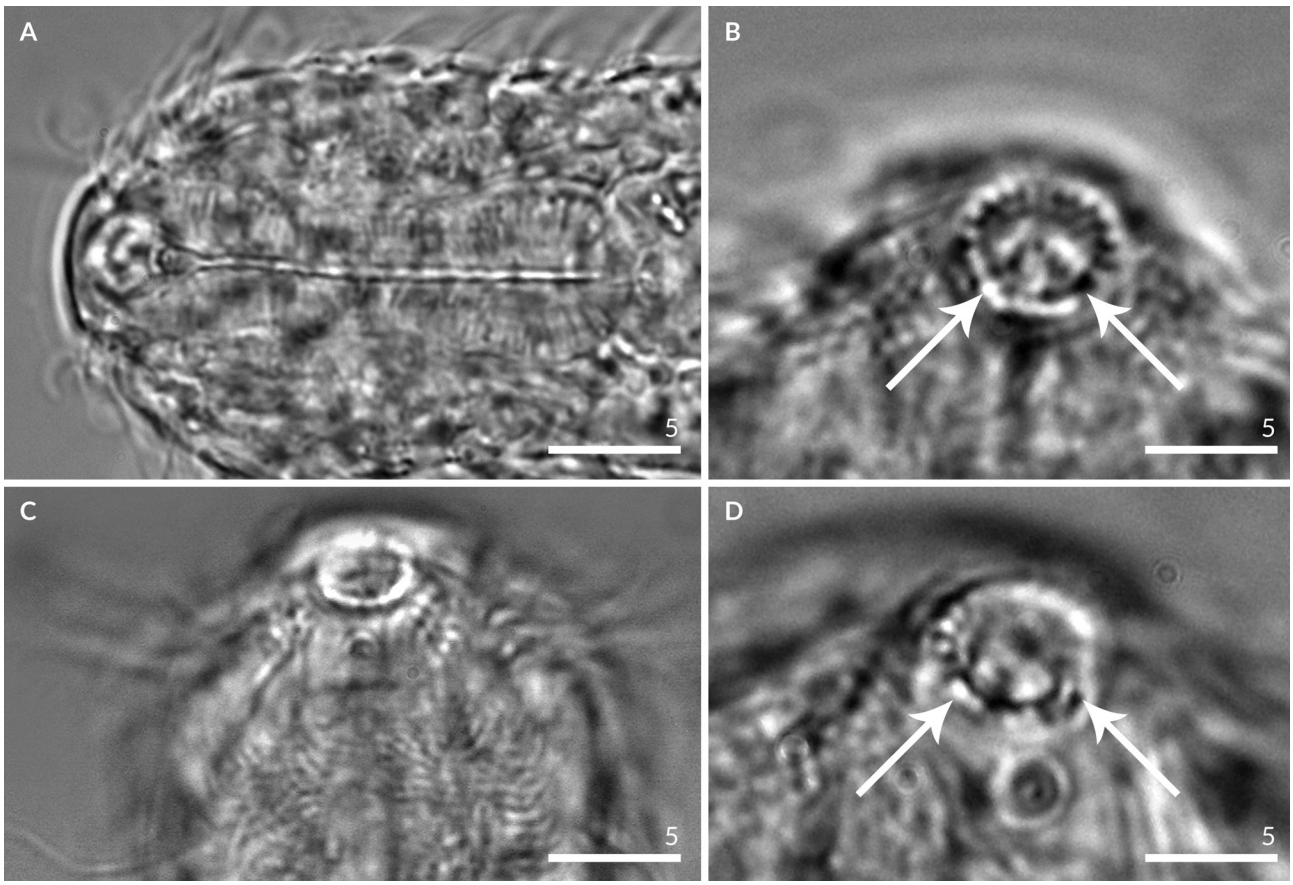


Figure 41. *Chaetonotus* (*Hystricochaetonotus*) *hornsundi* spec nov. Adult specimen. A – Detail of the pharynx. B, D – Detail of mouth ring with cuticular denticles. C – Detail of hypostomium.

the trunk spines. On the ventral body surface, spines emerging from scales arranged in two longitudinal rows located closest to the longitudinal bands are long, thin, hair-like and have narrow, delicate *lamellae* along their entire length. The *lamellae* are the widest here, *ca*. one-third of the length of the spine, and taper towards the end of the spines. These spines do not have a lateral denticle.

The species has three pairs of dorsal sensory bristles (Fig. 32). The first, anterior pair is located on the dorsal surface of the head at U5, near the dorsal epipleurae edge and emerge from small, spherical papillae. The second pair of sensory bristles is located on the dorsal surface of the neck at U26 and emerge from small, spherical papillae. The third, posterior pair of sensory bristles is located dorsally on the posterior trunk region and furcal base. The third pair of dorsal sensory bristles emerges from three-lobed scales with two strong keels situated at U79–U81. These scales' keels are connected in the centre of the scales. These scales are shaped like double, sharp triangles with long, narrow posterolateral lobes set very wide apart. These scales have a double, deep posterior notch (scales 15; Figs 32A, 34C).

On the ventral surface, the longitudinal ciliary bands begin at U11 and run back to U85 (Figs 32C, 36C, 37C, 38C, 40A). The ciliary bands are wider on the head than on the other parts of the body. The ventral interciliary field is covered with scales from beneath the end of the anterior pharynx dilatation (Figs 32C, 36C, 37C, 38C, 39B, 39D). Scales on the ventral interciliary field are one-lobed, plate-like and have a short keel. They are strongly differentiated in shape and size and are distributed in 5–7 total longitudinal rows with 17–20 scales in the central row (scales 24–29; Figs 32C, 34L–Q). On the anterior body the scales have a strongly rounded hexagonal shape and are located far from another (scales 24; Figs 32C, 34L). Posteriorly the scales become larger and their shape is less rounded (scales 25–29; Figs 34C, 36M–Q). Towards the posterior the keels on the scales become more distinct and the distance between the scales decreases so that the scales' edges start as adjacent to each other and soon start to overlap (Figs 32C, 36C, 38C, 39D). The scales

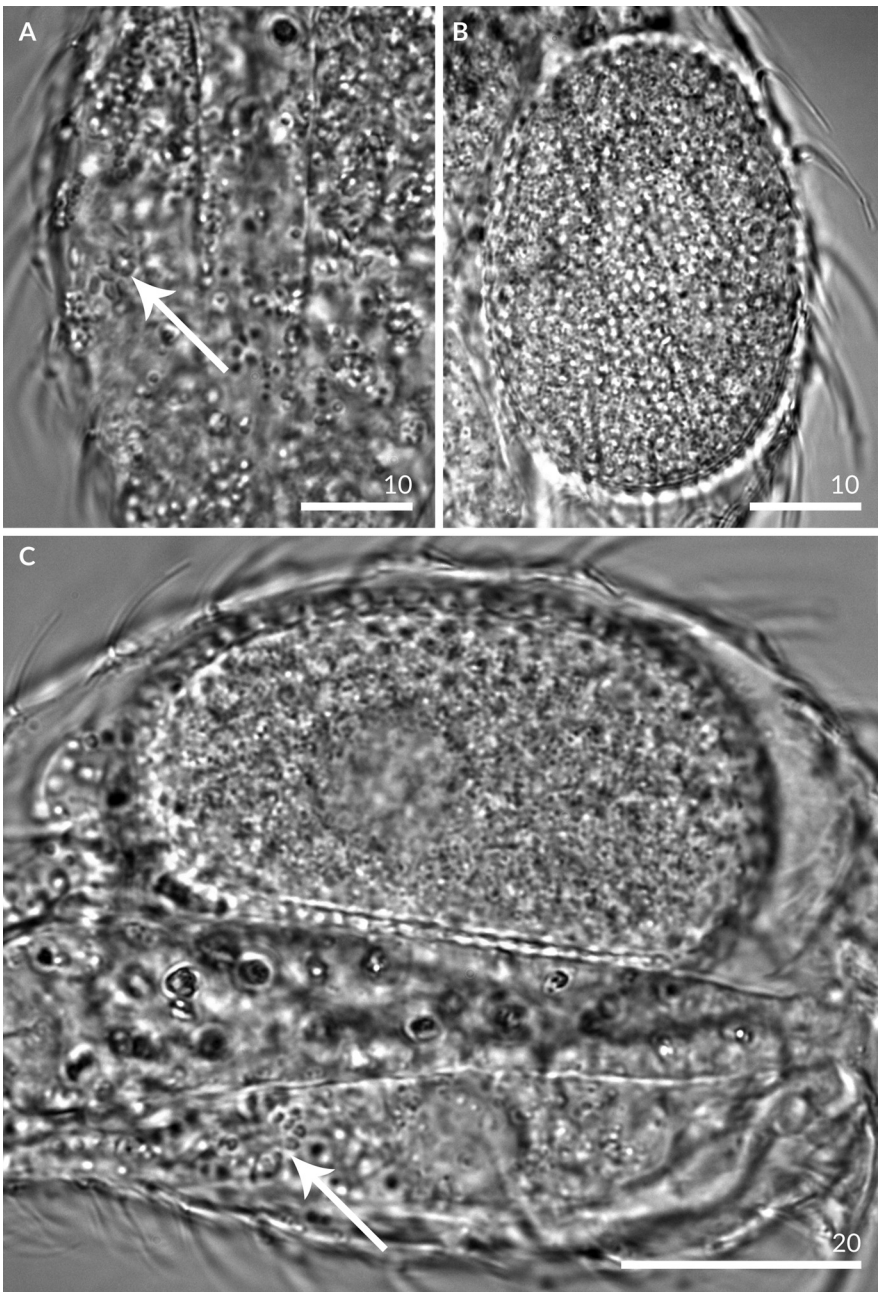


Figure 42. *Chaetonotus* (*Hystricochaetonotus*) *hornsundi* spec nov. Detail of the internal morfology. A – Sperm packet. B – Matured egg with horion. C – Matured egg and sperm packet.

located closest to the ventral longitudinal ciliary bands are smaller than the scales located in the centre of the ventral field (Fig. 32C). Four pairs of ventral interciliary field terminal scales are present. Each scale of the first pair (at U82–U87) is oval, expands toward the posterior end, has a long, strong keel and a long, straight spine (scales 30; Figs 32C, 34R). The second pair (at U87–U89) is elongated, shaped like rounded triangles and has straight, long spines extending towards the internal furcal indentation (scales 31; Figs 32C, 34S). The third and fourth pairs (at U89–U90 and U90–U91, respectively) are small, shaped like rounded triangles with weak keels and without spines. The second, third and fourth pairs of ventral interciliary field terminal scales are situated at the furcal appendages (scales 32; Figs 32C, 34T).

Table 17. Main morphometric characters of *Chaetonotus* (*Hystricochaetonotus*) *hornsundi* sp. nov; N – number of specimens or structures analysed, Range – the smallest and the largest structure found among all specimens measured. All measurements are given in micrometers (μm); all indicators are given as a percentage (%) and italicized.

Characters	N	Holotype	Range of adult paratypes
Body length	23	130.28	116.98–138.22
Furca length	15	25.79	21.28–26.86
Adhesive tube length	15	13.51	11.18–14.01
Number of scales in central longitudinal row	15	15	13–15
Total number of longitudinal alternating rows of scales	15	15	15–17
Head scales length x width	15	1.85–5.64 x 2.07–6.47	1.53–7.04 x 1.71–8.08
Neck scales length x width	15	3.03–6.94 x 3.45–7.28	2.07–8.67 x 2.32–9.09
Trunk scales length x width	15	4.74–11.57 x 4.71–12.52	3.24–13.27 x 3.20–14.36
Head spines length	15	3.56–9.89	2.78–14.09
Localization of lateral denticle on head spines	15	*6.576–12.470*	*6.44–12.648*
Neck spines length	15	6.96–12.36	5.43–15.23
Localization of lateral denticle on neck spines	15	*12.500–15.778*	*12.184–15.892*
Trunk spines length	15	8.98–13.38	6.97–16.51
Localization of lateral denticle on trunk spines	15	*15.813–18.862*	*15.638–19.055*
Length of posteriormost trunk lateral spines	15	13.51	11.61–15.62
Localization of lateral denticle on posteriormost trunk lateral spines	15	*10.369*	*9.818–11.426*
Length of posteriormost parafurcal lateral spines	15	7.81	6.71–8.55
Localization of lateral denticle on posteriormost parafurcal lateral spines	15	*5.995*	*5.386–6.400*
Mouth ring diameter	15	4.96	3.74–5.28
Pharynx length	23	33.26	29.62–34.35

Table 18. Detailed morphometric of *Chaetonotus* (*Hystricochaetonotus*) *hornsundi* sp. nov; N – number of specimens or structures analysed, Range – the smallest and the largest structure found among all specimens measured, SD – standard deviation. All measurements are given in micrometres (μm); all indicators are given as a percentage (%) and italicized.

Characters	N	Holotyp	Range of adult paratypes	SD
Cephalion length	15	13.59	12.17–16.32	1.058
Cephalion width	15	11.51	10.31–12.66	0.605
Hypostomium anterior plate length	15	2.92	2.49–3.42	0.252
Hypostomium anterior plate width	15	5.37	4.86–6.22	0.378
Hypostomium posterior plate length	15	3.97	3.16–4.02	0.287
Hypostomium posterior plate width	15	6.78	5.86–7.04	0.317
Length of cephalic cilia (anterior tuft)	15	3.89–22.19	(3.10–4.68)–(19.81–27.56)	0.400; 1.756
Length of cephalic cilia (posterior tuft)	15	6.32–19.13	(3.10–9.32)–(17.26–21.12)	1.279; 0.977
Width of cephalic cilia (anterior tuft)	15	0.95–0.98	(0.91–1.02)–(0.94–1.06)	0.029; 0.030
Width of cephalic cilia (posterior tuft)	15	1.00–1.02	(0.95–1.09)–(0.98–1.10)	0.033; 0.029
Width of adhesive tube	15	1.78	1.72–1.85	0.037
Ratio of scale distribution	23	*100.000*	*100.000–115.385*	7.466
Length of head dorsal scales (scales 1, 2 and 5)	15	4.98–5.64	(3.94–6.02)–(4.68–7.04)	0.619; 0.671
Width of head dorsal scales (scales 1, 2 and 5)	15	2.53–6.47	(2.00–3.06)–(5.37–8.08)	0.314; 0.769
Length of head dorsolateral scales (scales 2 and 5)	15	4.73–5.41	(3.74–5.72)–(4.49–6.75)	0.588; 0.643
Width of head dorsolateral scales (scales 2 and 5)	15	2.40–6.06	(1.90–2.90)–(5.03–7.56)	0.297; 0.720
Length of head lateral scales (scales 2 and 5)	15	3.67–4.76	(2.90–4.44)–(3.95–5.95)	0.473; 0.566
Width of head lateral scales (scales 2 and 5)	15	4.03–5.51	(3.18–4.88)–(4.57–6.87)	0.502; 0.652
Length of head ventrolateral scales (scales 3 and 6)	15	3.14–4.55	(2.49–3.80)–(3.10–5.69)	0.382; 0.626
Width of head ventrolateral scales (scales 3 and 6)	15	3.36–5.08	(2.66–4.07)–(3.46–6.35)	0.409; 0.699
Length of head ventral scales (scales 4 and 7)	15	1.85–2.87	(1.53–2.42)–(1.96–4.09)	0.269; 0.566
Width of head ventral scales (scales 4 and 7)	15	2.07–3.19	(1.71–2.71)–(2.18–4.55)	0.300; 0.629
Length of neck dorsal scales (scales 8)	15	5.51–6.94	(4.57–6.88)–(5.76–8.67)	0.656; 0.828
Width of neck dorsal scales (scales 8)	15	6.67–7.28	(5.53–8.29)–(6.04–9.09)	0.786; 0.868
Length of neck dorsolateral scales (scales 8)	15	5.23–6.71	(4.34–6.53)–(5.57–8.38)	0.623; 0.800
Width of neck dorsolateral scales (scales 8)	15	6.27–6.92	(5.20–7.83)–(5.74–8.64)	0.748; 0.820

Table 18. Continued.

Characters	N	Holotyp	Range of adult paratypes	SD
Length of neck lateral scales (scales 8)	15	5.01–6.58	(4.16–6.26)–(5.47–8.22)	0.598; 0.784
Width of neck lateral scales (scales 8)	15	5.91–6.69	(4.91–7.38)–(5.55–8.36)	0.704; 0.799
Length of neck ventrolateral scales (scales 9)	15	4.82–6.31	(4.00–6.03)–(5.24–7.88)	0.575; 0.752
Width of neck ventrolateral scales (scales 9)	15	5.58–6.34	(4.63–6.98)–(5.26–7.92)	0.666; 0.755
Length of neck ventral scales (scales 10)	15	3.03–4.52	(2.07–4.32)–(3.09–6.44)	0.595; 0.885
Width of neck ventral scales (scales 10)	15	3.45–4.50	(2.32–4.92)–(3.07–6.41)	0.682; 0.882
Length of trunk dorsal scales (scales 11)	15	7.19–11.57	(5.97–8.96)–(9.61–13.27)	0.857; 1.189
Width of trunk dorsal scales (scales 11)	15	7.41–12.52	(6.15–9.23)–(10.40–14.36)	0.882; 1.285
Length of trunk dorsolateral scales (scales 11)	15	6.94–9.87	(5.76–8.65)–(8.20–11.32)	0.825; 1.017
Width of trunk dorsolateral scales (scales 11)	15	7.09–10.44	(5.88–8.84)–(8.67–11.97)	0.845; 1.075
Length of trunk lateral scales (scales 11)	15	6.72–9.63	(5.58–8.38)–(8.00–11.04)	0.800; 0.989
Width of trunk lateral scales (scales 11)	15	6.80–9.97	(5.65–8.48)–(8.28–11.43)	0.810; 1.023
Length of trunk ventrolateral scales (scales 12)	15	6.51–9.18	(5.41–8.12)–(7.63–10.52)	0.779; 0.944
Width of trunk ventrolateral scales (scales 12)	15	6.53–9.34	(5.43–8.14)–(7.76–10.71)	0.775; 0.964
Length of trunk ventral scales (scales 13)	15	4.74–6.17	(3.24–6.58)–(4.22–8.60)	0.872; 1.043
Width of trunk ventral scales (scales 13)	15	4.71–6.21	(3.20–6.54)–(4.24–8.65)	0.869; 1.050
Length of posterior trunk spineless scales (scales 14)	15	6.83	5.53–8.70	0.946
Width of posterior trunk spineless scales (scales 14)	15	6.06	3.87–7.62	0.967
Length of rounded triangle-shaped scales on the furcal base (scales 16)	15	3.87	3.27–6.64	1.187
Width of rounded triangle-shaped scales on the furcal base (scales 16)	15	1.96	1.76–3.65	0.661
Length of lateral scales with parafurcal spines (scales 22)	15	9.24	7.93–10.96	0.977
Width of furcal lateral scales with parafurcal spines (scales 22)	15	9.56	8.20–11.34	1.013
Length of posteriormost trunk lateral scales (scales 21)	15	6.05	5.19–7.18	0.619
Width of posteriormost trunk last pair of lateral scales (scales 21)	15	6.23	5.31–7.39	0.641
Length of anterior pair of furcal base and furcal appendages dorsal elongated three-lobed scales (scales 17)	15	9.04	6.68–10.06	0.918
Width of of anterior pair furcal base and furcal appendages dorsal elongated three-lobed scales width (scales 17)	15	5.51	4.07–6.12	0.508
Length of posterior pair of furcal base and furcal appendages dorsal elongated three-lobed scales (scales 18)	15	7.16	5.98–8.33	0.746
Width of of posterior pair furcal base and furcal appendages dorsal elongated three-lobed scales width (scales 18)	15	3.64	2.74–4.25	0.423
Length of furcal appendages dorsal one-lobed scales length (scales 19)	15	6.89	5.35–8.14	0.787
Width of furcal appendages dorsal one-lobed scales width (scales 19)	15	1.97	1.58–3.12	0.374
Length of double-keeled scales on the furcal appendages (scales 20)	15	6.08	4.43–7.51	0.776
Width of double keeled scales on the furcal appendages (scales 20)	15	5.24	3.82–6.46	0.648
Length of intrafurcal scales (scales 23)	15	2.52–2.68	(1.94–3.09)–(2.13–3.37)	0.276; 0.287
Width of intrafurcal scales (scales 23)	15	1.92–2.05	(1.17–2.32)–(1.38–2.54)	0.276; 0.274
Length of head dorsal spines (scales 1, 2 and 5)	15	4.62–8.34	(3.61–5.07)–(6.51–9.12)	0.406; 0.822
Head dorsal spines length between lateral denticle and spine base (scales 1, 2 and 5)	15	0.32–1.04	(0.25–0.35)–(0.81–1.14)	0.028; 0.102
Length of head dorsolateral spines (scales 2 and 5)	15	4.41–7.51	(3.45–7.48)–(5.86–8.21)	0.389; 0.740
Head dorsolateral spines length between lateral denticle and spine base (scales 2 and 5)	15	0.29–0.90	(0.23–0.32)–(0.70–0.98)	0.023; 0.090
Length of head lateral spines (scales 2 and 5)	15	3.85–7.28	(3.01–4.24)–(5.68–7.96)	0.341; 0.718
Head lateral spines length between lateral denticle and spine base (scales 2 and 5)	15	0.43–0.87	(0.34–0.47)–(0.68–0.95)	0.038; 0.085
Length of head ventrolateral spines (scales 3 and 6)	15	3.56–6.79	(2.78–3.92)–(5.31–7.42)	0.317; 0.664
Head ventrolateral spines length between lateral denticle and spine base (scales 3 and 6)	15	0.39–0.81	(0.30–0.42)–(0.63–0.88)	0.033; 0.078

Table 18. Continued.

Characters	N	Holotyp	Range of adult paratypes	SD
Length of head ventral spines (scales 4 and 7)	15	8.61–9.89	(7.14–10.74)–(8.57–14.09)	0.909; 1.568
Lamellae width on the ventral head spines (scales 4 and 7)	15	0.68–0.94	(0.57–0.75)–(0.77–1.02)	0.055; 0.076
Length of neck dorsal spines (scales 8)	15	8.47–9.57	(6.61–9.23)–(7.47–10.63)	0.830; 0.957
Neck dorsal spines length between lateral denticle and spine base (scales 8)	15	1.07–1.51	(0.84–1.17)–(1.18–1.68)	0.103; 0.151
Length of neck dorsolateral spines (scales 8)	15	7.72–9.36	(6.03–8.41)–(7.31–10.40)	0.753; 0.936
Neck dorsolateral spines length between lateral denticle and spine base (scales 8)	15	0.97–1.47	(0.76–1.06)–(1.15–1.63)	0.095; 0.147
Length of neck lateral spines (scales 8)	15	7.42–8.91	(5.79–8.08)–(6.97–9.89)	0.721; 0.887
Neck lateral spines length between lateral denticle and spine base (scales 8)	15	0.93–1.39	(0.72–1.02)–(1.08–1.54)	0.091; 0.139
Length of neck ventrolateral spines (scales 9)	15	6.96–8.74	(5.43–7.58)–(6.84–9.70)	0.676; 0.870
Neck ventrolateral spines length between lateral denticle and spine base (scales 9)	15	0.87–1.36	(0.68–0.95)–(1.06–1.50)	0.084; 0.136
Length of neck ventral spines (scales 10)	15	10.04–12.36	(8.69–14.21)–(10.63–15.23)	1.569; 1.409
Lamellae width on the ventral neck spines (scales 10)	15	1.02–1.16	(0.80–1.06)–(0.92–1.26)	0.083; 0.110
Length of trunk dorsal spines (scales 11)	15	10.14–13.36	(7.88–11.23)–(10.45–15.09)	0.972; 1.256
Trunk dorsal spines length between lateral denticle and spine base (scales 11)	15	1.63–2.52	(1.27–1.81)–(1.97–2.85)	0.159; 0.238
Length of trunk dorsolateral spines (scales 11)	15	9.51–12.20	(7.39–10.53)–(9.54–13.78)	0.911; 1.149
Trunk dorsolateral spines length between lateral denticle and spine base (scales 11)	15	1.51–2.30	(1.17–1.67)–(1.80–2.60)	0.145; 0.217
Length of trunk lateral spines (scales 11)	15	9.15–11.93	(7.11–10.13)–(9.33–13.48)	0.877; 1.123
Trunk lateral spines length between lateral denticle and spine base (scales 11)	15	1.45–2.24	(1.13–1.61)–(1.75–2.53)	0.139; 0.215
Length of trunk ventrolateral spines (scales 12)	15	8.98–10.69	(6.97–9.94)–(8.36–12.09)	0.861; 1.011
Trunk ventrolateral spines length between lateral denticle and spine base (scales 12)	15	1.42–2.00	(1.09–1.57)–(1.56–2.26)	0.137; 0.190
Length of trunk ventral spines (scales 13)	15	12.54–13.38	(10.77–15.34)–(11.78–16.51)	1.400; 1.594
Lamellae width on the ventral trunk spines (scales 13)	15	1.20–1.41	(0.95–1.28)–(1.16–1.55)	0.109; 0.116
Length of rounded triangle-shaped scales of furcal base spines (scales 16)	15	0.62	0.61–1.63	0.295
Length of furcal base and furcal appendages anterior dorsal elongated three-lobed spines (scales 17)	15	1.65	1.36–2.16	0.262
Furcal base and furcal appendages dorsal elongated three-lobed spines length between lateral denticle and spine base (scales 17)	15	0.56	0.46–0.73	0.089
Length of furcal appendages posterior dorsal elongated three-lobed spines (scales 18)	15	0.87	0.71–1.36	0.188
Length of trunk posteriormost pair of lateral spines (scales 21)	15	13.51	11.61–15.62	0.991
Trunk posteriormost pair of lateral spines length between lateral denticle and spine base (scales 21)	15	2.12	1.82–2.44	0.151
Length of posteriormost parafurcal lateral spines (scales 22)	15	7.81	6.71–8.55	0.537
Posteriormost parafurcal lateral spines length between lateral denticle and spine base (scales 22)	15	1.62	1.39–1.78	0.110
Length of intrafurcal scales spines (scales 23)	15	1.02–1.21	(0.62–1.16)–(0.81–1.42)	0.151; 0.181
Length ratio of posteriormost trunk lateral spines (scales 21)	15	*10.369*	*9.818–11.426*	0.473
Length ratio of posteriormost parafurcal lateral spines (scales 22)	15	*5.995*	*5.386–6.400*	0.276
Localization of lateral denticle on dorsal head spines (scales 1, 2 and 5)	15	*6.926–12.470*	(*6.840–7.294*)–(*12.415–12.648*)	0.106; 0.064
Localization of lateral denticle on dorsolateral head spines (scales 2 and 5)	15	*6.576–11.984*	*(6.444–7.046)*–(*11.902–12.039*)	0.143; 0.044
Localization of lateral denticle on lateral head spines (scales 2 and 5)	15	*11.169–11.951*	(*10.986–11.296*)–(*11.878–12.007*)	0.086; 0.040
Localization of lateral denticle on ventrolateral head spines (scales 3 and 6)	15	*10.955–11.929*	(*10.632–11.074*)–(*11.712–11.994*)	0.131; 0.078

Table 18. Continued.

Characters	N	Holotyp	Range of adult paratypes	SD
Localization of lateral denticle on dorsal neck spines (scales 8)	15	*12.633–15.778*	(*12.576–12.761*)–(*15.734–15.892*)	0.056; 0.036
Localization of lateral denticle on dorsolateral neck spines (scales 8)	15	*12.565–15.705*	(*12.500–12.604*)–(*15.641–15.758*)	0.038; 0.031
Localization of lateral denticle on lateral neck spines (scales 8)	15	*12.534–15.600*	(*12.371–12.687*)–(*15.495–15.664*)	0.079–0.046
Localization of lateral denticle on ventrolateral head spines (scales 9)	15	*12.500–15.561*	(*12.184–15.464*)–(*15.460–15.584*)	0.786; 0.041
Localization of lateral denticle on dorsal trunk spines (scales 11)	15	*16.075–18.862*	(*15.721–16.122*)–(*18.820–19.055*)	0.099; 0.054
Localization of lateral denticle on dorsolateral trunk spines (scales 11)	15	*15.878–18.852*	(*15.832–15.961*)–(*18.807–18.898*)	0.035; 0.026
Localization of lateral denticle on lateral trunk spines (scales 11)	15	*15.847–18.776*	(*15.782–15.983*)–(*18.407–18.807*)	0.052–0.097
Localization of lateral denticle on ventrolateral trunk spines (scales 12)	15	*15.813–18.709*	(*15.638–15.813*)–(*17.702–18.785*)	0.054; 0.257
Localization of lateral denticle on anterior pair of furcal base and furcal appendages dorsal elongated three-lobed scales (scales 17)	15	*33.939*	*31.671–34.615*	0.693
Localization of lateral denticle on posteriormost trunk lateral spines (scales 21)	15	*15.692*	*15.621–16.333*	0.172
Localization of lateral denticle on posteriormost parafurcal lateral spines (scales 22)	15	*20.743*	*20.559–21.490*	0.213
Head dorsal sensory bristles length	15	14.62	13.56–17.01	1.164
Neck dorsal sensory bristles length	15	15.74	14.99–18.17	1.164
Posterior dorsal sensory bristles length	15	19	18.35–27.10	2.235
Number of scales in central longitudinal row on ventral interciliary field	23	20	17–20	0.626
Total number of longitudinal alternating rows of scales on ventral interciliary field	23	5	5–7	0.417
Length of ventral interciliary field scales (scales 24–29)	15	1.27–4.90	(1.04–1.36)–(3.73–6.07)	0.083; 0.640
Width of ventral interciliary field scales (scales 24–29)	15	1.23–3.46	(1.01–1.33)–(2.61–4.33)	0.080; 0.396
Length of terminal ventral interciliary field scales I pair (scales 30)	15	9.55	8.56–10.38	0.725
Width of terminal ventral interciliary field scales I pair (scales 30)	15	3.43	3.06–3.92	0.254
Length of terminal ventral interciliary field scales II pair (scales 31)	15	5.11	4.18–5.24	0.385
Width of terminal ventral interciliary field scales II pair (scales 31)	15	2.10	1.72–2.28	0.197
Length of terminal ventral interciliary field scales III pair (scales 32)	15	2.52	2.03–2.77	0.177
Width of terminal ventral interciliary field scales III pair (scales 32)	15	1.43	1.13–1.47	0.121
Length of terminal ventral interciliary field scales IV pair (scales 32)	15	2.48	1.97–2.72	0.178
Width of terminal ventral interciliary field scales IV pair (scales 32)	15	1.39	1.11–1.45	0.123
Length of terminal ventral interciliary field scales I pair spines (scales 30)	15	10.1	9.24–10.96	0.574
Length of terminal ventral interciliary field scales II pair spines (scales 31)	15	7.24	6.648.76	0.717
Width of anterior pharynx thickening (a)	15	10.67	9.41–10.97	0.499
Width of pharynx narrowing (n)	15	6.97	6.12–7.20	0.365
Width of middle pharynx (m)	15	7.42	6.64–8.04	0.402
Width of posterior pharynx thickening (p)	15	11.35	10.49–12.01	0.419
Width of pharyngeal-intestinal junction	15	3.73	3.31–3.90	0.175
Length of central intestine enzymatic section	15	2.05	1.91–2.90	0.306
Length of intestine anterior differ section	15	3.64	2.55–4.97	0.568
Intestine length	23	70.50	59.52–76.22	4.744
Pharynx formula a	15	*32.081*	*28.658–33.645*	1.559
Pharynx formula n	15	*20.956*	*18.83–22.891*	1.276
Pharynx formula m	15	*22.309*	*19.854–24.689*	1.419
Pharynx formula p	15	*34.125*	*32.544–37.225*	1.432
Pharynx-intestine ratio I	23	*47.177*	*44.135–54.570*	2.814
Length of matured egg	4	–	43.59–55.11	5.643
Width of matured egg	4	–	29.95–40.39	4.809

The pharynx (from U5 to U31) has marked anterior and no distinct posterior dilatations. The anterior dilatation has weak reinforcements composed of two short, thin and straight cuticular rods connected to each other at right angles (at U6–U7) (Fig. 41A). The pharynx is connected through the pharyngeal-intestinal junction to the straight intestine (running from U31 to U83). The pharyngeal-intestinal junction is clearly demarcated, short and narrow (U31–U32). The intestine has a short separate anterior section running from U31 to U32 (Figs 32B, 36B). The X-organ of this species (observed in one specimen) is located at U82–U83 near the terminal part of the intestine. It is small, bilobed, built from two extensions enveloped in a thin coat and connected by a thinner band located behind the intestine at the ventral surface. The extensions and bridge have a granular, grain-like structure. The sperm packets of this species are large, circular in shape and contain spermatozoids (per *ca.* 12–16 in one packet) in the form of a small spindle. They are located at *ca.* U62–U65 on both sides, near the intestine (Fig. 42A) .

Remarks. Total body length of juvenile and subadult specimens of *Chaetonotus* (*Hystricochaetonotus*) *hornsundi* sp. nov. varied from 84.2 to 110.1 μm; length of pharynx was between 29.6 to 35.2 μm; of the intestine from 37.4 to 55.4 μm; there were 13–17 total longitudinal rows of scales (5–7D+2DL+2L+2–4LV+2V) with 13–15 scales in the central row.

For a discussion on the phylogenetic affinities of *C.* (*H.*) *hornsundi* sp. nov., see the 'Remarks' section for *C.* (*H.*) *borealis* sp. nov.

Of the 23 adults, 8 individuals had a large, developing egg, 1 had an X-organ, sperm packet and a developing egg (Fig. 42C), 1 had two packages of sperm, and 3 had formations inside the integuments (probably some kind of microsymbiont structures).

Differential diagnosis. *Chaetonotus* (*Hystricochaetonotus*) *hornsundi* sp. nov. more closely resembles the other two new species from the Hornsund fjord area as described in this paper, namely *C.* (*H.*) *borealis* sp. nov. and *C.* (*H.*) *persimilis* sp. nov., as well as *C.* (*H.*) *hystrix* Mečnikow, 1865, *C.* (*H.*) *euhystrix* Schwank, 1990, *C.* (*H.*) *macrochaetus* Zelinka, 1889, *C.* (*H.*) *persetosus* Zelinka, 1889 and *Chaetonotus* (*Chaetonotus*) *benacensis* Balsamo *et* Fregni, 1995. The differential diagnosis for all new *Hystricochaetonotus* representatives is given in Table 21, below the *C.* (*H.*) *persimilis* sp. nov. description. See also 'Differential diagnosis' for *C.* (*H.*) *borealis* sp. nov.

***Chaetonotus* (*Hystricochaetonotus*) *persimilis* sp. nov.
(Figs 43–52; Tables 19–21)

Localities: 3, 4, 6, 12.

Material. Four samples, in total 22 specimens (15 adults, 2 subadults and 5 juveniles), all photographed. The photomicrographs are available in the Natural History Collections at Adam Mickiewicz University in Poznań under accession numbers NHC–GCHP––17–1–10/h (holotype) and NHC–GCHP–17–11–50/p (paratypes) and in the author's collection.

Etymology. From the Latin *persimilis*, "very similar", referring to similarities between species within the *Chaetonotus* (*Hystricochaetonotus*) *hystrix* group.

Diagnosis. Stocky body, measuring from 93.3 to 135.7 μm. Head five-lobed, cephalion narrow, epipleurae smaller than large hypopleurae. All cephalic plates demarcated in head outline. Hypostomium narrow and short, rounded, rectangular in shape. Ocellar granules absent. In mouth ring, two weak cuticular teeth arising from anterior pharynx region. Scales, except for one pair on the furcal appendages and scales on the ventral interciliary field, large, three-lobed with strong keel and spine. One pair of three-lobed, keeled scales without spines on dorsal posterior trunk region. One pair of small three-lobed scales, with posterior edges rotate toward each other located dorsally on furcal base. Three pairs of elongated, three-lobed, keeled and spined scales on dorsal surface of furcal base and furcal appendages. One pair of double-keeled, ovoid scales dorsolaterally on furcal appendages. Scales distributed in 15–17 total longitudinal rows (5–7D+2DL+2L+ +4LV+2V) with 12–13 scales in the central row. Scales in areas of head, neck and trunk differ morphologically. Spines on dorsal, dorsolateral, lateral and ventrolateral body surfaces strong and slightly curved, tapering towards end, with a lateral denticle. Spines on ventral body surface thin and hair-like, with narrow, delicate *lamellae* along entire length and without a lateral denticle. Length of spines gradually increases from head towards furcal base and decreases from dorsal, dorsolateral, lateral towards ventrolateral body surface. Ventral, hair-like spines longer than ventrolateral spines. Two pairs of posteriormost trunk lateral spines are the longest, strongest and most curved. Furcal appendages with one pair of scales with short, strong parafurcal spine with lateral denticle. Inside the furcal indentation, three pairs of small intrafurcal scales with keels and rudimetary spines. Ventral interciliary field from end of anterior pharynx dilatation to posterior body end covered with strongly differentiated scales; on trunk region, scales' edges meet but do not overlap. Three pairs of ventral interciliary field terminal scales. Pharynx with pronounced anterior and posterior dilatations. Two thin cuticular rods connected together in the centre inside anterior pharynx dilatation. Intestine straight with anterior section as a short band differing in form of morphology

Description. *Chaetonotus* (*Hystricochaetonotus*) *persimilis* sp. nov. has a stocky body (Figs 47, 50–53). The head is wide and wider than the neck, and

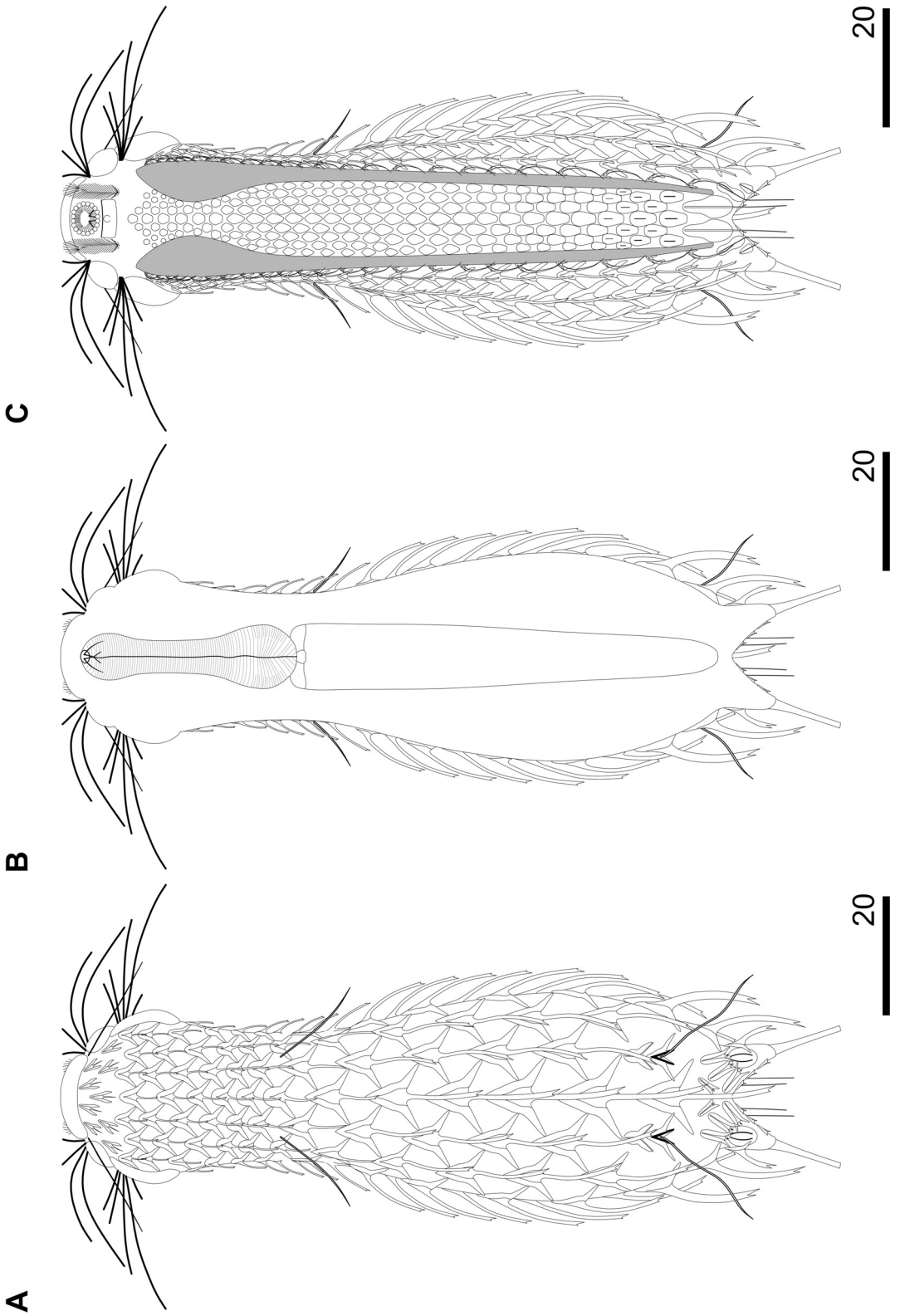


Figure 43. *Chaetonotus* (*Hystricochaetonotus*) *persimilis* sp. nov. Schematic drawings. A – Dorsal body view. B – View of internal morphology. C – Ventral body view. Light grey areas indicate the areas of the insertion of ciliary bands.

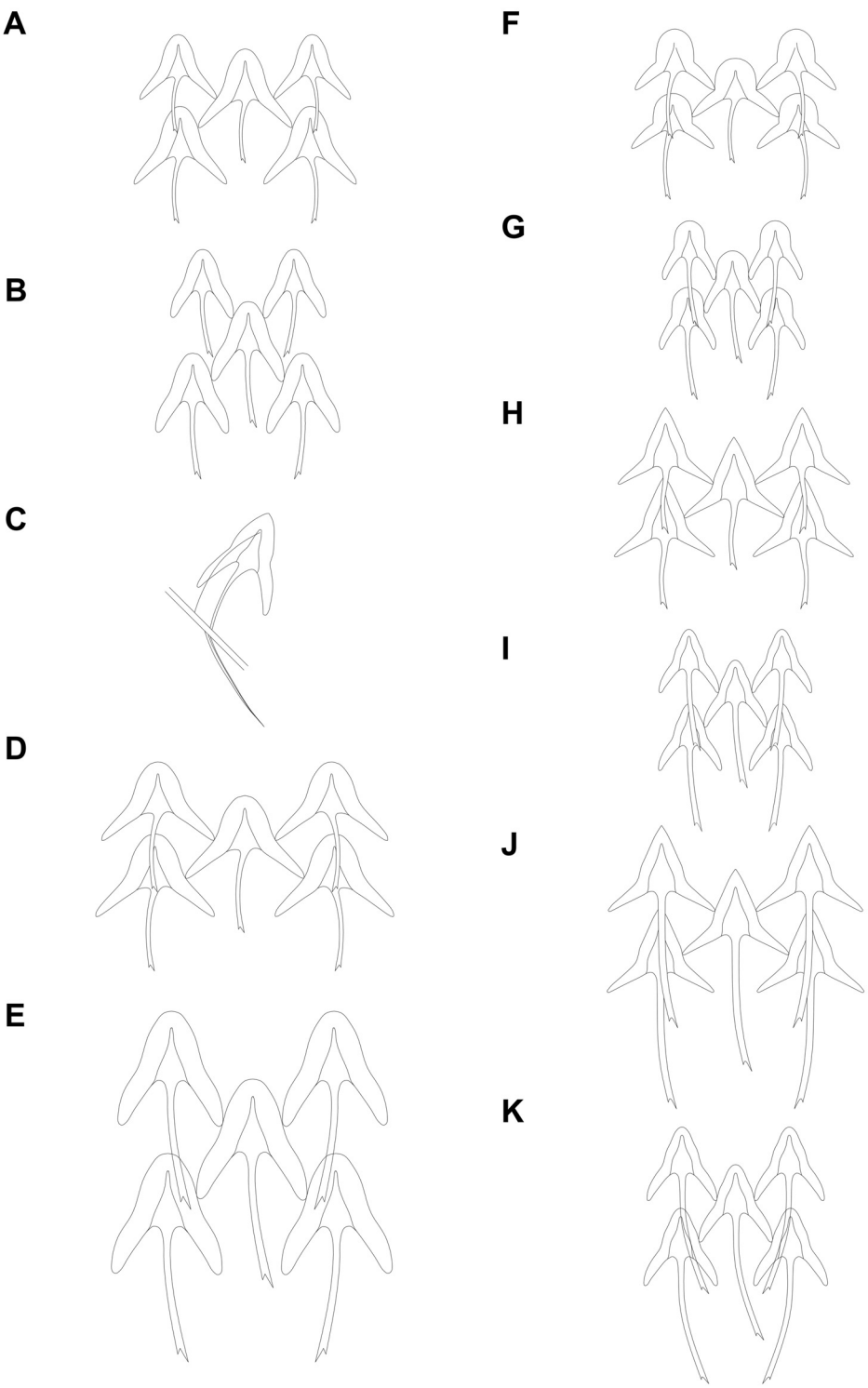


Figure 44. *Chaetonotus* (*Hystricochaetonotus*) *persimilis* sp. nov. Schematic scales drawings. A – Head anterior region dorsal, dorsolateral and lateral scales (scales 2). B – Head anterior region ventrolateral scales (scales 3). C – Ventral scales (scales 4). D – Head posterior region dorsal, dorsolateral, lateral scales (scales 5). E – Head posterior region ventrolateral scales (scales 6). F – Neck dorsal, dorsolateral, lateral scales (scales 7). G – Neck ventrolateral scales (scales 8). H – Trunk dorsal, dorsolateral, lateral scales (scales 9). I – Trunk anterior region ventrolateral scales (scales 10). J – Trunk anterior region ventral scales (scales 11). K – Trunk posterior region ventrolateral scales (scales 12). L – Trunk posterior region ventral scales (scales 13).

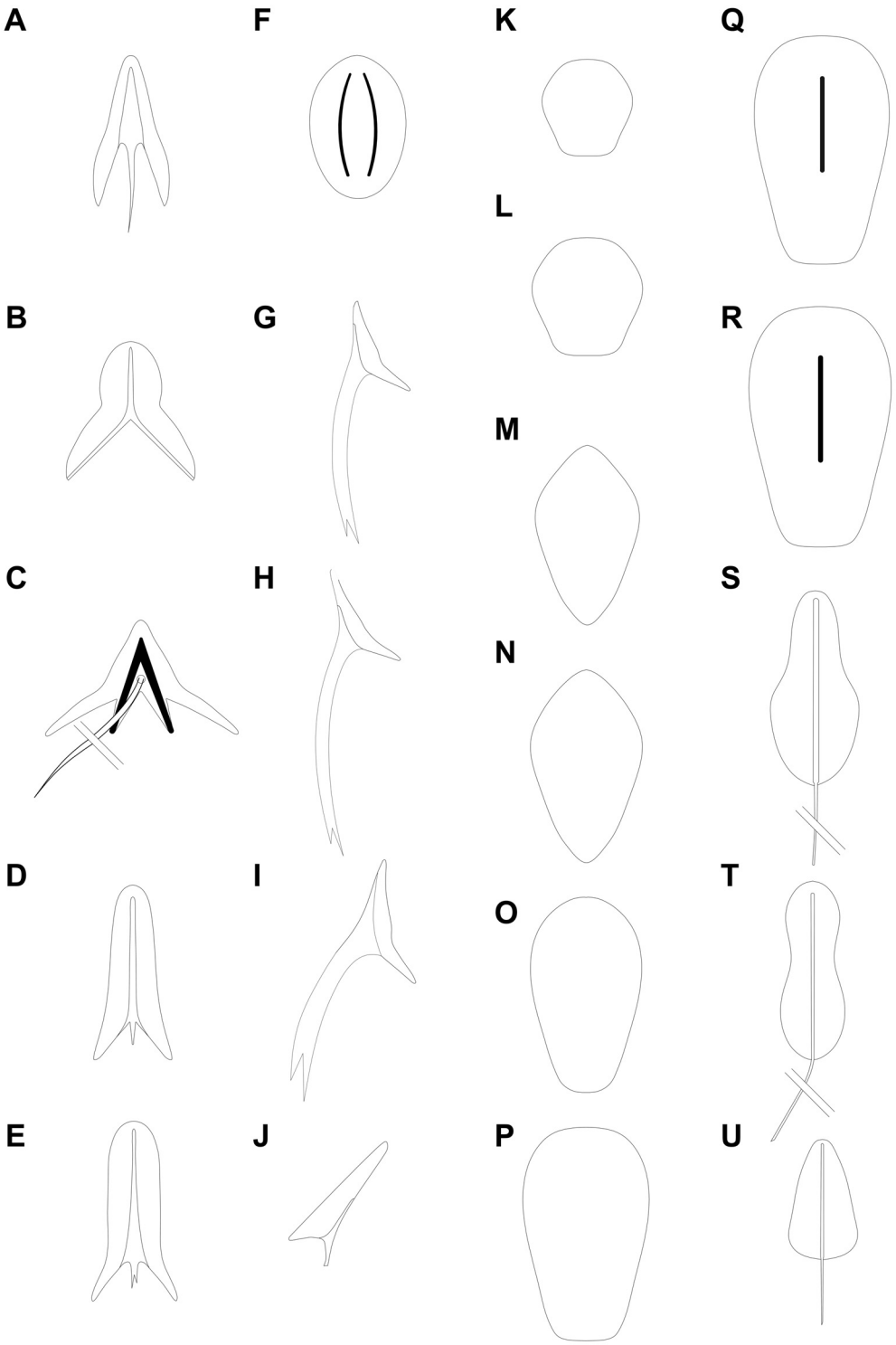


Figure 45. *Chaetonotus* (*Hystricochaetonotus*) *persimilis* sp. nov. Schematic scales drawings. A – Head anteriormost dorsal scale (scale 1). B – Spineless scale anterior to each double-keeled scale with dorsal sensory bristle (scale 14). C – Double-keeled scale with trunk dorsal sensory bristle (scale 15). D – Small furcal base dorsal scale (scale 16). E – Furcal base and furcal appendages dorsal scale (scale 17). F – Furcal appendages double-keeled scale (scale 18). G – Second to the posteriormost trunk lateral scales (scale 19). H – Posteriormost trunk lateral scale (scale 20). I – Scale with parafurcal spine (scale 21). J – Intrafurcal scale (scale 22). K–R – Ventral interciliary field scale (scales 23–30). S – First pair of ventral interciliary field terminal scale (scale 31). T – Second pair of ventral interciliary field terminal scale (scale 32). U – Third pair of ventral interciliary field terminal scale (scale 33).

the neck constriction is clearly marked. The neck gradually tapers from the head (from U21) to the beginning of the trunk (*ca*. U34) (Figs 43, 46–49). The trunk is wider than the head and gradually dilates from *ca*. U35 to *ca*. U63, where it is at its maximum width, and subsequently gradually tapers to the furcal base, which is clearly demarcated (from U82) (Figs 43, 46–49). The furcal indentation (intrafurcal space) is V-shaped. The furcal branches are narrowly set apart and point outwards (Figs 43, 46–49). The adhesive tubes are straight and taper towards their blunt ends (Figs 43, 46–49).

The head is five-lobed, long and semicircular in shape. The cephalion (U1–U4) is narrow, short and convex, and clearly demarcated in the body outline. It adheres to the head along its entire length and rapidly widens at the dorsal edge (Fig. 43A). The epipleurae (U4–U8) are pronounced and convex, *ca*. one-third the size of the hypopleurae. The hypopleurae (U7–U16) are large and long (Figs 43, 46–48). On the dorsal head surface, the lateral edges of the cephalion and epipleurae do not meet and there is a prominent space between them. Deep notches are present between the cephalion and epipleurae and between the epipleurae and hypopleurae. The hypostomium (U6–U8) is narrow and short, rectangular with slightly rounded edges (Figs 43C, 50B). It has a granular protuberance in the centre. Two pairs of cephalic ciliary tufts are present. The anterior tufts emerge in the area between the cephalion and epipleurae on the ventral head surface (at U4) and consist of 4 cilia. The anteriormost cilium in both anterior tufts is short. The length of the cilia in the posterior tuft gradually increases from the anteriormost to the posteriormost cilium. The posterior tufts (at U8–U9) have 5 cilia each and emerge in ventral notches between the epipleurae and hypopleurae. The length of the cilia in the posterior tuft gradually increases from the anteriormost to the fourth cilium. The posteriormost cilium is the shortest in the posterior tufts. All cilia in both tufts are thick. Ocellar granules are not present. The mouth ring is located subterminally at U2–U5 and has very strong, granular cuticular reinforcements and short inner hairs. Inside the mouth ring, two small and weak cuticular teeth arising from the anterior pharynx region are present (Figs

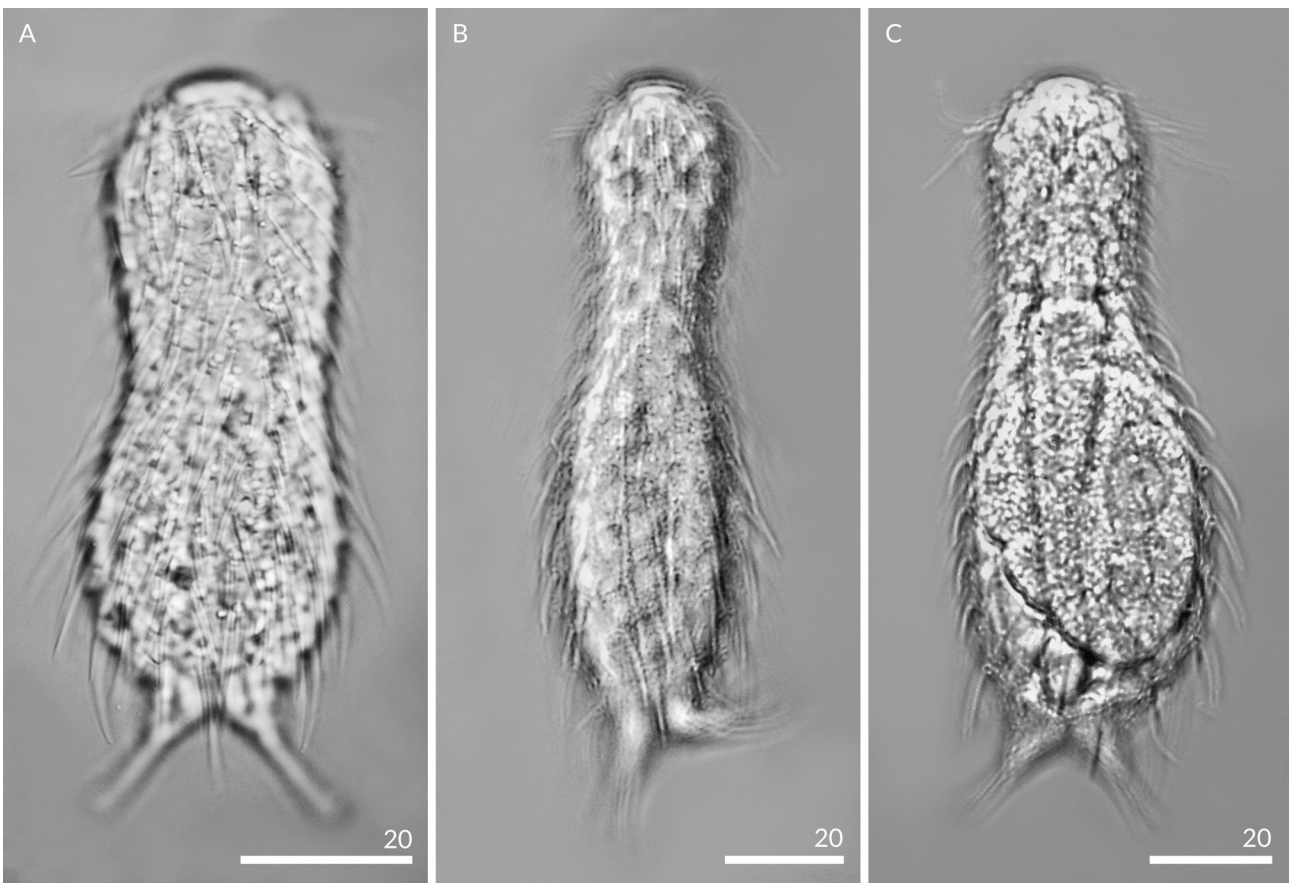


Figure 46. *Chaetonotus* (*Hystricochaetonotus*) *persimilis* sp. nov. A – Juvenile specimen. Habitus. B – Adult specimen. Habitus. C – Specimen with mature egg. Habitus.

50B, 51B–51D). Suboral bristles are located in two narrow and elongated tufts laterally to the mouth ring and hypostomium.

The entire body, except for one pair of scales on the furcal appendages, is covered with three-lobed scales that adhere to the cuticle along their entire surface. The scales have a strong, high, triangular keel and are shaped like triangles with a wide notch in the posterior edge. The scales are distributed in 15–17 total longitudinal alternating rows (5–7D+2DL+2L+4LV+2V) with 12–13 scales in the central row. The longitudinal rows of scales from the head to the posterior trunk region run parallel to another. The edges of the head, neck and trunk scales meet and are juxtaposed but never overlap, only the scales located on the dorsal surface of the furcal base and furcal appendages are overlapping (Figs 43, 47, 49–50). The longitudinal rows of scales begin on the head directly beneath the posterior edge of the cephalion, epipleurae and hypopleurae. The scales show strong morphological diversity between the various parts of the body: the length of the central lobe, the roundness of the edges and the size of the scales differ (scales 2–13; Fig. 44). The anteriormost scales of the head situated near the posterior edge of the cephalion and epipleurae are shaped like sharp triangles, long and narrow, with long and narrow posterolateral lobes, a keel and spine (scales 1; Figs 43A, 45A). The remaining scales of the head are wide and shaped like a triangle. The central lobes of these scales are rounded, the posterolateral lobes are slightly rounded, wide apart and strongly directed laterally (scales 2; Figs 43A, 44A). The neck scales have shorter, more rounded central lobes (scales 7; Figs 43A, 44F). On the neck, the central lobes of the scales gradually lengthen towards the anterior trunk region and their rounded edges gradually transition into sharp edges (scales 9; Figs 43A, 44H). Scales on the trunk have a sharp triangle shape with a long central lobe. The posterolateral lobes are clearly separated from the central lobe and directed laterally (scales 9; Figs 43A, 44H). Furthermore, the posterolateral lobes of the dorsolateral, lateral, ventrolateral and ventral scales become gradually directed downward and their edges gradually become slightly more rounded as compared to the

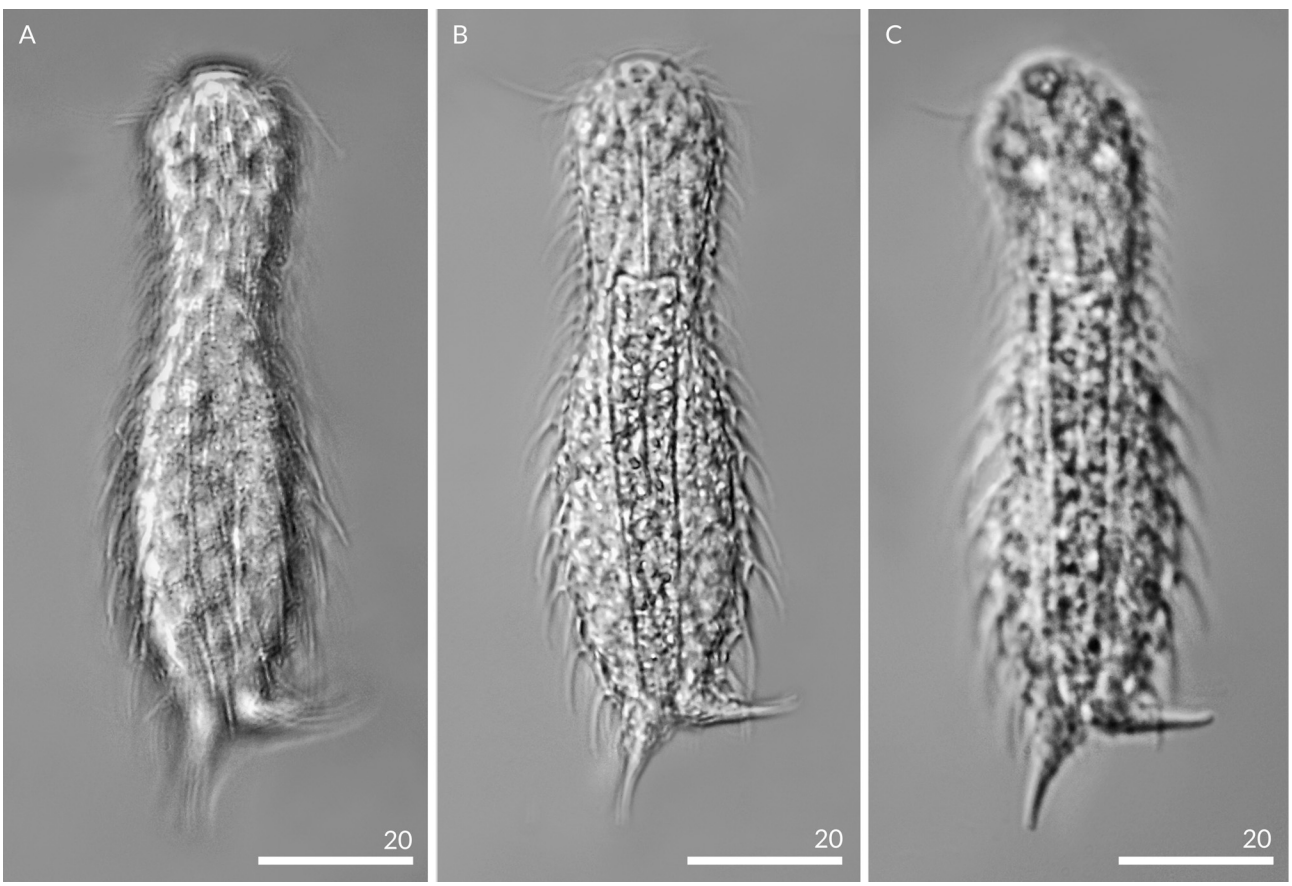


Figure 47. *Chaetonotus* (*Hystricochaetonotus*) *persimilis* sp. nov. Adult specimen. A – Dorsal body view. B – View of internal morphology. C – Ventral body view.

edges of the dorsal, dorsolateral, lateral, ventrolateral and ventral scales towards the ciliary bands (scales from 2 to 6, from 7 to 8; from 9 to 13; Figs 43, 44). The size of the scales gradually decreases from the dorsal towards the ventral ciliary bands (Table 20). The scales in the ventral longitudinal row located closest to the ciliary bands are small, much smaller than the scales of the other rows, and their central lobe is oriented towards the bands at an angle of *ca.* 30°. On the dorsal surface of the posterior trunk region at U75–U79, slightly anterior to the double-keeled scales with dorsal sensory bristles, is located one pair of three-lobed scales (scales 14; Figs 43A, 45B). These scales have wider, more rounded and shorter posterolateral lobes, have a long keel running from the central lobe's anterior part to the ends of the posterolateral lobes and do not have a spine (scales 14; Figs 43A, 45B). One pair of small, three-lobed scales shaped like elongated triangles with short posteriolateral lobes and a shallow posterior notch are located diagonally to the longitudinal body axis on the dorsal surface of the furcal base (at U82–U84) (scales 16; Figs 43A, 45D). These scales have a long, thin keel and short, simple spines. On the dorsal surface of the furcal base and furcal appendages (at U83–U88), three pairs of three-lobed, elongated and narrow scales are present. These scales are elongated, have a rounded, long central lobe and short posterolateral lobes. They have a long keel and a short spine with a lateral denticle (scales 17; Figs 43A, 45E). Dorsolaterally and laterally at the furcal appendages (at U86–U90), one pair of scales with two keels is present. These scales are oval in shape and have no posterior notches and spines. Their keels are long and slightly lateral bent (scales 18; Figs 43A, 45F). The edges of these scales slightly overlap the neighbouring dorsolateral and lateral scales. One pair scales with parafurcal spines of the same type as the trunk lateral scales is located at U86–U89 (scales 21; Figs 43, 45I). Thee pairs of intrafurcal scales are present on the inner surface of the furcal appendages at *ca*. U87–U91 (scales 22; Figs 43, 45J). These scales are small, three-lobed, almost triangular, have keels and short, rudimentary simple spines. The edges of these scales are curved, thus making the entire scale adhere to the

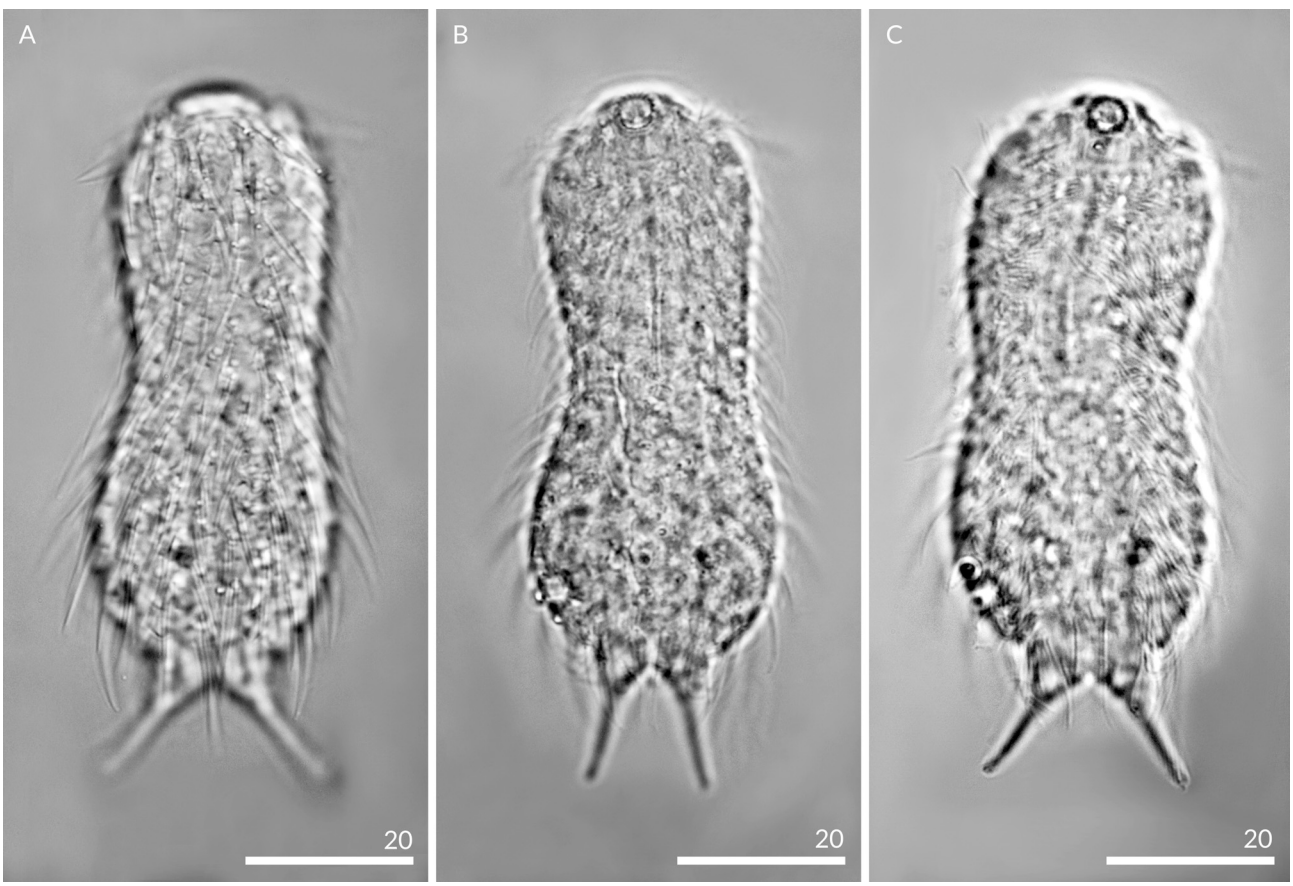


Figure 48. *Chaetonotus* (*Hystricochaetonotus*) *persimilis* sp. nov. Juvenile specimen. A – Dorsal body view. B – View of internal morphology. C – Ventral body view.

narrow surface of the inner furcal indentation (Figs 43, 59C).

Spines emerge from the posterior part of the scales. On the dorsal, dorsolateral, lateral and ventrolateral body surfaces the spines are strong, curved, have a lateral denticle near their end and gradually taper towards their pointed ends. The length and thickness of the spines gradually increase from the top of the head towards the furcal base. The lateral denticles on the spines become more pronounced from the head towards the furcal base. All dorsolateral, lateral, ventrolateral and ventral spines, except for the two posteriormost lateral pairs, are shorter than the dorsal spines and their length gradually decreases towards the ventrolateral body surface. The trunk's two posteriormost pairs of lateral spines are longer, stronger and more curved than the other body spines. The pair of parafurcal spines is strong and curved but shorter than the trunk spines. On the ventral body surface, spines emerging from scales arranged in two longitudinal rows located closest to the longitudinal bands are very long, thin, hair-like and have narrow, delicate *lamellae* along their entire length. The lamellae are widest at the base of the spine and gradually taper towards the spine's end. These spines do not have a lateral denticle.

The species has three pairs of dorsal sensory bristles (Fig. 43). The first, anterior pair is located on the dorsal surface of the head at U4, near the dorsal epipleurae edge. The bristles emerge from small, spherical papillae. The second pair of sensory bristles is located on the dorsal surface of the neck at U29 and emerges from small, spherical papillae. The third, posterior pair of sensory bristles is located dorsally and dorsolaterally on the posterior trunk region and furcal base. The third pair of dorsal sensory bristles emerges from the three-lobed scales with two strong keels situated at U78–U82. These scales have keels which are connected in the centre of the scales. The scales are shaped like double sharp triangles with long and narrow posterolateral lobes set very wide apart. These scales have a double deep posterior notch (scales 15; Figs 43A, 45C).

On the ventral surface, the longitudinal ciliary

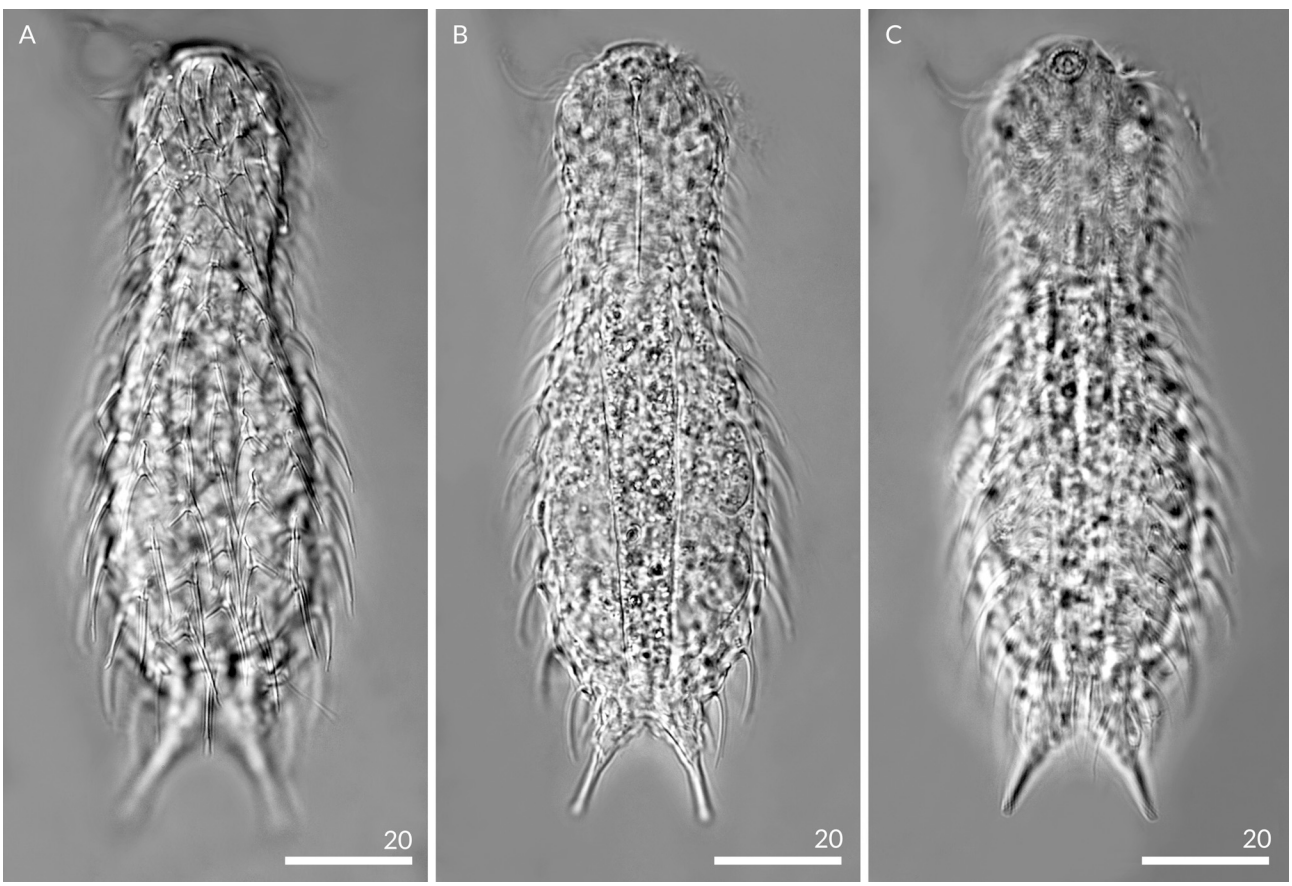


Figure 49. *Chaetonotus* (*Hystricochaetonotus*) *persimilis* sp. nov. Specimen with maturing eggs. A – Dorsal body view. B – View of internal morphology. C – Ventral body view.

bands begin at U11 and run back to U84 (Figs 43C, 47C, 48C, 49C, 50C). The ciliary bands are wider on the head and neck than in the other body parts. The entire ventral interciliary field is covered by scales which are one-lobed and strongly differentiated in shape and size. They are distributed in 7–9 longitudinal rows with 19–29 scales in the central row (Figs 43C, 47C, 48C, 49C, 50C). On the anterior body part the scales are round and located far from another (scales 23; Fig. 45K). Towards the posterior body region the scales gradually become elongated, larger and have a fusiform shape (scales 24–30; Figs 45L–45R). Towards the trunk posterior region the keels on the scales become more distinct and the distance between the scales decreases so that the scales' edges start adjacent to another but never overlap (Figs 43C, 47C, 48C, 49C,

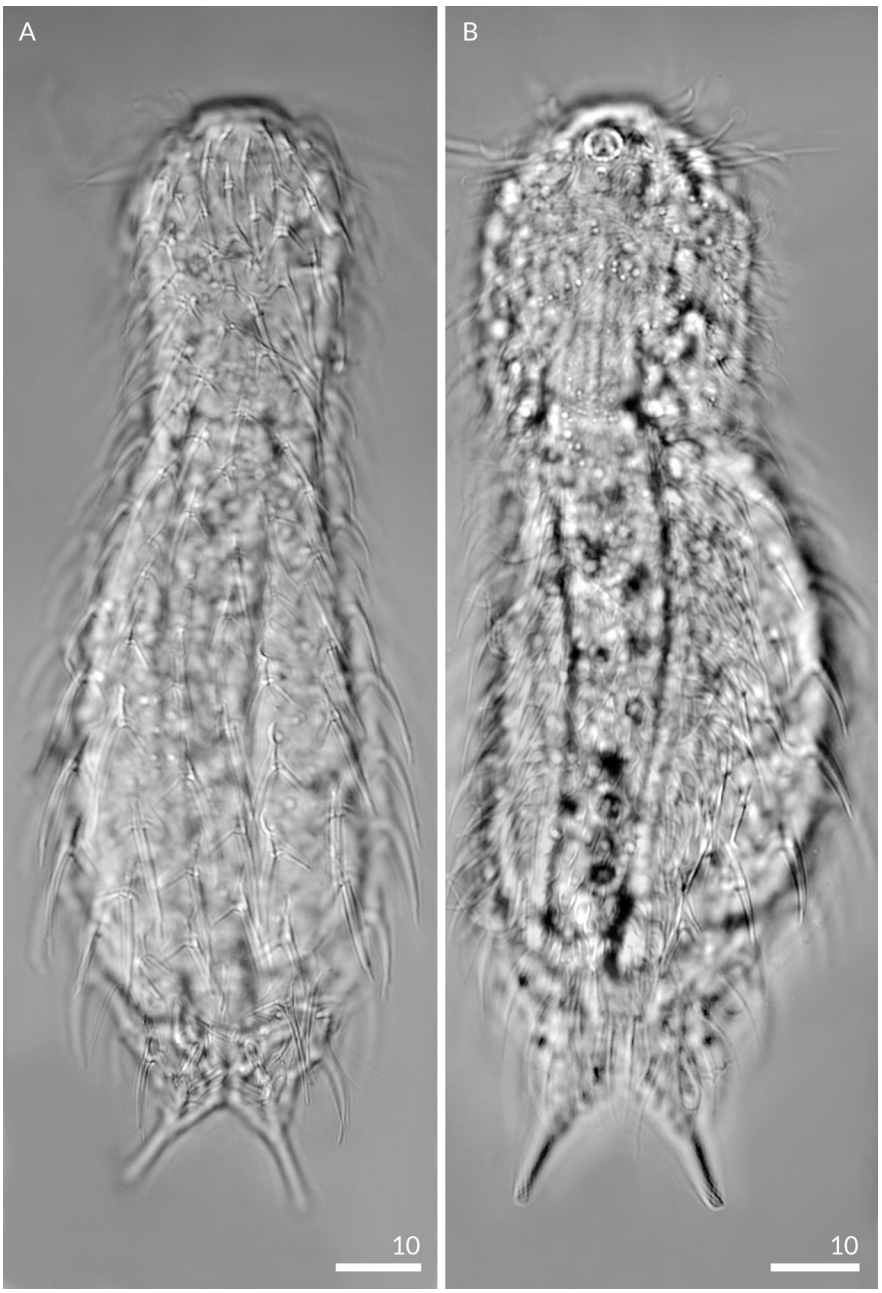


Figure 50. *Chaetonotus* (*Hystricochaetonotus*) *persimilis* sp. nov. Adult specimen. A – Dorsal view of scales and spines. B – Ventral view of scales and spines.

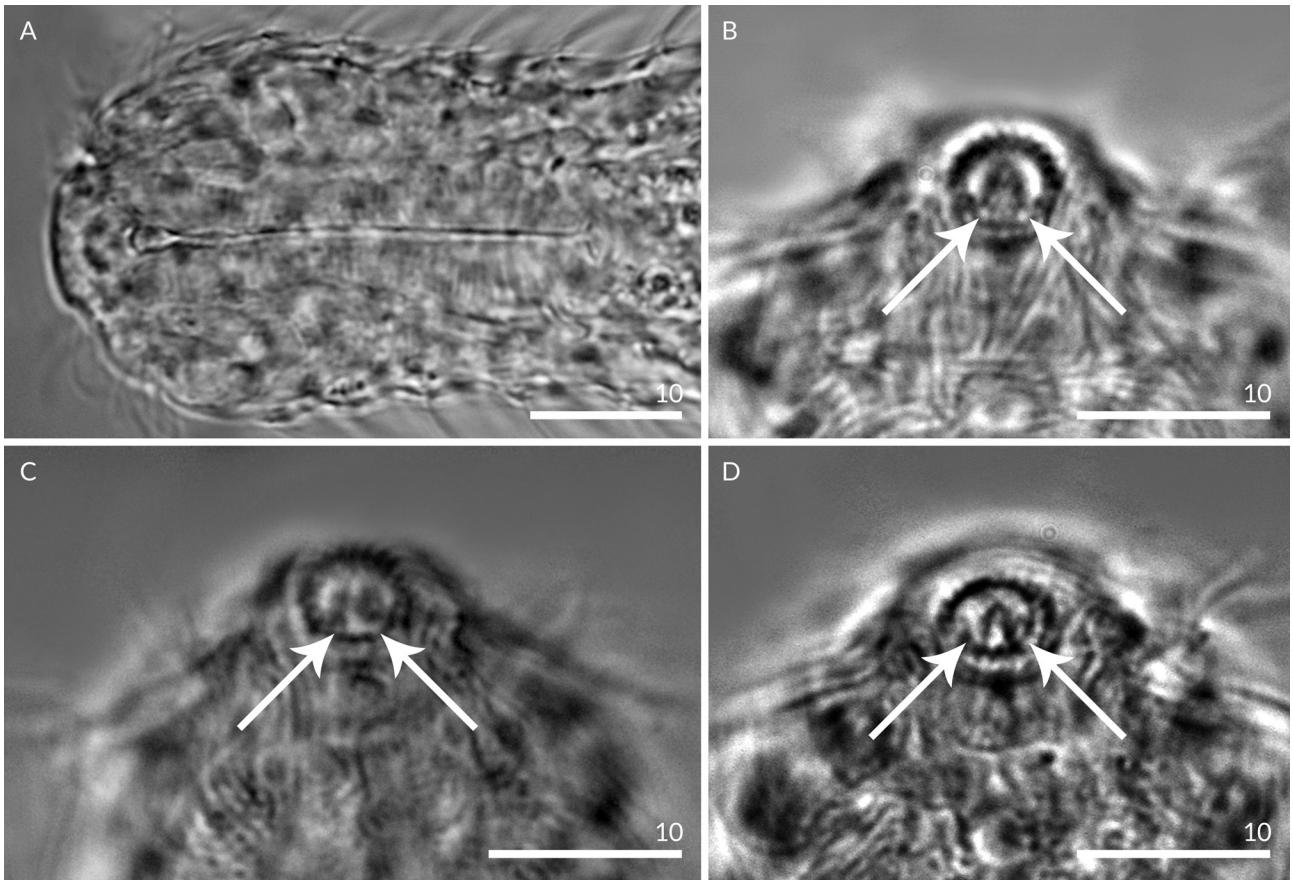


Figure 51. *Chaetonotus* (*Hystricochaetonotus*) *persimilis* spec nov. A – Detail of the pharynx. B–D – Detail of mouth ring with cuticular denticles.

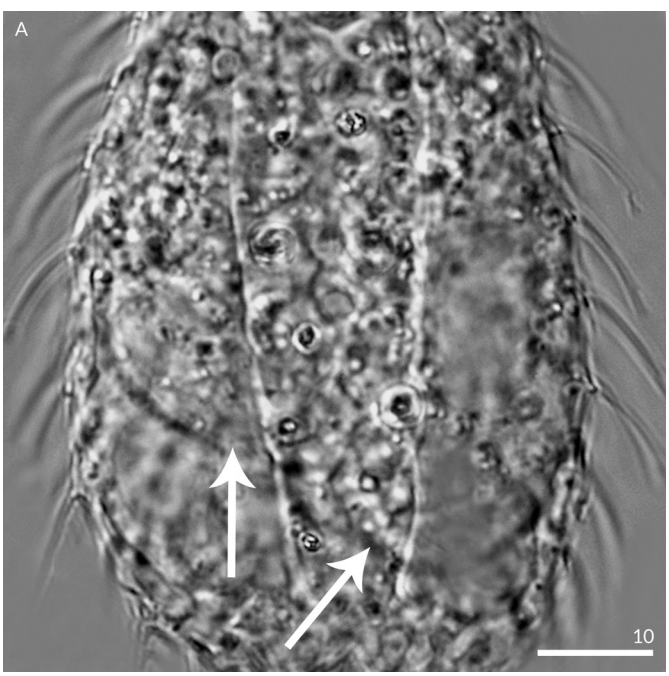


Figure 52. *Chaetonotus* (*Hystricochaetonotus*) *persimilis* spec nov. Adult specimen. Detail of the internal morfology. A – Two maturing eggs.

Table 19. Main morphometric characters of *Chaetonotus* (*Hystricochaetonotus*) *persimilis* sp. nov; N – number of specimens or structures analysed, Range – the smallest and the largest structure found among all specimens measured. All measurements are given in micrometers (μm); all indicators are given as a percentage (%) and italicized.

Characters	N	Holotype	Range of adult paratypes
Body length	15	132.16	114.72–135.72
Furca length	15	24.57	21.68–26.49
Adhesive tube length	15	12.46	11.10–13.58
Number of scales in central longitudinal row	15	13	12–13
Total number of longitudinal alternating rows of scales	15	15	15–17
Head scales length x width	15	1.71–6.12 x 1.47–5.89	1.44–6.42 x 1.24–6.23
Neck scales length x width	15	2.73–4.86 x 2.49–7.18	2.32–6.16 x 2.12–7.73
Trunk scales length x width	15	3.57–10.16 x 3.10–8.54	3.04–11.78 x 2.64–9.90
Head spines length	15	4.97–10.39	3.60–12.05
Localization of lateral denticle on head spines	15	*13.280–17.269*	*13.053–17.342*
Neck spines length	15	*6.59–12.70*	*4.78–14.72*
Localization of lateral denticle on neck spines	15	*16.692–17.302*	*16.510–17.980*
Trunk spines length	15	10.02–16.76	7.27–21.93
Localization of lateral denticle on trunk spines	15	*18.663–25.122*	*18.462–27.817*
Length of posteriormost trunk lateral spines	15	15.21	12.31–31.46
Localization of lateral denticle on posteriormost trunk lateral spines	15	*7.46*	*6.03–15.25*
Length of posteriormost parafurcal lateral spines	15	23.80	23.773–23.881
Localization of lateral denticle on posteriormost parafurcal lateral spines	15	*18.499*	*18.352–18.656*
Mouth ring diameter	15	5.36	4.31–5.51
Pharynx length	15	35.73	28.50–39.12

Table 20. Detailed morphometric of *Chaetonotus* (*Hystricochaetonotus*) *persimilis* sp. nov; N – number of specimens or structures analysed, Range – the smallest and the largest structure found among all specimens measured, SD – standard deviation. All measurements are given in micrometres (μm); all indicators are given as a percentage (%) and italicized.

Characters	N	Holotyp	Range of adult paratypes	SD
Cephalion length	15	10.1	9.12–10.96	0.476
Cephalion width	15	13.68	11.67–14.03	0.657
Hypostomium length	15	2.79	2.38–3.37	0.231
Hypostomium width	15	6.87	5.23–7.76	0.603
Length of cephalic cilia (anterior tuft)	15	5.08–28.52	(4.26–6.11)–(18.74–29.23)	0.464; 3.254
Length of cephalic cilia (posterior tuft)	15	6.71–30.1	(5.29–7.96)–(19.64–30.57)	0.685; 3.387
Width of cephalic cilia (anterior tuft)	15	1.06–1.10	(1.00–1.07)–(1.05–1.13)	0.024; 0.026
Width of cephalic cilia (posterior tuft)	15	1.08–1.14	(1.02–1.09)–(1.08–1.15)	0.023; 0.025
Width of adhesive tube	15	1.97	1.86–1.98	0.033
Ratio of scale distribution	15	*115.385*	*115.385–130.769*	3.972
Length of head dorsal scales (scales 1, 2 and 5)	15	4.82–6.12	(4.06–5.17)–(5.21–6.42)	0.305; 0.340
Width of head dorsal scales (scales 1, 2 and 5)	15	3.12–5.89	(2.66–3.27)–(4.84–6.23)	0.172; 0.415
Length of head dorsolateral scales (scales 2 and 5)	15	4.23–5.46	(3.56–4.54)–(4.65–5.73)	0.258; 0.304
Width of head dorsolateral scales (scales 2 and 5)	15	4.25–5.48	(3.58–4.56)–(4.67–5.75)	0.258; 0.304
Length of head lateral scales (scales 2 and 5)	15	4.01–5.11	(337–4.30)–(4.35–5.36)	0.245; 0.284
Width of head lateral scales (scales 2 and 5)	15	3.83–4.88	(3.22–4.11)–(4.15–5.12)	0.234; 0.272
Length of head ventrolateral scales (scales 3 and 6)	15	3.86–4.48	(3.24–4.14)–(3.81–4.70)	0.243; 0.249
Width of head ventrolateral scales (scales 3 and 6)	15	3.41–4.03	(2.86–3.65)–(3.43–4.23)	0.207; 0.225
Length of head ventral scales (scales 4)	15	1.71–2.69	(1.44–1.83)–(2.29–2.88)	0.103; 0.159
Width of head ventral scales (scales 4)	15	1.47–2.36	(1.24–1.57)–(2.01–2.53)	0.088; 0.140
Length of neck dorsal scales (scales 7)	15	3.21–4.86	(2.79–4.32)–(4.17–6.10)	0.413; 0.469
Width of neck dorsal scales (scales 7)	15	4.78–7.18	(4.15–5.63)–(5.73–7.73)	0.434; 0.536
Length of neck dorsolateral scales (scales 7)	15	3.14–4.74	(2.73–4.23)–(4.08–5.95)	0.405; 0.456

Table 20. Continued.

Characters	N	Holotyp	Range of adult paratypes	SD
Width of neck dorsolateral scales (scales 7)	15	4.57–6.79	(3.97–5.39)–(5.42–7.30)	0.416; 0.502
Length of neck lateral scales (scales 7)	15	3.09–4.68	(2.69–4.16)–(4.03–5.87)	0.397; 0.449
Width of neck lateral scales (scales 7)	15	4.26–6.56	(3.71–5.02)–(5.24–7.06)	0.385; 0.485
Length of neck ventrolateral scales (scales 8)	15	3.05–4.62	(2.66–4.10)–(3.98–5.79)	0.391; 0.443
Width of neck ventrolateral scales (scales 8)	15	4.08–6.17	(3.56–4.80)–(4.92–6.65)	0.367; 0.459
Length of neck ventral scales (scales 4)	15	2.73–3.54	(2.32–2.92)–(3.01–3.79)	0.161; 0.209
Width of neck ventral scales (scales 4)	15	2.49–3.97	(2.12–2.66)–(3.37–4.25)	0.146; 0.235
Length of trunk dorsal scales (scales 9)	15	4.94–10.16	(4.24–6.20)–(8.69–11.78)	0.477; 0.801
Width of trunk dorsal scales (scales 9)	15	6.82–8.54	(5.85–7.36)–(7.30–9.90)	0.421; 0.673
Length of trunk dorsolateral scales (scales 9)	15	4.83–8.22	(4.15–6.06)–(7.07–9.53)	0.465; 0.642
Width of trunk dorsolateral scales (scales 9)	15	6.52–6.87	(5.60–7.03)–(5.91–7.96)	0.401; 0.534
Length of trunk lateral scales (scales 9)	15	4.78–8.18	(4.11–6.00)–(7.04–9.48)	0.460; 0.639
Width of trunk lateral scales (scales 9)	15	6.37–6.71	(5.47–6.87)–(5.77–7.77)	0.392; 0.524
Length of trunk ventrolateral scales (scales 10 and 12)	15	4.71–8.06	(4.05–5.91)–(6.94–9.34)	0.453; 0.629
Width of trunk ventrolateral scales (scales 10 and 12)	15	6.18–6.52	(5.31–6.67)–(5.61–7.56)	0.379; 0.510
Length of trunk ventral scales (scales 11 and 13)	15	3.57–4.68	(3.04–3.82)–(3.67–5.02)	0.209; 0.326
Width of trunk ventral scales (scales 11 and 13)	15	3.10–3.73	(2.64–3.32)–(2.94–4.00)	0.181; 0.262
Length of trunk posteriormost pair of lateral scales (scales 20)	15	8.20	7.06–9.46	0.634
Width of trunk posteriormost pair of lateral scales (scales 20)	15	6.72	5.79–7.75	0.520
Length of posterior trunk spineles scales (scales 14)	15	8.12	4.95–10.48	1.297
Width of posterior trunk spineles scales (scales 14)	15	5.17	4.11–6.77	0.917
Length of triangle-scales on the furcal base (scales 16)	15	4.57	3.32–5.60	0.656
Width of triangle-scales on the furcal base (scales 16)	15	3.38	1.74–3.62	0.642
Length of furcal base and furcal appendages dorsal elongated three-lobed scales (scales 17)	15	6.28–7.20	(5.87–9.80)–(6.39–10.37)	0.959; 0.955
Width of furcal base and furcal appendages dorsal elongated three-lobed scales width (scales 17)	15	3.89–4.05	(2.97–5.37)–(3.16–5.84)	0.578; 0.613
Length of double keeled scales on the furcal appendages (scales 18)	15	7.27	5.73–9.64	0.844
Width of double keeled scales on the furcal appendages (scales 18)	15	5.92	4.03–7.42	0.722
Length of lateral scales with parafurcal spines (scales 21)	15	4.26	3.69–4.92	0.329
Width of furcal lateral scales with parafurcal spines (scales 21)	15	3.49	3.02–4.06	0.274
Length of intrafurcal scales (scales 22)	15	2.83–3.01	(1.84–2.93)–(2.05–3.17)	0.338; 0.368
Width of intrafurcal scales (scales 22)	15	2.01–2.13	(1.45–2.09)–(1.66–2.25)	0.220; 0.214
Length of head dorsal spines (scales 1, 2 and 5)	15	4.98–7.46	(3.61–5.76)–(5.41–8.62)	0.550; 0.826
Head dorsal spines length between lateral denticle and spine base (scales 1, 2 and 5)	15	0.86–1.27	(0.62–0.99)–(0.92–1.47)	0.095; 0.141
Length of head dorsolateral spines (scales 2 and 5)	15	5.63–7.14	(4.08–6.51)–(5.18–8.25)	0.622; 0.791
Head dorsolateral spines length between lateral denticle and spine base (scales 2 and 5)	15	0.78–1.21	(0.57–0.90)–(0.88–1.40)	0.084; 0.134
Length of head lateral spines (scales 2 and 5)	15	5.42–6.91	(3.93–6.27)–(5.01–7.98)	0.611; 0.767
Head lateral spines length between lateral denticle and spine base (scales 2 and 5)	15	0.74–1.16	(0.53–0.86)–(0.84–1.34)	0.085–0.129
Length of head ventrolateral spines (scales 3 and 6)	15	4.97–6.33	(3.60–5.75)–(4.59–7.31)	0.560; 0.703
Head ventrolateral spines length between lateral denticle and spine base (scales 3 and 6)	15	0.66–1.05	(0.48–0.76)–(0.76–1.21)	0.075; 0.117
Length of head ventral spines (scales 4)	15	8.97–10.39	(6.53–10.38)–(8.39–12.05)	0.986; 1.051
Lamellae width on the ventral head spines (scales 4)	15	1.03–1.26	(0.91–1.16)–(1.12–1.39)	0.068; 0.065
Length of neck dorsal spines (scales 7)	15	7.59–10.75	(5.50–8.77)–(7.79–12.42)	0.841; 1.189
Neck dorsal spines length between lateral denticle and spine base (scales 7)	15	1.30–1.86	(0.94–1.50)–(1.35–2.15)	0.145; 0.205
Length of neck dorsolateral spines (scales 7)	15	7.27–10.23	(5.27–8.40)–(7.41–11.82)	0.806; 1.132
Neck dorsolateral spines length between lateral denticle and spine base (scales 7)	15	1.24–1.76	(0.90–1.43)–(1.27–2.03)	0.137; 0.194

Table 20. Continued.

Characters		N Holotyp	Range of adult paratypes	SD
Length of neck lateral spines (scales 7)	15	7.12–10.04	(5.16–8.23)–(7.27–11.60)	0.794; 1.109
Neck lateral spines length between lateral denticle and spine base (scales 7)	15	1.20–1.72	(0.87–1.39)–(1.25–1.99)	0.135; 0.190
Length of neck ventrolateral spines (scales 8)	15	6.59–9.21	(4.78–7.62)–(6.69–10.64)	0.735; 1.026
Neck ventrolateral spines length between lateral denticle and spine base (scales 8)	15	1.10–1.57	(0.79–1.27)–(1.14–1.81)	0.124; 0.173
Length of neck ventral spines (scales 4)	15	10.48–12.70	(7.64–12.15)–(9.26–14.72)	1.186; 1.497
Lamellae width on the ventral neck spines (scales 4)	15	1.29–1.40	(1.15–1.42)–(1.23–1.57)	0.067; 0.086
Length of trunk dorsal spines (scales 9)	15	11.17–14.29	(8.09–18.92)–(10.35–29.56)	2.472; 4.416
Trunk dorsal spines length between lateral denticle and spine base (scales 9)	15	2.12–3.59	(1.54–3.59)–(2.60–7.43)	0.468; 1.111
Length of trunk dorsolateral spines (scales 9)	15	10.82–13.67	(7.84–18.33)–(9.90–28.28)	2.394; 4.225
Trunk dorsolateral spines length between lateral denticle and spine base (scales 9)	15	2.04–3.42	(1.48–3.46)–(2.48–7.08)	0.452; 1.058
Length of trunk lateral spines (scales 9)	15	10.56–14.96	(7.65–17.89)–(10.84–30.95)	2.340; 4.624
Trunk lateral spines length between lateral denticle and spine base (scales 9)	15	1.98–3.55	(1.43–3.35)–(2.57–7.34)	0.438; 1.096
Length of trunk ventrolateral spines (scales 10 and 12)	15	10.02–13.11	(7.27–16.98)–(9.76–28.12)	2.292; 4.036
Trunk ventrolateral spines length between lateral denticle and spine base (scales 10 and 12)	15	1.87–3.25	(1.35–3.17)–(2.42–6.72)	0.415; 1.000
Length of trunk ventral spines (scales 11 and 13)	15	12.81–16.76	(9.34–14.85)–(12.21–21.93)	1.506; 2.397
Lamellae width on the ventral trunk spines (scales 11 and 13)	15	1.43–1.62	(1.29–1.61)–(1.40–1.77)	0.085; 0.096
Length of triangle-shaped scales on furcal base spines (scales 16)	15	1.02	0.67–1.48	0.216
Length of trunk posteriormost pair of lateral spines (scales 20)	15	15.21	12.31–31.46	4.622
Trunk posteriormost pair of lateral spines length between lateral denticle and spine base (scales 20)	15	3.62	2.93–7.49	1.101
Length of furcal base and furcal appendages dorsal elongated three-lobed scales spines (scales 17)	15	2.02–2.21	(1.22–2.51)–(1.35–2.88)	0.328; 0.401
Furcal base and furcal appendages dorsal elongated three-lobed scales spines length between lateral denticle and spine base (scales 17)	15	0.69–0.87	(0.41–0.86)–(0.53–1.13)	0.115; 0.159
Length of posteriormost parafurcal lateral spines (scales 21)	15	7.46	6.03–15.25	2.222
Length of posteriormost parafurcal lateral spines length between lateral denticle and spine base (scales 21)	15	1.38	1.12–2.82	0.410
Length of intrafurcal scales spines (scales 22)	15	0.72–0.84	(0.52–1.03)–(0.65–1.19)	0.131; 0.139
Length ratio of posteriormost trunk lateral spines (scales 20)	15	*11.509*	*10.410–23.245*	3.143
Length ratio of posteriormost parafurcal lateral spines (scales 20)	15	*5.645*	*5.104–11.268*	1.506
Localization of lateral denticle on dorsal head spines (scales 1, 2 and 5)	15	*17.024–17.269*	(*16.933–17.175*)–(*17.164–17.342*)	0.058; 0.048
Localization of lateral denticle on dorsolateral head spines (scales 2 and 5)	15	*13.854–16.947*	(*13.774–13.971*)–(*16.916–17.028*)	0.065; 0.030
Localization of lateral denticle on lateral head spines (scales 2 and 5)	15	*13.653–16.787*	(*13.387–16.626*)–(*16.693–16.846*)	0.777; 0.040
Localization of lateral denticle on ventrolateral head spines (scales 2 and 5)	15	*13.280–16.588*	(*13.053–13.333*)–(*16.278–16.667*)	0.090; 0.116
Localization of lateral denticle on dorsal neck spines (scales 7)	15	*17.128–17.302*	(*17.063–17.194*)–(*17.267–17.336*)	0.038; 0.018
Localization of lateral denticle on dorsolateral neck spines (scales 7)	15	*17.056–17.204*	(*16.998–17.130*)–(*17.139–17.250*)	0.035; 0.034
Localization of lateral denticle on lateral neck spines (scales 7)	15	*16.854–17.131*	(16.797–16.930)–(*17.076–17.194*)	0.039; 0.042

Table 20. Continued.

Characters	N	Holotyp	Range of adult paratypes	SD
Localization of lateral denticle on ventrolateral neck spines (scales 8)	15	*16.692–17.047*	(*16.510–16.752*)–(*16.929–17.980*)	0.076; 0.250
Localization of lateral denticle on dorsal trunk spines (scales 9)	15	*18.979–25.122*	(*18.944–19.036*)–(*24.704–25.149*)	0.028; 0.109
Localization of lateral denticle on dorsolateral trunk spines (scales 9)	15	*18.854–25.018*	(*18.808–18.878*)–(*24.978–25.051*)	0.025; 0.020
Localization of lateral denticle on lateral trunk spines (scales 9)	15	*18.750–23.730*	(*18.692–18.803*)–(*23.705–23.806*)	0.039; 0.26
Localization of lateral denticle on ventrolateral trunk spines (scales 10 and 12)	15	*18.663–24.790*	(*18.462–18.719*)–(*24.748–27.817*)	0.064; 0.798
Localization of lateral denticle on posteriormost trunk lateral spines (scales 20)	15	*23.80*	*23.773–23.881*	0.029
Localization of lateral denticle of spines furcal base and furcal appendages dorsal elongated three-lobed scales (scales 17)	15	*34.158–39.367*	(*33.544–34.409*)–(*38.764–40.465*)	0.297; 0.365
Localization of lateral denticle on posteriormost parafurcal lateral spines (scales 21)	15	*18.499*	*18.352–18.656*	0.069
Head dorsal sensory bristles length	15	16.20	14.57–16.93	0.703
Neck dorsal sensory bristles length	15	12.73	11.67–14.16	0.826
Posterior dorsal sensory bristles length	15	14.23	12.69–15.89	0.802
Number of scales in central longitudinal row on ventral interciliary field	15	27	25–29	1.033
Total number of longitudinal alternating rows of scales on ventral interciliary field	15	9	8–9	0.258
Length of ventral interciliary field scales (scales 23–30)	15	1.15–4.28	(0.80–1.21)–(3.28–4.64)	0.120; 0.348
Width of ventral interciliary field scales (scales 23–30)	15	1.14–2.18	(0.78–1.19)–(1.82–2.57)	0.124; 0.231
Length of terminal ventral interciliary field scales I pair (scales 31)	15	9.37	7.66–10.07	0.675
Width of terminal ventral interciliary field scales I pair (scales 31)	15	4.11	3.46–4.64	0.373
Length of terminal ventral interciliary field scales II pair (scales 32)	15	4.93	4.09–5.64	0.480
Width of terminal ventral interciliary field scales II pair (scales 32)	15	2.10	1.82–2.57	0.201
Length of terminal ventral interciliary field scales III pair (scales 33)	15	3.07	2.41–3.32	0.248
Width of terminal ventral interciliary field scales III pair (scales 33)	15	1.62	1.39–1.83	0.104
Length of terminal ventral interciliary field scales I pair spines (scales 31)	15	9.07	8.19–9.69	0.356
Length of terminal ventral interciliary field scales II pair spines (scales 32)	15	8.13	7.16–8.64	0.928
Length of terminal ventral interciliary field scales III pair spines (scales 33)	15	1.92	1.69–2.32	0.162
Width of anterior pharynx thickening (a)	15	10.74	9.04–13.84	1.079
Width of pharynx narrowing (n)	15	7.55	6.45–9.60	0.718
Width of middle pharynx (m)	15	8.14	7.01–10.23	0.722
Width of posterior pharynx thickening (p)	15	12.97	10.86–17.19	1.407
Width of pharyngeal-intestinal junction	15	2.72	2.43–3.36	0.236
Length of intestine anterior differ section	15	3.23	2.69–4.31	0.388
Intestine length	15	71.05	57.66–74.13	4.236
Pharynx formula a	15	*30.059*	*26.719–35.378*	2.322
Pharynx formula n	15	*21.131*	*18.794–24.540*	1.446
Pharynx formula m	15	*22.782*	*20.425–26.150*	1.397
Pharynx formula p	15	*36.30*	*33.304–43.942*	2.658
Pharynx-intestine ratio I	15	*50.289*	*46.356–54.509*	2.415
Length of matured egg	1	–	48.39	0
Width of matured egg	1	–	35.92	0

Table 21. Comparison of the most important differentiating characters between species most similar to *Chaetonotus* (*Hystricochaetonotus*) *borealis* sp. nov., *C.* (*H.*) *hornusndi* sp. nov. and *C.* (*H.*) *persimilis* sp. nov. Measurements are given in micrometres (μm). NA – data not available; – – not applicable.

