Diversity and ecology of water bears (Tardigrada) in Svalbard archipelago

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CONTENTS

I STRESZCZENIE

Archipelag Svalbard położony jest w europejskiej części Arktyki (pomiędzy 74 a 81 stopniem szerokości północnej oraz 10 a 35 stopniem długości wschodniej) między Norwegią, a biegunem północnym (Coulson 2013, Stankowski 2013). W skład archipelagu wchodzi kilkadziesiąt wysp, największa z nich to Spitsbergen. W ciągu ostatnich kilku tysięcy lat Svalbard pokryty był kilkukrotnie lądolodem (Lonne i Lysa 2005). Panuje tam typowy klimat polarny, jednak temperatury są wyraźnie wyższe niż w innych częściach Arktyki położonych na tej samej szerokości geograficznej (Przybylak i in. 2014). Z tego też powodu duże obszary Svalbardu, oprócz lodowców, pokryte są tundrą. Rzeźba terenu, ze względu na znaczny udział lodowców oraz budowę geologiczną (liczne pasma górskie) jest bardzo różnorodna, a największe wyspy archipelagu charakteryzują się występowaniem rozległych i głębokich fiordów. Do specyficznych elementów tego obszaru, oprócz obecności licznych lodowców, zaliczyć należy (a) wpływ różnych prądów morskich na klimat, (b) istnienie silnego związku pomiędzy dostawą biogenów pochodzących od kręgowców, a zgrupowaniami bezkręgowców w tundrze oraz (c) znikomy wpływ działalności człowieka na ekosystem.

Archipelag Svalbard to jedno z najlepiej zbadanych miejsc w Arktyce pod kątem fauny lądowej i słodkowodnej. Obecnie z tego obszaru znanych jest ok. 1000 gatunków bezkręgowców (Coulson i in. 2014). Ponadto przez cały czas odnotowywane są kolejne gatunki, w tym nowe dla wiedzy (np. Kaczmarek i in. 2012, Gwiazdowicz i in. 2012, Kiedrowicz i in. 2016, Kolicka i in. 2016). Ze względu na obecność ośrodka uniwersyteckiego (University Centre in Svalbard) oraz sieci stacji badawczych, w których prowadzone są kompleksowe badania z różnych dziedzin, stale wzrasta międzynarodowe zainteresowanie naukowe, polityczne i przemysłowe tym obszarem. Z tego też powodu Svalbard traktowany jest jako „naturalne laboratorium” dla badań nad bezkręgowcami (Coulson 2013).

Lądowe i słodkowodne ekosystemy arktyczne są zwykle uważane za ubogie pod względem różnorodności gatunkowej. Związane jest to z warunkami środowiskowymi i klimatycznymi, takimi jak niskie temperatury i ograniczona dostępność składników odżywczych, krótki okres wegetacyjny i znaczne pokrycie obszaru lodem. Jednakże, większość publikowanych badań wskazuje na to, że zarówno tundra jak i lodowce są środowiskiem życia licznych gatunków mikroskopijnych zwierząt. Najwyższe zagęszczenia

i biomasa bezkręgowców zasiedlających tundrę na Svalbardzie są związane z obszarami kolonii ptaków planktonożernych (np. Zmudczyńska i in. 2012). Z kolei na lodowcach tzw. „oazami życia” dla bezkręgowców są kriokonity – czyli niewielkie zagłębienia w powierzchni lodowców wypełnione wodą (Hodson i in. 2008, Cook i in. 2015, Zawierucha i in. 2015b).

Lodowce oraz czapy lodowe pokrywają ok. 10% powierzchni naszej planety, dlatego też Międzynarodowy Panel do Spraw Zmian Klimatu (IPCC) podkreśla istotność lodowców jako ekosystemu będącego jednocześnie magazynem 70% wody słodkiej na świecie (ACIA 2006, Vaughan i in. 2013). Svalbard w 59% powierzchni pokryty jest lodowcami oraz czapami lodowymi (Hagen i in. 2003). Powierzchnia lodu jest zasiedlana przez bakterie (w większości są to sinice), glony, grzyby, pierwotniaki, ale także mikroskopijne zwierzęta (Hodson i in. 2008, Cook i in. 2015, Zawierucha i in. 2015b, Kaczmarek i in. 2016). Ze względu na charakterystyczne cechy krajobrazu i klimatu oraz unikatowe zgrupowania mikroorganizmów systemy glacjalne stanowią osobny biom (Anesio i Laybourn-Parry 2012). Pomimo długiej historii badań nad elementami biotycznymi lodowców rozpoczętych na przełomie XIX i XX wieku (Drygalski 1897) mało uwagi poświęcono zwierzętom, które pełnią funkcje konsumentów wyższego rzędu i kontrolują zagęszczenia innych organizmów w ekosystemach glacjalnych (Zawierucha i in. 2015b, Vonnahme i in. 2015). Pomimo tego, że bezkręgowce są roślinożercami jak i drapieżnikami i mogą wpływać na obieg węgla, a także przyspieszać topnienie powierzchni lodowców, stan wiedzy na temat ich różnorodności i ekologii jest niewielki (Zawierucha i in. 2015b).

Ptaki w ekosystemach arktycznych są łącznikiem pomiędzy bogatymi ekosystemami morskimi a ubogimi w składniki odżywcze ekosystemami lądowymi. Żywią się one w morzu i gniazdują na lądzie gdzie pozostawiają duże ilości guana, które użyźnia ubogie ekosystemy lądowe (Stempniewicz 1990, 1992). Szczególnie ważne w tym procesie są alczyki (*Alle alle* Linnaeus, 1758), które gniazdują na łagodnych zboczach górskich w głębi lądu, przez co nawożą obszar w sąsiedztwie kolonii a także tundrę między miejscem gniazdowania a morzem (Stempniewicz i in. 2007). Dla przykładu kolonia alczyków zlokalizowana w fiordzie Hornsund na Spitsbergenie dostarcza do 60 ton suchego guana na km^2 (Stempniewicz 1990, 1992). Efektem tego nawożenia jest zwiększenie różnorodności i biomasy roślin, ale także bezkręgowców (Eurola i Hakala 1977, Zmudczyńska i in. 2012). Ponadto miejsca nawożone przez ptaki pełnią funkcję żerowiska dla kręgowców takich jak renifery czy bernikle (Jakubas i in. 2008).

Obiektem moich badań są niesporczaki (Tardigrada) czyli bezkręgowce zasiedlające zarówno tundrę jak i lodowce. Tardigrada w systematyce stanowią osobny typ bezkręgowców wielkości od ok. 50 do 1200 µm. Obecnie dzielimy je na dwie gromady Heterotardigrada i Eutardigrada (Nelson i in. 2015). Do dziś opisano ok. 1200 gatunków niesporczaków (Degma i in. 2016, Vicente i Bertolani 2013). Zwierzęta te zasiedlają ekosystemy wodne i lądowe od głębin oceanów po szczyty górskie, włączając w to siedliska ekstremalne takie jak pustynie czy powierzchnie lodowców. Na lądzie niesporczaki żyją w mszakach, porostach, glebie i ściółce, z kolei w wodzie zasiedlają głównie osady i rośliny wodne (Ramazzotti i Maucci 1983, Nelson i in. 2015). Niesporczaki to jedna z liczniejszych grup bezkręgowców zasiedlających Svalbard (Coulson i in. 2014). Zagęszczenia niesporczaków sięgają kilkuset osobników na gram suchego materiału, a przeliczając ich zagęszczenie na powierzchnię obliczaną w m^2 liczebności sięgają kilkudziesięciu tysięcy (Dastych 1988, Zawierucha i in. 2016).

Badania niesporczaków na Svalbardzie mają długą historię i sięgają początku XX wieku (Scourfield 1897). Jednakże, pomimo wielu lat badań, wiedza na temat fauny Tardigrada tej części Arktyki wciąż jest niewielka. Większość prac nad różnorodnością Tardigrada Svalbardu dotyczy tylko największej wyspy archipelagu – Spitsbergenu (np. Węglarska 1965, Dastych 1985, Tumanov 2007). Fauna większości pozostałych wysp jest bardzo słabo poznana lub wręcz całkowicie niezbadana, a i sam Spitsbergen, jak pokazują najnowsze prace, zbadany jest bardzo nierównomiernie (np. Kaczmarek i in. 2012). Wiele gatunków wciąż wymaga formalnego potwierdzenia statusu taksonomicznego, inne wymagają redeskrypcji (Zawierucha i in. 2013). W rejonach polarnych niesporczaki wraz z wrotkami oraz nicieniami są głównymi elementami fauny (Porazińska i in. 2002, Sohlenius i Boström 2008). Pomimo ważnej funkcji niesporczaków w ekosystemach polarnych (np. Sohlenius i Boström 2008, Vonnahme i in. 2015), wiedza na temat ich ekologii jest wciąż znikoma.

W przeszłości jedyne kompleksowe badania nad niesporczakami prowadzone były przez Dastycha (1985). W części faunistycznej opisał on nowy podgatunek *Mesobiotus harmsworthi obscurus* (Dastych, 1985). Natomiast w części ekologicznej wykazał że różne gatunki niesporczaków występują na podłożu o różnej zawartości węgla. Dodatkowo opisał on zależność pomiędzy wysokością n.p.m., a różnorodnością gatunkową niesporczaków (Dastych 1985). Jednakże w świetle nowych badań okazuje się, że wpływ na rozmieszczenie i różnorodność Tardigrada w Arktyce mogą mieć także ptaki gniazdujące na górskich zboczach (Zawierucha i in. 2015c). Dostarczają one znaczną ilość guana do ekosystemów tundrowych, tym samym użyźniając glebę i wpływając pozytywnie na rośliny, czyli siedlisko dla bezkręgowców (Zmudczyńska i in. 2008, 2009, 2012). Badania pokazują, że niesporczaki w sąsiedztwie kolonii ptaków są istotnie większe od niesporczaków zasiedlających obszary będące poza bezpośrednim wpływem kolonii. Jest to związane prawdopodobnie z lepszą bazą pokarmową w postaci większej biomasy roślin (Zawierucha i in. 2015a). Niestety wciąż brak jest informacji na temat wpływu zróżnicowania siedliska na Tardigrada, które może mieć istotny związek z ich różnorodnością i liczebnością. Tundra na Svalbardzie wydaje się najlepszym miejscem do takich badań ze względu na różnorodność flory – setki gatunków mchów i porostów (Eurola i Hakala 1977, Øvstedal i in. 2011).

Jak dotąd nie poświęcono szczególnej uwagi niesporczakom zasiedlającym kriokonity Svalbardu (Zawierucha i in. 2015b). O Tardigrada wspominano w dwóch pracach bez szczegółowych analiz morfologicznych, zagęszczeń czy opisu ich funkcji (Dastych 1985, De Smet i Van Rompu 1994). Jedyne badania ekologiczne uwzględniające zwierzęta w kriokonitach arktycznych przeprowadził Vonnahme i in. (2015), jednakże autorzy w swoich badaniach nie uwzględnili morfologii badanych kriokonitów, zagęszczeń i różnorodności niesporczaków tym samym ich miejsca w sieciach troficznych ekosystemów glacjalnych.

W związku z poważnymi lukami w wiedzy na temat niesporczaków archipelagu Svalbard, ale też generalnie całego rejonu arktycznego, **główne cele mojej rozprawy doktorskiej to:**

- zbadanie różnorodności gatunkowej niesporczaków w ekosystemach tundrowych i glacjalnych,

- porównanie fauny Tardigrada tundry i lodowców,

- przetestowanie hipotezy, że morfologia oraz położenie kriokonitów wpływają na zagęszczenia Tardigrada,

- przetestowanie hipotezy, że guano z kolonii ptaków planktonożernych wpływa na zagęszczenie i różnorodność niesporczaków w tundrze,

- ocena wpływu zróżnicowania siedliska na zagęszczenie i różnorodność niesporczaków w porostach.

W pierwszej części pracy doktorskiej przedstawiam opis nowego dla wiedzy gatunku niesporczaka – *Isohypsibius karenae*. W dwóch próbach mchów zebranych z tundry na Spitsbergenie znalazłem 5 gatunków niesporczaków, w tym jeden nowy dla nauki. Łącznie z próbek wyizolowałem i spreparowałem 32 okazy oraz 9 wylinek należących do nowego taksonu. Opis morfologiczny uzupełniłem o dane morfometryczne dla 33 cech zmierzonych w zakresie od 1 do 15 osobników. Łącznie wykonałem 270 pomiarów, które zostały wykorzystane także w diagnozie różnicującej. Nowy gatunek różni się od innych opisanych do tej pory podobnych gatunków z rodzaju *Isohypsibius* Thulin, 1928 kombinacją następujących cech: obecnością retikularnej rzeźby na kutikuli oraz kutykularnych barów pod pazurami na trzech pierwszych parach odnóży, wzrastającą długością makroplakoidów w gardzieli (1<2<3), innym ułożeniem ząbków w gardzieli, a także szerszą i dłuższą rurką gardzielową. Opis taksonu zwiększa liczbę znanych gatunków z rodzaju *Isohypsibius* na Svalbardzie do 13. Jednak podkreślam także w pracy, że stare opisy, opierające się o analizy okazów na sprzęcie optycznym niskiej jakości, wymagają rewizji i potwierdzenia statusu taksonomicznego notowanych dotychczas gatunków w rodzaju *Isohypsibius*. W świetle opisu nowych, morfologicznie podobnych rodzajów (np. *Doryphoribius* Pilato, 1969, *Thulinius* Bertolani, 2003, czy *Pseudobiotus* Nelson, 1980) cechy definiujące wyższe taksony takie jak lamina brzuszna czy obecność i liczba lamelli, ze względu na brak odpowiednich narzędzi mikroskopowych mogły zostać pominięte w starych opisach gatunków przyporządkowanych do rodzaju *Isohypsibius*.

W drugiej części pracy przedstawiam wyniki analiz 28 prób mchów, porostów oraz gleby zebranych z wysp Nordaustlandet, Edgeøya i Prins Karls Forland. Z prób wyizolowałem 324 okazy, 15 wylinek oraz 132 jaja niesporczaków. Na wyspie Nordaustlandet niesporczaki zostały zbadane po raz pierwszy i odnotowałem tam pięć gatunków Tardigrada. Ponadto w analizowanym materiale znalazłem osiem nowych gatunków dla wyspy Edgeøya oraz cztery dla Prins Karls Forland. Najczęściej występującymi gatunkami w próbkach były: *Testechiniscus spitsbergensis* (Scourfield, 1897), *Mesobiotus harmsworthi harmsworthi* (Murray, 1907), i *Macrobiotus islandicus islandicus* Richters, 1904. Rozdział drugi ma także charakter kwerendy na temat niesporczaków w Archipelagu Svalbard. W pracy tej podsumowałem dotychczasową wiedzę (30 publikacji) na temat Tardigrada Svalbardu i przygotowałem zestawienie gatunków dla 11 dotychczas zbadanych wysp z komentarzami na temat ich obecnego statusu taksonomicznego. W pracy podkreśliłem fakt, że stare informacje na temat przedstawicieli grup gatunków takich jak m.in. grupa *hufelandi*, grupa *dujardini* i grupa *convergens* ze względu na rewizje taksonomiczne wymagają ponownego potwierdzenia. Liczba poprawnie oznaczonych gatunków niesporczaków na Svalbardzie wynosi 85, w tym pięć gatunków endemicznych: *Bryodelphax parvuspolaris* Kaczmarek i in. 2012, *Isohypsibius ceciliae* Pilato et Binda, 1987, *I. coulsoni* Kaczmarek i in. 2012, *I. karenae* Zawierucha, 2013, i *Tenuibiotus voronkovi* (Tumanov, 2007).

Część trzecia to integratywna redeskrypcja gatunku *Tenuibiotus voronkovi* (Tumanov, 2007) przy użyciu metod taksonomii klasycznej opartej na opisie morfologicznym i morfometrycznym, ale także przy użyciu metod biologii molekularnej. Głównym celem pracy było zbadanie zmienności morfometrycznej i molekularnej gatunku oraz jego ponowny opis. *Tenuibiotus voronkovi* oryginalnie został opisany na podstawie materiału obejmującego dwa osobniki i 5 jaj. Jednakże analizowane okazy miały uszkodzone tylne części ciała. Ponadto autor opisu oryginalnego nie uwzględnił istotnych taksonomicznie pomiarów cech morfologicznych. W pracy uzupełniam opis gatunku opierając swoje analizy o oryginalny paratyp wypożyczony z kolekcji autora gatunku, a także osobnikach i jajach znalezionych na wyspach: Spitsbergen, Nordaustlandet oraz Edgeøya. Opis taksonu uzupełniłem o informacje morfologiczne na temat obecności oczu, budowy pazurków na IV parze odnóży, wcięciu w drugim makroplakoidzie oraz różnicach w wielkości wypustek jaj. Dodatkowo uzupełniłem opis o dane morfometryczne dla: szerokości rurki gardzielowej, długości laminy brzusznej, pazurów na odnóżach I i II pary, długości wypustek, długości podstawy wypustki oraz dystansu między wypustkami jaj. Łącznie uzupełniłem opis o dane morfometryczne dla 33 cech zmierzonych w zakresie od 13 do 27 osobników oraz 5 cech zmierzonych w zakresie od 3 do 53 na jajach. Porównanie pomiarów długości ciała okazów zebranych w gradiencie szerokości geograficznej między wyspami (Edgeøya i Nordaustlandet) nie wykazało różnic. Znaleziono jednak istotne różnice między szerokością bazy wypustek na jajach oraz odległością pomiędzy wypustkami. Dane molekularne obejmowały cztery uniwersalnie używane markery molekularne w integratywnej taksonomii niesporczaków: COI mRNA, ITS rRNA, 28S rRNA w domenie D1-D3 oraz dodatkowy 18S rRNA. DNA wyizolowano z trzech dorosłych osobników oraz jednego jajka. W obrębie: 28S rRNA znaleziono dwa haplotypy, w COI i ITS po trzy haplotypy. Niski dystans genetyczny między haplotypami w obrębie COI i ITS (<3%) wskazuje, że wszystkie sekwencje należą do jednego gatunku. Dodatkowo przeanalizowałem opisy oraz fotografie gatunku *T. willardi* (Pilato, 1977) z pracy Dastycha (1985) oraz oryginalne fotografie okazów typowych tego gatunku (otrzymanych dzięki uprzejmości profesora Giovanni Pilato, Uniwersytet w Katanii, Włochy) oraz fotografie osobników z innych części Arktyki (otrzymanych dzięki uprzejmości profesora Reinhardta

Kristensena, Muzeum Historii Naturalnej w Kopenhadze, Dania). Pozwoliło to wykazać, że gatunek *T. willardi* został błędnie oznaczony przez Dastycha (1985) i jest to w rzeczywistości *T. voronkovi*, który może mieć znacznie szerszy zasięg w Arktyce niż dotychczas sądzono. Budowa jaj niesporczaków jest uznawana za kluczową cechę taksonomiczną. Wyniki mojej pracy pokazują, że jaja należące do jednego gatunku mogą różnić się morfologicznie i morfometrycznie (może to być efekt zmienności lub metod utrwalenia) dlatego też w opisach powinno się analizować jak największą liczbę jaj do zbadania ich zmienności wewnątrzgatunkowej. Dodatkowo pokazałem, że pomimo różnic w budowie pazurków między rodzajem *Tenuibiotus* Pilato et Lisi, 2011 a rodzajami *Macrobiotus* C.A.S. Schultze, 1834 i *Paramacrobiotus* Guidetti, Schill, Bertolani, Dandekar et Wolf, 2009, na poziomie molekularnym rodzaj *Tenuibiotus* powinien być klasyfikowany w obrębie rodziny Macrobiotidae.

W części czwartej zajmuję się fauną niesporczaków zasiedlających kriokonity. Głównym celem pracy było opisanie morfologii, różnorodności i rozmieszczenia niesporczaków oraz określenia ich funkcji w sieciach troficznych na lodowcach. W pracy tej sprawdzałem także czy ekosystemy glacjalne są zasiedlane przez unikatowe gatunki Tardigrada. Materiał do badań obejmował 33 próby osadów z kriokonitów z sześciu lodowców zlokalizowanych na Spitsbergenie. W 25 próbach znaleziono niesporczaki należące do pięciu taksonów, są to: *Hypsibius dujardini* (Doyère, 1840), *Hypsibius* sp. A, *Isohypsibius* sp. A., *Pilatobius recamieri* (Richters, 1911) i jeden przedstawiciel rodziny Ramazzottiidae. *Hypsibius dujardini* oraz *P. recamieri* to gatunki notowane wcześniej z mszaków, porostów oraz zbiorników wodnych w arktycznej tundrze. Pozostałe taksony to gatunki jak dotąd stwierdzone tylko w kriokonitach. *Hypsibius* sp. A, *Isohypsibius* sp. A i przedstawiciel rodziny Ramazzottiidae to taksony nowe dla wiedzy, jednakże ze względu na stare i niekompletne opisy podobnych gatunków w przeszłości zdecydowałem się nie opisywać znalezionych okazów jako nowych. Pozwoliło to uniknąć formułowania niepełnej diagnozy różnicującej, tym samym kumulowania nieścisłości w taksonomii Tardigrada. W celu ułatwienia rozpoznawania w przyszłości gatunków z lodowców arktycznych, umieściłem w pracy ich diagnozy morfologiczne, uwagi na temat statusu taksonomicznego i podobieństw do innych gatunków, a także zdjęcia. W analizach porównawczych wykorzystałem wcześniej opublikowaną literaturę na temat niesporczaków z arktycznych kriokonitów (Dastych 1985, Grongaard i in. 1999) oraz materiał z kriokonitów z Grenlandii zdeponowany w Muzeum Historii Naturalnej w Kopenhadze. Dodatkowo posłużyłem się okazami zdeponowanymi w mojej kolekcji (wyniki prac opublikowano w Kaczmarek i in. 2012, Zawierucha i in. 2013, 2015c, 2016). Porównanie okazów wyraźnie wskazuje, że *Hypsibius* sp. A, *Isohypsibius* sp. A i przedstawiciel rodziny Ramazzottiidae to taksony zasiedlające wyłącznie kriokonity. W dyskusji podkreśliłem także fakt, że gatunek „*Hypsibius* ? *arcticus*" notowany przez Dastycha (1985) z kriokonitów na Spitsbergenie to gatunek opisany przeze mnie jako *Hypsibius* sp. A, który nie powinien być mylony z gatunkiem *H. arcticus* ze względu na inną strategię składania jaj (jaja składane w wylince, a nie bezpośrednio do środowiska). Opierając się o budowę aparatu gębowo-gardzielowego (Guil i Sanchez-Moreno 2013) wykazałem, że okazy znajdowane w kriokonitach są przedstawicielami grup niesporczaków roślinożernych i bakteriożernych. Biorąc pod uwagę, że (a) znalazłem niesporczaki w większości analizowanych próbek, (b) tworzą one unikatowe zgrupowania i (c) są konsumentami najwyższego rzędu w kriokoniatch, mogę uznać, że są stałym i charakterystycznym elementem glacjalnego biomu. Ponadto pokazałem w pracy, że występowanie niesporczaków na lodowcach może mieć związek z koncentracją kationów takich jak Mg^{2+}, K^{+} i Ca^{2+}. Niniejszy rozdział podkreśla, że pomimo ekstremalnych warunków panujących na powierzchni lodowców, są one dogodnym siedliskiem dla mikroskopijnych zwierząt. Obecność unikatowych dla kriokonitów niesporczaków wyraźnie wskazuje, że są one charakterystycznymi siedliskami z innymi ich zgrupowaniami niż w tundrze. Ponadto zwierzęta te pełnią ważną funkcję jako konsumenci najwyższego rzędu w kriokonitach, tym samym niesporczaki mogą wpływać na obieg materii i konkurować o zasoby z pierwotniakami czy wrotkami.

W części piątej zbadałem zależność pomiędzy powierzchnią, głębokością i położeniem kriokonitów nad poziomem morza, a zagęszczeniami niesporczaków. W analizach wykorzystałem 38 prób osadów z kriokonitów z czterech lodowców zlokalizowanych na Spitsbergenie. Średnia (±SD): powierzchnia kriokonitów wynosiła 1287,21±2400,8 cm^2, głębokość 10,8±11,2 cm, położenie na wysokości 172,6±109,66 m. n.p.m. i zagęszczenie niesporczaków 24,9±33,0 osobników na gram mokrego osadu. Zagęszczenia niesporczaków osiągały maksymalne wartości dochodzące do 168 osobników/cm^3, 82 osobników/cm^2, 326 osobników/g^{-1} mokrej masy osadu i 624 osobniki/g^{-1} suchej masy osadu. W próbkach zidentyfikowałem pięć taksonów, są to: *Hypsibius dujardini, Hypsibius* sp. A, *Isohypsibius* sp. A., *Pilatobius recamieri* i jednego przedstawiciela rodziny Ramazzottiidae. Obliczony dla zmiennych model nie wyjaśnia zmienności zagęszczeń niesporczaków w kriokonitach. Wyniki pracy pokazują, że podstawowe założenia biogeograficzne mówiące, że wraz z wzrastającą powierzchnią obszaru wzrasta liczba gatunków i osobników danego taksonu (np. MacArthur i Wilson 1967, Root 1973) nie sprawdzają się w przypadku kriokonitów. Główną przyczyną braku zależności między zagęszczeniami Tardigrada a powierzchnią, głębokością i położeniem zbiorników wodnych na lodowcach w rejonach arktycznych może być ich charakterystyka. Te zlokalizowane w Arktyce są zwykle otwarte – bez powierzchniowej warstwy lodu. Intensywne topnienie lodowców w Arktyce wpływa na mieszanie osadów wewnątrz zbiornika i zaburzanie tego siedliska. Dodatkowo dochodzi tam do wypłukiwania osadów z dna kriokonitów (Mueller i in. 2001). Wszystkie te czynniki mogą mieć istotny wpływ na otrzymane wyniki. Jednakże pomimo zaburzeń spowodowanych intensywnym topnieniem niesporczaki zasiedlają arktyczne kriokonity w wyższych zagęszczeniach niż te na lodowcach w Antarktyce. Podsumowując, głównymi czynnikami, które mogą wpływać na zagęszczenia niesporczaków na lodowcach arktycznych jest wypłukiwanie osadów, ale mogą to być także kształt kriokonitów oraz ilość dostępnych składników odżywczych.

W części szóstej zbadałem zależność między kolonią ptaków planktonożernych *A. alle*, a niesporczakami zasiedlającymi tundrę. W pracy testuję następujące hipotezy badawcze:

H_1 *Zagęszczenia niesporczaków są wyższe w obszarach nawożonych przez ptaki,*

H_2 *Skład gatunkowy niesporczaków różni się między obszarami nawożonymi i nie nawożonymi przez ptaki.*

W celu przetestowania powyższych hipotez wyznaczyłem dwa transekty (każdy długości 1 km.) biegnące od brzegu morza do zboczy górskich w fiordzie Hornsund na Spitsbergenie. Transekt główny znajdował się pod bezpośrednim wpływem guana z kolonii alczyków, z kolei kontrolny zlokalizowany został poza wpływem kolonii i przelotu ptaków. W każdym transekcie co 100 metrów wyznaczyłem poprzeczny subtransekt, w którym zbierałem pięć próbek oddalonych od siebie o 5 metrów. Łącznie zebrałem 160 próbek mchów, porostów oraz próbek mieszanych – mchy i porosty (po 80 próbek w każdym transekcie). W sumie z badanych próbek wyizolowałem 1990 osobników należących do 32 taksonów z czego 25 oznaczyłem do poziomu gatunku. Zagęszczenie niesporczaków w transekcie głównym było istotnie wyższe od zagęszczenia w próbie kontrolnej. Znalazłem także różnice w zagęszczeniach między rodzajami próbek. Największa liczebność niesporczaków znaleziona została w próbkach mchów, niższa w próbkach mieszanych (mchy/porosty) i najniższa w porostach. Istotna statystycznie różnica w zagęszczeniach stwierdzona została pomiędzy mchami i porostami. Nie znalazłem istotnych różnic między różnorodnością gatunkową w badanych transektach. Analizy statystyczne wykazały także, że zmienne: transekt (czyli nawożenie lub jego brak) i rodzaj próbek (mchy, mchy/porosty, porosty) wyjaśniają istotne

16,2% zmienności w zgrupowaniach Tardigrada. Zmienna „transekt” wyjaśnia także zmienność w składzie gatunkowym niesporczaków. Z kolei rodzaj próbek nie wpływa istotnie na zgrupowania Tardigrada (mchy, mchy/porosty, porosty). Najczęściej występującym gatunkiem w próbkach był *P. recamieri* znaleziony w 35 próbkach w transekcie głównym i w 6 próbkach w kontrolnym. Gatunki wyraźnie związane z obszarem nawożonym to: *Isohypsibius elegans* Binda et Pilato, 1971, *Macrobiotus crenulatus* Richters, 1904 i *P. recamieri*. Gatunki silnie związane z transektem kontrolnym to: *Echiniscus granulatus* (Doyère, 1840), *Hypsibius convergens* (Urbanowicz,1925), *H. pallidus* Thulin, 1911 i *Testechiniscus spitsbergensis* (Scourfield, 1897). W analizowanym materiale znalazłem także cztery taksony nowe dla fauny Archipelagu Svalbard: *Microhypsibius bertolanii* Kristensen, 1982, *M.* cf. *formosus, Ramazzottiius* cf. *rupeus* i *Isohypsibius* cf. *reticulatus.* Niniejszy rozdział prezentuje pierwsze badania pokazujące pozytywny wpływ dostawy guana z kolonii alczyków na liczebność Tardigrada. Kolonia alczyków może mieć wpływ pośredni na niesporczaki – ptaki produkują guano i użyźniają obszary w sąsiedztwie kolonii przez co jest tam większa biomasa roślin, które z kolei są siedliskiem dla Tardigrada. Jest to istotne, ponieważ w obszarach nawożonych jest więcej mszaków, w których jak pokazuję w badaniach, zagęszczenia niesporczaków są wyższe. Wyniki opisane w tym rozdziale podkreślają istotność użyźniania tundry przez ptaki i konieczność uwzględniania czynnika jakim jest guano ptaków w badaniach nad różnorodnością i wzorcami rozmieszczenia bezkręgowców w Arktyce.

W części siódmej oceniałem różnorodność oraz zagęszczenia niesporczaków w porostach z archipelagu Svalbard. W pracy testuje dwie hipotezy:

H_1 *zróżnicowanie siedliska na przykładzie składu gatunkowego porostów w próbie wpływa na różnorodność gatunkową i zagęszczenia niesporczaków,*

H_2 *w porostach z obszarów znajdujących się pod wpływem kolonii ptaków zagęszczenia niesporczaków są wyższe niż w porostach występujących poza koloniami.*

Do analiz wykorzystałem 45 próbek porostów zebranych z wysp: Spitsbergen, Nordaustlandet, Prins Karls Forland, Danskøya, Fuglesongen, Phippsøya i Parrøya. W dwunastu próbkach oznaczono więcej niż jeden gatunek porostu (do pięciu gatunków w próbce). W sumie z 26 próbek wyizolowałem 321 okazów niesporczaków należących do 23 taksonów (17 zidentyfikowanych do poziomu gatunku). W niniejszym rozdziale stwierdziłem po raz pierwszy niesporczaki na wyspach Danskøya, Fuglesongen, Phippsøya i Parrøya. Najczęściej występującymi gatunkami w próbkach były *Hypsibius pallidus* Thulin, 1911 (obecny w 9 próbkach), *Diphascon pingue pingue* (Marcus, 1936) (obecny w czterech próbkach), i *Adropion prorsirostre* (Thulin, 1928) (obecny w czterech próbkach). Najwyższe zagęszczenie niesporczaków na 10 g. suchej masy próbki wynosiło 720 okazów i zostało stwierdzone w próbce złożonej z dwóch gatunków porostów: *Cladonia arbuscula* i *Cladonia rangiferina*. Zagęszczenie niesporczaków i ich różnorodność gatunkowa nie były istotnie skorelowane z różnorodnością porostów w próbce. Zagęszczenia niesporczaków w porostach zebranych z obszarów pod wpływem guana z kolonii ptaków nie były istotnie wyższe od tych zebranych z obszarów poza wpływem kolonii. Biorąc pod uwagę wyniki na temat zagęszczeń opublikowane w poprzednich moich pracach, zagęszczenia niesporczaków są istotnie wyższe w mchach niż w porostach. Najwyższe zagęszczenia niesporczaków zostały znalezione w próbkach porostów nie posiadających kory, która może być czynnikiem ograniczającym dostęp do glonów czyli pokarmu dla roślinożernych Tardigrada.

I SUMMARY

The Svalbard archipelago is located in the European part of the Arctic (between 74 and 81 latitude and 10 and 35 longitude), between Norway and the North Pole (Coulson 2013, Stankowski 2013). Svalbard constitute several dozen of islands. The biggest island is Spitsbergen. Within the last few thousand years Svalbard has got covered with extensive ice masses several times (Lonne and Lysa 2005). Even though Svalbard is characterized by a typical polar climate, local temperatures are higher than in the other parts of the Arctic located at the same latitude (Przybylak et al. 2014). Therefore, apart from glaciers, large areas of Svalbard are covered with tundra. Due to the presence of glaciers and its geological features Svalbard's landscape is diversified and characterized by vast and deep fjords. There is a number of specific elements of this part of the Arctic. Apart from numerous glaciers, these are: (a) the influence of different ocean currents on the climate, (b) the relation between vertebrates enrichment and invertebrates assemblages in tundra, and finally, (c) a small human impact on the ecosystem.

The Svalbard archipelago is one of the most investigated Arctic areas in terms of soil and freshwater fauna. So far, ca. 1000 invertebrate species have been known from this area (Coulson et al. 2014). Moreover, new records for this archipelago and new species for science are still being described (e.g. Kaczmarek et al. 2012, Gwiazdowicz et al. 2012, Kiedrowicz et al. 2016, Kolicka et al. 2016). The presence of the University (University Centre in Svalbard), a scientific station network and complex research into various domains led to an increase of international scientific, political and industrial attention. Due to that, Svalbard is like "natural laboratory" for studies on invertebrates (Coulson 2013).

Arctic terrestrial ecosystems are considered to be relatively species-poor, and this is mostly due to the harshness of environmental conditions like low temperatures and nutrient concentrations, extreme seasonality and a vast ice coverage. However, the published literature indicates that both tundra and ice constitute a habitat for a number of microscopic invertebrates. The highest density and biomass of invertebrates inhabiting Svalbard is connected to planktivorous bird colonies (Zmudczyńska et al. 2012). On the glaciers, "an oasis for live" are cryoconite holes – small water-filled reservoirs on the glacier's surface (Hodson et al. 2008, Cook et al. 2015, Zawierucha et al. 2015b).

Glaciers and ice sheets cover 10% of our planet, which is why the Intergovernmental Panel on Climate Change (IPCC) underlines the importance of glaciers as an ecosystem and a reservoir of 70% of World's water supply (ACIA 2006, Vaughan et al. 2013). About 59% of Svalbard's area is covered by glaciers and ice caps (Hagen et al. 2003). Glacier's surface is mostly inhabited by bacteria (mainly cyanobacteria), algae, fungi, protists and animals (Hodson et al. 2008, Cook et al. 2015, Zawierucha et al. 2015b, Kaczmarek et al. 2016). Because of a characteristic landscape and climatic features as well as unique organisms assemblages, glacial ecosystems constitute an independent biome (Anesio and Laybourn-Parry 2012). Despite there being a long history of studies on biotic elements of glaciers with the first from XIX/XX century (Drygalski 1897) little attention has been given to animals which play the role of higher trophic level consumers, and control the density of other organisms in glacial ecosystems (Zawierucha et al. 2015b, Vonnahme et al. 2015). In spite of the fact that invertebrates are herbivorous and they are predators which may exert an influence on carbon flow and increase glacier's surface melting, knowledge on their diversity and ecology on ice is poor (Zawierucha et al. 2015b).

Seabirds in Arctic ecosystems are a link between seas rich in nutrients and poor terrestrial ecosystems. They feed in seas and nest on the land where they leave huge quantities of guano, fertilizing poor terrestrial ecosystems (Stempniewicz 1990, 1992). Little auks (*Alle alle* Linnaeus, 1758) nesting on mountain hills inland and fertilizing both the colonies neighbourhood and the tundra between the colonised area and the sea are particularly important in the process (Stempniewicz et al. 2007). For example, one little auk colony in the Hornsund Fjord on Spitsbergen delivers up to 60 tons of dry guano per km^2 (Stempniewicz 1990, 1992). Guano exert an influence on higher plants diversity and biomass but it also has an impact on invertebrates density (Eurola and Hakala 1977, Zmudczyńska et al. 2012). Moreover, places influenced by those birds are feeding ground for vertebrates like reindeers or goose (Jakubas et al. 2008).

My scientific object are tardigrades (Tardigrada) – invertebrates which may inhabit both tundra and glaciers. Tardigrada is a phylum of micro animals ranging between 50-1200 μm in size. Currently, Tardigrada is divided into two orders: Heterotardigrada and Eutardigrada (Nelson et al. 2015). More than 1200 species have been described so far (Degma et al. 2016, Vicente and Bertolani 2013). Those animals inhabit aquatic and terrestrial ecosystems – from deep oceans to high mountains including extreme ecosystems like deserts or glacier's surface. On land they live in mosses, lichens, soil and leaf litter, whereas in water they inhabit sediments and plants (Ramazzotti and Maucci 1983, Nelson et al. 2015).

Tardigrades are one of the most abundant and frequent invertebrate groups which inhabit Svalbard (Coulson et al. 2014). The densities of tardigrades reach up to hundreds of individuals per gram of dry material. Tardigrade densities calculated per m^2 may reach up to tens of thousands of individuals (Dastych 1988, Zawierucha et al. 2016).

Studies on tardigrades in Svalbard have a long history, and started at the beginning of the 20th century (Scourfield 1897). However, in spite of many years of studies on tardigrades, the knowledge of these animals in this part of the Arctic is still poor. Most studies on Tardigrada diversity in Svalbard is connected to the biggest island - Spitsbergen (Węglarska 1965, Dastych 1985, Tumanov 2007). The fauna of the other islands is either poorly known or completely unknown, and in terms of tardigrades Spitsbergen itself has been investigated in a very irregular manner (Kaczmarek et al. 2012). There is a number of species that still need a revision and re-descriptions (Zawierucha et al. 2013). In polar regions tardigrades along with nematodes and rotifers are the main faunal element (Porazinska et al. 2002, Sohlenius and Boström 2008). Despite an important function tardigrades play in polar ecosystems (Sohlenius and Boström 2008, Vonnahme et al. 2015), the knowledge about them is still fragmentary.

In the past only Dastych (1985) conducted comprehensive studies on tardigrades. In the faunisitic part of his work a new sub-species, *Mesobiotus harmsworthi obscurus,* was described (Dastych 1985). In the ecological part, Dastych (1985) proved that different tardigrade species inhabit substratum with different carbon content. Moreover, he showed the relation between altitude and tardigrades diversity. However, in view of recent research, seabirds may influence the distribution and diversity of Tardigrada in the Arctic (Zawierucha et al. 2015c). Those birds deliver guano to tundra ecosystems, and therefore, they fertilize soils and positively influence plants – habitats for invertebrates (Zmudczyńska et al. 2008, 2009, 2012). One of the recent studies has shown that tardigrades in the areas of seabird colonies are bigger than tardigrades inhabiting outside of the colonies. It may prove that places under seabird impact serve as a better food source which is most likely due to a higher biomass of plants (Zawierucha et al. 2015a). Besides, still number of gaps in knowledge on tardigrades exist. There is still no evidence for the impact of habitat heterogeneity on Tardigrada. It may influence on their diversity and density. Tundra on Svalbard seems to be the best candidate to test such a relation due to flora diversity – hundreds of species of mosses and lichens (Eurola and Hakala 1977, Øvstedal et al. 2011).

Little attention has been given to tardigrades in cryoconite holes so far (Zawierucha et al. 2015b). Tardigrades in the cryoconite holes of Svalbard have been mentioned in two papers only. There have been no morphological analyses, studies on densities or the function of those animals in glacial ecosystems either (Dastych 1985, De Smet and Van Rompu 1994). Only Vonnahme et al. (2015) in their ecological paper took into consideration the animals. However, the authors included neither the morphology of the studied cryoconite holes, nor the densities and diversity of tardigrades, and therefore, their place in the food webs of glacial ecosystems has been omitted, too.

Due to significant knowledge gaps on tardigrades in Svalbard and Arctic regions in general, the **main aims of my PhD thesis are**:

- studies on the Tardigrada species diversity in tundra and glaciers,

- the comparison of Tardigrada communities in tundra and glaciers,

- testing the hypothesis that morphology and the location of cryoconite holes influence Tardigrada densities,

- testing the hypothesis that guano of planktivorous birds influences the density and diversity of tardigrades in tundra,

- studies on impact of habitat heterogeneity on densities and diversity of tardigrades in lichens.

In the first part of my PhD thesis I am presenting the description of a tardigrade species new to science – *Isohypsibius karenae* Zawierucha, 2013. I have found 5 tardigrade species in two moss samples collected from tundra on Spitsbergen, within one species new to science. In total, I have extracted from the samples 32 specimens and nine exuviae which belong to the new taxon. I have supplemented morphological descriptions regarding morphometric data for 33 characters, measured in ranges between 1 and 15 individuals. In total, I have measured 270 traits, which were used in a differential diagnosis. The new species differs from other similar species in *Isohypsibius* Thulin, 1928 by the combination of the following characteristics: the presence of a reticular sculpture on a cuticle and the presence of cuticular bars under claws I-III, an increasing macroplacoids length (1<2<3), a different composition of teeth in oral cavity as well as a longer and wider buccal tube. The description of a new species increased the number of *Isohypsibius* on Svalbard to thirteen. However, I have underlined that the old descriptions based solely on the analysis of specimens conducted with optical equipment of poor quality need a revision and a confirmation of taxonomical status with regards to genus *Isohypsibius*. In view of the descriptions of a new similar genus (e.g. *Doryphoribius* Pilato, 1969, *Thulinius* Bertolani, 2003, or *Pseudobiotus* Nelson, 1980) the characteristics defining higher taxa like a ventral lamina, the presence and a number of lamellae might have been overlooked in the old descriptions of *Isohypsibius* species due to the lack of a sufficient optical equipment.

In the second part of my thesis I am presenting the results of the analysis of 28 moss, lichen and soil samples collected from the following islands: Nordaustlandet, Edgeøya and Prins Karls Forland. From the samples I have extracted 324 specimens, 15 exuviae and 132 tardigrade eggs. It is the first record of tardigrades from Nordaustlandet with five species of Tardigrada. Moreover, in the material I have found eight new records for Edgeøya and four new records for Prins Karls Forland islands. The most frequent species were: *Testechiniscus spitsbergensis* (Scourfield, 1897), *Mesobiotus harmsworthi harmsworthi* (Murray, 1907), and *Macrobiotus islandicus islandicus* Richters, 1904. This chapter is also the summary of the current knowledge on the tardigrades of Svalbard. In this part I have summarized all the known literature (30 papers) on the tardigrades of Svalbard and I have prepared a list of species from 11 islands along with the comments on the species. I have underlined the fact that due to a taxonomic revision, the old information on species from groups like *hufelandi* group, *dujardini* group or *convergens* group needs confirmation. In the paper I have summarized a number of valid species from Svalbard which constituted 85 species, with five endemic species within them: *Bryodelphax parvuspolaris* Kaczmarek et al., 2012, *Isohypsibius ceciliae* Pilato et Binda, 1987, *I. coulsoni* Kaczmarek et al., 2012, *I. karenae* Zawierucha, 2013 and *Tenuibiotus voronkovi* (Tumanov, 2007).

