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EFFECTS OF THE EVOLUTION OF INTROMISSION ON COURTSHIP COMPLEXITY AND MALE AND FEMALE MORPHOLOGY: WATER MITES OF THE GENUS *ARRENURUS* (ACARI; HYDRACHNIDA) FROM EUROPE AND NORTH AMERICA

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1. Introduction

1. 1. Sexual selection in animals

Darwin's views:

Darwin (1859) proposed sexual selection theory as an explanation for sexual dimorphism. He distinguished between intrasexual and intersexual selection. The first type refers to situations in which individuals of one sex (usually males) compete for access to individuals of the other sex (usually females). In intersexual selection individuals of one sex (usually females) choose competing representatives of the other sex (usually males) (Darwin, 1871). Though Charles Darwin wrote that competition occurs among males and females are choosy, he was conscious that the roles of the sexes may be reversed (Arnqvist and Rowe, 2005). However, there are parts of his theory that became out of date with time. In his works sexual selection was considered a weaker force of diversification than natural selection which refer to struggle for existence. There is an experimental evidence that mating success can be a strong force of evolutionary change (Arnqvist and Rowe, 2005). Moreover, Charles Darwin was not aware of interactions between competitors after sperm transfer and stated that the outcome of sexual selection resulted from number of achieved matings by males (Arnqvist and Rowe, 2005).

There are several hypotheses that attempt to explain evolution of female preferences and male secondary sexual traits.

Direct benefits:

In this model females show preference for particular male traits that improve the female's viability and fecundity, e.g., through provision of food resources or parental care for offspring. Females may judge the value of resources directly, or indicator traits in males are involved (Arnqvist and Rowe, 2005). However, female choosiness is costly because of the risk of predation and being unfertilised (Kuijper et al., 2012).

Good genes:

The good genes model assumes that females choose males bearing secondary sexual traits that indicate the possession of genes that increase offspring survival (Proctor and Wilkinson, 2001; Kuijper et al., 2012). The 'handicap' model suggests that these costly secondary sexual traits are possessed only by males with 'high quality' genes, and

thus, females that select males with 'indicator' traits benefit with having high quality offspring (Arnqvist and Rowe, 2005).

Fisherian runaway:

In Fisherian process females choose attractive males that have the most exaggerated ornaments based exclusively on the possession of that ornament by males. In result choosy females should have attractive sons that will have higher mating rates (Kuijper et al., 2012). According to this model, the preference in females and the presence of ornamentation in males should strengthen over time as the proportion of females with the preference for this trait and males with the trait increase each generation (Futuyma, 2008). Furthermore, if male's ornamentation is non-adaptive, female choice may undermine natural selection. In contrary to 'good genes' this model focuses on self-reinforcing selection and does not include aspects associated with genetic quality of mates (Arnqvist and Rowe, 2005).

Sensory exploitation:

In the sensory exploitation model females have a pre-existing tendency to respond to particular sensory cues (Proctor 1992a). For example, selection on the female sensory system responsible for foraging may result in pleiotropic effects and affect mating success. In this process males exploit previously extant female sensitivities as it was described for the water mite *Neumania papillator* (Proctor, 1992a). In this species females responded to vibrations caused by males that imitate a copepod prey.

Sexual conflict:

However, males of some species overcome female choice by force, e.g. by grasping them with modified legs or piercing the female's body with a sharp intromittent organ (Arnqvist and Rowe, 2005). The struggle between males and females for control of fertilization of eggs explains sexual conflict theory. The exaggerated male genitalia and courtship dances are here interpreted as attempts that aim to force females to mate (Proctor and Wilkinson, 2001). In turn, females evolve counteradaptations that enable them to resist male harassment. The sexually antagonistic co-evolution leads to development of male strategies aiming to bypass female choosiness, and females increase their resistance to male manipulative traits.

Although these hypotheses are sometimes presented as exclusive explenations, sexual selection in a species may be the result of more than one factor. Good genes, Fisherian runaway, sensory exploitation, and sexual conflict may drive diversification of the sexes on different stages of their mutual evolution (Proctor and Wilkinson, 2001).

1. 2. Sexual conflict as a subset of sexual selection

The sexes differ in their investment in offspring as by definition, females produce larger, energetically costly gametes than those produced by males. Therefore, females are usually the more discriminating and choosy sex and males compete for them. However, these roles may be reversed, for instance when males invest more in parental care (e.g. dance fly *Rhamphomyia longicauda* females exploit male preference for large females, Arnqvist and Rowe, 2005). Sexual conflict is expected whenever there are differences in evolutionary interests between the sexes. It is worthwhile to note that sexual conflict theory is not limited to the animals with separate sexes, but also explains the battles between hermaphrodites over fertilization of a partner's eggs (Michiels and Newman, 1998).

Fitness of males increases with number of matings achieved, whereas females benefit from intermediate mating rates. There are different ways in which males overcome female choice, and females protect themselves from fitness costs resulting from elevated mating rates. These adaptations are displayed both prior to mating and after mating (Arnqvist and Rowe, 2005). Strategies that decrease costs of matings in females, and that increase rates of mating in males may be morphological, behavioural or physiological. Males may harass females prior to mating by grasping and mounting them. Morphological adaptations to grasp females are widespread in male animals (Eberhardt, 1985). Modified legs and antennae occur in water strider species, and males of diving beetles have adhesive structures on their foreleg tarsi (Bergsten and Miller, 2007). Males of several species of bedbugs pierce the female's body with their sharp intromittent organ and inject sperm into the female's hemolymph (Eberhardt, 1985). In turn, females of many species have evolved adaptations aiming to resist the male's harassment. Females of water striders make it difficult for males to engage their genitalia by having dorsally oriented spines on their abdomens. Elaborate sculpture on the backs of female diving beetles decrease the attachment ability of males. Moreover, females behaviourally resist male's attacks by dislodging them by vigorous swimming (Bergsten and Miller, 2007). Ather female

resistance strategy is shown in robber flies where grasped females display thanatosis (playing dead) (Dennis and Lavigne, 1976).

In addition to conflict associated with sperm transfer, postmating conflicts between the sexes are common in different groups of animals (Vahed et al., 2014). There are significant direct costs of delaying remating for females. This is because they may benefit from mating with additional males by for instance receiving sperm of 'higher quality'. The males attempt to prevent females from remating by transferring aggressive sperm, seminal toxins or antiaphrodisiacs (Arnqvist and Rowe, 2005). Polyandry in females may be restricted by the use of mating plugs, genital spines or claspers and mate-guarding behaviour. Conflicts over time spent in mating are expected because of differences in optimal duration of mating for males and females. Whereas females benefit from receiving viable sperm and nourishing seminal substances, sperm competition in males often requires prolonged postcopulatory associations (Arnqvist and Rowe, 2005). The chance for successful fertilization of eggs by sperm of a particular male is an increasing function of mating duration. This is because more sperm and accessory ejaculate substances is transferred over time, and because of advantage in sperm competition (Eberhardt, 1985). Moreover, males being in copula with females prevent them from having physical contact with other males. However, these strategies are costly to females that may suffer increased risk of injury, restriction of their own mate choice and predation risk or death (Arnqvist and Rowe, 2005). Therefore, females of many species have evolved counteradaptations: concealment of reproductive state, morphological antimale adaptations, struggling aiming to dislodge males, or choice of males that cause least harm (Arnqvist and Rowe, 2005; Bergsten and Miller, 2007).

Sexual selection and sexually antagonistic co-evolution are considered to be engines of evolutionary divergence (Kuijper et al., 2012). Female resistance as a response to male persistence may result in selection for particular male phenotypes. This can lead to reproductive isolation of different populations of the same species. The prediction that traits involved in the arms race between the sexes evolve faster than many other traits was confirmed by Bergsten and Miller (2007). They found that speciation in two species of diving beetles was probably driven by sexual conflict. Arnqvist et al. (2000) demonstrated that clades with the possibility for postmating sexual selection and sexual conflict show elevated levels of speciation in comparison to clades in which forces associated with sexual selection are more limited.

1. 3. Focal taxon: *Arrenurus*

With more than 6,000 species worldwide, water mites (Arachnida: Acariformes: Parasitengona: Hydrachnidia) are the most species-rich group of arachnids that occur in standing and flowing freshwater habitats (Smith et al., 2009). There is a great diversity of sperm transfer modes among water mites: complete dissociation where the sexes have no physical or chemical contact, incomplete dissociation involving chemoreception (pairing behaviour absent), pairing with indirect transfer in which females control sperm uptake, and pairing with direct transfer (copulation) where males introduce sperm in the reproductive tract of females (Proctor, 1992b).

The genus *Arrenurus* (Hydrachnidia: Arrenuridae) is considered, together with other Arrenuroidea, Lebertioidea, and Hygrobatoidea as belonging to more derived water mites (Di Sabatino et al., 2008). Representatives of the genus *Arrenurus* inhabit all types of standing and running freshwater habitats excluding thermal springs (Cook, 1974). They form the most species-rich genus of water mites, and the most species-rich genus of any arachnid, with more than 950 species worldwide (Smit, 2012). The genus *Arrenurus* consists of 11 putative subgenera worldwide, but the subject of this study are subgenera from the Palearctic and Nearctic regions: *Arrenurus* s. str. (*Arrenurus (Arrenurus)*, '*Arr.'*), *Megaluracarus* (*'Meg.'*), *Micrarrenurus* (*'Mic.'*), *Micruracarus* (*'Miu.'*) and *Truncaturus* (*'Tru.'*) (Tab. 1.3.1). Like most members of the Parasitengona, *Arrenurus* mites have a complex life cycle that includes a parasitic-phoretic larva, inactive protonymph and tritonymph, and predatory deutonymph and adult (Więcek et al., 2013a). The parasitic-phoretic larvae parasitize mostly odonates and dipterans, rarely coleopterans (*A. (Meg.) globator*; Böttger and Martin, 2003). This relationship enables *Arrenurus* individuals to disperse and colonize new habitats.

Table 1.3.1. The distribution of *Arrenurus* subgenera in the world. The subgenera examined in this study are *Arrenurus* s.str., *Megaluracarus*, *Micruracarus*, *Truncaturus* and *Micrarrenurus*.

Subgenus	Distribution	Author
Arrenurus	worldwide	Dugès, 1834
Megaluracarus	worldwide	K. Viets, 1911
Micruracarus	worldwide	K. Viets, 1911
Truncaturus	worldwide	Thor, 1901
Micrarrenurus	Palearctic	Cassagne-Méjean, 1966
Brevicaudaturus	Oriental, Australasia, Neotropic	Smit, 1997
Rhinophoracarus	Oriental, Afrotropic, Australasia	K. Viets, 1916
Dividuracarus	Australia	Smit, 1997
Dadayella	Neotropic	Koenike, 1907
Arrhenuropsis	Neotropic	K. Viets, 1954
Arrhenuropsides	Neotropic	K. Viets, 1954

1. 3. 1. Reproductive morphology

There are various degrees of sexual dimorphism among *Arrenurus* subgenera and species. The range of body modification in male *Arrenurus* is broad and starts from unmodified hindbody (=cauda) and legs to elongated and bumpy cauda and legs with grasping structure formed by the elongation of the distal end of one leg segment that opposes the subsequent leg segment. In contrast, the body of females shows almost no interspecific variation (Cook, 1974).

Male:

The males vary greatly in modifications of hindbody (=cauda), intromittent organ and, to a lesser extent of hind legs. The genital opening is located on the ventral side of the body and is associated with the area covered by genital acetabula (Fig. 1.3.1.1 A). The male cauda is the most posterior part of the idiosoma that extends from the end of dorsal or ventral shield (Cook, 1974; Fig. 1.3.1.1 A, Fig. 1.3.1.2 A, B). In the posterior part of male cauda occur four pairs of glandularia that produce an adhesive secretion during mating (Lundblad, 1930; Fig. 1.3.1.1 A). In the least modified male morphotype, the cauda is not clearly demarcated from the body proper, and pygal lobes (posterolateral extensions of the male cauda) and also medial cleft are absent (e.g. *A. (Tru.) fontinalis*, Fig. 1.3.1.3 E; see Materials and Methods section 3.1 for SEM methodology). The morphotype with short and complex hindbody with medial cleft, but without pygal lobes occurs e.g. in *A. (Miu.)*

biscissus (Fig. 1.3.1.3 D). The morphotype with exaggerated and very elongated cauda distinctly set off from the body proper and without pygal lobes and medial cleft occurs e.g. in *A. (Meg.) globator* (Fig. 1.3.1.3 B). The most complex morphotype has cauda equipped with various humps and well developed pygal lobes, but lacks a medial cleft (e.g. *A. (Arr.) magnicaudatus*, Fig. 1.3.1.3 A) (Cook, 1974). The intromittent organ (petiole) of males is a projecting sclerite associated with male cauda that varies greatly in size, shape and texture. This structure is most complex in the type subgenus *Arrenurus* s. str. and consists of a basal and a central piece (absent in several species) (Fig. 1.3.1.2 A, Fig. 1.3.1.4 A). The hyaline appendage is structure located at the base of the petiole in *Arrenurus* s. str. only (absent in several species; *A. (Arr.) bicuspidator*, Fig. 1.3.1.4 A). In other species, the petiole may be short and simple (*A. (Miu.) biscissus*, Fig. 1.3.1.3 D), short and complex (*A. (Miu.) sinuator*, Fig. 1.3.1.4 C) or rudimentary (*A. (Meg.) globator*, Fig. 1.3.1.4 D). Finally, this structure may be absent (*A. (Tru.) fontinalis*, Fig. 1.3.1.3 E). The petiole is predominantly sclerotized, however, in a few species a sclerite is covered with a membrane that may have a wrinkled texture (*A. (Miu.) sinuator*, Fig. 1.3.1.4 C) (Cook, 1974). The fourth legs in males may be simple and resemble legs of females (Fig. 1.3.1.5 B), but in other species there is a distal extension, the 'spur' located on the fourth segment (Fig. 1.3.1.5 A). The spur is equipped with long setae and functions as a grasping structure that is used in first stages of mating (Cook, 1974; Proctor and Wilkinson, 2001).

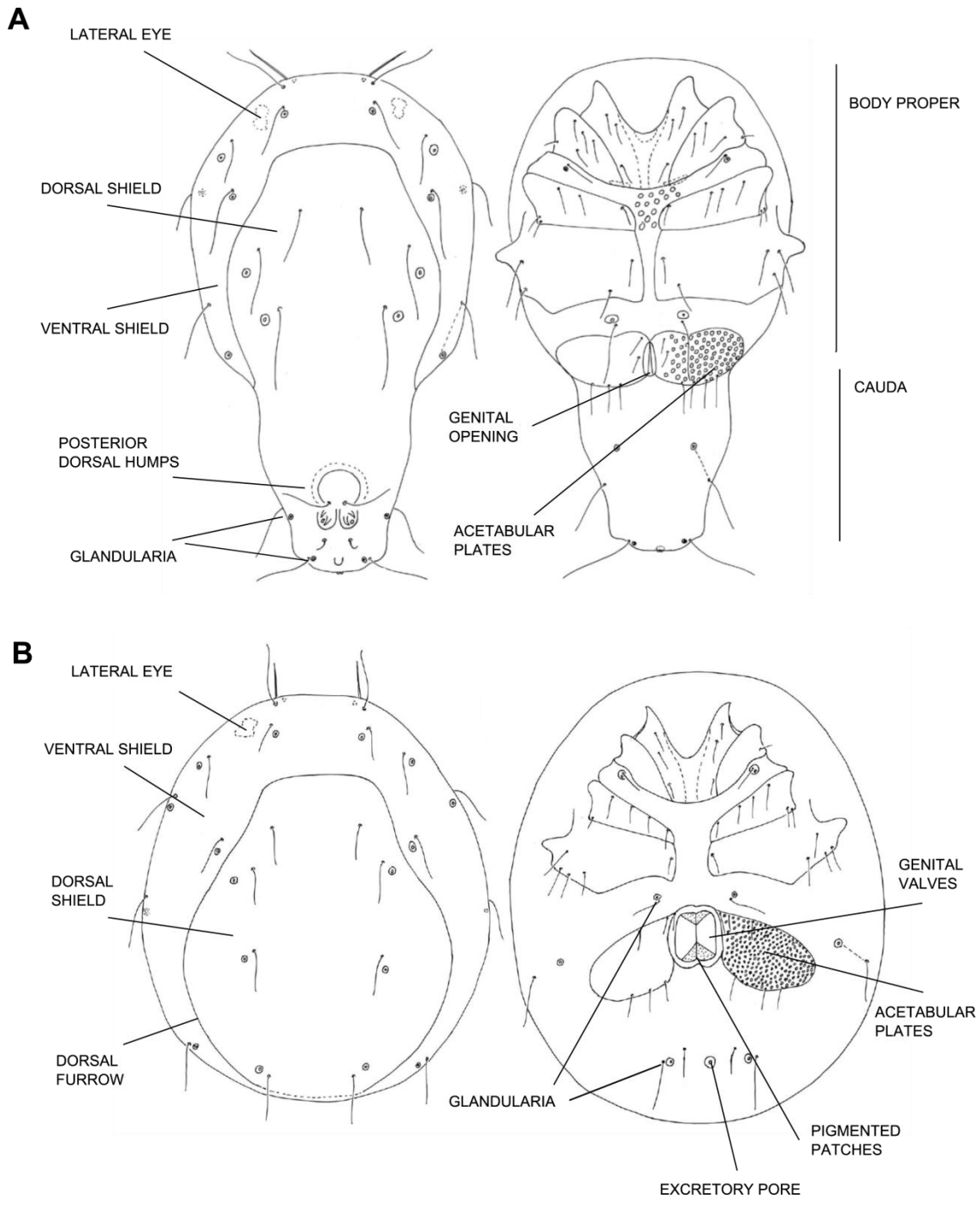

Figure 1.3.1.1. Morphology of *A. (Meg.) praeclarus*: A. male: dorsal view (left), ventral view (right); B. female: dorsal (left), ventral (right); after Tuzovsky (2012), modified.

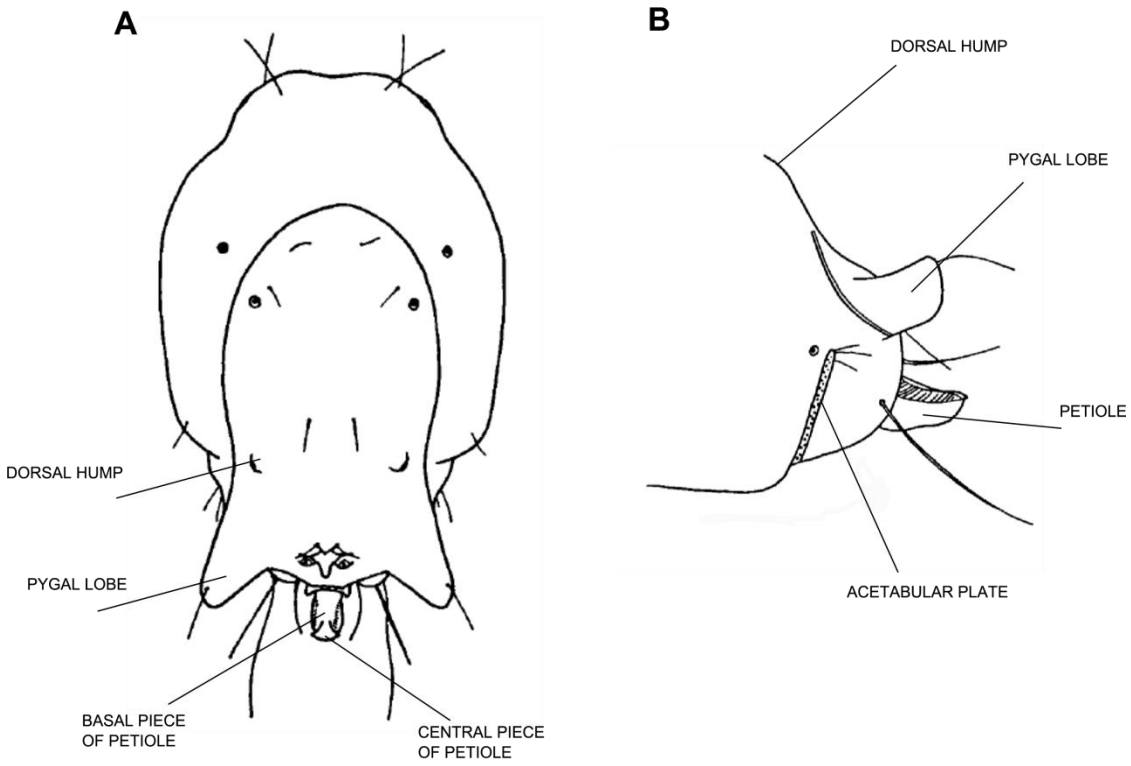

Figure 1.3.1.2. Morphology of *A. (Arr.) bruzelioides*: A. male, dorsal view; B. male, cauda, lateral view (after Smit, 1996, modified).

Female:

The bodies of females are much less morphologically diverse than those of males. Females lack cauda, but occasionally have enlarged glandularial tubercles (Cook, 1974; Fig. 1.3.1.1 B, Fig. 1.3.1.6 A). The fourth legs are not equipped with spurs (Fig. 1.3.1.6 C). In the ventral side of the female's body that contacts with a male cauda during mating are located two pairs of glandularia (Lundblad, 1930; Fig. 1.3.1.1 B). The genital area (genital field) includes gonopore with genital valves and the area covered by the genital acetabula (Fig. 1.3.1.1 B, Fig. 1.3.1.6 A, B). The genital valves are shaped as genital flaps without acetabula (Cook, 1974; Fig. 1.3.1.6 B). The acetabulum is a cup-like structure that lies on elongated sclerites (acetabular plates) and is supposed to be used in osmoregulation. In the upper and lower part of genital valves may occur cuticular fields ('pigmented patches on genital valves'; in German 'Lefzenflecken', Viets, 1936; see Fig. 1.3.1.1 B). This structure typically occurs in females of species with petiolate males (*Arrenurus* s. str.), but also in a few species with apetiolate males (*A. (Tru.) fontinalis*, *A. (Meg.) globator*). At the edges of the posterior part of the female body may occur rudimentary lobes (Fig. 1.3.1.6 A).

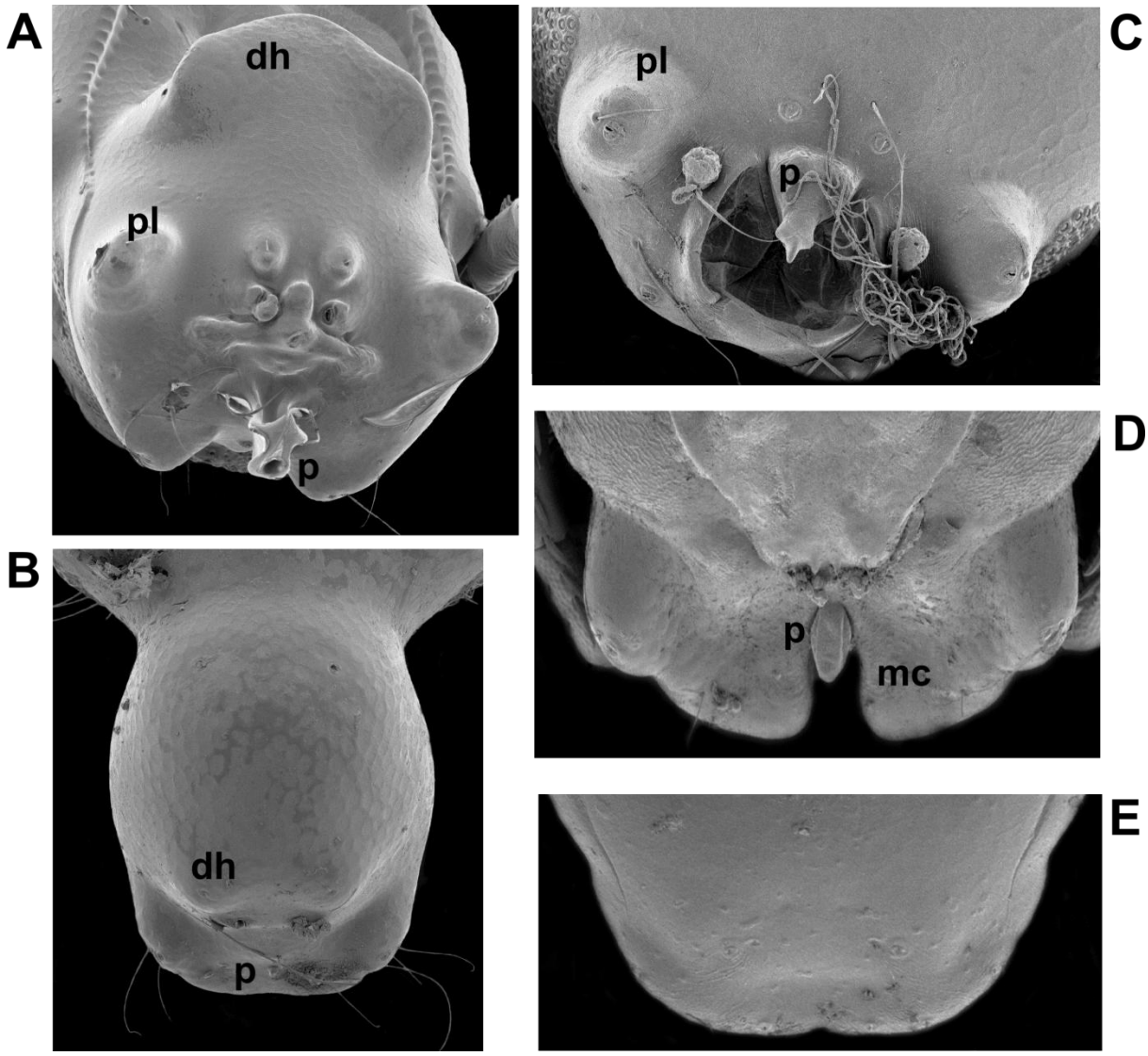

Figure 1.3.1.3. Male hindbody in males from different subgenera of *Arrenurus*; A. *A. (Arr.) magnicaudatus*, B. *A. (Meg.) globator*, C. *A. (Mic.) crassicaudatus*, D. *A. (Miu.) biscissus*, E. *A. (Tru.) fontinalis*; dh – dorsal hump, mc – medial cleft, p – petiole, pl – pygal lobe; see Materials and Methods section 3.1 for SEM methodology.