Character	*C.* (*H.*) *borealis* sp. nov.	*C.* (*H.*) *hornsundi* sp. nov.	*C.* (*H.*) *persimilis* sp. nov.	*C.* (*H.*) *hystrix*	*C.* (*H.*) *euhystrix*	*C.* (*H.*) *macrochaetus*	*C.* (*H.*) *persetosus*	*C.* (*C.*) *benacensis*
Body length	115.3–127.4	117.0–138.2	114.7–135.7	87.0–130.0	150.0–160.0	65.0–153.0	75.0–119	80.0–117.0
Cephalic pleurae	Cephalion short and narrow; epipleurae and hypopleurae large, similar in size. Epipleurae and hypopleurae located on dorsal, dorsolateral, lateral, ventrolateral and ventral head sides; deep notches between cephalion, epipleurae and hypopleurae. Hypostomium wide, short, pentagonal with granular perturbance on central part of anterior edges and reinforced posterior edge	Cephalion short and placed forward, epipleurae small, *ca*. three times smaller than hypopleurae; hypopleurae large. Epipleurae and hypopleurae located on dorsal, dorsolateral, lateral, ventrolateral and ventral head sides. Hypostomium composed of three plates: anterior plate rectangular in shape, short with granular perturbance in the centeral part of anterial edges, beyound pair of transverse tear-shaped plates	Cephalion short and narrow; epipleurae small, *ca*. three times smaller than hypopleurae. Epipleurae and hypopleurae located on dorsal, dorsolateral, lateral, ventrolateral and ventral head sides; deep notches between cephalion, epipleurae and hypopleurae. Hypostomium narrow, short, rectangular with granular perturbance on centre	Cephalion narrow; epipleurae and hypopleurae similar in size; epipleurae and hypopleurae located mostly laterally. Hypostomium not demarcated	Cephalion narrow; epipleurae and hypopleurae similar in size; epipleurae and hypopleurae located mostly laterally. Hypostomium not demarcated	Cephalion narrow; epipleurae and hypopleurae similar in size; epipleurae and hypopleurae located mostly laterally. Hypostomium not demarcated	Cephalion narrow; epipleurae and hypopleurae similar in size; epipleurae and hypopleurae located mostly laterally. Hypostomium not demarcated	Cephalion narrow; epipleurae and hypopleuriae similar in size; epipleurae and hypopleurae located mostly laterally. Hypostomium trapezoidal provided with two conic swellings

Table 21. Continued

Character	*C.* (*H.*) *borealis* sp. nov.	*C.* (*H.*) *hornsundi* sp. nov.	*C.* (*H.*) *persimilis* sp. nov.	*C.* (*H.*) *hystrix*	*C.* (*H.*) *euhystrix*	*C.* (*H.*) *macrochaetus*	*C.* (*H.*) *persetosus*	*C.* (*C.*) *benacensis*
Presence of additional hypostomium plates	Absent	Present	Absent	Absent	Absent	Absent	Absent	Absent
Place of arising cephalic cilia	Anterior tufts: on ventral side, in area between cephalion and epipleurae; posterior tufts: on the ventral side, in notches between epipleurae and hypopleurae	Anterior tufts: on dorsolateral side, between lateral cephalion edge and anterior epipleurae edge; posterior tufts: on the ventral side, in notches between epipleurae and hypopleurae	Anterior tufts: on ventral side, in area between cephalion and epipleurae; posterior tufts: on the ventral side, in notches between epipleurae and hypopleurae	Anterior tufts: dorsoterminal between cephalion and epipleurae; posterior tufts: laterally, between epipleurae and hypopleurae	Anterior tufts: dorsoterminal between cephalion and epipleurae; posterior tufts: laterally, between epipleurae and hypopleurae	Anterior tufts: dorsoterminal between cephalion and epipleurae; posterior tufts: laterally, between epipleurae and hypopleurae	Anterior tufts: dorsoterminal between cephalion and epipleuriae; posterior tufts: laterally, between epipleurae and hypopleurae	Anterior tufts: dorsoterminal between cephalion and epipleurae; posterior tufts: laterally, between epipleurae and hypopleurae
Presence of cuticular teeth in the mouth ring	Two, small, weak cuticular teeh	Two, small, weak cuticular teeh	Two, small, weak cuticular teeh	Absent	Absent	Absent	Absent	Absent
Pharynx length	31.1–34.8	29.6–34.4	28.5–39.1	21.0–32.0	50.0–55.0	27.0–30.0	22.0–25.0	22.8–33.6
Pharynx shape	Anterior and wider posterior dilatation	Anterior and wider posterior dilatation	Anterior and wider posterior dilatation	Anterior and wider posterior dilatation	Anterior and wider posterior dilatation	Anterior and wider posterior dilatation	Anterior and wider posterior dilatation	Weak anterior and posterior dilatation; posterior dilatation wider
Presence of cuticular reinforcements in the pharynx	Present (weak single cuticular curved rod)	Present (two connected short, thin and straight rods)	Present (two connected short, thin and straight rods)	Absent	Absent	Absent	Absent	Absent
Anterior intestine section differs in form of morphology	Present	Present	Present	Absent	Absent	Absent	Absent	Absent
Furca length	20.9–22.1	21.3–26.9	21.7–26.5	14.0	25.0–35.0	9.0–20.0	12.0–18.5	9.5–17.3
Adhesive tube length	10.6–12.1	11.2–14.0	11.1–13.6	10.0	15.0–16.0	9.0–10.0	NA	5.7–11.6

Table 21. Continued

Character	*C.* (*H.*) *borealis* sp. nov.	*C.* (*H.*) *hornsundi* sp. nov.	*C.* (*H.*) *persimilis* sp. nov.	*C.* (*H.*) *hystrix*	*C.* (*H.*) *euhystrix*	*C.* (*H.*) *macrochaetus*	*C.* (*H.*) *persetosus*	*C.* (*C.*) *benacensis*
Type of furca indentation	V-shaped	V-shaped	V-shaped	V-shaped	V-shaped	V-shaped	V-shaped	Parabolic in shape
Number of scales in central longitudinal row	12–14	13–15	12–13	12–13	13	14	12–14	13–14
Total number of longitudinal alternating rows of scales	15–17	15–17	15–17	11–13	NA	13–15	NA	11–13
Type of scales	Almost all three-lobed with strong, high, triangular keels and strong spines; shape strongly differentiated within body regions	Almost all three-lobed with strong, high, triangular keels and strong spines; shape strongly differentiated within body regions	Almost all three-lobed with strong, high, triangular keels and strong spines; shape strongly differentiated within body regions	Almost all three-obed with strong, l high, triangular keels and strong spines; shape strongly differentiated within body regions	All three-lobed with strong, high, triangular keels and strong spines; shape strongly differentiated within body regions	Almost all three-lobed with strong, high, triangular keels and strong spines; shape strongly differentiated within body regions	Almost all three-lobed with strong, high, triangular keels and strong spines; shape strongly differentiated within body regions	Almost all three-lobed with strong, high, triangular keels and strong spines; shape strongly differentiated within body regions
Scale size variation	Strongly differentiated within body regions	Strongly differentiated within body regions	Strongly differentiated within body regions	Strongly differentiated within body regions	Size increases gradually from the head towards the posterior trunk region	Strongly differentiated within body regions	Strongly differentiated within body regions	Size increases gradually from the head towards the posterior trunk region
Scale size	Head: 1.9–7.1 x 1.8–6.9 Neck: 2.8–8.6 x 2.7–8.3 Trunk: 3.5–13.1 x 3.7–11.8	Head: 1.5–7.0 x 1.7–8.1 Neck: 2.1–8.7 x 2.3–9.1 Trunk: 3.2–13.3 x 3.2–14.4	Head: 1.4–6.4 x 1.2–6.2 Neck: 2.3–6.1 x 2.1–7.7 Trunk: 3.0–11.8 x 2.6–9.9	Head: NA Neck: 4.5–5.5 Trunk: 5.0–10.0	7.0 x NA–15.0 x NA	6.0–13.0 x 5.0–9.0	4.0–11.0	Head: 2.2 x NA Neck: NA Trunk: 4.9–8.1
Alignment of scales	Scales do not meet and their edges do not overlap; only on the furcal base and furcal	Scales located far from another but posteriolatera lobes slightly overlap the adjacent scales	Scale edges on the head, neck and trunk meet and are juxtaposed but never overlap;	Scales located far from another but posteriolateral lobes slightly overlap the adjacent scales	Scales located far from another but posteriolateral lobes meet and are juxtaposed	Scales located far from another but posteriolateral lobes slightly overlap the adjacent scales	Scales located far from another but posteriolateral lobes slightly overlap the adjacent scales	Scales located far from another

Table 21. Continued

Character	*C.* (*H.*) *borealis* sp. nov.	*C.* (*H.*) *hornsundi* sp. nov.	*C.* (*H.*) *persimilis* sp. nov.	*C.* (*H.*) *hystrix*	*C.* (*H.*) *euhystrix*	*C.* (*H.*) *macrochaetus*	*C.* (*H.*) *persetosus*	*C.* (*C.*) *benacensis*
	appendage do the edges of the scales strongly overlap		only on dorsal surfaces of furcal base and furcal append-ages do the scales overlap					
Presence of a different type of scales on the dorsal and dorso-lateral side of the furcal base and furcal appendages	Present	Present	Present	Present	Present	Present	Present	Present
Presence of an intrafurcal scale	Present	Present	Present	Absent	Absent	Absent	Absent	Absent
Scale coverage of the ventral interciliary field	Covered from end of anterior dilatation of the pharynx to the furcal base	Covered from end of anterior dilatation of the pharynx to the furcal base	Entire ventral interciliary field covered by scales	Entire ventral interciliary field covered by scales	Entire ventral interciliary field covered by scales	Entire ventral interciliary field covered by scales	Entire ventral interciliary field covered by scales	Covered from end of anterior dilatation of the pharynx to the furcal base
Type of scales on ventral interciliary field	One-lobed, plate-like, strongly differentiated in shape and size; from anterior to posterior body region scales from small, rounded and located far from another to large, keels and strongly plate-like with overplapping edges	One-lobed, plate-like, scales strongly differentiated in shape, size; from anterior to posterior body region scales from strongly rounded, hexagonal in far from another shape and located to large, less rounded,	One-lobed, strongly differentiated in shape and size; from anterior to posterior body region scales from rounded and located far from another to large, elongated shape and fusiform in	One-lobed, rounded, without overlapping edges; anterior scales without keels, posterior with keels	One-lobed, rounded, keeless, without overlapping edges	One-lobed, rounded with weak keels, without overlapping edges	One-lobed, rounded, without overlapping edges; anterior scales without keels, posterior with keels	One-lobed, rounded, without overlapping edges; anterior scales without keels, posterior with keels

Table 21. Continued

Character	*C.* (*H.*) *borealis* sp. nov.	*C.* (*H.*) *hornsundi* sp. nov.	*C.* (*H.*) *persimilis* sp. nov.	*C.* (*H.*) *hystrix*	*C.* (*H.*) *euhystrix*	*C.* (*H.*) *macrochaetus*	*C.* (*H.*) *persetosus*	*C.* (*C.*) *benacensis*
		hexagonal with more pronounced keels and strongly overplapping edges						
Number of scales in central longitudinal row on the ventral interciliary field	20–25	17–20	25–29	30	NA	NA	40	NA
Total number of longitudinal alternating rows of the scales	9–11	5–7	8–9	3	NA	5	5	5
Number of terminal scales on the ventral interciliary field	3 pairs	4 pairs	3 pairs	2 pairs	1 pair	1 pair	4 pairs	3 pairs
Type of terminal scales on the ventral interciliary field	I pair: elongated and asymmetrical with anterior edges directed aside and without posterior notches, keeled and spined. II and III pairs: triange shape with strong rounded edges, without posterior notches, keeled and spined	I pair: Oval expanding to posterior end, keeled and spined. II pair: elongated, rounded triangle in shape, keeled and spined III and IV pairs: triangle in shape, keeled and spineless	I pair: elongated, widening oval in shape, keeled and spined; II pair: elongated, oval with constriction in half length, keeled and spined III pair: rounded triangle in shape, keeled and spined	I pair: oval, keeled and spined II pair: oval, keeled and spineless	Oval, keeled and spineless	Oval, keeled and spined	I–IV pairs: oval, keeled and spined	I and II pairs: oval, keeled and spineless; III pair: oval, keeled and spined
Spine length variation	Spine length and thickness	Spine length and thickness	Spine length and thickness	Spine length and thickness	Spine length and thickness	Spine length and thickness	Spine length and thickness	Spine length gradually increases

Table 21. Continued

Character	*C.* (*H.*) *borealis* sp. nov.	*C.* (*H.*) *hornsundi* sp. nov.	*C.* (*H.*) *persimilis* sp. nov.	*C.* (*H.*) *hystrix*	*C.* (*H.*) *euhystrix*	*C.* (*H.*) *macrochaetus*	*C.* (*H.*) *persetosus*	*C.* (*C.*) *benacensis*
	gradually increase from head top towards the furcal base. All dorsolateral, lateral and ventrolateral spines, except posteriormost lateral pair are shorter than the dorsal spines, their length gradually decreases towards the ventrolateral body sides. Ventral spines longer	gradually increase from head top towards the furcal base. All dorsolateral, lateral and ventrolateral spines, except for posteriormost lateral pair, are shorter than the dorsal spines, their length gradually decreases towards the ventrolateral body sides. Ventral spines longer	gradually increase from head top towards the furcal base. All dorsolateral, lateral and ventrolateral spines, except for two posteriormost lateral pairs, are shorter than the dorsal spines, their length gradually decreases towards the ventrolateral body sides. Ventral spines longer	gradually increase gradually from head top towards the trunk posterior region	gradually increase gradually from head top towards the posterior trunk region. All dorsolateral, lateral, ventrolateral and ventral spines shorter than the dorsal spines, their length gradually decreases towards the ventral body sides	gradually increase slightly from head top towards the neck end and increase rapidly from trunk anterior region towards the furcal base	gradually increase slightly from head top towards one third of trunk and next increase rapidly towards the furcal base	to dorsal trunk posterior region. All dorsolateral, lateral and ventrolateral spines are shorter than the central dorsal spines, their length gradually decreases towards the ventrolateral body sides.
Spine length	Head: 2.9–11.20 Neck: 6.0–14.0 Trunk: 7.9–20.2	Head: 2.8–14.1 Neck: 5.4–15.2 Trunk: 7.0–16.5	Head: 3.6–12.1 Neck: 4.8–14.7 Trunk: 7.3–31.0	Head and neck: 2.0–5.0 Trunk: 10.0–21.0	Head and neck: 5.0–7.0 Trunk: 50.0–70.0	Head and neck: 4.0–11.0 Trunk: 17.0–23.0	Head and neck: 2.5–7.0 Trunk: 12.0–23.0	Head: 2.4–4.4 Neck: 3.2–6.0 Trunk: 4.6–14.4
Presence of hair-like spines on the ventral body side ventral spines	Present	Present	Present	Present	Absent	Present	Present	Absent
Presence of *lamellae* on	Present	Present	Present	Absent	Absent	Absent	Present	Absent
Presence and distinctness of lateral denticle on the spines	All spines, except for ventral side spines, have lateral denticle;	All spines, except for ventral side spines, have lateral denticle;	All spines, except for ventral side spines, have lateral denticle;	All spines, except for ventral side spines, have lateral denticle;	All spines have lateral denticle; lateral denticle on the spines	All spines, except for ventral side spines, have lateral denticle;	All spines, except for ventral side spines, have lateral denticle;	All spines, except for ventral side spines, have lateral denticle

Table 21. Continued

Character	*C.* (*H.*) *borealis* sp. nov.	*C.* (*H.*) *hornsundi* sp. nov.	*C.* (*H.*) *persimilis* sp. nov.	*C.* (*H.*) *hystrix*	*C.* (*H.*) *euhystrix*	*C.* (*H.*) *macrochaetus*	*C.* (*H.*) *persetosus*	*C.* (*C.*) *benacensis*
	lateral denticle on the spines becomes more pronounced from head towards the furcal base	lateral denticle on the spines becomes more pronounced from head towards the furcal base	lateral denticle on the spines becomes more pronounced from head towards the furcal base	lateral denticle on the spines becomes more pronounced from head towards the furcal base	becomes more pronounced from head towards the posterior trunk region	lateral denticle on the spines becomes more pronounced from head towards the furcal base	lateral denticle on the spines becomes more pronounced from head towards the furcal base	
Localization of lateral denticle on the spines	Head: *11.96%–18.56%* Neck: *16.01%–18.89%* Trunk: *18.04%–25.82%*	Head: *6.44–12.65%* Neck: *12.18%–15.89%* Trunk: *15.64%–19.06%*	Head: *13.05–17.34%* Neck: *16.51%–17.34%* Trunk: *18.46%–25.15%*	Head: *33.00%* Neck: NA Trunk: *12.00%–17.00%*	NA	Head: NA Neck: NA Trunk: *20.00%–25.00%*	NA	NA
Type of posteriormost pair of trunk lateral spines	Longer, stronger and more curved than other body spines	Longer, stronger and more curved than other body spines	Longer, stronger and more curved than other body spines	Strong, curved, slightly shorter or similar to posterior trunk region spines	Shorter than other posterior trunk region spines	Strong, slightly curved, similar to posterior trunk region spines	Strong, curved, slightly shorter than posterior trunk region spines	Similar to posterior trunk region spines
Length ratio of posteriormost trunk lateral spines	*12.82–16.19%*	*9.82–11.43%*	*10.41–23.25%*	NA	NA	NA	NA	NA
Type of last pair lateral spines	Strong, curved and shorter than trunk spines	Strong, curved and shorter than trunk spines	Strong, curved and shorter than trunk spines	–	Shorter than posterior trunk region spines	–	–	–
Length ratio of posteriormost parafurcal lateral spines	*3.21–4.34%*	*5.39–6.40%*	*5.10–11.27%*	–	NA	–	–	–
Ventral locomotor cilia	Two longitudinal bands wider on the head and neck region	Two longitudinal bands wider on the head and neck region	Two longitudinal bands wider on the head and neck region	Two longitudinal bands merged on the head region	Two longitudinal bands	Two longitudinal bands	Two longitudinal bands on the head region	Two longitudinal bands wider on the head region
Number of dorsal sensory bristles	3 pairs	3 pairs	3 pairs	2 pairs	2 pairs	2 pairs	2 pairs	2 pairs

50C). Three pairs of ventral interciliary field terminal scales are present. The first pair (at U80–U87) is large, elongated, widening and oval in shape. They have a long keel and a long, thin and straight spine extending towards the internal furcal indentation (scales 31; Figs 43C, 45S). The second (at U85–U88) pair is elongated, oval, narrowing in shape, has a long, strong keel and a long, straight spine extending towards the internal furcal indentation (scales 32; Figs 43C, 45T). The third pair (at U88–U89) is small, rounded and triangular in shape. They have a long, straight keel and a short, straight spine (scales 33; Figs 43C, 45U). The second and third pairs of ventral interciliary field terminal scales are situated at the furcal appendages.

The pharynx (from U3 to U30) has marked anterior and distinct posterior dilatations. The anterior dilatation has weak reinforcements composed of two, short, thin and straight cuticular rods connected with each other at straight angles (at U5–U6). The posterior dilatation is wider than the anterior one (Figs 43B, 51A). The pharynx is connected through the pharyngeal-intestinal junction to the straight intestine, running from U31 to U85. The pharyngeal-intestinal junction is clearly demarcated, short and narrow (U31–U32). The intestine has a short separate anterior section running from U31 to U32 (Figs 43B, 47B, 48B, 49B). The X-organ of this species (observed in one specimen) is located at U83–U84 near the terminal part of the intestine. It is small, bilobed, built from two extensions enveloped in a thin coat and connected by a thinner band located ventral to the intestine. The extensions have a granular, grain-like structure. The thin coat and the cellular bridge connecting the extensions have a smooth and homogeneous structure. The sperm packets of this species are circular in shape and contain spermatozoids (per 4 in one packet) in the form of a short rod. They are located at U65–U66 on both sides, juxtaposed to the intestine.

Remarks. The total body length of juvenile and subadult specimens of *Chaetonotus* (*Hystricochaetonotus*) *persimilis* sp. nov. varied from 93.3 to 116.8 μm; the length of the pharynx was between 31.2 and 34.1 μm; of the intestine from 37.3 to 59.6 μm; there were 15 total longitudinal rows of scales (5D+2DL+2L+4LV+ +2V) with 13 scales in the central row. Of the 15 adults, 5 individuals had a large, developing egg, 1 had two eggs that were large and similar in size (Fig. 52A), 1 had an X-organ and two sperm packets, and 1 had spherical formations inside the integuments (probably some kind of microsymbiont structures).

For a discussion on the phylogenetic affinities *C.* (*H.*) *persimilis* sp. nov., see the 'Remarks' section for *C.* (*H.*) *borealis* sp. nov.

Differential diagnosis. *Chaetonotus* (*Hystricochaetonotus*) *persimilis* sp. nov. most closely resembles the other two new species from the Hornsund fjord area as described in this paper, namely *C.* (*H.*) *borealis* sp. nov. and *C.* (*H.*) *hornsundi* sp. nov. as well as *C.* (*H.*) *hystrix* Mečnikow, 1865, *C.* (*H.*) *euhystrix* Schwank, 1990, *C.* (*H.*) *macrochaetus* Zelinka, 1889, *C.* (*H.*) *persetosus* Zelinka, 1889 and *Chaetonotus* (*Chaetonotus*) *benacensis* Balsamo *et* Fregni, 1995. The differential diagnosis for all new *Hystricochaetonotus* representatives is given in Table 21. See also 'Differential diagnosis' for *C.* (*H.*) *borealis* sp. nov.

Genus ***Lepidodermella*** Blake, 1933

Typus generis. *Lepidodermella squamata* (Dujardin, 1841).

Terra typica. France.

The polyphyletic genus (Kånneby *et al*. 2012, 2013) comprises 13 nominal freshwater species and 1 nominal marine species (Todaro 2018). It is present in benthic, interstitial and periphytic habitats.

Lepidodermella polaris sp. nov.
(Figs 53–58; Tables 22–24)

Localities: 6, 9, 12.

Material. Three samples, in total 7 specimens (5 adults, 1 subadult and 1 juvenile), all photographed. The photomicrographs are available in the Natural History Collections at Adam Mickiewicz University in Poznań under accession numbers NHC–GLP–18– –1–10/h (holotype) and NHC–GLP–18–11–50/p (paratypes) and in the author's collection.

Etymology. Referring to the polar region where the species was found.

Diagnosis. Slender, bottle-shaped body measuring from 144.7 to 199.8 μm. Head five-lobed, cephalion narrow, epipleurae larger than hypopleurae. Cephalic plates weakly demarcated in head outline. Hypostomium small and kidney-shaped. Beneath the hypostomium, two additional plates. Ocellar granules absent. In mouth ring, two weak cuticular teeth arising from anterior pharynx region. Whole body covered with strongly overlapping scales. Scales large, strongly extraverted, visible as plates with double edges. Only anterior scale part connected with cuticle, remaining part of scale free. Scales distributed in 17–21 total longitudinal rows (3–5D+4DL+4L+2–4LV+4V) with 24–29 scales in the central row. Scales in areas of head, neck and trunk differ morphologically. Spines absent. Ventral interciliary field covered with semirectangular plates in pharynx area and scales of same type as scales on dorsal body surface in intestine area. Two pairs of ventral interciliary field terminal scales present. Pharynx narrow with anterior dilatations, expands gradually towards posterior end. A single, narrow, curved

cuticular rod inside anterior pharynx dilatation. Intestine straight with anterior section differing in form of morphology, demarcated as a narrow band.

Description. *Lepidodermella polaris* sp. nov. has a slender, bottle-shaped body. The head is slightly wider than the neck. The neck gradually tapers from the head (from U21) to the beginning of the trunk (*ca.* U32) (Figs 53, 55). The trunk gradually dilates from *ca.* U33 to *ca.* U61, where it is at its maximum width. Then it gradually tapers up to the beginning of the furcal base (at U83) (Figs 53, 55). The branches of the furca are set wide apart. The furcal indentation is V-shaped and the ends of the adhesive tubes point outwards. The adhesive tubes are relatively short in comparison to the length of the body and are straight, thick and slightly taper towards the ends. The adhesive tubes' ends are blunt with slightly rounded edges.

The head is five-lobed, long and parabolic in shape. The cephalion and pleurae are separated from each other by shallow notches. All plates are visible in the head outline, however, the presence of strongly extraverted scales on the head gives the impression that the plates are weakly demarcated and almost invisible in the body outline (Figs 53, 55–56). The cephalion (U1–U4) is narrow and adheres to the head along its entire length. The epipleurae (U4–U9) are *ca.* three times larger, more convex and more rounded than the hypopleurae (U9–U11) (Figs 53, 55–56). The epipleurae on the dorsolateral, lateral, ventrolateral and ventral side of the head are visible from each side of the body and clearly demarcated in the head outline (Figs 53, 55–56). The hypopleurae are smaller than the epipleurae, flat and mostly located on the ventrolateral and ventral side of the head. Only small parts of the hypopleurae are visible from the dorsal side (Figs 53, 55–56). The hypostomium (U4–U5) is small, kidney-shaped and has two small protuberances placed anterolaterally. One pair of additional plates is located posterolaterally to the hypostomium, at U6–U8. These plates are semitriangular in shape and their lateral edges extend to the edges of the ventral epipleurae (Fig. 58B). Two pairs of cephalic ciliary tufts are present. The anterior tufts have 4 cilia each. The first three cilia emerge on the ventral side near the anterior edges of the epipleurae. The posteriormost cilium from the anterior tuft emerges on the dorsal side in the indentation between the cephalion and epipleurae. The length of the cilia increases gradually from the anteriormost, very short cilium to the posteriormost, long cilium. The posterior tufts have 6 cilia each, and all of their cilia emerge on the ventral side in the indentation between the epipleurae and hypopleurae. The length of the cephalic cilia in the posterior tufts increases from the anteriormost to the posteriomost cilium. The posteriormost cilia's posterior tufts are the longest cephalic cilia. All cilia in both tufts are thick. Ocellar granules are not present. The mouth ring is narrow, located terminally at U1–U3 and has weakly marked, long and finger-like reinforcements as well as short cuticular inner hairs. Inside the mouth ring, two small and weak cuticular teeth arising from the anterior pharynx region are present. Laterally to the mouth ring, long suboral bristles emerge in lines near the posterior edge of the cephalion.

The entire body, including the furcal appendages, is covered with large plate-like scales. All of the scales on the dorsal, dorsolateral, lateral, ventrolateral and ventral body surfaces have a strongly extraverted and convex anterior part (scales 1–45; Fig. 54). Only this part is connected with the cuticle, the rest of the scales are free and look as if they have double edges (Figs 53, 55–56). The scales strongly overlap throughout the entire body and no part of the cuticle is naked, the body is fully covered with cuticular structures. All scales are devoid of spines and, except for the two pairs of posteriormost ventral interciliary field scales, are also devoid of keels. The scales are distributed in 17–21 longitudinal alternating rows (3–5D+4DL+4L+2–4LV+4V) with 24–29 scales in the central row. The longitudinal rows of scales run straight and in parallel from the top of the head to the trunk posterior region. The scales are arranged in rounded arcs and in a rosette only on the furcal base and furcal appendages (Figs 53, 55–56). The head scales adjacent to the cephalion and pleurae on the dorsum are of a different type and shape than the other head scales (scales 1; Fig. 54A). The medial dorsal scales adjacent to the cephalion are longer, quite narrower and with more convex anterior edges, located laterally with a pair of distinct horn-like protuberances. The scales located laterally to these scales are oval and asymmetrical (scales 2; Fig. 54B). The dorsal and dorsolateral scales adjacent to the epipleurae and hypopleurae are asymmetric and quite longer than the subsequent head scales; they have slightly more convex anterior edges with distinct horn-like protuberances (scales 3 and 4; Figs 53A, 54C–54D). The subsequent head scales are rounded, sub-oval in shape with a short, weakly elongated anterior scale edge (scales 7 and 8; Figs 54G–54H). The posterior head region scales gradually become more rectangular and smaller. The scales of the neck are rectangular in shape and are clearly smaller than the head scales (scales 9; Fig. 54I). The trunk scales are larger, and gradually increases from the anterior towards the widest body region less than one-half of the length of the trunk (*ca.* U61), after which gradually they decrease in size towards the furcal base. The trunk scales are rounded, sub-oval with a short anterior edge. Their anterior edges are located laterally with a pair of weak horn-like protuberances (Figs 54J–54K). The posterior trunk region, furcal base and furcal appendages are covered dorsally by smaller

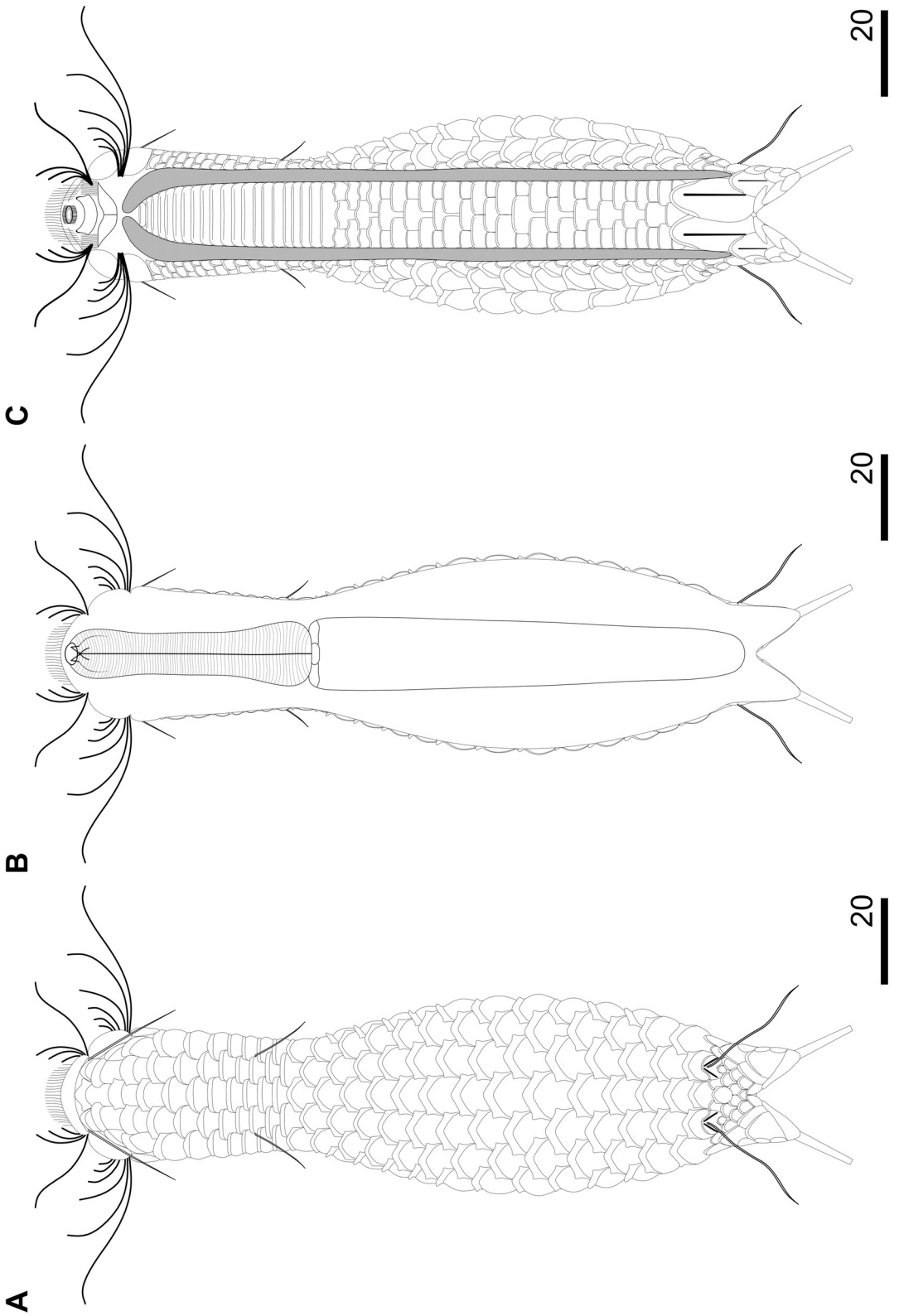


Figure 53. *Lepidodermella polaris* sp. nov. Schematic drawings. A – Dorsal body view. B – View of internal morphology. C – Ventral body view. Light grey areas indicate the areas of the insertion of ciliary bands.

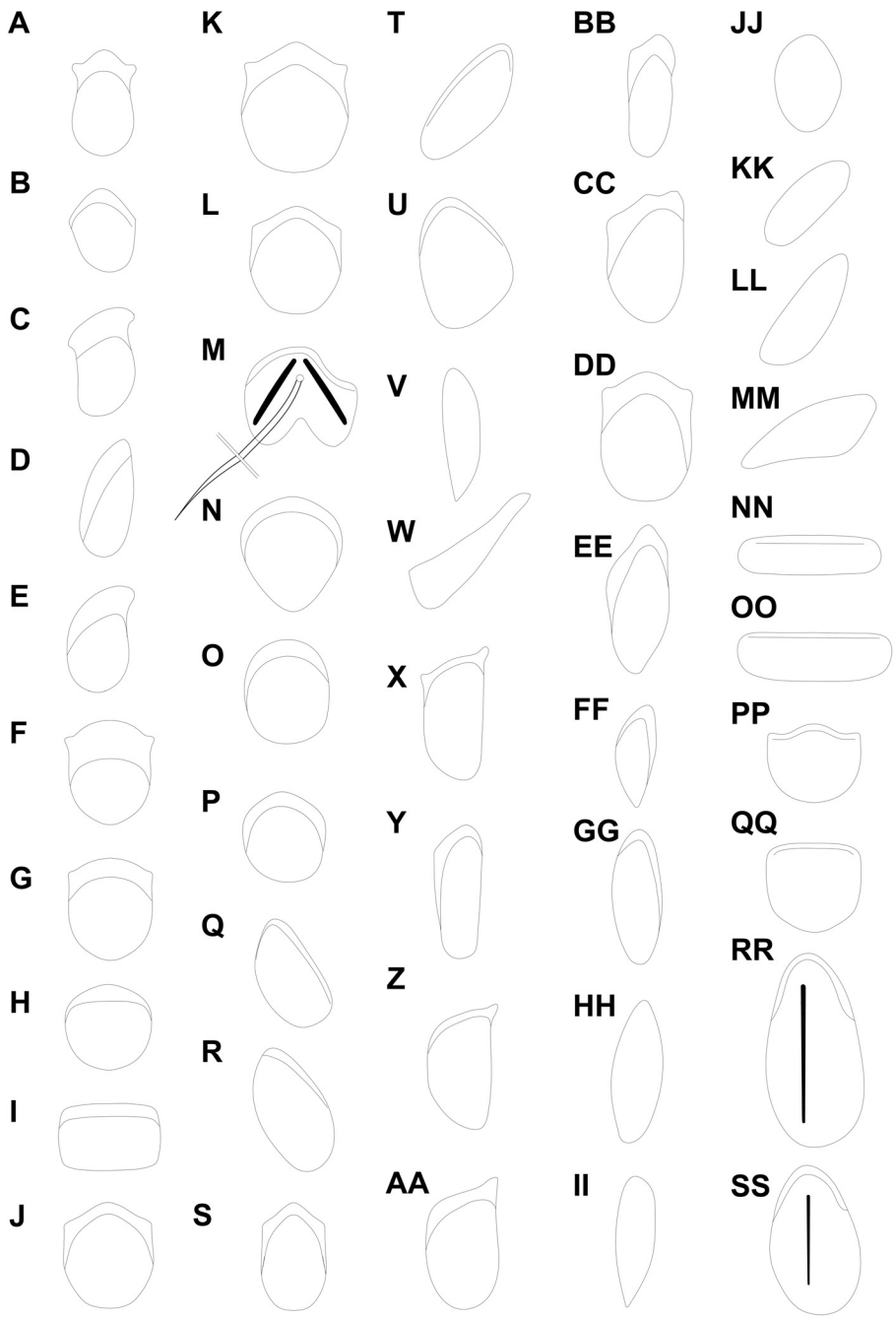


Figure 54. *Lepidodermella polaris* sp. nov. Schematic scales drawings. A – Head anteriormost dorsal central scale (scale 1). B – Head anteriormost dorsal lateral scale (scale 2). C – Head anterior dorsal central scale (scale 3). D – Head anterior dorsal central scale (scale 4). E – Head dorsal lateral scale (scale 5). F – Head dorsal central scale (scale 6). G – Head central region scale (scale 7).H – Head posterior region scale (scale 8). I – Neck dorsal, dorsolateral and lateral scale (scale 9). J – Trunk anterior region dorsal scale (scale 10) K – Trunk central and posterior dorsal, dorsolateral and lateral scale (scale 11). L – Furcal base dorsal central scale (scale 12). M – Double-keeled scale (scale 13). N – Small, tear–shaped scale located inside the posterior notch of double-keeled scale with dorsal sensory bristle (scale 14). O–P – Furcal base central dorsal scales (scales 15 and 16). Q– Furcal base dorsolateral scale (scale 17). R – Furcal base dorsolateral scale (scale 18). S – Furcal base small single central scale (scale 19). T – Furcal appendages dorsal central scale (scale 20). U – Furcal appendages dorsal, dorsolateral and lateral scale (scale 21). V – Furcal appendages lateral scale (scale 22). W – Dorsal intrafurcal scale (scale 23). X – Head ventrolateral scale (scale 24). Y – Head ventral scale (scale 25). Z – Neck ventrolateral scale (scale 26). AA–BB – Neck ventral scales (scales 27 and 28). CC – Trunk ventrolateral scale (scale 29). DD–EE – Trunk ventral scales (scale 30 and 31). FF–GG – Furcal base ventrolateral scales (scales 32 and 33). HH–JJ – Furcal appendages lateral scales (scales 34–36). KK–LL – Furcal appendages ventral scales (scales 37 and 38). MM – Ventral intrafurcal scale (scale 39). NN–QQ – Ventral interciliary field scales (scales 40–43). RR – First pair of ventral interciliary field scale (scale 44). SS – Second pair of ventral interciliary field scale (scale 45).

and more rounded scales forming a rounded arc in a rosette pattern (U80–U88) (scales 12; Figs 53A, 54L, 57). Posterior to the double-keeled scale with dorsal sensory bristles (at U86) is a pair of small, oval scales with weakly extraverted posterior edges that strongly overlap posterior parts of double-keeled scales (scales 14; Figs 53A, 54N). Smaller scales, shaped like oval, elongated tear drops, are also present dorsally, dorsolaterally, laterally, internally, ventrolaterally and ventrally on the furcal appendages. All furcal appendages are tightly covered with highly overlapping scales (scales 15–23; Figs 53, 54O–54W, 55–56).

The scales of the dorsal, dorsolateral and lateral surface of the head, neck and trunk are similar in size. The scales on the ventrolateral and ventral surface of the head, neck and trunk are similar in length but tend to become gradually and distinctly narrower towards the ventral ciliary bands. The scales in the ventral longitudinal row located closest to the ciliary bands are distinctly smaller and narrower than the other scales (Figs 53, 56C–56D). Moreover, all scales on the ventrolateral and ventral body surfaces are asymmetric, i.e. the sides of the scales differ noticeably from another (scales 24–31; Figs 53C, 54X–54EE, 55C, 56C-56D). The scales on the surface located further from the ciliary bands have a normal shape, like the other scales of the dorsal, dorsolateral and lateral body surfaces in this region. The scales on the other surface, located closer to the ciliary bands, are straight cut and narrower (Fig. 53C). Ventral scales have straight lateral edges adhering strictly to the longitudinal ciliary bands (scales 24–28 and 31; Figs 54X–54BB, 54EE).

The species has three pairs of dorsal sensory bristles. The first pair is located on the head, very near the lateral edges of the epipleurae (U4); each bristle and emerges from a small, round papilla. The second pair of sensory bristles is very short and located on the neck (U27); each bristle and emerges from a small, rounded papilla. The third posterior pair of sensory bristles emerges from the scales with double-keeled extraverted scales located on the furcal base at U85–U86. These scales are asymmetrical and shaped like trapezoids with strongly rounded edges and with a distinct posterior notch. The keels of these scales are not connected to one another (scales 13; Figs 53A, 54M).

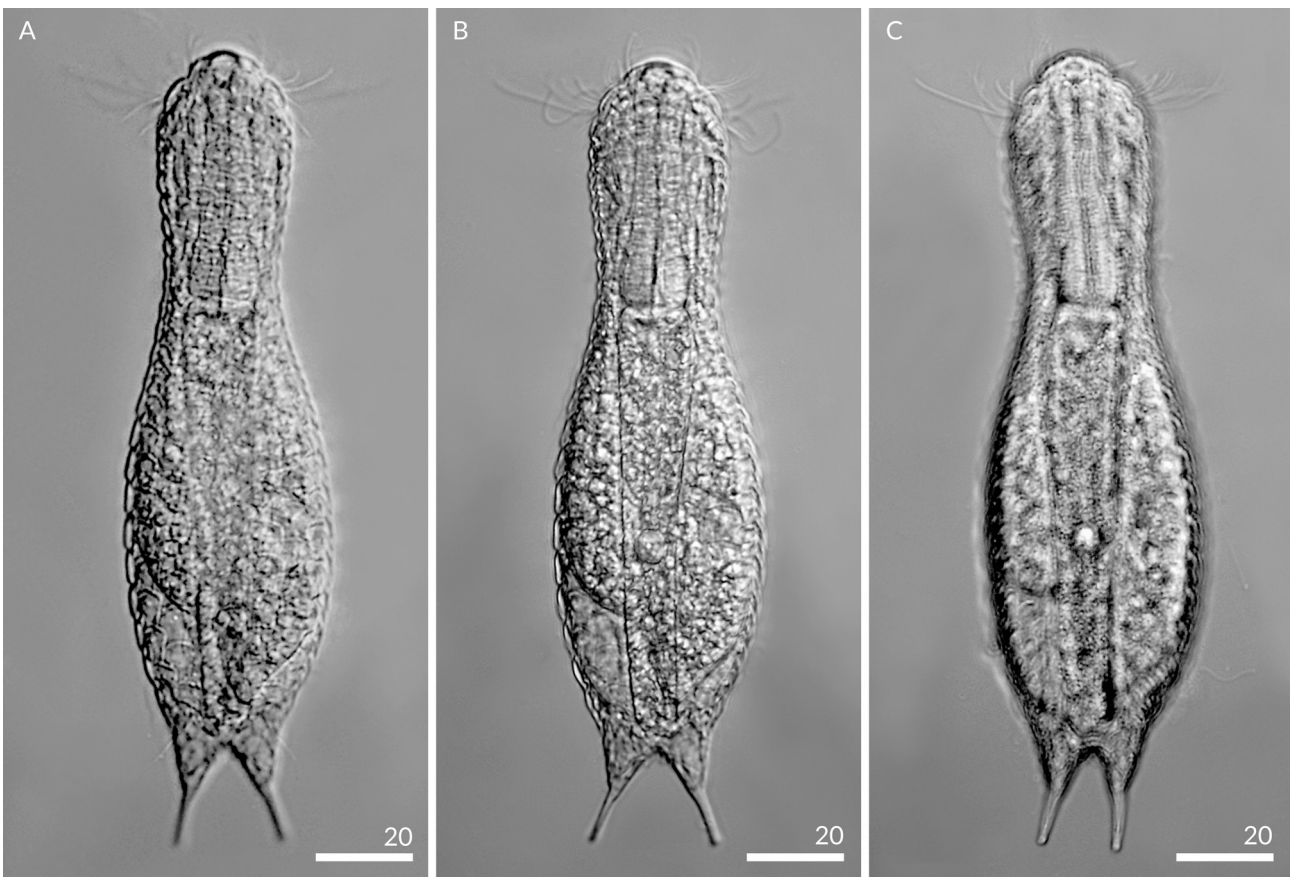


Figure 55. *Lepidodermella polaris* sp. nov. Adult specimen. A – Dorsal body view. B – View of internal morphology. C – Ventral body view.

On the ventral surface, the longitudinal ciliary bands begin at U9 and run back to U86. They are wider in the area of the head than in the other parts of the body, and converge towards each other in front of the first ventral field terminal scales but do not join or adhere (Figs 53C, 55C, 56C). In the remaining part of the body the bands are narrow and in parallel. The whole pharyngeal field and the anterior part of the intestinal field are covered with short, wide plates which are arranged in a single longitudinal row (scales 40 and 41; from U11 to U40) (Figs 53C, 54NN–54MM). The size of these scales gradually increases from the anterior to the posterior end. Starting from U41, the short, wide plates abruptly pass into scales that are similar in shape the dorsal, dorsolateral and la-teral scales (scales 41–43; Figs 53C, 54OO–54PP). Scales in the part of the intestinal ventral interciliary field differ from the other trunk scales by having weaker

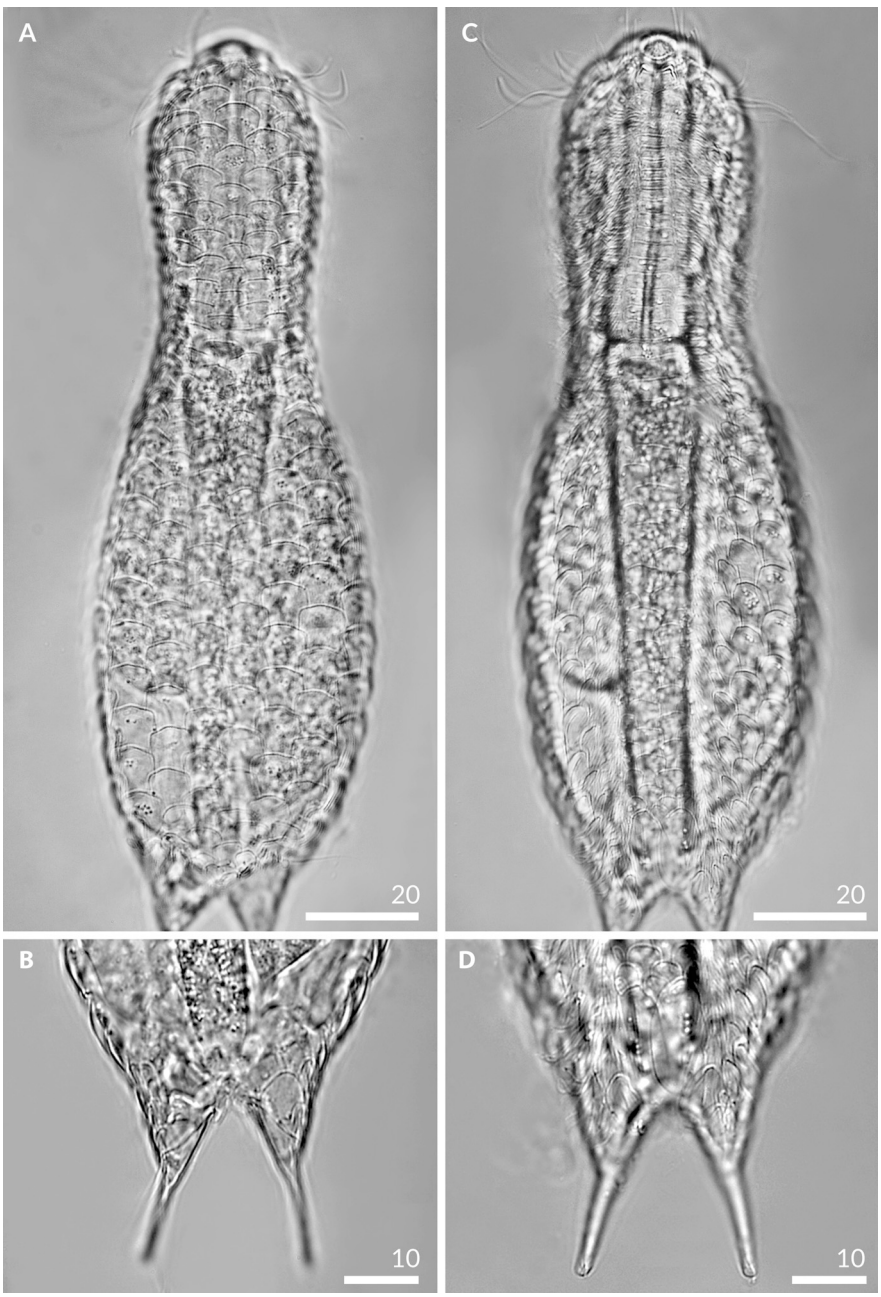


Figure 56. *Lepidodermella polaris* sp. nov. Adult specimen. A – Dorsal view of scales. B – Ventral view of scales. C – Posterior trunk region and furcal base dorsal view of scales. D – Posterior trunk region and furcal base dorsal view of scales.

extraversion of the scales and their weaker curvature, a more rectangular shape, smaller size and absence of a horn-like protuberance. The longitudinal row of ventral field scales closest to the ventral ciliary bands has significantly smaller scales than in the central rows. The first pair of terminal scales on the ventral interciliary field is very large and located at U78–U88. They have an oval shape and their edges overlap. They have a long, weak keel running asymmetrically near the lateral edge rather than in the centre (scales 44; Figs 53C, 54RR, 56D). The second pair of terminal scales on the ventral interciliary field is located on the furcal appendages (at U86–U90) (scales 45; Figs 53C, 54SS, 56D). These scales have an oval shape and a long, weak keel running asymmetrically as in the first pair.

The pharynx (from U3 to U32) has a weakly marked anterior dilatation, posterior to which the pharynx gradually dilates towards the posterior end (Fig. 58A; Table 22). In the anterior dilatation, weak cuticular reinforcements are located in the form of a single, strongly curved rod (at U5–U6). The pharynx connects to the straight intestine, which runs from U33 to U86. The pharyngeal-intestinal junction is clearly demarcated at U34 and is short and narrow. The intestine has an anterior section differing in form and morphology, demarcated as a narrow band running from U33 to U34 (Figs 53B, 55B).

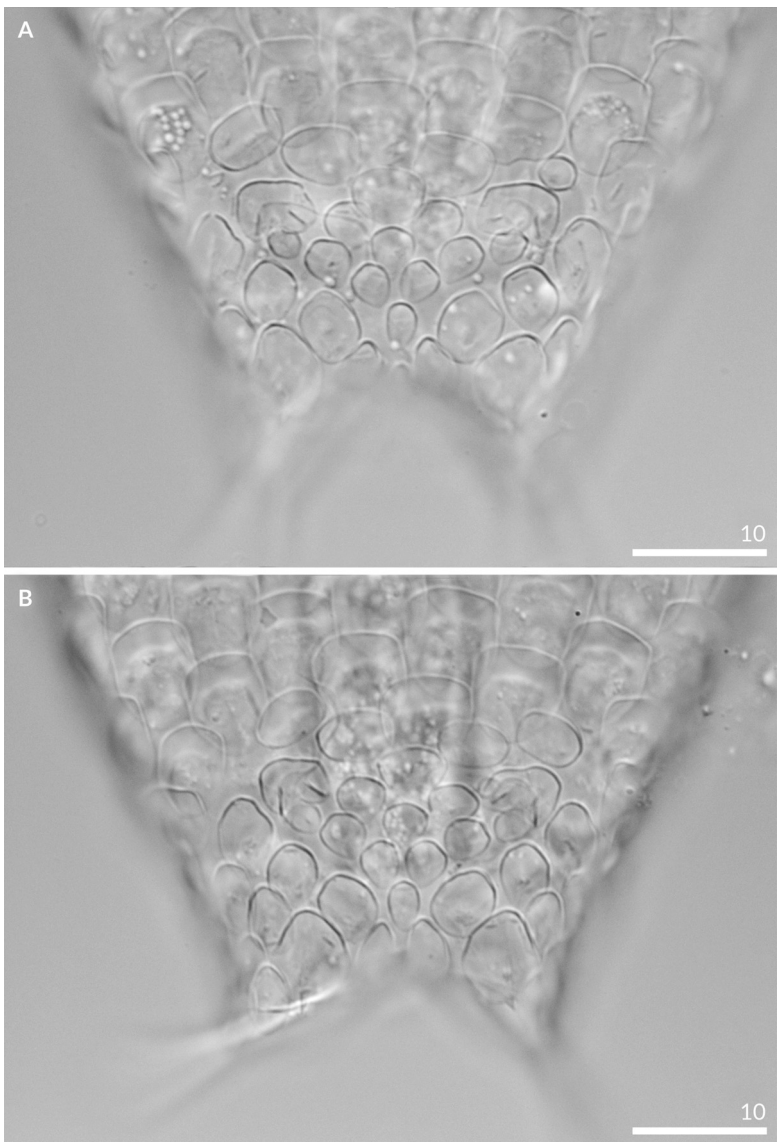


Figure 57. *Lepidodermella polaris* sp. nov. Adult specimen. A – Dorsal view of furcal base scales. B – Dorsal view of furcal appendages scales.

Remarks. Genus *Lepidodermella* Blake, 1933 groups 14 species of which one, *L. squamata* (Dujardin, 1841), is generally considered a complex of cryptic species or a set of very similar species in terms of morphology (*e.g.* Fregni *et al*. 1998; Kånneby *et al.* 2012, 2013). Among the "slender" species which have a body type like *L. squamata* that do not have readily conspicuous distinctive characteristics such as spikes, spines or supplementary plates near the hypostomium, it is difficult to make a description of a new taxon even if it is clearly different from the specimens of *L. squamata* that were originally described by Dujardin in the 19th century (Dujardin 1841). This difficulty results from the similar type of scales present in the genus representatives or from difficulties in describing the different diagnostic characters, such as the shape of the scales, the degree of overlapping or extraversion and convexity. These are features that do not have universal, measurable units and may be regarded as subjective, thus they constitute significant diagnostic characters that allow one to distinguish species which seem to look very similar but are, in fact, different species.

The total body length of the juvenile and subadult specimens of *Lepidodermella polaris* sp. nov. varies from 144.7 to 176.2 μm; the length of the pharynx is between 49.4 and 57.5 μm; of the intestine from 65.8 to 95.5 μm; and there are 21 total longitudinal rows of scales (5D+4DL+4L+4LV+4V) with 27–29 scales in the central row.

In our phylogenetic tree, *L. polaris* sp. nov. forms a monophyletic group with *L. squamata* 1 and *L. squamata* 2 within a well-supported clade grouping also *C.* (*C.*) *microchaetus*, *C.* (*C.*) *similis* and *C.* (*C.*) *maximus*. However, the other *Lepidodermella* species, viz. *L. minor minor* and *L. intermedia*, are recovered with maximal support as a sister group to *Aspidiophorus tetrachaetus*. Representatives of the genus *Lepidodermella* usually have a long pharynx, the relation of pharynx length to body and intestine length is different than in most chaetonotids and the pharynx-intestine ratio (I) also has higher values. The subadult specimens of *L. polaris* sp. nov. have a pharynx-intestine ratio (I) higher than 60%, and the juveniles individuals have the ratio higher than 70%, ratherhigher than 55%, as is usually observed in other genera.

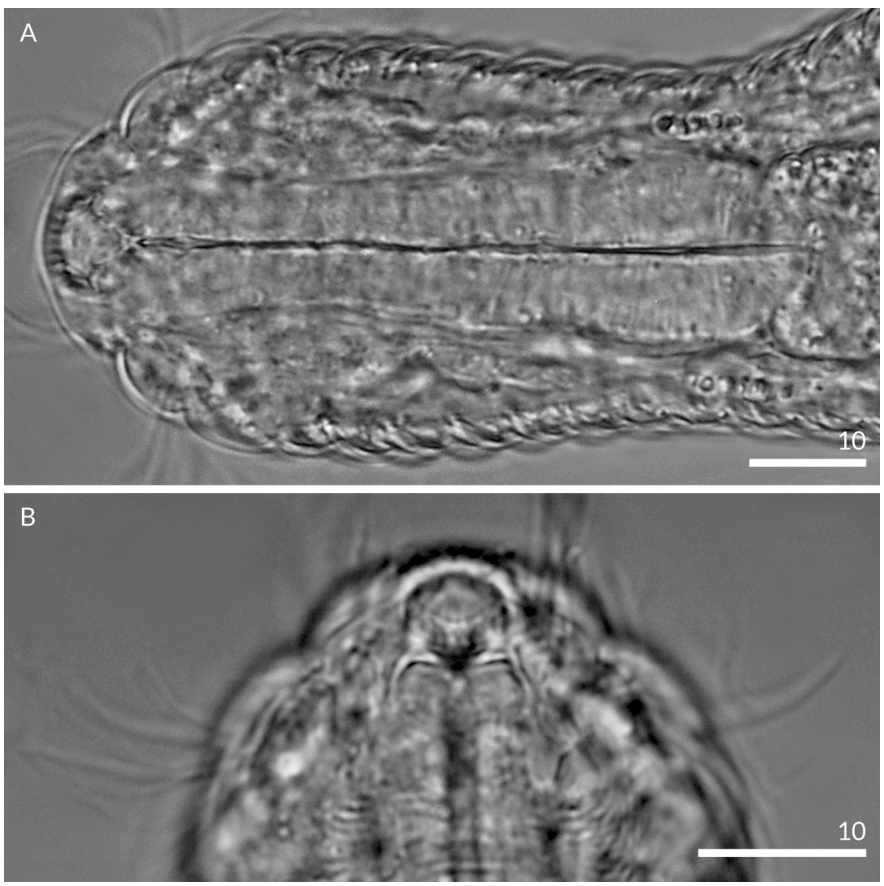


Figure 58. *Lepidodermella polaris* sp. nov. Adult specimen. A – Detail of the pharynx. B – Detail of hypostomium.

Table 22. Main morphometric characters of *Lepidodermella polaris* sp. nov; N – number of specimens or structures analysed, Range – the smallest and the largest structure found among all specimens measured. All measurements are given in micrometers (μm).

Characters	N	Holotype	Range of adult paratypes	N	Range of juvenile paratypes
Body length	4	182.36	167.09–199.79	2	144.7–176.18
Furca length	4	29.06	28.40–32.77	2	27.93–29.48
Adhesive tube length	4	13.81	13.18–14.56	2	13.65–14.04
Number of scales in central longitudinal row	4	26	24–29	2	27–29
Total number of longitudinal alternating rows of scales	4	19	17–21	2	21
Head scales length x width	4	4.96–15.69 x 1.92–10.10	4.23–16.04 x 1.55–10.57	2	2.98–13.87 x 1.59–9.46
Neck scales length x width	4	3.10–9.03 x 1.36–8.79	2.88–10.14 x 1.20–9.23	2	2.48–8.74 x 1.13–8.51
Trunk scales length x width	4	5.58–12.64 x 1.83–9.78	4.07–14.04 x 1.38–10.88	2	4.68–12.15 x 1.47–9.34
Mouth ring diameter	4	5.41	5.40–5.91	2	5.22–5.65
Pharynx length	4	55.02	47.85–59.01	2	49.40–57.54

Table 23. Detailed morphometric characters of *Lepidodermella polaris* sp. nov; N – number of specimens or structures analysed, Range – the smallest and the largest structure found among all specimens measured, SD – standard deviation. All measurements are given in micrometers (μm); all indicators are given as a percentage (%) and italicized.

Characters	N	Holotype	Range of adult paratypes	SD	N	Range of juvenile paratypes	SD
Cephalion length	5	10.66	9.27–11.28	0.741	2	9.88–10.03	0.106
Cephalion width	5	17.94	16.86–18.12	0.5563	2	16.68–17.01	0.233
Hypostomium length	5	8.65	6.75–8.91	0.944	2	7.21–7.32	0.078
Hypostomium width	5	4.01	2.80–4.92	0.758	2	3.78–3.82	0.064
Hypostomium additional plates length	5	9.05	8.39–9.37	0.395	2	8.84–8.99	0.106
Hypostomium additional plates width	5	5.21	4.98–5.96	0.389	2	4.79–4.98	0.134
Length of cephalic cilia (anterior tuft)	5	10.95–21.31	(7.59–12.51)–(19.82–27.87)	1.865; 3.332	2	(8.67–11.94)–(18.32–19.86)	2.312; 1.089
Length of cephalic cilia (posterior tuft)	5	13.26–32.22	(11.38–13.63)–(26.40–40.98)	0.861; 5.752	2	(12.10–12.11)–(20.96–26.19)	0.007; 3.698
Width of cephalic cilia (anterior tuft)	5	1.06–1.13	(1.00–1.08)–(1.07–1.13)	0.036; 0.026	2	(1.01–1.02)–(1.06–1.07)	0.007; 0.007
Width of cephalic cilia (posterior tuft)	5	1.11–1.18	(1.05–1.11)–(1.10–1.18)	0.022; 0.032	2	(1.04–1.05)–(1.07–1.11)	0.007; 0.028
Width of adhesive tube	5	2.07	2.03–2.28	0.094	2	1.97–2.08	0.078
Ratio of scale distribution	5	73.077	*65.517–73.077*	2.865	2	*72.414–77.778*	3.793
Length of head dorsal scales (scales 1–8)	5	6.96–15.69	(6.16–7.07)–(11.96–16.04)	0.352; 1.922	2	(5.41–6.49)–(12.31–13.87)	0.764; 1.103
Width of head dorsal scales (scales 1–8)	5	5.87–10.10	(5.63–6.28)–(9.07–10.57)	0.299; 0.667	2	(4.23–5.72)–(8.91–9.46)	1.054; 0.389
Length of head dorsolateral scales (scales 7 and 8)	5	6.88–11.41	(5.99–7.01)–(9.53–12.26)	0.396; 1.197	2	(5.34–6.43)–(9.79–10.51)	0.771; 0.509
Width of head dorsolateral scales (scales 7 and 8)	5	5.83–8.47	(5.58–6.24)–(7.46–9.33)	0.306; 0.681	2	(4.17–5.69)–(6.22–7.89)	1.075; 1.181
Length of head lateral scales (scales 7 and 8)	5	6.79–11.36	(5.89–6.87)–(9.48–12.20)	0.388; 1.092	2	(5.22–6.32)–(9.72–10.44)	0.778; 0.509
Width of head lateral scales (scales 7 and 8)	5	5.80–8.18	(5.54–6.19)–(7.14–8.97)	0.307; 0.796	2	(4.14–5.64)–(6.09–7.60)	1.061; 1.068
Length of head ventrolateral scales (scales 24)	5	6.57–10.29	(5.70–6.67)–(8.55–11.18)	0.397; 1.146	2	(5.10–6.10)–(9.03–9.39)	0.707; 0.255

Table 23. Continued.

Characters	N	Holotype	Range of adult paratypes	SD	N	Range of juvenile paratypes	SD
Width of head ventrolateral scales (scales 24)	5	2.88–8.14	(2.40–4.68)–(7.06–8.91)	0.934; 0.722	2	(2.63–2.69)–(5.95–7.52)	0.042; 1.110
Length of head ventral scales (scales 25)	5	4.96–8.10	(4.23–5.17)–(6.68–8.46)	0.411; 0.716	2	(2.98–4.51)–(5.12–7.28)	1.082; 1.527
Width of head ventral scales (scales 25)	5	1.92–3.86	(1.55–2.29)–(3.66–4.52)	0.272; 0.332	2	(1.59–1.82)–(2.34–3.33)	0.163; 0.700
Length of neck dorsal scales (scales 9)	5	5.55–9.03	(5.34–6.15)–(7.94–10.14)	0.393; 0.780	2	(4.07–5.83)–(6.96–8.74)	1.245; 1.259
Width of neck dorsal scales (scales 9)	5	5.50–8.79	(5.29–6.07)–(7.89–9.23)	0.382; 0.523	2	(3.98–5.87)–(6.78–8.51)	1.336; 1.223
Length of neck dorsolateral scales (scales 9)	5	5.51–8.98	(5.30–6.11)–(7.87–10.08)	0.395; 0.783	2	(4.01–5.79)–(6.89–8.69)	1.259; 1.273
Width of neck dorsolateral scales (scales 9)	5	4.42–7.53	(4.39–5.73)–(6.97–8.01)	0.596; 0.405	2	(3.13–4.48)–(5.18–7.28)	1.230; 1.485
Length of neck lateral scales (scales 9)	5	5.47–8.44	(5.28–6.06)–(7.55–9.45)	0.377; 0.689	2	(3.98–5.74)–(6.29–8.17)	1.245; 1.329
Width of neck lateral scales (scales 9)	5	4.32–6.62	(4.27–5.01)–(6.10–7.10)	0.382; 0.355	2	(3.11–4.78)–(4.06–6.41)	1.181; 1.662
Length of neck ventrolateral scales (scales 26)	5	5.41–8.37	(5.25–6.02)–(7.47–9.39)	0.366; 0.690	2	(3.94–5.70)–(6.24–8.09)	1.245; 1.08
Width of neck ventrolateral scales (scales 26)	5	3.55–6.23	(3.19–4.29)–(5.74–6.83)	0.414; 0.441	2	(2.39–3.94)–(3.78–6.06)	1.096; 1.612
Length of neck ventral scales (scales 27 and 28)	5	3.10–7.08	(2.88–4.13)–(6.15–8.25)	0.494; 0.871	2	(2.48–3.44)–(4.91–6.78)	0.679; 1.322
Width of neck ventral scales (scales 27 and 28)	5	1.36–6.11	(1.20–1.85)–(4.70–6.25)	0.270; 0.678	2	(1.13–1.64)–(3.51–5.66)	0.361; 1.520
Length of trunk dorsal scales (scales 10 and 11)	5	6.19–12.64	(5.78–6.72)–(11.03–14.04)	0.410; 1.251	2	(5.26–5.96)–(9.90–12.15)	0.495; 1.591
Width of trunk dorsal scales (scales 10 and 11)	5	6.00–9.78	(5.52–6.53)–(9.10–10.88)	0.403; 0.687	2	(5.07–5.78)–(6.96–9.34)	0.502; 1.683
Length of trunk dorsolateral scales (scales 10 and 11)	5	6.13–12.60	(5.73–6.68)–(10.93–13.93)	0.408; 1.243	2	(5.18–5.91)–(9.81–12.10)	0.516; 1.619
Width of trunk dorsolateral scales (scales 10 and 11)	5	5.84–9.41	(5.48–6.41)–(8.74–10.40)	0.382; 0.645	2	(4.95–5.60)–(6.77–9.01)	0.474; 1.584
Length of trunk lateral scales (scales 10 and 11)	5	6.07–12.57	(5.66–6.61)–(10.86–13.89)	0.407; 1.250	2	(5.12–5.89)–(9.75–12.04)	0.544; 1.619
Width of trunk lateral scales (scales 10 and 11)	5	5.71–9.07	(5.37–6.29)–(8.52–10.05)	0.388; 0.602	2	(456–4.81)–(6.26–8.78)	0.177; 1.782
Length of trunk ventrolateral scales (scales 29)	5	6.02–12.49	(5.51–6.56)–(10.80–13.63)	0.436; 1.192	2	(5.09–5.81)–(9.68–11.96)	0.509; 1.612
Width of trunk ventrolateral scales (scales 29)	5	4.82–8.03	(4.59–6.15)–(7.57–8.82)	0.672; 0.509	2	(3.83–3.93)–(5.29–7.81)	0.071; 1.782
Length of trunk ventral scales (scales 30)	5	5.58–11.01	(4.07–6.10)–(9.36–12.18)	0.753; 1.192	2	(4.68–5.41)–(8.06–10.48)	0.516; 1.711
Width of trunk ventral scales (scales 30)	5	1.83–6.60	(1.38–1.93)–(6.14–7.11)	0.210; 0.398	2	(1.47–1.71)–(3.17–5.96)	0.170; 1.973
Length furcal appendaged dorsal scales (scales 12,15 and 16)	5	4.96–9.65	(4.68–6.04)–(8.62–10.02)	0.518; 0.547	2	(3.42–4.16)–(8.33–9.41)	0.524; 0.764
Width of furcal appendages dorsal scales (scales 12,15 and 16)	5	4.22–5.11	(3.82–4.73)–(5.11–6.90)	0.324; 0.847	2	(2.79–3.45)–(4.71–4.92)	0.467; 0.148
Length of furcal appendages dorsolateral scales (scales 17, 18 and 21)	5	4.80–6.42	(4.52–5.04)–(6.09–7.14)	0.191; 0.428	2	(3.36–4.06)–(5.63–6.13)	0.495; 0.354

Table 23. Continued.

Characters	N	Holotype	Range of adult paratypes	SD	N	Range of juvenile paratypes	SD
Width of furcal appendages dorsolateral scales (scales 17, 18 and 21)	5	2.46–3.41	(2.46–2.98)–(3.41–4.19)	0.226; 0.349	2	(2.07–2.40)–(2.82–3.14)	0.233; 0.226
Length of furcal appendages lateral scales (scales 20 and 22)	5	4.28–5.37	(3.99–4.52)–(5.37–6.02)	0.194; 0.265	2	(2.97–3.91)–(4.80–4.98)	0.665; 0.127
Width of furcal appendages lateral scales (scales 20 and 22)	5	1.74–2.93	(1.63–2.19)–(2.86–3.51)	0.226; 0.262	2	(1.44–1.56)–(2.31–2.69)	0.085; 0.269
Head dorsal sensory bristles length	5	15.37	15.09–15.71	0.260	2	14.27–15.03	1.160
Neck dorsal sensory bristles length	5	17.06	16.35–17.29	0.374	2	16.38–16.67	0.205
Posterior dorsal sensory bristles length	5	18.94	17.58–19.19	0.651	2	17.31–18.40	0.771
Number of scales in central longitudinal row on ventral interciliary field	5	33	32–34	0.753	2	34–37	2.121
Total number of longitudinal alternating rows of scaleson ventral interciliary field	5	4	4	0	2	4	0
Length of ventral interciliary field scales on anterior body part (scales 40 and 41)	5	1.36–5.83	(1.29–1.79)–(4.42–7.11)	0.200; 0.952	2	(1.27–1.28)–(4.71–5.19)	0.007; 0.339
Width of ventral interciliary field scales on anterior body part (scales 40 and 41)	5	3.80–11.93	(3.51–3.87)–(9.37–12.06)	0.144; 1.163	2	(3.42–3.79)–(9.64–10.43)	0.261; 0.559
Length ventral interciliary field scales on posterior body part (scales 42 and 41)	5	3.38–8.91	(2.61–3.38)–(6.93–10.50)	0.316; 1.485	2	(2.59–2.71)–(6.80–8.55)	0.085; 1.237
Width of ventral interciliary field scales on posterior body part (scales 40 and 43)	5	2.18–9.06	(1.74–2.18)–(7.67–9.69)	0.182; 0.840	2	(2.02–2.03)–(6.80–9.14)	0.007; 1.655
Length of terminal ventral interciliary field scales I pair (scales 44)	5	16.69	13.35–17.34	1.732	2	11.71–16.27	3.224
Width of terminal ventral interciliary field scales I pair (scales 44)	5	8.52	7.48–9.07	0.577	2	5.54–7.74	1.556
Length of terminal ventral interciliary field scales II pair (scales 45)	5	8.70	4.73–8.78	1.833	2	5.39–8.06	1.888
Width of terminal ventral interciliary field scales II pair (scales 45)	5	5.74	2.82–5.74	1.177	2	3.23–4.87	1.160
Width of anterior pharynx thickening (a)	5	12.27	11.54–13.62	0.750	2	12.12–12.18	0.042
Width of pharynx narrowing (n)	5	9.58	8.10–10.93	1.010	2	8.44–10.69	1.591
Width of middle pharynx (m)	5	11.01	9.45–11.75	0.848	2	10.47–11.25	0.445
Width of posterior pharynx thickening (p)	5	14.81	14.35–15.78	0.549	2	13.72–14.35	0.325
Width of pharyngeal-intestinal junction	5	4.53	4.03–4.83	0.320	2	4.38–4.85	8.686
Length of intestine anterior differ section – in it central part	5	4.80	3.78–4.80	0.396	2	3.73–3.74	0.007
Length of intestine anterior differ section – in it lateralpart	5	3.27	2.96–3.27	0.144	2	2.55–2.78	0.163
Intestine length	5	98.60	82.91–101.22	7.201	2	65.83–95.54	21.008
Pharynx formula a	5	22.301	*22.301–24.176*	0.847	2	*21.168–24.534*	2.380
Pharynx formula n	5	17.412	*16.928–19.309*	0.945	2	*17.085–18.578*	1.056
Pharynx formula m	5	20.011	*19.749–20.703*	0.366	2	*19.552–21.194*	1.161
Pharynx formula p	5	26.917	*26.741–29.990*	1.379	2	*23.844–29.049*	3.680
Pharynx-intestine ratio I	5	55.801	*52.923–58.299*	2.104	2	*60.226–75.042*	10.476

Table 24. Condensed comparison of the most important differential characters between species most similar to *Lepidodermella polaris* sp. nov. Measurements are given in micrometres (μm). NA – data not available.

Character	*L. polaris* sp. nov.	*L. squamata*	*L. acantholepida*	*L. amazonica*
Body length	167.1–199.8	159.0–220.0	140.0–147.4	199.0–200.0
Cephalic pleurae	Cephalion narrow; epipleurae three times larger, more convex and more rounded than the hypopleurae. Epipleurae located on dorsal, lateral, ventrolateral and ventral head side, visible on each body side, clearly demarcated in head outline. Hypopleurae small, flat, mostly located on the ventrolateral and ventral side of the head and only small parts of them are visible from the dorsal side. Hypostomium small, kidney-shaped with two small anterolateral protuberances	Cephalion narrow; epipleurae and hypopleurae similar in size; located laterally. Hypostomium small, kidney-shaped with two small anterolateral protuberances	Cephalion narrow; epipleurae and hypopleurae similar in size; located laterally. Hypostomium composed of two narrow plates, equal length	Cephalion narrow; epipleurae smaller than the hypopleurae; located laterally. Hypostomium small, rectangular in shape, with two small anterolateral protuberances.
Presence of additional hypostomium plates	Present	Absent	Absent	Present
Place of emerging cephalic cilia	Anterior tufts: First three cilia emerge on ventral head side, near anterior edge of epipleurae, the fourth cilia emerge on dorsal side in an indentation between cephalion and epipleurae; posterior tufts: on ventral side in the indentation between epipleurae and hypopleurae	Anterior tufts: Lateral-terminal, betwen cephalion and epipleurae; posterior tufts: laterally, between epipleurae and hypopleurae	Anterior tufts: Lateral-terminal, betwen cephalion and epipleuria; posterior tufts: laterally, between epipleurae and hypopleurae	Anterior tufts: Lateral-terminal, betwen cephalion and epipleurae; posterior tufts: laterally, between epipleurae and hypopleurae
Presence of cuticular teeth in the mouth ring	Two small, weak cuticular teeh	Absent	Absent	Absent
Pharynx length	47.9–59.0	46.0–59.0	36.9	61.0–65.0
Pharynx shape	Pharynx with weakly marked anterior dilatation and expands gradually to the posterior end	Pharynx with weakly marked anterior and posterior dilatations and expands gradually towards the posterior end	Pharynx cylindrical	Pharynx with weakly marked anterior and posterior dilatations and slightly expands gradually towards the posterior end
Anterior intestine section differing in form of morphology	Present	Absent	Absent	Absent

Table 24. Continued.

Character	*L. polaris* sp. nov.	*L. squamata*	*L. acantholepida*	*L. amazonica*
Furca length	28.4–32.8	18.0–24.0	15.2–19.9	NA
Adhesive tube length	13.2–14.6	11.0–17.0	NA	17.0–19.0
Type of furcal indentation Number of scales in central longitudinal row	V-shaped 24–29	Helmet-like shaped 26–31	Helmet-like shaped 28–32	V-shaped 21–23
Total number of longitudinal alternating rows of scales	17–21	13–19	Overall: NA Dorsally: 5–7	9
Type of scales	Plate-like, strongly extraverted, “double edges” scales; shape very strongly differentiated within body regions; ventral scales in longitudinal rows located closest to the ciliary bands are assymetrical	Plate-like, extraverted, "double edges" scales; head scales more rounded than neck and trunk scales	Plate-like, weakly extraverted, "double edges" scales; shape undifferentiated within body regions	Almost all plate-like, extraverted, "double edges" scales; strongly differentiated within body regions; on the furcal base and furcal appendages no extraverted scales
Scale size variation	Strongly differentiated within body regions	Scale size increasing from head top towards the widest trunk region	Similar within body regions	Scale size increasing from head top towards the posterior trunk region
Scale size	Head: 4.2–16.0 x 1.6–10.6 Neck: 2.9–10.4 x 1.2–9.2 Trunk: 4.1–14.0 x 1.4–10.9	Head: NA Neck: 3.0–4.0 x NA Trunk: 6.0–9.0 x 6.5–9.0	Mean diameter: 5.8	Head: NA Neck: 10.5 x NA Trunk: 13.5–21.0 x 10.0–11.0
Alignment of scales	All scales strongly overlapping	All scales overlapping	All scales overlapping	Almost all scales overlapping
Extraversion of anterior scale edges	All scales have a strongly extraverted and convex anterior portion	Dorsal, dorsolateral, lateral, ventrolateral and ventral scales have an extraverted anterior portion	Dorsal, dorsolateral, lateral, ventrolateral and ventral scales have an extraverted anterior portion	Almost all dorsal, dorsolateral, lateral, ventrolateral and ventral scales have an extraverted anterior portion
Presence of a scale with spines	Absent	Absent	Present, one pair of scales on furcal appendages	Absent
Presence of a different type of scales on the dorsal and dorso-lateral side of the furcal base	Present	Present	Present	Present
Scale coverage of the ventral interciliary field	Entire ventral interciliary field covered with scales	Entire ventral interciliary field covered with scales	Entire ventral interciliary field covered with scales	Entire ventral interciliary field covered with scales
Type of scales of the ventral interciliary field	Pharyngeal and anterior part of intestinal field region: short, wide plates arranged in a single	Pharyngeal and anterior part of intestinal field region: short, wide plates arranged in a single	Pharyngeal and anterior part of intestinal field region: short, wide plates arranged in	Pharyngeal and anterior part of intestinal field region: short, wide plates arranged in a single

Table 24. Continued.

Character	*L. polaris* sp. nov.	*L. squamata*	*L. acantholepida*	*L. amazonica*
	longitudinal row, their dimension gradually increases from their anterior to posterior end. Posterior part of intestinal region: wide plates gradually change their shape and number of longitudinal rows and pass into scales similar in shape to those present on the dorsal, dorsolateral and lateral body side, differ from the other trunk scales by weaker extraversion of anterior scales section and curvature, more rectangular in shape, smaller size and absence of horne-like protuberance; longitudinal row of ventral field scales closest to the ventral ciliary bands has significantly smaller scales than in the central rows	longitudinal row. Posterior part of intestinal region: scales similar in shape to those present on the dorsal, dorsolateral and lateral body side, but without extraverted anterior edges	a single longitudinal row. Posterior part of intestinal region: scales similar in shape to those present on the dorsal, dorsolateral and lateral body side	longitudinal row. Posterior part of intestinal region: scales similar in shape to those present on the dorsal, dorsolateral and lateral body side, but without extraverted anterior edges; longitudinal row of ventral field scales closest to the ventral ciliary bands has significantly smaller scales than in the central rows.
Number of terminal scales of the ventral interciliary field	2 pairs	2 pairs	1 pair	1 pair
Type of terminal scales of the ventral interciliary field	Anterior pair: large, oval in shape, with strongly extraverted edges, with long, weak, keels running asymmetrically; posterior pair: large, oval in shape, with keels running asymmetrically	Anterior and posterior pair large, oval, without keels and spines	Large, oval, without keels and spines	Large, rectangular, without keels and spines
Ventral locomotor cilia	Two longitudinal bands wider on the head region; bands converge towards each other but do not join or adhere, remaining part narrow and expanding parallel	Two longitudinal bands	Two longitudinal bands merged on the head	Two longitudinal bands wider on the head region
Number of dorsal sensory bristles	3 pairs	2 pairs	2 pairs	1 pair

Differential diagnosis. *Lepidodermella polaris* sp. nov. more closely resembles *L. squamata* (Dujardin, 1841), *L. acantholepida* Suzuki *et* Furuya, 2013 and *L. amazonica* Kisielewski, 1991. *L. squamata* was selected for a comparison with the new species due to similarity in terms of: range of body length; head and body shape; range of pharynx length; pharynx shape; range of adhesive tube length; range of scale number in the central longitudinal row; type of scale alignment; absence of scales with spines; scale coverage of the ventral interciliary field; and number of terminal scales of the ventral interciliary field. *L. acantholepida* shares with *L. polaris* sp. nov. the following: body shape; alignment of scales; presence of a different type of scales on the dorsal and dorsolateral surfaces of the furcal base; and scale coverage of the ventral interciliary field. *L. amazonica* resembles the newly described taxa in terms of: head and body shape; presence of an additional hypostomium plate; type of furcal indentation; alignment of scales; absence of scales with spines; presence of a different type of scales on the dorsal and dorsolateral surfaces of the furcal base; and scale coverage of the ventral interciliary field. Despite the fact that of all the hitherto known taxa *L. squamata, L. acanthholepida* and *L. amazonica* have the highest number of common features with the newly described species, they are significantly different from *L. polaris* sp. nov., most strikingly by scale shape and variation in differnt body region. A comparison between the new species and morphologically similar taxa has been summarised in Table 24.

Order **Macrodasyida** Remane, 1925

Family **Turbanellidae** Remane, 1926

Genus ***Turbanella*** Schultze, 1853

Typus generis. *Turbanella hyalina* Schultze, 1853.

Terra typica. North Sea.

The genus regarded as monophyletic and cosmopolitan, comprises 29 nominal marine species, usually with a very wide range of ecological tolerance (*e.g.* Boaden *et* Erwin 1971, Kieneke *et al.* 2012, Todaro *et al.* 2015, Todaro 2018).

Turbanella pilosum sp. nov.
(Figs 59–65; Tables 25–26)

Localities: II, III.

Material. Three samples, in total 80 specimens (55 adults, 8 subadults and 17 juveniles), 51 photographed. The photomicrographs are available in the Natural History Collections at Adam Mickiewicz University in Poznań under accession numbers NHC–GTP–19–1–10/h (holotype) andNHC–GTP–19–11–50/p (paratypes) and in the author's collection.

Etymology. From the Latin *pilosum*, "hairiness", referring to the very numerous sensory cilia located over the entire body.

Diagnosis. Relatively slender, strap-shaped body, measuring from 203.1 to 650.8 μm. Head sculptured in front without distinctly demarcated conical appendages (conical cones, palpal organs) and with only a flat protrusion. This species has three circumcephalic bands of long sensory cilia cross around whole head: around a snout-like oral protuberance; in top head corners; in the line from the top of the conical appendages. Ocellar granules absent. Mouth ring oval, narrow, protruding, located terminally and surrounded by sensory cilia. Neck constriction slight, starting just anterior to the anterior adhesive tubes. Trunk widest *ca.* halfway down body length, tapering gradually towards caudal lobes. Caudum small and narrower than trunk, without medial cone. Body outline relatively regular, without strong wave appearance. Anterior adhesive tubes located on back of head on prominent fleshy hands, in adult specimens 5–9 tubes in one tuft; the most internal from anterior adhesive tube significantly shorter than other adhesive tubes. Dorsal, dorsolateral and lateral adhesive tubes arranged relatively symmetrically on both body sides. In adults, 7–11 pairs of dorsal adhesive tubes, 12–20 pairs of dorsolateral adhesive tubes, and 7–11 pairs of lateral adhesive tubes. In adults, only 2 pairs of dorsal, 1 pair of dorsolateral and 1 pair of lateral adhesive tubes on the neck. Adhesive tubes more numerous on the trunk. Posterior adhesive tubes located on posterior and posterolateral margin of caudal lobes. In adults, each caudal lobe bears 5 to 14 adhesive tubes of various lengths. Central tubes are the longest, the most internal tubes are the shortest. All adhesive tubes naked and without supporting structures. Dorsal, dorsolateral and lateral adhesive tubes bear apical cilia; anterior and posterior adhesive tubes without cilia. Many cilia not connected with adhesive tubes arranged dorsally and laterally on neck and trunk; caudal lobes without cilia. Ventral locomotory ciliature on anterior neck region merged on rectangular field; from central neck region ciliature in form of two parallel to each other longitudinal bands running to anus. Very numerous epidermal glands, from small to medium, are usually distributed in two main columns; a single, median epidermal gland is located between the caudal lobes. Pharynx cylindrical and expanding very slightly posteriorly. Pharyngeal pores distinct, near posterior end of pharynx. Pharynx connected via pharyngeal-intestinal junction to straight intestine.

Description. *Turbanella pilosum* sp. nov. has a relatively slender, strap-shaped body (Figs 59, 61).

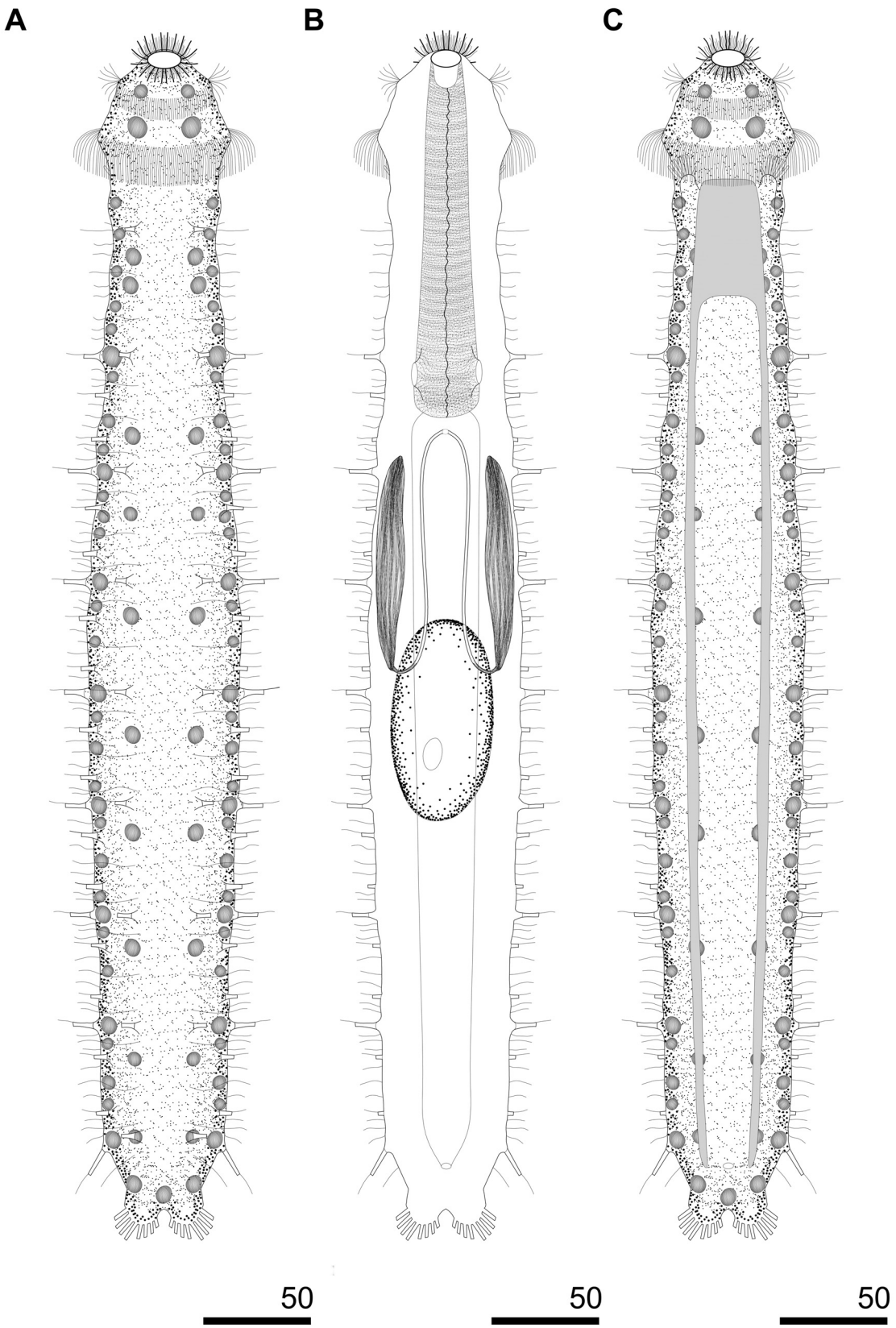


Figure 59. *Turbanella pilosum* sp. nov. Schematic drawings of adutl specimen. A – Dorsal body view. B – View of internal morphology. C – Ventral body view. Light grey areas indicate the areas of the insertion of ciliary bands.

The head is wider than the neck, the neck constriction starts from the head at *ca.* U11 and runs towards the beginning of the wider trunk (*ca.* U32). The trunk is widest halfway down the body length (at *ca.* U50) and gradually tapers towards the caudum. The caudum (from *ca.* U95) is small and clearly narrower than the trunk. The caudal lobes are distinct, semicircular, and not directed outwards. No medial caudal cones are present between the caudal lobes. The body outline is relatively regular, without a strong wave appearance. Small protuberances are present only at the base of the dorsal, dorsolateral and lateral adhesive tubes.

The head is short, similar to a rounded trapezoid in shape, sculptured in front without distinctly demarcated conical appendages (conical cones, palpal organs, auricles) and with only a slight protrusion (at U7–U8). The conical appendages only slightly demarcated in the body outline (Figs 59, 61). Instead of cephalic ciliary tufts, three circumcephalic bands of long sensory cilia encircle the whole head (Figs 59, 61). The first circumcephalic band is located around the snout-like oral protuberance (at U1–U2), and includes both thin cilia and stronger, longer vibratile cilia. The second circumcephalic band is located in the anterior head region at U3–U4, and includes short cilia that are and not too densely arranged. The third circumcephalic band form a line and is located on the anterior part of the conical appendages (at U7–U8), and includes long and very densely located. Ocellar granules are not present. The mouth ring is located terminally (at U1–U2), and is oval, narrow and only slightly protruding. The buccal (mouth) cavity is parabolic in shape, as wide as the anterior pharynx region and has weakly cuticularised walls (Figs 59, 61).

The anterior adhesive tubes are located on the back of the head in distinct, long, movable, fleshy hands at *ca.* U11–U14. In this species, flashy hands are not a false impression caused by the presence of strong longitudinal musculature underlying the anterior adhesive tubes (Kieneke *et* Nikoukar 2017) since these structures are in different positions during the specimen's movement. In adult specimens, 5–9 adhesive tubes are grouped on each tuft (Figs 59, 63B–63C). They are thin, vary in length, and the internal ones are

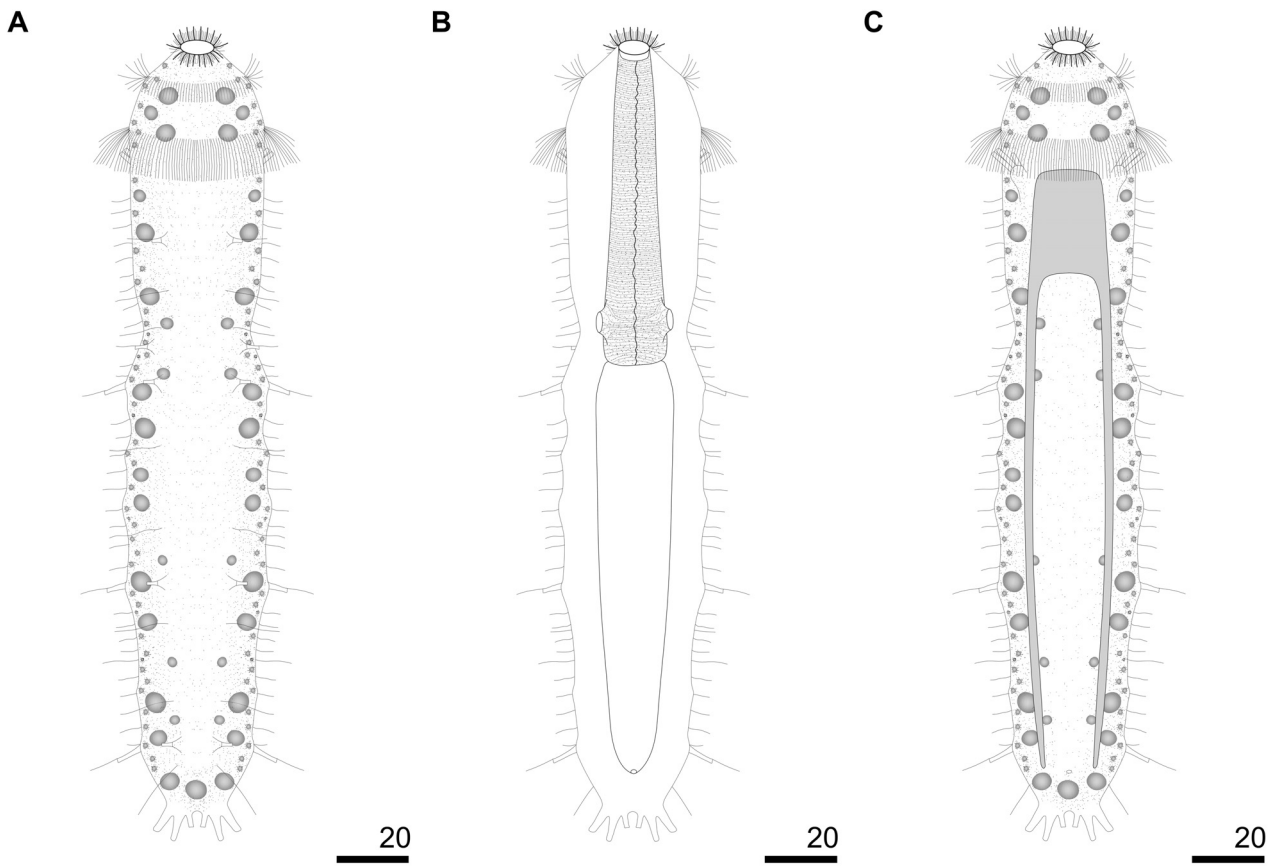


Figure 60. *Turbanella pilosum* sp. nov. Schematic drawings of juvenile specimen. A – Dorsal body view. B – View of internal morphology. C – Ventral body view. Light grey areas indicate the areas of the insertion of ciliary bands.

significantly shorter (*ca*. half) than the remaining ones and are inserted slightly behind the others. In the typical direction of the flashy hands, one or two from the external anterior adhesive tubes project over the body margin. The dorsal, dorsolateral and lateral adhesive tubes are arranged relatively symmetrically in longitudinal rows on both sides of the body. The dorsal adhesive tubes are short and vary slightly length (Figs 59–61, 63E–63F). The first pair of dorsal adhesive tubes located at the neck (at *ca*. U15) is the shortest. They are thin, straight and gradually taper slightly towards their blunt ends. The dorsolateral adhesive tubes are the most numerous, are longer and slightly wider than the dorsal ones. They are straight and slightly gradually tapering towards their blunt ends. Their ends project over the body margin. The lateral adhesive tubes are thick and straight, and do not taper towards their blunt ends. They vary slightly in length: the length of the adhesive tubes gradually increases from the anterior to the posterior part of the body. In adults, 2 pairs of dorsal, 1 pair of dorsolateral and 1 pair of lateral adhesive tubes are present on the neck only (Figs 59, 61D). Adhesive tubes are more numerous on the trunk. The posterior adhesive tubes are located on the posterior and posterolateral margin of the caudal lobes (at *ca*. U98–U100). In adults, each caudal lobe has from 5 to 14 adhesive tubes of various lengths. On each caudal lobe, the central tubes are the longest and the internal tubes are the shortest. When the number of caudal adhesive tubes is even, then they are grouped in pairs of very similar length (Figs 59, 61D, 63H–63I). The difference in length between the pairs is very clear. All adhesive tubes are naked and do not bear any supporting structures (subtube). All dorsal, dorsolateral and lateral adhesive tubes bear apical cilia. The anterior and posterior adhesive tubes do not have apical cilia.

Many sensory cilia, not connected with the adhesive tubes, are present over the entire body. They are arranged in longitudinal, relatively regular parallel rows on the dorsal and lateral body sides of the neck and trunk. The cilia are of different lengths, with the longest present in the dorsal longitudinal rows. There are so many cilia that the body looks hairy (Figs 59–61). Sensory cilia are not present on the caudal lobes.

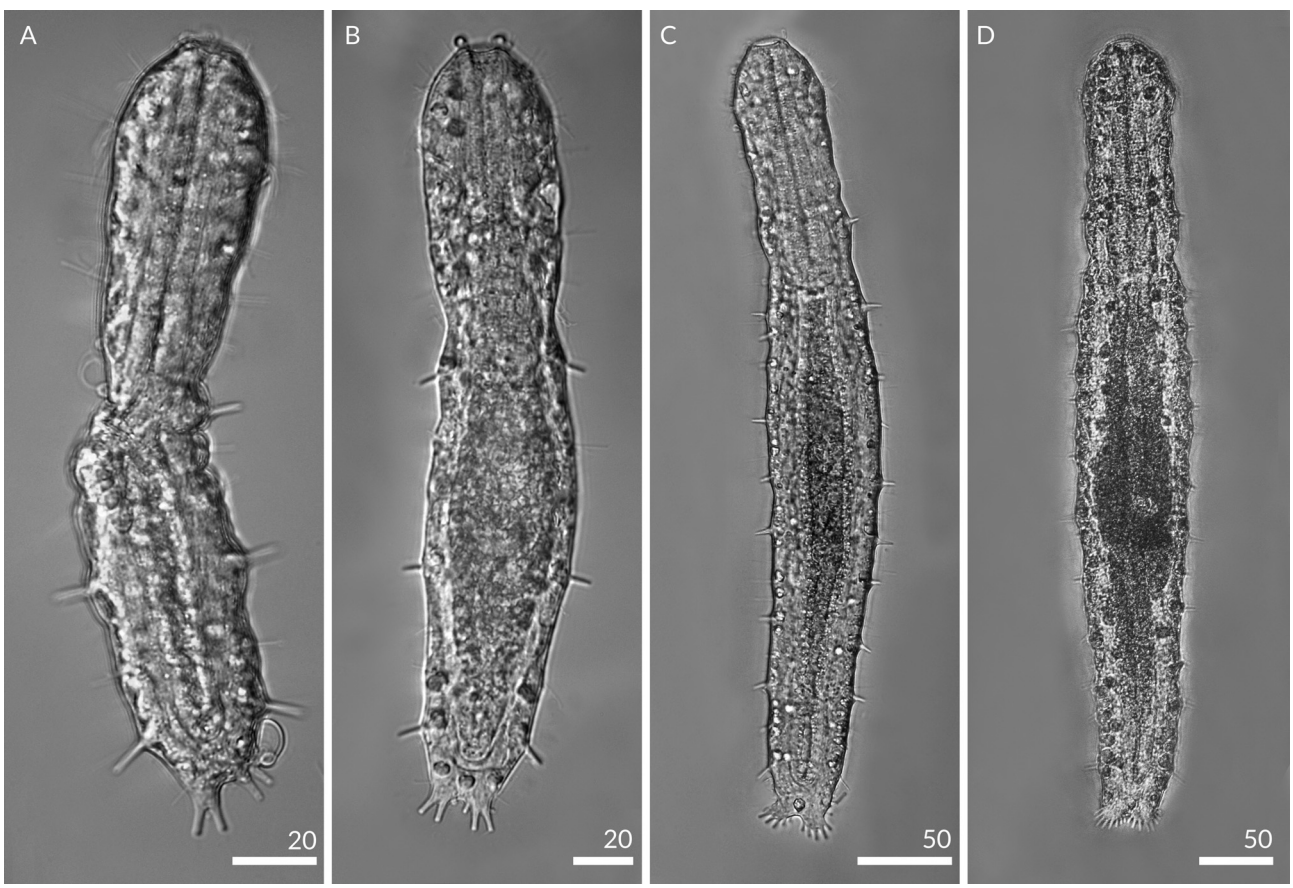


Figure 61. *Turbanella pilosum* sp. nov. A–B – Juvenile specimen. Habitus. C – Subadult specimen. Habitus. D – Adult specimen. Habitus.

On the ventral body, the locomotory ciliature area starts at U12 and runs to U94 (Fig. 59C, 60C). On the anterior neck region, locomotory cilia are located in a wide field (from U12 to U21). Posterior to this region the locomotory cilia are arranged in parallel longitudinal bands that run to the anus.

The epidermal glands, which are usually from circular to irregular in shape, are very numerous and vary strongly in size in this species (Figs 59, 61, 64C). The majority and largest epidermal glands are arranged in regular paired longitudinal rows located on the dorsolateral, lateral and ventrolateral body. A single, large median epidermal gland is situated on the caudum between the caudal lobes (Figs 63G–63I). On the head, epidermal glands are fewer in number in comparison to the neck and trunk. On the head, usually only two pairs of large epidermal glands (*ca*. at U3–U4 and U6–U7; Fig. 62) are present. The smaller epidermal glands are very numerous and arranged less regularly. They are most abundant near the body outline.

The pharynx (from U2 to U94) is cylindrical and expands very slightly towards its posterior end (Figs 59, 64A). Along the entire length and width of the pharynx, abundant, small and coloured (greenish and brownish) vesicle-like inclusions are arranged irregularly. The pharyngeal pores are distinct, disc-shaped and located at U27–U28 (Figs 59, 62B). The pharynx is connected via the pharyngeal-intestinal junction to the straight intestine (at U31). The intestine's end is clearly visible on the ventral body surface as an anus at U94.

The new species is hermaphroditic. It has paired elongated testes located dorsolaterally at *ca*. U35–U52, on both sides of the intestine (Figs 59B, 65A). The thin vas deferens extends anteriorly and connects at U33, where a male genital opening is located on the ventral body surface. Near the posterior edges of each testis, a small residual body, either oval or irregular in shape, is located. This residual body is filled with numerous (*ca*. 10–24 in each) spherical inclusions (*ca*. U49–U52). The bilateral ovaries are located posterior to the testes (between U57 and U74). Both ovaries fuse into the uterus located centrally between U49 and U65. The developing eggs are located in the middle trunk region. The accesory reproductive organ (frontal organ) is

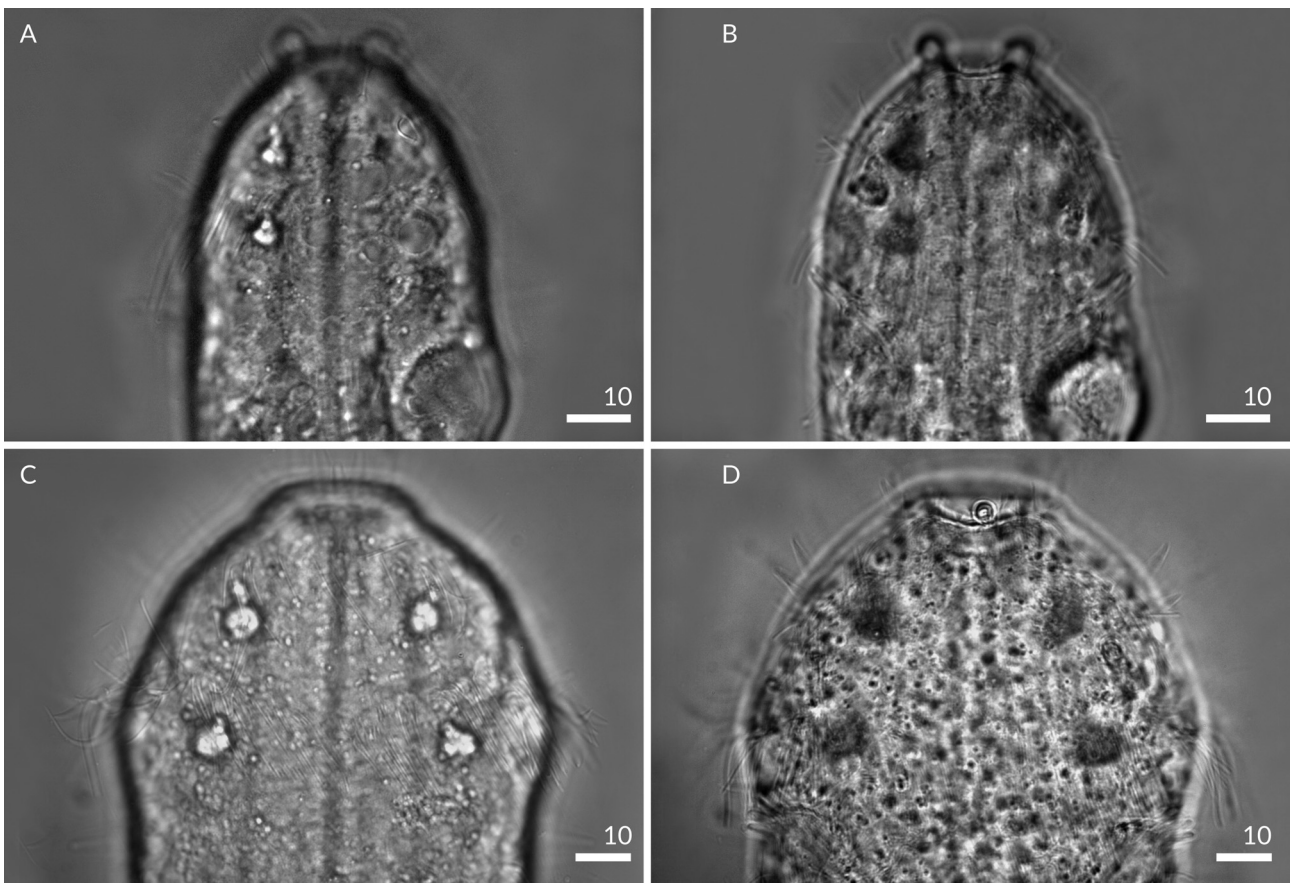


Figure 62. *Turbanella pilosum* sp. nov. A – Juvenile specimen. Dorsal view of head region. B – Juvenile specimen. Ventral view of head region. C – Adult specimen. Dorsal view of head region. D – Adult specimen. Ventral view of head region.

located at *ca*. U64–U69, posterior to the large, matured egg that is located either centrally or slightly laterally. The frontal organ is tear-shaped and subdivided in two different compartments (Fig. 65B). The anterior compartment contains numerous vesicle-like secretion droplets. The posterior compartment has an inconspicuous lumen that contains few strongly coiled spermatozoa. None of the specimens possessed a noticable caudal organ.

Remarks. Different numbers of posterior adhesive tubes in two caudal lobes were observed in two adults from all of the observed specimens. One specimen had 5 adhesive tubes in one lobe and 6 adhesive tubes in the second lobe. The other specimen had 7 adhesive tubes in one lobe and 8 posterior adhesive tubes in the second lobe. Perhaps this difference had resulted from the environmental conditions during the post-embryonic stage or during the on-going process of growth and development, after which the complete number of posterior adhesive tubes is equal. A similar asymmetry in the number of adhesive tubes was observed by Kieneke and Nikoukar (2017) in *Turbanella lutheri* Remane, 1952 (*sensu* Kieneke *et* Nikoukar 2017).

Of the 55 reported adults, 19 individuals had a large developing egg (Fig. 65C) and 7 had a distinct frontal organ (Fig. 65B).

In our phylogenetic tree, *Turbanella pilosum* sp. nov. form a monophyletic group with *T. lutheria* and *T. lutheri* 2, which is consistent with the morphological similarity (see the 'Differential diagnosis' section below). As a sister group – to this clade, three other *Turbanella* representatives are located, namelly *T. cornuta* 4, *T.* sp., *T. cornuta* and *T. cornuta* 2 (Fig. 5).

Differential diagnosis. The genus *Turbanella* Schultze, 1853 currently contains 29 nominal species (Todaro 2018), which are mostly similar and could be confusing at first glance (see Kieneke etet Nikoukar, 2017). All *Turbanella* taxapossessing a strap-shaped, relatively regular body without a strong wavy body outline, without a distinct protrusion, or without any conical appendages demarcated, was chosen for

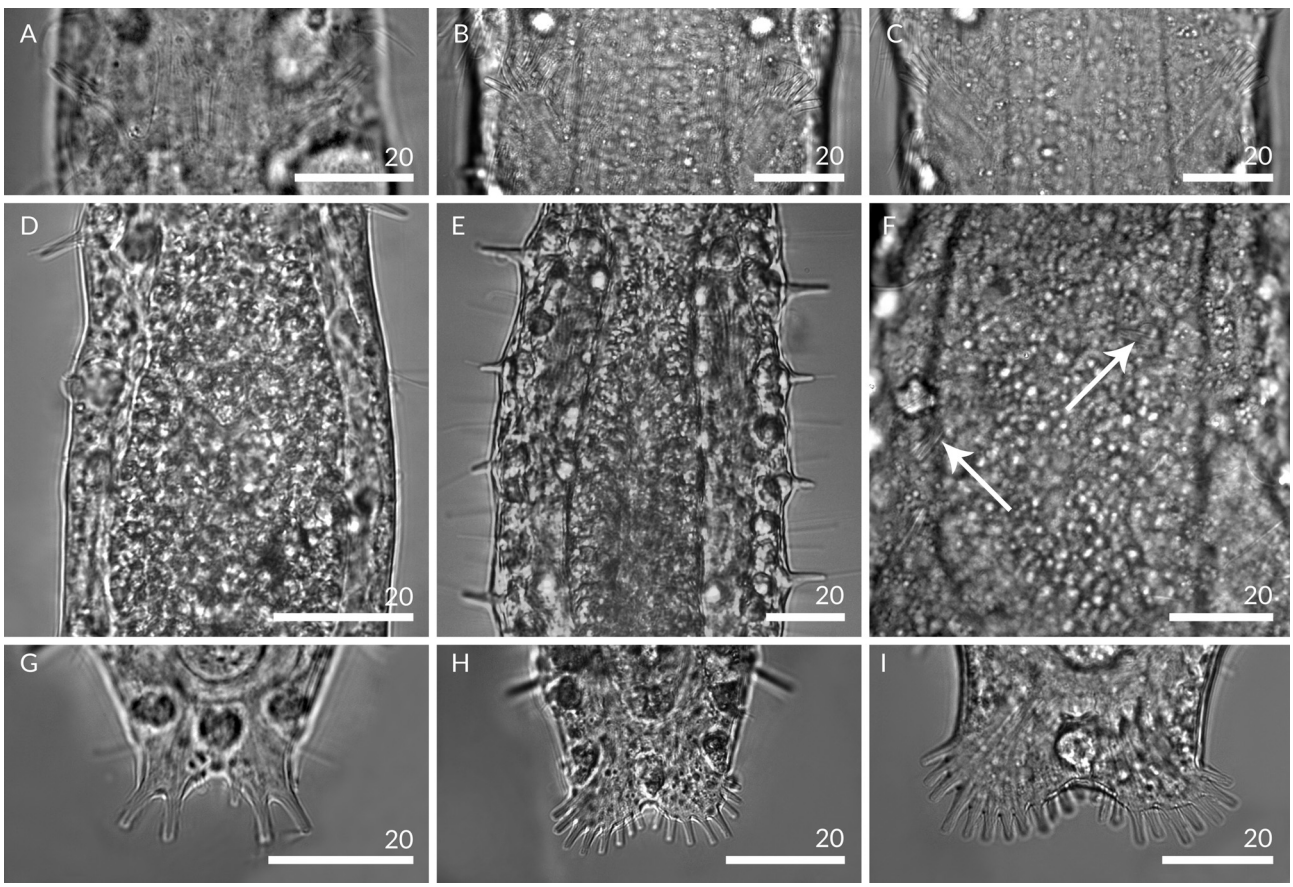


Figure 63. *Turbanella pilosum* sp. nov. A – Juvenile specimen. Anterior adhesive tubes. B–C – Adult specimen. Anterior adhesive tubes. D – Juvenile specimen. Dorsolateral and lateral adhesive tubes. E – Adult specimen. Dorsolateral and lateral adhesive tubes. F – Adult specimen. Dorsal adhesive tubes. G – Juvenile specimen. Caulad adhesive tubes. H–I – Adult specimen. Caulad adhesive tubes.

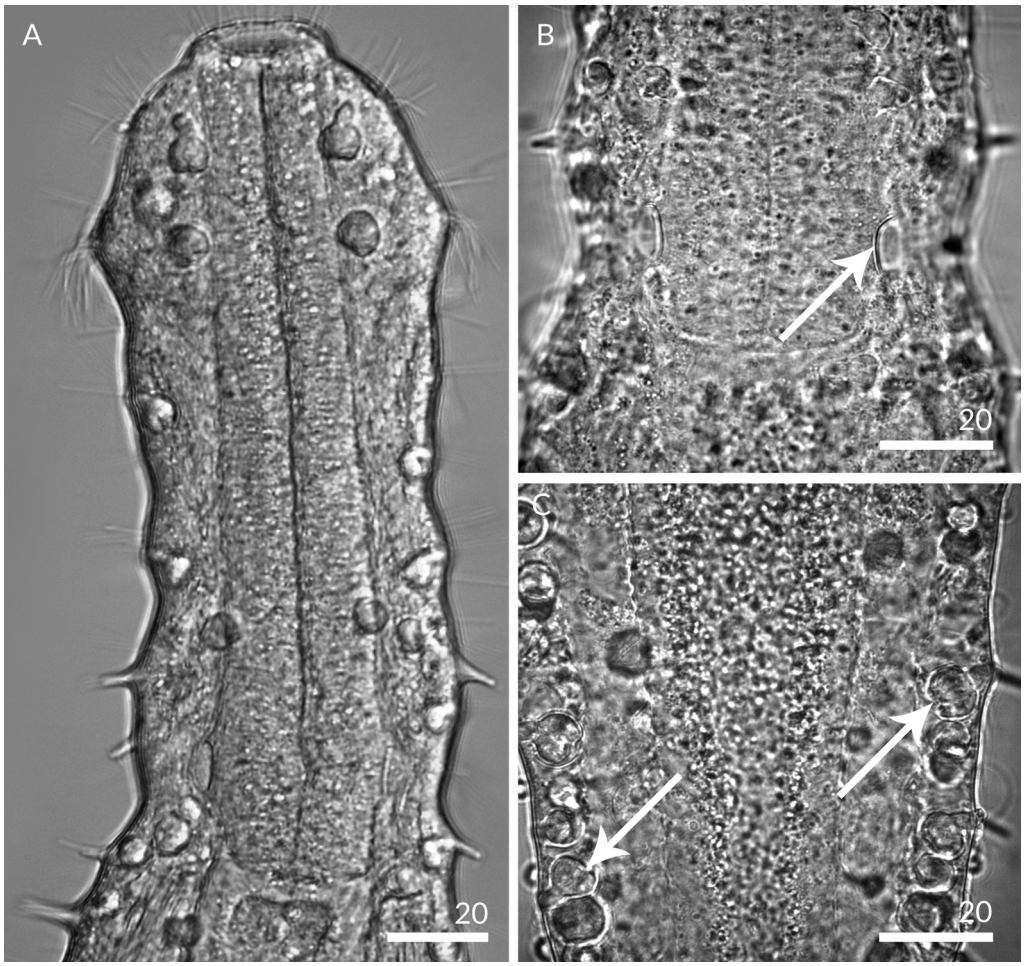


Figure 64. *Turbanella pilosum* sp. nov. Adult specimen. A – Detail of the pharynx. B – Detail of pharyngeal pores. B – Detail of epidermal glands.

comparison with the new species. It turned out that adult specimens of *T. pilosum* sp. nov. more closely resemble *T. lutheri* Remane, 1952 (*sensu* Kieneke *et* Nikoukar, 2017), *T. multidigitata* Kisielewski, 1987, *T. varians* Maguire, 1976, *T. brusci* Hochberg, 2002, *T. caledoniensis* Hummon, 2008 and *T. scilionensis* Hummon, 2008. The comparison between *T. pilosum* sp. nov. and the more morphologically similar taxa is summarised in Table 26.

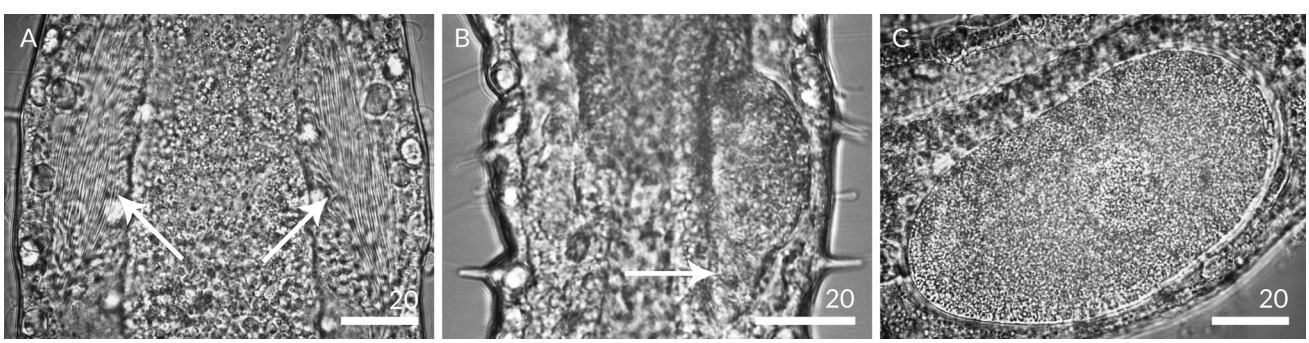


Figure 65. *Turbanella pilosum* sp. nov. Adult specimen. A – Detail of testis. B – Detail of frontal organ. C – Matured egg.

Table 25. Morphometric characters of *Turbanella pilosum* sp. nov; N – number of specimens or structures analysed, Range – the smallest and the largest structure found among all specimens measured, SD – standard deviation. All measurements are given in micrometres (μm).

Characters	N	Holotype	Range of adult paratypes	SD	N	Range of juvenile paratypes	SD
Body length	47	556.23	414.74–650.75	52.516	4	203.06–458.42	124.601
Diameter of mouth ring	20	18.27	16.62–23.73	2.023	4	12.39–17.26	2.232
Suboral hairs length	20	7.37–7.59	(6.67–9.32)–(6.97–9.61)	0.812; 0.822	4	(7.13–7.92)–(7.49–8.07)	0.373; 0.252
Head sensory cilia length	20	12.45–23.08	(11.40–14.19)–(20.68–28.03)	0.730; 2.144	4	(10.54–11.74)–(19.49–23.36)	0.557; 1.687
Dorsolateral sensory cilia length	20	20.03–24.32	(16.91–21.47)–(21.18–29.01)	1.024; 2.278	4	(14.06–20.83)–(19.89–22.36)	2.802; 1.077
Lateral sensory cilia length	20	7.55–14.63	(6.56–11.92)–(11.10–17.25)	1.789; 1.691	4	(5.29–7.09)–(9.29–12.63)	0.788; 1.432
Anterior adhesive tubes length	20	3.19–7.69	(3.19–4.88)–(7.07–10.95)	0.494; 0.962	4	(1.86–4.13)–(5.65–8.16)	1.044; 1.209
Dorsal adhesive tubes length	20	4.53–5.29	(4.28–5.75)–(4.89–6.19)	0.401; 0.364	4	(3.43–4.03)–(4.08–5.66)	0.284; 0.725
Dorsolateral adhesive tubes length	20	5.03–6.54	(4.73–6.62)–(6.24–9.89)	0.504; 0.957	4	(4.43–6.06)–(5.31–7.55)	0.707; 0.961
Lateral adhesive tubes length	20	6.21–13.32	(5.49–7.89)–(10.33–13.64)	0.639; 1.018	4	(4.97–6.33)–(9.04–12.10)	0.663; 1.283
Caudal adhesive tubes length	20	4.96–8.54	(3.43–5.63)–(8.01–11.75)	0.621; 1.129	4	(1.94–6.81)–(6.88–8.52)	2.177; 0.799
Number of anterior adhesive tubes per side	47	7	5–9	0.707	4	2–6	1.826
Number of dorsal adhesive tubes per side	47	9	7–11	0.907	4	4–8	2.309
Number of dorsolateral adhesive tubes per side	47	14	12–20	1.861	4	3–12	5.196
Number of lateral adhesive tubes per side	47	8	6–10	0.914	4	3–7	2.309
Number of caudal adhesive tubes in one caudal lobe	47	8	5–14	1.558	4	2–5	1.500
Epidermal glands diameter	20	1.31–10.02	(0.88–1.62)–(8.05–14.04)	0.202; 1.178	4	(0.74–1.18)–(7.05–9.49)	0.199; 1.135
Pharynx length	20	159.26	150.15–200.20	13.217	4	95.45–137.75	21.889
Anterior pharynx width	20	21.03	18.17–28.74	2.988	4	12.95–18.79	2.491
Posterior pharynx width	20	32.50	26.92–46.21	5.644	4	14.53–27.61	5.503
Testes length	20	82.23	61.14–82.23	6.183	–	–	–
Single testis width	20	16.63	13.29–18.91	1.359	–	–	–
Frontal organ length	7	31.90	20.77–41.21	6.610	–	–	–
Frontal organ width	7	17.34	11.75–21.47	3.076	–	–	–
Egg length	10	97.61	81.51–115.15	10.824	–	–	–
Egg width	10	58.75	45.44–71.40	8.414	–	–	–

Table 26. Condensed comparison of the most important differential characters between species most similar to *Turbanella pilosum* sp. nov. Measurements are given in micrometres (μm). NA – data not available.