In part three I am presenting an integrative redescription of *Tenuibiotus voronkovi* (Tumanov, 2007) based on classical taxonomic methods (morphology and morphometry) combined with molecular biology techniques. The main aim of the study was a survey on the morphometric and molecular variability and a redescription of the species. *Tenuibiotus voronkovi* was originally described on the material consisting of two individuals and five eggs. However, the analysed individuals have had their caudal part of the body destroyed. What is more, the author did not present the important taxonomic measurements. In this chapter I have supplemented the description of the species based on the original paratype loaned from the author’s collection as well as the individuals and eggs from the islands: Spitsbergen, Nordaustlandet and Edgeøya. I have supplemented the redescription of the species with the morphological characteristics regarding the presence of eyes, the morphology of claws of IV pair of legs, the incision in a second macroplacoid, and the differences in eggs processes size. Additionally, I have supplemented the morphometric with: the buccal tube’s width, the length of a ventral lamina, the length of claws I and II, processes and the base of processes length as well as the distance between processes base. In total, I have supplemented the description with morphometric data regarding 33 characteristics which were measured in a range between 13-27 of specimens, and five characteristics in a range between 3-53 of eggs. No effect was detected in the comparison of body length between individuals collected in latitudinal gradient between islands Edgeøya and Nordaustlandet. However, I have found significant differences between processes base length and the distance between processes. Molecular data comprised four universal markers used in tardigrade integrative taxonomy: COI mRNA, ITS rRNA, 28S rRNA for domains: D1-D3, and an additional one 18S rRNA. DNA was isolated from three adults and one egg. In 28S rRNA I have found two haplotypes, and in COI and ITS three haplotypes. A low genetic distance between COI and ITS haplotypes (<3%) indicates that all the sequences belong to one species. Moreover, I have analysed the description and pictures of *T. willardi* (Pilato, 1977) from the paper of Dastych (1985), and original pictures of the type material of the same species (by the courtesy of professor Giovanni Pilato, University in Catania, Italy), and pictures of the individuals determined as *T. willardi* from other parts of the Arctic (by the courtesy of professor Reinhardt Kristensen, Natural History Museum in Copenhagen, Denmark). The comparison of pictures indicates that *T. willardi* was mistakenly identified by Dastych (1985), and in fact it is *T. voronkovi* which may have a broader distribution in the Arctic than it has been thought so far. Tardigrades egg's shell morphology is considered to be a key taxonomic characteristic. The results of my study showed that eggs belonging to one species may differ morphologically and morphometrically (it could be affected by species variability or preservation methods). Thus, in the descriptions of the new species the highest possible number of eggs should be analysed in terms of their variability. Additionally, I have shown that despite there being differences in claws between *Tenuibiotus* Pilato et Lisi, 2011, and genera *Macrobiotus* C.A.S. Schultze, 1834 and *Paramacrobiotus* Guidetti et al., 2009, at a molecular level *Tenuibiotus* should be classified within the Macrobiotidae family.

In part four I am presenting data on the fauna of tardigrades inhabiting cryoconite holes. The main aim of the paper was to provide the description of Tardigrada morphology, diversity and distribution as well as the determination of tardigrade's function in trophic nets on glaciers. Additionally, in this chapter I have also checked if tardigrades inhabiting glacial ecosystems are unique. The material for studies comprised of 33 samples of cryoconite material from six glaciers on Spitsbergen. In 25 samples five taxa of tardigrades have been identified: *Hypsibius dujardini* (Doyère, 1840), *Hypsibius* sp. A, *Isohypsibius* sp. A., *Pilatobius recamieri* (Richters, 1911), and one representative of Ramazzottiidae. *Hypsibius dujardini* and *P. recamieri* both of which have been previously recorded from mosses, lichens and freshwater ponds in Arctic tundra. So far, the rest of taxa have been noted in cryoconite holes only. *Hypsibius* sp. A, *Isohypsibius* sp. A. and one representative of Ramazzottiidae are taxa new to science. However due to old and incomplete descriptions of similar species in the past, I have decided not describe them as new species. This allowed to avoid an incomplete differential diagnosis and, at the same, the accumulation of errors and inaccuracies in Tardigrada taxonomy. In order to identify individuals from Arctic glaciers, in my work I have included morphological diagnosis, remarks on a taxonomic status and similarities to other species as well as figures. In comparative analyses I have used a previously published literature on the tardigrades from Arctic cryoconite holes (Dastych 1985, Grongaard et al. 1999), and the material from Greenlandic cryoconite holes deposited in the Natural History Museum in Copenhagen. What is more, I have used specimens deposited in my collection (the results of slide analysis were published in Kaczmarek et al. 2012, Zawierucha et al. 2013, 2015c, 2016). The comparison of specimens indicates that *Hypsibius* sp. A, *Isohypsibius* sp. A., and one representative of Ramazzottiidae only inhabit cryoconite holes. In discussion I have underlined the fact that "*Hypsibius* ? *arcticus*" noted by Dastych (1985) from cryoconite holes on Spitsbergen is a species diagnosed by me as *Hypsibius* sp. A. *Hypsibius arcticus* should not be misidentified as "*Hypsibius* ? *arcticus"* due to a different strategy of eggs laying (eggs laid in exuviae instead directly to environment). Based on the morphology of bucco-pharyngeal apparatus (Guil and Sanchez-Moreno 2013) I have proved that taxa found in cryoconite holes are the representatives of herbivorous and microbivores tardigrades. Taking into consideration that (a) I have found tardigrades in most of the analysed samples, (b) they form unique assemblages, and (c) they are higher level trophic consumers in cryoconite holes, I have recognized that they are a constant and characteristic element of a glacial biome. Moreover, I have shown that, the presence of tardigrades in cryoconite holes is connected to cations Mg^{2+}, K^{+} and Ca^{2+}. In this chapter I have underlined that despite there being extreme environmental conditions, glacier's surface is habitable for micro animals. The presence of tardigrade species unique for cryoconite holes indicates that they are a distinct habitat and Tardigrada with their assemblages being different than in tundra. What is more, those animals play an important role as higher level consumers in cryoconite holes. Thus, tardigrades may influence matter cycle, and compete for food with protists and rotifers.

In the fifth part of the thesis I have investigated the relations between area, depth and location (a.s.l.) of cryoconite holes and tardigrades densities. In the analysis I have used 38 samples of cryoconite sediments from four glaciers on Spitsbergen. The mean (±SD) of cryoconite area was 1287.21±2400.8 cm^2, while the depth was on average 10.8±11.2 cm, the elevation 172.6±109.66 m a.s.l., and tardigrade density amounted to 24.9±33.0 individuals per gram of wet material. The densities of tardigrades on the Hans Glacier reached values of up to 168 ind. cm^3, 104 ind. g^{-1} wet weight, and 275 ind. g^{-1} dry weight. I have found 5 taxa in cryoconite holes and these are: *Hypsibius dujardini*, *Hypsibius* sp. A, *Isohypsibius* sp. A, *Pilatobius recamieri*, and one species of Ramazzottiidae. The model including area, depth and elevation can not explain Tardigrada density in cryoconite holes. The results of the study showed that basic biogeographical assumptions claiming that the number of species and individuals increases along with land's area (MacArthur and Wilson 1967, Root 1973) are not valid for cryoconite holes. The main factor which influences the lack of relation between Tardigrada densities, and area, depth and location of cryoconite holes in the Arctic may be their special characteristics. Those holes located in the Arctic are open – without a surface layer of ice. An intense melting of glaciers in the Arctic influences sediment mixing in water reservoirs and the disturbance within this habitat. Additionally, sediments in the Arctic are being flushed from the bottom of cryoconite holes (Mueller et al. 2001). All these factors may have a significant impact on the results. However, in spite of the disturbances affected by a rapid melting, tardigrades inhabit cryoconite holes in higher densities than in the Antarctic. To summarise, the main factors which potentially may influence Tardigrada densities on Arctic glaciers are the flushing of sediments, the shape of cryoconite holes and the availability of nutrients.

In part six I have investigated the relation between a colony of planktivorous *A. alle,* and tardigrades in tundra. I have tested the hypotheses that:

H_1 *tardigrades are more abundant in the areas affected (in the vicinity of little auk colony) as opposed to those non-affected by seabird guano,*

H_2 *species composition differs between these two areas*.

In order to test those hypotheses I have established two transects (each 1 km long) from the sea to mountain hills in the Hornsund Fjord on Spitsbergen. The main transect was under the influence of a seabird colony, while the control transect was beyond the impact of seabirds. In each transect, every 100 m, a transverse sub-transect was established from which eight samples at five-meter intervals were collected. In total, I have collected 160 subsamples of mosses, lichens and mixed (moss, lichens) – 80 subsamples in each transect. Overall, I have extracted 1990 specimens belonging to 32 taxa (25 determined to a species level). The total average density of tardigrades (all species together) was higher in the seabird transect as compared to the control one. I have also found significant differences in the densities between different vegetation types. The highest tardigrade density has been observed in the moss samples, a lower density in the mixed (moss/lichen) samples and the lowest in the lichen ones. However, only the difference between mosses and lichens was statistically significant. There were no significant differences in the Shannon diversity index, the evenness Jaccard index and the number of species between the seabird and control transects. The statistical analysis revealed that both variables tested (transect and vegetation) explained significant 16.2 % of the non-random variability of the Tardigrada assemblages. The transect also had a significant unique contribution to explaining the tardigrades composition. However, the plant type did not significantly influence the tardigrade communities. The most frequent tardigrade species in the samples was *Pilatobius recamieri* (Richters, 1911) which was present in 35 samples (in 29 and 6 samples from the seabird and control transects, respectively). Species positively associated with the seabird transect were *I. elegans*, *M. crenulatus* Richters, 1904 and *P. recamieri*; while those related to the control one were *E. granulatus* (Doye`re, 1840), *Hypsibius convergens* (Urbanowicz, 1925), *H. pallidus* Thulin, 1911 and *Testechiniscus spitsbergensis*. I have found four taxa, i.e. *M. bertolanii* Kristensen, 1982, *M. cf. formosus*, *Ramazzottiius cf. rupeus* and *Isohypsibius cf. reticulatus*, in the Svalbard archipelago for the first time. This chapter presents the first studies on the positive effect of guano delivery from little auk colony on the densities of Tardigrada. The effect of little auk colony may be indirect – birds produce guano and fertilize areas in the colony’s neighbourhood. Thus, plants’ biomass – the habitat for tardigrades – is higher. It is of particular importance since there are more bryophytes in the fertilized areas, and, as it has been shown in my studies, these are inhabited by tardigrades in higher densities. The results presented in this study underline the importance of seabirds fertilization and the necessity of including factors such as guano into studies on the diversity and invertebrate species distribution pattern in the Arctic.

In part seven I have studied the diversity and densities of tardigrades in Svalbard lichens. In this work I have tested two hypotheses:

H_1 *Habitat heterogeneity on the example of lichens influences tardigrade diversity and density,*

H_2 *In lichens collected from areas influenced by seabirds, densities of tardigrades are higher than in the lichens collected from beyond the colonies.*

For the analyses I have used 45 lichen samples collected from the following islands: Spitsbergen, Nordaustlandet, Prins Karls Forland, Danskøya, Fuglesongen, Phippsøya and Parrøya. Twelve samples consisted of more than one lichen species per sample (with up to five species). In total, 321 specimens of Tardigrada belonging to 23 taxa (with 17 determined to species level) have been found in 26 samples. Tardigrades for the islands of Danskøya, Fuglesongen, Phippsøya and Parrøya are reported for the first time. The most frequent species in the samples were *Hypsibius pallidus* (present in nine samples), *Diphascon pingue pingue* (Marcus, 1936) (present in four samples), and *Adropion prorsirostre* (Thulin, 1928) (present in four samples). The highest number of tardigrades calculated per 10 g of dry material was 720 specimens, which have been found in a mixed lichen sample of *Cladonia arbuscula* and *Cladonia rangiferina*. Tardigrade abundance and species diversity were not significantly related to the number of lichen species in a single sample. Tardigrades in lichens have not been more abundant in samples collected from areas enriched in nutrients by seabird colonies as compared with samples collected from non-enriched areas. Furthermore, regardless of the seabirds influence of lichens, tardigrades have been more abundant in moss samples. The highest number of tardigrades has been found in those lichen samples which did not have a thick surface layer potentially limiting herbivorous tardigrades' access to the photobiont layer and algal cells of the lichen.

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II DOCTORAL DISSERTATION

List of original publications

1. **Zawierucha, K.** (2013) Tardigrada from Arctic tundra (Spitsbergen) with description of *Isohypsibius karenae* sp. n. (Isohypsibiidae). *Polish Polar Research*, 34(4): 383-396.

2. **Zawierucha, K**., Coulson, S., Michalczyk, Ł. & 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.

3. **Zawierucha, K**., Kolicka, M. & Kaczmarek, Ł. (2016) Re-description of the Arctic tardigrade *Tenuibiotus voronkovi* (Tumanov, 2007) (Eutardigrada; Macrobiotidea), with the first molecular data for the genus. *Zootaxa*, 4196(4): 498-510.

4. **Zawierucha, K**., Ostrowska, M., Vonnahme, T.R., Devetter, M., Nawrot, A.P., Lehmann, S. & Kolicka, M. (2016) Diversity and distribution of Tardigrada in Arctic cryoconite holes. *Journal of Limnology*, 75(3): 545-559.

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Oświadczenie

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Tardigrada from Arctic tundra (Spitsbergen) with description of *Isohypsibius karenae* sp. n. (Isohypsibiidae)

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Abstract: Five species of Tardigrada were found in two moss samples collected from the Hornsund area (Ariekammen, Spitsbergen) including one new to science. The new species, *Isohypsibius karenae* sp. n., differs from the other similar congeners mainly by having a different type of cuticular sculpture, a different macroplacoid length sequence, by the presence of lunules and cuticular bars under claws as well as by some morphometric characters. The current study increases the number of *Isohypsibius* species known from Svalbard to thirteen.

Key words: Arctic, Hornsund, Ariekammen, Eutardigrada, moss fauna, taxonomy.

Introduction

Tardigrada, also known as water bears, is a phylum with *ca.* 1100 known species of micrometazoans (50–2112 μm in size) that inhabit a great variety of ecosystems throughout the globe (Ramazzotti and Maucci 1983; Nelson 2002; Guil 2008; Degma *et al.* 2012). On the Arctic archipelago of Svalbard, tardigrades are found in marine environments (*e.g.* Smykla *et al.* 2011), areas of terrestrial tundra (*e.g.* Węglarska 1965; Dastych 1985; Maucci 1996; Kaczmarek *et al*. 2012), freshwater bodies (*e.g.* Janiec 1996), as well as in extreme glacier microecosystems such as cryoconite holes (*e.g.* Dastych 1985; De Smet and Van Rompu 1994). Despite earlier, more than a century ago, polar exploration and associated reports of water bears (*e.g.* Richters 1911), new tardigrades are still being discovered in both the Arctic and the Antarctic (*e.g.* Miller *et al.* 2005; Tumanov 2007; McInnes 2010; Dastych 2011; Kaczmarek *et al.* 2012; Kaczmarek *et al.* 2013).

The genus *Isohypsibius* Thulin, 1928 has a worldwide distribution and can be found both in freshwater and terrestrial habitats, in all climatic zones, including polar regions (McInnes 1994).

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Previously, twelve *Isohypsibius* species have been reported from the Svalbard archipelago (Scourfield 1897; Murray 1907; Richters 1911; Marcus 1936; Węglarska 1965; Pilato *et al*. 1982; Dastych 1985; Pilato and Binda 1987; Van Rompu and De Smet 1988; De Smet and Van Rompu 1994; Janiec 1996; Maucci 1996; Kaczmarek *et al*. 2012). These are: *I. annulatus annulatus* (Murray, 1905), *Isohypsibius ceciliae* Pilato *et* Binda, 1987, *I. coulsoni* Kaczmarek *et al.* 2012, *I. dastychi* Pilato, Bertolani *et* Binda, 1982, *I. elegans* Binda *et* Pilato, 1971, *I. granulifer granulifer* Thulin, 1928, *I. papillifer bulbosus* (Marcus, 1928), *I. p. papillifer* (Murray, 1905), *I. prosostomus prosostomus* Thulin, 1928, *I. sattleri* (Richters, 1902), *I. schaudinni* (Richters, 1909), *I. tuberculatus* (Plate, 1888).

In this study, two moss samples collected from the Ariekammen area near a little auk (*Alle alle* (Linnaeus, 1758)) colony were examined. This resulted in five species, including one new for science, which are reported and described below.

Material and methods

Two moss samples were collected from the Ariekammen area, near a large colony of little auks (*Alle alle*) located on the northern coast of Hornsund (Spitsbergen, Svalbard archipelago), 77°00' N, 15°32' E, *ca.* 7 m asl. The samples were collected by K. Wojczulanis-Jakubas and D. Jakubas, and later examined for tardigrades according to Ramazzotti and Maucci (1983) and Dastych (1985).

All specimens and eggs were mounted in microscopic slides in Hoyer's medium and then examined and photographed with a Phase Contrast Microscope (PCM) (Olympus BX 40). Species were identified using the key to the world Tardigrada (Ramazzotti and Maucci 1983) and later original descriptions (Binda 1988; Bertolani and Balsamo 1989). All measurements (performed with Quick-Photo Camera 2.3 software) are given in micrometers [μm]. Structures were measured only if their orientations were suitable. Body length was measured from the anterior to the posterior end of the body, excluding the hind legs. Macroplacoid length sequence is given according to the Tardigrada Register (Michalczyk and Kaczmarek 2013). Claws were measured according to Beasley *et al.* (2008). In eutardigrades, the *pt* ratio is the ratio of the length of a given structure to the length of the buccal tube, expressed as a percentage (Pilato 1981). Morphometric data were handled using the "Hypsibioidea and Isohypsibioidea" ver. 1.1 template available from the Tardigrada Register (Michalczyk and Kaczmarek 2013).

The new species has also been compared with two paratypes of *I. laevis* and measurements and microphotographs of *I. marii*. The investigated material is held in the Department of Animal Taxonomy and Ecology, Adam Mickiewicz University, Poznań, Poland. Raw data underlying the description of *Isohypsibius karenae* sp. n. are deposited in the Tardigrada Register (Michalczyk and Kaczmarek 2013, under http://www.tardigrada.net/register/0008.htm).

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Taxonomy

Phylum Tardigrada (Spallanzani, 1777)
Class Eutardigrada Richters, 1926
Order Parachela Schuster, Nelson, Grigarick *et* Christenberry, 1980
Superfamily Hypsibioidae Pilato, 1969 in Marley *et al.* 2011
Family Hypsibiidae Pilato, 1969
Subfamily Diphasconinae Dastych, 1992
Genus *Diphascon* Plate, 1888
Diphascon (*Diphascon*) *recamieri* Richters, 1911

Material examined. — 39 specimens + 13 exuvia.

Remarks. — Holarctic, recorded from sparsely distributed localities in Europe, Asia and North America (McInnes 1994). Previously reported from Svalbard archipelago for Spitsbergen (Richters 1911; Marcus 1936; Węglarska 1965; Dastych 1985; De Smet and Van Rompu 1994; Janiec 1996; Maucci 1996; Kaczmarek et al. 2012) and for Hopen (Richters 1911; Van Rompu and De Smet 1996).

Family Hypsibiidae Pilato, 1969
Subfamily Hypsibiinae Pilato, 1969
Genus *Hypsibius* Ehrenberg, 1848
Hypsibius dujardini (Doyère, 1840)

Material examined. — 90 specimens + 19 exuvia.

Remarks. — Species belongs to the cosmopolitan *convergens–dujardini* complex of species (McInnes 1994; Miller *et al.* 2005; Kaczmarek and Michalczyk 2009). The original description is inadequate and unsatisfactory therefore the examined specimens were compared with the later descriptions (*e.g.* Ramazzotti and Maucci 1983; Dastych 1988). Previously reported from Svalbard archipelago for Spitsbergen (Richters 1903, 1904, 1911; Marcus 1936; Węglarska 1965; Dastych 1985; Janiec 1996; Maucci 1996; Kaczmarek *et al.* 2012), for Prins Karls Forland (Murray 1907), for Bjørnøya (Van Rompu and De Smet 1988), for Barentsøya (Van Rompu and De Smet 1991), for Edgeøya (De Smet *et al.* 1988), Hopen (Van Rompu and De Smet 1996), and for Amsterdamøya (Richters 1911).

Subfamily Itaquasconinae Rudescu, 1964
Genus *Mesocrista* Pilato, 1987
Mesocrista spitzbergensis (Richters, 1903)

Material examined. — 1 specimen.

Remarks. — Holarctic, recorded from localities in Europe, Asia and North America (McInnes 1994). Previously reported from Svalbard archipelago for Spitsbergen (Marcus 1936, recounting Richters 1903 and 1904; Węglarska 1965; Dastych 1985; Klekowski and Opaliński 1989), for Prins Karls Forland (Murray 1907), for Edgeøya (De Smet *et al.* 1988), for Bjørnøya (Van Rompu and De Smet 1988), and for Amsterdamøya (Richters 1903, 1904).

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Superfamily Isohypsibioidea Marley, McInnes *et* Sands, 2011
Family Isohypsibiidae Marley, McInnes *et* Sands, 2011
Genus *Isohypsibius* Thulin, 1928
Isohypsibius karenae sp. n.

(Figs 1–3, Table 1)

Material. — Holotype (slide 86.8/4), 31 paratypes and 9 exuvia (including two with eggs) (slides numbers: 86.7/1, 86.7/2, 86.7/3, 86.7/5, 86.8/1, 86.8/2, 86.8/3, 86.8/4, 86.8/6, 86.8/7, 86.8/11), deposited in the Department of Animal Taxonomy and Ecology at Adam Mickiewicz University, Poznań, Poland.

Type locality. — 77°00' N 15°32' E, *ca.* 7 m. asl, Svalbard Archipelago, Spitsbergen, Hornsund, Ariekammen area, coll. K. Wojczulanis-Jakubas and D. Jakubas.

Etymology.— Karen means Katarzyna in Norwegian. I dedicate this new species to Katarzyna Wojczulanis-Jakubas from the University of Gdańsk (Poland) who collected the material.

Description (measurements in Table 1). — Body colourless or slightly yellow (also after preparation) (Fig. 1a–b). Eyes were present in 75% of the examined specimens (mounted in Hoyer medium). Dorso-lateral cuticle, including outer portions of legs IV, covered with a faint reticular pattern. The reticulum with irregular ridges, mesh 1.4–3.1 in diameter (Fig. 1c–d). Diameter of reticular mesh slightly increasing towards caudal end of the body. Ventral cuticle smooth (*i.e.* without sculpturing).

Mouth antero-ventral. Peribuccal lamellae absent. Oral cavity armature comprises a single ventral and dorsal band of small round teeth in the posterior portion of the oral cavity (barely visible in some specimens) (Fig. 2a, insert). Bucco-pharyngeal apparatus of the *Isohypsibius* type. Buccal tube without the ventral lamina. Buccal tube walls very thick along the entire length. Pharyngeal bulb with apophyses, with three rod-shaped macroplacoids, all without constrictions. Macroplacoid length sequence 1<2<3, microplacoid and septulum absent (Fig. 2a).

Claws of the *Isohypsibius* type (Figs 2b–c, 3a–c), similar in shape and size on all legs, sometimes with a very wide basal part. External claws I–III and posterior claws IV slightly larger than internal claws I–III and anterior claws IV, respectively (see Remarks). All primary branches with accessory points, but those on the internal and external claws I–III are usually very poorly visible. The primary branches of anterior and posterior claws IV with better developed and visible accessory points (Fig. 3a). Small smooth lunules present on legs, but sometimes very hard to observe (Figs 2b–c, 3b) or invisible (Fig. 3a, c), especially on internal claws (see Remarks) (Fig. 2c). Oblique cuticular bars near the bases of claws I–III present (Fig. 3c).

Smooth eggs (3–4 in number) deposited in the exuvium (Fig. 1b).

Remarks. — In the majority of specimens instead of proper lunules only widened claw bases can be observed. Usually lunules are evident on the external claws and rather poorly visible on the internal claws (Fig. 2b–c). Sometimes they are vis-

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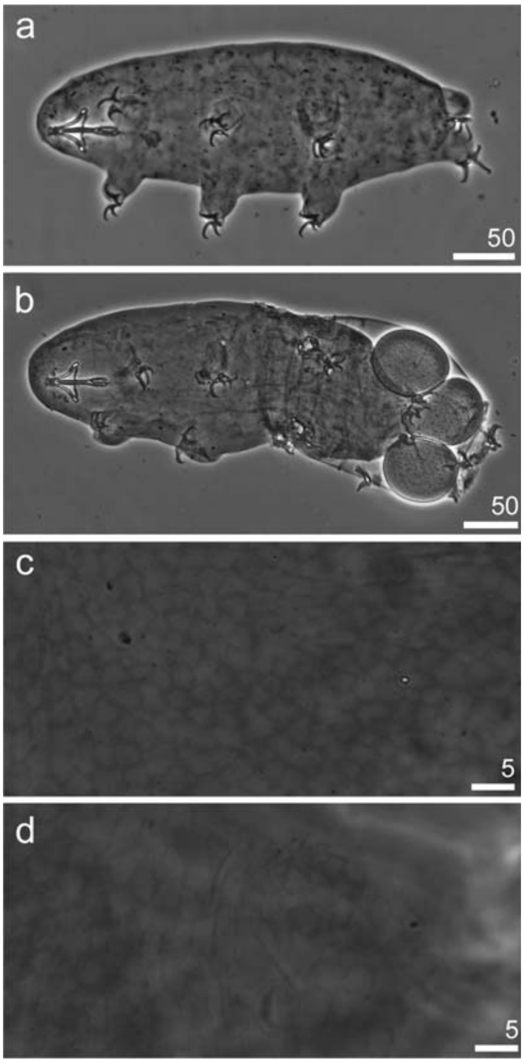

Fig. 1. *Isohypsibius karenae* sp. n.: **a**, habitus (dorsoventral view, holotype); **b**, an individual in the final stage of oviposition (three smooth eggs have been laid into the exuvium, lateral view, paratype); **c**, dorsal cuticle (paratype); **d**, cuticle on legs IV (paratype). Scale in μm.

ible only on the external claws (Figs 2b, 3b). Presence of lunules in this species could be confused, I have not observed lunules on the anterior claws IV which certainly are absent (Fig. 3a). Presence of lunules is variable character in *I. karenae* sp. n. Thus, this species could have lunules on internal and external claw I–III, but sometimes they are invisible.

In two paratypes small, short cuticular thickenings are present on outer (lateral) side of legs I–III. They are most likely folds of cuticule and they are under proper cuticular bars.

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Table 1

Measurements and *pt* values of selected morphological structures of fifteen specimens (including the holotype) from the type population of *Isohypsibius karenae* sp. n.: N – number of specimens or structures measured; Range – the smallest and the largest structure found among all specimens measured, SD – standard deviation; pt – ratio of the length of a given structure to the length of the buccal tube, expressed as a percentage; ? – structure oriented unsuitably for measurement.

Character	N	Range		Mean		SD		Holotype	
		µm	pt	µm	pt	µm	pt	µm	pt
Body length	9	169–395	634–1046	274	825	77	139	341	928
Buccopharyngeal tube									
Buccal tube length	15	25.9–37.8	–	33.1	–	3.6	–	36.7	–
Stylet support insertion point	15	17.2–24.2	63.8–67.3	21.7	65.5	2.2	1.2	24.1	65.7
Buccal tube external width	15	2.4–4.3	9.0–11.7	3.4	10.2	0.5	0.9	3.5	9.5
Buccal tube internal width	15	1.2–2.5	4.3–6.8	1.8	5.4	0.4	0.7	2.1	5.7
Placoid lengths									
Macroplacoid 1	15	1.4–4.5	5.0–12.3	2.7	8.0	0.8	1.8	4.5	12.3
Macroplacoid 2	15	2.1–4.6	8.1–12.5	3.3	9.9	0.7	1.3	4.6	12.5
Macroplacoid 3	15	2.8–6.3	10.4–17.2	4.4	13.2	0.9	1.7	6.3	17.2
Macroplacoid row	14	8.1–15.3	30.6–41.4	12.0	36.2	2.2	3.3	15.2	41.4
Claw 1 lengths									
External base	1	8.8–8.8	24.0–24.0	8.8	24.0	?	?	8.8	24.0
External primary branch	3	9.8–16.7	35.3–44.4	13.9	40.5	3.6	4.7	15.3	41.7
External secondary branch	3	7.8–10.3	24.5–28.1	9.1	26.9	1.3	2.1	10.3	28.1
Internal base	4	3.7–8.0	12.2–22.6	6.7	19.4	2.0	4.9	7.7	21.0
Internal primary branch	4	7.6–13.6	25.1–37.1	11.0	32.2	2.5	5.3	13.6	37.1
Internal secondary branch	4	6.6–10.1	21.8–30.3	8.4	24.7	1.4	3.8	8.6	23.4
Claw 2 lengths									
External base	3	8.2–10.5	24.6–27.8	9.7	26.7	1.3	1.8	?	?
External primary branch	3	13.6–18.9	40.8–50.0	16.4	45.0	2.7	4.6	?	?
External secondary branch	3	12.2–14.5	32.3–38.6	13.1	36.2	1.2	3.4	?	?
Internal base	2	6.0–7.3	16.3–21.9	6.7	19.1	0.9	3.9	6.0	16.3
Internal primary branch	3	7.8–13.3	25.4–39.9	11.2	33.2	3.0	7.3	12.6	34.3
Internal secondary branch	3	6.7–10.2	21.8–30.6	8.8	26.2	1.9	4.4	9.6	26.2
Claw 3 lengths									
External base	3	4.5–8.5	17.4–26.3	7.1	23.1	2.2	4.9	?	?
External primary branch	3	9.1–14.1	35.1–43.9	12.3	40.5	2.8	4.7	?	?
External secondary branch	3	6.9–12.2	26.6–36.6	9.8	32.0	2.7	5.0	?	?
Internal base	5	4.3–8.4	14.0–22.2	6.2	17.8	1.5	3.0	6.4	17.4
Internal primary branch	5	9.2–16.0	30.0–42.3	12.5	35.8	2.5	4.6	12.9	35.1
Internal secondary branch	5	6.9–12.6	22.5–33.3	9.8	28.3	2.2	5.0	8.6	23.4
Claw 4 lengths									
Anterior base	4	5.7–10.5	18.8–27.8	8.1	23.3	2.0	3.7	8.3	22.6
Anterior primary branch	4	10.0–22.2	31.3–58.7	14.8	42.6	5.6	11.9	16.0	43.6
Anterior secondary branch	3	6.7–15.0	21.0–39.7	10.1	29.6	4.4	9.4	?	?
Posterior base	6	6.3–11.5	19.5–30.6	8.6	24.4	1.9	4.6	8.0	21.8
Posterior primary branch	6	13.3–20.6	43.9–54.8	16.7	47.4	2.4	4.3	16.3	44.4
Posterior secondary branch	6	8.2–16.4	27.1–45.3	12.6	35.5	3.2	7.9	11.0	30.0

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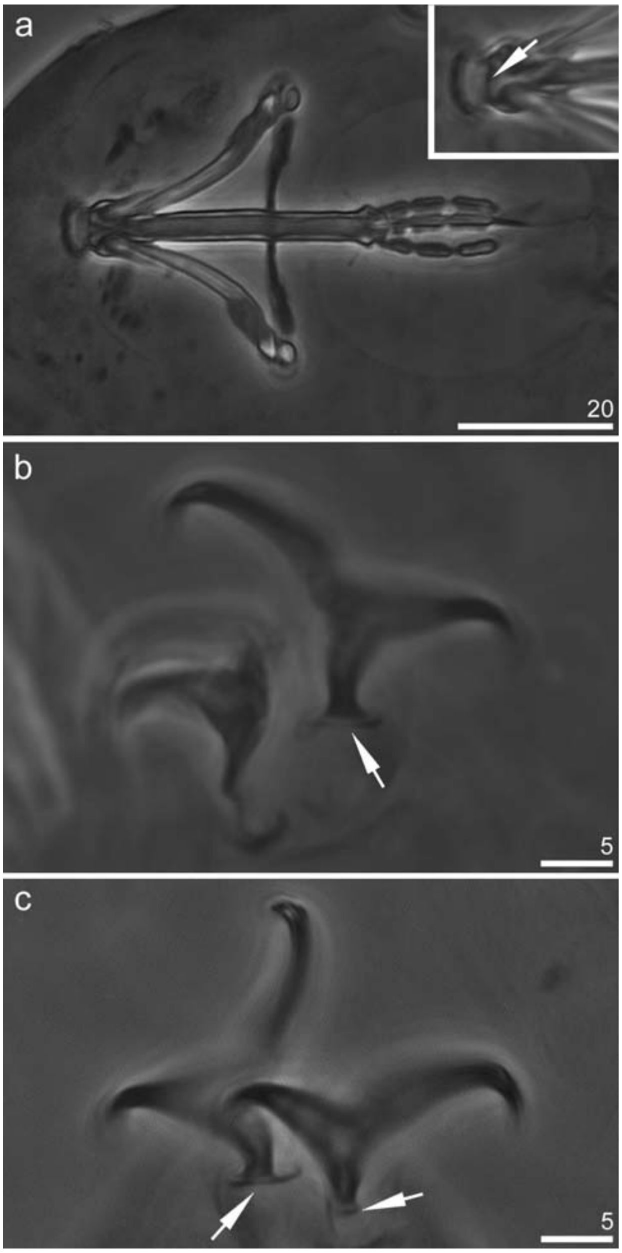

Fig. 2. *Isohypsibius karenae* sp. n.: **a**, buccal apparatus (the insert shows the oral cavity armature composed of a single ventral band of small teeth, holotype); **b**, external claw I with a well developed lunule (holotype); **c**, claw III with visible lunules (paratype). Scale in µm.

Accessory points at primary branches are usually poorly visible, such similarity is known from the description of *I. ladogensis* Tumanov, 2003. In one specimen the primary anterior branch is slightly longer than the primary posterior branch.

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Differential diagnosis. — *Isohypsibius karenae* sp. n. has been found in samples collected from a very wet habitat located near small water bodies. Thus, like many of its congeners, it is potentially a hygrophilous terrestrial or an aquatic species (copepods were also found in the samples). The new species should be therefore compared with both aquatic and terrestrial species of the genus *Isohypsibius* with sculptured dorsal cuticle.

By having three macroplacoids in the pharynx and by sculptured dorsal cuticle the new species is most similar to the following aquatic *Isohypsibius* species: *I. asper* (Murray, 1906), *I. baldii* (Ramazzotti, 1945), *I. baldiioides* Tumanov, 2003, *I. granulifer granulifer* Thulin, 1928, *I. g. koreanensis* (Iharos, 1971), *I. kotovae* Tumanov, 2003, *I. ladogensis* Tumanov, 2003, *I. laevis* McInnes, 1995, *I. marii* Bertolani, 1982, *I. monoicus* Bertolani, 1982, *I. pushkini* Tumanov, 2003 and *I. tubereticulatus* Pilato *et* Catanzaro, 1989, but specifically differs from:

- *I. asper*, by a different type of cuticular sculpturing (reticular pattern with irregular polygons of various size in the new species *vs.* hemispherical thickenings in *I. asper*) and by the presence of cuticular bars under claws I–III;
- *I. baldii*, by a different type of cuticular sculpturing (reticular pattern with irregular polygons of various size in the new species *vs.* reticular pattern composed of small tubercles in *I. baldii*), a different macroplacoid length sequence (1<2<3 in the new species *vs.* 2<1<3 in *I. baldii*), the presence of cuticular bars under claws I–III, presence of oral cavity armature, and by a wider buccal tube (2.4–4.3 µm in the new species *vs. ca.* 1.6–1.8 µm in *I. baldii*);
- *I. baldiioides*, by a different type of cuticular sculpturing in caudal region (reticular pattern with irregular polygons of various size in the new species *vs.* reticular pattern composed of small partially fused tubercles in *I. baldiioides*), the absence of ventral sculpture, a different macroplacoid length sequence (1<2<3 in the new species *vs.* 2<1<3 in *I. baldiioides*), different oral cavity armature and by a lower *pt* ratio of the stylet support insertion point (63.8–67.3 in the new species *vs*. 67.5–73.8 in *I. baldiioides*);
- *I. granulifer granulifer*, by a different type of cuticular sculpturing (reticular pattern with irregular polygons of various size in the new species *vs.* irregularly distributed thickenings in *I. g. granulifer*), absence of granulation on the legs, a longer buccal tube (32.2 µm in the new species (specimen 230 µm long) *vs.* 26 µm in *I. g. granulifer* (specimen 230 µm long)), and by the presence of cuticular bars under claws I–III;
- *I. granulifer koreanensis*, by absence of ventral sculpture, a different type of dorsal cuticular sculpturing (reticular pattern with irregular polygons of various size in the new species *vs.* irregularly distributed granules in *I. g. koreanensis*), absence of granulation on the legs, the shape of macroplacoids (rods in the new species *vs.* round macroplacoids in *I. g. koreanensis*), and by the presence of cuticular bars under claws I–III;

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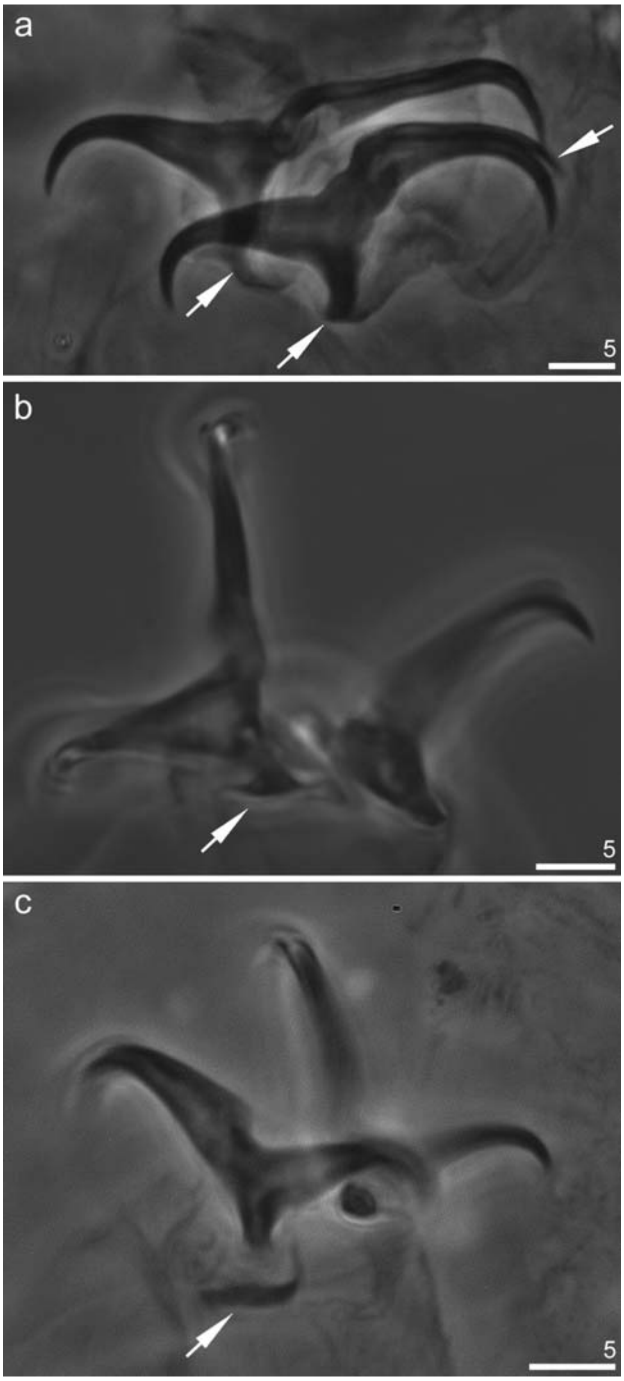

Fig. 3. *Isohypsibius karenae* sp. n.: **a**, claws IV with not developed lunules (paratype), anterior claw with well visible accessory point; **b**, anterior claw IV with a lunule (holotype); **c**, cuticular bar under claws III (paratype). Scale in µm.

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- *I. kotovae*, by the presence of cuticular bars under claws I–III, presence of oral cavity armature, a lower *pt* ratio of the stylet support insertion point (63.8–67.3 in the new species *vs.* 67.2–68.8 in *I. kotovae*), and by presence of eyes;
- *I. ladogensis*, by a different type of cuticular sculpturing (reticular pattern with irregular polygons of various size in the new species *vs.* indistinct granules fused laterally which form reticulate pattern in *I. ladogensis*), a different macroplacoid length sequence (1<2<3 in the new species *vs.* 2<1<3 in *I. ladogensis*), and by different oral cavity armature;
- *I. laevis*, by the presence of cuticular bars under claws I–III and the presence of lunules (but see the Remarks above) (McInnes pers. comm.);
- *I. marii*, by a different macroplacoid length sequence (1<2<3 in the new species *vs.* 2<1<3 in *I. marii*), a thicker buccal tube (2.1 μm internal and 4.1 μm external diameter in the new species (specimen 395 μm in length) *vs.* 5.2 μm internal and 5.9 μm external diameter in *I. marii* (specimen 483 μm in length)), a lower *pt* ratio of the stylet support insertion point (63.8 in the new species (specimen 395 μm in length) *vs.* 67.1 in *I. marii* (specimen 483 μm in length)) (Bertolani pers. comm.), and by the presence of lunules (but see also Remarks above);
- *I. monoicus*, by a different type of cuticular sculpturing (reticular pattern with irregular polygons of various size in the new species *vs.* wrinkles in *I. monoicus*), the shape of macroplacoids (rods in the new species *vs.* granules in *I. monoicus*), and by the presence of cuticular bars under claws I–III;
- *I. pushkini*, by a different type of cuticular sculpturing (reticular pattern with irregular polygons of various size in the new species *vs.* reticular pattern with granules connected by thin, winding ridges), a different macroplacoid length sequence (1<2<3 in the new species *vs.* 2<1<3 in *I. pushkini*), presence of lunules on internal claws (but see the Remarks above), and by the slightly smaller third macroplacoid (4.4 μm in the new species (specimen 395 μm long) *vs*. 5.5 μm in *I. pushkini* (specimen 405 μm long));
- *I. tubereticulatus*, by a different type of cuticular sculpturing (reticular pattern with irregular polygons of various size in the new species *vs.* reticular pattern with granules connected by thin, winding ridges in *I. tubereticulatus*), a different macroplacoid length sequence (1<2<3 in the new species *vs.* 2<1<3 in *I. tubereticulatus*) the absence of a constriction in the third macroplacoid, the presence of cuticular bars under claws I–III, and by a lower *pt* ratio of the stylet support insertion point (63.8–67.3 in the new species *vs.* 67.2–69.5 in *I. tubereticulatus*).

Additionally, by having three macroplacoids in the pharynx and a sculptured dorsal cuticle the new species is most similar to the following terrestrial species of the genus *Isohypsibius*: *I. brulloi* Pilato *et* Pennisi, 1976, *I. irregibilis* Biserov, 1992, *I. kenodontis* Kendall-Fite *et* Nelson, 1996, *I. liae* X. Li *et* L. Wang, 2006, *I. palmai* Pilato, 1996, and *I. yunnanensis* Yang, 2002, but differs in particular from:

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- *I. brulloi*, by absence of ventral sculpture, a longer buccal tube (32.2 µm in the new species (specimen 230 µm in length) *vs.* 25 µm in *I. brulloi* (specimen 230 µm in length)), a different macroplacoid length sequence (1<2<3 in the new species *vs.* 2<1<3 in *I. brulloi*), presence of oral cavity armature, and by the presence of eyes;
- *I. irregibilis*, by a different type of cuticular sculpturing (reticular pattern with irregular polygons of various size in the new species *vs.* wrinkled cuticle in *I. irregibilis*), a different macroplacoid length sequence (1<2<3 in the new species *vs.* 2<1<3 in *I. irregibilis*), a lower *pt* ratio of the stylet support insertion point (63.8–67.3 in the new species *vs.* 67.8–75.0 in *I. irregibilis*), and by the lack of a projections on the primary branches on legs I–IV;
- *I. kenodontis*, by a different type of cuticular sculpture (reticular pattern with irregular polygons of various size in the new species *vs.* partially fused granules in *I. kenodontis*), a different macroplacoid length sequence (1<2<3 in the new species *vs.* 2<1<3 in *I. kenodontis*), the presence of cuticular bars under claws I–III, and by the presence of the oral cavity armature;
- *I. liae*, by the absence of cuticular undulations, absence of ventral sculpture, a different shape of macroplacoids (rods in the new species *vs.* granules in *I. liae*), a longer buccal tube (32.2 µm in the new species (specimen 230 µm in length) *vs.* 26.1 µm in *I. liae* (specimen 232 µm in length)), and by the presence of eyes;
- *I. palmai*, by a different macroplacoid length sequence (1<2<3 in the new species *vs.* 1=2=3 in *I. palmai*), a different oral cavity armature (a ventral band of small round teeth in the new species *vs.* three transverse ventral and three dorsal ridges in *I. palmai*), a lower *pt* ratio of the stylet support insertion point (66.8 in the new species (specimen 230 µm in length) *vs.* 73.0 in *I. palmai* (specimen 222 µm in length)) and by the presence of eyes;
- *I. yunnanensis*, by a different type of cuticular sculpturing (reticular pattern with irregular polygons of various size in the new species *vs.* poriform sculpture in *I. yunnanensis*) and by the presence of lunules (but see also Remarks above).