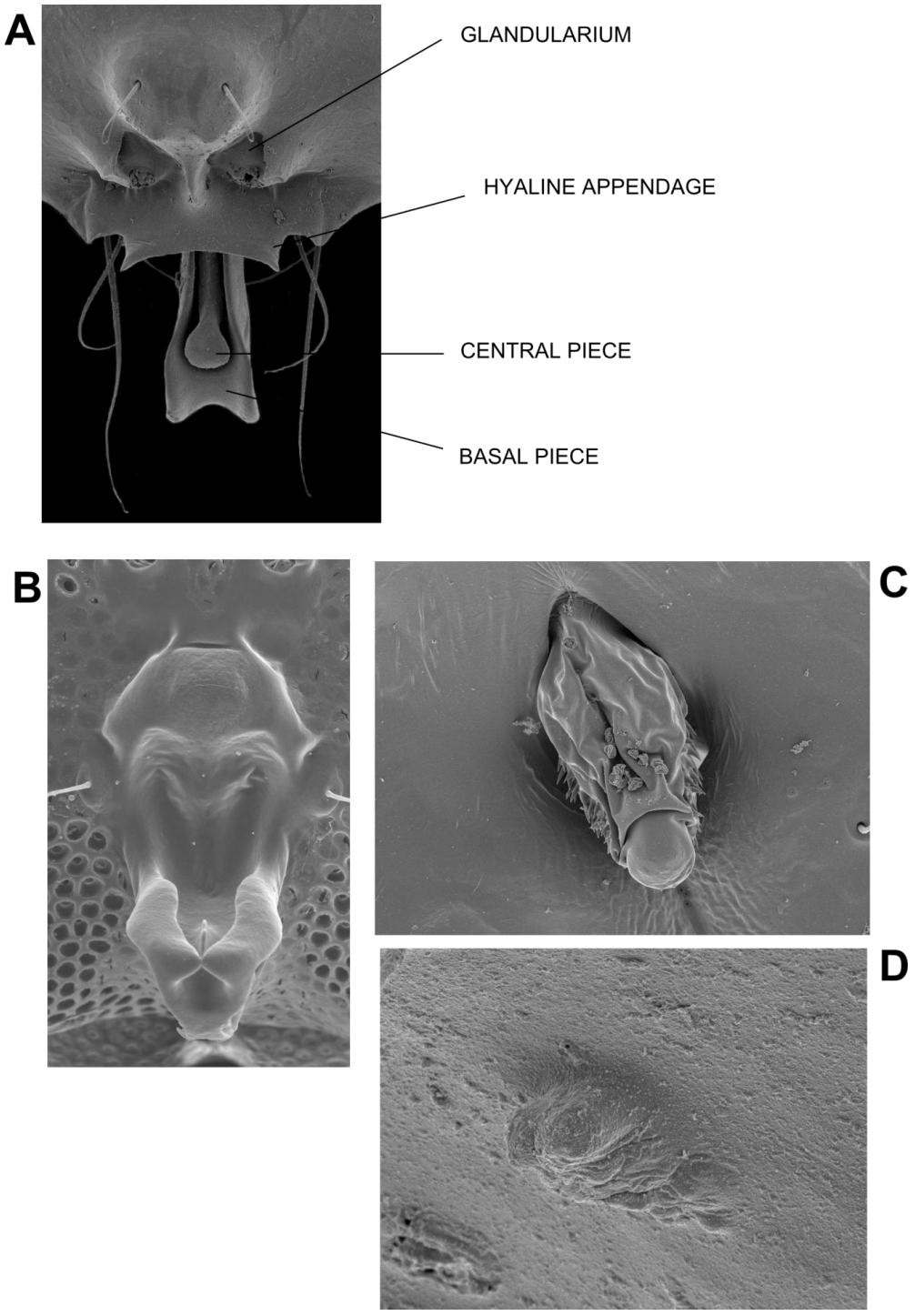

Figure 1.3.1.4. Intromittent organ (petiole) in different *Arrenurus* males; A. *A. (Arr.) bicuspidator*, well developed petiole with a central piece and a hyaline appendage at the base of petiole, B. *A. (Arr.) pustulator*, well developed petiole without central piece and hyaline appendage at the base of petiole, C. *A. (Miu.) sinuator*, short petiole (sklerite covered with a wrinkled membrane), D. *A. (Meg.) globator*, peg-like petiole.

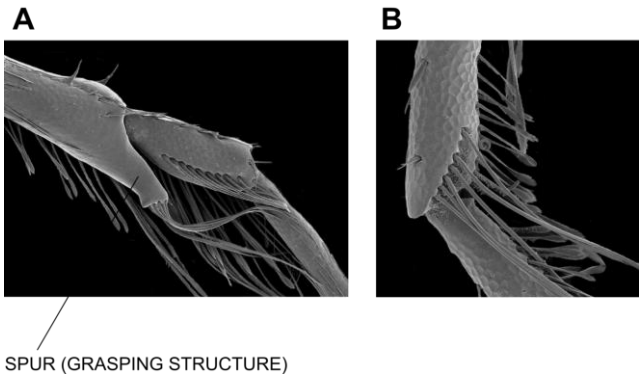

Figure 1.3.1.5. Fourth and fifth segment of IV-L in *Arrenurus* males: A. *A. (Arr.) bicuspidator*, spur (grasping structure) visible on fourth segment; B. *A. (Mic.) albator*, spur absent on fourth segment.

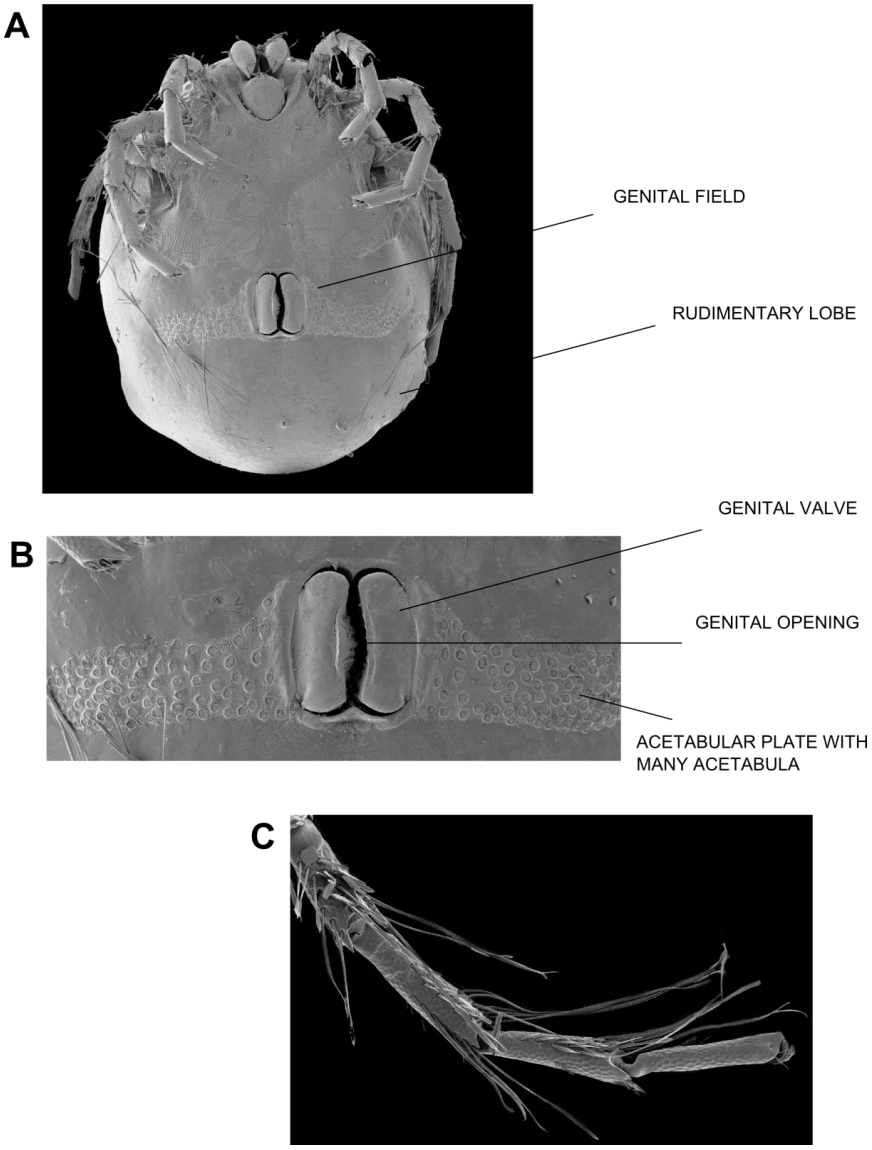

Figure 1.3.1.6. Morphology of *A. (Mic.) albator* female: A. ventral view, B. genital area, C. IV-L without spur.

1. 3. 2. Mating behaviour

There are two major types of copulation in different *Arrenurus* species. In the first one males use the intromittent organ (the petiole) to insert sperm into the genital tract of the female (male control), whereas in the second type males simply press the female's genital area on the sperm mass of the spermatophore and the female subsequently pushes the sperm in (female control). Thus, there are expected differences in degree of conflict between the sexes among different species of *Arrenurus*, because females appear to have differential control over sperm uptake. It is predicted that in species with males equipped with well developed intromittent organ sexual conflict underlies the evolution of morphology and behaviour, and in species with males that lack this structure female choice is assumed to be the stronger current force of selection.

Despite the diversity and broad distribution of the genus, relatively little has been published about mating behaviour or chemical communication in *Arrenurus*. Communication via sex pheromones among differently related *Arrenurus* species was examined by Smith and Hagman (2002), and Smith and Florentino (2004). Smith and Hagman presented experimental evidence for a nonpolar and water-borne sex pheromone produced by females that elicits 'readiness posture' in males. In this posture the male crooks his fourth legs at the fourth distal segment and holds them flat over his back (typically displayed in the close proximity of females). Subsequently, Smith and Florentino examined male responses to water conditioned by conspecific and heterospecific females. They showed that communication via sex pheromones occurs in subgenera *Arrenurus*, *Megaluracarus* and *Truncaturus*. In these experiments males responded with arrestant behavior (male freezes in a close proximity of female), leg fanning (moving fourth legs in a rotary motion) and readiness posture to conspecific cues, but in a few cases also to heterospecific cues. However, the authors stated that cross-attraction occurs only between representatives of the same species group, and not between members of different species groups and subgenera. They concluded that sex pheromones may be not decisive in species recognition, since most species with cross-attraction co-occur in natural habitats. Chemoreception in *Arrenurus (Micruracarus) acutus* was studied by Baker (1996) with the use of ultrastructural methods and in behavioural experiments. Baker (1996) found that chemosensory sensilla located on the palpi, tarsi and tibiae of legs I and II in *A. acutus* have a porous cuticle and contain dendrites. In addition, behavioural experiments showed that these sensilla are involved in the perception of chemical cues of mates.

Proctor (1992b) states that in all *Arrenurus* subgenera occurs pairing with direct transfer (copulation), in which males play an active role in placing sperm in a female's sperm-receiving structure. Sperm transfer behaviour of species from the subgenus *Megaluracarus* has been described for *A. (Meg.) globator* (Lundblad, 1929; Böttger, 1962), *A. (Meg.) manubriator* (Proctor and Smith, 1994), *A. (Meg.) marshalli* (Proctor, 1992b) and *A. (Meg.) birgei* (Proctor, 1992b). In *A. globator* and *A. manubriator*, males display readiness posture and present their cauda to female in the first stage of courtship (Lundblad, 1929; Proctor and Smith, 1994). Female may take the active part in mounting male's hindbody in *A. globator* (Böttger, 1962). Proctor and Smith (1994) noted that females of *A. manubriator* touch males with palps and forelegs prior to mounting. In *Megaluracarus* females are manoeuvred with hind legs of males and glued to cauda with the use of a sticky and transparent secretion (Lundblad, 1929, 1930; Proctor and Smith, 1994). In *A. globator* and *A. manubriator* males do not insert sperm into the female reproductive tract. Females of both species push in the sperm placed by males on their genital flaps. The males of the two species display slow lateral waving, vigorously jerk cauda side to side and sharply jerk their backs upwards. The vigorous side-jerking of the body was observed also in *A. marshalli* and *A. birgei*, and was thought to encourage female to take up sperm on her genital valves (Böttger, 1962; Proctor, 1992b). To disconnect, males of *Megaluracarus* push their fourth legs against the female's venter (Proctor, 1992b), or shake their cauda vigorously (Proctor and Smith, 1994). The females of *A. manubriator* may separate from males through grabbing substratum (Proctor and Smith, 1994). After separation male of *A. manubriator* may engage in mating with the same female, which is assumed to make her less inclined to seek out another male to mate with (Proctor, 2002). The duration of mating has been measured for *A. globator* (2-4 hours, Lundblad, 1929) and for *A. manubriator* (on average about 2 hours; Proctor and Wilkinson, 2001). There are differences in time spent on behaviours in different stages of mating. *Arrenurus globator* spend less time on pre-deposition behaviours than *A. manubriator*. However, for the post-deposition stage was observed the opposite pattern (Böttger, 1962; Proctor and Smith, 1994).

In the pre-pairing stage of mating, males of the subgenus *Arrenurus* move their fourth legs in a rotary motion or hold them crooked over their backs (e.g. *A. (Arr.) sp. nr. reflexus*, Proctor and Wilkinson, 2001). In these species, the male presents his cauda to passing female and attempts to put it under her and grasp her with spurs on fourth legs. Males of *A. (Arr.) valdiviensis* and *A. (Arr.) dentipetiolatus* grasp females that

subsequently enter a state of rigidity (Böttger, 1965; Proctor, 1992b; respectively). In contrary, females of *A. (Arr.) cuspidifer* may take the active role in climbing onto the male's cauda (Cassagne-Mejean, 1966). In the spermatophore deposition stage of mating, males of *A. valdiviensis* (Böttger, 1965) and *A. sp. nr. reflexus* (Proctor and Wilkinson, 2001) lift their cauda, presumably drawing out a spermatophore, then lean forward to pick up sperm on petiole and slightly rock hindbody. Subsequently, the petiole with load of sperm is inserted in to the female's genital tract. Furthermore, the vigorous sideways jerking of male's hind back with glued female is displayed by *A. cuspidifer* (Cassagne-Mejean, 1966) and *A. sp. nr. reflexus* (Proctor and Wilkinson, 2001). Moreover, in the post-sperm-transfer stage of mating long periods of motionlessness occur in mating of *A. valdiviensis* and *A. sp. nr. reflexus* (Böttger, 1965; Proctor and Wilkinson, 2001; respectively). In addition, in male of *A. valdiviensis* this behaviour is accompanied by trembling third legs near the genital area of female. Proctor (1992b) summarizes that separation is achieved in *A. valdiviensis* and *A. cuspidifer* by pressing fourth legs against the female's venter. However, *Arrenurus (Arr.) planus* differs strongly in mating behaviour from other *Arrenurus* s. str. Although in this species males are equipped with petiole, sperm is not gathered from substrate-deposited spermatophores onto the head of the petiole but is rather transferred along the petiole into the female's genital opening via legs (Proctor and Wilkinson, 2001). Male of *A. planus* brushes ventral side of his body with forelegs presumably transferring sperm from his genital opening on to petiole, and female seems to push sperm with her fourth legs in to her genital opening (Proctor and Wilkinson, 2001). The total duration of mating in *Arrenurus* s. str. seems to be longer than in other *Arrenurus*, which results from the time spent in the stage following the deposition and collection of spermatophores. *Arrenurus valdiviensis* spends 3-4 hours on post transfer behaviours (Böttger, 1965), *A. cuspidifer* even up to 7 hours, and *A. sp. nr. reflexus* on average about 5 hours (Proctor and Wilkinson, 2001).

The mating behaviour of *Truncaturus* mites is known from observations for *A. (Tru.) stecki* (Lundblad, 1929) and *A. (Tru.) rufopyriformis* (Proctor and Wilkinson, 2001). Though the particular behavioural events are similar in both species, the courtship of *A. rufopyriformis* seems to be more complex than that of *A. stecki*. In the pre-pairing stage of mating, males of both species crook their hind legs and hold them flat over their backs (Lundblad, 1929; Proctor and Wilkinson, 2001). In the deposition stage of mating in the two species male with female glued to the hindbody jerks cauda up, leans his body slowly to the left by bending left legs I to III, and then to the right by bending right legs I to III,

and strokes fourth legs along sides of female's body (Lundblad, 1929; Proctor and Wilkinson, 2001). Separation seems to be achieved in *A. stecki* and *A. rufopyriformis* either by sharp vertical jerking or vigorous swimming (see Lundblad, 1929), or grabbing substratum by female (*A. rufopyriformis*, Proctor and Wilkinson, 2001). The duration of mating in both *Truncaturus* seems to be shorter than in other *Arrenurus*. *Arrenurus stecki* spend on mating from 0.5 to 1 hour (Lundblad, 1929), and *A. rufopyriformis* on average 1 hour (Proctor and Wilkinson, 2001).

Knowledge about mating of *Micruracarus* mites is based only on the partial mating sequence of *A. (Micruracarus) forpicatus* (Lundblad, 1929). Male and female of *A. forpicatus* swim rapidly and crash repeatedly with ventral sides of their bodies. They touch with palpi and legs when being turned towards ventral sides of their bodies, but do not show ready position. The male of *A. forpicatus* maneouvres female on to his back and glues her with the sticky secretion (Lundblad, 1929). The first stage of mating in this species resembles wrestling of the sexes in *A. (Arr.) planus* (Proctor and Wilkinson, 2001). Sperm transfer in *A. forpicatus* was not observed (Lundblad, 1929). Moreover, there are no data on mating duration in *Micruracarus*.

1. 3. 3. Taxonomy

Although the genus *Arrenurus* consists of 11 putative subgenera worldwide, the subject of this study are subgenera of the Palearctic and Nearctic regions: *Arrenurus* s. str., *Megaluracarus, Micrarrenurus, Micruracarus* and *Truncaturus* (Tab. 1.3.1). The current subgeneric classification of the genus is based predominantly on male reproductive morphology since females are morphologically very similar (Smit, 2012). The main distinguishing characters used in systematics of the genus (including species delimitation) pertain to presence or absence of the intromittent organ (the petiole) and modifications of the hindbody (cauda) and fourth legs (Cook, 1974). In the genus *Arrenurus*, males can be grouped according to morphological adaptations for mating. Species with males equipped with elaborate cauda with well developed pygal lobes and petiole, modified fourth legs and dorsum can be found in the subgenus *Arrenurus* (see Fig. 1.3.1.3 A, Fig. 1.3.1.4 A, B, Fig. 1.3.1.5 A). In contrary males from the subgenus *Micrarrenurus* have shorter cauda and lack a spur on hind legs and a hyaline appendage at the base of petiole (see Fig. 1.3.1.3 C, Fig. 1.3.1.5 B). Males that have a very elongated cauda (set off from the body proper) and either lack a petiole or have a small peg-like petiole belong to the subgenus *Megaluracarus*

(Fig. 1.3.1.3 B, Fig. 1.3.1.4 D). Males with a short hindbody with a deep medial cleft, and that either lack a petiole or have a short petiole without a central piece are grouped in the subgenus *Micruracarus* (Fig. 1.3.1.3 D, Fig. 1.3.1.4 C). Males that lack significant body modifications with cauda that are only slightly elongated and not set off from the body proper, and therefore resemble females, belong to the subgenus *Truncaturus* (see Fig. 1.3.1.3 E; Cook, 1974, Proctor, 1992b).

2. Goals of the thesis and expected results

The main goal of the study is to reconstruct the evolution of mating behaviour and external morphological structures associated with reproduction, and to test hypotheses about the driving forces of diversification in *Arrenurus* (Arrenuridae) in a phylogenetic context.

There are species of the genus *Arrenurus* in which sperm is placed on or near the female's genital valves, and subsequently pushed in by female (female control), and species in which the male loads sperm on an intromittent organ (the petiole) and inserts it into the female (male control). This raises the question whether sexual conflict underlies the evolution of behaviour and morphology of species with well developed intromittent organ, and female choice is the stronger current force of selection in species with males that lack this structure.

The scientific problems aimed to be solved are:

- testing the status of species with the application of DNA barcodes,
- resolving phylogenetic relationships in *Arrenurus* from Europe and North America with the use of molecular markers from the nuclear and mitochondrial genome,
- testing pheromone responses between species differing in the degree of relatedness,
- describing the relationship between the strength of behavioural responses of males to female cues, and phylogenetic distance,
- describing complexity and duration of mating in differently related species,
- mapping of evolution of male and female morphological structures associated with mating on to phylogenetic tree,
- mapping of evolution of mating behaviour on to phylogenetic tree.

3. Materials and methods

3. 1. Mite collection, identification and morphological analyses

Mites were collected in Europe and North America from standing and running waters including rivers, streams, springs, lakes, ponds, temporary water bodies and wetlands. European mites were collected in Germany, Poland, Austria and the Netherlands. North American species came mostly from areas located around the Queen's University Biological Station (Ontario, Canada) and Elk Island National Park (Alberta, Canada), but also from United States (Texas). The mites were collected during field surveys in years 2011-2014 (Tab. 3.1.1).The research material from Germany, Austria and the Netherlands was kindly provided by Dr. Reinhard Gerecke (Tübingen, Germany), Dr. Peter Martin (Zoological Institute, Limnology, University of Kiel, Germany) and Dr. Harry Smit (Naturalis Biodiversity Center, the Netherlands).

The samples were collected with the use of an aquatic net (mesh size 250 μm) and light traps (for design see p. 651, Proctor et al., 2015). Water mites were sorted in the laboratory under a stereomicroscope and preserved in 96% ethyl alcohol. Mites collected in Europe were determined to species level using Viets (1936), Cassagne-Méjean (1966), Davids et al. (2007) and Di Sabatino et al. (2010). Species from North America were determined with Cook (1954a, 1954b, 1955). In questionable cases the correctness of identifications were checked by Prof. Bruce Smith (Ithaca College, Ithaca, NY, U.S.A) and Dr. Ian Smith (The Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa).

Morphological adaptations for mating were characterized based on more than 200 scanning electron micrographs of 28 *Arrenurus* species (see Appendix 1-28). The images were taken with the use of a JEOL field emission scanning electron microscope (SEM) in the Department of Earth and Atmospheric Sciences (University of Alberta), and in the Faculty of Biology (Adam Mickiewicz University). For SEM studies the mites were dehydrated through an alcohol-HMDS (hexamethyldisilazane) series, mounted on stubs with double-sided tape and sputter coated with gold. The SEM images were adjusted (background cleaning) in Photoshop 6.0. Morphological data concerning further 13 species were taken from literature for European taxa (Viets, 1936; Cassagne-Méjean, 1966) and from unpublished data from Bruce P. Smith for North American taxa.

Table 3.1.1. Species included in this study. GenBank accession numbers are given for species represented by unique haplotypes.

				GenBank Acc. no.	
Subgenus	Species	Locality	DNA voucher	28S rDNA	COI
Arrenurus s. str.	*A. major* Marshall, 1908	Ontario, Canada	AMUmw255	KP836122	KP836187
	A. tricuspidator (O. F. Müller, 1776)	Germany	AMUmw164	KP836133	KP836199
	A. tricuspidator (O. F. Müller, 1776)	Germany	AMUmw167	-	KP836200
	A. bruzelii Koenike, 1885	Germany	AMUmw104	KP836113	KP836177
	A. bruzelii Koenike, 1885	Germany	AMUmw105	-	KP836178
	A. neumani Piersig, 1895	Poland	AMUmw115	KP836125	KP836190
	A. neumani Piersig, 1895	Poland	AMUmw122	KP836126	KP836191
	A. neumani Piersig, 1895	The Netherlands	AMUmw274	KP836127	KP836192
	A. robustus Koenike, 1894	Germany	AMUmw143	KP836131	KP836197
	A. cuspidator, (O. F. Müller, 1776)	Poland	AMUmw116	KP836132	KP836198
	A. affinis Koenike, 1887	Germany	AMUmw130	KP836109	KP836172
	A. affinis Koenike, 1887	The Netherlands	AMUmw264	KP836110	-
	A. compactus Piersig, 1894	Poland	AMUmw111	-	KP836180
	A. compactus Piersig, 1894	Poland	AMUmw110	KP836114	KP836179
	A. compactus Piersig, 1894	Austria	AMUmw192	KP836115	-
	A. cuspidifer Piersig, 1896	Germany	AMUmw161	KP836116	KP836181
	A. maculator (O. F. Müller, 1776)	Poland	AMUmw120	KP836120	-
	A. pustulator (O. F. Müller, 1776)	Poland	AMUmw152	KP836128	KP836194
	A. bicuspidator Berlese, 1885	Germany	AMUmw101	KP836111	KP836174
	A. americanus (red) Marshall, 1908	Ontario, Canada	AMUmw258	-	KP836171
	A. americanus (green) Marshall, 1908	Ontario, Canada	AMUmw051	-	KP836173
	A. hungerfordi Cook, 1954	Alberta, Canada	AMUmw093	-	KP836185
	A. reflexus Marshall, 1908	Ontario, Canada	AMUmw013	KP836129	KP836195
	A. reflexus Marshall, 1908	Ontario, Canada	AMUmw017	KP836130	KP836196
	A. bleptopetiolatus Cook, 1954	Ontario, Canada	AMUmw001		KP836175
	A. bleptopetiolatus Cook, 1954	Ontario, Canada	AMUmw007	KP836112	KP836176
	A. magnicaudatus Marshall, 1908	Ontario, Canada	AMUmw031	KP836121	KP836186
	A. maryellenae Cook, 1954	Ontario, Canada	AMUmw250	KP836123	KP836188
	A. planus Marshall, 1908	Ontario, Canada	AMUmwpla1	-	KP836193
	A. mucronatus Levers, 1945	Ontario, Canada	AMUmw048	KP836124	KP836189
	A. fissicornis Marshall, 1908	Ontario, Canada	AMUmw008	KP836117	KP836182
	A. fissicornis Marshall, 1908	Ontario, Canada	AMUmw011	KP836118	KP836183
	A. fissicornis Marshall, 1908	Ontario, Canada	AMUmw012	KP836119	KP836184
	A. claviger Koenike 1885	Poland	-	-	-
Micrarrenurus	*A. crassicaudatus* Kramer 1875	Poland	AMUmw235	KP836156	KP836225
	A. albator (O.F. Müller, 1776)	Germany	AMUmw098_100	KP836155	KP836224
	A. fimbriatus Koenike, 1885	Poland	AMUmw225	KP836157	KP836226
Micruracarus	*A. biscissus* Lebert, 1879	Germany	AMUmw140	KP836158	KP836227
	A. sinuator (O. F. Müller, 1776)	Germany	AMUmw171	-	KP836232
	A. sinuator (O. F. Müller, 1776)	Poland	AMUmw234	KP836164	KP836233
	A. sinuator (O. F. Müller, 1776)	Germany	AMUmw159	KP836163	KP836231
	A. perforatus George, 1881	Germany	AMUmw157	KP836162	KP836230
	Arrenurus sp1	Poland	AMUmw237	KP836165	KP836234
	Arrenurus sp1	Poland	AMUmw238	-	KP836235
	A. inexploratus Viets, 1930	Poland	AMUmw232	KP836159	KP836228
	A. lyriger Marshall, 1908	Ontario, Canada	AMUmw046	KP836161	KP836229
	A. setiger Koenike, 1895	Ontario, Canada	AMUmw039	KP836166	KP836236
	A. setiger Koenike, 1895	Ontario, Canada	AMUmw040	-	KP836237
	A. setiger Koenike, 1895	Ontario, Canada	AMUmw042	-	KP836238
	A. cf. lyriger	Ontario, Canada	AMUmw251	KP836160	-

Table 3.1.1 (continued). Species included in this study. GenBank accession numbers are given for species represented by unique haplotypes.