Character	*T. pilosum* sp. nov.	*T. lutheri*	*T. multidigitata*	*T. varians*	*T. brusci*	*T. caledoniensis*	*T. scilionensis*
Body length	414.7–650.8	700.0–800.0	900.0–1230.0	550.0–800.0	<1002.0	650.0–700.0	700.0–800.0
Body shape	Strap-shaped body; head wider than the neck, trunk widest halfway down body length and tapering gradually towards the caudum; caudum small, clearly narrower than the trunk. Body outline relatively regular, without strong wave appearance; small protuberance at the base of the dorsal, dorsolateral and lateral adhesive tubes	Strap-shaped body; head wider than the neck, both narrower than the trunk, trunk widest halfway down body length, caudum the same width as the trunk. Body outline relatively regular, without strong wave appearance; small protuberance at the base of the dorsal, dorsolateral and lateral adhesive tubes	Strap-shaped body; similar width throughout entire length, only caudum wider. Body outline relatively regular, without strong wave appearance and protuberance	Strap-shaped body; head, neck and trunk similar in width; caudum small, clearly narrower than the trunk. Body outline relatively regular, without strong wave appearance	Strap-shaped body; similar width throughout entire length, caudum the same width as trunk. Body outline relatively regular, without strong wave appearance and protuberance	Strap-shaped body; body narrowing gradually from head to caudum; caudum widespread. Body outline relatively regular, without strong wave appearance	body; head wider than the neck, trunk widest halfway down its length and tapering gradually towards the caudum; caudum widespread. Body outline relatively regular, without strong wave appearance
Conical appendages	Not distinctly demarcated, only a flat protrusion	Not distinctly demarcated, only a flat protrusion	Absent	Not distinctly demarcated	Not distinctly demarcated	Absent	Absent
Cephalic sensory cilia	Three circumcephalic ciliary bands crossing around whole head, from dorsal to ventral body sides (1) around snout-like oral protuberance; 2) in the top head	Two circumcephalic ciliary bands crossing around whole head, from dorsal to ventral body sides (1) around snout-like oral protuberance; 2) in the line from	Two circumcephalic ciliary bands (1) crossing around snout-like oral protuberance; 2) dorsally in the top head corners and one pair of lateral tufts	Two pairs of tufts with short sensory cilia (1) on each side of snout-like oral protuberance; 2) anteriorly to the neck indentation on ventral side and one ciliary band crossing anterior	Two circumcephalic ciliary bands (1) crossing around snout-like oral protuberance; 2) dorsally in the top head corners and one pair of lateral tufts	Two circumcephalic ciliary bands (1) crossing around snout-like oral protuberance with tufts, longer cilia; 2) around the top head corners and one pair of lateral tufts	Two circumcephalic ciliary bands (1) crossing around snout-like oral protuberance with tufts, longer cilia; 2) around the top head corners and one

Table 26. Continued

Character	*T. pilosum* sp. nov.	*T. lutheri*	*T. multidigitata*	*T. varians*	*T. brusci*	*T. caledoniensis*	*T. scilionensis*
	corners; 3) in the line from the top of the conical appendages	the top of conical appendages and one pair of lateral tufts		part of the head			pair of single, long cilia
Body sensory cilia	Apical cilia connected with dorsal, dorsolateral and lateral tubes and numerous free cilia; numerous on the neck and trunk, absent on caudal lobes	Apical cilia connected with dorsal and lateral tubes and only single free cilia; absent on caudal lobes	Apical cilia connected with part of dorsal, dorsolateral and lateral tubes and numerous free cilia; numerous on the neck and trunk, one pair on caudal lobes	Apical cilia connected with dorsal and lateral tubes and numerous free cilia; numerous on the neck and trunk, absent on caudal lobes	Apical cilia connected with dorsal, lateral, ventrolateral and posterior tubes and numerous free cilia; numerous on the neck and trunk, present on caudal lobes	Only free cilia not associated with adhesive tubes; in two longitudinal rows in trunk, 9–10 per side	Only free cilia not associated with adhesive tubes; in two longitudinal rows in trunk, last dorsal cilia longer than the others, 5–7 per side
Pharynx length	150.5–200.2	162.0–220.0	215.0–277	250.0–290.0	131.0–260.0	171.0 (in subadult)	NA
Pharynx width	Anterior: 18.2–28.7 Posterior: 26.9–46.2	27	NA	NA	19.0–35.0	NA	NA
Pharynx shape	Cylindrical and expands very slightly towards its posterior end; pharyngeal pores distinct, near the posterior end of pharynx	Cylindrical, does not expand to the posterior end; pharyngeal pores distinct, near the posterior end of pharynx	Cylindrical, does not expand to the posterior end; pharyngeal pores distinct, near the posterior end of pharynx	Cylindrical, does not expand to the posterior end; pharyngeal pores distinct, near the posterior end of pharynx	Cylindrical, does not expand to the posterior end; pharyngeal pores present as lateral outgrowths, near the posterior end of pharynx	Narrowing behind the buccal cavity and expands to the conspicuous basal pharyngeal pores; pharyngeal pores distinct, near the posterior end of pharynx	Cylindrical and expands very slightly towards its posterior end; pharyngeal pores distinct, near the posterior end of pharynx
Number of anterior adhesive tubes per side	5–9	4–7	9–15	5–13	6–12	4	11
Number of dorsal adhesive tubes per side	7–11	–	*ca*. 30	–	<22	7	4

Table 26. Continued

Character	*T. pilosum* sp. nov.	*T. lutheri*	*T. multidigitata*	*T. varians*	*T. brusci*	*T. caledoniensis*	*T. scilionensis*
Number of dorso-lateral adhesive tubes per side	12–20	4–6	*ca*. 30	15–19	–	–	–
Number of lateral adhesive tubes per side	6–10	6–12	*ca*. 30	28–32	10–25	7	6
Number of ventrolateral adhesive tubes per side	–	–	–	–	<24	–	–
Number of ventral posterior adhesive tubes per side	–	7–12	–	–	–	–	–
Number of caudal adhesive tubes in one caudal lobe	5–14	6–11	11–15	7–11	6–9	5	7
Length of anterior adhesive tubes per side	3.2–11.0	2.4–9.8	9.0–18.0	*ca*. 9.0	6.0–9.0	7.0–11.0	5.0–10.0
Length of dorsal adhesive tubes per side	4.3–6.2	6.5–19.1	16.0–24.0	–	*ca*. 9.0	NA	NA
Length of dorso-lateral adhesive tubes per side	4.7–9.9	6.3–20.5	16.0–24.0	12.0–16.0	–	–	–
Length of lateral adhesive tubes per side	5.5–13.6	6.3–20.5	16.0–24.0	12.0–16.0	10.0–13.0	NA	NA
Length of ventro-lateral adhesive tubes per side	–	–	–	–	6.0–8.0	–	–
Length of caudal adhesive tubes in one caudal lobe	3.4–11.8	2.6–10.7	4.5–14.0	*ca*. 10.0	3.0–8.0	4.0–8.0	8.0–10.0

Table 26. Continued

Character	*T. pilosum* sp. nov.	*T. lutheri*	*T. multidigitata*	*T. varians*	*T. brusci*	*T. caledoniensis*	*T. scilionensis*
Shape of fleshy hands with anterior adhesive tubes	Distinct, long, movable fleshy hands; adhesive tubes thin, vary in length, internal ones significantly shorter than remaining ones and inserted slightly behind the others	Distinct, short, movable fleshy hands; all adhesive tubes of similar length	Distinct, short, movable fleshy hands; adhesive tubes thin, varying in length, internal ones significantly shorter than remaining ones and inserted slightly behind the others	Not distinct, very short fleshy hands; all adhesive tubes of similar length	Distinct, long with internal support rod on medial base, movable fleshy hands; adhesive tubes thin, similar length, internal ones inserted much lower than the others	Distinct, long, movable fleshy hands; all adhesive tubes of similar length	Distinct, long, movable fleshy hands; length of adhesive tubes increasing from internal to external
Type and arrangement of dorsal, dorsolateral and lateral adhesive tubes	Arranged relatively symmetrically on both body sides; on neck 2 pairs of dorsal, 1 pair of dorsolateral and 1 pair of lateral adhesive tubes; on trunk adhesive tubes more numerous	Arranged more or less asymmetrically on both body sides; on trunk adhesive tubes more numerous than in the neck region	Arranged relatively symmetrically on both body sides; on neck 3–5 pairs of dorsal, dorsolateral and lateral adhesive tubes; on trunk adhesive tubes more numerous	Arranged relatively symmetrically on both body sides; on neck and trunk same, numerous	Arranged relatively symmetrically on both body sides; in the pharyngeal region lateral adhesive tubes are spaced closer together as compared to the trunk	Arranged symmetrically on both body sides; on the neck 1 pair and at the pharyngeal-junction both dorsal and lateral, 5 pairs of dorsal and lateral tubes evenly spaced along trunk, only to anal opening	Arranged symmetrically on both body sides; on neck 2 pairs of dorsal, 3 pairs of lateral adhesive tubes; 2 pairs of dorsal, 3 pairs of lateral adhesive tubes
Shape of caudal lobes and posterior adhesive tubes arrangement	Caudal lobes small, distinct, semicircular in shape and not directed outwards. Median cone absent. Adhesive tubes located on the posterior and posterolateral margin of caudal lobes; central tubes the longest, internal tubes the shortest; when number of caudal adhesive tubes is even they are grouped in pairs of very similar length, difference in length between pairs very clear	Caudal relatively large, distinct, semicircular in shape and slightly directed outwards. Median cone absent. Adhesive tubes located on the posterior and posterolateral margin of caudal lobes; adhesive tubes only slightly varying in length, central tubes the longest, internal tubes the shortest	Caudal lobes long, slender, triangle-shaped and directed wide outwards. Small median cone present. First three external tubes located on the margin and inner located dorsally on the caudal lobes; first external adhesive tubes the longest and thickest, subsequent shorter towards the inner part of the lobe	Caudal lobes small, triangle-shaped and directed outwards. Long median cone present. Adhesive tubes located on the posterior margin of caudal lobes; two first external adhesive tubes the longest, subsequent gradually shorter towards the inner part of the lobe	Caudal lobes small, triangle-shaped and directed outwards. Median cone present. Adhesive tubes located on the posterior margin of caudal lobes; first external adhesive tubes the longest, subsequent gradually shorter towards the inner part of the lobe	Caudal lobes small, triangle-shaped, with a concave surface between lobes; lobes directed outwards. Median cone absent. Adhesive tubes located on the posterior and posterolateral margin of caudal lobes; central tubes the longest, internal tubes the shortest	Caudal lobes small, triangle-shaped, with a concave surface between lobes; lobes directed outwards. Median cone absent. Adhesive tubes forming a series around the lobe with the main 2 located posterolaterally, 2 others on the lateral or external edge and 3 more on the medial or internal edge; all tubes similar in
Number of epidermal glands	Very numerous	Numerous	*ca.* 20 per side	Very numerous	4 in head region; 25 (laterally) and 10 (along intestine merging) per side	17–19 per side	*ca.* 75 per side

Discussion

A proper identification of gastrotrich species is the first step to understanding their diversity, abundance and population structures. Improper determination or definition of a taxon can make it difficult to understand its ecology and evolution, which is associated with problems in comprehension of the formation and functioning of entire communities in a given habitat. Without detailed taxonomic identification, the recording of higher level taxa only provides data that are uninformative and may constitute misleading indicators of biodiversity and environmental changes. A key issue in deliberations on species is the boundry between intra– and interspecific variability, which is related to the classification of an organism/specimen as separate taxa, sets of morphologically resembling species or a complex of cryptic species. This problem is of particular significance since in many groups of aquatic organisms, due to insufficient knowledge about the range of phenotypic variability, separate ecotypes have been regarded as distinct taxa, or independent species have been classified within one taxon, *e.g.* in the cladocerans' *Daphnia longispina* complex (Petrusek *et al*. 2008). Therefore, it is extremely important to combine detailed morphological (qualitative and quantitative) and molecular analyses, which will contribute to obtaining more information on the plasticity ranges of different species and help to dispel doubts about the validity of taxonomic studies, and which cannot be provided by one of the methods alone (*e.g.* Tautz *et al*. 2003; Kipling *et* Rubinoff 2004; Wirta *et al*. 2015).

During the last glacial period, nearly the entire Svalbard Archipelago was completely occupied by a glacier, although some areas remained free from ice and may have provided refuge to some species (Coulson *et al*. 2014). Currently, about 60% of Svalbard is covered by ice, and the areas where a retreat of the glacier has been observed form a new habitat that is undergoing the succession process (*e.g*. Gislason 2005; Coulson 2013). The present study revealed no correlation between the distance from the front of the glacier and species diversity and gastrotrich abundance. The number of species and specimens at the sampling stations, both freshwater and marine, varied widely without any specific distribution pattern.

Freshwater gastrotrich densities are strongly varied, on some station are very high, and reach up to 810 individuals per 1 cm^3 of bottom sediments. The similar density was noted only once from the Isfjorden fjord area (West Spitsbergen) (Kolicka 2017) and reached more than 1026 individuals per 1 cm^3 of bottom sediments. In comparison, the highest density reported from a temperate climate was 101 individuals per 1 cm^3 of bottom sediments (Kisielewska *et* Kisielewski 1986). In the case of marine species, densities are very different and depend on sediment type and structure, depth, tide regime and water temperature (see *e.g*. Kieneke *et* Schmidt-Rhaesa 2015).

The relatively high number of Gastrotricha species and specimens seems to contradict the general notion of the Arctic as a region of poverty caused by its „young age", i.e. the relatively recent retreat of glaciation and harsh environmental conditions which are unfavourable to living organisms for most of the year as well as separation from a continental area (Pirożnikow 2013). Moreover, all of the listed in this work freshwater taxa have some features, regarded as a rare and not commonly presented in other species description, such as: ventral displacement of the point from which the cephalic cilia emerge, a weak cuticular reinforcement in the anterior pharynx dilatation. The intestine anterior section differing from the remaining part, and the presence of three pairs of dorsal sensory bristles.Most probably the long-term in situ evolution within an isolated and confined area, such as the Svalbard Archipelago, may lead to the appearance of new endemic species, often characterised by phenotypic adaptation to local ecological conditions (Hermant *et al*. 2013). Marine gastrotrichs might have met relatively stable conditions without episodes of high mortality, whereas individuals in the inland waters could be strongly influenced by random death rate, which might have favoured genetic diversity and speciation through numerous mosaic-dying separate populations (di Prisco *et* Verde 2011). All these morphological traits of recorded freshwater gastrotrichs could be caused by adaptation to specific Arctic conditions (such as chronic low temperatures, freezing and desiccation stress, environmental variability) or may stem from evolutionary parallelism or convergence (see also Kolicka 2017). This could be connected with these populations which inhabit a harsh environment and must be able to survive in demanding conditions, as well as with the type of gastrotrich reproduction. Considering the shared characteristics, high diversity and abundance of freshwater chaetonotids can in the Hornsund fjord area, we must take into account their high developmental rate and their ability to inhabit all types of aquatic and semi-aquatic habitats (*e.g.* Kisielewski 1997, Balsamo *et al.* 2014, Kieneke *et* Schmidt-Rhaesa 2015). In our research we took DNA sequences from 26 specimens belonging to new or newly recorded taxa. Only one haplotype was found in each of the analysed species, which may suggest a small founding population of these taxa. Probably all species of freshwater chaetonotids reproduce by parthenogenesis, and the typical process is apomictic parthogenesis. In this way, representatives of this order can begain to colonize a new habitat even from a small number of individuals (*e.g.* Kisielewski 1997, Balsamo *et al*. 2014). The second

manner of reproduction occurring among freshwater Chaetonotida is amictic reproduction by cross-fertilisation suggested by the presence of sperm and X-organ. These two ways of sexual reproduction allow freshwater Chaetonotida to produce three types of eggs. Two of these types are the result of apomictic parthenogenesis, i.e. tachyblastic eggs which develop immediately after laying, and opsiblastic eggs (the so-called resting or dormant eggs), which do not develop immediately after laying and which are able to spread after exposure to unfavourable environmental conditions, such as low or high temperatures, drying up, *etc*. These eggs are likely to create a reservoir of individuals in habitats with highly changing environmental conditions (such as astatic reservoirs) and to rebuild entire populations. However, the factors determining the process of laying opsiblastic eggs and their development remain, to date, almost unknown. There are also no empirical data on the conditions and factors regarding the type of eggs that can survive and still be able to successfully hatch. As shown by Nesteruk (2007), after a complete drying up of peatbog reservoirs, during one season the invertebrates were able to reconstruct 70% of their initial abundance once water returned. That reconstruction may have been at least partially promoted by the presence of resting eggs. It is supposed that dormant eggs need a several-month period of rest before they start to develop (Kisielewski 1997). The third type includes eggs derived from amictic reproduction; these remarkably differ from tachyblastic and opsiblastic eggs. It is believed that these are eggs that are laid in the post-parthenogenetic phase of life of the individual; however, there are no reliable data on this subject (Levy *et* Weiss 1980, Hummon 1984, 1986, Balsamo *et* Todaro 1987, 1988, Kisielewski 1997, Balsamo *et al*. 2014). It is possible that both reproduction strategies during the life of an individual are of adaptational importance and serve to shape the differentialpopulation response to conditions in a given area. Tachyblastic eggs allow for a rapid increase in the number of individuals and colonisation of a new habitat, whereas opsiblastic eggs constitute a reservoir of genetic material in the event of a sudden deterioration in the occupied habitat and may result in a population recovery after a rapid decline in its abundance or complete extinction. Opsiblastic eggs can also bemore resistant to unfavourable conditions than adults, thus providing material for dispersal to other environments. The eggs from hermaphroditic reproduction, in turn, ensure genetic variability within the population, which may increase the range of environmental response plasticity and may provide better chances for adaptation to future changes in the occupied ecosystem (Balsamo 1992, Hummon *et* Hummon 1992, Balsamo *et al*. 2014). The presence and commonness of gastrotrichs on the astatic and shallow water reservoirs frozen in winter in demanding environmental Arctic conditions may result from this complex process of reproduction and the ability to lay different types of eggs, as well as high reproductive and colonisaction rate (see also Kolicka *et al*. 2016, Kolicka 2017). This could be crucial in the polar regions, which are extreme and highly variable environments characterised by a combination of low winter temperature, the length of the winter season and high summer microhabitat inconstancy (Convey *et al*. 2012). Also during the rapid climate change in the Arctic, the ability to lay different kinds of eggs could play a crucial role and promote the remodelling of polar gastrotrich communities.

In the case of marine species, our research suggests the opposite situation to that observed in freshwater taxa. The only one species, viz. *Turbanella pilosum* sp. nov., was relatively abundant; it belongs to the genus known as cosmopolitan, which occurs in water with highly differing salinity (from freshwater to 60 PSU) and temperatures (*e.g*. Jansson 1968, Boaden *et* Erwin 1971, Kisielewski 1987, Balsamo *et* Tongiorgi 1995, Kieneke *et al*. 2012). However, the limiting factor in species diversity does not seem to be only the presence of extreme seasonality in a light regime (Convey *et al*. 2012). Berge *et al*. (2015a, 2015b) proved the existence of biological activity in bottom sediments and the pelagic zone during the polar night. Moreover, gastrotrichs are mostly bacteriophagous and detritusophagous, and primary production is not strictly limited for them (*e.g*. Kisielewski 1997, Balsamo *et al*. 2014). The presence of species which probably have a high range of environmental tolerance is understandable when we consider variable freshwater run-off during the polar summer and winter from the retreating glaciers (Beszczynska-Moller *et al*. 1997).

Coulson (2007) suggested that the invertebrate fauna that is currently present on Spitsbergen recolonised the island during the Holocene, after the retreat of the last glaciation 10,000 years ago. According to this hypothesis, gastrotrichs should have reached the island, although it is unknown how. The vast majority of freshwater Gastrotricha species spend nearly their entire lives either in or on the surface of bottom sediments, or on submerged vegetation, while marine taxa mainly inhabit interstitial spaces. They have the ability to move over the substrate and actively push the substrate particles; however, their dispersive abilities seem to be limited by small body size, delicate structure and lack of any free-swimming life stadium, such as, *e.g*. among planktonic larvae (*e.g*. Kisielewski 1997; Balsamo *et al*. 2014; Kieneke *et* Schmidt-Rhaesa 2015). Regardless of how gastrotrichs survived during the glaciations in Svalbard or/and reached the archipelago during the glaciers regression, they represent now a very common and important element

of Arctic meiobenthic communities and are an excellent model for further research on speciation and adaptation to demanding/challenging environmental conditions.

ACKNOWLEDGEMENTS

We thank Ziemowit Olszanowski and James Kirk for their kind advice and his insightful and helpful comments to earlier versions of the manuscript. Special thanks to Alicja Kubicka for preparing the drawings of the new species and figures used in this paper. Last but not least, the authors are grateful to members of the XXXVII Polish Polar Expedition for their kind help and support during sample collection. Also, special thanks to Piotr Bałazy and Maciej Grubiak for their kind help in transporting the equipment to the Polish Polar Station in Hornsund. All samples were collected in public spaces. Collection and transportation of material was made possible under authorisation number RIS–ID 6783 as part of the research project "Glaciers as Arctic ecosystem refugia". This permit was granted by the Governor of Svalbard in 2014. Further research on Gastrotricha did not require any additional permits. The study was funded by the Polish MSHE grant DI 2014 013844 titled "Gastrotricha diversity and phylogenetic relationships in the foreland of the Arctic glaciers – a consequence of climate changes" for MK. MK is a beneficiary of the Adam Mickiewicz Foundation scholarship scheme.

REFERENCES

Balsamo, M. 1978. Prime ricerche sui Gastrotrichi dulciacquicoli italiani. Atti della Societa Toscana di Scienze Naturali Memorie Serie B, 84: 87–150. 1977.

Balsamo, M. 1983. Gastrotrichi. *In*: Consiglio Nazionale delle Ricerche. Guide per il riconoscimento delle specie animali delle acque interne italiane, 20: 1–92.

Balsamo, M. 1992. Hermafroditism and partenogenesis in lower Bilateria: Gnathostomulida and Gastrotricha. *In*: R. Dallai (Ed.), Sex: Origin and Evoluction. Mucchu, Modena, 309–327.

Balsamo, M., d'Hondt, J. L., Kisielewski, J., Todaro, M. A., Tongiorgi, P., Gudi, L., Grilli, P., Y. de Jong. 2015. Fauna Europea: Gastrotricha. Biodiversity Data Journal 3: e5800.

Balsamo, M., E. Fregni. 1995. Gastrotrichs from interstitial fresh water, with a description of four new species. Hydrobiologia, 302: 163–175. http://dx.doi.org/10.1007/bf00027040.

Balsamo, M., Grilli, P., Guidi, L., J. L. d'Hondt. 2014. Gastrotricha: Biology, Ecology and Systematics: Families Dasydytidae, Dichaeturidae, Neogosseidae, Proichthydiidae. *In*: Identification Guides to the Plankton and Benthos of Inland Waters. 24 (Ed. H. J. F. Dumont), 1–187 pp.

Balsamo, M., Guidi, L., J. L. d'Hondt. 2013. Phylum Gastrotricha. *In*: Zhang, Z.-Q. (Ed.) Animal Biodiversity: An Outline of Higher-level Classification and Survey of Taxonomic Richness. Zootaxa, 3703(1): 79–82.

Balsamo, M., Guidi, L., Ferraguti, M., Pierboni, L., R. M. Kristensen. 2010. *Diuronotus aspetos* (Gastrotricha): new morphological data and description of the spermatozoon. Helgoland Marine Research, 64: 27–34.

Balsamo, M., M. A. Todaro. 1987. *Aspidiophorus polystictos*, a new marine species (Gastrotricha, Chaetonotida) and its life cycle. Bollettino di Zoologia, 54: 147–153.

Balsamo, M., M. A. Todaro. 1988. Life history traits of two chaetonotids (Gastrotricha) under different experimental conditions. Invertebrate Reproduction and Development, 14: 161–176.

Balsamo, M., M. A. Todaro. 1995. Gastrotrichi del Trentino: le Viotte del Monte Bondone (Trento). Studi trentini di scienze naturali, 70: 9–22.

Balsamo, M., P. Tongiorgi. 1995. Gastrotricha. *In*: Minelli A., Ruffo S., La Posta S. (Eds.), Checklist delle specie della fauna italiana, 7. Calderini, Bologna, 1–11 pp.

Berge, J., Daase, M., Renaund, P. E., Ambrose, W. G. Jr., Darnis, G., Last, K. S., Leu, E., Cohen, J. H., Johnsen, G., Moline, M. A., Cottier, F., Varpe, Ø., Shuratova, N., Bałazy, P., Morata, N., Massobuan, J. C., Falk-Petersen, S., Kosobokova, K., Hopp, C. J. M., Węsławski, J. M., Kukliński, P., Legeżyńska, J., Nikishina, D., Cusa, M., Kędra, M., Włodarska-Kowalczuk, M., Vogedes, D., Camus, L., Tran, D., Michaud, E., Gabrielsen, T. M., Grenovitsch, A., Gonchar, A., Krapp, R., T. A. Callesen. 2015a. Unexpected Levels of Biological Activity during the Polar Night Offer New Perspectives on a Warming Arctic. Current Biology, http://dx.doi.org/10.1016/j.cub.2015.08.024.

Berge, J., Renaund, P. E. Darnis, G., Cottier, F., Last, K., Gabrielsen, T. M., Johnsen, G., Seuth, L., Weslawski, J. M., Leu, E., Moline, M., Nehrgang J., Søreide, J. E., Varpe, Ø., Lønne, O. J., Daase, M., Falk-Petersen, S. 2015b. In the dark: A review of ecosystem processes during the Arctic polar night. Progress in Oceanography, 139: 258–271.

Beszczyńska-Moller, A., Węsławski, J. M., Walczowski, W., M. Zajaczkowski. 1997. Estimation of glacial meltwater discharge into Svalbard coastal waters. Oceanologia, 39: 289–298.

Blake, C. H. 1933. Nomenclatorial notes on Gastrotricha. Science, 77: 606.

Błaszczyk, M., Jania, J. A., L. Kolondra. 2013. Fluctuations of tidewater glaciers in Hornsund Fjord (Southern Svalbard) since the beginning of the 20th century. Polish Polar Research, 34(4): 327–352.

Blott, S., K. Pye. 2001. Gradistat: A grain size distribution and statistics package for the analysis of unconsolidated sediments. Earth Surface Processes and Landforms, 26: 1237–1248.

Brehm, V. 1917. Ergebnisse einiger im Franzensbader Moor unternommener Exkursionen. Archives of Hydrobiology, 11: 306–323.

Boaden, P. J. S., D. G. Erwin. 1971. *Turbanella hyalina* versus *Protodriloides symbioticus*: A study in interstitial ecology. Vie *et* Milieu, 22 (Supplement): 479–492.

Boaden, P. J. S. 1960. Three new thiobiotic Gastrotricha. Cahiers de Biologie Marine, 15: 367–378.

Buchanan, J. B. 1984. Sediment analysis. *In*: Holme N.A., McIntyre A.D. (Eds) Methods for the study of marine benthos, Blackwell Scientific Publications, Boston, MA, 41–65 pp.

Convey, P., Aitken, S., di Prisco, G., Gill, M. J., Coulson, S. J., Barry, T., Jónstóttir, I. S., Dang, P. T., Hik, D., Kulkarni, T., G. Lewis. 2012. The impacts of climate change on circumpolar biodiversity. Biodiversity, 13(3–4): 134–143.

Coulson, S. J. 2007. The terrestrial and freshwater invertebrate fauna of the High Arctic archipelago of Svalbard. Zootaxa, 1448: 41–58.

Coulson, S. J. 2013. The terrestrial invertebrate fauna of the Svalbard archipelago in a changing world: history of research and challenges. The Canadian Entomologist, 145: 131–146.

Coulson, S. J., Convey, P., Aakra, K., Aarvik, L., Avila-Jimenez, M. L., Babenko, A., Biersma, E. M., Bostrom, S., Brittain, J. E., Carlsson, A. M., Christoffersen, K., De Smet, W. H., Ekremj, T., Fjellberg, A., Fureder, L., Gustafssonm, D., Gwiazdowicz, D. J., Hansen, L. O., Holmstrup, M., Hulle, M., Kaczmarek, Ł., Kolicka, M., Kuklin, V., Lakka, H. K., Lebedeva, N., Makarova, O., Maraldo, K., Melekhina, E., Odegaard, F., Pilskog, H. E., Simon, J. C., Sohlenius, B., Solhoy, T., Soli, G., Sture, E., Tanasevitch, A., Taskaeva, A., Velle, G., Zawierucha, K., Zmudczyńska-Skarbek, K. 2014. The terrestrial and freshwater invertebrate biodiversity of the archipelagoes of the Barents Sea; Svalbard, Franz Josef Land and Novaya Zemlya. Soil Biology and Biochemistry, 68: 440–470.

Dabert, J., Ehrnsberger, R., M. Dabert. 2008. *Glaucalges tytonis* sp. nov. (Analgoidea: Xolalgidae) from the barn owl *Tyto alba* (Strigiformes: Tytonidae): compiling morphology with DNA barcode data for taxa descriptions in mites (Acari). Zootaxa, 1719: 41–52.

Dabert, M., Proctor, H. C., J. Dabert. 2016. Higher-level molecular phylogeny of the water mites (Acariformes: Prostigmata: Parasitengonina: Hydrachnidiae). Molecular Phylogenetics and Evolution, 101: 75–90. http://dx.doi.org/10.1016/j.ympev.2016.05.004.

Dabert, M., Witalinski, W., Kaźmierski, A., Olszanowski, Z., J. Dabert. 2010. Molecular phylogeny of acariform mites (Acari, Arachnida): strong con ict between phylogenetic signal and long branch attraction artifacts. Molecular Phylogenetics and Evolution, 56: 222–241. http://dx.doi.org/10.1016/j.ympev.2009.12.020.

Daday, E. V. von. 1905. Untersuchungen uber die susswasser mikrofauna Paraguays. Zoology of Bulgaria, 44: 72–86.

De Smet, W. H., Van Rompu, E. A., L. Beyens. 1987. Rotifera, Gastrotricha en Tardigradauit Shetland, de Faroër en Spitsbergen (Rotifera, Gastrotricha et Tardigrada-provenant du Shetland, les îles Féroé et du Spitsberg). Natuurwetten Tijdschrift, 69: 81–102.

De Smet, W. H. 1993. Report on rotifers from Barentsoya, Svalbard (78°30'N). Fauna Norvegica Serie A, 14: 1–26.

Dujardin, F. 1841. Histoire Naturelle des Zoophytes, Infusoires. Librairie Encyclopedique de Roret, Paris, 684 & atlas 12.

Ehrenberg, C. G. 1830. Organisation, Systematik, und geographisches Verhältnis der Infusionstierchen. F. Dümmler, Berlin, 1–108pp. http://dx.doi.org/10.5962/bhl.title.2077.

Ehrenberg, C. G. 1838. Die Infusionstierchen als vollkommene Organismen. Ein Blick in des tiefere organische Leben der Natur. Leipzig: Leopold Voss.

Folk, R. L., W. C. Ward. 1957. Brazos River bar: a study in the significance of grain size parameters. Journal of Sedimentary Research, 27: 3–26.

Folmer, O., Black, M., Hoeh, W., Lutz, R., R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology, 3: 294–299.

Fregni, E., Balsamo, M., P. Tongiorgi. 1998. Interstitial gastrotrichs from lotic Italian fresh waters. Hydrobiologia, 368: 175–187.

Garaffoni, A. R. S., Araújo T. Q., Lourenço, A. P., Guidi, L., M. Balsamo. 2016. A new genus and new species of freshwater Chaetonotidae (Gastrotricha: Chaetonotida) from Brazil with phylogenetic position inferred from nuclear and mitochondrial DNA sequences. Systematics and Biodiversity, http://dx.doi.org/10.1080/14772000.2016.1214189.

Gilbert, R. 2000. Environmental assessment from the sedimentary record ofhigh-latitude fjords. Geomorphology, 32: 295–314.

Gislason, G. M. 2005. Origin of freshwater fauna of the North-Atlantic islands: present distribution in relaction to climat and posible migraction routes. Verhandlungen des Internationalen Verein Limnologie, 29: 198–203.

Giżejewski, J., A. Karczewski. 2013. Landforms. *In*: Zwoliński, Z., Kostrzewski, A., Puliny, M. (Eds.) Acient and modern geoecosystems of Spitsbergen. Polish geomorphological research. Bogucki Wydawnictwo Naukowe, Poznań, 65–71.

Giżejewski, J., Nawrot, A., Birkenmajer, K., Głowacki, P., Karczewski, A., Luks, B., Migała, K., Pirożnikow, E., Puczko, M., Skiba, S., Stempniewicz, L., T. Wawrzyniak. 2013. Geographical rnvironment in the vicinity of the Stanisław Siedlecki Polar Station in Hornsund. *In*: Zwoliński, Z., Kostrzewski, A., Puliny, M. (Eds.) Acient and modern geoecosystems of Spitsbergen. Polish geomorphological research. Bogucki Wydawnictwo Naukowe, Poznań, 57–100.

Gosse, P. H. 1851. A catalogue of Rotifera found in Britain with descriptions of five new genera and thirty-two new species. Annals and Magazine of Natural History, 2: 197–203.

Gosse, P. H. 1864. The natural history of the hairy-backed animalcules (Chaetonotidae). The Intellectual Observer, 5: 387–406.

Gerlach, S. A. 1953. Gastrotrichen aus dem Küstengrundwasser des Mittelmeeres. Zoologischer Anzeiger, 150: 203–211.

Greuter, A. 1917. Beiträge zur Systematik der Gastrotrichen in der Schweiz. Revue Suisse de Zoologie, 25: 35–76.

Grünspan, T. 1908. Beitraege zur Systematik der Gastrotrichen. Zoologische Jahrbuecher Systematik, 26: 214–256.

Grzelak, K., L. Kotwicki. 2012. Meiofaunal distribution in Hornsund fjord, Spitsbergen. Polar Biology, 35: 269–280.

Hermant, M., Prinzing, A., Vernon, P., Convey, P., F. Heunion. 2013. Endemic species have highly integrated phenotypes, environmental distributions and phenotype-environment relationships. Journal of Biogeography, 40(8): 1583–1594.

Hochberg, R. 2002. Two new species of Turbanellidae (Gastrotricha: Macrodasyida) from a high-energy beach on North Stradbroke Island, Australia. New Zealand Journal of Marine and Freshwater Research, 36(2): 311–319.

Hochberg, R., M. K. Litvaitis. 2000. Phylogeny of Gastrotricha: a Morphology-Based Framework of Gastrotricha Relationships. Biological Bulletin, 198: 299–305.

d'Hondt, J. L. 1971. Gastrotricha. *In*: Barnes, H. (Ed.), Oceanography and marine biology: an annual review, 9, 141–192 pp. [George Allen and Unwin Ltd.].

Humlum, O., Instanes, A., J. L. Sollid. 2003. Permafrost in Svalbard: a review of research history, climatic background and engineering challenges. Polar Research, 22(2): 191–215.

Hummon, W. D. 1974. Gastrotricha from Beaufort, North Carolina, U.S.A. Cahiers de Biologie Marine, 15: 431–446.

Hummon, M. R. 1984. Reproduction and sexual development in a freshwater gastrotrich. 3. Postparthenogenic development of primary oocytes and the X-body. Cell and Tissue Research, 236: 629–636.

Hummon, M. R. 1986. Reproduction and Sexual Development in a Fresh-water Gastrotricha. 4. Life History Traits and the Possibility of Sexual Reproduction. Transactions of the American Microscopical Society, 105(2): 97–109.

Hummon, M. R., W. D. Hummon. 1992. Gastrotricha. *In*: Adiyodi K.G., Adiyodi A.G. (Eds), Reproductive Biology of Invertebrates, Vol. V. Sexual Differentiation and Behaviour, Oxfors et IBH, New Delhi, 137–146.

Hummon, W. D., Balsamo, M., M. A. Todaro. 1992. Italian marine Gastrotricha: I. Six new and one redescribed species of Chaetonotida. Bollettino di Zoologia, 59: 499–516.

Hummon, W. D. 2008. Gastrotricha of the North Atlantic Ocean: 1. Twenty four new and two redescribed species of Macrodasyida. Meiofauna Marina, 16: 117–174.

Hummon, W. D., M. A. Todaro. 2010. Analytic taxonomy and notes on marine, brackish-water and estuarine Gastrotricha. Zootaxa, 2392: 1–32.

Hlava, S. 1904. Die systematische Stellung von *Polyarthra fusiformis*. Zoologischer Anzeiger, 28: 335.

International Commission on Zoological Nomenclature. 1999. International Code of Zoological Nomenclature, Fourth Edition. The International Trust for Zoological Nomenclature, The Natural History Museum, Cromwell Road, London.

Jania, J. 1988. Dynamiczne procesy glacjalne na Południowym Spitsbergenie (w świetle badań fotointerpretacyjnych i fotogrametrycznych). Prace Naukowe Uniwersytetu Śląskiego w Katowicach nr 955. [In Polish].

Jansson, B. O. 1968. Quantitative and experimental studies of the interstitial fauna of four Swedish sandy beaches. Ophelia, 5: 1–71.

Katoh, K., D. M. Standley. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution, 30(4): 772–780. https://doi.org/10.1093/molbev/mst010.

Kaplan, G. 1958. Primieres observations sur les Gastrotriches psammophiles des cotes du Calvados. Archives de Zoologie Expérimentale et Générale, 96: 27–37.

Kånneby, T., Todaro, M. A., U. Jondelius. 2009. One new species and records of *Ichthydium* Ehrenberg, 1830 (Gastrotricha: Chaetonotida) from Sweden with a key to the genus. Zootaxa, 2278: 26–46.

Kånneby, T. 2013. New species and records of freshwater *Chaetonotus* (Gastrotricha: Chaetonotidae) from Sweden. Zootaxa, 3701: 551–588.

Kånneby, T., J. J. Kirk. 2017. A new species of *Redudasys* (Gastrotricha: Macrodasyida: Redudasyidae) from the United States. Proceedings of the Biological society of Washington, 130: 129–139.

Kånneby, T., Todaro, M. A., U. Jondelius. 2012. A phylogenetic approach to species delimitation in freshwater Gastrotricha from Sweden. Hydrobiologia, 683: 185–202.

Kånneby, T., Todaro, M. A., U. Jondelius. 2013. Phylogeny of Chaetonotidae and other Paucitubulatina (Gastrotricha: Chaetonotida) and colonization of aquatic ecosystems. Zoologica Scripta, 42: 88–105.

Kånneby, T., M. Wicksten. 2014. First record of the enigmatic genus *Redudasys* Kisielewski, 1987 (Gastrotricha: Macrodasyida) from the Northern hemisphere. Zoosystema, 36(4): 723–734.

Kieneke, A., Martinez Arbizu, P. M., D. Fontaneto. 2012. Spatially structured populations with a low level of cryptic diversity in European marine Gastrotricha. Molecular Ecology, 21: 1239–1254.

Kieneke, A., Riemann, O., W. H. Ahlrichs. 2008. Novel implications for the basal internal relationschips of Gastrotricha revealed by an analysis of morphological characters. Zoologica Scripta, 37: 429–460. http://dx.doi.org/10.1111/j.1463–6409.2008.00334.x.

Kieneke, A., A. Schmidt-Rhaesa. 2015. Gastrotricha. *In*: Schmidt-Rhaesa (Ed.) Handbook of Zoology. Vol. 3 Gastrotricha and Gnathifera, 1–134 pp. (Berlin, Boston: De Gruyter).

Kieneke, A., H. Nikoukar. 2017. Integrative morphological and molecular investigation of *Turbanella hyalina* Schultze, 1853 (Gastrotricha: Macrodasyida), including a redescription of the species. Zoologischer Anzaiger, 267: 168–186.

Kipling, W. W., D. Rubinoff. 2003. Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. Cladistics, 20: 47–55.

Kisielewska, G., Kolicka, M., K. Zawierucha. 2015. Prey or parasite? The first observations of live Euglenida in the intestine of Gastrotricha. European Journal of Protistology, 51: 138–141.

Kisielewska, G., J. Kisielewski. 1986. Freshwater Gastrotricha of Poland. III. Gastrotricha from the Białowieża Forest and the Białowieża Glade. Fragmenta Faunistica, 30: 195–215.

Kisielewski, J. 1981. Gastrotricha from raised and transitional peat bogs in Poland. Monografie Fauny Polski, 11. Polska Akademia Nauk, Kraków, 143 pp.

Kisielewski, J. 1984. Three new freshwater gastrotrichs of the genus *Chaetonotus* Ehrenberg (Chaetonotida) from Poland. Bulletin des L'Academie Polonaise des Sciences, Serie des Sciences Biologiques, 32: 293–301.

Kisielewski, J. 1986. Taxonomic notes on freshwater gastrotrichs of the genus *Aspidiophorus* Voigt (Gastrotricha, Chaetonotoidea) with description of four new species. Fragmenta Faunistica, 30: 139–156.

Kisielewski, J. 1987. New records of marine Gastrotricha from the French coasts of Manche and Atlantic I. Macrodasyida, with descriptions of seven new species. Bulletin du Museum national d'histoire naturelle, 9: 837–877.

Kisielewski, J. 1991. Inland-water Gastrotricha from Brazil. Annales Zoologici, Warszawa, 43: 1–168.

Kisielewski, J. 1997. Brzuchorzęski (Gastrotricha). Fauna Słodkowodna Polski, Zeszyt 31. Wydawnictwo Uniwersytetu Łódzkiego, Łódź, 157 pp.

Kolicka, M. 2016. Gastrotrichs in bromeliads – newly recorded *Chaetonotus* (*Hystricochaetonotus*) *furcatus* Kisielewski, 1991 (Chaetonotida) from the Łódź Palm House. Zoosystema, 38(1): 141–155. http://dx.doi.org/10.5252/z2016n1a5.

Kolicka, M. 2017. New Arctic Gastrotricha – three *Chaetonotus* (Chaetonotidae) from Longyearbyen (Spitsbergen, Svalbard Archipelago). Annales Zoologici, 67(1): 131–180.

Kolicka, M., Dabert, M., Dabert, J., Kånneby, T., J. Kisielewski. 2016. *Bifidochaetus*, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. Invertebrate Systematics, 30(4): 398–419. http://dx.doi.org/10.1071/IS16001.

Kolicka, M., Gadawski, P., M. Dabert. 2017a. A new species of freshwater Chaetonotidae (Gastrotricha, Chaetonotida) from Obodska Cave (Montenegro) based on morphological and molecular characters. European Journal of Taxonomy, 354: 1–30. https://doi.org/10.5852/ejt.2017.354.

Konsuloff, S. 1913. Notizen über die Gastrotrichen Bulgariens. Zoologischer Anzeiger, 43: 255–260.

Leasi, F., M. A. Todaro. 2008. The muscular system of *Musellifer delamarei* (Renaud-Mornant, 1968) and other chaetonotidans with implications for the phylogeny and systematization of the Paucitubulatina (Gastrotricha). Biological Journal of the Linnean Society, 94: 379–398. http://dx.doi.org/10.1111/j.1095–8312.2008.00974.x.

Luporini, P., Magagnini, G., P. Tongiorgi. 1973. Gastrotrichi macrodasioidei delle coste della Toscana. Pubblicazioni Della Stazione Zoologica di Napoli, 38: 267–288.

Levy, D. P., M. J. Weiss. 1980. Sperm in the life cycle of the freshwater gastrotrich, *Lepidodermella squamata*. American Zoologist, 20: 749.

Lanfear, R., Calcott, B., Ho, S. Y. W., S. Guindon. 2012. PartitionFinder: Combined Selection of Partitioning Schemes and Substitution Models for Phylogenetic Analyses. Molecular Biology and Evolution, 29(6): 1695–1701.

Maguire, C. 1976. *Turbanella varians* n.sp., a marine gastrotrich with local morphological variants. Cahiers de Biologie Marine, 17: 185–194.

Marcolongo, I. 1910. Primo contributo allo studio dei Gastrotrichi del lago-stagno craterico di Astroni. Monitore Zoologico Italiano, 21: 315–318.

Mečnikow, E. 1865. Über einige wenig bekannte niedere Thierformen. Zeitschrift für Wissenschaftliche Zoologie, 15: 450–463.

Migała, K., T. Wawrzyniak. 2013. Climate. *In*: Zwoliński, Z., Kostrzewski, A., Puliny, M. (Eds.) Acient and modern geoecosystems of Spitsbergen. Polish geomorphological research. Bogucki Wydawnictwo Naukowe, Poznań, 71–76.

Müller, O. F. 1773. Vermium terrestrium et fluviatilium saeu animalium infosoriorum, helminthicorum et testaceorum non marinorum, succincta historia. Heineck et Faber, Havniae et Lipsiae, 135 pp. http://dx.doi.org/10.5962/bhl.title.12733.

Navajas, M., Lagnel, J., Gutierrez, J., Boursot, P. 1998. Species-wide homogeneity of nuclear ribosomal ITS2 sequences in the spider mite *Tetranychus urticae* contrasts with extensive mitochondrial COI polymorphism. Heredity, 80: 742–752.

Nesteruk, T. 2007. Recolonization of two dried peat-hags by gastrotrich fauna. Teka Komisji Ochrony i Kształtowania Środowiska Przyrodniczego [Teka Commission of Protection and Formation of Natural Environment], 4: 192–197.

Palli, A., Moore, J. C., Jania, J., P. Glowacki. 2003. Glacier changes in southern Spitsbergen, Svalbard, 1901–2000. Annals of Glaciology, 37: 219–225.

Paps, J., M. Riutort. 2012. Molecular phylogeny of the phylum Gastrotricha: New data brings together molecular and morphology. Molecular Phylogenetic and Evolution, 63: 208–212. http://dx.doi.org/10.1016/j.ympev.2011.12.010.

Petrusek, A., Hobæk, A., Nilssen, J. P., Skage, M., Černý, M., Brede, N., K. Schwenk. 2008. A taxonomic reappraisal of the European *Daphnia longispina* complex (Crustacea, Cladocera, Anomopoda). Zoologica Scripta, 37(5): 507–519.

Pflannkuche, O., H. Thiel. 1987. Meiobentchic Stocks and Benthic Activity on the NE-Svalbard Shelf and in the nansen Basin. Polar Biology, 7: 253–266.

Pirożnikow, E. 2013. Flora. *In*: Zwoliński, Z., Kostrzewski, A., Puliny, M. (Eds.) Acient and modern geoecosystems of Spitsbergen. Polish geomorphological research. Bogucki Wydawnictwo Naukowe, Poznań, 79–81.

Preobrajenskaja, E. N. 1926. Zur Verbreitung der Gastrotrichen in den Gewässern der Umgebung von Kossino. Arbeiten der Biologischen Station zu Kossino (Moskau), 4: 1–14.

di Prisco, G. C. Verde. 2011. Evolution and Biodiversity in the Antarctic – The Response of Life to Change (EBA): Role and legacy. Oecologia Australis, 15 (1): 23–31. https://doi.org/10.4257/oeco.2011.1501.02.

Rao, G. C., C. Clausen. 1970. *Planodasys marginalis* gen. et. sp. nov. and *Planodasyidae* fam. nov. (Gastrotricha Macrodasyoidea). Sarsia, 42: 73–82.

Rambaut, A. 2014. FigTree v. 1.4.2. Available from http://tree.bio.ed.ac.uk/software/figtree/ [accessed 12 Sep. 2017].

Remane, A. 1924. Neue aberrante Gastrotrichen. I. *Macrodasys buddenbrocki* nov. gen. nov. spec. Zoologischer Anzeiger, 61: 289–297.

Remane, A. 1925. Organisation und systematische Stellung der aberranten Gastrotrichen.Verhandlungen der deutschen zoologischen. Gesellschaft, 30: 121–128.

Remane, A. 1926. Marine Gastrotrichen aus der Ordung der Chaetonotoidea. Zoologischer Anzeiger, 66: 243–252.

Remane, A. 1927a. Beiträge zur Systematik der Süsswassergastrotrichen. Zoologische Jahrbücher Abteilung für Systematik. Oekologie und Geographie der Tiere, 53: 269–320.

Remane, A. 1927b. Neue Gastrotricha Macrodasyidea. Zoologische Jahrbücher. Abteilung für Systematik, Geographie und Biologie der Tier, 54: 203–242.

Remane, A. 1934. Die Gastrotrichen des Küstengrundwassers von Schilksee. Schriften des Naturwissenschaftliches Vereins für Schleswig-Holstein, 20: 473–478.

Remane, A. 1936. Gastrotricha. *In*: 'Klassen Ordnungen des Tierreichs, Band 4, Abteilung II, Buch I, Teil 2, Liefrungen 1–2'. (Ed. H. G. Bronns.): 1–385pp. (Akademie Verlagsgesellschaft: Berlin, Germany).

Remane, A. 1952. Zwei neue *Turbanella*-Arten aus dem marinen Kuestengrundwasser. Kieler Meeresforsch, 9: 62–65.

Roszczak, R. 1969. Brzuchorzęski (Gastrotricha) środkowej Wielkopolski. Poznańskie Towarzystwo Przyjaciół Nauk. Wydział matematyczno-przyrodniczy. Prace Komisji Biologicznej, 32: 1–92 (1968).

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., H hra, S., Larget, B., Liu, L., Suchard, M. A., J. P. Huelsenback. 2012. MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. Systematic Biology, 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029.

Rudescu, L. 1967. Gastrotricha. *In*: Fauna Republicii Socialiste Romania, Trochelminthes, 2(3). Academia Republicii Socialiste Romania, Bucharest, 292 pp. http://dx.doi.org/10.1016/0016–7185(71)90018–2.

Schultze, M. 1853. Über *Chaetonotus* und Ichthydium (Ehrb.) und eine neue verwandte Gattung *Turbanella*. Müller's Archiv für Anatomie und Physiologie, 6: 241–254.

Schwank, P. 1990. Gastrotricha. *In*: Schwoerbel, J., Zwick, P. (Eds.), Süsswasserfauna von Mitteleuropa, Band 3. Gastrotricha und Nemertini. Gustav Fischer Verlag, Stuttgart, Jena, New York, 1–252 pp.

Schrom, H. 1972. Nordadriatische Gastrotrichen. Helgoländer wissenschaftlicher Meeresuntersuchungen, 23: 286–351.

Scourfield, D. J. 1897. Contributions to the non-marine fauna of Spitsbergen. Part I. Preliminary notes, and reports on the Rhipozoda, Tardigrada, Entomostraca, etc. Proceedings of the Zoological Society of London, 784–792.

Sonnenberg, R., Nolte, A. W., D. Tautz. 2007. An evaluation of LSU rDNA D1–D2 sequences for their use in species identification. Frontiers in Zoology, 4: 6, https://doi.org/10.1186/1742–9994–4–6.

Stamatakis, A. 2006. RAxML–VI–HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics, 22(21): 2688–2690. https://doi.org/10.1093/bioinformatics/btl446.

Stokes, A. C. 1887. Observations on a new *Dasydytes* and a new *Chaetonotus*. The Microscope (Detroit), 7: 261–265.

Spencer, T. 1890. On a new rotifer. Journal of the Quekett Microscopical Club, Series 2, 4: 59+pl. 5.

Suzuki, T. G., Maeda, M., H. Furuya. 2013. Two new Japanese species of Gastrotricha (Chaetonotida, Chaetonotidae, *Lepidodermella* and Dichaeturidae, *Dichaetura*), with comments on the diversity of gastrotrichs in rice paddies. Zootaxa, 3691: 229–239.

Swedmark, B. 1954. Description de *Paraturbanella teissieri*, n. sp. (Gastrotriche Macrodasyoide). Bulletin de la Société zoologique de France, 79: 46–49.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30(12): 2725–2729. doi:10.1093/molbev/mst197.

Tautz, D., Arctander, P., Minelli, A., Thomas, R. H., A. P. Vogler. 2003. A plea for DNA taxonomy. Trends in Ecology and Evolution, 18(2): 70–74.

Telford, M. J., Lockyer, A. E., Cartwright-Finch, C., D. T. J. Littlewood. 2003. Combined large and small subunit ribosomal RNA phylogenies support a basal position of the acoelomorph flatworms. Proceedings of the Royal Society B – Biological Sciences, 1519(270): 1077–1083.

Todaro M. A., Dal Zotto M., F. Leasi. 2015. An integrated morphological and molecular approach to the description and systematisation of a novel genus and species of Macrodasyida (Gastrotricha). PLoS ONE 10(7): e0130278. https://doi.org/doi:10.1371/journal.pone.0130278.

Todaro, M. A., Dal Zotto, M., Maiorova, A. S., A. V. Adrianov. 2009. A new species of *Aspidiophorus* (Gastrotricha, Chaetonotida) from the Russian Far East with a key to marine species of the genus. Marine Biology Research, 5: 297–303.

Todaro, M. A. 2018. Gastrotricha. World Register of Marine Species. Available from: http://www.marinespecies.org/aphia.php?p=taxdetailsetid=2078 (accessed 15 November 2017).

Todaro, M. A., Balsamo, M., R. M. Kristensen. 2005. A new genus of marine chaetonotids (Gastrotricha), with a description of two new species from Greenland and Denmark. Journal of the Marine Biological Association of the United Kingdom, 85: 1391–1400.

Todaro, M. A., Balsamo, M., P. Tongiorgi. 1992. Marine gastrotrichs from the Tuscan Archipelago (Tyrrhenian Sea): I. Macrodasyida, with description of three new species. Bollettino di Zoologia, 59: 471–485.

Todaro, M. A., Dal Zotto, M., Jondelius, U., Hochberg, R., Hummon, W. D., Kånneby, T., C. E. F. Rocha. 2012. Gastrotricha: a marine sister for a freshwater puzzle. PLoS One, 7(2), e31740.

Todaro, M. A., Dal Zotto, M., F. Leasi. 2015. An integrated morphological and molecular approach to the description and systematisation of a novel genus and species of Macrodasyida (Gastrotricha). PLoS One 10(7), e0130278.

Todaro, M. A., W. D. Hummon. 2008. An overview and a dichotomous key to genera of the phylum Gastrotricha. Meiofauna Marina, 16: 3–20.

Todaro, M. A., Leasi, F., R. Hochberg. 2014. A new species, genus and family of marine Gastrotricha from Jamaica, with a phylogenetic analysis of Macrodasyida based on molecular data. Systematics and Biodiversity, 12: 473–488.

Todaro, M. A., Perissinotto, R., S. J. Bownes. 2013. Neogosseidae (Gastrotricha, Chaetonotida) from the iSimangaliso Wetland Park, KwaZulu-Natal, South Africa. ZooKeys, 315: 77–94.

Todaro, M. A., Telford, J., Lockyer, A. E., D. T. J. Littlewood. 2006. Interrelationships of the Gastrotricha and their place among the Metazoa inferred from 18S rRNA genes. Zoologica Scripta, 35: 251–259. http://dx.doi.org/10.1080/00222930310001561 69

Todaro, M. A., Dal Zotto, M., F. Leasi. 2015. An integrated morphological and molecular approach to the description and systematisation of a novel genus and species of Macrodasyida (Gastrotricha). PLoS ONE 10(7): e0130278. doi:10.1371/journal.pone.0130278.

Urban-Malinga, B., Kotwicki, L., Gheskiere, T. L. A., Jankowska, K., Opaliński, K., M. Malinga. 2004. Composition and distribution of meiofauna, including nematode genera, in two contrasting Arctic beaches. Polar Biology, 27(8): 447–457.

Urban-Melinga, B., Wiktor, J., Jabłońska, A., T. Moens. 2005. Intertidial meiofauna of high-latitude glacial Arctic fjord (Kongsfjorden, Svalbard) with emphasis on the structure of free-living Nematoda communities. Polar Biology, 28: 940–950.

Valkanov, A. 1937. Rotatorien und Gastrotrichen der Umgebung von Plönsbiotopen. Forschungsberichte aus der Biologishen Station zu Plön, 11: 1–178.

Voigt, M. 1901. Mittheilungen aus der Biologischen Station zu Plön, Holstein. Über einige bisher unbekannte Süsswasserorganismen. Zoologischer Anzeiger, 24: 191–195.

Voigt, M. 1902. Drei neue *Chaetonotus*-Arten aus Plöner Gewässern. Zoologischer Anzeiger, 25: 116–118.

Węsławski, J. M., Włodarska-Kowalczuk, M., Kedra, M., Legeżyńska, J., L. Kotwicki. 2012. Eight species that rule today's European Arctic fjord benthos. Polish Polar Research, 33(3): 225–238.

Węsławski, J. M., Zajączkowski, M., Wiktor, J., M. Szymelfening. 1997. Interditial Zone of Svalbard. 3. Littoral of subartic, oceanic island: Bjornoya. Polar Biology, 18: 45–52.

Wieser, W. 1957. Gastrotricha Macrodasyoidea from the intertidal of Puget Sound. Transactions of the American Microscopical Society, 76: 372–381.

Wilke, U. 1954. Mediterrane Gastrotrichen. Zoologischer Jahrbücher Abteilung für Systematik (Jena), 82: 497–550.

Wirta, H., Várkonyi, G., Rosmussen, C., Kaartinen, R., Schmidt, N. M., Hebert, P. D. N., Barták, M., Blogeove, G., Disney, H., Ertl, S., Gjelstrup, P., Gwiazdowicz, D. J., Huldén, L., Ilmonen, J., Jakovlev, J., Jaschhof, M., Kahanpää, J., Kankaanpää, T., Nielsen, T. R., Paasivirta, L., Padersen, S., Pohjoimsmäki, J., Salmela, J., Vilkamaa, P., Väre, H., Tschinnhaus, von M., T. Roslin. 2015. Establishing a community-wide DNA barcode library as a new tool for arctic research. Molecular Ecology Resources, http://dx.doi.org/10.1111/1755–0998.12489.

Włodarska-Kowalczuk, M., Szymfeling, M., L. Kotwicki. 1999. Macro- and meiobenthic fauna of Yaldiabucta glaciar bay (Isfjorden, Spitsbergen). Polish Polar Research, 4: 367–386.

Zelinka, C. 1889. Die Gastrotrichen. Eine monographische Darstellung ihrer Anatomie, Biologie und Systematik. Zeitschrift für Wissenschaftliche Zoologie, 49: 209–384.

Zwickl, J. 2006. Genetic algorithm approaches for thr phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Dissertation, The University of Texas at Austin, 1–125.

Received: February 14, 2018
Accepted: September 1, 2018

Poznań, 16.12.2019r.

Małgorzata Kolicka
Adam Mickiewicz University, Poznań
Faculty of Biology
Department of Animal Taxonomy and Ecology
Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Declaration

I declare that the publication: **Kolicka M**. 2019. From saline to salty? *Heterolepidoderma sinus* (Chaetonotida, Chaetonotidae) from subsaline coal mine settling ponds. *Zootaxa*, 4559 (3): 568-572, has been prepared as a part of my doctoral dissertation under the scientific supervision of Prof. UAM, dr hab. Ziemowit Olszanowski.

Yours faithfully,

Poznań, 16.12.2019r.

Małgorzata Kolicka
Uniwersytet im. Adama Mickiewicza, Poznań
Wydział Biologii
Zakład Taksonomii i Ekologii Zwierząt
ul. Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Oświadczenie

Oświadczam, że publikacja: **Kolicka M**. 2019. From saline to salty? *Heterolepidoderma sinus* (Chaetonotida, Chaetonotidae) from subsaline coal mine settling ponds. *Zootaxa*, 4559 (3): 568-572, została wykonana na poczet mojej rozprawy doktorskiej pod opieką promotora Prof. UAM dra hab. Ziemowita Olszanowskiego.

Z wyrazami szacunku,

Zootaxa 4559 (3): 568–572
https://www.mapress.com/j/zt/
Copyright © 2019 Magnolia Press

Correspondence

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.4559.3.7
http://zoobank.org/urn:lsid:zoobank.org:pub:A39FE2EB-A4DD-4E84-ADD9-BDBCC3E10982

From saline to salty? *Heterolepidoderma sinus* (Chaetonotida, Chaetonotidae) from subsaline coal mine settling ponds

MAŁGORZATA KOLICKA

Department of Animal Taxonomy and Ecology, Institute of Environmental Biology, Adam Mickiewicz University, Poznan. Umultowska 89, 61–614 Poznań, Poland. E-mail: kolicka@amu.edu.pl

Gastrotrichs are a permanent and common element of meiofauna communities. They inhabit wide range of aquatic and semi-aquatic niches, including a variety of anthropogenic habitats, such as the sand filters of a cold marine mesocosm (Parent *et al*. 2001) or palm houses (*e.g.* Kolicka *et al.* 2013), and extreme natural habitats like hot springs (Guerne 1888) or hydrothermal vent zones (Kieneke & Zekely 2007).

The presence of Gastrotricha in coal mine settling ponds that are used for temporary retention of saline mine waters should not be surprising. The material for the gastrotrich studies presented here was taken from a settling pond located in Knurów in southern Poland. This pond is regarded as subsaline, forming part of a water drainage system created in the 1970s to drain salt water from the Knurów-Szczygłowice coal mine (Bielańska-Grajner *et al*. 2014; Sowa *et al*. 2018). The pond is situated in an area of hard bituminous coal mining and the bottom sediments are of the gravel type (Bielańska-Grajner *et al*. 2014). The presence of salt water in the coal mine is connected with sodium chloride deposition in rock beds and dissolution it by the underground water. Water conductivity at the time of sampling was 3418 µS/cm and salinity was 1.7 PSU, but in this pond it is significantly variable and closely connected with discharges of salt water from the operating mine. Water conductivity in the years 2016-2017 fluctuated in the range of 2060-5200 µS/cm (Krodkiewska, personal communication). The samples were collected on May 18, 2018 from three sites (Fig. 1): Site 1: N 50.202094, E 18.634122; Site 2: N 50.201856, E 18.635411; Site 3: N 50.201269, E 18.636636. Each sample consisted of three subsamples. All bottom sediment samples were collected manually in such a manner that a 5 cm upper layer of sediment and approximately 5 cm of ambient water were placed in a 200 cm^3 plastic container (Kiesielewski 1997). Additionally, from Site 3, samples of submerged vegetation (*Myriophyllum spicatum*) were collected by picking the plants and thoroughly squeezing water out of them (together with the organisms) into a container of 200 cm^3 (Kisielewski 1997; Balsamo *et al*. 2014).

A total of 0.3 cm^3 of sediment from each bottom sediment subsample and 15 cm^3 of the submerged plant material were analysed under an Olympus SZ51 stereomicroscope. Gastrotrichs were picked out with a micropipette, mounted on glass slides and studied alive (Kisielewski 1997; Balsamo *et al.* 2014). All specimens were observed under an Olympus BX53 compound microscope equipped with differential interference contrast (DIC) and photographed with an Olympus DS50 digital camera. The identification of gastrotrichs, their morphological study and terminology follow Kisielewski (1981, 1991, 1997) and Kolicka *et al*. (2016). Total genomic DNA from a single specimen of *Heterolepidoderma sinus* was extracted as previously described (Kolicka *et al.* 2016). The amplification, sequencing and sequence analyses follows Kolicka *et al*. (2018).

In total, 124 specimens belonging to 6 species, namely *Chaetonotus (Chaetonotus)* aff. *maximus* Ehrenberg, 1838 (4 adults), *C. (C.)* aff. *sphagnophilus* Kisielewski, 1981 (1 adult), *C. (Hystricochaetonotus)* aff. *hystrix* Mečnikow, 1865 (81 adults, 9 juveniles), *C. (Zonochaeta)* sp. (17 adults, 1 juvenile), *Ichthydium (Ichthydium)* aff. *podura* (Müller, 1773) (5 adults) and *Heterolepidoderma sinus* Kolicka, 2015 (3 adults, 2 juveniles), were found. All taxa, except for *H. sinus* and *C. (Zonochaeta)* sp., were recorded as species with an unclear taxonomic position because they corresponded well with the nominal species but possessed some characters that differed from the original description and previous records. It is not excluded that these taxa belong to new, undescribed taxa or represent a highly variable phenotypical form of known species. However, despite their unclear taxonomic status, *C. (Chaetonotus)* aff. *maximus*, *C. (C.)* aff. *sphagnophilus*, *C. (H)* aff. *hystrix*, *C. (Zonochaeta)* sp. and *I. (I.)* aff. *podura* exhibited a set of features typical for freshwater species, such as one- or thee-lobed scales with simple spines or spines with lateral denticle for *Chaetonotus* and delicate, naked body for *Ichthydium*. The recorded specimens of *H. sinus* corresponded exactly with the features and measurements included in the original description (Fig. 2) (Kolicka *et al*. 2015). The specimens from *locus typicus* have not been sequenced, thus the

 Accepted by W. Sterrer: 24 Dec. 2018; published: 21 Feb. 2019

comparison with molecular type material will have to be left to further investigation. A complete 18S rRNA (1970 bp) and complete 28S rRNA (3594 bp) were sequenced and analysed. Both sequences are deposited in GenBank under accession numbers, MK302474 (18S) and MK302475 (28S). The nucleotide blast search of 18S sequence of *Heterolepidoderma sinus* suggested *Heterolepidoderma* sp. 1 TK-2012, as the most similar taxon, with similarity equal to 99%. Whereas the nucleotide blast search of 28S sequence in D1-D3 domain show *Halichaetonotus aculifer* (Gerlach, 1953), *Ha. paradoxus* and *Ha.* sp. 2 TK-2012, as the most similar taxa, all with similarity equal to 91% identity. All these taxa are marine.

H. sinus was originally found in Puck Bay of the Baltic Sea where salinity is 6-8 PSU; it was thus regarded as a brackish or tolerant marine species. The brackish or marine origin of this species was additionally confirmed by its morphological features, such as the distinct cuticular reinforcements in the anterior dilatation of the pharynx and the presence of two types of lamellate scales: scales with small lamellae on the lateral sides and scales with large lamellae on the ventral sides, which are present mostly on species inhabiting saline and brackish waters, and only rarely on freshwater taxa (see Kolicka *et al*. 2015). In the subsaline settling pond investigated here, 5 specimens of *H. sinus* were observed from Site 1 and on submerged plants from Site 3, including juvenile individuals and one specimen with a large, mature egg, which suggests the presence of this taxon as a stable population. The coal mine settling ponds provide specific conditions for the presence of both fauna and flora (Sowa *et al*. 2018 and citation therein). Organisms inhabiting this reservoir type have been very poorly investigated (Bielańska-Grajner *et al*. 2014; Sowa *et al*. 2018 and citation therein), and the present work is the first report on the occurrence of gastrotrichs in such a habitat.

The anthropogenic origin, specific type of sediment (bituminous coal mining) and significant fluctuations of the salinity concentration make this environment an extreme habitat. The coexistence of freshwater taxa and a single species described from the brackish shore of Baltic Sea in one reservoir allow hypotheses about their colonization by fauna.

Freshwater *C. (C.)* aff. *maximus*, *C. (C.)* aff. *sphagnophilus*, *C. (H)* aff. *hystrix*, *C. (Zonochaeta)* sp. and *I. (I.)* aff. *podura* may possess a high range of tolerance to salinity. However, freshwater taxa are considered stenohaline, with a limited ability to survive in saline waters (*e.g.* Tongiorgi *et al.* 1999). The presence of these species in the reservoir investigated here might also be due to frequent passive dispersion from nearby natural freshwater reservoirs (lakes, fish ponds) in the form of resting eggs, and to a rapid population growth. The temporary presence of freshwater gastrotrichs in this subsaline pond with cyclical mortality episodes when the fluctuating salinity concentration exceeds their tolerance range cannot be excluded as an explanation.

The presence of *H. sinus* constitutes another issue. Despite the relatively good knowledge on Gastrotricha from Poland, this species has not been reported in fresh water habitats. The pond where *H. sinus* was recorded during the current study is at a distance of about 550 km from the type locality. It is possible that *H. sinus* has the ability to produce resting eggs, just as the freshwater representatives of Chaetonotida do. In this framework, the population we found in the pond results from resting eggs arrived by that are resistant to unfavourable environmental conditions passive dispersal *e.g.* by birds, surface runoff or wind (*e.g.* Kolicka *et al*. 2015). Conversely, *H. sinus* could have inhabited this particular pond, which is separated from neighbouring coal mine settling ponds belonging to the same drainage system. This is quite probable, as submerged macrophytes (*Ruppia maritima*) are present in an adjacent saline pond (Halabowski *et al.* 2018). *R. maritima* is a very tolerant plant for various salinity ranges and is present mainly in marine and brackish, but also in freshwater habitats (Sowa *et al*. 2018 and citation therein). It is a species that is commonly present in Puck Bay (Baltic Sea) (*e.g.* Węsławski *et al*. 2013), thus small invertebrate eggs such as those of gastrotrichs could easily have been brought to inland saline water together with these submerged macrophyte propagules, where they found favourable conditions for population development.

FIGURE 1. A—Study area with marked sampling sites. B—Studied coal mine settling pond.

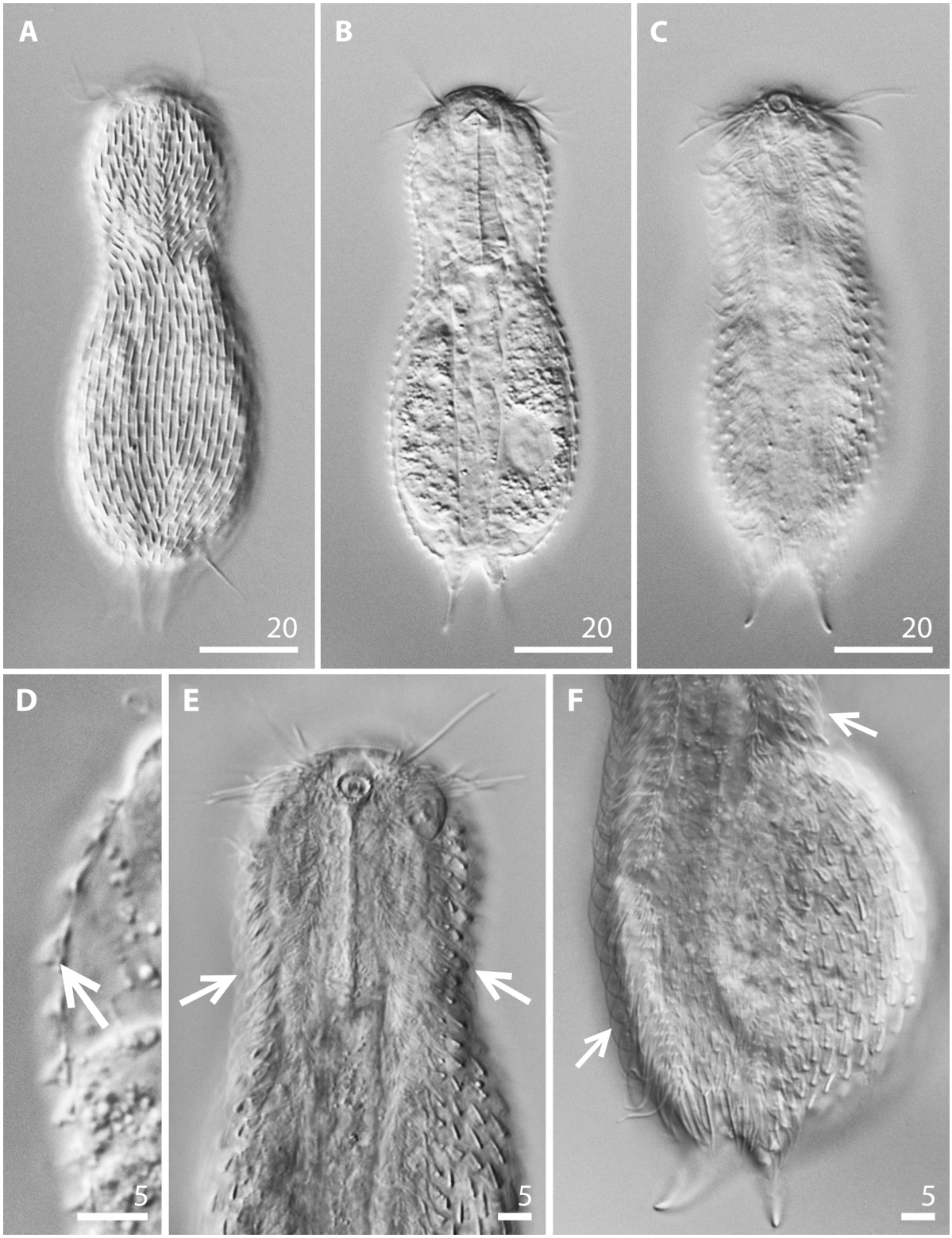


FIGURE 2. *Heterolepidoderma sinus* Kolicka, 2015. **Scales are in µm.** A—Dorsal body view. B—View of internal morphology. C—Ventral body view. D—Detail of head with lateral lamellae. E—Detail of head, neck and anterior trunk ventral view with visible lamellae. F—Detail of trunk, furca base and furcal appendages ventral view with visible ventral lamellae.

Artificial saline reservoirs provide suitable conditions for various groups of organisms (Bielańska-Grajner *et al*. 2014). Due to the fluctuating salinity, they can be considered the inland equivalent of the brackish water of the coastal lagoons and estuaries. Yet these artificial water reservoirs are not directly connected to natural ground saline waters such

as seas or oceans and constitute separate, isolated ecosystems. Generally, marine species can reach these artificial reservoirs only via long-distance dispersion. For this reason, these reservoirs can be treated as an excellent place for research looking for clues on the dispersal capabilities of the organisms and on their ability to occupy new ecological niches, and their capacity to adapt to changing conditions. These environments may also allow for studies on possible morphological variations caused by stressful conditions. Further research into these types of ecosystems may provide also a significant contribution to knowledge on ecological traits of artificial populations made up of introduced species separated from their natural source habitats.

Acknowledgements

I sincerely thank Professor Mariola Krodkiewska for making available her unpublished research data, Dr Agnieszka Sowa and Dr Dariusz Halabowski for additional information about research area. The studies were supported by the National Science Centre, grant no. 2015/17/N/NZ8/01568.

References

Balsamo, M., Grilli, P., Guidi, L. & d'Hondt, J.-L. (2014) Gastrotricha: biology, ecology and systematics: families Dasydytidae, Dichaeturidae, Neogosseidae, Proichthydiidae. *In*: Dumont, H.J.F. (Ed.), *Identification Guides to the Plankton and Benthos of Inland Waters.* 24. Backhuys Publishers, Margraf Publishers GmbH: Weikeirsheim, Germany, pp. 1–187.

Bielańska-Grajner, I. & Cudak, A. (2014) Effects of salinity on species diversity of rotifers in anthropogenic water bodies. *Polish Journal of Environmental Studies*, 23, 27–34.

Bielańska-Grajner, I., Cudak, A., Biała, A., Szymańczak, R. & Sell, J. (2014) Role of spatial and environmental factors in shaping the rotifer metacommunity in anthropogenic water bodies. *Limnology,* 15, 173–183. https://doi.org/10.1007/s10201-014-0428-1

Ehrenberg, C.G. (1830) *Organisation, Systematik, und geographisches Verhältnis der Infusionstierchen.* F. Dümmler, Berlin, 108 pp. https://doi.org/10.5962/bhl.title.2077

Guerne, J. (1888) Campagnes scientifiques du Yacht monégasque l'Hirondelle, troisième année 1887. [Scientific surveys of the Monaco Yacht Hirondelle, third year 1887]. *Paris Gauthier-Villars*, 10 (26), 72–78.

Halabowski, D., Sowa, A. & Krodkiewska, M. (2018) Inland coal mine settling pond as a habitat for the brackishwater plant *Ruppia maritima*, 66, 301–308. http://dx.doi.org/10.3161/15052249PJE2018.66.3.009

Kieneke, A. & Zekely, J. (2007) *Desmodasys abyssalis* sp. nov. – first record of a deep-sea gastrotrich from hydrothermal vents. *Marine Biodiversity Records*, 1 (88), 1–7.

Kisielewski, J. (1981) *Gastrotricha from raised and transitional peat bogs in Poland. Monografie Fauny Polski, 11*. Polska Akademia Nauk, Kraków, 143 pp.

Kisielewski, J. (1986) Freshwater Gastrotricha of Poland. VII. Gastrotricha of extremely eutrophicated water bodies. *Fragmenta Faunistica*, 30, 267–295. https://doi.org/10.3161/00159301FF1986.30.16.267

Kisielewski, J. (1991) Inland–water Gastrotricha from Brazil. *Annales Zoologici, Warszawa*, 43, 1–168.

Kisielewski, J. (1997) *Brzuchorzeski (Gastrotricha). Fauna Słodkowodna Polski, Zeszyt 31.* Wydawnictwo Uniwersytetu Łódzkiego, Łódź, 157 pp.

Kolicka, M., Dabert, M., Dabert, J., Kånneby, T. & Kisielewski, J. (2016) *Bifidochaetus*, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. *Invertebrate Systematics*, 30, 398–419. https://doi.org/10.1071/IS16001

Kolicka, M., Jankowska, E. & Kotwicki, L. (2015) Baltic Sea Gastrotricha – one new species and one new record of Chaetonotida from Poland. *Zootaxa*, 4027 (4), 487–508. https://doi.org/10.11646/zootaxa.4027.4.2

Kolicka, M., Kisielewski, J., Nesteruk, T. & Zawierucha, K. (2013) Gastrotricha from the Poznan Palm House – one new subgenus and three new species of freshwater Chaetonotida (Gastrotricha). *Zootaxa*, 3717 (2), 231–279. http://dx.doi.org/10.11646/zootaxa.3717.2.7

Kolicka, M., Kotwicki, L. & Dabert, M. (2018) Diversity of Gastrotricha on Spitsbergen (Svalbard Archipelago, Arctic) with a description of seven new species. *Annales Zoologici,* 68 (4), 609–739. https://doi.org/10.3161/00034541ANZ2018.68.4.001

Mečnikow, E. (1865) Über einige wenig bekannte niedere Thierformen. *Zeitschrift für Wissenschaftliche Zoologie,* 15, 450–463.

Müller, O.F. (1773) *Vermium terrestrium et fluviatilium saeu animalium infusoriorum, helminthicorum et testaceorum non marinorum, succincta historia.* Heineck et Faber, Havniae et Lipsiae, 135 pp. http://doi.org/10.5962/bhl.title.12733

Parent, S., Morion, A. & Gon, D. (2001) Are meiofauna transient or resident in sand filters of marine aquariums? *Water Resources*, 33 (15), 3625–3634.

Sowa, A., Krodkiewska, M. & Halabowski, D. (2018) Macroinvertebrate communities on various microhabitats of a saline coal mine

settling pond. *Oceanological and Hydrobiological Studies*, 47 (1), 50–59.
https://doi.org/10.1515/ohs-2018-0006

Tongiorgi, P., Fregni, E. & Balsamo, M. (1999) Gastrotricha from Italian brackish environment with description of a new species of *Chaetonotus*. *Journal of the Marine Biological Association of the United Kingdom,* 79, 585–592.
https://doi.org/10.1017/S0025315498000757

Węsławski, J.M., Kryl-Stroszewska, L., Piwowarczyk, J., Urbański, J., Warzcha, J., Kotwicki, L., Włodarska-Kowalczuk, M. & Wiktor, J. (2013) Habitat modelling limitations – Puck Bay, Baltic Sea – a case study. *Oceanologia,* 55 (1), 167–183.
http://doi.org/10.5697/oc.55-1.167

Poznań, 16.12.2019r.

Małgorzata Kolicka
Adam Mickiewicz University, Poznań
Faculty of Biology
Department of Animal Taxonomy and Ecology
Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Declaration

I declare that the publication: **Kolicka M**. 2019. Gastrotricha – not only in sediments: new epiphytic species of Chaetonotida from the Jubilee Greenhouse of the Botanical Garden in Kraków. *European Journal of Taxonomy*, 511 (511):1-100, has been prepared as a part of my doctoral dissertation under the scientific supervision of Prof. UAM, dr hab. Ziemowit Olszanowski.

Yours faithfully,

Poznań, 16.12.2019r.

Małgorzata Kolicka
Uniwersytet im. Adama Mickiewicza, Poznań
Zakład Taksonomii i Ekologii Zwierząt
ul. Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Oświadczenie

Oświadczam, że publikacja: **Kolicka M**. 2019. Gastrotricha – not only in sediments: new epiphytic species of Chaetonotida from the Jubilee Greenhouse of the Botanical Garden in Kraków. *European Journal of Taxonomy*, 511 (511):1-100, została wykonana na poczet mojej rozprawy doktorskiej pod opieką promotora Prof. UAM dra hab. Ziemowita Olszanowskiego.

Z wyrazami szacunku,

European Journal of Taxonomy 511: 1–100
https://doi.org/10.5852/ejt.2019.511
ISSN 2118-9773
www.europeanjournaloftaxonomy.eu
2019 · Kolicka M.

This work is licensed under a Creative Commons Attribution Licence (CC BY 4.0).

Monograph

urn:lsid:zoobank.org:pub:8FDAD45D-1B7D-446F-8B34-026EDF192210

Gastrotricha – not only in sediments: new epiphytic species of Chaetonotida from the Jubilee Greenhouse of the Botanical Garden in Kraków

Małgorzata KOLICKA

Department of Animal Taxonomy and Ecology, Institute of Environmental Biology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland.
Email: kolicka@amu.edu.pl

urn:lsid:zoobank.org:author:550BCAA1-FB2B-47CC-A657-0340113C2D83

Abstract. Although Gastrotricha have previously been recorded in bottom sediments of greenhouses as well as in micro-reservoirs of Bromeliaceae, palm houses provide a more specific microhabitat for these animals that often originate from different regions of the world. This paper presents an investigation of gastrotrich assemblages associated with aquatic plants. Eight species of the epiphytic chaetonotids were found in the Jubilee Greenhouse of the Botanical Garden in Kraków (Poland), including three species new to science, *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov., *C.* (*Hystricochaetonotus*) *horridus* sp. nov. and *C.* (*H.*) *inaequabilis* sp. nov. Two other species are new to Poland, *C.* (*C.*) *paucisquamatus* Kisielewski, 1991 and *C.* (*Zonochaeta*) *cestacanthus* Balsamo, 1990, both recorded here for the first time outside their *terra typica*. These observations confirm that greenhouses and palm houses provide many ecological niches and favourable conditions for the development of a number of unintentionally introduced species which cannot be found outside their original climate conditions.

Keywords. Alien species, *Chaetonotus*, new species, new records, palm house.

Kolicka M. 2019. Gastroticha – not only in sediments: new epiphytic species of Chaetonotida from the Jubilee Greenhouse of the Botanical Garden in Kraków. *European Journal of Taxonomy* 511: 1–100.
https://doi.org/10.5852/ejt.2019.511

Introduction

The beginning of the 20th century can be described as a period of very dynamic growth of urban areas throughout the world. This process is directly connected not only with the growing number of areas covered by compact housing but also with globalisation and the unification of environments (Ignatieva 2010). Palm houses and greenhouses are a frequent and common element in many major European cities. Many such structures were built as early as in the 18th and 19th centuries, and they hosted exhibitions of exotic vegetation species and ornamental elements (e.g., volcanic lava, boulders, logs), which were often brought from different natural environments (Britton 1896; Ignatieva 2010). New, modern palm houses and greenhouses were also created in the 20th and 21st centuries, using plant species from own agriculture, botanical stores or exchanges between palm houses. Regardless of the type of greenhouse, many animals species (both local and foreign) could have been brought in a random and uncontrolled manner together with the plants, seeds, soil, litter and other components of the exhibitions (e.g., Kolicka

et al. 2015). Palm houses provide these accidentally introduced organisms with specific conditions for colonisation and habitation that are different from outside anthropic habitats, e.g., municipal parks or squares (e.g., Duggan & Duggan 2011; Zawierucha *et al*. 2013). Due to the high temperatures and humidity kept at a constant level, they create favourable conditions for the formation of stable and numerous populations of invertebrates which function in an unchanged form for many years (e.g., Kolicka *et al*. 2013). Because of that, palm houses and greenhouses are often regarded as 'tropical islands' in a moderate climate (e.g., Zawierucha *et al.* 2013). These 'islands', as habitats that are distant and isolated from their natural counterparts and other greenhouses, could be a perfect place for research on newly introduced, alien communities of species and the new areas they occupy as well as on variability within and among isolated populations separated from their source habitats.

Studies on palm house gastrotrichs have previously been conducted in Poznań, Łódź (Poland) and Copenhagen (Denmark) (Kolicka *et al.* 2013; Kolicka 2014, 2016), and these microinvertebrates seem to be a constant element of these artificial habitats. However, previous research always concerned the bottom sediments or microreservoirs formed in the axils of Bromeliaceae. Species of Gastrotricha are components of benthic, epibenthic and epiphytic communities. All these habitats need to be the subjects of conducted analyses, as otherwise a false conclusion might be reached that greenhouse faunas do not reflect all the types of environments that these animals occupy in natural conditions. In inland waters, gastrotrichs are a common, abundant group of invertebrates, crucial in meiofauna communities of all types of aquatic and semi-aquatic ecosystems (e.g., Kisielewski 1997a). In nature, they are an important element of the benthic communities (they occur in particularly large abundance in bottom sediments that are very rich in organic matter) as well as of the epiphytic fauna (e.g., Balsamo & Todaro 2002; Balsamo *et al.* 2014).

Taxonomic studies of Gastrotricha have so far mainly focused on the species of benthic communities, both from greenhouses and natural environments. Investigations focusing only on communities of submerged plant fauna remain scarce (e.g., Kisielewski 1981, 1991; Nesteruk 2000, 2004, 2010, 2011). However, analyses of bottom sediments from reservoirs or stations with a rich aquatic vegetation reveal a much higher abundance and species richness of gastrotrichs (e.g., Kisielewski 1986). This may be caused by mixing sediment and vegetation faunas or it may be linked with an increased production of organic matter from decaying plants. Analyses of mixed material (collected, for example, by using a net from the surface of the bottom and submerged plants) also indicate a large number of recorded species (e.g., Balsamo *et al.* 2014). Nonetheless, comparative studies on the gastrotrichofauna of submerged plants and bottom sediments show distinct differences in species composition and the dominance structure of various taxa. The number of common taxa between these two habitats is low, the dominant taxa are different, and the species number and diversity are higher in bottom sediments than in aquatic plants (Nesteruk 2004, 2011).

In my research, I have focused on analysing the composition of the gastrotrich epiphytic fauna forming in an anthropic habitat of the Jubilee Greenhouse of the Botanical Garden in Kraków (Poland). I would like to see whether this artificial habitat provides favourable conditions for Gastrotricha communities and to compare the results of this observation with previous data on palm house chaetonotids from bottom sediments as well as from microreservoirs formed in the axils of bromeliads (Kisielewski 1991; Kolicka *et al*. 2013, Kolicka 2014, 2016).

Material and methods

Study area

Samples were collected at the Jubilee Greenhouse of the Botanical Garden in Kraków (50°03′38″ N, 19°57′30″ E). This greenhouse was built in the years 1959–1966 in the southern part of the Botanical

Garden of Jagiellonian University. The Jubilee Greenhouse comprises 8 departments, viz. the section of the palm house, of carnivorous plants, of aquatic and semi-aquatic plants, of useful tropical plants, of cycads, of desert and semi-desert plants and the nursery section. The research area was the aquatic and semi-aquatic plant section, with large water reservoirs (Fig. 1). These reservoirs with bottom sediments are an exposition place for many plants and some small, ornamental fish species. The submerged plants in this tank come from seeds imported from natural habitats, exchanged between palm houses or from the Botanical Garden's own nursery, whereas the fish come from aquarium shops. Most of the vegetation is cultivated directly in the bottom sediments, but some of the plant species grow in pots immersed in the reservoirs. Manure is added to these pots twice a year. The reservoirs are cleaned once a year, the plants are at that time transferred to a tank in the nursery section, while the water and half of the sediments are exchanged for new ones. The sediments in the aquatic reservoir and pots come from the local area whereas the fresh water for the tank is taken from a municipal water supply. The temperature in this section does not fall below 23°C during the year.

Fig. 1. Study area. **A**. The inside of Jubilee Greenhouse at the Botanical Garden in Kraków. **B**. Sampling site.

Sampling and documentation

Samples of aquatic plants were taken two times (15 November 2013 and 17 April 2014) from water reservoirs at the Jubilee Greenhouse of the Botanical Garden in Kraków. During both sampling sessions, the material was collected at the same spots from three stations within the reservoirs. However, between these sessions the plant arrangement was changed and the samples were collected from different plants during the first and second sampling (see Table 1). Samples of submerged vegetation were collected by picking the plants and squeezing water out of them thoroughly into a container, rinsing and squeezing them one more time, and then picking fresh, non-squeezed plants and placing them in a 1000 cm^3 container. Water plant samples were collected using the standard method for gastrotrichofauna research due to the limited effectiveness of other methods (Kisielewski 1997a). Within 5 days of collecting the material, a total of 5 cm^3 of water from the vegetation was analysed under an Olympus SZ51 stereo microscope. All specimens were observed, photographed and documented alive under an Olympus BX41 compound microscope (Tokyo, Shinjuku, Japan) equipped with phase contrast and an Array Artcam 300 digital camera (Tokyo, Shinjuku, Japan). Morphometric characters were measured in cellSens Entry 1.12 software (Olympus). All measurements are given in micrometres (µm) and all formulas used are given as a percentage (%) (Kisielewski 1981, 1991, 1997a; Hummon *et al.* 1992).

Table 1. Sampling sites with specified aquatic plant species.

Site	Session 1 (15 Nov. 2013)	Session 2 (17 Apr. 2014)
I	*Myriophyllum verticillatum* L.	*Myriophyllum verticillatum* L.
II	*Elodea nuttallii* (Planch.) H.St.John	*Myriophyllum verticillatum* L.
III	*Limnophila sessiliflora* Blume	*Elodea nuttallii* (Planch.) H.St.John

Morphological analyses

Morphological characters were only measured if they were amenable to accurate measurement. Because of the generally low taxonomic usefulness of preserved gastrotrichs, each specimen was documented using a set of photomicrographs and each holotype was additionally documented with detailed schematic drawings (all deposited in the Natural History Collections at Adam Mickiewicz University, Poznań = NHC). The International Code of Zoological Nomenclature allows for such a documentation of types in article 73.1.4 (The International Commission on Zoological Nomenclature 1999), even if the documented specimen no longer exists. The taxon descriptions follow the convention of Hummon *et al.* (1992), in which the distance of a morphological character from the anterior end is expressed as percentage units (U) of the animal's total length. In the descriptions of new species in this paper, the measurements of width of different body sections were omitted because they would be strongly influenced by the pressure of the coverglass on the studied live specimens. The identification of gastrotrichs, their morphological study and terms follow Kisielewski (1981, 1991, 1997a) and Kolicka *et al.* (2016). The terms describing the shape of the furcal branches and furcal indentation follow Roszczak (1969). This paper uses the following formula to describe the distribution of scales:

$$\text{Ratio of scale distribution} = \frac{\text{total number of longitudinal alternating rows of scales}}{\text{number of scales in central longitudinal rows}} \times 100\%$$

as well as the pharynx formulae according to Kisielewski (1991):

$$a = \frac{\text{width of anterior thickening}}{\text{pharynx length}} \times 100\% \qquad n = \frac{\text{width of pharyngeal narrowing}}{\text{pharynx length}} \times 100\%$$

$$m = \frac{\text{width of middle pharynx}}{\text{pharynx length}} \times 100\% \qquad p = \frac{\text{width of posterior thickening}}{\text{pharynx length}} \times 100\%$$

and the pharynx-to-intestine length ratio according to Kolicka *et al.* (2016):

$$I = \frac{\text{pharynx length}}{\text{intestine length}} \times 100\%$$

The use of these formulae allows for the objective comparison of these morphological features between different taxa and the demonstration of scale distribution and detailed pharynx shape in a numerical manner. The pharynx-to-intestine length ratio helps estimate the age of specimens, thus avoiding misclassification by analysing juvenile, subadult and adult individuals together. Juvenile and subadult specimens have a significantly higher I-ratio than adults, usually for representatives of *Chaetonotus* at > 70% and 55%–70%, respectively. In this paper, the following abbreviations were used: D = dorsal; DL = dorsolateral; L = lateral; LV = ventrolateral; V = ventral, sensu Kolicka *et al*. (2016, see S1 and S2).

Results

The analysed material consisted of 208 specimens belonging to three genera, two subgenera and nine species, including three species that are new to science, namely *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov., *C.* (*Hystricochaetonotus*) *inaequabilis* sp. nov. and *C.* (*H.*) *horridus* sp. nov. Two other species

recorded at the Jubilee Greenhouse of the Botanical Garden in Kraków turned out to be new to Poland; the first species, *C.* (*C.*) *paucisquamatus* Kisielewski, 1991, was described from and is only known from Brazil; the second newly reported species, *C.* (*Zonochaeta*) *cestacanthus* Balsamo, 1990, was described from Italy and was previously recorded only in that country. Another species that was found, namely *C.* (*H.*) *hystrix* Mečnikow, 1865, is well known in Poland and is considered as a taxon with a worldwide distribution. The last two of the recorded species were identified as *Lepidodermella* aff. *squamata* (Dujardin, 1841) and *Heterolepidoderma* aff. *majus* Remane, 1927. These taxa were defined as species with an unclear taxonomic rank because they corresponded well with the original description, but possessed a single character that differed from previous records (see the 'Remarks' section for these species). Live euglenids were present inside the intestines of 4 out of 208 specimens (see Kisielewska *et al*. 2015). In all the samples, rotifers (representatives of Monogononta and Bdelloidea), ostracods and rare polychaetes were observed as accompanying fauna.

Phylum Gastrotricha Mečnikow, 1865
Order Chaetonotida Remane, 1925 [Rao & Clausen 1970]
Suborder Paucitubulatina d'Hondt, 1971
Family Chaetonotidae Gosse, 1864 (*sensu* Leasi & Todaro 2008)
Subfamily Chaetonotinae Gosse, 1864 (*sensu* Kisielewski 1991)

Genus ***Chaetonotus*** Ehrenberg, 1830

Type species

Trichoda larus Müller, 1773 (= *Chaetonotus larus* (Müller, 1773)).

Terra typica

Denmark.

Remarks on genus

This is a polyphyletic taxon, with only one apparently monophyletic subgenus (*Chaetonotus* (*Zonochaeta*) Remane, 1927); it encompasses 175 nominal freshwater species and 44 nominal marine species (Kånneby *et al*. 2012, 2013; Todaro 2018). Present in benthic, interstitial, periphytic and rarely semi-planktonic habitats (Kisielewski 1997a).

Subgenus ***Chaetonotus*** s. str. Ehrenberg, 1830

Type species

Trichoda larus Müller, 1773 (= *Chaetonotus larus* (Müller, 1773)).

Terra typica

Denmark.

Remarks on subgenus

This is clearly a polyphyletic subgenus in terms of molecular data (Kieneke *et al.* 2008; Kånneby *et al.* 2012, 2013); it encompasses 84 nominal freshwater and 4 nominal marine species (Kieneke & Schmidt-Rhaesa 2015; Todaro 2018). Present in benthic, interstitial, periphytic and rarely also in semi-planktonic habitats (Kisielewski 1997a).

Chaetonotus* (*Chaetonotus*) *invitatus sp. nov.
urn:lsid:zoobank.org:act:5350662C-4565-4EB6-96B0-4742ED038286
Figs 2–11, A1–A2; Tables 2, A1–A2

Diagnosis

Slender, bottle-shaped, body measuring from 174.6 to 194.7 μm in length. Head five-lobed, cephalion, epipleurae and hypopleurae clearly demarcated in head outline. Hypostomium kidney-shaped with horn-like protuberances anterolaterally and pair of reinforcements near anterior edge. Two additional plates beyond posterior hypostomium edge. Ocellar granules absent. In mouth ring, two weak cuticular teeth arising from anterior pharynx region. All scales one-lobed, with shallow to deep posterior notches, keeled and spined. Scales distributed in 23–25 single longitudinal rows (5–7D+4DL+6L+4LV+4V) with 25–27 scales in central row. Scales strongly differing morphologically in body areas. Scales located close to one another and with overlapping edges on furcal base and furcal appendages. Spines basally bent and thick, subsequently strongly and gradually tapering towards hair-like end, ventral spines hair-like along entire length. Lateral spines more strongly curved than dorsal and dorsolateral ones. Length of spines gradually increases from dorsal, dorsolateral, lateral and ventrolateral surfaces towards ventral surface and from head towards widest trunk region. Dorsally on posterior trunk region, two or three scales with longer and thicker spines. Dorsally and dorsolaterally on posterior trunk region, on furcal base and furcal appendages, scales with shorter spines, with rudimentary spines or without any spine. Dorsolaterally on furcal appendages, one pair of scales with long, rigid and spike-like spines reaching to furca inner indentation. Furcal appendages with two pairs of scales with long, thick parafurcal spines tapering to their ends. Ventral interciliary field covered with semirectangular plates on pharyngeal region and one-lobed, keeled scales on intestine region. Four pairs of ventral interciliary field terminal scales. Pharynx narrow, with anterior and posterior dilatations. Intestine straight without anterior section differing in form and morphology.

Etymology

From the Latin '*invitatus*' = 'invited', referring to the artificial place to which it was transferred.

Material examined

Holotype

POLAND • adult; Kraków, Botanical Garden, Jubilee Greenhouse, site 1; 50°03′38″ N, 19°57′30″ E; 15 Nov. 2013; M. Kolicka leg.; NHC-GCCI-20-1-25/h (photomicrographs, also in the author's collection).

Paratypes

POLAND • 24 adults, 5 subadults, 5 juveniles; same collection data as for holotype; NHC-GCCI-20-26-100/p (photomicrographs, also in the author's collection).

Description

Habitus. *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov. has a slender, bottle-shaped body (Figs 2, 5). The head is wide and the neck constriction is strongly marked. The neck gradually tapers to the beginning of the trunk (ca U38) (Figs 2, 5). The trunk is only slightly wider than the head and gradually dilates from ca U39 to ca U59, where it is at its maximum width. Then it gradually tapers slightly towards a weakly demarcated furcal base (from U85) (Fig. 2). The furcal indentation is parabolic in shape. The furcal branches are set apart and point slightly outwards (Figs 6G–H, 8). The adhesive tubes are long, slightly bent, narrow and very gradually taper slightly towards their ends. The adhesive tube ends are blunt (Figs 2, 6G–H, 8).

Fig. 2. *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov. Schematic drawings. **A**. Dorsal body view. **B**. View of internal morphology. **C**. Ventral body view. Light grey areas indicate the areas of the insertion of ciliary bands.

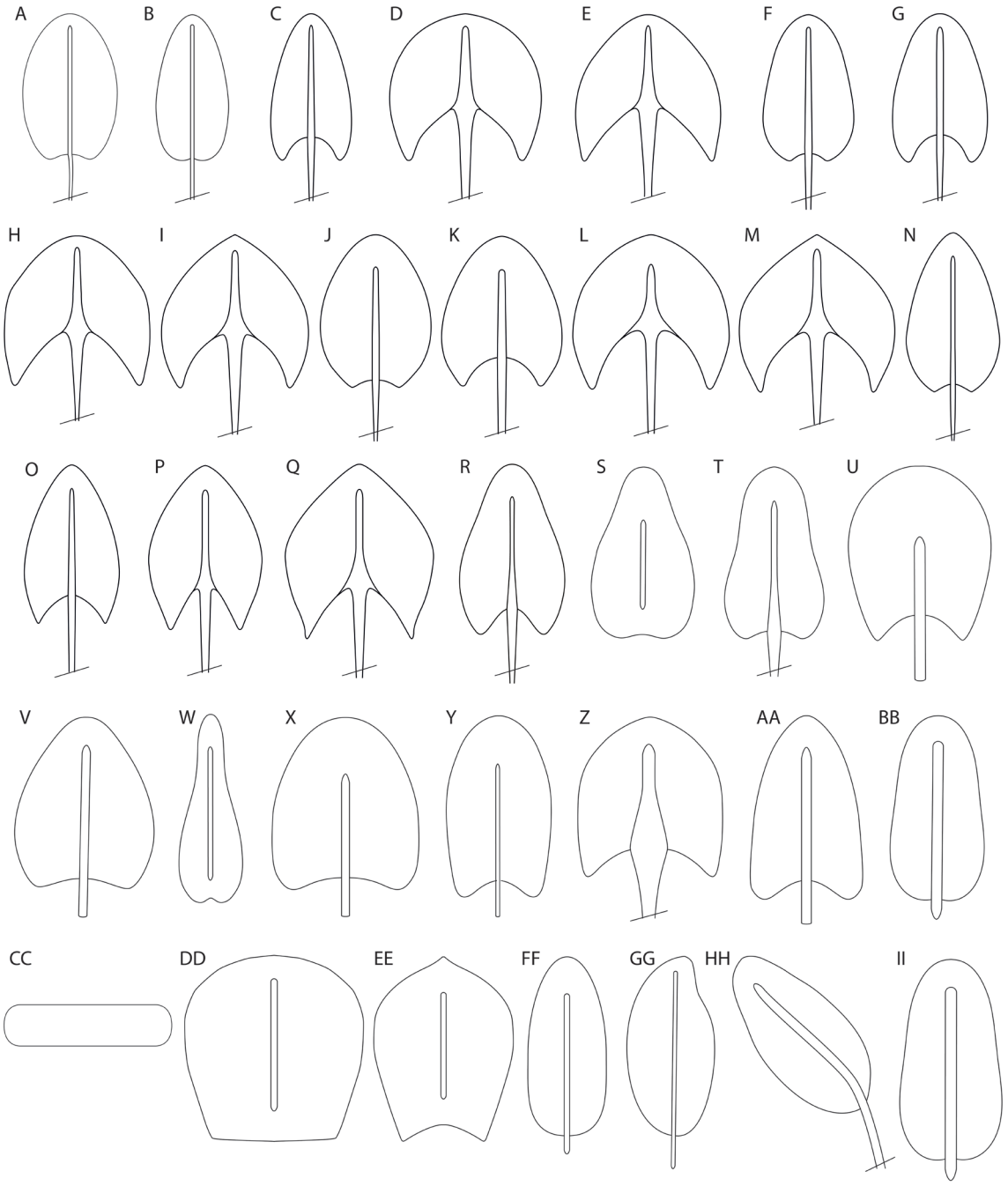


Fig. 3. *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov. Schematic drawings of the scales. **A**. Head anteriormost dorsal scale (scale 1). **B**. Head dorsal scale (scale 2). **C**. Head dorsolateral scale (scale 3). **D**. Head lateral and ventrolateral scale (scale 4). **E**. Head ventral scale (scale 5). **F**. Anterior neck dorsal scale (scale 6). **G**. Anterior neck dorsolateral scale (scale 7). **H**. Anterior neck lateral and ventrolateral scale (scale 8). **I**. Anterior neck ventral scale (scale 9). **J**. Posterior neck dorsal scale (scale 10). **K**. Posterior neck dorsolateral scale (scale 11). **L**. Posterior neck lateral and ventrolateral scale (scale 12). **M**. Posterior neck ventral scale (scale 13). **N**. Trunk dorsal scale (scale 14). **O**. Trunk dorsolateral scale (scale 15). **P**. Trunk lateral and ventrolateral scale (scale 16). **Q**. Trunk ventral scale (scale 17). **R**. Scale 18. **S**. Scale 19. **T**. Scale 20. **U**. Scale 21. **V**. Scale 22. **W**. Scale 23. **X**. Scale 24. **Y**. Scale 25. **Z**. Scale 26. **AA**. Scale 27. **BB**. Scale 28. **CC**. Scale 29. **DD**. Scale 30. **EE**. Scale 31. **FF**. Scale 32. **GG**. Scale 33. **HH**. Scale 34. **II**. Scale 35.

Head. The head is five-lobed, long and parabolic. The cephalion (U1–U7) is long and wide, it is visible on the dorsal head surface as an inverted, rounded triangle. It is clearly demarcated in the body outline and its dorsal edge is free and does not adhere to the head surface (Fig. 7A–B). The pleurae are large, convex and clearly demarcated in the head outline, the hypopleurae are only slightly larger than the epipleurae (Fig. 2). Between the epipleurae and hypopleurae, deep notches are present. The epipleurae (U3–U7) are located on the dorsal, dorsolateral, lateral and ventrolateral surfaces, whereas the hypopleurae (U8–U12) cover the dorsal, dorsolateral, lateral, ventrolateral and ventral surfaces in such a manner that their greatest portion is located on the ventral side. The hypostomium is kidney-shaped and has two horn-like protuberances placed anterolaterally and a pair of strong reinforcements near its anterior edge (U3–U6) (Figs 2C, 6B). One pair of additional plates is located posterolaterally to the hypostomium, at U6–U7. These plates are wide and short and their lateral edges extend to the edges of the ventral epipleurae (Fig. 6B). Two pairs of cephalic ciliary tufts are present. The anterior tufts emerge laterally between the cephalion edge and the dorsal edge of the epipleurae (at U3). The anterior tufts consist of 5 cilia which are bent and short, and their length increases from the first to the last cilium (Table A1). The posterior tufts have 5 cilia each and emerge laterally between the epipleurae and the hypopleurae (at U7–U8). Cilia in the posterior tufts are long and nearly straight and their length increases from the first cilium to the last, long cilium (Table A1). Ocellar granules are absent. The mouth ring is located terminally at U1–U3, is wide and has long, strong finger-like reinforcements as well as very long cuticular inner hairs (Figs 2, 9A–B). Inside the mouth ring, two small and weak cuticular teeth arising from the anterior pharynx region are present (Fig. 9B–C). Suboral bristles emerge in three pairs of tufts, laterally to the mouth ring, near the lateral edges of the hypostomium and in lines below the additional hypostomium plates (Fig. 2C).

Scales. The body is covered with one-lobed scales that adhere along their entire surface to the cuticle (Figs 3, 6–7). The scales have a straight keel and are egg-shaped with very weak posterior notches to very deep posterior notches. The scales are distributed in 23–25 longitudinal alternating rows

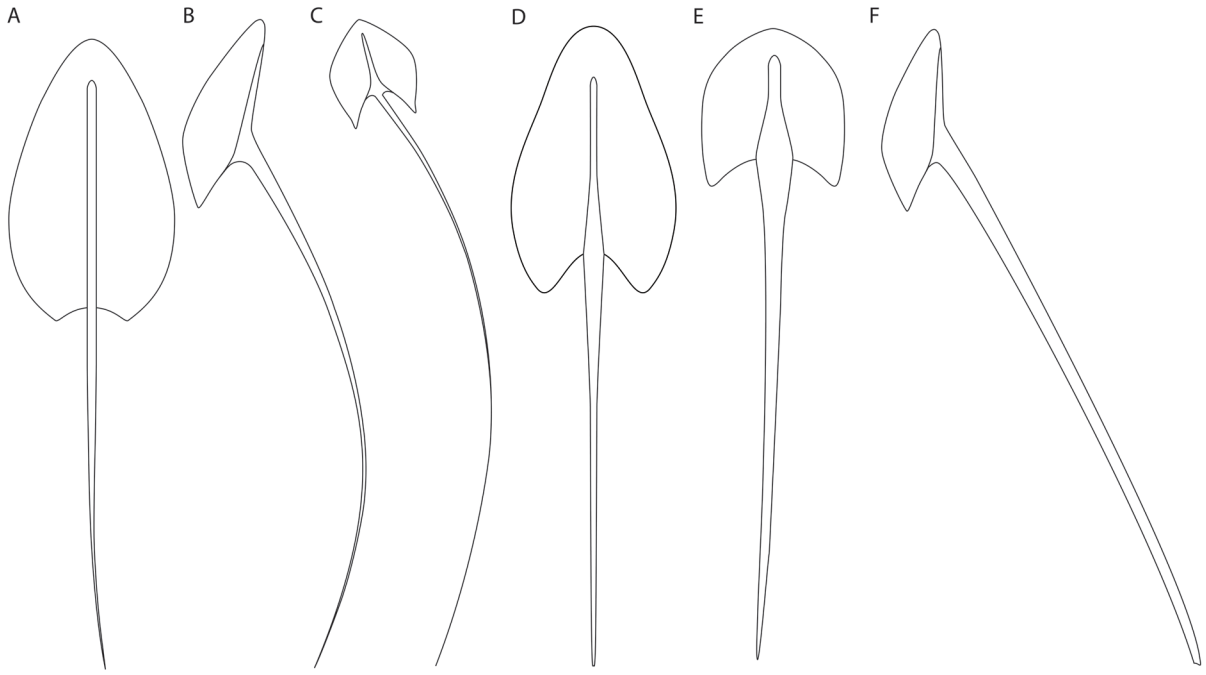


Fig. 4. *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov. Schematic drawings of the spine types. **A**. Dorsal and dorsolateral spine type. **B**. Lateral and ventrolateral spine type. **C**. Ventral spine type. **D**. Scale 18 spine type. **E**. Dorsolateral furcal appendage spine type. **F**. Parafurcal spine type.

Table 2. Main morphometric characters of *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov. N = number of specimens analysed; Range = the smallest and the largest structure found among all specimens measured. All measurements in micrometers (μm).