Superfamily Macrobiotoidea Thulin, 1928 in Marley *et al.* 2011
Family Macrobiotidae Thulin, 1928
Genus *Macrobiotus* C.A.S. Schultze, 1834
Macrobiotus crenulatus Richters, 1904

Material examined. — 2 specimens + 1 exuvium.

Remarks. — Holarctic (McInnes 1994). Specimens correspond perfectly to the description by Binda (1988). Previously reported from Svalbard archipelago for Spitsbergen (Maucci 1996; Kaczmarek *et al.* 2012), for Prins Karls Forland (Murray 1907), for Edgeøya (Maucci 1996), for Hopen (Van Rompu and De Smet 1996), and for Amsterdamøya (Richters 1904).

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Discussion

Recent systematic revision (Marley *et al*. 2011), placed the genus *Isohypsibius* Thulin, 1928 within the family Isohypsibiidae, along with *Doryphoribius* Pilato, 1969, *Thulinius* Bertolani, 2003, *Pseudobiotus* Nelson,1980, *Halobiotus* Kristensen, 1982, *Ramajendas* Pilato *et* Binda, 1990, and *Eremobiotus* Biserov, 1992. *Isohypsibius* comprises 131 valid species (Degma *et al.* 2012); though further work is required on many older descriptions as characters defining the more recently described genera may have been missed or omitted from the original descriptions. For example the presence/absence of the ventral lamina on the buccal tube, a distinguishing character of *Doryphoribius*, was omitted in older descriptions as *D. vietnamensis* (Iharos, 1969) and *D. zyxiglobus* (Horning, Schuster *et* Grigarick, 1978) (Beasley *et al.* 2006 and Claxton *et al.* 2010, respectively). Similarly, the presence of twelve peribuccal lamellae around the mouth in *Thulinius*, or 30 peribuccal lamellae of *Pseudobiotus*, can be difficult to identify in poorly fixed specimens (*e.g. Thulinius augusti* (Murray, 1907), *Thulinius saltursus* (Schuster, Toftner *et* Grigarick, 1978), *Thulinius itoi* (Tsurusaki, 1980) or *Pseudobiotus kathmanae* Nelson, Marley *et* Bertolani, 1999 (Bertolani *et al.* 1999; Nelson *et al*. 1999; Marley *et al*. 2008; Kaczmarek and Michalczyk 2009; Kaczmarek *et al.* 2010). It is therefore important to provide as detailed descriptions as possible for new *Isohypsibius* species, with clear statements on the absence of the above mentioned characters as ventral lamina and peribuccal lamellae.

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Krzysztof Zawierucha

Polar Research

RESEARCH/REVIEW ARTICLE

Current knowledge of the Tardigrada of Svalbard with the first records of water bears from Nordaustlandet (High Arctic)

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Keywords
Checklist; Edgeøya; new species; Prins Karls Forland; soil fauna; Svalbard biodiversity.

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Abstract

The first investigations of the tardigrades of Svalbard took place in the early 20th century and 30 papers on the subject have been published to date. In this article, we summarize available information on the distribution of tardigrades in this Arctic archipelago with remarks on the dubious species and records. Additionally, we examined 28 new moss, lichen and soil samples collected from the islands of Nordaustlandet, Edgeøya and Prins Karls Forland. These samples yielded 324 specimens, 15 exuvia and 132 free-laid eggs belonging to 16 limnoterrestrial species (Heterotardigrada and Eutardigrada). These include five first records of water bears from Nordaustlandet, eight new records for Edgeøya and four for Prince Karls Forland. The most dense population of tardigrades was found in a sample with 253 specimens/10 g of dry material and the least dense population in a sample with three specimens/10 g of dry material. The most frequently recorded species in samples collected in this study were *Testechiniscus spitsbergensis* Scourfield, 1897, *Macrobiotus harmsworthi harmsworthi* Murray, 1907, and *M. islandicus islandicus* Richters, 1904. This article also provides the first ever scanning electron microscope photomicrographs of *Tenuibiotus voronkovi* Tumanov, 2007.

To access the supplementary material for this article, please see Supplementary files under Article Tools online.

Tardigrada are a phylum of microscopic animals (typically 50–2100 µm in size) inhabiting a great majority of ecosystems throughout the world (Ramazzotti & Maucci 1983; Guil 2008). About 1170 species have been described worldwide (Guidetti & Bertolani 2005; Degma & Guidetti 2007; Degma et al. 2009–2013). Limnoterrestrial tardigrades are found mainly in mosses, lichens and soil habitats, whereas aquatic species live both in freshwater and marine environments. Tardigrades are recognized as possessing great environmental stress tolerance and are able to survive in extreme conditions on Earth and, through experimental exposure in low Earth orbit, to the space environment (Wełnicz et al. 2011; Guidetti et al. 2012). In addition to their typical microhabitats (e.g., mosses, lichens or soil), they are also able to dwell in cryoconite holes in alpine and polar glaciers (e.g., De Smet & Van Rompu 1994; Grongaard et al. 1999; Dastych 2004; Porazińska et al. 2004). It has also recently been shown that tardigrades inhabit detached moss balls ("glacier mice") that roll free on ice surfaces in the Arctic (Coulson & Midgley 2012). Despite the fact that studies of the tardigrades of the Svalbard islands were conducted by a number of researchers over a long period (e.g., Richard 1898; Richters 1903, 1904, 1911a, b; Murray 1907; Summerhayes & Elton 1923; Marcus 1936; De Smet et al. 1987, 1988; Van Rompu & De Smet 1988, 1991, 1994; Maucci 1996), only the tardigrade fauna of the largest island of the archipelago, Spitsbergen, is relatively well surveyed. In contrast, our knowledge of water bears of other islands of the Svalbard Archipelago is still very poor.

In this article, we list all the species found on every investigated island in the Svalbard Archipelago.

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We exclude species mistakenly listed by Coulson & Refseth (2004) in a previous survey of the literature (e.g., *Isohypsibius fuscus* [Mihelčič, 1971]). Moreover, we offer remarks on dubious species and records (e.g., *Acutuncus antarcticus* [Richters, 1904], *Hypsibius arcticus* [Murray, 1907]). Revision of the inventory of Svalbard is urgently required because of likely errors in the current inventories (Coulson 2013). Additionally, we also present new records of water bears from the islands of Edgeøya and Prins Karls Forland and the first records from Nordaustlandet.

Materials and methods

In the summers of 2009 and 2010, 28 moss, lichen and soil samples were collected from the islands of Prins Karls Forland, Nordaustlandet and Edgeøya in Svalbard (Fig. 1). These were examined for the tardigrade fauna using standard methods (Ramazzotti & Maucci 1983; Dastych 1985). Twenty samples (71%) contained tardigrades (Table 1). All tardigrades collected were mounted on microscope slides in Hoyer's medium. Species identification was based on the key to the world fauna of Tardigrada by Ramazzotti & Maucci (1983) as well as later descriptions and diagnostic keys (Dastych 1985, 1988; Bertolani & Rebecchi 1993; Miller et al. 2005; Kaczmarek & Michalczyk 2009; Kaczmarek et al. 2011; Kaczmarek, Zawierucha et al. 2012).

Only specimens determined to the species level are provided in the list of species from Prins Karls Forland, Edgeøya and Nordaustlandet. We decided to do so because records identified only to the generic level do not provide any additional information about the biodiversity range if other species of a genus have been already reported from a given area. In other words, it is not possible to designate a dubious record as a new record or another record of a species that has already been reported and identified to the species level. Doing so could lead to inaccurate estimates of biodiversity and confusion in faunistic checklists and biogeographic studies. In the list of species found during this study, the Roman numerals

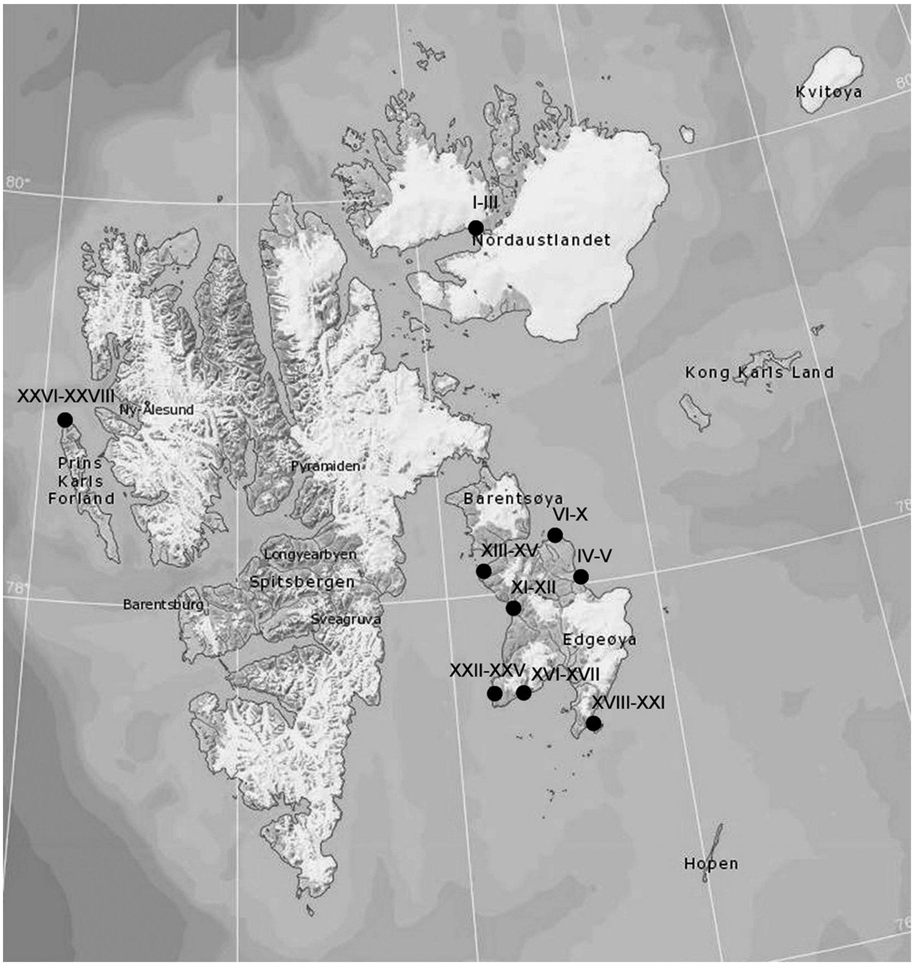

Fig. 1 Map of Svalbard with sampling sites (map from Norwegian Polar Institute).

Table 1 List of samples investigated in this study, with data on the total number of specimens (S), the number of exuvia (E), the number of eggs (e) found and average density of specimens (calculated as the number of specimens per 10 g of dry sample).

Sample code	Island	Region	Latitude, longitude	Date	S, E, e	Sample type (dry weight)	Density
I	Nordaustlandet	Oxfordhalvøya	79°78′04′′N 21°73′04′′E	07/2009	S: 13 E: 22 e: 0	Moss, soil (1.93 g)	67
II	Nordaustlandet	Oxfordhalvøya	79°78′04′′N 21°73′04′′E	07/2009	S: 110 E: 1 e: 51	Moss, soil (10.09 g)	109
III	Nordaustlandet	Oxfordhalvøya	79°78′04′′N 21°73′04′′E	07/2009	S: 4 E: 0 e: 0	Moss, soil (1.97 g)	20
IV	Edgeøya	Blåbukta	78°03′27′′N 22°52′53′′E	07/2010	S: 8 E: 0 e: 1	Moss (2.26 g)	35
V	Edgeøya	Blåbukta	78°03′27′′N 22°52′53′′E	07/2010	S: 18 E: 1 e: 6	Moss (3.24 g)	56
VI	Edgeøya	Kapp Heuglin	78°15′02′′N 22°50′40′′E	07/2010	S: 3 E: 1 e: 1	Moss (1.24 g)	24
VII	Edgeøya	Kapp Heuglin	78°15′02′′N 22°50′40′′E	07/2010	S: 0 E: 0 e: 0	Moss, soil (15.12 g)	0
VIII	Edgeøya	Kapp Heuglin	78°14′46′′N 22°48′21′′E	07/2010	S: 0 E: 0 e: 0	Moss (1.99 g)	0
IX	Edgeøya	Kapp Heuglin	78°14′46′′N 22°48′21′′E	07/2010	S: 10 E: 0 e: 0	Moss (1.91 g)	52
X	Edgeøya	Kapp Heuglin	78°14′46′′N 22°48′21′′E	07/2010	S: 4 E: 0 e: 0	Moss (1.65 g)	24
XI	Edgeøya	Diskobukta	77°58′25′′N 21°19′08′′E	07/2009	S: 0 E: 0 e: 0	Moss, soil (3.92 g)	0
XII	Edgeøya	Diskobukta	77°58′25′′N 21°19′08′′E	07/2009	S: 3 E: 1 e: 0	Moss, soil (2.66 g)	11
XIII	Edgeøya	Kapp Lee	78°06′45′′N 14°50′58′′E	07/2009	S: 15 E: 1 e: 8	Moss (2.62 g)	57
XIV	Edgeøya	Kapp Lee	78°06′45′′N 14°50′58′′E	07/2009	S: 26 E: 0 e: 19	Moss (2.03 g)	128
XV	Edgeøya	Kapp Lee	78°06′45′′N 14°50′58′′E	07/2009	S: 2 E: 0 e: 0	Moss (3.20 g)	6
XVI	Edgeøya	Kraussbukta	77°50′09′′N 20°85′30′′E	07/2009	S: 1 E: 1 e: 0	Moss (3.62 g)	3
XVII	Edgeøya	Kraussbukta	77°50′09′′N 20°85′30′′E	07/2009	S: 0 E: 0 e: 0	Moss (1.57 g)	0
XVIII	Edgeøya	Negerdalen	77°17′42′′N 22°53′37′′E	07/2010	S: 0 E: 0 e: 0	Moss, soil (6.24 g)	0
XIX	Edgeøya	Negerdalen	77°17′42′′N 22°53′37′′E	07/2010	S: 6 E: 0 e: 6	Moss (3.45 g)	17

Table 1 *Continued*

Sample code	Island	Region	Latitude, longitude	Date	S, E, e	Sample type (dry weight)	Density
XX	Edgeøya	Negerdalen	77°19′25″N 22°50′02″E	07/2010	S: 5 E: 0 e: 0	Moss (1.8 g)	28
XXI	Edgeøya	Negerdalen	77°19′25″N 22°50′02″E	07/2010	S: 0 E: 0 e: 0	Moss (4.46 g)	0
XXII	Edgeøya	Russebukta	77°32′22″N 20°50′53″E	07/2009	S: 0 E: 0 e: 0	Moss, soil (2.83 g)	0
XXIII	Edgeøya	Russebukta	77°32′22″N 20°50′53″E	07/2009	S: 44 E: 6 e: 2	Moss (1.74 g)	253
XXIV	Edgeøya	Russebukta	77°32′22″N 20°50′53″E	07/2009	S: 0 E: 0 e: 0	Moss (2.43 g)	0
XXV	Edgeøya	Russebukta	77°32′22″N 20°50′53″E	07/2009	S: 13 E: 2 e: 1	Moss (1.7 g)	76
XXVI	Prins Karls Forland	Fuglehuken	78°53′29″N 10°28′07″E	08/2010	S: 8 E: 0 e: 0	Moss (1.21 g)	66
XXVII	Prins Karls Forland	Fuglehuken	78°53′29″N 10°28′07″E	08/2010	S: 41 E: 1 e: 11	Moss (2.16 g)	190
XXVIII	Prins Karls Forland	Fuglehuken	78°53′29″N 10°28′07″E	08/2010	S: 3 E: 0 e: 4	Moss, lichen (2.28 g)	13

indicate locality code, the first Arabic number indicates the number of specimens, the second Arabic number (in brackets) refers to exuvia and the third Arabic number (preceded by a plus sign) to eggs. Short zoogeographic comments for the recorded species from the Svalbard Archipelago are also provided.

To assess tardigrade population densities in our samples, we used the quotient of specimen number per 10 g of dry weight of the material (i.e., soil, moss or lichen). The samples and slides are deposited in the Department of Animal Taxonomy and Ecology at Adam Mickiewicz University in Poznań, Poland.

Results

In this study, 16 Tardigrada species of two orders, Eutardigrada and Heterotardigrada, were found. New records for Nordaustlandet, Edgeøya and Prins Karls Forland are marked below with asterisks. The samples (Roman numbers) are described in Table 1.

The average density in samples containing tardigrades was 62 specimens per 10 g of dry material. A density greater than 100 specimens per 10 g was found only in four samples. The most dense population of tardigrades was found in sample XXIII (253 specimens/10 g) and the least dense population in sample XVI (three specimens/ 10 g). In eight samples (29%), no tardigrades or their eggs were found.

In the Supplementary file, we list all tardigrade records from Svalbard known from the literature as well as from the present study: Spitsbergen, Prins Karls Forland, Bjørnøya, Barentsøya, Edgeøya, Hopen, Amsterdamøya, Svenskøya, Ryke Yseøyane, Kong Ludvigøyane and Nordaustlandet.

Diphascon (Adropion) prorsirostre Thulin, 1928*. Samples II: 1, X: 4, XIII: 1. These are new records for Nordaustlandet and Edgeøya. The *Diphascon* (*Adropion*) *prorsirostre* complex is cosmopolitan (McInnes 1994); however, the majority of these records are old and need be confirmed.

Diphascon (Diphascon) recamieri Richters, 1911*. Samples XIV: 1, XXVI: 5, XXVII: 2 (1), XXVIII: 1. These are new records for Prins Karls Forland and Edgeøya. This is a Holarctic species, recorded from sparse localities in Europe, Asia and North America (McInnes 1994).

Echiniscus merokensis Richters, 1904*. Sample XXVIII: 1. This is a new record for Prins Karls Forland. The species is Palaearctic (McInnes 1994).

Hypsibius dujardini (Doyère, 1840). Samples IX: 8, XIV: 4. Species belongs to the cosmopolitan *convergens–dujardini* complex of species (McInnes 1994; Miller et al. 2005; Kaczmarek & Michalczyk 2009). Because the original description is inadequate and unsatisfactory, the examined specimens were compared with recent descriptions (e.g., Ramazzotti & Maucci 1983; Dastych 1988; Miller et al. 2005).

Hypsibius convergens (Urbanowicz, 1925)*. Sample XXIII: 17 (1). This is a new record for Edgeøya. See remarks accompanying *H. dujardini*, above.

Isohypsibius coulsoni Kaczmarek et al. 2012*. Sample XXVII: 14 (1). This is a new record for Prins Karls Forland, previously known only from Rotjesfjellet (Spitsbergen) (Kaczmarek, Zawierucha et al. 2012).

Isohypsibius cf. *marcellinoi* Binda & Pilato, 1971*. Sample XII: 3 (1 with eggs). This is a new record for Edgeøya. Holoarctic (McInnes 1994). This species is very similar to *Isohypsibius dastychi* Pilato, Bertolani & Binda, 1982 but differs from it by the lack of sculpturing on the dorsal cuticle and by a different arrangement of dorsal teeth in the oral cavity. Our specimens clearly do not have sculptured cuticle, but oral cavities are not well preserved and do not allow the determination of teeth numbers. Although we are sure that our animals do not belong to *I. dastychi*, we are not able to confidently determine them to the species level.

Macrobiotus crenulatus Richters, 1904. Samples XXVII: 7+7, XIV: 3, XV: 2, XVII: 1. This species is Holarctic (McInnes 1994).

Macrobiotus harmsworthi harmsworthi Murray, 1907. Samples XXVI: 2, XXVII: 9+4, XXVIII: 1+4, XIV: 3+3, XXIII: 23 (5)+2, XXV: 14 (2)+1. This is the nominal taxon for a cosmopolitan species complex (McInnes 1994; Kaczmarek et al. 2011).

Macrobiotus hufelandi C.A.S. Schultze 1833*. Samples VI: 0+1, XIV: 10+15. This is a new record for Edgeøya. The *M. hufelandi* group of species is cosmopolitan (McInnes 1994); however, the majority of older records need to be confirmed (Bertolani & Rebecchi 1993).

Macrobiotus islandicus islandicus Richters, 1904*. Samples I: 0+4, II: 1+9, IV: 3+1, V: 2+1, XIX: 0+6. These are new records for Nordaustlandet and Edgeøya. Holarctic (McInnes 1994).

Murrayon hibernicus Murray, 1911*. Sample IX: 1. This is a new record for Edgeøya. Holarctic, Indomalayan (McInnes 1994).

Platicrista angustata Murray, 1905*. Sample XXVI: 1. This is a new record for Prins Karls Forland. The species is Holarctic (McInnes 1994).

Pseudechiniscus suillus Ehrenberg, 1853*. Samples II: 1, XX: 1. This is a new record for Nordaustlandet. The *P. suillus* group of species is cosmopolitan, but the distribution of the nominal species is unknown (McInnes 1994).

Tenuibiotus voronkovi Tumanov, 2007*. Samples I: 4+18, II: 16+24, III: 10+7, V: 8 (1)+3, XIII: 10 (1)+8. Eggs of this species are presented in Fig. 2. These are new records for Nordaustlandet and Edgeøya. The species has been previously recorded only from Spitsbergen (Tumanov 2007).

Testechiniscus spitsbergensis Scourfield, 1897*. Samples I: 8, II: 90, III: 4, IV: 3, V: 8, VI: 3. These are new records for

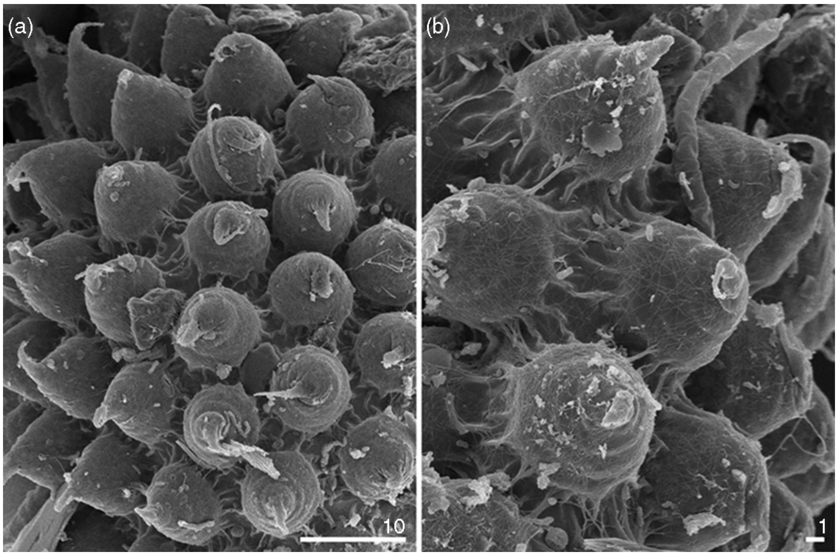

Fig. 2 *Tenuibiotus voronkovi*: details of the eggshell, shown for the first time with scanning electron microscopy (scale bar in micrometres).

Nordaustlandet and Edgeøya. The species is Holarctic (McInnes 1994).

Discussion

Tardigrade surveys carried out in Svalbard have been fragmentary and the numbers of species known from different islands of the archipelago are proportional to the number of papers concerning these islands. Because the majority of studies have been performed on the largest island—Spitsbergen—this island has also the longest tardigrade species list: 80 (excluding dubious records; see Supplementary file), with 95% of all species known from the entire archipelago. Second, in terms of species diversity, is Prins Karls Forland 23 (27%), followed by Hopen with 22 (26%), Bjørnøya with 20 (24%) species and Edgeøya with 20 (24%) species. Fifteen (18%) species are known from Barentsøya, nine (11%) from Amsterdamøya, five (6%) from Nordaustlandet and five (6%) from Kong Ludvigøyane, one (1%) from Svenskøya and one species (1%) from Ryke Yseøyane (all figures exclude dubious records; see Supplementary file).

The total number of valid water bear species known from Svalbard is 85. However, given the poor sampling coverage, it is very likely that the real number of species inhabiting the islands is far greater. For example, the tardigrade fauna of the archipelago's second largest island, Nordaustlandet, has not been assayed until this study, in which we found five species in only three samples. Moreover, among tardigrades only two marine species (*Halobiotus arcturulius* Crisp & Kristensen, 1983 and *H. crispae* Kristensen, 1982) have been recorded so far (Smykla et al. 2011) and they are probably a small fraction of the real biodiversity of this area. It should also be borne in mind that many older records date from the time when species complexes, such as the *Macrobiotus harmsworthi harmsworthi*, *M. hufelandi*, *Hypsibius dujardini* and *H. convergens* groups, were considered single cosmopolitan species and, since the archipelago is isolated from the mainland Europe by about 700 km and from Greenland by about 450 km of sea a careful examination of the Svalbardian tardigrade fauna would probably reveal more endemic species instead the five already described (*Bryodelphax parvuspolaris* Kaczmarek, Zawierucha et al. 2012, *Isohypsibius ceciliae* Pilato & Binda, 1987, *I. coulsoni* Kaczmarek Zawierucha et al. 2012, *I. karenae* Zawierucha 2013; and *Tenuibiotus voronkovi* [Tumanov, 2007]).

The most frequently recorded species in samples collected in this study were *Testechiniscus spitsbergensis* and *M. harmsworthi harmsworthi* (each found in six samples), and *M. islandicus islandicus* (recorded in five samples). All of these species were also abundant in previous faunistic surveys conducted on Spitsbergen. Dastych (1985) reported *T. spitsbergensis* and *M. islandicus. islandicus* whereas Kaczmarek, Zawierucha et al. (2012) reported *Diphascon* (*Diphascon*) *recamieri*, *T. spitsbergensis*, *M. islandicus islandicus* and *M. harmsworthi harmsworthi* as species that occurred most frequently in their analysed samples. Thus, both earlier studies and that reported here show the dominance of these species, with *T. spitsbergensis* clearly the most prevalent.

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Re-description of the Arctic tardigrade *Tenuibiotus voronkovi* (Tumanov, 2007) (Eutardigrada; Macrobiotidea), with the first molecular data for the genus

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Abstract

Tardigrada is phylum of micrometazoans widely distributed throughout the world, because of old descriptions and insufficient morphometric data, many species currently need revision and re-description. *Tenuibiotus voronkovi* (Tumanov, 2007) is tardigrade previously only recorded from the Svalbard archipelago. This species' original description was based on two individuals with destroyed claws on the fourth pair of legs and a lack of complete morphometric data for buccal tube and claws. In this paper, we present a re-description of *T. voronkovi*, supplementing the original description using the original paratype and additional material from Svalbard: Spitsbergen, Nordaustlandet and Edgeøya. This species is characterised by two macroplacoids and a microplacoid, claws of *Tenuibiotus* type, dentate lunules under claw IV, and faint granulation on legs I–III and strong granulation on the legs IV. We include a new morphological description with microphotographs, morphometric, and molecular data (including: mitochondrial cytochrome *c* oxidase subunit I (COI), internal transcribed spacers (ITS1–5.8S rDNA–ITS2), and nuclear ribosome subunits 28S rRNA and 18S rRNA). These are the first published molecular data for the genus *Tenuibiotus* Pilato and Lisi, 2011, analysis of which indicated an affiliation of *Tenuibiotus* to the family Macrobiotidae. We found no differences in body size between individuals from different islands (Nordaustlandet and Edgeøya), but did observe variability in the eggs. After revision of the literature and the published figures, we concluded that Dastych's (1985) report of *T. willardi* (Pilato, 1976) from Svalbard, was actually *T. voronkovi*, which has the greater distribution in Svalbard, and other Arctic locations, than previously believed.

Key words: biodiversity, DNA barcodes, COI, ITS, 28S rRNA, 18S rRNA, egg variability, morphometry, Tardigrada, *Tenuibiotus willardi*, Svalbard

Introduction

The Svalbard archipelago is located in the European part of the Arctic between latitudes 74˚N and 81˚N and longitudes 10˚E and 35˚E. As a consequence of the complex terrestrial Arctic ecology and intense international attention (i.e. scientific, political and industrial), Svalbard represents an important natural laboratory for polar invertebrate studies (Coulson 2013). Currently, terrestrial invertebrate fauna of Svalbard is one of the best investigated in the High Arctic, and consists of *ca.* 1000 species (Coulson *et al.* 2014). Moreover, new invertebrate species and new records are still being discovered in this region (*e.g.* Gwiazdowicz *et al.* 2012; Kiedrowicz *et al.* 2016; Kolicka *et al.* 2016). However, some older records, due to insufficient data, need confirmation and many species require re-description (*e.g.* Zawierucha *et al.* 2013; Coulson *et al.* 2014).

One of the most diverse and widely distributed invertebrates in Svalbard are the tardigrades, also known as water bears (Zawierucha *et al.* 2013). Tardigrada is a phylum of micrometazoans inhabiting a wide variety of environments, including polar ecosystems (Węglarska 1965; Dastych 1985; Zawierucha *et al.* 2016a). Limno–terrestrial tardigrades inhabit soil, leaf–litter, mosses, lichens and hepatics (Nelson *et al.* 2015). They play an important role in food webs (Hohberg and Traunspurger 2005; Sohlenius and Boström 2008; Zawierucha *et al.* 2016b) and can reach very high densities, up to hundreds per 1 gram of dry sample (Zawierucha *et al.* 2016a). Despite being investigated on Svalbard for over a hundred years, they are still rather poorly known and the

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presence of some requires confirmation, while others need a re-description (Zawierucha *et al.* 2013). For example, *Tenuibiotus voronkovi* (Tumanov, 2007), which was relatively recently described from Spitsbergen (Kongsfjorden) (Tumanov, 2007), but based on scarce material (two incomplete specimens and five eggs).

The genus *Tenuibiotus* was erected, based on the specific claws morphology, by Pilato and Lisi (2011) for 12 species, which had previously been attributed to the genus *Macrobiotus* C.A.S. Schultze, 1834. Currently, the genus *Tenuibiotus* consists of 13 species reported from various polar and temperate climates, and geographical regions (McInnes 1994; Tumanov 2007; Degma *et al.* 2009–2015; Kaczmarek *et al.* 2015). To date, two *Tenuibiotus* species have been reported from Svalbard: *T. willardi* (Pilato, 1977) and *T. voronkovi* (Dastych 1985; Tumanov 2007). In Tumanov's (2007) original description of *T. voronkovi*, the two type specimens were damaged at the caudal end and claws IV destroyed. Thus Tumanov (2007) was unable to provide the characteristics and measurements of claws IV, nor did he measure the internal diameter of the buccal tube, ventral lamina, distance between egg processes, or make any distinction between external and internal claws measurements.

While Hebert *et al.* (2003) proposed DNA barcoding as a perfect tool for taxonomy (i.e. the determination of species is based on a single gene sequences), it cannot be used alone for tardigrade taxonomy. However, it is becoming common to combine classical alpha taxonomy with molecular techniques (*e.g.* Schill 2007; Cesari *et al.* 2009; Stec *et al.* 2015). In our study, we used a method of DNA extraction proposed by Dabert *et al.* (2008) and Mironov *et al.* (2012), which leaves the exoskeleton for reference. This method was successfully used in a recent tardigrade study (Dabert *et al.* 2014), and by using this method, we have the remaining exoskeletons for analysis and egg shells for identification (available in slide collections).

An integrative approach to taxonomy such as this provides the best method for describing the complexity and diversity of life. Thus, in our present re-description of *T. voronkovi* we have adapted this to provide the first molecular data for the species and by inference for the genus. To avoid future confusion in studies on the tardigrade biogeography we also discuss the presence of *T. willardi* in Svalbard.

Material and methods

General. Specimens of *T. voronkovi* and eggs were extracted from Nordaustlandet (three moss/soil samples), from Edgeøya (two moss samples), and Spitsbergen (one lichen sample) (for collection details see Zawierucha *et al.* 2013, 2016a). Examples of tardigrades and eggs were mounted on microscope slides in Hoyer's medium. Specimens were observed using a phase contrast (PCM) or differential interference contrast (DIC) microscope. Images were taken using ARTCAM 500, and measurements collected via Quick−Photo Camera 2.3 software. All figures were assembled in *Corel Photo-Paint 9*. For deep structures that could not be fully focused in a single photograph, a series of images were taken and then assembled manually into a single deep focus image in *Corel Photo-Paint 9*.

Species identification was based on the original paper by Tumanov (2007) and the paratype (one adult and one egg) on loan from the Denis Tumanov collection (slide number 205 (2), slide description: Spitzbergen, Konigsfjorden, Ny–Alesund (in English), island in fjord direction, moss with soil (in Russian), 15.09.1996 coll. A. Воронков).

Systematics follows Bertolani *et al.* (2014).

Morphometric analysis. All measurements are given in micrometres [μm], and structures were only measured if their orientation was suitable. Body length was measured from the anterior extremity to the end of the body, excluding the hind legs. Buccal tube length and the level of the stylet support insertion point were measured according to Pilato (1981). Buccal tube width was measured as the external and internal diameter at the level of the stylet support insertion point. Lengths of the claw branches, were measured from the base of the claw to the top of the branch. The *pt* ratio is the ratio of the length of a given structure to the length of the buccal tube expressed as a percentage (Pilato 1981). Macroplacoid length sequence is given according to Kaczmarek *et al.* (2014). Morphometric data were handled using the 'Parachela' template available from the Tardigrada Register (www.tardigrada.net/register, Michalczyk and Kaczmarek 2013).

Additional measurements of the paratype, not provided by Tumanov (2007), i.e. internal buccal width, ventral lamina length, and measurements of primary and secondary branches of external and internal claws I, II are in *italic* and **bold** in Table 1. Measurements of individuals and eggs from Nordaustlandet and Edgeøya are presented in

Table 3. Measurements of one egg from the type series, i.e. processes length and bases width, and distance between processes are presented in the complementary description below.

Molecular analysis. All specimens used for DNA analysis were collected from Nordaustlandet (12.07.2012., coll. Stephen Coulson). Total genomic DNA was extracted individually from three single adult specimens and from a single egg using the method described in Dabert *et al.* (2008) and Mironov *et al.* (2012). After extraction, exoskeletons of adults and eggs were mounted on microscope slides for further analysis. DNA was isolated using the DNeasy Blood and Tissue Kit (Qiagen GmbH, Hilden, Germany) as following the protocol in Dabert *et al.* (2008). A fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was amplified with a bcdF01 forward primer (CGATGRTTTTTTCHACWAACCAYAARGATATCGG) (Dabert *et al.* 2008), and bcdR04 reverse primer (TATAAACYTCDGGATGNCCAAAAAA) (Dabert *et al.* 2008); fragment coding for both internal transcribed spacers (ITS1–5.8S rDNA–ITS2) was amplified with ITS1_18S (AGAGGAAGTAAAAGTCGTAACAAG) and ITS2_28S (ATATGCTTAAATTCAGGGGG) primers (Navajas *et al*. 1998). A complete sequence of 18S rRNA was amplified in two overlapping fragments using 18Sfw (CTTGTCTCAAAGATTAAGCCATGCA) with rev960 (GACGGTCCAAGAATTTCAC) and fw390 (AATCAGGGTTCGATTCCGGAGA) with rev18S (TGATCCTTCCGCAGGTTCACCT) primer pairs (Dabert *et al*. 2016), respectively. D1–D3 region of the nuclear 28S rRNA was amplified with primers 28SF0001 (ACCCVCYNAATTTAAGCATAT) and 28SF440 (ACAAGTACCGTGAGGGAAAGTTG). PCRs 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 40 steps of 30 s each 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 amplicons. 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 v 3.1 kit and the ABI Prism 3130xl Genetic Analyzer (Applied Biosystems), following the manufacturer's instructions.

The identity of 28S rRNA and 18S rRNA sequences were verified using Basic Local Alignment Search Tool (BLAST; Altschul *et al*. 1990) to find the most similar taxa. To calculate molecular distances for the 28S rRNA fragments, three sequences were obtained from GenBank: *Macrobiotus furcatus* Ehrenberg, 1859 (GenBank accession number: FJ435760.1), *Paramacrobiotus richtersi* (Murray, 1911) (FJ435757.1) and *Macrobiotus pallarii* Maucci, 1954 (FJ435756.1) deposited by Guil and Giribet (2012). To calculate molecular distances for the 18S rRNA fragments, two sequences were obtained from GenBank: *Paramacrobiotus tonollii* (Ramazzotti, 1956) (DQ839605.1) and *Paramacrobiotus richtersi* (Murray, 1911) (EU038078.1) deposited by Schill and Steinbrueck (unpublished paper) and Guidetti *et al*. (2009). Sequences were processed in *BioEdit* ver. 7.2.5 (Hall 1997). Pairwise distances between nucleotide sequences were calculated using a distance model for all codon positions as implemented in MEGA 7 (Tamura *et al*. 2013).

Statistical analysis. The eutardigrade body is soft and, therefore, its length is not a reliable estimate of the body size. However, rigid, sclerified structures, such as the bucco–pharyngeal apparatus, have been shown to be well correlated with body size and are ideal for morphometry (*e.g.* Higgins 1959; Bartels *et al*. 2011). Measurements were tested between 'body length' versus 'buccal tube length' and they proved to be correlated (Spearman rank correlations, $p<0.05$, $r = 0.817$). We therefore used 'buccal tube length' as the equivalent of 'body length' to test for differences in the size of individuals collected on Edgeøya (N=13) and Nordaustlandet (N=14). The nonparametric Mann–Whitney test was used (Shapiro–Wilk test, for individuals from Nordaustlandet $p>0.05$, and from Edgeøya $p<0.05$). For each egg, three measurements of process length (height), process base diameter, and egg shell distances between processes were made. Eggs from Nordaustlandet and Edgeøya, were tested for character differences as: process height—parametric T–test (Shapiro–Wilk test, for eggs from Nordaustlandet (N=34) and from Edgeøya (N=15) $p>0.05$), process base diameter—nonparametric Mann–Whitney test (Shapiro–Wilk test, for eggs from Nordaustlandet (N=53) $p<0.05$, and from Edgeøya (N=15) $p>0.05$), and egg shell distance between processes—parametric T–test (Shapiro–Wilk test, for eggs from Nordaustlandet (N=34) and from Edgeøya (N=15) $p>0.05$). As only three eggs were suitably oriented (not broken) for the measurement of egg diameter, we decided to omit this character from statistical comparisons. In total, 27 individuals and 18 eggs were measured. These data were processed using STATISTICA 10.0 (StatSoft, Inc. 2011).

Results

Taxonomic account

Phylum: Tardigrada Doyère, 1840
Class: Eutardigrada Richters, 1926
Order: Parachela Schuster, Nelson, Grigarick & Christenberry, 1980
Superfamily: Macrobiotoidea Thulin, 1928 in Marley *et al.* 2011
Family: Macrobiotidae Thulin, 1928
Genus: *Tenuibiotus* Pilato & Lisi, 2011

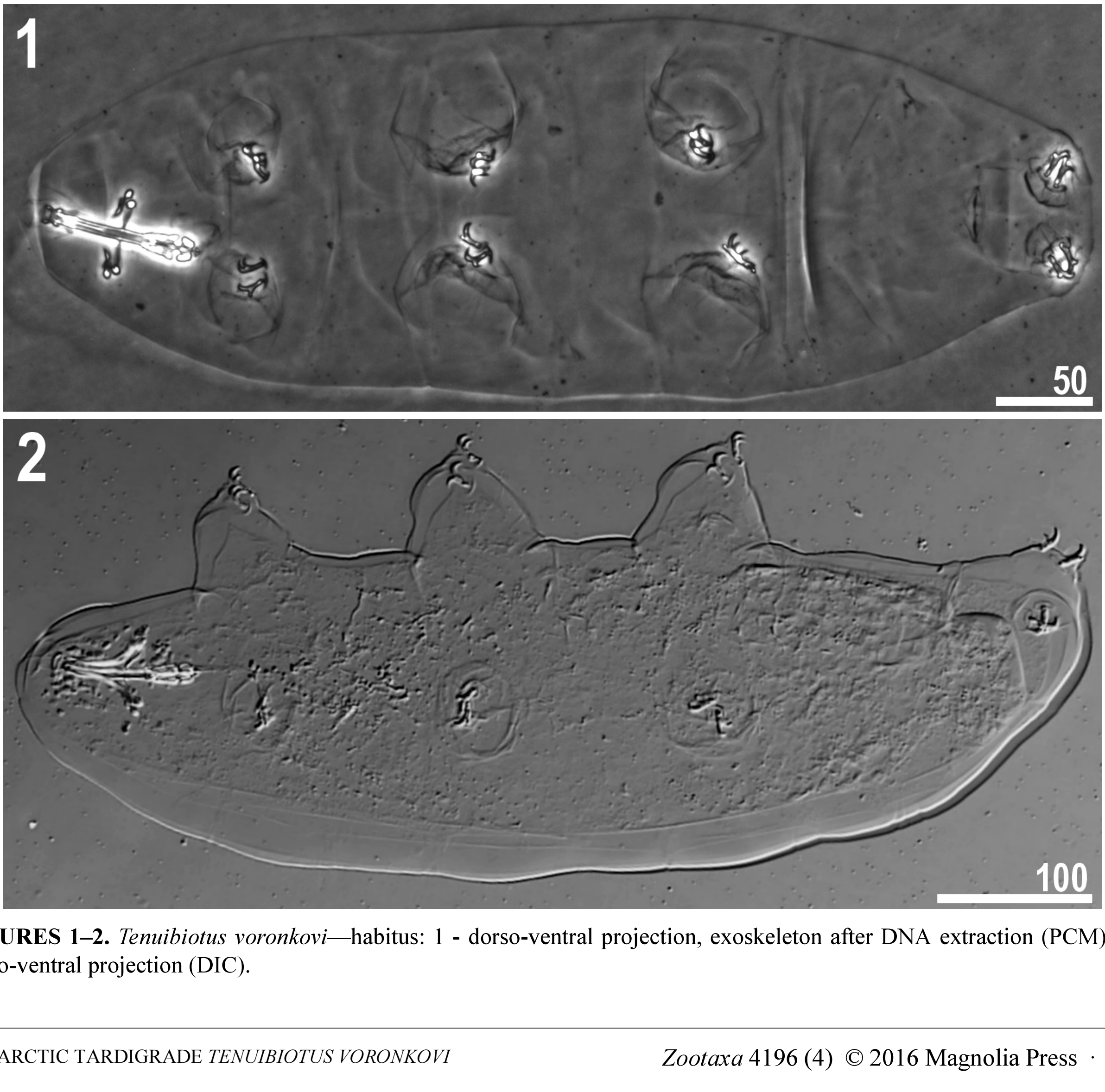
***Tenuibiotus voronkovi* (Tumanov, 2007)**
(Figs. 1–18, Tables 1–3)

Macrobiotus voronkovi in Tumanov (2007)

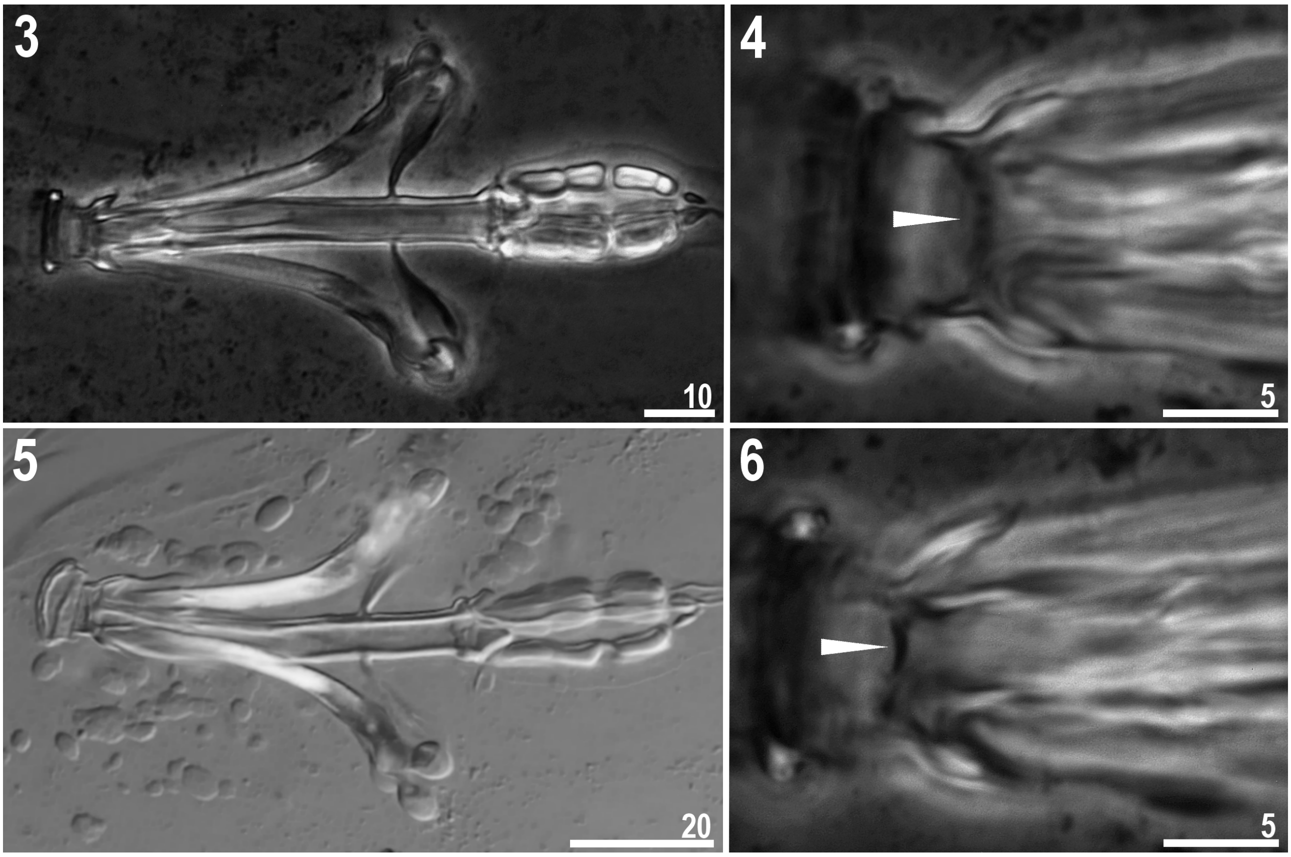
Material examined: *Type material:* Paratype from Spitsbergen, Kongsfjorden (1 adult, 1 egg).