				GenBank Acc. no.	
Subgenus	Species	Locality	DNA voucher	28S rDNA	COI
Truncaturus	*A. stecki* Koenike, 1894	Poland	AMUmw223	KP836170	KP836242
	A. stecki Koenike, 1894	Poland	AMUmw200	-	KP836241
	A. fontinalis Viets, 1920	Germany	AMUmw141	KP836168	-
	A. truncatellus (O. F. Müller, 1776)	Poland	AMUmw201	KP836167	KP836239
	Arrenurus sp3	Ontario, Canada	AMUmw303	KP836169	KP836240
	A. rufopyriformis Habeeb, 1954	USA	-	-	-
Megaluracarus	*A. cylindratus* Piersig, 1896	Germany	AMUmw165	KP836138	KP836206
	A. securiformis Piersig, 1894	Germany	AMUmw124	-	KP836218
	A. securiformis Piersig, 1894	Germany	AMUmw139	KP836150	KP836219
	A. securiformis Piersig, 1894	Germany	AMUmw156	KP836151	KP836220
	A. mediorotundatus Thor, 1898	Germany	AMUmw142	KP836146	KP836215
	A. scutiliformis Garms, 1961	Ontario, Canada	AMUmw256	KP836149	-
	A. cardiacus Marshall, 1903	Ontario, Canada	AMUmw259	KP836137	KP836205
	A. globator (O. F. Müller, 1776)	Poland	AMUmw211	KP836139	KP836207
	A. buccinator (O.F. Müller, 1776)	Germany	AMUmw106	KP836136	-
	A. apetiolatus (blue) Piersig, 1904	Ontario, Canada	AMUmw034	-	KP836201
	A. apetiolatus (blue) Piersig, 1904	Ontario, Canada	AMUmw036	KP836135	KP836202
	A. apetiolatus (blue) Piersig, 1904	Ontario, Canada	AMUmw074	-	KP836203
	A. apetiolatus (blue) Piersig, 1904	Ontario, Canada	AMUmw082	-	KP836204
	A. apetiolatus (red) Piersig, 1904	Ontario, Canada	AMUmw248	KP836134	-
	A. marshallae Piersig, 1904	Ontario, Canada	AMUmw247	KP836148	KP836217
	A. intermedius (blue) Marshall, 1940	Ontario, Canada	AMUmw306	KP836140	KP836208
	A. intermedius (blue) Marshall, 1940	Ontario, Canada	AMUmw307	KP836141	KP836209
	A. intermedius (blue) Marshall, 1940	Ontario, Canada	AMUmw308	KP836142	KP836210
	A. intermedius (red) Marshall, 1940	Alberta, Canada	AMUmw263	KP836152	KP836221
	A. megalurus Marshall, 1903	Ontario, Canada	AMUmw249	KP836147	KP836216
	A. manubriator (blue) Marshall, 1903	Ontario, Canada	AMUmw028	KP836143	KP836211
	A. manubriator (blue) Marshall, 1903	Ontario, Canada	AMUmw030	-	KP836212
	A. manubriator (red) Marshall, 1903	Texas, USA	AMUmw020	KP836144	KP836213
	A. manubriator (red) Marshall, 1903	Texas, USA	AMUmw023	KP836145	KP836214
	A. wardi Marshall, 1940	Ontario, Canada	AMUmw301	KP836153	KP836222
	A. wardi Marshall, 1940	Ontario, Canada	AMUmw309	KP836154	KP836223

3. 2. Molecular analyses

3. 2. 1. DNA amplification and sequencing

Genomic DNA was extracted from single mites using a nondestructive method (Dabert et al., 2008). The following primers were applied for COI gene fragment amplification (Dabert et al., 2010):

- bcdF01 (5'-CATTTTCHACTAAYCATAARGATATTGG-3'),
- bcdR04 (5'-TATAAACYTCDGGATGNCCAAAAAA-3').

For amplification of the D2 region of the 28S rDNA were applied (Mironov et al., 2012):

- 28SF0001 (5'-ACCCVCYNAATTTAAGCATAT-3'),
- 28SR0990 (5'-CCTTGGTCCGTGTTTCAAGAC-3').

PCR amplifications were carried out in 10 µl reaction volumes with 4 µl (1-5 ng) of DNA, 5 µl Type-it Microsatellite PCR Kit (Qiagen, Hilden, Germany) and 0.5 µM of primer, and with the use of a thermocycling profile of one cycle of 5 min at 95 °C followed by 35 steps of 30 sec at 95 °C, 1 min at 50 °C, 1 min at 72 °C, with a final step of 5 min at 72 °C. The PCR reactions were diluted after amplification with 5 µl of water and directly sequenced using 1 µl of the diluted PCR reaction and 50 pmoles of sequencing primer. Sequencing was conducted with a BigDye Terminator v3.1 on an ABI Prism 3130XL Analyzer (Applied Biosystems). The total number of generated sequences was 219 (134, COI; 85, D2 28S rDNA). The 134 unique haplotypes have been uploaded to GenBank with Accession Nos. KP836109 - KP836170 (for D2 28S rDNA) and KP836171 - KP836242 (for COI) (see Tab. 3.1.1). The COI and D2 28S rDNA sequences of *Horreolanus orphanus* were taken from the GenBank (Tab. 3.1.1).

3. 2. 2. Dataset

COI and D2 28S rDNA sequences were assembled with Chromas Lite 2.0 (http://chromas-lite.software.informer.com/). COI sequences were aligned manually using GeneDoc v. 2.7.0 (Nicholas and Nicholas, 1997). Contigs of the D2 28S rDNA were preliminary aligned using Clustal X 2.0.10 (Larkin et al., 2007) and subsequently justified manually in GeneDoc. Pairwise distances were calculated for COI and D2 sequences in

MEGA 5.0 (Tamura et al., 2011) with the application of the Kimura 2-parameter model (Kimura, 1980).

3. 2. 3. Tree building procedure

Trees were built for 45 named *Arrenurus* species, 4 initially unclassified taxa and 3 apparent colour variants of *A. (Meg.) apetiolatus*, *A. (Meg.) intermedius* and *A. (Arr.) americanus* with application of maximum likelihood (ML) method. An outgroup species was *Horreolanus orphanus*. Trees were constructed for two molecular markers, separately and together: cytochrome oxidase I gene fragment (COI) from mitochondrial DNA (537 nucleotide positions), and the gene coding for the 28S rRNA (large subunit ribosomal RNA, D2 domain, 691 nucleotide positions) from the nuclear genome. The concatenated dataset COI+D2 included 1228 nucleotide positions. The fast mutation rate of COI enables discrimination of closely related species being at the same time relatively conserved among conspecifics. In contrast, the D2 region of 28S rDNA is conservative enough to reveal ancient relationships (Dabert, 2006). Therefore, the ML tree based on COI shows species boundaries, and the ML tree obtained based on D2 28S rDNA resolves deeper phylogenetic relationships within the genus *Arrenurus*. The ML tree built based on the concatenated dataset COI+D2 combines information from both markers and thus, was used as a hypothesis for phylogenetic relationships of this set of *Arrenurus* species. There were 454 variable characters in the combined datamatrix (175 for COI, 279 for D2 28S rDNA). In all analyses, for D2 28S rDNA the best model of DNA evolution chosen by jModelTest 0.1.1 (Guindon and Gascuel, 2003; Darriba et al., 2012) was GTR + I + G, and Codon model was selected for COI sequences. ML analyses were performed with 10 search replications in Garli 0.96 (Zwickl, 2006). Support values of the nodes were obtained in Garli with non-parametric 100 bootstrap replicates. Trees were edited in Inkscape 0.48.4-1 (Harrington, 2004-2005) and MEGA5 (Tamura et al., 2011).

3. 2. 4. Species delimitation methods

I was interested in testing whether there was statistical support for *a priori* defined species, and whether color morphs of what appeared to be the same species were actually cryptic species. Pairwise distances between COI and D2 nucleotide sequences were computed with the Kimura 2-parameter model (Kimura, 1980) in MEGA5 (Tamura et al., 2011). The COI distances between *a priori* identified species are expected to be at least 10

x the intracluster variation (a rule of thumb for recognizing new species) (Hebert et al., 2004). Moreover, since the monophyly of taxa does not always result from differential selection, but can be caused by stochastic processes of gene coalescence within a panmictic population (genetic drift) (Rosenberg, 2007), the probability of reciprocal monophyly under the null model of random coalescence was computed using Geneious 6.1.6 (Masters et al., 2011). The probability of species distinctiveness was assessed with Randomly Distinct P_{RD} (Rodrigo et al., 2008) and reciprocal monophyly P_{AB} (Rosenberg, 2007) for *A. (Megaluracarus) manubriator*, which had the largest sample size of individuals from two geographically distant populations (Ontario, Texas) that also displayed different colours (blue vs red). Randomly Distinct P_{RD} values from 0.05 to 1 indicate groups characterized by branching events expected under the coalescent model, while values less than 0.05 show that the presence of a cryptic species is possible. Rosenberg's P_{AB} reflects the probability of reciprocal monophyly under the null model of random coalescence. In addition, gene genealogies were estimated based on COI sequences in TCS 1.21 using statistical parsimony (cladogram estimation method; Templeton et al., 1992). Each statistical parsimony network represents a single species, and COI sequences that do not form networks represent separate species. The probability of parsimony was computed for COI pairwise differences until the probability exceeded 0.95.

3. 2. 5. Mapping of character evolution

The evolution of morphology of male reproductive structures was mapped for 13 characters from 41 *Arrenurus* species. Evolutionary changes in mating behaviour were mapped for 13 characters from 13 *Arrenurus* species for which full mating sequences are known. The morphological and behavioural traits were plotted onto the pruned ML tree (COI+D2). The likelihood Markov k-state 1 parameter model was applied for mapping evolutionary changes of both morphological and behavioural characters and was performed in the MESQUITE 3.01 software package (Maddison and Maddison, 2014). The matrix for behavioural and morphological characters is presented in Table 3.2.5.1 and Table 3.2.5.2, respectively.

Table 3.2.5.1. Character matrix for *Arrenurus* species and *Horreolanus orphanus* (outgroup); a dash means that the character is inapplicable to the taxa studied; the symbol '?' indicates that the character state is unknown for a particular taxon; behavioural characters used in tracing of character evolution: 1 - male crooked his hind legs at the fourth distal segment and placed them over his back when the female was in a close proximity (ready position), 2 - touching female's body with claws of first and second legs in first stages of mating, 3 - spermatophores are deposited on the substratum, 4 - male jerks sharply back end upwards (vertical jerking), 5 - when courtship is completed female lies in a state of motionless rigidity at the bottom, 6 - male crawls around female, touches her with his first and second legs, displays ready position and attempts to start courtship again (mate attendance), 7 - Sperm is transferred with the use of legs, 8 - male is attached under the standing female facing in the opposite direction as her being dragged by her around, 9 - male leaned his body slowly to the left by bending left legs I to III, and then to the right by bending right legs I to III (sideways leaning), 10 - trembling third legs throughout mating by male, 11 - long periods of motionlessness when spermatophore deposition and collection are completed, 12 - male jerks sharply hind body side to side (side jerking), 13 - time spent in post-transfer behaviours (in %).

TAXON/CHARACTER	1	2	3	4	5	6	7/8	9	10	11	12	13
Horreolanus orphanus	?	?	?	?	?	?	?	?	?	?	?	?
A. (Meg.) manubriator	present	absent	present	present	present	?	absent	absent	absent	absent	present	<40
A. (Tru). rufopyriformis	present	absent	present	present	present	?	absent	present	absent	absent	absent	40-100
A. (Tru.) stecki	present	absent	present	present	absent	absent	absent	present	absent	absent	absent	<40
A. (Mic.) crassicaudatus	absent	absent	absent	absent	absent	absent	present	absent	absent	absent	absent	-
A. (Arr.) reflexus	present	absent	present	absent	present	?	absent	absent	present	present	present	40-100
A. (Meg.) globator	present	present	present	present	present	present	absent	absent	absent	absent	present	<40
A. (Arr.) cuspidator	present	present	present	absent	present	present	absent	present	present	present	present	40-100
A. (Arr.) tricuspidator	present	present	present	absent	present	absent	absent	absent	present	present	absent	?
A. (Arr.) planus	absent	present	absent	absent	absent	?	present	absent	absent	present	absent	-
A. (Arr.) bruzelii	present	absent	present	absent	present	present	absent	absent	present	present	present	40-100
A. (Arr.) bicuspidator	present	absent	present	absent	present	present	absent	absent	present	present	present	40-100
A. (Arr.) maculator	present	present	present	absent	absent	absent	absent	present	present	present	present	40-100
A. (Arr.) claviger	present	absent	present	present	present	present	absent	absent	present	present	absent	40-100

Table 3.2.5.2. Character matrix for *Arrenurus* species and *Horreolanus orphanus* (outgroup); a dash means that the character is inapplicable to the taxa studied; the symbol '?' indicates that the character state is unknown for a particular taxon; morphological characters used in tracing of character evolution: 1- the presence of the spur on leg IV, 2 - the presence of petiole, 3 - the shape of petiole, if present, 4 - the texture of petiole, 5 - the presence of central piece of the petiole, 6 - the shape of cauda, 7 - the presence of humps in the posterior part of the cauda, 8 - the presence of anterior dorsal humps, 9 - the number of anterior dorsal humps, if present, 10 - the shape of pygal lobes, 11 - the angle of petiole (if present) in relation to the main axis of the body. 12 - the presence of hyaline appendage, 13 - the presence of pigmented patches on the valves of the female genital opening.

TAXON/CHARACTER	1	2	3	4	5	6
Horreolanus orphanus	absent	absent	petiole absent	-	-	cauda absent
A. (Meg.) apetiolatus	present	present	peg-like	sclerotized	absent	very elongated and tubular
A. (Meg.) marshallae	present	absent	petiole absent	-	-	very elongated and tubular
A. (Med.) intermedius	present	absent	petiole absent	-	-	very elongated and tubular
A. (Meg.) manubriator	present	absent	petiole absent	-	-	very elongated and tubular
A. (Tru.) fontinalis	absent	absent	petiole absent	-	-	elongated, sclerotized and shallow concavity
A. (Arr.) robustus	present	present	well developed with central piece	sclerotized	present	elaborate with pygal lobes
A. (Arr.) major	present	present	well developed with central piece	sclerotized	present	elaborate with pygal lobes
A. (Arr.) americanus	present	present	well developed with central piece	sclerotized	present	elaborate with pygal lobes
A. (Arr.) maculator	present	present	well developed with central piece	sclerotized	present	elaborate with pygal lobes
A. (Arr.) affinis	present	present	well developed with central piece	sclerotized	present	elaborate with pygal lobes
A. (Arr.) compactus	present	present	well developed with central piece	sclerotized	present	elaborate with pygal lobes
A. (Arr.) neumani	present	present	well developed with central piece	sclerotized	present	elaborate with pygal lobes
A. (Arr.) bicuspidator	present	present	well developed with central piece	sclerotized	present	elaborate with pygal lobes
A. (Arr.) cuspidifer	present	present	well developed with central piece	sclerotized	present	elaborate with pygal lobes
A. (Arr.) bruzelii	present	present	well developed with central piece	sclerotized	present	elaborate with pygal lobes
A. (Arr.) tricuspidator	present	present	well developed with central piece	sclerotized	present	elaborate with pygal lobes
A. (Arr.) planus	present	present	well developed without central piece	sclerotized	absent	elaborate with pygal lobes
A. (Arr.) pustulator	present	present	well developed without central piece	sclerotized	absent	elaborate with pygal lobes
A. (Arr.) magnicaudatus	present	present	well developed without central piece	sclerotized	absent	elaborate with pygal lobes
A. (Arr.) maryellenae	present	present	well developed without central piece	sclerotized	absent	elaborate with pygal lobes
A. (Arr.) bleptopetiolatus	present	present	well developed with central piece	sclerotized	present	elaborate with pygal lobes
A. (Arr.) fissicornis	present	present	well developed with central piece	sclerotized	present	elaborate with pygal lobes
A. (Arr.) reflexus	present	present	well developed with central piece	sclerotized	present	elaborate with pygal lobes
A. (Meg.) globator	present	present	peg-like	sclerotized	absent	very elongated and tubular
A. (Tru.) truncatellus	present	absent	petiole absent	-	-	elongated, sclerotized and shallow concavity
Arrenurus (Tru.) sp3	present	present	peg-like	sclerotized	absent	elongated, sclerotized and shallow concavity
A. (Miu.) perforatus	absent	present	peg-like	sclerotized	absent	short with deep cleft
A. (Mic.) albator	absent	present	well developed without central piece	sclerotized	absent	short with pygal lobes and membranous sub-petiolar cavity
A. (Mic.) crassicaudatus	absent	present	well developed without central piece	sclerotized	absent	short with pygal lobes and membranous sub-petiolar cavity
A. (Miu.) biscissus	absent	present	small, partly membranous	membranous and simple	absent	short with deep cleft
A. (Miu.) sinuator	absent	present	small, partly membranous	membranous and complex	absent	short with deep cleft
A. (Mic.) fimbriatus	absent	present	well developed without central piece	sclerotized	absent	elongated, sclerotized and shallow concavity
A. (Tru.) stecki	absent	present	peg-like	sclerotized	absent	elongated, sclerotized and shallow concavity
A. (Miu.) inexploratus	absent	present	peg-like	sclerotized	absent	elongated, sclerotized and shallow concavity
A. (Meg.) mediorotundatus	present	absent	petiole absent	-	-	very elongated and tubular
A. (Meg.) cardiacus	present	absent	petiole absent	-	-	very elongated and tubular
A. (Meg.) cylindratus	present	present	peg-like	sclerotized	absent	very elongated and tubular
A. (Meg.) securiformis	present	absent	petiole absent	-	-	very elongated and tubular
A. (Meg.) scutiliformis	present	absent	petiole absent	-	-	very elongated and tubular
A. (Meg.) buccinator	present	present	peg-like	sclerotized	absent	very elongated and tubular
A. (Meg.) wardi	present	present	peg-like	sclerotized	absent	very elongated and tubular

Table 3.2.5.2 (continued). Character matrix for *Arrenurus* species and *Horreolanus orphanus* (outgroup); a dash means that the character is inapplicable to the taxa studied; the symbol '?' indicates that the character state is unknown for a particular taxon; morphological characters used in tracing of character evolution: 1- the presence of the spur on leg IV, 2 - the presence of petiole, 3 - the shape of petiole, if present, 4 - the texture of petiole, 5 - the presence of central piece of the petiole, 6 - the shape of cauda, 7 - the presence of humps in the posterior part of the cauda, 8 - the presence of anterior dorsal humps, 9 - the number of anterior dorsal humps, if present, 10 - the shape of pygal lobes, 11 - the angle of petiole (if present) in relation to the main axis of the body. 12 - the presence of hyaline appendage, 13 - the presence of pigmented patches on the valves of the female genital opening.

TAXON/CHARACTER	7	8	9	10	11	12	13
Horreolanus orphanus	absent	absent	-	absent	-	absent	absent
A. (Meg.) apetiolatus	present	absent	-	rudimentary	<180°	absent	absent
A. (Meg.) marshallae	present	absent	-	rudimentary	-	absent	absent
A. (Med.) intermedius	present	absent	-	rudimentary	-	absent	absent
A. (Meg.) manubriator	present	absent	-	rudimentary	-	absent	absent
A. (Tru.) fontinalis	absent	-	-	rudimentary	-	absent	present
A. (Arr.) robustus	present	present	two	well developed	parallel to main axis	present	present
A. (Arr.) major	present	present	two	well developed	parallel to main axis	present	present
A. (Arr.) americanus	present	present	two	well developed	parallel to main axis	present	present
A. (Arr.) maculator	present	present	two	well developed	parallel to main axis	present	present
A. (Arr.) affinis	present	present	two	well developed	parallel to main axis	present	present
A. (Arr.) compactus	present	present	two	well developed	parallel to main axis	present	present
A. (Arr.) neumani	present	present	two	well developed	parallel to main axis	present	present
A. (Arr.) bicuspidator	present	present	two	well developed	parallel to main axis	present	present
A. (Arr.) cuspidifer	present	present	two	well developed	parallel to main axis	present	present
A. (Arr.) bruzelii	present	present	two	well developed	parallel to main axis	present	present
A. (Arr.) tricuspidator	present	present	two	well developed	parallel to main axis	present	present
A. (Arr.) planus	present	absent	-	rudimentary	>180°	absent	absent
A. (Arr.) pustulator	present	absent	-	well developed	parallel to main axis	absent	present
A. (Arr.) magnicaudatus	present	present	one	well developed	parallel to main axis	absent	present
A. (Arr.) maryellenae	present	present	one	well developed	parallel to main axis	absent	present
A. (Arr.) bleptopetiolatus	present	present	two	well developed	parallel to main axis	present	present
A. (Arr.) fissicornis	present	present	two	well developed	parallel to main axis	present	present
A. (Arr.) reflexus	present	present	two	well developed	parallel to main axis	present	present
A. (Meg.) globator	present	absent	-	rudimentary	<180°	absent	present
A. (Tru.) truncatellus	absent	absent	-	rudimentary	-	absent	absent
Arrenurus (Tru.) sp3	absent	absent	-	rudimentary	<180°	absent	absent
A. (Miu.) perforatus	present	absent	-	rudimentary	<180°	absent	absent
A. (Mic.) albator	absent	absent	-	rudimentary	parallel to main axis	absent	absent
A. (Mic.) crassicaudatus	absent	absent	-	rudimentary	parallel to main axis	absent	absent
A. (Miu.) biscissus	present	absent	-	rudimentary	<180°	absent	absent
A. (Miu.) sinuator	present	absent	-	rudimentary	<180°	absent	absent
A. (Mic.) fimbriatus	present	present	two	rudimentary	parallel to main axis	absent	absent
A. (Tru.) stecki	absent	absent	-	rudimentary	<180°	absent	absent
A. (Miu.) inexploratus	present	absent	-	rudimentary	<180°	absent	absent
A. (Meg.) mediorotundatus	present	absent	-	rudimentary	-	absent	absent
A. (Meg.) cardiacus	present	absent	-	rudimentary	-	absent	absent
A. (Meg.) cylindratus	present	absent	-	rudimentary	<180°	absent	absent
A. (Meg.) securiformis	present	absent	-	rudimentary	-	absent	absent
A. (Meg.) scutiliformis	present	absent	-	rudimentary	-	absent	?
A. (Meg.) buccinator	present	absent	-	rudimentary	<180°	absent	absent
A. (Meg.) wardi	present	absent	-	rudimentary	<180°	absent	absent

3. 3. Experimental methods: responses to sex pheromones

3. 3. 1. Responses to sex pheromones among *Arrenurus* species of different relatedness

The aim of the experiment was to test pheromone responses between species differing in degree of relatedness, as judged by D2 28S rDNA distance, and to determine whether there is a relationship between the strength of behavioural response and phylogenetic distance. Arrestant behaviour and leg fanning displayed by males were used as indicators of the presence of pheromones that elicited a sexual response. In the experiment, I used 6 species representing different evolutionary lineages: *A. (Arr.) bicuspidator, A. (Arr.) bruzelii, A. (Meg.) globator, A. (Mic.) crassicaudatus* and *A. (Miu.) biscissus*. Mites were sampled from ponds near the University Campus of AMU in Poznań. Only freshly collected and well-fed mites were used in the experiment since pheromone production and male responsiveness decrease when mites are not in good condition (Smith and Florentino, 2004). Adults used in the experiment were fed with living ostracods and copepods from laboratory colonies. Female-conditioned water was produced by storing from 19 to 31 mites in tissue culture plates for 24 h at room temperature. However, only 9 females of *A. (Arr.) bicuspidator* were available. Tap water destined for use as a control was stored in a plastic beaker at same conditions. The test arena was a plastic dish 2 cm in diameter and 1 cm deep. The same volume of control water and female-conditioned water was added using a pipette in to containers with single males. The pipettes used for female-treated water from a particular species were used for that species throughout all sets of comparisons. Each male was first tested with control water, then the same individuals were tested with a sequence of water conditioned by females of different *Arrenurus* species. Conspecific female-conditioned water was added as the last treatment. There was a 5 minute break after addition of control water or water conditioned with females of a particular species before water from another species was introduced. Male responses were noted immediately after adding control water or female conditioned water (males were watched for 2 minutes). In each treatment 15-30 males per species (one male at a time in a single container) were used (Tab. 4.3.1.1). For *A. (Arr.) bicuspidator* and *A. (Miu.) biscissus* where males responded negatively to water conditioned with their own females the treatment with conspecific females was repeated on the second day. This was a double check for the presence of sex pheromones in these species because of the anomalous initial response.

3. 3. 2. Responses to sex pheromones among closely related *Arrenurus* s. str. species

The aim of the experiment was to check the strength of premating reproductive isolation, and therefore the possibility of interspecific crosses in closely related, geographically proximate *Arrenurus* s. str.: *A. (Arr.) bicuspidator, A. (Arr.) compactus, A. (Arr.) cuspidator* and *A. (Arr.) neumani*. Mites were collected in the Western-Pomeranian Lakeland from two dystrophic lakes (Bagnisko Lake, Brachowo Lake), and additionally from ponds near the University Campus of AMU in Poznań. The mites were maitained under the same conditions as in the experiment 'Responses to sex pheromones among *Arrenurus* species of different relatedness'. The strength of behavioural response was measured as altering previous form of locomotion, swimming or crawling towards the tip of the pipette and fanning fourth legs. However, the order of introducing control water and water from containers with females of different species was random. Ten males were tested separately (1 per container) in each trial of each experiment (exceptions: *A. (Arr.) bicuspidator*, 6 males; no males of *A. (Arr.) cuspidator* available) with water conditioned with 3 to 5 females (Tab. 4.3.2.1). No females of *A. (Arr.) compactus* were available, but nevertheless males were tested with water conditioned with females of other species. Male responses were noted after adding control water or female conditioned water (males were watched for 5 minutes).

3. 4. Mating observations: maintenance of mites, videotaping and behavioural events

Arrenurus mites in the deutonymphal and adult stage that were destined for observation of mating behaviour were maintained in the laboratory in tissue culture plates at room temperature. Each deutonymph was kept separately in its own well until it transformed into an adult. After transformation mites were sorted by sex and maintained together in microaquaria. The mites, both deutonymphs and adults, were fed with ostracods, copepods and cladocerans from native water bodies and laboratory colonies.

Male and female specimens (whenever possible, virgin adults) were maintained separately in their own wells 24 h before the observation. A plastic container 2 cm in diameter and 1 cm deep was half filled with water and the bottom was scratched to create a substrate for the mites to grip during mating. A moveable light source was turned on in later stages of observation so as not to disturb mites at the initial phase of mating. The trial began when a male was introduced to a container with a conspecific female.