Characters	N	Holotype	Range of adult paratypes
Body length	23	193.18	174.64–194.66
Furca length	22	33.17	31.14–34.45
Adhesive tube length	22	18.97	16.39–19.20
Number of scales in central longitudinal row	25	27	25–27
Total noumber of longitudinal alternating rows of scales	25	25	23–25
Head scales length × width	22	1.94–4.94 × 1.91–4.23	1.58–5.19 × 1.60–4.91
Neck scales length × width	22	2.55–6.29 × 3.02–5.71	2.08–7.09 × 2.46–6.44
Trunk scales length × width	22	3.57–8.78 × 2.79–5.97	2.84–9.38 × 2.22–7.39
Head spines length	22	3.88–24.11	3.31–26.39
Neck spines length	22	6.37–27.41	5.03–31.86
Trunk spines length	22	10.09–31.06	7.84–34.26
Mouth ring diameter	22	8.43	7.58–9.47
Pharynx length	23	56.84	51.02–59.64

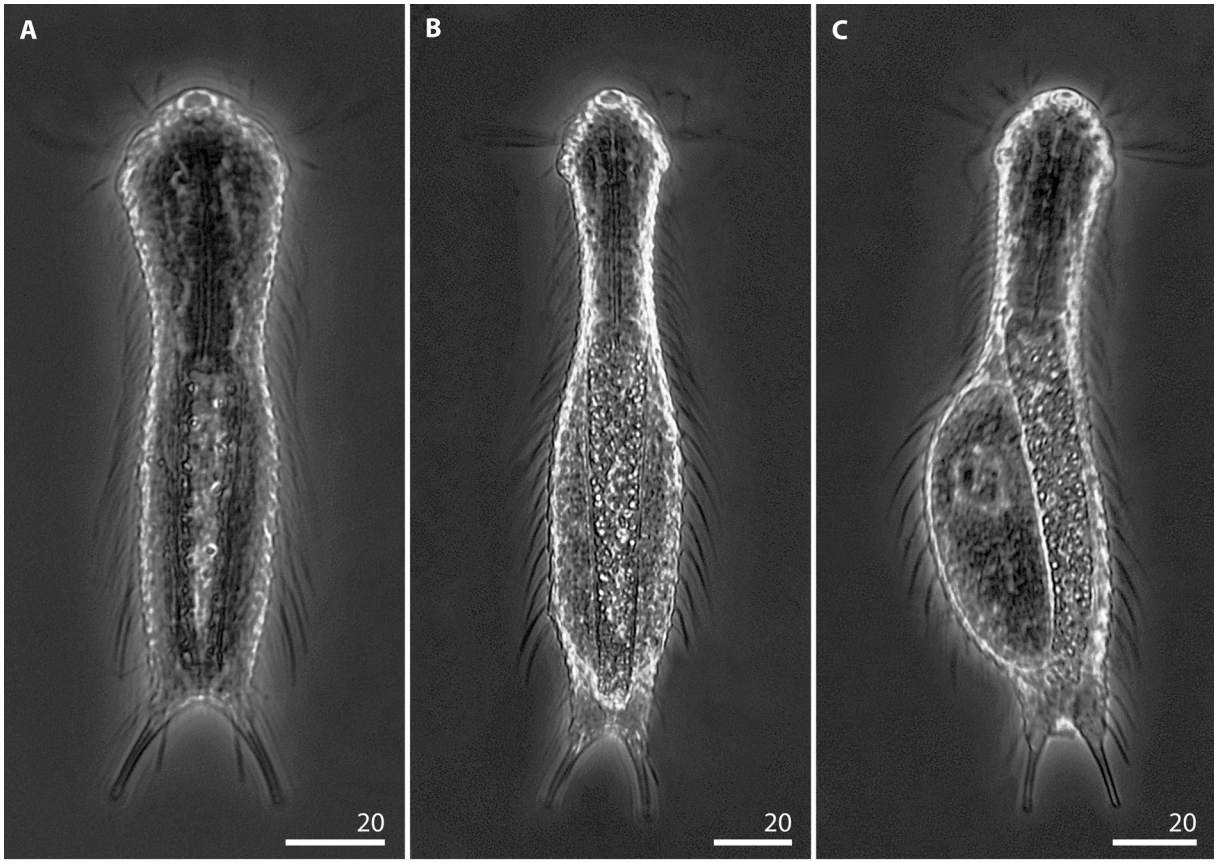


Fig. 5. *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov. Habitus. Phase contrast microphotographs. **A**. Juvenile specimen. **B**. Adult specimen. **C**. Specimen with mature egg.

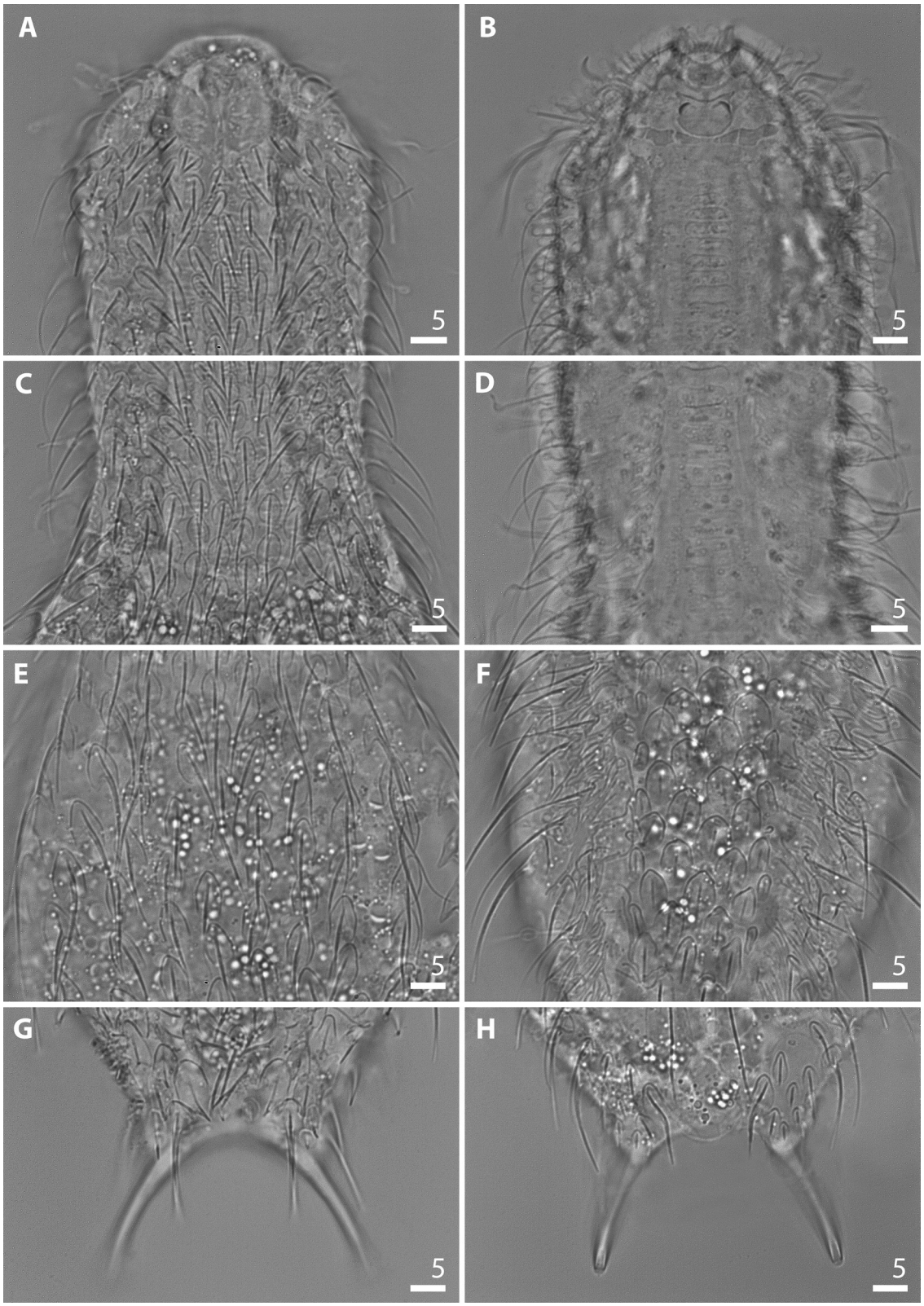


Fig. 6. *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov. Bright field microphotographs. **A**. Head, dorsal view. **B**. Head, ventral view. **C**. Neck, dorsal view. **D**. Neck, ventral view. **E**. Trunk, dorsal view. **F**. Trunk, ventral view. **G**. Furca base and furcal appendages, dorsal view. **H**. Furca base and furcal appendages, ventral view.

(5–7D+4DL+6L+4LV+4V) with 25–27 scales in the central row. The longitudinal rows of scales run straight and are arranged in parallel to one another from the top of the head to the widest body region. Dorsally and dorsolaterally on the posterior trunk region, furca base and furcal appendages, the scales are arranged in rounded arcs and in a rosette (Figs 2, 7C–D, 8A). The scales are located close to one another, but on the head, neck and anterior and central trunk part they do not overlap. The edges of the scales overlap only on the furcal base and furcal appendages (Figs 2, 8A). The scales show morphological diversity throughout the particular body regions in terms of shape and size (Fig. A1). The head anteriormost scales are situated near the posterior edge of the cephalion. These scales are oval with shallow posterior notches (scale 1; Fig. 3A). The remaining head dorsal scales (scale 2; Fig. 3B) are more elongated and have shallower posterior notches. The head dorsolateral scales (scale 3; Fig. 3C) become shorter than the dorsal scales and have deeper posterior notches. The head lateral and ventrolateral scales (scale 4; Fig. 3D) become more semi-rounded, shorter and wider than the dorsolateral ones and possess deep posterior notches, whereas the ventral scales are smaller, wide and have more pointed anterior edges (scale 5; Fig. 3E). On the neck, the scales are shorter and wider than the scales on the head area (scales 6–9; Fig. 3F–I) and their size gradually increases towards the trunk region (scales 10–13; Fig. 3J). The neck dorsal scales (scales 6 and 10; Fig. 3F, J) are egg-shaped and possess weak posterior notches. The neck dorsolateral scales (scales 7 and 11; Fig. 3G, K) become gradually larger than the dorsal scales and have deeper posterior notches. The neck lateral and ventrolateral scales (scales 8 and 12; Fig. 3H, L) become more semi-rounded and deeply posteriorly notched. They are wider and gradually larger than the dorsolateral ones, whereas the ventral scales (scales 9 and 13; Fig. 3I, M) are

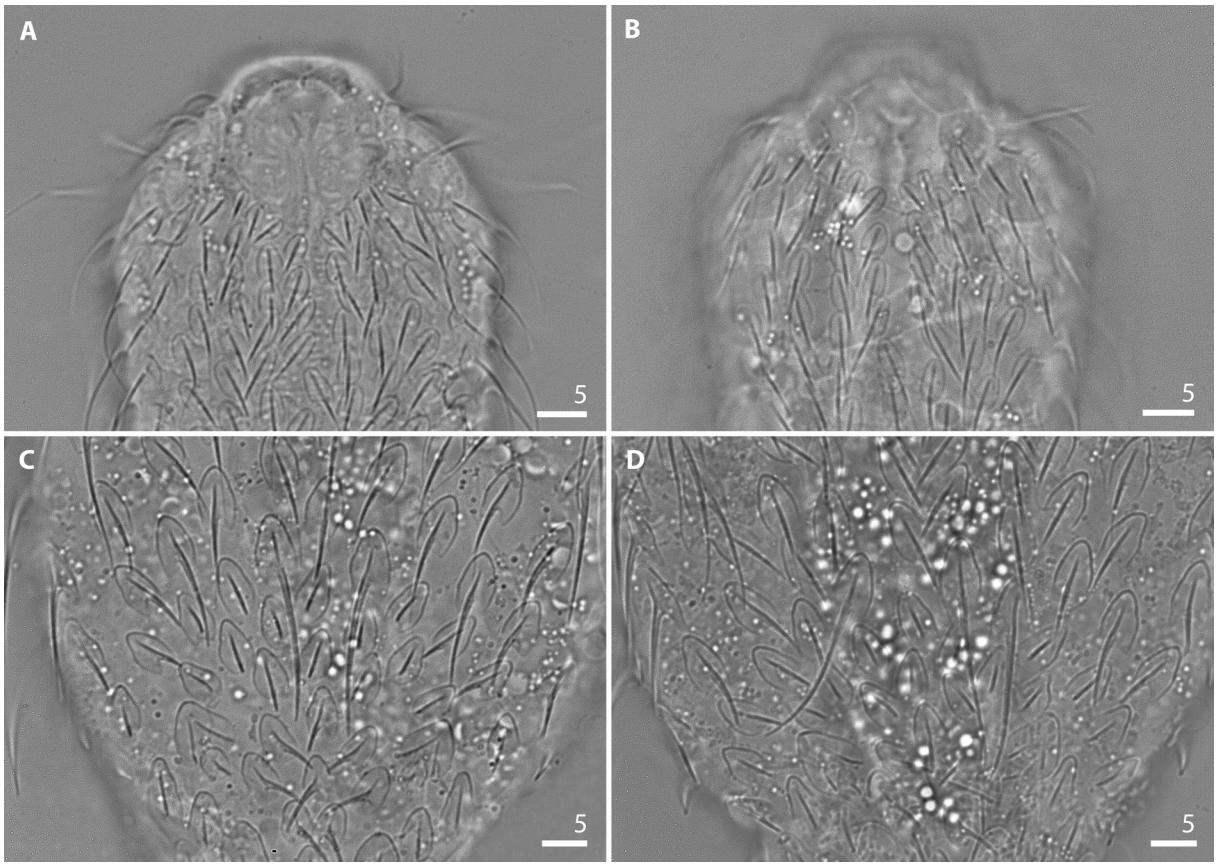


Fig. 7. *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov. Bright field microphotographs. **A**. Detail of dorsal head view of adult specimen. **B**. Detail of dorsal head view of juvenile specimen. **C**. Dorsal view of posterior trunk region of specimen with two scales 17. **D**. Dorsal view of posterior trunk region of specimen with three scales 17.

smaller, wide and have more pointed anterior edges (Fig. 2C). The trunk dorsal scales (scale 14; Fig. 3N) from the anterior to posterior trunk part are egg-shaped, but with a more tapered anterior edge than the neck scales. These scales have weak posterior notches. The trunk dorsolateral scales (scale 15; Fig. 3O) become gradually larger than the dorsal scales and have deeper posterior notches. The trunk lateral and ventrolateral scales (scale 16; Fig. 3P) become more oval, gradually larger and wider than the dorsolateral ones, have a more pointed anterior edge and possess deep posterior notches. The trunk ventral scales (scale 17; Fig. 3Q) are smaller, shorter and wider, have more pointed anterior edges and a very deep posterior notch (Figs 2A, 6F). The scales in the ventral longitudinal row located closest to the ciliary band are distinctly smaller than the other scales and their anterior edge is oriented towards the bands at an angle of ca 40° (Figs 2C, 6B, D, F, H). Dorsally on the trunk posterior region (at U73–U77), two or three slightly larger scales with deeper posterior notches and much longer and stronger spines are present (scale 18; Fig. 3R). Near the scales with longer and stronger spines and posteriorly to them (at U75–U80), smaller, trifle pear-shaped scales are located with weak posterior notches, straight keels and without spines (scale 19; Fig. 3S). Subsequently, on U80–U85, dorsally on the posterior trunk region, 8 scales, that are triangle-shaped with strongly rounded edges, with clear posterior notches and a straight keel and spine, are situated (scale 20; Fig. 3T). On the dorsal posterior trunk region, one pair of small, semirounded scales with a straight keel and a short, straight spine is located in the notch of a pair of double keeled scales with trunk sensory bristles (at U82–U83) (scale 21; Fig. 3U). Dorsolaterally on the posterior trunk region and furcal base, two pairs of small semi-triangular scales with strongly rounded edges and shallow posterior notches, straight keels and straight rudimental spines are present (at U84–

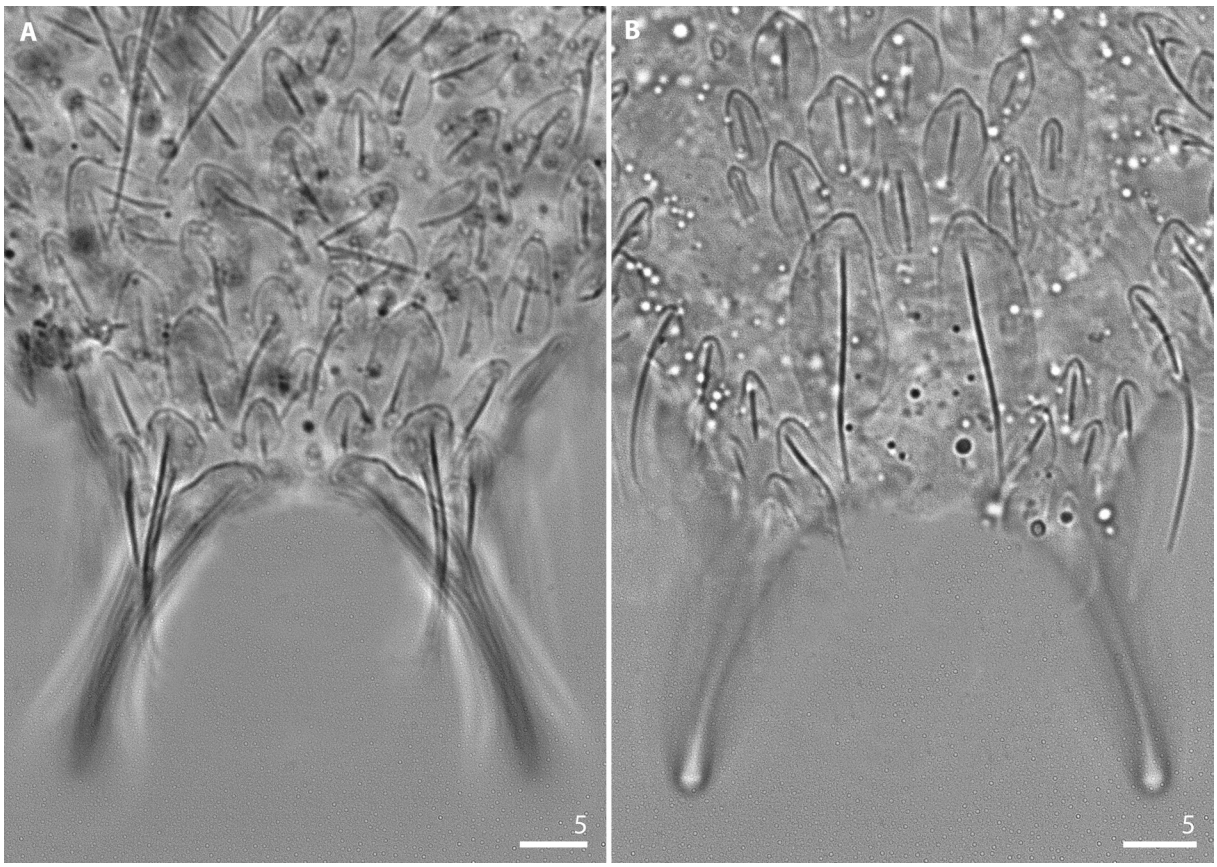


Fig. 8. *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov. Bright field microphotographs. **A**. Dorsal view of the posterior trunk region, furca base and furcal appendage scales. **B**. Ventral view of the posterior trunk region, furca base and furcal appendage scales.

U87) (scale 22; Fig. 3V). Laterally on the posterior trunk region and on the furcal base, at U82–U88, two pairs of narrow, elongated pear-shaped scales with very weak posterior notches are present. These scales have long straight keels but are not spined (scale 23; Fig. 3W). Dorsally on the furcal base and furcal appendages at U85–U88, three semi-rounded scales with weak posterior notches, straight keels and short spines are present (scale 24; Fig. 3X). Laterally to them (to scales 24), on the dorsal furcal base, one pair of large (5.4–9.1 × 3.2–5.3 μm), oval scales with a shallow posterior notch, straight keel and a short, straight spine is situated (at U84–U87) (scale 25; Fig. 3Y). Dorsolaterally on the furcal appendages at U87–U89, one pair of large, rounded scales with prominent posterior notches is present (scale 26; Fig. 3Z). These scales have straight keels and long, rigid spines. Laterally to this pair of scales (to scales 26) at U88–U89, two pairs of small (2.6–6.1 × 1.8–4.1 μm), oval-shaped scales with a shallow indentation and straight keels and spines are present (scale 27; Fig. 3AA). On the lateral surface of the furcal appendage, two pairs of scales with parafurcal spines are present. The first pair is located at U88–U89 and the second one, i.e. the last pair of furcal lateral scales, is located at U90–U91. Both pairs are similar in shape to the posterior trunk scales (Figs 2, 6G). Ventrolaterally and ventrally on the furcal appendages and furcal base, two pairs of small (2.2–5.3 × 1.4–2.8 μm) oval scales without any posterior notches are present (at U85–U88). These scales have straight keels and straight rudimentary spines (scale 28; Fig. 3BB).

Spines. All spines emerging from the posterior scale region have no lateral denticles. The dorsal, dorsolateral (Fig. 4A), lateral and ventrolateral (Fig. 4B) spines are basally bent and thick, and subsequently strongly and gradually taper towards the slightly bent hair-like ends. The spines arising from the lateral,

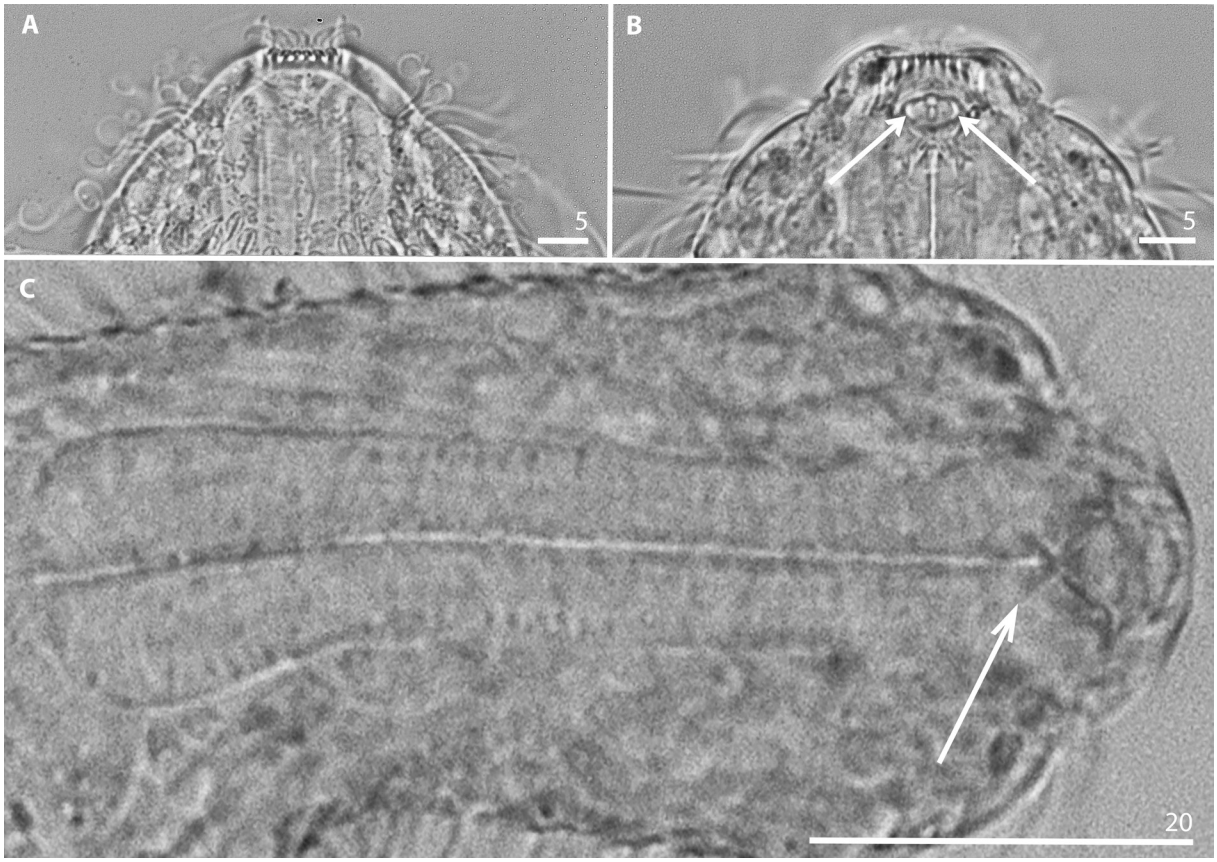


Fig. 9. *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov. Adult specimen. Bright field microphotographs. **A**. Detail of the mouth ring, dorsal view. **B**. Detail of the mouth ring, internal view. **C**. Detail of the pharynx.

ventrolateral and ventral scales are much more strongly bent than the dorsal and dorsolateral spines. The ventral scale spines are hair-like along their entire length (Fig. 4C). The length of the spines gradually increases from the head towards the widest body region (from 3.9–24.1 to 10.1–31.1 μm). Then the length of the dorsal spines gradually shortens to the posterior trunk region. Moreover, the length of the spines gradually increases from the dorsal surface, along the dorsolateral, lateral, ventrolateral and ventral surfaces towards the ciliary bands (head: from 1.6–7.4 to 13.3–26.4 μm; neck: from 5.0–10.7 to 15.9–31.9 μm; trunk: from 7.8–16.5 to 19.6–34.3 μm) (Table A1). On the dorsal posterior trunk region, two or three spines of different types are present (arising from scales 18). These spines are much longer (16.6–20.0 μm) and stronger than the other dorsal spines and taper to blunt ends (Figs 2A, 6C–D). The spines arising from the dorsal and dorsolateral posterior trunk and furcal base scales (namely from scales 20, 22, 24, 27) are thick and straight, tapering to blunt ends (Fig. 4D). The posteriormost lateral trunk spines (16.1–25.1 μm) do not vary in length from the other lateral spines in the posterior trunk region. From the dorsolateral furcal appendage scales (scale 26) arise very long (14.5–18.4 μm), thick, rigid, basally bent and spike-like spines that extend to the inner furcal indentation (Figs 2A, 4E, 6G, 8A). These spines taper slightly to pointed ends. Two pairs of parafurcal spines, emerging from the lateral scales of the furcal appendages (at U88–U89 and at U90–U91), are thicker and longer (11.3–13.5 μm) than the other lateral body spines (Fig. 4F). These spines are basally bent and taper slightly towards their pointed ends (Figs 6G, 8A).

Dorsal sensory bristles. This species has three pairs of dorsal sensory bristles. The first pair is located on the head, directly between the lateral edge of the cephalion and the epipleurae (at U4), and emerges from small, round papillae. The second pair of sensory bristles is located on the neck (U19) and emerges

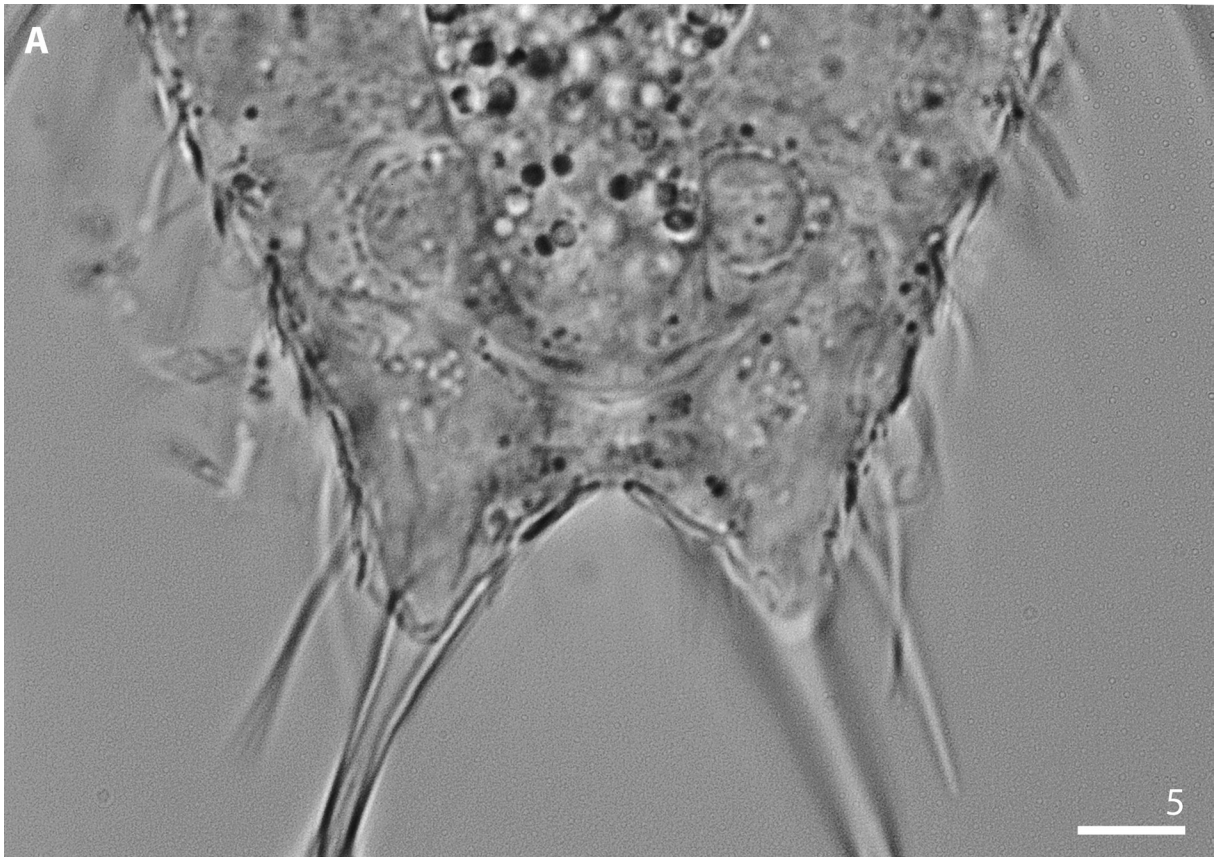


Fig. 10. *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov. Detail of internal morphology. Bright field microphotography. X-organ.

from small, rounded papillae. The third posterior pair of sensory bristles emerges from the double-keeled scales located on the posterior trunk region at U81–U83. These scales are shaped like triangles with strongly rounded edges and a deep posterior notch. The keels of these scales are slightly bent, not connected and pass into rudimentary spines (Figs 2A, 8A).

Ventral ciliary bands and ventral interciliary field. Longitudinal ventral ciliary bands begin at U8 and run back to U86 (Figs 2C, 6B, D, F, H). On the head, at ca U8–U10, the ciliary bands are merged and lie close to the hypopleurae. The ventral interciliary field scales are distributed in single (on the pharyngeal region) and 5–7 (on the intestinal region) longitudinal alternating rows, with 29–33 scales in the central row, including 15–18 plate-like scales and 14–17 one-lobed scales. The entire pharyngeal region (from U10 to U31) is covered with short, wide plates (scale 29; Fig. 3CC) and their size gradually increases from the anterior to the posterior end (from 1.0–1.7 × 1.9–3.5 μm to 2.6–5.2 × 7.1–11.4 μm). On the intestinal region, one-lobed scales with overlapped edges are present (from U32 to U84). On the anterior part of the intestinal region the scales are semi-rectangular in shape, have weak keels and their posterior edges are straight, without posterior notches (scale 30, Fig. 3DD). Towards the furcal base the keels become more pronounced and on the edges of the anterior scales small, horn-like perturbances begin to appear; the posterior edges become notched (scale 31; Fig. 3EE). The last alternating row of interciliary field scales is of a different type, i.e., they are oval in shape without posterior notches, have straight keels and present rudimentary spines (scale 32; Fig. 3FF). The longitudinal row of ventral field scales closest to the ventral ciliary bands has significantly smaller and narrower scales than in the central rows. The first pair of terminal scales on the ventral interciliary field is large (14.8–17.1 × 6.6–8.1) and located at U84–U87. They are oval, asymmetrical in shape, possess a straight keel and a straight,

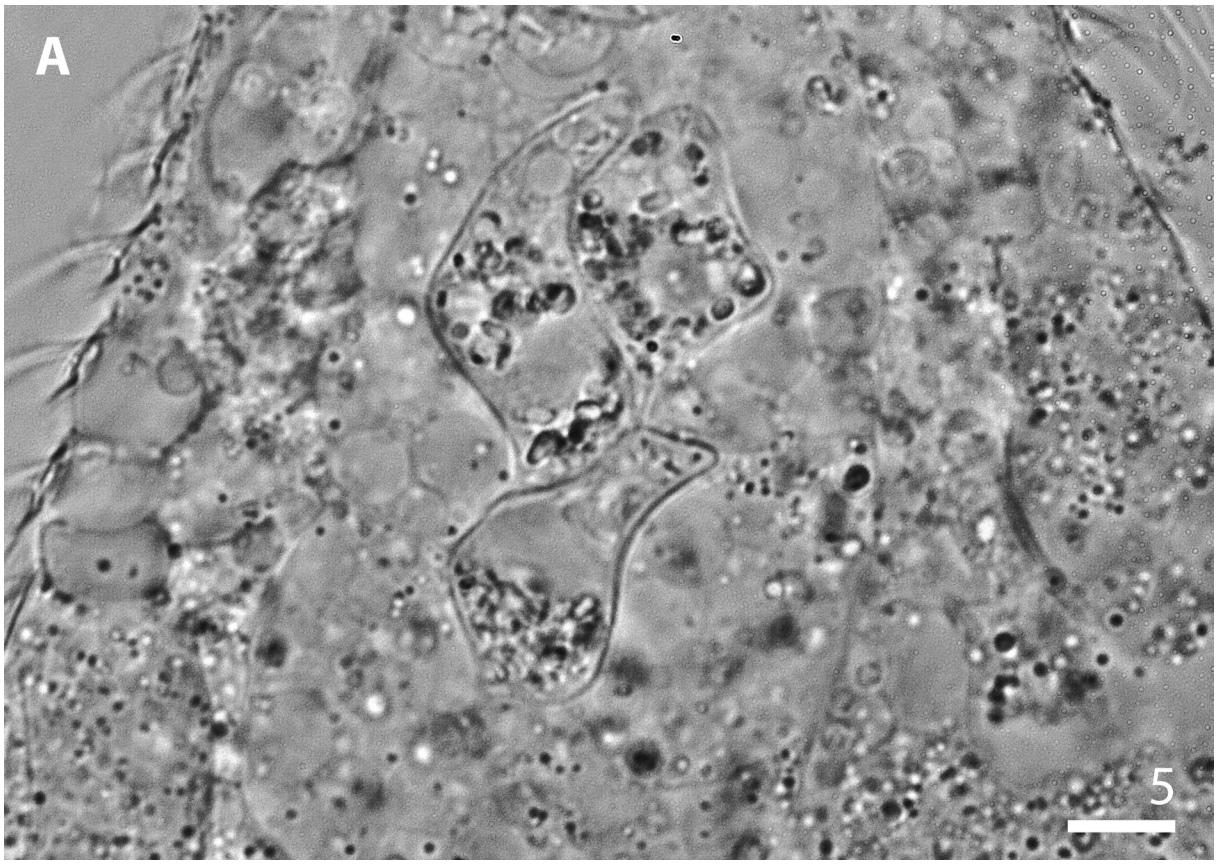


Fig. 11. *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov. Adult specimen. Bright field microphotography. Living euglenids inside intestine.

thick spine (scale 33; Fig. 3GG). The second pair of terminal scales on the ventral interciliary field is located on the furcal appendages (at U86–U88). These scales are oval in shape, have long, straight keels and thick, curved spines reaching to the internal furcal indentation (scale 34; Fig. 3HH). The first and second pairs slightly overlap. The third (at U87–U88) and fourth (at 88–U89) pairs are definitely small (2.0–4.8 × 1.3–3.0 μm), egg-shaped, are located on the furcal appendages, and have straight keels and rudimentary spines (scale 35; Fig. 3II).

Internal morphology. The pharynx (from U2 to U31) is narrow and has marked anterior and posterior dilatations. The posterior dilatation is stronger than the anterior one (Fig. 9C). In the anterior dilatation, weak cuticular reinforcements are located in the form of two straight, connected rods (at U4). The pharynx is connected through the pharyngeal–intestinal junction by the straight intestine (running from U32 to U86). The pharyngeal–intestinal junction is clearly demarcated, short and wide (U32) (Fig. 2B). The intestine does not have a separate, different anterior section. The X-organ of this species (observed in one specimen) is located at U84–U86 near the terminal part of the intestine. It is bilobed, built from two extensions enveloped in a thin coat and connected by a thinner band located below the intestine end, at the ventral surface (Fig. 10). The extensions have a granular appearance. The thin coat and the cellular bridge connecting the extensions have a smooth and homogeneous structure. The sperm packets of this species were not observed.

Remarks

Because of the long pharynx in relation to the length of the body and intestine, the pharynx–intestine ratio (I) has high values in this species. Juvenile and subadult specimens have a pharynx–intestine ratio (I) higher than 65%, rather than higher than 55%, as is usually observed in other species. In juvenile specimens, the first head scales from each dorsal longitudinal row are located under the free dorsal cephalion edges, whereas in adults they are nto located under the cephalion but below their dorsal edges (Fig. 7A–B). Of the 25 reported adults, one individual had a large, developing egg (Fig. 5C) and another possessed a distinct X-organ (Fig. 10). Four specimens had live, motile euglenids inside their intestine (Fig. 11) and were listed in an earlier paper as *Chaetonotus* (*Chaetonotus*) sp. 1 (Kisielewska *et al.* 2015).

Differential diagnosis

Of all the 92 currently known nominal representatives of the subgenus *Chaetonotus* (*Chaetonotus*) Ehrenberg, 1830, *C.* (*C.*) *invitatus* sp. nov. most closely resembles *C.* (*C.*) *maximus* Ehrenberg, 1830, *C.* (*C.*) *microchaetus* Preobrajenskaja, 1926, *C.* (*C.*) *similis* Zelinka, 1889, *C.* (*C.*) *heterospinosus* Balsamo, 1978, *C.* (*C.*) *laroides* Marcolongo, 1910 and *C.* (*C.*) *polyspinosus* Greuter, 1917. Of all 32 species belonging to the subgenus *Hystricochaetonotus* Schwank, 1990, *C.* (*H.*) *trispinosus* Balsamo, 1990 seems to be the most similar to the newly described taxon, whereas of all the currently known 22 nominal representatives of the subgenus *C.* (*Primochaetus*) Kisielewski, 1997b, the new species most closely resembles *C.* (*P.*) *mutinensis* Balsamo, 1978. *Chaetonotus* (*C.*) *maximus, C.* (*C.*) *microchaetus, C.* (*C.*) *similis, C.* (*C.*) *heterospinosus, C.* (*C.*) *laroides, C.* (*C.*) *polyspinosus* and *C.* (*P.*) *mutinensis* are the most similar to the newly described species in terms of: body shape similarity, range of body length, range of pharynx length, range of adhesive tube length, presence of a scale of a different type on the dorsal and dorsolateral surfaces on the furcal base, presence of rigid, long spines on the furcal appendages and a similar type of terminal scales on the ventral interciliary field. *Chaetonotus* (*H.*) *trispinosus* was selected for comparison with *C.* (*C.*) *invitatus* sp. nov. due to its similarity in terms of having three larger scales with longer and stronger spines present on the dorsal posterior trunk region, length variation of large spines on different body sides and by the presence of scales of a different type on the dorsal and dorsolateral surfaces of the furcal base. Despite the fact that these eight species have the highest number of common features with the newly described species, they are significantly different from *C.* (*C.*) *invitatus* sp. nov. – most strikingly by scale type and shape variation, spine types

and length variation. Comparisons between the new species and the most morphologically similar taxa are summarised in Table A2. Taxa chosen for the differential diagnosis often occur in different regions of the world and are presented very differently by various researchers. Thus, a different description/specification or taxonomic classification of theoretically the same species may suggest that we are dealing with a very large range of plasticity of these taxa or with a larger number of undiagnosed, separate species. To avoid any doubts in comparisons with *C.* (*C.*) *invitatus* sp. nov., only the original descriptions of these species and three of the most detailed papers with morphological data were used (Balsamo 1983; Schwank 1990; Kisielewski 1997a). For information regarding *C.* (*P.*) *mutinensis*, only original data are considered in the differential diagnosis.

Chaetonotus* (*Chaetonotus*) *paucisquamatus Kisielewski, 1991
Figs 12–13; Table 3

Chaetonotus (*Chaetonotus*) *paucisquamatus* Kisielewski, 1991: 40–42, figs 47–50.

Locus typicus

Brazil.

Material examined

POLAND • 1 adult; Kraków, Botanical Garden, Jubilee Greenhouse, site 1; 50°03′38″ N, 19°57′30″ E; 15 Nov. 2013; M. Kolicka leg.; NHC (photomicrographs, also in the author's collection).

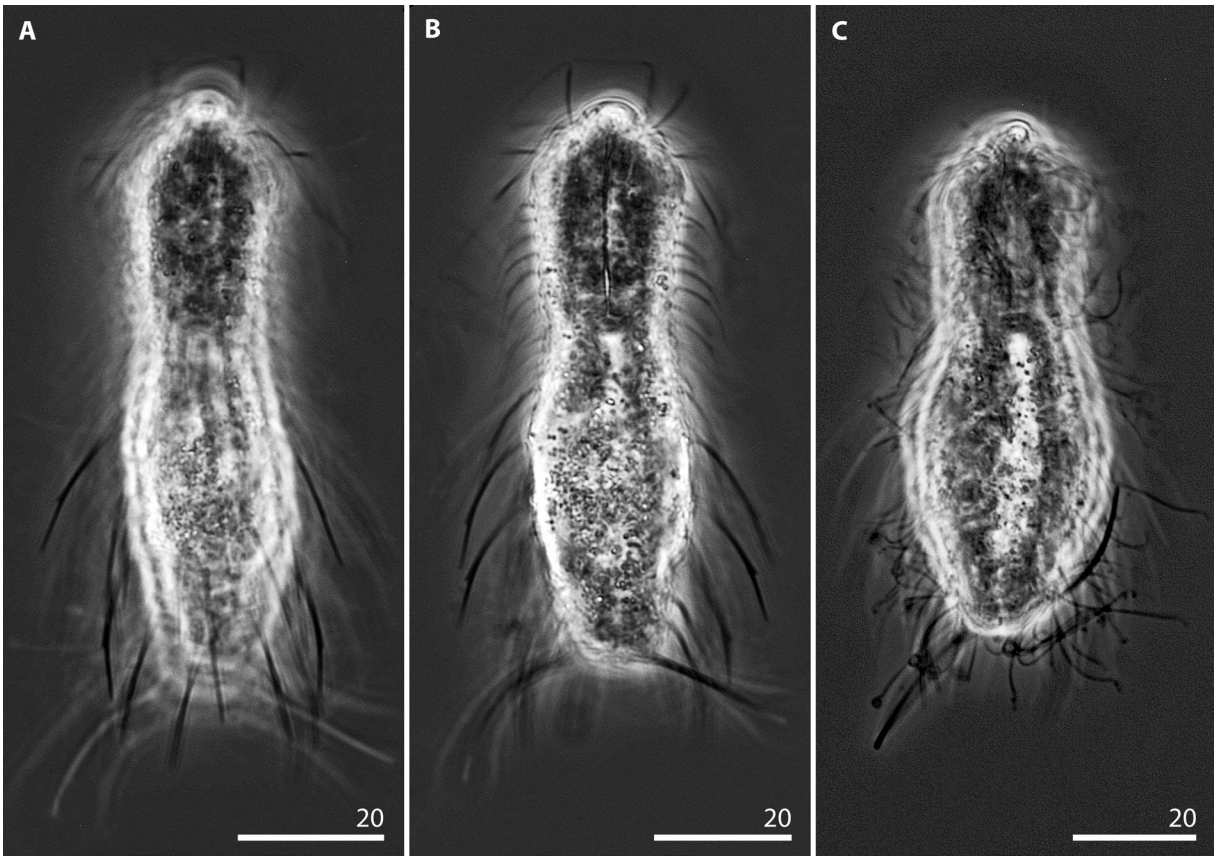


Fig. 12. *Chaetonotus* (*Chaetonotus*) *paucisquamatus* Kisielewski, 1991. Adult specimen. Phase contrast microphotographs. **A**. Dorsal body view. **B**. View of internal morphology. **C**. Ventral body view.

Table 3. Main morphometric characters of *Chaetonotus* (*Chaetonotus*) *paucisquamatus* Kisielewski, 1991. All measurements in micrometers (μm). NA = data not available.

Characters	According Kisielewski (1991)	Adult specimen
Body length	121.00–154.00	114.76
Furca length	NA	36.74
Adhesive tube length	25.00–40.00	27.82
Number of scales in central longitudinal row	9 (head and neck: 5; trunk: 4)	14 (head and neck: 9; trunk: 5)
Total number of longitudinal alternating rows of scales	9	9
Length of cephalic cilia (anterior tuft)	NA	9.49–21.98
Length of cephalic cilia (posterior tuft)	NA	12.97–24.34
Head scales length × width	NA	3.09–4.37 × 3.13–4.14
Neck scales length × width	NA	4.62–6.56 × 3.90–6.26
Trunk scales length × width	8.00–12.50 x 10.00	7.31–13.33 × 7.01–11.72
Head dorsal spines length	NA	1.24–4.03
Head ventral spines length	NA	6.28–8.49
Neck dorsal spines length	4.00–4.50	4.18–14.70
Neck ventral spines length	NA	9.83–20.06
Trunk dorsal spines length	23.00–35.00	23.70–26.70
Trunk ventral spines length	NA	20.86–24.29
Length of rearmost lateral spines	12.00–21.50	19.05
Neck dorsal sensory bristles length	NA	19.96
Posterior dorsal sensory bristles length	NA	23.36
Number of scales in central longitudinal row on ventral interciliary field	NA	13
Total number of longitudinal alternating rows of scales on ventral interciliary field	NA	7
Ventral interciliary field scales length × width	NA	2.83–4.62 × 2.20–3.18
Terminal ventral interciliary field scales length × width	NA	7.41 × 4.02
Mouth ring diameter	3.20–3.50	3.39
Pharynx length	27.50–34.00	30.81
Intestine length	NA	52.90

Distribution

This species was previously known only from Brazil, namely from water reservoirs, silt sediments and aquatic vegetation (Kisielewski 1991).

Remarks

The specimen found in the Jubilee Greenhouse in Kraków is consistent with the detailed description of *Chaetonotus* (*Chaetonotus*) *paucisquamatus* Kisielewski, 1991. The presence of only one specimen does not allow for a thorough comparison and for conclusions to be drawn; however, the individual observed in Poland differed slightly from the original description by: its smaller body size (Table 3); a larger number of scales in the central longitudinal row than in the original description (Table 3); the presence of thin, rounded scales on the ventral interciliary field, increasing in size and isolation from the cuticle towards the posterior trunk region, where the scales possessed weak keels; and the presence of one pair of elongated, elliptical and keeled ventral terminal scales. These differences may result from

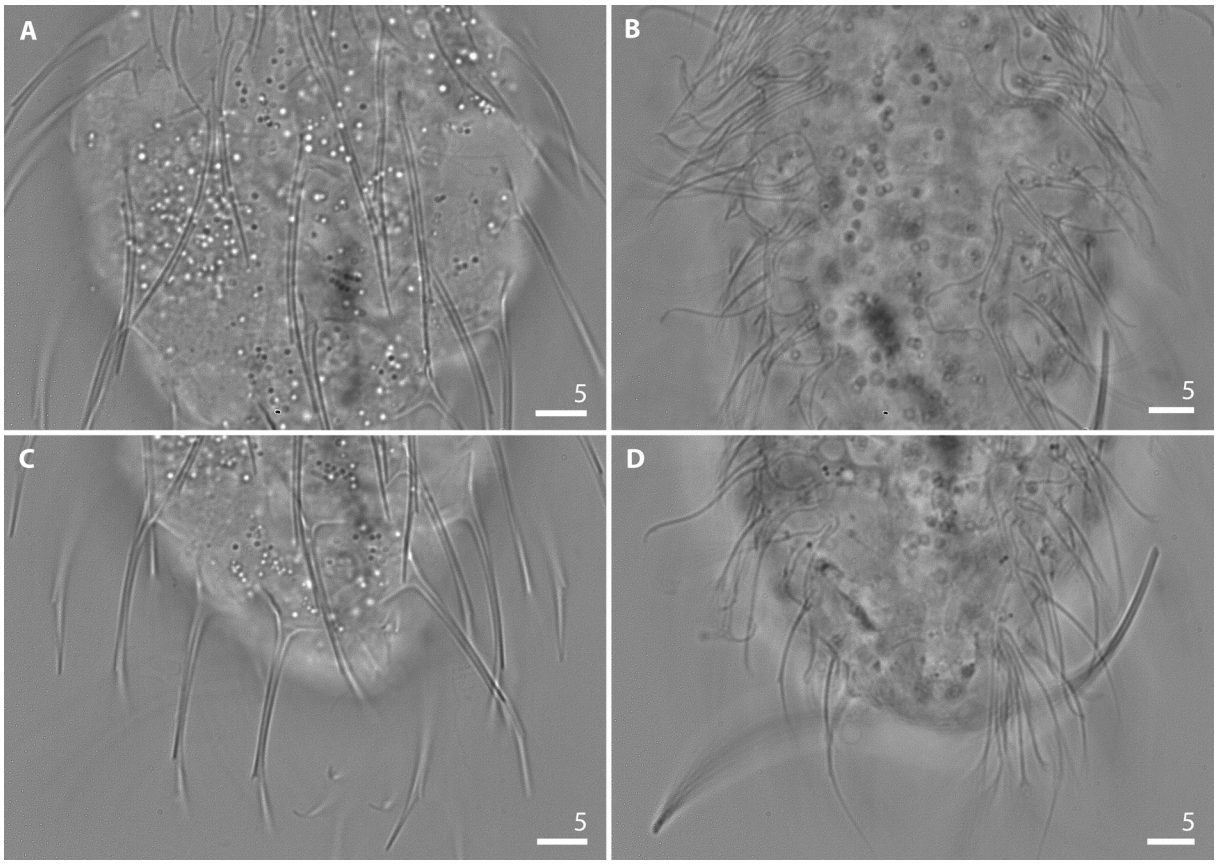


Fig. 13. *Chaetonotus* (*Chaetonotus*) *paucisquamatus* Kisielewski, 1991. Adult specimen. Bright field microphotographs. **A**. Anterior trunk, dorsal view. **B**. Anterior trunk ventral view. **C**. Posterior trunk dorsal view. **D**. Posterior trunk, ventral view.

phenotypic variation caused, e.g., by different developing conditions. Due to the lack of any differences in the other taxonomic characteristics, there are no grounds to conclude that the recorded individual represents a separate species.

Subgenus ***Hystricochaetonotus*** Schwank, 1990

Type species

Chaetonotus hystrix Mečnikow, 1865.

Terra typica

Russia.

Remarks on subgenus

A polyphyletic subgenus in terms of molecular data (Kånneby *et al.* 2013), it encompasses 28 nominal freshwater species and 10 marine species (Todaro 2018). Present in benthic and periphytic habitats (e.g., Kisielewski 1997a).

Chaetonotus* (*Hystricochaetonotus*) *hystrix Mečnikow, 1865
Figs 14–15

Chaetonotus hystrix Mečnikow, 1865: 451, fig. 7.

Locus typicus

Russia.

Material examined

POLAND • 21 adults, 3 subadults, 7 juveniles; Kraków, Botanical Garden, Jubilee Greenhouse, sites 1–3; 50°03′38″ N, 19°57′30″ E; 17 Apr. 2014; M. Kolicka leg.; NHC (photomicrographs, also in the author's collection).

Distribution

Chaetonotus (*Hystricochaetonotus*) *hystrix* Mečnikow, 1865 is considered a cosmopolitan and common species recorded worldwide from highly differing habitats (lakes with different trophic structures, mountain springs, fertile ponds, peat bogs, brackish estuarine waters) (Kisielewski 1991). Outside Europe, it is known from Brazil (Kisielewski 1991), Israel (Kisielewski 1997b), Japan (Saito 1937) and Paraguay (Daday 1905).

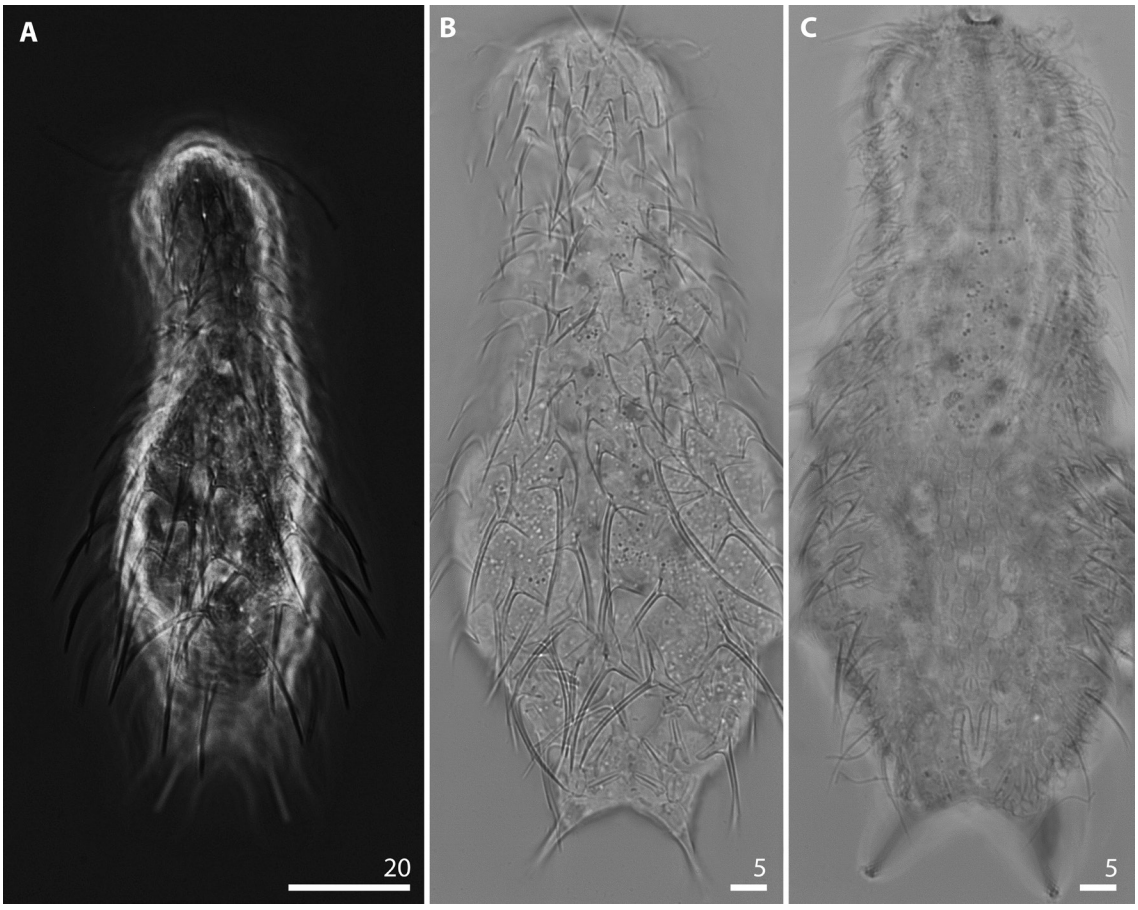


Fig. 14. *Chaetonotus* (*Hystricochaetonotus*) *hystrix* Mečnikow, 1865. Adult specimen. **A**. Habitus, phase contrast microphotograph. **B**. Dorsal scales, bright field microphotograph. **C**. Ventral scales, bright field microphotograph.

Remarks

The specimens examined here correspond very well with the original description (Figs 14–15). All their morphometric characteristics are within the ranges given in the literature for this species (Rudescu 1967; Roszczak 1969; Balsamo 1983; Schwank 1990; Kisielewski 1984, 1997b). The total body length of adult specimens varied from 107.4 to 128.6 μm; length of pharynx was between 27.6

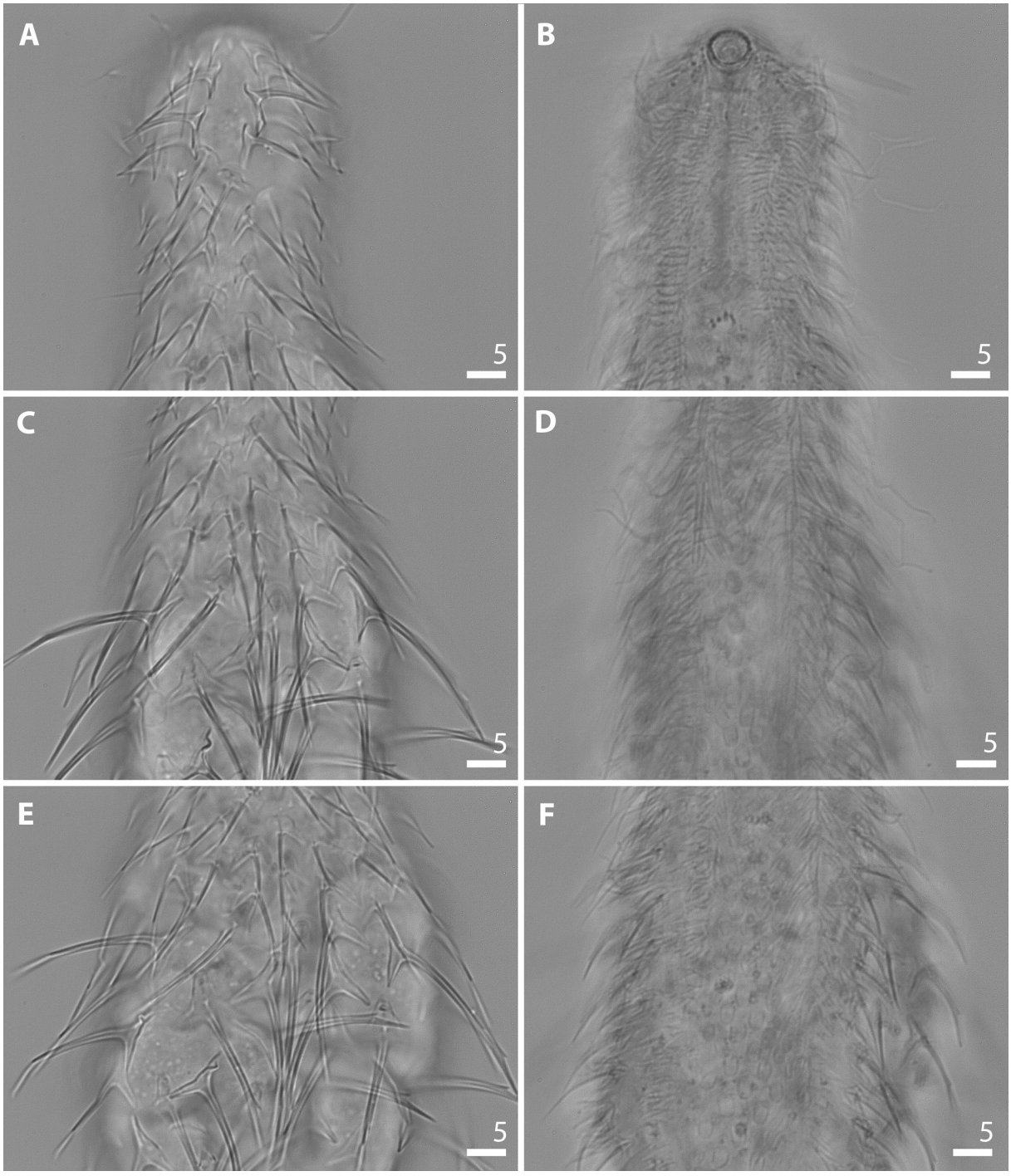


Fig. 15. *Chaetonotus* (*Hystricochaetonotus*) *hystrix* Mečnikow, 1865. Adult specimen. Bright field microphotographs. **A**. Head dorsal spines. **B**. Head ventral spines. **C**. Neck and trunk dorsal spines. **D**. Neck and trunk ventral spines. **E**. Trunk dorsal spines. **F**. Trunk ventral spines.

and 32.7 µm; length of intestine from 56.1 to 81.9 µm; there were 15–17 total longitudinal rows of scales (5–7D+2DL+2L+4LV+2V), with 13–17 scales in the central row. The total body length of juvenile specimens varied from 86.9 to 105.4 µm; length of pharynx was between 28.6 and 32.4 µm; length of intestine from 37.9 to 52.6 µm; there were 13–17 total longitudinal rows of scales (5–7D+2DL+2L+2–4LV+2V), with 13–17 scales in the central row. Among the adults, two had a large, developing egg, while one had an X-organ and two packages of sperm.

Chaetonotus* (*Hystricochaetonotus*) *horridus sp. nov.
urn:lsid:zoobank.org:act:478175BF-E4D8-48BC-AE73-2032127AB69D
Figs 16–25, A3–A6; Tables 4, A3–A4

Diagnosis

Stocky body, measuring from 107.8 to 134.0 µm in length. Head five-lobed, cephalion narrow, hypopleurae slightly larger in size than epipleurae. All cephalic plates weakly demarcated in head outline. Hypostomium small, rectangular in shape. Ocellar granules absent. Almost all scales three-lobed and keeled. Scales distributed in 15–21 total longitudinal rows (3D+2–4DL+4–6L+4LV+2–4V), with 13–15 scales in central row. Scales strongly differ morphologically in various body areas. On dorsal trunk area, large scales with very long, thick spines with a strong, very prominent lateral denticle. Two pairs of posteriormost trunk lateral scales with long, thick spines with strong lateral denticle. Remaining scales with simple, shorter and thinner spines or on central dorsal trunk area and on furcal appendages with rudimentary spines or without spines. Parafurcal spines absent. Spines on ventral surface hair-like, with narrow, delicate lamellae along entire length. Entire ventral interciliary field covered with plate-like scales. Three pairs of ventral interciliary field terminal scales. Pharynx wide, with pronounced anterior and posterior dilatations. Intestine straight without anterior section differing in form and morphology.

Etymology

From the Latin '*horridus*' = 'hairy', referring to the very long and strong trunk spines.

Material examined

Holotype

POLAND • adult; Kraków, Botanical Garden, Jubilee Greenhouse, site 1; 50°03′38″ N, 19°57′30″ E; 15 Nov. 2013; M. Kolicka leg.; NHC-GCHH-22-1-25/h (photomicrographs, also in the author's collection).

Paratypes

POLAND • 80 adults, 30 subadults, 9 juveniles; same locality as holotype; sites 1–3; 15 Nov. 2013 and 17 Apr. 2014; M. Kolicka leg.; NHC-GCHH-22-21-60/p (photomicrographs, also in the author's collection).

Description

Habitus. *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov. has a stocky body that is slightly bottle-shaped (Figs 16, 19). Its head is only slightly wider than the neck and the neck constriction is weakly demarcated. The neck gradually tapers from the head (from U17) to the beginning of the trunk (ca U31). The trunk is slightly wider than the head and gradually dilates from ca U32 to ca U59, where it is at its maximum width. Then it gradually tapers towards the narrow furcal base (from U79) (Figs 16, 19). The furcal base is clearly demarcated and narrow. The furcal indentation is V-shaped. The furcal branches are set widely apart and point outwards to the widest trunk region (Figs 16, 19). The adhesive tubes are straight, fairly thick and slightly tapered towards the blunt ends (Figs 16, 19).

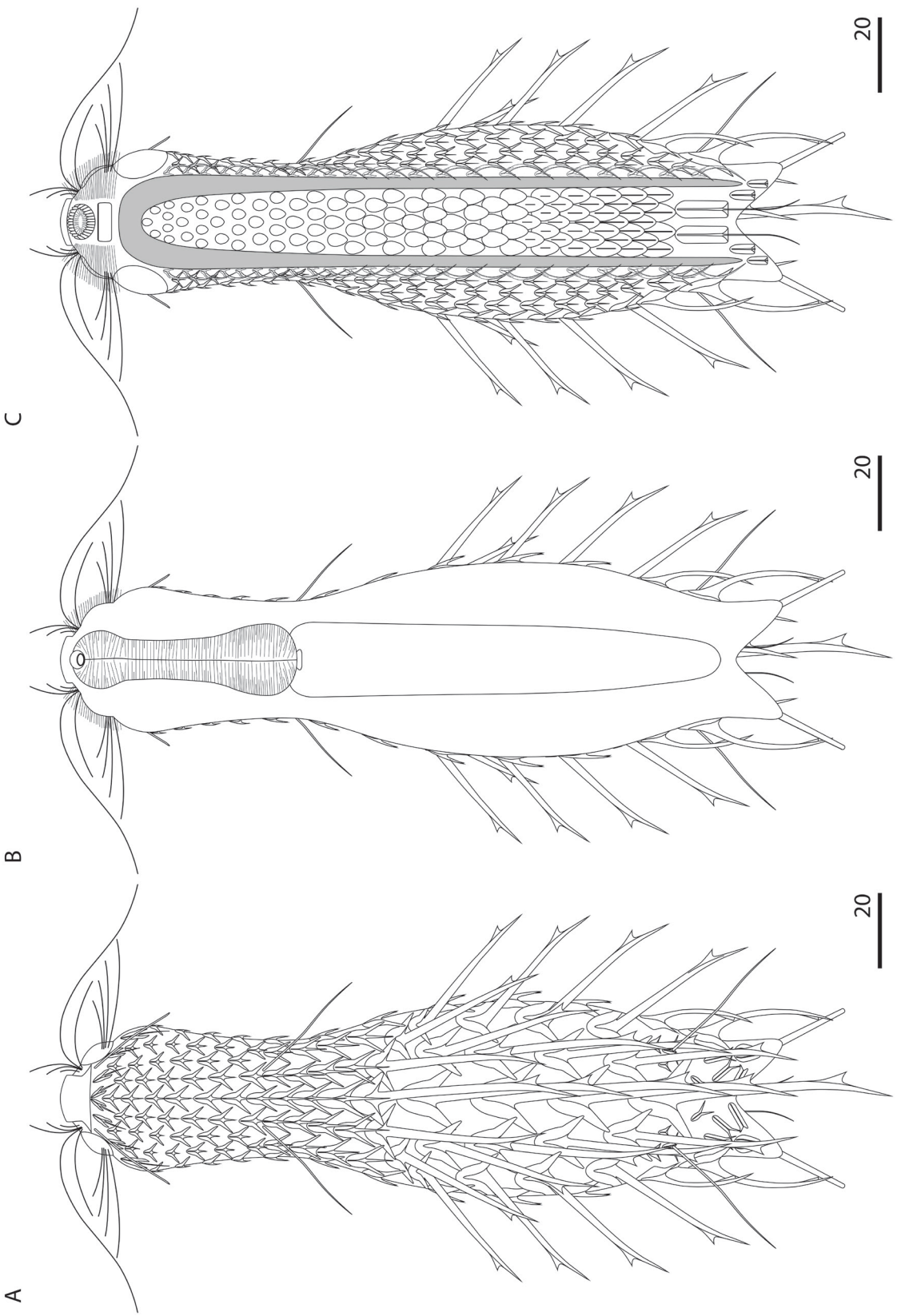


Fig. 16. *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov. Schematic drawings. **A**. Dorsal view. **B**. View of internal morphology. **C**. Ventral view. Light grey areas indicate the areas of insertion of ciliary bands.

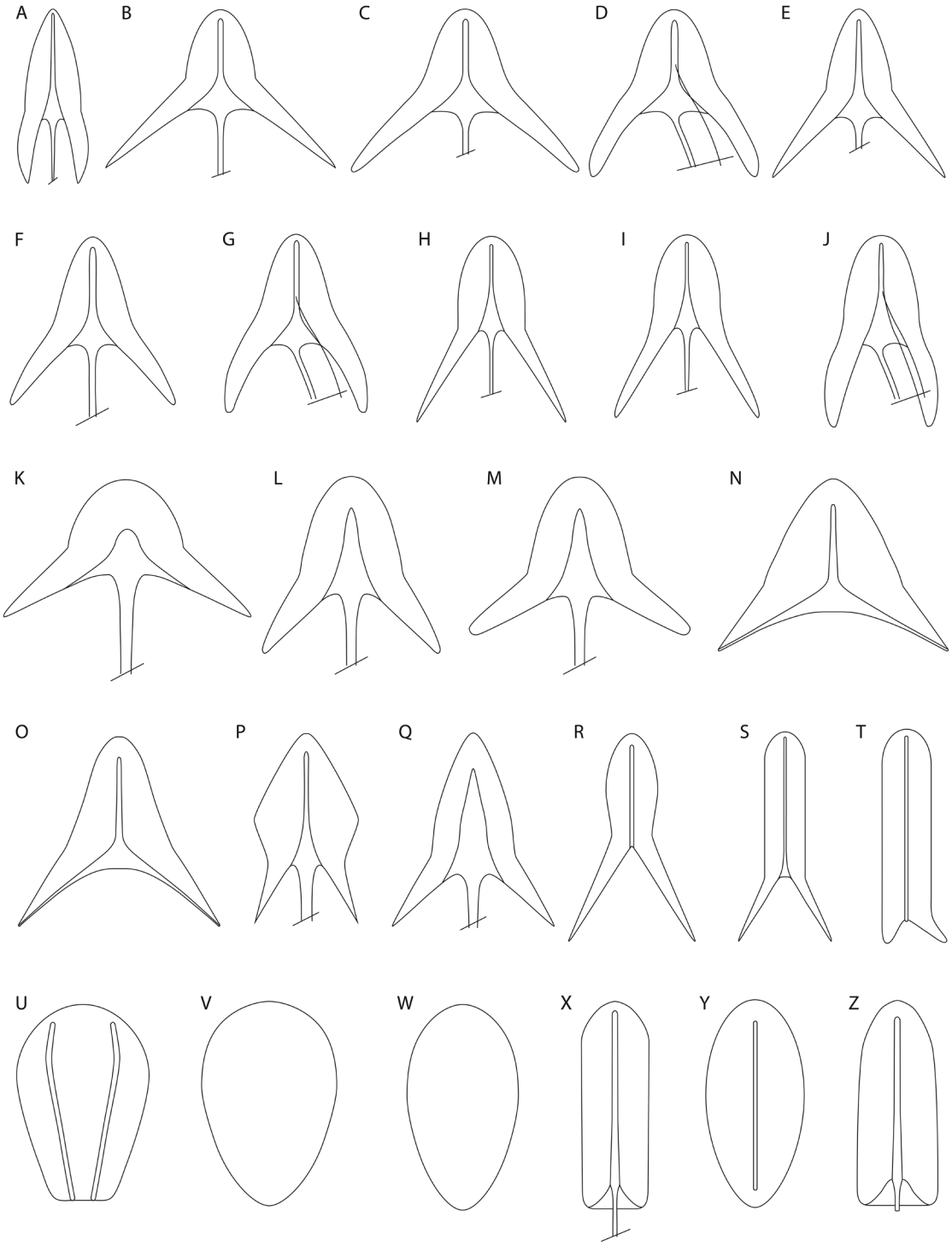


Fig. 17. *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov. Schematic drawings of the scales. **A**. Head anteriormost scale (scale 1). **B**. Head dorsal scale (scale 2). **C**. Head dorsolateral, lateral and ventrolateral scale (scale 3). **D**. Head ventral scale (scale 4). **E**. Neck dorsal scale (scale 5). **F**. Neck dorsolateral, lateral and ventrolateral scale (scale 6). **G**. Neck ventral scale (scale 7). **H**. Trunk dorsal scale (scale 8). **I**. Trunk dorsolateral, lateral and ventrolateral scale (scale 9). **J**. Trunk ventral scale (scale 10). **K**. Scale 11. **L**. Scale 12. **M**. Scale 13. **N**. Scale 14. **O**. Scale 15. **P**. Scale 16. **Q**. Scale 17. **R**. Scale 18. **S**. Scale 19. **T**. Scale 20. **U**. Scale 21. **V**. Scale 22. **W**. Scale 23. **X**. Scale 24. **Y**. Scale 25. **Z**. Scale 26.

Head. The head is five-lobed, short and semicircular in shape. The cephalion (U1–U4) is narrow, short, with straight dorsal edge. It adheres to the head along its entire length and rapidly extends near the dorsal edge (Fig. 20A). The epipleurae (U4–U7) are slightly arched and weakly demarcated in the head outline. They are located on the dorsal, dorsolateral and lateral sides and are slightly visible on the ventrolateral head side. The hypopleurae (U8–U14) are slightly larger in size than the epipleurae and located entirely on the ventrolateral and ventral head sides (Figs 16, 20A–B). They are not visible in dorsal body view. On the dorsal head surface, between the lateral edges of the cephalion and epipleurae there is a prominent space, their edges meeting only in the cephalion extension place. Deep notches are present between the cephalion and epipleurae and between the epipleurae and hypopleurae. The hypostomium (U6–U7) is short, rectangular, with a slightly reinforced anterior edge (Figs 16C, 20B). Two pairs of cephalic ciliary tufts are present. The anterior tufts emerge in the area between the cephalion and epipleurae on the dorso-terminal head surface (ca U3) and consist of 5 cilia. The anteriormost cephalic cilium is very short and the length of the cilia in the anterior tuft gradually increases from the anteriormost to the fourth cilium. The fourth cilium in the anterior tufts is very long, the longest in both pairs of tufts, while the fifth, posteriormost cilium is much shorter. The posterior tufts (at U6–U7) have 4 cilia each and emerge on the ventral head side, above the anterior edge of the hypopleurae. The length of the cilia in the posterior tuft gradually increases from the anteriormost to the fourth cilium. Ocellar granules are not present. The mouth ring is large, wide and located subterminally at U2–U5. It has very strong, long reinforcements and long inner hairs (Figs 16C, 20B). Suboral bristles are located in two tufts located beneath and laterally to the mouth ring and reaching beyond the hypostomium end (between U3 and U7).

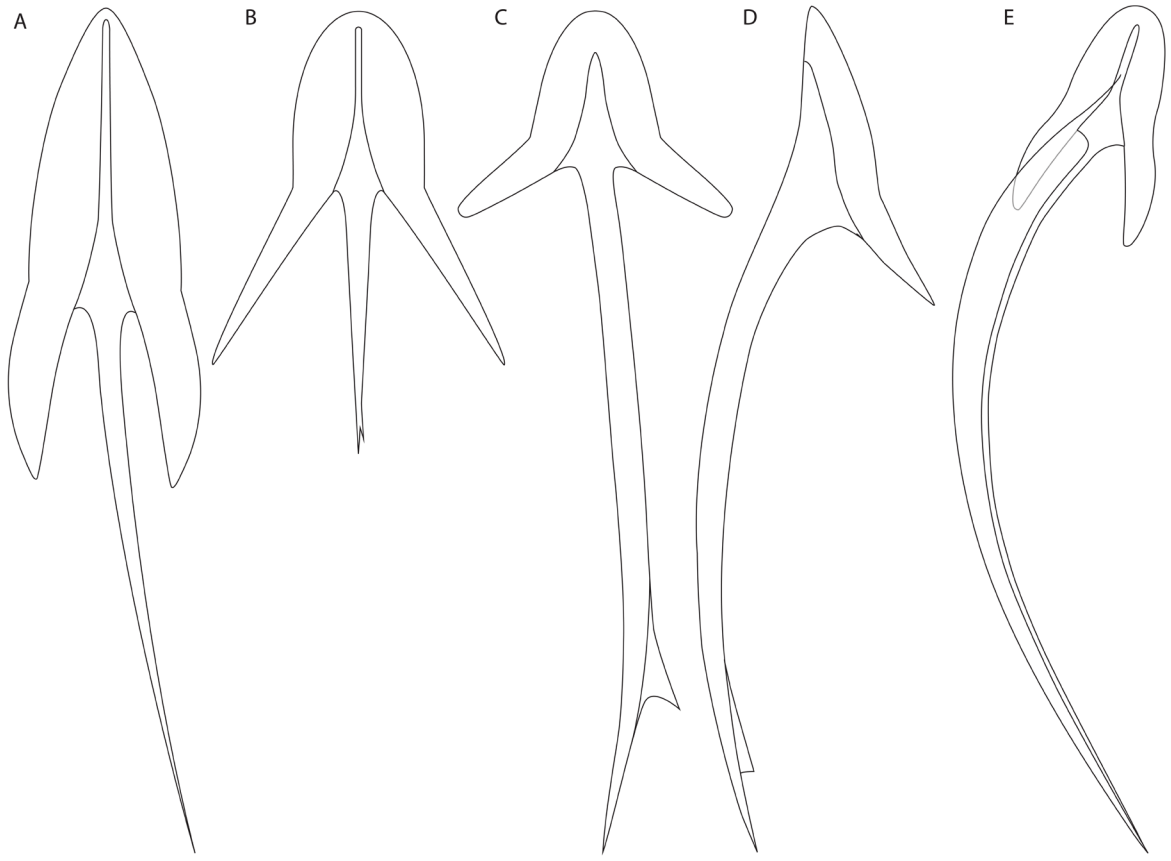


Fig. 18. *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov. Schematic drawings of the spine types. **A**. First type of spines. **B**. Second type of spines. **C**. Third type of spines. **D**. Fourth type of spines. **E**. Fifth type of spines.

Scales. The entire body, except for one pair of scales on the furcal appendages, is covered with three-lobed scales that adhere to the cuticle along their entire surface (Fig. A6). All scales have a strong keel and are shaped like triangles. The scales are distributed in 15–21 total longitudinal alternating rows (3D+2–4DL+4–6L+4LV+2–4V), with 13–15 scales in the central row. The central longitudinal rows of scales begin on the head directly beneath the posterior edge of the cephalion. On the head and anterior neck region the scales are located far from one another, on the posterior neck and anterior trunk region the scales are located gradually closer, but still at a distance to the other scales and not juxtaposed. The dorsolateral, lateral, ventrolateral and ventral trunk scales are located at a distance from the other scales, whereas the dorsal trunk scales and scales on the furcal appendages have slightly overlapping posterolateral edges (Fig. 20). The head anteriormost scales are situated near the posterior edge of the cephalion and epipleurae. The dorsal anterior scales are located aslant, whereas the dorsolateral scales are arranged parallel to the longitudinal body axis. These scales are elongated and their central lobes are long, trilobed and have very long keels. Their posterolateral lobes are slightly separated, narrow, slanted downward and create a narrow, deep V-shaped posterior scale notch (scale 1; Fig. 17A). The remaining scales of the head are significantly wider. The central lobes of these scales become shorter and simultaneously the posterolateral lobes become slightly longer, whereas their keels are shorter. The posterolateral lobes are directed diagonally downward and laterally; they create a deep and wide posterior scale notch (scale 2; Fig. 17B). The anterior neck scales become gradually longer than the head scales. Their central lobes become longer and the posterolateral lobes are more separated and directed more downward and create a wide and deep V-shaped posterior scale notch (scale 5; Fig. 17E). From the neck towards the trunk, the scale size gradually increases (from 2.8–3.5 × 2.4–3.2 to 5.2–6.4 × 5.1–5.9 μm). All of the anterior trunk scales and dorsolateral, lateral, ventrolateral and ventral trunk scales on the

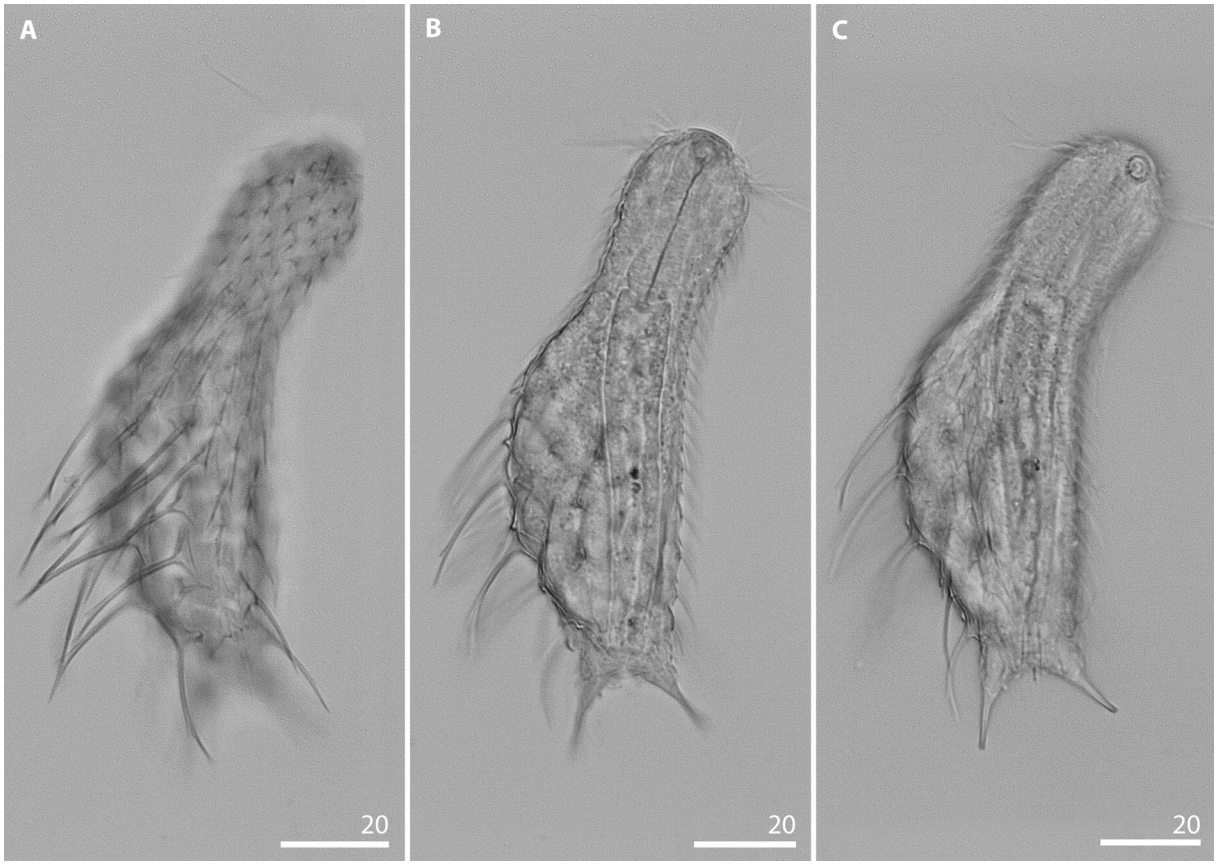


Fig. 19. *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov. Adult specimen. Bright field microphotographs. **A**. Dorsal body view. **B**. View of internal morphology. **C**. Ventral body view.

central and posterior body region have a wide and rounded anterior central lobe and clear, separate, long, narrow and sharp posterolateral lobes. The posterolateral lobes are directed diagonally downward and are slightly lateral; they create a deep V-shaped posterior scale notch (scale 8; Fig. 17H). The size of these scales increases from the anterior trunk region to the widest body region (from 3.0–4.1 × 2.1–3.3 to 5.6–9.7 × 4.68–8.43 μm) (Figs 20, 23). Dorsally and dorsolaterally from ca U37 to U73 and dorsally to U81, larger and thick, different scales are located with very long and strong spines (Figs 16, 20, 22). The first of the different, larger scales from each longitudinal dorsal and dorsolateral row are triangular in shape, their central lobes are short, wide and pass seamlessly into posterolateral lobes that are directed very wide apart. The posterior edge of this scale is almost straight and only very narrowly notched. Its keel starts from the half-length of the central lobe and is very pronounced and high, strongly triangular in shape (scale 11; Fig. 17K). The subsequent large scales (located from U41 to U73) are triangular in shape, with rounded edges, and their central lobes are longer and have longer keels. The posterolateral lobes of these scales are more rounded, directed slightly more downward and create a wide V-shaped posterior scale notch (scale 12; Fig. 17L). The posteriormost scale of the dorsal and dorsolateral large scales with long, strong spines is located in the central longitudinal row of scales at U78 to U81 (Figs 17A, 19A, 22B). This scale is wide and its central and posterolateral lobes are rounded. The posterior edge of this scale is almost straight and only very narrowly notched. Its keel is very pronounced and high, strongly triangular in shape (scale 13; Fig. 17M). Anteriorly to the double keeled scales with trunk dorsal sensory bristles, on the dorsal and dorsolateral trunk region, three pairs of keeled and spineless scales are located at U73–U77. Two of these three pairs are located more anteriorly and laterally than the third pair and are triangular in shape, their posterolateral lobes are weakly separated, and their keels are strong and reach from the anterior part of the central lobes to the ends of the

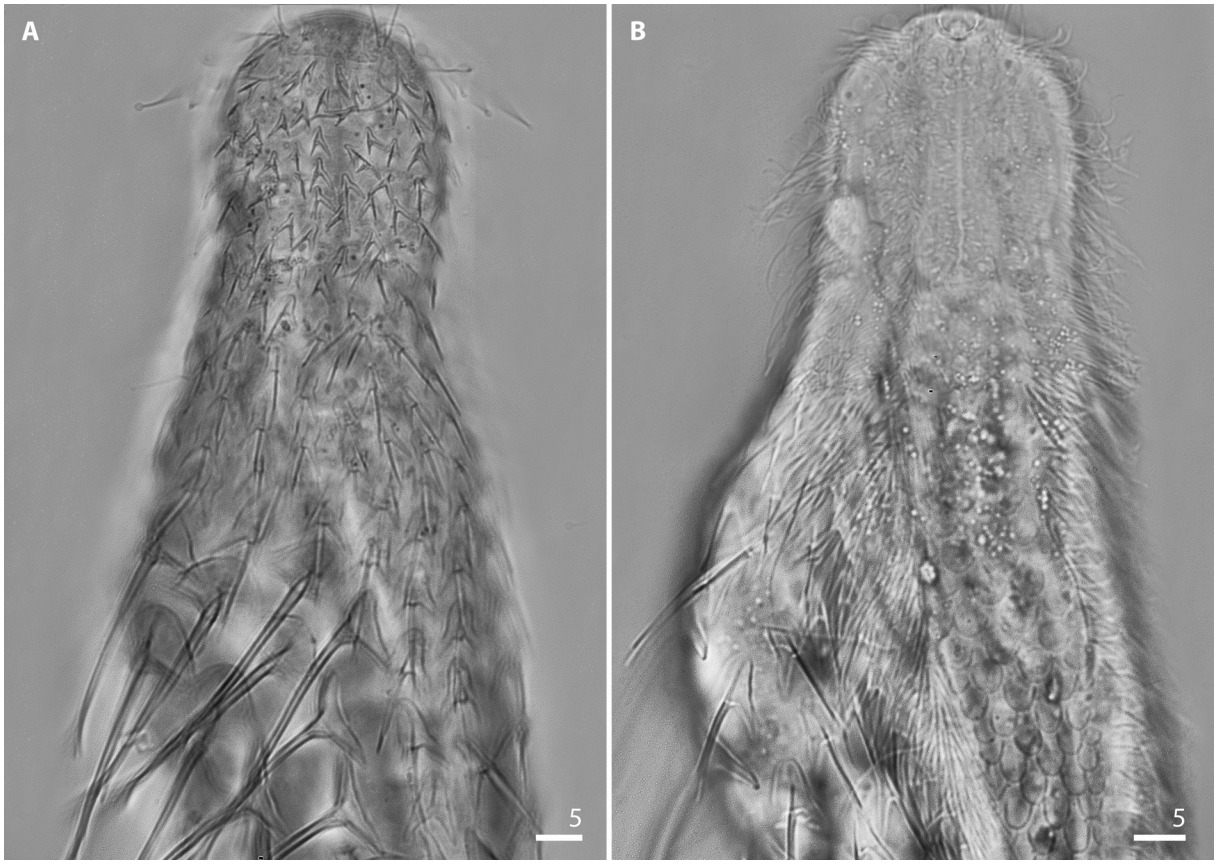


Fig. 20. *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov. Adult specimen. Bright field microphotographs. **A**. Scales, dorsal view. **B**. Scales, ventral view.

posterolateral lobes. The posterior notches of these scales are shallow and semicircular (scale 14; Fig. 17N). The third pair of these scales, located at U75–U77, is wider and possesses shallower posterior notches (scale 15; Fig. 17O). On the lateral surface of the posterior trunk region, one pair of the second to last trunk scales is located at U69–U75. These scales are large and have a long, wide, and anteriorly clearly pointed central lobes with strongly rounded lateral edges. Their posterolateral lobes are clearly separated from the central lobes, are long and have sharp edges. These posterolateral lobes are directed diagonally downward (scale 16; Fig. 17P). The scales have strong keels and strong, long spines. Posteriorly to them, on the posterior trunk region and furcal base, the last pair of trunk lateral scales is situated at U76–U82. This pair of scales is large (7.4–11.7 × 5.3–8.9 μm); its scales have a long, wide and anteriorly strongly pointed central lobes and straight posterolateral lobes that are triangular. The posterolateral lobes of these scales are directed diagonally wide apart, downward and laterally, creating a wide and shallow V-shaped posterior scale notch. These scales have a very strong keel and strong, long spines (scale 17; Fig. 17Q). One pair of small (2.2–7.0 × 1.6–4.5 μm) three-lobed scales, shaped like triangles with strongly rounded anterior lobes and with straight, narrow and long posterolateral lobes, is located diagonally on the dorsal surface of the furcal base (at U81–U83). These scales have long, thin keels and are spineless. Their posterior edge notches are V-shaped and very deep (scale 18; Fig. 17R). Two pairs of three-lobed, elongated and narrow scales are located on the dorsal surface of the furcal base and furcal appendages (at U82–U86). These scales have wide and rounded central lobes and straight, narrow posterolateral lobes located diagonally downward which create narrow, deep V-shaped posterior

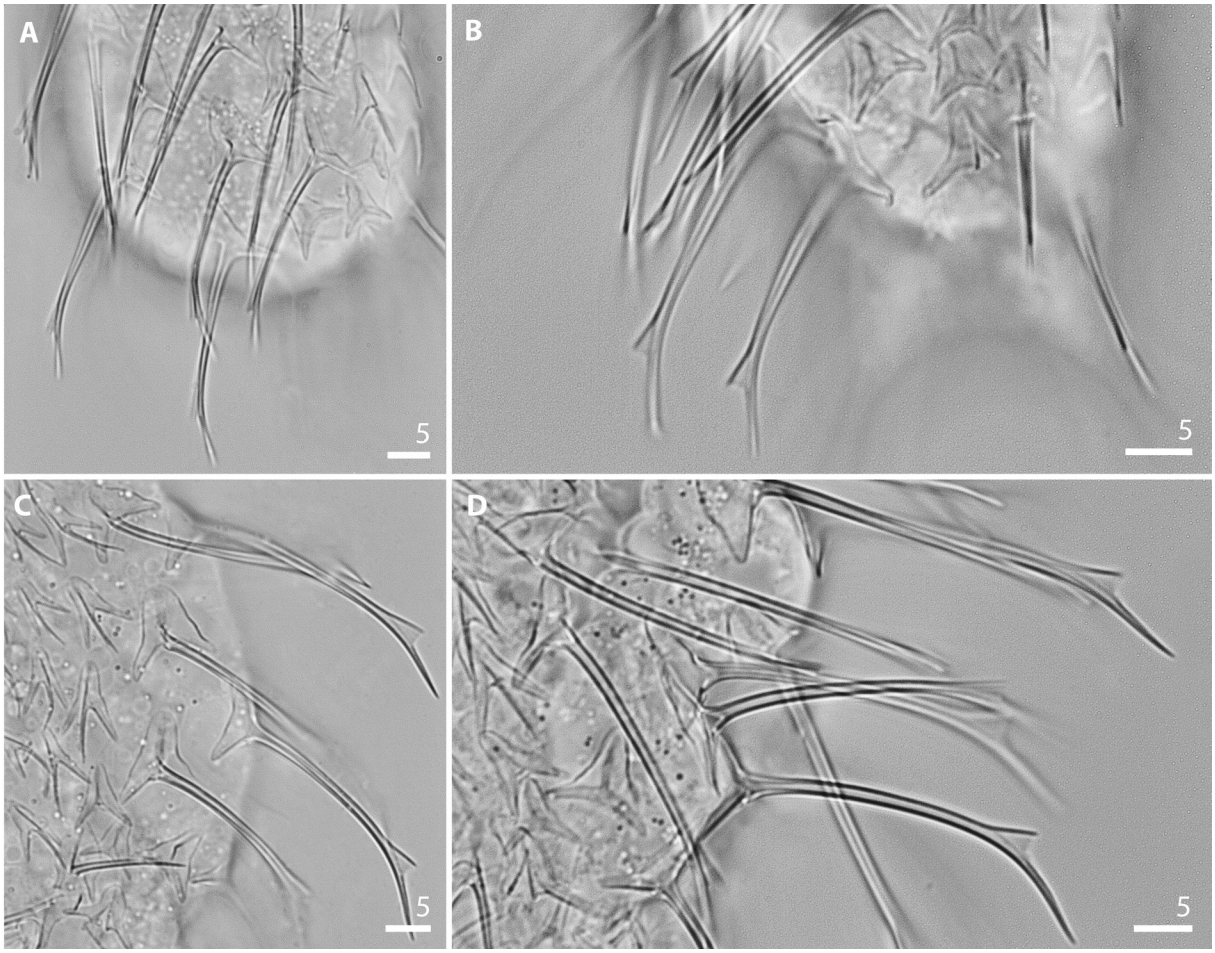


Fig. 21. *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov. Adult specimen. Bright field microphotographs. **A**. Trunk spines, dorsal view. **B**. Trunk spines, dorsolateral view. **C–D**. Trunk spines, lateral view.

scale notches. Their keels are long and thin and the scales are spineless (Fig. 22B). The scales are diagonally arranged and directed towards the central body axis (scale 19; Fig. 17S). One pair of slightly three-lobed, narrow, asymmetrical scales is located on the dorsal surface of the furcal appendages. These scales are shaped like strongly elongated ovals, with a slightly triangular posterior notch. This pair of scales has long keels running along their length and does not have a spine (scale 20; Fig. 17T). They are located at U86–U88, slightly diagonally to the central body axis. Dorsolaterally at the furcal appendages, one pair of scales with two keels is located (at U86–U89). These scales are oval in shape and do not have any posterior notches or spines. Their keels are long and slightly bent (scale 21; Fig. 17U). The edges of the furcal appendage scales are slightly overlapping (Fig. 22). The size of the scales gradually decreases slightly from the dorsal, dorsolateral, lateral, ventrolateral and ventral surfaces towards the ciliary bands (head: from 3.2–5.2 × 3.2–6.2 to 2.2–4.1 × 2.1–3.9 μm; neck: from 4.5–6.4 × 4.1–5.9 to 2.8–4.6 × 2.4–4.1 μm, trunk: from 5.3–9.7 × 4.1–8.4 to 3.0–5.5 × 2.1–4.6 μm) (Table A3). Furthermore, the edges of the dorsolateral, lateral, ventrolateral and ventral scales gradually become slightly more rounded towards the ciliary bands as compared to the edges of the dorsal scales (Figs 16, 20). Scales arranged in the ventral longitudinal row located closest to the ciliary bands are smaller than the scales of the other rows, and their anterior edge is oriented towards the bands at an angle of ca 20°.

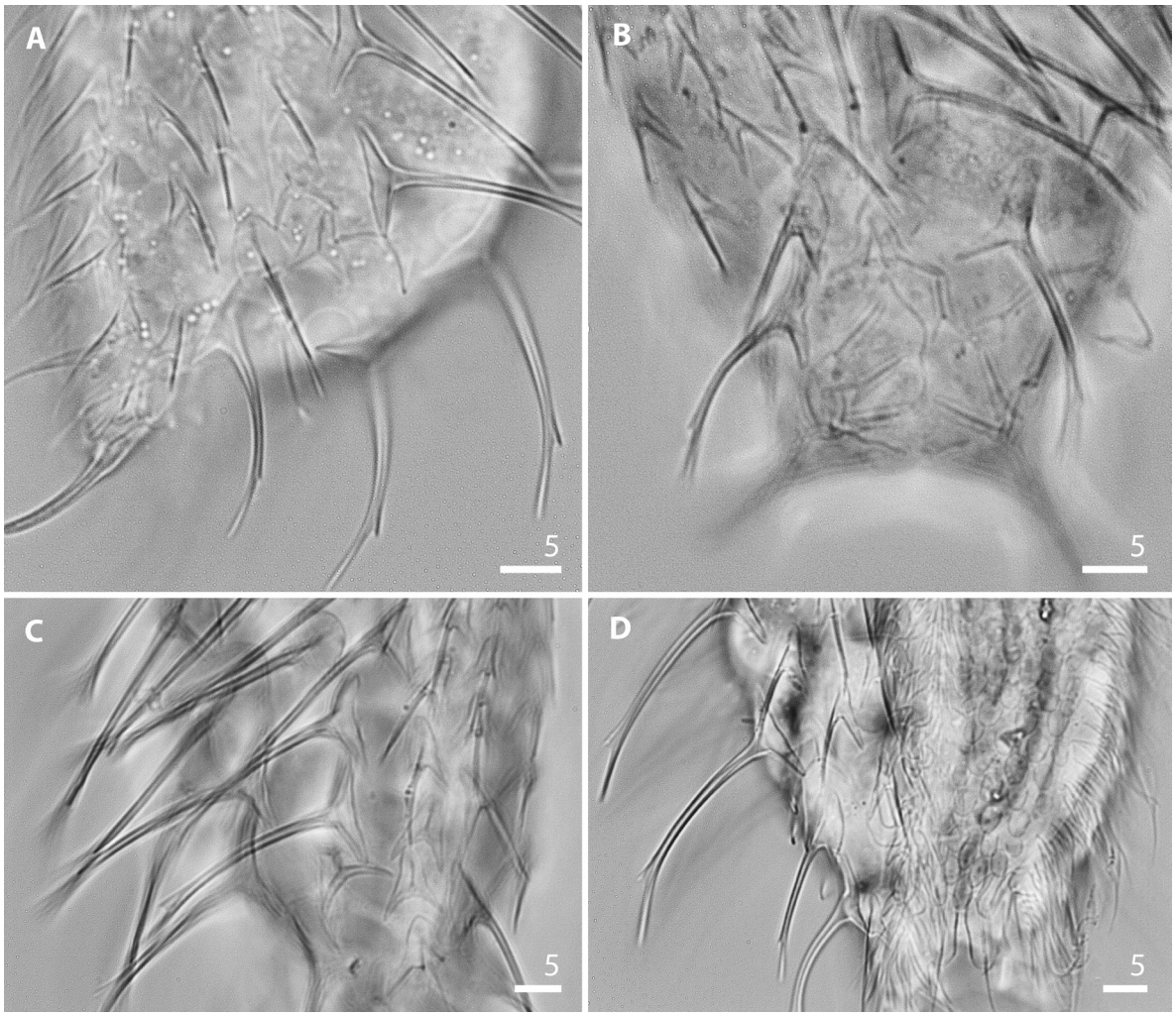


Fig. 22. *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov. Adult specimen. Bright field microphotographs. **A**. Posterior trunk, furca base and furcal appendage, scales lateral view. **B**. Posterior trunk, furca base and furcal appendage scales, dorsal view. **C**. Central and posterior trunk scales, dorsal view. **D**. Posterior trunk, furca base and furcal appendage scales, ventral view.

Table 4. Main morphometric characters of *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov. N = number of specimens analysed; Range = the smallest and the largest structure found among all specimens measured. All measurements in micrometers (μm); all indicators given as a percentage (%) and italicized.

Characters	N	Holotype	Range of adult paratypes
Body length	70	131.69	107.77–134.01
Furca length	70	25.51	21.74–25.90
Adhesive tube length	70	12.43	9.67–13.04
Number of scales in central longitudinal row	81	15	13–15
Total number of longitudinal alternating rows of scales	81	21	15–21
Head scales length × width	15	2.82–5.23 × 2.66–5.20	2.17–5.30 × 2.05–6.20
Neck scales length × width	15	3.24–6.37 × 2.75–5.91	2.77–6.37 × 2.35–5.91
Trunk scales length × width	15	3.49–13.43 × 2.62–13.37	3.03–13.43 × 2.12–13.37
Head spines length	15	1.86–13.73	0.44–13.78
Localization of lateral denticle on head spines	15	*15.054–32.630*	*13.699–32.653*
Neck spines length	15	5.12–16.49	2.45–16.49
Localization of lateral denticle on neck spines	15	*25.502–29.323*	*19.818–29.448*
Trunk spines length	15	7.88–19.58	4.97–19.54
Localization of lateral denticle on trunk spines	15	*21.053–31.124*	*21.053–32.937*
Long trunk spines length	15	16.65–31.75	11.59–34.14
Localization of lateral denticle on trunk long spines spines	15	*23.039–31.937*	*17.969–34.785*
Length of trunk posteriormost pair of lateral spines	15	20.09	13.87–20.09
Localization of lateral denticle on trunk posteriormost pair of lateral spines	15	*31.210*	*24.942–34.785*
Mouth ring diameter	70	6.45	5.48–7.04
Pharynx length	70	33.20	27.03–33.58

Spines. In this species, five main types of spines may be distinguished (Fig. 18). The first type of spine bends slightly along its entire length and tapers towards the end, without lateral denticles. This type of spine emerges from the anteriormost head dorsal scales (Fig. 18A). The second type of spine is basally bent, tapering towards their ends, with a weak lateral denticle. These spines emerge from the dorsal, dorsolateral, lateral and ventrolateral head, neck and anterior trunk region scales and laterally and ventrolaterally on the remaining scales of the trunk part (Fig. 18B). The length and thickness of the spines gradually increase from the head towards the widest body region (ca U59) (from 1.8–13.7 to 7.9–19.6 μm). Moreover, their length gradually increases towards the ventrolateral surface (head: from 0.4–5.6 to 0.7–6.1 μm; neck: from 2.5–8.3 to 3.4–10.1 μm; trunk: 5.0–11.1 to 5.7–17.0 μm) (Table A3). The third type of spine is very long and thick, straight, with a prominent, deeply notched lateral denticle. The spines emerge from large (scales 11, 12 and 13), thick dorsal and dorsolateral trunk scales (from ca U37 to ca U73), are the longest (18.9–35.5 μm) and thickest body spines and have the most prominent lateral denticle (Fig. 18C). From scales 13, located on the posterior trunk region (at U78–U81), arise the last long dorsal and dorsolateral long spines (Fig. 21). These spines are slightly shorter than the preceding long spines and their lateral denticle is less prominent (18.9–27.2 μm). The fourth type of spine is long, thick, slightly bent and with a clearly marked lateral denticle (Fig. 18D). This type of spine arises from the second to posteriormost trunk pair of lateral scales located at U69–U75 and from the posteriormost trunk pair of lateral scales located at U76–U82 (Fig. 22A–B). The posteriormost pair of trunk spines is longer (13.9–20.1 μm) and thicker than the second to posteriormost pair of lateral

Fig. 23. *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov. Adult specimen. **A**. Spines lateral view, bright field microphotograph. **B**. Spines with lamellae, phase contrast microphotograph.

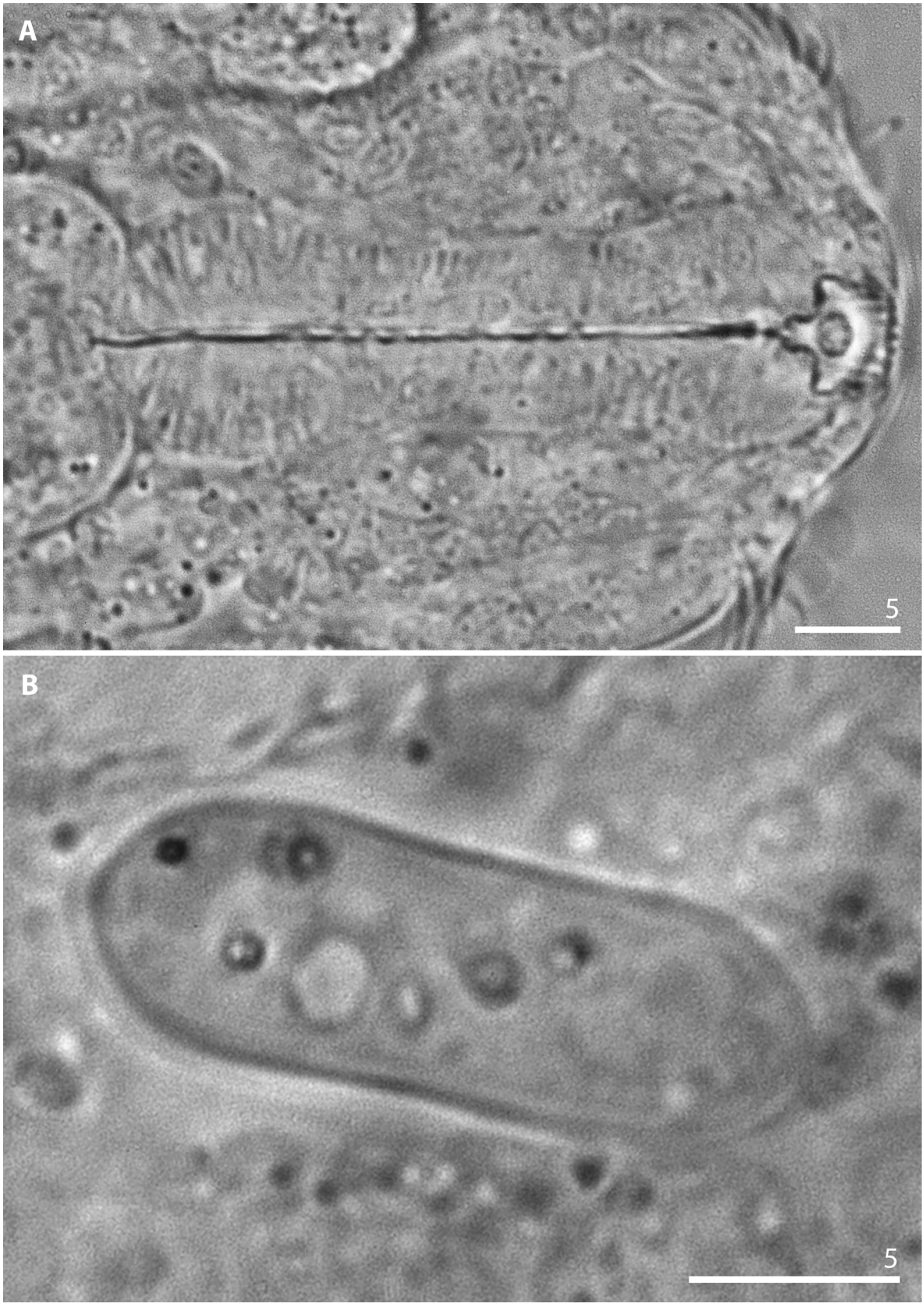


Fig. 24. *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov. Adult specimen. Bright field microphotographs. **A**. Detail of pharynx. **B**. Crescent-like formation inside body integument.

spines (11.6–16.7 μm) (Table A3), and their lateral denticles are more prominent and deeply notched (Fig. 22A–B). The fifth type are long (7.2–19.5 μm), thin, hair-like spines with narrow, delicate lamellae along their entire length. These spines arise on the ventral surface, from scales arranged in one pair of longitudinal rows located closest to the ciliary bands (Fig. 18E). The lamellae are the widest here, ca one-third of the length of the spine, and taper towards the end of the spines (Fig. 21B).

Dorsal sensory bristles. This species has three pairs of dorsal sensory bristles (Fig. 16A). The first, anterior pair is located on the dorsal surface of the head at U5, beyond the dorsolateral cephalion edges, whereas the second pair of sensory bristles is located on the dorsal surface of the posterior neck region at U27. The first and the second pairs emerge from small, spherical papillae. The third, posterior pair of sensory bristles is located dorsally and dorsolaterally on the posterior trunk and emerges from the three-lobed, double-keeled scales at U76–U79. The keels of these scales are connected in the centre of the scales, and the scales are shaped like double, sharp triangles with long, narrow posterolateral lobes set very wide apart and a double, deep posterior notch (Figs 16A, 22B).

Ventral ciliary bands and ventral interciliary field. On the ventral surface, the longitudinal ciliary bands begin at U8 and run back to U87 (Fig. 16C). On the head region (from ca U8 to ca U11) the ciliary bands are merged (Fig. 16C) and subsequently are divided into two separate bands arranged parallel to one another. The entire ventral interciliary field is covered by plate-like scales. They are distributed in 7 longitudinal rows with 14–23 scales in the central row (Figs 16C, 19C, 22D). The ventral interciliary field anterior scales are partially recessed on the cuticle and isolation from the cuticle increases towards the posterior body part. The anterior scales are rounded to inverted egg-shaped (scale

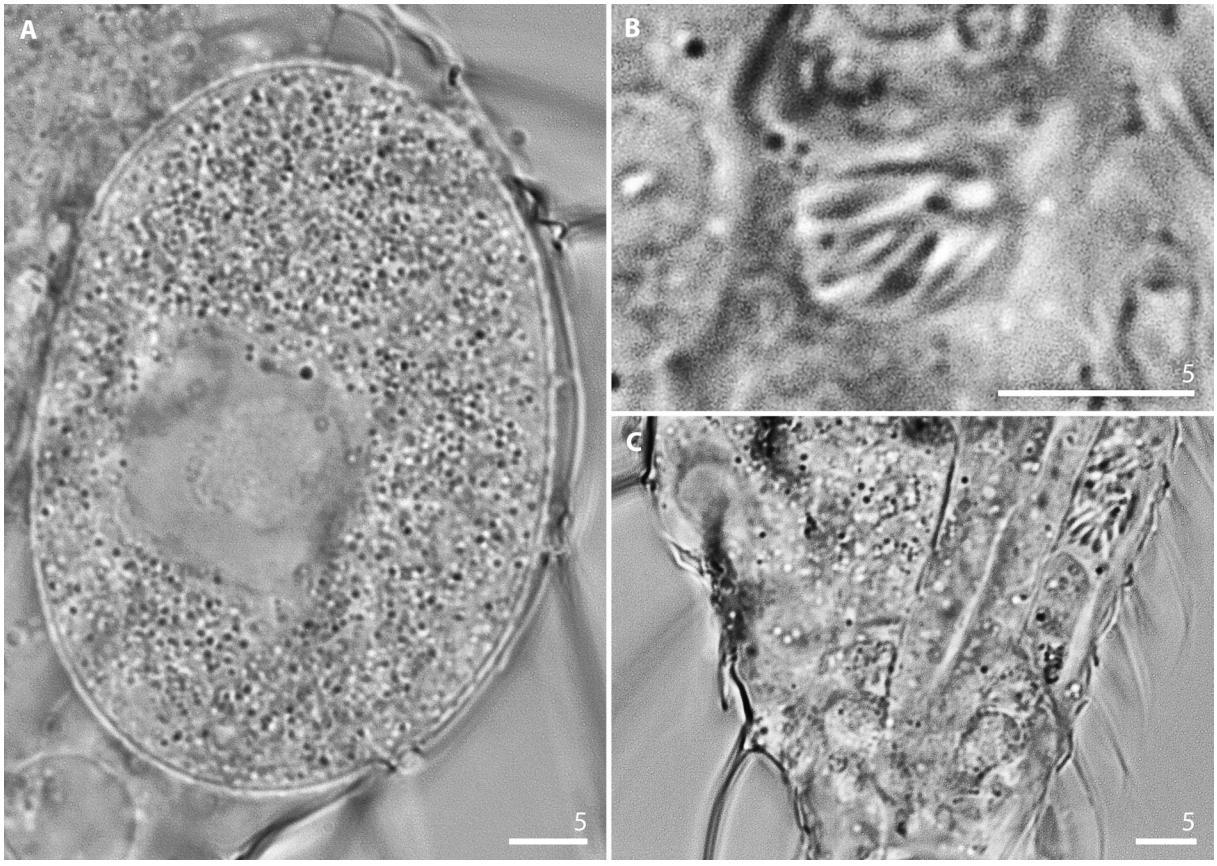


Fig. 25. *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov. Detail of the internal morphology. Bright field microphotographs. **A**. Mature egg. **B**. Sperm pocket. **C**. X-organ and sperm pocket.

22; Figs 17V, 20B), and towards the posterior body region the scales become gradually larger and more elongated (scales 23–24; Fig. 17W–X). The posterior ventral interciliary field region scales (at U73–U79) are elongated, oval in shape, and have straight and thin keels (Figs 20F, 22D). From the anterior to posterior body region, the distances between the scales decrease, and beyond half the trunk length the scales start to overlap (Fig. 20D, F). Three pairs of ventral interciliary field terminal scales are present. The first pair (at U79–U85) is elongated and rectangular, with rounded anterior edges (scale 25; Fig. 17Y). They have a long, narrow keel and a long, thin and straight spine extending beyond the internal furcal indentation. The second and third pairs (at U86–U89) are elongated and rectangular, with rounded anterior edges (scale 26; Fig. 17Z). These pairs have long, narrow keels and possess short, rudimentary spines that only slightly extend towards the posterior scale edge (Fig. 22D).