Additional material: Spitsbergen: 1 specimen, 1 egg, Nordaustlandet: 23 specimens and 51 eggs, Edgeøya: 22 specimens and 18 eggs.

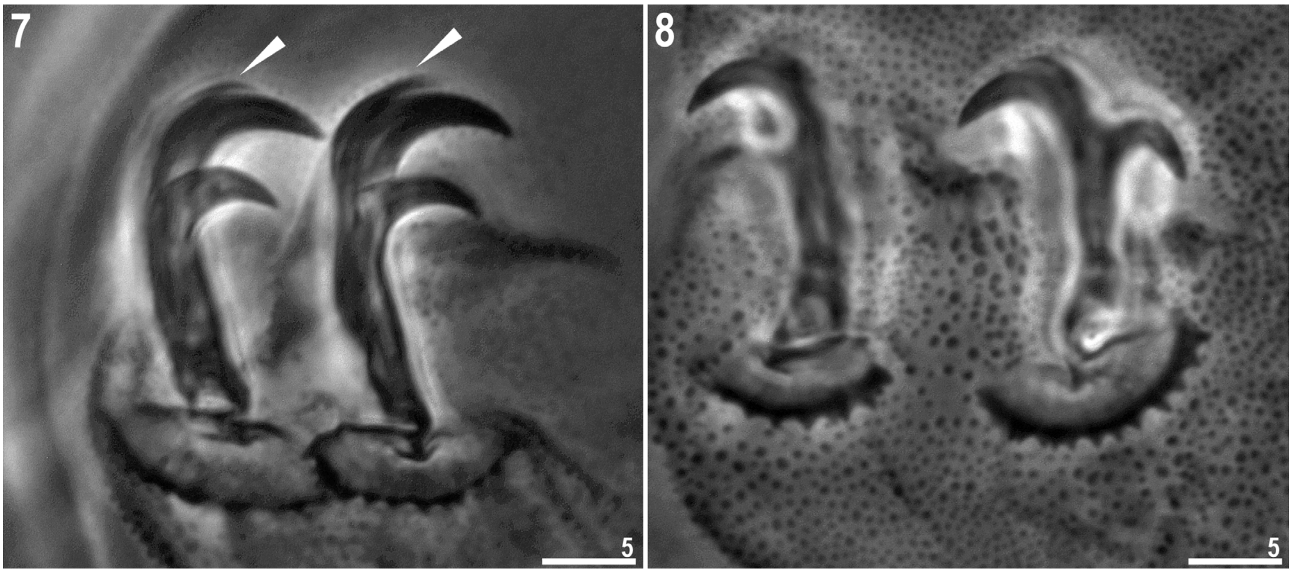
Description: Adults. Body transparent or light yellow in living specimens and transparent after fixation in Hoyer's medium (Figs 1–2). Faint granulation visible on leg I–III and obvious granulation present on legs IV (Figs 7–8). Dorsal and ventral cuticle without gibbosities, papillae, spines or sculpturing.



FIGURES 1–2. *Tenuibiotus voronkovi*—habitus: 1 - dorso-ventral projection, exoskeleton after DNA extraction (PCM); 2 - dorso-ventral projection (DIC).



FIGURES 3–6. *Tenuibiotus voronkovi*—buccal apparatus: 3—buccal apparatus, dorso-ventral projection (PCM); 4—ventral view of the buccal armature, arrowhead indicate row of teeth (PCM); 5—buccal apparatus, dorso-ventral projection (DIC); 4—dorsal view of the buccal armature, arrowhead indicate single teeth (PCM).



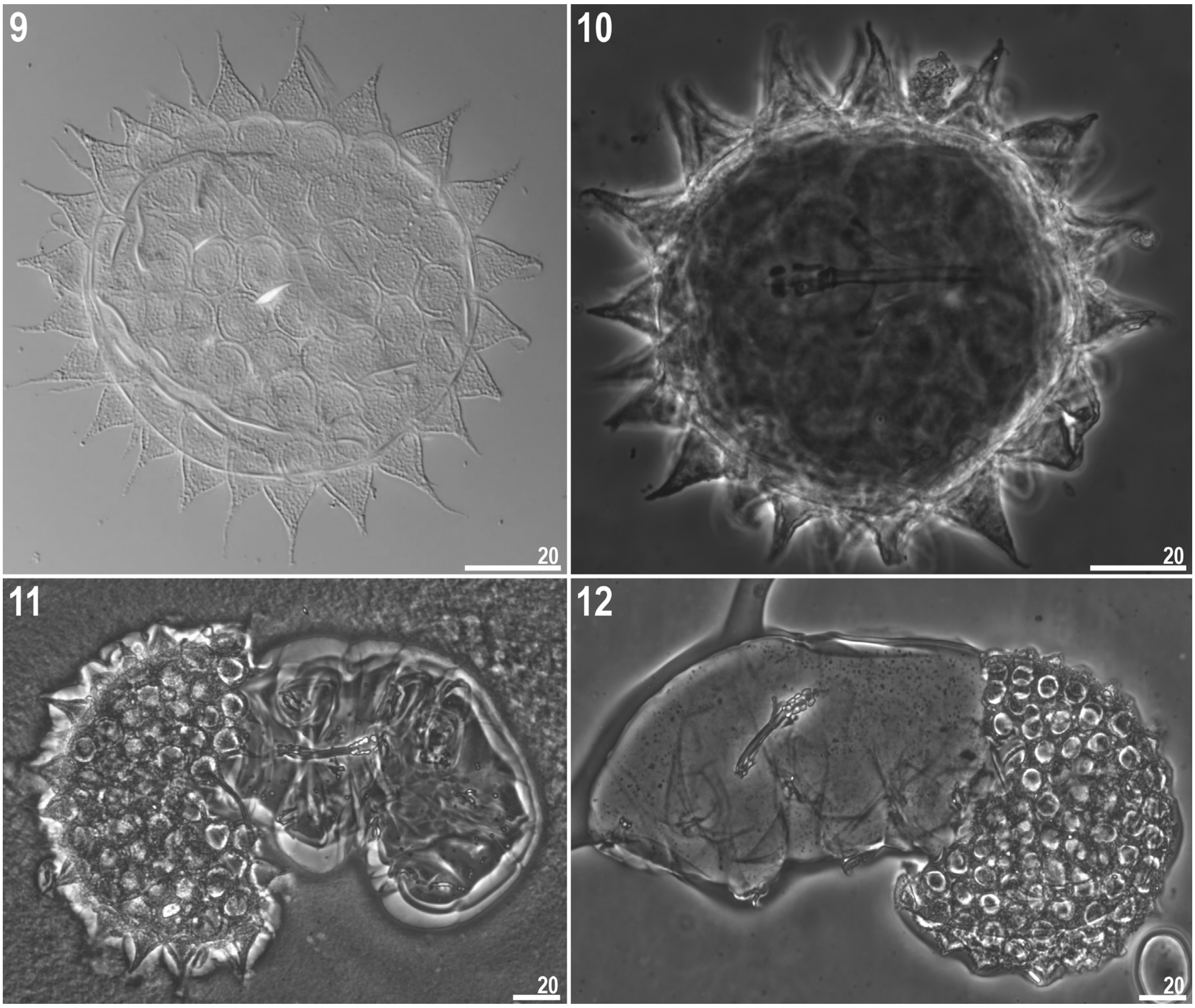
FIGURES 7–8. *Tenuibiotus voronkovi*—claws of leg IV seen in PCM: 7—arrowhead indicate accessory points; 8—dentate lunules and granulation.

Bucco–pharyngeal apparatus of the *Macrobiotus* type (Figs 3–6), with ventral lamina and poorly visible peribuccal lamellae. Mouth antero–ventral. First and second row of teeth in the oral cavity armature, absent (or not visible under PCM). Third row of teeth composed dorsally of "three transverse ridges (medial ridge arched with the convexity turned backwards and very thin lateral ridges" (Fig. 3) and ventrally of a "single medial ridge arched with the convexity turned backwards and a pair of lateral ridges fragmented into granules" Tumanov (2007) (Fig. 4). Pharyngeal bulb with apophyses, two rod–shaped macroplacoids and a triangular microplacoid. Macroplacoid length sequence 2<1. The first macroplacoid with a central constriction (Figs 3, 5).

Claws of the *Tenuibiotus* type (Figs 7–8). Primary branches with distinct accessory points (Figs 7–8). Lunules under claws on legs I–III smooth and dentate on legs IV (Figs 7–8). Other cuticular structures on legs absent.

Eggs. Spherical and ornamented, with conical processes. Processes have blunt or attenuate apices with reticulation. Egg shell between processes with poorly visible short irregular ridges.

Complementary description. Eyes are present in some individuals preserved on the slides. Claws on leg IV of the *Tenuibiotus* type (Figs 7–8). Primary branches of claw IV with distinct accessory points (Fig. 7). Less clear constriction is present prior to the caudal end of the second macroplacoid. Eggs with short or long conical processes (Figs 9–12).



FIGURES 9–12. *Tenuibiotus voronkovi*—eggs and juveniles: 9—egg midsection (DIC); 10—egg midsection with embryo (PCM); 11, 12—juveniles and eggs.

Remarks. Some of our observed live individuals were yellowish. Additional measurements of paratype (adult): internal buccal width, ventral lamina length, and measurements of primary and secondary branches of external and internal claws I, and II are given in Table 1. Supplementary measurement of egg (paratype) are: 11.2, 11.3, 10.3 for processes length (height), 13.6, 11.4, 11.4 for process base diameters, 3.2, 2.2, 1.8 for egg shell distances between processes. Supplementary measurements of all important taxonomic traits for specimens and eggs of *T. voronkovi* from Nordaustlandet and Edgeøya are presented in Tables 2 and 3.

TABLE 1. Measurements [in µm] of selected morphological structures of holotype and paratype of *T. voronkovi* from Tumanov (2007). New measurements not included in the original description are **bolded** and *italicized*.

CHARACTER	µm	*pt*	µm	*pt*
	Holotype		Paratype	
Body length	532	*876*		
Buccal tube length	60.7	-	63.6	-
Stylet support insertion point		*74.4*		*76.7*
Buccal tube external width	6.7	*11.0*	7.4	*11.6*
Buccal tube internal width			***5.1***	*8.0*
Ventral lamina length			***46.4***	*73.0*
Macroplacoid 1	14.1	*23.2*	16.3	*25.6*
Macroplacoid 2	8.1	*13.3*	14.1	*22.2*
Microplacoid	2.2	*3.6*	3.3	*5.2*
Macroplacoid row	22.2	*36.6*	28.1	*44.2*
Placoid row	25.2	*41.5*	32.2	*50.6*
Claw 1 lengths				
External primary branch			***17.1***	*26.9*
External secondary branch			***12.3***	*19.3*
Internal primary branch			***16.9***	*26.6*
Internal secondary branch			***12.3***	*19.3*
Claw 2 lengths				
External primary branch			***18.2***	*28.6*
External secondary branch			***14.6***	*23.0*
Internal primary branch			***18.0***	*28.3*
Internal secondary branch			***13.5***	*21.2*

We have complemented the original description of *T. voronkovi* with claw IV description, variability of egg processes (Figs 9–12, 13–18) and other morphometric traits of adults (internal width of buccal tube, ventral lamina, claws II and IV). Our measurements increase the morphometric range (min, max) in all measured traits of *T. voronkovi*.

No significant differences in body length were found between individuals from Edgeøya and Nordaustlandet (Mann–Whitney test, $z = -0.582$, $p = 0,560$). We therefore present the morphometric data in a single table (Table 1). Statistical analysis of morphometric traits of the eggs collected from Edgeøya and Nordaustlandet showed no significant differences in process height between the eggs (T–test, $t = -0.061$, $p < 0.951$), but there was a statistical difference in process base diameter (Mann–Whitney test, $z = -2.61$, $p = 0.008$) and the egg shell distances between processes (T–test, $t = -5.44$, $p < 0.005$). However, as only five eggs were measured from Edgeøya, and the raw measurements from both islands overlapped, we decided to put all eggs measurements in a single table (Table 3).

Type depositories: The holotype (Slide No. 205 (1)) is preserved at the Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia. The paratype (adult and egg) has been given to the tardigrade collection at the Department of Animal Taxonomy and Ecology, Institute of Environmental Biology, Adam Mickiewicz University in Poznań (Slide No. 205 (2)). Additional material from Spitsbergen (slide code: 102.8/1), Nordaustlandet (slide codes: 103.1/1, 2, 4, 5, 7, 9, 103.2/1, 3, 5, 6, 7, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42), and Edgeøya (slide codes: 104/2, 113.1/2, 3, 4, 118.1/2, 4, 5, 7, 8, 9, 10) are preserved in the tardigrade collection at the Department of Animal Taxonomy and Ecology, Institute of Environmental Biology, Adam Mickiewicz University in Poznań.

TABLE 2. Measurements [in µm] of selected morphological structures of individuals of *T. voronkovi* mounted in Hoyer's medium (N—number of specimens/structures measured, RANGE refers to the smallest and the largest structure among all measured specimens; SD—standard deviation).

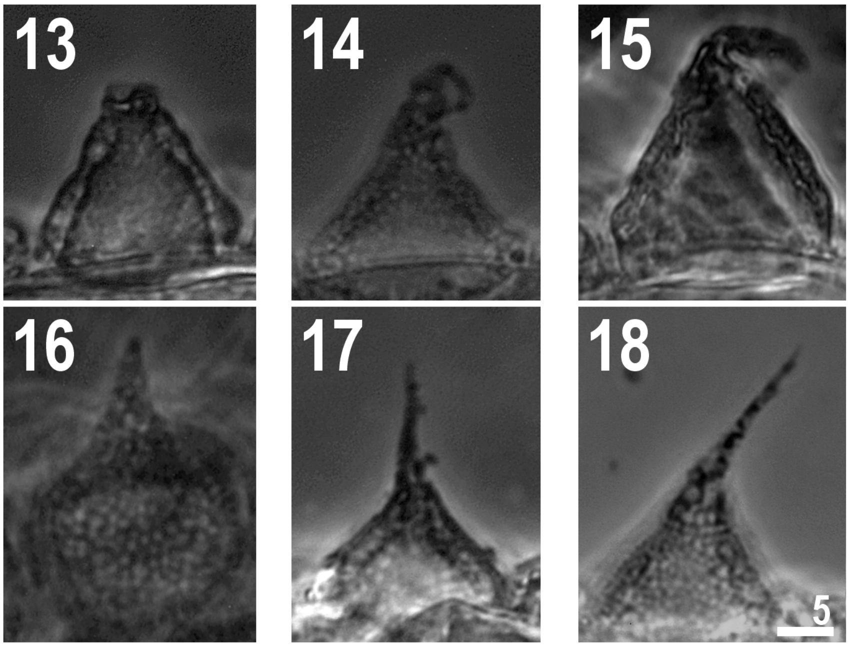
CHARACTER	N	RANGE		MEAN		SD	
		µm	*pt*	µm	*pt*	µm	*pt*
Body length	23	407–769	871–1534	646	1241	111	131
Buccopharyngeal tube							
Buccal tube length	27	33.5–56.5	-	50.5	–	6.0	–
Stylet support insertion point	27	24.2–42.6	72.2–77.1	37.9	75.0	4.6	1.4
Buccal tube external width	27	4.5–7.2	11.0–14.7	6.3	12.6	0.7	0.9
Buccal tube internal width	27	2.2–4.7	5.6–9.2	3.6	7.1	0.7	0.9
Ventral lamina length	19	18.0–32.7	50.2–64.1	27.4	56.0	3.9	3.9
Placoid lengths							
Macroplacoid 1	27	8.8–17.6	21.9–33.2	14.0	27.7	2.6	3.1
Macroplacoid 2	27	4.9–9.2	12.5–18.7	7.9	15.6	1.6	1.7
Microplacoid	27	1.5–3.6	4.2–8.2	3.0	5.8	0.7	1.0
Macroplacoid row	27	14.3–27.7	35.7–53.5	23.9	47.0	4.3	4.7
Placoid row	26	17.1–32.8	42.6–63.5	27.9	54.8	5.2	6.0
Claw 1 lengths							
External primary branch	13	12.2–17.5	25.3–36.1	15.5	29.9	2.0	3.1
External secondary branch	13	8.5–12.5	18.2–26.1	11.2	21.6	1.4	2.3
Internal primary branch	14	11.5–17.0	25.4–33.4	15.0	29.2	2.5	2.6
Internal secondary branch	14	9.0–12.4	17.1–26.0	11.2	22.0	1.4	2.1
Claw 2 lengths							
External primary branch	23	9.5–18.5	23.6–34.8	15.6	30.9	2.6	2.7
External secondary branch	23	8.6–16.9	21.4–32.7	12.7	25.2	2.0	2.4
Internal primary branch	19	12.5–18.5	26.9–36.4	15.9	31.0	2.0	2.7
Internal secondary branch	19	9.2–14.2	19.7–29.2	12.4	24.3	1.7	2.4
Claw 3 lengths							
External primary branch	21	11.8–20.0	26.9–41.2	16.8	32.6	3.1	3.4
External secondary branch	20	9.0–14.2	19.2–27.3	12.7	24.8	1.9	2.2
Internal primary branch	23	12.4–21.0	26.5–38.5	16.3	31.5	2.3	3.5
Internal secondary branch	23	9.1–17.0	19.4–37.5	13.1	25.2	2.9	3.7
Claw 4 lengths							
Anterior primary branch	21	9.7–21.7	28.8–41.3	17.1	34.1	3.3	3.4
Anterior secondary branch	21	7.6–15.9	21.1–31.3	12.9	25.8	2.3	2.6
Posterior primary branch	19	7.1–20.0	21.2–40.9	16.4	33.3	3.3	4.2
Posterior secondary branch	19	8.5–14.9	20.1–30.7	12.5	25.6	2.3	3.4

Sequences data

All sequences for *T. voronkovi* obtained in this study were unique and distinct from all other sequences deposited in GenBank. The 28S rRNA alignment comprised 1004–bp with two haplotypes (two sequences from two adults) that differed in one nucleotide position (the uncorrected p-distances = 0.2%) (sequences deposited in GenBank under accession numbers: KX810049–50 respectively). The 18S rRNA comprised 1702–bp (one adult, sequence deposited in GenBank under accession number: KX810045). The COI alignment comprised 432–bp with three haplotypes (three sequences from two adult and one egg) that differed in eight nucleotide positions (the uncorrected p-distances varied from 0.9% to 1.9%) (sequences deposited in GenBank under accession numbers: KX810042–44 respectively). The ITS2 alignment comprised 1044-bp with three haplotypes (three sequences from three adult) that differed in six nucleotide positions (the uncorrected p-distances varied from 0.1% to 0.6%) (sequences deposited in GenBank under accession numbers: KX810046–48 respectively).

TABLE 3. Measurements [in µm] of selected morphological structures of 18 eggs of *T. voronkovi* from Nordaustlandet (13 eggs) and Edgeøya (5 eggs) mounted in Hoyer's medium (N—number of eggs/structures measured, RANGE refers to the smallest and the largest structure among all measured traits; SD—standard deviation).

CHARACTER	N	RANGE	MEAN	SD
Diameter of egg without processes	3	86.6–123.2	104.0	18.4
Diameter of egg with processes	2	137.0–182.0	159.5	31.8
Process height	49	12.3–37.3	21.8	5.7
Process base width	53	9.7–25.4	15.6	3.5
Process base/height ratio	49	44%–124%	75%	18%
Distance between processes	49	0.6–4.2	1.8	0.8
Number of processes on the egg circumference	2	17–22	19.5	3.5



FIGURES 13–18. *Tenuibiotus voronkovi*—egg process details, different shape of processes seen in PCM.

The low genetic distance between the COI and ITS–2 haplotypes indicate that, at the molecular level, all individuals and egg should be classified as a single species.

Nucleotide blast searches using *T. voronkovi* 28S rRNA and 18S rRNA sequences, suggested that this taxon is most closely related to *Macrobiotus furcatus* Ehrenberg, 1859*, Paramacrobiotus richtersi* (Murray, 1911)*, M. pallarii* Maucci, 1954 (28S rRNA), *P. tonollii* (Ramazzotti, 1956) and *P. richtersi* (Murray, 1911) (18S rRNA). These results placed the genus *Tenuibiotus* in the family Macrobiotidae. The analysis of 28S rRNA involved four nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 1052 positions in the final dataset. The p-distances for 28S rRNA between *T. voronkovi* (GenBank accession number: KX810049–50), *M. furcatus* (FJ435760.1), *P. richtersi* (FJ435757.1) and *M. pallarii* (FJ435756.1) varied from 6.7% to 7%. The analysis of 18S rRNA involved three nucleotide sequences. As before, all positions containing gaps and missing data were eliminated, providing a total of 1699 positions in the final dataset. The p-distances for 18S rRNA between *T. voronkovi* (GenBank accession number: KX810045), *P. tonollii* (DQ839605.1) and *P. richtersii* (EU038078.1) varied from 1.3% to 2.1%.

Discussion

In the original description of *T. willardi*, Pilato (1977) reported three macroplacoids in the pharynx, but also added "some specimens have only two macroplacoids and in that case the first placoid has a very evident constriction". Photomicrographs of the original *T. willardi* (kindly sent us by Prof. Giovanni Pilato) clearly indicate three macroplacoids in this species, with the first and second macroplacoid relatively close and the third distinctly further from the second. There was no mention of dentate lunules either in the text nor indicated in the figure (Pilato 1977; Fig. 1(w) and (t)), though the cuticle is described as "clearly granulated, especially in the posterior legs, and forms a true tubercule dorsal to each claw" (Pilato 1977; Fig. 1(w)). However, when Pilato and Lisi (2011) erected the genus *Tenubiotus*, they emended the description with the information that lunules on the fourth pair of legs are dentate.

Before *T. voronkovi* had been recognised, Dastych (1985) reported *T. willardi* as a new record for the Svalbard archipelago. In the short diagnosis he noted the, "specimens and eggs developed typically (Pilato, 1977; Dastych, 1979)", but also mentioned that his specimens had only two macroplacoids (Dastych 1985). From the figures provided with this brief description it is possible to see the granulation and obvious dentate lunules of claw IV (Dastych 1985; plate VIII, Fig. f). This evidence, in particular the presence of two macroplacoids, suggests that the specimens observed by Dastych (1985) from Svalbard would now be attributed to *T. voronkovi*, instead of *T. willardi*. However, analysis of the slide collection from Dastych's (1985) study would be required for confirmation of this hypothesis. We would also observe that during our studies we have examined more than 300 samples from the Svalbard archipelago and have only found *T. voronkovi* (see: Kaczmarek et al. 2012, Zawierucha 2013, Zawierucha et al. 2013, 2015, 2016a,b), which suggests *T. willardi* is not present.

It appears similar misidentifications have been detected in material described as *T. willardi* from Greenland (Grøngaard *et al.* 1990), where two examples were found, one with three and the other with two macroplacoids, but both attributed to *T. willardi* (Prof. R.M. Kristensen pers. com.). Specimens attributed to *T. willardi* were also reported from Canada (Igloolik) (Jørgensen and Kristensen 1989), however, in the light of our information these do not belong to either *T. willardi* or *T. voronkovi* (Prof. R.M. Kristensen pers. com.).

Egg shell ornamentation has provided one of the key taxonomic characters for the determination of many eutardigrade species. Indeed, for some, a definitive species identification is not possible if the eggs are absent (see*:* Bertolani and Rebecchi 1993; Kaczmarek *et al.* 2011). In our study, we found some distinct variation in the egg processes of *T. voronkovi* (Figs. 9–12, 13–18), which need to be accounted for if this character is to be used sensibly as a taxonomic character. A large differences in the size of processes observed in *T. voronkovi* may be affected by external factors damaging the upper parts of the processes (Figs. 11–12). However, we also found the eggs of *T. voronkovi* (egg with embryo, Fig. 10) with clearly differently shaped processes (wider bases and more elongated upper parts), which does indicate this species has eggs with variable processes (Figs. 9–18). Further studies, to explore how different factors might influencing egg shell morphology, are required before we can reliably explain these observations. Variations in the egg shell morphology have also been detected in other macrobiotids were a high number of eggs were analysed (Mapalo *et al.* 2016). Thus, although embryonate eggs may help to link adults and eggs, the description of new macrobiotids indicating slight differences in eggs morphology should be considered carefully.

With the advantage of having collected specimens of *T. voronkovi* from different habitats and two islands, located on a latitudinal gradient, it was possible to explore the potential influence that latitude might have on body size (*e.g.* Blackburn *et al.* 1999). While we suspected that location of the islands might influence body length in *T. voronkovi* specimens, our results showed a statistical lack of difference. This may a have been affected by small sample size (N=27), or the relatively small differences in latitude.

The superfamily Macrobiotoidea Thulin, 1928, erected by Marley *et al.* (2011), is one of the most species rich within the phylum Tardigrada (Degma *et al.* 2016) and currently consists of 19 genera grouped into two families: Macrobiotidae Thulin, 1928 and Murrayidae Guidetti, Rebecchi & Bertolani, 2000. The morphological and molecular data presented by Guil *et al.* (2013) confirmed the monophyletic status of Macrobiotoidea. However, molecular data for most macrobiotids are still scarce, and at generic level there is still evidence of polyphyly (*e.g.* Vecchi *et al.* 2016). In this study, we have shown that despite the limited number of morphological differences (mainly in claw structure) between *Tenuibiotus* and the macrobiotid genera *Macrobiotus* and *Paramacrobiotus*, at the molecular level differences in 28S rRNA (6.7% to 7%) and 18S rRNA (1.3% to 2.1%) indicate the generic distinction of *Tenuibiotus* and association within the family Macrobiotidae.

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ORIGINAL ARTICLE

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Diversity and distribution of Tardigrada in Arctic cryoconite holes

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ABSTRACT

Despite the fact that glaciers and ice sheets have been monitored for more than a century, knowledge on the glacial biota remains poor. Cryoconite holes are water-filled reservoirs on a glacier's surface and one of the most extreme ecosystems for micro-invertebrates. Tardigrada, also known as water bears, are a common inhabitant of cryoconite holes. In this paper we present novel data on the morphology, diversity, distribution and role in food web of tardigrades on Arctic glaciers. From 33 sampled cryoconite holes of 6 glaciers on Spitsbergen, in 25 tardigrades were found and identified. Five taxa of Tardigrada (Eutardigrada) were found in the samples, they are: Hypsibius dujardini*,* Hypsibius *sp. A,* Isohypsibius *sp. A.,* Pilatobius recamieri*, and one species of Ramazzottiidae.* H. dujardini *and* P. recamieri *were previously known from tundra in the Svalbard archipelago. Despite the number of studies on Arctic tundra ecosystems,* Hypsibius *sp. A, one species of Ramazzottiidae and* Isohypsibius *sp. A are known only from cryoconite holes. Tardigrade found in this study do not falsify the hypothesis that glaciers and ice sheets are a viable biome (characteristic for biome organisms assemblages - tardigrades). Diagnosis of* Hypsibius *sp. A,* Isohypsibius *sp. A, and species of Ramazzottiidae with discussion on the status of taxa, is provided. To check what analytes are associated with the presence of tardigrades in High Arctic glacier chemical analyses were carried out on samples taken from the Buchan Glacier. pH values and the chemical composition of anions and cations from cryoconite holes water from the Buchan Glacier are also presented. The current study on the Spitsbergen glaciers clearly indicates that tardigrade species richness in cryoconite holes is lower than tardigrade species richness in Arctic tundra ecosystems, but consists of unique cryoconite hole species. As cryoconite tardigrades may feed on bacteria as well as algae, they are primary consumers and grazers - secondary consumers of the decomposer food chain in this extreme ecosystem.*

Key words: Buchanbreen; Hansbreen; glacial biome; Svalbard; Tardigrada; unique species.

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INTRODUCTION

The most recent Intergovernmental Panel on Climate Change assessment (Vaughan *et al.*, 2013) emphasized the importance of glaciers in terms of the Earth's ecosystem and underlined their containing a significant amount (70%) of the Earth's water in the frozen state (ACIA, 2006). Besides, glaciers also constitute an important habitat for microorganisms (Porazińska *et al*., 2004; Hodson *et al.*, 2008; Edwards *et al.*, 2011; Kaczmarek *et al.*, 2016; Zawierucha *et al.*, 2015a), which is why glaciers and ice sheets can be treated as a distinct glacial biome where cryoconite holes make up small ecosystems (Anesio and Laybourn-Parry, 2012). Cryoconite holes are water-filled reservoirs that occur on glacier surfaces throughout the world. The development and functioning of these habitats is possible because of the decrease in albedo on the surfaces of mineral and organic dust-covered glaciers, the presence of cryophilic microorganisms and the existence of simple trophic webs (McIntyre, 1984; Takeuchi *et al.*, 2001; Hodson *et al.*, 2008; Zawierucha *et al.*, 2015a; Cook *et al.*, 2016b). Cryoconite holes are still poorly known extreme habitats, therefore the interest in them is increasing, especially in biological sciences (Dabert *et al*., 2015; Bagshaw *et al*., 2016a, 2016b; Cook *et al*., 2016a, 2016b; Kaczmarek *et al*., 2016). Svalbard archipelago is the most glaciated region of the European Arctic (Hagen, 1993). The history of studies the high Arctic Svalbard archipelago fauna starts at beginning of the twentieth century, and, to date, ca. 1000 limnoterrestrial species have been identified from this area, including new species for science (Zawierucha, 2013; Coulson *et al*., 2014; Kiedrowicz *et al*., 2016). Surprisingly, faunistic studies on glacial fauna in this region are limited (Dastych, 1985; De Smet and Van Rompu, 1994; Zawierucha *et al*., 2015a).

One of the taxa known to inhabit glaciers in the Arctic, Antarctic, Himalayas and Alps are tardigrades, which are well adapted to survive extreme conditions, including glacial environments (Dastych *et al*., 2003; Zawierucha *et* *al*., 2015a). The phylum, Tardigrada, presently consists of over 1200 species (Degma *et al*., 2011; Vicente and Bertolani, 2013) that inhabit terrestrial (soil, plants, leaf litter) and aquatic (freshwater and marine sand, mud and plants) environments across the globe (Ramazzotti and Maucci, 1983; Nelson *et al*., 2015). The understanding of the diversity and distribution of glacial tardigrades is limited (Zawierucha *et al*., 2015a). So far, research on Svalbard has focused primarily on limno-terrestrial tardigrades (Węglarska, 1965; Dastych, 1985) rather than on the typically aquatic ones (De Smet *et al*., 1987). Thus, till now, just four tardigrade species have been found from cryoconite holes on Svalbard (Dastych, 1985; De Smet and Van Rompu, 1994).

A biome is a large independent category of ecosystems with characteristic animal and plant assemblages adapted to the general conditions of the region (Purves, 2007). A glacial biome is typified by truncated food webs and distinctive biogeographical structures (landscape features). This biome encompasses habitats with low temperatures, such as the ice surface, subglacial and englacial environments, and snow (Anesio and Laybourn-Parry, 2012). Unexpectedly, data on the animal assemblages in a glacial biome are extremely limited (Zawierucha *et al*., 2015a).

In this paper, the diversity and distribution of tardigrades inhabiting glaciers in Spitsbergen, with discussion on the food web and the glacial biome hypothesis, is presented.

METHODS

Study area

The Svalbard archipelago (Fig. 1) is situated at 74-81°N and 10-30°E in the European High Arctic. This region lies at the boundary between the Greenland Sea and the Barents Sea. During the last few hundred thousand years, Svalbard has been covered by extensive ice sheets several times (Lonne and Lysa, 2005). During the last ice age, almost the entire archipelago was covered by ice. After the glacial retreat, a landscape without soil or veg-

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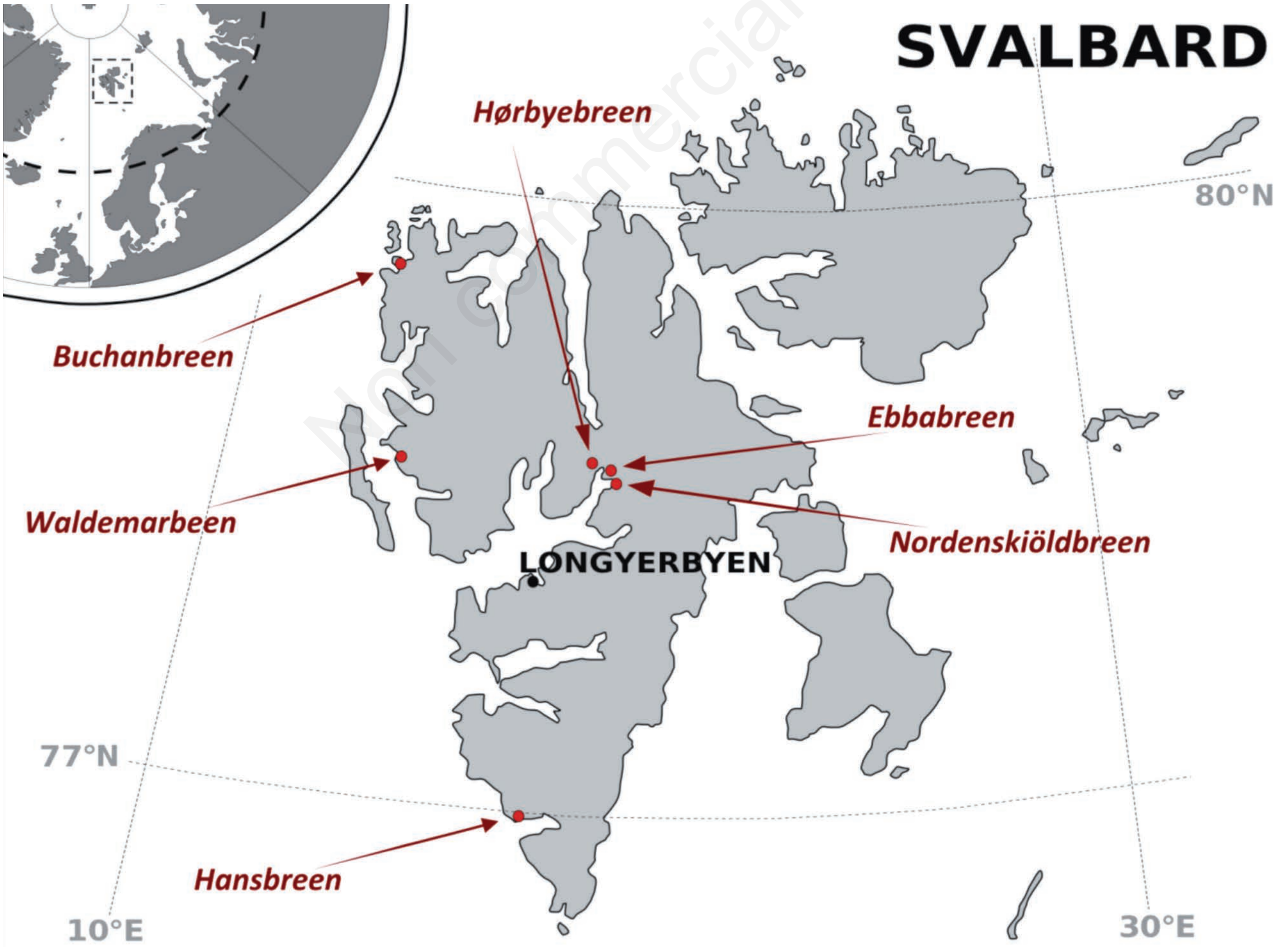

Fig. 1. Svalbard archipelago with sampled glaciers.

etation was left behind (Mangerud and Svendsen, 1992; Hjelle, 1993).

Presently, glaciers and ice caps cover 59% (36.6 km^2) of the 61.7 km^2 area of the Svalbard archipelago (Hagen *et al*., 2003). Rugged mountains and coastal lowlands are typical for this geologically young region where the present summer landscape is dominated by running water and mass wasting (Owczarek *et al*., 2014). Svalbard has a treeless landscape, composed of tundra ecosystems that are simple and fragile based on the harsh climate and low energy budget (Jonsdottir, 2005). The increases in temperature and changes in precipitation influence both the biotic and abiotic components of fluvial systems (Callaghan and Tweedie, 2011; Owczarek *et al*., 2014). Małecki *et al*. (2013) stated that climatic conditions on Svalbard are relatively mild compared to other high Arctic sites - the mean temperature of the summer period (June-August) at Svalbard Airport (Longyearbyen) is 4.9°C (1981-2010), while during the coldest month (February), mean temperatures of -13.5°C have been recorded (based on data from the Norwegian Meteorological Institute). As a result of the West Spitsbergen Current, the west part of the archipelago is warmer and more humid than other areas of similar latitude (Głowacki, 2007).

Glaciers

All samples were taken from Spitsbergen, which is the largest island of the Svalbard archipelago (Figs. 1, 2). The Hans Glacier is located in the Hornsund Fjord, where, because of the West Spitsbergen Current, local oceanic climate has more precipitation and higher air temperatures then in the area of Isfjord and Dickson Land (Głowacki, 2007).

The Dickson Land glaciers, like the Ebba Glacier, Hørbye Glacier and Nordenskiöld Glacier, are situated in the central part of Spitsbergen, in Billefjorden (Petuniabukta). This region is characterized by an inner-fjord climate type, meaning arid conditions and higher annual thermal contrasts (Rachlewicz and Styszyńska, 2007; Rachlewicz, 2009). Local glaciers may be therefore more exposed to high temperatures and less precipitation, including the coast of the Spitsbergen island, between the Oscar II Land and the Prins Karls Forland island. Compared to the central part of Spitsbergen, precipitation is twice as high. Mean air temperature is slightly higher (by 0.1°C) than at Svalbard Airport and significantly lower (by 1.1°C) than at Hornsund (Przybylak and Araźny, 2005).

The Waldemar Glacier is situated in the northern part of the Oscar II Land, Kaffiøyra, northwestern Spitsbergen (Sobota, 2009). The mean summer air temperature (1975-2010) is 4.9°C and the average sum of precipitation (1975-2010) is 46.5 mm (Przybylak *et al*., 2011).

The Buchan Glacier is located at the northern site of the Magdalenefjorden. Based on the air temperature distribution maps provided by Przybylak *et al.* (2014), the annual air temperature in Magdalenefjorden is lower by approximately 1.5°C than at Svalbard Airport.

Sample collection, preservation and specimen determination

Cryoconite material was collected from six glaciers on Spitsbergen: 10 samples from Hans Glacier (July 2014), three samples from Ebba Glacier (August 2012), five from Hørbye Glacier (August 2013, July and August, 2014), ten from Nordenskiöld Glacier (July and August, 2014), three samples from Buchan Glacier (28 July, 2013), and two samples from Waldemar Glacier (4th August, 2013) (Figs. 1, 2). Samples from Hans Glacier were collected with disposable plastic Pasteur pipettes and transferred to 15 cm^3 plastic test tubes. Samples were collected in such a manner that the sediment from the bottom of the cryoconite holes was taken. Following collection, the samples were preserved using 96% ethylene alcohol. From each sample, 1 cm^3 of sediment was scanned for tardigrades with a stereomicroscope.

Cryoconite holes on three glaciers around Billefjorden were collected employing a plastic siphon, sucking all the cryoconite sediment within a 4.5 cm plastic ring (area 15 cm^2) from a second plastic siphon into a 0.5 dm^{-3} polyethylene bottle. All sampling equipment was washed with meltwater of the sampling site prior to the sampling. In the laboratory, the samples were sedimented, the supernatant was removed and a subsample was counted in a counting chamber under stereomicroscope. Cryoconite holes on the Buchan Glacier and Waldemar Glacier were collected by hand and placed in plastic boxes, then fixed with formaldehyde. The number of samples (N), GPS coordinates, and elevation above sea level (ASL) are presented in Tabs. 1 to 3. Reference system of the GPS coordinates is WGS 84.

Densities of the tardigrades from Hans Glacier and glaciers in Billefjorden are presented in Zawierucha *et al.* (2016b). However, the number of analysed samples from Billefjorden in the present study is different. Some of the samples that were analysed in terms of abundance are currently not available for taxonomic and faunistic analysis. Because of losing a piece of material during sampling, quantitative data from cryoconite holes from Buchan and Waldemar Glaciers were omitted in Zawierucha *et al*., (2016b). In total, we analysed 33 samples in terms of tardigrade diversity (instead of the 48 mentioned in Zawierucha *et al*., 2016b).

All tardigrades and their exuvia with eggs were mounted on microscopic slides in Hoyer's medium and polyvinyl alcohol, and then examined with a phase contrast microscope (PCM), Olympus, model BX53. The species were identified using the key to the World Tardigrada (Ramazzotti and Maucci, 1983) and compared

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with other original papers (Morgan, 1975; Dastych, 1985, 1988; Miller *et al.,* 2005). Macroplacoid length sequences are given according to Kaczmarek *et al*. (2014). Tardigrade taxonomy is presented according to Bertolani *et al*. (2014). Trophic groups were established based on the scheme presented in Guil and Sanchez-Moreno (2013). The abbreviation ZMUC in the text refers to the Museum of Natural History, Copenhagen, Denmark.