The full mating sequences of the following species were videotaped: *A.* (*Arr.*) *bicuspidator*, *A.* (*Arr.*) *bruzelii*, *A.* (*Arr.*) *claviger*, *A.* (*Arr.*) *cuspidator*, *A.* (*Arr.*) *maculator*, *A.* (*Arr.*) *tricuspidator*, *A.* (*Meg.*) *globator*, *A.* (*Mic.*) *crassicaudatus* and *A.* (*Tru.*) *stecki*. An ethogram (chronological description of behavioural steps) was built for each species, and types and durations of behaviours were described. The descriptions of mating behavior of *A.* (*Arr.*) *planus*, *A.* (*Arr.*) *nr. reflexus*, *A.* (*Meg.*) *manubriator* and *A.* (*Tru.*) *rufopyriformis* were taken from Proctor and Wilkinson (2001).

The observations of mating sequences were made using a Zeiss Stemi 2000-C stereomicroscope and recorded with DIC illumination and digital camera Olympus DP71 with CellËD 2.8 software (Olympus Soft Imaging Solutions GmbH).

3. 5. Statistical analyses

Pheromone experiments: a Friedman's Test was run separately for each species in the two pheromone experiments 'Responses to sex pheromones among *Arrenurus* species of different relatedness' and 'Responses to sex pheromones among closely related *Arrenurus* s. str. species'. In cases where the same species was tested twice for pheromone responses, separate Friedman's Tests were run. The considered variables were dependent since female conditioned water and control water were added subsequently to containers with single males (repeated measures design). The variables were not continuous but ordinal, and thus, normal distribution of data was not achieved. Therefore, nonparametric Friedman's Test for ordinal dependent variables was applied to test for an overall difference in behavioural responses of males to water conditioned by conspecific and heterospecific females and control water. The significant p-values of the Friedman's Test for a treatment with subsequent adding cues of differently related females showed that at least one statistically significant difference between male responses to female cues or control water was found. Whenever Friedman's Test indicated that there were significant differences, a post-hoc test (Dunn's Test) was performed to find out which male reactions were significantly different from each other. The relationship between phylogenetic distance and bahavioural responses of males of differently related species was presented with the use of Graph 4.4.2.

Mating sequences: the difference in percentage of time spent on post-spermatophore-transfer behaviours between petiolate and apetiolate species was compared with the parametric two-tailed t-Test ($\alpha = 0.05$). The Shapiro-Wilk Test was applied to test

the null hypothesis that the data come from normally distributed populations. The Shapiro-Wilk Test showed $p > 0.05$ which indicated that the data for duration of post-transfer behaviours were distributed normally (Shapiro-Wilk Test, $p > 0.059$, apetiolate species; Shapiro-Wilk Test $p > 0.591$, petiolate species). Since this test is used for data which are continuously variable (no fixed limits), the percentages were changed to proportions and log transformed prior to analysis. All analyses were run using STATISTICA software.

4. Results

4. 1. Inferred phylogeny

I resolved phylogenetic relationships among 52 taxa of *Arrenurus* from Palearctic and Nearctic regions rooted with the outgroup species *Horreolanus orphanus*. Phylogenetic analysis reconstructed two main clades, A and B (Fig. 4.1.1). Clade A groups North American species from the subgenus *Megaluracarus* (bootstrap support 100%, all analyses) with the *A. apetiolatus* (blue and red) clade basal to the clade containing *A. intermedius* (blue and red), *A. manubriator*, *A. marshallae* and *A. megalurus*. Clade B clusters all European and all other North American species, but ML analysis was weakly supported (BS 58%).

Within clade B, the basalmost clade (C) includes the remaining North American and European *Megaluracarus* species with the exception of *A. (Meg.) globator*. The last species formed a clade with *A. (Truncaturus)* sp3 and *A. (Tru.) truncatellus* (clade E) which was sister to all *Arrenurus* s. str. (clade F). *Arrenurus (Tru.) fontinalis* was basal to the clade with *Micrarrenurus*, *Arrenurus* s. str., *Micruracarus*, other *Truncaturus* and *A. globator* (clades D-F). Clade D consisted of species from the subgenera *Micrarrenurus* and *Micruracarus*, and also of *A. (Truncaturus) stecki*. However, the last species group is weakly supported. Moreover, two representatives of the subgenus *Micrarrenurus*, *A. albator* and *A. crassicaudatus* clustered together in all trees (BS 100%), but the third, *A. fimbriatus*, grouped with *A. (Tru.) stecki*. *Arrenurus* (*Arrenurus*) species formed a well-supported monophyletic group (clade F) (BS 86%).

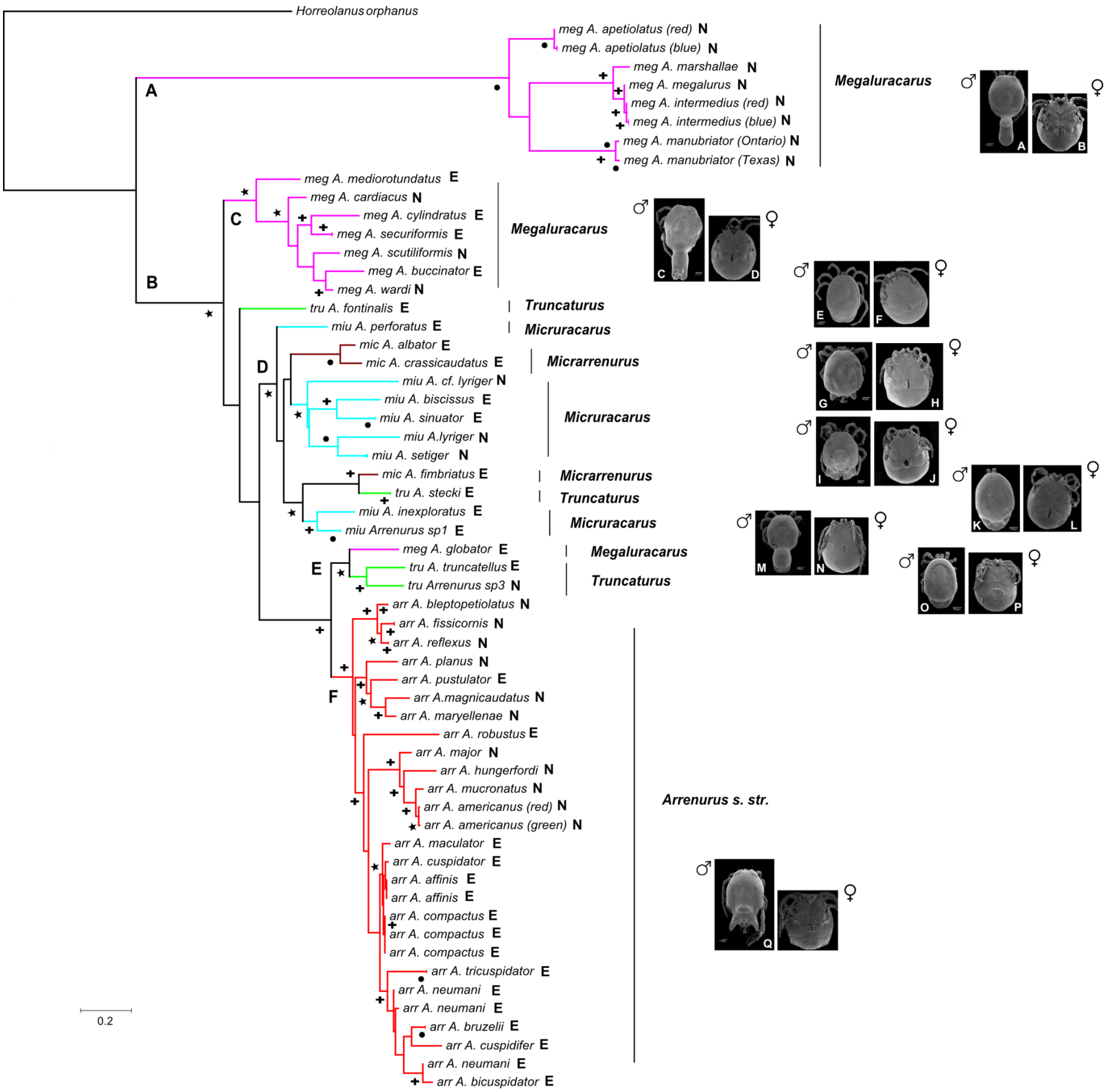

Figure 4.1.1. Maximum Likelihood tree calculated for concatenated D2 28S + COI dataset. Bootstrap support values are next to branches; • perfect support (100%) , + strong support (≥70%), ⋆ intermediate support (≥50%, <70%); arr *Arrenurus* s. str. (red), meg *Megaluracarus* (violet), mic *Micrarrenurus* (brown), miu *Micruracarus* (blue), tru *Truncaturus* (green), Hor *Horreolanus orphanus*; abbreviations after species names: E – Europe, N – North America; SEM photos illustrate sexual dimorphism: *A.* (*Meg.*) *apetiolatus*, male (A), female (B); *A.* (*Meg.*) *wardi*, male (C), female (D); *A.* (*Tru.*) *fontinalis*, male (E), female (F); *A. (Mic.) albator*, male (G), female (H); *A.* (*Miu.*) *biscissus*, male (I), female (J); *A.* (*Tru.*) *stecki*, male (K), female (L); *A.* (*Meg.*) *globator*, male (M), female (N); *Arrenurus* (*Tru.*) sp3, male (O), female (P); *A.* (*Arr.*) *bicuspidator*, male (Q), female (R).

4. 2. Species boundaries

The clades on the phylogenetic tree in most part show distinct species (Fig. 4.1.1). However, in a few monophyletic clades the distance from the species-defining node to the tip was very short. The low distinctiveness occurred in two groups of *Megaluracarus* from North America, some of which differed primarily in body colour: in *A. intermedius* (blue), *A. intermedius* (red) and *A. megalurus*, and *A. apetiolatus* (blue, Lake Opinicon, Ontario) and *A. apetiolatus* (red, Hebert's Bog, Ontario). Similarly, the distance from the species-defining node to the tip was small in green *A. (Arr.) americanus*, red *A. (Arr.) americanus* and *A. (Arr.) mucronatus* from *Arrenurus* s.str. (see Fig. 4.1.1). Moreover, a distinct phylogenetic structure was found in *A.* (*Meg.*) *manubriator.* The clade with *A.* (*Meg.*) *manubriator* was divided into two subclades (Fig. 4.1.1). The first one contained red individuals from San Marcos River (Texas, US) and the second one blue individuals from Lake Opinicon (Ontario). Furthermore, *Arrenurus (Arr.) affinis, A. (Arr.) bicuspidator, A. (Arr.) compactus, A. (Arr.) cuspidator* and *A. (Arr.) neumani* shared the same COI type (Type 1). In addition, a second type of COI (Type 2) occurred in *A. (Arr.) bicuspidator* and *A. (Arr.) neumani* (Fig. 4.2.1). In the ML tree based on the nuclear marker each of the five species is monophyletic (Fig. 4.2.2).

Intraspecific distances (COI) were calculated for the 28 putative species that were represented by more than 1 individual, and ranged from 0% (*A. (Arr.) compactus*, *A. (Arr.) magnicaudatus, A. (Arr.) robustus, A. (Meg.) globator*, *A. (Miu.) biscissus*, *Arrenurus (Tru.)* sp3) to 6.1% (*A. neumani*) (Fig. 4.2.3). The interspecific K2P distances between sister species pairs obtained for COI sequences ranged from 8.6% to 19.6% (Fig. 4.2.3). In the North American *Megaluracarus* species group that consisted of blue and red *A. intermedius* and *A. megalurus* genetic distances were typical for intraspecific variation, being 0.9% to 1.6% (Fig. 4.2.3). Similarly, in the species group of green and red *A. (Arr.) americanus* and *A. (Arr.) mucronatus* there were genetic distances typical for a within-species variability, being 0.8% to 5.1% (Fig. 4.2.3). Very low genetic diversification in mitochondrial markers also occurred among the *Arrenurus* s.str. species *A. affinis, A. bicuspidator, A. compactus, A. cuspidator* and *A. neumani* (Fig. 4.2.3).

Statistical parsimony (SP) analysis for COI data revealed that the 134 specimens had 88 unique haplotypes, and 23 distinct networks were identified (Fig. 4.2.4, Fig. 4.2.5). Seventeen networks corresponded to single defined species, and the other 6 networks were composed of haplotypes from more than one species. The SP analysis showed that the

haplotypes of *A. (Arr.) americanus* (red) and *A. (Arr.) americanus* (green) formed a single network (95% connection limit for species boundary; Network 6, Fig. 4.2.5). However, *Arrenurus (Arr.) mucronatus* was unconnected. In the network analysis of *A. (Meg.) manubriator* both populations grouped as one consistent network (Network 4, Fig. 4.2.4). Statistical parsimony showed that the haplotypes of *A. (Meg.) intermedius* (red, Alberta), *A. (Meg.) intermedius* (blue, Ontario) and *A. (Meg.) megalurus* created a single network (Network 3, Fig. 4.2.5). The SP analysis conducted for *A. (Arr.) affinis, A. (Arr.) bicuspidator, A. (Arr.) compactus, A. (Arr.) cuspidator, A. (Arr.) neumani* revealed that these species split into Network 1 which contained Type 1 COI sequence (*A. (Arr.) affinis, A. (Arr.) compactus, A. (Arr.) cuspidator, A. (Arr.) neumani*) and Network 2 with Type 2 COI sequence (*A. (Arr.) bicuspidator, A. (Arr.) neumani*) (Fig. 4.2.5). In addition, one haplotype of *A. (Arr.) neumani* was unconnected.

The analysis of species delimitation in two populations of *A. (Meg.) manubriator* showed that the observed divergence was not a result of a random coalescent process (P_{RD} <0.05, Rosenberg's P_{AB} =9.7 E-5). The probability measures for species delimitation could not be applied for *A. (Meg.) intermedius, A. (Meg.) megalurus, A. (Arr.) americanus* and *A. (Arr.) mucronatus* because of insufficient number of available COI sequences. In *A. (Arr.) affinis, A. (Arr.) bicuspidator, A. (Arr.) compactus, A. (Arr.) cuspidator* and *A. (Arr.) neumani* probability measures were not applied since these species did not form a monophyletic group (see Fig. 4.1.1).

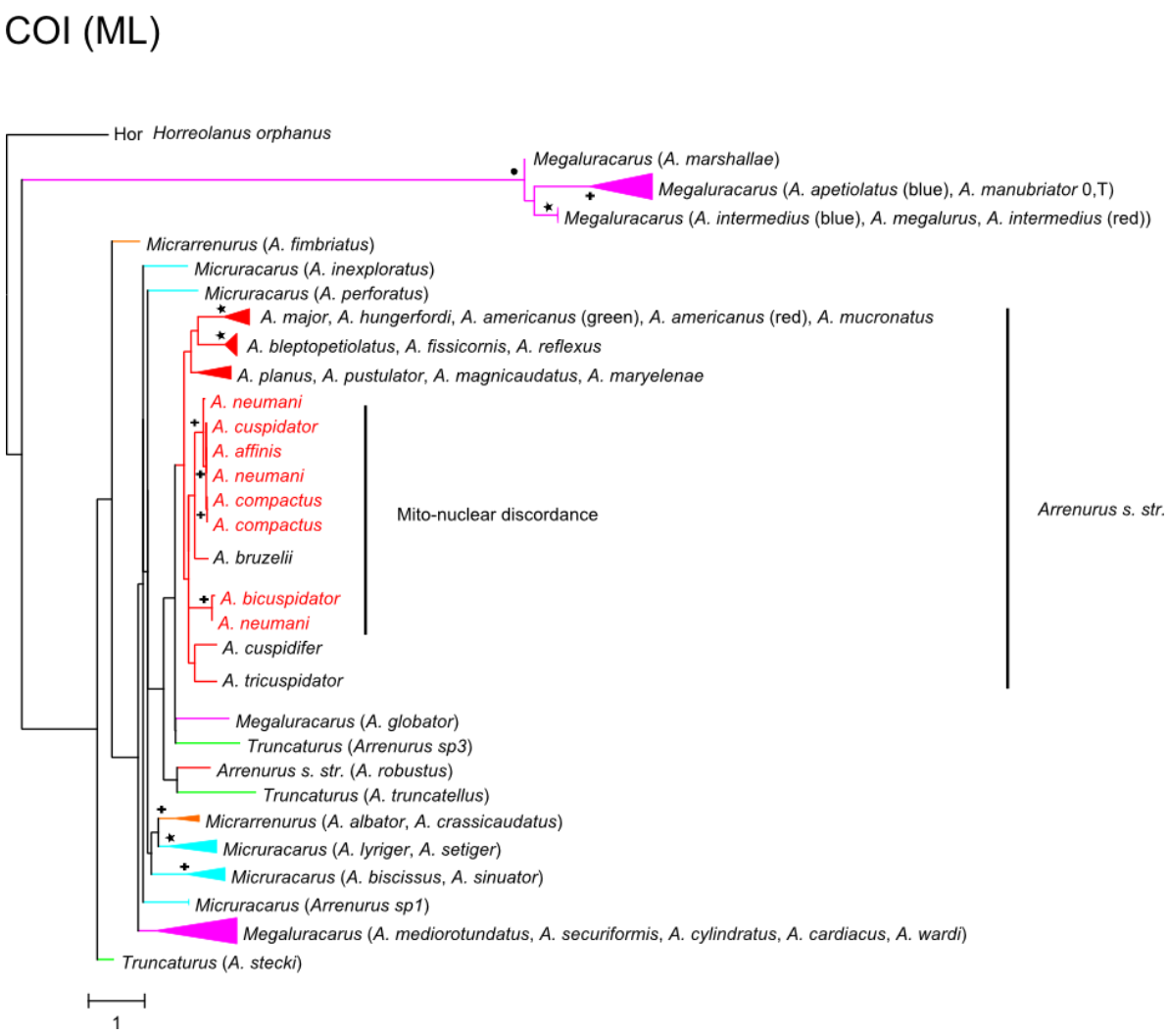

Figure 4.2.1. Maximum Likelihood tree obtained for COI gene fragment. Values next to branches show bootstrap support – abbreviations as in Figure 4.1.1; in red are species suspected of mitochondrial transfer.

28S (ML)