Internal morphology. The pharynx (from U2 to U30) is wide and has distinct, marked, rounded anterior and posterior dilatations. The posterior dilatation is wider than the anterior one (Fig. 24A). The pharynx is connected through the pharyngeal–intestinal junction to a straight intestine, running from U30 to U84. The pharyngeal–intestinal junction is clearly demarcated, short and narrow (U31). The X-organ of this species (observed in five specimens) is located at U83–U86 near the terminal part of the intestine. It is bilobed, built from two extensions enveloped in a thin coat and connected by a thinner band located below the intestine, at the ventral side. The extensions and the thin coat and the cellular bridge connecting the extensions have a grain-like structure (Fig. 25C). The pair of sperm packets of this species are circular in shape and contain spermatozoids (8–16 per packet) in the form of a short rod. The pair is located at U60–U62 on both sides, juxtaposed to the intestine. When only a single sperm pocket is present, it is irregular in shape, less compact and contains 8–16 spermatozoids. Unpaired sperm pockets, when present, are located at U64–U66, very near the internal trunk wall (Fig. 25C).

Remarks

The total body length of juvenile specimens of *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov. varied from 88.8 to 122.9 μm; length of pharynx between 27.6 and 34.2 μm; length of intestine from 36.4 to 58.2 μm; there were 15–21 total longitudinal rows of scales (3D+2–4DL+4–6L+4LV+2–4V), with 11–15 scales in the central row. Out of 69 adults, nine had a large, developing egg, one had a developing egg, an X-organ and a single sperm packet, one had an X-organ and a single sperm packet, whereas two others had an X-organ and two sperm packets and the next one only had a single sperm packet; one had large, crescent-like formations inside the integuments (Fig. 25B) and another had diffuse formations (in both case probably some kind of parasitic structures).

Differential diagnosis

Of all 32 species belonging to the subgenus *Hystricochaetonotus* Schwank, 1990, *Chaetonotus* (*H.*) *horridus* sp. nov. most closely resembles *C.* (*H.*) *acanthophorus* Stokes, 1888, *C.* (*H.*) *balsamoae* Kisielewski, 1997, *C.* (*H.*) *euhystrix* Schwank, 1990 and *C.* (*H.*) *novenarius* Greuter, 1917 (Table A4). *Chaetonotus* (*H.*) *acanthophorus* shares the following characters with *C.* (*H.*) *horridus* sp. nov.: long and strong spines on the dorsal and dorsolateral trunk surfaces; two strong and long anteriormost lateral trunk spines. *Chaetonotus* (*H.*) *balsamoae* and *C.* (*H.*) *novenarius* were selected for comparison with the new species due to their similarity in possessing very long and strong spines with strong, prominent lateral denticles on the dorsal trunk surface. *Chaetonotus* (*H.*) *euhystrix* was selected for comparison with the new species due to its similarity in possessing very long and strong spines with strong, prominent lateral denticles on the dorsal and dorsolateral trunk surfaces; a large mouth ring; a similar number of scales in the central longitudinal row of scales; and type of covering of the ventral interciliary field. Despite the fact that among all of the hitherto known species in this subgenus, those listed above have the highest number of common features with *C.* (*H.*) *horridus* sp. nov., they are significantly different from it – most strikingly by scale type and shape, spine length variation, as well as arrangements of the long trunk dorsal spines and lateral denticle type. In order to avoid any doubts in comparisons with

the new species, only the original descriptions of these species and three of the most detailed papers with morphological data were used (Balsamo 1983; Schwank 1990; Kisielewski 1997a). Comparisons between the new species and the most morphologically similar taxa have been summarised in Table A4.

Chaetonotus* (*Hystricochaetonotus*) *inaequabilis sp. nov.
urn:lsid:zoobank.org:act:82BC94AE-15DD-4A5D-A727-B34013BC6B7A
Figs 26–32, A7; Tables 5, A5–A6

Diagnosis

Stocky body, measuring from 98.2 to 108.5 µm in length. Head five-lobed, cephalion narrow, epipleurae and hypopleurae small. All cephalic plates weakly demarcated in head outline. Hypostomium small, rectangular in shape. Ocellar granules absent. Almost all scales three-lobed. Scales distributed in 15–17 total longitudinal rows (5–7D+2DL+2L+4LV+2V), with 12–13 scales in central row. Scales strongly differ morphologically in various body areas. Nine significantly larger scales on dorsal trunk area with very long, thick spines with a strong lateral denticle. Two pairs of posteriormost trunk lateral scales with long, thick spines with strong lateral denticle. Remaining scales with simple, shorter and thinner spines, or with rudimentary spines, or without spines on central dorsal trunk area and on furcal appendages. Scales with parafurcal spines absent. Spines on ventral surface hair-like, with narrow, delicate lamellae along entire length. Entire ventral interciliary field covered with small scales. Four pairs of ventral interciliary field terminal scales. Pharynx wide, with pronounced anterior and posterior dilatations. Intestine straight without anterior section differing in form and morphology.

Etymology

From the Latin '*inaequabilis*' = 'heterogeneous', referring to the very numerous types of scales on the body.

Type material

Holotype

POLAND • adult; Kraków, Botanical Garden, Jubilee Greenhouse, site 2; 50°03′38″ N, 19°57′30″ E; 15 Nov. 2013; M. Kolicka leg.; NHC-GCHI-23-1-25/h (photomicrographs, also in the author's collection).

Paratypes

POLAND • 6 adults; same locality as holotype; sites 2–3; 15 Nov. 2013 and 17 Apr. 2014; M. Kolicka leg.; NHC-GCHI-23-26-60/p (photomicrographs, also in the author's collection).

Description

Habitus. *Chaetonotus* (*Hystricochaetonotus*) *inaequabilis* sp. nov. has a stocky body (Figs 26, 29). Its head is only slightly wider than the neck, and the neck constriction is weakly demarcated. The neck gradually tapers from the head (from U20) to the beginning of the trunk (ca U30) (Figs 26, 29). The trunk is slightly wider than the head and gradually dilates from ca U31 to ca half of its length (U56), where it is at its maximum width. Then it gradually tapers towards the narrow furcal base (from U84) (Figs 26, 29). The furcal base is clearly demarcated and narrow. The furcal indentation is V-shaped. The furcal branches are set narrowly apart and point slightly outwards (Figs 26, 29). The adhesive tubes are relatively short in comparison to the whole body length (Table 5), straight, fairly thick and not tapered towards the blunt ends (Figs 26, 29).

Head. The head is five-lobed, short and semicircular in shape. The cephalion (U1–U3) is narrow, short and straight, on the dorsal body side. It adheres to the head along its entire length and rapidly extends

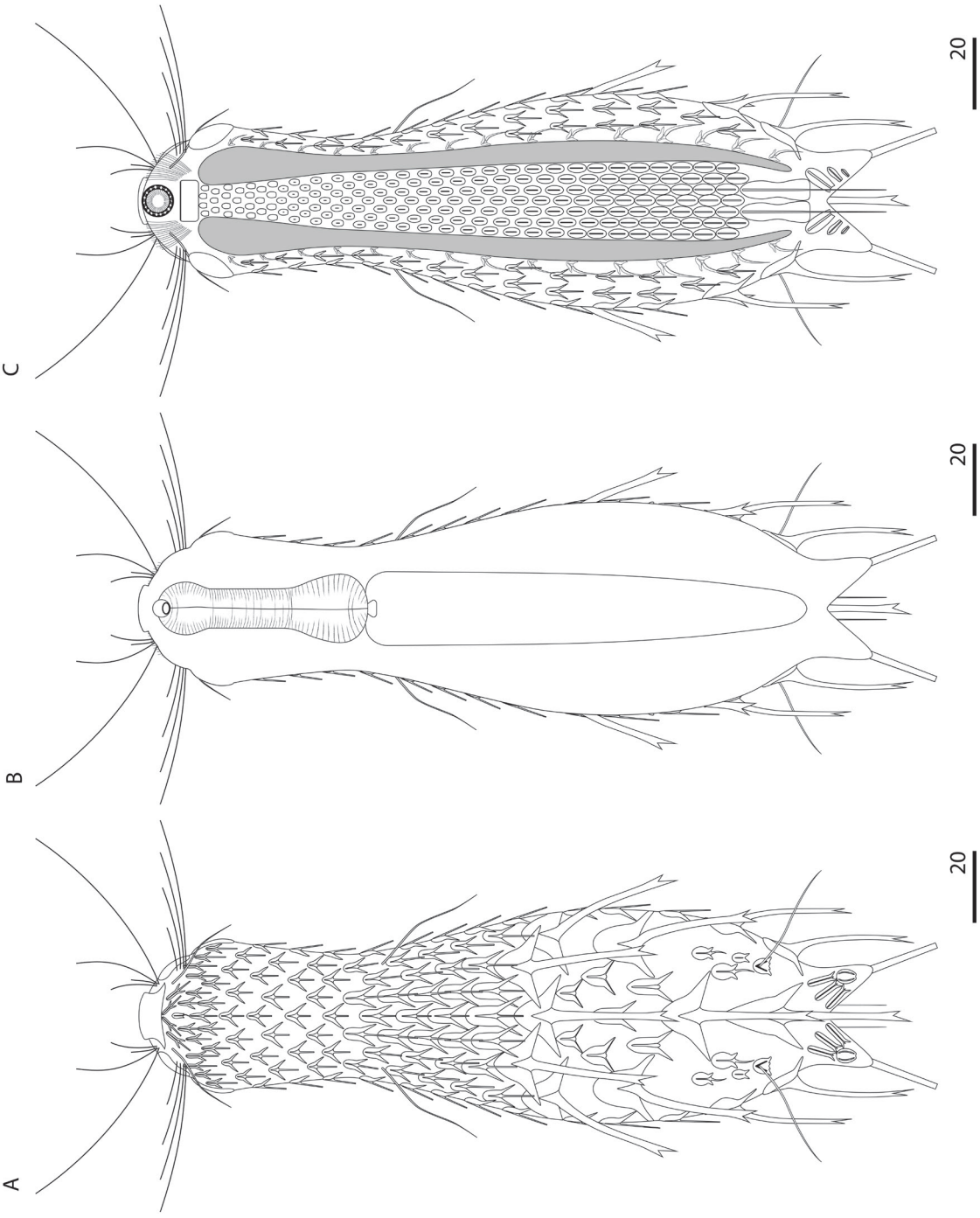


Fig. 26. *Chaetonotus* (*Hystricochaetonotus*) *inaequabilis* sp. nov. Schematic drawings. **A**. Dorsal body view. **B**. View of internal morphology. **C**. Ventral body view. Light grey areas indicate the areas of insertion of ciliary bands.

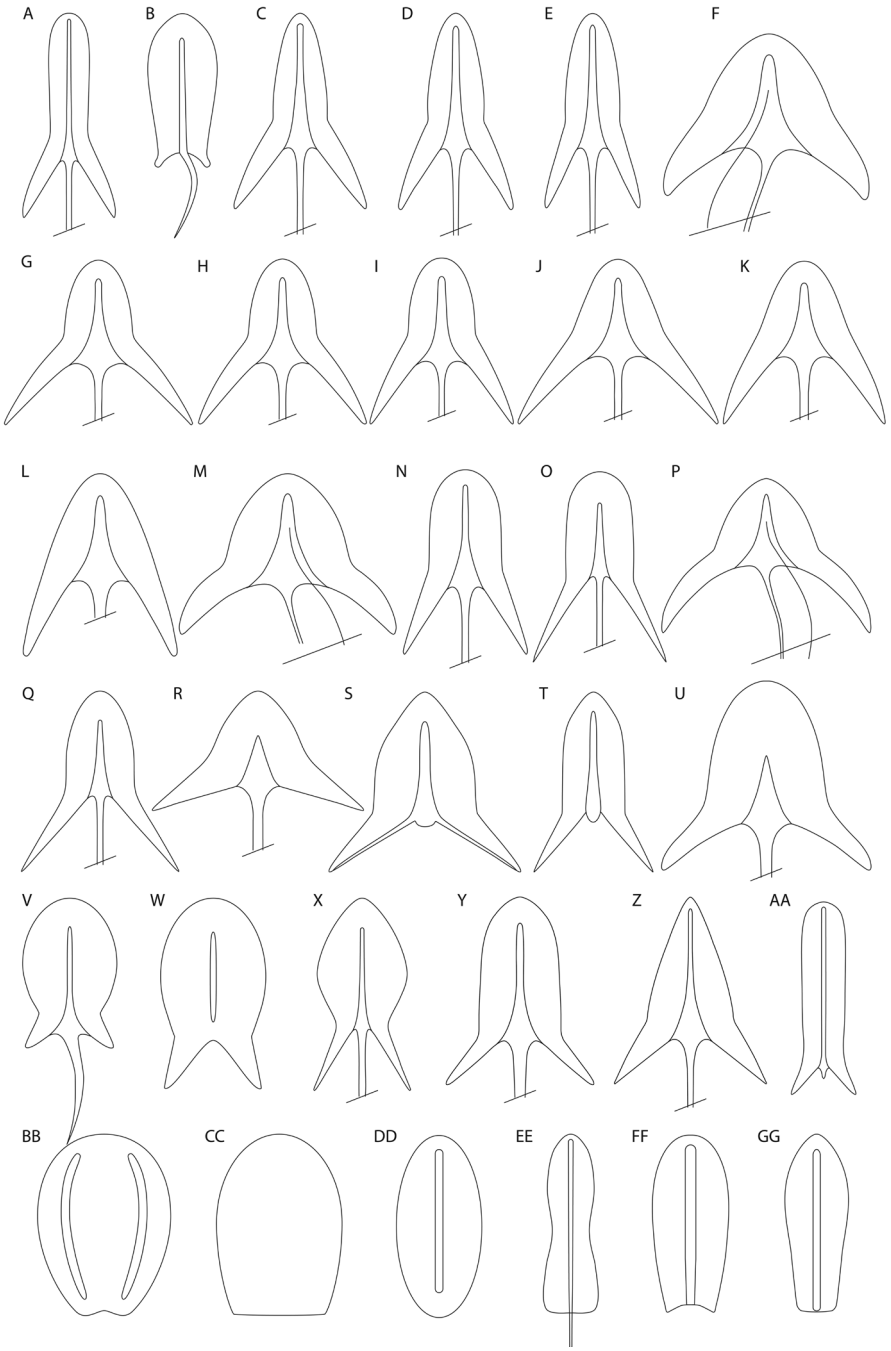


Fig. 27. *Chaetonotus* (*Hystricochaetonotus*) *inaequabilis* sp. nov. Schematic drawings of the scales. **A**. Head anteriormost scale (scale 1). **B**. Head anteriormost central scale (scale 2). **C**. Anterior head dorsal scale (scale 3). **D**. Anterior head dorsolateral and lateral scale (scale 4). **E**. Anterior head ventrolateral scale (scale 5). **F**. Head ventral scale (scale 6). **G**. Posterior head dorsal scale (scale 7). **H**. Posterior head dorsolateral and lateral scale (scale 8). **I**. Posterior head ventrolateral scale (scale 9). **J**. Anterior neck dorsal scale (scale 10). **K**. Neck dorsolateral and lateral scale (scale 11). **L**. Neck ventrolateral scale (scale 12). **M**. Neck ventral scale (scale 13). **N**. Posterior neck dorsal scale (scale 14). **O**. Anterior trunk dorsal, dorsolateral, lateral and ventrolateral scale (scale 15). **P**. Trunk ventral scale (scale 16). **Q**. Posterior trunk dorsal, dorsolateral, lateral and ventrolateral scale (scale 17). **R**. Scale 18. **S**. Scale 19. **T**. Scale 20. **U**. Scale 21. **V**. Scale 22. **W**. Scale 23. **X**. Scale 24. **Y**. Scale 25. **Z**. Scale 26. **AA**. Scale 27. **BB**. Scale 28. **CC**. Scale 29. **DD**. Scale 30. **EE**. Scale 31. **FF**. Scale 32. **GG**. Scale 33.

Table 5. Main morphometric characters of *Chaetonotus* (*Hystricochaetonotus*) *inaequabilis* sp. nov. N = number of specimens analysed; Range = the smallest and the largest structure found among all specimens measured. All measurements in micrometers (μm); all indicators given as a percentage (%) and italicized.

Characters	N	Holotype	Range of adult paratypes
Body length	5	107.91	98.16–108.49
Furca length	5	20.96	20.08–22.05
Adhesive tube length	5	10.05	9.92–10.50
Number of scales in central longitudinal row	5	14	14–15
Total number of longitudinal alternating rows of scales	5	19	19–21
Head scales length × width	5	1.87–5.22 × 1.49–4.86	1.49–5.91 × 1.23–5.01
Neck scales length × width	5	2.39–6.45 × 2.77–5.21	2.07–6.65 × 2.40–5.37
Trunk scales length × width	5	2.82–11.04 × 3.31–13.03	2.28–11.39 × 2.24–13.03
Head spines length	5	0.34–11.42	0.27–12.03
Neck spines length	5	1.76–12.41	1.62–13.60
Trunk spines length	5	3.32–14.62	3.09–15.23
Trunk long spines length	5	11.61–21.61	10.16–21.61
Localization of lateral denticle on trunk long spines spines	5	*10.288–17.399*	*8.780–17.958*
Length of trunk posteriormost pair of lateral spines	5	13.69	11.42–14.02
Localization of lateral denticle on trunk posteriormost pair of lateral spines	5	13.587	12.610–15.149
Mouth ring diameter	5	6.04	5.14–7.07
Pharynx length	5	28.05	26.21–28.29

laterally near the dorsal edge (Figs 26A, 31A). The epipleurae (U3–U7) are small, narrow, slightly arched and weakly demarcated in the head outline. They are visible on the dorsal, dorsolateral and lateral head sides. The hypopleurae (U7–U12) are similar in size to the epipleurae and located entirely on the ventrolateral and ventral head sides (Fig. 26). On the dorsal head surface, between the lateral edges of the cephalion and epipleurae, is a prominent space; their edges meet only in the cephalion extension place. Deep notches are present between the cephalion and epipleurae and between the epipleurae and hypopleurae. The hypostomium (U6–U8) is small, rectangular, with a slightly reinforced anterior edge (Figs 26C, 31B). Two pairs of cephalic ciliary tufts are present. The anterior tufts emerge in the area between the cephalion and epipleurae on the dorso-terminal head surface (at U2–U3) and consist of four cilia. The anteriormost cilium in both anterior tufts is fairly short. The second cilium is short, shorter than the anteriormost one. The third cilium is longer than either of the preceding cilia. The fourth, last cilium in the anterior tufts is very long, the longest in both pairs of tufts. The posterior tufts (at U6–U7) have four straight cilia each and emerge on the ventral head surface, above the anterior edge of the hypopleurae. The length of the cilia in the posterior tuft gradually increases from the anteriormost to the fourth cilium. Ocellar granules are not present. The mouth ring is large and subterminal at U2–U5. It has very strong, granular cuticular reinforcements and short inner hairs (Figs 26C, 29C). Suboral bristles are located in two tufts located beneath and laterally to the mouth ring and reaching half the length of the lateral hypostomium edges (between U5 and U7).

Scales. The entire body, except for one pair of scales on the furcal appendages, is covered with three-lobed scales that adhere to the cuticle along their entire surface (Fig. A7). All scales have a strong, triangular keel and are shaped like triangles with a deep posterior notch. The scales are distributed in 15–17 total longitudinal alternating rows (5–7D + 2DL + 2L + 4LV + 2V), with 12–13 scales in the central

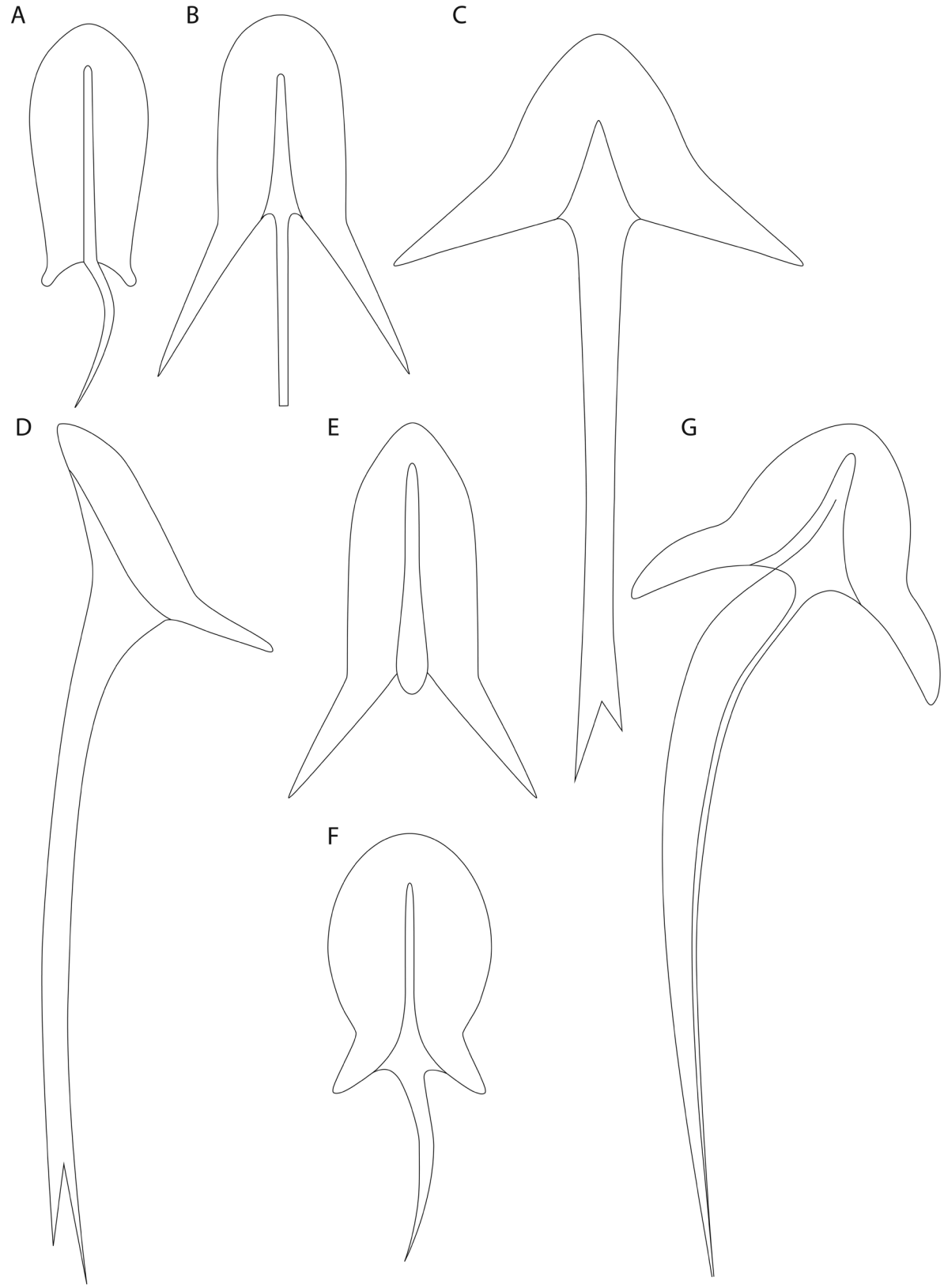


Fig. 28. *Chaetonotus* (*Hystricochaetonotus*) *inaequabilis* sp. nov. Schematic drawings of the spine types. **A**. First type of spines. **B**. Second type of spines. **C–D**. Third type of spines. **E–F**. Fourth type of spines. **G**. Fifth type of spines.

row. The longitudinal rows of scales begin on the head directly beneath the posterior edge of the cephalion, epipleurae and hypopleurae. The scales show a strong diversity in size and shape between the various parts of the body. On the head and anterior neck region the scales are located far from each other, while on the posterior neck and anterior trunk region the scales become gradually juxtaposed but do not overlap. The dorsolateral, lateral, ventrolateral and ventral trunk scales are juxtaposed, whereas the dorsal trunk scales and scales on the furcal appendages have overlapping posterolateral edges (Fig. 30). The head anteriormost scales are situated near the posterior edge of the cephalion and epipleurae. The dorsal anterior scales are located aslant, whereas the dorsolateral scales are arranged parallel to the longitudinal body axis. These scales are elongated, their central lobes are long and wide, and they have very long keels. The posterolateral lobes are narrow, slanted downward and create a narrow, deep V-shaped posterior scale notch (scale 1; Fig. 27A). The central anteriormost head scales are located at U5–U6, beneath the aslant arranged scales, are smaller, more rounded and have weaker separate posterolateral lobes (scale 2; Fig. 27B). The remaining scales of the head are wider. The central lobes of these scales become shorter, and simultaneously the posterolateral lobes become slightly longer, whereas their keels are shorter. The posterolateral lobes are directed diagonally downward and laterally and create a deep and wide posterior scale notch (scales 3 and 7; Fig. 27C, G). The anterior neck scales are shorter than the head scales. Their central lobes are wide and short. Their posterolateral lobes are weaker and separate from the central lobes (scale 10; Fig. 27J). The subsequent neck scales become larger but relatively narrower than the preceding neck scales (Table A5). Their central lobes are wide, with strongly rounded anterior edges, whereas their postero-lateral lobes are more separated and directed more downward and create a deep V-shaped posterior scale notch (scale 14; Fig. 27N). The posterolateral lobes of the dorsolateral, lateral and ventrolateral scales of the head and neck become gradually more

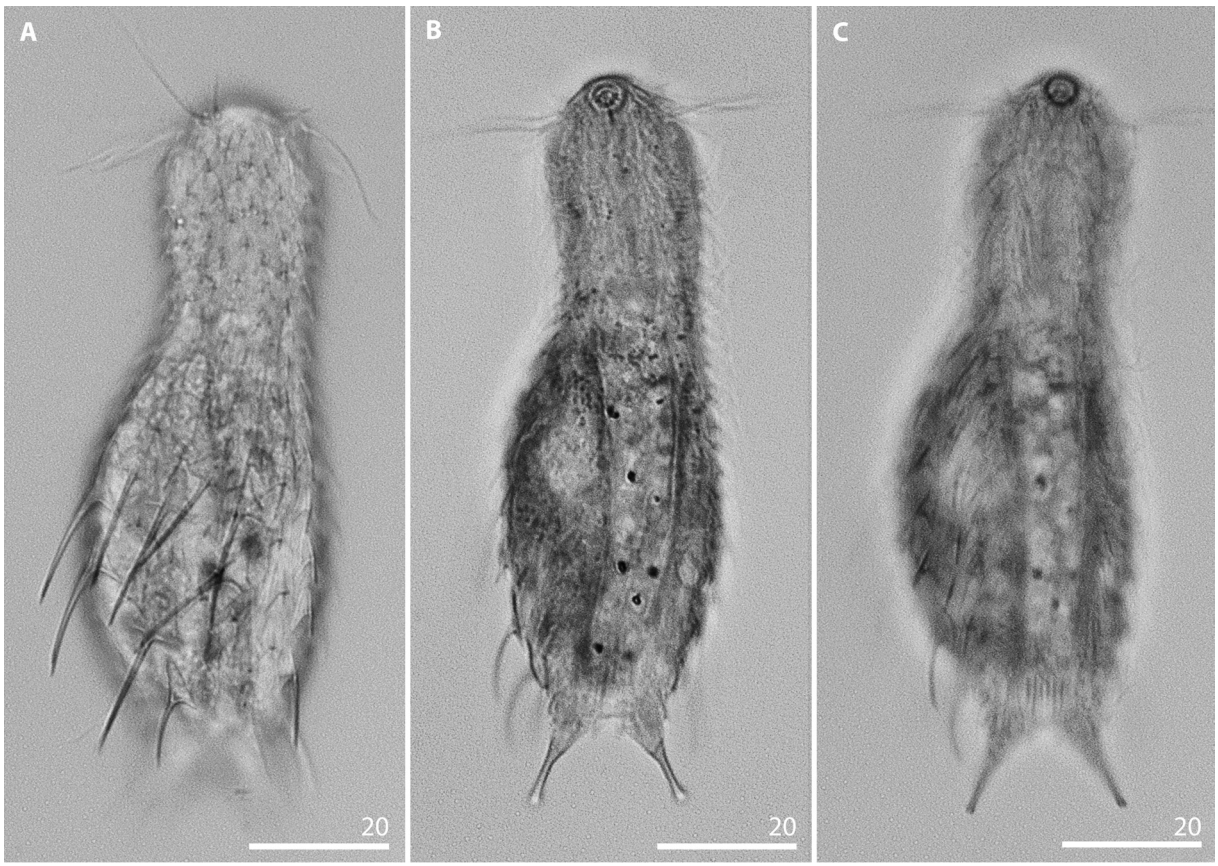


Fig. 29. *Chaetonotus* (*Hystricochaetonotus*) *inaequabilis* sp. nov. Adult specimen. Bright field microphotographs. **A**. Dorsal body view. **B**. View of internal morphology. **C**. Ventral body view.

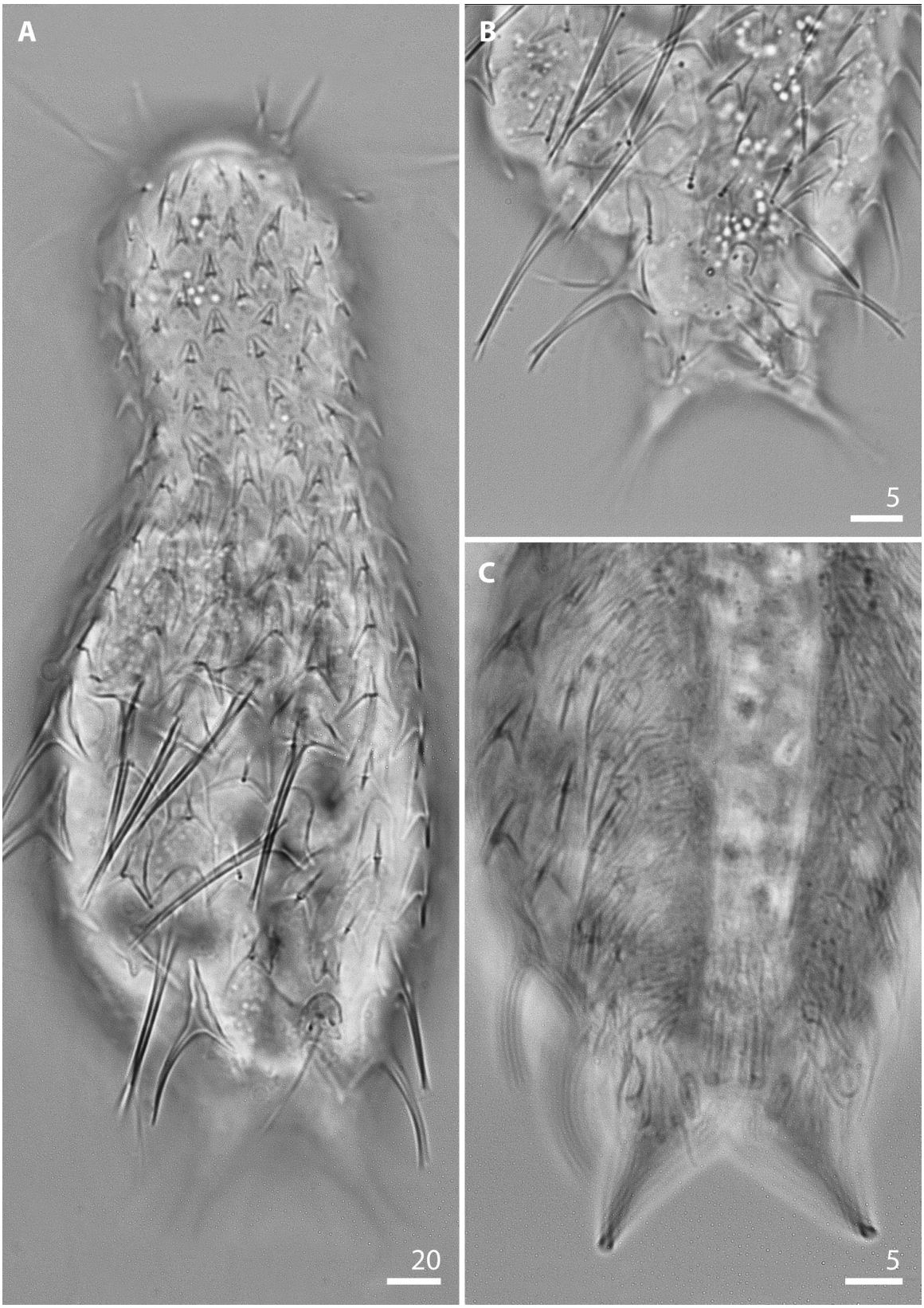


Fig. 30. *Chaetonotus* (*Hystricochaetonotus*) *inaequabilis* sp. nov. Adult specimen. Bright field microphotographs. **A**. Scales, dorsal view. **B**. Posterior trunk region, furca base and furcal appendage scales, dorsal view. **C**. Posterior trunk region, furca base and furcal appendage scales, ventral view.

directed downward towards the ciliary bands as compared to the dorsal scales (scales 3–14; Fig. 27C–N). From the neck towards the trunk, the size of the scales gradually increases (from 2.1–2.5 × 2.4–2.9 to 4.2–5.4 × 5.1–6.5 μm). The anterior trunk scales have a wide and rounded anterior central lobe and clearly separate, long, narrow and sharp posterolateral lobes. The posterolateral lobes are directed diagonally downward and slightly laterally, and they create a deep, narrow V-shaped posterior scale notch (scale 15; Fig. 27O). The dorsolateral, lateral and ventrolateral trunk scales, arranged from the central to posterior trunk region (U50 to U83), become gradually larger than the anterior trunk scales (from 2.3–7.1 × 2.7–5.1 to 3.5–8.8 × 4.1–7.1 μm) and they have a more pointed anterior edge of the central lobe and longer, more sharp posterolateral lobes directed more apart and diagonally downward (scale 17; Fig. 27Q). Dorsally, on the central trunk part (at U47–U57), five different scales with very long and strong spines are located in one transverse row. These scales are very large (6.4–13.0 × 5.2–8.3 μm) and very thick. They are triangular, their central lobes are short, wide and pass seamlessly into wide, posterolateral lobes directed very far apart. The posterior edges of these scales are almost straight and only very narrowly notched. Their keels start from the half length of the central lobes and are very pronounced, strongly triangular in shape (scale 18; Fig. 27R). Directly beneath the row of large scales, two pairs of smaller scales with long keels and spiny processes are located (at ca U55–U61). These scales are triangular, have longer central lobes with pointed anterior edges and narrower posterolateral lobes directed slightly more downward (scale 19; Fig. 27S). Posteriorly to these scales, at the centre of the dorsal trunk region, three scales with long keels and spiny processes are located (at U60–U68). These scales are narrower and have longer central lobes than the preceding scales. Their posterolateral

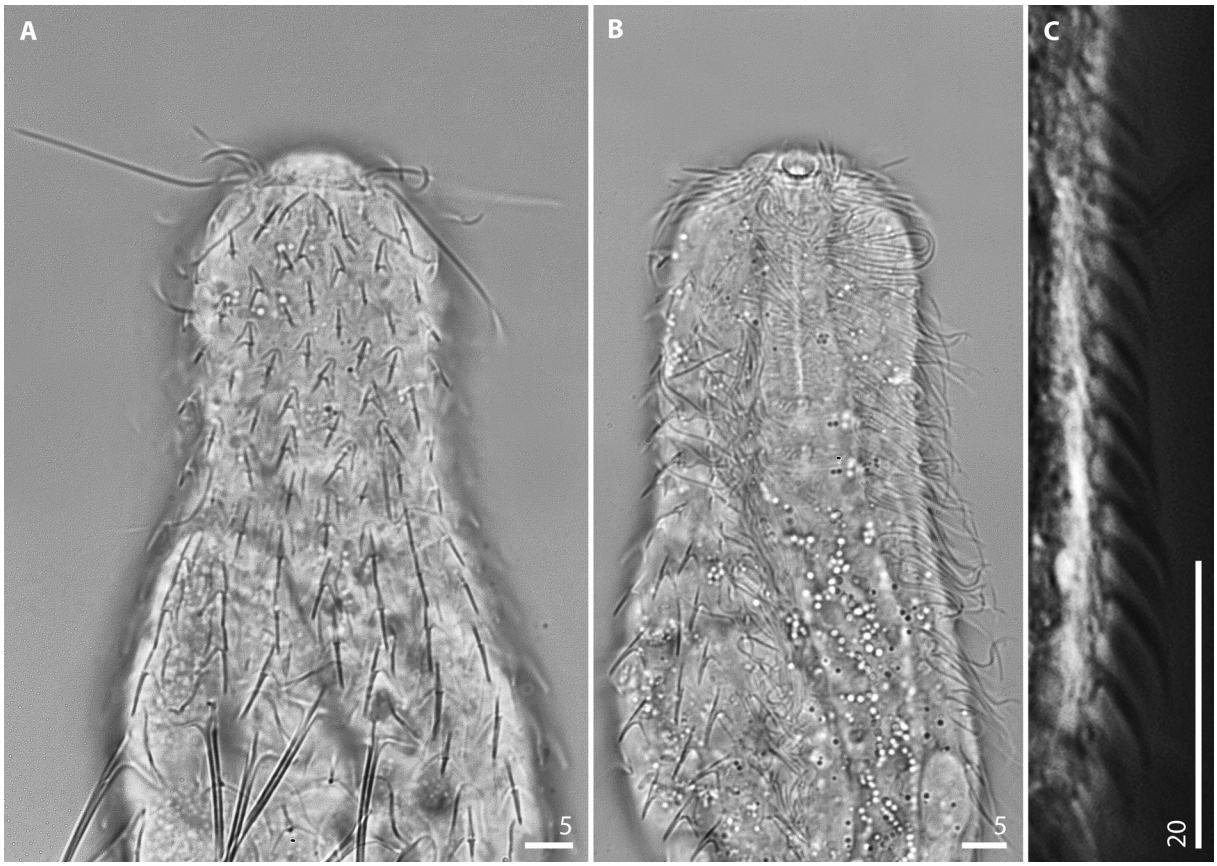


Fig. 31. *Chaetonotus* (*Hystricochaetonotus*) *inaequabilis* sp. nov. Adult specimen. **A**. Head, neck, anterior and central trunk spines, dorsal view, bright field microphotograph. **B**. Head, neck, anterior and central trunk spines, ventral view, bright field microphotograph. **C**. Ventrolateral spines with lamellae, phase contrast microphotograph.

lobes are directed clearly downward, creating a V-shaped posterior scale notch (scale 20; Fig. 27T). Laterally to these three scales and posteriorly to the transverse row with wide, large scales, another pair of large scales with very long and strong spines is located (at U60–U66). These scales have longer central lobes, narrower posterolateral lobes directed more downward (7.3–9.2 × 7.2–9.5 μm) and more rounded edges than the preceding large scales (scale 21; Fig. 27U). In the central dorsal longitudinal row, at U67–U74, a single scale of the same type as the large scales from the transverse row at U55–U59 is present (scale 18; Fig. 27R). Laterally to it, at U70–U74, two pairs of smaller scales are located, with one pair after another in the longitudinal row. These scales have a long and strongly rounded central lobe and clearly separated, short and wide posterolateral lobes (Fig. 30B). Of these two scale pairs, the pair situated below (U72–U74) is larger than the upper pair (U70–U72) (scale 22; Fig. 27V). On the posterior trunk region laterally to these rounded scales, directed above the double-keeled scales with trunk dorsal sensory bristles, one pair of small (3.3–4.9 × 2.2–3.3 μm) scales is arranged at U74–U75 (scale 23; Fig. 27W). These scales have a strongly rounded central lobe and posterolateral lobes that are weakly separated from the central lobes, wide, short and directed downward, creating a V-shaped posterior notch. Their keels are strong but short and pass into short spines which do not extend to the posterior edge of the scales. On the lateral surface of the posterior trunk region, one pair of second to last trunk scales is located (at U71–U78). These scales are large (7.4–11.4 × 4.1–6.1 μm) and have a long, wide, strongly rounded and clearly pointed anterior central lobe. Their postero-lateral lobes are strongly separated from the central lobe and have long, narrow and sharp edges that are directed diagonally downward. These scales have strong keels and strong, long spines (scale 24; Fig. 27X). Posteriorly to them, the last pair of trunk lateral scales is situated at the furcal base (at U78–U84). This pair of scales is large (6.4–10.8 × 4.9–8.3 μm); these scales have a long, wide and strongly anteriorly rounded central

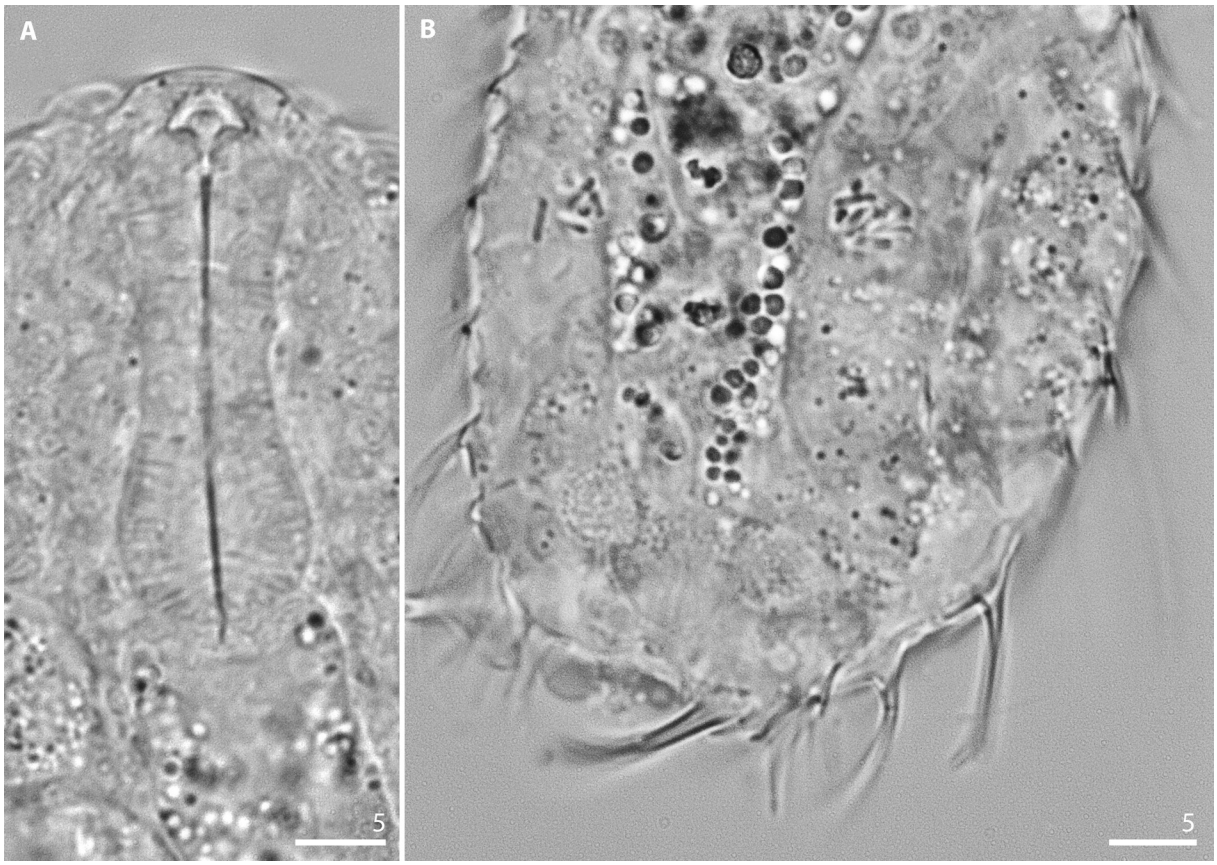


Fig. 32. *Chaetonotus* (*Hystricochaetonotus*) *inaequabilis* sp. nov. Adult specimen. Bright field microphotographs. **A**. Detail of pharynx. **B**. X-organ and sperm pockets.

lobe with a very strong and high keel and strong, long spines and short, narrow posterolateral lobes. The posterolateral lobes of these scales are directed diagonally wide apart, downward and laterally, creating a wide and shallow V-shaped posterior scale notch (scale 25; Fig. 27Y). On the dorsal posterior trunk region and furcal base, at U76–U83, one median large scale that is different in type is located. This scale is triangular, has a long and pointed central lobe with a long keel and a long, strong spine, and clearly separate, relatively short postero-lateral lobes directed diagonally downwards (scale 26; Fig. 27Z). On the dorsal surface of the furcal appendages (at U84–U89), three pairs of three-lobed, elongated and narrow scales with long central and short posterolateral lobes are present. These scales have a long keel with a spiny process (scale 27; Fig. 27AA). Dorsolaterally and laterally to the furcal appendages (at U88–U90), one pair of scales with two long and slightly bent keels is present. These scales are oval in shape and have very shallow posterior notches (scale 28; Fig. 27BB). The edges of the scales on the furcal appendage are slightly overlapping (Fig. 30B). The size of the scales gradually decreases from the dorsal, dorsolateral and lateral surfaces towards the ventrolateral surface. The scales in the ventral longitudinal row located closest to the ciliary bands are short and wide, their central lobes are short and their posterolateral lobes are weakly separated. From the head to the widest body region the length of the posterolateral lobes of these scales, as well as their separation from the central lobes, gradually increase. The notches of the posterior edges of these scales are semicircular in shape. The ventral scales are much smaller than the scales of the other rows and their anterior edge is oriented towards the bands at an angle of ca 20°.

Spines. In this species, five main types of spines may be distinguished (Fig. 28). The first type are basally bent spines tapering towards the end, without any lateral denticle (Fig. 28A). These spines emerge from single, central anteriormost head scales located at U5–U6 (Figs 27A, 31A) and from two pairs of dorsal posterior trunk region scales located at U70–U74 (scale 12) (Fig. 27A). The second type are simple spines that do not taper towards the blunt ends and are without any lateral denticle (Fig. 28B). These spines emerge from the dorsal, dorsolateral, most of the lateral and ventrolateral head, neck and anterior trunk region scales and dorsolaterally, laterally and ventrolaterally on the remaining trunk part scales. The length and thickness of the spines gradually increase from the head top towards the widest body region (from 0.3–12.0 to 3.1–15.2 μm) (ca U57). Moreover, their length gradually decreases towards the ventrolateral surface (head: from 0.5–3.1 to 0.3–2.4 μm; neck: from 2.1–5.7 to 1.6–4.4 μm; trunk: from 3.9–8.2 to 3.1–7.1 μm) (Table A5). The third type are very long (11.4–21.6 μm) and thick, straight spines with a prominent lateral denticle (Fig. 28C). Together, nine dorsal spines and four (two pairs) lateral spines of this type are present. Five of them arise from large dorsal trunk scales located in one transverse row at U47–U57 (scale 18) (Figs 29A, 31A–B). The next two spines arise from one pair of dorsal trunk scales located at U60–66 (scale 21) (Figs 29A, 31A–B). The eighth of these long spines arises from the central dorsal scale located at U67–U74 (scale 18) (Figs 29A, 31A–B). This spine is the longest (11.4–21.6 μm) and the thickest body spine and it has the most prominent lateral denticle. The ninth and last of these long dorsal spines arises from the central dorsal scale located at the posterior trunk region and on the furcal base (at U84–U89) (scale 26) (Fig. 31A–B). This spine is slightly shorter than the preceding long spine (11.4–14.2 μm). The two pairs of long lateral spines arise from the trunk second to posteriormost pair of lateral scales (at U71–U78) (scale 24) and from the trunk posteriormost pair of lateral scales (at U78–U84) (scale 25; Figs 28D, 31A–B). The posteriormost pair is longer (11.4–14.0 μm) and thicker than the second to posteriormost pair of lateral spines (10.1–12.7 μm long). The fourth type are short, simple spines (Fig. 28E) or spiny processes without any lateral denticle (Fig. 28F). These spines arise from the central dorsal trunk scales located at U55–U59 and U60–U68 (Fig. 31A), from one pair of trunk dorsal scales located at U74–U75, above the double-keeled scales with the dorsal sensory bristle, and from three pairs of elongated scales located on the dorsal and dorsolateral surface of the furcal appendages at U84–U89 (Fig. 31A). The fifth type are long (7.3–15.2 μm), thin and hair-like, with narrow, delicate lamellae along their entire length (Fig. 28G). These spines arise on the ventral

surface, from scales arranged in one pair of a longitudinal row located closest to the ciliary bands. The lamellae on these spines are widest near the spine base and gradually taper towards the end of the spine (Fig. 31C).

Dorsal sensory bristles. This species has three pairs of dorsal sensory bristles (Fig. 26A). The first, anterior pair is located on the dorsal surface of the head at U5, beyond the dorsolateral cephalion edges, where the second pair of sensory bristles is located on the dorsal surface of the posterior neck at U31. The first and the second pairs emerge from small, spherical papillae. The third, posterior pair of sensory bristles is located dorsolaterally on the posterior trunk region and furcal base and emerges from the small three-lobed scales with two strong keels situated at U77–U79. These scales have keels which are connected in the centre of the scales and have a strongly rounded, wide central lobe and short, weakly separated posterolateral lobes. These scales have a double, shallow posterior notch (Fig. 31A).

Ventral ciliary bands and ventral interciliary field. On the ventral surface, the longitudinal ciliary bands begin at U9 and run back to U82 (Fig. 29C). The ciliary bands are wide, and wider on the head region than on other parts of the body. The entire ventral interciliary field is covered with thin, small scales. They are distributed in seven longitudinal rows, with 21–25 scales in the central row (Figs 30C, 31B). The ventral interciliary field anterior scales are partially recessed on the cuticle and isolation from the cuticle increases towards the posterior body part. On the anterior region the scales have an oval shape with a straight posterior edge and are located far from each other (scale 29; Fig. 27CC). Towards the posterior body region the scales become gradually larger (from 1.4–1.9 × 0.6–1.1 to 3.8–5.2 × 2.7–4.1 μm), more elongated and with a rounded posterior edge (scale 30; Fig. 27DD). The anterior scales are keelless and spineless, but towards the trunk posterior region the keels on the scales become more distinct and the distance between the scales decreases (Figs 26C). Four pairs of ventral interciliary field terminal scales are present. The first pair (at U76–U85) is elongated and has a narrowing halfway along the length of the scale (Fig. 30C). They have a long, narrow keel and a long, thin and straight spine extending beyond the internal furcal indentation (scale 31; Fig. 27EE). The second pair (at U84–U87) is elongated, has a pointed anterior edge and gradually tapers to the posterior end. This pair has long, narrow keels and is spineless (scale 32; Fig. 27FF). The third (at U87–U88) and fourth (at U88–U89) pairs are small, elongated and oval-shaped, with a shallow posterior notch (scale 33; Fig. 27GG). They have a long, straight keel and are spineless. The second, third and fourth pairs of ventral interciliary field terminal scales are situated at the furcal appendages.

Internal morphology. The pharynx (from U3 to U29) is wide and has distinct, marked, rounded anterior and posterior dilatations. The posterior dilatation is wider than the anterior one (Fig. 32A). The pharynx is connected through the pharyngeal–intestinal junction to a straight intestine, running from U29 to U84. The pharyngeal–intestinal junction is clearly demarcated, short and narrow (U29–U30). The X-organ of this species (observed in one specimen) is located at U80–U83 near the terminal part of the intestine. It is bilobed, built from two extensions enveloped in a thin coat and connected by a thinner band located behind the intestine at the ventral side. The extensions, the thin coat and the cellular bridge connecting the extensions have have a grain-like structure. The pair of sperm packets of this species are circular in shape and contain spermatozoids (12 per packet) in the form of a short rod. They are located at U62–U64 on both sides, juxtaposed to the intestine (Fig. 32B).

Remarks

Juvenile specimens of *Chaetonotus* (*Hystricochaetonotus*) *inaequabilis* sp. nov. were not observed; furthermore, specimens with large, developing eggs were not present. Out of seven adults, one had an X-organ and two had sperm packets.

Differential diagnosis

Within the subgenus *Hystricochaetonotus*, *Chaetonotus* (*H.*) *inaequabilis* sp. nov. most closely resembles *C.* (*H.*) *enormis* Stokes, 1887, *C.* (*H.*) *schlitzensis* Schwank, 1990, *C.* (*H.*) *octonarius* Stokes, 1887, *C.* (*H.*) *novenarius* Greuter, 1917, *C.* (*H.*) *aemilianus* Balsamo, 1978, *C.* (*H.*) *ferrarius* Schwank, 1990, *C.* (*H.*) *spinulosus* Stokes, 1887 and *C.* (*H.*) *longispinosus* Stokes, 1887. All of these species were chosen for comparison because they possess a set of similar features such as: range of body length; body shape; presence of very long spines on dorsal and/or dorsolateral trunk surfaces; presence of lateral denticle only on the long spines (for *C.* (*H.*) *ferrarius* see Table A6); and absence of parafurcal spines. Moreover, *C.* (*H.*) *enormis* is similar to the new species in having two pairs of furcal base lateral spines which are longer and stronger than the remaining lateral spines. *Chaetonotus* (*H.*) *schlitzensis*, *C.* (*H.*) *octonarius*, *C.* (*H.*) *novenarius*, *C.* (*H.*) *aemilianus*, *C.* (*H.*) *ferrarius* and *C.* (*H.*) *spinulosus* share with *C.* (*H.*) *inaequabilis* sp. nov. the presence of scales without spines or with spiny processes on the central dorsal trunk region. *Chaetonotus* (*H.*) *novenarius* and *C.* (*H.*) *aemilianus* also possess the same number of scales with long spines as the newly described species. Despite the fact that among the hitherto known species in this subgenus, those listed above have the highest number of common features with the newly described species, they are significantly different from *C.* (*H.*) *inaequabilis* sp. nov. – most strikingly by size, type, and shape of the scale coverage as well as the arrangement of scales with long spines. In order to avoid any doubts in comparisons with the new species, only the original descriptions of these species and three of the most detailed papers with morphological data were used (Balsamo 1983; Schwank 1990; Kisielewski 1997a). Comparisons between the new species and the morphologically most similar taxa are summarised in Table A6.

Subgenus ***Zonochaeta*** Remane, 1927

Type species

Chaetonotus succinctus Voigt, 1902.

Terra typica

Germany.

Remarks on subgenus

This is the only subgenus of *Chaetonotus* that seems to be monophyletic in terms of molecular data (Kånneby *et al.* 2013); it encompasses 13 nominal freshwater species (Todaro 2018). It is present in benthic and periphytic habitats (e.g., Kisielewski 1997a).

Chaetonotus* (*Zonochaeta*) *cestacanthus Balsamo, 1990
Figs 33–34; Table 6

Chaetonotus (*Zonochaeta*) *cestacanthus* Balsamo, 1990: 168, fig. 4.

Locus typicus

Italy.

Material examined

POLAND • 3 adults, 1 juvenile; Kraków, Botanical Garden, Jubilee Greenhouse, sites 1–2; 50°03′38″ N, 19°57′30″ E; 15 Nov. 2013 and 17 Apr. 2014; M. Kolicka leg.; NHC-GCZC-24-1-10 (photomicrographs, also in the author's collection).

Distribution

Chaetonotus (*Z.*) *cestacanthus* Balsamo, 1990 was originally described from Italian lakes, namely Lake Chiusi and Lake Bolsena (Balsamo 1990), and next recorded from the volcanic Lake Nemi, also in Italy (Balsamo & Fregni 1995). It has not previously been listed outside of this country.

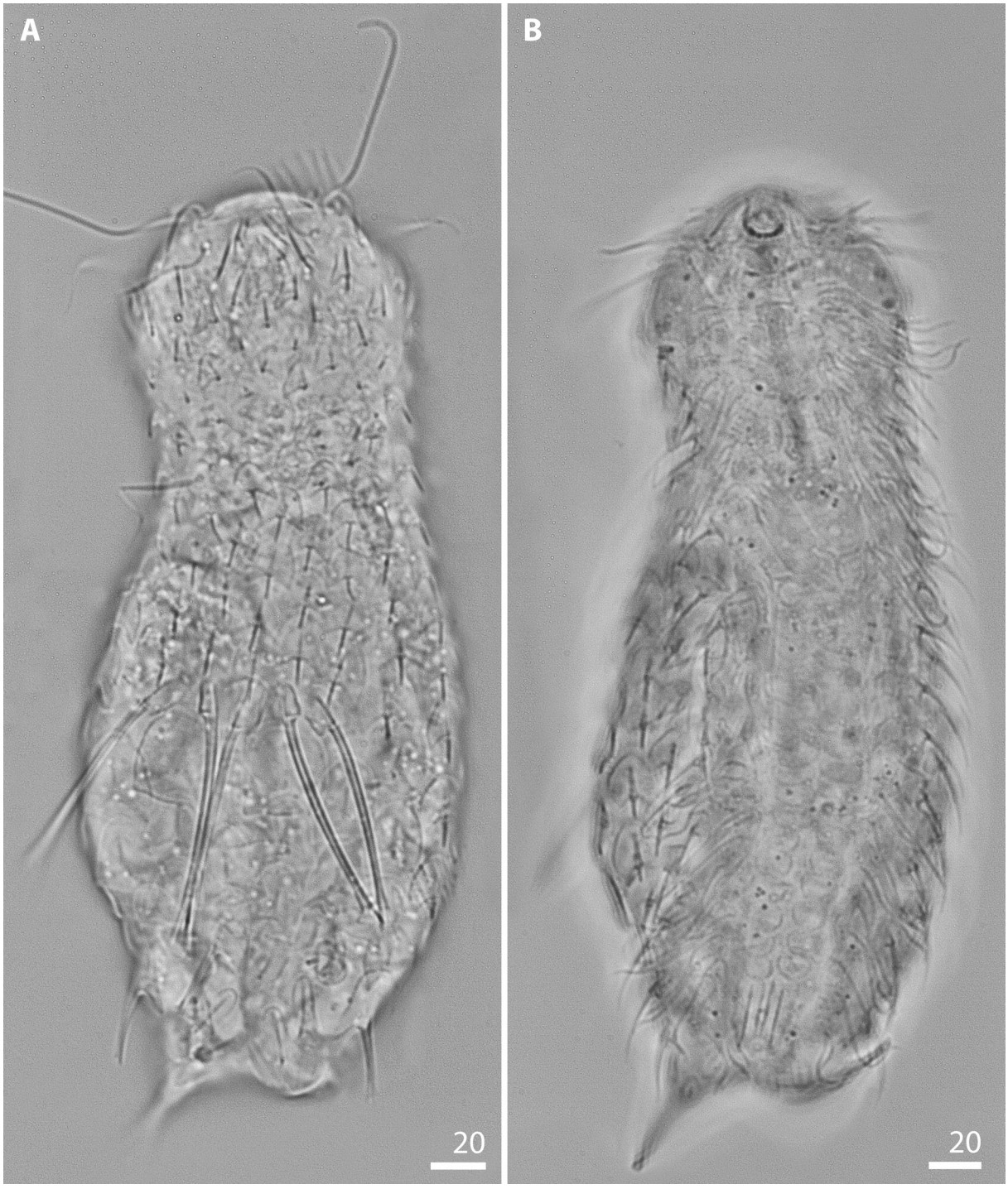


Fig. 33. *Chaetonotus* (*Zonochaeta*) *cestacanthus* Balsamo, 1990. Adult specimen. Bright field microphotographs. **A**. Scales and spines, dorsal view. **B**. Scales and spines, ventral view.

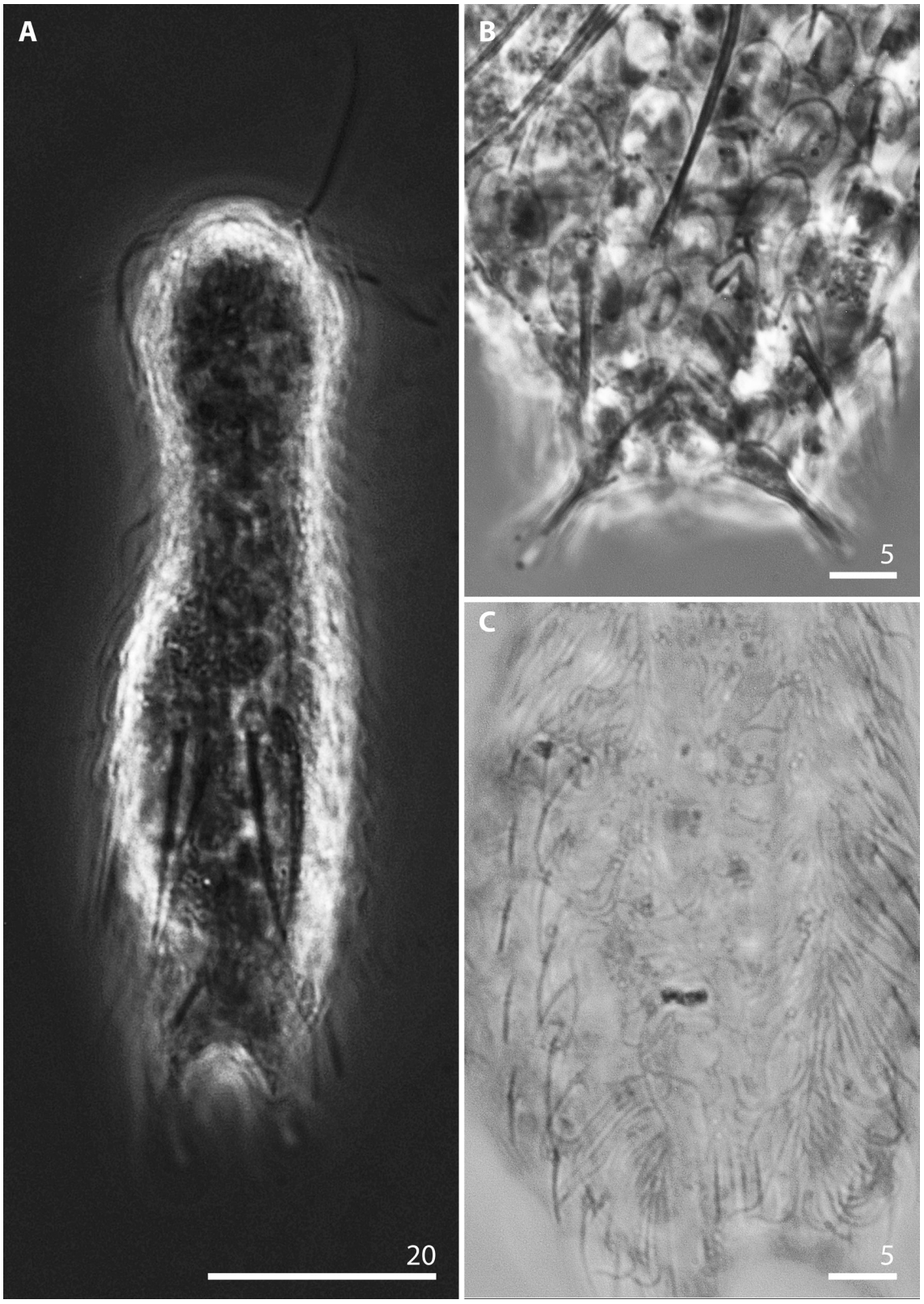


Fig. 34. *Chaetonotus* (*Zonochaeta*) *cestacanthus* Balsamo, 1990. Adult specimen. **A**. Habitus, phase contrast microphotograph. **B**. Posterior trunk region, furca base and furcal appendages, dorsal view, phase contrast microphotograph. **C**. Posterior trunk region, furca base and furcal appendages, dorsal view, bright field microphotograph.

Table 6 (continued on next page). Main morphometric characters of *Chaetonotus* (*Zonochaeta*) *cestacanthus* Balsamo, 1990. N = number of specimens analysed; NA = data not available; Range = the smallest and the largest structure found among all specimens measured. All measurements in micrometers (μm).

Characters	According to Balsamo (1990)	N	Range of adult specimens	Juvenile specimen
Body length	64.50–80.00	3	87.19–89.11	70.12
Furca length	8.50–11.00	3	14.91–15.77	15.05
Adhesive tube length	NA	3	7.50–8.13	8.06
Number of scales in central longitudinal row	13–14	3	14	13
Total number of longitudinal alternating rows of scales	15	3	15	13
Length of cephalic cilia (anterior tuft)	(8.50–10.50)–(18.50–20.0)	3	(7.85–8.11)–(20.89–22.46)	7.34–18.16
Length of cephalic cilia (posterior tuft)	8.50–10.50	3	(8.24–9.55)–(11.73–16.54)	8.15–11.37
Head scales length × width	3.50–4.20 × 2.50–3.20	3	(2.98–3.19)–(4.67–5.02) × (2.18–2.34)–(3.63–3.83)	2.89–4.22 × 2.09–3.32
Neck scales length × width	3.00 × 2.80	3	(2.56–2.91)–(4.19–4.67) × (2.77–3.19)–(4.10–4.31)	2.34–3.83 × 2.67–3.86
Trunk scales length × width	6.50–8.70 × 5.30–6.50	3	(3.89–4.05)–(9.46–9.59) × (3.26–3.91)–(7.20–7.85)	3.68–5.94 × 3.10–4.72
Scales with long dorsal spines length × width	3.70–4.60 × 3.20–3.70	3	(4.24–4.78)–(4.69–4.95) × (2.89–3.30)–(3.19–3.53)	4.19–4.81 × 3.22–3.46
Median and dorsolateral scales with long spines on posterior trunk region length × width	6.00 × 4.00–6.00	3	(5.68–6.70)–(5.86–7.19) × (5.22–5.88)–(5.42–6.47)	5.03–5.72 × 4.41–4.76
Double keeled scales on furcal appendages length × width	NA	3	4.36–4.93 × 4.04–4.51	3.85 × 3.52
Head dorsal spines length	1.30–2.30	3	(0.39–0.51)–(3.17–4.61)	0.34–3.97
Head ventral spines length	2.00–7.80	3	(3.02–3.81)–(6.14–6.54)	2.84–5.79
Neck dorsal spines length	1.30–2.30	3	(0.31–0.37)–(2.66–2.74)	0.27–2.30
Neck ventral spines length	2.00–7.80	3	(6.23–6.82)–(7.28–7.79)	6.08–7.14
Trunk dorsal spines length	1.30–2.30	3	(2.34–2.49)–(2.81–2.92)	2.12–2.92
Trunk ventral spines length	2.00–7.80	3	(7.47–8.06)–(9.16–10.12)	7.36–9.69
Long dorsal spines length	13.50–17.80	3	19.69–20.61	18.28
Median spine on posterior trunk region length	9.30–11.00	3	8.20–9.66	7.8
Dorsolateral spines on posterior trunk region length	7.20–8.70	3	7.18–7.69	6.61
Head dorsal sensory bristles length	NA	3	9.47–11.21	10.29
Neck dorsal sensory bristles length	8.70–10.20	3	12.95–14.29	12.7
Posterior dorsal sensory bristles length	8.70–10.20	3	14.23–15.62	13.94
Number of scales in central longitudinal row on ventral interciliary field	25–26	3	25–26	23
Total number of longitudinal alternating rows of scales on ventral interciliary field	2	3	2	2

Table 6 (continued). Main morphometric characters of *Chaetonotus* (*Zonochaeta*) *cestacanthus* Balsamo, 1990.

Characters	According to Balsamo (1990)	N	Range of adult specimens	Juvenile specimen
Ventral interciliary field scales length × width	2.50–2.60 × 2.80–3.00	3	(1.93–2.26)–(3.28–3.72) × (2.26–3.25)–(4.59–4.66)	1.72–3.16 × 2.04–4.05
Terminal ventral interciliary field scales I pair length × width	7.60 × 2.7	3	7.25–8.04 × 2.24–2.80	6.63 × 2.43
Terminal ventral interciliary field scales II pair length × width		3	3.10–3.50 × 1.43–1.89	2.79 × 1.39
Mouth ring diameter	NA	3	3.79–3.95	3.82
Pharynx length	20.70–27.00	3	23.26–24.09	24.28
Intestine length	NA	3	45.24–46.70	30.51

Remarks

The original description of *C.* (*Z.*) *cestacanthus* is very detailed (Balsamo 1990) and specimens found in the Jubilee Greenhouse in Kraków correspond well with it. However, the present specimens differ from the original data in being slightly larger (Table 6) and in having three pairs of dorsal sensory bristles instead of two (the additional pair arising on the head, near the dorsal cephalion edge); the first scales of the dorsal longitudinal rows, located near the cephalion and epipleurae, are more elongated and have longer spines than the remaining head scales in comparison to the original description and figures (Figs 33–34); the ventrolateral and ventral scales are similar to the dorsal ones (Fig. 33B), and dorsolaterally on the furcal appendages they have one pair of double-keeled scales, rounded in shape, with shallow posterior notches (Fig. 34B).

Chaetonotus (*Zonochaeta*) *cestacanthus* has previously been recorded only as an element of benthic assemblages in sandy sediments, as well as in sediments rich in detritus, at a station with rich vegetation (Balsamo 1990; Balsamo & Fregni 1995). There have been no reports of this taxon being found in epiphytic or other periphyton communities, although their presence in samples collected near rich aquatic vegetation may suggest that *C.* (*Z.*) *cestacanthus* could also survive in this habitat type.

Genus ***Heterolepidoderma*** Remane, 1927

Type species

Ichthydium ocellatum Mečnikow, 1865 (= *Heterolepidoderma ocellatum* (Mečnikow, 1865)).

Terra typica

Russia.

Remarks on genus

This is regarded as a polyphyletic genus (Kieneke *et al*. 2008; Kånneby *et al*. 2013), which encompasses 22 nominal freshwater species and 10 nominal marine species. It is present in benthic, interstitial and periphytic habitats.

Heterolepidoderma aff. ***majus*** Remane, 1927
Fig. 35

Heterolepidoderma majus Remane, 1927: 313–315, fig. 10.

Locus typicus

Germany.

Material examined

POLAND • 8 adults; Kraków, Botanical Garden, Jubilee Greenhouse, sites 1–2; 50°03′38″ N, 19°57′30″ E; 15 Nov. 2013; M. Kolicka leg.; NHC (photomicrographs, also in the author's collection).

Distribution

Nominal *Heterolepidoderma majus* Remane, 1927 is a species that is widely distributed in Europe, *inter alia* reported from: Bulgaria (Valkanov 1937), France (Kisielewski 1997a), Germany (Remane 1927), Great Britain (Martin 1990), Poland (e.g., Roszczak 1936; Kisielewska & Kisielewski 1986a, 1986b, 1986c), Russia (Tretjakova 1989) and Romania (Rudescu 1967); it has also been reported from Argentina (Grosso & Drahg 1984), Brazil (Kisielewski 1991), Canada (Schwank 1990) and Japan (Sudzuki 1971).

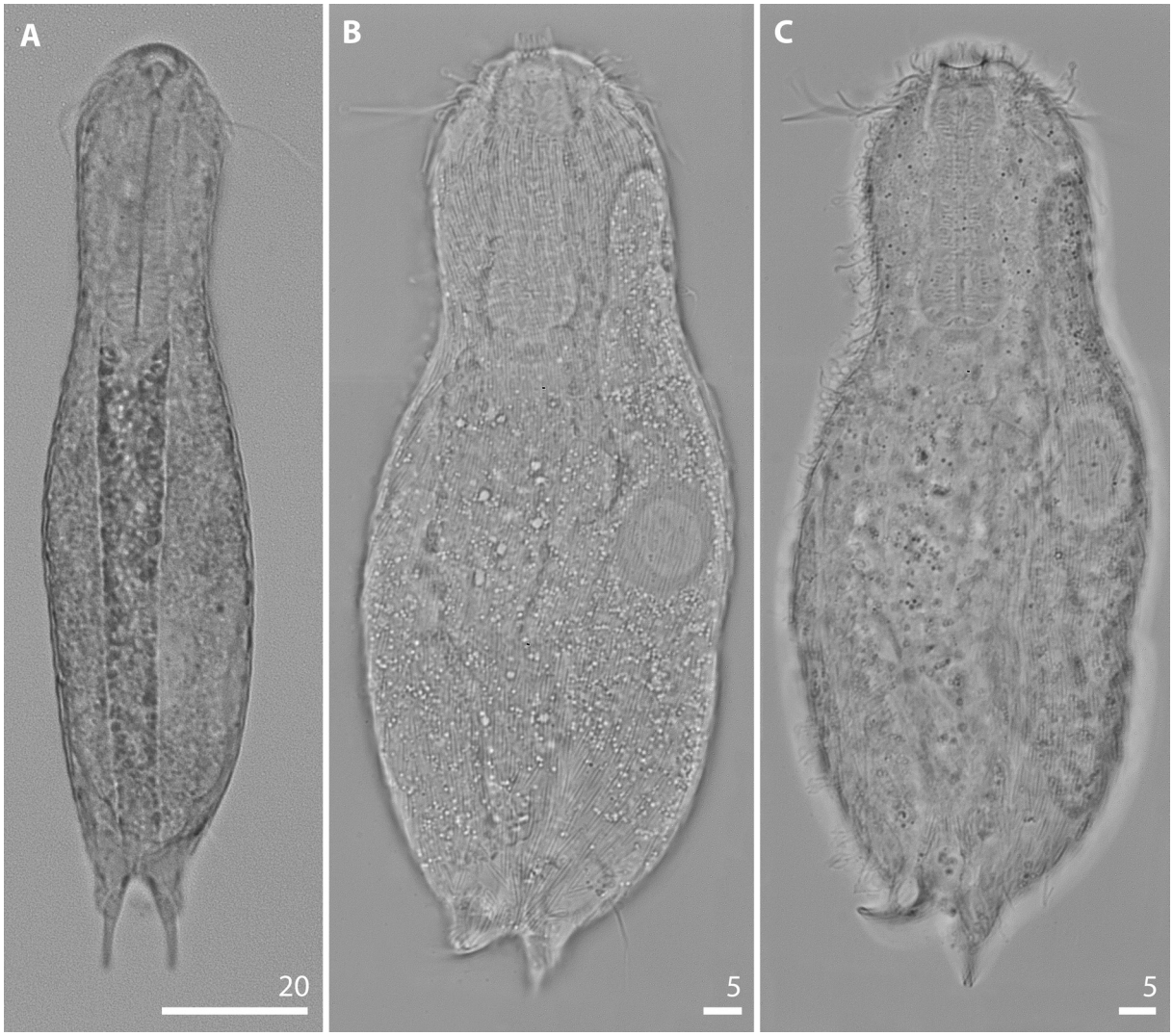


Fig. 35. *Heterolepidoderma* aff. *majus* Remane, 1927. Adult specimen. Bright field microphotographs. **A**. Habitus. **B**. Dorsal view. **C**. Ventral view.

Remarks

The body length of adult specimens of *Heterolepidoderma* aff. *majus* Remane, 1927 varied from 111.1 to 131.4 μm; length of pharynx was between 31.9 and 40.3 μm; intestine length from 60.7 to 72.6 μm; 53–57 total longitudinal rows of scales (19–21D + 10DL + 8L + 8–10LV + 8V), with 23–27 scales in the central row (Fig. 35).

In a previous paper, these specimens were reported as *Heterolepidoderma majus* Remane, 1927 (Kisielewska *et al*. 2015). However, although the specimens seemed to fall within the range of variability as reported by Schwank (1990) and Kisielewski (1997a), the presence of one pair of scales with four keels, nearly parallel to one another, did not allow, after a thorough analysis, the identification of the specimens from the Jubilee Greenhouse as representatives of the nominal species *H. majus*. The question of the species affinities of these individuals demands further analyses and studies of intra- and interspecific variability within *Heterolepidoderma*.

Genus ***Lepidodermella*** Blake, 1933

Type species

Chaetonotus squamatus Dujardin, 1841 (= *Lepidodermella squamata* (Dujardin, 1841)).

Terra typica

France.

Remarks on genus

This polyphyletic genus (Kånneby *et al.* 2012, 2013) comprises 12 nominal freshwater species and one nominal marine species. It is present in benthic, interstitial and periphytic habitats.

Lepidodermella aff. ***squamata*** (Dujardin, 1841)
Fig. 36

Chaetonotus squamatus Dujardin, 1841: 664, fig. 18.

Locus typicus

France.

Material examined

POLAND • 2 adults; Kraków, Botanical Garden, Jubilee Greenhouse, site 2; 50°03′38″ N, 19°57′30″ E; 15 Nov. 2013; M. Kolicka leg.; NHC (photomicrographs, also in the author's collection).

Distribution

Lepidodermella squamata is a widely distributed species or, more precisely, in the light of the current data, it represents sets of cryptic or morphologically similar species noted in Europe, *inter alia* reported from Bulgaria (Valkanov 1937), Germany (Remane 1935–36), Great Britain (Martin 1981), Italy (Mola 1932), Poland (Roszczak 1936; Kisielewska & Kisielewski 1986a, 1986b, 1986c), Romania (Rudescu 1967), Sweden (Kånneby 2011) and Switzerland (Greuter 1917); also reported from Argentina (Grosso & Drahg 1984), Australia (Hochberg 2005), Brazil (Kisielewski 1991), Canada (Schwank 1990), East Africa (Daday 1910), India (Naidu & Rao 2004), Israel (Kisielewski 1999), Japan (Sudzuki 1971), South Korea (Lee & Chang 2000), the United States (Bryce 1924) and Uruguay (Cordero 1918).

Remarks

Lepidodermella squamata (Dujardin, 1841) is one of the four earliest described gastrotrich species (Balsamo *et al*. 2014). This taxon is distributed worldwide and is very common in very different habitats (from freshwater and brackish psammon to micro-reservoirs in bromeliad leaves); however, in light of molecular data and on the basis of morphological evidence, it constitutes not one but at least a few morphologically very similar species, i.e., a complex of pseudocryptic or cryptic species (Fregni *et al*. 1998; Kånneby *et al*. 2012).

Both of the specimens reported here corresponded well with the original species description (Dujardin 1841) and with later records and the emended descriptions by Balsamo (1983) and Kisielewski (1984, 1997a) (Fig. 36). A detailed discussion of the shape of the scales and comparisons between the studied specimens and *L. squamata* in the literature are considerably impeded by the noticeable differences in the shape and distribution of the scales that were presented by various authors and by intraspecific variability (e.g., Rudescu 1967; Roszczak 1969; Balsamo 1983; Schwank 1990; Kisielewski 1997a), but the type and shape of the scales in the main covering remain consistent with Balsamo (1983)

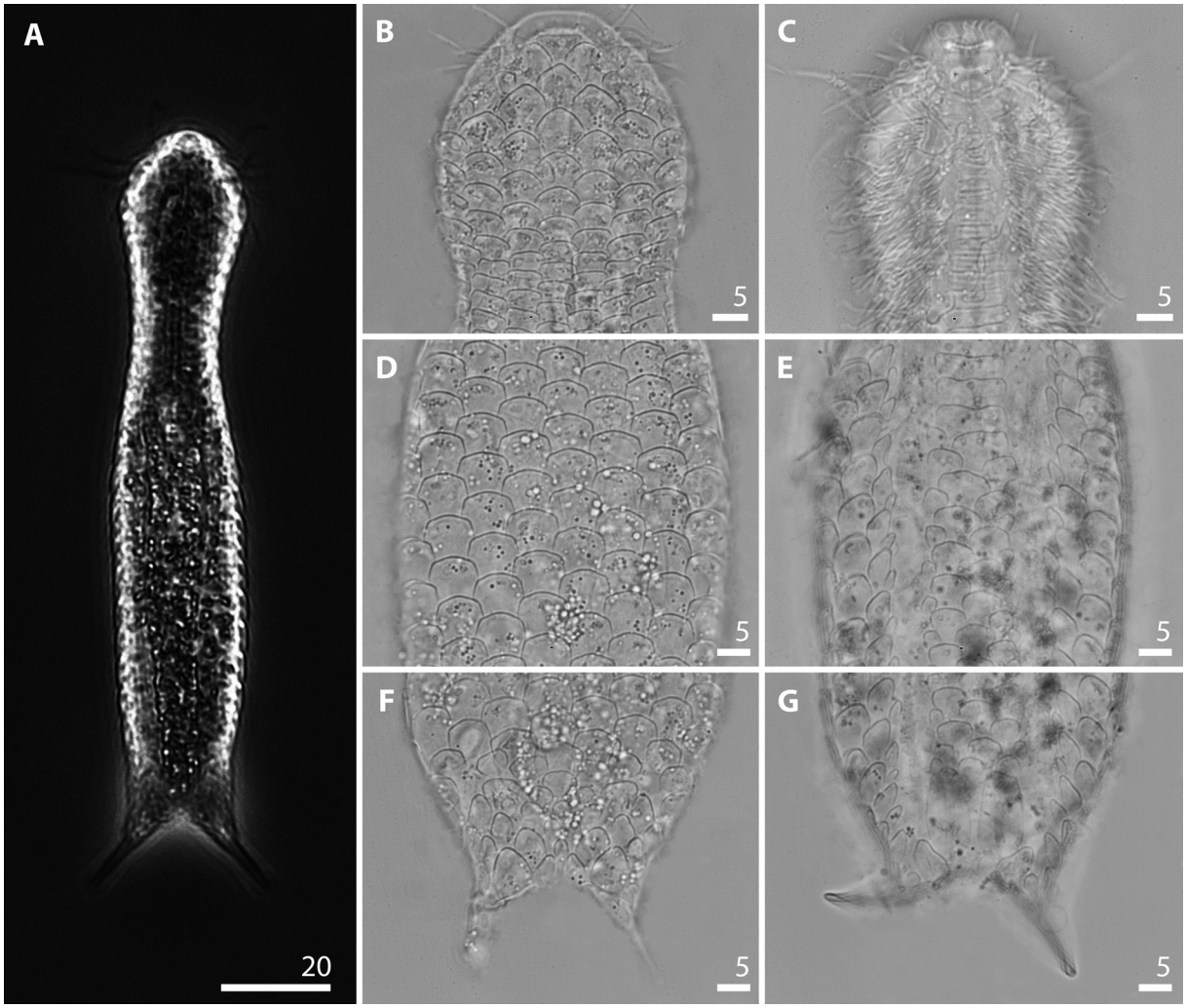


Fig. 36. *Lepidodermella* aff. *squamata* (Dujardin, 1841). Adult specimen. **A**. Habitus, phase contrast microphotograph. **B–G**. Bright field microphotographs. **B**. Head, dorsal view. **C**. Head, ventral view. **D**. Trunk, dorsal view. **E**. Trunk, ventral view. **F**. Posterior trunk region, furca base and furcal appendages, dorsal view. **G**. Posterior trunk region, furca base and furcal appendages, ventral view.

and Kisielewski (1984, 1997a). All of the main morphometric characteristics of *L*. aff. *squamata* are within the range given in the literature (Rudescu 1967; Roszczak 1969; Balsamo 1983; Schwank 1990; Kisielewski 1984, 1997a). The body length of studied adult specimens varied from 146.2 to 153.5 μm; the length of the pharynx from 45.7 to 47.3 μm; the intestine length from 75.5 to 82.1 μm; there were 19 total longitudinal rows of scales (5D+4DL+4L+4LV+2V), with 29–31 scales in the central row. The main clear differences between the studied specimens and *L. squamata* are the possession of one pair of scales with keels and rudimental but rigid spines on the dorsolateral area of the furcal appendages. Scales with keels or spines were listed neither in the original description nor in the main taxonomic reports on *L. squamata*; thus, I could not determine that the specimens undoubtedly belonged to this nominal taxon. Moreover, the present specimens possessed three pairs of dorsal sensory bristles, but this features cannot be a valid diagnostic character, since it was omitted in the original description and most previous works.

Discussion

The epiphytic species found in the Jubilee Greenhouse in Kraków increase the number of Gastrotricha known from Poland to 105 and those known from palm houses to 24 species (see Kolicka 2016). This is also the first recorded presence of gastrotrichs in aquatic plants in an artificial habitat. It is worth emphasising that these chaetonotids were found on vegetation cultivated from seeds or small seedling shoots in reservoirs that were cleaned on a yearly basis, including a partial replacement of the bottom sediment. The species composition changed between the first and second sample collections. The presence of different species could have been caused by two factors, namely the reservoir cleaning which took place between the collection of the samples and the change of plant species arrangement. Perhaps some of the gastrotrich species did not survive the seasonal cleaning and replacement of sediments, or some new taxa from the second sample collection were ones that were unintentionally imported into the Jubilee Greenhouse or were present on different plant species brought from another part of the reservoir. Observations of epiphytic gastrotrichs and species community changes may increase our knowledge of the colonisation abilities of Gastroticha because they suggest three ways of inhabitation of specific plants: 1) the gastrotrichs were brought in their resting egg stages on seeds or seedlings to the reservoir and survived the transfer to the nursery tank during the annual cleaning procedure; 2) gastrotrichs were introduced to the reservoir with the bottom sediments, they grew in abundance and subsequently they also moved to the submerged plants from the benthic communities; 3) the resting eggs of various gastrotrich species were brought yearly to the reservoirs in uncontrolled ways and some taxa found appropriate conditions to live in the bottom sediments while others were able to live only on aquatic vegetation.

Kisielewski (1990) suggested that submerged plants were the last freshwater habitat to be colonised by gastrotrichs after their expansion from marine waters. Possible routes of gastrotrich transfer from sediments to aquatic plants or from aquatic plants to sediments remain unconfirmed. Chaetonotids, as small benthic or epibenthic invertebrates without any free-swimming life stages, possess very limited abilities to actively move long distances. However, the active movement of Gastrotricha into vegetation rooted in a reservoir bottom does not seem to be impossible. On the other hand, transport by the water column itself seems to be a task requiring external factors in the form of water tides or larger, more actively swimming animals which could drag the gastrotrichs or their eggs from the bottom sediments to the submerged plants. The very long spines on the trunk of *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov. and *C.* (*H.*) *inaequabilis* sp. nov., as well as the very long lateral to ventral spines in *C.* (*C.*) *invitatus* sp. nov., may be a crucial adaptation to an epibenthic or epiphytic lifestyle. The presence of a long spine could increase the likelihood of elevation from sediments to vegetation and allow this species to stay on submerged plants by increasing the bearing surface (Kisielewski 1991).

The presence of as many as three species that are new to science and two newly recorded species in such a relatively well-studied country as Poland may suggest the foreign origin of the newly found taxa. Although we do not know the place of origin of the new species, the newly observed *Chaetonotus* (*Chaetonotus*) *paucisquamatus* Kisielewski, 1991 and *C.* (*Zonochaeta*) *cestacanthus* Balsamo, 1990 were previously known only from their *terra typica* in Brazil and Italy, respectively. Both regions have a relatively high annual temperature and more stable climatic conditions than those observed in natural Polish habitats (e.g., Hijmans *et al*. 2005). It is possible that these species were accidentally imported to the Jubilee Greenhouse along with the subsoil (potted plants, sand, peat or, less probably, in the manure) or with the water containing fish, and they found appropriate living conditions. It cannot be excluded that the resting eggs of these species are widely spread in a temperate climate zone via natural (air currents, surface runoffs, dispersion with larger animals) or anthropogenic (long-distance transport of goods and people) means, but do not find favourable conditions for development and/or the creation of a stable population (e.g., due to long, cold winters). On the other hand, the taxa recorded in the Kraków greenhouse were not found in any previously studied palm houses (see Kolicka *et al*. 2013; Kolicka 2014, 2016), which may suggest that the aquatic vegetation plays a crucial, habitat-forming role for these chaetonotids. This research could confirm that urban greenhouses may be considered as biodiversity hot-spots in a strongly industrialised world and that they provide conditions for communities composed of species from different habitats and world regions.

Acknowledgements

I thank Prof. Ziemowit Olszanowski and two anonymous reviewers for their kind advice and insightful and helpful comments regarding earlier versions of the manuscript. Special thanks to Małgorzata Kuźnik and Beata Starczewska for preparing the drawings of the new species and the figures used in this paper. I also sincerely thank Mrs. Dorota Wróbel and the staff of the Botanical Garden of Jagiellonian University in Kraków for their kindness and support during collection of the samples. My studies were supported by National Science Centre grant no. 2015/17/N/NZ8/01568 titled 'Gastrotricha in palm houses as a model for research on phenotypic variability and cryptic diversity.' I was also a beneficiary of the Adam Mickiewicz Foundation scholarship scheme.

References

Balsamo M. 1983. Gastrotrichi. *In*: Consiglio Nazionale delle Ricerche (ed.) *Guide per il Riconoscimento delle Specie animali delle Acque interne italiane* 20: 1–92.

Balsamo M. 1990. Gastrotrichs from Lakes Bolsena, Chiusi and Montepulciano (central Italy), with the description of four new species. *Bolletino di Zoologia* 57 (2): 165–178. https://doi.org/10.1080/11250009009355693

Balsamo M. & Fregni E. 1995. Gastrotrichs from interstitial fresh water, with a description of four new species. *Hydrobiologia* 302: 163–175. https://doi.org/10.1007/BF00027040

Balsamo M. & Todaro M.A. 2002. Gastrotricha. *In*: Rundle S.D., Robertson A.L & Schmid-Araya J.M. (eds) *Freshwater Meiofauna: Biology and Ecology*: 45–61. Backhuys Publishers, Leiden.

Balsamo M., Grilli P., Guidi L. & d'Hondt J.-L. 2014. Gastrotricha: biology, ecology and systematics: families Dasydytidae, Dichaeturidae, Neogosseidae, Proichthydiidae. *In*: Dumont H.J.F. (ed.) *Identification Guides to the Plankton and Benthos of Inland Waters* 24. Backhuys Publishers and Margraf Publishers, Weikersheim, Germany.

Britton N.L. 1896. Botanical Gardens. *Science* 4 (88): 284–293.

Bryce D. 1924. The Rotifera and Gastrotricha of Devils and Stump Lakes, North Dakota, USA. *Journal of the Quekett Microscopical Club* 15: 81–108.

Cordero E.H. 1918. Notes sur les Gastrotriches. *Physis* 4: 241–244.

Daday E.V. von. 1905. Untersuchungen über die Süsswasser-Mikrofauna Paraguays. *Zoology of Bulgaria* 44: 72–86.

Daday E.V. von. 1910. Die Süßwasserfauna Deutsch-Ostafrikas. *Zoologica* 23: 56–59.

Duggan I.C. & Duggan K.S. 2011. Are botanical gardens a risk for zooplankton invasions? *Biological Invasions* 13 (12): 2997–3003. https://doi.org/10.1007/s10530-011-9984-z

Dujardin F. 1841. *Histoire naturelle des Zoophytes, Infusoires*. Librairie Encyclopedique de Roret, Paris.

Fregni E., Balsamo M. & Tongiorgi P. 1998. Interstitial gastrotrichs from lotic Italian fresh waters. *Hydrobiologia* 368: 175–187. https://doi.org/10.1023/A:1003250217483

Greuter A. 1917. Beiträge zur Systematik der Gastrotrichen in der Schweiz. *Revue suisse de Zoologie* 25: 35–76. Available from https://biodiversitylibrary.org/page/10710334 [accessed 28 Jan. 2019].

Grosso L.E. & Drahg F. 1984. Gastrotricos dulceacuicolas de la provincia de Tucuman. II. *Chaetonotus majestuosus* n. sp. *Acta Zoologica, Lilloana* 37: 227–230.

Hijmans R.J., Cameron S.E., Parra J.L., Jones P.G. & Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1965–1978. https://doi.org/10.1002/joc.1276

Hochberg R. 2005. First record of *Polymerurus* (Gastrotricha, Chaetonotida) from Australia with the description of a new species from Queensland and of cuticular ultrastructure in *P. nodicaudus*. *Invertebrate Biology* 124: 119–130. https://doi.org/10.1111/j.1744-7410.2005.00014.x

Hummon W.D., Balsamo M. & Todaro M.A. 1992. Italian marine Gastrotricha: I. Six new and one redescribed species of Chaetonotida. *Bollettino di Zoologia* 59: 499–516.

Ignatieva M. 2010. Design and future of urban biodiversity. *In*: Müller N., Werner P. & Kelcey J.G. (eds) *Urban Biodiversity and Design*: 118–144. Blackwell Publishing, Chichester.

International Commission on Zoological Nomenclature. 1999. *International Code of Zoological Nomenclature*, Fourth Edition. The International Trust for Zoological Nomenclature, The Natural History Museum, London.

Kånneby T. 2011. New species and new records of freshwater Chaetonotida (Gastrotricha) from Sweden. *Zootaxa* 3115: 29–55.

Kånneby T., Todaro M.A. & Jondelius U. 2012. A phylogenetic approach to species delimitation in freshwater Gastrotricha from Sweden. *Hydrobiologia* 683: 185–202. https://doi.org/10.1007/s10750-011-0956-1

Kånneby T., Todaro M.A. & Jondelius U. 2013. Phylogeny of Chaetonotidae and other Paucitubulatina (Gastrotricha: Chaetonotida) and colonization of aquatic ecosystems. *Zoologica Scripta* 42: 88–105. https://doi.org/10.1111/j.1463-6409.2012.00558.x

Kieneke A. & Schmidt-Rhaesa A. 2015. Gastrotricha. *In*: Schmidt-Rhaesa A. (ed.) *Handbook of Zoology 3. Gastrotricha and Gnathifera*: 1–134. De Gruyter, Berlin, Boston.

Kieneke A., Riemann O. & Ahlrichs W.H. 2008. Novel implications for the basal internal relationschips of Gastrotricha revealed by an analysis of morphological characters. *Zoologica Scripta* 37: 429–460. https://doi.org/10.1111/j.1463-6409.2008.00334.x

Kisielewska G. & Kisielewski J. 1986a. Freshwater Gastrotricha of Poland. II. Gastrotricha from the seaside lakes in the Słowiński National Park. *Fragmenta Faunistica* 30: 183–182.

Kisielewska G. & Kisielewski J. 1986b. Freshwater Gastrotricha of Poland. III. Gastrotricha from the Białowieża Forest and the Białowieża Glade. *Fragmenta Faunistica* 30: 195–215.

Kisielewska G. & Kisielewski J. 1986c. Freshwater Gastrotricha of Poland. V. Gastrotricha of alder woods. *Fragmenta Faunistica* 30: 235–250.

Kisielewska G., Kolicka M. & Zawierucha K. 2015. Prey or parasite? The first observations of live Euglenida in the intestine of Gastrotricha. *European Journal of Protistology* 51: 138–141. https://doi.org/10.1016/j.ejop.2014.12.003

Kisielewski J. 1981. Gastrotricha from raised and transitional peat bogs in Poland. *Monografie Fauny Polski* 11: 1–143.

Kisielewski J. 1984. Three new freshwater gastrotrichs of the genus *Chaetonotus* Ehrenberg (Chaetonotida) from Poland. *Bulletin de l'Académie polonaise des Sciences, Série des Sciences biologiques* 32: 293–301.

Kisielewski J. 1986. Freshwater Gastrotricha of Poland. VII. Gastrotricha of extremely eutrophicated water bodies. *Fragmenta Faunistica* 30: 267–295.

Kisielewski J. 1990. Origin and phylogenetic significance of freshwater psammic Gastrotricha. *Stygologia* 5: 87–92.

Kisielewski J. 1991. Inland-water Gastrotricha from Brazil. *Annales Zoologici* 43: 1–168.

Kisielewski J. 1997a. Brzuchorzęski (Gastrotricha). *Fauna Słodkowodna Polski, Zeszyt* 31: 1–157.

Kisielewski J. 1997b. On the subgeneric division of the genus *Chaetonotus* Ehrenberg (Gastrotricha). *Annales Zoologici* 46: 145–151.

Kisielewski J. 1999. A preliminary study of the inland-water Gastrotricha of Israel. *Israel Journal of Zoology* 45: 135–157.

Kolicka M. 2014. Gastrotricha Mečnikow, 1865 from Copenhagen Palm House – contribution to the knowledge of *Lepidodermella intermedia* Kånneby, Todaro & Jondelius, 2012 (Chaetonotida, Gastrotricha). *Zoosystema* 34 (4): 713–722. https://doi.org/10.5252/z2014n4a1

Kolicka M. 2016. Gastrotrichs in bromeliads – newly recorded *Chaetonotus* (*Hystricochaetonotus*) *furcatus* Kisielewski, 1991 (Chaetonotida) from the Łódź Palm House. *Zoosystema* 38 (1): 141–155. https://doi.org/10.5252/z2016n1a5

Kolicka M., Kisielewski J., Nesteruk T. & Zawierucha K. 2013. Gastrotricha from the Poznań Palm House – one new subgenus and three new species of freshwater Chaetonotida (Gastrotricha). *Zootaxa* 3717 (2): 231–279. https://doi.org/10.11646/zootaxa.3717.2.7

Kolicka M., Dziuba M.K., Zawierucha K., Kuczyńska-Kippen N. & Kotwicki L. 2015. Palm house – biodiversity hotspot or risk of invasion? Aquatic invertebrates: The special case of Monogononta (Rotifera) under greenhouse conditions. *Biologia* 70 (1): 94–103. https://doi.org/10.1515/biolog-2015-0012

Kolicka M., Dabert M., Dabert J., Kånneby T. & Kisielewski J. 2016. *Bifidochaetus*, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. *Invertebrate Systematics* 30: 398–419. https://doi.org/10.1071/IS16001

Leasi F. & Todaro M.A. 2008. The muscular system of *Musellifer delamarei* (Renaud-Mornant, 1968) and other chaetonotidans with implications for the phylogeny and systematization of the Paucitubulatina (Gastrotricha). *Biological Journal of the Linnean Society* 94: 379–398. https://doi.org/10.1111/j.1095-8312.2008.00974.x

Lee J.M. & Chang C.Y. 2000. Freshwater chaetonotid gastrotrichs in Korea. *The Korean Journal of Systematic Zoology* 16: 87–104.

Martin L.V. 1981. Gastrotrichs found in Surrey. *Microscopy* 34: 286–300.

Martin L.V. 1990. Further observations on gastrotrichs in Surrey and a provisional British list. *Microscopy* 36: 415–425.

Mečnikow E. 1865. Über einige wenig bekannte niedere Thierformen. *Zeitschrift für Wissenschaftliche Zoologie* 15: 450–463. Available from https://biodiversitylibrary.org/page/45004241 [accessed 28 Jan. 2019].

Mola P. 1932. Gastrotricha delle acque dolci italiane. *International Review of Hydrobiology* 26 (5–6): 397–423. https://doi.org/10.1002/iroh.19320260504

Naidu K.V. & Rao G.C. 2004. *The Fauna of India and the Adjacent Countries – Gastrotricha*. Zoological Survey of India, Kolkata.

Nesteruk T. 2000. Epiphytic Gastrotricha – species composition and dominance. *Acta Hydrobiologica* 42: 53–57.

Nesteruk T. 2004. Benthic and epiphytic fauna of Gastrotricha in littoral of mesotrophic lake in Łęczna-Włodawa Lakeland, Poland. *Fragmenta Faunistica* 47 (1): 1–6.

Nesteruk T. 2010. Species structure, density and biomass of gastrotrich fauna of elodeids in two lakes of different trophy. *Teka Komisji Ochrony i Kształtowania Środowiska Przyrodniczego* [Teka Commission of Protection and Formation of Natural Environment] 7: 280–289.

Nesteruk T. 2011. Comparison of gastrotrich fauna on elodeids and in bottom sediments of different trophic status (the region Polesie Lubelskie, Estern Poland). *Oceonological and Hydrobiological Studies* 40: 13–21. https://doi.org/10.2478/s13545-011-0012-9

Rao G.C. & Clausen C. 1970. *Planodasys marginalis* gen. et. sp. nov. and Planodasyidae fam. nov. (Gastrotricha Macrodasyoidea). *Sarsia* 42: 73–82.

Remane A. 1927. Beiträge zur Systematik der Süsswassergastrotrichen. *Zoologische Jahrbücher, Abteilung für Systematik, Ökologie und Geographie der Tiere* 53: 269–320.

Remane A. 1935–1936. Gastrotricha und Kinorhyncha. *In*: Bronn H.G. (ed.) *Klassen und Ordnungen des Tierreichs, Vol. 4, Section 2, Book 1, Part 2, Issues 1–2*. Akademie Verlagsgesellschaft, Leipzig.

Roszczak R. 1936. Brzuchorzęski (Gastrotricha) zbiorników wodnych okolic Poznania. *Sprawy, Poznańskie Towarzystwo Przyjaciół Nauk* 9: 109–111.

Roszczak R. 1969. Brzuchorzęski (Gastrotricha) środkowej Wielkopolski. *Prace Komisji Biologicznej* 32: 1–92.

Rudescu L. 1967. Gastrotricha. *In*: *Fauna Republicii Socialiste Romania, Trochelminthes* 2 (3). Academia Republicii Socialiste Romania, Bucharest.

Saito I. 1937. Neue und bekannte Gastrotrichen der Umgebung von Hiroshima (Japan). *Journal of Science of the Hiroshima University* 5: 245–265.

Schwank P. 1990. Gastrotricha. *In*: Schwoerbel J. & Zwick P. (eds) *Süsswasserfauna von Mitteleuropa, 3. Gastrotricha und Nemertini*. Gustav Fischer Verlag, Stuttgart, Jena, New York.

Sudzuki M. 1971. Die das Kapillarwasser des Lückensystems bewohnenden Gastrotrichen Japans II. *Bulletin of the Biogeographical Society of Japan* 27 (5): 37–41.

Todaro M.A. 2018. *Gastrotricha*. Available from http://www.marinespecies.org/aphia.php?p=taxdetailsetid=2078 [accessed 2 Aug. 2018].

Tretjakova E.I. 1989. *K poznaniju Fauny presnovodnych Gastrotrich evropejskoj Casti SSSR*. Gosudarstvennyj Komitet SSSR po Narodnomu Obrazovaniju, Redkollegija Zhurnala. *Biologieeskie Nauki* (typescript, 21 pp, ID: udk 595.132).

Valkanov A. 1937. Über die Gastrotrichen Bulgariens. *Zoologische Jahrbücher, Abteilung für Systematik, Ökologie und Geographie der Tiere* 70: 171–176.

Zawierucha K., Szymkowiak P., Dabert M. & Harvey M.S. 2013. First record of the schizomid *Stenochrus portoricensis* (Schizomida: Hubbardiidae) in Poland with DNA barcode data. *Turkish Journal of Zoology* 37: 357–361. https://doi.org/10.3906/zoo-1210-9

Manuscript received: 5 August 2018
Manuscript accepted: 13 December 2018
Published on: 2 April 2019
Topic editor: Rudy Jocqué
Desk editor: Danny Eibye-Jacobsen

Printed versions of all papers are also deposited in the libraries of the institutes that are members of the *EJT* consortium: Muséum national d'Histoire naturelle, Paris, France; Meise Botanic Garden, Belgium; Royal Museum for Central Africa, Tervuren, Belgium; Royal Belgian Institute of Natural Sciences, Brussels, Belgium; Natural History Museum of Denmark, Copenhagen, Denmark; Naturalis Biodiversity Center, Leiden, the Netherlands; Museo Nacional de Ciencias Naturales-CSIC, Madrid, Spain; Real Jardín Botánico de Madrid CSIC, Spain; Zoological Research Museum Alexander Koenig, Bonn, Germany.

Appendices

Figs A1–A2: *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov.

Figs A3–A6: *Chaetonotus* (*Chaetonotus*) *horridus* sp. nov.

Fig. A7: *Chaetonotus* (*Chaetonotus*) *inaequabilis* sp. nov.

Tables A1–A2: *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov.

Tables A3–A4: *Chaetonotus* (*Chaetonotus*) *horridus* sp. nov.

Tables A5–A6: *Chaetonotus* (*Chaetonotus*) *inaequabilis* sp. nov.

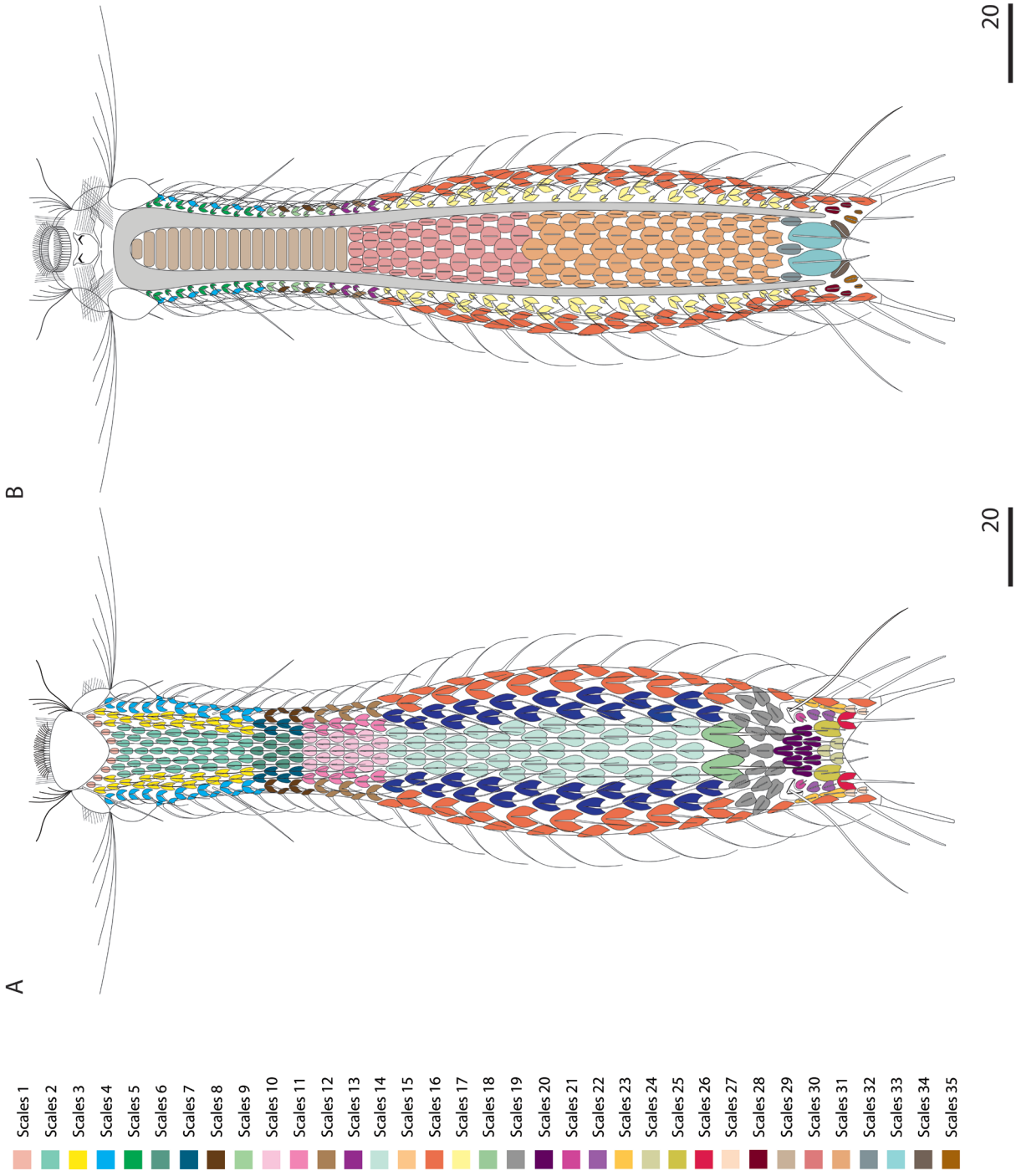


Fig. A1. *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov. Schematic drawings with various types of scales indicated. **A**. Dorsal body view. **B**.Ventral body view. Light grey areas indicate the areas of insertion of ciliary bands.

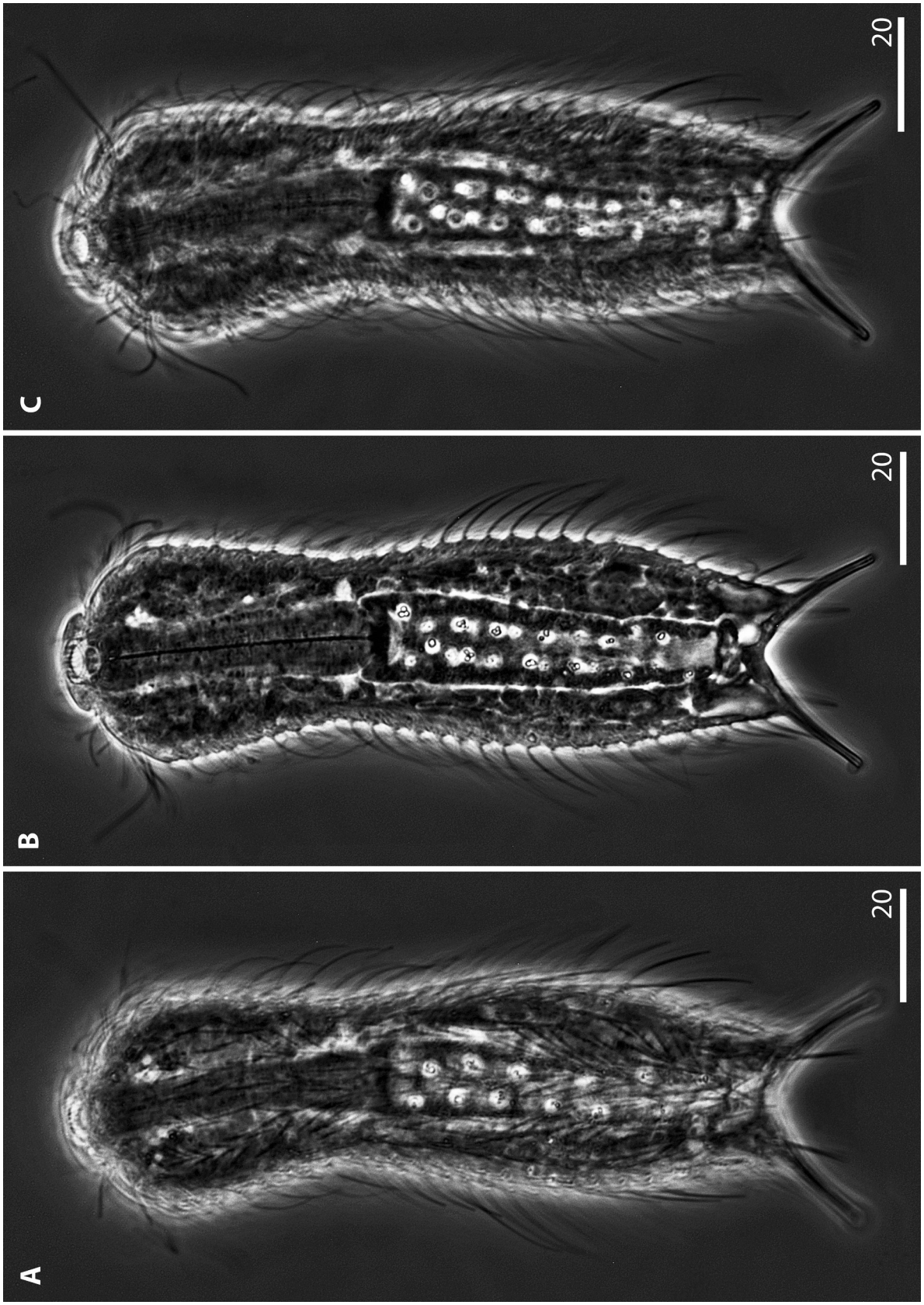


Fig. A2. *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov. Juvenile specimen. Phase contrast microphotographs. **A**. Dorsal body view. **B**. View of internal morphology. **C**. Ventral body view.