Chemical analysis

Chemical analyses were made strictly for samples taken from the Buchan Glacier, to check what analytes are associated with the presence of tardigrades in High Arctic glacier and to compare our results with data from Antarctic presented in Porazińska *et al*. (2004). Water samples were collected in 0.12 dm^{-3} bottles and stored at 3°C in

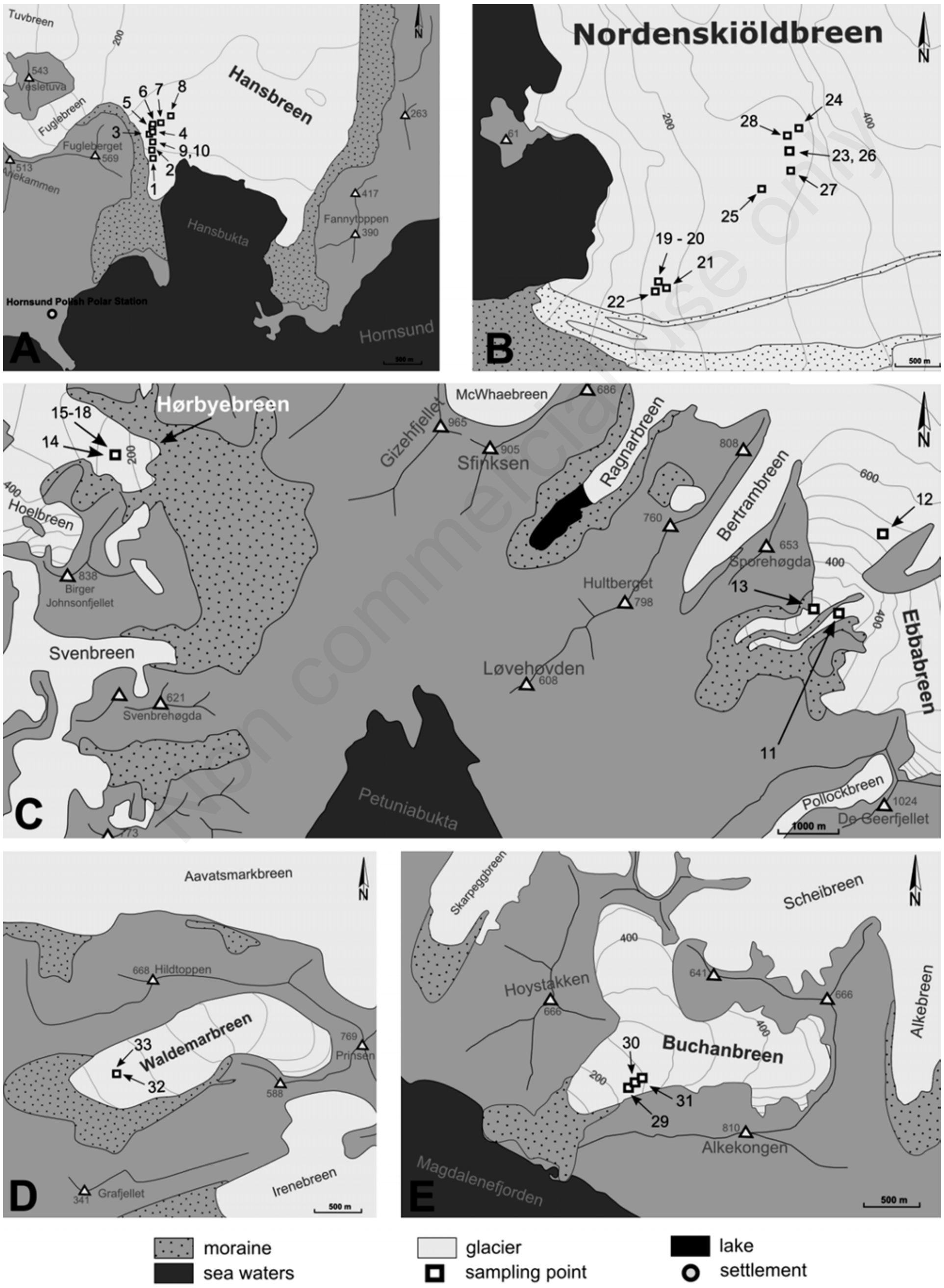

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Fig. 2. Glaciers with sampled points. A, Hans Glacier; B, Nordenskiöld Glacier; C, Ebba Glacier, Hørbye Glacier; D, Waldemar Glacier; E, Buchan Glacier.

Tab. 1. List of cryoconite samples from Hans Glacier with GPS coordinates, elevation, number of individuals determined to species/genus level. All samples were collected on 30.07.2014. For sampling sites localities see Figs. 1 and 2.

N	GPS	Elevation (m)	*P. recamieri*	*Hypsibius* sp. A
1	N77° 01.204’ E15° 35.740’	23	2	2
2	N77° 01.247’ E15° 35.707’	70	1	
3	N77° 01.349’ E15° 35.647’	92	1	
4	N77° 01.358’ E15° 35.641’	87	23	12
5	N77° 01.378’ E15° 35.702’	86		
6	N77° 01.377’ E15° 35.707’	85	153	14
7	N77° 01.417’ E15° 35.956’	91	67	2
8	N77° 01.464’ E15° 36.254’	101	22	6
9	N77° 01.302’ E15° 35.716’	81	1	2
10	N77° 01.302’ E15° 35.716’	81	8	

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Tab. 2. List of cryoconite samples from Ebba Glacier (11-13), Hørbye Glacier (14-18), Nordenskiöld Glacier (19-28) with date of collection, GPS coordinates, elevation, number of individuals determined to species/genus level. For sampling sites localities see Figs. 1 and 2. The table presents faunistic analysis of randomly selected for slides tardigrades.

N	Date	GPS	Elevation (m)	*P. recamieri*	*H. dujardini*	*Hypsibius* sp. A	*Isohypsibius* sp. A	Undetermined species of Ramazzottiidae
11	8 August 2014	N78°43.823’ E16°52.458’	306			1		
12	8 August 2014	N78°44.499’ E16°54.630’	525	2	1	2		
13	8 August 2014	N78°43.866’ E16°51.268’	286	4				2
14	21 July 2014	N78°45.421’ E16°19.516’	230	2	3	1	1	
15	17 August 2014	N78°45.413’ E16°19.487’	230	1	1			
16	17 August 2014	N78°45.412’ E16°19.498‘	230	1				
17	14 August 2013	N78°45.414’ E16°19.473’	190	1	3			
18	14 August 2013	N78°45.413’ E16°19.459’	203	2	6			
19	31 July 2014	N78°38.338’ E16°59.357’	150	2				
20	22 August 2014	N78°38.339’ E16°59.358’	150	1		1		
21	16 July 2014	N78°38.325’ E16°59.484’	150	2				
22	31 July 2014	N78°38.288’ E16°59.450’	150	2	2			
23	13 July 2014	N78°39.103’ E17°03.306’	200					
24	13 July 2014	N78°39.250’ E17°03.744’	200					
25	13 July 2014	N78°38.892’ E17°02.521’	200					
26	16 July 2014	N78°39.103’ E17°03.393’	200					
27	22 July 2014	N78°39’ E17°03.4’	200					
28	22 July 2014	N78°39.2’ E17°03.4’	200					

Tab. 3. List of cryoconite samples from Buchan Glacier (29-31) and Waldemar Glacier (32-33) with date of collection, GPS coordinates, elevation, and number of individuals determined to species/genus level. For sampling sites localities see Figs. 1 and 2.

N	Date	GPS	Elevation (m)	*P. recamieri*	*H. dujardini*	*Hypsibius* sp. A	*Isohypsibius* sp. A
29	4 August 2013	N79°34.764’ E 11°6.019’	228	15		26	
30	4 August 2013	N79°34.769’ E11°6.043’	230				
31	4 August 2013	N79°34.778’ E 11°6.1’	234	1	1	5	6
32	28 July 2013	N78°40.578’ E12°01.614’	195		4	4	6
33	28 July 2013	N78°40.578’ E12°01.617’ 201				1	1

the dark. After two weeks, physicochemical analysis was conducted in the chemical laboratories of the Hornsund Polish Polar Station (Spitsbergen). Conductivity and pH were measured with an Elmetron CX-505 and glass electrodes. Water samples were filtered in a vacuum by a Millipore filtration kit with rinsed Whatmann nylon filters (0.22 µm). Subsequent water samples were analysed for their chemical composition, including SO_4^{-2}, Cl^-, NO_2^-, NO_3^-, F^-, Br^-, Ca^{2+}, Mg^{+2}, Na^+, K^+, and NH_4^+. Anions and cations were determined by high performance liquid chromatography (HPLC) through two separate Metrohm 761 Compact IC Systems. Alkalinity was determined by the titration method (Metrohm 702 SM Titrino) (Stachnik *et al*., 2016). The quality of analyses were determine by charge balance error for each sample: 1.4% (sample 29); 22% (sample 30), and 3.3% (sample 31). Ion balance criteria met the standards of UNEP and WMO (Bartram and Balance, 1996; Allan, 2015). Samples from the Buchan Glacier were replicated, three samples were collected for tardigrade fauna analysis and three from the same cryoconite holes for chemical analysis. As only three samples were collected, statistical analysis are omitted and remarks on the links between tardigrade and chemistry are presented in the 'Discussion' section.

Chemical analysis on the cryoconite holes on Dickson Land glaciers were conducted by Vonnahme *et al*. (2015). However, concentration of all analytes are given in mmol/kg what makes unreasonable the comparison of these values with our results. Thus, we decided to omit this comparison in Tab. 4.

RESULTS

Tardigrades were found in 25 samples. In those samples, specimens belonging to five tardigrade taxa were discovered. They represent 2 superfamilies, 4 families and 4 genera, being *Hypsibius dujardini*, *Hypsibius* sp. A, *Isohypsibius* sp. A, *Pilatobius recamieri*, and one undetermined species of Ramazzottiidae (for taxonomic and zoogeographic remarks, see Taxonomic Account). Data for samples with sample number, coordinates, altitude above sea level, are presented in Tabs. 1, 2 and 3. All sampling sites are detailed in Fig. 2. *Pilatobius recamieri* was the most frequent species, present in 22 (88%) samples. Except for the undetermined species of Ramazzottiidae that was found on one glacier only, other species more frequently inhabited cryoconite holes. Tardigrades reported from Buchan Glacier are northernmost record of tardigrades from cryoconite holes. Densities of the tardigrades from Hans Glacier and glaciers in Billefjorden presented in this paper, in terms of cryoconite hole area, depth and elevation are covered in Zawierucha *et al*. (2016b).

Chemical determined pH values were in the range 6.50-7.45. Mean concentrations of cations, such as Ca^{2+},

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Tab. 4. Mean values of physicochemical parameters/analytes determined in cryoconites samples collected from Buchan Glacier and results of the research performed by Porazińska *et al.* (2004) on glaciers in Antarctica.

Analytes	**Buchanbreen**				**Antarctic glaciers (Commonwealth, Canada, Howard, Hughes, Taylor)**
	Mean values	**29**	**30**	**31**	**Ranges of mean values**
pH	6.93	7.45	6.84	6.5	5.80-8.10
EC (µS)	20.82	52.5	4.8	5.15	60-125
		(mg/L)			**(mg/L)**
Cations					
Ca^{2+}	0.26	0.65	0.10	0.05	2.2-7.1
Mg^{2+}	0.29	0.80	0.03	0.03	0.5-2.0
Na^{2+}	2.47	6.19	0.27	0.94	3.0-7.7
K^+	5.57	4.26	2.03	10.42	0.5-1.9
NH^{4+}	0.99	2.94	<LOD	0.04	0.035-0.11
Anion					
$^*F^-$	<LOD	<LOD	<LOD	<LOD	
C^{l-}	3.86	9.79	0.61	1.19	4.8-14.7
$^*NO^{2-}$	<LOD	<LOD	<LOD	<LOD	
$^*Br^-$	<LOD	<LOD	<LOD	<LOD	
$^*NO^{3-}$	<LOD	<LOD	0.01	<LOD	0.010-0.400
PO_4^{3-}	2.42	1.02	2.65	3.58	
SO_4^{2-}	0.49	1.18	0.12	0.16	2.8-7.0
HCO^{3-}	11.2	18.9	1.2	13.4	???

*<LOD - below limit of detection for F^-, NO^{2-}, Br^-, NO^{3-}=0.010 mg/L; LOQ=0.030 mg/L. Both the limit of detection (LOD) and the limit of quantitation (LOQ) were calculated based on the standard deviation of the response (s) and the slope of the calibration curve (b) according to the formulas: LOD=3.3 (s/b), LOQ=10 (s/b).

Mg^{2+}, and NH_4^+, did not exceed 1mg L^{-1}, while the mean concentrations of Na^{2+} and K^+ were, respectively, 2.47mg L^{-1} and 5.57mg L^{-1}. In the case of mean concentrations of anions in the examined samples, HCO_3^-, Cl^-, PO_4^{3-} and SO_4^{3-} were, respectively, 11.2 mg L^{-1}, 3.86 mg L^{-1}, 2.42 mg L^{-1}, and 0.486 mg L^{-1}, whereas no presence of F^-, Br^-, NO_2^-, NO_3^-, or Br^- was observed (Tab. 4).

Taxonomic account

Phylum Tardigrada (Spallanzani, 1777)
Class Eutardigrada Richters, 1926
Order Parachela Schuster, Nelson, Grigarick *et* Christenberry, 1980
Superfamily Hypsibioidea Pilato, 1969 in Marley *et al.* (2011)
Family Hypsibiidae Pilato, 1969
Subfamily Hypsibiinae Pilato, 1969
Genus *Hypsibius* Ehrenberg, 1848

1. *Hypsibius dujardini* (Doyère, 1840) (Fig. 3 A-B)
Localities: 12, 14, 15, 17, 18, 22, 31, 32.
Remarks: Species belongs to the cosmopolitan *convergens-dujardini* complex (McInnes, 1994; Miller *et al.*, 2005; Kaczmarek and Michalczyk, 2009). The original description is inadequate and incomplete in the context of modern taxonomy methods, and so the examined specimens were compared with the later descriptions (Ramazzotti and Maucci, 1983; Dastych, 1988). This species is characterized by claws of *Hypsibius* type, two macroplacoids, septulum, and a short cuticular bar between claws IV. The species was previously reported from the Svalbard archipelago (Zawierucha *et al.*, 2013).

2. *Hypsibius* sp. A (Fig. 4 A-E)
Localities: 1, 4, 6, 7, 8, 9, 11, 12, 14, 20, 29, 31, 32, 33.
Diagnosis: The body transparent/white, with eyes present in the examined specimens. The cuticle smooth in the PCM (Fig. 4A). The buccal apparatus of the *Hypsibius* type without ventral lamina (Fig. 4B). Peribuccal lamellae absent. Oral cavity armature either absent or not visible in the PCM (Fig. 4B). The pharyngeal bulb with apophyses, with two rod-shaped macroplacoids, second in lateral position broader at the end (Fig. 4C). The macroplacoid length sequence 2<1, microplacoid and septulum absent. Stylet supports located in the posterior position. Typically shaped stylet furcae present. The apophyses clearly separated from the 1st macroplacoids. All macroplacoids clearly separated (Fig. 4 B,C). Their usual claws of the *Hypsibius* type but with variation within even single specimens - claws of the *Isohypsibius* type or the *Mixibius* type (Fig. 4 D, E). All main branches with accessory points (Fig. 4 D,E). Cuticular bars or thickening under and between the claws absent. Proper lunulae absent.
Remarks. This species belongs to a large group of hypsibids with two macroplacoids and a smooth cuticle. It was previously found in cryoconite holes on Spitsbergen (Dastych, 1985). Dastych (1985) had previously reported *Hypsibius* ? *arcticus* from cryoconite holes on Spitsbergen. However, that determination was uncertain because of the absence of eggs in the samples (Dastych, 1985). The current findings are evidence that *Hypsibius* sp. A (noted by Dastych (1985) as *Hypsibius* ? *arcticus*) laid eggs to exuvia, thus, it is not *Hypsibius arcticus* (Murray, 1907). This species, by presence of smooth cuticle, two macroplacoids, and the macroplacoid length sequence, is the most similar to *H. arcticus*, and *H. convergens* (Urbanowicz, 1925), known before from non-glacial habitats (Ramazzotti and Maucci, 1983). Up to now, tardigrades recorded from the Arctic tundra were not morphologically the same as *Hypsibius* sp. A. However, erection of the *Hypsibius* sp. A to the new species without redescriptions of the other hypsibids (Kaczmarek *et al.*, 2015) made exact differential diagnosis difficult. To avoid taxonomic confusion, if additional new fresh material will available together with individuals for exact comparison, *Hypsibius* sp. A will be described together with morphometric data in a future paper.

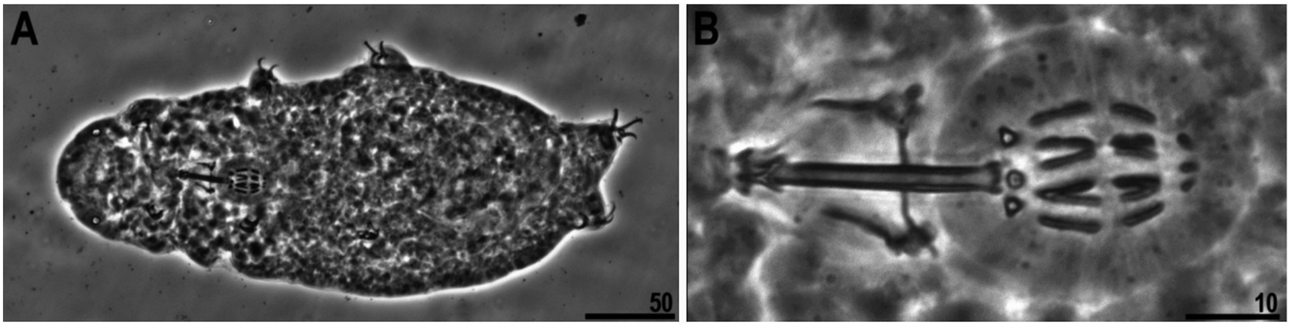

Fig. 3. *Hypsibius dujardini*. A) Habitus. B) Buccal apparatus.

Family Pilatobiinae Bertolani, Guidetti, Marchioro, Altiero, Rebecchi & Cesari, 2014
Genus *Pilatobius* Bertolani, Guidetti, Marchioro, Altiero, Rebecchi & Cesari, 2014

3. *Pilatobius recamieri* Richters, 1911 (Fig. 5 A-B)
Localities: 1, 2, 3, 4, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 29, 31.
Remarks: Holarctic species, recorded from localities in Europe, Asia and North America (McInnes, 1994). Previously reported from Svalbard archipelago (Zawierucha *et al*., 2013). It comes from cryoconite holes reported from Spitsbergen, Greenland and Norway (Zawierucha *et al*., 2015a).

4. Family Ramazzottiidae Sands, McInnes, Marley, Goodall-Copestake, Convey & Linse, 2008
Locality: 13.
Diagnosis: Black pigmented cuticle present (Fig. 6A). Peribuccal lamellae absent. The oral cavity armature absent or not visible in the PCM. The pharyngeal bulb with apophyses, with two rod-shaped macroplacoids, the first with constriction. The macroplacoid length sequence 2<1, and the microplacoid and septulum absent (Fig. 6B). Stylet supports were located in the posterior position. Typical stylet furcae present. The apophyses clearly separated from the 1st macroplacoids. All macroplacoids are clearly separated (Fig. 6B). Claws of *Ramazzottius* type with

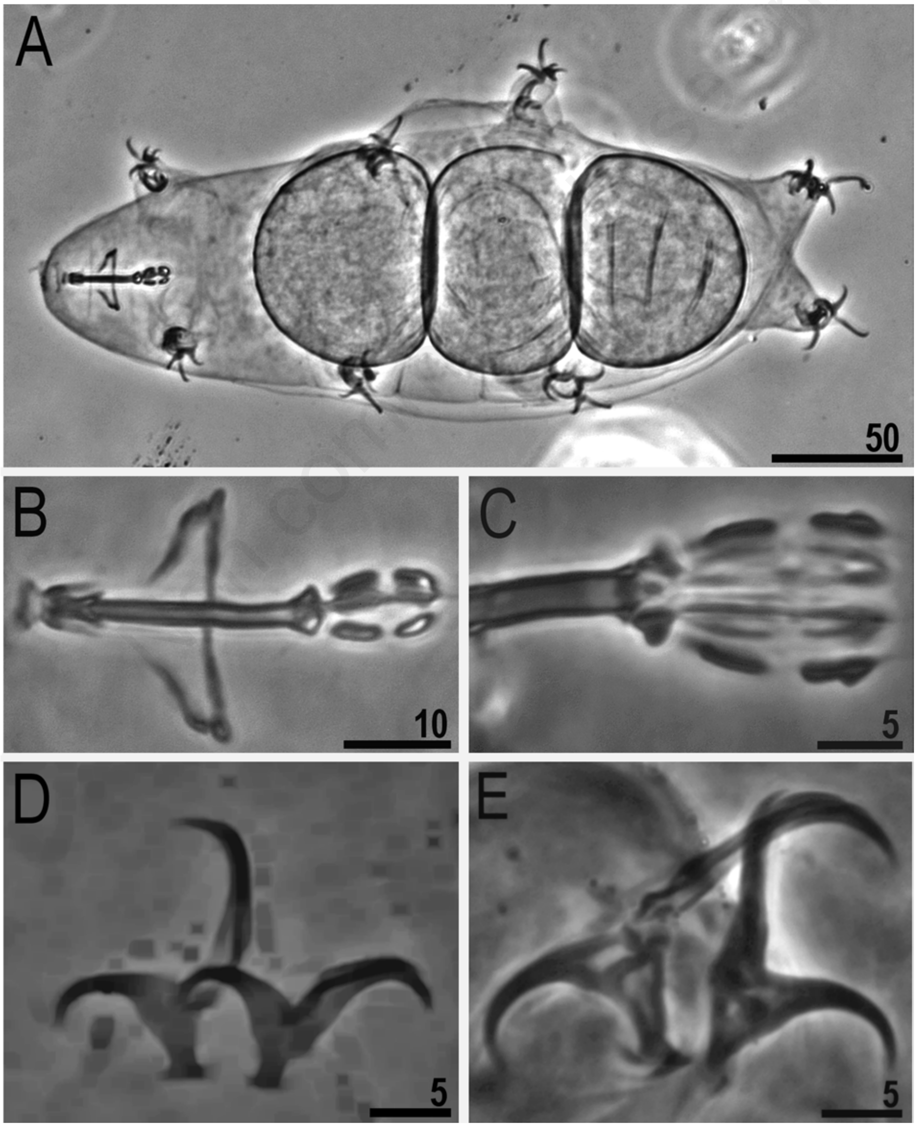

Fig. 4. *Hypsibius* sp. A. A) Habitus in moulting state. B) Buccal apparatus. C) Macroplacoids in lateral position. D) Claws III. E) Claws IV.

strongly thin main branches of external claws. All branches without accessory points (Fig. 6C). Cuticular bars or thickening under and between the claws absent or not visible in destroyed specimens preserved in formaldehyde and analysed under PCM. Proper lunules absent or not visible in destroyed specimens preserved in formaldehyde and analyzed under PCM.

Remarks: Only two black pigmented individuals collected from the Ebba Glacier have been analysed. Individuals were destroyed because of long preservation in formaldehyde. Proper delimitation at the genus level was difficult. The generic feature, the apophyses for the insertion of the stylet muscles, are completely invisible. The claws are of *Ramazzottius* type but with a rare feature - a lack of accessory points. Despite the fact that they were conserved in formaldehyde and then prepared in Hoyer medium, they did not lose the dark pigment in the cuticle. Other glacier tardigrades in the Alps and Himalayas (Dastych *et al*., 2003; Dastych, 2004) were noted to have black pigment that protected them from high doses of UV radiation (Greven *et al*., 2005). Strong black pigmentation of the examined individuals may be a unique adaptation to glacier environment. Without more individuals preserved in better condition, with suitable character for proper morphological and morphometric analysis, discussion on the status of this species is unfounded. Morphologically similar specimens have been found in cryoconites in Tien and the Quilian Mountains (personal observation). Based on problems with proper identification at the genus level, we decided to delimit individuals at the family level.

Superfamily Isohypsibioidea Sands, McInnes, Marley, Goodall-Copestake, Convey & Linse, 2008
Family Isohypsibiidae Sands, McInnes, Marley, Goodall-Copestake, Convey & Linse, 2008
Genus *Isohypsibius* Thulin, 1928

5. ***Isohypsibius*** **sp. A** (Fig. 7 A-C)

Localities: 14, 31, 32, 33.

Diagnosis: The body transparent/white, with eyes present in the examined specimens. The cuticle smooth in the PCM (Fig. 7A). The buccal apparatus of the *Isohypsibius* type without ventral lamina (Fig. 7B). Peribuccal lamellae absent. The oral cavity armature comprised of a single ventral and dorsal band of small round teeth in the posterior portion of the oral cavity (barely visible in certain specimens). The pharyngeal bulb with apophyses, with three rod-shaped macroplacoids, all without constrictions. Macroplacoid length sequence 1<2<3 and microplacoid and septulum absent (Fig. 7B). Stylet supports located in the posterior position. Typically, stylet furcae present. The apophyses clearly separated from the 1st macroplacoid. All macroplacoids were clearly separated. Claws of the *Isohypsibius* type (Fig. 7C). All external and all internal claws similar in shape and all main branches with accessory points. Short, cuticular bars present on legs I-III

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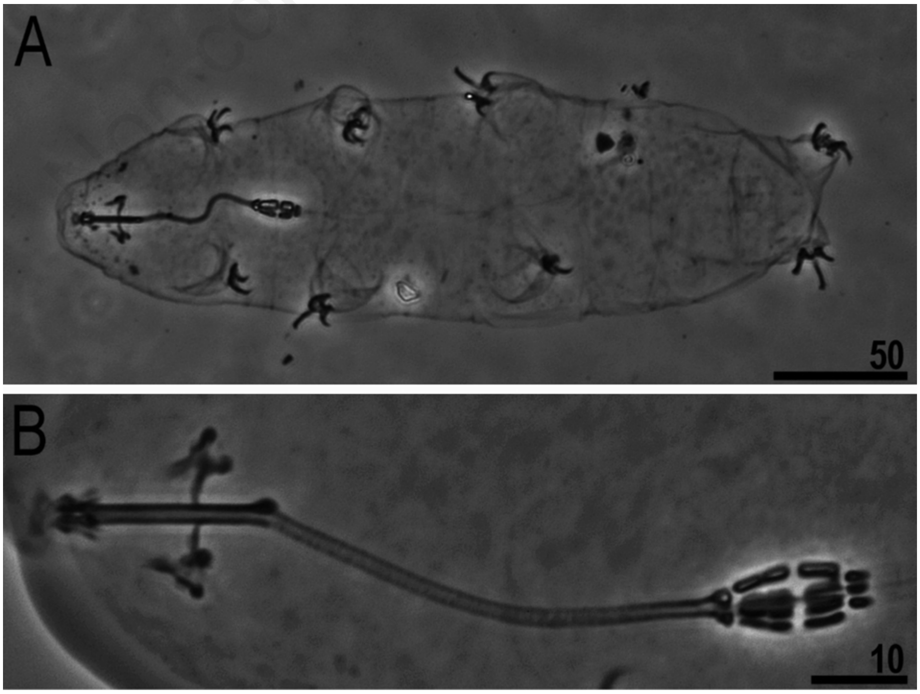

Fig. 5. *Pilatobius recamieri*. A) Habitus. B) Bucco-pharyngeal apparatus.

(sometimes poorly visible). Other cuticular structures or thickening between claws absent. Proper lunules absent, and only certain areoles visible under the claw bases but needed clarification in better preserved individuals. Eggs smooth and deposited in exuvia.

Remarks: This species belongs to a large group of isohypsibids with three macroplacoids, smooth cuticle and cuticular bars. This species, based on the presence of smooth cuticle, three macroplacoids, and macroplacoid length sequence is the most similar to *I. tetradactyloides* (Richters, 1907) and *I. schaudinni* (Richters, 1909), known before from non-glacial habitats (Ramazzotti and Maucci, 1983). Up to now, tardigrades recorded from the Arctic tundra were not morphologically the same as *Isohypsibius* sp. A. However, erection of the *Isohypsibius* sp. A, to the new species without redescriptions of other isohypsibids (*e.g.*, *I. schaudinni, I. tetradactyloides;* Kaczmarek *et al*., 2015) made precise differential diagnosis difficult. Moreover, more well-preserved material for morphometry and confirmation of presence/absence of lunules is required. To avoid taxonomic confusion, if additional new fresh material will be available together with individuals for exact comparison, *Isohypsibius* sp. A will be described together with morphometric data in a future paper.

DISCUSSION

Tardigrada diversity and distribution

In this study, five tardigrade taxa in cryoconite holes sediment were discovered and are presented as the first comprehensive data regarding their distribution on glaciers in Spitsbergen. Current data from Spitsbergen, along with previous surveys, clearly indicates that tardigrade diversity in cryoconite holes is low in comparison to Arctic terrestrial ecosystems in which up to 10 species per moss sample may be found (Dastych, 1985). However, some species are

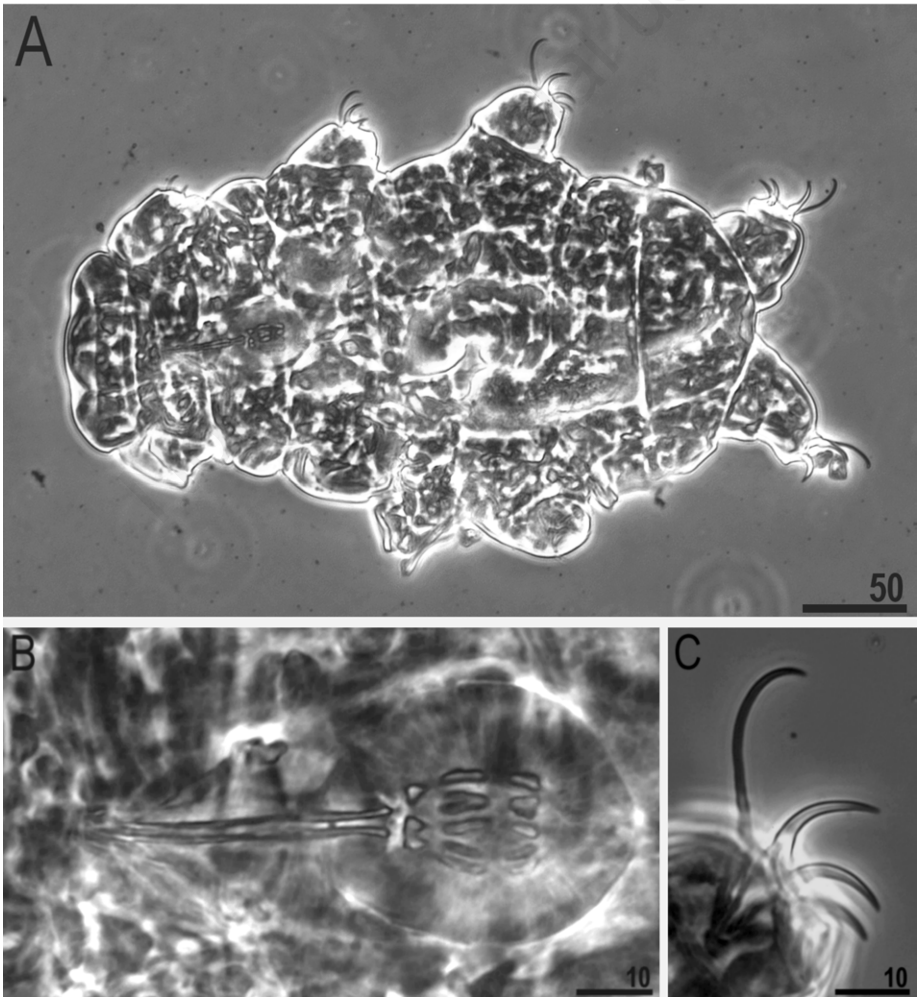

Fig. 6. Undetermined species of Ramazzottiidae. A) Habitus. B) Buccal apparatus. C) Claws III.

unique to this environment and known only from glaciers, like *Hypsibius* sp. A, *Isohypsibius* sp. A, found in this study, or other tardigrades in the Alps *e.g.*, *Hypsibius klebelsbergi* Mihelčič, 1959 or Himalayas, *H. janetscheki* Ramazzotti, 1968 and *H. thaleri* Dastych, 2004.

In recent papers published by Kaczmarek *et al*. (2012) and Zawierucha *et al*. (2013, 2015b, 2016a), mosses, lichens, soil, and mixed samples (moss/lichen) were analysed and observed such that previously recorded species were not morphologically similar to *Hypsibius* sp. A, species of Ramazzottiidae and *Isohypsibius* sp. A from cryoconite holes (all collected samples and specimens are deposited at the Department of Animal Taxonomy and Ecology, Adam Mickiewicz University in Poznań). Dastych (1985) found *Hypsibius* sp. A only in cryoconite samples and noted it as *Hypsibius* ? *arcticus* (see 'Remarks' for details). The picture was included in that work (Plate VII) with buccal apparatus of this species. We also found *Hypsibius* sp. A exclusively in cryoconite holes, the buccal apparatus is the same and it is also similar to *Hypsibius arcticus*. That is why we are certain that it is the same species as in the work of Dastych (1985). Moreover, Dastych (1991) questioned the determination of *Hypsibius arcticus* and discussed validation. Dastych (1985) did not find *Isohypsibius* sp. A and species of Ramazzottiidae in cryoconite holes. *Isohypsibius* sp. A, *Hypsibius* sp. A and *P. recamieri* were discovered by Grøngaard *et al*. (1999) in the Greenland ice caps and deposited at ZMUC. Material from the present study was compared with specimens deposited at ZMUC, and *Isohypsibius* sp. A, *Hypsibius* sp. A and *P. recamieri* are morphologically identical (KZ personal observation). Taking in to consideration i) the huge sample size of terrestrial material analysed so far and the absence of species found in cryoconite holes, as well as this being ii) the same species found in Greenland cryoconite holes, *Isohypsibius* sp. A and *Hypsibius* sp. A may be deemed unique glacier inhabitants.

Only Dastych (1985) and De Smet and Van Rompu (1994) conducted studies on the glacier tardigrades in Svalbard. They investigated three glaciers (Sörbreen, Tryggvebreen, Hyrnebreen) and their found four species: *H. dujardini*, *Hypsibius* sp. A, *P. recamieri* and *Isohypsibius granulifer granulifer* (Thulin, 1928), with the most frequent *P. recamieri* (Dastych, 1985; De Smet and Van Rompu, 1994). Knowledge on the glacier tardigrades from Svalbard archipelago include totally nine glaciers

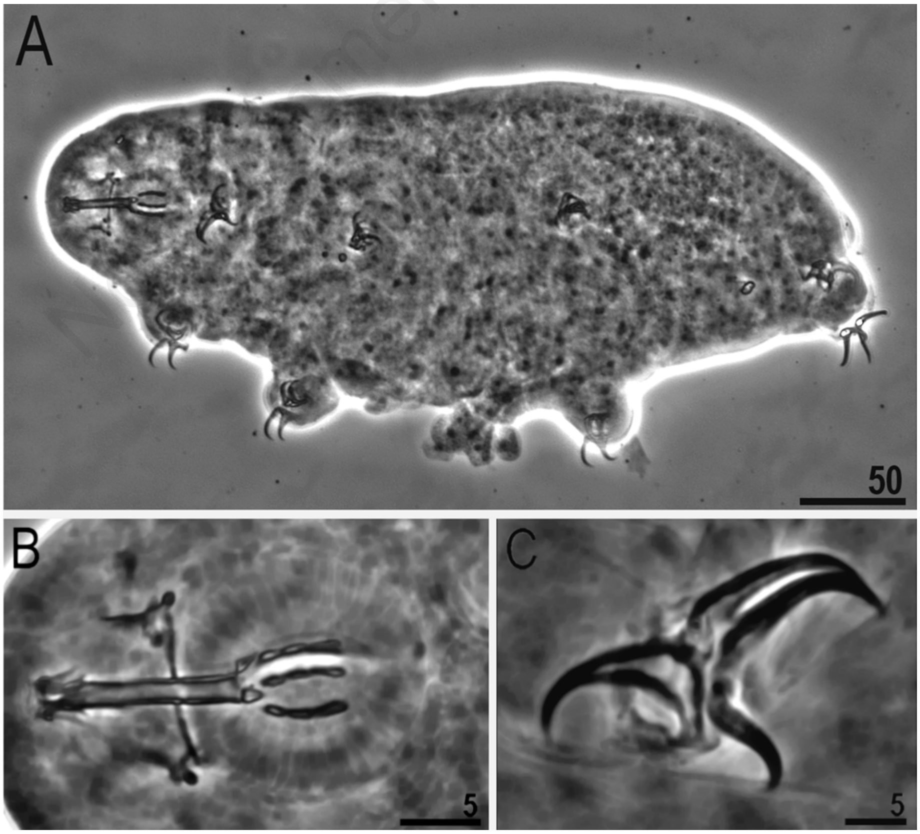

Fig. 7. *Isohypsibius* sp. A. A) Habitus; B) Buccal apparatus; C) Claws I.

(six analysed in this study and three in previous). Distribution of tardigrades seems to be homogenous - with the most dominant species *P. recamieri*, then *Hypsibius* sp. A, *H dujardini* and less frequent *Isohypsibius* sp. A. Up to now, undetermined species of Ramazzottidae and *I. g. granulifer* are known only from single glaciers.

Biochemistry

Porazińska *et al*. (2004) discussed the relationship between invertebrates (rotifers and tardigrades) inhabiting Antarctic cryoconites and the chemistry of the environment in which they live. Authors suggest that higher pH and lower NO^{3-} concentration favour higher densities of tardigrades in cryoconite sediments (Porazińska *et al*., 2004). These parameters determined in the Buchan Glacier samples were in the following ranges: 6.5-7.45 (pH) and NO$_3^-$ < LOD (limit of detection), indicating favourable conditions for life and development of tardigrades. It is also stated that tardigrade variation may be explained by the concentration of primary macronutrients (NH$_4^+$ and NO$_3$-) as well as secondary macronutrients (Mg^{2+}) (Porazińska *et al*., 2004). Ammonium present in samples collected from the Buchan glacier was on the order of magnitude <LOD of 2.94 mg L^{-1}. The mean value of NH$_4^+$ was about nine times greater than the highest value detected by Porazińska *et al*. (2004) in Antarctic glacier samples (Table 4). Cryoconite holes with the highest concentrations of NH$_4^+$ were also characterized by the highest levels of magnesium (0.800 mg dm^{-3}). Occurrence of tardigrades seems to be related to concentrations of Mg^{2+} and other cations, including K$^+$ and Ca^{2+}.

The highest conductivity, and highest concentration of the most of ions can be found in sample 29, which was located lower (228 m asl) than the two other samples (230 and 234 m asl; Tab. 4). Differences in chemical composition can be because of i) the shorter distance to the sea and influence of the sea aerosols; or ii) the result of the flow of matter through water flow on the Buchan Glacier surface.

Glacial biome - unique species and food web

This study presents evidence for the biotic background of a glacial biome hypothesis. Firstly, despite the extreme conditions of ice environments, organisms were found in most of samples. Thus, they cannot be fortuitous elements on glaciers and certainly have adaptations and strategies like other glacier inhabitants (Price, 2000; Zawierucha *et al*., 2015a; Singh *et al*., 2014). Secondly, the presence of different trophic groups (herbivores, microbivores, and higher-level consumers) is not possible without the presence of autotrophs, which commonly inhabit glaciers (Kaczmarek *et al*., 2016). Therefore, glacial ecosystems on Spitsbergen are typified by truncated food webs. This is supported by other environmental studies conducted on Svalbard glaciers (Vonnahme *et al*., 2015). Finally, a strong selection pressure resulting from extreme conditions on the ice influences the unique character of organisms inhabiting glaciers (species which were found strictly in cryoconite holes). Similar to cryoconite holes, ephemeral micro-ecosystems are formed in the axils of Bromeliaceae, where species specialists but also species known for their wide tolerance of habitats have been discovered. It seems that a number of the species living in them are endemic, while others cosmopolitan or they constitute complexes of morphologically similar taxa or cryptic species (Balke *et al*., 2008; Kolicka 2016; Kolicka *et al*., 2016). Observations on the species in bromeliads are analogous to those in cryoconite holes - there is a wide distribution of *P. recamieri* and a limited distribution within cryoconite holes of *e.g*., *Isohypsibius* sp. A.

Vonnahme *et al*. (2015) showed that grazing by tardigrades has a positive influence on eukaryotic microalgae by nutrient recycling in cryoconite holes. Similar observations on the trophic web at the microscale have been demonstrated on decaying caribou antlers in the Arctic (Sutcliffe and Blake, 2000). In these unusual ecosystems, mosses, lichens and algae are photoautotrophic producers and tardigrades (with mites and dipterans) are primary consumers (Sutcliffe and Blake, 2000). Trophic webs in Bromeliad tanks also present analogies to cryoconite holes. However, grazers are less abundant, and more fortuitous species can be observed in bromeliads (Brouard *et al*., 2011; Kolicka *et al*., 2016).

CONCLUSIONS

Despite extreme conditions and high ice melt, tardigrades can inhabit cryoconite sediments in high densities (Zawierucha *et al*., 2016b). Glacier organisms may influence the size of cryoconite holes (McIntyre, 1984). Therefore, the presence or absence of invertebrates (which are considerably larger than bacteria, viruses or algae), could affect the melting of glacier surfaces and should be discussed in future literature as a part of bicryomorphological studies (Zawierucha *et al*., 2015a; Cook *et al*., 2015). The presence of unique cryoconite tardigrade species indicate cryoconites as distinctive extreme habitats and tardigrades as a constant element in a glacial biome, with different communities structure than in tundra. Most likely, tardigrades adapted to life on a glacier rather than being transported there by accident as hypothesized earlier by Grøngaard *et al*. (1999). The important role of tardigrades in a trophic web (the herbivorous genus, *Hypsibius*, or the microbivore genus, *Pilatobius*), greatly corroborates the hypothesis of a multitrophic food web in cryoconites. Various tardigrade species may recycle limiting nutrients to microalgae and bacteria, and compete with or suppress bacterivorous and grazing rotifers and ciliates.

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Area, depth and elevation of cryoconite holes in the Arctic do not influence Tardigrada densities

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Abstract: Water bears (Tardigrada) are known as one of the most extremophile animals in the world. They inhabit environments from the deepest parts of the oceans up to the highest mountains. One of the most extreme and still poorly studied habitats which tardigrades inhabit are cryoconite holes. We analysed the relation between area, depth, elevation and tardigrades densities in cryoconite holes on four glaciers on Spitsbergen. The mean (±SD) of cryoconite area was 1287.21±2400.8 cm^2, while the depth was on average 10.8±11.2 cm, the elevation 172.6±109.66 m a.s.l., and tardigrade density 24.9±33.0 individuals per gram of wet material (n = 38). The densities of tardigrades on Hans Glacier reached values of up to 168 ind. cm^3, 104 ind. g^{-1} wet weight, and 275 ind. g^{-1} dry weight. The densities of tardigrades of the three glaciers in Billefjorden were up to 82 ind. cm^2, 326 ind. g^{-1} wet weight and 624 ind. g^{-1} dry weight. Surprisingly, although the model included area, depth and elevation as independent variables, it cannot explain Tardigrada density in cryoconite holes. We propose that due to the rapid melting of the glacier surface in the Arctic, the constant flushing of cryoconite sediments, and inter-hole water-sediment mixing, the functioning of these ecosystems is disrupted. We conclude that cryoconite holes are dynamic ecosystems for microinvertebrates in the Arctic.

Key words: Arctic, Svalbard, Tardigrada, cryoconite holes, ecology, glaciers.

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Introduction

Cryoconite holes are small, water-filled, cylindrical reservoirs occurring on the surface of glaciers throughout the world (*e.g.* Wharton *et al.* 1983, 1985; Mueller *et al.* 2001). Currently these holes are considered to be extreme micro-ecosystems (*e.g.* Mueller *et al.* 2001; Fountain *et al.* 2004; Hodson *et al.* 2008). The functioning of such habitats is possibly caused by a decrease in the albedo on the surface of a cryoconite-covered glacier and by the presence of various groups of microorganisms (McIntyre 1984; Takeuchi *et al.* 2001; Hodson *et al.* 2010; Kaczmarek *et al.* 2016). Pioneer observations and studies of cryoconite holes took place in Greenland between the 19th and 20th century (Drygalski 1897) and have continued through present day. So far, representatives of Rotifera, Nematoda, Annelida, Tardigrada and Arthropoda have been found in cryoconite holes in various zoogeographical regions (*e.g.* Zawierucha *et al.* 2015a). One of the most common taxa known to inhabit glaciers in polar regions are tardigrades (Dastych 1985; Zawierucha *et al.* 2015b).