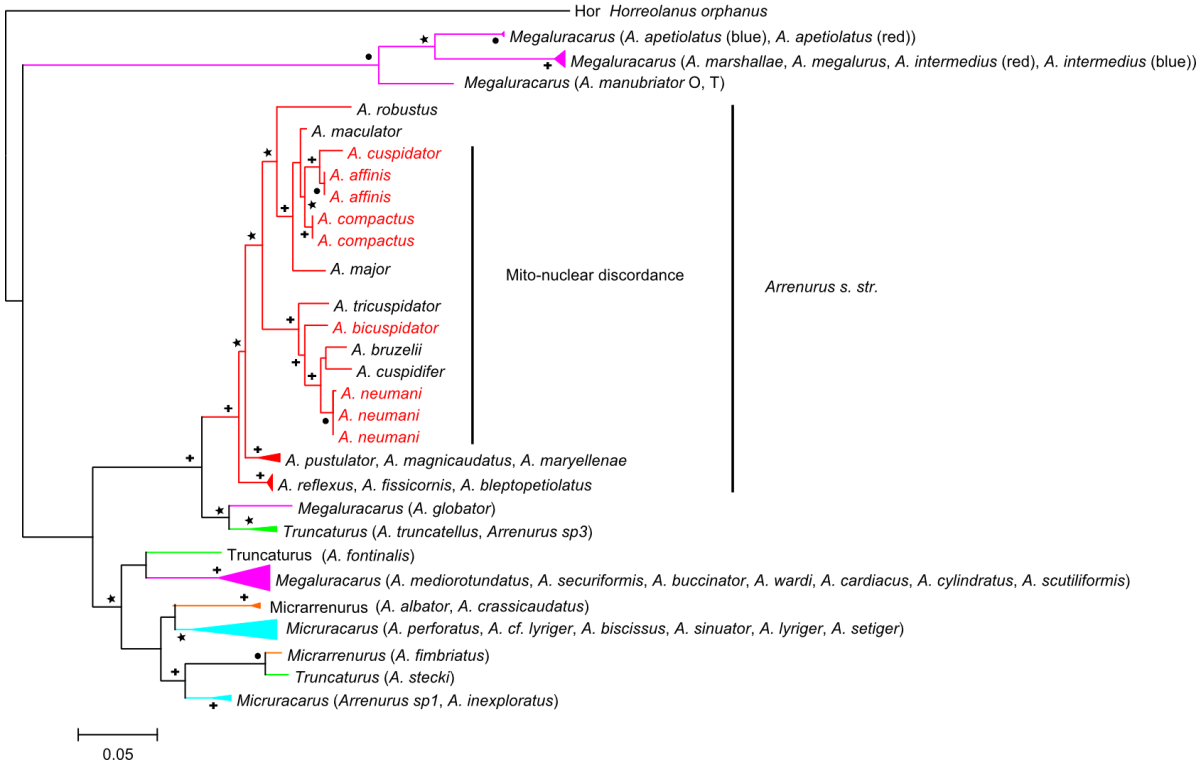

Figure 4.2.2. Maximum Likelihood tree obtained for D2 28S rDNA. Values at branches indicate bootstrap support - abbreviations as in Figure 4.1.1; in red are species suspected of mitochondrial transfer.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
1	*Horreolanus orphanus*	-																																														
2	*A. (Arr.) americanus (red)*	30.2	-																																													
3	*A. (Arr.) affinis*	29.0	17.1	-																																											
4	*A. (Arr.) americanus (green)*	30.0	0.8	17.4	0.1																																										
5	*A. (Arr.) bicuspidator*	29.4	18.1	14.3	19.1	-																																									
6	*A. (Arr.) bleptopetiolatus*	30.1	19.0	16.0	19.9	21.0	0.1																																								
7	*A. (Arr.) bruzelii*	30.0	19.3	9.7	19.6	14.8	18.2	2.1																																							
8	*A. (Arr.) compactus*	29.4	17.4	0.6	17.7	15.2	16.9	9.9	0.0																																						
9	*A. (Arr.) cuspidifer*	26.3	19.7	15.6	19.4	14.9	20.5	14.2	15.9	-																																				
10	*A. (Arr.) fissicornis*	30.5	15.1	18.2	16.0	21.4	11.6	19.9	18.5	18.8	0.9																																			
11	*A. (Arr.) hungerfordi*	32.7	14.9	19.4	14.7	18.8	21.1	18.7	19.1	18.1	19.4	0.4																																		
12	*A. (Arr.) magnicaudatus*	31.1	21.2	18.3	21.5	20.0	18.2	17.7	19.3	18.4	17.8	19.7	0.0																																	
13	*A. (Arr.) major*	33.0	13.8	18.8	14.8	20.8	18.3	19.3	18.5	18.0	18.0	14.0	17.6	-																																
14	*A. (Arr.) maryellenae*	31.1	22.5	17.3	22.2	18.7	18.3	17.3	17.6	17.3	18.1	19.5	14.3	18.7	-																															
15	*A. (Arr.) mucronatus*	31.0	5.1	18.0	5.1	18.3	18.2	17.9	18.3	19.6	16.2	17.2	19.3	14.3	19.9	-																														
16	*A. (Arr.) neumani*	29.2	17.7	3.5	18.1	11.8	17.1	10.8	3.9	15.6	18.7	19.0	18.9	19.0	17.8	18.4	6.1																													
17	*A. (Arr.) planus*	27.3	20.0	15.5	19.6	16.5	19.5	16.9	15.2	16.3	18.0	18.6	17.8	20.7	14.9	19.9	15.7	-																												
18	*A. (Arr.) pustulator*	31.5	18.5	16.7	18.8	17.9	19.2	15.2	17.0	15.3	18.5	18.2	17.3	16.4	16.3	17.8	16.8	12.8	1.3																											
19	*A. (Arr.) reflexus*	31.6	17.3	15.1	17.5	20.1	11.0	18.6	16.0	17.2	8.6	19.0	17.2	19.3	19.6	17.4	16.3	16.4	15.8	0.9																										
20	*A. (Arr.) robustus*	30.4	25.7	22.5	26.1	22.3	27.5	23.5	23.6	24.2	25.2	24.8	24.5	23.3	24.7	28.7	22.5	27.1	24.7	22.3	0.0																									
21	*A. (Arr.) cuspidator*	28.6	17.4	0.2	17.7	14.6	16.3	9.6	0.4	15.3	17.9	19.1	18.6	18.5	17.6	18.3	3.5	15.2	17.0	15.4	22.8	-																							
22	*A. (Arr.) tricuspidator*	27.2	22.9	14.5	22.2	17.9	20.3	13.5	14.8	14.4	17.7	19.6	19.9	21.6	20.9	19.7	14.9	18.2	19.1	18.1	24.7	14.2	0.4																						
23	*A. (Meg.) apetiolatus*	37.4	33.6	36.2	32.7	36.2	39.7	33.0	36.8	32.1	36.0	32.9	41.3	37.5	35.0	33.2	36.1	39.0	33.5	37.3	39.0	35.8	35.1	0.6																					
24	*A. (Meg.) cardiacus*	30.6	23.8	28.2	24.3	25.0	25.8	26.6	28.6	26.1	25.0	29.8	25.0	20.0	25.9	22.2	27.3	29.3	27.3	22.8	25.3	28.6	27.3	35.5	-																				
25	*A. (Meg.) cylindratus*	33.5	25.9	23.8	26.2	24.6	25.9	25.6	24.2	23.4	26.9	29.2	28.2	27.6	27.8	24.1	24.1	25.4	22.9	23.4	31.5	23.5	23.7	34.4	26.6	0.9																			
26	*A. (Meg.) globator*	33.8	18.8	16.9	18.4	18.4	17.6	17.1	17.2	19.3	20.0	21.8	18.4	18.8	18.5	19.1	17.6	18.8	17.6	18.0	29.6	17.2	19.6	33.7	26.2	23.1	0.0																		
27	*A. (Meg.) intermedius (blue)*	31.7	32.8	33.2	32.3	31.5	33.8	30.3	33.7	29.0	32.6	31.9	30.9	31.2	32.9	31.2	33.0	30.4	29.3	35.2	37.7	32.8	30.4	25.2	31.4	32.4	34.2	0.2																
28	*A. (Meg.) manubriator*	34.0	27.2	29.2	27.5	25.9	35.0	28.5	29.5	26.2	32.0	29.2	32.5	28.2	30.2	27.9	28.4	31.0	27.4	32.7	34.1	28.8	30.6	24.2	31.4	34.0	29.7	22.6	0.6															
29	*A. (Meg.) mediorotundatus*	29.7	23.3	23.2	23.6	25.9	22.7	23.4	23.6	23.0	23.2	25.2	23.8	22.5	28.0	22.5	23.3	25.8	26.4	21.9	27.7	22.9	22.1	31.8	26.4	23.0	27.0	32.4	31.9	-														
30	*A. (Meg.) megalurus*	30.5	32.7	31.5	32.2	32.1	32.3	30.2	31.9	28.0	31.9	31.2	30.6	30.6	31.8	31.1	31.9	30.2	29.0	34.9	35.7	31.1	30.2	24.8	31.9	30.9	33.5	1.6	22.2	31.3	-													
31	*A. (Meg.) marshallae*	33.4	35.6	31.0	35.1	30.2	36.7	30.6	31.4	30.9	33.3	29.5	30.2	33.0	31.6	35.2	30.6	31.6	31.2	35.9	37.5	30.6	30.3	27.5	35.8	34.0	35.4	12.2	20.5	32.6	11.8	-												
32	*A. (Meg.) securiformis*	30.3	25.5	24.5	25.0	24.1	26.0	26.2	24.9	25.4	24.6	28.2	24.8	26.0	28.1	25.1	24.2	20.8	28.1	22.0	28.5	24.1	23.2	42.4	21.4	18.2	29.1	29.2	34.4	24.4	29.5	29.6	1.0											
33	*A. (Meg.) intermedius (red)*	32.2	32.7	33.2	32.2	31.3	33.6	29.5	33.6	28.8	32.4	31.6	31.0	30.9	32.6	31.1	32.9	30.2	29.0	34.7	37.4	32.8	29.4	25.6	31.5	32.6	33.9	1.0	22.5	31.7	0.9	11.8	29.1	-										
34	*A. (Meg.) wardi*	32.8	26.2	26.5	25.4	26.5	28.6	30.3	26.3	26.3	22.9	30.0	25.2	29.8	26.6	25.0	26.5	24.4	26.9	21.6	31.0	26.1	24.6	33.9	25.5	21.3	26.8	37.3	34.0	27.1	37.2	37.0	23.9	38.2	0.9									
35	*A. (Mic.) albator*	29.9	23.8	21.6	23.5	16.8	25.2	23.1	21.9	18.2	21.1	23.1	25.9	23.0	18.8	23.5	20.7	21.4	20.1	21.5	25.8	21.9	19.3	38.3	26.8	29.0	18.6	31.6	29.7	26.2	31.7	34.0	28.2	32.1	29.0	-								
36	*A. (Mic.) crassicaudatus*	30.4	25.9	23.3	24.8	21.0	27.3	21.1	23.7	19.0	24.0	22.4	24.5	23.6	18.2	23.5	22.6	24.0	22.0	24.0	27.2	23.0	20.2	35.3	27.7	31.0	21.6	30.4	28.5	26.6	29.8	29.0	29.3	30.6	31.1	14.8	0.9							
37	*A. (Mic.) fimbriatus*	29.1	25.5	24.8	25.1	26.4	25.0	26.3	24.5	25.9	27.5	24.8	28.3	26.4	25.7	26.3	25.2	27.4	29.2	24.1	26.1	24.5	24.6	35.4	33.3	31.8	23.0	34.1	36.5	25.8	33.7	31.6	26.8	34.6	31.6	24.0	20.1	0.4						
38	*A. (Miu.) biscissus*	27.5	21.9	24.9	22.6	24.5	24.2	22.2	26.0	23.9	24.0	24.1	23.4	22.6	24.2	20.9	24.9	25.8	25.7	24.4	29.3	25.3	22.4	34.5	26.2	28.9	24.6	30.9	34.3	22.3	30.8	33.4	29.9	31.2	29.0	24.4	23.5	26.8	0.0					
39	*A. (Miu.) inexploratus*	31.3	24.4	21.6	25.1	23.0	24.6	18.4	21.2	21.3	21.5	21.5	20.4	21.0	21.6	22.3	21.5	21.7	22.2	24.3	26.0	21.2	20.2	35.6	25.1	31.5	20.9	32.5	28.8	23.6	32.8	30.4	28.3	32.4	28.7	20.8	18.9	23.1	20.7	-				
40	*A. (Miu.) lyriger*	30.4	27.0	21.8	26.7	21.4	24.7	20.8	22.8	21.2	25.9	25.7	21.7	23.6	21.2	26.3	21.9	21.1	20.7	23.8	26.3	22.1	21.6	37.6	31.2	27.9	20.0	34.8	31.6	25.4	33.2	33.5	26.7	34.5	31.3	21.0	20.1	23.7	21.5	22.4	-			
41	*A. (Miu.) perforatus*	27.8	25.6	22.5	25.3	23.5	26.0	22.0	22.1	22.7	21.4	23.6	26.5	24.7	22.7	23.7	22.4	22.1	20.6	22.4	26.9	22.1	20.8	31.1	28.7	29.3	18.7	32.3	30.2	27.9	32.6	32.0	25.2	33.0	28.6	19.4	20.7	23.8	24.3	21.4	20.4	1.1		
42	*A. (Miu.) sinuator*	29.7	24.9	22.7	25.4	23.4	22.4	23.8	23.0	22.8	24.2	22.2	17.7	23.5	21.6	23.1	22.6	21.4	22.2	22.6	24.0	23.0	23.2	41.8	28.9	29.1	24.3	33.1	35.9	24.7	31.7	31.7	24.6	31.7	31.8	26.7	23.5	24.8	18.9	21.4	19.9	21.0	0.4	
43	*Arrenurus (Miu.)* sp1	31.3	25.9	22.7	25.6	23.1	21.8	20.8	22.8	23.8	23.6	23.3	21.4	23.3	20.7	23.0	22.7	22.4	22.8	23.6	26.1	23.0	21.6	39.7	27.3	26.6	21.5	35.5	33.1	23.9	34.8	31.3	22.9	35.7	24.3	21.6	20.8	23.7	21.4	19.6	20.8	19.9	21.5	0.3	
44	*A. (Miu.) setiger*	27.2	24.5	20.6	24.2	23.8	21.7	20.1	21.1	20.7	19.6	21.9	21.3	24.3	21.6	22.5	21.0	21.1	22.5	19.7	26.4	20.4	19.2	35.2	30.0	30.3	24.3	35.3	34.0	25.3	34.2	30.7	29.6	35.1	30.5	21.4	21.3	20.8	21.2	21.0	16.8	19.3	18.4	20.8	1.3	
45	*A. (Tru.) truncatellus*	34.0	20.9	19.4	21.6	23.0	20.5	21.6	19.7	21.6	18.8	19.7	21.6	21.3	20.9	21.2	20.4	19.0	18.0	18.1	22.9	19.7	19.9	36.9	31.3	30.6	19.3	36.1	33.8	26.7	35.8	33.4	26.4	36.2	29.4	22.2	24.7	23.0	26.6	21.2	22.1	21.3	23.0	23.8	20.9	-	
46	*Arrenurus (Tru.)* sp3	33.7	20.8	21.7	21.1	22.8	20.2	21.6	22.1	19.9	20.3	19.5	21.3	23.0	20.9	20.5	22.0	19.8	20.3	19.7	28.9	22.1	22.2	39.3	30.1	25.7	19.0	34.1	33.7	25.2	33.4	34.7	27.2	34.3	25.6	21.5	25.0	25.7	26.5	24.2	23.8	22.7	24.8	21.6	19.9	19.2	0.0	
47	*A. (Tru.) stecki*	27.8	27.3	27.0	27.2	25.6	26.0	24.7	27.4	24.9	26.8	27.2	28.2	28.1	24.2	25.8	26.1	25.2	28.9	25.4	29.6	26.6	21.3	36.7	29.9	28.6	26.8	31.0	35.2	23.1	31.2	33.1	26.2	31.6	28.0	25.0	22.2	19.2	24.3	24.3	25.1	25.2	28.7	23.6	24.1	27.5	28.5	0.4

Figure 4.2.3. Genetic distances obtained based on COI barcode region between and within (in grey) species using Kimura 2-parameter. The intraspecific distances were only calculated for putative species that had more than one specimen sequenced for COI.

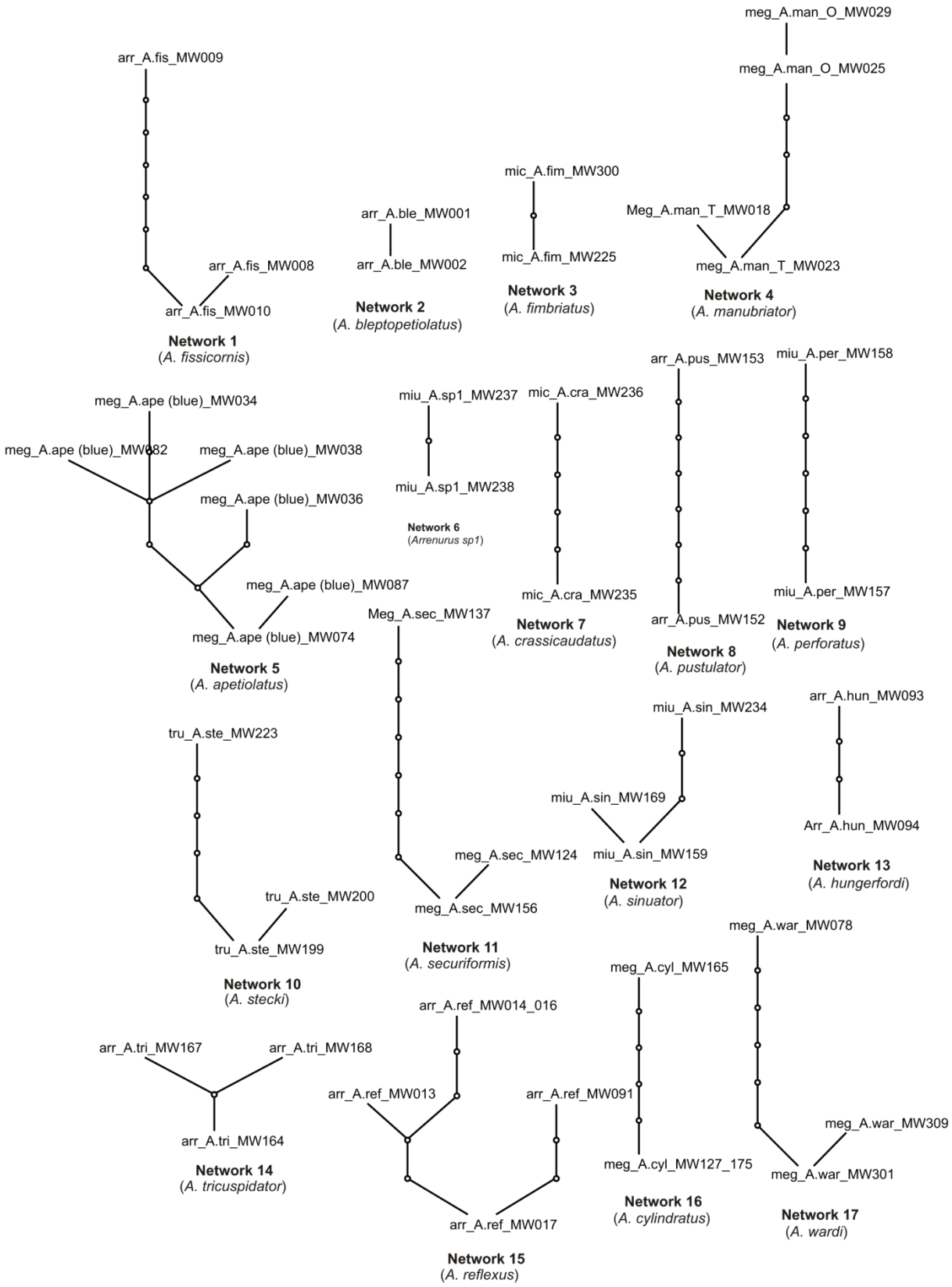

Figure 4.2.4. Haplotype networks for barcode region of COI. The connection limit for species boundary is 95%. Statistical parsimony networks corresponding with defined species are presented.

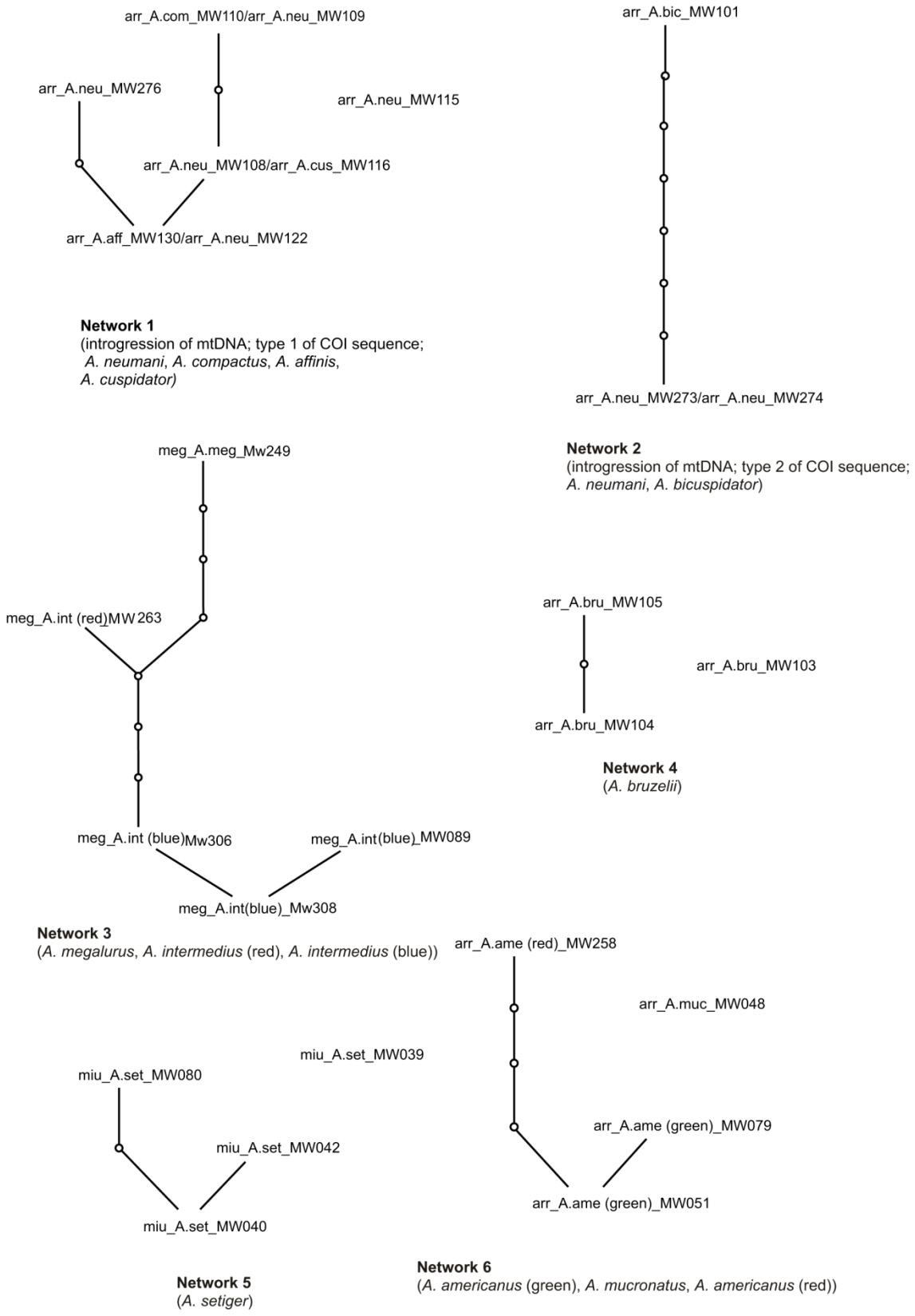

Figure 4.2.5. Haplotype networks for barcode region of COI. The connection limit for species boundary is 95%. Statistical parsimony networks composed of more than one species are shown.

4. 3. Communication via sex pheromones

4. 3. 1. Responses to sex pheromones among *Arrenurus* species of different relatedness

Female-conditioned water elicited the strongest responses in conspecific males, but in a few cases also in heterospecific males, e.g., water conditioned by *A.* (*Arr*.) *tricuspidator* females caused strong responses in male *A.* (*Arr*.) *bicuspidator* and *A.* (*Miu*.) *biscissus* (Fig. 4.3.1.1). There was an overall and statistically significant difference among reactions of males to female cues (at least one statistically significant difference between male responses to female cues or control water was found) in *A. (Arr.) trucuspidator* (Friedman's test, $X^2(5) = 25.807$, $p < 0.001$), *A. (Arr.) bruzelii* (Friedman's test, $X^2(6) = 27.869$, $p < 0.001$), *A. (Miu.) biscissus* (Friedman's test, $X^2(5) = 16.656$, $p = 0.005$) and *A. (Meg.) globator* (Friedman's tests; $X^2(4) = 15.837$, $p = 0.003$; $X^2(2) = 8.400$, $p = 0.015$). However, no significant differences occurred in reactions of *A. (Mic.) crassicaudatus* males (Friedman's tests; $X^2(3) = 3.409$, $p = 0.333$; $X^2(3) = 2.793$, $p = 0.425$). Two treatments were conducted to determine reactions of males of *A. (Arr.) bicuspidator* to female cues since *A. bicuspidator* males failed to response to water conditioned with conspecific females in the first trial. However, there was an overall difference among reactions of males to female cues in the first treatment (Friedman's tests, $X^2(2) = 1.600$, $p = 0.449$; $X^2(4) = 27.140$, $p < 0.001$). In the second treatment conducted the day after, males of *A. bicuspidator* responded positively to water conditioned by conspecific females (with control; sign test, $p < 0.008$).

Dunn's post-hoc test was run to reveal differences between responses of males to water conditioned by females of different species. This test showed that differences with $p < 0.05$ occurred only in selected pairwise comparisons in treatments with males of *A. (Arr.) tricuspidator, A. (Meg.) globator, A. (Arr.) bicuspidator,* and were close to this value in *A. (Arr.) bruzelii* (response to conspecific cues vs. pure water, $p = 0.053$):

- responses of males of *A. (Arr.) tricuspidator* to conspecific cues vs. responses to pure water ($p < 0.001$),
- responses of *A. (Meg.) globator* males to conspecific females vs. responses to water conditioned by females of *A. (Mic.) crassicaudatus* ($p < 0.002$),
- responses of males of *A. (Arr.) bicuspidator* to water conditioned by females of *A. tricuspidator* vs. responses to water from females of *A. (Arr.) bruzelii* ($p = 0.034$),

- responses of males of *A. (Arr.) bicuspidator* to water conditioned by *A. (Arr.) tricuspidator* females vs. responses to water conditioned by females of *A. (Mic.) crassicaudatus* ($p = 0.034$),
- responses of males of *A. (Arr.) bicuspidator* to water conditioned by *A. (Arr.) tricuspidator* females vs. responses to pure water ($p = 0.034$).

Dunn's post-hoc test showed no differences between treatments for males of *A. (Mic.) crasicaudatus* and *A. (Miu.) biscissus*.

When sign test of association was applied for revealing differences between responses of males to water conditioned by females of different species and responses to control water, responses of *A. (Arr.) bicuspidator*, *A. (Arr.) bruzelii*, *A. (Arr.) tricuspidator* and *A. (Meg.) globator* to conspecific stimuli were statistically significant (sign test, all cases $p < 0.05$; for average mean scores and p-values see Tab. 4.3.1.1). However, males of *A. (Arr.) bicuspidator* reacted to heterospecific cues of *A. (Arr.) tricuspidator* more strongly than to their own cues (sign test, $p < 0.008$). Water from containers with females of *A. (Arr.) tricuspidator* elicited in males of *A. (Arr.) bicuspidator* a high but statistically non-significant response (sign test, $p = 0.070$). Although males of *A. (Miu.) biscissus* were tested twice for responses to conspecific cues, in neither case were the responses statistically significant from that to control water (first trial, sign test, $p = 0.617$; second trial, $p = 0.450$). However, males of *A. (Miu.) biscissus* yielded positive responses to water from *A. (Arr.) tricuspidator* females (sign test, $p < 0.041$). Males of *A. (Arr.) tricuspidator* showed very little response to water conditioned with *A. (Miu.) biscissus* females. Males of *A. (Mic.) crassicaudatus* responded most strongly to conspecific cues, but results were statistically not significant. Males of *A. (Arr.) tricuspidator* showed statistically significant (sign test, $p < 0.043$) response to water with *A. (Mic.) crassicaudatus* females. The average mean scores and p-values of the sign test are presented in Table 4.3.1.1.

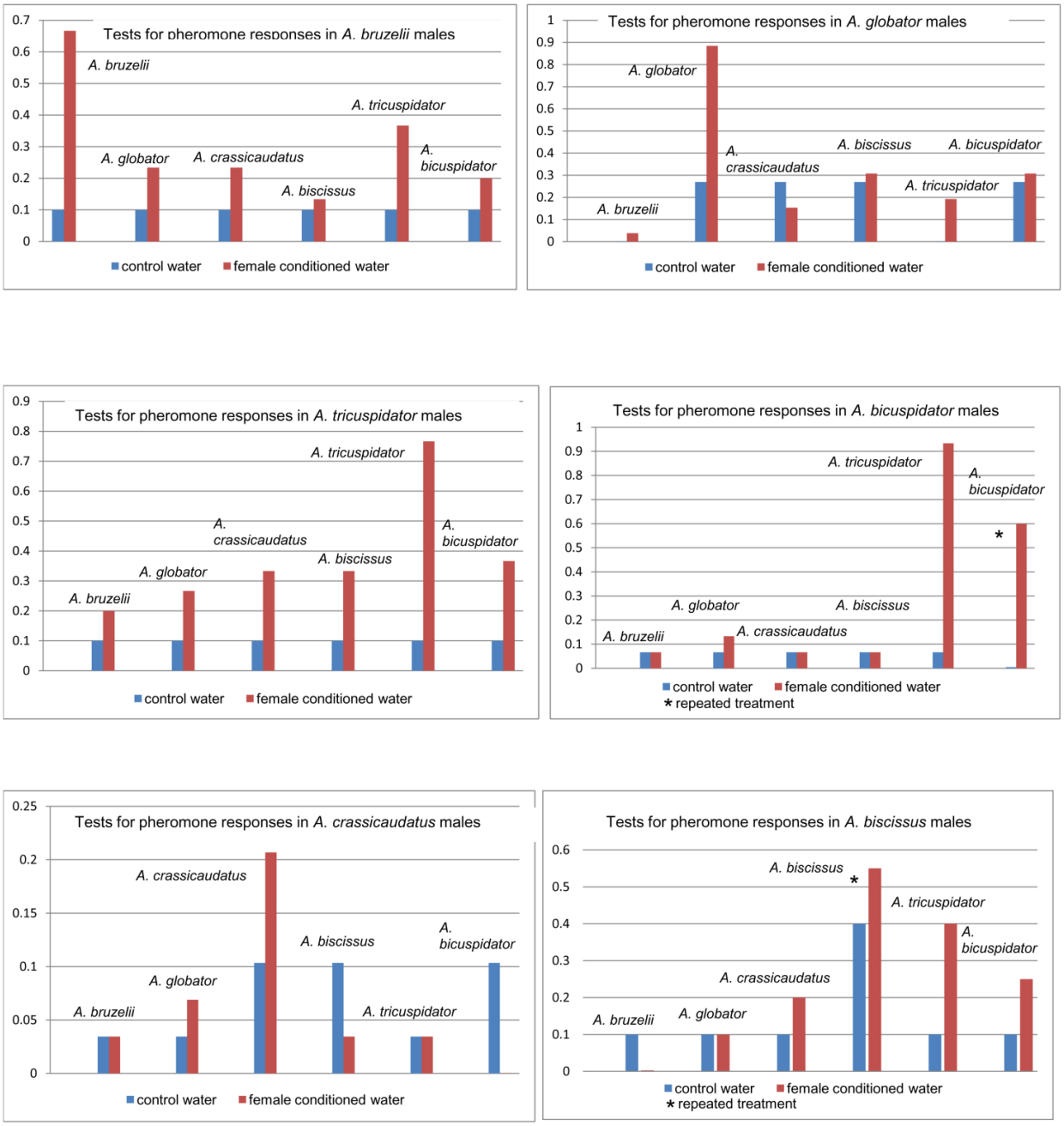

Figure 4.3.1.1. Tests for pheromone responses as indicated by male arrestant behaviour and fanning hind legs (axis y, average mean scores). The species tested are *A. (Meg.) globator*, *A. (Arr.) bruzelii*, *A. (Arr.) bicuspidator*, *A. (Arr.) tricuspidator)*, *A. (Miu.) biscissus* and *A. (Mic.) crassicaudatus*; * repeated treatment conducted on the second day after treatment with negative conspecific responses.

Table 4.3.1.1. Tests for pheromone responses as indicated by male arrestant behaviour and fanning hind legs among *Arrenurus* species. The conspecific reactions are underlined. Abbreviations: [1] treatment with negative conspecific male responses (first day), [2] repeated treatment (on the second day after treatment with negative conspecific responses); column 'mean score (crosses)' - responses of males to female conditioned water, column 'mean score (control)' – responses of males to control water; in red are given statistically significant reactions.