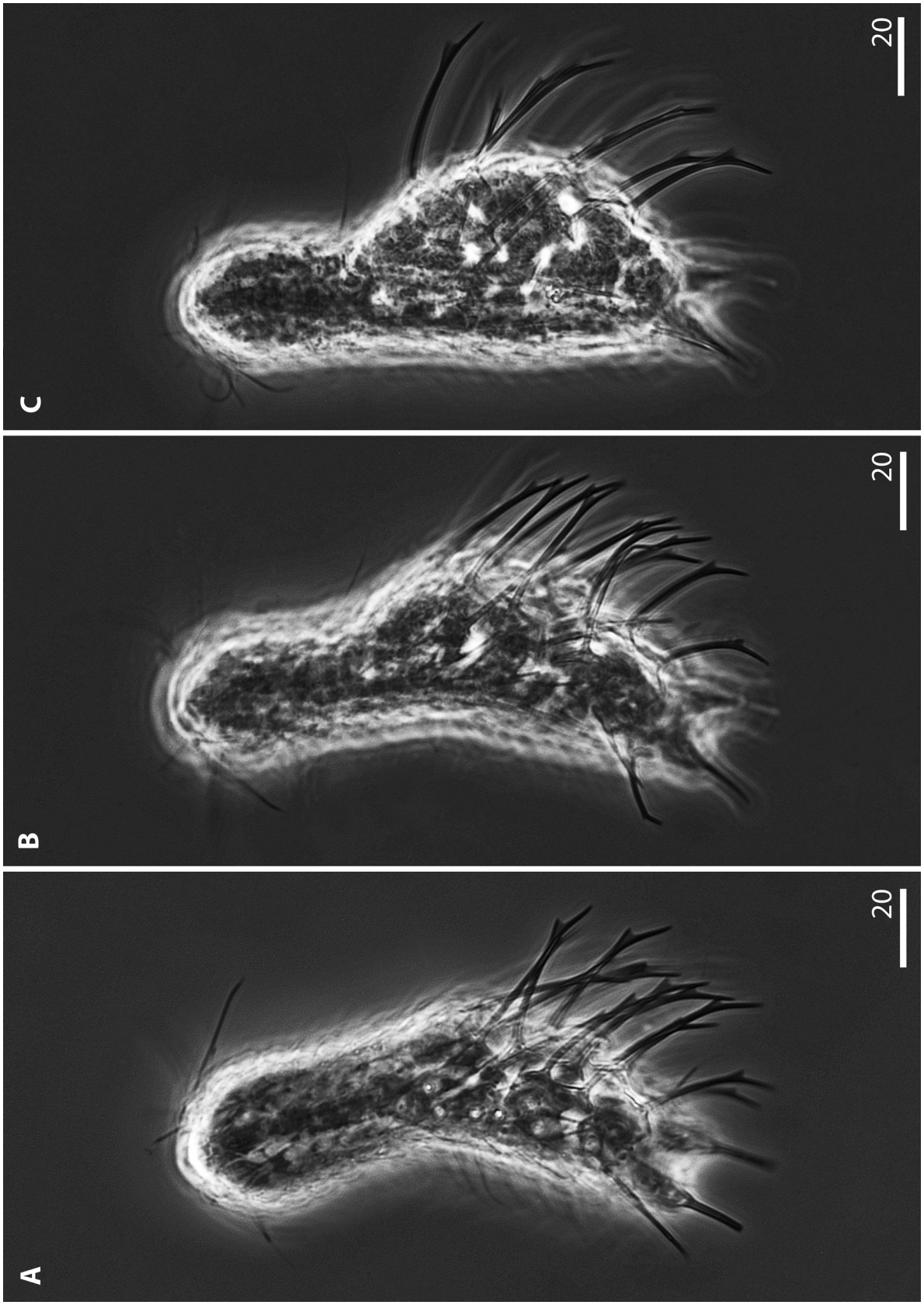


Fig. A3. *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov. Habitus. Phase contrast microphotographs. **A**. Juvenile specimen. **B**. Adult specimen. **C**. Specimen with mature egg.

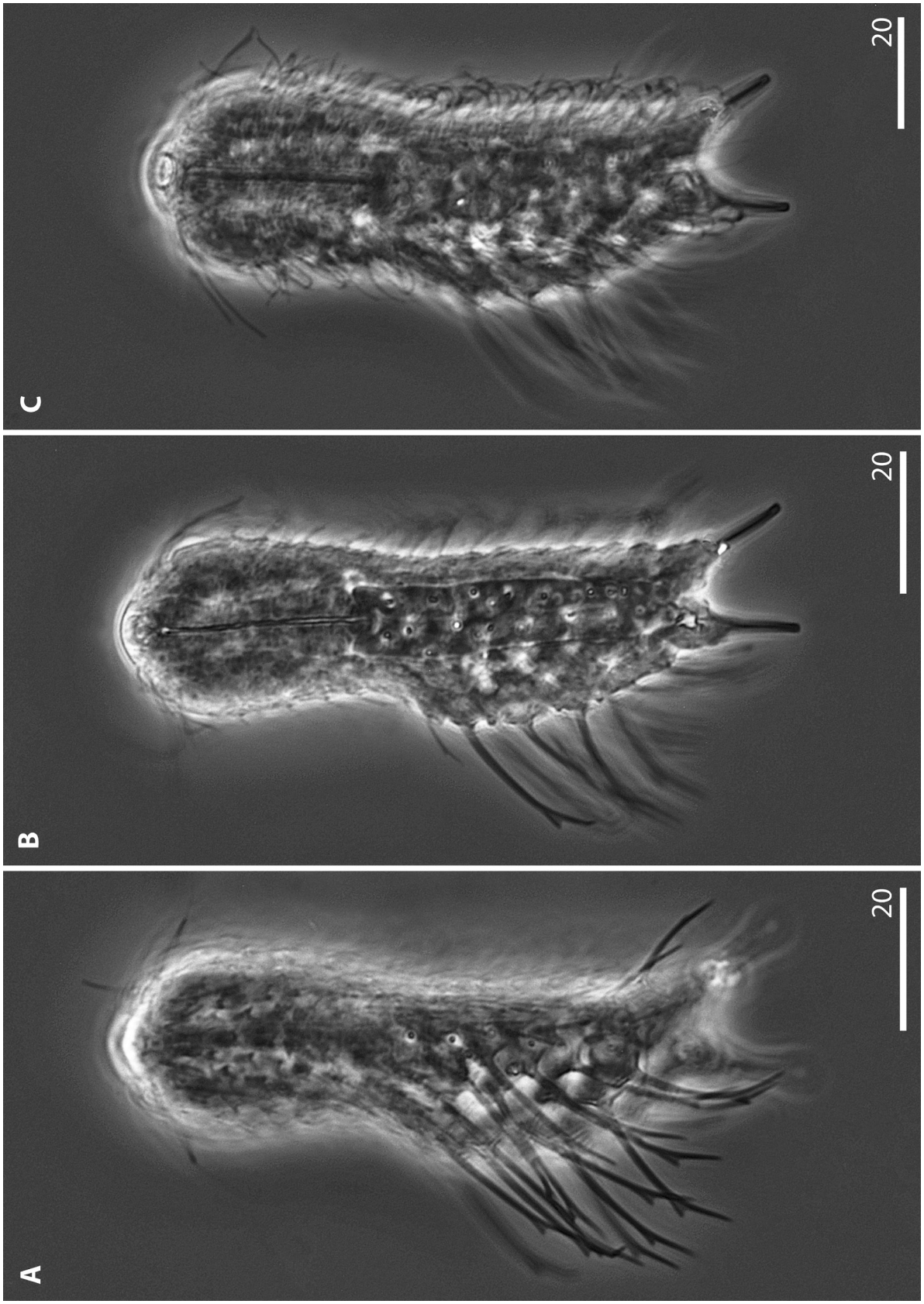


Fig. A4. *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov. Juvenile specimen. Phase contrast microphotographs. **A**. Dorsal body view. **B**. View of internal morphology. **C**. Ventral body view.

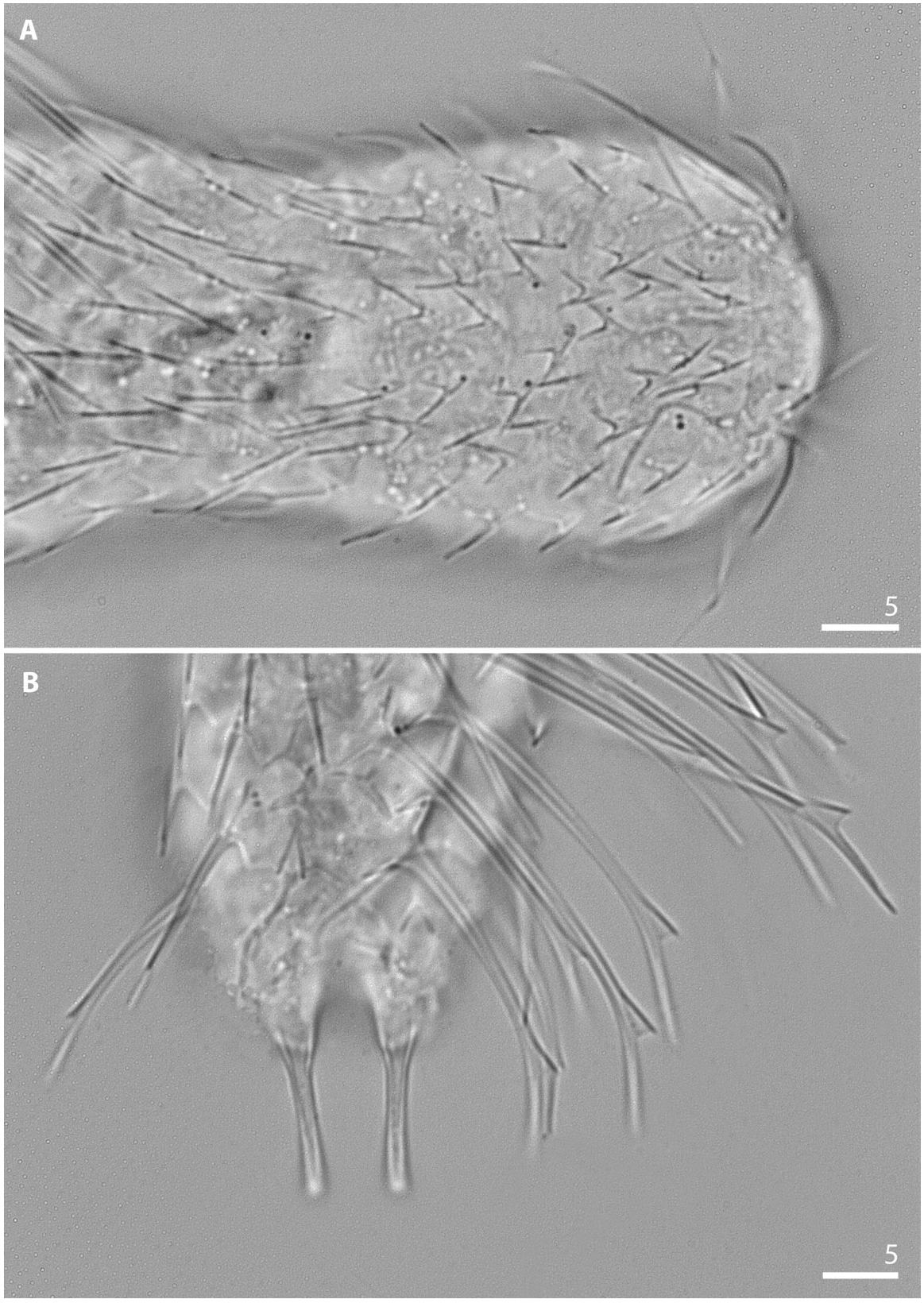


Fig. A5. *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov. Juvenile specimen. Bright field microphotographs. **A**. Head, neck and anterior trunk scales, dorsal view. **B**. Posterior trunk, furca base and furcal appendage scales and spines, dorsal view.

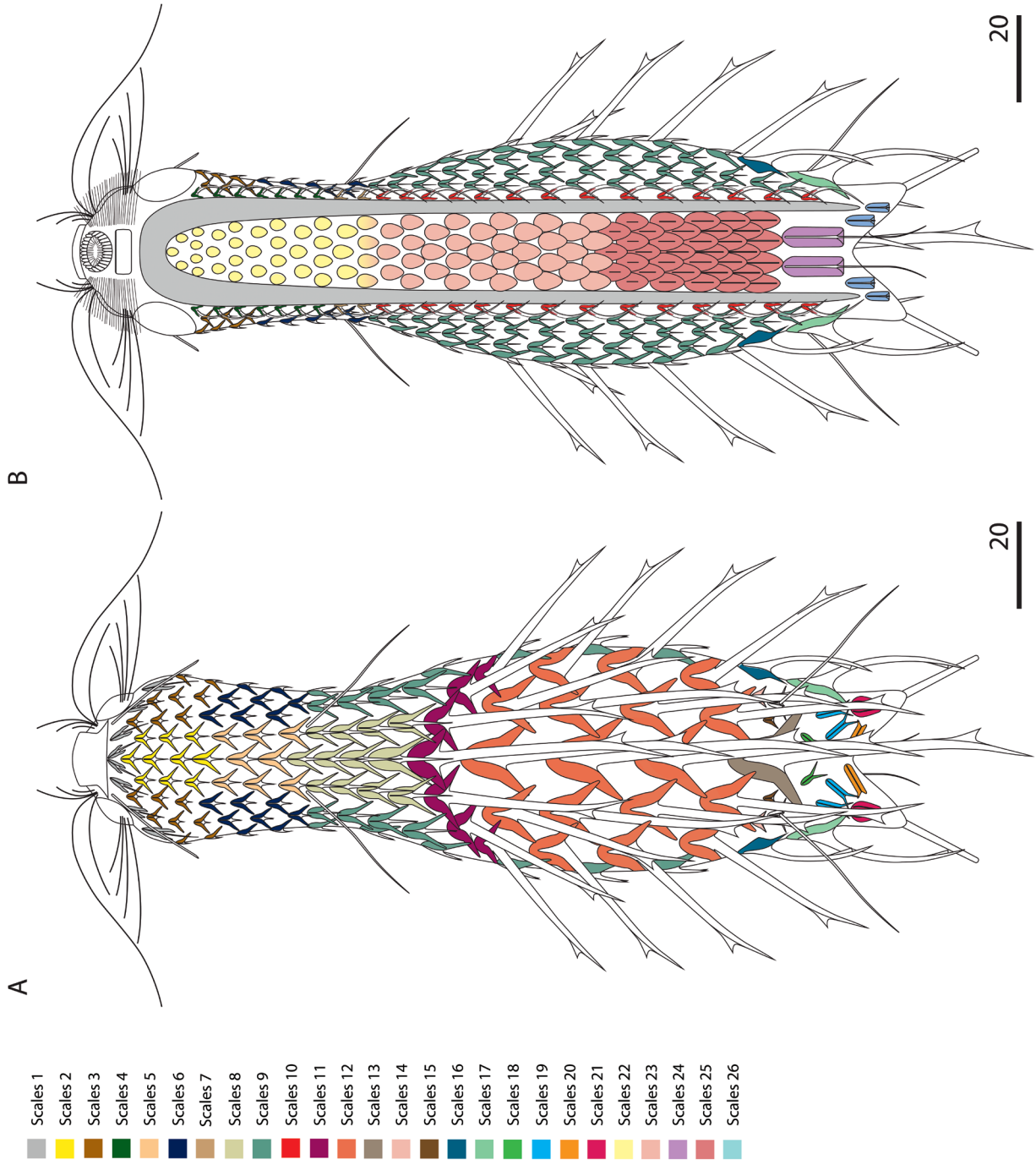


Fig. A6. *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov. Schematic drawings with various types of scales indicated. **A**. Dorsal body view. **B**. Ventral body view. Light grey areas indicate the areas of insertion of ciliary bands.

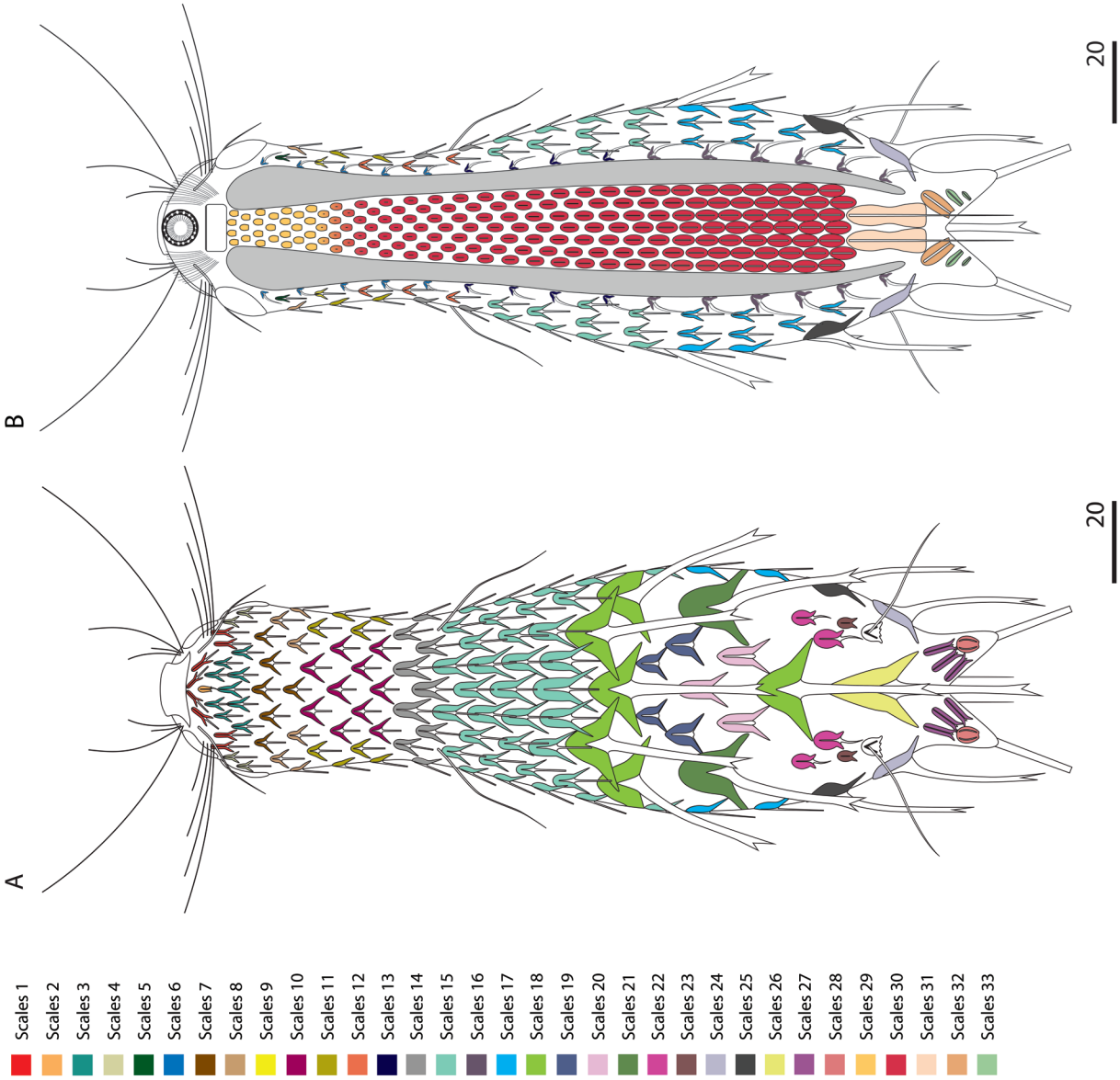


Fig. A7. *Chaetonotus* (*Hystricochaetonotus*) *inaequabilis* sp. nov. Schematic drawings with various types of scales indicated. **A**. Dorsal body view. **B**. Ventral body view. Light grey areas indicate the areas of insertion of ciliary bands.

Table A1 (continued on next 5 pages). Detailed morphometric characters of *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov. N = number of specimens analysed; Range = the smallest and the largest value found among all specimens measured; SD = standard deviation. All measurements in micrometers (μm); all indicators given as a percentage (%) and italicized. When the range of plural structures in many specimens is given, the first parentheses contain the range of smallest/shortest structures in analysed individuals; the second parentheses contain the range of the largest/longest structures in analysed individuals.

Characters	N	Holotype	Range of adult paratypes	SD	N	Range of juvenile and subadult paratypes	SD
Cephalion length	22	17.15	15.09–18.23	0.856	10	14.49–18.14	0.939
Cephalion width	22	19.11	16.34–20.64	1.205	10	15.41–19.38	1.082
Hypostomium length	22	6.94	4.04–9.11	0.955	10	5.24–8.26	0.910
Hypostomium width	22	9.15	8.75–12.55	1.062	10	8.61–12.58	1.151
Length of cephalic cilia (anterior tuft)	22	6.36–10.28	(4.67–7.27)–(9.47–15.28)	0.644; 1.233	10	(4.67–7.49)–(9.47–12.92)	0.756; 1.015
Length of cephalic cilia (posterior tuft)	22	17.33–33.58	(11.32–18.15)–(24.35–33.58)	1.675; 1.989	10	(11.32–15.28)–(24.35–30.06)	1.295; 1.782
Width of cephalic cilia (anterior tuft)	22	0.58–0.65	(0.49–0.65)–(0.54–0.72)	0.049; 0.049	10	(0.51–0.65)–(0.56–0.69)	0.054; 0.048
Width of cephalic cilia (posterior tuft)	22	0.67–0.72	(0.54–0.81)–(0.58–0.85)	0.061; 0.062	10	(0.57–0.72)–(0.61–0.76)	0.060; 0.059
Width of adhesive tube	23	1.95	1.89–1.97	0.029	10	1.85–1.95	0.037
Ratio of scale distribution	25	*92.593*	*92.000–100.00*	1.546	11	*92.000–109.523*	5.895
Length of head dorsal scales (scales 1 and 2)	22	2.73–4.94	(2.23–2.87)–(4.27–5.19)	0.176; 0.229	10	(2.06–2.37)–(3.74–4.29)	0.106; 0.191
Width of head dorsal scales (scales 1 and 2)	22	1.96–2.82	(1.60–2.06)–(1.83–2.96)	0.125; 0.252	10	(1.48–1.70)–(2.14–2.45)	0.077; 0.107
Length of head dorsolateral scales (scales 3)	22	2.66–4.81	(2.17–2.80)–(4.17–5.05)	0.173; 0.221	10	(2.01–2.31)–(3.26–4.18)	0.102; 0.294
Width of head dorsolateral scales (scales 3)	22	1.91–2.80	(1.56–2.01)–(2.58–4.78)	0.127; 0.543	10	(1.44–1.66)–(1.90–2.43)	0.075; 0.172
Length of head lateral scales (scales 4)	22	2.89–3.74	(2.36–3.04)–(3.37–3.93)	0.189; 0.155	10	(2.18–2.51)–(2.83–3.25)	0.111; 0.159
Width of head lateral scales (scales 4)	22	3.28–4.23	(2.68–3.44)–(3.81–4.45)	0.212; 0.174	10	(2.47–2.85)–(3.20–3.68)	0.127; 0.178
Length of head ventrolateral scales (scales 4)	22	2.81–3.68	(2.28–2.96)–(3.32–4.37)	0.187; 0.252	10	(2.12–2.44)–(2.78–3.20)	0.109; 0.159
Width of head ventrolateral scales (scales 4)	22	3.22–4.14	(2.61–3.39)–(3.74–4.91)	0.214; 0.281	10	(2.43–2.80)–(3.13–3.60)	0.128; 0.180
Length of head ventral scales (scales 5)	22	1.94–2.97	(1.58–2.05)–(2.69–3.53)	0.133; 0.202	10	(1.46–1.69)–(2.24–3.56)	0.078; 0.403
Width of head ventral scales (scales 5)	22	2.21–3.32	(1.80–2.34)–(2.95–3.48)	0.152; 0.129	10	(1.66–1.93)–(2.50–3.98)	0.089; 0.450
Length of neck dorsal scales (scales 6 and 10)	22	3.64–5.83	(3.15–3.82)–(5.04–6.13)	0.174; 0.275	10	(2.76–3.16)–(4.42–5.06)	0.138; 0.221
Width of neck dorsal scales (scales 6 and 10)	22	3.41–4.45	(2.95–3.58)–(3.85–4.66)	0.159; 0.203	10	(2.59–2.96)–(3.37–3.86)	0.129; 0.169
Length of neck dorsolateral scales (scales 7 and 11)	22	4.11–5.98	(3.55–4.32)–(5.17–6.29)	0.190; 0.284	10	(3.12–3.57)–(4.53–5.19)	0.154; 0.227

Table A1 (continued). Detailed morphometric characters of *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov.

Characters	N	Holotype	Range of adult paratypes	SD	N	Range of juvenile and subadult paratypes	SD
Width of neck dorsolateral scales (scales 7 and 11)	22	4.21–5.22	(3.64–4.43)–(4.47–5.49)	0.194; 0.288	10	(3.20–3.66)–(3.95–4.53)	0.157; 0.199
Length of neck lateral scales (scales 8 and 12)	22	4.23–6.29	(3.65–4.45)–(5.44–7.09)	0.197; 0.377	10	(3.21–3.67)–(4.76–5.46)	0.158; 0.239
Width of neck lateral scales (scales 8 and 12)	22	4.38–5.71	(3.78–4.61)–(4.94–6.44)	0.204; 0.348	10	(3.32–3.80)–(4.32–4.96)	0.166; 0.216
Length of neck ventrolateral scales (scales 8 and 12)	22	3.91–5.97	(3.37–4.11)–(5.16–6.73)	0.182; 0.366	10	(2.97–3.39)–(4.52–5.18)	0.146; 0.226
Width of neck ventrolateral scales (scales 8 and 12)	22	4.05–5.40	(3.49–4.26)–(4.67–6.10)	0.190; 0.333	10	(3.08–3.63)–(4.09–4.69)	0.181; 0.206
Length of neck ventral scales (scales 9 and 13)	22	2.55–3.49	(2.08–2.68)–(2.77–3.93)	0.153; 0.263	10	(1.94–2.21)–(2.64–3.03)	0.094; 0.133
Width of neck ventral scales (scales 9 and 13)	22	3.02–3.94	(2.46–3.16)–(3.13–4.44)	0.178; 0.297	10	(2.30–2.61)–(2.98–3.42)	0.108; 0.151
Length of trunk dorsal scales (scales 14)	22	5.97–8.08	(5.16–6.53)–(7.32–8.66)	0.324; 0.366	10	(4.53–5.18)–(6.13–7.01)	0.228; 0.307
Width of trunk dorsal scales (scales 14)	22	4.21–4.89	(3.64–4.60)–(4.43–5.18)	0.223; 0.214	10	(3.19–3.65)–(3.71–4.24)	0.161–0.186
Length of trunk dorsolateral scales (scales 15)	22	6.42–8.58	(5.56–7.02)–(7.77–9.20)	0.347; 0.390	10	(4.87–5.76)–(6.51–7.44)	0.294; 0.327
Width of trunk dorsolateral scales (scales 15)	22	4.61–5.19	(3.99–5.04)–(4.70–5.57)	0.247; 0.242	10	(3.50–4.14)–(3.94–4.50)	0.212; 0198
Length of trunk lateral scales (scales 16)	22	6.60–8.78	(5.72–7.23)–(7.95–9.38)	0.358; 0.404	10	(5.01–5.92)–(6.66–7.61)	0.305; 0.340
Width of trunk lateral scales (scales 16)	22	4.88–5.97	(4.23–5.35)–(5.41–6.38)	0.266; 0.276	10	(3.70–4.38)–(4.53–5.17)	0.226; 0.229
Length of trunk ventrolateral scales (scales 16)	22	6.43–8.02	(5.56–7.02)–(7.25–8.59)	0.345; 0.371	10	(4.88–5.77)–(6.10–6.95)	0.298; 0.307
Width of trunk ventrolateral scales (scales 16)	22	4.97–5.93	(4.30–5.43)–(5.36–7.39)	0.267; 0.502	10	(3.76–4.47)–(4.51–5.14)	0.234; 0.228
Length of trunk ventral scales (scales 17)	22	3.57–5.10	(2.84–3.87)–(4.08–5.46)	0.243; 0.381	10	(2.71–3.21)–(3.88–4.42)	0.166; 0.197
Width of trunk ventral scales (scales 17)	22	2.79–3.81	(2.22–3.03)–(3.05–4.08)	0.191; 0.302	10	(2.12–2.51)–(2.90–3.30)	0.125; 0.149
Length of scales 18	22	9.52	6.97–9.81	0.824	10	6.97–8.57	0.467
Width of scales 18	22	5.78	4.23–5.93	0.499	10	4.13–5.20	0.325
Length of scales 19	22	6.22–7.56	(5.44–6.33)–(6.27–7.70)	0.249; 0.369	10	(4.26–5.15)–(4.81–6.08)	0.321; 0.430
Width of scales 19	22	3.91–5.18	(3.32–4.21)–(3.95–5.26)	0.214; 0.415	10	(2.77–3.24)–(3.30–4.17)	0.166; 0.284
Length of scales 20	22	5.21–5.28	(4.51–6.77)–(4.97–6.90)	0.555; 0.524	10	(3.69–4.59)–(3.75–5.07)	0.315; 0.434
Width of scales 20	22	3.67–3.84	(3.15–4.49)–(3.47–4.55)	0.312; 0.269	10	(2.60–3.37)–(2.73–3.69)	0.258; 0.303
Length of scales 21	22	3.68	2.42–3.95	0.375	10	2.42–3.95	0.435
Width of scales 21	22	3.10	2.05–3.34	0.351	10	2.05–3.17	0.326
Length of scales 22	22	5.18–5.82	(4.29–6.04)–(4.68–6.42)	0.497; 0.518	10	(3.67–4.61)–(4.12–4.98)	0.331; 0.299
Width of scales 22	22	3.62–4.02	(3.26–3.98)–(3.49–4.74)	0.203; 0.293	10	(2.56–3.23)–(2.85–3.44)	0.235; 0.207

Table A1 (continued). Detailed morphometric characters of *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov.

Characters	N	Holotype	Range of adult paratypes	SD	N	Range of juvenile and subadult paratypes	SD
Length of scales 23	22	6.32–7.20	(5.64–7.89)–(6.07–8.08)	0.559; 0.614	10	(5.26–6.17)–(5.82–6.53)	0.318; 0.261
Width of scales 223	22	3.15–3.56	(2.61–4.06)–(2.86–4.31)	0.378; 0.427	10	(2.62–3.09)–(2.89–3.24)	0.161; 0.129
Length of scales 24	22	4.11–4.32	(3.42–4.74)–(3.76–5.20)	0.299; 0.375	10	(2.85–3.93)–(2.92–4.38)	0.379; 0.460
Width of scales 24	22	3.28–3.42	(2.72–3.79)–(2.89–4.25)	0.243; 0.306	10	(2.27–3.18)–(2.43–3.45)	0.323; 0.351
Length of scales 25	22	8.67	5.37–9.12	1.123	10	5.37–7.16	0.548
Width of scales 25	22	5.20	3.23–5.31	0.623	10	3.23–4.32	0.336
Length of scales 26	22	5.91	4.52–6.17	0.436	10	4.52–5.79	0.371
Width of scales 26	22	5.71	4.37–5.84	0.381	10	4.37–5.46	0.369
Length of scales 27	22	3.06–3.93	(2.60–3.61)–(3.35–6.14)	0.247; 0.663	10	(1.98–2.93)–(2.41–3.62)	0.377; 0.348
Width of scales 27	22	1.97–2.49	(1.84–2.53)–(2.19–4.08)	0.268; 0.472	10	(1.32–1.85)–(1.55–2.18)	0.211; 0.226
Length of pairs of scales lateral to the furcal appendages	22	4.36–4.53	(3.71–4.55)–(3.88–5.08)	0.231; 0.277	10	(3.39–3.92)–(3.64–4.27)	0.175; 0.220
Width of pairs of scales lateral to the furcal appendages	22	3.88–3.92	(3.31–4.05)–(3.39–4.40)	0.210; 0.246	10	(3.02–3.49)–(3.16–3.70)	0.157. 0.190
Length of scales 28	22	3.68–4.81	(2.18–3.68)–(3.51–5.26)	0.513; 0.533	10	(2.08–3.02)–(2.72–3.65)	0.302; 0.325
Width of scales 28	22	2.04–2.51	(1.37–2.17)–(1.92–2.79)	0.239; 0.249	10	(1.15–1.71)–(1.43–1.90)	0.185; 0.166
Length of head dorsal spines (scales 1 and 2)	22	1.82–6.08	(1.59–2.24)–(4.76–7.38)	0.154; 0.647	10	(1.37–1.64)–(4.58–5.47)	0.090; 0.301
Length of head dorsolateral spines (scales 3)	22	3.72–13.41	(3.25–4.54)–(10.41–16.24)	0.306; 1.442	10	(2.80–3.36)–(10.11–12.06)	0.188; 0.659
Length of head lateral spines (scales 4)	22	12.93–19.09	(10.87–15.78)–(15.37–23.09)	1.158; 1.970	10	(9.73–11.66)–(14.39–17.17)	0.650; 0.941
Length of head ventrolateral spines (scales 4)	22	14.97–20.22	(12.59–18.21)–(16.25–24.28)	1.332; 2.062	10	(11.27–13.50)–(15.24–18.18)	0.752; 0.996
Length of head ventral spines (scales 5)	22	15.76–24.11	(13.25–19.14)–(17.88–26.39)	1.393; 2.431	10	(11.86–14.21)–(18.17–21.63)	0.790; 1.177
Length of neck dorsal spines (scales 6 and 10)	22	6.37–8.81	(5.03–7.75)–(6.86–10.73)	0.676; 0.984	10	(4.82–5.73)–(6.72–7.92)	0.309; 0.411
Length of neck dorsolateral spines (scales 7 and 11)	22	11.33–15.62	(8.95–13.75)–(12.14–19.01)	1.200; 1.747	10	(8.57–10.18)–(11.91–14.04)	0.550; 0.728

Table A1 (continued). Detailed morphometric characters of *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov.

Characters	N	Holotype	Range of adult paratypes	SD	N	Range of juvenile and subadult paratypes	SD
Length of neck lateral spines (scales 8 and 12)	22	17.00–23.17	(13.46–20.62)–(18.01–28.18)	1.803; 2.504	10	(12.86–15.28)–(17.66–20.37)	0.825; 1.004
Length of neck ventrolateral spines (scales 8 and 12)	22	18.65–25.45	(14.77–22.30)–(19.81–30.54)	1.922; 2.674	10	(14.11–16.79)–(19.39–22.38)	0.903; 1.111
Length of neck ventral spines (scales 9 and 13)	22	20.07–27.41	(15.86–23.75)–(21.27–31.86)	2.041; 2.720	10	(15.19–18.05)–(20.88–24.11)	0.969; 1.191
Length of trunk dorsal spines (scales 14)	22	10.09–13.62	(7.84–12.27)–(10.57–16.48)	1.122; 1.494	10	(7.69–9.07)–(10.38–12.25)	0.471; 0.636
Length of trunk dorsolateral spines (scales 15)	22	15.07–19.91	(11.70–18.29)–(15.36–23.11)	1.668; 2.022	10	(11.49–13.56)–(15.17–17.91)	0.710; 0.934
Length of trunk lateral spines (scales 16)	22	20.76–24.62	(15.93–25.08)–(19.02–27.87)	2.294; 2.383	10	(15.84–18.69)–(18.76–22.14)	0.978; 1.174
Length of trunk ventrolateral spines (scales 16)	22	22.28–27.12	(17.09–26.34)–(20.90–30.24)	2.373; 2.460	10	(17.02–20.02)–(20.63–24.34)	1.037; 1.281
Length of trunk ventral spines (scales 17)	22	25.58–31.06	(19.63–30.17)–(23.06–34.26)	2.696; 2.887	10	(19.54–22.93)–(23.63–27.86)	1.173; 1.465
Length of spines of scales 18	22	18.52	16.56–20.04	0.976	10	13.43–20.04	1.617
Length of spines of scales 20	22	4.31–5.91	(3.05–5.81)–(5.51–7.35)	0.684; 0.538	10	(2.85–4.21)–(4.59–6.74)	0.439; 0.782
Length of spines of scales 21	22	1.76	1.28–1.82	0.168	10	0.94–1.82	0.233
Length of spines of scales 22	22	1.23–1.43	(1.15–1.38)–(1.25–1.76)	0.083; 0.139	10	(0.90–1.19)–(1.13–1.39)	0.91; 0.077
Length of spines of scales 24	22	0.86–1.03	(0.58–1.48)–(0.84–2.84)	0.279; 0.640	10	(0.56–2.09)–(0.74–0.55)	0.453; 0.528
Length of spines of scales 25	22	1.71	1.10–2.42	0.328	10	1.11–1.51	0.11
Length of spines of scales 26	22	17.47	14.48–18.39	1.046	10	13.04–16.21	1.221
Length of spines of scales 27	22	0.40–0.63	(0.25–0.98)–(0.47–1.43)	0.205; 0.312	10	(0.23–0.75)–(0.45–0.86)	0.186; 0.151
Length of trunk posteriormost pair of lateral spines	22	21.10	16.05–25.10	2.237	10	16.36–19.01	0.854
Length of parafurcal spines	22	12.37–13.14	(11.30–12.98)–(12.04–13.45)	0.421; 0.401	10	(9.61–12.11)–(10.34–12.25)	0.771; 0.552
Length of spines of scales 28	22	0.40–0.63	(0.25–0.98)–(0.47–1.43)	0.205; 0.312	10	(0.23–0.75)–(0.45–0.86)	0.186; 0.151
Head dorsal sensory bristles length	22	18.2	17.31–21.57	1.327	10	15.61–20.99	1.628

Table A1 (continued). Detailed morphometric characters of *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov.

Characters	N	Holotype	Range of adult paratypes	SD	N	Range of juvenile and subadult paratypes	SD
Neck dorsal sensory bristles length	22	19.84	18.42–24.17	1.507	10	17.70–22.40	1.443
Posterior dorsal sensory bristles length	22	22.61	20.33–27.96	1.939	10	19.83–23.01	0.973
Number of scales in central longitudinal row on ventral interciliary field	22	33	29–33	0.831	10	25–32	1.038
Total number of longitudinal alternating rows of scales on ventral interciliary field	22	2	5–7	0.770	10	5–7	0.850
Length of ventral interciliary field anterior scales (scales 28)	22	1.21–2.96	(1.03–1.72)–(2.55–5.19)	0.175; 0.674	10	(0.81–1.50)–2.29–2.82)	0.201; 0.174
Width of ventral interciliary field anterior scales (scales 28)	22	2.98–8.53	(1.89–3.48)–(7.13–11.40)	0.457; 1.034	10	(2.06–2.87)–(6.68–8.01)	0.256; 0.446
Length of ventral interciliary field posterior scales (scales 29–31)	22	4.25–8.28	(2.07–4.68)–(6.47–9.85)	0.759; 0.868	10	(2.48–3.56)–(6.42–7.74)	0.323; 0.420
Width of ventral interciliary field posterior scales (scales 30–32)	22	1.96–7.21	(1.09–2.39)–(5.67–11.26)	0.368; 1.216	10	(1.14–1.69)–(4.74–6.82)	0.195; 0.636
Length of terminal ventral interciliary field scales I pair (scales 33)	22	16.28	14.83–17.14	0.619	10	12.23–15.06	0.812
Width of terminal ventral interciliary field scales I pair (scales 33)	22	7.96	6.57–8.06	0.461	10	5.78–6.99	0.437
Length of terminal ventral interciliary field scales II pair (scales 34)	22	6.65	6.17–7.58	0.443	10	5.36–6.46	0.365
Width of terminal ventral interciliary field scales II pair (scales 34)	22	3.32	3.17–4.58	0.346	10	2.87–3.49	0.195
Length of terminal ventral interciliary field scales III pair (scales 35)	22	3.15	2.91–4.80	0.552	10	2.51–3.51	0.266
Width of terminal ventral interciliary field scales III pair (scales 35)	22	1.94	1.79–2.95	0.329	10	1.42–1.96	0.174
Length of terminal ventral interciliary field scales IV pair (scales 35)	22	2.23	2.06–3.56	0.402	10	1.72–2.25	0.174

Table A1 (continued). Detailed morphometric characters of *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov.

Characters	N	Holotype	Range of adult paratypes	SD	N	Range of juvenile and subadult paratypes	SD
Width of terminal ventral interciliary field scales IV pair (scales 35)	22	1.51	1.29–2.03	0.212	10	0.93–1.50	0.155
Length of terminal ventral interciliary field scales I pair spines (scales 33)	22	3.66	3.32–5.76	0.748	10	3.09–5.33	0.657
Length of terminal ventral interciliary field scales II pair spines (scales 34)	22	8.22	5.84–9.29	1.033	10	6.15–6.91	0.268
Length of terminal ventral interciliary field scales III pair spines (scales 35)	22	0.67	0.59–0.98	0.132	10	0.47–1.02	0.166
Length of terminal ventral interciliary field scales IV pair spines (scales 35)	22	0.41	0.27–0.64	0.101	10	0.23–0.39	0.053
Width of anterior pharynx thickening (a)	22	10.39	9.78–11.26	0.342	10	8.92–10.99	0.574
Width of pharynx narrowing (n)	22	8.6	7.29–8.98	0.470	10	6.46–8.72	0.557
Width of middle pharynx (m)	22	9.72	8.45–10.50	0.511	10	7.83–9.89	0.568
Width of posterior pharynx thickening (p)	22	12.48	11.67–13.83	0.523	10	11.54–13.62	0.606
Width of intestine junction	22	5.26	4.63–5.91	0.306	10	4.21–5.51	0.386
Intestine length	23	106.04	90.07–106.04	3.990	10	63.54–81.21	5.503
Pharynx formula a	22	18.279	*17.486–21.345*	0.835	10	*18.795–21.427*	0.807
Pharynx formula n	22	15.13	*12.643–15.705*	0.867	10	*13.584–17.001*	1.000
Pharynx formula m	22	17.101	*14.168–18.738*	1.123	10	*15.934–19.283*	1.055
Pharynx formula p	22	21.956	*20.787–26.009*	1.235	10	*21.339–25.568*	1.554
Pharynx–intestine ratio I	22	53.602	*52.173–61.974*	2.548	10	*66.162–80.563*	5.165
Length of mature egg	1	–	74.05	0	–	–	–
Width of mature egg	1	–	33.17	0	–	–	–
Length of X-organ	1	–	13.04	0	–	–	–
Width of X-organ	1	–	21.83	0	–	–	–

Table A2 (continued on next 5 pages). Condensed comparison of the most important differentiating characters among species most similar to *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov. All measurements in micrometres (μm). NA = data not available; – = not applicable.

Character	*C.* (*C.*) *invitatus* sp. nov.	*C.* (*C.*) *maximus*	*C.* (*C.*) *microchaetus*	*C.* (*C.*) *similis*	*C.* (*C.*) *heterospinosus*	*C.* (*C.*) *laroides*	*C.* (*C.*) *polyspinosus*	*C.* (*H.*) *trispinosus*	*C.* (*P.*) *mutinensis*
Body length	174.6–194.7	112.0–330.0	130.0–283.0	176.0–232.0	138.0–210.0	180.0–220.0	194.0–355.0	91.0–99.0	111.0–143.0
Cephalic pleurae	cephalion long and wide, with free dorsal edge; pleurae large, convex and clearly demarcated, hypopleurae slightly larger than epipleurae; epipleurae located on dorsal, dorsolateral, lateral and ventrolateral surfaces, hypopleurae located on dorsal, dorsolateral, lateral, ventrolateral and ventral, greatest portion present on the ventral side; hypostomium with two horn–like anterolateral protuberances and pair of strong anterior edge reinforcements	cephalion narrow; pleurae convex and clearly demarcated, epipleurae and hypopleurae similar in size, located mostly laterally but clearly visible on the dorsal body side; hypostomium with pair of anterior edge reinforcements	cephalion short and wide, pleurae convex and clearly demarcated, hypopleurae slightly larger than epipleurae; epipleurae located on dorsal, dorsolateral, lateral and ventrolateral surfaces, hypopleurae located on dorsal, dorsolateral, lateral, ventrolateral and ventral, greatest portion present on the ventral side; hypostomium with pair of anterior edge reinforcements	cephalion short and wide, pleurae large, convex, clearly demarcated and similar in size; epipleurae and hypopleurae located on mostly laterally; hypostomium with two horn-like anterolaterally protuberances	cephalion short and wide, pleurae convex and clearly demarcated, hypopleurae slightly larger than epipleurae; epipleurae and hypopleurae located mostly laterally; hypostomium not visible	cephalion short and wide, slightly smaller than epipleurae and hypopreuria; all pleurae convex and clearly demarcated, hypopleurae slightly larger than epipleurae; pleurae located mostly laterally but clearly visible on the dorsal body side; hypostomium with two horn–like anterolateral protuberances	cephalion long and wide, convex and clearly demarcated, hypopleurae slightly larger than epipleurae; epipleurae and hypopleurae located mostly laterally; hypostomium with pair of anterior edge reinforcements	cephalion short, wide and slightly extended near the dorsal edge; pleurae flat and clearly demarcated, hypopleurae three times larger than epipleurae; epipleurae located on dorsal, dorsolateral, lateral and ventrolateral surfaces, hypopleurae located on dorsal, dorsolateral, lateral and ventrolateral and ventral, greatest portion present on the ventrolateral side; hypostomium short, semirectangular in shape	cephalion long and wide; pleurae large, convex and clearly demarcated, hypopleurae larger than epipleurae; epipleurae and hypopleurae located on mostly laterally; hypostomium with pair of anterior edge reinforcements
Presence of additional hypostomium plates	present	absent	absent	absent	absent	present	absent	absent	absent
Place of emerging cephalic cilia	anterior tufts: laterally between cephalion edge and the dorsal edge of the epipleurae; posterior tufts: laterally between epipleurae and hypopleurae	anterior tufts: laterally between cephalion and epipleurae; posterior tufts: laterally between epipleurae and hypopleurae	anterior tufts: laterally between cephalion and epipleurae; posterior tufts: laterally between epipleurae and hypopleurae	anterior tufts: laterally between cephalion and epipleurae; posterior tufts: laterally between epipleurae and hypopleurae	anterior tufts: laterally between cephalion and epipleurae; posterior tufts: laterally between epipleurae and hypopleurae	anterior tufts: laterally between cephalion and epipleurae; posterior tufts: laterally between epipleurae and hypopleurae	anterior tufts: laterally between cephalion and epipleurae; posterior tufts: laterally between epipleurae and hypopleurae	anterior tufts: laterally between cephalion and epipleurae; posterior tufts: laterally between epipleurae and hypopleurae	anterior tufts: anterolateral between cephalion and epipleurae; posterior tufts: laterally between epipleurae and hypopleurae

Table A2 (continued). Comparison of species most similar to *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov.

Character	*C.* (*C.*) *invitatus* sp. nov.	*C.* (*C.*) *maximus*	*C.* (*C.*) *microchaetus*	*C.* (*C.*) *similis*	*C.* (*C.*) *heterospinosus*	*C.* (*C.*) *laroides*	*C.* (*C.*) *polyspinosus*	*C.* (*H.*) *trispinosus*	*C.* (*P.*) *mutinensis*
Presence of cuticular teeth in the mouth ring	two small, weak cuticular teeth	absent	absent	absent	absent	absent	absent	absent	absent
Pharynx length	51.0–59.6	50.0–66.0	47.0–63.0	35.0–75.0	46.0–66.0	68.0–70.0	55.0–90.0	26.7–29.0	41.0–58.0
Pharynx shape	pharynx narrow; anterior and slightly wider posterior dilatation	anterior and wider posterior dilatation	anterior and wider posterior dilatation	anterior and wider posterior dilatation	slightly anterior and posterior dilatation	cylindrical, gradually widening to the posterior end	anterior and wider posterior dilatation	cylindrical with weak posterior dilatation	cylindrical with posterior dilatation
Presence of cuticular reinforcements in the pharynx	present (two straight, connected rods)	absent	absent	absent	absent	absent	absent	absent	absent
Anterior intestine section differing in form of morphology	absent	absent	absent	absent	absent	absent	present	absent	absent
Furca length	31.1–34.5	15.0–30.0	16.0–17.0	19.0–39.0	21.0–30.0	27.0–30.0	30.0–55.0	13.6–14.3	16.0–26.0
Adhesive tube length	16.4–19.2	16.0–18.0	16.0–19.0	21.0–23.0	15.0–22.5	15.0–19.0	15.0–26.0	NA	NA
Type of furcal indentation	parabolic in shape	parabolic in shape	parabolic in shape	V-shaped	parabolic in shape	V-shaped	parabolic in shape	parabolic in shape	V-shaped
Number of scales in central longitudinal row	25–27	21–26	31–35	17–27	18–22	28–32	33–70	15–16	22–25
Total number of longitudinal alternating rows of scales	23–25	17–21	30–36	15–23	9–15	11–17	38–47	13	9–12
Type of scales	one-lobed, oval and egg-shaped with very weak posterior notches to deep posterior notches, all scales keeled and spined; strongly varied within body regions	one-lobed with keels and spines, all with posterior notches; shape differentiated within body regions	one-lobed small, with posterior notches, with keels and spines	one-lobed small, with shallow posterior notches, with keels and spines	one-lobed with shallow posterior notches, all scales with keels, some without spines, remaining with spines	one-lobed with wide and concave posterior edge, all scales keeled and spined; varied within body regions	one-lobed, small, triangle-shaped with posterior notches, with keels and spines	one-lobed, trilobate in shape, keeled and spined scales; varied within body regions	one-lobed, oval in shape without posterior notches, with keels and spines

Table A2 (continued). Comparison of species most similar to *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov.

Character	*C.* (*C.*) *invitatus* sp. nov.	*C.* (*C.*) *maximus*	*C.* (*C.*) *microchaetus*	*C.* (*C.*) *similis*	*C.* (*C.*) *heterospinosus*	*C.* (*C.*) *laroides*	*C.* (*C.*) *polyspinosus*	*C.* (*H.*) *trispinosus*	*C.* (*P.*) *mutinensis*
Scales size variation	neck scales smaller, shorter and wider than the head scales; trunk scales become larger and more elongated than neck scales; from dorsal, dorsolateral, lateral to the ventrolateral body surface, scales become shorter and wider; ventral scales much smaller than remaining scales	size gradually increases from the head towards the widest trunk region; on the furcal base and furcal appendage scales strongly varied in size	size similar on the head, neck and trunk; on the furcal base and furcal appendage scales differentiated in size	size gradually increase from the head towards the widest trunk region	strongly varied within body regions. Size gradually increases from the head towards the widest trunk region, on posterior trunk region and furcal base and furcal appendage scales smaller	size gradually increases from the head towards the widest trunk region; on the furcal base and furcal appendage scales strongly varied in size	similar size all over the body	size gradually increases from the head towards the trunk; three scales with long spines on the posterior trunk region larger	size gradually increases from the head towards the posterior trunk region; on the furcal base and furcal appendage scales smaller; ventral scales much smaller than remaining scales
Scale size	head: 1.6–5.2 × 1-6–4.9 neck: 2.1–7.1 × 2.5–6.4 trunk: 2.8–9.4 × 2.2–7.4	6.0–10 × NA	head: NA neck: 3.0 × NA trunk: 3.0–6.0 × NA	head: NA neck: 4.0–6.0 × NA trunk: 11.0–12.0 × NA	head and neck: 5.0–6.0 × NA trunk: 8.0–8.5 × NA	head and neck: 2.0–3.0 × NA trunk: 5.0–8.0 × NA	head and neck: 2.0–4.0 × 3.0–4.0 trunk: 3.0–7.0 × NA	head: 2.9 × 3.1 neck: NA trunk: 3.8 × 3.4	3.0–8.0 × NA
Alignment of scales	located close to another; on the head, neck and trunk do not overlap, scale edges overlap only on the furcal base and furcal appendages	located close to another; their posterolateral parts overlap adjacent scales	located close to another; their posterolateral parts overlap adjacent scales	located close to another; their posterolateral parts overlap adjacent scales	edges of scales overlapping	edges of scales overlapping	located close to another; their posterolateral parts overlap adjacent scales	located far from another; do not overlap	located close to another; their posterolateral parts overlap adjacent scales
Presence of a scale of a different type on the dorsal and dorsolateral surfaces on the furcal base	present	present	present	present	present	present	present	present	present
Scale coverage of the ventral interciliary field	entire ventral interciliary field covered with scales	entire ventral interciliary field covered with scales	entire ventral interciliary field covered with scales	entire ventral interciliary field covered with scales	entire ventral interciliary field covered with scales	entire ventral interciliary field covered with scales	entire ventral interciliary field covered with scales	ventral interciliary field naked except for one pair of ventral terminal scales	entire ventral interciliary field covered with keels

Table A2 (continued). Comparison of species most similar to *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov.

Character	*C.* (*C.*) *invitatus* sp. nov.	*C.* (*C.*) *maximus*	*C.* (*C.*) *microchaetus*	*C.* (*C.*) *similis*	*C.* (*C.*) *heterospinosus*	*C.* (*C.*) *laroides*	*C.* (*C.*) *polyspinosus*	*C.* (*H.*) *trispinosus*	*C.* (*P.*) *mutinensis*
Type of scales on the ventral interciliary field	on pharyngeal region semirectangular plates;on intestine region one-lobed, keeled scales	on anterior pharyngeal region semirectangular plates;on posterior pharyngeal region and intestine region one-lobed, keeled scales with rudimental spines	on pharyngeal region and anterior intestine region semirectangular plates;on intestine region central and posterior region one-lobed, keeled, semirectangular-shaped scales	small, rounded keeled and spined scales	on anterior pharyngeal region small semirectangular in shape, keeled scales;on posterior pharyngeal region and intestine region round in shape, keeled scales	small, rounded keeled scales with rudimental spines	small rounded or elongated keeled scales with rudimental spines from intestine region	–	from the head to posterior trunk region scales small rounded, keelless and spineless; on posterion trunk region scales semitriangular in shape, keeled and with rudimental spines
Number of terminal scales of the ventral interciliary field	4 pairs	1 pair	4 pairs	3 pairs	3 pairs	4 pairs	6 pairs	1 pair	3 pairs
Type of terminal scales on the ventral interciliary field	I pair: large, oval shaped, asymmetrical, without posterior notches, keeled and spined; II pair: oval shaped, without posterior notches, keeled and spined; III and IV pairs: egg-shaped keeled with rudimental spines	oval shaped, asymmetrical, without posterior notches, keeled, spined	I pair: large, oval shaped, asymmetrical, without posterior notches, keeled and spineless; II pair: rounded triangular in shape, with posterior notches, keeled and spined; III and IV pairs: rounded triangular in shape, keeled with rudimental spines	I pair: large, oval shaped, asymmetrical, without posterior notches, keeled and spineless; II pair: rounded triangular in shape, with shallow posterior notches, keeled and spined; III pair: rounded triangular in shape, keeled with rudimental spines	I pair: large, oval shaped, asymmetrical, without posterior notches, keeled and spineless; II pair: rounded triangular in shape, with shallow posterior notches, keeled and spined; III pair: rounded triangular in shape, keeled and spineless	I pair: large, oval shaped, asymmetrical, without posterior notches, keeled and spined; II–IV pairs: rounded triangular in shape, with shallow posterior notches, keeled and spined	I–III pairs: elongated, with very shallow posterior notches, keeled and spined; IV–VI pairs: small, rounded triangular in shape, keeled with rudimental spines	large, semirectangular in shape, keeled and spined	I pair: large, oval shaped, without posterior notches, keeled and spineless; II–III pairs: rounded triangular in shape, with posterior notches, keeled and spined

Table A2 (continued). Comparison of species most similar to *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov.

Character	*C.* (*C.*) *invitatus* sp. nov.	*C.* (*C.*) *maximus*	*C.* (*C.*) *microchaetus*	*C.* (*C.*) *similis*	*C.* (*C.*) *heterospinosus*	*C.* (*C.*) *laroides*	*C.* (*C.*) *polyspinosus*	*C.* (*H.*) *trispinosus*	*C.* (*P.*) *mutinensis*
Spine length variation	spine length gradually increases from dorsal, dorsolateral, lateral and ventrolateral towards the ventral surface and from the head towards the widest trunk region; dorsally on the posterior trunk region two or three longer and thicker spines than neighbouring scale spines	spine length gradually increases from head towards the widest trunk region	spine length slightly, gradually increases from head towards the posterior trunk region; on furcal base and furca appendages spines longer	spine length gradually increases from head towards the widest body region	spine length gradually increases from dorsal, dorsolateral and lateral towards the ventrolateral surface and from the head towards the widest trunk region; on the trunk spines much longer	spine length gradually increases from head towards the widest body region	spine length gradually increases slightly from head towards the widest body region	spine length gradually increases from dorsal, dorsolateral, lateral and ventrolateral towards the ventral surface and from the head towards the trunk; dorsally on the posterior trunk region three longer and thicker spines than neighbouring scale spines; remaining spines on the posterior trunk region, furcal base and furca appendages short	spine length gradually increases from head towards the trunk posterior region
Spine length	head: 3.3–26.4 neck: 5.0–31.9 trunk: 7.8–34.3 (long dorsal spines: 16.6–20.0)	head: 5.0–11.0 neck: 6.0–15.0 trunk: 8.0–17.0	head: NA neck: 2.0–4.0 trunk: 3.0–5.0	head: NA neck: 4.0–6.0 trunk: 18.0–30.0	head and neck: 2.0–9.0 trunk: 14.0–20.5	head and neck: 12.0–16.0 trunk: 20.0–22.0	head: NA neck: 2.0–5.0 trunk: 3.0–10.0	2.0–8.3 (long dorsal spines: 12.5–13.0)	head and neck: 4.0–7.0 trunk: 9.0–22.0
Presence of lateral denticle on the spine	absent	absent	absent	present	present	absent	absent	absent	absent
Presence of hair–like spines on the ventral body surface	present	absent	absent	absent	absent	absent	absent	present	present
Type of posteriormost pair of trunk lateral spines	do not vary from other posterior trunk spines, not longer or thicker	slightly longer and thicker than the other lateral spines	slightly longer than the other lateral spines	slightly longer and thicker than the other lateral spines	shorter than the other lateral spines	slightly longer and thicker than the other lateral spines	longer and thicker than the other spines	longer and thicker than the other spines	slightly shorter than the other posterior trunk lateral spines
Number of rigid, long spines on furcal appendages	1 pair	1–3 pairs	1 pair	2 pairs	2 pairs	2–3 pairs	4–10 pairs	Absent	2 pairs

Table A2 (continued). Comparison of species most similar to *Chaetonotus* (*Chaetonotus*) *invitatus* sp. nov.

Character	*C.* (*C.*) *invitatus* sp. nov.	*C.* (*C.*) *maximus*	*C.* (*C.*) *microchaetus*	*C.* (*C.*) *similis*	*C.* (*C.*) *heterospinosus*	*C.* (*C.*) *laroides*	*C.* (*C.*) *polyspinosus*	*C.* (*H.*) *trispinosus*	*C.* (*P.*) *mutinensis*
Type of parafurcal spines	two pairs of parafurcal spines on lateral surface of furca appendages; long, thick and tapering to their end	longer and thicker than the other spines	longer and thicker than the other spines	longer and thicker than the other spines	similar in length to the trunk spines but more straight	longer and thicker than the other spines	longer and thicker than the other spines	longer and thicker than the other spines	similar in length to posterior trunk lateral spines
Ventral locomotor cilia	two longitudinal bands merged on the head region	two longitudinal bands wider on the head region	two longitudinal bands	two longitudinal bands wider on the head region	two longitudinal bands wider on the head region	two longitudinal bands	two longitudinal bands	two longitudinal bands wider on the head region	two longitudinal bands wider on the head region
Number of dorsal sensory bristles	3 pairs	2 pairs	2 pairs	2 pairs	2 pairs	2 pairs	2 pairs	2 pairs	2 pairs

Table A3 (continued on next 5 pages). Detailed morphometric characters of *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov. N = number of specimens analysed; Range = the smallest and the largest structure found among all specimens measured; SD = standard deviation. All measurements in micrometers (μm); all indicators given as a percentage (%) and italicized. When the range of plural structures in many specimens is given, the first parentheses contain the range of smallest/shortest structures in analysed individuals; the second parentheses contain the range of the largest/longest structures in analysed individuals.

Characters	N	Holotype	Range of adult paratypes	SD
Cephalion length	20	8.01	7.23–8.87	0.554
Cephalion width	20	9.41	7.64–9.72	0.555
Cephalion maximum width	20	13.98	12.82–14.21	0.322
Hypostomium length	20	2.92	2.36–3.12	0.183
Hypostomium width	20	6.31	5.48–6.63	0.276
Length of cephalic cilia (anterior tuft)	20	3.75–29.59	(3.21–4.48)–(23.75–29.59)	0.349; 1.678
Length of cephalic cilia (posterior tuft)	20	5.59–20.25	(4.79–7.61)–(17.38–21.72)	0.683; 0.965
Width of cephalic cilia (anterior tuft)	20	0.67–0.72	(0.61–0.69)–(0.68–0.78)	0.021; 0.023
Width of cephalic cilia (posterior tuft)	20	0.76–0.83	(0.70–0.79)–(0.79–0.90)	0.020; 0.029
Width of adhesive tube	20	1.83	1.67–1.83	0.041
Ratio of scale distribution	81	140	12.104–16.626	1.169
Length of anteriormost head scales (scales 1)	81	4.39–5.23	(3.37–4.39)–(4.47–5.23)	0.239; 0.189
Width of anteriormost head scales (scales 1)	15	2.19–2.54	(1.68–3.04)–(2.17–3.22)	0.293; 0.272
Length of head dorsal scales (scales 2)	15	4.10–5.17	(3.16–4.10)–(4.43–5.17)	0.216; 0.189
Width of head dorsal scales (scales 2)	15	4.15–5.20	(3.20–5.26)–(4.46–6.20)	0.438; 0.403
Length of head dorsolateral scales (scales 3)	15	3.97–4.96	(3.06–3.97)–(4.25–4.96)	0.211; 0.191
Width of head dorsolateral scales (scales 3)	15	3.98–4.94	(3.07–4.84)–(4.23–5.53)	0.375; 0.304
Length of head lateral scales (scales 3)	15	3.85–4.84	(2.97–3.85)–(4.11–4.84)	0.209; 0.202
Width of head lateral scales (scales 3)	15	3.79–4.78	(2.92–4.37)–(4.10–5.20)	0.307; 0.265
Length of head ventrolateral scales (scales 3)	15	3.49–4.57	(2.69–3.49)–(3.73–4.57)	0.194; 0.212
Width of head ventrolateral scales (scales 3)	15	3.36–4.43	(2.59–4.05)–(3.81–4.72)	0.309; 0.226
Length of head ventral scales (scales 4)	15	2.82–4.13	(2.17–2.82)–(3.39–4.13)	0.152; 0.188
Width of head ventral scales (scales 4)	15	2.66–3.95	(2.05–3.38)–(3.39–3.98)	0.284; 0.166
Length of neck dorsal scales (scales 5)	15	5.30–6.37	(4.54–5.30)–(5.18–6.37)	0.197; 0.308
Width of neck dorsal scales (scales 5)	15	4.78–5.91	(4.09–4.78)–(5.06–5.91)	0.186; 0.236

Table A3 (continued). Detailed morphometric characters of *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov.

Characters	N	Holotype	Range of adult paratypes	SD
Length of neck dorsolateral scales (scales 6)	15	5.13–6.01	(4.32–5.13)–(4.88–6.01)	0.218; 0.292
Width of neck dorsolateral scales (scales 6)	15	4.56–5.53	(3.90–4.56)–(4.74–5.53)	0.168; 0.221
Length of neck lateral scales (scales 6)	15	5.07–5.78	(4.10–5.07)–(4.62–5.78)	0.240; 0.291
Width of neck lateral scales (scales 6)	15	4.43–5.27	(3.79–4.43)–(4.38–5.27)	0.172; 0.245
Length of neck ventrolateral scales (scales 6)	15	4.66–5.44	(3.93–4.66)–(4.48–5.44)	0.197; 0.253
Width of neck ventrolateral scales (scales 6)	15	4.02–4.92	(3.44–4.02)–(4.17–4.92)	0.147; 0.212
Length of neck ventral scales (scales 7)	15	3.24–4.57	(2.77–3.46)–(3.92–4.57)	0.167; 0.185
Width of neck ventral scales (scales 7)	15	2.75–4.09	(2.35–3.22)–(3.51–4.09)	0.200; 0.154
Length of trunk dorsal scales (scales 8)	15	7.02–9.71	(5.28–7.02)–(5.64–9.71)	0.424; 0.953
Width of trunk dorsal scales (scales 8)	15	5.52–8.43	(4.10–5.52)–(4.68–8.43)	0.343; 0.878
Length of trunk dorsolateral scales (scales 9)	15	6.88–8.55	(5.00–6.88)–(5.52–8.55)	0.450; 0.711
Width of trunk dorsolateral scales (scales 9)	15	5.34–7.37	(3.87–5.34)–(4.34–7.37)	0.352; 0.709
Length of trunk lateral scales (scales 9)	15	6.41–7.81	(4.87–6.41)–(5.34–7.81)	0.376; 0.582
Width of trunk lateral scales (scales 9)	15	4.93–6.68	(3.69–4.93)–(4.17–6.68)	0.300; 0.587
Length of trunk ventrolateral scales (scales 9)	15	6.29–7.47	(4.64–6.29)–(5.11–7.47)	0.391; 0.558
Width of trunk ventrolateral scales (scales 9)	15	4.79–6.34	(3.47–4.79)–(4.06–6.34)	0.310; 0.535
Length of trunk ventral scales (scales 10)	15	3.49–5.48	(3.03–4.08)–(4.60–5.48)	0.251; 0.267
Width of trunk ventral scales (scales 10)	15	2.62–4.61	(2.12–3.33)–(3.64–4.61)	0.270; 0.259
Length of scales 11	15	8.21–9.02	(5.34–8.21)–(5.56–9.06)	0.885; 0.899
Width of scales 11	15	12.43–13.37	(7.05–12.43)–(7.42–13.37)	1.379; 1.301
Length of scales 12	15	10.74–13.43	(7.44–10.74)–(7.64–13.43)	0.942; 1.354
Width of scales 12	15	10.51–13.22	(7.20–10.51)–(7.42–13.22)	0.874; 1.359
Length of scales 13	15	13.46	7.56–13.46	1.627
Width of scales 13	15	14.24	7.70–14.24	1.869
Length of scales 14	15	7.94–8.46	(4.30–7.94)–(4.54–8.46)	0.893; 0.950
Width of scales 14	15	6.29–6.98	(4.73–6.77)–(4.90–7.16)	0.632; 0.672
Length of scales 15	15	8.38	5.13–8.38	0.839
Width of scales 15	15	10.02	5.05–10.02	1.181

Table A3 (continued). Detailed morphometric characters of *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov.

Characters	N	Holotype	Range of adult paratypes	SD
Length of scales 16	15	10.5	6.35–10.50	1.038
Width of scales 16	15	8.21	4.65–8.21	0.942
Length of scales 17	15	11.72	7.41–11.72	1.206
Width of scales 17	15	8.86	5.28–8.86	1.048
Length of scales 18	15	6.98	2.21–6.98	1.291
Width of scales 18	15	4.46	1.58–4.46	0.869
Length of scales 19	15	8.16–9.24	(5.96–8.16)–(6.63–9.24)	0.588; 0.652
Width of scales 19	15	3.03–3.93	(2.04–3.22)–(2.67–3.93)	0.332; 0.310
Length of scales 20	15	7	5.72–7.00	0.441
Width of scales 20	15	2.28	1.76–2.44	0.203
Length of scales 21	15	6.05	3.42–6.05	0.671
Width of scales 21	15	5.88	3.07–5.88	0.637
Length of head anteriormost spines (scales 1)	15	3.26–4.39	(2.24–3.45)–(3.30–5.24)	0.360; 0.550
Length of head dorsal spines (scales 2)	15	1.86–5.04	(0.44–1.86)–(2.38–5.04)	0.394; 0.783
Head dorsal spines length between lateral denticle and spine top (scales 2)	15	0.28–1.63	(0.07–0.28)–(0.54–1.63)	0.061; 0.282
Length of head dorsolateral spines (scales 3)	15	2.03–5.21	(0.48–2.03)–(2.55–5.21)	0.445; 0.800
Head dorsolateral spines length between lateral denticle and spine top (scales 3)	15	0.34–1.70	(0.09–0.34)–(0.57–1.70)	0.067; 0.294
Length of head lateral spines (scales 3)	15	2.48–5.53	(0.59–2.48)–(2.80–5.53)	0.529; 0.819
Head lateral spines length between lateral denticle and spine top (scales 3)	15	0.47–1.77	(0.12–0.47)–(0.64–1.77)	0.096; 0.290
Length of head ventrolateral spines (scales 3)	15	3.05–6.11	(0.73–3.05)–(3.09–6.11)	0.643; 0.869
Head ventrolateral spines length between lateral denticle and spine top (scales 3)	15	0.53–1.84	(0.14–0.53)–(0.70–1.84)	0.103; 0.307
Length of head ventral spines (scales 4)	15	12.31–13.73	(7.16–12.31)–(8.44–13.78)	1.467; 1.479
Lamella width on the ventral head spines (scales 4)	15	0.80–1.44	(0.42–0.94)–(0.65–1.44)	0.143; 0.216
Length of neck dorsal spines (scales 5)	15	5.12–7.39	(2.45–5.72)–(4.66–8.26)	0.916; 1.051
Neck dorsal spines length between lateral denticle and spine top (scales 5)	15	1.48–1.91	(0.66–1.65)–(1.20–2.13)	0.268; 0.270
Length of neck dorsolateral spines (scales 6)	15	5.32–7.97	(2.70–5.94)–(5.03–8.91)	0.921; 1.136
Neck dorsolateral spines length between lateral denticle and spine top (scales 6)	15	1.56–2.05	(0.72–1.74)–(1.29–2.29)	0.281; 0.292
Length of neck lateral spines (scales 6)	15	5.98–8.47	(3.03–6.6.8)–(5.35–9.47)	1.019; 1.204

Table A3 (continued). Detailed morphometric characters of *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov.

Characters	N	Holotype	Range of adult paratypes	SD
Neck lateral spines length between lateral denticle and spine top (scales 6)	15	1.72–2.16	(0.81–1.92)–(1.36–2.42)	0.308; 0.309
Length of neck ventrolateral spines (scales 6)	15	6.65–8.98	(3.37–7.43)–(5.68–10.04)	1.097; 1.251
Neck ventrolateral spines length between lateral denticle and spine top (scales 6)	15	1.81–2.22	(0.87–2.02)–(1.40–2.48)	0.321; 0.313
Length of neck ventral spines (scales 7)	15	14.26–16.49	(8.76–14.26)–(10.12–16.49)	1.464; 1.584
Lamella width on the ventral neck spines (scales 7)	15	1.49–1.72	(0.71–1.49)–(1.01–1.72)	0.210; 0.206
Length of trunk dorsal spines (scales 8)	15	7.88–9.96	(4.97–8.82)–(7.06–11.14)	1.121; 1.341
Trunk dorsal spines length between lateral denticle and spine top (scales 8)	15	2.09–3.10	(1.32–2.34)–(2.20–3.47)	0.296; 0.418
Length of trunk dorsolateral spines (scales 9)	15	8.41–10.45	(5.30–9.39)–(7.41–11.69)	1.190; 1.326
Trunk dorsolateral spines length between lateral denticle and spine top (scales 9)	15	2.23–3.25	(1.41–2.49)–(2.30–3.64)	0.314; 0.414
Length of trunk lateral spines (scales 9)	15	8.68–11.12	(5.47–9.68)–(7.89–12.44)	1.233; 1.409
Trunk lateral spines length between lateral denticle and spine top (scales 9)	15	2.30–3.32	(1.45–2.57)–(2.36–3.71)	0.325; 0.425
Length of trunk ventrolateral spines (scales 9)	15	9.11–16.15	(5.74–10.16)–(8.71–16.98)	1.293; 2.436
Trunk ventrolateral spines length between lateral denticle and spine top (scales 9)	15	2.38–3.40	(1.50–2.65)–(2.46–3.84)	0.335; 0.437
Length of trunk ventral spines (scales 10)	15	17.19–19.54	(10.56–17.19)–(12.04–19.54)	1.617; 1.799
Lamella width on the ventral trunk spines (scales 10)	15	1.86–2.14	(1.06–1.86)–(1.24–2.14)	0.224; 0.255
Length of spines of scales 11	15	28.17–29.35	(19.88–29.70)–(22.36–34.14)	2.856; 3.789
Trunk scales 11 spines length between lateral denticle and spine top	15	6.49–7.52	(4.41–7.47)–(5.35–9.08)	0.920; 0.998
Length of spines of scales 12	15	27.07–31.75	(20.14–28.92)–(23.37–35.52)	2.568; 3.945
Trunk scales 12 spines length between lateral denticle and spine top	15	7.08–10.14	(3.75–7.08)–(6.14–10.59)	0.896; 1.211
Length of spines of scales 13	15	24.89	18.94–27.15	1.925
Trunk scales 13 spines length between lateral denticle and spine top	15	6.91	5.19–8.23	0.757
Length of spines of scales 16	15	16.65	11.59–16.65	1.284
Trunk scales 16 spines length between lateral denticle and spine top	15	4.68	2.32–4.68	0.613
Length of trunk posteriormost pair of lateral spines (scales 17)	15	20.09	13.87–20.09	1.564
Trunk posteriormost pair of lateral spines length between lateral denticle and spine base (scales 17)	15	6.27	4.15–6.32	0.698
Length ratio of posteriormost trunk lateral spines	15	15.256	12.104–16.626	1.169
Localization of lateral denticle on dorsal head spines (scales 2)	15	*15.054–32.341*	*(13.699–17.949)–(22.689–32.642)*	1.158; 2.477
Localization of lateral denticle on dorsolateral head spines (scales 3)	15	*16.749–32.630*	*(14.773–23.711)–(22.353–32.653)*	2.357; 2.641

Table A3 (continued). Detailed morphometric characters of *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov.

Characters	N	Holotype	Range of adult paratypes	SD
Localization of lateral denticle on lateral head spines (scales 3)	15	*18.952–32.007*	*(15.657–22.689)–(21.053–32.143)*	1.422; 2.830
Localization of lateral denticle on ventrolateral head spines (scales 3)	15	*17.377–30.115*	*(15.745–20.161)–(17.632–30.233)*	1.124; 3.213
Localization of lateral denticle on dorsal neck spines (scales 5)	15	*25.846–28.906*	*(23.558–28.861)–(25.789–29.290)*	1.013; 0.927
Localization of lateral denticle on dorsolateral neck spines (scales 6)	15	*25.721–29.323*	*(24.828–29.268)–(25.691–29.448)*	0.963; 1.227
Localization of lateral denticle on lateral neck spines (scales 6)	15	*25.502–28.763*	*(23.209–25.581)–(27.147–28.861)*	0.594; 0.421
Localization of lateral denticle on ventrolateral head spines (scales 6)	15	*24.722–27.218*	*(19.818–24.799)–(25.486–27.310)*	1.268; 0.521
Localization of lateral denticle on dorsal trunk spines (scales 8)	15	*26.523–31.124*	*(25.796–26.606)–(31.005–31.452)*	0.202; 0.098
Localization of lateral denticle on dorsolateral trunk spines (scales 9)	15	*26.516–31.100*	*(25.221–26.604)–(30.979–31.808)*	0.338; 0.190
Localization of lateral denticle on lateral trunk spines (scales 9)	15	*26.498–29.856*	*(25.00–26.571)–(29.755–32.937)*	0.389; 0.801
Localization of lateral denticle on ventrolateral trunk spines (scales 9)	15	*21.053–26.125*	*(21.053–26.198)–(21.053–32.814)*	2.213; 3.092
Localization of lateral denticle on spines of scales 11	15	*23.039–25.622*	*(17.969–25.152)–(20.395–27.370)*	1.815; 1.844
Localization of lateral denticle on spines of scales 12	15	*26.154–31.937*	*(18.620–26.449)–(23.057–31.937)*	2.056–2.706
Localization of lateral denticle on spines of scales 13	15	*27.762*	*24.213–33.204*	2.580
Localization of lateral denticle on spines of scales 16	15	*28.108*	*18.026–30.015*	3.285
Localization of lateral denticle on posteriormost trunk lateral spines (scales 17)	15	*31.21*	*24.942–34.785*	2.530
Head dorsal sensory bristles length	15	24.53	15.86–24.53	2.053
Neck dorsal sensory bristles length	15	21.56	18.49–24.36	1.532
Posterior dorsal sensory bristles length	15	28.48	19.22–28.48	2.160
Number of scales in central longitudinal row on ventral interciliary field	81	18	15–23	1.992
Total number of longitudinal alternating rows of scales on ventral interciliary field	81	7	7	0
Length of ventral interciliary field scales (scales 22–24)	15	2.43–4.68	(1.48–2.73)–(3.15–5.37)	0.379; 0.622
Width of ventral interciliary field scales (scales 22–24)	15	1.87–4.12	(1.05–2.46)–(2.87–5.14)	0.361; 0.530
Length of terminal ventral interciliary field scales I pair (scales 25)	15	8.71	7.10–8.71	0.419
Width of terminal ventral interciliary field scales I pair (scales 25)	15	3.18	2.44–3.31	0.257
Length of terminal ventral interciliary field scales II pair (scales 26)	15	4.17	2.69–4.69	0.569
Width of terminal ventral interciliary field scales II pair (scales 26)	15	2.7	1.63–2.70	0.288
Length of terminal ventral interciliary field scales III pair (scales 26)	15	3.11	1.82–3.18	0.401
Width of terminal ventral interciliary field scales III pair (scales 26)	15	1.66	1.13–1.67	0.192

Table A3 (continued). Detailed morphometric characters of *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov.

Characters	N	Holotype	Range of adult paratypes	SD
Length of terminal ventral interciliary field scales I pair spines (scales 25)	15	14.45	9.78–14.51	1.496
Length of terminal ventral interciliary field scales II pair spines (scales 26)	15	0.61	0.37–1.48	0.281
Length of terminal ventral interciliary field scales III pair spines (scales 26)	15	0.31	0.23–1.47	0.078
Width of anterior pharynx thickening (a)	20	10.14	8.49–10.97	0.614
Width of pharynx narrowing (n)	20	6.42	6.00–7.48	0.356
Width of middle pharynx (m)	20	6.96	6.54–7.77	0.334
Width of posterior pharynx thickening (p)	20	10.32	10.22–11.96	0.539
Width of pharyngeal–intestinal junction	20	3.78	3.17–3.97	0.242
Intestine length	70	74.91	54.58–74.91	3.622
Pharynx formula a	20	*30.542*	*27.378–34.367*	1.768
Pharynx formula n	20	*19.337*	*18.991–23.317*	1.147
Pharynx formula m	20	*20.964*	*20.936–24.221*	1.123
Pharynx formula p	20	*31.084*	*31.084–39.298*	2.288
Pharynx–intestine ratio I	70	*44.32*	*43.759–54.732*	2.515
Length of matured egg	4	–	41.89–46.16	1.907
Width of matured egg	4	–	21.12–29.34	3.815
Length of X-organ	4	–	7.96–10.56	1.209
Width of X-organ	4	–	14.71–17.68	1.521

Table A4 (continued on next 3 pages). Comparison of the most important differentiating characters among species most similar to *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov. All measurements in micrometres (µm). NA = data not available; – = not applicable.

Character	*C.* (*H.*) *horridus* sp. nov.	*C.* (*H.*) *acanthophorus*	*C.* (*H.*) *balsamoae*	*C.* (*H.*) *euhystrix*	*C.* (*H.*) *novenarius*
Body length	107.8–134.0	80.0–110.0	147.0–172.0	150.0–160.0	106.0–193.0
Cephalic pleurae	cephalion short and narrow; straight cut and extended on the dorsal body side; epipleurae slightly arched and located on the dorsal, dorsolateral, lateral side and slightly visible on the ventrolateral head side; hypopleurae slightly larger than the epipleurae and located entirely on the ventrolateral and ventral head sides, not visible from the dorsal body view; between the cephalion and epipleurae and between the epipleurae and hypopleurae deep notches; hypostomium short, rectangular-shaped with a slightly reinforced anterior edge	cephalion short and narrow; epipleurae and hypopleurae similar in size; epipleurae and hypopleurae located mostly laterally; hypostomium not marked	cephalion short and narrow; epipleurae and hypopleurae similar in size; epipleurae and hypopleurae located mostly laterally; hypostomium not marked	cephalion narrow; epipleurae and hypopleurae similar in size; epipleurae and hypopleurae located mostly laterally; hypostomium not marked	cephalion wide and narrow; epipleurae smaller than hypopleurae; epipleurae and hypopleurae located mostly laterally; hypostomium not marked
Place of arising cephalic cilia	anterior tufts: dorso-terminally, in area between the cephalion and epipleurae; posterior tufts: ventrally, above the anterior edge of the hypopleurae	anterior tufts: dorsoterminal between cephalion and epipleurae; posterior tufts: laterally, between epipleurae and hypopleurae	anterior tufts: dorsoterminal between cephalion and epipleurae; posterior tufts: laterally, between epipleurae and hypopleurae	anterior tufts: dorsoterminal between cephalion and epipleurae; posterior tufts: laterally, between epipleurae and hypopleurae	anterior tufts: dorsoterminal between cephalion and epipleurae; posterior tufts: laterally, between epipleurae and hypopleurae
Pharynx length	27.0–33.6	30.0	40.0–46.0	50.0–55.0	27.5–51.0
Pharynx shape	wide; round anterior and wider posterior dilatations	cylindrical expanding towards posterior end	narrow; small, similar in size anterior and posterior dilatations	anterior and wider posterior dilatation	cylindrical expanding towards posterior end
Furca length	21.7–25.9	16.0	25.0–32.0	25.0–35.0	17.0–32.5
Adhesive tube length	9.7–13.0	8.0	19.0–21.0	15.0–16.0	*ca.* 11.0–21.5
Type of furca indentation	V-shaped	V-shaped	helmet-shaped	V-shaped	helmet-shaped
Number of scales in central longitudinal row	13–15	11	16	13	19
Total number of longitudinal alternating rows of scales	15–21	NA	NA	NA	14–15
Type of scales	almost all three-lobed with strong, high, triangular keels, with from strong towards rudimentary spines; shape strongly differentiated within body regions	small, reduced to the triangle, high keels	all three-lobed with triangular keels and spines	all three-lobed with strong, high, triangular keels and strong spines; shape strongly differentiated within body regions	scales one-lobed and three-lobed; with strong, high, triangular keels, with strong or rudimentary spines; shape strongly differentiated within body regions

Table A4 (continued). Comparison of species most similar to *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov.

Character	*C.* (*H.*) *horridus* sp. nov.	*C.* (*H.*) *acanthophorus*	*C.* (*H.*) *balsamoae*	*C.* (*H.*) *euhystrix*	*C.* (*H.*) *novenarius*
Scale size variation	strongly differentiated within body regions; dorsal and dorsolatral scales much larger	NA	scales similar in size	size increases gradually from the head towards the posterior trunk region	strongly differentiated within body regions
Scale size	Head: 2.2–5.3 × 2.0–6.2 Neck: 2.8–6.4 × 2.4–5.9 Trunk: 3.0–13.7 × 2.1–13.4	NA	4.0 × NA	7.0 × NA–15.0 × NA	3.6–6.0 × NA
Alignment of scales	scales on the head and anterior neck region located far from another; on posterior neck and anterior trunk region located gradually closer but still at a distance to the other scales and not juxtaposed; scales on dorsolateral, lateral, ventrolateral and ventral trunk region located at a distance from the other scales, dorsal trunk scales and furcal appendages scales slightly overlap	scales located far from another	scales located far from another	scales located far from another but posteriolateral lobes meet and are juxtaposed	scales on the head located far from another; on neck and anterior trunk region located gradually closer and juxtaposed; trunk scales slightly overlap, furcal base scales juxtaposed and not overlap
Number of scales with long spines	21–29	20	7–9	–	9
Location of scales with long spines	on the trunk and furcal base; dorsally and dorsolaterally from *ca*. U37 to U73 and dorsally to U81, laterally two pairs at U69–U75 and U76–U82	dorsally and dorsolaterally on the trunk, posteriormost trunk lateral spines on the furcal base	dorsally on anterior trunk region	dorsally and dorsolaterally on the trunk	dorsally on anterior trunk region
Presence of a different type of scales on the dorsal and dorsolateral surfaces of the furcal base and furcal appendages	present	absent	absent	present	present
Scale coverage of the ventral interciliary field	entire ventral interciliary field covered by scales	NA	naked pharyngeal region; keels on intestine region s	entire ventral interciliary field covered by scales	entire ventral interciliary field covered by scales
Type of scales on ventral interciliary field	one-lobed; anterior scales egg-shaped, rounded and are rounded towards the posterior body region become gradually larger and more elongated, posterior scales elongated, oval-shaped with straight, thin keels; from the anterior to posterior body region, the distances between the scales decrease, and beyond half the trunk's length the scales start to overlap	NA	only keels visible	one-lobed, rounded, keelless, without overlapping edges	one-lobed, rounded, keelless and spineless

Table A4 (continued). Comparison of species most similar to *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov.

Character	*C.* (*H.*) *horridus* sp. nov.	*C.* (*H.*) *acanthophorus*	*C.* (*H.*) *balsamoae*	*C.* (*H.*) *euhystrix*	*C.* (*H.*) *novenarius*
Number of scales in central longitudinal row on the ventral interciliary field	15–23	NA	NA	NA	40
Total number of longitudinal alternating rows of scales on the ventral interciliary field	7	NA	6–8	NA	4
Number of terminal scales on the ventral interciliary field	3 pairs	NA	2–5 scales	1 pair	1 pair
Type of terminal scales on the ventral interciliary field	I pair: elongated, rectangular-shaped with rounded anterior edges, keeled and with with a long, thin, straight spine; II and III pairs: elongated, rectangular-shaped with rounded anterior edges, keeled and rudimentary spined	NA	scales with keels and simple, long spines	oval, keeled and spineless	elongated, oval-shaped keeled and spined
Spine length variation	anteriormost spines slightly longer than subsequent head spines; spine length and thickness increase gradually from head towards the widest body region; head, neck and anterior trunk spines length gradually increase from dorsal, dorsolateral, lateral and ventrolateral towards to the ventral surface; ventral hair–like spines very long; dorsal and dorsolateral trunk spines very long, thick, rigid; distinctly longer and stronger than remaining spines	head and neck spines short, trunk dorsal and dorsolateral, and posteriormost trunk lateral spines very long	spine length gradually increase slightly from head towards the posterior body region; ventral hair–like spines longer than remaning spines; anterior trunk dorsal spines very long, thick, rigid; distinctly longer and stronger than remaining spines	spine length and thickness increase gradually from head top towards the posterior trunk region; all dorsolateral, lateral, ventrolateral and ventral spines shorter than the dorsal spines, their length gradually decreases towards the ventral surface	anteriormost spines longer than subsequent head spines; spine length and thickness increase gradually from head posterior region towards the anterior trunk region; anterior dorsal trunk spines very long, thick, rigid; distinctly longer and stronger than remaining spines; central and posterior trunk spines short or rudimentary; from lateral and ventrolateral towards ventral surface spines much longer than dorsolateral spines
Spine length	head: 0.4–13.8 neck: 2.5–16.5 trunk: 5.0–19.5 long spines: 11.6–34.1	head: 4.0 long spines: 16.0–24.0	head and neck: 2.5–19.0 posterior trunk: 12.0–32.0 long spines: 30.0–76.0	head and neck: 5.0–7.0 trunk: 50.0–70.0	head: 9.0 neck: NA trunk: 9.0–19.0 long spines: 60.0–85.0
Presence of hair-like spines on the ventral body surface	present	NA	present	absent	absent
Presence of lamellae on ventral spines	present	NA	absent	absent	absent

Table A4 (continued). Comparison of species most similar to *Chaetonotus* (*Hystricochaetonotus*) *horridus* sp. nov.

Character	*C.* (*H.*) *horridus* sp. nov.	*C.* (*H.*) *acanthophorus*	*C.* (*H.*) *balsamoae*	*C.* (*H.*) *euhystrix*	*C.* (*H.*) *novenarius*
Presence and distinctness of lateral denticle on the spines	all spines, except for anteriormost head dorsal spines, furcal appendages spines and ventral surface spines, have lateral denticle; lateral denticle on spine becomes more pronounced from head towards the widest body region; the most prominent, very distinct and deeply notched lateral denticle present on dorsal and dorsolateral trunk spines	dorsal, dorsolateral and posteriormost trunk lateral spines have lateral denticle; lateral denticle on remaining spines not observed	double lateral denticle arranged in single file present only on long, anterior trunk dorsal spines; lateral denticle on remaining spines absent	all spines have lateral denticle; lateral denticle on the spines becomes more pronounced from head towards the posterior trunk region	single or double lateral denticle arranged in single file present only on long, anterior trunk dorsal spines; lateral denticle on remaining spines absent
Localization of lateral denticle on the spines	head: *13.699–32.653* neck: *19.818–29.448* trunk: *21.053–32.937* long spines: *17.969–34.785*	NA	long spines: smaller denticle: *12.000–14.000* larger denticle: *23.000–31.000*	NA	NA
Type of posteriormost pair of trunk lateral spines	longer, stronger and more curved than other lateral spines but shorter thank the trunk dorsal and dorsolateral long spines	longer than other lateral spines	the same as other lateral trunk spines	shorter than other posterior trunk region spines	longer than other lateral spines
Length ratio of posteriormost trunk lateral spines	*24.942–34.785*	NA	NA	NA	NA
Type of last pair lateral spines	–	–	–	shorter than posterior trunk region spines	–
Length ratio of posteriormost parafurcal lateral spines	–	–	–	NA	–
Ventral locomotor cilia	two longitudinal bands merged on the head region	NA	two longitudinal bands almost merged on the head region	two longitudinal bands	NA
Number of dorsal sensory bristles	3 pairs	2 pairs	1 pair	2 pairs	2 pairs

Table A5 (continued on next 4 pages). Detailed morphometric characters of *Chaetonotus* (*Hystricochaetonotus*) *inaequabilis* sp. nov. N = number of specimens analysed; Range = the smallest and the largest structure found among all specimens measured; SD = standard deviation. All measurements in micrometers (μm); all indicators given as a percentage (%) and italicized. When the range of plural structures in many specimens is given, the first parentheses contain the range of smallest/shortest structures in analysed individuals; the second parentheses contain the range of the largest/longest structures in analysed individuals.

Characters	N	Holotype	Range of adult paratypes	SD
Cephalion length	5	6.11	6.11–6.94	0.307
Cephalion width	5	7.56	7.03–8.29	0.449
Cephalion maximum width	5	11.68	11.68–12.93	0.492
Hypostomium length	5	2.55	2.55–2.92	0.152
Hypostomium width	5	6.10	5.55–7.19	0.601
Length of cephalic cilia (anterior tuft)	5	4.58–26.81	(4.36–4.99)–(22.66–26.81)	0.239; 1.719
Length of cephalic cilia (posterior tuft)	5	7.41–20.05	(5.22–8.56)–(19.44–20.95)	1.213; 0.562
Width of cephalic cilia (anterior tuft)	5	0.67–0.78	(0.62–0.73)–(0.71–0.81)	0.040; 0.039
Width of cephalic cilia (posterior tuft)	5	0.80–0.91	(0.073–0.83)–(0.80–0.91)	0.036; 0.045
Width of adhesive tube	5	1.43	1.43–1.48	0.019
Ratio of scale distribution	5	*135.714*	*135.714–140.000*	*1.620*
Length of anteriormost head scales (scales 1)	5	4.15–5.22	(3.77–4.54)–(4.13–5.91)	0.305; 0.651
Width of anteriormost head scales (scales 1)	5	2.07–2.65	(1.77–2.26)–(2.12–3.02)	0.187; 0.330
Length of head dorsal cental scales (scales 2)	5	2.76	2.42–3.19	0.305
Width of head dorsal central scales (scales 2)	5	1.49	1.23–1.72	0.189
Length of head dorsal scales (scales 3 and 7)	5	3.81–4.72	(3.08–3.92)–(3.82–4.86)	0.391; 0.470
Width of head dorsal scales (scales 3 and7)	5	3.57–4.86	(2.89–3.67)–(3.93–5.01)	0.364; 0.486
Length of head dorsolateral scales (scales 4 and 8)	5	3.68–4.57	(2.97–3.79)–(3.70–4.71)	0.380; 0.456
Width of head dorsolateral scales (scales 4 and 8)	5	3.34–4.55	(2.70–3.44)–(3.68–4.69)	0.345; 0.456
Length of head lateral scales (scales 4 and 8)	5	3.60–4.24	(2.90–3.71)–(3.43–4.37)	0.375; 0.424
Width of head lateral scales (scales 4 and 8)	5	3.22–4.15	(2.59–3.32)–(3.36–4.28)	0.335; 0.415
Length of head ventrolateral scales (scales 5 and 9)	5	3.28–4.09	(2.64–3.38)–(3.31–4.22)	0.341; 0.435
Width of head ventrolateral scales (scales 5 and 9)	5	2.89–3.87	(2.33–2.98)–(3.13–3.99)	0.305; 0.388
Length of head ventral scales (scales 6)	5	1.87–2.21	(1.49–1.93)–(1.91–2.28)	0.194; 0.152
Width of head ventral scales (scales 6)	5	2.03–2.44	(1.62–2.11)–(2.12–2.52)	0.206; 0.158

Table A5 (continued). Detailed morphometric characters of *Chaetonotus* (*Hystricochaetonotus*) *inaequabilis* sp. nov.

Characters	N	Holotype	Range of adult paratypes	SD
Length of neck dorsal scales (scales 10 and 14)	5	4.11–6.45	(3.33–4.24)–(5.23–6.65)	0.420; 0.654
Width of neck dorsal scales (scales 10 and14)	5	3.92–5.21	(3.18–4.04)–(4.22–5.37)	0.398; 0.528
Length of neck dorsolateral scales (scales 11)	5	3.90–6.29	(3.16–4.02)–(5.10–6.49)	0.398; 0.640
Width of neck dorsolateral scales (scales 11)	5	3.66–4.85	(2.97–3.77)–(3.93–5.00)	0.369; 0.493
Length of neck lateral scales (scales 11)	5	3.72–6.07	(3.01–3.83)–(4.92–6.27)	0.380; 0.620
Width of neck lateral scales (scales 11)	5	3.40–4.56	(2.75–3.50)–(3.70–4.71)	0.348; 0.465
Length of neck ventrolateral scales (scales 12)	5	3.51–5.54	(2.84–3.64)–(4.49–5.72)	0.359; 0.565
Width of neck ventrolateral scales (scales 12)	5	3.14–4.09	(2.54–3.26)–(3.31–4.22)	0.320; 0.418
Length of neck ventral scales (scales 13)	5	2.39–3.94	(2.07–2.48)–(3.19–4.09)	0.167; 0.364
Width of neck ventral scales (scales 13)	5	2.77–4.48	(2.40–2.87)–(3.64–4.66)	0.192; 0.411
Length of trunk dorsal scales (scales 15 and 17)	5	6.90–8.48	(5.59–7.11)–(6.87–8.82)	0.701; 0.882
Width of trunk dorsal scales (scales 15 and 17)	5	4.98–6.85	(4.03–5.13)–(5.55–7.14)	0.507; 0.716
Length of trunk dorsolateral scales (scales 15 and 17)	5	6.74–7.86	(5.46–6.95)–(6.37–8.19)	0.687; 0.820
Width of trunk dorsolateral scales (scales 15 and 17)	5	4.74–6.27	(3.84–5.09)–(5.08–6.53)	0.532; 0.653
Length of trunk lateral scales (scales 15 and 17)	5	6.36–7.60	(5.15–6.56)–(6.16–7.92)	0.650; 0.793
Width of trunk lateral scales (scales 15 and 17)	5	4.39–5.97	(3.58–4.53)–(4.84–6.22)	0.441; 0.623
Length of trunk ventrolateral scales (scales 15 and 17)	5	6.08–7.22	(4.92–6.27)–(5.82–7.52)	0.623; 0.763
Width of trunk ventrolateral scales (scales 15 and 17)	5	4.10–5.49	(3.32–4.23)–(4.43–5.72)	0.420; 0.579
Length of trunk ventral scales (scales 16)	5	2.82–4.34	(2.28–2.93)–(3.51–4.51)	0.263; 0.404
Width of trunk ventral scales (scales 16)	5	3.31–5.02	(2.68–3.44)–(4.06–5.22)	0.307; 0.467
Length of scales 18	5	6.78–8.37	(5.04–6.78)–(6.13–8.37)	0.759; 0.955
Width of scales 18	5	10.89–13.03	(6.44–10.89)–(8.37–13.03)	1.729; 1.882
Length of scales 19	5	7.38–8.26	(5.15–7.38)–(6.12–8.26)	0.898; 0.886
Width of scales 19	5	8.10–8.86	(6.26–8.10)–(7.29–8.86)	0.779; 0.777
Length of scales 20	5	9.14–10.40	(6.23–9.14)–(7.53–10.40)	1.118; 1.143
Width of scales 20	5	6.37–7.09	(4.90–6.37)–(5.44–7.09)	0.635; 0.667
Length of scales 21	5	8.97	7.32–9.16	0.740
Width of scales 21	5	8.86	7.20–9.53	0.728
Length of scales 22	5	4.77–6.97	(4.31–4.84)–(5.88–7.11)	0.249; 0.572

Table A5 (continued). Detailed morphometric characters of *Chaetonotus* (*Hystricochaetonotus*) *inaequabilis* sp. nov.

Characters	N	Holotype	Range of adult paratypes	SD
Width of scales 22	5	3.84–4.93	(3.48–3.92)–(4.20–5.03)	0.212; 0.330
Length of scales 23	5	4.85	3.27–4.85	0.644
Width of scales 23	5	3.32	2.24–3.32	0.481
Length of scales 24	5	11.04	7.42–11.39	1.765
Width of scales 24	5	5.86	4.13–6.05	0.872
Length of scales 25	5	10.63	6.37–10.80	1.900
Width of scales 25	5	8.19	4.93–8.32	1.453
Length of scales 26	5	10.52	8.12–10.68	1.065
Width of scales 26	5	8.69	6.71–9.03	0.916
Length of scales 27	5	5.89–8.77	(4.43–6.47)–(6.42–8.77)	0.771; 0.925
Width of scales 27	5	2.21–2.67	(1.61–2.28)–(2.05–3.19)	0.284; 0.438
Length of scales 28	5	5.96	4.22–5.96	0.689
Width of scales 28	5	4.18	3.15–4.41	0.489
Length of head anteroiormost spines (scales 1)	5	2.03–3.19	(1.63–2.47)–(2.61–3.45)	0.313; 0.309
Length of head scales 2 spines	5	1.66	1.53–2.92	0.552
Length of head dorsal spines (scales 3 and 7)	5	0.74–2.14	(0.54–1.93)–(1.96–3.07)	0.568; 0.505
Length of head dorsolateral spines (scales 4 and 8)	5	0.68–1.92	(0.50–1.77)–(1.76–2.75)	0.519; 0.452
Length of head lateral spines (scales 4 and 8)	5	0.56–1.80	(0.41–1.48)–(1.66–2.53)	0.439; 0.421
Length of head ventrolateral spines (scales 5 and 9)	5	0.34–1.63	(0.27–0.92)–(1.59–2.35)	0.268; 0.362
Length of head ventral spines (scales 6)	5	8.06–11.42	(7.32–8.95)–(9.59–12.03)	0.679; 0.985
Lamella width on the ventral head spines (scales 6)	5	0.68–1.09	(0.57–0.91)–(0.87–1.09)	0.149; 0.103
Length of neck dorsal spines (scales 10 and 14)	5	2.27–3.96	(2.09–3.26)–(3.67–5.69)	0.533; 0.917
Length of neck dorsolateral spines (scales 11)	5	2.10–3.58	(1.93–3.02)–(3.32–5.14)	0.495; 0.828
Length of neck lateral spines (scales 11)	5	1.88–3.35	(1.73–2.70)–(3.11–4.81)	0.442; 0.775
Length of neck ventrolateral spines (scales 12)	5	1.76–3.08	(1.62–2.53)–(2.86–4.42)	0.414; 0.712
Length of neck ventral spines (scales 13)	5	11.68–12.41	(9.84–12.31)–(10.98–13.60)	0.969; 0.956
Lamella width on the ventral neck spines (scales 13)	5	1.17–1.80	(0.94–1.17)–(1.07–1.80)	0.111; 0.300
Length of trunk dorsal spines (scales 15 and 17)	5	4.21–5.93	(3.91–6.02)–(5.52–8.24)	0.964; 1.280

Table A5 (continued). Detailed morphometric characters of *Chaetonotus* (*Hystricochaetonotus*) *inaequabilis* sp. nov.

Characters	N	Holotype	Range of adult paratypes	SD
Length of trunk dorsolateral spines (scales 15 and 17)	5	3.98–5.52	(3.70–5.68)–(5.14–7.67)	0.908; 1.192
Length of trunk lateral spines (scales 15 and 17)	5	3.81–5.37	(3.55–5.44)–(5.02–7.56)	0.868; 1.189
Length of trunk ventrolateral spines (scales 15 and 17)	5	3.32–5.04	(3.09–4.74)–(4.71–7.11)	0.757; 1.120
Length of trunk ventral spines (scales 16)	5	12.68–14.62	(11.19–13.93)–(13.26–15.23)	0.990; 0.868
Lamella width on the ventral trunk spines (scales 16)	5	1.88–2.07	(1.16–1.88)–(1.24–2.18)	0.282; 0.374
Length of spines of scales 18	5	18.08–21.61	(16.15–18.08)–(18.31–21.61)	0.801; 1.436
Trunk scales 18 spines length between lateral denticle and spine top	5	1.86–2.83	(1.49–1.86)–(2.02–2.86)	0.163; 0.348
Length of spines of scales 19	5	0.64–0.72	(0.30–0.64)–(0.51–0.84)	0.128; 0.118
Length of spines of scales 20	5	0.61–0.66	(0.31–0.61)–(0.36–0.97)	0.121; 0.236
Length of spines of scales 21	5	18.79	15.92–18.79	1.260
Trunk scales 21 spines length between lateral denticle and spine top	5	2.28	1.67–2.28	0.222
Length of spines of scales 22	5	3.41–4.75	(2.32–3.41)–(3.44–4.75)	0.430; 0.520
Length of spines of scales 24	5	12.58	10.16–12.73	1.078
Trunk scales 24 spines length between lateral denticle and spine top	5	1.63	1.37–1.71	0.156
Length of trunk posteriormost pair of lateral spines (scales 25)	5	13.69	11.42–14.02	1.028
Trunk posteriormost pair of lateral spines length between lateral denticle and spine base (scales 25)	5	1.86	1.58–1.97	0.147
Length of spines of scales 26	5	11.61	11.39–14.20	1.148
Trunk scales 26 spines length between lateral denticle and spine top	5	2.02	1.66–2.55	0.355
Length of spines of scales 27	5	0.31–0.45	(0.27–0.42)–(0.42–0.55)	0.068; 0.056
Length ratio of posteriormost trunk lateral spines (scales 25)	5	*12.686*	*11.094–12.923*	0.750
Localization of lateral denticle on spines of scales 1	5	*10.288–13.096*	*(8.780–10.504)–(9.839–15.068)*	0.733; 2.136
Localization of lateral denticle on spines of scales 19	5	*12.134*	*10.127–12.477*	1.086
Localization of lateral denticle on spines of scales 24	5	*12.957*	*12.398–13.682*	0.533
Localization of lateral denticle on posteriormost trunk lateral spines (scales 25)	5	*13.587*	*12.610–15.149*	0.938
Localization of lateral denticle on spines of scales 26	5	*17.399*	*12.691–17.958*	2.154
Head dorsal sensory bristles length	5	10.45	9.24–11.76	1.036
Neck dorsal sensory bristles length	5	14.92	14.24–17.93	1.508
Posterior dorsal sensory bristles length	5	18.57	18.28–19.71	0.578

Table A5 (continued). Detailed morphometric characters of *Chaetonotus* (*Hystricochaetonotus*) *inaequabilis* sp. nov.

Characters	N	Holotype	Range of adult paratypes	SD
Number of scales in central longitudinal row on ventral interciliary field	5	25	21–25	1.574
Total number of longitudinal alternating rows of scales on ventral interciliary field	5	7	7	0
Length of ventral interciliary field scales (scales 29–30)	5	1.66–4.42	(1.39–1.91)–(3.82–5.15)	0.190; 0.531
Width of ventral interciliary field scales (scales 29–30)	5	0.79–3.29	(0.62–1.05)–(2.73–4.12)	0.158; 0.552
Length of terminal ventral interciliary field scales I pair (scales 31)	5	8.23	6.92–8.57	0.627
Width of terminal ventral interciliary field scales I pair (scales 31)	5	2.79	2.62–3.04	0.152
Length of terminal ventral interciliary field scales II pair (scales 32)	5	3.98	3.14–4.05	0.401
Width of terminal ventral interciliary field scales II pair (scales 32)	5	1.81	1.41–1.93	0.210
Length of terminal ventral interciliary field scales III pair (scales 33)	5	2.31	2.25–2.68	0.188
Width of terminal ventral interciliary field scales III pair (scales 33)	5	1.27	1.20–1.32	0.044
Length of terminal ventral interciliary field scales VI pair (scales 33)	5	1.84	1.75–2.06	0.113
Width of terminal ventral interciliary field scales VI pair (scales 33)	5	0.90	0.85–0.98	0.053
Length of terminal ventral interciliary field scales I pair spines (scales 31)	5	9.41	7.71–10.69	1.065
Width of anterior pharynx thickening (a)	5	7.98	7.58–9.09	0.610
Width of pharynx narrowing (n)	5	5.31	4.92–7.13	0.929
Width of middle pharynx (m)	5	5.64	5.33–7.40	0.890
Width of posterior pharynx thickening (p)	5	9.83	9.22–11.08	0.758
Width of pharyngeal–intestinal junction	5	2.92	2.51–3.09	0.234
Intestine length	5	61.59	54.61–61.70	3.164
Pharynx formula a	5	*28.449*	*26.965–32.887*	2.180
Pharynx formula n	5	*18.93*	*18.771–25.796*	3.127
Pharynx formula m	5	*20.107*	*20.107–26.773*	2.993
Pharynx formula p	5	*35.045*	*35.045–40.087*	2.281
Pharynx–intestine ratio I	5	*45.543*	*45.543–49.147*	1.561
Length of X-organ	1	–	6.13	–
Width of X-organ	1	–	16.39	–

Table A6 (continued on next 4 pages). Comparison of the most important differentiating characters among species most similar to *Chaetonotus* (*Hystricochaetonotus*) *inaequabilis* sp. nov. All measurements in micrometres (µm). NA = data not available; – = not applicable.

Character	*C.* (*H.*) *inaequabilis* sp. nov.	*C.* (*H.*) *enormis*	*C.* (*H.*) *schlitzensis*	*C.* (*H.*) *octonarius*	*C.* (*H.*) *novenarius*	*C.* (*H.*) *aemilianus*	*C.* (*H.*) *ferrarius*	*C.* (*H.*) *spinulosus*	*C.* (*H.*) *longispinosus*
Body length	98.16–108.49	85.0	125.0	70.0–110.0	106.0–193.0	80.0–103.0	90.0–110.0	60.0–90.0	70.0–160.0
Cephalic pleurae	cephalion short and narrow; straight cut and extended on the dorsal body side; epipleurae and hypopleurae similar in size; epipleurae slightly arched and located on the dorsal, dorsolateral, lateral side and slightly visible on the ventrolateral head side; hypopleurae located entirely on the ventrolateral and ventral head sides, not visible from the dorsal body view; between the cephalion and epipleurae and between the epipleurae and hypopleurae deep notches; hypostomium short, rectangular–shaped with a slightly reinforced anterior edge	cephalion short and narrow; epipleurae and hypopleurae similar in size; epipleurae and hypopleurae located mostly laterally; hypostomium not marked	cephalion short and narrow; epipleurae smaller than hypopleurae; epipleurae and hypopleurae located mostly laterally; hypostomium not marked	cephalion short and narrow; epipleurae smaller than hypopleurae; epipleurae and hypopleurae located mostly laterally; hypostomium not marked	cephalion wide and narrow; epipleurae smaller than hypopleurae; epipleurae and hypopleurae located mostly laterally; hypostomium not marked	cephalion short and narrow; epipleurae and hypopleurae similar in size; epipleurae and hypopleurae located mostly laterally; hypostomium not marked	cephalion short and narrow; epipleurae and hypopleurae similar in size; epipleurae and hypopleurae located mostly laterally; hypostomium not marked	cephalion short and narrow; epipleurae, hypopleurae and hypostomium unrecognizable	cephalion wide and narrow; epipleurae smaller than hypopleurae; epipleurae and hypopleurae located mostly laterally; hypostomium not marked
Place of arising cephalic cilia	anterior tufts: dorso–terminaly, in area between the cephalion and epipleurae; posterior tufts: ventrally, above the anterior edge of the hypopleurae	anterior tufts: dorsoterminal between cephalion and epipleurae; posterior tufts: laterally, between epipleurae and hypopleurae	anterior tufts: dorsoterminal between cephalion and epipleurae; posterior tufts: laterally, between epipleurae and hypopleurae	anterior tufts: dorsoterminal between cephalion and epipleurae; posterior tufts: laterally, between epipleurae and hypopleurae	anterior tufts: dorsoterminal between cephalion and epipleurae; posterior tufts: laterally, between epipleurae and hypopleurae	anterior tufts: dorsoterminal between cephalion and epipleurae; posterior tufts: laterally, between epipleurae and hypopleurae	anterior tufts: dorsoterminal between cephalion and epipleurae; posterior tufts: laterally, between epipleurae and hypopleurae	anterior tufts: dorsoterminal between cephalion and epipleurae; posterior tufts: laterally, between epipleurae and hypopleurae	anterior tufts: dorsoterminal between cephalion and epipleurae; posterior tufts: laterally, between epipleurae and hypopleurae
Pharynx length	26.2–28.3	NA	21.0–23.0	20.0–28.0	27.5–51.0	24.0–29.0	ca 22.5–37.0	20.0–26.0	
Pharynx shape	anterior and wider posterior dilatation	cylindrical expanding towards posterior end	cylindrical with posterior dilatation	cylindrical with posterior dilatation	cylindrical expanding towards posterior end	cylindrical expanding towards posterior end	cylindrical expanding towards posterior end	cylindrical expanding towards posterior end	cylindrical with slight posterior dilatation
Furca length	20.1–22.1	NA	20.0	14.0–18.0	17.0–32.5	10.0–15.0	10.0–15.0	8.0–10.0	15.0
Adhesive tube length	9.9–10.5	NA	10.0	7.0–9.0	ca 11.0–21.5	NA	5.0–7.0	ca 2.0–5.0	6.0–7.0
Type of furca indentation	V-shaped	V-shaped	V-shaped	V-shaped	helmet-shaped	V-shaped	V-shaped	V-shaped	V-shaped
Number of scales in central longitudinal row	14–15	15–16	14	3	19	7–11	7–10	NA	NA

Table A6 (continued). Comparison of species most similar to *Chaetonotus* (*Hystricochaetonotus*) *inaequabilis* sp. nov.

Character	*C.* (*H.*) *inaequabilis* sp. nov.	*C.* (*H.*) *enormis*	*C.* (*H.*) *schlitzensis*	*C.* (*H.*) *octonarius*	*C.* (*H.*) *novenarius*	*C.* (*H.*) *aemilianus*	*C.* (*H.*) *ferrarius*	*C.* (*H.*) *spinulosus*	*C.* (*H.*) *longispinosus*
Total number of longitudinal alternating rows of scales	19–21	NA	NA	3	14–15	7–17	19–20	NA	NA
Type of scales	almost all three-lobed with strong, high, triangular keels, with spines from strong towards rudimentary; shape strongly differentiated within body regions	NA	one-lobed, keeled and spined	one-lobed, rounded in shape with very shallow or straight posterior edge, keeled and with long spines; sub-three-lobed, keeled and spineless	scales one-lobed and three-lobed; with strong, high, triangular keels, with spines strong or rudimentary; shape strongly differentiated within body regions	almost all three-lobed with strong, high, triangular keels, with spines from strong towards rudimentary; shape strongly differentiated within body regions	almost all sub-three-lobed with strong, high, triangular keels, with spines from strong towards rudimentary; shape strongly differentiated within body regions	all three-lobed or sub-three-lobed with keels, with spines from strong towards rudimentary; shape differentiated within body regions	scales sub-three-lobed with keels, with spines from strong towards short; shape differentiated within body regions
Scale size variation	strongly differentiated within body regions; dorsal and dorsolatral scales much larger	NA	size gradually increases from the head towards the widest body region	trunk scales similar in size	strongly differentiated within body regions	strongly differentiated within body regions; dorsal scales much larger	strongly differentiated within body regions; dorsal scales much larger	dorsal trunk scales much larger than remaining scales	NA
Scale size	head: 1.49–5.91 × 1.23–5.01 neck: 2.07–6.65 × 2.40–5.37 trunk: 2.28–10.40 × 2.24–13.03	NA	head and neck: 3.0–5.0 trunk: 7.0–9.0	head: – neck: – trunk: NA	3.6–6.0 × NA	head and neck: 2.0–6.0 × NA trunk: 4.5–8.0 × NA	head and neck: 1.0–5.0 × NA trunk: 1.0–7.0 × NA	NA	NA
Alignment of scales	scales on the head and anterior neck region located far from another; on posterior neck and anterior trunk region located gradually closer but still at a distance to the other scales and not juxtaposed; scales on dorsolateral, lateral, ventrolateral and ventral trunk region located at a distance from the other scales, dorsal trunk scales and furcal appendages scales slightly overlap	scales located far from each other	scales located far from another but dorsally on posterior trunk region juxtaposed	head and neck naked, trunk scales located far from another	scales on the head located far from another; on neck and anterior trunk region located gradually closer and juxtaposed; trunk scales slightly overlap, furcal base scales juxtaposed and not overlap	head or head and neck entirely or partially naked; on remaining body parts scales located far from another	scales located far from another but dorsally on posterior trunk region located closer	body almost naked, scales if present located far from another	body almost naked, scales present only laterally on head, neck, trunk and dorsally and dorsolaterally on trunk centre
Number of scales with long spines	9	13	7	8	9	7–9	5	4–8	7–8

Table A6 (continued). Comparison of species most similar to *Chaetonotus* (*Hystricochaetonotus*) *inaequabilis* sp. nov.