Tardigrada, also known as water bears, are small microinvertebrates (*ca.* 50–2000 µm) inhabiting terrestrial and aquatic environments (Ramazzotti and Maucci 1983; Nelson *et al.* 2015). Because of their adaptations to unfavorable conditions they are known as one of the toughest animals on earth. They can survive at the bottom of the deep sea, on high mountains and even in space (Guidetti *et al.* 2012; Nelson *et al.* 2015). In cryoconite holes they may act as grazers and top predators in a multi-trophic food web (Vonnahme *et al.* 2016; Zawierucha *et al.* 2015a). Tardigrades and rotifers are proposed to have an impact on the size of microalgae colonies and to be important for nutrient recycling by feeding and digestion of their prey in cryoconite holes (Vonnahme *et al.* 2016). Studies designed to explore tardigrade fauna in cryoconite holes were conducted by *e.g.* De Smet and Van Rompu (1994), Grøngaard *et al.* (1999), Séméria (2003), Dastych *et al.* (2003) and Dastych (2004). Only Porazinska *et al.* (2004) conducted well-designed ecological studies on cryoconite-dwellng tardigrades in the Antarctic. Despite the papers which have been published so far, our knowledge of tardigrade ecology in cryoconite holes is still severely limited (Zawierucha *et al.* 2015a).

The relationship between area and species richness have been frequently discussed in the literature (*e.g.* Gaston and Blackburn 2000). The first studies to explore the relationship between area and population density were done by MacArthur and Wilson (1967), MacArthur *et al.* (1972) and Root (1973). However, little attention has been given to the relationship between area and individuals of higher taxon (*e.g.* phylum) densities. Connor *et al.* (2000) showed that the population density (insects, birds) is positively correlated with the area, but the authors did not discuss the correlation between generally individual densities of higher taxa and the area in general. According to Gaston and Matter (2002), individuals–area relationships need more detailed discussion and the relationship should always include densities per

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accompanying area. In glacier ecology only Porazinska *et al*. (2004) have proved that the diameter, the amount of sediment, and the concentrations of Na^+ were able to predict tardigrade abundances in Antarctica. In cryoconite holes at lower elevations the concentration of sediment is higher than in those located at higher elevations (Porazinska *et al*. 2004). Significant variations in tardigrade abundance between lower and higher elevations have also been observed in Antarctic cryoconite holes with different sediment content (Porazinska *et al*. 2004).

Since glaciers and ice sheets are forgotten biomes and one of the fastest disappearing habitats (Anesio and Laybourn-Parry 2012), studies on the ecology of animals in these habitats are urgently needed (Zawierucha *et al*. 2015a). So far, the most comprehensive ecological studies of cryoconite holes were conducted on bacterial communities (*e.g*. Stibal *et al*. 2006, 2008; Edwards *et al*. 2014) and knowledge of the larger organisms and top predators in cryoconite holes – microanimals – is seriously limited (Zawierucha *et al*. 2015a). In this study the relationship between area, depth and elevation of cryoconite holes and tardigrade densities is presented and some differences between Arctic and Antarctic systems are discussed.

Material and methods

Cryoconite material was collected from four glaciers on Spitsbergen (48 samples): I – ten samples from Hans Glacier (July, 2014) located in Hornsund; II – twenty-two from Nordenskiöld Glacier (July, August, 2014); III – four samples from Ebba Glacier (August, 2012) and IV – twelve from Hørbye Glacier (August, 2013, July, August, 2014) located in Billefjorden (Fig. 1).

Cryoconite samples from Hans Glacier were collected with disposable plastic Pasteur pipettes from the bottom of selected cryoconite holes and transferred to 15 cm^3 plastic test tubes. After collection, the samples were preserved in 96% ethyl alcohol. From each sample 1 cm^3 of sediments was scanned for tardigrades with a stereomicroscope. Then the wet sediments were weighed and again after drying them at room temperature for two days, the number of tardigrades were calculated per one gram of material.

Cryoconite holes on three glaciers around Billefjorden (Ebba Glacier, Hørbye Glacier, Nordenskiöld Glacier) were collected randomly using a large (500 cm^3) pooter (Southwood and Henderson 2000) within a 4.5 cm plastic ring (15 cm^2 area). All sampling equipment was washed with meltwater from the sampling site prior to sampling. In the lab, the samples were allowed to settle, the supernatant was removed and subsamples of sediment were counted in a counting chamber under a stereomicroscope. The sediments were weighed in a wet state and again after drying them at 50°C for 12 h. Taxa were identified using the key to the World Tardigrada (Ramazzotti and Maucci 1983).

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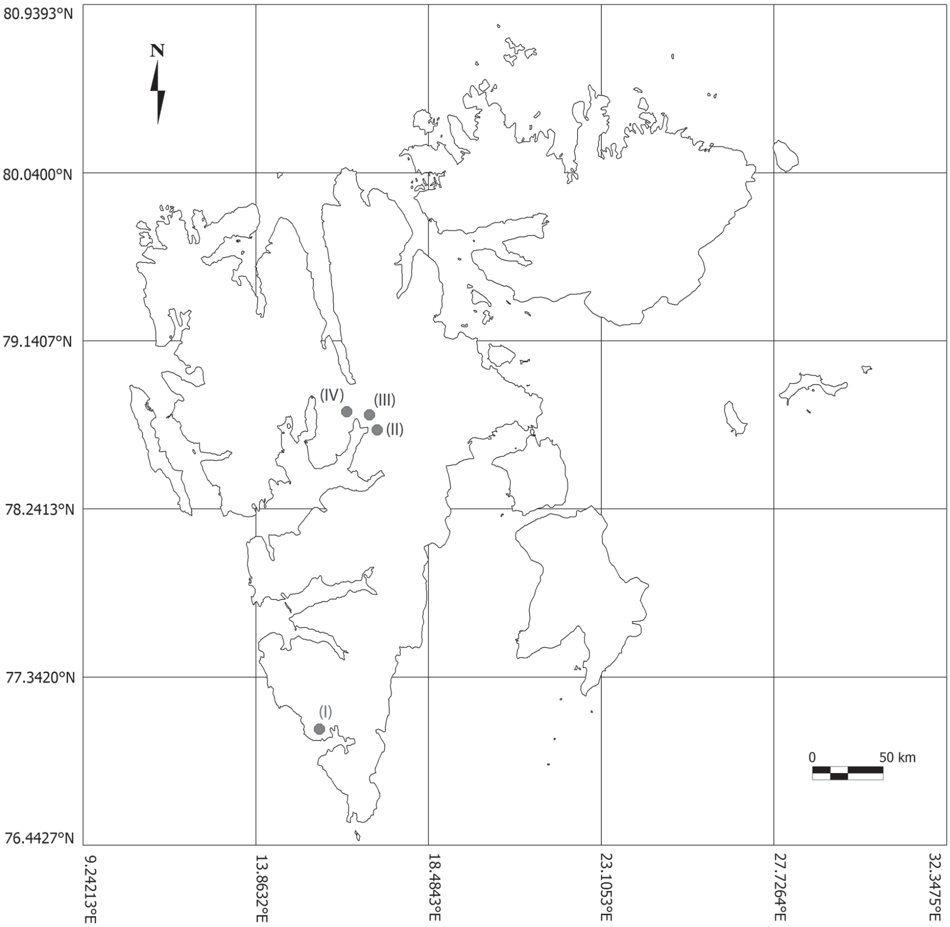

Fig. 1. Svalbard archipelago with sampled glaciers. I – Hansbreen, II – Nordenskiöldbreen, III – Ebbabreen, IV – Hørbyebreen.

The depth of cryoconite holes on all glaciers was measured on site with a ruler. The area of each cryoconite hole was calculated from calibrated photographic documentation with the Olympus cellSens Entry 1.11 software.

Data analysis

Full data, *i.e*. depth, area, elevation and the calculated number of tardigrades per gram of wet material, were obtained for 41 samples. However, three samples were excluded from all the analyses because of the extremely high: tardigrade densities (326 ind. g^{-1} sediment), depth (49 cm) and area (162831.9 cm^2). Thus,

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in analysis 38 samples have been used. For each cryoconite hole the relative area, depth, elevation and tardigrade density were calculated as the residual expressed as the differences between observed values and mean value. Thus, all the variables define how much the observed values (area, depth and elevation) in each cryoconite hole deviate from the average estimated for all samples. For the calculations we used General Linear Models (GLM) and backward selection of non-significant factors. All the calculations were performed with R 2.12.0 (R Development Core Team 2010). GLMs were developed for standarized area, depth and elevation as independent variables and for standarized tardigrade abundances as a dependent variable (n = 38). Among 48 cryoconite samples data for: tardigrade densities per gram of wet sediments, depth and area for six, three and seven samples respectively, were not available. Thus, in Table 1, data for 41 samples are presented.

Results and discussion

Tardigrada were present in 42 from 48 samples, which is 87.5% of all the collected samples. Five taxa were found in cryoconite holes, they are: *Hypsibius dujardini* (Doyère, 1840), *Hypsibius* sp. A, *Pilatobius recamieri* (Richters, 1911), one species of Ramazzottiidae Sands, McInnes, Marley, Goodall-Copestake, Convey and Linse, 2008, *Isohypsibius* sp. A. The mean (±SD) of cryoconite area was 1287.21±2400.8 cm^2, while the depth was on average 10.8±11.2 cm, the elevation 172.6±109.66 m a.s.l., and tardigrade density 24.9±33.0 ind. per g^{-1} wet weight (n = 38). The average numbers of tardigrades were 32 ind. cm^3, 23 ind. g^{-1} wet weight, 58 ind. g^{-1} dry weight in Hornsund and 6.13 ind. cm^2, 24.43 ind. g^{-1} wet weight, 46 ind. g^{-1} dry weight in Billefjorden (for all collected samples in Billefjorden). The densities of tardigrades on Hans Glacier reached values of up to 168 ind. cm^3, 104 ind. g^{-1} wet weight and 275 ind. g^{-1} dry weight. At Billefjorden up to 82 ind. cm^2, 326 ind. g^{-1} wet weight and 624 ind. g^{-1} dry weight were found. The model, which included area, depth and elevation as independent variables, cannot explain the Tardigrada density in cryoconite holes (GLM, $F = 0.71$, $p = 0.54$, $R^2 = 0.059$). Mean values of a.s.l., area, depth and number of tardigrades for each glacier (41 cryoconite samples) are presented in Table 1.

Quantitative data on the ecology and diversity of aquatic tardigrades are limited and different methods of collection and calculation were used in previous studies (*e.g.* Kathman and Nelson 1987), which makes the comparison of water bear assemblages in freshwater habitats impossible. The ecology of glacier tardigrades is almost unknown with only three papers published on this topic so far (De Smet and Van Rompu 1994; Dastych *et al.* 2003; Porazinska *et al.* 2004). But quantitative data for tardigrades were presented in only two of these papers (Dastych *et al.* 2003; Porazinska *et al.* 2004). According to Dastych *et al.* (2003) the abundance of tardigrades in glacier pools of water in the Alps

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Table 1

Mean values of a.s.l., area, depth and number of tardigrades for Hansbreen, Nordenskiöldbreen, Ebbabreen, Hørbyebreen.

	N	Mean a.s.l. [m] (95% CL)	Mean area [cm^2] (95% CL)	Mean depth [cm] (95% CL)	Mean no. of tardigrades [ind g^{-1} of sediment] (95% CL)
Hansbreen	10	79.7 (64.3 – 95.0)	358.5 (55.6 – 661.4)	8.5 (4.2 – 12.7)	23.4 (0 – 49.4)
Nordenskiöld-breen	17	148.2 (115.0 – 181.4)	11875 (8199.3 – 31949.7)	19.0 (10.9 – 27.1)	22.8 (8.2 – 37.3)
Ebbabreen	4	319.2 (77.9 – 560.5)	1317.0 (137.0 – 2771.0)	12.9 (1.5 – 24.3)	136.1 (71.9 – 344.2)
Hørbyebreen	10	265.1 (211.0 – 319.1)	626.9 (412.4 – 841.4)	4.0 (0.5 – 7.5)	9.2 (1.8 – 16.6)

consisted of up to 75 individuals and up to 3.60 ind. cm^3 (360 ind. 100 cm^3) of meltwater current (Dastych *et al*. 2003). However, the data presented in Dastych *et al*. (2003) is not comparable to ours due to a different quantitative methodology (estimation of tardigrades per cryoconite hole or pooled samples in Dastych *et al*. (2003)). The densities of tardigrades in the present study are higher than in the studies conducted in the Antarctic (McMurdo Dry Valley) by Porazinska *et al*. (2004). In the Antarctic the highest concentration of tardigrades were *ca*. 12.5 ind. g^{-1} (1250 individuals per 100 g of dry sediment). However, Everitt (1981) reported that maximum tardigrades abundance in wet algal mats in tundra pond can reach 470 specimens g^{-1}, which is higher than the numbers currently observed in cryoconite holes.

Significant differences in tardigrade abundance between lower and higher elevations have also been observed in the Antarctic cryoconite holes with variations in the amount of sediments (Porazinska *et al*. 2004). Tardigrades were not significantly more abundant in cryoconite holes located in lower elevations in the present study.

Surprisingly, *ca*. 90% of samples examined in the present study contained tardigrades. This number of positive samples is similar to the results of previous studies conducted in tundra in the Svalbard – 71.7%–80% (Dastych 1985; Zawierucha *et al*. 2015b, 2016).

The lack of a significant relationship between tardigrade abundances and the abiotic factors examined can be explained by a more dynamic supragla-

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cial hydrological system, frequent melting events, and less stable cryoconite holes in the Arctic, which can influence organism assemblages (Mueller *et al*. 2001; Fountain *et al*. 2004; Mueller and Pollard 2004). The hydrological system between cryoconite holes is important for the transport of nutrients and for the development of a drainage system near their surface (Gajda 1958). In the initial state, cryoconite holes are capable of storing water. Combined with supraglacial channels, they form a dynamic drainage system, which has conseqences for the internal morphology of the cryoconite holes, for example by enhancing heat transport or altering chemical properties (MacDonell and Fitzsimons 2008). On the glacier surface, stripping events take place, during which the cryoconite holes are melted away in a few days (Fountain *et al*. 2004; MacDonell and Fitzsimons 2008; Vonnahme *et al*. 2016). Additionally, partial stripping, which can impact the transport of fresh or relocated sediments, has been observed (MacDonell and Fitzsimons 2008).

Cryoconite holes in the Antarctic are wider, deeper and mostly covered with ice, which makes them more stable than the Arctic ones (Mueller *et al*. 2001; Fountain *et al*. 2004). High meltwater production and open cryoconite holes in the Arctic may negatively affect cryoconite communities because of inter-hole water-sediment mixing (Mueller and Pollard 2004). In comparison to the Antarctic ones, the Arctic cryocnite holes have a lower concentration of nutrients, which may be caused by the flushing or dilution effect (Mueller *et al*. 2001).

In the Arctic, factors depending on direct surroundings are most likely the important factors determining tardigarde abundances in cryoconite holes. Vonnahme *et al*. (2016) found a positive relationship between tardigrade abundances and the impact of guano input by sea birds on the same glaciers in Billefjorden. This very local phenomenon supplies a small, hydrologically connected area on the glacier with a large amount of nutrients (Zárský *et al*. 2013). These nutrients can fuel the base of the food web, which can act as a food source for higher trophic levels, such as tardigrades.

A new term – biocryomorphology – was embedded by Cook *et al*. (2015), which describes ice-organisms interaction. Glacier organisms can influence the size of cryoconite holes (McIntyre 1984), therefore, despite perturbations on the ice surface, high densities of tardigrades may influence speed of ice melting and cryoconite hole size. The shape of cryoconite hole together with glacier hydrology may be also a factor, which influences tardigrade communities. Thus, our data are the first attempt to complement the gaps in term of ice (area, depth, elevation of cryoconite holes) – invertebrate densities.

It could be stated that area, depth, and elevation do not influence tardigrade densities in cryoconite holes in the Arctic as much as the amount of nutrients, value of meltwater and the flushes of sediment.

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ORIGINAL PAPER

The influence of a seabird colony on abundance and species composition of water bears (Tardigrada) in Hornsund (Spitsbergen, Arctic)

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Abstract Seabirds in the Arctic are known to link marine and terrestrial ecosystems. They feed in the sea and breed on land, where they deposit enormous amounts of guano. Soil in the vicinity of seabird colonies is much more enriched with nutrients as compared to areas beyond their impact, which positively affects primary and secondary production. Water bears (Tardigrada) are microinvertebrates which constitute a permanent and ubiquitous faunal component of polar regions. Here, we tested the influence of seabird guano on Tardigrada communities; we established two transects in Hornsund (SW Spitsbergen): (1) SEABIRD where little auks (*Alle alle*) nested, and (2) CONTROL, an area without a little auk colony. In total, we collected 160 moss, lichen and mixed (moss/lichen) samples from those areas. In total, we found 1990 specimens belonging to 32 taxa (25 identified to species level). The average density of water bears was higher in the SEABIRD transect (9.31 ind g^{-1}), where mosses predominated over lichens, in comparison with the CONTROL transect (5.83 ind g^{-1}), where more lichens occurred. Thus, ornithogenic enrichment of soil and locally facilitated development of mosses over lichens might be important factors responsible for the increase in invertebrate abundance. According to canonical correspondence analysis, the little auk colony effect explained 13.2 % of the tardigrade species composition, and this factor appeared to be more important than the vegetation type itself. Four taxa, i.e. *Isohypsibius* cf. *reticulatus*, *Microhypsibius bertolanii*, *Minibiotus* cf. *formosus* and *Ramazzottius* cf. *rupeus*, have been recorded in the Svalbard archipelago for the first time.

Keywords Little auk · Moss fauna · Lichen fauna · New records · Ornithogenic nutrients · Svalbard · Tundra

Introduction

Arctic terrestrial ecosystems are considered to be relatively species-poor, and this is mostly because of the harshness of the environmental conditions (e.g. low temperatures and nutrient concentrations, extreme seasonality). Limnoterrestrial Arctic invertebrate fauna are currently estimated at ca. 1000 named species (e.g. Coulson et al. 2014), while the counterpart number of other archipelagos is estimated to be twice that number and more; for instance, in non-Arctic archipelagos, on the Galapagos (located in tropical zone) and the Azores (located in temperate zone), the number of invertebrate species exceeds 2000 (Herrera and Roque-Álbelo 2014; Base de Dados da Biodiversidade Dos Açores 2015). However, the low number of invertebrates identified may also be related to the fact that the Arctic has been poorly investigated (Coulson et al. 2014). Therefore, faunistic studies on invertebrate diversity are of great interest (Hodkinson 2013).

A growing amount of evidence suggests that some invertebrate species may be connected with very distinct microhabitats in the Arctic and, with conditions permitting,

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they may be locally abundant (e.g. Byzova et al. 1995; Sømme and Birkemoe 1999; Zmudczyńska et al. 2012; Zawierucha et al. 2015b). Such habitats are, for example, formed in the vicinity of seabird colonies. The supply of ornithogenic nutrients around these colonies, originating mainly from guano, significantly enhances soil conductivity, nitrogen, potassium and phosphate ion concentrations and decreases soil pH values, all of which dramatically change the environmental conditions (Mulder et al. 2011; Zmudczyńska et al. 2012; Zwolicki et al. 2013; Klimaszyk et al. 2015a, b). In polar regions, such fertilisation results in the development of exceptionally lush, compact and diverse plant and microbiota communities (Eurola and Hakala 1977; Odasz 1994; Ellis 2005; Zmudczyńska et al. 2008, 2009; Zmudczyńska-Skarbek et al. 2013), which in turn attracts populations of herbivores, predators, scavengers and decomposers (Croll et al. 2005; Jakubas et al. 2008; Zmudczyńska et al. 2012).

In this study, we examined assemblages of water bears (Tardigrada) with respect to the presence of a breeding colony of a pelagic seabird, i.e. the little auk *Alle alle* Linnaeus, 1758. Terrestrial tardigrades are a cosmopolitan phylum of microscopic metazoans ranging from ca. 100 to 2200 µm in size. They inhabit various microhabitats within mosses, lichens, liverworts and soil. Many species are known to live in extreme conditions, e.g. on the highest mountain peaks and on the surface of glaciers (e.g. Ramazzotti and Maucci 1983; Nelson 2002; Guil 2008; Zawierucha et al. 2015a). Up to now, about 1200 tardigrade species have been described from different habitats throughout the world (Guidetti and Bertolani 2005; Degma and Guidetti 2007; Vicente and Bertolani 2013; Degma et al. 2009–2015). Terrestrial water bears are also a constant element of polar ecosystems, where they inhabit tundra and polar deserts (e.g. McInnes 1994; Convey and McInnes 2005; Zawierucha 2013; Zawierucha et al. 2013; Coulson et al. 2014). The number of tardigrade species from Svalbard consists of ca. 90 described species (Zawierucha et al. 2013).

Despite the water bears' ubiquitous distribution in polar regions (e.g. Ramazzotti and Maucci 1983; Dastych 1985; McInnes 1994) and the important role they play in food webs (Sohlenius and Boström 2008), ecological studies on this group have rarely been conducted in these ecosystems. Almost all previous research in Svalbard has focused mainly on descriptions of new species and/or the addition of new records (e.g. Węglarska 1965; Kaczmarek et al. 2012). The only two ecological studies that were conducted on the abundance and distribution of tardigrades were by Dastych (1985) and Zawierucha et al. (2015b), who showed the negative influence of altitude on tardigrade diversity. The impact of seabird guano on Tardigrada communities has been reported sporadically. In the Antarctic, penguin guano negatively affected invertebrate assemblages, including tardigrades (Porazińska et al. 2002; Smykla et al. 2012). In the Arctic, no such an investigation has been performed; however, a recent study has shown that seabird guano positively influenced the water bears' body size (Zawierucha et al. 2015c).

Little auks are small planktivorous seabirds breeding exclusively in the High Arctic zone and are considered a keystone species of both marine and terrestrial ecosystems (Stempniewicz et al. 2007). With an estimated number of 37 million breeding pairs, these are the most abundant seabirds in the Atlantic Ocean (including Svalbard), and probably one of the most numerous seabirds in the world (Wojczulanis-Jakubas et al. 2011). Little auks are important consumers of sea resources, and due to the deposition of guano, feathers, egg shells and carcasses, while on land they are responsible for significant soil fertilisation, tundra productivity and diversity enhancement around their breeding colonies (Eurola and Hakala 1977; Jakubas et al. 2008; Zwolicki et al. 2013). This, in turn, is expected to be of an advantage to numerous invertebrates, including tardigrades.

The present research is focused on influence of seabird guano on tardigrade abundance and species composition. Specifically, we tested two hypotheses that: (1) tardigrades are more abundant in areas affected (in the vicinity of a little auk colony) as compared to areas non-affected by seabird guano (control area); and (2) species composition differs between these two areas.

Moreover, we report four taxa: (1) *Microhypsibius bertolanii* Kristensen, 1982, (2) *Minibiotus* cf. *formosus* (3) *Ramazzottius* cf. *rupeus* and (4) *Isohypsibius* cf. *reticulatus*, which have been recorded in the Svalbard archipelago for the first time.

Materials and methods

Study area and field sampling

The study was conducted in August 2011 on the northern coast of Hornsund (Wedel Jarlsberg Land, West Spitsbergen), within the limits of South Spitsbergen National Park (Fig. 1). The average annual temperature in the studied area is −3.8 °C (Migała and Wawrzyniak 2013), and the soils are lithosols and frost-deformed regosols (Skiba 2013).

Two transects were established, each 1 km long: (1) SEABIRD—spanning from the seashore to the centre of the little auk colony situated on the slope of Ariekammen [ca. 77°01′N; 15°31′E; ca. 15,000 nesting pairs (Puczko and Stepniewicz 2013)], and (2) CONTROL—spanning from the seashore up the Fugleberget slope (77°00′N,

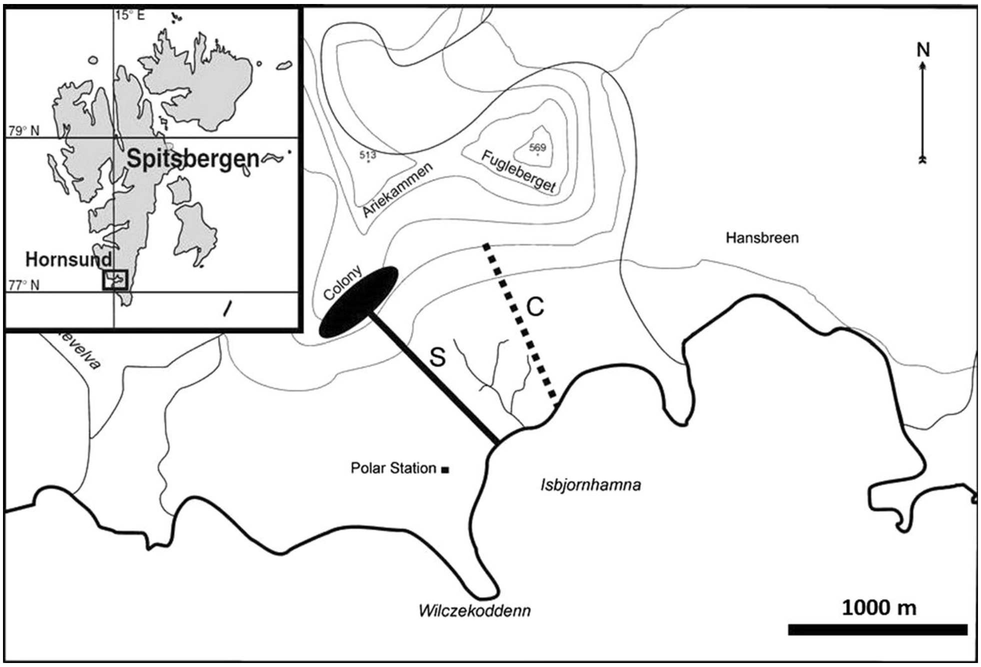

Fig. 1 Study area. Northern part of Hornsund, S—SEABIRD transect, C—CONTROL transect

15°33′E) (Fig. 1). The whole SEABIRD transect was an area being under influence of the colony; it consisted of vegetation-covered rock debris and a near-horizontal tundra approaching the seashore. Plant communities occurring there are characteristic of well-fertilised bird-cliff vegetation (Rønning 1996), with vegetation cover of typically 90–95 %, except for the seashore. All samples of that transect were collected within the zone of vegetation modified by the birds. The CONTROL transect was situated outside the little auk colony and was not impacted directly by the birds' guano. It was situated on the site topographically similar to the SEABIRD transect, with patchy vegetation, mostly lichens. Both transects were oriented towards the S–E and covered an altitudinal range from the sea level to ca. 72 m asl.

At each 100 m, along both 1-km-long transects, a transverse sub-transect was established from which eight samples at five-metre intervals were collected ($N = 160$ in total) and classified as moss (M), lichen (L) or mixed (moss/lichen) (ML) samples. Each sample had size of ca. 1/3 of the paper envelope (A4). All samples were later stored in the same envelopes, which allowed them to dry up slowly. Samples were weighed with a precision of 0.01 g.

Material extraction and species identification

All samples were treated following standard methods for tardigrade examination (e.g. Dastych 1980; Ramazzotti and Maucci 1983). Each sample was soaked in water for ca. 5 h. Water containing tardigrades and detritus particles was decanted and examined under a stereomicroscope. All extracted specimens, simplex, exuvia and eggs were mounted on microscope slides in Hoyer's medium and then examined using a Phase Contrast Microscope (PCM) (Olympus BX40) associated with an ART-CAM 300 MI photo camera and QuickPhoto Camera 2.3 software. Species were identified using the key to World Tardigrada (Ramazzotti and Maucci 1983) and later original descriptions, re-descriptions and keys: Dastych (1985, 1988), Binda (1988), Bertolani and Rebecchi (1993), Tumanov (2007), Fontoura and Pilato (2007), Kaczmarek and Michalczyk (2009), Fontoura and Morais (2010), Kaczmarek et al. (2011a; 2012), Zawierucha (2013) and Zawierucha et al. (2014). All samples and microscope slides were deposited at the Department of Animal Taxonomy and Ecology at Adam Mickiewicz University in Poznań, Poland.

Statistical analyses

The number of tardigrades found in each sample was extrapolated for 1 g of dry mass of vegetation in order to calculate the total density, i.e. for all species together and for densities of particular species. Prior to this extrapolation, possible correlations were tested between a sample weight *versus* the number of individuals and number of species. No such correlation was found (Spearman rank correlations, $p > 0.05$ for both individuals and species). All individuals which were not identified to species level (due to the lack of important taxonomic traits) were also included in comparisons of total density, but disregarded in species composition analyses. One outlying sample taken from the mossy-lichen habitat of the SEABIRD area was excluded from all the analyses because of the extremely high density of *Isohypsibius coulsoni* Kaczmarek et al., 2012 (955 ind g^{-1}, the standardised value more than 12). Since five individuals of *Ramazzottius* cf. *rupeus* occurred only in this sample, the species was excluded from further analyses. We treated *Paramacrobiotus richtersi* (Murray, 1911) and *Paramacrobiotus* sp. as one taxon in the diversity and species composition analyses. Since a similar number of empty samples (with no individuals recorded) were found in the SEABIRD (25) and CONTROL (30) transects (Chi-square test, $\chi^2 = 0.69$, $p = 0.41$), they were omitted from all analyses. The frequency of different vegetation type occurrences differed between the SEABIRD and CONTROL transects ($\chi^2 = 13.54$, $p = 0.001$). There were more M samples within the SEABIRD area ($n_S = 43$ vs. $n_C = 26$), while more L samples were within the CONTROL area ($n_C = 12$ vs. $n_S = 1$). The number of ML samples in both areas was similar ($n_S = 10$ vs. $n_C = 12$).

The Shannon diversity index was used to describe tardigrade species diversity [$H' = \Sigma_i(n_i N^{-1}) \log (n_i N^{-1})$], where n_i is the number of individuals of species i and N is the total number of individuals, and evenness [$J' = H' (\log S)^{-1}$, where S is the number of species] in the samples with the help of DIVer 10.o1 (AZB analysis and software 2010). To test for differences in total density and H', J' and the number of species between both transects and vegetation types, the nonparametric Mann–Whitney test and Kruskal–Wallis with post hoc Dunn procedures were used (due to distinct deviations from normal distributions of the data; Shapiro–Wilk test, $p < 0.05$ in all cases). These data were processed using STATISTICA 10.0 (StatSoft, Inc. 2011).

Numerical ordination methods were used to describe the total (qualitative and quantitative) variability of Tardigrada assemblages: (1) based only on species composition data to describe the general pattern of variability in the studied community (unimodal unconstrained analysis—detrended correspondence analysis, DCA; gradient length = 7.50 SD); and (2) in relation to two nominal environmental variables: transect—representing the presence (SEABIRD) or absence (CONTROL) of a seabird colony in the vicinity of the sampling sites, and vegetation—determining the type of vegetation from which the invertebrates were collected (unimodal constrained analysis—canonical correspondence analysis, CCA). Species occurring in only one sample were excluded from the analyses [besides the *R.* cf. *rupeus* as mentioned above, these were also: *Echiniscus merokensis merokensis* Richters, 1904, *E. testudo* (Doyère, 1840), *Hebesuncus conjungens* (Thulin, 1911), *Microhypsibius bertolani* Kristensen, 1982, *Minibiotus* cf. *formosus*, *Pseudechiniscus victor* (Ehrenberg, 1853), *Richtersius coronifer* (Richters, 1903), *Tenuibiotus* sp. and *Tenuibiotus voronkovi* (Tumanov, 2007)]. All species data were log-transformed in order to normalise their distributions. After the CCA, a Monte Carlo test was performed with 499 permutations to identify which of the factors significantly influenced the model. The variation partitioning test was used to calculate the factors' unique contribution in order to explain variability in the tardigrade species composition. The variation value was adjusted using the number of degrees of freedom as suggested by Peres-Neto et al. (2006) to provide a more accurate estimation of the variation explained with the use of the CCA. Each time the results of constrained ordination were compared with those of unconstrained ordination (% variability explained by an environmental factor was divided by % variability explained by the first three axes of DCA). Thus, the efficiency of the environmental factor(s) was obtained in explaining the non-random variability existing in the data (%). *T* value biplots (Van Dobben circles) which approximated the *t* values of the regression coefficients of a weighted multiple regression were employed to explore significant relationships between individual tardigrade species and the environmental factors. These data were processed using CANOCO 5.0 software (ter Braak and Šmilauer 2012).

Results

Density of Tardigrada

The total average density of tardigrades (all species together) was higher in the SEABIRD transect (median = 9.31 ind g^{-1}) as compared to the CONTROL (5.83 ind g^{-1}), and the distribution of this parameter's values differed between the two areas (Mann–Whitney test, $z = 2.01$, $p = 0.044$; Fig. 2a). There were also significant differences in the density value distribution between different vegetation types (Kruskal–Wallis test, $H = 12.73$, $p = 0.002$; Fig. 2b). The highest tardigrade density was observed in the moss samples (vegetation type M, median = 9.52 ind g^{-1}); a lower density was in the mixed (moss/lichen) samples (ML, 5.35 ind g^{-1}); and the lowest was in the lichen samples (L, 3.39 ind g^{-1}). However, only the difference between M and L was statistically significant (post hoc Dunn's test, $z = 3.41$, $p = 0.002$).

Species composition

In total, 1990 specimens belonging to 32 taxa (25 determined to species level) were found (Table 1). Due to the lack of eggs, some of the specimens were determined only to genus or species group level. Twenty-two taxa were

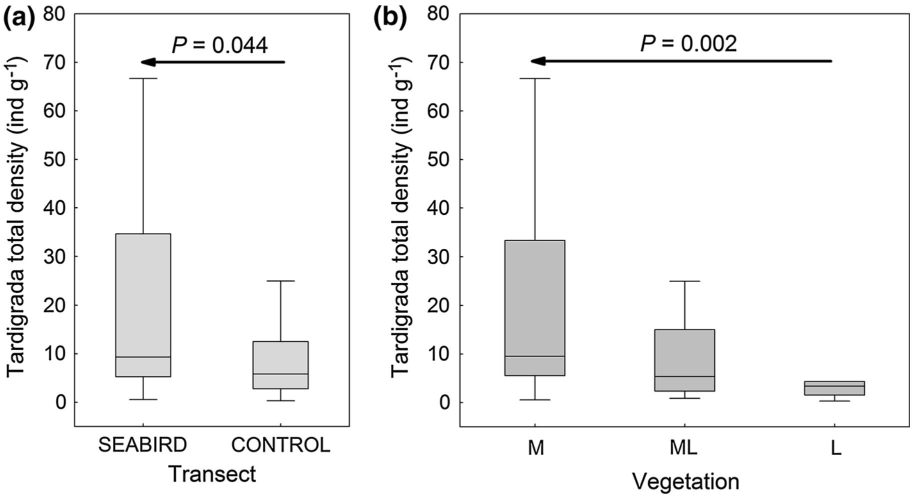

Fig. 2 Comparison of total Tardigrada density [medians with interquartile ranges (*boxes*) and non-outliers (*whiskers*)] between the transects (**a**) and vegetation types (**b**). Statistically significant differences marked (Mann–Whitney test)

found in the SEABIRD transect and 24 in the CONTROL transect. Fifteen taxa were found in both transects; thus, seven were revealed only in the SEABIRD transect and ten only in the CONTROL. The most frequent tardigrade species in all of the samples was *Pilatobius recamieri* (Richters, 1911), which was present in 35 samples (in 29 and 6 samples from the SEABIRD and CONTROL transects, respectively).

There were no significant differences in the Shannon H' diversity index, the evenness J' index and the number of species between the SEABIRD and CONTROL transects, or between different vegetation types ($p > 0.05$). The CCA analysis revealed that both of the variables tested (transect and vegetation) together explained 16.2 % of the non-random (i.e. described with DCA theoretical gradients: axis 1—10.5 %, axis 2—7.8 %, axis 3—5.1 %) variability of the Tardigrada assemblages (all canonical axes were significant; Monte Carlo permutation test, *pseudo-F* = 2.3, $p = 0.002$). The transect factor was responsible for 13.2 % ($F = 4.0$, $p = 0.002$) of the tardigrade variability, and 1.3 % of this variation was partitioned with the vegetation factor (Fig. 3). The transect also had a significant unique contribution to explaining the tardigrades' composition (11.9 %, $F = 3.7$, $p = 0.002$). However, the vegetation factor did not significantly differentiate the tardigrade communities either independently of the transect (3.4 %, $F = 1.5$, $p = 0.078$) or in addition to it (4.3 %, $F = 1.4$, $p = 0.12$). Figure 4 presents 10 species best fitted to the ordination based on both variables, with their percentage occurrences in different transects. Species significantly positively associated with the SEABIRD transect were *I. elegans*, *M. crenulatus* Richters, 1904 and *P. recamieri*; while those related to the CONTROL were *E. granulatus* (Doyère, 1840), *Hypsibius convergens* (Urbanowicz, 1925), *H. pallidus* Thulin, 1911 and *Testechiniscus spitsbergensis* (Scourfield, 1897).

Four taxa, i.e. *M. bertolanii*, *M.* cf. *formosus*, *R.* cf. *rupeus* and *I.* cf. *reticulatus*, were recorded in the Svalbard archipelago for the first time.

Discussion

Tardigrada abundance and species composition

This is the first study that examined the influence of a planktivorous seabird colony on tardigrades in the Arctic, confirming the hypothesis presented in introduction. It was found that tardigrades were more abundant in the vicinity of the little auk colony as compared to areas not occupied by these birds. The density of Tardigrada was higher in the area influenced by the little auk colony as compared to the CONTROL transect. Unfortunately, it was not possible to compare the absolute values that were obtained here with results obtained in polar regions by other authors as these authors had calculated the densities of tardigrades in a different manner: (a) per area (e.g. Dastych 1985), (b) per weight of wet material (Everitt 1981) or (c) they completely omitted such calculations (e.g. Johansson et al. 2013). However, the average density (9 ind g^{-1}) recorded in the SEABIRD transect was comparable with our previous results from the Ariekammen and Rotjesfjellet slopes (Hornsund), which were also inhabited by little auks (9 ind g^{-1}), although the elevation range sampled there (14–524 m asl, Zawierucha et al. 2015b) was much wider than in the present study (only 0–72 m asl). The average densities of water bears in the samples collected in Nordaustlandet and Edgøya and the northern part of Prins Karl Forland were only 6 ind g^{-1} (Zawierucha et al. 2013). Furthermore, the maximum number of specimens collected there was very low (only 25 ind g^{-1}), while in the CONTROL and SEABIRD transects, it was much higher (253

Table 1 List of taxa found in study with division on the vegetation type (M—moss, L—lichens, ML—moss/lichens) and transect (S—SEABIRD, C—CONTROL)

Taxa	Vegetation			Transect	
	M	L	M/L	S	C
Diphascon pingue pingue (Marcus, 1936)	+	+	+	+	+
Echiniscus granulatus (Doyère, 1840)	+	+			+
E. merokensis merokensis Richters, 1904	+			+	
E. quadrispinosus quadrispinosus Richters, 1902	+		+	+	+
E. testudo (Doyère, 1840)			+	+	
E. wendti Richters, 1903		+	+	+	+
Hebesuncus conjungens (Thulin, 1911)			+		+
Hypsibius convergens (Urbanowicz, 1925)	+		+		+
H. dujardini (Doyère, 1840)	+	+	+	+	+
H. pallidus Thulin, 1911	+	+	+	+	+
Isohypsibius coulsoni Kaczmarek et al., 2012	+		+	+	+
I. elegans Binda and Pilato, 1971	+		+	+	
I. karenae Zawierucha 2013	+			+	
I. cf. *reticulatus*	+	+			+
I. sattleri (Richters, 1902)	+			+	+
I. cf. *tuberculatus*	+	+	+	+	+
Macrobiotus crenulatus Richters, 1904	+		+	+	+
M. islandicus islandicus Richters, 1904	+		+	+	+
M. harmsworthi harmsworthi Murray, 1907	+	+	+	+	+
M. h. obscurus Dastych 1985	+			+	
M.hufelandi group	+		+	+	+
Microhypsibius bertolanii Kristensen 1982	+			+	
Minibiotus cf. *formosus*			+		+
Paramacrobiotus richtersi (Murray, 1911)	+				+
Paramacrobiotus sp.	+			+	
Pilatobius recamieri (Richters, 1911)	+		+	+	+
Pseudechiniscus victor (Ehrenberg, 1853)	+				+
Ramazzottius cf. *rupeus*			+	+	
Richtersius coronifer (Richters, 1903)	+				+
Testechiniscus spitsbergensis (Scourfield, 1897)	+	+	+	+	+
Tenuibiotus sp.	+				+
Tenuibiotus voronkovi (Tumanov, 2007)		+			+

and 1304 ind g^{-1}, respectively). This is the first study that shows the positive effect of polar seabirds' guano on the abundance of tardigrades. In contrast, Porazinska et al. (2002) and Smykla et al. (2012) showed that guano deposited in penguin colonies in Antarctica caused a decrease in tardigrade abundance. The most probable explanations for this could be an extremely high concentration of guano and the accumulation of ornithogenic nutrients around the penguin nests due to their location on flat terrains, as well as considerable devastation of the vegetation by bird trampling (Tatur 1989; Smykla et al. 2007). Similarly, Zmudczyńska et al. (2012) showed that springtails abundance was generally much higher in the ornithogenic tundra in Hornsund as compared to areas far from the seabird nests. However, in the centre of the little auk colony as well as directly beneath the cliff inhabited by guillemots and kittiwakes, density values were lower than the maximum recorded there.

The effect of the little auk colony may be indirect, e.g. it could have been created through the deposition of guano and soil fertilisation that amended the conditions for plant growth in the vicinity of the colony which, in turn, favoured the formation of microhabitats that were attractive for the tardigrade communities. Specifically, ornithogenic enrichment of the soil facilitated the development of bryophytes over the lichens (otherwise commonly occurring in the Arctic tundra, Thomas et al. 2008), which appear to be important factors for tardigrade density. Nevertheless, it was found that tardigrades were more numerous in mosses than in lichens regardless of the

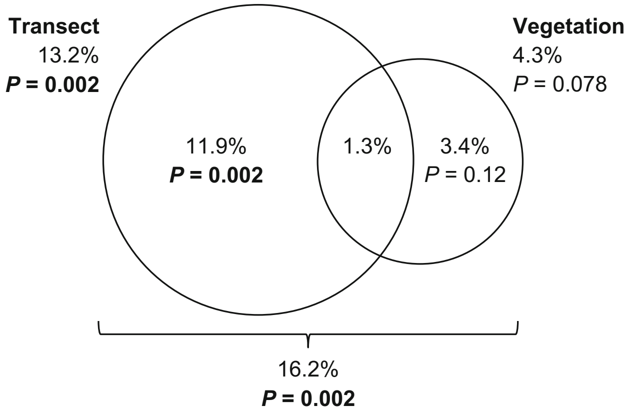

Fig. 3 Unique and shared fractions of the total variation in Tardigrada species composition explained by transect (SEABIRD and CONTROL) and vegetation (M—moss, L—lichens, ML—moss/lichens)

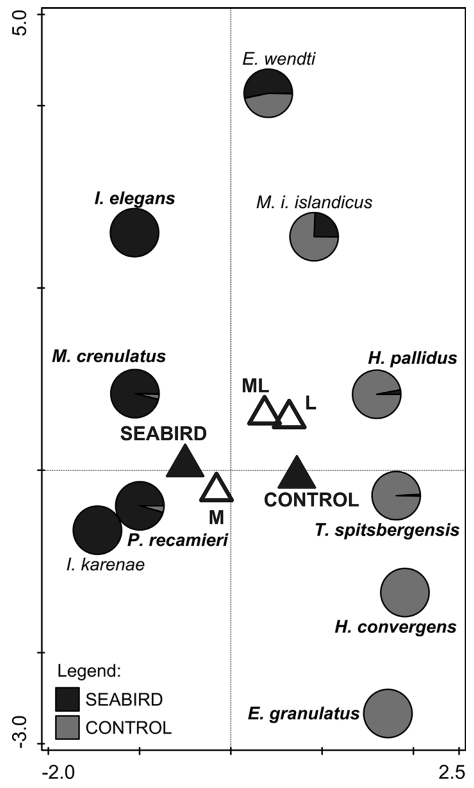

Fig. 4 CCA ordinations of 10 best fitted species to the transect (*full triangles*; SEABIRD and CONTROL) and vegetation (empty triangles; M—moss, L—lichens, ML—moss/lichens) factors, with pie slices based on the species percentage occurrence in different transects. *Bold* indicates species that significantly reacted to the transect (on the basis of *t* value biplot). Diagram scaling focused on case distances

transect that was considered. Some authors have shown that tardigrades have species-specific preferences towards the different types of material that were examined, e.g. mosses, lichens and leaf litter (e.g. Dastych 1980, 1988; Wright 1991; Guidetti et al. 1999; Jönsson 2003). Jönsson (2003) also demonstrated that the growth form of mosses might have some influence on tardigrade density, with "wefts" (mats) containing more individuals than other growth forms. Factors relating to the moss growth form, e.g. water-retention capacity, probably influence its suitability as a habitat for tardigrades (Jönsson 2003). However, the relationship between tardigrades and moss species (e.g. Kathman and Cross 1991), or even moss genera (Romano et al. 2001), has not been confirmed until today. Bartels and Nelson (2006) observed that tardigrade species' richness was always lower in lichens than in mosses in Great Smoky Mountains National Park (North Carolina, Tennessee, USA). Additionally, a study conducted in Costa Rican rainforests revealed that plant type had the weakest influence on tardigrade distribution (Kaczmarek et al. 2011b).