Species of males	Female water	No. of males	Mean score (crosses)	Mean score (control)	Sign test
A.(Arr.) bicuspidator[1]	*A.(Arr.) bicuspidator*	15	0.2	0.07	p = 0.617
A.(Arr.) bicuspidator[2]	*A.(Arr.) bicuspidator*	15	0.6	0.0	p < 0.008
A.(Arr.) bicuspidator	*A.(Arr.) bruzelii*	15	0.07	0.07	p = 0.480
A.(Arr.) bicuspidator	*A.(Arr.) tricuspidator*	15	0.93	0.07	p < 0.008
A.(Arr.) bicuspidator	*A.(Meg.) globator*	15	0.13	0.07	-
A.(Arr.) bicuspidator	*A. (Mic.) crassicaudatus*	15	0.07	0.07	p = 0.480
A.(Arr.) bicuspidator	*A. (Miu.) biscissus*	15	0.07	0.07	p = 0.480
A.(Arr.) bruzelii	*A.(Arr.) bruzelii*	30	0.67	0.1	p < 0.002
A.(Arr.) bruzelii	*A.(Arr.) bicuspidator*	30	0.20	0.1	p = 0.450
A.(Arr.) bruzelii	*A.(Arr.) tricuspidator*	30	0.37	0.1	p = 0.070
A.(Arr.) bruzelii	*A. (Miu.) biscissus*	30	0.13	0.1	p = 1.000
A.(Arr.) bruzelii	*A. (Mic.) crassicaudatus*	30	0.23	0.1	p = 0.220
A.(Arr.) bruzelii	*A.(Meg.) globator*	30	0.23	0.1	p = 0.683
A.(Arr.) tricuspidator	*A.(Arr.) tricuspidator*	30	0.77	0.1	p = 0.000
A.(Arr.) tricuspidator	*A.(Arr.) bicuspidator*	30	0.37	0.1	p = 0.070
A.(Arr.) tricuspidator	*A.(Arr.) bruzelii*	30	0.2	0.1	p = 0.505
A.(Arr.) tricuspidator	*A. (Mic.) crassicaudatus*	30	0.33	0.1	p < 0.043
A.(Arr.) tricuspidator	*A. (Miu.) biscissus*	30	0.33	0.1	p = 0.077
A.(Arr.) tricuspidator	*A.(Meg.) globator*	30	0.27	0.1	p = 0.289
A.(Meg.) globator	*A.(Meg.) globator*	26	0.88	0.27	p < 0.009
A.(Meg.) globator	*A.(Arr.) bicuspidator*	26	0.31	0.27	p = 0.724
A.(Meg.) globator	*A.(Arr.) bruzelii*	26	0.04	0.0	-
A.(Meg.) globator	*A.(Arr.) tricuspidator*	26	0.19	0.0	p = 0.074
A.(Meg.) globator	*A. (Miu.) biscissus*	26	0.31	0.27	p = 1.000
A.*(Meg.) globator*	*A. (Mic.) crassicaudatus*	26	0.15	0.27	p = 0.505
A. (Mic.) crassicaudatus	*A. (Mic.) crassicaudatus*	29	0.21	0.03	p = 0.505
A. (Mic.) crassicaudatus	*A.(Arr.) bicuspidator*	29	0.0	0.03	p = 0.250
A. (Mic.) crassicaudatus	*A.(Arr.) bruzelii*	29	0.03	0.03	p = 0.480
A. (Mic.) crassicaudatus	*A.(Arr.) tricuspidator*	29	0.10	0.03	p = 0.480
A. (Mic.) crassicaudatus	*A.(Meg.) globator*	29	0.07	0.03	p = 1.000
A. (Mic.) crassicaudatus	*A. (Miu.) biscissus*	29	0.03	0.03	p = 0.617
A. (Miu.) biscissus[1]	*A. (Miu.) biscissus*	20	0.15	0.15	p = 0.617
A. (Miu.) biscissus[2]	*A. (Miu.) biscissus*	20	0.55	0.4	p = 0.450
A. (Miu.) biscissus	*A.(Arr.) bicuspidator*	20	0.25	0.1	p = 0.617
A. (Miu.) biscissus	*A.(Arr.) bruzelii*	20	0.0	0.1	-
A. (Miu.) biscissus	*A.(Arr.) tricuspidator*	20	0.4	0.1	p < 0.041
A. (Miu.) biscissus	*A.(Meg.) globator*	20	0.1	0.1	-
A. (Miu.) biscissus	*A. (Mic.) crassicaudatus*	20	0.2	0.1	p = 1.000

4. 3. 2. Responses to sex pheromones among closely related *Arrenurus* s. str. species

There was an overall difference among reactions of males to female cues in males of *A. (Arr.) neumani* (Friedman's test, $X^2(3) = 8.477$, $p = 0.037$), but not in males of *A. (Arr.) compactus* (Friedman's test, $X^2(3) = 0.966$, $p = 0.809$) or of *A. (Arr.) bicuspidator* (Friedman's test, $X^2(3) = 0.600$, $p = 0.896$). The Dunn's post-hoc test revealed that statistically significant differences occurred only between two pairs of treatments: responses of *A. (Arr.) neumani* males to water conditioned with *A. (Arr.) cuspidator* and responses of males of *A. (Arr.) neumani* to pure water ($p < 0.028$). The stronger response of males of *A. (Arr.) neumani* to stimuli of *A. (Arr.) cuspidator* than to water conditioned with their own females was also confirmed by sign test ($p < 0.008$). The species examined reacted in general more strongly to conspecific and heterospecific cues than to control water (Fig. 4.3.2.1). The average mean scores and p-values of the sign test are presented in Table 4.3.2.1.

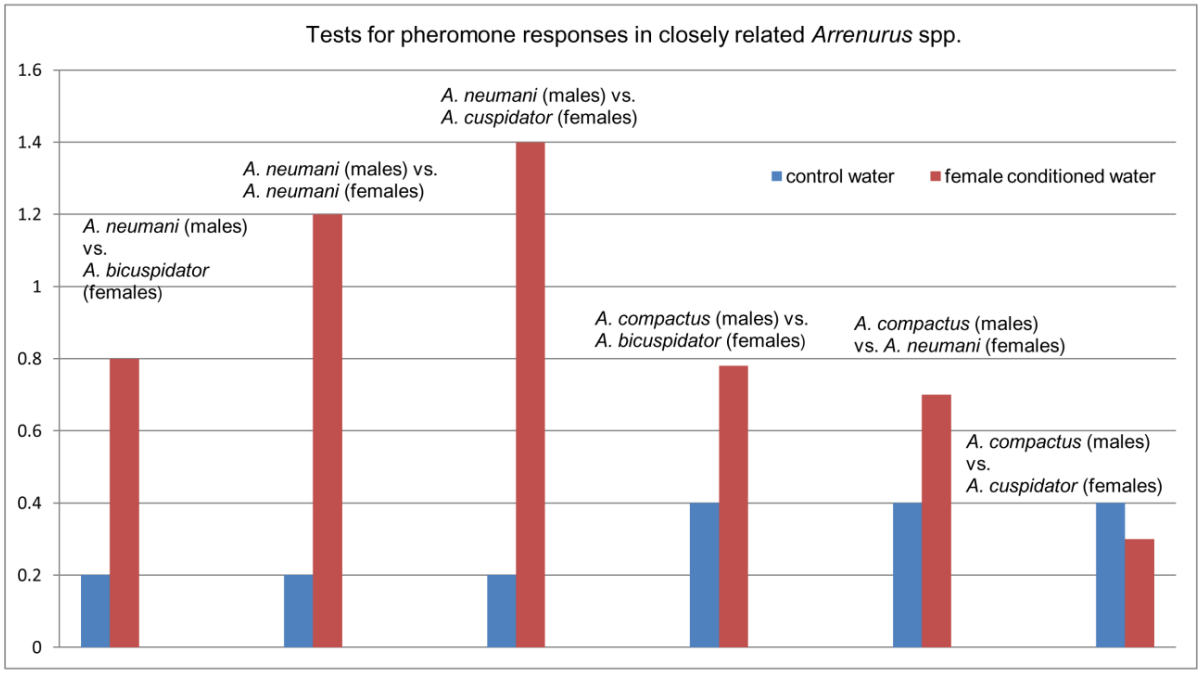

Figure 4.3.2.1. Tests for pheromone responses among *A. (Arr.) neumani, A. (Arr.) compactus, A. (Arr.) cuspidator, A. (Arr.) bicuspidator* suspected of mitochondrial transfer. Altering previous form of locomotion, swimming or crawling towards the tip of the pipette and fanning fourth legs were considered a positive reaction.

Table 4.3.2.1. Tests for pheromone responses in closely related *Arrenurus* s. str. spp. Altering previous form of locomotion, swimming/crawling towards the pipette and fanning IV-L were considered a positive reaction; underlined - conspecific reactions; 'mean score (crosses)' - responses of males to female conditioned water, 'mean score (control)' – responses of males to control water.

No. of males	Species of males	Female water	Mean score (crosses)	Mean score (control)	Sign test
10	*A.(Arr.) neumani*	*A.(Arr.) bicuspidator*	0.80	0.20	p = 0.450
10	*A.(Arr.) neumani*	*A.(Arr.) neumani*	1.20	0.20	p = 0.221
10	*A.(Arr.) neumani*	*A.(Arr.) cuspidator*	1.40	0.20	p < 0.008
10	*A.(Arr.) compactus*	*A.(Arr.) bicuspidator*	0.78	0.40	p = 1.000
10	*A.(Arr.) compactus*	*A.(Arr.) neumani*	0.70	0.40	p = 0.450
10	*A.(Arr.) compactus*	*A.(Arr.) cuspidator*	0.30	0.40	p = 1.000
6	*A.(Arr.) bicuspidator*	*A.(Arr.) bicuspidator*	0.33	0.17	-
6	*A.(Arr.) bicuspidator*	*A.(Arr.) neumani*	0.33	0.17	-
6	*A.(Arr.) bicuspidator*	*A.(Arr.) cuspidator*	0.33	0.17	-

4. 3. 3. Male responses to female-conditioned water and phylogenetic distance

The intensity of male responses to female cues of differently related species was shown in relation to phylogentic distance (based on D2 28S rDNA) (Fig. 4.3.3.1). In Fig. 4.3.3.1 A (data from the Experiment 'Responses to sex pheromones among *Arrenurus* species of different relatedness') two peaks occurred. The first and strongest peak was explained by responses to conspecific cues. The second and weaker peak reflected responses to sex pheromones of more distantly related species. The male reactions obtained in the Experiment 'Responses to sex pheromones among closely related *Arrenurus* s. str. species' formed a curve with a peak indicating stronger responses to heterospecific cues than to conspecific cues (Fig. 4.3.3.1 B).

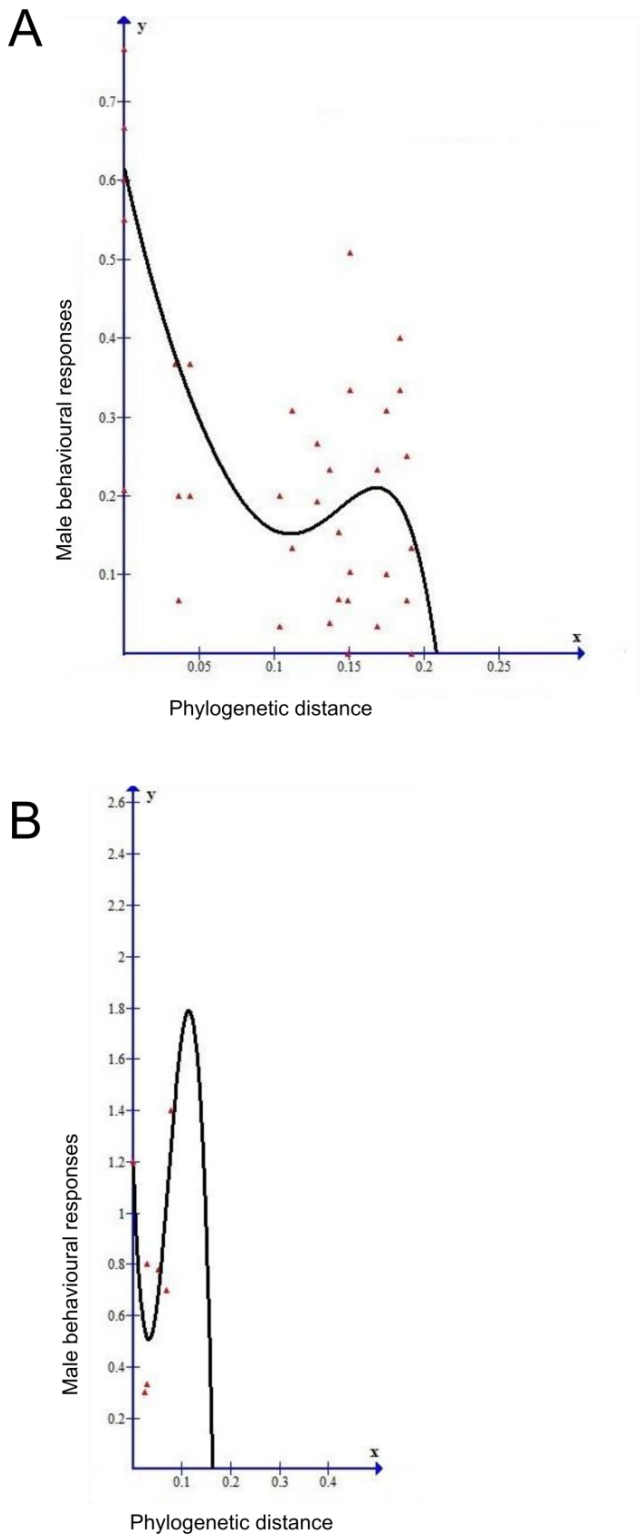

Figure 4.3.3.1. The relationship between male behavioural responses to conspecific and heterospecific cues (average mean scores) and phylogenetic distance (D2 28S rDNA) (A) among species representing different evolutionary lineages (*A. (Meg.) globator, A. (Arr.) bruzelii, A. (Arr.) bicuspidator, A. (Arr.) tricuspidator, A. (Miu.) biscissus, A. (Mic.) crassicaudatus*), and (B) among species suspected of mitochondrial transfer (*A. (Arr.) neumani, A. (Arr.) compactus, A. (Arr.) cuspidator, A. (Arr.) bicuspidator*).

4. 4. Mating behaviour

4. 4. 1. Ethograms

Mating behaviour of 30 pairs from 9 *Arrenurus* species representing 4 subgenera (*Arrenurus* s. str., *Megaluracarus*, *Micrarrenurus*, *Truncaturus*) was videotaped. In sum, more than 200 hours of tape were obtained and analysed. The following outlines of behavioural sequences (ethograms) describe different stages of mating where the introduction of a single male to the container containing a single female was considered the beginning of mating, and physical separation of male and female after sperm transfer was considered the terminus; however, if the male displayed leg fanning, ready position or touching the female with palpi and legs after an already completed mating sequence, observations were continued (copulatory positions of *Arrenurus* spp. are shown in Fig. 4.4.1.1). Data on duration of mating behaviour in studied species are given in Table 4.4.1.1. I was not able to see spermatophores, and thus the timing of spermatophore deposition was assumed based on the male's movements.

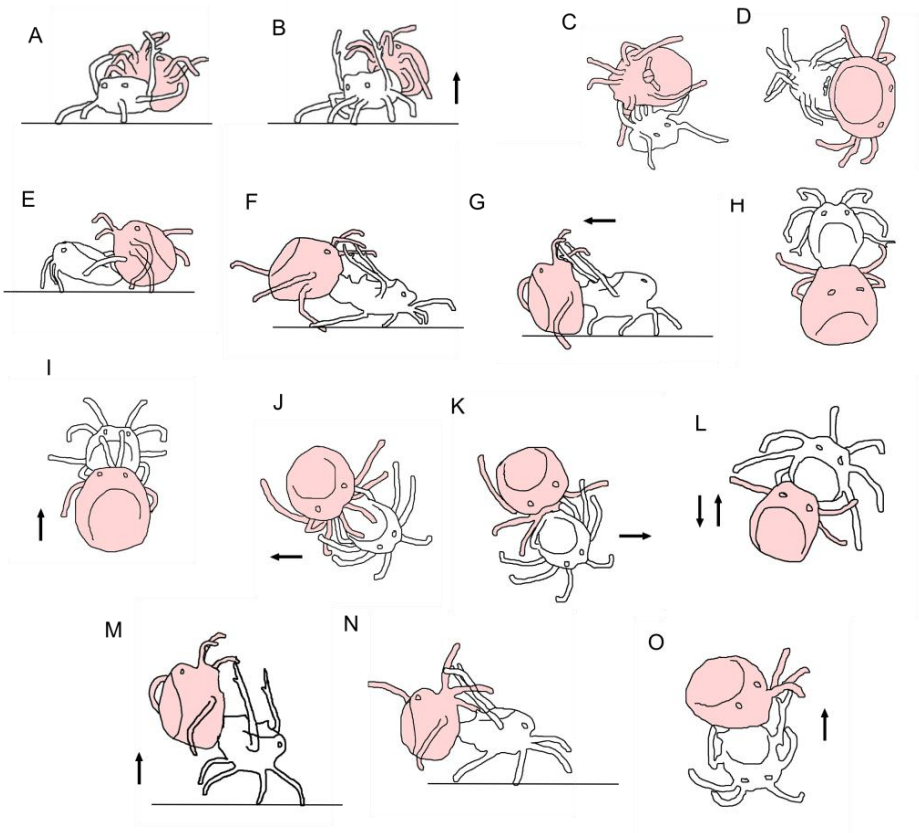

Figure 4.4.1.1. Spermatophore deposition and transfer by *Arrenurus* water mites. (A, B) Copulatory position of *A. (Meg.) globator*; (A) female is glued to male's cauda, (B) male lifts his hindbody to draw out spermatophore. (C) Female of *A. (Mic.) crassicaudatus* lies on the well bottom in a state of motionless rigidity; male touches ventral side of her (including genital area) with his palpi and forelegs. (D) Male of *A. (Mic.) crassicaudatus* is attached under the standing female, facing in the opposite direction as her; she manipulated her first, second and third legs presumably to transfer sperm into her genital tract. (E) Copulatory position of *A. (Tru.) stecki*; male deposits spermatophore by pressing cauda to the substratum (slightly weaving his cauda with glued female). Copulatory position of *A. (Arr.) tricuspidator* (F) and *A. (Arr.) bicuspidator* (G); (F) female is glued to the male's cauda and male pushes her back with his IV-L equipped with spur; in (G) female of *A. (Arr.) bicuspidator* is pushed back and the petiole with load of sperm is inserted in to her genital tract. (H-K) Spermatophore deposition and sperm transfer in *A. (Arr.) cuspidator* (the same behavioural steps occur in *A. (Arr.) maculator*); male pushes his venter to the substratum (presumably to deposite spermatophore) (H) and then lifts his cauda with attached female (drawing out spermatophore) (I); male tilts his body slowly to the right by bending right legs I to III (J), and then to the left by bending left legs I to III (K) (sideways leaning). (L, M) Copulatory positions of *A. (Arr.) claviger*; (L) male jerks sharply cauda upwards with glued female; (M) male lifts his cauda with attached female presumably drawing out spermatophore. (N) Male of *A. (Arr.) bruzelii* with glued to his cauda female; (O) female is held by hindlegs of male as he lifts his cauda with her upwards. Abbreviations - individuals in pink are females; some leg pairs are omitted for clarity.

***Arrenurus (Megaluracarus) globator*, N=4**

I. Walking: Male and female walked around the well bottom with fourth legs held up over their backs. Male moved hind legs in a rotary motion (fanning).

II. Ready position: Male crooked his hind legs at the fourth distal segment (flexed at the joint between genu and tibia) and placed them over his back when the female was in a close proximity. He often directed his cauda towards the female and touched her with first and second legs.

III. Mounting and gluing: In some cases, the female climbed on to the male's cauda. In other cases the male grasped her with leg spur that clamped onto the female's front legs when she passed by him. She was then glued by her venter in mating position on male's back via secretions from his caudal glands (Fig. 4.4.1.1 A; for SEM micrographs see Fig. 4.4.1.2 A, B). Sometimes the male appeared uninterested in mating even when female actively climbed onto his hind body.

IV. Attachment: Male walked around with female on his back. Female often flailed her legs.

V. Spermatophore deposition and sperm transfer: Male held on to the substratum with the claws of his first three leg pairs, pressed venter against substratum and rocked from side to side. He lifted his hind body (presumably to draw out a spermatophore, Fig. 4.4.1.1 B) and jerked cauda vigorously side to side. Sometimes male sharply jerked his back upwards. This was repeated several times. The male lowered the female down onto the putative spermatophore occasionally. This was interspersed with periods of tapping female with male's fourth legs. Fourth leg spurs were used as forked support to hold up female in mating position.

VI. Separation: This was achieved through pushing female off using male's fourth legs and vigorous swimming around the well.

VII. Grooming: Both brushed own body from front to back with hind legs.

VIII. Mate attendance: After detachment the female sometimes lay in a state of motionless rigidity on the well bottom. Male crawled to the female and touched her with his first and second legs repeatedly. He slowly walked around her with crooked fourth legs and displayed ready position. The male presented his cauda to the female, and attempted to push his back end under her. Both sexes sometimes started full courtship sequence again. However, one female that already mated was resistant to male's harassment. She did not keep balance and fell down when a male

approximated her. She behaved like she was attracted to the male because she crawled on to his cauda. However, she attempted to escape after a while.

***Arrenurus (Micrarrenurus) crassicaudatus*, N=1**

I. Walking: After introduction to well, both male and female walked around with their fourth legs raised high, or moved them in a rotary motion. Fanning hind legs was accompanied in males by sudden changes in walking direction.

II. Wrestling: Male 'wrestled' with the female climbing over and around her body. Both sexes were turned towards ventral sides of their bodies. Male touched female's venter in genital area and gnathosoma with his palpi repeatedly, and she appeared to touch his back end with palpi and first legs. Sometimes female lay in a state of motionless rigidity at the well bottom and male climbed around her body (Fig. 4.4.1.1 C).

III. Mounting and gluing: Male placed himself under female's body and maneuvered her into mating position by gluing her onto his cauda. Though the male of *A. (Mic.) crassicaudatus* lacks a spur on IV-L, he seemed to manipulate female with hind legs when gluing her. The female was attached to male's cauda with her venter faced in the same direction as male.

IV. Walking: Male walked around shaking, with female attached to him. This was interspersed with periods of motionlessness. They often lost balance and lay for a while on female's back. Male brushed his venter with first, second and third legs. He pushed female backwards using his fourth legs.

V. Motionlessness and sperm transfer: Male was attached under the standing female, facing in the opposite direction as her (Fig. 4.4.1.1 D). She manipulated her first, second and third legs presumably to transfer sperm into her genital tract.

VI. Walking: Female walked around – male was dragged around on his back and tried to detach.

VII. Separation: Pair separated when male detached himself using his fourth legs.

VIII. Grooming: After separation, both brushed own body with fourth legs.

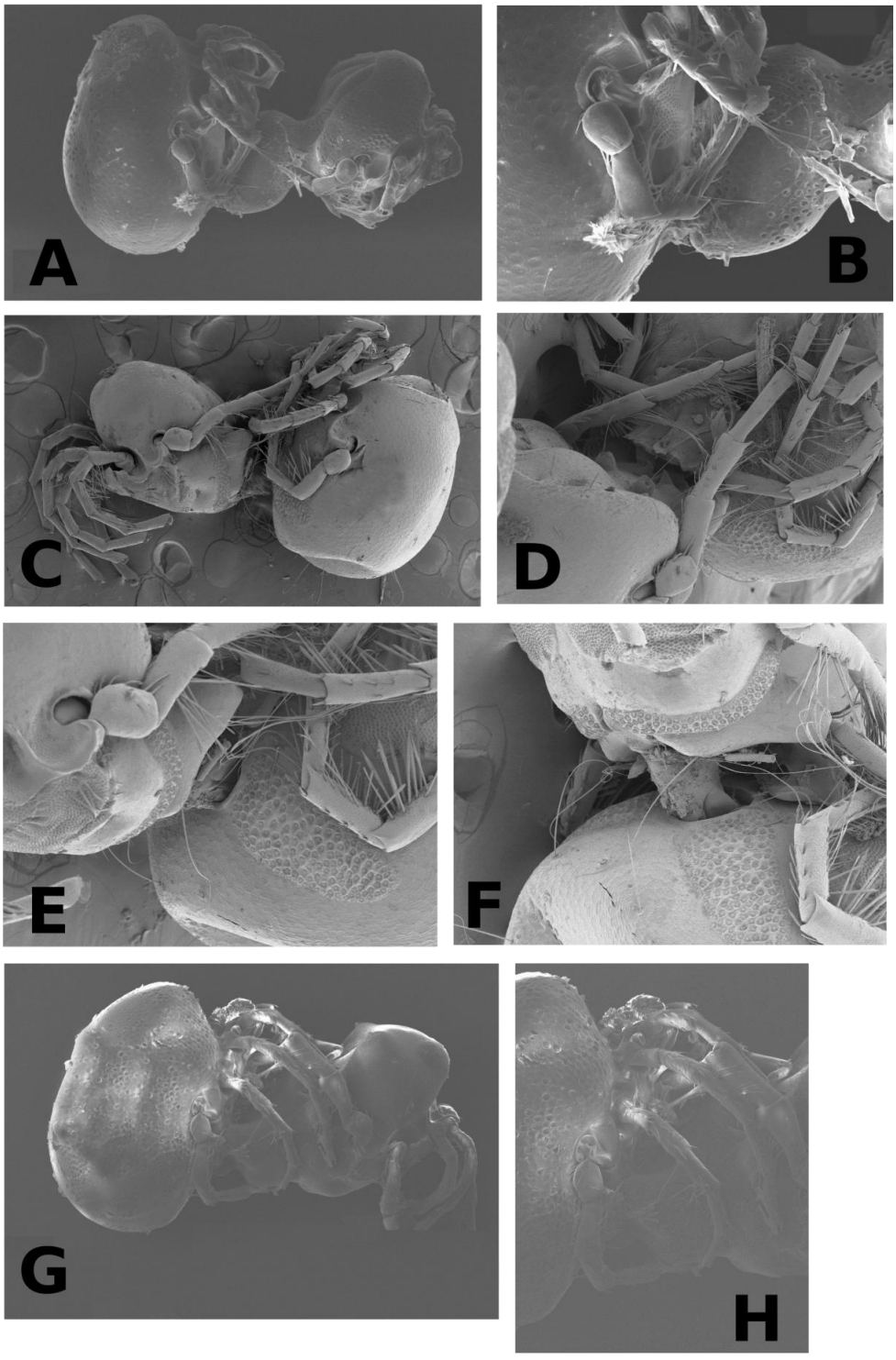

Figure 4.4.1.2. Scanning electron micrographs of petiolate and apetiolate *Arrenurus* in copula. A, B. Female of *A. (Meg.) globator* in mating position glued to males cauda (no petiole), lateral view. C, D. Female of *Arrenurus (Arr.)* sp glued to males hindbody; the petiole is inserted in to female genital opening (E,F). G, H. Female of *A. (Arr.) tricuspidator* glued to males dorsum (petiole present).

Arrenurus (Truncaturus) stecki, N=2

I. Walking: Male and female walked around the well bottom with fourth legs held up over their hind body. Male sometimes move hind legs in a rotary motion which was accompanied by sudden changes in walking direction.

II. Ready position: Though male had no spurs on fourth legs, he crooked his fourth legs and held them flat over his back. He often directed his back towards the female.

III. Mounting and gluing: Male placed his cauda under the standing female repeatedly and attempted to glue her many times before both sexes were successfully paired.

IV. Walking: Male walked around with female on his back. Female often flailed her legs.

V. Spermatophore deposition and sperm transfer: Male leaned forward (presumed to be drawing out a spermatophore). Subsequently, male pressed venter against substratum (presumed to be placing sperm onto female's venter) and slightly and slowly rocked his cauda from side to side (Fig. 4.4.1.1 E). Moreover, male leaned his body slowly to the left by bending left legs I to III, and then to the right by bending right legs I to III (sideways leaning). This was interspersed with sharp vertical jerking. Sideways leaning could be displayed until the end of mating.

VI. Separation: This was achieved presumably by sharp vertical jerking.

VII. Grooming: When separated both sexes brushed their own bodies from front to back with fourth legs.

***Arrenurus (Arrenurus) tricuspidator*, N=5**

I. Walking: Male and female walked around the well bottom. Male moved fourth legs in a rotary motion (leg fanning).

II. Ready position: male held hind legs crooked over his back when he was in close proximity to female. He often directed his cauda towards the female and touched her with his first legs. Male grabbed legs of female using his fourth leg spurs.

III. Wrestling: Both were directed towards their ventral sides and wrestled. However, male of *A. (Arr.) tricuspidator* did not touch genital area and gnathosoma of female with palpi and legs as was observed in *A. (Mic.) crassicaudatus*.

IV. Mounting: Female was maneuvered into mating position by gluing on male's hindbody (for SEM micrographs see Fig. 4.4.1.2 G, H). Sometimes she climbed unassisted on to the male's cauda.

V. Walking: Male walked around with female on his back and maneuvered her with his fourth legs. Female often flailed her legs.

VI. Spermatophore deposition and collection: Male lifted back end (presumably drawing out a spermatophore) and afterwards leaned forward, presumably gathering sperm from the top of the spermatophore onto his petiole. In addition, he displayed high vertical movements (by lifting his cauda very high on straight legs with glued female) throughout mating. Moreover, male pushed the female backwards (Fig. 4.4.1.1 F). He rocked his body (slightly and slowly rocked his cauda from side to side) but did not display vigorous side-jerking and sideways leaning. Male was trembling his third legs.