Character	*C.* (*H.*) *inaequabilis* sp. nov.	*C.* (*H.*) *enormis*	*C.* (*H.*) *schlitzensis*	*C.* (*H.*) *octonarius*	*C.* (*H.*) *novenarius*	*C.* (*H.*) *aemilianus*	*C.* (*H.*) *ferrarius*	*C.* (*H.*) *spinulosus*	*C.* (*H.*) *longispinosus*
Location of scales with long spines	dorsally, dorsolaterally and laterally on the trunk and furcal base; five scales dorsally at U47–U57, one pair dorsolaterally at U76–U83, one scaes dorsally at U76–U83, two pairs laterally at U71–U78 and U78–U84, schematic trunk scale arrangement: 5 + 2 + 2 +1	dorsally, dorsolaterally and laterally on the trunk and furcal base; scales arrangement in five alternating rows, schematic trunk scale arrangement: 3 + 4 + 2 +3 +1	dorsally on the trunk, schematic trunk scale arrangement: 2 + 1 + 2 +2	dorsally on the trunk, schematic trunk scale arrangement: 3 + 2 + 3	dorsally on anterior trunk region	dorsally on the trunk in various arrangements	dorsally on the trunk, schematic trunk scale arrangement: 3 + 2	dorsally on the trunk in various arrangements	dorsally and dorsolaterally on the trunk; scales arrangement in two alternating rows, schematic trunk scale arrangement: 3–4 + 4–5
Presence of a scales without or with rudimentary spines on the central dorsal trunk region	present	NA	present	present	present	present	present	present	absent
Presence of a different type of scales on the dorsal and dorsolateral surfaces of the furcal base and furcal appendages	present	NA	present	present	present	present	present	absent	absent
Scale coverage of the ventral interciliary field	entire ventral interciliary field covered with scales	NA	NA	ventral interciliary field naked	entire ventral interciliary field covered by scales	ventral interciliary field naked or covered by scales	covered from end of anterior dilatation of the pharynx to the furcal base	ventral interciliary field naked	ventral interciliary field naked
Type of scales on ventral interciliary field	anteriorly oval-shaped with straight posterior edge, keelless and spineless; posteriorly more elongated, keeled and spineless	NA	NA	–	one-lobed, rounded, keelless and spineless	scale if present, small, one-lobed, oval-shaped, keelless and spineless	one-lobed, rounded, keelless and spineless	–	–
Number of scales in central longitudinal row on the ventral interciliary field	21–25	NA	NA	–	40	7–10	*ca.* 32	–	–
Total number of longitudinal alternating rows of the ventral scales	7	NA	NA	–	4	NA	8–10	–	–

Table A6 (continued). Comparison of species most similar to *Chaetonotus* (*Hystricochaetonotus*) *inaequabilis* sp. nov.

Character	*C.* (*H.*) *inaequabilis* sp. nov.	*C.* (*H.*) *enormis*	*C.* (*H.*) *schlitzensis*	*C.* (*H.*) *octonarius*	*C.* (*H.*) *novenarius*	*C.* (*H.*) *aemilianus*	*C.* (*H.*) *ferrarius*	*C.* (*H.*) *spinulosus*	*C.* (*H.*) *longispinosus*
Number of terminal scales on the ventral interciliary field	4 pairs	NA	NA	absent	1 pair	absent or 1 pair	1 pair	absent	absent
Type of terminal scales on the ventral interciliary field	I pair: elongated with narrowing in half of its length, keeled and spined; II pair: elongated, with the pointed anterior edge, gradually tapers to the posterior end, keeled and spineless; III and IV pairs: elongated, oval-shaped with shallow posterior notch, keeled and spineless	NA	NA	–	elongated, oval-shaped keeled and spined	NA	elongated, oval-shaped keeled and spineless	–	–
Spine length variation	anteriormost spines slightly longer than subsequent head spines; spine length and thickness increase gradually from head towards the widest body region; head, neck and anterior trunk spines length gradually decrease towards to the ventrolateral surface; ventral hair-like spines very long; dorsal and dorsolateral trunk spines very long, thick, rigid; distinctly longer and stronger than remaining spines	head and neck spines short, on lateral surface longer; trunk dorsal, dorsolateral and furcal base lateral spines very long, thick, rigid; distinctly longer and stronger than remaining spines	head and neck spines short, on lateral surface longer; trunk dorsal, spines very long, thick, rigid; distinctly longer and stronger than remaining spines	all spines similar in length	anteriormost spines longer than subsequent head spines; spine length and thickness increase gradually from head posterior region towards the anterior trunk region; anterior dorsal trunk spines very long, thick, rigid; distinctly longer and stronger than remaining spines; central and posterior trunk spines short or rudimentary; from lateral towards ventral surface spines much longer than dorsolateral spines	all spines short except 7–9 distinctly longer and thicker dorsal trunk spines	head, neck and anterior trunk region spines short, on lateral surface longer; central trunk dorsal spines very long, thick, rigid; distinctly longer and stronger than remaining spines	all spines short except 4–8 distinctly longer and thicker dorsal trunk spines	lateral spines short and thin, dorsal and dorsolateral trunk spines very long, the spines located in second alternating row ca two times longer than spine arrangement in first row
Spine length	head: 0.3–12.0 neck: 1.6–13.6 trunk: 3.1–15.2 long spines: 10.2–21.6	head and neck: 2.0–9.0 trunk: NA long spines: NA	head and neck: 2.0–9.0 trunk: NA long spines: 25.0–30.0	long spines: 24.0–26.0	head: 9.0 neck: NA trunk: 9.0–19.0 long spines: 60.0–85.0	head, neck, trunk: 1.8–5.0 long spines: 18.0–26.0	head, neck, trunk: 2.0–3.0 long spines: 11.0–35.0	long spines: 12.0–25.0	long spines: 20.0–90.0

Table A6 (continued). Comparison of species most similar to *Chaetonotus* (*Hystricochaetonotus*) *inaequabilis* sp. nov.

Character	*C.* (*H.*) *inaequabilis* sp. nov.	*C.* (*H.*) *enormis*	*C.* (*H.*) *schlitzensis*	*C.* (*H.*) *octonarius*	*C.* (*H.*) *novenarius*	*C.* (*H.*) *aemilianus*	*C.* (*H.*) *ferrarius*	*C.* (*H.*) *spinulosus*	*C.* (*H.*) *longispinosus*
Presence of hair-like spines on the ventral surface	present	NA	NA	absent	absent	absent	present	absent	absent
Presence of lamellae on ventral spines	present	NA	NA	absent	absent	absent	present	absent	absent
Presence and distinctness of lateral denticle on the spines	dorsal, dorsolateral and posteriormost trunk lateral spines have lateral denticle; lateral denticle on remaining spines absent	dorsal, dorsolateral and posteriormost trunk lateral spines have lateral denticle; lateral denticle on remaining spines absent	dorsal trunk lateral spines have lateral denticle; lateral denticle on remaining spines absent	dorsal trunk spines have lateral denticle; lateral denticle on remaining spines absent	single or double lateral denticle arranged in single file present only on long, anterior trunk dorsal spines; lateral denticle on remaining spines absent	dorsal trunk and lateral furcal base spines have lateral denticle; lateral denticle on remaining spines absent	long dorsal and lateral or all spines with lateral denticle	long dorsal spines with lateral denticle; lateral denticle on remaining spines absent	dorsal and dorsolateral trunk spines have distinct, strong lateral denticle; lateral denticle on lateral spines absent
Localization of lateral denticle on the spines	long spines: *8.780–17.959*	NA	NA	NA	NA	NA	NA	NA	NA
Type of posteriormost pair of trunk lateral spines	longer than the other lateral spines	longer than the other lateral spines	the same as other lateral spines	–	longer than other lateral spines	the same as or longer than other lateral spines	the same as other lateral spines	the same as or longer than other lateral spines	the same as or longer than other lateral spines
Length ratio of posteriormost trunk lateral spines	*11.094–12.923*	NA	NA	–	NA	NA	NA	NA	NA
Type of last pair lateral spines	–	–	–	–	–	–	–	–	–
Length ratio of posteriormost parafurcal lateral spines	–	–	–	–	–	–	–	–	–
Ventral locomotor cilia	two longitudinal bands wider on the head region	two longitudinal bands	two longitudinal bands	two longitudinal bands	two longitudinal bands	two longitudinal bands	two longitudinal bands	two longitudinal bands	two longitudinal bands
Number of dorsal sensory bristles	3 pairs	2 pairs	2 pairs	2 pairs	2 pairs	2 pairs	2 pairs	2 pairs	2 pairs

Poznań, 16.12.2019r.

Małgorzata Kolicka
Adam Mickiewicz University, Poznań
Faculty of Biology
Department of Animal Taxonomy and Ecology
Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Declaration

I declare that the publication: **Kolicka M**. 2019. New *Chaetonotus* (*Wolterecka*) *semovitus* sp. nov. (Gastrotricha: Chaetonotida: Chaetonotidae) from a Palm House in Vienna (Austria). *Annales Zoologici*, 69 (2):447-475, has been prepared as a part of my doctoral dissertation under the scientific supervision of Prof. UAM, dr hab. Ziemowit Olszanowski.

Yours faithfully,

Poznań, 16.12.2019r.

Małgorzata Kolicka
Uniwersytet im. Adama Mickiewicza, Poznań
Wydział Biologii
Zakład Taksonomii i Ekologii Zwierząt
ul. Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Oświadczenie

Oświadczam, że publikacja: **Kolicka M**. 2019. New *Chaetonotus* (*Wolterecka*) *semovitus* sp. nov. (Gastrotricha: Chaetonotida: Chaetonotidae) from a Palm House in Vienna (Austria). *Annales Zoologici*, 69 (2):447-475, została wykonana na poczet mojej rozprawy doktorskiej pod opieką promotora Prof. UAM dra hab. Ziemowita Olszanowskiego.

Z wyrazami szacunku,

ANNALES ZOOLOGICI (Warszawa), 2019, 69(2): 447-475

NEW *CHAETONOTUS* (*WOLTERECKA*) *SEMOVITUS* SP. NOV. (GASTROTRICHA: CHAETONOTIDA: CHAETONOTIDAE) FROM A PALM HOUSE IN VIENNA (AUSTRIA)

MAŁGORZATA KOLICKA

Department of Animal Taxonomy and Ecology, Institute of Environmental Biology, Adam Mickiewicz University, Poznan. Umultowska 89, 61-614 Poznań, Poland; e-mail: kolicka@amu.edu.pl

Abstract.— Gastrotricha is a monophyletic phylum of small invertebrates inhabiting any kind of aquatic and semi-aquatic environments. Currently, *ca*. 850 gastrotrich species have been described, although the species numbers are not evenly distributed among the families and genera. The family Chaetonotidae Gosse, 1864 (*sensu* Leasi & Todaro, 2008) is considered the most numerous and diverse, with the most abundant genus being *Chaetonotus* Ehrenberg, 1830. This genus has more species grouped together than all of the remaining genera of this family. Nevertheless, when the subgenera division within Chaetonotus is analysed it is noticeable that the differences in species number are also very large among these groups. One of the smallest and rarest is the subgenus *Wolterecka* Mola, 1932, which currently includes only three nominal species. The main characteristics of this subgenus are short, reduced hook-shaped adhesive tubes and long, rigid spines on dorsal or dorsolateral surfaces of the furcal base and furcal appendages. In this paper, a new representative of *Wolterecka* is described, namely *Chaetonotus* (*W*.) *semovitus* **sp. nov.** This taxon was found in the Schönbrunn Palm House in Vienna (Austria) and is distinguished from the other *Chaetonotus* (*Wolterecka*) species mainly by the presence of three pairs of cephalic cilia, a seven-lobed head outline and scales with a concave posterior edge and diversified shape around the body. For this new species, detailed morphological and molecular analyses including 18S rRNA, ITS1–5.8S rRNA–ITS2, 28S rRNA and COI mtDNA are presented.

Key words.— Alien species, greenhouse, DNA barcode, gastrotrichs, new species

INTRODUCTION

Gastrotricha is a monophyletic phylum of invertebrates measuring from 50 to 3500 μm. Gastrotrichs inhabit any kind of aquatic and semi-aquatic environments, both marine and freshwater (*e.g.* Hochberg *et* Litvaitis 2000, Kisielewski 1997a, Todaro *et al*. 2006, Balsamo *et al*. 2014, 2015, Kieneke & Schmidt-Rhaesa 2015). Currently, *ca.* 850 nominal species are described within two orders: Chaetonotida Remane, 1925 [Rao & Clausen, 1970] and Macrodasyida Remane, 1925 [Rao & Clausen, 1970]. Of the some 490 known chaetonotidan species, more than 70% are present in freshwater ecosystems, whereas the remaining taxa inhabit brackish and marine waters (Balsamo *et al.* 2013, Garraffoni *et al.* 2016, Kolicka *et al.* 2016, Todaro 2019). Macrodasyidans group together approximately 360 species, which are almost exclusively observed within salty waters, with the exception of 3 described freshwater taxa from two genera (Hummon *et* Todaro 2010, Kånneby *et* Wicksten 2014, Todaro *et al.* 2014, 2015, Kånneby *et* Kirk 2017, Todaro 2019).

The order Chaetonotida is divided into two suborders: Multitubulatina d'Hondt, 1971, with only one

PL ISSN 0003-4541 © Museum and Institute of Zoology PAS
doi: 10.3161/00034541ANZ2019.69.2.011

family, namely Neodasyidae Remane, 1929, and Paucitubulatina d'Hondt, 1971, containing 7 families. Of the paucitubulatin families, four are exclusively freshwater (Dasydytidae Daday, 1905, Dichaeturidae Remane, 1927, Neogosseidae Remane, 1927, Proichthydiidae Remane, 1927) and two are exclusively marine (Muselliferidae Leasi *et* Todaro, 2008, Xenotrichulidae Remane, 1927), whereas only one family, viz. Chaetonotidae Gosse, 1864 (*sensu* Leasi & Todaro, 2008), groups together marine as well as freshwater species (Balsamo *et al*. 2013, Todaro 2019). Single-family Chaetonotidae dominate in the number of species and contain more taxa than all of the remaining families within the chaetonotidans. Within these, the largest family situation regarding genera seems to be the same, namely genus *Chaetonotus* Ehrenberg, 1830 is the most numerous and contains more species than all of the 12 remaining genera summed up (Balsamo *et al*. 2014, Todaro 2019). The main diagnostic character of this genus are scales with any kind of single spines and without extraverted anterior scale edges, however these features are very common and strongly varied among species (*e.g.* Kisielewski 1997b; Balsamo *et al*. 2014). On base on the molecular phylogenetic analysis, genus *Chaetonotus* is considered to be an artificial, polyphyletic group created as an aggregated category for separate, not closely correlated evolutionary lines (*e.g.* Kånneby *et al*. 2012, 2013, Balsamo *et al*. 2014). As it is currently recognized, *Chaetonotus* comprises 222 species grouped within 9 subgenera containing marine (*Marinochaetus* Kisielewski, 1997b), freshwater (*Captochaetus* Kisielewski, 1997b, *Primochaetus* Kisielewski, 1997b, *Tristratachaetus* Kolicka, Kisielewski, Nesteruk *et* Zawierucha, 2013, *Wolterecka* Mola, 1932, *Zonochaeta* Remane, 1927) and taxa from both of these habitats together (*Chaetonotus sensu stricto* Ehrenberg, 1830, *Hystricochaetonotus* Schwank, 1990, *Schizochaetonotus* Kisielewski, 1997b). The most common and species-rich is subgenus *Chaetonotus sensu stricto*, whereas the rarest and smallest, excluding the monotypical *Tristratachaetus* found only in its *locus typicus*, is *Wolterecka*, which groups only three nominal species. This subgenus was established mainly due to the presence of more or less reduced furca with rudimentary, hook-shaped adhesive tubes and some hooked or rigid spines dorsally or dorsolaterally on the furcal base and furcal appendages (Mola 1932, Schwank 1990, Kisielewski 1997b). From the morphological point of view, *Wolterecka* is regarded as one of the most evolutionarily advanced *Chaetonotus* lines, with reduced adhesive tubes considered as an apomorphic state of character (Kisielewski 1997b). This supposition is partially supported by molecular analyses, in which *Wolterecka* is found as one of the most derived clades, (*e.g.* Kånneby *et al*. 2012, 2013, Kolicka *et al*. 2016, 2018). However, it is still unclear which ancestral line this subgenus derives from, although the morphological character of usually small, one-lobed scales suggests *Chaetonotus sensu stricto* (Kisielewski 1997b).

The representatives of *Wolterecka* have been recorded only a few times and always in low numbers from three different continents (Europe, South America and Asia). Because the number of representatives of this subgenus is low, the finding of a new species is particularly interesting because it can provide additional information regarding the origin and evolution of the *Wolterecka* subgenus.

Gastrotrichs from various habitats (bottom sediments, bromeliad micro-reservoirs and aquatic plants) within different greenhouses have been researched a few times (Kolicka *et al*. 2013, Kolicka 2014, 2016, 2018). To date, 24 species constitute the results of the above analyses. The recorded taxa belong to genera *Heterolepidoderma* Remane, 1927, *Ichthydium* Ehrenberg, 1830, *Lepidodermella* Black, 1933 and subgenera *Chaetonotus sensu stricto* Ehrenberg, 1830, *Hystricochaetonotus* Schwank, 1990, *Primochaetus* Kisielewski, 1997b, and *Tristratachaetus* Kolicka, Kisielewski, Nesteruk *et* Zawierucha, 2013 within genus *Chaetonotus* Ehrenberg, 1830. No representatives of subgenus *Wolterecka* were recorded in the pevious surveys carried out in palm houses. The new *Wolterecka* species represent also the first record of this subgenus in Austria, a country where the gastrotrichofauna has been poorly investigated (Vornatscher 1938, Ruttner-Kolisko 1995, Balsamo *et al*. 2014).

Material and methods

Study area

The sampling took place at the Schönbrunn Palm House in Vienna (48°11'2.99"N, 16°18'10.35"E). The palm house was built in the years 1880–1882 and opened on 19 July 1882. It is 111 meters long, 25 meters wide and 28 meters high, and its area is 2,500 square meters. When it was opened it was the largest palm house in the world. During World War II, namely on 7 February 1945, the palm house was seriously damaged, and its renovation and reconstruction lasted until 14 January 1953. Another renovation took place in 1986–1990, with the most recent renovation taking place in the years 2011–2014.

The Schönbrunn Palm House has primary forest vegetation from three continents and is divided, according to type of climate, into three pavilions with plants from: a subarctic climate (a minimum temperature of 5°C), a moderate climate (a minimum temperature of 12°C) and a tropical climate

(a minimum temperature of 18°C). A large part of the plants currently presented in the greenhouse comes from the preserved historical collections; the remaining part of the collection comes from exchanges with other European palm houses, mainly with the University of Vienna (Austria), The Royal Botanic Gardens, Kew (Great Britain) and the Botanischer Garten und Botanisches Museum Berlin (German). The remaining, relatively small part of plants comes from self-cultivation of seeds carried out in the greenhouse. The soil and bottom sediments for cultivation of plants come from local Austrian resources, whereas the ornamental fish bred in some larger reservoirs come from aquarium shops (personal comunication).

In total, 24 samples were collected from different palm house pavilions and various habitats; eighteen samples contained bottom sediments from water reservoirs and six samples came from micro-reservoirs formed in the axils of Bromeliaceae. The new species, described below was found in a single sample collected from the water inflow to a reservoir, which contained aquatic plants (*e.g. Victoria cruziana* Orb.) located in the Moderate Climate Pavilion. This reservoir and the water inflow system were built during the last palm house renovation in the years 2011–2014. The water in this tank is filtered in a closed system, and the inflow in the form of an open stream leads purified water from the filtration system back to the reservoir (Fig. 1). This inflow is shallow and its water flow rate is low; it is additionaly slowed down by the presence of sediments and plant roots, mainly the large palm *Trachycarpus fortunei* (Hook.) in the riverbed.

Sampling and documentation

The material for the gastrotrich studies was collectred from the Schönbrunn Palm House on 8 July 2017. All bottom sediment samples were collected manually in such a manner that a 5 cm upper layer of sediment and approximately 5 cm of ambient water were placed in a 200 cm^3 plastic container. The samples from the bromeliads were collected using Pasteur pipettes in such a manner as to collect a volume of sediments and water no less than 20 cm^3. The collected samples were subsequently placed in an isothermal box to maintain the ambient temperature and transported to the laboratory as soon as it was possible. In the laboratory the samples were oxygenated and placed in a thermal chamber and temperature set at the collecting temperature. The material was examined within 10 days from sampling. A total of 0.3 cm^3 of sediment from each bottom sediments sample and 2 cm^3 of bromeliads material were analysed under a Olympus SZ51 stereomicroscope and all specimens were extracted with a micropipette and studied alive (Kisielewski 1997a, Balsamo *et al.* 2014). All specimens were observed, photographed and documented alive under a Olympus BX53 microscope compound microscope equipped with differential interference contrast (DIC) and an Olympus DS 50 digital camera. Metric data were obtained with a cellSens Entry 1.12 software (Olympus). All measurements are given in micrometres (μm) and all formulas used here are given as a percentage (%).

Morphological analyses

A morphological character was measured only if the orientation of that feature allowed an accurate measurement. The ranges of measurements are presented below in Tables 2–3. each specimen was documented by means of a set of photomicrographs and holotype was additionally documented by detailed schematic. Such documentation of types is allowed by The International Code of Zoological Nomenclature, namely in article 73.1.4 (The International Commission on Zoological Nomenclature 1999), even if the specimen documented no longer exists. The taxon descriptions follow the convention of Hummon *et al.* (1992), in which the longitudinal distance of a morphological character from the anterior end is expressed as percentage units (U) of the animal's total length (i.e. the distance of the character from the anterior end divided by the total body length and multiplied by 100). In this work, measurements of width of different body sections were omitted because they would be strongly influenced by the pressure of the coverglass on the live specimens being studied. The identification of gastrotrichs, their morphological study and the terms using to the new species description follow Kisielewski (1981, 1991, 1997a) and Kolicka *et al.* (2016). The terms describing the shape of the furcal branches and furcal indentation of new species follow Roszczak (1969). In this paper, the following formulae describing the distribution of scales were used, namely:

$$\textit{Ratio of scales distribution} = \frac{\text{total number of longitudinal alternating rows of scales}}{\text{number of scales in central longitudinal row}} \times 100\%$$

and the pharynx shape, namely:

$$\alpha = \frac{\text{width of anterior thickening}}{\text{pharynx length}} \times 100\%$$

and pharynx formula

$$n = \frac{\text{width of pharynx narrowing}}{\text{pharynx length}} \times 100\%$$

$$m = \frac{\text{width of middle pharynx}}{\text{pharynx length}} \times 100\%$$

$$p = \frac{\text{width of posterior thickening}}{\text{pharynx length}} \times 100\%$$

according to Kisielewski (1991), and the pharynx-to-intestine length ratio: according Kolicka *et al.*

(2016). In this work, the following abbreviations were applied:

D – dorsal,
DL – dorsolateral,
L – lateral,
LV – ventrolateral,
V – ventral *sensu* Kolicka *et al*. (2016, see S1 and S2).

Molecular analyses

Total genomic DNA was extracted from three single specimens of the new species using the DNeasy Blood and Tissue Kit (Qiagen GmbH, Hilden, Germany) as described by Dabert *et al*. (2008). A 695 bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified with a 1:1 mixture of two forward

Figure 1. Study area. (A) the Schönbrunn Palm House in Vienn; (B–D) sampling site.

primers, bcdF08 and bcdF09 (Kolicka *et al*. 2016), and reverse primer bcdR04 (Dabert *et al*. 2010). A fragment coding for internal transcribed spacers (ITS1–5.8S rRNA–ITS2) was amplified with ITS1_18S and 28SGasR (these study). A complete sequence of 18S rDNA was amplified in two overlapping fragments using 18Sfw/rev960 and fw390/rev18S primer pairs (Dabert *et al*. 2010), respectively. A 3681 bp fragment of 28S rRNA was amplified using the forward primer 28SF0001 (Dabert *et al*. 2016) with a 1:1 mixture of two reverse primers, R3950 and R3980 (Kolicka *et al*. 2018). For primer details see Table 1. PCRs were carried out in 5 μl reaction volumes containing 3 μl of the Type–it Microsatellite PCR Kit (Qiagen), 0.5 μM of each primer and 1 μl of the DNA template using a thermocycling profile with one cycle of 5 min at 95°C followed by 40 steps of 30 s at 95°C, 90 s at 50°C, 1–3 min at 72°C, and with a final step of 10 min at 72°C for all reactions. After amplification, the PCR products were diluted with 5 μl of MQ water; 2 μl of the diluted PCR reaction was analysed by electrophoresis on 1% agarose gel. Samples containing visible bands were purified with exonuclease I and Fast alkaline phosphatase (Thermo Fisher Scientific Inc.) and sequenced using the BigDye Terminator v3.1 kit and the ABI Prism 3130xl Genetic Analyzer (Applied Biosystems), following the manufacturer's instructions. Individual sequence reads were aligned and manually assembled into contigs in Geneious 11.1.2 (Biomatters Ltd.).

Results

The analysed material contained 53 specimens belonging to four genera, four subgenera and 13 species. One of the new species from the rare subgenus *Chaetonotus* (*Wolterecka*) Mola, 1932 is described below. All of the details of the remaining results will be included in a future publication.

Table 1. PCR primers used in this study; A and S refer to amplifying and sequencing, respectively.

Primer name	Sequence (5'–3')	Product	Use	Source
bcdF08	CGATGRTTTTTTTCHACWAACCAYAARGATATCGG	COI	A, S	Kolicka *et al*. 2016
bcdF09	CGATGRTTTTTTTCHACWAACCAYAARGACATTGG	COI	A, S	Kolicka *et al*. 2016
bcdR04	TATAAACYTCDGGATGNCCAAAAAA	COI	A, S	Dabert *et al*. 2008
ITS1_18S	AGAGGAAGTAAAAGTCGTAACAAG	ITS	A, S	Navajas *et al*. 1998
28SFgast	GTACGTGAAACCGTTCAGAGG	28S	S	This study
28SRgast	GTCACTGCCGYYGCGGCGA	ITS, 28S	A, S	This study
18Sfw	CTTGTCTCAAAGATTAAGCCATGCA	18S rDNA	A, S	Dabert *et al*. 2010
rev480	GTTATTTTTCGTCACTACCT	18S rDNA	S	Dabert *et al*. 2010
fw390	AATCAGGGTTCGATTCCGGAGA	18S rDNA	A, S	Dabert *et al*. 2010
rev960	GACGGTCCAAGAATTTCAC	18S rDNA	A, S	Dabert *et al*. 2010
for1300	TGCATGGCCGTTCTTAGTTG	18S rDNA	S	Dabert *et al*. 2010
rev1460	CATCACAGACCTGTTATTGC	18S rDNA	S	Dabert *et al*. 2010
rev18S	TGATCCTTCCGCAGGTTCACCT	18S rDNA	A, S	Dabert *et al*. 2010
F0001	ACCCVCYNAATTTAAGCATAT	28S rDNA	A	Dabert *et al*. 2016
300R	CAACTTTCCCTCACGGTACTTG	28S rDNA	S	Telford *et al*. 2003
F440	ACAAGTACCGTGAGGGAAAGTTG	28S rDNA	S	Sonnenberg *et al*. 2007
1200R	GCATAGTTCACCATCTTTCGG	28S rDNA	S	Telford *et al*. 2003
R1634	ATTCGGCAGGTGAGTTGTTACA	28S rDNA	A, S	Telford *et al*. 2003
1200F	CCCGAAAGATGGTGAACTATGC	28S rDNA	A, S	Telford *et al*. 2003
1600F	AGCAGGACGGTGGCCATGGAAG	28S rDNA	S	Telford *et al*. 2003
R2450	GCTTTGTTTTAATTAGACAGTCGGA	28S rDNA	A, S	Telford *et al*. 2003
F2120	GCCAGGCCGTACCCATATCC	28S rDNA	A, S	Kolicka *et al*. 2018
F2840	CTTTTATCGTTTCTTTACTTATTCAG	28S rDNA	S	Kolicka *et al*. 2018
F2960	CTTTTATCGTTTCTTTACTTATTCAG	28S rDNA	S	Kolicka *et al*. 2018
R3950	TGCTCTACTAAGTACAACACC	28S rDNA	A, S	Kolicka *et al*. 2018
R3980	CAGTGTGGTGGCTGCTCTACT	28S rDNA	A, S	Kolicka *et al*. 2018

Taxonomic accounts

Phylum **Gastrotricha** Mečnikow, 1865

Order **Chaetonotida** Remane, 1925
[Rao & Clausen, 1970]

Suborder **Paucitubulatina** d'Hondt, 1971

Family **Chaetonotidae** Gosse, 1864
(sensu Leasi & Todaro, 2008)

Subfamily **Chaetonotinae** Gosse, 1864
(sensu Kisielewski, 1991)

Genus ***Chaetonotus*** Ehrenberg, 1830

Typus generis. *Chaetonotus larus* (Müller, 1773)
Terra typica. Denmark.

A polyphyletic taxon, with only one apparently monophyletic subgenus namelly *Chaetonotus* (*Zonochaeta*) Remane, 1927a from all included in molcular analyses; encompasses 178 nominal freshwater species and 44 nominal marine species (Kånneby *et al*. 2012, 2013; Todaro 2019). Present in benthic, interstitial, periphytic and rarely semi-planktonic habitats (Kisielewski 1997a).

Subgenus ***Chaetonotus*** (***Wolterecka***) Mola, 1932

Type species. *Chaetonotus* (*Wolterecka*) *uncinus* Voigt, 1902.
Terra typica. Germany.

Small subgenus within *Chaetonotus* grouping so far only three freshwater species (Todaro 2019), up today only one species included in molecular phylogenies (*e.g*. Kånneby *et al*. 2013, Kolicka *et al*. 2016). Present in benthic and interstitial habitats (Kisielewski 1997a).

Chaetonotus (***Wolterecka***) ***semovitus*** sp. nov.
(Figs 2–13; Table 2–4)

Localities. Site 4.
Material. One sample, in total 3 adult specimens. The photomicrographs are available in the Natural History Collections at Adam Mickiewicz University in Poznań under accession numbers NHC–GCWS–25–1–50/h (holotype) and NHC–GCWS–25–51–80/h (paratypes) and in the author's collection.
Etymology. "Semovitus" is Latinised form of first name "Ziemowit", and this species is named to honour Professor Ziemowit Olszanowski, a prominent Polish acarologist and taxonomist, and at the same time a great, wise man, always offering advice and help.

Diagnosis. Slender, bottle-shaped body. Body length from 216.4 to 245.1 μm. Head seven-lobed, cephalic plates clearly demarcated in head outline. Cephalion with a free posterior edge. Hypostomium rhomboid with anterior edges reinforced, centrally, near posterior edges cambered reinforcement and bar. Ocellar granules absent. Mouth ring wide, with strong, long reinforcements with two strong cuticular teeth arising from anterior pharynx region. All scales one-lobed, spined, with a concave posterior end. Scales distributed in 37–43 single longitudinal rows (13–15D+6–8DL+6–8L+6–8LV+4V) with 39–43 scales in central row and strongly differing morphologically in the body areas. On the dorsal head and anterior neck region and on dorsal posterior trunk region scales juxtaposed and overlapping, on the remaining body surfaces scales located close to each other. Spines basally straight, thick, strong and conical in shape, subsequently strongly tapering towards the ends. Length of spines gradually decreases from dorsal to ventrolateral and ventral body side, and increase from the head towards trunk posterior region. Dorsally, on posterior trunk region spines much stronger and longer than remaining trunk scales. Dorsally and dorsolaterally on furcal base and furcal appendages, present scales with shorter or with rudimentary spines and without any spines. Dorsally, dorsolaterally on furcal appendages and partially in internal furcal indentation, four pairs of strongly bent scales with long, rigid and strongly curved, hook-shaped spines reaching beyond the adhesive tubes. Laterally, ventrolaterally, ventrally on furcal appendages and in internal furcal indentation small, thick scales with conical shapedspines, tapering to their ends. The adhesive tubes short, hook-like, with swollen base and narrow, blunt ends. Ventral interciliary field entirely covered by small, one-lobed scales which posterior edges entirely crossing into conical, rigid, simple spines. One pairs of ventral interciliary field terminal scales. Pharynx robust, conical in shape, with distinct lateral edges corrugation and with slightly pronounced anterior and posterior dilatations. Intestine straight with short anterior section differing in form and morphology.

Description. *Chaetonotus* (*Wolterecka*) *semovitus* sp. nov. has a slender, slightly bottle-shaped body (Figs 2, 7). The head is wide, but the neck constriction is slightly marked. The neck gradually tapers to the beginning of the trunk (from *ca*. U18 to *ca*. U35) (Figs 2, 7). The trunk is only slightly wider than the head and gradually widens from *ca*. U36 to *ca*. U53, where it is at its maximum width. Then it gradually tapers down to a clearly demarcated furcal base (from *ca*. U87 to *ca*. U90) (Figs 2, 7, 11). The furca is short and the furcal indentation is V-shaped. The furcal branches are thick, swollen-like and are set close together and slightly

point outwards (Figs 2, 7, 11). The adhesive tubes are very short, hook-like, strongly bentand thick. Their bases are swollen-like and subsequently, adhesive tubes gradually taper towards their blunt ends (Figs 2, 7, 11).

The head is seven-lobed. The cephalion (U1–U10) is long and wide. It is convex terminally and clearly demarcated in the body outline. Its posterior edge is free and does not adhere to the head surface (Figs 2A, 3, 8A, 8B, 9). The epipleurae are slightly convex and merged to the lateral edges of cephalion. They are present only on the dorsal head sides at U3–U6. The two pairs of hypopleurae are present, both pairs are the arrangement in a manner that any of its parts are not located on lateral head sides. The first pair is located at U4–U11 on dorsal, dorsolateral and ventrolateral

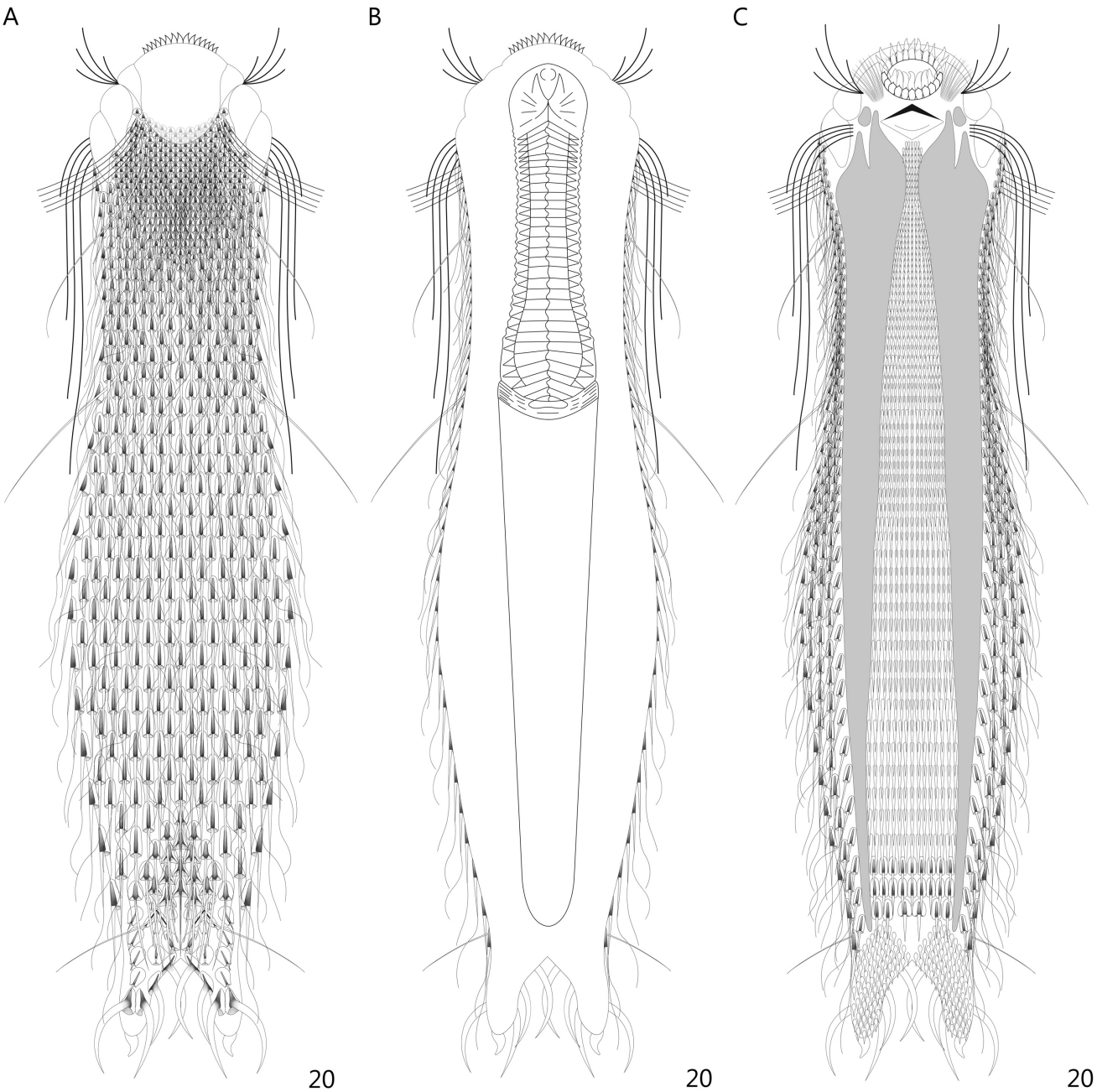


Figure 2. *Chaetonotus* (*Wolterecka*) *semovitus* sp. nov. Schematic drawings. (A) Dorsal body view; (B) view of internal morphology; (C) ventral body view. Light grey areas indicate the areas of the insertion of ciliary bands.

Table 2. Main morphometric characters of *Chaetonotus* (*Wolterecka*) *semovitus* sp. nov.; N – number of specimens or structures analyzed, Range – the smallest and the largest structure found among all specimens measured. All measurements are given in micrometers (μm).

Characters	N	Holotype	Range of adult paratypes
Body length	3	245.06	216.43–245.06
Furca length	3	30.12	30.12–32.41
Adhesive tube length	3	4.76	3.67–6.09
Number of scales in central longitudinal row	3	43	39–43
Total number of longitudinal alternating rows of scales	3	39	37–43
Head scales length x width	3	2.17–4.54 x 1.58–2.87	2.10–4.76 x 1.52–3.01
Neck scales length x width	3	2.97–7.14 x 1.81–3.11	2.91–7.31 x 1.77–3.18
Trunk scales length x width	3	3.82–10.96 x 1.51–3.73	3.71–10.96 x 1.45–3.73
Head spines length	3	9.86–14.65	8.85–14.65
Neck spines length	3	12.42–18.92	11.97–18.92
Trunk spines length	3	16.20–25.23	15.24–25.23
Mouth ring diameter	3	14.47	13.07–14.47
Pharynx length	3	80.92	79.99–80.92

body sides. On the dorsal side, they precisely juxtaposed to the epipleurae. This pair is much larger than epipleurae, strongly convex and clearly demarcated in the head outline (Figs 2A, 8A, 8B, 9). The second pair of hypopleurae are located at U8–U13 are distributed on the dorsal, dorsolateral and ventrolateral surfaces in such a manner that their greatest portion is located on the ventral side. On the dorsal and dorsolateral head sides, they are precisely juxtaposed to the first pair of hypopleurae. This pair is smaller than the first hypopleurae pair and less convex. All cephalic plates are strong and massive. The hypostomium is located at U7–U10, rhomboidand has anterior edge reinforced and centrally, near posterior edge cambered reinforcement bar (Figs 2C, 8C, 8D). Three pairs of cephalic ciliary tufts are present. The anterior tufts emerge ventrally between the cephalion edge and the anterior edge of the first hypopleurae pair (at U5). The anteriormost cilium in both anterior tufts is the shortest. The second cilium is much longer, and the longest in the tuft. The third cilium is shorter than the second one and the fourth is longer than third but shorter than second. The second pair of cephalic tufts have four cilia each and emerge ventrally in notches between the first and the second pair of hypopleurae (at U9–U10). Cilia in the posterior tufts are longer than in the first pair of tufts and their length increase from the anteriormost to the posteriormost cilium (Table 3). The last, forth cilium is very long and reaches the end of the neck region. The third pair of cephalic tufts have six cilia each and emerge in line dorsally in the area between cephalion and the first pair of hypopleurae (at U8). The anteriormost cilium in both third tufts is short, whereas remaining are longe and similar in length. All cilia in these tufts have different construction than cilia arrangements in the first and the second tufts. The cilia from the third pair of tufts more resemble in structures ventral locomotory cilia and may not be homologous to remaining cephalic cilia (Figs 2, 8, 9 and see 'Remark' section). Ocellar granules are absent. The mouth ring is located subterminal at U2–U6, is wide and has long, strong, thick finger-like reinforcements as well as very long cuticular inner lanceolate lamellas and long, strong bent inner hairs (Figs 2C, 8D, 9). Inside the mouth ring, two strong cuticular teeth arise from the anterior pharynx region (Figs 8C, 12). Suboral cilia emerge in one pair of tufts, located laterally to the mouth ring, at *ca.* U5–U6 (Figs 2C, 7D).

The body is covered by one-lobed scales with the concaved posterior edge that adhere along their entire surface to the cuticle (Figs 1–10). All scales are keeled, and the keels run straight by almost the entire scale length; in anterior scales region the keels are smoothly arising from the remaining scales surface and towards to the posterior end of scales become gradually higher, stronger and more distinct. The scales are distributed in 37–43 longitudinal alternating rows (13–15D+6–8DL+6–8L+6–8LV+4V) with 39–43 scales in the central row. The scales on the dorsal head and anterior neck region and on dorsal posterior trunk region have slightly overlapping edges or are juxtaposed, whereas on the remaining body parts scales do not overlap and are located close to another (Figs 2–4, 7–11). Scales varing in shape and size may be present in the same body region; scales diversity is highest on the posterior trunk region. The one or two anteriormost scales in the central longitudinal rows on the dorsal head region are arrangement underneath the free

cephalion posterior edge (Figs 2–3, 7–8), whereas scales on dorsolateral, lateral and ventrolateral surfaces adhere directly to the cephalic plates. The dorsal head scales are round-shaped with deep, semi-circular concaved posterior edge and without any posterior notches (scales 1) (Fig. 5A). This type of scales become gradually, slightly, more oval in shape towards to the dorsal neck anterior region (scales 2) (Fig. 5B). The scales arrangement on head median anterior neck region, located from *ca*. U8 to *ca*. U22, are smaller than surrounding scales, but they are thick, strong and posses a prominent, pointed, high keels. The scales of this area clearly stands out from those of the other body regions because where scales are thin, delicate and with lower keels (Figs 2–3, 7–8). The dorsolateral, lateral and ventrolateral head and remaining dorsal, dorsolateral, lateral and ventrolateral anterior neck region scales are more elongated, oval in shape and possess a shallower concaved posterior edges (scales 3) (Fig. 5C). From central to posterior neck region dorsal, dorsolateral, lateral and ventrolateral scales become gradually more semitriangular with rounded edges (scales 4) (Fig. 5D). Anterior trunk region scales are more elongated and are more rectangular with rounded, slightly pointed anterior edges (scales 5) (Fig. 5E), whereas from central (*ca*. U47) to the posterior trunk region scales became gradually more elongated and rectangular-shaped with slightly rounded edges (scales 6) (Fig. 5F). On the dorsal side of posterior trunk region, from *ca*. U73 to *ca*. U83, in triangle area expanding to the furca base (Fig. 4), two types of different scales with overlapping edges are arrangement.

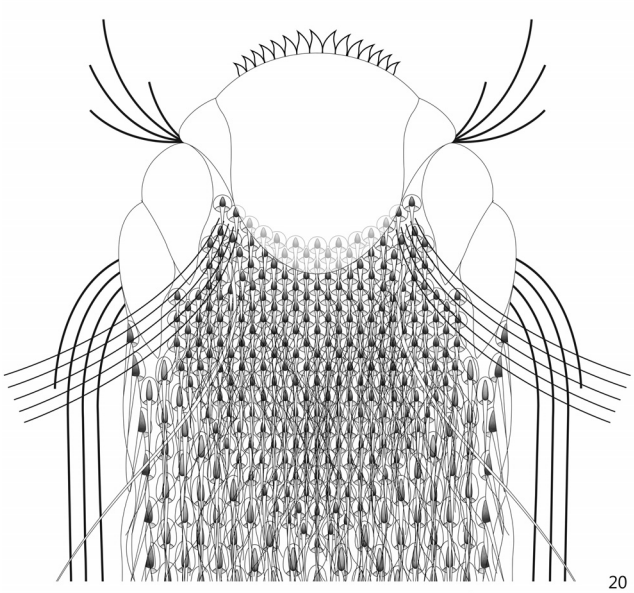


Figure 3. *Chaetonotus* (*Wolterecka*) *semovitus* sp. nov. Schematic drawings of the dorsal head and neck region.

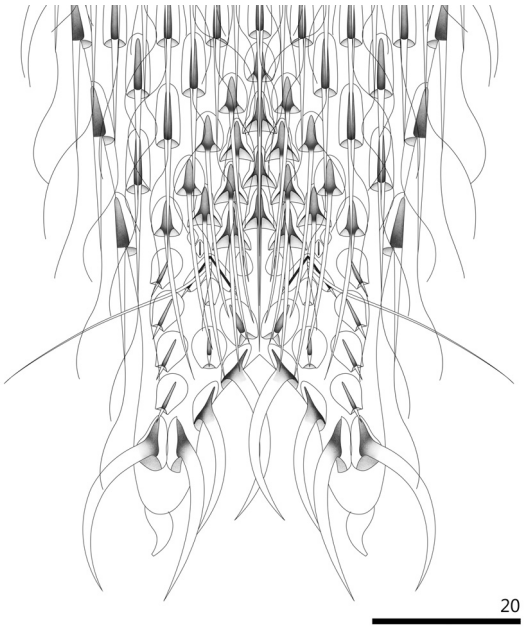


Figure 4. *Chaetonotus* (*Wolterecka*) *semovitus* sp. nov. Schematic drawings of the posterior trunk region, furcal base and furcal appendages.

In anterior and lateral parts of this region, scales are semioval with deep, semicircle posterior concave and straight posterior edges. These scales have prominent, high, semitriangulare keels (scales 7) (Fig. 5G). In the center of this area, arrangement in the shape of smaller, internal triangle (from U75 to U82), 12 larger and stronger scales with very long spines are located (scales 8) (Fig. 5H). These scales are triangle and have high, strong triangle keels and very deep semicircle concaved posterior edges (Figs 1–2, 5H, 9C). On the dorsal posterior trunk side, laterally to these scales, two pairs of small, keeled and spineless scales are arrangement. The first pair is located at U81, the second at U82; scales from both these pairs are semirhomboid and without concaved posterior edges and without notches (scales 9) (Fig. 5I). Posteriorly to these scales in the center of the dorsal surface, at *ca*. U83, one pair of small, very thin scales is arrangement. Scales from this pair are egg-shaped without any concaves of posterior edges;posses straight, weak keels and is spineless (scales 10) (Fig. 5J). Laterally to these scales, but still dorsally on the posterior trunk region (at U82–U83) double keeled scales with trunk sensory bristles are present (scales 11) (Fig. 5K). One pair of small, thin, oval tapering to the straight posterior edge scales with a straight keel and a short, straight, conical shaped spine is located in the posterior notches of double keeled scales (at U83–U84) (scale 12) (Figs 4, 5L). In the center of the furcal base dorsal surface (at U85–U85) one pair of small, thin, ovoid scales with

Table 3. Detailed morphometric characters of *Chaetonotus* (*Wolterecka*) *semovitus* sp. nov; N – number of specimens or structures analyzed, Range – the smallest and the largest structure found among all specimens measured, SD – standard deviation. All measurements are given in micrometers (μm); all indicators are given as a percentage (%) and italicized.

Characters	N	Holotype	Range of paratypes	SD
Cephalion length	3	25.31	22.93–26.01	1.615
Cephalion width	3	19.54	19.39–20.46	0.579
Hypostomium length	3	6.37	6.37–10.24	2.153
Hypostomium width	3	10.69	10.57–18.68	4.648
Length of cephalic cilia (first pair tuft)	3	5.75–18.66	(5.51–5.93)–(17.38–18.75)	0.211; 0.766
Length of cephalic cilia (second pair tuft)	3	19.24–53.45	(18.15–23.89)–(46.26–53.45)	3.048; 3.816
Length of cephalic cilia (third pair tuft)	3	5.34–20.26	(5.02–5.89)–(18.82–20.26)	0.440; 0.780
Width of cephalic cilia (first pair tuft)	3	1.00–1.11	(0.92–1.00)–(0.96–1.11)	0.046; 0.075
Width of cephalic cilia (second pair tuft)	3	1.13–1.35	(1.01–1.13)–(1.28–1.35)	0.060; 0.038
Width of cephalic cilia (third pair tuft)	3	0.65–0.71	(0.59–0.65)–(0.71–0.77)	0.031; 0.032
Width of adhesive tube on the base	3	2.35	2.32–2.17	0.217
Width of adhesive tube on the end	3	1.03	0.88–1.03	0.075
Ratio of scale distribution	3	90.698	90.698–104.878	7.287
Length of head dorsal scales (scales 1. 2 and 3)	3	2.17–2.93	(2.10–2.22)–(2.87–3.07)	0.060; 0.103
Width of head dorsal scales (scales 1. 2 and 3)	3	2.21–2.87	(2.14–2.26)–(2.81–3.01)	0.060; 0.103
Length of head dorsolateral scales (scales 3)	3	2.64–4.54	(2.56–2.70)–(4.45–4.76)	0.070; 0.159
Width of head dorsolateral scales (scales 3)	3	1.78–1.96	(1.73–1.82)–(1.92–2.05)	0.045; 0.067
Length of head lateral scales (scales 3)	3	2.60–4.32	(2.51–2.66)–(4.23–4.53)	0.075; 0.154
Width of head lateral scales (scales 3)	3	1.66–1.91	(1.60–1.70)–(1.87–2.00)	0.050; 0.067
Length of head ventrolateral scales (scales 3)	3	2.49–4.17	(2.40–2.55)–(4.09–4.37)	0.075; 0.144
Width of head ventrolateral scales (scales 3)	3	1.58–1.86	(1.52–1.62)–(1.82–1.95)	0.050; 0.067
Length of neck dorsal scales (scales 2. 3 and 4)	3	2.97–7.14	(2.91–3.04)–(6.97–7.31)	0.065; 0.170
Width of neck dorsal scales (scales 2. 3 and 4)	3	1.81–3.11	(1.77–1.85)–(3.04–3.18)	0.040; 0.070
Length of neck dorsolateral scales (scales 3 and 4)	3	4.51–6.93	(4.42–4.62)–(6.77–7.10)	0.100; 0.165
Width of neck dorsolateral scales (scales 3 and 4)	3	2.03–2.86	(1.99–2.08)–(2.79–2.93)	0.045; 0.070
Length of neck lateral scales (scales 3 and 4)	3	4.42–6.26	(4.33–4.54)–(6.12–6.41)	0.105; 0.145
Width of neck lateral scales (scales 3 and 4)	3	1.99–2.68	(1.95–2.04)–(2.62–2.74)	0.045; 0.060
Length of neck ventrolateral scales (scales 3 and 4)	3	4.31–6.05	(4.22–4.43)–(5.91–6.08)	0.105; 0.091
Width of neck ventrolateral scales (scales 3 and 4)	3	1.90–2.33	(1.86–1.95)–(2.28–2.34)	0.045; 0.032
Length of trunk dorsal scales (scales 5 and 6)	3	7.23–10.96	(7.06–7.41)–(10.70–10.96)	0.175; 0.135
Width of trunk dorsal scales (scales 5 and 6)	3	3.17–3.73	(3.10–3.26)–(3.64–3.73)	0.080; 0.045
Length of trunk dorsolateral scales (scales 5 and 6)	3	7.04–9.15	(6.88–7.22)–(8.93–9.15)	0.172; 0.114
Width of trunk dorsolateral scales (scales 5 and 6)	3	2.96–3.58	(2.89–3.04)–(3.49–3.58)	0.075; 0.046
Length of trunk lateral scales (scales 5 and 6)	3	6.54–8.82	(6.38–6.71)–(8.60–8.82)	0.165; 0.112
Width of trunk lateral scales (scales 5 and 6)	3	2.80–3.33	(2.73–2.87)–(3.25–3.33)	0.070; 0.040
Length of trunk ventrolateral scales (scales 5 and 6)	3	6.19–8.36	(6.04–6.35)–(8.14–8.36)	0.155; 0.111
Width of trunk ventrolateral scales (scales 5 and 6)	3	2.43–3.02	(2.37–2.49)–(2.84–3.02)	0.060; 0.093
Length of trunk ventral scales (scales 5 and 6)	3	3.82–6.28	(3.71–3.92)–(6.09–6.28)	0.105; 0.095
Width of trunk ventral scales (scales 5 and 6)	3	1.51–2.47	(1.45–1.55)–(2.29–2.47)	0.050; 0.095
Length of scales 7	3	5.84–8.36	(5.72–5.84)–(8.08–8.36)	0.061; 0.141
Width of scales 7	3	4.49–6.38	(4.20–4.49)–(6.16–6.38)	0.150; 0.110
Length of scales 8	3	9.90–11.10	(7.84–9.90)–(10.15–11.10)	1.030; 0.517
Width of scales 8	3	7.84–8.80	(6.22–7.84)–(8.05–8.80)	0.810; 0.410
Length of scales 9	3	2.63–3.37	(2.37–2.63)–(3.09–3.37)	0.132; 0.147
Width of scales 9	3	2.32–2.98	(2.24–2.37)–(2.47–2.98)	0.066; 0.284

Table 3. Continued

Characters	N	Holotype	Range of paratypes	SD
Length of scales 10	3	3.03	3.03–4.01	0.500
Width of scales 10	3	2.26	2.26–3.55	0.686
Length of scales 12	3	3.49	3.49–5.05	0.785
Width of scales 12	3	2.4	2.40–3.77	0.718
Length of scales 13	3	5.17	5.17–6.36	0.598
Width of scales 13	3	4.08	4.08–5.02	0.472
Length of scales 14	3	5.61	5.42–6.07	0.334
Width of scales 14	3	4.38	4.29–4.99	0.381
Length of scales 15	3	4.54–4.76	(4.47–6.29)–(4.76–6.90)	1.031; 1.208
Width of scales 15	3	3.14–3.43	(3.10–3.53)–(3.43–3.89)	0.238; 0.242
Length of scales 16	3	8.37–9.72	(7.79–8.46)–(8.78–9.72)	0.364; 0.483
Width of scales 16	3	5.63–6.27	(5.27–5.70)–(5.69–6.27)	0.231; 0.291
Length of scales 17	3	4.92–5.57	(4.56–4.92)–(5.31–5.57)	0.195; 0.147
Width of scales 17	3	3.67–3.99	(3.38–3.67)–(3.80–3.99)	0.155; 0.107
Length of scales 18	3	1.49–2.91	(1.42–1.57)–(2.91–3.15)	0.075; 0.120
Width of scales 18	3	0.76–1.71	(0.72–0.79)–(1.71–1.80)	0.035; 0.047
Length of head dorsal spines (scales 1. 2 and 3)	3	11.67–14.65	(10.44–11.67)–(13.72–14.65)	0.615; 0.466
Length of head dorsolateral spines (scales 3)	3	10.46–13.09	(9.39–10.46)–(10.26–13.09)	0.535; 0.415
Length of head lateral spines (scales 3)	3	10.11–12.81	(9.08–10.11)–(12.01–12.81)	0.515; 0.401
Length of head ventrolateral spines (scales 3)	3	9.86–12.17	(8.85–9.86)–(11.43–12.17)	0.505; 0.371
Length of neck dorsal spines (scales 2. 3 and 4)	3	14.94–18.92	(14.03–14.94)–(17.77–18.92)	0.457; 0.577
Length of neck dorsolateral spines (scales 3 and 4)	3	13.97–17.89	(13.12–13.97)–(16.82–17.89)	0.427; 0.538
Length of neck lateral spines (scales 3 and 4)	3	13.18–17.12	(12.71–13.39)–(16.17–17.12)	0.348; 0.480
Length of neck ventrolateral spines (scales 3 and 4)	3	12.42–16.64	(11.97–12.59)–(15.75–16.64)	0.320; 0.452
Length of trunk dorsal spines (scales 5 and 6)	3	19.44–25.23	(18.26–19.44)–(23.70–25.23)	0.593; 0.769
Length of trunk dorsolateral spines (scales 5 and 6)	3	18.19–24.27	(17.09–18.19)–(22.81–23.40)	0.553; 0.417
Length of trunk lateral spines (scales 5 and 6)	3	17.71–23.22	(16.62–17.71)–(21.82–23.22)	0.547; 0.704
Length of trunk ventrolateral spines (scales 5 and 6)	3	17.05–22.37	(16.04–17.05)–(21.03–22.37)	0.509; 0.674
Length of trunk ventral spines (scales 5 and 6)	3	16.20–21.06	(15.24–16.02)–(19.82–21.06)	0.483; 0.625
Length of scales 7 spines	3	28.58–30.19	(24.88–28.58)–(29.37–30.19)	1.858; 0.416
Length of scales 8 spines	3	30.36–37.29	(30.10–30.36)–(34.56–37.29)	0.135; 1.446
Length of scales 9 spines	3	15.56–17.42	(15.56–17.45)–(17.42–20.69)	0.958; 1.648
Length of scales 12 spines	3	0.99	0.81–1.04	0.121
Length of scales 13 spines	3	1.03	1.03–1.24	0.110
Length of scales 14 spines	3	1.46	1.38–1.82	0.234
Length of scales 15 spines	3	2.26–2.71	(2.11–2.26)–(2.71–3.07)	0.075; 0.197
Length of scales 16 spines	3	12.45–14.24	(12.18–12.45)–(12.39–14.24)	0.135; 1.043
Length of scales 17 spines	3	14.82–15.98	(13.41–14.82)–(15.98–21.21)	0.779; 2.748
Length of scales 18 spines	3	0.48–1.91	(0.48–0.74)–(1.86–1.97)	0.131; 0.055
Length of trunk posteriormost pair of lateral spines	3	23.06	21.22–23.06	0.920
Head dorsal sensory bristles length	3	43.15	41.33–43.15	0.945
Neck dorsal sensory bristles length	3	49.08	43.64–49.08	3.027
Posterior dorsal sensory bristles length	3	30.32	30.32–31.42	0.603
Number of scales in central longitudinal row on ventral interciliary field	3	62	60–62	1.155
Total number of longitudinal alternating rows of scales on ventral interciliary field	3	15	15–17	1.155

Table 3. Continued

Characters	N	Holotype	Range of paratypes	SD
Length of ventral interciliary field scales (scales 19 and 20)	3	1.51–5.14	(1.43–1.54)–(5.11–5.56)	0.057; 0.252
Width of ventral interciliary field anterior scales (scales 19 and 20)	3	0.52–2.36	(0.52–0.68)–(2.28–2.64)	0.085; 0.189
Length of ventral interciliary field terminal scales pair (scales 22)	3	5.25	5.25–5.67	0.229
Width of ventral interciliary field terminal scales pair (scales 22)	3	3.21	3.04–3.61	0.293
Length of ventral interciliary field scales spines (scales 19 and 20)	3	2.57–7.48	(2.57–2.84)–(7.41–7.58)	0.135; 0.085
Length of ventral interciliary field terminal scales pair spines (scales 22)	3	6.38	6.21–6.94	0.382
Width of anterior pharynx thickening (a)	3	18.68	18.68–19.56	0.492
Width of pharynx narrowing (n)	3	14.76	14.76–15.76	0.519
Width of middle pharynx (m)	3	16.44	16.44–17.11	0.347
Width of posterior pharynx thickening (p)	3	20.98	20.58–21.48	0.451
Width of intestine junction	3	6.33	6.33–9.19	1.489
Intestine length	3	130.20	110.47–130.20	11.362
Length of intestine anterior differ section	3	3.22	3.16–4.07	0.509
Pharynx formula a	3	*23.085*	*23.085–24.380*	0.653
Pharynx formula n	3	*18.240*	*18.24–19.544*	0.710
Pharynx formula m	3	*20.316*	*20.316–21.218*	0.506
Pharynx formula p	3	*25.927*	*25.728–26.637*	0.478
Pharynx-intestine ratio I	3	*62.151*	*61.983–72.409*	5.972
Ratio of scale distribution	3	*90.698*	*90.698–104.878*	7.287

a shallow concaved posterior edge and very shallow posterior notches are present (scale 13) (Figs 3, 5M). These scales have narrow, slightly triangle shaped keels and very short, conical shaped spines tapering to their ends, which not extend beyond the posterior edge of scales. Laterally and slightly posteriorly to ovoid scales (to scales 13), dorsally on furcal base and partially on the furcal appendages, one pair of small, thin, apple-shaped scales is located (scales 14) (Fig. 5N). These scales have narrow, weakly triangle keels and a short, conical spines tapering to the their ends and a shallow concaved posterior edges (at U86–U87). Dorsolaterally on the furca base and furcal appendages from *ca*. U83 to *ca*. U90, four pairs of small and thin scales are arrangement. These scales are ovoid gradually tapering to the straight and narrow posterior edge with shallow concave (scales 15) (Fig. 5O). These dorsolateral scales possessing a narrow, slightly triangle keels and short, conical spines tapering to their ends. On furcal appendages, four pairs of thickest body scales bearing long, rigid, hook-shaped spines are present. The first (located at U87–U88) and second (located at U88–U90) pairs of these scales are located on inner edges of furca braches – partially on dorsal and partially on inner furcal indentation surface (Figs 2, 4, 7, 11). These scales are elongated romboid with rounded edges and posses a very strong and high triangle keels and posterior concavity. These scales are large and and their edges are strongly curved, thus making the entire scale adhere to the rounded surfaces of inner edges of furcal branches (scales 16) (Fig. 5P). The third (at U89–U91) and the fourth (at U90–91) of these pairs are situated on the dorsolateral surfaces of the furcal appendages. These scales are smaller than scales from first and second pairs of scales bearing hook-shaped spines and are bell-sheaped, have wide, high semitriangle keels and wide semicircular concaves (scales 17) (Fig. 5Q). The lateral edges of these scales from third and fourth pairs closely adhere to each other. Laterrally, ventrolaterally, ventrally on the furcal base and furcal appendages as well as in inner furcal indentation, small oval scales are arrangemen. These scales are convex and keeless, their short cylindrical spines arrising directly from posterior high scales edges and tappering to their ends (scales 18) (Fig. 5R). The size of these scales increase gradually

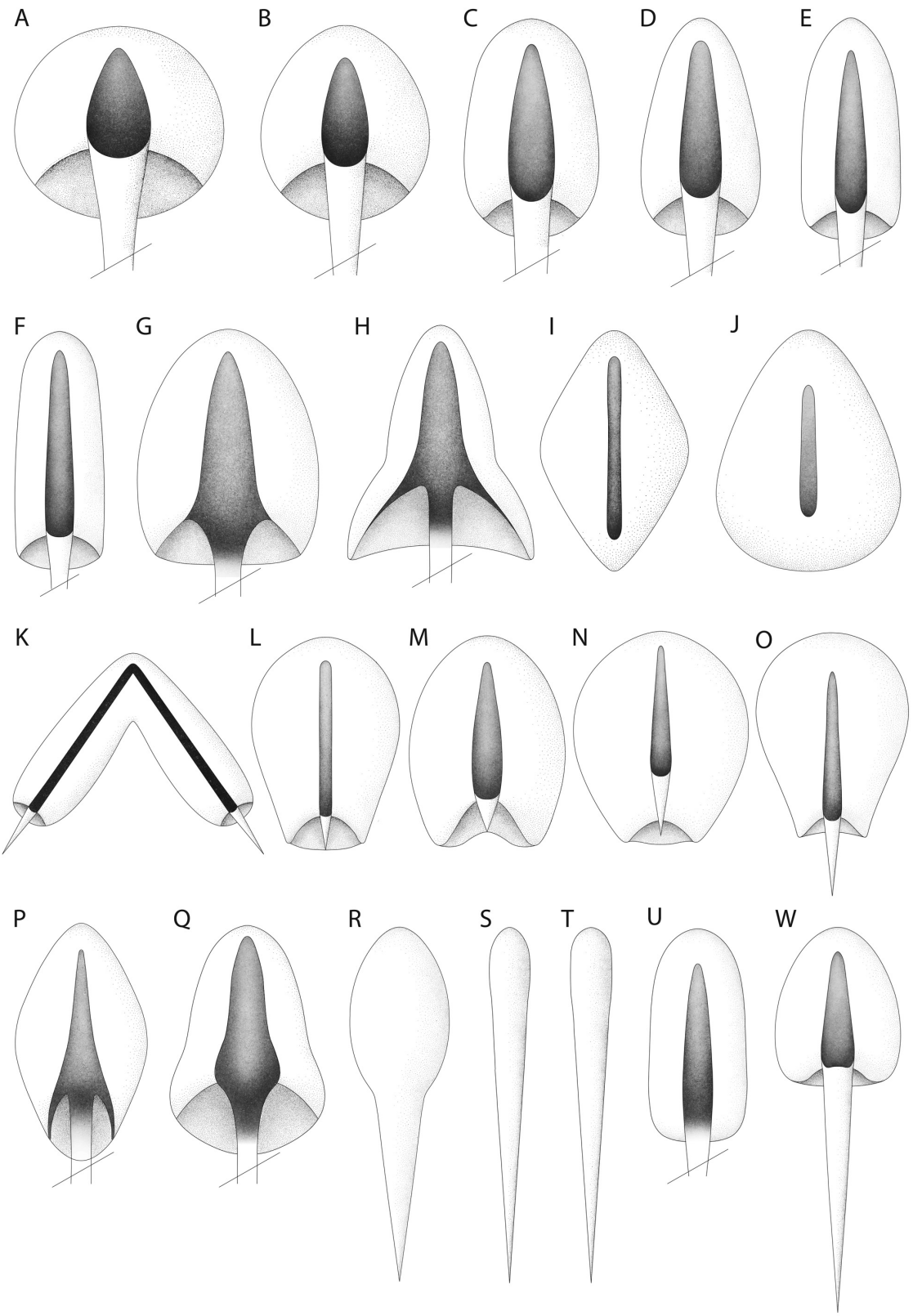


Figure 5. *Chaetonotus* (*Wolterecka*) *semovitus* sp. nov. Schematic drawings of the scales. A – Scale 1; B – scale 2; C – scale 3; D – scale 4; E – scale 5; F – scale 6. G – Scale 7. H – Scale 8. I – Scale 9. J – Scale 10. K – Scale 11. L – Scale 12. M – Scale 13. N – Scale 14. O – Scale 15; P – scale 16; Q – scale 17; R – scale 18; S, T – scale 19–20; U – scale 21; W – scale 22.

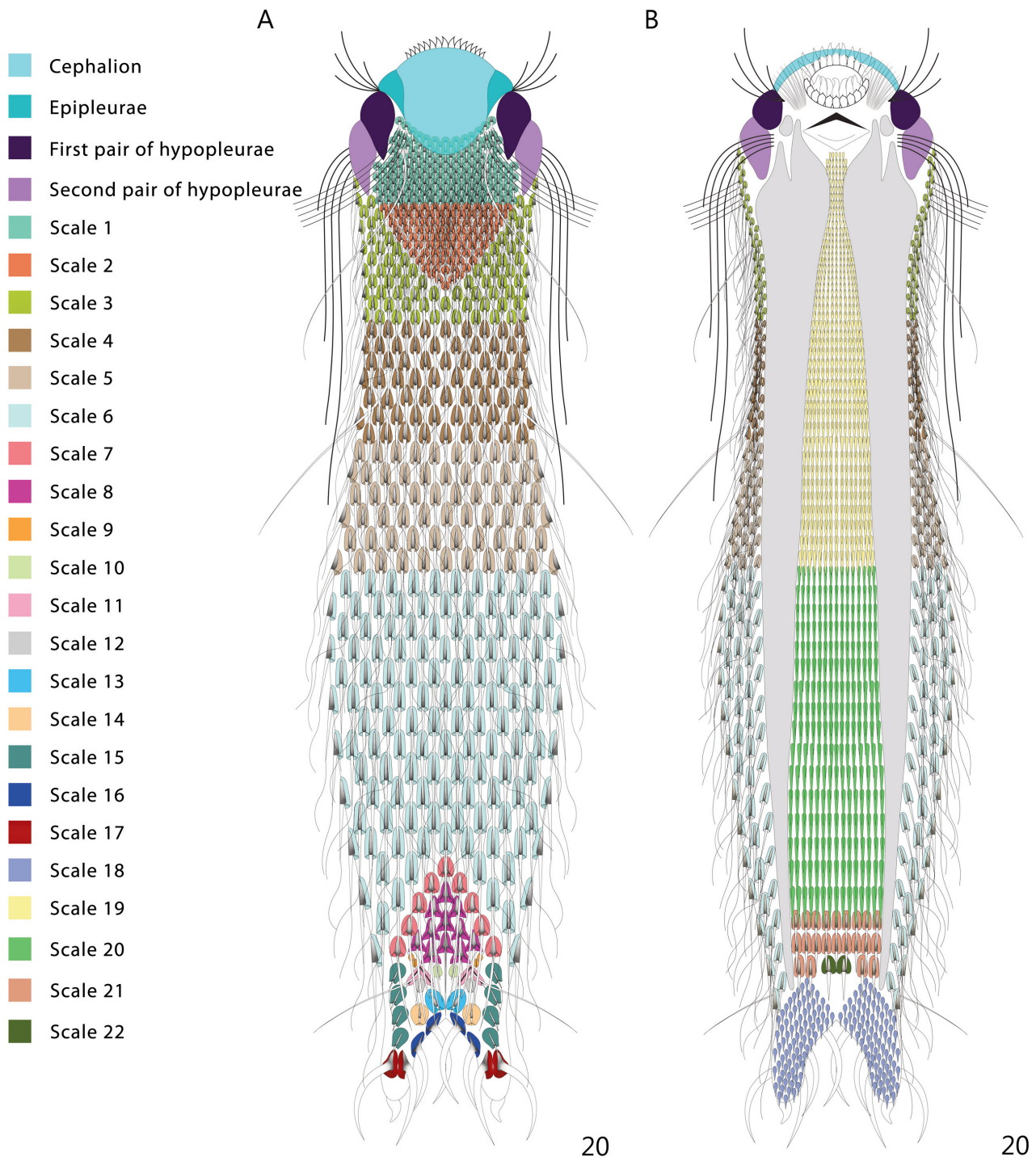


Figure 6. *Chaetonotus* (*Wolterecka*) *semovitus* sp. nov. Schematic drawings with various type of scales and characters marked. (A) Dorsal body view; (B) ventral body view. Light grey areas indicate the areas of the insertion of ciliary bands.

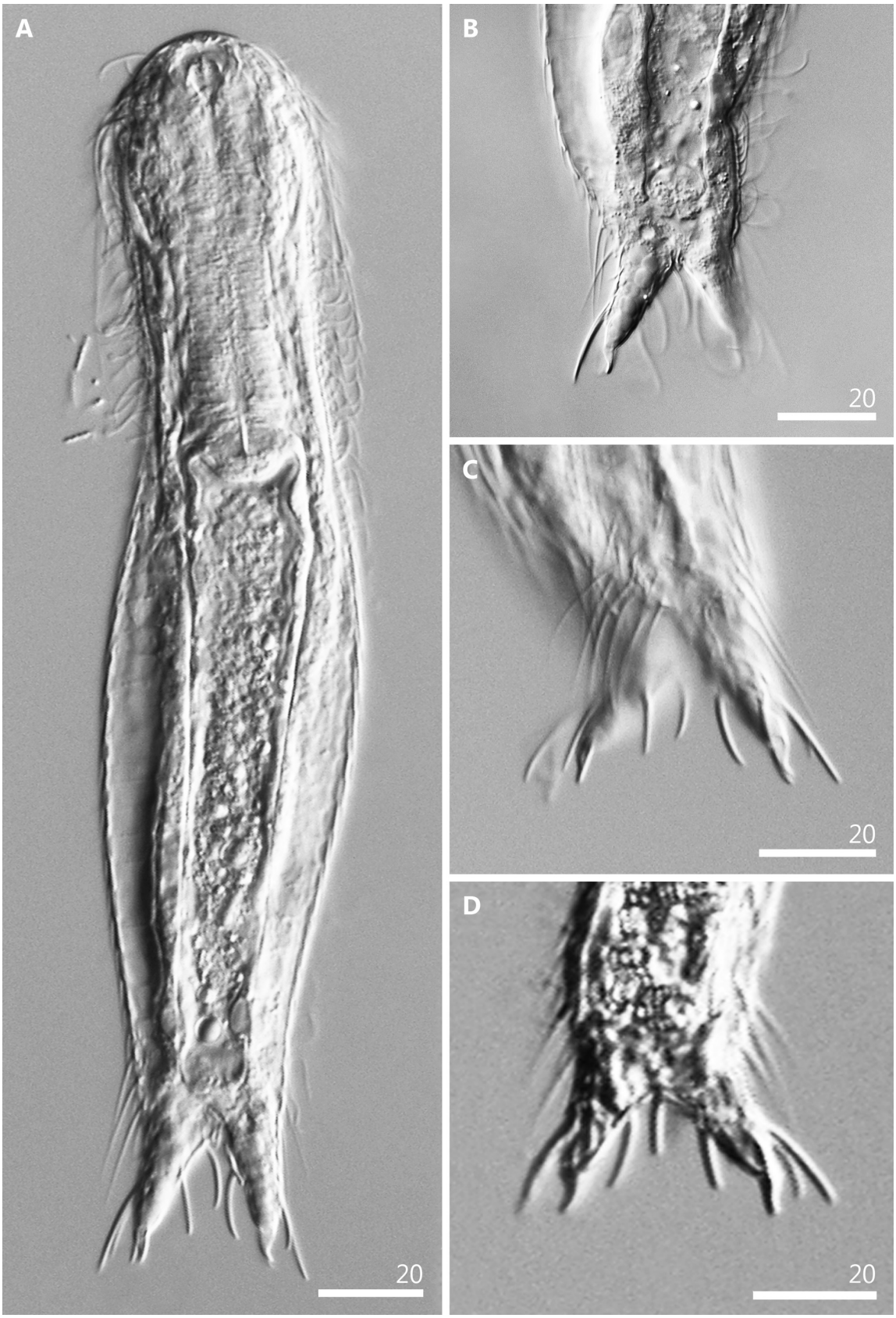


Figure 7. *Chaetonotus* (*Wolterecka*) *semovitus* sp. nov. (A) Habitus; (B, C, D) view of posterior body end.

from the furca base to the inner furcal indentation (Figs 2, 7).

The size of the scales gradually and very slightly increases from the head towards the posterior trunk region. Moreover, the scales of the dorsal, dorsolateral, lateral, ventrolateral and ventral surface of the head, neck, and trunk gradually decreases slightly, insignificantly in size towards the ventral ciliary bands (Tables 2–3). However, the scales in the ventral longitudinal row located closest to the ciliary band are only slightly smaller than the other scales and their anterior edge is oriented towards the bands at an angle of *ca.* 5° (Figs 2, 10). Scales from dorsal, dorsolateral, lateral to ventrolateral head and neck surfaces and from dorsal, dorsolateral, lateral, ventrolateral to ventral body surfaces of the anterior and central trunk region do not vary in shape.

All spines emerging from keels on posterior region of scales and have no lateral denticles. Spines arising from the highest part of the keels and basally run straight, are thick and conical in shape, subsequently spines strongly but gradually tapering to their hair-like, pointed ends and beginning more bent and flexible (Figs 2, 7, 9–11). On the dorsal surface of posterior trunk region, from larger scales (scales 7 and 8) located from *ca*. U73 to *ca*. U83, in the triangle area, arising stronger and longer spines. These spines are thick, conical in shape and basally bent and gradually tapers to their pointed ends, but the posterior segment of these spines are not hair-like or flexible.

The length of spines gradually increases from the head towards the posterior trunk region. Moreover, the length of spines gradually decreases slightly from the dorsal surface, along with the dorsolateral, lateral, ventrolateral and ventral body surfaces towards the ciliary bands (Table 3). The last pair of trunk lateral spines is not longer nor thicker than remaining posterior lateral spines. On the furcal appendages, from four pairs of scales 16 and 17 arising rigid, thick and strongly curved spines. These spines are hook-shaped and gradually tappers to their slightly rounded ends. The length of these spines increases from the first to the last, fourth pair located dorsolaterally. The length of spines arising from third and fourth scales pairs reach beyond the posterior ends of furcal branches (Figs 2, 4, 11).

This species has three pairs of dorsal sensory bristles. The first and the second pairs emerge from small, rounded papillae. The first pair of sensory bristles is located on the head at U10 and the second pair is located on the neck posterior region (U30) and emerges from small, rounded papillae. The third pair of sensory bristles emerges from a double-keeled scales located on the posterior trunk region at U82–U83. These scales are trianglular with strongly rounded edges and a deep, trianglular posterior notches. The keels of these scales are straight and connected and they pass into short, conical shaped spines (Figs 2, 4, 5K).

The ventral locomotory cilia are arranged in two longitudinal bands that begin at U7 and run back to U84 (Fig. 2C). On the head and the neck the ciliary bands are very wide, and occupy the entire ventral field (Figs 8–9). From the anterior trunk region the longitudinal ciliary bands start to narrow becoming very thin at the posterior end. Additional locomotory cilia are present as paired patch between first pair of hypopleurae and the lateral edges of the longitudinal ciliary band, at U7–U8. The entire ventral interciliary field is covered by small oval to semirectangular scales. These scales are keeless, convex and become higher toward their posterior edges which smoothly pass into the thick conical shaped spines tapering to their ends (scales 19 and 20) (Figs 5S, 5T). Towards the furcal base, the scales become more pronounced and the separated keels begin to appear and the spines become arising from keels instead all posterior edge (scales 21) (Fig. 5U). The scales size and spines length gradually increases from the anterior to the posterior body end. The ventral interciliary field scales arrangement in the longitudinal rows located closest to the ciliary bands are significantly smaller and narrower than the scales in the central rows. The scales in single longitudinal rows on ventral interciliary field are arrangement in straight lines, one after another (Figs 2C, 10), and are located close to another and juxtaposed but they do not have overlapping edges. The pair of terminal scales on the ventral interciliary field is small and only slightly larger than neighboring scales from posterior interciliary field region. These scales are located at U79–U82 and are ovoid with straight posterior edges and with shallow concaved posterior ends. These scales possess narrow, slightly triangle keels and a straight, thick, conical shaped spines tapering to their ends (scales 22) (Figs 2C, 5W). The spines are short and do not reach to the furcal indentation. Posteriory to the pair of ventral interciliary field terminal scale are located the necked area without any scales. This area has semipentagonal shape and expands from *ca*. U83 to *ca*. U86. On these nacked area the anus opening are present.

The pharynx (from U3 to U55) is long, wide and massive, with regularly corrugated lateral edges. It is conical in shape and has only insensibly demarcated anterior and slightly stronger posterior dilatations. In the anterior dilatation, cuticular reinforcements are located in the form of two, straight rods merged with each other at straight angles (at U7–U8). The pharynx is connected through the pharyngeal-intestinal junction to the straight intestine (running from U56 to U88). The pharyngeal-intestinal junction is clearly demarcated, short and wide (U56) (Fig 2B, 12). The intestine has a short separate anterior section running from U56 to

U57 (Figs 2B, 12). The X-organ and sperm packets of this species were not observed.

Remarks. *Chaetonotus* (*Wolterecka*) *semovitus* sp. nov. has the seven-lobed head, with present two pairs of hypopleura. In others Chaetonotidae representatives, head usually is five-lobed or three-lobed or is rarely devoid cephalic plates. The seven-lobed head seems to be uncommon character and is pointed for the first time. Also, the presence of three pairs of cephalic cilia is noted for the first time and not mentioned before. The first and the second pair of cephalic cilia have construction and arrangement typical for chaetonotidian species, whereas the third pair is arrangement on the head dorsal side, and it cilia are thin and flexible, similar in construction to the ventral locomotive cilia in longitudinal bands (Figs 3, 9A). This diferences may suggest that the third pair of cephalic cilia is not homologus to the two remanin pair but consist

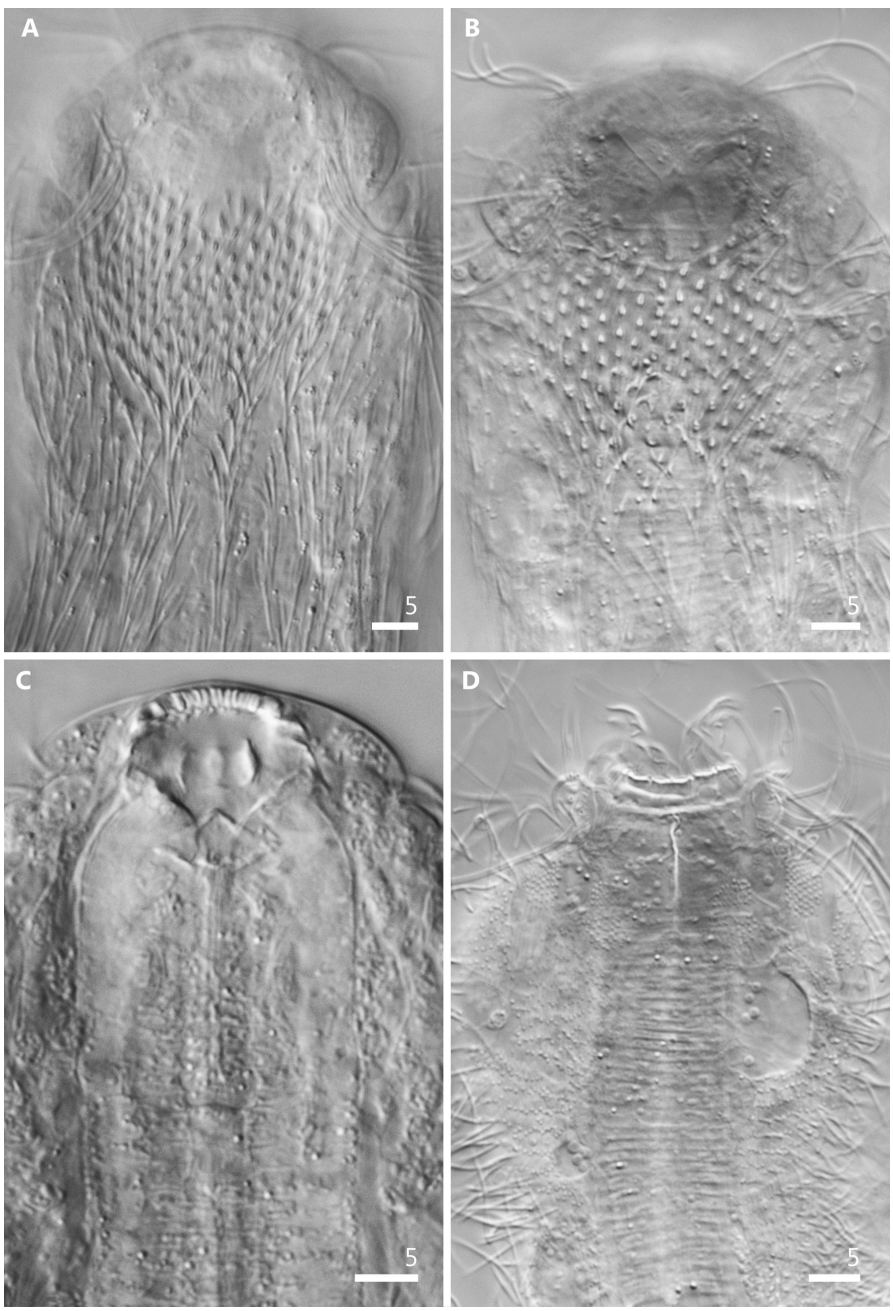


Figure 8. *Chaetonotus* (*Wolterecka*) *semovitus* sp. nov. (A) habitus; (B, C) dorsal view of head and neck region; (D) internal view of head and neck region; (E) ventral view of head and neck region.

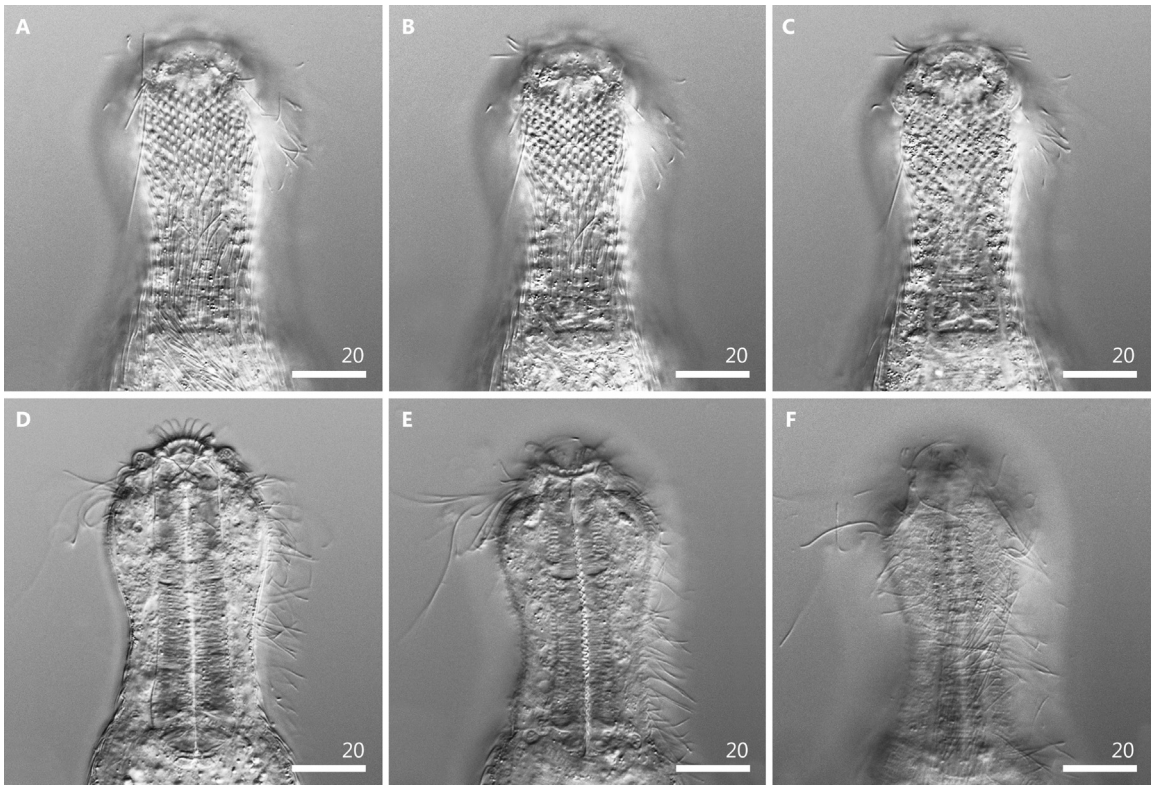


Figure 9. *Chaetonotus* (*Wolterecka*) *semovitus* sp. nov. (A, B, C) Dorsal view of head and neck region; (D, E) Internal view of head and neck region; (F) ventral view of head and neck region.

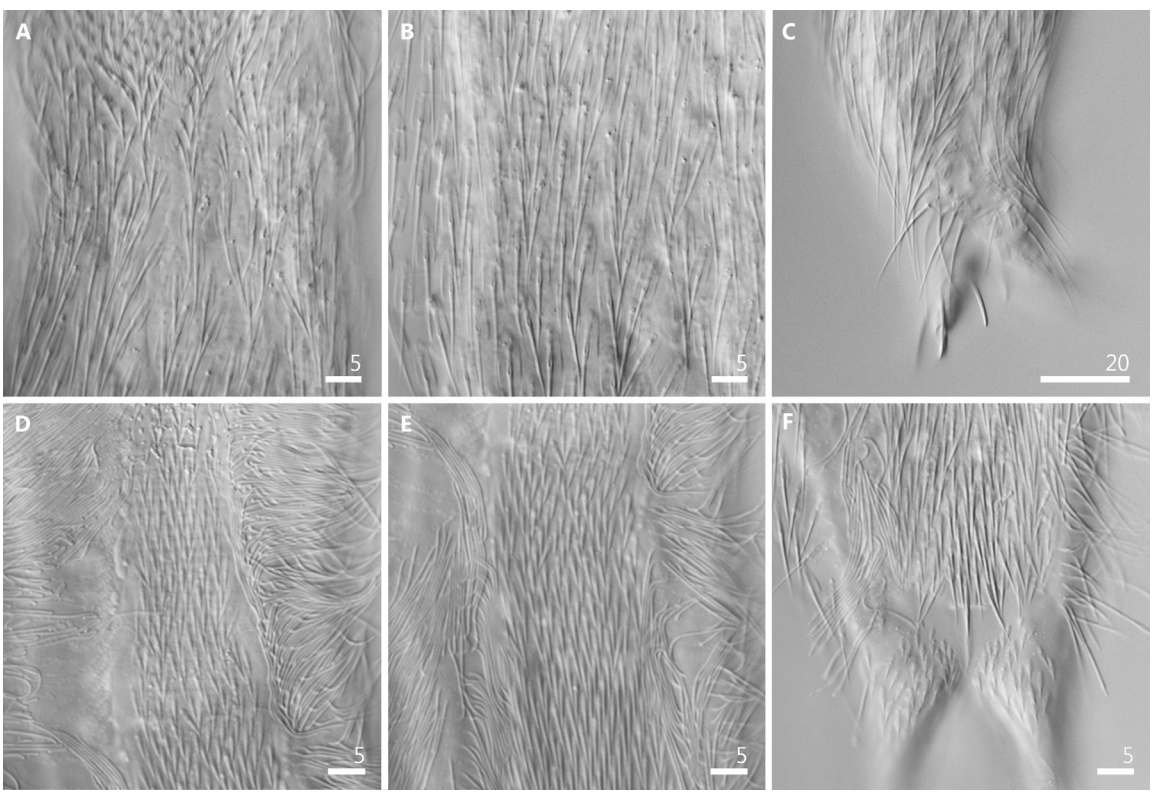


Figure 10. *Chaetonotus* (*Wolterecka*) *semovitus* sp. nov. (A) Dorsal head and neck region scales and spines; (B) dorsal anterior trunk region scales and spines; (C) dorsal posterior trunk region, furca base and furcal appendages scales and spines; (D) ventral head and neck region scales and spines; (E) ventral anterior trunk region scales and spines; (F) ventral posterior trunk region, furca base and furcal appendages scales and spines.

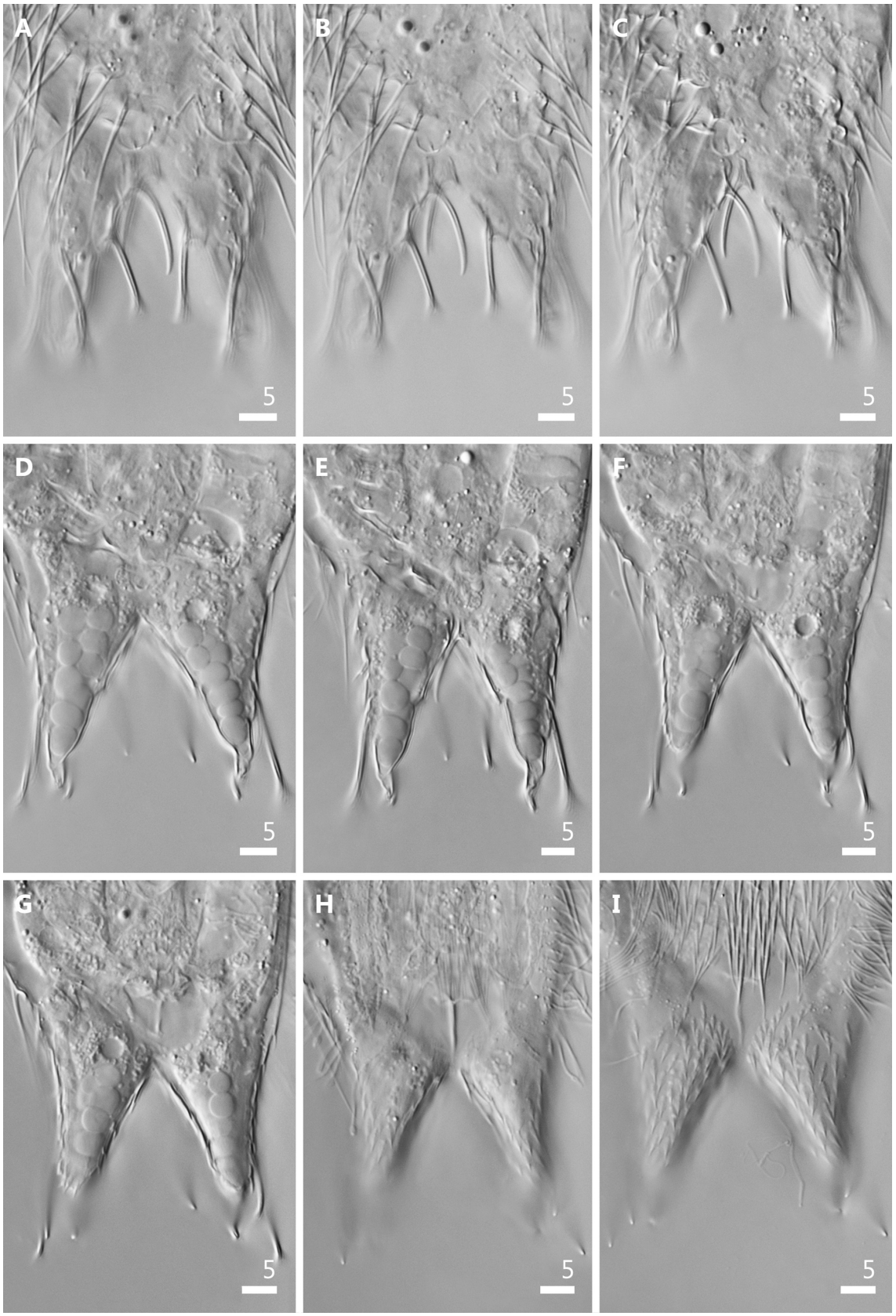


Figure 11. *Chaetonotus* (*Wolterecka*) *semovitus* sp. nov. (A–J) Section by the Posterior trunk region, furca base and furcal appendages.

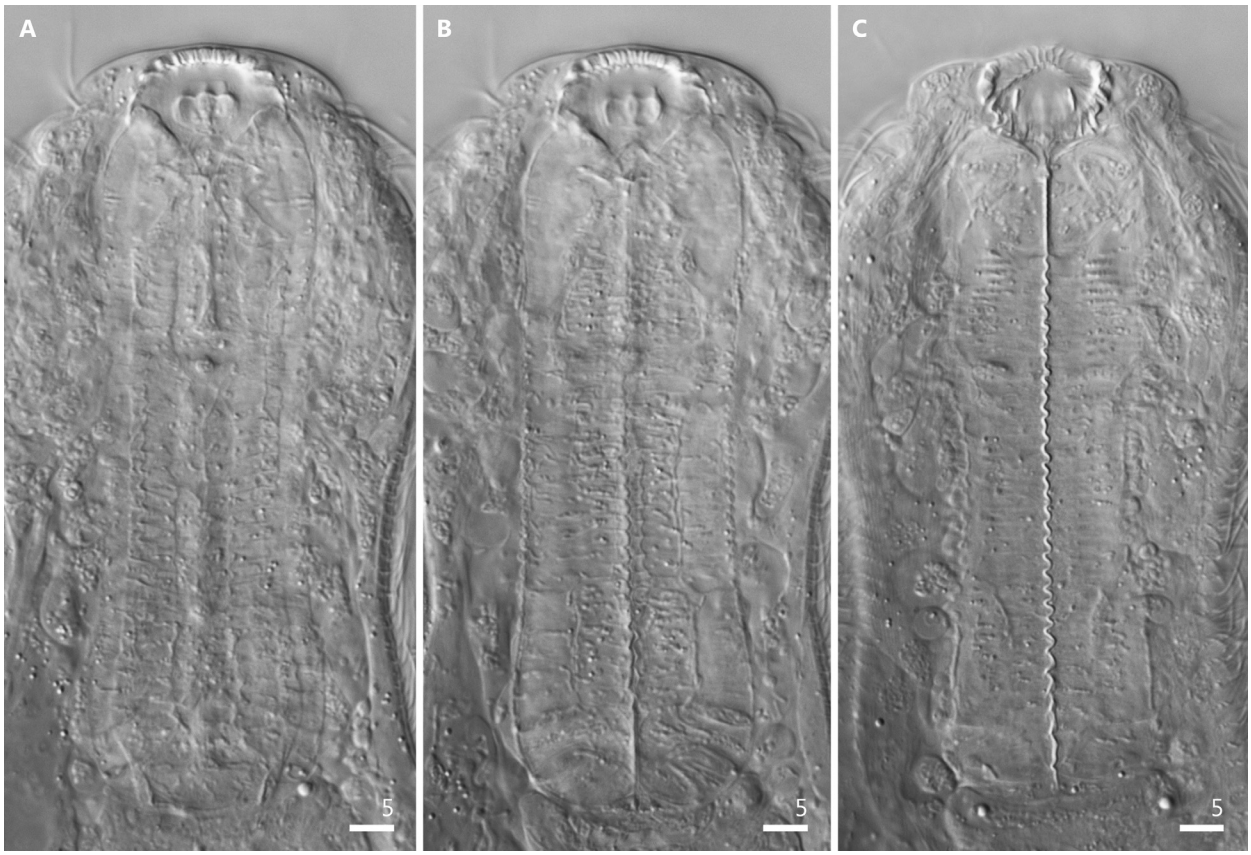


Figure 12. *Chaetonotus* (*Wolterecka*) *semovitus* sp. nov. (A–C) Detail of the pharynx.

a new observe, most likely, sensory structureof different origin. The precise of function this new observed characted need more detailed investigation like this conducted on sensory cilia by Hochberg (2001). This features may be more common and observed also in other species from *Wolterecka* line but overlooked or not considered in previous analyzes (see Fig. 13). The cephalic cilia modification have been observed in Chaetonotidae only once, in *Undula paraensis* Kisielewski, 1991 (subfamily Undulinae Kisielewski, 1991) where cepahlic cilia are varied on three different types. In other familise viz. Dasydytidae Daday, 1905 and Neogosseidae Remane, 1927 modified cephalic cilia have a locomotor function, and in Xenotrichulidae Remane, 1927 where cephalic cilia have a typical sensory function but posses another, mostly merged construction.

Because of the long pharynx in relation to the length of the body and intestine, the pharynx-intestine ratio (I) has higher values in this species than in most of genus *Chaetonotus* representatives.

All individuals were in partenogenethic life phase.

Sequence diversity and phylogenetic relationships. The mitochondrial and nuclear data, including the DNA region coding for COI mtDNA (695 bp), 18S rRNA (1780 bp), ITS1, 5.8S, ITS2 (1309 bp) and 28S rRNA (3681 bp), showed no intraspecific variation. All sequences are deposited in GenBank under accession numbers, holotype: MH211121 (COI), MH166749 (18S), MH166754 (ITS) and MH166746 (28S); and paratypes: MH211119–MH211120 (COI), MH166747–MH166748 (18S), MH166752–MH166753 (ITS) and MH166744–MH166745 (28S).

The nucleotide blast search of 18S and 28S rRNA sequences of *Chaetonotus* (*Wolterecka*) *semovitus* sp. nov. suggested *Chaetonotus* (*Wolterecka*) *uncinus* MT62, which was collected and identified by M. Antonio Todaro from Brazil in 2008, as the most similar taxon. The similarity within 18S rRNA was equal to 100%, whereas within 28S rRNS it reached 99% identity. The ITS rRNA and COI mtDNA data are not available for the Brasilian specimen. The very high similarity scores suggest that *C.* (*W.*) *uncinus* MT62 and *C.* (*W.*) *semovitus* sp. nov. belong to the same taxon or are very closely related species (see Fig. 13 and the 'Differential diagnosis' section below).

Differential diagnosis. In subgenus *Wolterecka* Mola, 1932, includes so far only threenominal species, namely *C.* (*W.*) *caudalspinosus* Visvesvara, 1964, *C.* (*W.*) *sudeticus* Kisielewski, 1984 and *C.* (*W.*) *uncinus* Voigt, 1902. All of them are similar to the newly

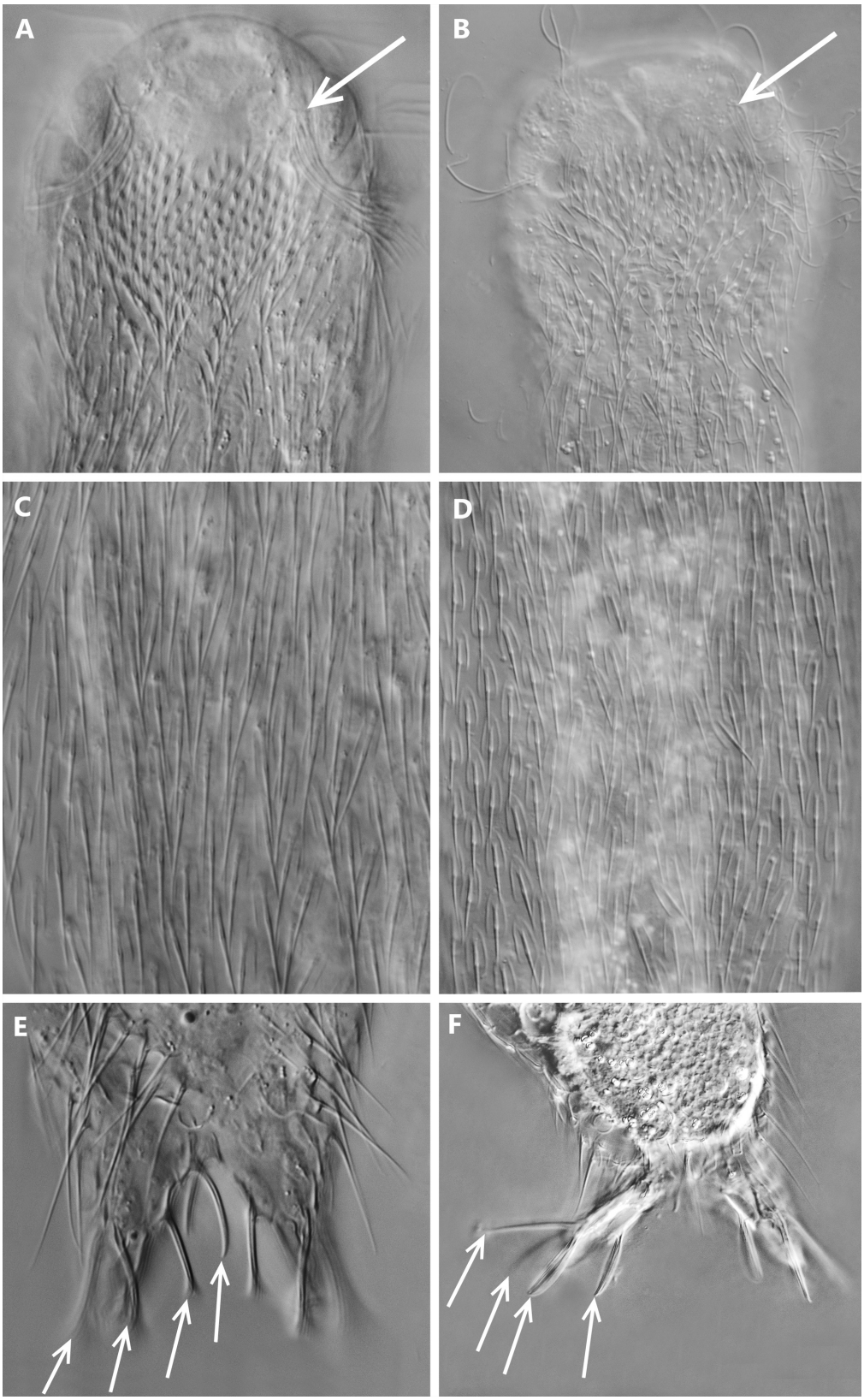


Figure 13. Comparition between *Chaetonotus* (*Wolterecka*) *semovitus* sp. nov. (A, C, E) and *Chaetonotus* (*Wolterecka*) *uncinus* MT62 (B, D, F). (A–B) Dorsal head and neck region scales and spines; (C–D) trunk dorsal scales; (E, F) furcal appendages. Arrows pointing the furcal hook-like spines. Fotomicrographs B, D and F after M. Antonio Todaro with permission.

Table 4. Condensed comparison of the most important differential characters between species most similar to *Chaetonotus* (*Wolterecka*) *semovitus* sp. nov. Measurements are given in micrometres (μm). NA – data not available; – – not applicable.

Character	*C.* (*W.*) *semovitus* sp. nov.	*C.* (*W.*) *caudalspinosus*	*C.* (*W.*) *sudeticus*	*C.* (*W.*) *uncinus*	*C.* (*W.*) *uncinus* MT62
Body length	216.4–245.1	185.0–220.0	211.0–221.0	258.0–340.0	NA
Cephalic pleurae	Head seven-lobed. Cephalion long and wide, strongly covex with free posterior edge. Epipleurae adhered to lateral cephalion edges, located on dorsal head side, weakly convex; two pairs of hypopleurs located on dorsal, dorsolateral and ventrolateral body sides, both convex and significantly larger than epipleurae. Hypostomium wide, short, rhomboidal with reinforced anterior edge and with reinforced cambered bar near the posterior edge	Head five-lobed. Cephalion long and wide, strongly convex with free posterior edge. Epipleurae and hypopleurae similar in size, located mostly laterally but clearly visible on the dorsal body side. Hypostomium – NA	Head outline three-lobed, but with five cephalic plates. Cephalion large, wide. Epipleurae and hypopleurae very small and moved to the dorsal side. Hypostomium with selvage	Head five-lobed. Cephalion short and flat. Epipleurae and hypopleurae small. Hypostomium present	Head seven-lobed. Cephalion long and wide, slightly covex with free posterior edge. Epipleurae adhered to lateral cephalion edges, located on dorsal head side, weakly convex; two pairs of hypopleurs located on dorsal, dorsolateral and ventrolateral body sides, both slightly convex and significantly larger than epipleurae. Hypostomium wide, short, rhomboidal shaped
Number of cephalic cilia tuft	3 pairs	2 pairs	1 pair	2 pairs	3 pairs
Place of emerging cephalic cilia	First tufts pair: ventro-terminally between cephalion edge and anterior edge of first hypopleurae pair; second tufts pair: ventrally in notches between first and second pair of hypopleurae; third tufts pair: dorsally, in line in area between cephalion and first pair of hypopleurae	Anterior tufts: dorsoterminal between cephalion and epipleurae; posterior tufts: laterally, between epipleurae and hypopleurae	Dorsally, between epipleurae and hypopleurae	Anterior tufts: dorsoterminal between cephalion and epipleurae; posterior tufts: laterally, between epipleurae and hypopleurae	First tufts pair: ventro-terminally between cephalion edge and anterior edge of first hypopleurae pair; second tufts pair: ventrally in notches between first and second pair of hypopleurae; third tufts pair: dorsally, in line in area between cephalion and first pair of hypopleurae

Character	*C.* (*W.*) *semovitus* sp. nov.	*C.* (*W.*) *caudalspinosus*	*C.* (*W.*) *sudeticus*	*C.* (*W.*) *uncinus*	*C.* (*W.*) *uncinus* MT62
Presence of cuticular teeth in the mouth ring	Present; two distinct, large teeth	NA	Absent	Absent	Present; two teeth
Pharynx length	80.0–80.9	75.0–82.0	46.0	80.0–100.0	NA
Pharynx shape	Conical with corrugated lateral edges and weakly marked anterior and wider posterior dilatation	Cylindrical with weakly marked anterior and wider posterior dilatation	Conical, without anterior dilatation and with extended posterior ends	Cylindrical with weakly marked anterior and wider posterior dilatation	Conical with corrugated lateral edges and weakly marked anterior and wider posterior dilatation
Presence of cuticular reinforcements in the pharynx	Present (two straight connected rods)	NA	Present (pair of longitudinal selvages located latterally in anterior pharynx region)	Absent	Present (two straight connected rods)
Anterior intestine section differing in form of morphology	Present	NA	Absent	Absent	NA
Furca length	30.1–32.4	25.0–27.0	28.0	23.0–27.0	NA
Adhesive tube length	3.7–6.1	NA	9.0–10.0	5.0	NA
Type of furcal indentation	V-shaped; swollen-shaped furcal branches	V-shaped	Eliptical in shape	V-shaped; slightly swollen-shaped furcal branches	V-shaped; swollen-shaped furcal branches
Number of scales in central longitudinal row	39–43	>30	NA	25–32	40
Total number of longitudinal alternating rows of scales	37–43	16	NA	36	NA
Type of scales	Scales small, one-lobed; almost all with concaved posterior edge, keeled and spined; shape differentiated between head, neck and trunk regions	NA	On the head region small cuticular formation resembling peduncular scales, neck and trunk regions covered by spined scales with hardly noticeably borders	Small, one-lobed, arrowhead-shaped with posterior notches, keeled and spined	Scales small, one-lobed; almost all with concaved posterior edge, keeled and spined; shape differentiated between head, neck and trunk regions
Scales size variation	Size increases gradually from the head towards the trunk posterior region; scale size slightly	NA	Body scales similar in size except one pair of large scales covered inner borders of basal parts of	Similar size all over the body	Size increases gradually from the head towards the trunk posterior region; scale size slightly

Table 4. Continued

Character	*C.* (*W.*) *semovitus* sp. nov.	*C.* (*W.*) *caudalspinosus*	*C.* (*W.*) *sudeticus*	*C.* (*W.*) *uncinus*	*C.* (*W.*) *uncinus* MT62
	decreases gradually from the dorsal body side of the head, neck and trunk towards the direction of the ventral ciliary bands		in caudal furcal appendages		decreases gradually from the dorsal body side of the head, neck and trunk towards the direction of the ventral ciliary bands
Scale size	Head: 2.1–4.8 x 1.5–3.0 Neck: 2.9–7.3 x 1.8–3.2 Trunk: 3.7–11.0 x 1.5–3.7	NA	NA	NA	NA
Alignment of scales	On dorsal head and neck anterior regions scales overlapping or adher one another, on remaning body parts scales edges juxtaposed but do not overlap	NA	NA	Located far from one another, edges do not overlap	On dorsal head region scales overlapping or adher one another, on remaning body parts scales edges juxtaposed but do not overlap
Presence of a scale of a different type on the dorsal and dorsolateral side on the furcal base	Present	NA	NA	Absent	Present
Scale coverage of the ventral interciliary field	Entire ventral interciliary field covered with scales	NA	Covered from the central part of pharyngeal region to the trunk posterior region	NA	Entire ventral interciliary field covered with scales
Type of scales on the ventral interciliary field	Scales small, oval in shape with thick, conical spines tappering to their ends	NA	Only smal spines visible	NA	Scales small, oval in shape with thick, conical spines tappering to their ends
Number of terminal scales of the ventral interciliary field	1 pair	NA	1 pair	NA	NA
Type of terminal scales on the ventral interciliary field	Small, oval with straight, concaved posterior edge, without posterior notches, keeled and spined	NA	Spined scales	NA	NA

Character	*C.* (*W.*) *semovitus* sp. nov.	*C.* (*W.*) *caudalspinosus*	*C.* (*W.*) *sudeticus*	*C.* (*W.*) *uncinus*	*C.* (*W.*) *uncinus* MT62
Spines type	Almost all basaly straight, strong, thick, Conical gradually tappering to the flexible, bent hair-like ends or on dorsal posterior trunk region tappering to the narrow, pointed ends; on the furcal appendages very strong, rigid, hook-shaped spines	Almost all simple and straight; on furcal appendages longer and curved	Almost all simple, straight, short spines; on furcal appendages one pair of thick, hook-shaped spines	Simple spines	On the head dorsal region from scales arrising flat, strongly extended spines, like oval-shaped lamellae arrising from keels and goes pararell to the scales and after this oval basal segments subsequently narrowing to the hair-like ends. Remaning spines almost all basaly straight, strong, thick, Conical gradually tappering to the flexible, bent hair-like ends or on dorsal posterior trunk region tappering to the narrow, pointed ends; on the furcal appendages very strong, rigid, hook-shaped spines
Spine length variation	Spines length gradually, slightly increases from the head towards the posterior trunk region; spine length decrease slightly and gradually from dorsal body side towards the ciliary bands	Spines lenght increas from the head to the posterior body region	Spines short, longer only on dorsal trunk posteriormost region	All spines of similar length, only furcal appendages spines are longer	Spines length gradually, slightly increases from the head towards the posterior trunk region; spine length decrease slightly and gradually from dorsal body side towards the ciliary bands
Spine length	Head: 8.9–14.7 Neck: 12.0–18.9 Trunk: 15.2–25.2	Anterior region: 10.0–12.0 Posterior region: 20.0–22.0	Head, neck, trunk: 3.5 Dorsal trunk posteriormost region: 8.0–11.0	Head, neck, trunk: < 10	NA
Type of posteriormost pair of trunk lateral spines Presence of hook-shaped spines on furcal appendages	The same as other lateral spines Present	The same as other lateral spines Absent	The same as other lateral spines Present	The same as other lateral spines Absent	The same as other lateral spines Present

Table 4. Continued

Character	*C.* (*W.*) *semovitus* sp. nov.	*C.* (*W.*) *caudalspinosus*	*C.* (*W.*) *sudeticus*	*C.* (*W.*) *uncinus*	*C.* (*W.*) *uncinus* MT62
Number and arrangement of long spines on furcal appendages	8 spines (4 pairs); 2 pairs dorsaly and internaly, 2 pairs dorsolateraly	9 spines; 3 spines dorsaly and internaly, 1 pair dorsaly, 2 pairs dorsolateraly	2 spines (1 pair); dorsaly and internaly	8 spines (4 pairs); 1 pair dorsaly and internaly, 3 pairs dorsolateraly	8 spines (4 pairs); 1 pair dorsaly and internaly on furcal appendages, 2 pairs dorsolaterally, 1 pair lateraly
Length of long spines on furcal appendages	12.2–21.2	15.0–25.0	5.5–7.0	19.0–20.0	NA
Type of parafurcal spines	Thick, short, conical spines tappering to the pointed ends	NA	NA	3 pairs, longer and thicker than laterall posterior trunk region scales	1 pair long, rigid, hook-shaped spines
Ventral locomotor cilia	Two longitudinal bands and one pair of separeted patches; bands much wider on the head and neck region, narrowing to the trunk posterior region	NA	Two longitudinal bands very wide on the head, very narrow on the trunk	Two longitudinal bands and two pair of separeted patches; bands branched and wide on the head region, narrowing to the trunk posterior region	Two longitudinal bands and one pair of separeted patches; bands much wider on the head and neck region, narrowing to the trunk posterior region
Number of dorsal sensory bristles	3 pairs	2 pairs	1 pair	NA	3 pairs

described taxon in terms of body size and shape, very short adhesive tubes, the presence of rigid spines on the furcal appendages, shape of ventral ciliary bands on the head region. Despite the high similarity, the new species shows a striking diversity of scales and spines not described for the other taxa. The comparison between the new species and the others is summarised in Table 4. *C.* (*W.*) *caudalspinosus* and *C.* (*W.*) *sudeticus* have been found only once and data come from the original descriptions; *C.* (*W.*) *uncinus* has been recorded several time in different European countries: Germany (Voigt 1902, 1904), Italy (Mola 1932, Balsamo 1983), Poland (Kisielewski 1986, Kisielewski *et* Kisielewska 1986), Romania (Reduscu 1967) and Ukraine (Jakubski 1919.