The effect of the seabird colony *per se* was responsible for 13.2 % of the variability in the tardigrade assemblage. This suggests that additional factors, besides plant communities' alteration, accounted for species composition of the tardigrades around the seabird colony. One of these factors could be soil pH, which was recognised to be lowered near little auk guano (Wait et al. 2005; Mulder et al. 2011; Zwolicki et al. 2013). Dastych (1985, 1988) divided tardigrade species into five categories with respect to their preferences for pH values: eucalciphil, polycalciphil, mesocalciphil, oligocalciphil and acalciphil. The SEABIRD transect should be classified as oligocalciphil or acalciphil (higher acidity), while the control area represented habitats of lower acidity (eucalciphil or polycalciphil). Most of the species found in the present study occurred in accordance with Dastych's (1985, 1988) assignment, and some of the species inhabit SEABIRD, and other CONTROL transect—oligocalciphil and eucalciphil, respectively (Fig. 4). However, three species were found in areas where they would not be expected given their assumed pH preferences and the soil pH within the transects. These were: *E. testudo* and *I. elegans* (apparently eucalciphil) found in the SEABIRD transect, and *P. recamieri* (acalciphil) found in both transects. While *E. testudo* can simply be a fortuitous faunal element in the SEABIRD transect (only one specimen recorded), the other two species were found in a higher number and could probably form stable populations.

A total of 32 taxa (25 determined to species level) were recorded that belonged to all ecological groups of tardigrades as distinguished by Guil and Sanchez-Moreno (2013): carnivores (e.g. species from genera *Macrobiotus* or *Paramacrobiotus*), herbivores (e.g. *Echiniscus* or *Hypsibius*) and microbivores (e.g. *Diphascon*). These records, along with previous studies carried out in the Ariekammen slopes and nearby areas (Węglarska 1965;

Dastych 1985; Zawierucha et al. 2015b), comprise the list of 49 known species, up until today, from this region. This is very high number given the fact that biodiversity indices (Chao 2 and ICE) have estimated that the number of tardigrade species inhabiting the slopes of Rotjesfjellet and Ariekammen should range from 41 to 69 taxa (Zawierucha et al. 2015b). In fact, this is more than half of the total tardigrade species known from the entire Svalbard archipelago (i.e. almost ca. 90 species). It cannot be excluded that the high number of tardigrade species in the Ariekammen area is a result of the little auk colony. However, it may also indicate that the currently reported diversity of Svalbard tardigrades is remarkably underestimated. Many areas within the Svalbard archipelago have never been sampled or were sampled unsystematically. This clearly shows that there is a great need for studies on the tardigrade distribution in the Arctic in order to fully comprehend their ecology and to recognise the true biodiversity of Arctic ecosystems.

New records

Studies on Ariekammen slopes conducted in the past (Węglarska 1964; Dastych 1985) provided records of five species which were not found in the present study, i.e. *R. oberhaeuseri* (Doyère 1840), *Tenuibiotus willardi* (Pilato 1977), *Milnesium tardigradum* Doyère, 1840 sensu lato, *Macrobiotus ariekammensis* (Węglarska 1965) and *Macrobiotus echinogenitus* Richters, 1904 (Węglarska 1964; Dastych 1985). However, due to some taxonomic confusions (see, e.g. Michalczyk et al. 2012a, b), all of these species (excluding *M. ariekammensis*, which was described from this area) could have been misidentifications of the species found in the recent studies (e.g. *Milnesium asiaticum* Tumanov, 2006, *M. eusrystomum* Maucci, 1991). Zawierucha et al. (2015b) reported an additional sixteen species from this region: *Adropion belgicae* (Richters, 1911), *A. prorsirostre* (Thulin, 1928), *A. scoticum scoticum* (Murray, 1905), *Bryodelphax parvulus* Thulin, 1928, *Calohypsibius ornatus* (Richters, 1900), *Diphascon tenue* Thulin, 1928, *Echiniscus blumi blumi* Richters, 1903, *E. capillatus* Ramazzotti, 1956, *Hypsibius microps* Thulin, 1928, *Isohypsibius prosostomus prosostomus* Thulin, 1928, *Macrobiotus hufelandi hufelandi* Schultze, 1834, *Milnesium asiaticum* Tumanov, 2006, *M. eusrystomum* Maucci, 1991, *Paramacrobiotus areolatus* (Murray, 1907), *Platicrista angustata* (Murray, 1905) and *Pseudechiniscus suillus* (Ehrenberg, 1853).

Another four taxa were also found: *Microhypsibius bertolanii* (Kristensen 1982), *Minibiotus* cf. *formosus*, *Ramazzottius* cf. *rupeus* and *Isohypsibius* cf. *reticulatus*, which has never been recorded in the Svalbard archipelago. Thus, the total number of taxa from Ariekammen area is now 49.

The genus *Microhypsibius* Thulin, 1928 was recorded in Svalbard for the first time. *Microhypsibius bertolanii* was originally described from a homothermic spring and ponds in Greenland (Kristensen 1982). Here it was found in a moss sample collected within the SEABIRD transect, on the coastal plain (11 m asl). Other species from the genus *Microhypsibius* are common in Arctic soils (Kristensen 1982), but the present findings extend *M. bertolanii* microhabitat preferences also to mosses.

So far, two species of the genus *Minibiotus* R.O. Schuster, 1980 have been reported from the Svalbard archipelago (Zawierucha et al. 2013). The second new record, *Minibiotus* cf. *formosus*, was found in the mixed, moss/lichen habitat within the CONTROL transect, and nominal species has been reported only from type locality in Estonia (Zawierucha et al. 2014). Although eggs were not found, the specimens corresponded well to the description presented by Zawierucha et al. (2014). This probably means that all older reports of species belonging to *Minibiotus* [especially *M. intermedius* (Plate, 1888)] reported from the Arctic need to be confirmed because of the increased number of new species being recorded for this genus. What is more, at present taxonomists are describing new species using taxonomic traits that were not considered important at the time when the *Minibiotus* representatives known from the Svalbard were reported (e.g. Claxton 1998; Fontoura et al. 2009). Thus, the number of species belonging to *Minibiotus* from the Svalbard archipelago can be much higher.

The genus *Isohypsibius* Thulin, 1928 is the most species-rich on the Svalbard archipelago (Zawierucha et al. 2013). Three species are endemic to Svalbard, and these are: *I. ceciliae* (Pilato and Binda, 1987), *I. karenae* (Zawierucha, 2013) and *I. coulsoni* (Pilato and Binda, 1987; Kaczmarek et al. 2012; Zawierucha 2013; Zawierucha et al. 2013). *Isohypsibius reticulatus* was previously known only from Italy (Sicily) and the former Yugoslavia (McInnes 1994), and so far, it was reported only from aquatic habitats (Pilato 1973). We found *I.* cf. *reticulatus* in mosses and lichen samples in the CONTROL transect. All records of *I. reticulatus* are based solely on morphological traits, but if molecular data in the future reveal great and discontinuous genetic variance between different populations of this taxon, the current opinion that the same species is known from the southern Europe and Spitsbergen may be refuted in favour of a species complex hypothesis.

Up to date, three species of the genus *Ramazzottius* Binda and Pilato, 1986 have been reported from the Svalbard archipelago (Zawierucha et al. 2013). *Ramazzottius rupeus* was originally described from lichens on Novaya Zemlya (Biserov 1999) and was known only from this locality. Although the eggs were not found, the specimens found in this study corresponded perfectly to the original description. *Ramazzottius* cf. *rupeus* was found in one mixed (moss/lichen) sample of the SEABIRD transect. All this together indicates that this species can be endemic to the Arctic region.

Every new record may underline the basic problem of knowing whether a species is newly establishing, alien/invasive or a mere vagrant, or was previously recorded from a given area but, due to the lower quality of optical equipment or certain taxonomic problems, it was misidentified. Again, this highlights the great importance of conducting studies on the present sort by recognising biodiversity in the polar regions.

Conclusions

This is the first comprehensive investigation of the impact of a seabird colony on tardigrade communities. It showed the clear response of these invertebrates to the seabird colony which was expressed as increased abundance and differentiated species composition. The findings of the study highlight the ecological importance of ornithogenic nutrients for a terrestrial ecosystem in the Arctic, and the necessity of considering this factor in investigations of invertebrate diversity and distribution patterns. Moreover, four taxa that are new to the Svalbard archipelago were discovered. Such a number revealed in a limited area illustrates the poor state of knowledge on tardigrade assemblages in the Arctic. The Conservation of Arctic Flora and Fauna report underlines that available data on Arctic invertebrates are sparse and that there is still a lack of complex information on behavioural and physiological divergence among species or the food web (Hodkinson 2013). Thus, the present study constitutes a good background for a discussion about the factors influencing invertebrate diversity in the Arctic.

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ORIGINAL PAPER

Tardigrada in Svalbard lichens: diversity, densities and habitat heterogeneity

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Abstract Tardigrades in lichens have been poorly studied with few papers published on their ecology and diversity so far. The aims of our study are to determine the (1) influence of habitat heterogeneity on the densities and species diversity of tardigrade communities in lichens as well as the (2) effect of nutrient enrichment by seabirds on tardigrade densities in lichens. Forty-five lichen samples were collected from Spitsbergen, Nordaustlandet, Prins Karls Forland, Danskøya, Fuglesongen, Phippsøya and Parrøya in the Svalbard archipelago. In 26 samples, 23 taxa of Tardigrada (17 identified to species level) were found. Twelve samples consisted of more than one lichen species per sample (with up to five species). Tardigrade densities and taxa diversity were not correlated with the number of lichen species in a single sample. Moreover, the densities of tardigrades was not significantly higher in lichens collected from areas enriched with nutrients by seabirds in comparison to those not enriched. The incorporation of previously published data on the tardigrades of Spitsbergen into the analysis showed that tardigrade densities was significantly higher in moss than it was in lichen samples. We propose that one of the most important factors influencing tardigrade densities is the cortex layer, which is a barrier for food sources, such as live photosynthetic algal cells in lichens. Finally, the new records of Tardigrada and the first and new records of lichens in Svalbard archipelago are presented.

Keywords High Arctic · Biodiversity · Ecology · Microhabitat heterogeneity · Mosses · Tardigrada

Introduction

Terricolous lichens are one of the main components of the vegetation of polar regions (Węgrzyn et al. 2011). The Arctic climate, with its low annual temperatures, reduces vascular plant colonization (Olech et al. 2011). Lichens are pioneer organisms, and some of them have the ability to assimilate atmospheric nitrogen, which allows them to colonize areas completely devoid of vegetation due to a lack of nutrients in the soil (Millbank 1978; Rychert et al. 1978; Belnap 2002; Węgrzyn and Wietrzyk 2015). Moreover, lichens provide an important habitat for various invertebrates including water bear species (Ramazzotti and Maucci 1983; Nelson 2002; Nelson et al. 2015).

Tardigrada, also known as water bears, is a phylum, whose representatives inhabit various ecosystems, from the deepest seas to the highest parts of mountains (Ramazzotti and Maucci 1983; Kaczmarek et al. 2015). Terrestrial tardigrades inhabit mosses, lichens, hepatics, rosette-type higher plants, algae, leaf litter and soil, while aquatic species inhabit either freshwater or marine habitats (e.g., Ramazzotti and Maucci 1983; Dastych 1988). Currently, more than 1200 tardigrade species have been described

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(Degma and Guidetti 2007; Vicente and Bertolani 2013; Degma et al. 2009–2016).

Various aspects of the diversity of lichen-dwelling micrometazoans have been discussed by different authors (e.g., Gerson and Seaward 1977; Bates et al. 2012; Šatkauskienė 2012; Hauck et al. 2014). First, Gerson and Seaward (1977) presented a complex discussion of the associations between invertebrates and lichens. The authors underlined the poor knowledge of the interactions between these lichens and their accompanying biota (i.e., micrometazoans) and suggested avenues for future research. Recent papers concerning tardigrades in lichens include taxonomical, ecological or physiological aspects (e.g., Greven 1972; Morgan and Lampart 1986; Rebecchi et al. 2006; Guil et al. 2009). In the scientific browsers Web of Science and Scopus, only 67/81 papers use the keywords “Tardigrada” and “lichens,” which is low in comparison to the keywords “Tardigrada” and “moss,” with 121/143 papers (http://apps.webofknowledge.com/, https://www.scopus.com/ accessed on 21 July 2016). Although studies of Arctic tardigrades have taken place since the early the twentieth century (Scourfield 1897) and the first comprehensive monographs were published more than 3 decades ago (Marcus 1936; Ramazzotti and Maucci 1983), lichens are less studied than mosses in terms of their tardigrade ecology. Most of the previous ecological studies have focused on the distribution of tardigrades within moss samples (e.g., Nelson 1975; Nelson and Adkins 2001; Jönsson 2003; Degma et al. 2011; Guil et al. 2009) and only Morgan and Lampard (1986), Miller et al. (1988), Bartels and Nelson (2007) and Guil et al. (2009) have presented studies on the relationship between lichens and tardigrades. Morgan and Lampard (1986) found seven species of tardigrade in four species of lichen. Miller et al. (1988) found four species of tardigrade in eight species of lichen. Bartels and Nelson (2007) found 35 tardigrade species in lichens and estimated the species richness of water bears, providing a maximum estimation of 37–45 tardigrade species in lichens. Lastly, Guil et al. (2009) described the species diversity and abundance of tardigrades in lichens collected from trees and rocks and found 17 tardigrade species.

Assemblages of invertebrates in the Arctic are usually more abundant in seabird colony areas because of the higher nutrient content (Zmudczyńska-Skarbek et al. 2015). It is well known that seabirds in polar regions strongly influence the composition of the ground and in many ways enhance the abiotic characteristics of the soil (Myrcha and Tatur 1991; Simas et al. 2007). In the Arctic, this results in greater plant diversity in the areas of bird cliffs than in the places where birds are absent (Dubiel and Olech 1992; Węgrzyn et al. 2011). As a consequence, such areas of greater plant diversity and biomass, mainly mosses, also provide a habitat for numerous invertebrate species (Zmudczyńska et al. 2012), including Tardigrada (Zawierucha et al. 2016).

Ecological analyses of the tardigrades from the Arctic tundra have been conducted by Dastych (1985), Johansson et al. (2013), Zawierucha et al. (2015a, b) and Zawierucha et al. (2016), who surveyed the influence of altitude, type of bedrock, seabird guano and substratum on tardigrade distribution, abundance, and species diversity. In this article, we provide novel information about Arctic tardigrade densities, species diversity and distribution with respect to environmental variables in Svalbard lichen samples, thus increasing our knowledge about this poorly studied tardigrade habitat.

Materials and methods

In total, we analyzed 45 samples collected in the summer of 2011 from Hornsund, Spitsbergen (Zawierucha et al. 2016), and in 2013 from Spitsbergen (Nissenfjella), Nordaustlandet, Prins Karls Forland, Danskøya, Fuglesongen, Phippsøya and Parrøya. Lichen samples were collected along a latitudinal gradient from the southern to the northern part of the Svalbard archipelago (Fig. 1), and samples were collected from 14 to 72 m a.s.l. Twenty samples were collected from areas influenced by seabird colonies (areas enriched in guano) and 23 samples from areas not influenced by seabirds. For two samples the presence/absence of seabird enrichment is unknown because this information was not noted during sampling. For a list of the samples and their GPS coordinates, see Online Resource 1.

The materials were stored in small paper bags, which allowed them to dry slowly, and were then weighed within a precision of ±0.01 g.

Statistical analysis

The number of tardigrades found in each sample was extrapolated for 10 g of dry mass of the vegetation to calculate the total density. Pearson correlation coefficients were used to assess the relationship between the tardigrade species diversity (number of taxa) and their densities in the lichens (determined in this study). Incorporating previously published data (Zawierucha et al. 2015a), we used the non-parametric Mann–Whitney test to test for the difference in the total density of water bears between lichens collected from areas enriched in nutrients ($N = 33$) and areas not enriched ($N = 23$) by seabird colonies. The Mann–Whitney test was also used to check for differences in Tardigrada abundance between moss ($N = 56$) and lichen samples ($N = 56$). Mann–Whitney tests were used because of the distinct deviations from normal distributions of the data; Shapiro–Wilk test, $p < 0.05$. For this comparison we combined previous data published by Zawierucha et al. (2015a) and Zawierucha et al. (2016) and randomly selected moss samples for comparison. Statistical analyses were performed with STATISTICA 10.0 (StatSoft, Inc., 2011).

Species identification

Tardigrada

All samples were treated following the standard methods for tardigrade examination (e.g., Dastych 1980; Ramazzotti and Maucci 1983; Stec et al. 2015). Each sample was soaked in water for ca. 5 h. Water containing tardigrades and detritus particles was then decanted and examined under a stereomicroscope. All extracted specimens, exuviae and eggs were mounted on microscope slides in Hoyer's medium and were then examined with a phase contrast microscope (PCM) (Olympus BX41) associated with an ART-CAM 300 MI photo camera and QuickPhoto Camera 2.3 software. Species were identified with the keys of Ramazzotti and Maucci (1983) and later original descriptions, re-descriptions and keys of Dastych (1985, 1988), Binda (1988), Fontoura and Pilato (2007), Kaczmarek and Michalczyk (2009) and Michalczyk et al. (2012ab). Nomenclature and new generic names follow Bertolani et al. (2014) and Vecchi et al. (2016). All the microscope slides were deposited in the Department of Animal Taxonomy and Ecology at Adam Mickiewicz University in Poznań, Poland.

Lichens

Lichens were identified by traditional taxonomic methods and standard microscopy. *Cladonia* species were determined using thin-layer chromatography (TLC) in solvent C, following the technique of Orange et al. (2001). Apothecial handmade sections were mounted in water, and their anatomical structures were measured in ethyl alcohol. Spot test reactions for apothecial pigments were examined in KOH and NaClO. For species determination, the following guides were used: Thomson (1984, 1997), Brodo et al. (2001), Smith et al. (2009) and Wirth (2013). The nomenclature follows the MycoBank Fungal Database (MycoBank 2015).

Results

In total, 321 specimens of Tardigrada belonging to 23 taxa (with 17 determined to species level) were found in 26 samples (42% of all samples collected). Analyzed lichen material included 20 lichen taxa (Online Resource 1). Due to the lack of tardigrade eggs, some of the specimens were determined to the species group level (see Online Resource 2). The most frequent species in the samples were *Hypsibius pallidus* Thulin, 1911 (present in nine samples), *Diphascon pingue pingue* (Marcus, 1936) (present in four samples) and *Adropion prorsirostre* (Thulin, 1928) (present in four samples). The highest number of tardigrades calculated per 10 g of dry material was 720 specimens (for samples analyzed in this study), which were found in a mixed lichen sample of *Cladonia arbuscula* (Wallr.) Flot. and *Cladonia rangiferina* (L.) Weber ex F.H. Wigg. The highest tardigrade diversity was found in two samples, each with four Tardigrada species (see Online Resource 2). It is worth noting that lichens with the highest tardigrade densities have a characteristic cottony-tomentose thallus surface composed of loose hyphae, and thus they do not have a typical cortical layer (e.g., *Cladonia arbuscula, C. mitis, C. rangiferina*). The average number of tardigrades calculated per 10 g of dry material for samples was 66 individuals.

Twenty samples were collected from areas that were enriched in nutrients because of seabird colonies, with 13 of these samples containing tardigrades (ca. 65 %). In comparison, 23 samples were collected from areas that were not influenced by seabirds, with 11 of these samples containing tardigrades (ca. 47.8 %) (see Online Resource 2). For two samples, data on the influence of guano were not available. Including previously published data, tardigrades in lichens were not more abundant in the samples collected from areas that were enriched in nutrients because of seabird colonies than in the samples collected from non-enriched areas (Mann–Whitney, $p = 0.13$). Furthermore, regardless of the nutrient status of lichens, tardigrades were more abundant in moss

Table 1 Statistics (mean, min., max., SE) for tardigrade densities in lichen samples analyzed in the present study and Zawierucha et al. (2015a)

	N	Mean	Min	Max	SE
All lichen samples	56	78.21	0	1196.26	27.20
Lichen from seabird colonies	33	118.90	0	1196.26	44.80
Lichens outside seabird colonies	23	19.82	0	125.00	7.13

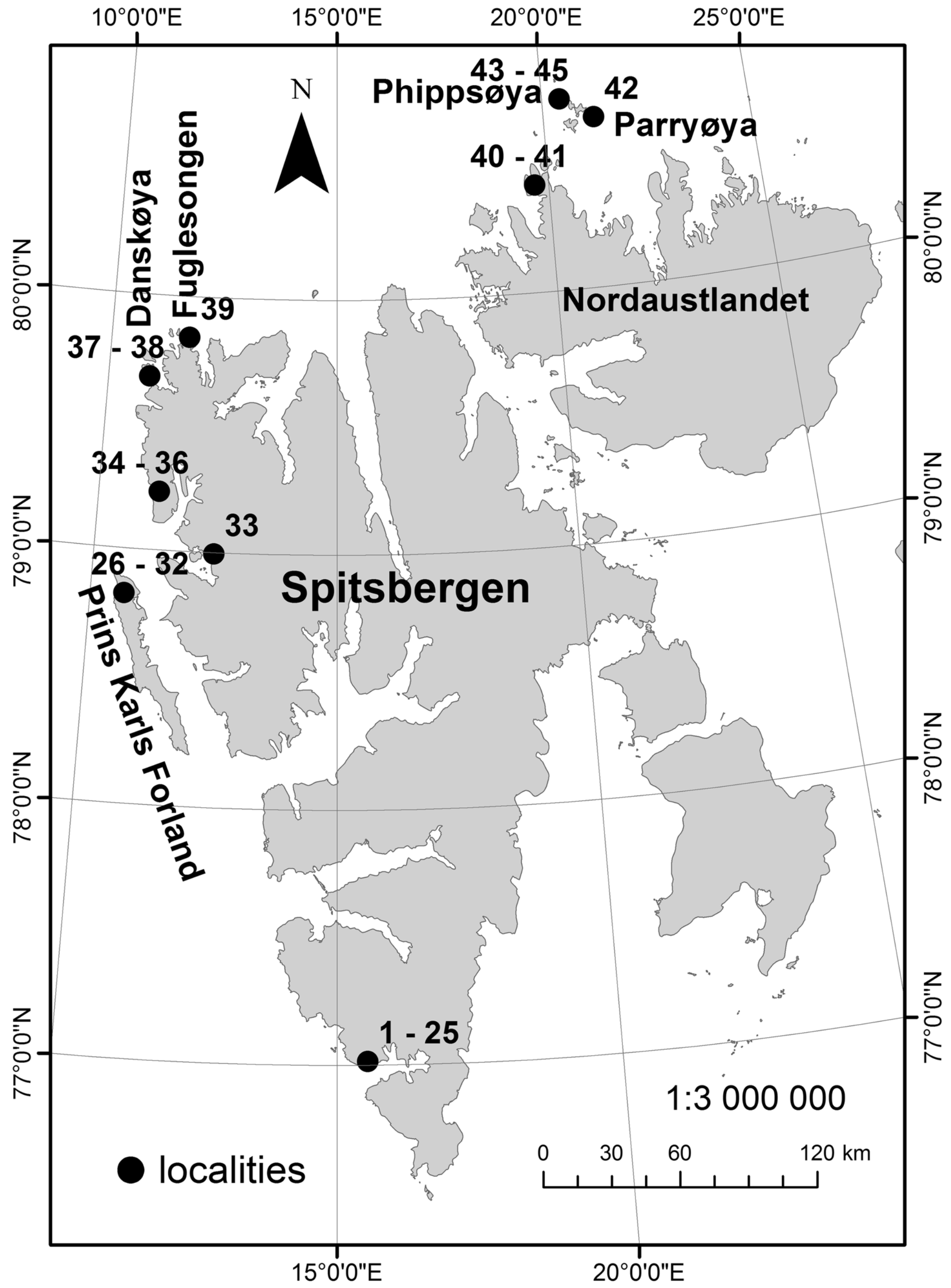

Fig. 1 Map of Svalbard with sampling sites

samples (Mann–Whitney, $p < 0.005$) (Fig. 2). The diversity of lichens species in single samples ranged from one to five species. Twelve of the 45 lichen samples consisted of more than one lichen species. Tardigrade densities and taxa diversity were not significantly related to the number of lichen species in a single sample (Pearson, $p = 0.72$, $r = -0.05$ and $p = 0.82$, $r = 0.04$, respectively).

Statistics (mean, minimum, maximum and standard error) of tardigrade density for combined lichen samples analyzed during this study as well as those of Zawierucha et al. (2015a) are presented in Table 1.

Tardigrades for the islands of Danskøya, Fuglesongen, Phippsøya and Parrøya are new records (Online Resource 2). The presences of tardigrades *A. prorsirostre, D. p. pingue* and *H. pallidus* from Prins Karls Forland, *A. prorsirostre, Echiniscus merokensis merokensis* Richters, 1904, and *Macrobiotus crenulatus* Richters, 1904, from Nordaustlandet are new records for these islands (see Online Resource 2). The first record of the lichen *Stereocaulon nanodes* Tuck. from Svalbard is reported, along with new records of lichens from Prins Karls Forland (*Cladonia amaurocrocea, C. macroceras, C. mitis, C. rangiferina*),

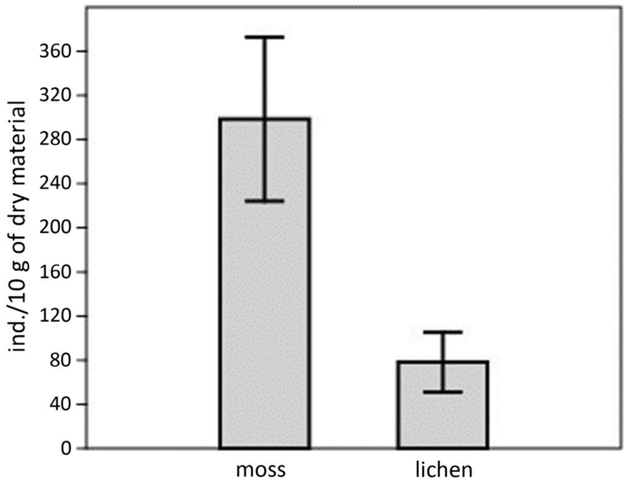

Fig. 2 Comparison of total Tardigrada density between vegetation types (abundances per 10 g of dry material). The error bar intervals represent the 95% confidence interval for the estimate of the mean (based on the standard error)

Danskøya (*Cladonia crispata* var. *cetrariformis*), Fuglesongen (*Cetraria hepatizon, Cladonia crispata* var. *cetrariformis, Sphaerophorus globosus, Stereocaulon alpinum*) and Phippsøya (*Cladonia crispata* var. *cetrariformis*); see Online Resource 1.

Discussion

Lichens are pioneers, but are also the most fragile and fastest disappearing organisms in the Arctic because they are affected by trampling and winter grazing by reindeer and face competition from vascular plants (Cornelissen et al. 2001; Storeheier et al. 2002; Węgrzyn et al. 2011). This study is an attempt to investigate factors such as habitat heterogeneity and nutrient enrichment of lichens by seabirds in terms of tardigrade density and species diversity in lichen thalli. Morgan and Lampard (1986) previously presented a study on the relationship between supralittoral lichens and tardigrades from Great Cumbrae Island in the Firth of Clyde, UK, and found seven species of tardigrades in four species of lichen along seashore. Unfortunately, the authors did not present their methods of calculation for the tardigrades in the samples; thus a comparison with the present study is not possible. Bartels and Nelson (2007) focused on species diversity estimators and predicted a lower number of tardigrade species in lichens than in mosses in Great Smoky National Park, USA. Guil et al. (2009) reported that *Echiniscus* species are more abundant in mosses and lichens collected from rocks than in leaf litter or in mosses and lichens collected from trees in the Sierra de Guadarrama, Central Spain. Moreover, Guil et al. (2009) noted significantly higher abundance and species richness in lichens collected from rocks because of the contribution of heterotardigrades (Guil et al. 2009). In our study the most frequent water bears were eutardigrades, and only three (not frequent) heterotardigrade species were found.

Despite the fact that lichens can constitute the only source of energy in cold desert areas, such as polar regions (Gerson and Seaward 1977), previous studies on Arctic tardigrades either did not focus on species diversity and ecology in lichens or noted a low number of specimens in lichens (e.g., Zawierucha et al. 2016). Studies conducted on the distribution and abundance of tardigrades in the Hornsund fjord (Spitsbergen) showed that one of the most significant factors influencing tardigrade abundance was vegetation type, with mosses having the most abundant individuals (Zawierucha et al. 2016).

Faunistic data for Svalbard are available only from limited areas (Węglarska 1965; Kaczmarek et al. 2012). Until now, extensive faunistic data on tardigrades in the Spitsbergen have only been published by Dastych (1985) and for the Svalbard archipelago by Zawierucha et al. (2013), with the authors analyzing different kinds of habitat samples, but predominantly mosses. The most frequently recorded species in the samples collected from our study were *H. pallidus* (present in 9 out of 26 samples) and *D. p. pingue* and *A. prorsirostre* (both present in 4 out of 26 samples) (Online Resource 2). Bartels and Nelson (2007) found 35 taxa of tardigrades in lichens collected from Great Smoky National Park. In contrast, only five of the same species were recorded in our study: *Adropion s. scoticum* (Murray, 1905), *Diphascon p. pingue* (Marcus, 1936), *Hypsibius dujardini* (Doyère, 1840), *Platicrista angustata* (Murray, 1905) and *Mesobiotus harmsworthi harmsworthi* (Murray, 1907). Guil et al. (2009) found 17 and 14 species in lichens collected from rocks and trees, respectively. *Echiniscus bisetosus, E. canadensis, E. m. merokensis* and *R. oberhaeuseri* were highly abundant in lichen samples from Spain (Guil et al. 2009). Only one species, *E. m. merokensis*, was recorded in both Svalbard and Spain. Tardigrade species composition in different biogeographical zones may be influenced by kind of substratum, general climatic conditions (e.g., humidity and temperature) or topographic features.

In the Arctic, seabirds are the link between marine and terrestrial ecosystems. They feed in the sea and nest on the land where they produce guano, which results in greater plant diversity in the areas surrounding bird colonies (Isaksen and Gavrilo 2000; Stempniewicz et al. 2007). Jennings (1976a, b) showed that in samples collected around vertebrate guano deposits from seabirds and seals, tardigrade densities are higher than in areas not affected by these nutrients in the Antarctic. However, Porazinska et al. (2002) and Smykla et al. (2012) showed that plant diversity and thus tardigrade abundance are decreased on flat regions of the Antarctic because of the high-salinity guano and trampling by penguins. Conversely, in the Arctic, seabird guano positively affected the biomass of mosses in which tardigrade abundance is higher than it is in lichens (Zawierucha et al. 2016). The positive influence of the Arctic seabirds on invertebrate densities and biomass has also been confirmed for other invertebrate groups (e.g., Zmudczyńska et al. 2012, 2015). However, to the best of our knowledge comparative tardigrade abundance in lichens with and without guano has never been studied previously. Here we show that the effect of seabird nutrients on tardigrade abundance in lichens was not significant and tardigrades were more abundant in mosses than in lichens.

The 12 collected samples consisted of more than 1 lichen species per sample (with up to 5 species per sample). In previous ecological studies, the diversity of invertebrates increased with the heterogeneity of the environment on various scales, e.g., forest age classes and complex and simple forms of plants (Niemelä et al. 1996; Benton et al. 2003; Taniguchi et al. 2003). But here, the tardigrade species diversity and abundance were not correlated to the number of lichen species in a single sample. It is possible that the biodiversity of tardigrades is affected by the availability of food sources, such as live photosynthetic algal cells in lichens. Water bears may feed on living, photosynthesizing eukaryotic cells (e.g., Altiero and Rebecchi 2001; Guidetti et al. 2012). Therefore, it is possible that the greater number of water bears noted in bryophytes than in lichens may be associated with easier access to photosynthetic cells in mosses. Alternatively, it may be related to the shape of the lichen (e.g., foliose, fruticose or crustose) and exposure to sunlight.

Conclusion

The results of this study underline the difficulties of surveying habitat associations of tardigrade species with single species of lichens in the Arctic. The 12 collected samples consisted of more than 1 lichen species per sample (with up to 5 species per sample). Contrary to expectation, the tardigrade taxa diversity and densities did not increase with the number of lichen species in a single sample. Additionally, we show that the nutrients from seabird colonies did not influence tardigrade densities in lichens; generally, tardigrades were more abundant in mosses than in lichens. In our study, we have presented higher numbers of tardigrades associated with some of the lichens, mainly *Cladonia arbuscula, C. mitis* and *C. rangiferina* (see Online Resource 1). These lichens, in addition to *S. alpinum*, do not have a thick surface layer and thus potentially provide the tardigrades with easier access to the photobiont layer and algal cells of the lichen. Usually, the most abundant tardigrades within samples are herbivores, with buccal tube morphology as discussed by Guidetti et al. (2012) and Guil and Sanchez-Moreno (2013). Thus, the availability of algal cells could influence tardigrade abundance in lichens. Nevertheless, factors such as pH, AMP (anti-microbial proteins) and the presence of other invertebrates (predators) in the sample should also be considered in future studies of the High Arctic lichen tardigrade assemblages.

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III SUPPLEMENTS

Supplementary file for: Zawierucha K., Coulson S.J., Michalczyk Ł. & Kaczmarek Ł. 2013. Current knowledge of the Tardigrada of Svalbard with the first records of water bears from Nordaustlandet (High Arctic). *Polar Research 32*. Correspondence: Krzysztof Zawierucha, Department of Animal Taxonomy and Ecology, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, PL-61-614 Poznań, Poland. E-mail: k.p.zawierucha@gmail.com.

A taxonomical list of Tardigrada known from the assayed islands of the Svalbard Archipelago. Dubious records are denoted with parentheses. A reference list follows the table. The total number of tardigrades from Svalbard consists of 97 taxa with 85 valid species (Coulson et al. 2014 mistakenly listed 92 taxa).

Species	Spitsbergen	Prins Karls Forland	Hopen	Bjørnøya	Edgeøya	Barentsøya	Amsterdamøya	Kong Ludvigøyane	Nordaustlandet	Svenskøya	Ryke Yseøyane	References and remarks
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	
Bryodelphax parvulus Thulin, 1928	+											^{a} Łagisz 1999
Bryodelphax parvuspolaris Kaczmarek, Zawierucha et al. 2012	+											^{a} Kaczmarek, Zawierucha et al. 2012
Bryodelphax sinensis (Pilato, 1974)	(+)											^{a} Dastych 1985 (probably a misidentified *B. parvuspolaris*)
Diploechiniscus oihonnae (Richters, 1903)	+			+			+	+				^{a} Richters 1911a, b ^{d} Richters 1911b ^{g} Richters 1904
Echiniscus arctomys Ehrenberg, 1853	+			+								^{a} Scourfield 1897; Richters 1903; Richters 1904; Murray 1907; Marcus 1936 ^{d} Summerhayes & Elton 1923
Echiniscus blumi blumi Richters, 1903	+											^{a} Richters 1903; Richters 1904; Marcus 1936; Dastych 1985; Łagisz 1999; Bernardová & Košnar 2012 (mentioned as cf.; extracted from Holocene sediments)
Echiniscus capillatus Ramazzotti, 1956	+											^{a} Dastych 1985; Łagisz 1999
Echiniscus columinis Murray, 1911	+											^{a} Węglarska 1965
Echiniscus granulatus (Doyère, 1840)	+			+								^{a} Dastych 1985; Maucci 1996; Łagisz 1999 ^{d} Richters 1911b

Species	Spitsbergen	Prins Karls Forland	Hopen	Bjørnøya	Edgeøya	Barentsøya	Amsterdamøya	Kong Ludvigøyane	Nordaustlandet	Svenskøya	Ryke Yseøyane	References and remarks
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	
Echiniscus merokensis merokensis Richters, 1904	+	+			+		+					[a] Marcus 1936; Dastych 1985; Maucci 1996; Kaczmarek, Zawierucha et al. 2012 [b] present study [e] Maucci 1996 [g] Richters 1904
Echiniscus quadrispinosus quadrispinosus Richters, 1902	+											[a] Marcus 1936; Kaczmarek, Zawierucha et al. 2012
Echiniscus spiculifer Schaudinn, 1901	+											[a] Marcus 1936
Echiniscus spinulosus (Doyère, 1840)	+											[a] Marcus 1936
Echiniscus testudo (Doyère, 1840)	+			+								[a] Richters 1904; Marcus 1936; Dastych 1985; Maucci 1996; Kaczmarek, Zawierucha et al. 2012 [d] Richters 1904; Summerhayes & Elton 1923, Marcus 1936
Echiniscus trisetosus Cuénot, 1932				(+)								[d] Marcus 1936 (author indicated that the Bjørnøya record was uncertain)
Echiniscus wendti Richters, 1903	+						+	+				[a] Richters 1911a; Marcus 1936; Węglarska 1965; Dastych 1985; Łagisz 1999; Kaczmarek, Zawierucha et al. 2012 [g] Richters 1903, 1904 [h] Richters 1911b
Pseudechiniscus islandicus (Richters, 1904)			+									[c] Van Rompu & De Smet 1996
Pseudechiniscus suillus (Ehrenberg, 1853)	+	+	+		+	+			+			[a] Richters 1911a; Marcus 1936; Węglarska 1965; Dastych 1985; Kaczmarek, Zawierucha et al. 2012 [b] Murray 1907 [c] Van Rompu & De Smet 1996 [e] Maucci 1996 [f] Van Rompu & De Smet 1991 [i] present study
Pseudechiniscus victor (Ehrenberg, 1853)	+							+		+	+	[a] Marcus 1936; Węglarska 1965; Dastych 1985; Łagisz 1999 [h, j, k] Richters 1911b

Species	Spitsbergen	Prins Karls Forland	Hopen	Bjørnøya	Edgeøya	Barentsøya	Amsterdamøya	Kong Ludvigøyane	Nordaustlandet	Svenskøya	Ryke Yseøyane	References and remarks
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	
Testechiniscus spitsbergensis (Scourfield, 1897)	+		+	+	+	+			+			^{a} Scourfield 1897; Richters 1903; Richters 1904; Richters 1911a; Marcus 1936; Węglarska 1965; Dastych 1985; Łagisz 1999; Kaczmarek, Zawierucha et al. 2012 ^{c} Richters 1911a; Van Rompu & De Smet 1996 ^{d} Richters 1911b; Marcus 1936 ^{e} present study ^{f} Van Rompu & De Smet 1991 ^{i} present study
Milnesium asiaticum Tumanov, 2006	+											^{a} Kaczmarek, Zawierucha et al. 2012
Milnesium eurystomum Maucci, 1991	+											^{a} Kaczmarek, Zawierucha et al. 2012
Milnesium tardigradum	(+)				(+)							^{a} Richters 1903; Richters 1904; Marcus 1936; Węglarska 1965; Dastych 1985; Maucci 1996; Łagisz 1999 ^{e} Maucci 1996 General remark: given all reports of *M. tardigradum* Doyère, 1840 in Svalbard are prior to the redescription of the species (Michalczyk et al. 2012a, b), they should be considered as dubious (i.e., listed as *Milnesium* sp.) until verified against the neotype series.
Bertolanius nebulosus (Dastych, 1983)	+											^{a} Dastych 1983, 1985
Bertolanius smreczynskii (Węglarska, 1970)	+				+							^{a} Klekowski & Opaliński 1989; Janiec 1996 ^{e} De Smet et al. 1988
Bertolanius weglarskae (Dastych, 1972)			+	+		+						^{c} Van Rompu & De Smet 1996 ^{d} Van Rompu & De Smet 1988 ^{f} Van Rompu & De Smet 1991
Calohypsibius ornatus (Richters, 1900)	+			+			+					^{a} Murray 1907; Richters 1911a; Marcus 1936; Dastych 1985; Kaczmarek, Zawierucha et al. 2012 ^{d} Richters 1904; Murray 1907; Summerhayes & Elton 1923; Marcus 1936 ^{g} Richters 1903, 1904

Species	Spitsbergen	Prins Karls Forland	Hopen	Bjørnøya	Edgeøya	Barentsøya	Amsterdamøya	Kong Ludvigøyane	Nordaustlandet	Svenskøya	Ryke Yseøyane	References and remarks
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	
Diphascon (*Diphascon*) *alpinum* Murray, 1906	+	+		+	+							[a] Marcus 1936; Węglarska 1965; Łagisz 1999 (according to Dastych [1985], Marcus [1936] and Węglarska [1965] mistakenly determined this species as *Diphascon* [*D.*] *pingue pingue*) [b] Murray 1907 [d] Van Rompu & De Smet 1988 [e] De Smet et al. 1988
Diphascon (*Diphascon*) *chilenense* Plate, 1888	+	+										[a] Marcus 1936 [b] Murray 1907
Diphascon (*Diphascon*) *oculatum oculatum* Murray, 1906	+											[a] Dastych 1985
Diphascon (*Diphascon*) *pingue pingue* (Marcus, 1936)	+		+			+						[a] Dastych 1985 (Maucci [1996] suggested that Dastych [1985] mistakenly determined this species and that the specimens belong to *D. [D.] chilenense*); Kaczmarek, Zawierucha et al. 2012 [c] Van Rompu & De Smet 1996 [f] Van Rompu & De Smet 1991
Diphascon (*Diphascon*) *recamieri* Richters, 1911	+	+	+		+							[a] Richters 1911a; Marcus 1936; Węglarska 1965; Dastych 1985; De Smet & Van Rompu 1994; Janiec 1996; Maucci 1996; Łagisz 1999; Kaczmarek, Zawierucha et al. 2012; Zawierucha 2013 [b,e] present study [c] Richters 1911a; Van Rompu & De Smet 1996
Diphascon (*Diphascon*) *stappersi* Richters, 1911	+		+	+		+						[a,d] Richters 1911a; Marcus 1936 [c] Richters 1911a [f] Van Rompu & De Smet 1991
Diphascon (*Diphascon*) *tenue* Thulin, 1928	+											[a] Węglarska 1965; Dastych 1985; Maucci 1996
Diphascon (*Adropion*) *arduifrons* Thulin, 1928	+											[a] Dastych 1985

Species	Spitsbergen	Prins Karls Forland	Hopen	Bjørnøya	Edgeøya	Barentsøya	Amsterdamøya	Kong Ludvigøyane	Nordaustlandet	Svenskøya	Ryke Yseøyane	References and remarks
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	
Diphascon (Adropion) behanae Dastych, 1987	+											[a] Dastych 1985 (reported as *Diphascon* sp. nov. ? and later described based on material from Spitsbergen and additional specimens from Canada [Dastych 1987])
Diphascon (*Adropion*) *belgicae* Richters, 1911	+		+	+								[a] Richters 1911a; Marcus 1936; Dastych 1985, Maucci 1996 [c] Richters 1911a [d] Richters 1911b; Marcus 1936
Diphascon (*Adropion*) *prorsirostre* Thulin, 1928	+								+			[a] Kaczmarek, Zawierucha et al. 2012 [i] present study
Diphascon (*Adropion*) *scoticum scoticum* Murray, 1905	+	+	+		+	+						[a] Murray 1907; Węglarska 1965; Dastych 1985; Łagisz 1999; Kaczmarek, Zawierucha et al. 2012 [b] Murray 1907 [c] Van Rompu & De Smet 1996 [e] De Smet et al. 1988 [f] Van Rompu & De Smet 1991
Acutuncus antarcticus (Richters, 1904)						(+)						[f] Van Rompu & De Smet 1991 General remark: according to Dastych (1991) all reports of *A. antarcticus* from the Northern Hemisphere are misidentifications.
Borealibius zetlandicus (Murray, 1907)	+	+										[a] Marcus 1936; Łagisz 1999 [b] Murray 1907
Hypsibius arcticus (Murray, 1907)	+	+	+									[a] Richters 1911a; Marcus 1936; Węglarska 1965; Dastych 1985 (author did not find eggs which made it impossible to determine his specimens to the species level) [b] Murray 1907 [c] Richters 1911a General remarks: Dastych (1991) suggested that status of *H. arcticus* should be verified due to insufficient morphological information. According to R. M. Kristensen (pers. comm.) most of the records of *H. arcticus* from Arctic are valid.
Hypsibius biscuitiformis Bartoš, 1960	(+)											[a] Łagisz 1999 (author signalised that this identification was uncertain)