VII. Motionlessness: Male was motionless except for flailing his third legs. He pushed female backwards with his fourth legs repeatedly.

VIII. Separation: Male pushed female off using his fourth legs and swam vigorously. In result both separated.

IX. Grooming: Male and female brushed own body. Female could lie in a state of motionless rigidity on the well bottom.

***Arrenurus (Arrenurus) bicuspidator*, N=2**

I. Walking: Male and female walked around the well bottom with fourth legs held up over their backs. Male displayed often arrestant posture (male freezes in a close proximity of female) or moved hind legs in a rotary motion (fanning).

II. Ready position: male held hind legs crooked over his back. In such a position the last two segments of the fourth leg equipped with a spur with long hairs vibrated.

In some cases the male vigorously put his cauda under female's venter and grasped her legs using his fourth legs. In other cases, the female actively climbed onto the male's back.

III. Mounting: Female was maneuvered into mating position and was glued on male's hindbody. In cases of unsuccessful gluing, detached females could not keep balance and behaved like they were in a state of motionless rigidity.

Females that were not successfully glued to male's hindbody and separated from him prematurely did not want to continue courtship and mount this particular male again.

IV. Walking: Male walked around with female on his back. Female often flailed her legs.

V. Spermatophore deposition and collection. Male lifted back end (presumably drawing out a spermatophore). He then leaned forward, presumably gathering sperm on petiole that was subsequently inserted in to female's genital tract. Male sometimes trembled his third legs.

VI. Side-jerking: Interspersed with deposition and collection; male jerked hindbody side to side and tapped female with hind legs. Male sometimes trembled his third legs.

VII. Tapping: Male used fourth legs to push female back onto petiole with sperm (Fig. 4.4.1.1 G); he jerked from side to side and tapped female.

VIII. Motionlessness. Male was motionless and trembled his third legs. He could display high vertical movements (by lifting his back on straight legs with attached female). Female sometimes flailed.

IX. Separation: Male pushed female off using his fourth legs.

X. Grooming: after separation, male and female brushed own body from front to back with hind legs.

XI. Mate attendance: In one case after separation the female did not move and lay in a state of motionless rigidity on the well bottom. Male crawled to the female and touched her with his first and second legs.

***Arrenurus (Arrenurus) cuspidator*, N=1**

I. Walking: Male appeared to sense female in well immediately, crawled to her and touched with his first and second legs repeatedly.

II. Ready position: male held hind legs equipped with spurs crooked over his back being in close proximity to female. He displayed ready position and walked around female.

III. Mounting: Male grabbed female and maneuvered her onto his back. He grasped her legs with his fourth legs when she passed by.

IV. Walking: Male walked around with female on his back and maneuvered her with his fourth legs. Female often flailed her legs.

V. Spermatophore deposition and sperm transfer: Male pushed his venter to the substratum (presumably to deposite spermatophore) and then lifted back end (presumably drawing out a spermatophore) (Fig. 4.4.1.1 H, I). He then leaned forward, presumably gathering sperm on petiole that was subsequently inserted in to female's genital tract. Moreover, male leaned his body slowly to the left by bending left legs I to III and then to the right by bending right legs I to III (Fig. 4.4.1.1 J, K). Male jerked hindbody side to side and tapped female with hind legs. Male flailed his third legs.

VI. Motionlessness: Male was motionless and trembled his third legs. He used fourth legs to push female backwards. Female flailed.

VII. Separation: Male pushed female off using his fourth legs and started to swim vigorously. As a result male and female separated.

VIII. Mate attendance: after separation, male brushed own body and female lay in a state of motionless rigidity on the well bottom. Male walked slowly around female and stood in a close proximity to her.

***Arrenurus (Arrenurus) maculator*, N=1**

I. Walking: Male and female walked around the well bottom. Male fanned his fourth legs and touched female with claws of his first and second legs repeatedly.

II. Ready position: male sat in a close proximity to female and held hind legs equipped with spurs crooked over his back. He walked with crooked legs around female.

III. Mounting: Male touched female with his first and second legs and female appeared to climb onto the male's cauda without being grasped by the male. She was maneuvered into mating position and glued on male's hindbody.

IV. Walking: Male walked with female on his back around and maneuvered her with his fourth legs. Female often flailed her legs.

V. Spermatophore deposition and collection: Male pushed his venter to the substratum (presumably to deposite spermatophore) and then lifted back end (presumably drawing out a spermatophore) (Fig. 4.4.1.1 H, I). He then leaned

forward, presumably gathering sperm on petiole that was subsequently inserted in to female's genital tract. Male leaned his body slowly to the left by bending left legs I to III and then to the right by bending right legs I to III (Fig. 4.4.1.1 J, K). Male jerked hind body side to side and tapped female with hind legs. In the meantime male was trembling his third legs. He displayed high vertical movements (by lifting cauda with glued female).

VI. Motionlessness. Male was motionless and trembled his third legs. Female sometimes flailed.

VII. Separation: This was achieved by pushing female off using male's fourth legs.

VIII. Grooming: Male and female brushed own body.

***Arrenurus (Arrenurus) claviger*, N=4**

I. Walking: Male and female walked around the well bottom with fourth legs held up over their backs. Male displayed arrestant posture and also leg fanning when introduced in to well. Male showed interest in mating 15-20 minutes after having been placed in well.

II. Ready position: Male crooked his fourth legs equipped with spurs at the fourth distal segment (between genu and tibia) and placed them over his back when the female came into contact with or ran by him.

III. Mounting and gluing: Male grabbed female's leg using his fourth legs and maneuvered her onto his back. Female was then glued in mating position on male's back. Sometimes as a result of unsuccessful gluing, female detached and lay in a state of motionless rigidity on the well bottom.

IV. Swimming/Walking: Male swam vigorously (or walked) with female glued on his back.

V. Spermatophore deposition and collection: Male sharply jerked cauda upwards repeatedly (Fig. 4.4.1.1 L). He lifted back end (presumably drawing out a spermatophore, Fig. 4.4.1.1 M) and leaned forward, presumably gathering sperm on petiole. He rocked slightly from side to side and lifted his cauda. Male trembled his third legs.

VI. Motionlessness: Male was motionless and trembled his third legs. He pushed female backwards with his fourth legs. Female sometimes flailed.

VII. Separation: This was achieved through pushing female off using male's fourth legs and swimming around the well.

VIII. Grooming: Once separate, male and female brushed own body from front to back with hind legs.

IX. Mate attendance: Sometimes female behaved like she was in a state of motionless rigidity. Male walked around her with crooked fourth legs and displayed ready position. He grabbed female's leg with his fourth legs and maneuvered her onto his back. Female that already mated was resistant to male's harassment, detached and escaped.

***Arrenurus (Arrenurus) bruzelii*, N=10**

I. Walking: Male and female walked around the well bottom. Male (and sometimes female) moved fourth legs in a rotary motion (leg fanning). Male appeared to immediately sense female that did not move in well and started displaying mating behavior.

II. Ready position: male held hind legs equipped with spur crooked over his back when he was in a close proximity to female. He often directed his cauda towards the female and grabbed her with fourth leg spurs.

III. Mounting: Female sometimes sat in a close proximity to male and fanned her fourth legs. In some cases, she appeared to climb unassisted on to the male's cauda. She could do this repeatedly even if male showed no interest in mating. In other cases male grabbed legs of female with fourth leg spurs and maneuvered her into mating position and glued her onto his cauda (Fig. 4.4.1.1 N). Male sometimes rejected female that showed interest in mating with him.

IV. Walking: Male walked around with female on his back and maneuvered her with his fourth legs. Female often flailed her legs.

V. Spermatophore deposition and sperm transfer: Male lifted back end (presumably drawing out a spermatophore). He leaned forward, presumably gathering sperm on petiole. Male displayed vertical movements with vigorous side-jerking (vertical movements, Fig. 4.4.1.1 O). He rocked his body slightly. Male pushed female backwards with hind legs.

VI. Motionlessness and side-jerking: Male was motionless and trembled his third legs. He used fourth legs to push female backwards. Male may rarely display side-jerking throughout mating. Female may flail.

VII. Separation: This was achieved by male pushing female off using his fourth legs and side-jerking.

VIII. Grooming: Male and female brushed own body.

IX. Mate attendance: Female sometimes lay in a state of motionless rigidity on the well bottom. Male stayed in close proximity of female, displayed leg fanning and ready position. Mating did not re-occur between recently mated pairs.

Table 4.4.1.1. Duration of mating behaviour in the 13 *Arrenurus* species. Data are means ± S.E. The post-deposition behaviour is included in pairing behaviour and is expressed as percentage of the total time spent on mating. The total duration of mating includes pre-pairing stage. Duration of post deposition behaviour could not be estimated in *A.(Arr.) tricuspidator* because of difficulties with interpretation of behavioural events in post-deposition stage. *Arrenurus (Mic.) crassicaudatus* and *A. (Arr.) planus* transfer sperm via legs, therefore their behaviour could not be partitioned in the same manner as it was done in the other species. Data regarding *A. (Arr.) maculator* and *A. (Arr.) cuspidator* base on observations of single pairs.

		pre-pairing behaviour (min)	pairing behaviour (min)	post-deposition behaviour (%)	total duration of mating (min)
A. (Arr.) maculator	N = 1	16.00 ± N.A.	478.00 ± N.A.	60.93 ± N.A.	494.00 ± N.A.
A. (Arr.) cuspidator	N = 1	111.00 ± N.A.	574.00 ± N.A.	41.61 ± N.A.	685.00 ± N.A.
A. (Arr.) bicuspidator	N = 2	35.00 ± 5.00	640.00 ± 84.00	62.09 ± 29.83	675.00 ± 89.00
A. (Arr.) tricuspidator	N = 5	15.00 ± 5.00	352.00 ± 37.15	N.A.	358.25 ± 32.69
A. (Arr.) bruzelii	N = 10	26.00 ± 11.50	316.00 ± 18.84	51.38 ± 8.53	338.90 ± 21.37
A. (Arr.) claviger	N = 4	18.00 ± 4.15	447.75 ± 16.97	90.15 ± 2.58	466.00 ± 18.77
*A. (Arr.) planus**	N = 7	5 ± 1.39	81.35±N.A.	N.A.	86.35 ± 18.02
A. (Mic.) crassicaudatus	N = 1	5.00 ± N.A.	137± N.A.	N.A.	142.00 ± N.A.
A. (Meg.) globator	N = 4	75.00 ± 60.00	164.00 ± 22.82	25.20 ± 12.98	201.75 ± 34.78
A. (Tru.) stecki	N = 2	30.00 ± 20.00	27.00 ± 4.00	19.56 ± 19.57	57.00 ± 24.00
*A. (Arr.) sp. nr. reflexus**	N = 8	9.85 ± 4.97	315.82 ± N.A.	94.3 ± 0.45	325.67 ± 19.65
*A. (Meg.) manubriator**	N = 7	N.A.	N.A.	21.2 ± 3.91	132.6 ± 19.97
*A. (Tru.) rufopyriformis**	N = 9	3.73 ± 1.05	52.9 ± N.A.	77.00 ± 2.5	56.63 ± 4.98

*data from Proctor and Wilkinson (2001)

4. 4. 2. Mating duration

The longest durations of mating were displayed by *Arrenurus* s.str. spp. with males equipped with a well developed petiole and that deposit spermatophores on the substratea (e.g. well bottom) (Tab. 4.4.1.1). The average mating duration ranged in these *Arrenurus* s.str. from 338.90 ± 21.37 minutes in *A. (Arr.) bruzelii* to 675.00 ± 89.00 minutes in *A. (Arr.) bicuspidator* (and in *A. (Arr.) cuspidator* 685.00 minutes, single observation). *Arrenurus (Mic.) crassicaudatus* with males that transfer sperm with legs and have short cauda and petiole without central piece spend less time on mating (142.00 minutes, single observation). The average mating duration in 'apetiolate species' (males lack petiole or have peg-like petiole that does not seem to function as an intromittent organ) ranged from 56.63 ± 4.98 minutes in *A. (Tru.) rufopyriformis* (in *A. (Tru.) stecki* 57.00 ± 24.00 minutes) to 201.75 ± 34.78 minutes in *A. (Meg.) globator*. Moreover, the difference in percent of time spent on post-transfer behaviours between apetiolate (N = 4) and petiolate (N = 6) species was statistically significant (two-tailed t test, p = 0.035; see also Fig. 4.4.2.1). The 'apetiolate species' spent less time in post-transfer stage of mating than *Arrenurus* s. str. with fully developed petiole (called'petiolate species') (Tab. 4.4.1.1). The time spent on behaviours in the pre-pairing stage seem to be dependent on the age, hunger, condition of mites, and also on female's willingness to mate. Since the age and hunger of mites were not standardized before trying to pair the mites, the pre-pairing duration was not used in statistical analyses and should be interpreted with caution (Tab. 4.4.1.1).

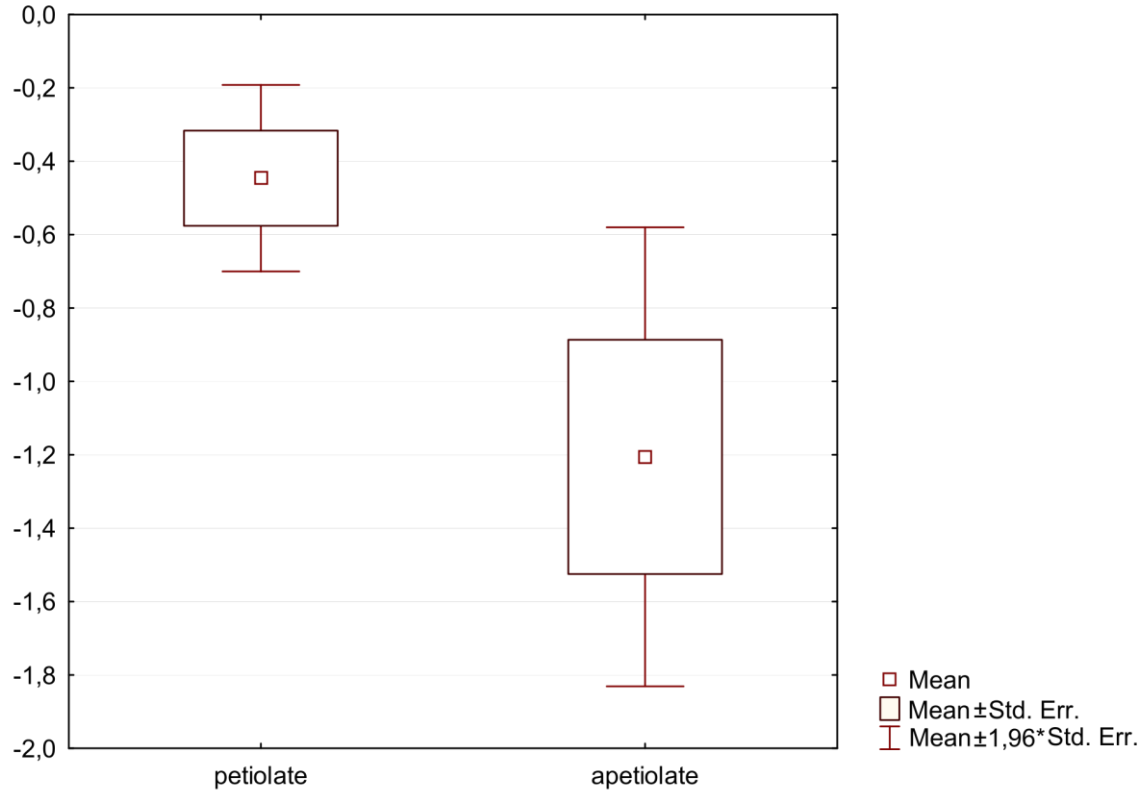

Figure 4.4.2.1. Box and whisker plots of the percent of time spent on post-transfer behaviours (log-transformed) in petiolate and apetiolate species.

4. 4. 3. Evolution of mating behaviour

I reconstructed evolution of mating behaviour across 13 *Arrenurus* species on the optimal ML tree with the likelihood Markov k-state 1 parameter model (Figs. 4.4.3.1 - 4.4.3.13). The analysis included mating behavior of the 9 species observed by the author (*A. (Arr.) bicuspidator, A. (Arr.) bruzelii, A. (Arr.) claviger, A. (Arr.) cuspidator, A. (Arr.) maculator, A. (Arr.) tricuspidator, A. (Meg.) globator, A. (Mic.) crassicaudatus, A. (Tru.) stecki*), and the behavior of further 4 species (*A. (Arr.) planus, A. (Arr.) sp. nr. reflexus, A. (Meg.) manubriator, A. (Tru.) rufopyriformis*) was taken from Proctor and Wilkinson (2001). The character matrix for observed behaviours is shown in Table 3.2.5.1.

The first two behaviors considered are related to the mode of sperm transfer. The first one 'spermatophore deposition on the substratum' occurred both in apetiolate species with elongated hindbody (*A. (Meg.) globator, A. (Meg.) manubriator, A. (Tru.) rufopyrformis, A. (Tru.) stecki*), and species equipped with a well developed petiole and an

elaborated cauda (*A. (Arr.) bicuspidator*, *A. (Arr.) bruzelii*, *A. (Arr.) claviger*, *A. (Arr.) cuspidator*, *A. (Arr.) maculator*, *A. (Arr.) sp. nr. reflexus*, *A. (Arr.) tricuspidator*) (Fig. 4.4.3.1). The second behavior 'sperm transferred with the use of legs' is characteristic for species with a short cauda, and a simple petiole without a hyaline appendage i.e. *A. (Arr.) planus* and *A. (Mic.) crassicaudatus* (Fig. 4.4.3.2).

In the pre-pairing stage, two behaviours were considered. In the first one male crooked his hind legs at the fourth distal segment and placed them over his back when the female was in a close proximity. The ready position occurred in species in which sperm was deposited on the substratum (Fig. 4.4.3.3). These species were those that had hind legs equipped with spurs (*A. (Arr.) bicuspidator*, *A. (Arr.) bruzelii*, *A. (Arr.) claviger*, *A. (Arr.) cuspidator*, *A. (Arr.) maculator*, *A. (Arr.) sp. nr. reflexus*, *A. (Arr.) tricuspidator*, *A. (Meg.) manubriator*, *A. (Meg.) globator*, *A. (Tru.) rufopyrformis*), and those lacking this structure (*A. (Tru.) stecki*). This behaviour is absent in species which transfer sperm via legs i.e. *A. (Arr.) planus* (spur present) and *A. (Mic.) crassicaudatus* (spur absent). The second behaviour, touching female's body with claws of first and second legs was found in *A. (Arr.) cuspidator*, *A. (Arr.) maculator*, *A. (Arr.) planus*, *A. (Arr.) tricuspidator* and *A. (Meg.) globator* which come from different clades (Fig. 4.4.3.4).

I distinguished four behaviours displayed during pairing and associated with spermatophore deposition and collection. In vertical jerking, the male jerked his hindbody sharply upwards. This behaviour occurred in all species that lack petioles or that have only a peg-like petiole and have elongated hindbody without pygal lobes i.e. in *A. (Meg.) manubriator*, *A. (Meg.) globator*, *A. (Tru.) rufopyriformis*, *A. (Tru.) stecki*; however, it also occurred in *A. (Arr.) claviger*, which has a well developed petiole and hindbody (Fig. 4.4.3.5). In side jerking, the male jerked sharply his hindbody from side to side. This occurred in *A. (Arr.) bicuspidator*, *A. (Arr.) bruzelii*, *A. (Arr.) cuspidator*, *A. (Arr.) maculator*, *A. (Arr.)* sp. *nr. reflexus*, *A. (Meg.) manubriator*, *A. (Meg.) globator* (Fig. 4.4.3.6). In sideways leaning, the males tilt to right side by bending right legs I to III and then to the left side by bending left legs I to III with female on his back (Fig. 4.4.3.7). This was displayed both by apetiolate (*A. (Tru.) rufopyriformis* and *A. (Tru.) stecki*), and petiolate species (*A. (Arr.) cuspidator*, *A. (Arr.) maculator*). The last behaviour, male being dragged by female while being attached under her and facing in the opposite direction, occurred exclusively in species which transfer sperm via legs (*A. (Arr.) planus*, *A. (Mic.) crassicaudatus*) (Fig. 4.4.3.8).

There are behaviours associated with the post-deposition stage of mating. Two behaviours were noted exclusively in the clade consisted of *Arrenurus* s.str.: long periods of motionlessness when spermatophore deposition and collection are presumably completed, and trembling third legs by male in the last stages of mating (Fig. 4.4.3.9, Fig. 4.4.3.10). The latter behaviour was not observed in *A. (Arr.) planus*. Moreover, the duration of mating after spermatophore deposition and collection was considered. The proportion of duration of post-transfer behaviours to the entire time spent on pairing varied among species. The percent of time spent on post-transfer behaviours ranged from 41.61 % to 94.3 % in all *Arrenurus* s.str. species and in *A. (Tru.) rufopyriformis* (Fig. 4.4.3.11, see Tab. 4.4.1.1). The percentage of time spent on post-transfer behaviours in *A. (Meg.) manubriator*, *A. (Tru.) stecki* and *A. (Meg.) globator* ranged from 19.56 % to 25.20 % (Fig. 4.4.3.11, Tab. 4.4.1.1). In general, the petiolate species spent significantly more time on post-transfer behaviours than apetiolate species (two-tailed t test, $p = 0.035$, Fig. 4.4.2.1, Tab. 4.4.1.1). The duration of this stage of mating was not given for *A. (Arr.) planus* and *A. (Mic.) crassicaudatus* because their behaviour could not be partitioned in the same manner as behaviour of the other species. Similarly, this character could not be considered in *A. (Arr.) tricuspidator* because of ambiguities in interpretation of behavioural events.

After mating was completed I sometimes observed two other behaviours. In the first one, a female detached from the male's back lay in a state of motionless rigidity at the bottom. This behaviour was observed across *Arrenurus* species (*A. (Arr.) bicuspidator*, *A. (Arr.) bruzelii*, *A. (Arr.) claviger*, *A. (Arr.) cuspidator*, *A. (Arr.) sp. nr. reflexus*, *A. (Arr.) tricuspidator*, *A. (Meg.) manubriator*, *A. (Meg.) globator*, *A. (Tru.) rufopyriformis*) (Fig. 4.4.3.12). In the second behaviour, after courtship male crawled around female, touched her with his first and second legs, displayed ready position and attempted to start courtship again. This behaviour was reported in *Arrenurus* s.str. (in *A. bicuspidator*, *A. bruzelii*, *A. cuspidator*) and in the clade consisted of *A. (Meg.) globator* (Fig. 4.4.3.13).

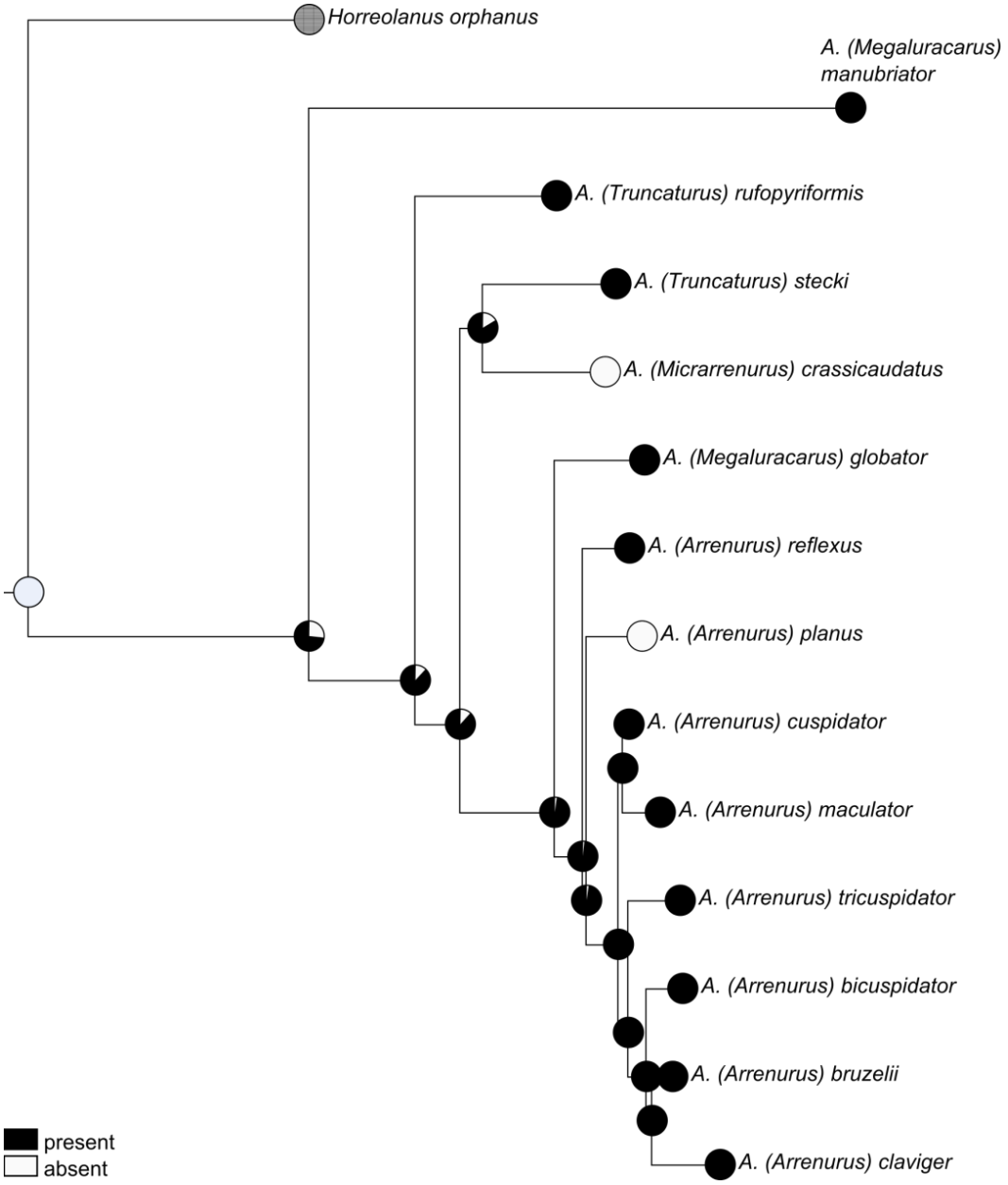

Figure 4.4.3.1. Results of the ancestral reconstruction analysis for character 'spermatophores deposited on the substratum'. The analysis was reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model. With grey circle unknown character states are indicated.