Chaetonotus (*Wolterecka*) *uncinus* MT62 Todaro, 2008 used in the phylogenetic analyses performed by Kånneby *et al*. (2013) deserves special treatment. As was mentioned above (see the 'Sequence diversity and phylogenetic relationships' section), the specimen found in Brazil and the species found in Vienna have identical 18S rRNA and very similar 28S rRNA sequences. The photomicrograph documentation available by M. Antonio Todaro (personal comunication) shows significant differences between *C.* (*W.*) *uncinus* MT62 and nominal *C.* (*W.*) *uncinus, e.g*. type and shape of scales and their diversity, type of spines, number of cephalic plates, number of cephalic cilia and number of dorsal sensory bristles, which suggests that we are dealing with two separate species (see Fig. 13). In Table 4 are reported the morphometry data of the two species.

Discussion

Chaetonotus (*Wolterecka*) *semovitus* sp. nov. belongs to the rare subgenus *Wolterecka* Mola, 1932 and increases the number of species belonging to it to four. Probably, the low number of known *Wolterecka* representatives does not reflect their real diversity but is caused by the low abundance of specimens and their scarce distribution, thus the low probability of finding them during gastrotrichological studies. Higher species richness could probably be provided by the distribution of different *Wolterecka* species among world regions *C.* (*W.*) *uncinus* MT62 comes from South America (Kånneby *et al*. 2013), *C.* (*W.*) *caudalspinosus* has been described from Asia (Visvesvara 1964), and *C.* (*W.*) *uncinus* and *C.* (*W.*) *sudeticus* are known only from Europe (*e.g.* Voigt 1902, Balsamo 1983, Schwank 1990, Kisielewski 1984, 1997a). The new species was recorded from Austria, a European country, but because it was found inside the Schönbrunn Palm House the area of its natural origin remains uncertain. The gastrotrichofauna of Austria is virtually unexplored (Vornatscher 1938, Ruttner-Kolisko 1955, Balsamo *et al*. 2014), thus the new *Wolterecka* representatives might be an autochthonous taxon brought to the greenhouse from neighboring regions. On the other hand, the high morphological and molecular similarities between *C.* (*W.*) *semovitus* sp. nov. and Brazilian *C.* (*W.*) *uncinus* MT62 may suggest that both these species are closely related and that their radiation came via one ancestor from a single geographical area, perhaps from South America. The unintentional introduction of into the palm house together with plants from exotic natural habitats is possible (see Kolicka *et al*. 2013, Kolicka 2014, 2016, 2018). Greenhouses are considered to be inland islands with a moderate climate which can potentially provide favorable conditions for population development and long-term existence of alien species (Zawierucha *et al*. 2013). However, the Schönbrunn Palm House was reconstructed and renovated thrice, and the reservoirs and water inflow in which *C.* (*W.*) *semovitus* sp. nov. was found were built during the last renovation, (2011–2014). In this framework, *C.* (*W.*) *semovitus* sp. nov. could have been brought into the palm house from a natural, exotic habitat during collection of vegetation that was conducted in the 19th and first decade of the 20th century, or perhaps it came from more recent material exchanges with other palm houses. A further investigation of gastrotrichofauna from different world regions and subsequently greenhouses could help us recognise the natural ranges of the occurrence of species together with their routes of both natural and anthropogenic introduction.

Acknowledgements

I thank Beata Starczewska for preparing the drawings of the new species and the figures used in this paper. I also sincerely thank Mr. Daniel Rohrauer and all of the staff members of the Schönbrunn Palm House in Vienna, particularly Mr. Norbert Lehrl, for their kindness and support during collection of the samples and Mr. Michael Knaack, for additional information about collection and building. I sincerely thank Professor M. Antonio Todaro for making available the photomicrographs of *Chaetonotus* (*Wolterecka*) *uncinus* MT62 with permission for using them in this work and for a very constructive discussion. I would also like to thank Professor Maria Balsamo for sharing the literature with me. The studies were supported by National Science Centre grant no. 2015/17/N/NZ8/01568 titled "Gastrotricha in palm houses as a model for research on phenotypic variability and cryptic diversity".

References

Balsamo, M. 1983. Gastrotrichi. *In*: Consiglio Nazionale delle Ricerche. Guide per il riconoscimento delle specie animali delle acque interne italiane, 20: 1–92.

Balsamo, M., d'Hondt, J-.L, Kisielewski, J., Todaro, M. A., Tongiorgi, P., Gudi, L., Grilli, P., and Y. de Jong. 2015. Fauna Europea: Gastrotricha. Biodiversity Data Journal, 3, e5800.

Balsamo, M., Grilli, P., Guidi, L., and J-.L. d'Hondt. 2014. Gastrotricha: biology, ecology and systematics: families Dasydytidae, Dichaeturidae, Neogosseidae, Proichthydiidae. In: 'Identification Guides to the Plankton and Benthos of Inland Waters. 24'. (Ed. H. J. F. Dumont.), pp. 1–187. (Backhuys Publishers, Margraf Publishers GmbH: Weikeirsheim, Germany.).

Balsamo, M., Guidi, L., and J-.L. d'Hondt. 2013. Phylum Gastrotricha. *In*: Zhang, Z.-Q. (Ed.) Animal Biodiversity: An Outline of Higher-level Classification and Survey of Taxonomic Richness. Zootaxa, 3703(1): 79–82.

Blake, C. H. 1933. Nomenclatorial notes on Gastrotricha. Science, 77: 606.

Dabert, J., Ehrnsberger, R., and M. Dabert. 2008. *Glaucalges tytonis* sp. nov. (Analgoidea: Xolalgidae) from the barn owl *Tyto alba* (Strigiformes: Tytonidae): compiling morphology with DNA barcode data for taxa descriptions in mites (Acari). Zootaxa, 1719: 41–52.

Dabert, M., Proctor, H. C., and J. Dabert. 2016. Higher-level molecular phylogeny of the water mites (Acariformes: Prostigmata: Parasitengonina: Hydrachnidiae). Molecular Phylogenetics and Evolution, 101, 75–90. http://dx.doi.org/10.1016/j.ympev.2016.05.004.

Dabert, M., Witalinski, W., Kaźmierski, A., Olszanowski, Z., and J. Dabert. 2010. Molecular phylogeny of acariform mites (Acari, Arachnida): strong conflict between phylogenetic signal and long branch attraction artifacts. Molecular Phylogenetics and Evolution, 56, 222–241. http://dx.doi.org/10.1016/j.ympev.2009.12.020.

Daday, E. V. von. 1905. Untersuchungen uber die susswasser mikrofauna Paraguays. Zoology of Bulgaria, 44: 72–86.

Ehrenberg, C. G. 1830. Organisation, Systematik, und geographisches Verhältnis der Infusionstierchen. F. Dümmler, Berlin, 108 pp. http://dx.doi.org/10.5962/bhl.title.2077.

Garaffoni, A. R. S., Araújo, T. Q., Lourenço, A. P., Guidi, L., and M. Balsamo. 2016. A new genus and new species of fresh-water Chaetonotidae (Gastrotricha: Chaetonotida) from Brazil with phylogenetic position inferred from nuclear and mitochondrial DNA sequences. Systematic Biodiversity, http://dx.doi.org/10.1080/14772000.2016.1214189.

Gosse, P. H. 1864. The natural history of the hairy-backed animalcules (Chaetonotidae). The Intellectual Observer, 5, 387–406.

Hochberg, R. 2001. A special form of sensory cilia in *Lepidodermella squamata* (Gastrotricha: Chaetonotida). Ophelia, 55: 137–139.

Hochberg, R., and M. K. Litvaitis. 2000. Phylogeny of Gastrotricha: a Morphology-Based Framework of Gastrotricha Relationships. Biological Bulletin, 198: 299–305.

d'Hondt, J-.L. 1971. Gastrotricha. *In*: Barnes, H. (Ed.), Oceanography and marine biology: an annual review, 9, pp. 141–192. [George Allen and Unwin Ltd.].

Hummon, W. D., Balsamo, M., and M. A. Todaro. 1992. Italian marine Gastrotricha: I. Six new and one redescribed species of Chaetonotida. Bollettino di Zoologia, 59: 499–516.

Hummon, W. D., and M. A. Todaro. 2010. Analytic taxonomy and notes on marine, brackish-water and estuarine Gastrotricha. Zootaxa, 2392: 1–32.

International Commission on Zoological Nomenclature 1999. International Code of Zoological Nomenclature, Fourth Edition. The International Trust for Zoological Nomenclature, The Natural History Museum, Cromwell Road, London.

Jakubski, A. W. 1919. Fauna wrotków (Rotatoria) i brzuchorzęsków (Gastrotricha) Stawu Gródeckiego i okolicy. Kosmos, 43: 20–42.

Kånneby, T. and J. J. Kirk. 2017. A new species of *Redudasys* (Gastrotricha: Macrodasyida: Redudasyidae) from the United States. Proceedings of the Biological Society of Washington, 130: 129–139.

Kånneby, T., Todaro, M. A. and U. Jondelius. 2012. A phylogenetic approach to species delimitation in freshwater Gastrotricha from Sweden. Hydrobiologia, 683: 185–202.

Kånneby, T., Todaro, M. A. and U. Jondelius. 2013. Phylogeny of Chaetonotidae and other Paucitubulatina (Gastrotricha: Chaetonotida) and colonization of aquatic ecosystems. Zoologica Scripta, 42: 88–105.

Kånneby, T. and M. Wicksten. 2014. First record of the enigmatic genus Redudasys Kisielewski, 1987 (Gastrotricha: Macrodasyida) from the Northern hemisphere. Zoosystema, 36(4): 723–734.

Kieneke, A. and A. Schmidt-Rhaesa. 2015. Gastrotricha. *In*: Schmidt-Rhaesa (Ed.) Handbook of Zoology. Vol. 3 Gastrotricha and Gnathifera. Pp. 1–134. (Berlin, Boston: De Gruyter).

Kisielewska, G., Kolicka, M. and K. Zawierucha. 2015. Prey or parasite? The first observations of live Euglenida in the intestine of Gastrotricha. European Journal of Protistology, 51: 138–141.

Kisielewski, J. 1981. Gastrotricha from raised and transitional peat bogs in Poland. Monografie Fauny Polski, 11. Polska Akademia Nauk, Kraków, 143 pp.

Kisielewski, J. 1984. Three new freshwater gastrotrichs of the genus *Chaetonotus* Ehrenberg (Chaetonotida) from Poland. Bulletin De l'Academie Polonaise Des Sciences. Serie Des Sciences Biologiques, 32: 293–301.

Kisielewski, J. 1986. Freshwater Gastrotricha of Poland. VII. Gastrotricha of extremely eutrophicated water bodies. Fragmenta Faunistica, 30: 267–295.

Kisielewski, J. 1991. Inland-water Gastrotricha from Brazil. Annales Zoologici, Warszawa, 43: 1–168.

Kisielewski, J. 1997a. Brzuchorzęski (Gastrotricha). Fauna Słodkowodna Polski, Zeszyt 31. Wydawnictwo Uniwersytetu Łódzkiego, Łódź, 157 pp.

Kisielewski, J. 1997b. On the subgeneric division of the genus *Chaetonotus* Ehrenberg (Gastrotricha). Annales Zoologici, 46: 145–151.

Kisielewski, J. and G. Kisielewska. 1986. Freshwater Gastrotricha of Poland. I. Gastrotricha from the Tatra and Karkonosze Mountains. Fragmenta Faunistica, 30: 158–295.

Kolicka, M. 2014. Gastrotricha Mečnikow, 1865 from Copenhagen Palm House – contribution to the knowledge of

Lepidodermella intermedia Kånneby, Todaro & Jondelius, 2012 (Chaetonotida, Gastrotricha). Zoosystema, 34(4): 713–722. http://dx.doi.org/10.5252/z2014n4a1.

Kolicka, M. 2016. Gastrotrichs in bromeliads – newly recorded *Chaetonotus* (*Hystricochaetonotus*) *furcatus* Kisielewski, 1991 (Chaetonotida) from the Łódź Palm House. Zoosystema, 38(1): 141–155. http://dx.doi.org/10.5252/z2016n1a5.

Kolicka, M., Dabert, M., Dabert, J., Kånneby, T., and J. Kisielewski. 2016. Bifidochaetus, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. Invertebrate Systematics, 30, 398–419. http://dx.doi.org/10.1071/IS16001.

Kolicka, M., Gadawski, P. and M. Dabert. 2017. A new species of freshwater Chaetonotidae (Gastrotricha, Chaetonotida) from Obodska Cave (Montenegro) based on morphological and molecular characters. European Journal of Taxonomy, 354, 1–30. https://doi.org/10.5852/ejt.2017.354.

Kolicka, M., Kisielewski, J., Nesteruk, T. and K. Zawierucha. 2013. Gastrotricha from the Poznań Palm House — one new subgenus and three new species of freshwater Chaetonotida (Gastrotricha). Zootaxa, 3717(2), 231–279. http:// dx.doi.org/10.11646/zootaxa.3717.2.7.

Kolicka, M., Kotwicki, L. and M. Dabert. 2018. Diversity of Gastrotricha on Spitsbergen (Svalbard Archipelago, Arctic) with a description of seven new species. Annales Zoologici, 68(4): 609–739.

Leasi, F. and M. A. Todaro. 2008. The muscular system of *Musellifer delamarei* (Renaud-Mornant, 1968) and other chaetonotidans with implications for the phylogeny and systematization of the Paucitubulatina (Gastrotricha). Biological Journal of the Linnean Society, 94: 379–398. http:// dx.doi.org/10.1111/j.1095–8312.2008.00974.x.

Mečnikow, E. 1865. Über einige wenig bekannte niedere Thierformen. Zeitschrift für Wissenschaftliche Zoologie, 15: 450–463.

Mola, P. 1932. Gastrotricha delle acque dolci italiane. International Review of Hydrobiology, 26: 397–423.

Müller, O. F. 1773. Vermium terrestrium et fluviatilium saeu animalium infosoriorum, helminthicorum et testaceorum non marinorum, succincta historia. Heineck et Faber, Havniae et Lipsiae, 135 pp. http://dx.doi.org/10.5962/bhl.title.12733.

Navajas, M., Lagnel, J., Gutierrez, J. and P. Boursot. 1998. Species-wide homogeneity of nuclear ribosomal ITS2 sequences in the spider mite *Tetranychus urticae* contrasts with extensive mitochondrial COI polymorphism. Heredity, 80: 742–752.

Rao, G. C. and C. Clausen. 1970. *Planodasys marginalis* gen. et. sp. nov. and Planodasyidae fam. nov. (Gastrotricha Macrodasyoidea). Sarsia, 42: 73–82.

Remane, A. 1925. Organisation und systematische Stellung der aberranten Gastrotrichen. Verhandlungen der deutschen zoologischen. Gesellschaft, 30: 121–128.

Remane, A. 1927a. Beiträge zur Systematik der Süsswassergastrotrichen. Zoologische Jahrbücher Abteilung für Systematik. Oekologie und Geographie der Tiere, 53: 269–320.

Remane, A. 1927b. *Xenotrichula velox* nov. gen. nov. sp., ein chaetonotoides Gastrotrich mit maennlichen Geschlectsorganen. Zoologisher Anzaiger, 71: 289–294.

Remane, A. 1929. Gastrotricha, *In*: W. Kükenthal & T. Krumback, eds. Handbuch der Zoologie, Band 2, Liefrung 6. Walter de Gruyter, Berlin.

Roszczak, R. 1969. Brzuchorzski (Gastrotricha) środkowej Wielkopolski. Poznańskie Towarzystwo Przyjaciół Nauk. Wydział matematyczno-przyrodniczy. Prace Komisji Biologicznej, 32: 1–92 (1968).

Rudescu, L. 1967. Gastrotricha. *In*: Fauna Republicii Socialiste Romania, Trochelminthes, 2(3). Academia Republicii Socialiste Romania, Bucharest, 292 pp. http://dx.doi.org/10.1016/0016–7185(71)90018–2.

Ruttner-Kolisko, A. 1955. Rheomorpha neiswestnovae and Marinella flagellata, zwei Phylogenetisch interessante Würmtypen aus dem Süsswasserpsammon. Österreichische Zoologische Zeitschrift, 6, 55–69.

Schwank, P. 1990. Gastrotricha. *In*: Schwoerbel, J., Zwick, P. (eds.), Süsswasserfauna von Mitteleuropa, Band 3. Gastrotricha und Nemertini. Gustav Fischer Verlag, Stuttgart, Jena, New York, pp. 1–252.

Sonnenberg, R., Nolte, A. W. and D. Tautz. 2007. An evaluation of LSU rDNA D1–D2 sequences for their use in species identification. Frontiers in Zoology, 4, 6, https://doi.org/10.1186/1742–9994–4–6.

Telford, M. J., Lockyer, A. E., Cartwright-Finch, C. and D. T. J. Littlewood. 2003. Combined large and small subunit ribosomal RNA phylogenies support a basal position of the acoelomorph flatworms. Proceedings of the Royal Society B – Biological Sciences, 1519(270): 1077–1083.

Todaro, M. A., Dal Zotto, M. and F. Leasi. 2015. An integrated morphological and molecular approach to the description and systematisation of a novel genus and species of *Macrodasyida* (Gastrotricha). PLoS ONE, 10(7), e0130278. https://doi.org/doi:10.1371/journal.pone.0130278.

Todaro, M. A. 2019. Gastrotricha. Accessed through: World Register of Marine Species at http://www.marinespecies.org/aphia.php?p=taxdetailsetid=2078 (accessed 25 May 2019).

Todaro, M. A., Leasi, F. and R. Hochberg. 2014. A new species, genus and family of marine Gastrotricha from Jamaica, with a phylogenetic analysis of *Macrodasyida* based on molecular data. Systematics and Biodiversity, 12: 473–488.

Todaro, M. A., Telford, J., Lockyer, A. E. and D. T. J. Littlewood. 2006. Interrelationships of the Gastrotricha and their place among the Metazoa inferred from 18S rRNA genes. Zoologica Scripta, 35: 251–259. http://dx.doi.org/10.1080/0022293031000156169.

Voigt, M. 1902. Drei neue *Chaetonotus*-Arten aus Plöner Gewässern. Zoologischer Anzeiger, 34: 717–722.

Vornatscher, J. 1938. Faunistische Untersuchung des Lusthauswassers in Wiener Prater. Internationale Revue der gesamten Hydrobiologie und Hydrographie, 37: 320–363.

Received: July 19, 2018
Accepted: December 20, 2018
Printed: June 30, 2019

View publication stats

Poznań, 16.12.2019r.

Małgorzata Kolicka
Adam Mickiewicz University, Poznań
Faculty of Biology
Department of Animal Taxonomy and Ecology
Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Declaration

I declare that the publication: Garraffoni A. R. S., Kieneke A., **Kolicka M.**, Corgosinho P. H. C., Prado J., Nihei S. S., Freitas A. V. L. 2019. ICZN Declaration 45: a remedy for the nomenclatural and typification dilemma regarding soft-bodied meiofaunal organisms? *Marine Biodiversity*, https://doi.org/10.1007/s12526-019-00983-7, has been prepared as a part of my doctoral dissertation under the scientific supervision of Prof. UAM, dr hab. Ziemowit Olszanowski.

Yours faithfully,

Poznań, 16.12.2019r.

Małgorzata Kolicka
Uniwersytet im. Adama Mickiewicza, Poznań
Wydział Biologii
Zakład Taksonomii i Ekologii Zwierząt
ul. Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Oświadczenie

Oświadczam, że publikacja: Garraffoni A. R. S., Kieneke A., **Kolicka M.**, Corgosinho P. H. C., Prado J., Nihei S. S., Freitas A. V. L. 2019. ICZN Declaration 45: a remedy for the nomenclatural and typification dilemma regarding soft-bodied meiofaunal organisms? *Marine Biodiversity*, https://doi.org/10.1007/s12526-019-00983-7, została wykonana na poczet mojej rozprawy doktorskiej pod opieką promotora Prof. UAM dra hab. Ziemowita Olszanowskiego.

Z wyrazami szacunku,

ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/334382567

ICZN Declaration 45: a remedy for the nomenclatural and typification dilemma regarding soft-bodied meiofaunal organisms?

Article *in* Marine Biodiversity · July 2019

DOI: 10.1007/s12526-019-00983-7

CITATION
1

READS
387

7 authors, including:

André R. S. Garraffoni
University of Campinas
81 PUBLICATIONS **487** CITATIONS
SEE PROFILE

Alexander Kieneke
Senckenberg Research Institute
46 PUBLICATIONS **481** CITATIONS
SEE PROFILE

Małgorzata Kolicka
Adam Mickiewicz University
33 PUBLICATIONS **236** CITATIONS
SEE PROFILE

Paulo Henrique Costa Corgosinho
Universidade Estadual de Montes Claros
64 PUBLICATIONS **263** CITATIONS
SEE PROFILE

Some of the authors of this publication are also working on these related projects:

O pensamento filogenético no ensino de biologia View project

Molecular phylogeny, systematics and the evolution of caterpillar-ant interactions in metalmark butterflies. View project

All content following this page was uploaded by André R. S. Garraffoni on 14 July 2019.

The user has requested enhancement of the downloaded file.

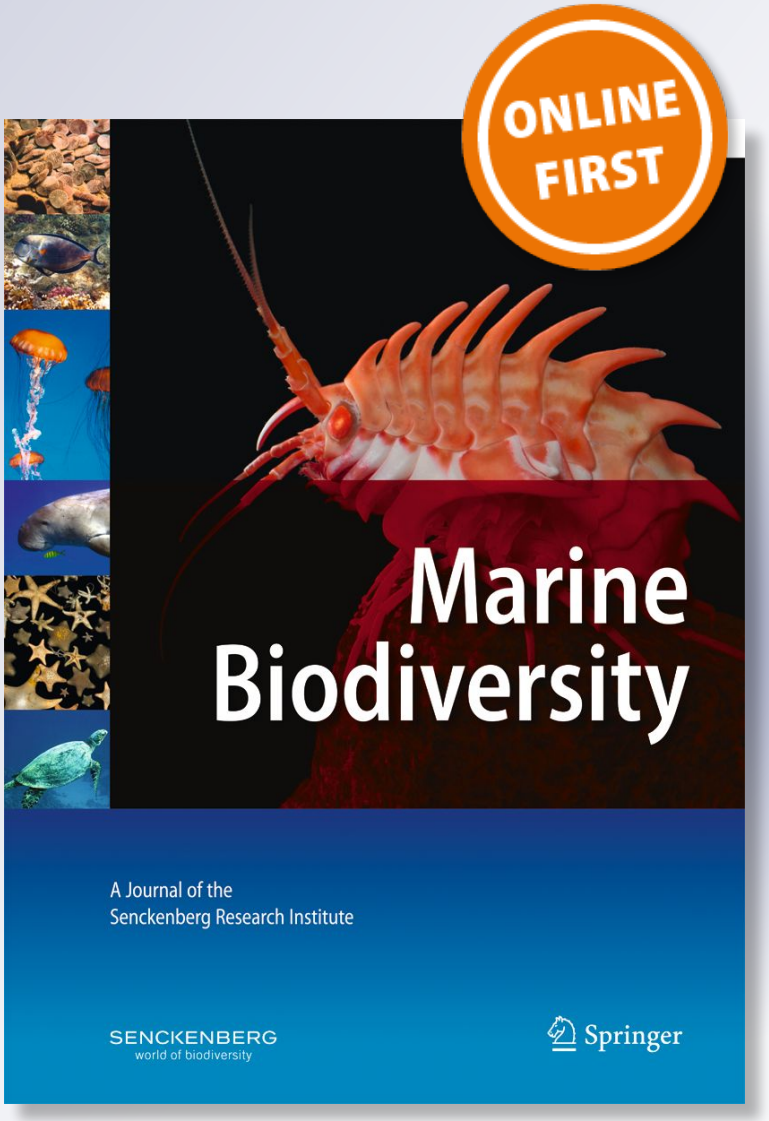
ICZN Declaration 45: a remedy for the nomenclatural and typification dilemma regarding soft-bodied meiofaunal organisms?

André R. S. Garraffoni, Alexander Kieneke, Małgorzata Kolicka, Paulo H. C. Corgosinho, Jefferson Prado, Silvio S. Nihei, et al.

Marine Biodiversity

ISSN 1867-1616

Mar Biodiv
DOI 10.1007/s12526-019-00983-7



Springer

Your article is protected by copyright and all rights are held exclusively by Senckenberg Gesellschaft für Naturforschung. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".

Author's personal copy

Marine Biodiversity
https://doi.org/10.1007/s12526-019-00983-7

SENCKENBERG

REVIEW

ICZN Declaration 45: a remedy for the nomenclatural and typification dilemma regarding soft-bodied meiofaunal organisms?

André R. S. Garraffoni[1] · Alexander Kieneke[2] · Małgorzata Kolicka[3] · Paulo H. C. Corgosinho[4] · Jefferson Prado[5] · Silvio S. Nihei[6] · André V. L. Freitas[1]

Received: 20 March 2019 / Revised: 30 May 2019 / Accepted: 14 June 2019
© Senckenberg Gesellschaft für Naturforschung 2019

Abstract
Although ICZN Declaration 45 already shed some light to the issue regarding "typeless species descriptions," we here explore the assignment of new species-group taxa in the absence of a physical type-specimen in more detail. The focus is on taxonomical studies of so-called soft-bodied meiofaunal organisms, in which a deposition of physical type specimens is frequently either not possible or even not advisable at all, because they are often lost before their formal designation as a name-bearing type. We emphasize some arguments and recommendations concerning photomicrography-based taxonomy on these delicate organisms. Furthermore, we discuss the designation of illustrations as nomenclatural types in the International Code of Nomenclature for algae, fungi, and plants, a practice that is applied for some time now and without any noticeable drawbacks regarding the quality of taxonomic acts.

Keywords International Code of Zoological Nomenclature · Photography-based taxonomy · Name-bearing type · Onymophoront type specimen

Background

Today, one of the major goals of systematic biology is to create and make an accessible and complete catalog of life (Zhang and Shear 2007). To achieve this, researchers interested in unraveling the biotic diversity must (1) recognize that certain individuals share a common evolutionary history and need to be united in a specific taxon (scope of taxonomy), and (2) make use of an obligatory system for the typification of this taxon by assigning a unique scientific name (scope of nomenclature, see Dubois 2007). Although taxonomy and nomenclature are distinct disciplines, both are deeply linked to each other since associating scientific names unequivocally with specified taxa is pivotal for establishing a reliable reference for biological information (Padial et al. 2010). To achieve this aim, the specimens are the unique objective connections between the scientific name and the natural population that they belonged to (Ceríaco et al. 2016; Dubois 2017).

Previous discussions revolving around the necessity of deposited vouchers (type specimens) for the availability of new species names (Dubois and Nemésio 2007) was recently brought into focus again, triggered by the description of a new insect species without designation of a preserved specimen as holotype (Marshall and Evenhuis 2015). According to a strict interpretation of the so-called photography-based taxonomy (hereafter PBT) (Ceríaco et al. 2016), when no physical type is deposited as voucher material, its nomenclatural function as the name-bearing type or onymophoront (i.e., a specimen serving as objective reference determining the

Communicated by M. Curini-Galletti

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12526-019-00983-7) contains supplementary material, which is available to authorized users.

✉ André R. S. Garraffoni
arsg@unicamp.br

[1] Departamento de Biologia Animal, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, SP, Brazil

[2] Deutsches Zentrum für Marine Biodiversitätsforschung, Senckenberg am Meer, Wilhelmshaven, Germany

[3] Department of Animal Taxonomy and Ecology, Institute of Environmental Biology, Adam Mickiewicz University, Poznan, Poland

[4] Departamento de Biologia Geral, Universidade Estadual de Montes Claros, Montes Claros, MG, Brazil

[5] Herbário SP, Instituto de Botânica, São Paulo, SP, Brazil

[6] Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, SP, Brazil

Published online: 10 July 2019

Author's personal copy

taxonomic allocation of a scientific name [see Dubois 2005, 2017]) is inexistent. This postulated method of depositing photomicrographs instead of holotypes and paratypes is nothing new for those organisms prone to damage or those where fixation methods are insufficient, and the advantages and disadvantages of this approach have been considered oftentimes (Frizzell 1933; Corliss 1962). However, shortly after the Marshall and Evenhuis (2015) publication, systematic biologists divided into two groups, either advocating (Pape et al. 2016; Garraffoni and Freitas 2017; Krell and Marshall 2017), or refusing (Amorim et al. 2016; Ceríaco et al. 2016; Santos et al. 2016; Dubois 2017; Gutiérrez and Pine 2017; Rogers et al. 2017) the assignment of a scientific name to a new taxon without a physical type-specimen. Besides the taxonomic bias (most authors and signatories against the PBT are specialists on vertebrates and arthropods—easily preservable taxa), the division among taxonomists was caused by differing interpretations of the nomenclatural and taxonomic functions of a type-specimen (Dubois 2005, 2017).

Regarding the nomenclatural function, taxonomists had varying interpretations of Articles 16.4.2 and 73.1.4 of the International Code of Zoological Nomenclature (ICZN 1999), which point out the designation of photomicrographs as voucher material, or the assignment of names to species in the absence of the physical type-specimen. Furthermore, various authors argued against the use of images and/or videos ("proxy types," Krell and Marshall 2017), pointing out that such an approach will "abandon commendable taxonomic practices," will enable "sub-standard science," a "fundamental misunderstanding of taxonomy," or have "harmful effects in taxonomic descriptions" (Amorim et al. 2016; Ceríaco et al. 2016; Santos et al. 2016; Dubois 2017; Gutiérrez and Pine 2017; Rogers et al. 2017). These strong statements were uttered by some authors fearing that the taxonomic function of "the type" could be reduced, as authors of a scientific name use morphological characters present in the type-specimen (that could be missing or insufficiently detailed in photomicrographs) as diagnostic features of the new taxon (Dubois 2005, 2017).

Our intention is to carefully explore the case of fragile meiofaunal animals, initially addressed by Garraffoni and Freitas (2017), and to assess the impact of International Committee on Zoological Nomenclature Declaration 45 (ICZN 2017) for the study of these taxa. Meiobenthologists often do not know that a specimen currently under investigation will later serve as the holotype of a new species. This applies especially for taxa covering fragile species as many potential type-specimens are frequently destroyed or even lost during investigation, i.e., before they can officially be designated as name-bearing types (Fig. 1a, b). Furthermore, preserved specimens of these microscopic and fragile animals will inevitably deteriorate over time and most of their diagnostic characteristics usually dissolve or are lost immediately after preservation (Fig. 1 c–h; supplementary material). Finally, we also present comments on how the International Code of Nomenclature for algae, fungi, and plants deals with similar problems (Turland et al. 2018).

A soft-bodied meiofauna taxonomist's everyday routine: investigation and imaging of living specimens

By definition, meiofauna is the assemblage of in- and epifaunal organisms that may pass through a 0.5 to 1-mm sieve but is retained by 0.044–0.063 mm mesh (Higgins and Thiel 1988; Giere 2009). Animals with epi- or periphytical lifestyles are frequently considered as meiofauna, too. In fact, meiofauna constitutes a discrete ecological guild with diverse interactions, having various degrees of effects on biodiversity, with trophic as well as energetic links to macrofauna and plankton communities (Higgins and Thiel 1988; Giere 2009). As characteristic feature, many meiofaunal lineages have a rigid and armored integument which is typical for arthropod taxa (e.g., Harpacticoida, Ostracoda, Mystacocarida, Halacarida) and can also be found in some non-arthropod taxa such as Nematoda, Kinorhyncha, and Loricifera. Other meiofaunal groups such as Cnidaria, Platyhelminthes, Gnathostomulida, Annelida, and Gastrotricha display a more flexible and fragile integument. For taxonomic identification of meiofauna, notably the just mentioned "soft-bodied meiofauna" (hereafter SBM), detailed microscopic investigations of living individuals are required. Hence, the investigated specimens often get lost. However, we emphasize that there might occur similar problems with "hard-bodied meiofauna," especially if the number of available specimens is low or even limited to a single specimen, e.g., when the samples were obtained from remote biotopes such as the deep sea (e.g., George and Plum 2009).

We do have to acknowledge that physical specimens indeed have several advantages (e.g., disposability for reinvestigations with current or new methodology). So why not documenting a SBM specimen by light microscopy first and then fixing it for further purposes or just for museum deposition? One has to know the *modus operandi* on how to study SBM organisms in order to understand why the aforementioned approach hardly works. Balsamo et al. (2014) and Kånneby (2016) gave a detailed instruction for preparation and preservation of gastrotrichs, though it is almost the same for any SBM taxon: single specimens are transferred from the raw sample to a small amount of water or anesthetization liquid (e.g., 7% or 1–2% of aqueous $MgCl_2$ solution for marine and freshwater taxa, respectively, see Balsamo et al. 2014) on a microscopic slide by means of a micropipette. Once the organism is on the slide, the liquid drop is carefully covered with a cover slip. Flat and perfectly aligned

Author's personal copy

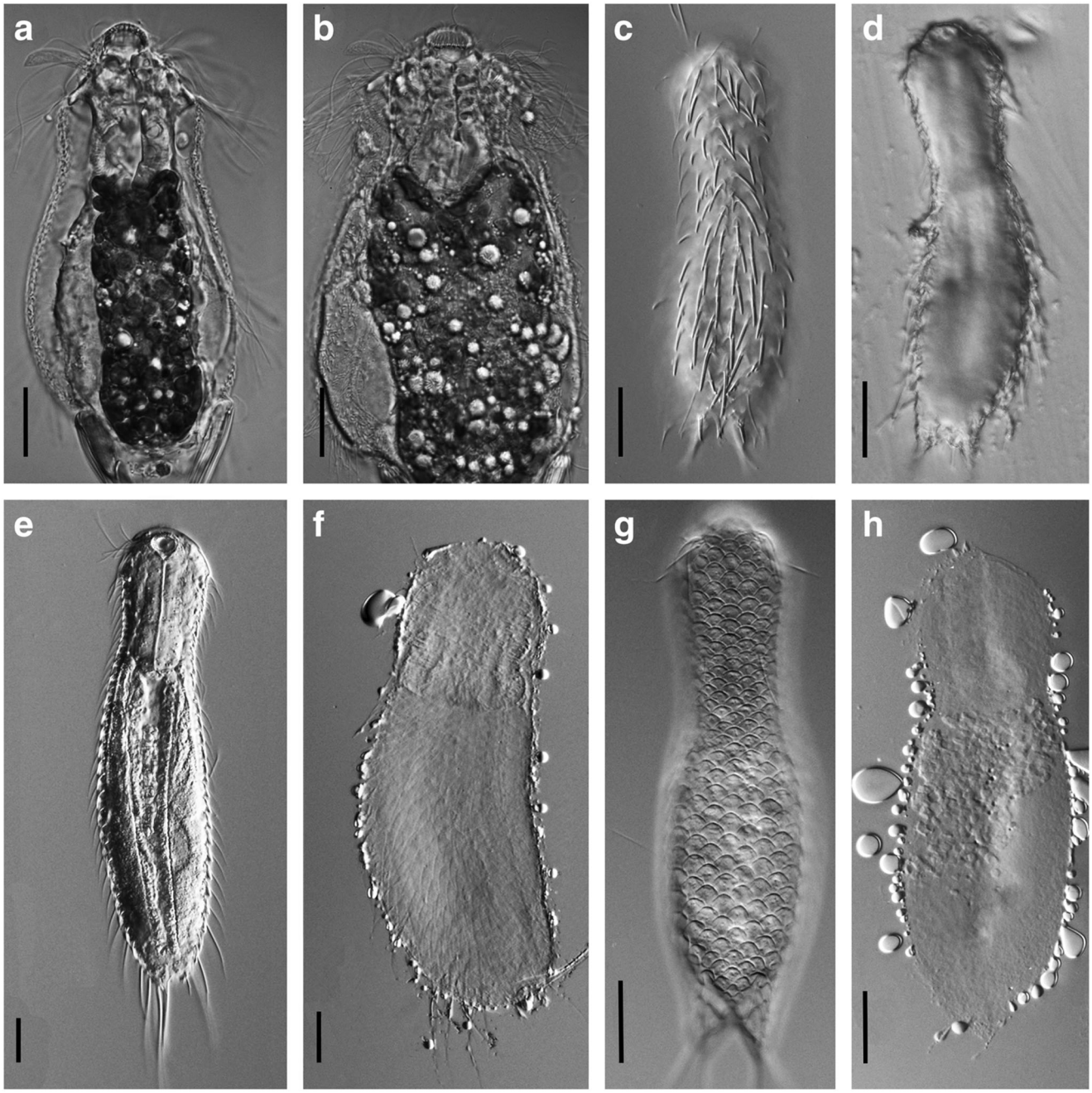


Fig. 1 Digital photomicrographs of living (**a**, **b**, **c**, **e**, **g**) and fixed preserved (**d**, **f**, **h**) specimens of soft-bodied meiofauna (Gastrotricha). Two distinct situations are illustrated here: (**a**, **b**) specimen of *Neogossea* sp. deteriorating during the observation in light microscopy; comparison between living and fixed specimens of *Chaetonotus* (*Hystricochaetonotus*) *furcatus* Kisielewski, 1991 (**c**, **d**), *Lepidochaetus* sp. (**e**, **f**), *Lepidoderma* sp. (**g**, **h**). Scales: **a**, **b** 30 μm; **c**–**h** 20 μm

mountings with non-tilted, dorso-ventral, or ventro-dorsal orientations are needed for high-quality photomicrographs that display diagnostic internal and external feature important for taxonomic identification (Kieneke and Nikoukar 2017). For recovery of the specimen after microscopic investigation, drops of liquid are added along the edges of the cover slip in order to float it. Thereby, the SBM specimen can be sucked up with a micropipette. Careful floating and removing of the coverslip is an alternative procedure for recovery of the specimen (Kieneke et al. 2012). However, receipt of an undamaged SBM specimen after microscopic investigation and documentation rarely succeeds. In most instances, the specimen is either completely destroyed, i.e., useless for any morphological investigation, or mostly just a clump of tissue, but at least suitable for extraction of genomic DNA. One advantage of such an approach is the well-known higher success rate of

Author's personal copy

gene amplifications via PCR when using template DNA of fresh instead of conserved (e.g., with ethanol) specimens (Kieneke et al. 2012). Furthermore, DNA sequence data are thereby linked to image data of an identical specimen (e.g., Kieneke and Nikoukar 2017; Kolicka et al. 2016).

The only alternative to an investigation of living specimens is a careful fixation and preparation for either light microscopy or further imaging techniques. However, it is worth considering the durableness of preserved specimens as well as other drawbacks. The most common way to deposit preserved SBM specimens is either as permanent slides of fixed animals or coated with gold for scanning electron microscopy (SEM). Concerning the first case, the list of available mounting media is endless and not regulated but it is well known that such microscope slides may deteriorate over time, sometimes rather quickly (Neuhaus et al. 2017). Optical properties of the mostly transparent and non-pigmented animals may change dramatically after chemical fixation or conservation (Balsamo et al. 2014). Thereby, a useful study of taxonomically relevant internal organs may be impeded or even impossible (Fig. 1d, f, h; supplementary material). Muscular contractions of dying animals during fixation are even more of a hindrance for proper identification as they cause various artifacts like shrinkage, deformation, and torsion of the body (Balsamo et al. 2014; Garraffoni and Freitas 2017). Concerning SEM as an additional imaging method, data have proven to have a considerable value in the course of recent descriptions of new SBM species (e.g., Sørensen and Sterrer 2002; Martínez et al. 2013; Garraffoni and Melchior 2015; Kieneke et al. 2015; Todaro et al. 2015; Garraffoni et al. 2017; Minowa and Garraffoni 2017). However, some disadvantages are definitely coupled with SEM. First, since specimens have to be coated with gold and/or palladium, internal organs cannot be displayed or investigated. Second, since specimens are mounted on aluminum stubs, observations of external structures of the attached side by SEM are not possible. Furthermore, specimens could be damaged or lost during the elaborate preparation process. Hence, it is not surprising that SEM micrographs are mostly derived from paratypes.

Taxonomically useful characters versus preserved (type) specimens in soft-bodied meiofauna: a dilemma

Taxonomically important features are predominantly lineage or group specific; however, they often include structures of, e.g., the reproductive system, the alimentary system, and external formations like cuticular differentiations, ciliation patterns, setae, spines, and adhesive tubes (e.g., Sterrer 1972; Littlewood and Bray 2001; Todaro and Hummon 2008) (Fig. 1a, c, e; supplementary material). To get profound information, detailed investigations of living specimens using light microscopy with high-resolution objectives equipped with differential interference contrast (DIC) are required (Todaro and Hummon 2008) (Fig. 1a, c, e; supplementary material). The only way to preserve such important taxonomic characters for subsequent analyses, or to "fixate" them, is photomicrography (Balsamo et al. 2014). In almost every case of PBT that was reviewed by Krell and Marshall (2017), the images of the type-specimens were at best as good as the physical specimen of which they were taken. However, as we have asserted above, image data of living, often anesthetized SBM specimens usually contain many more details than one can obtain by microscopic observation of fixed specimens (see also Todaro and Hummon 2008). This situation perfectly matches a statement of Marshall and Evenhuis (2015), as cited in Krell and Marshall (2017): "more characters can be extracted from good images than from bad specimens."

A crucial consequence of the above-demonstrated *modus operandi* in the study of SBM is an unusual temporal order of the designation of a name-bearing type specimen and its loss (Fig. 2a, b) compared with "normal" animals: if a new SBM species is described on the basis of microscopic images of a particular specimen, the pictured specimen is in fact the name-bearing type—an individual that definitely existed. However, the physical specimen was essentially lost before it was formally designated as a name-bearing type in the course of a scientific publication (Figs. 1a, b and 2a). Although Article 73.1.4 of the International Commission on Zoological Nomenclature (ICZN 1999) explicitly strengthens validity of the designation of an illustration of a single specimen if only illustrations remained and the specimen "no longer exists" ("illustrations" may also cover, e.g., digital photomicrographs), this regulation did not cover the case of SBM species as described above. Article 73.1.4 of the ICZN refers exclusively to a loss of a physical type specimen, e.g., due to the destruction of a whole collection, that was formally designated as such before its loss (Figs. 1d, f, h, 2b; supplementary material). Therefore, the common sense practice of the past decades in SBM taxonomy and nomenclature was not covered by the rules of the ICZN in the strict sense!

Meiofauna specialists, especially those working with SBM, definitely get into a *dilemma* when observing living specimens in their fresh sediment samples. One has to decide whether the specimens should be fixed and conserved for subsequent use as permanent slides, specimens for SEM or other morphological investigations (e.g., fluorescence or transmission electron microscopy), or if they shall be studied alive by means of light microscopy. In the first case, the retention of (a) physical type specimen(s) is guaranteed at the expense of a sufficient use for taxonomic purposes. In the second case, all necessary taxonomic details of the observed specimens can be retained by microscopic imaging; however, the retention of undestroyed physical specimens rarely succeeds.

Author's personal copy

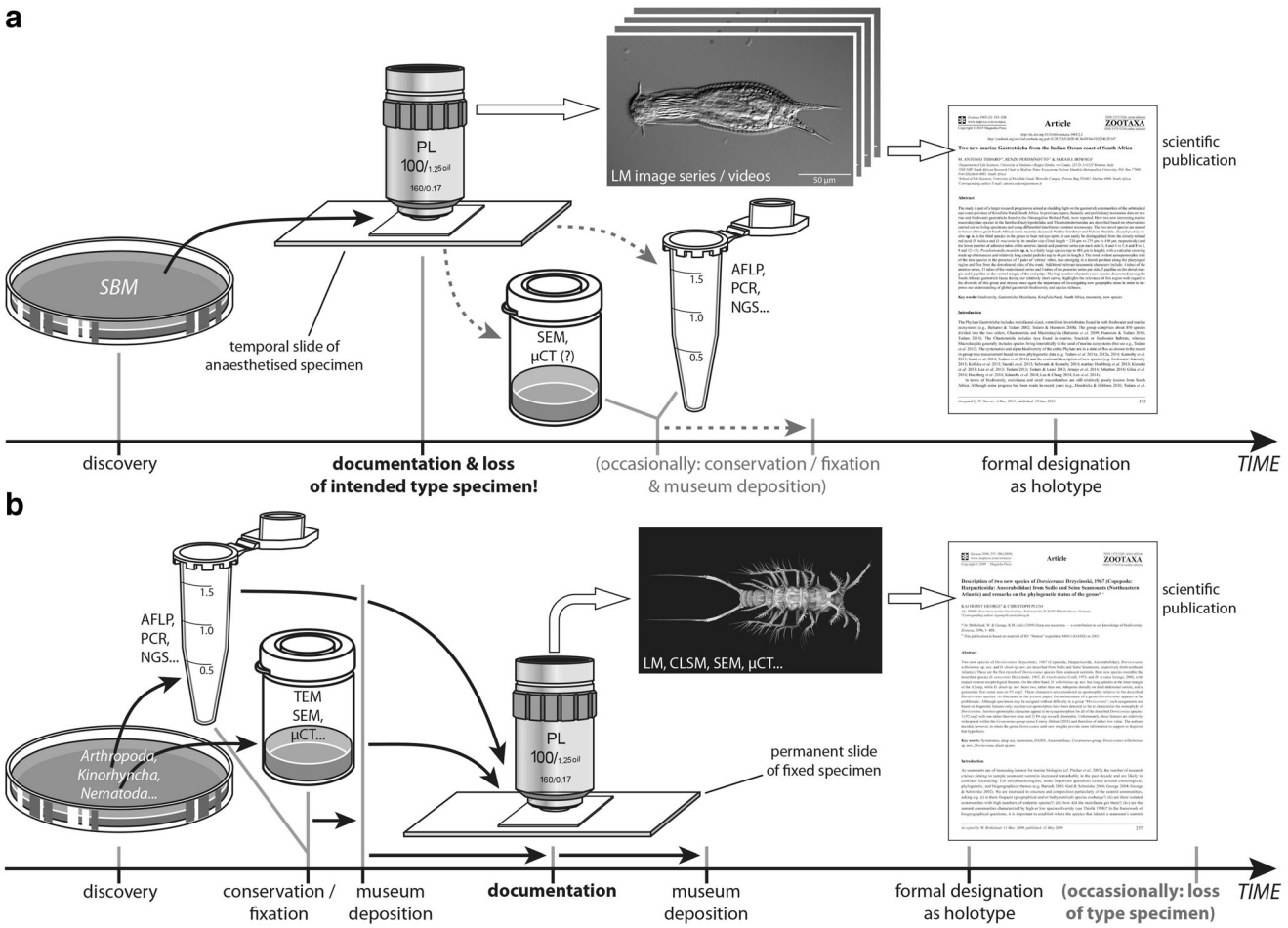


Fig. 2 Comparison between taxonomic processing of **a** soft-bodied meiofauna (SBM) and **b** "common" meiofauna. The fundamental difference concerns the temporal order. The usual processing of SBM results in the loss of an intended type specimen caused by microscopic observation and documentation. Specimens of "common" meiofaunal taxa (e.g., Nematoda, Crustacea, Kinorhyncha…) can be fixed and/or preserved prior to microscopic observation as a permanent slide. AFLP, amplified fragment length polymorphism; CLSM, confocal laser scanning microscopy; LM, light microscopy; NGS, next-generation sequencing; PCR, polymerase chain reaction; SBM, soft-bodied meiofauna; SEM, scanning electron microscopy; TEM, transmission electron microscopy; µCT, micro computed tomography. The micrographs show the gastrotrich *Heteroxenotrichula affinis* (Remane, 1934) in **a** (original) and the harpacticoid copepod *Dendropsyllus* sp. in **b** (modified from Brandt et al. 2018, with permission of Springer)

New recommendations for Article 73 of the ICZN

The review of the most recent accounts of fervid discussion on the pros and cons regarding PBT led to Declaration 45 of the International Commission on Zoological Nomenclature, adding four new recommendations for Article 73 (ICZN 2017). This declaration was triggered by the debate and is intended, among other objectives, to provide guidance in "disciplines dealing with organisms that are difficult or impossible to preserve" (Krell and Marshall 2017). In the following, we reproduce the new Recommendations 73G–73J to Article 73 of the ICZN and assess their feasibility for SBM organisms.

"Recommendation 73G. Specific reasons for designation of an unpreserved specimen as the name-bearing type. An author should provide detailed reasoning why at least one preserved specimen, whether a complete individual organism or a part of such an individual, was not used as the name-bearing type for the new taxon and why the formal naming of the taxon is needed at a point in time when no preserved name-bearing type will be available."

"Recommendation 73H. Assertion of due diligence. When establishing a new species-group taxon without a preserved name-bearing type, steps taken by an author to capture and preserve a physical specimen of the new taxon and/or locate an existing preserved specimen in natural history collections should be recounted."

In SBM taxonomy, living specimens have several advantages compared with fixed specimens; i.e., only living specimens (possibly anesthetized) display all characters that are mandatory for a proper taxonomic description. Furthermore, taxonomists of SBM taxa have to use the whole putative type specimen for extraction of genomic DNA and sequencing due to the tiny size of these animals. As studies integrating DNA

Author's personal copy

sequence data and morphological features to delineate SBM species boundaries are increasing (Fontaneto et al. 2015), an essential procedure is the photographic documentation of the identical putative type specimen, before it is used for DNA extraction and later sequencing (e.g., Larsson et al. 2008). Hence, in cases where only single individuals could be captured (frequently occurring in remote biotopes such as the deep sea), the taxonomist in charge will mostly decide to do a microscopic documentation of that specimen rather than fixing it for subsequent applications (e.g., as permanent slide or as specimen for SEM). The specific reasoning according to recommendation 73G is therefore a much better usability of fresh SBM specimens which are usually lost after investigation.

If a studied biotope provides multiple individuals of a putative new species, or if subsequent sampling efforts yield further specimens, we encourage the researches to preserve some of them as potential physical type specimens. However, due to the above-demonstrated deficiency of preserved SBM specimens regarding proper taxonomy, even those specimens will mostly not contribute substantial data to the knowledge of the respective species.

"Recommendation 73I. Consultation with specialists. Before the designation of an unpreserved specimen as a name-bearing type, an author should consult with specialists in the group in question."

Most groups of SBM and their sub-taxa are considered as "lesser known taxa," because they are problematic, treated superficially, and for some of these groups there may be very few active specialists (e.g., Garey 2002; Jenner and Littlewood 2010). Thus, this recommendation could be unfeasible due to the low number of specialists who could be consulted. However, photomicrographic documentation of holotypes and paratypes enables easy, fast, and inexpensive transfer of information (e.g., without loan arrangements and mailings). This does not exclusively apply to the few specialists, but also to other researchers that are aware of taxonomy practices in conjunction with the description of new species, though they might not be familiar with SBM per se. Hence, such professional consultations, discussions, and comparisons can easily be performed and they will inevitably favor better taxonomic practices and descriptions, as many more taxonomists can offer suggestions before submission of manuscripts. This will result in a high-quality assurance especially in the case of species descriptions using PBT.

"Recommendation 73J. Comprehensive iconography and measurements. When establishing a new species-group taxon without a preserved name-bearing type, the author should provide extensive documentation (e.g., multiple original high-resolution images, DNA sequences, etc.) of potentially diagnostic characters as completely as possible."

The provision of "multiple original high-resolution images" is exactly our claim. As already explained, image series and/or digital video files of SBM organisms are mostly the best basis for taxonomic processing, including reliable morphometric and meristic measurements (see also Aguiar et al. 2017). If feasible, one should also try to recover the documented specimens in order to store at least their tissues for subsequent extraction and amplification of genomic DNA (see also our comments concerning *Recommendation 73G* and *73H*).

The above-presented reasoning can be applied to most future descriptions of SBM species without an existing preserved type-specimen. In our opinion, the arguments in conjunction with Recommendation 73G (image data of living SBM specimens are much more suitable than any preserved physical specimen) are most important. Concerning Recommendation 73J, we now present a catalog of "quality locking" procedures. We cannot define these as mandatory provisions; however, the more tasks are applied, the better reproducibility of the taxonomic act should be:

- Usage of high-definition light microscopy with high numerical aperture objectives and suitable contrast techniques such as DIC or phase contrast
- Extensive documentation: series of high-resolution image stacks and videos
- Deposition of digital image data in a scientific collection
- Multiple depositions of digital image data in other repositories as backup
- Assignment of accession numbers to those digital data
- If possible, extraction of DNA from the documented type-specimen(s) and making DNA sequences or other molecular markers publicly available

Lessons from the botanists: the Shenzhen Code

The rules that govern the scientific naming of algae, fungi, and green plants are revised at Nomenclature Section meetings at successive International Botanical Congresses. The editions carry the name of the city where the Nomenclature Session was held, hence the "Shenzhen Code." The rules were modified by vote of the researchers attending the conference. Afterwards, the current Editorial Committee (with 16 commissioners) prepared and edited the Code text following the provisions as changed and defined by the conference participants. The International Code of Nomenclature for algae, fungi, and plants has currently 18 editions (Turland et al. 2018). According to the "Shenzhen Code," the only purpose of a name-bearing type is to attach a name to a taxon and the name is no more than a label. Eleven distinct definitions of types are included in the "Shenzhen Code," but in any case, the holotype is a specimen or an illustration used or designated by the author as the nomenclatural type.

Similar difficulties arise if delicate specimens that are deemed to be the types of taxa shall be preserved. However, this was already solved by the Code for algae, fungi, and

Author's personal copy

plants. The current code (Turland et al. 2018) provides a rule (Article 40.5) to support illustrations as types of names of new species or infraspecific taxa of microscopic algae or fungi. Article 40.5 (see transcription below) pinpoints the cases for which an illustration can be a type. This provision has worked very well for those microorganisms covered by the "Shenzhen Code" (Turland et al. 2018).

"Art. 40.5. For the purpose of Art. 40.1, the type of a name of a new species or infraspecific taxon of microscopic algae or microfungi (fossils excepted: see Art. 8.5) may be an effectively published illustration if there are technical difficulties of preservation or if it is impossible to preserve a specimen that would show the features attributed to the taxon by the author of the name".

With regard to content, ICN Article 40.5 corresponds to Article 73.1.4 of the ICZN in conjunction with the new Recommendations 73G–J (ICZN 2017). The application of this rule in microalgae and microfungi has shown to be very successful over the years since the publication of the Berlin Code (Greuter et al. 1988). We predict a comparably responsible usage of Article 73.1.4 of the ICZN and its Recommendations G–J, and do not expect possible flaws regarding an excess of bad-quality non-type descriptions by non-experts based on these regulations as evoked by Löbl et al. (2016). Quite the contrary, we are convinced that these revised nomenclatural rules will even foster better taxonomic studies in the area of SBM and eventually other hardly preservable metazoan groups which were not mentioned and exemplified here.

Conclusions

Despite that designation of illustrations as name-bearing types is not as absurd as previously claimed, as we can learn from practical experiences implemented by the "botanical" "Shenzhen Code," it is not our intention to propose that the image itself should be considered as the type. The name-bearing type will always be the actual specimen that was documented. We really would like to highlight that SBM specimens are frequently destroyed during the investigation and before their formal name-bearing type designation. Furthermore, images of such type-specimens can provide considerable information, usually as good as and often much better than can be extracted from any physical specimen. We regard the new Recommendations of Article 73.1.4 of the ICZN as a valuable extension of the zoological nomenclatural rules, something that has been needed for quite some time. Taxonomists that describe new species solely based on image data and without any physical type-specimen (i.e., PBT) still have to justify their reasons in detail according to recommendations 73G–73J. At least in the case of SBM, species descriptions without any preserved specimens have always been and will still represent a significant part of taxonomic work. However, taxonomists now have the genuine choice whether photomicrographic documentation (i.e., PBT) or a preserved physical type specimen yields more and/or better taxonomic information, without ignoring the compulsory designation of the name-bearing specimen for species-series taxa. We have to point out that the latter (physical specimen) still represents the standard approach while the former (PBT) is now conditionally accepted under the ICZN. The new recommendations and the herein suggested quality locking steps will facilitate a high quality of future taxonomic acts.

Acknowledgments Many thanks to Daniel Lahr and the Graduate Program in Zoology IB/USP to organize the symposium about typification methods in modern taxonomy on July 2017, which was the starting point of this text. Jacek Kisielewski is kindly acknowledged for providing photomicrographs of *Chaetonotus* (*Hystricochaetonotus*) *furcatus* from his private collection. Many thanks to Brett Christopher Gonzalez and Yvonne Kieneke for editing the English text and Marco Curini-Galletti and anonymous reviewers for their insightful comments.

Financial support This work was supported by grants from Biota Program/Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP: 2014/23856-0) and Brazilian Research Council (CNPq: 478825/2013-7) for ARSG, National Science Centre in Poland (2015/17/N/NZ8/01568) for MK, Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG: CRA-BPV-00547-17) for PHCC, Fundação de Amparo à Pesquisa do Estado (FAPESP: 2015/10788-0, 2016-50387-7) and Brazilian Research Council (CNPq: 403165/2016-4) for SSN, and Biota Program/Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP: 2011/50225-3), Brazilian Research Council (CNPq: fellowship 302585/2011-7, 303834/2015-3, 303615/2015-2), and National Science Foundation (DEB-1256742) for AVLF.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval No animal testing was performed during this study.

Sampling and field studies All necessary permits for sampling and observational field studies have been obtained from the competent authorities by the authors.

Data availability statement All data generated or analyzed during this study are included in this published article.

References

Aguiar JJM, Santos JC, Urso-Guimarães MV (2017) On the use of photography in science and taxonomy: how images can provide a basis for their own authentication. Bionomina 12:44–47. https://doi.org/10.11646/bionomina.12.1.4

Amorim DS, Santos CMD, Krell FT, Dubois A, Nihei SS, Oliveira OMP, Pont AC, Song H, Verdade VK, Fachin D, Klassa B, Lamas CJE, Oliveira SS, Carvalho CJB, Mello-Patiu C, Hajdu E, Couri M, Silva VC, Capellari RS, Falaschi R, Feitosa R, Prendini L, Pombal JP Jr,

Author's personal copy

Fernandez F, Rocha RM, Lattke J, Caramaschi U, Duarte M, Marques AC, Reis RE, Kurina O, Takiya DM, Tavares MDS, Silva DF, Franco FL, Cuezzo F, Paulson DR, Guenard B, Schlick-Steiner B, Arthofer W, Steiner F, Fisher BL, Johnson RA, Delsinne TD, Donoso DA, Mulieri PR, Patitucci LD, Carpenter JM, Herman L, Grimaldi D (2016) Timeless standards for species delimitation. Zootaxa 4137:121–128. https://doi.org/10.11646/zootaxa.4137.1.9

Balsamo M, Grilli P, Guidi L, d'Hondt JL (2014) Gastrotricha: biology, ecology and systematics. In: Families Dasydytidae, Dichaeturidae, Neogosseidae, Proichthydiidae, vol 24. Backhuys Publishers, Leiden

Brandt A, Scholz J, Allspach A, Brenke N, Brix S, George KH, Hörnschemeyer T, Holst S, Hoppenrath M, Iwan F, Janssen A, Janssen R, Janussen D, Jeskulke K, Fiege D, Kaiser S, Kieneke A, Kihara TC, Kröncke I, Krupp F, Martha SO, Martínez Arbizu PM, Meißner K, Miljutina M, Miljutin D, Renz J, Riehl T, Saeedi H, Siegler V, Sonnewald M, Stuckas H, Veit-Köhler G (2018) 200 years of marine research at Senckenberg: selected highlights. Mar Biodivers 48:159–178. https://doi.org/10.1007/s12526-017-0839-4

Ceríaco LMP, Gutiérrez EE, Dubois A (2016) Photography-based taxonomy is inadequate, unnecessary, and potentially harmful for biological sciences. Zootaxa 4196:435–445. https://doi.org/10.11646/zootaxa.4196.3.9

Corliss JO (1962) Taxonomic-nomenclatural practices in protozoology and the new international code of zoological nomenclature. J Protozool 9:307–324

Dubois A (2005) Proposed rules for the incorporation of nomina of higher-ranked zoological taxa in the International Code of Zoological Nomenclature. 1. Some general questions, concepts and terms of biological nomenclature. Zoosystema 27:365–426

Dubois A (2007) Phylogeny, taxonomy and nomenclature: the problem of taxonomic categories and of nomenclatural. Zootaxa 1519:27–68. https://doi.org/10.11646/zootaxa.1519.1.3

Dubois A (2017) The need for reference specimens in zoological taxonomy and nomenclature. Bionomina 12:4–38. https://doi.org/10.11646/bionomina.12.1.2

Dubois A, Nemésio A (2007) Does nomenclatural availability of nomina of new species or subspecies require the deposition of vouchers in collections. Zootaxa 1409:1–22. https://doi.org/10.11646/zootaxa.1409.1.1

Fontaneto D, Flot JF, Tang CQ (2015) Guidelines for DNA taxonomy, with a focus on the meiofauna. Mar Biodivers 45:433–451. https://doi.org/10.1007/s12526-015-0319-7

Frizzell DL (1933) Terminology of types. Amer Midl Nat 14:637–668

Garey JR (2002) The lesser-known protostome taxa: an introduction and a tribute to Robert P. Higgins. Integr Comp Biol 42:611–618. https://doi.org/10.1093/icb/42.3.611

Garraffoni ARS, Freitas AVL (2017) Photos belong in the taxonomic code. Science 355:805. https://doi.org/10.1126/science.aam7686

Garraffoni ARS, Melchior MP (2015) New species and new records of freshwater *Heterolepidoderma* (Gastrotricha: Chaetonotidae) from Brazil with an identification key to the genus. Zootaxa 4057:551–568. https://doi.org/10.11646/zootaxa.4057.4.5

Garraffoni ARS, Araújo TQ, Lourenço AP, Guidi L, Balsamo M (2017) A new genus and new species of freshwater Chaetonotidae (Gastrotricha: Chaetonotida) from Brazil with phylogenetic position inferred from nuclear and mitochondrial DNA sequences. Syst Biodivers 15:49–62. https://doi.org/10.1080/14772000.2016.1214189

George KH, Plum C (2009) Description of two new species of *Dorsiceratus* Drzycimski, 1967 (Copepoda: Harpacticoida: Ancorabolidae) from Sedlo and Seine Seamounts (northeastern Atlantic) and remarks on the phylogenetic status of the genus. Zootaxa 2006:257–286

Giere O (2009) Meiobenthology. The microscopic motile Fauna of aquatic sediments. Springer, Berlin

Greuter W, Burdet VHM, Chaloner WG, Demoulin V, Grolle R, Hawksworth DL, Nicolson DH, Silva PC, Stafleu FA, Voss EG, McNeill J (1988) International Code of Botanical Nomenclature. Adopted by the fourteenth international congress, Berlin, July-august 1987. Reg Veg 118:1–328

Gutiérrez EE, Pine RH (2017) Specimen collection crucial to taxonomy. Science 355:1275. https://doi.org/10.1126/science.aan0926

Higgins RP, Thiel H (1988) Introduction to the study of meiofauna. Smithsonian Institution, Washington

International Commission on Zoological Nomenclature (1999) International Code of Zoological Nomenclature, 4th edn. International Trust for Zoological Nomenclature, London

International Commission on Zoological Nomenclature (2017) Declaration 45 – addition of recommendations to article 73 and of the term "specimen, preserved" to the Glossary. Bull Zool Nom 73:96–97. https://doi.org/10.21805/bzn.v73i2.a2

Jenner RA, Littlewood DTJ (2010) Invertebrate Problematica: kinds, causes, and solutions. In: Telford MJ, Littlewood DTJ (eds) Animal evolution – genomes, fossils and trees. Oxford University Press, Oxford, pp 107–126

Kånneby T (2016) Phylum Gastrotricha. In: Thorp JH, Rogers DC (eds) Key to Nearctic Fauna: Thorp and Covich's freshwater invertebrates, vol 2. Elsevier, Amsterdam, pp 115–130

Kieneke A, Nikoukar H (2017) Integrative morphological and molecular investigation of *Turbanella hyalina* Schultze, 1853 (Gastrotricha: *Macrodasyida*), including a redescription of the species. Zool Anz 267:168–186. https://doi.org/10.1016/j.jcz.2017.03.005

Kieneke A, Martínez Arbizu PM, Fontaneto D (2012) Spatially structured populations with a low level of cryptic diversity in European marine Gastrotricha. Mol Ecol 21:1239–1254. https://doi.org/10.1111/j.1365-294X.2011.05421.x

Kieneke A, Schmidt-Rhaesa A, Hochberg R (2015) A new species of *Cephalodasys* (Gastrotricha, *Macrodasyida*) from the Caribbean Sea with a determination key to species of the genus. Zootaxa 3947:367–385. https://doi.org/10.11646/zootaxa.3947.3.4

Kolicka M, Dabert M, Dabert J, Kånneby T, Kisielewski J (2016) *Bifidochaetus*, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. Invert Syst 30:398–419. https://doi.org/10.1071/IS16001

Krell F-T, Marshall SA (2017) New species described from photographs: yes? No? Sometimes? A fierce debate and a new declaration of the ICZN. Insect Syst Divers 1:3–19. https://doi.org/10.1093/isd/ixx004

Larsson K, Ahmadzadeh A, Jondelius U (2008) DNA taxonomy of Swedish Catenulida (Platyhelminthes) and a phylogenetic framework for catenulid classification. Org Divers Evol 8:399–412. https://doi.org/10.1016/j.ode.2008.09.003

Littlewood DTJ, Bray RA (2001) Interrelationships of the Platyhelminthes. The systematics association special volume series 60. Taylor and Francis, London and New York

Löbl I, Cibois A, Landry B (2016) Describing new species in the absence of sampled specimens: a taxonomist's own-goal. Bull. Zool. Nom. 73:83–86. https://doi.org/10.21805/bzn.v73i1.a2

Marshall SA, Evenhuis NL (2015) New species without dead bodies: a case for photo-based descriptions, illustrated by a striking new species of *Marleyimyia* Hesse (Diptera, Bombyliidae) from South Africa. ZooKeys 525:117–127. https://doi.org/10.3897/zookeys.525.6143

Martínez A, Di Domenico M, Jörger K, Norenburg J, Worsaae K (2013) Description of three new species of *Protodrilus* (Annelida, Protodrilidae) from Central America. Mar Biol Res 9:676–691. https://doi.org/10.1080/17451000.2013.765574

Minowa AK, Garraffoni ARS (2017) A new species of *Haltidytes* Remane, 1936 (Gastrotricha: Chaetonotida: Dasydytidae) from an urban lagoon in Brazil with a phylogenetic reconstruction of the

Author's personal copy

genus based on morphological data. Zool Anz 269:100–109. https://doi.org/10.1016/j.jcz.2017.08.003

Neuhaus B, Schmid T, Riedel J (2017) Collection management and study of microscope slides: storage, profiling, deterioration, restoration procedures, and general recommendations. Zootaxa 4322(1):173. https://doi.org/10.11646/zootaxa.4322.1.1

Padial JM, Miralles A, De la RI, Vences M (2010) The integrative future of taxonomy. Front Zool 7:16. https://doi.org/10.1186/1742-9994-7-16

Pape T, Allison A, Bickel DJ, Carlton JT, Dikow T, Donegan T, Duszynski DW, El-Hawagry MS, Evenhuis NL, Fautin DG (2016) Species can be named from photos. Nature 537:307. https://doi.org/10.1038/537307b

Rogers DC, Ahyong ST, Boyko CB, D'acoz CD (2017) Images are not and should not ever be type specimens: a rebuttal to Garraffoni and Freitas. Zootaxa 4269:455–459. https://doi.org/10.11646/zootaxa.4269.4.3

Santos CMD, Amorim DS, Klassa B, Fachin DA, Nihei SS, de Carvalho CJB, Falaschi RL, Mello-Patiu CA, Couri MS, Oliveira SS (2016) On typeless species and the perils of fast taxonomy. Syst Ent 41:511–515. https://doi.org/10.1111/syen.12180

Sørensen MV, Sterrer W (2002) New characters in the gnathostomulid mouth parts revealed by scanning electron microscopy. J Morphol 253:310–334. https://doi.org/10.1002/jmor.10006

Sterrer W (1972) Systematics and evolution within the Gnathostomulida. Syst Zool 21:151–173. https://doi.org/10.1093/sysbio/21.2.151

Todaro MA, Hummon WD (2008) An overview and a dichotomous key to genera of the phylum Gastrotricha. Meiofauna Mar 16:3–20

Todaro MA, Dal Zotto M, Leasi F (2015) An integrated morphological and molecular approach to the description and systematisation of a novel genus and species of Macrodasyida (Gastrotricha). PLoS One 10:e0130278. https://doi.org/10.1371/journal.pone.0130278

Turland NJ, Wiersema JH, Barrie FR, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Kusber W-H, Li D-Z, Marhold K, May TW, McNeill J, Monro AM, Prado J, Price MJ, Smith GF (2018) International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the nineteenth international botanical congress Shenzhen, China, July 2017. Reg Veg 159:1–254

Zhang ZQ, Shear WA (2007) Linnaeus tercentenary and invertebrate taxonomy: an introduction. Zootaxa 1668:7–10

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

View publication stats

Authorship statements of PhD candidate
/
Oświadczenia doktoranta o wkładzie w powstanie artykułów

Poznań, 16.12.2019r.

Małgorzata Kolicka
Adam Mickiewicz University, Poznań
Faculty of Biology
Department of Animal Taxonomy and Ecology
Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article: **Kolicka M.**, Dabert M., Dabert J., Kånneby T. Kisielewski J. 2016. *Bifidochaetus*, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. *Invertebrate Systematics*, 30 (4): 398-419, was as follows: conceptualization, sample analyses and specimens extraction, morphological and molecular analyses of specimens, species identification, species and genus description, writing the first draft of the manuscript and revising the manuscript. I am first and corresponding author.

My total contribution to the following article is at 70%.

Yours faithfully,

Poznań, 16.12.2019r.

Małgorzata Kolicka
Uniwersytet im. Adama Mickiewicza, Poznań
Wydział Biologii
Zakład Taksonomii i Ekologii Zwierząt
ul. Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Oświadczenie określające wkład w powstanie artykułu

Niniejszym oświadczam, że mój wkład w powstanie poniższego artykułu: **Kolicka M.**, Dabert M., Dabert J., Kånneby T. Kisielewski J. 2016. *Bifidochaetus*, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. *Invertebrate Systematics*, 30 (4): 398-419, polegał na: zaprojektowaniu badań, analizie prób i izolacji z nich brzuchorzęsków, analizie morfologicznej i molekularnej okazów, identyfikacji gatunków, przygotowaniu opisu nowego gatunku i nowego rodzaju, napisaniu manuskryptu oraz poprawie manuskryptu po ocenie recenzentów. W tym artykule jestem pierwszym i korespondencyjnym autorem.

Mój całkowity wkład w pracę wynosi 70%.

Z wyrazami szacunku,

Poznań, 16.12.2019r.

Małgorzata Kolicka
Adam Mickiewicz University, Poznań
Faculty of Biology
Department of Animal Taxonomy and Ecology
Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article: **Kolicka M.**, Dabert M., Gadawski P. 2017. A new species of freshwater Chaetonotidae (Gastrotricha, Chaetonotida) from Obodska Cave (Montenegro) based on morphological and molecular characters. *European Journal of Taxonomy*, 354: 1-30, was as follows: conceptualization, samples analyses and specimens extraction, morphological and molecular analyses of specimens, species identification, species descriptions, writing the first draft of the manuscript and revising the manuscript. I am first and corresponding author.

My total contribution to the following article is at 70%.

Yours faithfully,

Poznań, 16.12.2019r.

Małgorzata Kolicka
Uniwersytet im. Adama Mickiewicza, Poznań
Wydział Biologii
Zakład Taksonomii i Ekologii Zwierząt
ul. Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Oświadczenie określające wkład w powstanie artykułu

Niniejszym oświadczam, że mój wkład w powstanie poniższego artykułu: **Kolicka M.**, Dabert M., Gadawski P. 2017. A new species of freshwater Chaetonotidae (Gastrotricha, Chaetonotida) from Obodska Cave (Montenegro) based on morphological and molecular characters. *European Journal of Taxonomy*, 354: 1-30, polegał na: zaprojektowaniu badań, analizie prób i izolacji z nich brzuchorzęsków, analizie morfologicznej i molekularnej okazów, identyfikacji gatunków, przygotowaniu opisu nowego gatunku, napisaniu manuskryptu oraz poprawie manuskryptu po ocenie recenzentów. W tym artykule jestem pierwszym i korespondencyjnym autorem.

Mój całkowity wkład w pracę wynosi 70%.

Z wyrazami szacunku,

Poznań, 16.12.2019r.

Małgorzata Kolicka
Adam Mickiewicz University, Poznań
Faculty of Biology
Department of Animal Taxonomy and Ecology
Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article: **Kolicka M**. 2017. New Arctic Gastrotricha – Three *Chaetonotus* (Chaetonotidae) from Longyearbyen (Spitsbergen, Svalbard Archipelago). *Annales Zoologici*, 67 (1): 131-180, was as follows: conceptualization, samples analyses and specimens extraction, morphological analyses of specimens, species identification, species descriptions, writing the first draft of the manuscript and revising the manuscript. I am the only author of this publication.

My total contribution to the following article is at 100%.

Yours faithfully,

Poznań, 16.12.2019r.

Małgorzata Kolicka
Uniwersytet im. Adama Mickiewicza, Poznań
Wydział Biologii
Zakład Taksonomii i Ekologii Zwierząt
ul. Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Oświadczenie określające wkład w powstanie artykułu

Niniejszym oświadczam, że mój wkład w powstanie poniższego artykułu: **Kolicka M**. 2017. New Arctic Gastrotricha – Three *Chaetonotus* (Chaetonotidae) from Longyearbyen (Spitsbergen, Svalbard Archipelago). *Annales Zoologici*, 67 (1): 131-180, polegał na: zaprojektowaniu badań, analizie prób i izolacji z nich brzuchorzęsków, analizie morfologicznej okazów, identyfikacji gatunków, przygotowaniu opisów nowych gatunków, napisaniu manuskryptu oraz poprawie manuskryptu po ocenie recenzentów. Jestem jedynym autorem tej publikacji.

Mój całkowity wkład w pracę wynosi 100%.

Z wyrazami szacunku,

Poznań, 16.12.2019r.

Małgorzata Kolicka
Adam Mickiewicz University, Poznań
Faculty of Biology
Department of Animal Taxonomy and Ecology
Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article: **Kolicka M.**, Kotwicki L., Dabert M. 2018. Diversity of Gastrotricha on Spitsbergen (Svalbard Archipelago, Arctic) with a Description of Seven New Species. *Annales Zoologici*, 68 (4): 609-739, was as follows: conceptualization, samples collection, samples analyses and specimens extraction, morphological and molecular analyses of specimens, species identification, species description, writing the first draft of the manuscript and revising the manuscript. I am first and corresponding author.

My total contribution to the following article is at 85%.

Yours faithfully,

Poznań, 16.12.2019r.

Małgorzata Kolicka
Uniwersytet im. Adama Mickiewicza, Poznań
Wydział Biologii
Zakład Taksonomii i Ekologii Zwierząt
ul. Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Oświadczenie określające wkład w powstanie artykułu

Niniejszym oświadczam, że mój wkład w powstanie poniższego artykułu: **Kolicka M.**, Kotwicki L., Dabert M. 2018. Diversity of Gastrotricha on Spitsbergen (Svalbard Archipelago, Arctic) with a Description of Seven New Species. *Annales Zoologici*, 68 (4): 609-739, polegał na: zaprojektowaniu badań, zbiorze prób, analizie prób i izolacji z nich brzuchorzęsków, analizie morfologicznej i molekularnej okazów, identyfikacji gatunków, przygotowaniu opisów nowych gatunków, napisaniu manuskryptu oraz poprawie manuskryptu po ocenie recenzentów. W tym artykule jestem pierwszym i korespondencyjnym autorem.

Mój całkowity wkład w pracę wynosi 85%.

Z wyrazami szacunku,

Poznań, 16.12.2019r.

Małgorzata Kolicka
Adam Mickiewicz University, Poznań
Faculty of Biology
Department of Animal Taxonomy and Ecology
Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article: **Kolicka M**. 2019. From saline to salty? *Heterolepidoderma sinus* (Chaetonotida, Chaetonotidae) from subsaline coal mine settling ponds. *Zootaxa*, 4559 (3): 568-572, was as follows: conceptualization, samples collection, samples analyses and specimens extraction, morphological and molecular analyses of specimens, species identification, writing the first draft of the manuscript and revising the manuscript. I am the only author of this publication.

My total contribution to the following article is at 100%.

Yours faithfully,

Poznań, 16.12.2019r.

Małgorzata Kolicka
Uniwersytet im. Adama Mickiewicza, Poznań
Wydział Biologii
Zakład Taksonomii i Ekologii Zwierząt
ul. Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Oświadczenie określające wkład w powstanie artykułu

Niniejszym oświadczam, że mój wkład w powstanie poniższego artykułu: **Kolicka M**. 2019. From saline to salty? *Heterolepidoderma sinus* (Chaetonotida, Chaetonotidae) from subsaline coal mine settling ponds. *Zootaxa*, 4559 (3): 568-572, polegał na: zaprojektowaniu badań, zbiorze materiału, analizie prób i izolacji z nich brzuchorzęsków, analizie morfologicznej i molekularnej okazów, identyfikacji gatunków, napisaniu manuskryptu oraz poprawie manuskryptu po ocenie recenzentów. Jestem jedynym autorem tej publikacji.

Mój całkowity wkład w pracę wynosi 100%.

Z wyrazami szacunku,

Poznań, 16.12.2019r.

Małgorzata Kolicka
Adam Mickiewicz University, Poznań
Faculty of Biology
Department of Animal Taxonomy and Ecology
Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article: **Kolicka M**. 2019. Gastrotricha – not only in sediments: new epiphytic species of Chaetonotida from the Jubilee Greenhouse of the Botanical Garden in Kraków. *European Journal of Taxonomy*, 511 (511): 1-100, was as follows: conceptualization, samples collection, samples analyses and specimens extraction, morphological analyses of specimens, species identification, species descriptions, writing the first draft of the manuscript and revising the manuscript. I am the only author of this publication.

My total contribution to the following article is at 100%.

Yours faithfully,

Poznań, 16.12.2019r.

Małgorzata Kolicka
Uniwersytet im. Adama Mickiewicza, Poznań
Zakład Taksonomii i Ekologii Zwierząt
ul. Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Oświadczenie określające wkład w powstanie artykułu

Niniejszym oświadczam, że mój wkład w powstanie poniższego artykułu: **Kolicka M**. 2019. Gastrotricha – not only in sediments: new epiphytic species of Chaetonotida from the Jubilee Greenhouse of the Botanical Garden in Kraków. *European Journal of Taxonomy,* 511 (511): 1-100, polegał na: zaprojektowaniu badań, zbiorze materiału, analizie prób i izolacji z nich brzuchorzęsków, analizie morfologicznej okazów, identyfikacji gatunków, przygotowaniu opisów nowych gatunków, napisaniu manuskryptu oraz poprawie manuskryptu po ocenie recenzentów. Jestem jedynym autorem tej publikacji.

Mój całkowity wkład w pracę wynosi 100%.

Z wyrazami szacunku,

Poznań, 16.12.2019r.

Małgorzata Kolicka
Adam Mickiewicz University, Poznań
Faculty of Biology
Department of Animal Taxonomy and Ecology
Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article: **Kolicka M**. 2019. New *Chaetonotus* (*Wolterecka*) *semovitus* sp. nov. (Gastrotricha: Chaetonotida: Chaetonotidae) from a Palm House in Vienna (Austria). *Annales Zoologici*, 69 (2): 447-475, was as follows: conceptualization, samples collection, samples analyses and specimens extraction, morphological analyses of specimens, species identification, species description, writing the first draft of the manuscript and revising the manuscript. I am the only author of this publication.

My total contribution to the following article is at 100%.

Yours faithfully,

Poznań, 16.12.2019r.

Małgorzata Kolicka
Uniwersytet im. Adama Mickiewicza, Poznań
Wydział Biologii
Zakład Taksonomii i Ekologii Zwierząt
ul. Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Oświadczenie określające wkład w powstanie artykułu

Niniejszym oświadczam, że mój wkład w powstanie poniższego artykułu: **Kolicka M**. 2019. New *Chaetonotus* (*Wolterecka*) *semovitus* sp. nov. (Gastrotricha: Chaetonotida: Chaetonotidae) from a Palm House in Vienna (Austria). *Annales Zoologici*, 69 (2):447-475, polegał na: zbiorze materiału, analizie prób i izolacji z nich brzuchorzęsków, analizie morfologicznej i molekularnej okazów, identyfikacji gatunków, przygotowaniu opisu nowego gatunku, napisaniu manuskryptu oraz poprawie manuskryptu po ocenie recenzentów. Jestem jedynym autorem tej publikacji.

Mój całkowity wkład w pracę wynosi 100%.

Z wyrazami szacunku,

Poznań, 16.12.2019r.

Małgorzata Kolicka
Adam Mickiewicz University, Poznań
Faculty of Biology
Department of Animal Taxonomy and Ecology
Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article: Garraffoni A. R. S., Kieneke A., **Kolicka M.**, Corgosinho P. H. C., Prado J., Nihei S. S., Freitas A. V. L. 2019. ICZN Declaration 45: a remedy for the nomenclatural and typification dilemma regarding soft-bodied meiofaunal organisms? *Marine Biodiversity*, https://doi.org/10.1007/s12526-019-00983-7, was as follows: investigation, resources, data curation, writing the first draft of the manuscript and revising the manuscript.

My total contribution to the following article is at 20%.

Yours faithfully,

Poznań, 16.12.2019r.

Małgorzata Kolicka
Uniwersytet im. Adama Mickiewicza, Poznań
Wydział Biologii
Zakład Taksonomii i Ekologii Zwierząt
ul. Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Oświadczenie określające wkład w powstanie artykułu

Niniejszym oświadczam, że mój wkład w powstanie poniższego artykułu: Garraffoni A. R. S., Kieneke A., **Kolicka M.**, Corgosinho P. H. C., Prado J., Nihei S. S., Freitas A. V. L. 2019. ICZN Declaration 45: a remedy for the nomenclatural and typification dilemma regarding soft-bodied meiofaunal organisms? *Marine Biodiversity*, https://doi.org/10.1007/s12526-019-00983-7, polegał na analizie i zgromadzeniu materiału oraz uczestnictwie w napisaniu manuskryptu i poprawie manuskryptu po ocenie recenzentów.

Mój całkowity wkład w pracę wynosi 20%.

Z wyrazami szacunku,

Authorship statements of co-authors of the article/ Oświadczenia współautorów o wkładzie w powstanie artykułów

Bifidochaetus, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach.

Invertebrate Systematics, 30 (4): 398-419

Poznań, 16.12.2019r.

Prof. dr hab. Mirosława Dabert
Adam Mickiewicz University, Poznań
Faculty of Biology
Molecular Biology Techniques Laboratory
Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: mirkad@amu.edu.pl

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:
Kolicka M., Dabert M., Dabert J., Kånneby T., Kisielewski J. 2016. *Bifidochaetus*, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. *Invertebrate Systematics*, 30 (4): 398-419 was as follows:
I contributed to the analysis and interpretation of data and writing the paper.

My total contribution to the following article is at 10%

Sincerely yours,

Poznań, 16.12.2019r.

Prof. dr hab. Mirosława Dabert
Uniwersytet im. Adama Mickiewicza, Poznań
Wydział Biologii
Wydziałowa Pracownia Technik Biologii Molekularnej
ul. Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: mirkad@amu.edu.pl

Oświadczenie określające wkład w powstanie artykułu

Niniejszym oświadczam, że mój wkład w powstanie poniższego artykułu:
Kolicka M., Dabert M., Dabert J., Kånneby T., Kisielewski J. 2016. *Bifidochaetus*, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. *Invertebrate Systematics*, 30 (4): 398-419 polegał na: uczestniczeniu w analizie i interpretacji danych i w pisaniu pracy.

Swój całkowity wkład oceniam na 10%.

Z wyrazami szacunku,

Poznań, 16.12.2019r.

Prof. dr hab. Jacek Dabert
Adam Mickiewicz University, Poznań
Faculty of Biology
Department of Animal Morphology
Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: kolicka@amu.edu.pl

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:
Kolicka M., Dabert M., Dabert J., Kånneby T., Kisielewski J. 2016. *Bifidochaetus*, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. *Invertebrate Systematics*, 30 (4): 398-419 was as follows: participation of the phylogenetic analysis.

My total contribution to the following article is at 5%

Sincerely yours,

Poznań, 16.12.2019r.

Prof. dr hab. Jacek Dabert
Uniwersytet im. Adama Mickiewicza, Poznań
Wydział Biologii
Zakład Morfologii Zwierząt
ul. Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: dabert@amu.edu.pl

Oświadczenie określające wkład w powstanie artykułu

Niniejszym oświadczam, że mój wkład w powstanie poniższego artykułu:
Kolicka M., Dabert M., Dabert J., Kånneby T., Kisielewski J. 2016. *Bifidochaetus*, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. *Invertebrate Systematics*, 30 (4): 398-419 polegał na: współudziale w przeprowadzeniu analizy filogenetycznej.

Swój całkowity wkład oceniam na 5%.

Z wyrazami szacunku,

Gävle, 19.12.2019r.

Prof. Tobias Kånneby
Swedish Museum of Natural History
Department of Zoology,
PO Box 50007,
SE–104 05 Stockholm
e-mail: tobiaskanneby@gmail.com

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:
Kolicka M., Dabert M., Dabert J., Kånneby T., Kisielewski J. 2016. *Bifidochaetus*, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. *Invertebrate Systematics*, 30 (4): 398-419 was as follows: contributed by supplying (i), additional information on *Bifidochaetus veronicae*; (ii), additonal information to the discussion; and (iii), correction of language and grammar.

My total contribution to the following article is at 5%

Sincerely yours,

Toronto, 27.12.2019r.

Dr hab. Jacek Kisielewski
Adam Mickiewicz University, Poznań
Faculty of Biology
Department of Animal Taxonomy and Ecology
Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: jjkisielewski@gmail.com

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:
Kolicka M., Dabert M., Dabert J., Kånneby T., Kisielewski J. 2016. *Bifidochaetus*, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. *Invertebrate Systematics*, 30 (4): 398-419

was at 10%

Sincerely yours,

Toronto, 27.12.2019r.

Dr hab. Jacek Kisielewski
Uniwersytet im. Adama Mickiewicza, Poznań
Wydział Biologii
Zakład taksonomii i Ekologii Zwierząt
ul. Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: jjkisielewski@gmail.com

Oświadczenie określające wkład w powstanie artykułu

Niniejszym oświadczam, że mój wkład w powstanie poniższego artykułu:
Kolicka M., Dabert M., Dabert J., Kånneby T., Kisielewski J. 2016. *Bifidochaetus*, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. *Invertebrate Systematics*, 30 (4): 398-419

oceniam na 10%.

Z wyrazami szacunku,

Authorship statements of co-authors of the article/ Oświadczenia współautorów o wkładzie w powstanie pracy

A new species of freshwater Chaetonotidae (Gastrotricha, Chaetonotida) from Obodska Cave (Montenegro) based on morphological and molecular characters. *European Journal of Taxonomy*, 354: 1-30

Poznań, 16.12.2019r.

Prof. dr hab. Mirosława Dabert
Adam Mickiewicz University, Poznań
Faculty of Biology
Molecular Biology Techniques Laboratory
Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: mirkad@amu.edu.pl

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:
Kolicka M., Dabert M., Dabert J., Kånneby T., Kisielewski J. 2016. *Bifidochaetus*, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. *Invertebrate Systematics*, 30 (4): 398-419 was as follows:
I contributed to the analysis and interpretation of data and writing the paper.

My total contribution to the following article is at 10%

Sincerely yours,

Poznań, 16.12.2019r.

Prof. dr hab. Mirosława Dabert
Uniwersytet im. Adama Mickiewicza, Poznań
Wydział Biologii
Wydziałowa Pracownia Technik Biologii Molekularnej
ul. Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: mirkad@amu.edu.pl

Oświadczenie określające wkład w powstanie artykułu

Niniejszym oświadczam, że mój wkład w powstanie poniższego artykułu:
Kolicka M., Dabert M., Dabert J., Kånneby T., Kisielewski J. 2016. *Bifidochaetus*, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. *Invertebrate Systematics*, 30 (4): 398-419 polegał na: uczestniczeniu w analizie i interpretacji danych i w pisaniu pracy.

Swój całkowity wkład oceniam na 10%.

Z wyrazami szacunku,

Guelph, 18.12.2019r.

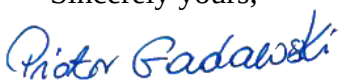
MSc Piotr Gadawski
University of Lodz
Faculty of Biology and Environmental Protection
Department of Invertebrate Zoology and Hydrobiology
Katedra Zoologii Bezkręgowców i Hydrobiologii
Banacha Street 12/16
90-237 Lodz
e-mail: Piotr.gadawski@biol.uni.lodz.pl

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:
Kolicka M., Gadawski, P., Dabert M. 2017. A new species of freshwater Chaetonotidae (Gastrotricha, Chaetonotida) from Obodska Cave (Montenegro) based on morphological and molecular characters. *European Journal of Taxonomy*, 354: 1-30 was as follows: sample collection, figures preparation, partially developing "Material and methods" chapter.

My total contribution to the following article is at 25%

Sincerely yours,



Guelph, 18.12.2019r.

Mgr Piotr Gadawski
Uniwersytet Łódzki
Wydział Biologii i Ochrony Środowiska
Department of Animal Morphology
Katedra Zoologii Bezkręgowców i Hydrobiologii
ul. Banacha 12/16
90-237 Łódź
e-mail: Piotr.gadawski@biol.uni.lodz.pl

Oświadczenie określające wkład w powstanie artykułu

Niniejszym oświadczam, że mój wkład w powstanie poniższego artykułu:
Kolicka M., Gadawski, P., Dabert M. 2017. A new species of freshwater Chaetonotidae (Gastrotricha, Chaetonotida) from Obodska Cave (Montenegro) based on morphological and molecular characters. *European Journal of Taxonomy*, 354: 1-30 polegał na: zebraniu materiału, przygotowaniu części rycin, oraz pomoc w opracowaniu rozdziału „Materiały i metody".

Swój całkowity wkład oceniam na 25%.

Z wyrazami szacunku,

Piotr Gadawski

Authorship statements of co-authors of the article/ Oświadczenia współautorów o wkładzie w powstanie pracy

Diversity of Gastrotricha on Spitsbergen (Svalbard Archipelago, Arctic) with a Description of Seven New Species. *Annales Zoologici*, 68 (4): 609-739

Sopot, 16.12.2019r.

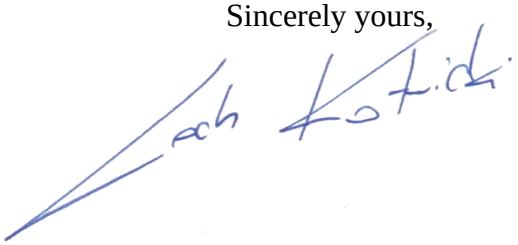
dr hab. Lech Kotwicki prof. IOPAN
Institute of Oceanology, Polish Academy of Sciences
Department of Marine Ecology
Powstańców Warszawy 55
81–712 Sopot
e-mail: lechk@iopan.gda.pl

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:
Kolicka M., Kotwicki L., Dabert M. 2018. Diversity of Gastrotricha on Spitsbergen (Svalbard Archipelago, Arctic) with a Description of Seven New Species. *Annales Zoologici*, 68 (4): 609-739 was as follows: the environmental aspect description.

My total contribution to the following article is at 5%

Sincerely yours,



Poznań, 16.12.2019r.

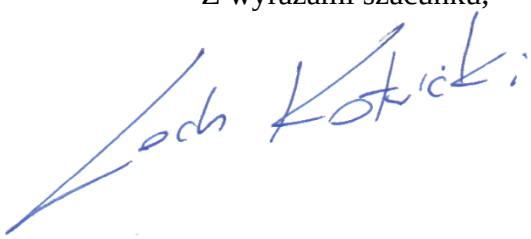
dr hab. Lech Kotwicki prof. IOPAN
Instytut Oceanologii Polskiej Akademii Nauk
Zakład Ekologii Morza
ul. Powstańców Warszawy 55
81–712 Sopot
e-mail: lechk@iopan.gda.pl

Oświadczenie określające wkład w powstanie artykułu

Niniejszym oświadczam, że mój wkład w powstanie poniższego artykułu:
Kolicka M., Kotwicki L., Dabert M. 2018. Diversity of Gastrotricha on Spitsbergen (Svalbard Archipelago, Arctic) with a Description of Seven New Species. *Annales Zoologici*, 68 (4): 609-739 polegał na: opisie aspektu ekologicznego.

Swój całkowity wkład oceniam na 5%.

Z wyrazami szacunku,



Poznań, 16.12.2019r.

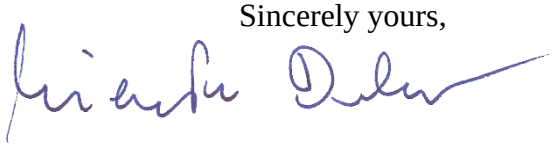
Prof. dr hab. Mirosława Dabert
Adam Mickiewicz University, Poznań
Faculty of Biology
Molecular Biology Techniques Laboratory
Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: mirkad@amu.edu.pl

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:
Kolicka M., Kotwicki L., Dabert M. 2018. Diversity of Gastrotricha on Spitsbergen (Svalbard Archipelago, Arctic) with a Description of Seven New Species. *Annales Zoologici*, 68 (4): 609-739 was as follows: I contributed to the analysis and interpretation of data and writing the paper.

My total contribution to the following article is at 10%

Sincerely yours,



Poznań, 16.12.2019r.

Prof. dr hab. Mirosława Dabert
Uniwersytet im. Adama Mickiewicza, Poznań
Wydział Biologii
Wydziałowa Pracownia Technik Biologii Molekularnej
ul. Uniwersytetu Poznańskiego 6
61-614 Poznań
e-mail: mirkad@amu.edu.pl

Oświadczenie określające wkład w powstanie artykułu

Niniejszym oświadczam, że mój wkład w powstanie poniższego artykułu:
Kolicka M., Kotwicki L., Dabert M. 2018. Diversity of Gastrotricha on Spitsbergen (Svalbard Archipelago, Arctic) with a Description of Seven New Species. *Annales Zoologici*, 68 (4): 609-739 polegał na: uczestniczeniu w analizie i interpretacji danych i w pisaniu pracy.

Swój całkowity wkład oceniam na 10%.

Z wyrazami szacunku,

Authorship statements of co-authors of the article/ Oświadczenia współautorów o wkładzie w powstanie pracy

ICZN Declaration 45: a remedy for the nomenclatural and typification dilemma regarding soft-bodied meiofaunal organisms? *Marine Biodiversity*, https://doi.org/10.1007/s12526-019-00983-7

Campinas, 16.12.2019

Prof. André R. S. Garraffoni
Universidade Estadual de Campinas
Instituto de Biologia
Departamento de Biologia Animal
Monteiro Lobato, 255
e-mail: arsg@unicamp.br

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:
Garraffoni, A.R.S., Kieneke A., **Kolicka M.**, Corgosinho P.H.C., Prado J., Nihei S.S., Freitas A.V.L. 2019. ICZN Declaration 45: a remedy for the nomenclatural and typification dilemma regarding soft-bodied meiofaunal organisms? *Marine Biodiversity*, https://doi.org/10.1007/s12526-019-00983-7.

My total contribution to the following article is at 25% and my contributions were Conceptualization; investigation; resources, data curation; writing—original draft preparation; writing—review and editing

Also, as a first author of this article, I confirm the participation of other authors in this publication as is listed below:

Kieneke A. 20%, contributions: investigation; resources, data curation; writing—original draft preparation; writing—review and editing

Kolicka M. 20%, contributions: investigation; resources, data curation, writing—original draft preparation; writing—review and editing

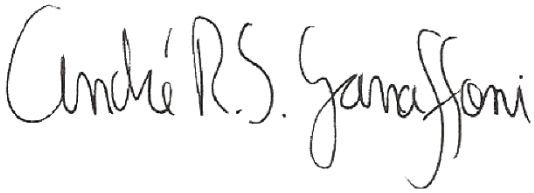
Corgosinho P.H.C. 10%, contributions: investigation; original draft preparation, writing—review and editing; writing—review and editing

Prado J. 10%, contributions: investigation; writing—original draft preparation; writing—review and editing

Nihei S.S. 7,5%, contributions: writing—original draft preparation; writing—review and editing

Freitas A.V.L., 7,5%, contributions: writing—original draft preparation; writing—review and editing.

Sincerely yours,



ACKNOWLEDGEMENTS

When developing my scientific career, I met plenty of great people, and I would not have been where I am today without their help and support. And even though finalising the dissertation changes nothing, it is a good moment to express my gratitude to the people who made it possible for the PhD thesis to be completed.

At first, I would like to thank Krzysztof Zawierucha, PhD, for our shared journey we have been on and all support given during it.

In my acknowledgements I also cannot ignore the Invertebrate Studies Section – a part of the AMU Naturalists' Scientific Club in which I found the company and scientific space that allowed me to develop and realise my passion.

I would like to pass my sincere thanks to Prof. Jacek Junosza Kisielewski who provided me with invaluable substantive support in the field of gastrotrichs taxonomy, and owing to whom I had a chance to discuss potential evolutionary paths of these animals as well as their morphology and ecology. I wholeheartedly thank you for all your passion, heart and devotion to the animals. It was particularly valuable in difficult and demanding moments. You have set an unsurpassed example to follow.

I would like to thank Prof. UAM, dr hab. Wojciech Magowski for all your substantive support, independence you let me maintain and your understanding for my habits, customs and work style.

I sincerely thank Prof. dr hab. Michał Grabowski for all the inspiring discussions and scientific support for my National Science Centre Preludium grant.

I would like to thank Prof. dr hab. Jan Marcin Węsławski for offering scientific support to my research and the Ministry of Science and Higher Education's Diamond Grant.

I am enormously grateful to Prof. dr hab. Lech Kotwicki for all the inspiring discussions as well as teaching me a wealth of new skills indispensable when exploring marine meiofauna. Thank you for all the scientific expeditions, for your help while sampling and for lending me the equipment.

To the members of the 37th and the 39th Polish Polar Expeditions as well as all the researchers from the Polish Polar Station, Hornsund: thank you for your kind help during sampling and analysis of the arctic material. I would like to offer my most sincere thanks to Joanna Przytarska, PhD who took excellent care of me and whose supervision was invaluable during my first weeks in Hornsund.

I would also like to thank all the workers and students (including the PhD ones) of the Department of Marine Ecology of the Institute of Oceanology of the Polish Academy of Sciences in Sopot for allowing me to gain experience, acquire new skills and broaden my horizons during numerous visits and scholarships in the Institute.

I would like to thank Prof. dr hab Andrzej Kaźmierski and Prof. dr hab. Maciej Skoracki for all the taxonomic discussions we shared and for your understanding of my taxonomic dilemmas – even if they were related to a different group of organisms.

I thank Michał Oźmiński for friendship and all the scientific expeditions we made together.

I am expressing my gratitude to Mateusz Zmudziński. Thank you for our friendship and inspiring sharing of ideas.

I would like to thank Katarzyna Frątczak-Łagiewska, Justyna Liberska, Paweł Podkowa, Artur Trzebny and Natalia Szudarek-Trepto for sharing research efforts, for all the interesting discussions and all the support I received in various aspects.

The workers from the Department of Invertebrate Zoology and Hydrobiology at the University of Łódź: thank you for all the interesting discussions in pleasant company and circumstances, for sharing your thoughts and showing me, it is not only work itself one can enjoy but life, too.

I would like to sincerely thank Małgorzata Kuźnik, Alicja Kubicka and Beata Starczewska for their help in preparing taxonomic illustrations for the new taxa.

I am offering special thanks to all the workers of the Molecular Biology Techniques Laboratory at the Faculty of Biology for their support and help during the molecular part of my studies.

I would also like to pass special thanks to all the Co-authors of my papers for exploring gastrotrichs and for constructive disputes of contradictory ideas.

Almost last but not least, I thank the Department of Animal Taxonomy and Ecology which has been my home for all those years. A home I was always eager to return to from various expeditions and journeys, and where I could have always found what I appreciate the most: scientific independence.

Finally, I would like to express my sincere gratitude to my Dissertation Supervisor, the late Prof. UAM, dr hab. Ziemowit Olszanowski. I would like to thank you for your understanding, the self-confidence you instilled in me, your silent yet permanent presence brought relief.

PODZIĘKOWANIA

Na swojej naukowej drodze spotkałam wielu wspaniałych ludzi, bez pomocy i wsparcia których nie znajdowałabym się teraz tu gdzie jestem. Choć sama finalizacja rozprawy nie zmieni niczego, jest to dobry moment by wyrazić swoją wdzięczność wobec tych którzy sprawili, że ta dysertacja miała szansę powstać.

Pragnę na samym wstępie podziękować Dr. Krzysztofowi Zawierusze za tą naszą wspólną drogę jaką przemierzyliśmy i całe udzielone podczas niej wsparcie.

W moich podziękowaniach, nie może także zabraknąć Sekcji Badania Bezkręgowców Koła Naukowego Przyrodników w której to znalazłam towarzystwo i naukową przestrzeń pozwalające na rozwój i realizację pasji.

Serdecznie podziękowania kieruję do Prof. Jacka Junoszy Kisielewskiego za nieocenioną pomoc merytoryczną w dziedzinie taksonomii brzuchorzęsków oraz bezcenne dyskusje o potencjalnych drogach ewolucji tych zwierząt, o ich morfologii oraz ekologii. Ogromnie dziękuję za całą Pańską pasję, serce i oddanie dla tych zwierząt które we wszystkich trudnych i wymagających chwilach były dla mnie niedoścignionym przykładem.

Wyrazy podziękowania należą się Prof. UAM dr. hab. Wojciechowi Magowskiemu za wsparcie merytoryczne, pozostawioną swobodę i wyrozumiałość dla moich nawyków, przyzwyczajeń oraz stylu pracy.

Prof. dr. hab. Michałowi Grabowskiemu serdecznie dziękuję za wszystkie inspirujące dyskusje oraz opiekę naukową nad moim grantem Preludium przyznanym przez Narodowe Centrum Nauki.

Prof. dr. hab. Janowi Marcinowi Węsławskiemu dziękuję za przyjęcie mnie pod swoją opiekę i pieczę naukową nad moim Diamentowym Grantem przyznanym przez Ministerstwo Nauki i Szkolnictwa Wyższego.

Jestem ogromnie wdzięczna prof. dr. hab. Lechowi Kotwickiemu za wszystkie inspirujące dyskusje oraz za nauczenie mnie wielu umiejętności potrzebnych w badaniu mejofauny morskiej. Dziękuję za te wszystkie wyprawy badawcze, za pomoc przy zbiorze prób i użyczenie sprzętu.

Członkom XXXVII i XXXIX Polskiej Ekspedycji Polarnej oraz wszystkim badaczom przebywającym w Stacji im. Stanisława Siedleckiego w Hornsundzie dziękuję za pomoc udzieloną przy zbiorze i analizie prób arktycznych. Szczególnie serdeczne podziękowania kieruję do Dr Joanny Przytarskiej która troskliwie się mną opiekowała i sprawowała nade mną pieczę przez pierwsze tygodnie pobytu w Hornsundzie.

Podziękowania składam również wszystkim pracownikom, doktorantom i studentom Zakładu Ekologii Morza, Instytutu Oceanologii PAN w Sopocie za możliwość zdobycia doświadczenia, nowych umiejętności i poszerzenia horyzontów podczas moich licznych wizyt i staży u Was w Instytucie.

Prof. dr. hab. Andrzejowi Kaźmierskiemu oraz Prof. dr. hab. Maciejowi Skorackiemu bardzo dziękuję za wszystkie wspólne dyskusje taksonomiczne i zrozumienie dla taksonomicznych rozterek, nawet jeśli dotyczyły one zupełnie innej grupy organizmów.

Dziękuję Michałowi Ożmińskiemu za przyjaźń i wszystkie wspólne wyprawy naukowe.

Mateuszowi Zmudzińskiemu składam wyrazy wdzięczności za przyjaźń i zrozumienie, nawet w tych niezrozumiałych chwilach oraz inspirujące wymiany idei.

Katarzynie Frątczak-Łagiewskiej, Justynie Liberskiej, Pawłowi Podkowie, Arturowi Trzebnemu oraz Natalii Szudarek-Trepto dziękuję za wspólne zmagania z trudami badawczymi, ciekawe dyskusje i całe udzielone na różnych płaszczyznach wsparcie.

Pracownikom Katedry Zoologii Bezkręgowców i Hydrobiologii Uniwersytetu Łódzkiego dziękuję za wszystkie ciekawe dyskusje w miłym towarzystwie i okolicznościach, za wymianę myśli oraz pokazanie, że można cieszyć się nie tylko pracą ale też życiem.

Małgorzacie Kuźnik, Alicji Kubickiej oraz Beacie Starczewskiej serdecznie dziękuję za pomoc w przygotowaniu ilustracji taksonomicznych dla nowych taksonów.

Specjalne podziękowania kieruję do wszystkim pracowników Wydziałowej Pracowni Technik Biologii Molekularnej za wsparcie i pomoc podczas realizacji molekularnej części moich badań.

Szczególne podziękowania też kieruję do wszystkich Współautorów artykułów za wspólne zgłębianie tajemnic brzuchorzęsków oraz konstruktywne ścieranie się odmiennych idei.

Niemalże na końcu dziękuję Zakładowi Taksonomii i Ekologii Zwierząt który przez te wszystkie lata był mi prawdziwym domem. Domem do którego zawsze chętnie wracałam z różnych wypraw i wyjazdów, a w którym zawsze odnajdowałam to co cenię najbardziej – naukową wolność.

Na koniec pragnę wyrazić wdzięczność za wszystko mojemu nieodżałowanemu Promotorowi śp. Prof. UAM dr. hab. Ziemowitowi Olszanowskiemu. Dziękuję szczególnie za otuchę, zrozumienie, oparciem i niesłabnącą wiarę w moje możliwości.

Research funding

The research, whose results are presented in this dissertation, was financed under the program of the Ministry of Science and Higher Education (DIAMOND GRANT number DI2014 013844), by the National Science Center (grant PRELUDIUM number 2015/17/N/NZ8/01568, and ETIUDA number 2018/28/T/NZ8 / 00013). During my PhD studies I also benefited from financial support in the form of a scholarship from the UAM Foundation.

Finansowanie badań

Badania, których wyniki przedstawiono w niniejszej rozprawie zostały sfinansowane w ramach programu Ministerstwa Nauki i Szkolnictwa Wyższego (DIAMENTOWY GRANT numer DI2014 013844), przez Narodowe Centrum Nauki (grant PRELUDIUM numer 2015/17/N/NZ8/01568, oraz ETIUDA numer 2018/28/T/NZ8/00013). W czasie studiów doktoranckich korzystałam też ze wsparcia finansowego w postaci stypendium Fundacji UAM.