Species	Spitsbergen	Prins Karls Forland	Hopen	Bjørnøya	Edgeøya	Barentsøya	Amsterdamøya	Kong Ludvigøyane	Nordaustlandet	Svenskøya	Ryke Yseøyane	References and remarks
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	
Hypsibius convergens (Urbanowicz, 1925)	+				+	+						[a] Marcus 1936 (author signalised that the Spitsbergen record is uncertain); Węglarska 1965; Dastych 1985; Janiec 1996; Łagisz 1999 [e] present study [f] Van Rompu & De Smet 1991
Hypsibius dujardini (Doyère, 1840)	+	+	+	+	+	+	+	+				[a] Richters 1903; Richters 1904; Richters 1911a; Marcus 1936; Węglarska 1965; Dastych 1985; Janiec 1996; Maucci 1996; Łagisz 1999; Kaczmarek, Zawierucha et al. 2012; Zawierucha 2013 [b] Murray 1907 [c] Van Rompu & De Smet 1996 [d] Van Rompu & De Smet 1988 [e] De Smet et al. 1988 [f] Van Rompu & De Smet 1991 [g] Richters 1904; De Smet et al. 1987 [h] Richters 1911b
Hypsibius microps Thulin, 1928	+											[a] Maucci 1996; Kaczmarek, Zawierucha et al. 2012
Hypsibius pallidus Thulin, 1911	+											[a] Węglarska 1965; Dastych 1985; Janiec 1996; Łagisz 1999; Kaczmarek, Zawierucha et al. 2012
Hypsibius sp.				(+)								[d] Marcus 1936 (author indicated that the Bjørnøya record was uncertain)
Mesocrista spitzbergensis (Richters, 1903)	+	+	+	+	+		+					[a] Marcus 1936; Węglarska 1965; Dastych 1985; Klekowski & Opaliński 1989; Zawierucha 2013 [b] Murray 1907 [c] Van Rompu & De Smet 1996 [d] Van Rompu & De Smet 1988 [e] De Smet et al. 1988 [g] Richters 1903, 1904
Platicrista angustata (Murray, 1905)	+	+										[a] Murray 1907; Marcus 1936; Dastych 1985; Maucci 1996; Kaczmarek, Zawierucha et al. 2012 [b] Murray 1907
Hebesuncus conjungens (Thulin, 1911)	+					+						[a] Dastych 1985; Kaczmarek, Zawierucha et al. 2012 [f] Van Rompu & De Smet 1991
Ramazzottius cataphractus (Maucci, 1974)	+											[a] Dastych 1985

Species	Spitsbergen	Prins Karls Forland	Hopen	Bjørnøya	Edgeøya	Barentsøya	Amsterdamøya	Kong Ludvigøyane	Nordaustlandet	Svenskøya	Ryke Yseøyane	References and remarks
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	
Ramazzottius montivagus (Dastych, 1983)	+											^{a} Datych 1983, Dastych 1985
Ramazzottius oberhaeuseri (Doyère, 1840)	+	+		+								^{a} Richters 1903; Richters 1904; Richters 1911b; Marcus 1936; Węglarska 1965; Dastych 1985 ^{b} Murray 1907 ^{d} Richters 1911b, Marcus 1936
Doryphoribius macrodon Binda et al. 1980	+											^{a} Binda et al. 1980; Dastych 1985
Halobiotus arcturulius Crisp & Kristensen, 1983	+											^{a} Mokievsky 1992
Halobiotus crispae Kristensen, 1982	+											^{a} Smykla et al. 2011
Isohypsibius annulatus annulatus (Murray, 1905)				+								^{d} Murray 1907; Marcus 1936
Isohypsibius ceciliae Pilato & Binda, 1987	+											Pilato & Binda 1987
Isohypsibius coulsoni Kaczmarek, Zawierucha et al. 2012	+	+										^{a} Kaczmarek, Zawierucha et al. 2012 ^{b} present study
Isohypsibius dastychi Pilato et al. 1982	+											^{a} Pilato et al. 1982; Dastych 1985
Isohypsibius elegans Binda & Pilato, 1971	+		+									^{a} Dastych 1985 ^{c} Van Rompu & De Smet 1996
Isohypsibius granulifer granulifer Thulin, 1928	+		+	+	+	+						^{a} Dastych 1985; De Smet & Van Rompu 1994; Janiec 1996), ^{c} Van Rompu & De Smet 1996 ^{d} Van Rompu &De Smet 1988 ^{e} De Smet et al. 1988 ^{f} Van Rompu & De Smet 1991
Isohypsibius karenae Zawierucha, 2013	+											^{a} Zawierucha 2013
Isohypsibius marcellinoi Binda and Pilato, 1971	(+)				(+)							^{a} Łagisz 1999 (author indicated that her identification was uncertain) ^{e} present study
Isohypsibius papillifer bulbosus (Marcus, 1928)				+		+						^{d} Van Rompu & De Smet 1988 ^{f} Van Rompu & De Smet 1991

Species	Spitsbergen	Prins Karls Forland	Hopen	Bjørnøya	Edgeøya	Barentsøya	Amsterdamøya	Kong Ludvigøyane	Nordaustlandet	Svenskøya	Ryke Yseøyane	References and remarks
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	
Isohypsibius papillifer papillifer (Murray, 1905)	+		+	+		+						[a] Richters 1911a; Marcus 1936 [d] Van Rompu & De Smet 1988 [c] Van Rompu & De Smet 1996 [f] Van Rompu & De Smet 1991
Isohypsibius prosostomus prosostomus Thulin, 1928	+											[a] Węglarska 1965; Dastych 1985; Maucci 1996
Isohypsibius sattleri (Richters, 1902)	+											[a] Dastych 1985
Isohypsibius schaudinni (Richters, 1909)	(+)											[a] Richters 1909; Marcus 1936; Węglarska 1965. General remark: according to Dastych (1988) species requires rediscription.
Isohypsibius tetradactyloides (Richters, 1907)	+											[a] Węglarska 1965; Łagisz 1999 (author indicated that her identification was uncertain).
Isohypsibius tuberculatus (Plate, 1888)	+	+										[a] Scourfield 1897; Marcus 1936 [b] Murray 1907 General remark: according to Dastych (1988) species ought to be considered as species *dubia et inquirenda*.
Thulinius augusti (Murray, 1907)	(+)											[a] Mihelčič 1971 General remark: according to Bertolani et al. (1999) it is not possible to determine which record actually refers to true *T. augusti.*
Macrobiotus ariekammensis Węglarska, 1965	+											[a] Węglarska 1965; Dastych 1985; Łagisz 1999
Macrobiotus crenulatus Richters, 1904	+	+	+		+		+					[a] Maucci 1996; Kaczmarek, Zawierucha k et al. 2012; Zawierucha 2013 [b] Murray 1907 [c] Van Rompu & De Smet 1996 [e] Maucci 1996 [g] Richters 1904

Species	Spitsbergen	Prins Karls Forland	Hopen	Bjørnøya	Edgeøya	Barentsøya	Amsterdamøya	Kong Ludvigøyane	Nordaustlandet	Svenskøya	Ryke Yseøyane	References and remarks
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	
Macrobiotus echinogenitus Richters, 1904	+	+	+	+	+	+	+					[a] Richters 1904; Richters 1911a, b; Marcus 1936; Węglarska 1965; Dastych 1985; Klekowski & Opaliński 1989; Łagisz 1999 [b] Murray 1907 [c] Richters 1911a; Van Rompu & De Smet 1996 [d] Richters 1911b; Marcus 1936 [e] De Smet et al. 1988 [f] Van Rompu & De Smet 1991 [g] Richters 1903, 1904
Macrobiotus grandis Richters, 1911	+											[a] Richters 1911a
Macrobiotus harmsworthi harmsworthi Murray, 1907	+	+	+		+			+				[a] Richters 1911a; Marcus 1936; Węglarska 1965; Dastych 1985; Klekowski & Opaliński 1989; Maucci 1996; Łagisz 1999; Kaczmarek, Zawierucha et al. 2012; Bernardová & Košnar 2012 (mentioned as cf.*;* extracted from Holocene sediments) [b] Murray 1907 [c] Van Rompu & De Smet 1996 [e] Maucci 1996 [h] Richters 1911b
Macrobiotus harmsworthi obscurus Dastych, 1985	+											[a] Dastych 1985, Kaczmarek, Zawierucha et al. 2012
Macrobiotus hufelandi hufelandi C.A.S. Schultze, 1833	+	+	+	+	+							[a] Scourfield 1897; Richters 1904; Murray 1907; Richters 1911a; Marcus 1936; Węglarska 1965; Dastych 198; Łagisz 1999; Kaczmarek, Zawierucha et al. 2012; Bernardová & Košnar 2012 (mentioned only in figure 5 but omitted in the text; extracted from Holocene sediments) [b] Murray 1907 [c] Richters 1911a [d] Van Rompu & De Smet 1988; Summerhayes & Elton 1923 [e] present study

Species	Spitsbergen	Prins Karls Forland	Hopen	Bjørnøya	Edgeøya	Barentsøya	Amsterdamøya	Kong Ludvigøyane	Nordaustlandet	Svenskøya	Ryke Yseøyane	References and remarks
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	
Macrobiotus islandicus islandicus Richters, 1904	+	+			+				+			*[a]* Murray 1907; Marcus 1936; Węglarska 1965; Dastych 1985; Klekowski & Opaliński 1989; Maucci 1996; Łagisz 1999; Kaczmarek, Zawierucha et al. 2012 *[b]* Murray 1907 *[e]* present study
Macrobiotus occidentalis occidentalis Murray, 1910	+											*[a]* Węglarska 1965
Macrobiotus spectabilis Thulin, 1928	+											*[a]* Klekowski & Opaliński 1989; Łagisz 1999
Minibiotus crassidens (Murray, 1907)	+											*[a]* Węglarska 1965
Minibiotus intermedius (Plate, 1888)	(+)	(+)										*[a]* Richters 1904; Richters 1911a; Marcus 1936; Dastych 1985; Łagisz 1999 *[b]* Murray 1907 General remark: many older reports of this species need to be confirmed because of a number of new species similar to *M. intermedius* that have been described using taxonomic traits that were not considered important at the time when *M. intermedius* was being reported from all over the world (e.g., Claxton 1998, Li et al. 2008, Fontoura et al. 2009).
Paramacrobiotus areolatus (Murray, 1907)	+		+									*[a]* Murray 1907; Richters 1911a; Marcus 1936; Węglarska 1965; Dastych 1985; Maucci 1996; Łagisz 1999 *[c]* Richters 1911a
Paramacrobiotus richtersi (Murray, 1911)	+											*[a]* Węglarska 1965; Mihelcic 1971; Dastych 1985; Łagisz 1999
Richtersius coronifer (Richters, 1903)	+											*[a]* Richters 1903; Richters 1904; Marcus 1936; Węglarska 1965; Dastych 1985; Maucci 1996; Łagisz 1999; Bernardová & Košnar 2012 (mentioned as cf.; extracted from Holocene sediments)
Tenuibiotus voronkovi (Tumanov, 2007)	+				+				+			*[a]* Tumanov 2007 *[e]* present study *[i]* present study
Tenuibiotus willardi (Pilato, 1977)	+											*[a]* Dastych 1985; Maucci 1996

Species	Spitsbergen	Prins Karls Forland	Hopen	Bjørnøya	Edgeøya	Barentsøya	Amsterdamøya	Kong Ludvigøyane	Nordaustlandet	Svenskøya	Ryke Yseøyane	References and remarks
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	
Dactylobiotus ambiguus (Murray, 1907)	+	+		+	+							[a] Janiec 1996 [b] Murray 1907 [d] Van Rompu & De Smet 1988 [e] De Smet et al. 1988
Dactylobiotus dispar (Murray, 1907)	+	+	+			+						[a] Marcus 1936; Klekowski & Opaliński 1989 [b] Murray 1907 [c] Van Rompu & De Smet 1996 [f] Van Rompu & De Smet 1991
Dactylobiotus macronyx (Dujardin, 1851)	(+)		(+)	(+)								[a] Marcus 1936 (author indicated that the Spitsbergen record is uncertain) [d] Summerhayes & Elton 1923 [c] Richard 1898 General remark: despite the fact that the species is nominal for the genus, it is considered *nomen dubium* because of its poor original description and the lack of the type series (Binda & Pilato 1999; Kaczmarek, Schabetsberger et al. 2012).
Murrayon dianeae (Kristensen, 1982)			+			+						[c] Van Rompu & De Smet 1996 [f] Van Rompu & De Smet 1991
Murrayon hastatus (Murray, 1907)	(+)											[a] Bernardová & Košnar 2012 (mentioned as cf.; extracted from Holocene sediments)
Murrayon hibernicus (Murray, 1911)	+				+							[a] Dastych 1985; Łagisz 1999 [e] present study
Murrayon pullari (Murray, 1907)	+	+			+		+					[a] Marcus 1936; De Smet et al. 1987 [b] Murray 1907 [e] De Smet et al. 1988 [g] De Smet et al. 1987
Total number of taxa	89	23	23	23	22	16	9	5	5	1	1	
Number of valid species	80	22	22	20	20	15	9	5	5	1	1	

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Tardigrada in Svalbard lichens: diversity, densities and habitat heterogeneity. Polar Biology. Krzysztof Zawierucha, Michał Węgrzyn, Marta Ostrowska, Paulina Wietrzyk. Department of Animal Taxonomy and Ecology, Faculty of Biology, Adam Mickiewicz University in Poznań, Umultowska 89, PL-61-614 Poznań, Poland, corresponding author: k.p.zawierucha@gmail.com

Online Resource 1. List of lichens with: sample ID – number of a sample, Island: S – Spitsbergen, PKF – Prins Karls Forland, D – Danskøya, F – Fuglesongen, N – Nordaustlandet, P – Parrøya, Ph – Phippsøya; weight of dry material; substratum: R – rock, S – soil, P – peat; presence /absence of seabird colony (area is/is not nutrient enriched by seabird guano): 1 – presence, 0 – absence, ? – lack of data; calculation of tardigrades per 10 gram of dry material; GPS coordinates. Samples 1-25 were previously analyzed in Zawierucha et al. (2016).

Sample ID	**Island**	**Weight of dry mterial**	**Substratum**	**Presence /absence of seabird colony**	***Cetrariella delisei* (Bory ex Schaer.) Kärnefelt & A. Thell**	***Ochrolechia frigida* (Sw.) Lynge**	***Cladonia pyxidata* (L.) Hoffm**	***Cetraria ericetorum* Opiz**	***Stereocaulon alpinum* Laurer**	***Cladonia crispata var. cetrariformis* (Delise) Vain.**	***Cladonia crispata* (Ach.) Flot.**	***Sphaerophorus globosus* (Huds.) Vain.**	***Flavocetraria nivalis* (L.) Kärnefelt & A. Thell**	***Cladonia mitis* Sandst.**	***Melanelia hepatizon* (Ach.) Vain.**	***Lecidea ramulosa* Th. Fr.**	***Cladonia amaurocraea* (Flörke) Schaer.**	***Flavocetraria cucullata* (Bellardi) Kärnefelt**	***Cladonia arbuscula* (Wallr.) Flot.**	***Cladonia rangiferina* (L.) Weber ex F.H. Wigg.**	***Cladonia macroceras* (Delise) Ahti**	***Parmelia omphalodes* (L.) Ach.**	***Umbilicaria decussata* (Vill.) Zahlbr.**	***Stereocaulon nannodes* Tuck.**	**Tardigrades per 10 g/dry material**	**coordinates**
1.	S	1,69	S	1	1	1	1																		0	N 77° 0' 17.7"; E 15° 33' 5.94"
2.	S	1,42	R	1				1																	0	N 77° 0' 17.7"; E 15° 33' 5.94"
3.	S	1,17	R	1				1																	9	N 77° 0' 21.48"; E 15° 33' 37.98"
4.	S	0,63	S	0				1	1																0	N 77° 0' 21.48"; E 15° 33' 37.98"
5.	S	0,82	R	0				1																	0	N 77° 0' 24.24"; E 15° 33' 30.36"
6.	S	0,42	S	0				1	1																24	N 77° 0' 26.82"; E 15° 33' 20.82"
7.	S	0,48	R	0	1																				0	N 77° 0' 26.82"; E 15° 33' 20.82"
8.	S	0,27	S	0				1																	37	N 77° 0' 26.82"; E 15° 33' 20.82"
9.	S	0,5	R	0	1	1			1																0	N 77° 0' 29.82"; E 15° 33' 14.7"
10.	S	0,63	R	0	1																				0	N 77° 0' 29.82"; E 15° 33' 14.7"

Sample ID	**Island**	**Weight of dry mterial**	**Substratum**	**Presence /absence of seabird colony**	**_Cetrariella delisei_ (Bory ex Schaer.) Kärnefelt & A. Thell**	**_Ochrolechia frigida_ (Sw.) Lynge**	**_Cladonia pyxidata_ (L.) Hoffm**	**_Cetraria ericetorum_ Opiz**	**_Stereocaulon alpinum_ Laurer**	**_Cladonia crispata var. cetrariformis_ (Delise) Vain.**	**_Cladonia crispata_ (Ach.) Flot.**	**_Sphaerophorus globosus_ (Huds.) Vain.**	**_Flavocetraria nivalis_ (L.) Kärnefelt & A. Thell**	**_Cladonia mitis_ Sandst.**	**_Melanelia hepatizon_ (Ach.) Vain.**	**_Lecidea ramulosa_ Th. Fr.**	**_Cladonia amaurocraea_ (Flörke) Schaer.**	**_Flavocetraria cucullata_ (Bellardi) Kärnefelt**	**_Cladonia arbuscula_ (Wallr.) Flot.**	**_Cladonia rangiferina_ (L.) Weber ex F.H. Wigg.**	**_Cladonia macroceras_ (Delise) Ahti**	**_Parmelia omphalodes_ (L.) Ach.**	**_Umbilicaria decussata_ (Vill.) Zahlbr.**	**_Stereocaulon nannodes_ Tuck.**	**Tardigrades per 10 g/dry material**	**coordinates**
11.	S	0,57	R	0	1																				0	N 77° 0' 29.82"; E 15° 33' 14.7"
12.	S	0,56	R	0				1																	18	N 77° 0' 32.7"; E 15° 33' 8.1"
13.	S	0,48	R	0	1																				0	N 77° 0' 32.7"; E 15° 33' 8.1"
14.	S	0,92	R	0	1																				43	N 77° 0' 35.64"; E 15° 33' 2.4"
15.	S	0,88	R	0	1																				0	N 77° 0' 35.64"; E 15° 33' 2.4"
16.	S	0,65	R	0	1																				15	N 77° 0' 35.64"; E 15° 33' 2.4"
17.	S	0,69	R	0	1																				0	N 77° 0' 38.58"; E 15° 32' 56.22"
18.	S	3,22	R	0	1																				3	N 77° 0' 38.58"; E 15° 32' 56.22"
19.	S	3,39	R	0	1																				6	N 77° 0' 38.58"; E 15° 32' 56.22"
20.	S	2,99	R	0																					0	N 77° 0' 38.58"; E 15° 32' 56.22"
21.	S	0,46	R	0	1																				107	N 77° 0' 41.52"; E 15° 32' 50.1"
22.	S	0,59	R	0				1																	34	N 77° 0' 41.52"; E 15° 32' 50.1"
23.	S	0,4	R	0	1																				0	N 77° 0' 41.52"; E 15° 32' 50.1"
24.	S	0,88	R	0	1																				125	N 77° 0' 47.52"; E 15° 32' 39.84"
25.	S	1,92	R	0						1															42	N 77° 0' 47.52"; E 15° 32' 39.84"
26.	PKF	1,13	R	1														1							477	N 78° 53' 33.8"; E 10° 28' 36"
27.	PKF	1,51	R	1															1						390	N 78° 53' 34.18"; E 10° 28' 36.3"
28.	PKF	1,24	R	1													1								0	N 78° 53' 34.84"; E 10° 28' 35"

Sample ID	Island	Weight of dry mterial	Substratum	Presence /absence of seabird colony	*Cetrariella delisei* (Bory ex Schaer.) Kärnefelt & A. Thell	*Ochrolechia frigida* (Sw.) Lynge	*Cladonia pyxidata* (L.) Hoffm	*Cetraria ericetorum* Opiz	*Stereocaulon alpinum* Laurer	*Cladonia crispata var. cetrariformis* (Delise) Vain.	*Cladonia crispata* (Ach.) Flot.	*Sphaerophorus globosus* (Huds.) Vain.	*Flavocetraria nivalis* (L.) Kärnefelt & A. Thell	*Cladonia mitis* Sandst.	*Melanelia hepatizon* (Ach.) Vain.	*Lecidea ramulosa* Th. Fr.	*Cladonia amaurocraea* (Flörke) Schaer.	*Flavocetraria cucullata* (Bellardi) Kärnefelt	*Cladonia arbuscula* (Wallr.) Flot.	*Cladonia rangiferina* (L.) Weber ex F.H. Wigg.	*Cladonia macroceras* (Delise) Ahti	*Parmelia omphalodes* (L.) Ach.	*Umbilicaria decussata* (Vill.) Zahlbr.	*Stereocaulon nannodes* Tuck.	Tardigrades per 10 g/dry material	coordinates
29.	PKF	1,11	R	1																	1				18	N 78° 53' 34.84"; E 10° 28' 35"
30.	PKF	1,53	R	1										1			1								7	N 78° 53' 35.38"; E 10° 28' 33"
31.	PKF	0,75	R	1															1	1					720	N 78° 53' 43.24" E 10° 28' 12.89"
32.	PKF	1,17	S	1										1							1				0	N 78° 53' 45.73" E 10° 28' 35"
33.	S	1,86	R	?																			1	1	113	N 78° 59' 44.16"; E 12° 24' 21"
34.	S	1,11	P	1										1											0	N 79° 24' 0"; E 11° 0' 0"
35.	S	0,75	R	1																					13	N 79° 24' 0"; E 11° 0' 0"
36.	S	1,21	R	1										1											0	N 79° 24' 0"; E 11° 0' 0"
37.	D	1,78	R	1								1													11	N 79°43' 10.44"; E 10°44' 10.98"
38.	D	1,44	R	1						1			1	1											42	N 79° 43' 11.64"; E 10° 44' 31"
39.	F	0,77	R	?					1	1		1			1										52	N 79°51' 00"; E 11°20' 00"
40.	N	1,15	R	1		1			1			1				1						1			9	N 80° 26'; E 19° 24'
41.	N	1,97	R	1		1	1		1			1									1				10	N 80° 26'; E 19° 24'
42.	P	0,84	R	1							1														274	N 80° 40'; E 20° 38'
43.	Ph	1,4	S	1						1															379	N 80° 42' 11.82"; E 20° 50' 45.6"
44.	Ph	0,75	S	0	1																				0	N 80° 41' 13.02"; E 20° 50' 36.3"
45.	Ph	1,24	S	1					1																0	N 80° 42' 18.66"; E 20° 50' 30"

Tardigrada in Svalbard lichens: diversity, densities and habitat heterogeneity. Polar Biology. Krzysztof Zawierucha, Michał Węgrzyn, Marta Ostrowska, Paulina Wietrzyk. Department of Animal Taxonomy and Ecology, Faculty of Biology, Adam Mickiewicz University in Poznań, Umultowska 89, PL-61-614 Poznań, Poland, corresponding author: k.p.zawierucha@gmail.com

Online Resource 2. List of tardigrades. Sample ID – number of sample, equivalent of sample ID in Online Resource 1. Island: S – Spitsbergen, PKF – Prins Karls Forland, D – Danskøya, F – Fuglesongen, N – Nordaustlandet, P – Parrøya, Ph – Phippsøya. Samples 1-25 were previously analyzed in Zawierucha et al. (2016).

Sample ID	**Island**	***A. prorsirostre* (Thulin, 1928)**	***A. scoticum* (Murray, 1905)**	***D. pingue* (Marcus, 1936)**	***D. tenue* Thulin, 1928**	***E. merokensis* Richters, 1904**	***E. wendti* Richters, 1903**	***H. dujardini* (Doyère, 1840)**	***H. pallidus* Thulin, 1911**	***I. coulsoni Kaczmarek et al., 2012***	***I. cf. reticulatus* Pilato, 1973**	***I. cf. tuberculatus* (Plate, 1888)**	***M. crenulatus* Richters, 1904**	***M. h. harmsworthi* (Murray, 1907)**	***M. harmsworthi* group**	***M. hufelandif* group**	***M. asiaticum* Tumanov, 2006**	***M. spitzbergensis* (Richters, 1903)**	***P. recamieri* (Richters, 1911)**	***P. angustata* (Murray, 1905)**	***R.cf. rupeus* Biserov, 1999**	***Tenuibiotus sp.***	***T. voronkovi* (Tumanov, 2007)**	***T. spitsbergensis* (Scourfield, 1897)**	**Unidentified specimens**	**Diversity of taxon per sample**
1.	S																									0
2.	S																									0
3.	S														1											1
4.	S																									0
5.	S																									0
6.	S																								1	1
7.	S																									0
8.	S														1											1
9.	S																									0
10.	S																									0
11.	S																									0
12.	S							1																		1

Sample ID	Island	*A. prorsirostre* (Thulin, 1928)	*A. scoticum* (Murray, 1905)	*D. pingue* (Marcus, 1936)	*D. tenue* Thulin, 1928	*E. merokensis* Richters, 1904	*E. wendti* Richters, 1903	*H. dujardini* (Doyère, 1840)	*H. pallidus* Thulin, 1911	*I. coulsoni Kaczmarek et al., 2012*	*I. cf. reticulatus* Pilato, 1973	*I. cf. tuberculatus* (Plate, 1888)	*M. crenulatus* Richters, 1904	*M. h. harmsworthi* (Murray, 1907)	*M. harmsworthi* group	*M. hufelandif* group	*M. asiaticum* Tumanov, 2006	*M. spitzbergensis* (Richters, 1903)	*P. recamieri* (Richters, 1911)	*P. angustata* (Murray, 1905)	*R.cf. rupeus* Biserov, 1999	*Tenuibiotus sp.*	*T. voronkovi* (Tumanov, 2007)	*T. spitsbergensis* (Scourfield, 1897)	Unidentified specimens	Diversity of taxon per sample
13.	S																									0
14.	S											3													1	2
15.	S																									0
16.	S			1																						1
17.	S																									0
18.	S								1																	1
19.	S						1				1															2
20.	S																									0
21.	S								2					2								1				3
22.	S								1					1												2
23.	S																									0
24.	S						1							10												2
25.	S								3						3								1	1		3
26.	PKF	38		8					6	1															1	4
27.	PKF	1							53							4									1	3
28.	PKF																									0
29.	PKF			1												1										2
30.	PKF	1																								1
31.	PKF								47																7	1

Sample ID	Island	*A. prorsirostre* (Thulin, 1928)	*A. scoticum* (Murray, 1905)	*D. pingue* (Marcus, 1936)	*D. tenue* Thulin, 1928	*E. merokensis* Richters, 1904	*E. wendti* Richters, 1903	*H. dujardini* (Doyère, 1840)	*H. pallidus* Thulin, 1911	*I. coulsoni Kaczmarek et al., 2012*	*I. cf. reticulatus* Pilato, 1973	*I. cf. tuberculatus* (Plate, 1888)	*M. crenulatus* Richters, 1904	*M. h. harmsworthi* (Murray, 1907)	*M. harmsworthi* group	*M. hufelandif* group	*M. asiaticum* Tumanov, 2006	*M. spitzbergensis* (Richters, 1903)	*P. recamieri* (Richters, 1911)	*P. angustata* (Murray, 1905)	*R.cf. rupeus* Biserov, 1999	*Tenuibiotus sp.*	*T. voronkovi* (Tumanov, 2007)	*T. spitsbergensis* (Scourfield, 1897)	Unidentified specimens	Diversity of taxon per sample
32.	PKF																									0
33.	S																1				20					2
34.	S																									0
35.	S		1																							1
36.	S																									0
37.	D												2													1
38.	D		2						1											3						2
39.	F																		4							1
40.	N												1													1
41.	N	1				1																				2
42.	P			8				1	13									1								4
43.	Ph					50													3							2
44.	Ph																									0
45.	Ph																									0

IV Authorship statements

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

Krzysztof Zawierucha
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Poznań, 03.01.2017r.

Oświadczenie określające wkład w powstanie artykułu

Niniejszym oświadczam, że mój wkład w powstanie poniższego artykułu:

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

polegał na: zbiorze materiału, izolacji niesporczaków z próbek, spreparowaniu i oznaczeniu okazów, przygotowaniu pomiarów, napisaniu manuskryptu i poprawie manuskryptu po ocenie recenzentów.

Mój całkowity wkład w pracę wynosi 100%.

Krzysztof Zawierucha

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Poznań, 03.01.2017.

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:

Zawierucha K, Coulson SJ, Michalczyk Ł, 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

was as follows: species identification, writing the first draft of the manuscript, revising the manuscript. I'm first and corresponding author.

My total contribution to the following article is at 85%.

Krzysztof Zawierucha

Krzysztof Zawierucha
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Poznań, 03.01.2017r.

Oświadczenie określające wkład w powstanie artykułu

Niniejszym oświadczam, że mój wkład w powstanie poniższego artykułu:

Zawierucha K, Kolicka M, Kaczmarek Ł. 2015. Re-description of the Arctic tardigrade *Tenuibiotus voronkovi* (Tumanov, 2007) (Eutardigrada; Macrobiotidea), with the first molecular data for the genus. *Zootaxa* 4196 (4): 498–510

polegał na: zaplanowaniu pracy, izolacji niesporczaków z próbek, spreparowaniu i oznaczeniu okazów, przygotowaniu pomiarów, analizach laboratoryjnych tj. przygotowaniu reakcji PCR oraz sekwencjonowaniu odcinków COI i 28S rRNA, napisaniu pierwszej wersji manuskryptu, poprawie manuskryptu po ocenie recenzentów. W pracy jestem pierwszym i korespondencyjnym autorem.

Mój całkowity wkład w pracę oceniam na 90%.

Krzysztof Zawierucha

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Poznań, 03.01.2017.

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:

Zawierucha K, Ostrowska M, Vonnahme TR, Devetter M, Nawrot AP, Lehmann S, Kolicka M. 2016. Diversity and distribution of Tardigrada in Arctic cryoconite holes. *Journal of Limnology* 75(3): 545–559. DOI: 10.4081/jlimnol.2016.1453

was as follows: designing the study, collection of samples from Buchan and Waldemar Glaciers, species identification, interpretation of results, writing the first draft of the manuscript (sections: Introduction, Taxonomic accounts, Discussion), revising the manuscript. I'm first and corresponding author.

My total contribution to the following article is at 60%.

Krzysztof Zawierucha

Poznań, 03.01.2017.

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Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:

Zawierucha K, Vonnahme TR, Devetter M, Kolicka M, Ostrowska M, Chmielewski S, Kosicki JZ. 2016. Area, depth and elevation of cryoconite holes in the Arctic do not influence Tardigrada densities. *Polish Polar Research* 37(2): 325–334. DOI 10.1515/popore-2016-0009

was as follows: designing the study, interpretation of results, writing the first draft of the manuscript, revising the manuscript. I'm first and corresponding author.

My total contribution to the following article is at 50%.

Krzysztof Zawierucha

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Poznań, 03.01.2017r.

Oświadczenie określające wkład w powstanie artykułu

Niniejszym oświadczam, że mój wkład w powstanie poniższego artykułu:

Zawierucha K, Zmudczyńska-Skarbek K, Kaczmarek Ł, Wojczulanis-Jakubas K, 2016. The influence of a seabird colony on abundance and species composition of water bears (Tardigrada) in Hornsund (Spitsbergen, Arctic). *Polar biology* 39: 713–723.

polegał na: postawieniu hipotez, zbiorze materiału, izolacji niesporczaków z próbek, spreparowaniu i oznaczeniu okazów, przygotowaniu baz danych, interpretacji wyników i napisaniu pierwszej wersji manuskryptu. W pracy jestem pierwszym i korespondencyjnym autorem.

Mój całkowity wkład w pracę wynosi 60%.

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Cambridge, 11.02.2017r.

Oświadczenie określające wkład w powstanie artykułu

Niniejszym oświadczam, że mój wkład w powstanie poniższego artykułu:

Zawierucha K, Węgrzyn M, Ostrowska M, Wietrzyk P. 2017. Tardigrada in Svalbard lichens: diversity, densities and habitat heterogeneity. *Polar Biology*, DOI 10.1007/s00300-016-2063-2.

polegał na: zaplanowaniu badań, zbiorze materiału, izolacji i spreparowaniu niesporczaków z dwudziestu pięciu próbek, oznaczeniu zwierząt, liczeniu części analiz statystycznych, interpretacji wyników i pisaniu manuskryptu. W pracy jestem pierwszym i korespondencyjnym autorem.

Mój całkowity wkład w pracę oceniam na 70%.

Krzysztof Zawierucha

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

Current knowledge of the Tardigrada of Svalbard with the first records of water bears from Nordaustlandet (High Arctic). *Polar Research*, 32: 20886

Prof. S.J. Coulson
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08.12.2016.

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:

Zawierucha K, Coulson SJ, Michalczyk Ł, 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

was as follows: sample collection, proof read of manuscript.

Stephen J. Coulson

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Kraków, 19.09.2016

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:

Zawierucha K, Coulson SJ, Michalczyk Ł, 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

was as follows: co-writing of the manuscript.

Łukasz Michalczyk

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Poznań, 14.10.2016

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:

Zawierucha K, Coulson SJ, Michalczyk Ł, 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

was as follows: co-identification of species, co-writing of the manuscript.
I estimate my total contribution to the following article at 10%.

Łukasz Kaczmarek

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

Re-description of the Arctic tardigrade *Tenuibiotus voronkovi* (Tumanov, 2007) (Eutardigrada; Macrobiotidea), with the first molecular data for the genus. *Zootaxa*, 4196(4): 498-510

Małgorzata Kolicka
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Poznań, 03.12.2016r.

Oświadczenie określające wkład w powstanie artykułu

Niniejszym oświadczam, że mój wkład w powstanie poniższego artykułu:

Zawierucha K, Kolicka M, Kaczmarek Ł. 2015. Re-description of the Arctic tardigrade *Tenuibiotus voronkovi* (Tumanov, 2007) (Eutardigrada; Macrobiotidea), with the first molecular data for the genus. *Zootaxa* 4196 (4): 498–510
polegał na: analizach laboratoryjnych tj. przygotowaniu reakcji PCR oraz sekwencjonowaniu odcinków 28S rDNA, ITS1–5.8S rDNA–ITS2, a także formatowaniu manuskryptu.
Swój całkowity wkład oceniam na 2%.

Małgorzata Kolicka

Małgorzata Kolicka

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Poznań, 05.12.2016r.

Oświadczenie określające wkład w powstanie artykułu

Niniejszym oświadczam, że mój wkład w powstanie poniższego artykułu:

Zawierucha K, Kolicka M, Kaczmarek Ł. 2015. Re-description of the Arctic tardigrade *Tenuibiotus voronkovi* (Tumanov, 2007) (Eutardigrada; Macrobiotidea), with the first molecular data for the genus. *Zootaxa* 4196 (4): 498–510
polegał na: korektach manuskryptu oraz przygotowaniu tablic.
Swój całkowity wkład oceniam na 8%.

Łukasz Kaczmarek

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

Diversity and distribution of Tardigrada in Arctic cryoconite holes. *Journal of Limnology*, 75(3): 545-559

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Poznań, 27.09.2016

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:

Zawierucha K, Ostrowska M, Vonnahme TR, Devetter M, Nawrot AP, Lehmann S, Kolicka M. 2016. Diversity and distribution of Tardigrada in Arctic cryoconite holes. *Journal of Limnology*, 75(3): 545-559. DOI: 10.4081/jlimnol.2016.1453

was as follows: animal isolation from samples collected on Buchan Glacier and Waldemar Glaciers, preparation of figures 3-7.
I estimate my total contribution to the following article at 5%.

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Marta Ostrowska

Tobias Vonnahme
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Bremen, 09.11.2016

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:

Zawierucha K, Ostrowska M, Vonnahme TR, Devetter M, Nawrot AP, Lehmann S, Kolicka M. 2016. Diversity and distribution of Tardigrada in Arctic cryoconite holes. *Journal of Limnology*, 75(3): 545-559. DOI: 10.4081/jlimnol.2016.1453

was as follows: collection of samples in Billefjorden, co-writing of manuscript (section Introduction).
I estimate my total contribution to the following article at 10%.

Tobias Vonnahme

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Č. Budějovice, 19.09.2016

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:

Zawierucha K, Ostrowska M, Vonnahme TR, Devetter M, Nawrot AP, Lehmann S, Kolicka M. 2016. Diversity and distribution of Tardigrada in Arctic cryoconite holes. *Journal of Limnology*, 75(3): 545-559. DOI: 10.4081/jlimnol.2016.1453

was as follows: collection of samples in Billefjorden, preparation of slides with tardigrades found in Billefjorden.

I estimate my total contribution to the following article at 5%.

Miloslav Devetter

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Warszawa, 12.10.2016

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:

Zawierucha K, Ostrowska M, Vonnahme TR, Devetter M, Nawrot AP, Lehmann S, Kolicka M. 2016. Diversity and distribution of Tardigrada in Arctic cryoconite holes. *Journal of Limnology*, 75(3): 545-559. DOI: 10.4081/jlimnol.2016.1453

was as follows: co-writing of manuscript (section Methods: Study area, Glaciers, Chemical analysis), preparation of figures 1-2, collection of water samples from Buchan Glacier, chemical analysis.

I estimate my total contribution to the following article at 10%.

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Gdańsk, 12.10.2016

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:

Zawierucha K, Ostrowska M, Vonnahme TR, Devetter M, Nawrot AP, Lehmann S, Kolicka M. 2016. Diversity and distribution of Tardigrada in Arctic cryoconite holes. *Journal of Limnology*, 75(3): 545-559. DOI: 10.4081/jlimnol.2016.1453

was as follows: co-writing of manuscript (section Discussion: Biochemistry), preparation of table 4.

I estimate my total contribution to the following article at 5%.

Sara Lehmann-Konera

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Poznań, 14.10.2016

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:

Zawierucha K, Ostrowska M, Vonnahme TR, Devetter M, Nawrot AP, Lehmann S, Kolicka M. 2016. Diversity and distribution of Tardigrada in Arctic cryoconite holes. *Journal of Limnology*, 75(3): 545-559. DOI: 10.4081/jlimnol.2016.1453

was as follows: samples collection from Hans Glacier, co-preparation of tables 1-3.
I estimate my total contribution to the following article at 5%.

Małgorzata Kolicka

Małgorzata Kolicka

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

Area, depth and elevation of cryoconite holes in the Arctic do not influence Tardigrada densities. *Polish Polar Research*, 37(2): 325-334

Tobias Vonnahme
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Bremen, 09.11.2016

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:

Zawierucha K, Vonnahme TR, Devetter M, Kolicka M, Ostrowska M, Chmielewski S, Kosicki JZ. 2016. Area, depth and elevation of cryoconite holes in the Arctic do not influence Tardigrada densities. *Polish Polar Research*, 37(2): 325-334. DOI 10.1515/popore-2016-0009

was as follows: collection of samples in Billefjorden, measurements in the field, animal isolation from the samples (glaciers in Billefjorden), co-writing of manuscript (section Results and discussion).

I estimate my total contribution to the following article at 20%.

Tobias Vonnahme

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Č. Budějovice, 19.09.2016

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:

Zawierucha K, Vonnahme TR, Devetter M, Kolicka M, Ostrowska M, Chmielewski S, Kosicki JZ. 2016. Area, depth and elevation of cryoconite holes in the Arctic do not influence Tardigrada densities. *Polish Polar Research*, 37(2): 325-334. DOI 10.1515/popore-2016-0009

was as follows: collection of samples in Billefjorden.
I estimate my total contribution to the following article at 3%.

Miloslav Devetter

Małgorzata Kolicka
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Poznań, 10.02.2017r.

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:

Zawierucha K, Vonnahme TR, Devetter M, Kolicka M, Ostrowska M, Chmielewski S, Kosicki JZ. 2016. Area, depth and elevation of cryoconite holes in the Arctic do not influence Tardigrada densities. *Polish Polar Research*, 37(2): 325-334. DOI 10.1515/popore-2016-0009

was as follows: collection of samples from Hans Glacier, making of measurements in the field.

I estimate my total contribution to the following article at 5%.

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Poznań, 14.10.2016r.

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:

Zawierucha K, Vonnahme TR, Devetter M, Kolicka M, Ostrowska M, Chmielewski S, Kosicki JZ. 2016. Area, depth and elevation of cryoconite holes in the Arctic do not influence Tardigrada densities. *Polish Polar Research*, 37(2): 325-334. DOI 10.1515/popore-2016-0009

was as follows: database preparation.
I estimate my total contribution to the following article at 5%.

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Zawierucha K, Vonnahme TR, Devetter M, Kolicka M, Ostrowska M, Chmielewski S, Kosicki JZ. 2016. Area, depth and elevation of cryoconite holes in the Arctic do not influence Tardigrada densities. *Polish Polar Research*, 37(2): 325-334. DOI 10.1515/popore-2016-0009

was as follows: isolation of tardigrades from samples (collected from Hans Glacier).
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Poznań 14.10.2016

Declaration of contribution to the article

Hereby I confirm that my contribution to the following article:

Zawierucha K, Vonnahme TR, Devetter M, Kolicka M, Ostrowska M, Chmielewski S, Kosicki JZ. 2016. Area, depth and elevation of cryoconite holes in the Arctic do not influence Tardigrada densities. *Polish Polar Research*, 37(2): 325-334. DOI 10.1515/popore-2016-0009

was as follows: statistical analysis, preparation of figure and table.
I estimate my total contribution to the following article at 10%.

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

The influence of a seabird colony on abundance and species composition of water bears (Tardigrada) in Hornsund (Spitsbergen, Arctic).
Polar Biology, 39(4): 713-723

Gdańsk, dnia: 26. 09. 2016 r.

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Oświadczenie określające wkład w powstanie artykułu

Niniejszym oświadczam, że mój wkład w powstanie poniższego artykułu:

Zawierucha K, **Zmudczyńska-Skarbek K**, Kaczmarek Ł, Wojczulanis-Jakubas K, 2016. The influence of a seabird colony on abundance and species composition of water bears (Tardigrada) in Hornsund (Spitsbergen, Arctic). *Polar Biology* 39, 713-723

polegał na: analizach statystycznych, interpretacji wyników oraz pisaniu manuskryptu. Całkowity udział w powstawanie powyższego artykułu oceniam na 25%.

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polegał na: pomocy przy identyfikacji gatunków.
Swój całkowity udział w powstawanie powyższego artykułu oceniam na 5%.

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Zawierucha K, Zmudczyńska-Skarbek K, Kaczmarek Ł, **Wojczulanis-Jakubas K**, 2016. The influence of a seabird colony on abundance and species composition of water bears (Tardigrada) in Hornsund (Spitsbergen, Arctic). *Polar Biology* 39, 713-723

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Tardigrada in Svalbard lichens: diversity, densities and habitat heterogeneity. *Polar Biology*, DOI 10.1007/s00300-016-2063-2

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polegał na: identyfikacji porostów, pisaniu podsekcji *Lichens* w Material and methods, pisaniu części sekcji Results i sekcji Discussion.

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polegał na: izolacji i spreparowaniu niesporczaków z dwudziestu próbek, przygotowaniu bazy danych, liczeniu części analiz statystycznych i formatowaniu manuskryptu.

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Zawierucha K, Węgrzyn M, Ostrowska M, Wietrzyk P. 2017. Tardigrada in Svalbard lichens: diversity, densities and habitat heterogeneity. *Polar Biology*, DOI 10.1007/s00300-016-2063-2.

polegał na: identyfikacji porostów, pisaniu podsekcji *Lichens* w Material and methods, pisaniu części sekcji Results i sekcji Discussion.

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