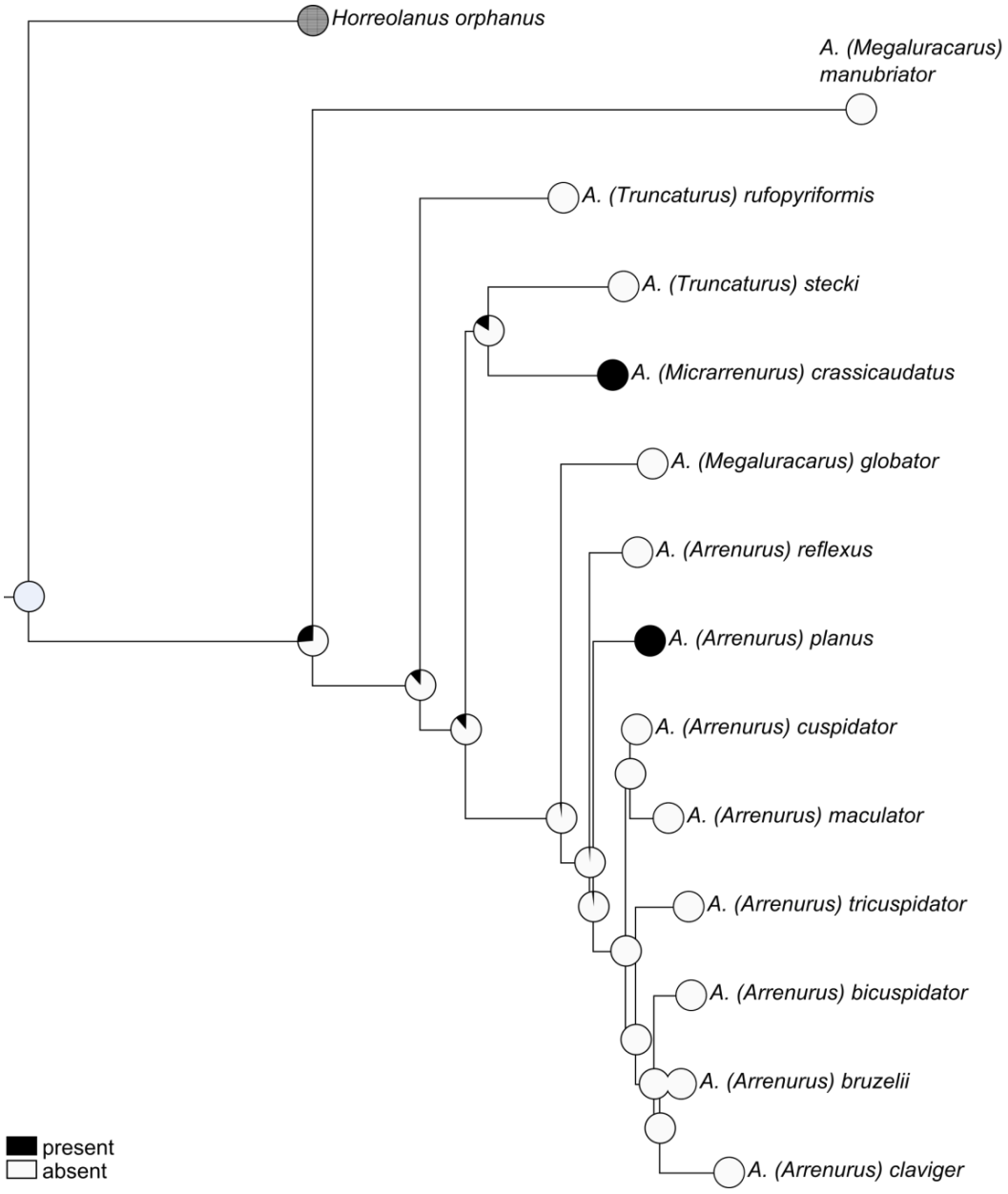

Figure 4.4.3.2. Results of the ancestral reconstruction analysis for character 'sperm transferred with the use of legs'. The analysis was reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model. With grey circle unknown character states are indicated.

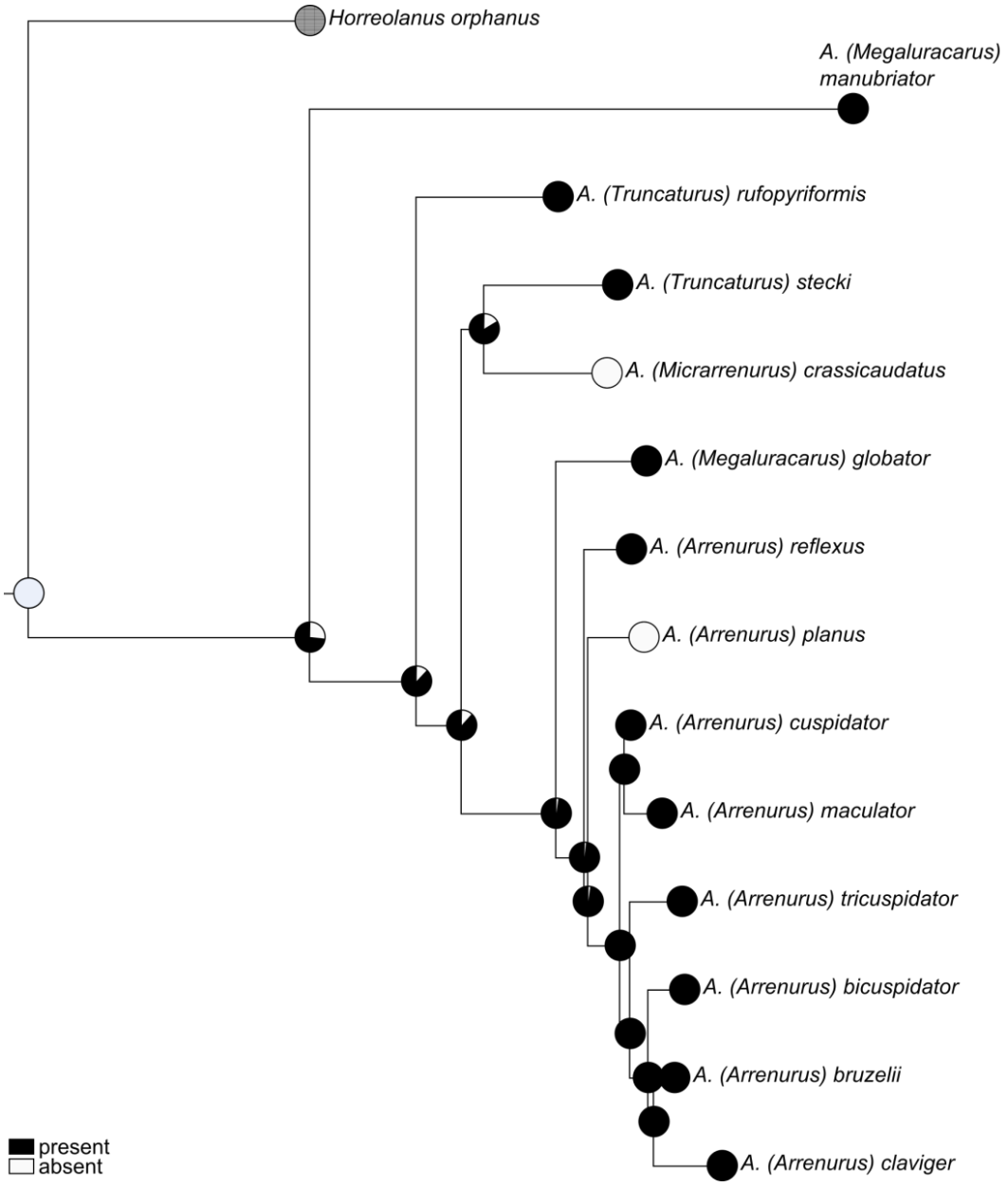

Figure 4.4.3.3. Results of the ancestral reconstruction analysis for character 'ready position'. The analysis was reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model. With grey circle unknown character states are indicated.

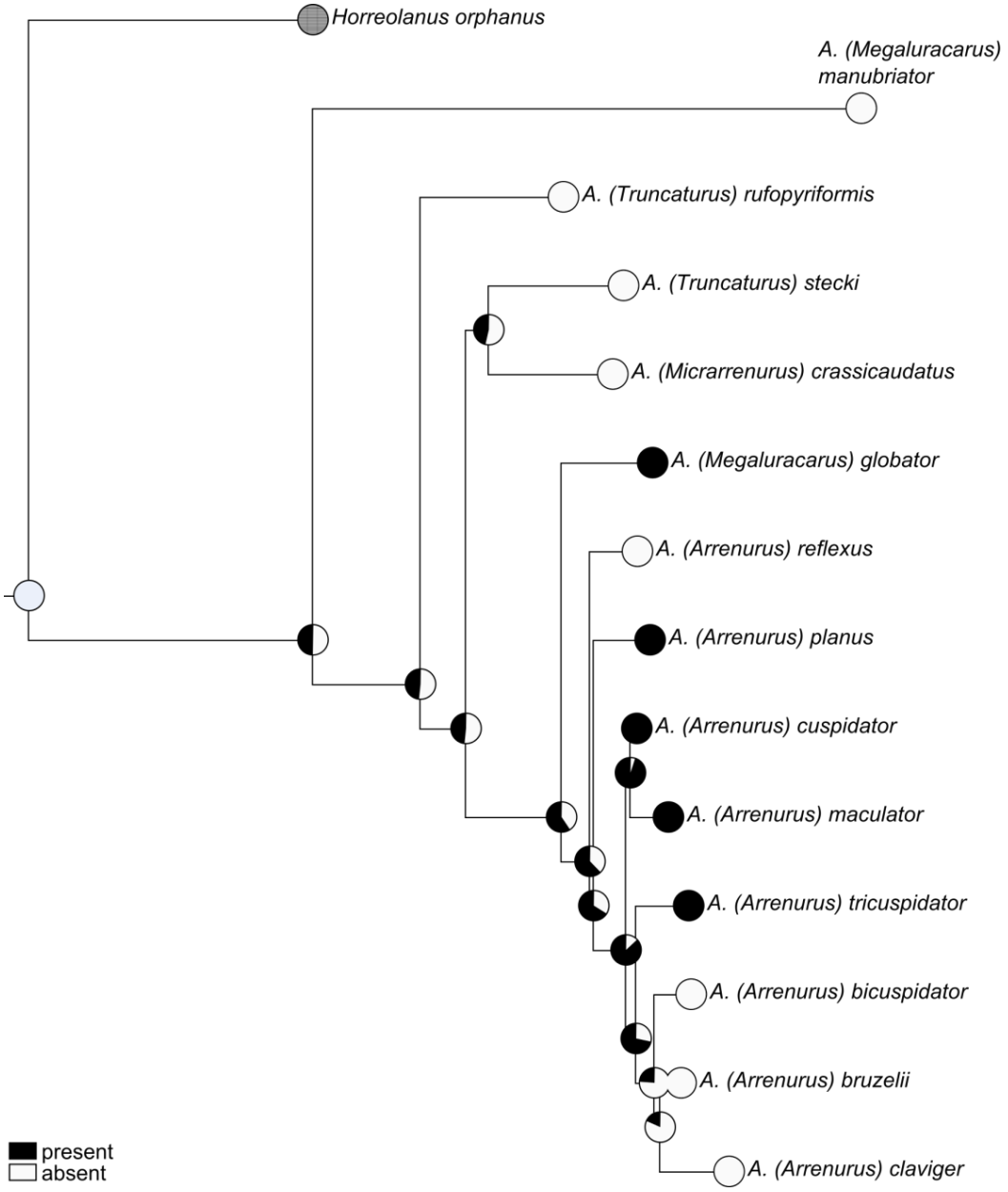

Figure 4.4.3.4. Results of the ancestral reconstruction analysis for character 'touching females body with claws of first and second legs of males in first stages of mating'. The analysis was reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model. With grey circle unknown character states are indicated.

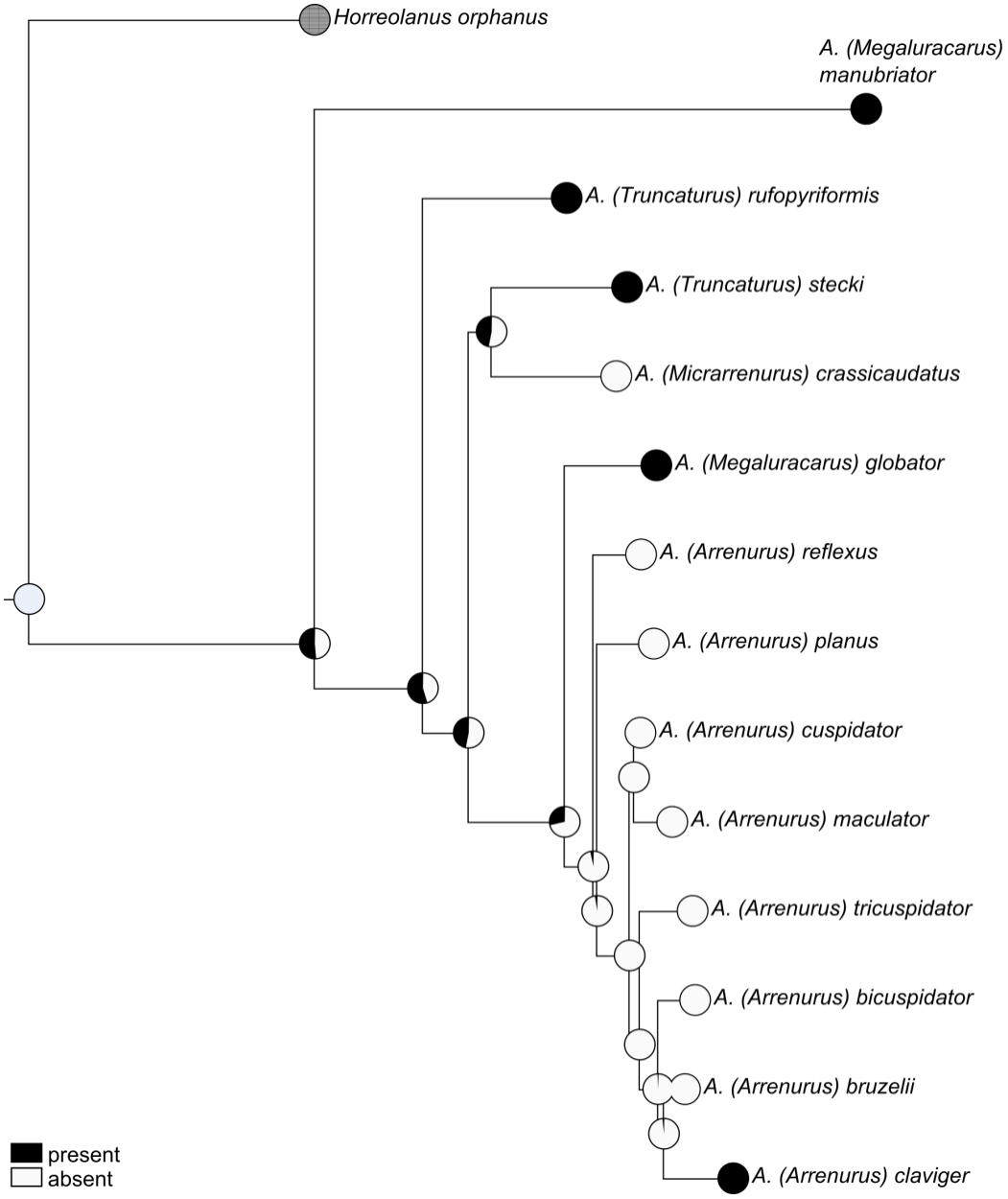

Figure 4.4.3.5. Results of the ancestral reconstruction analysis for character 'vertical jerking'. The analysis was reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model. With grey circle unknown character states are indicated.

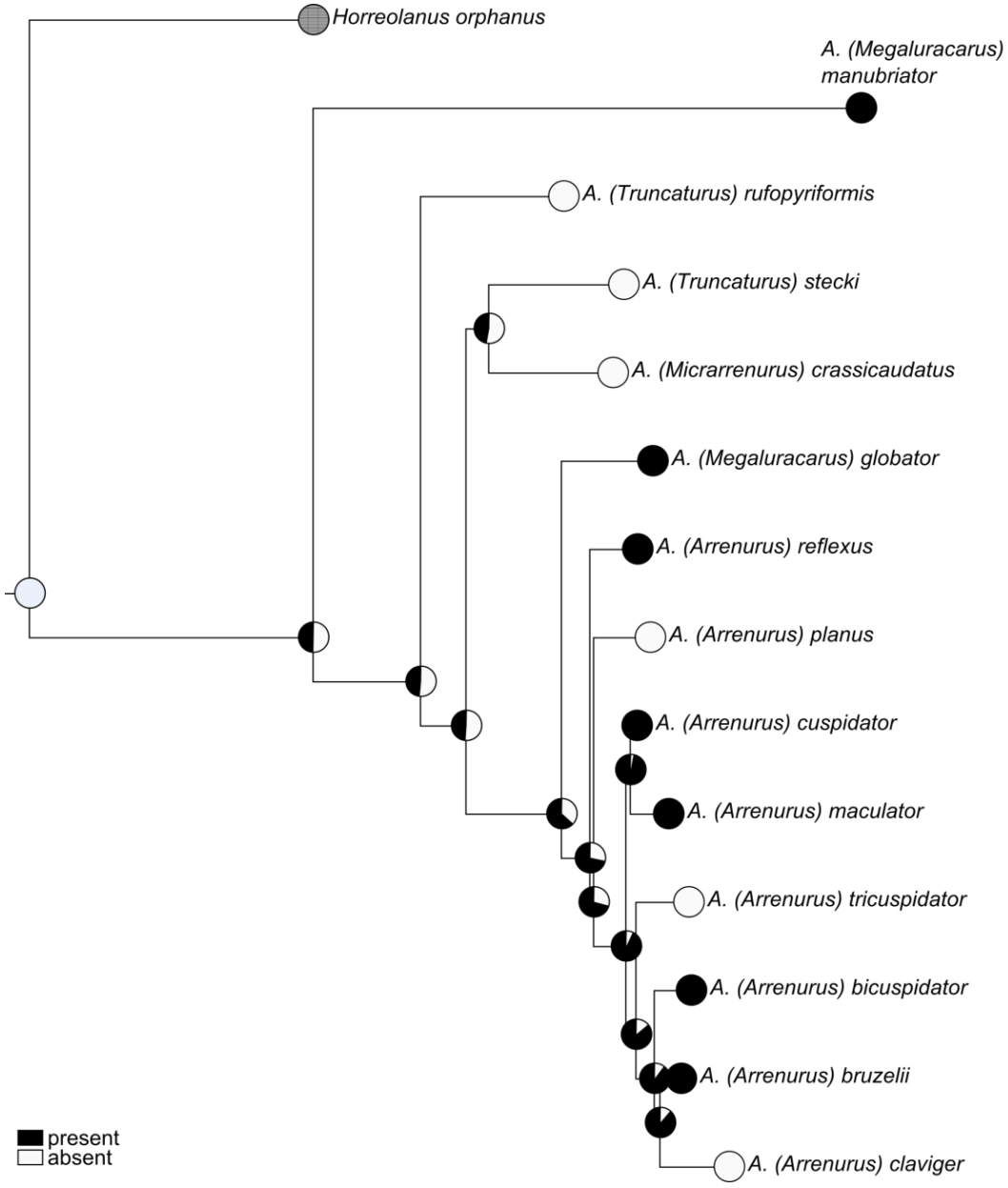

Figure 4.4.3.6. Results of the ancestral reconstruction analysis for character 'side jerking'. The analysis was reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model. With grey circle unknown character states are indicated.

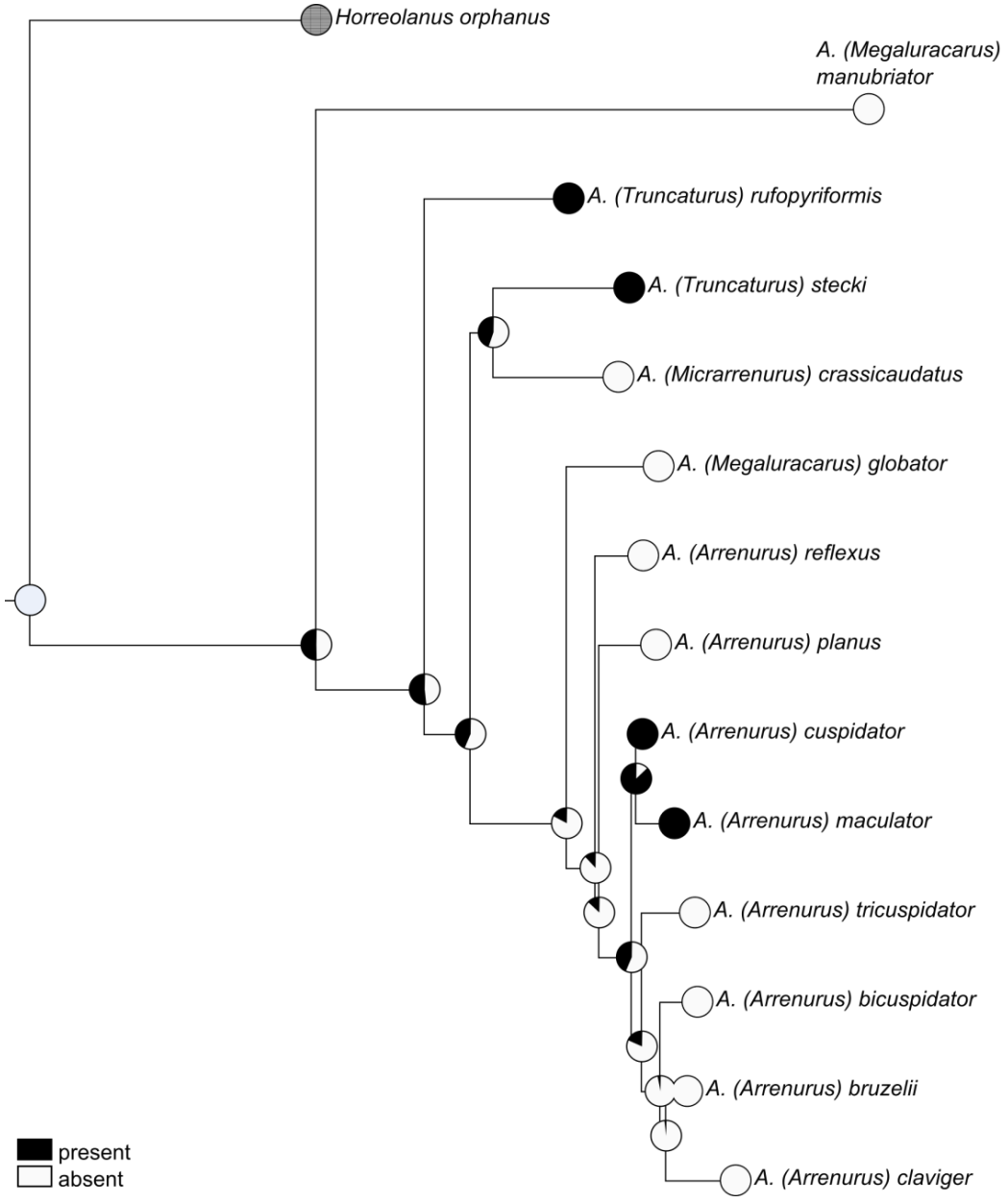

Figure 4.4.3.7. Results of the ancestral reconstruction analysis for character 'sideways leaning'. The analysis was reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model. With grey circle unknown character states are indicated.

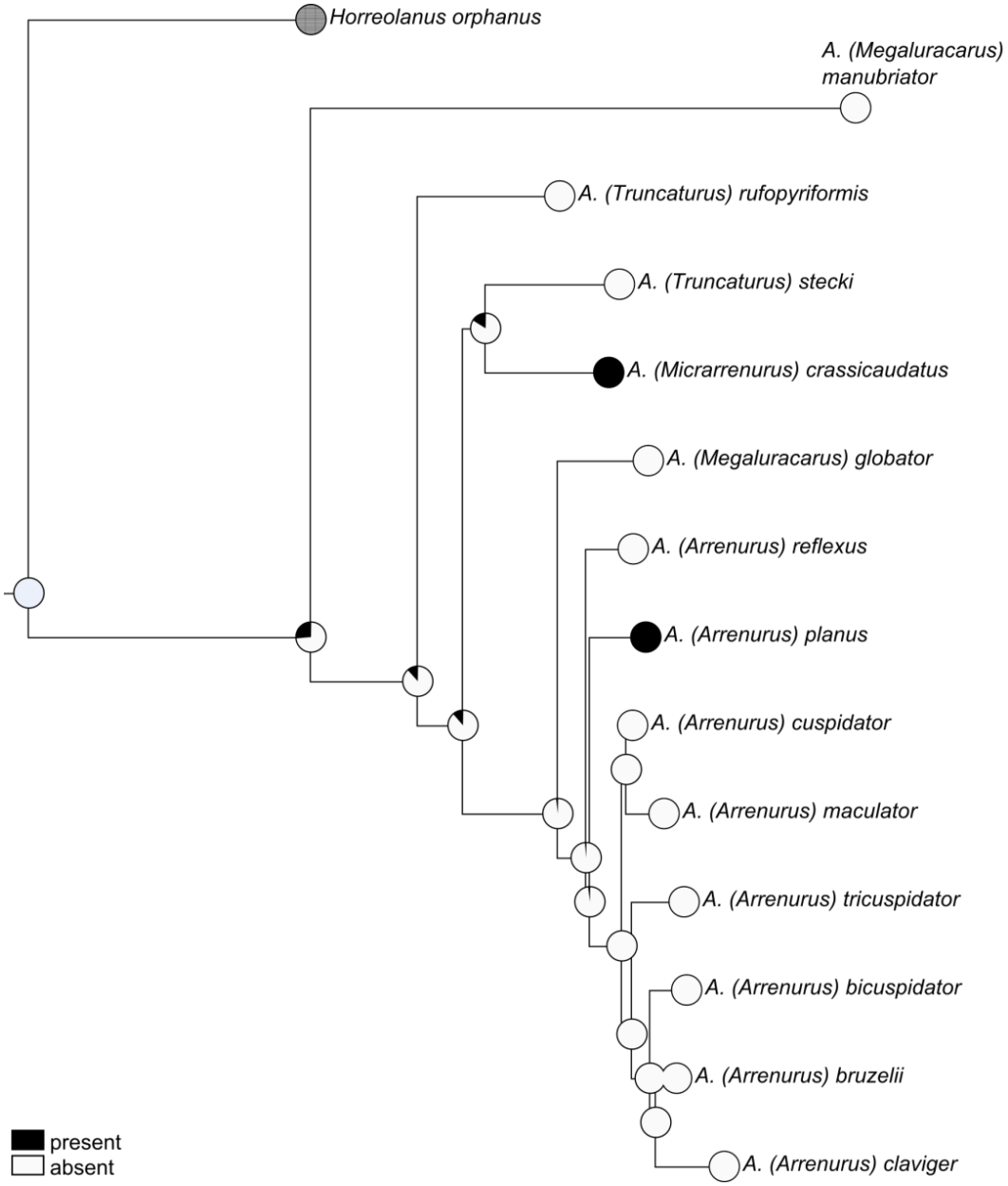

Figure 4.4.3.8. Results of the ancestral reconstruction analysis for character 'male attached under the standing female facing in the opposite direction as her and being dragged by her around'. The analysis was reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model. With grey circle unknown character states are indicated.

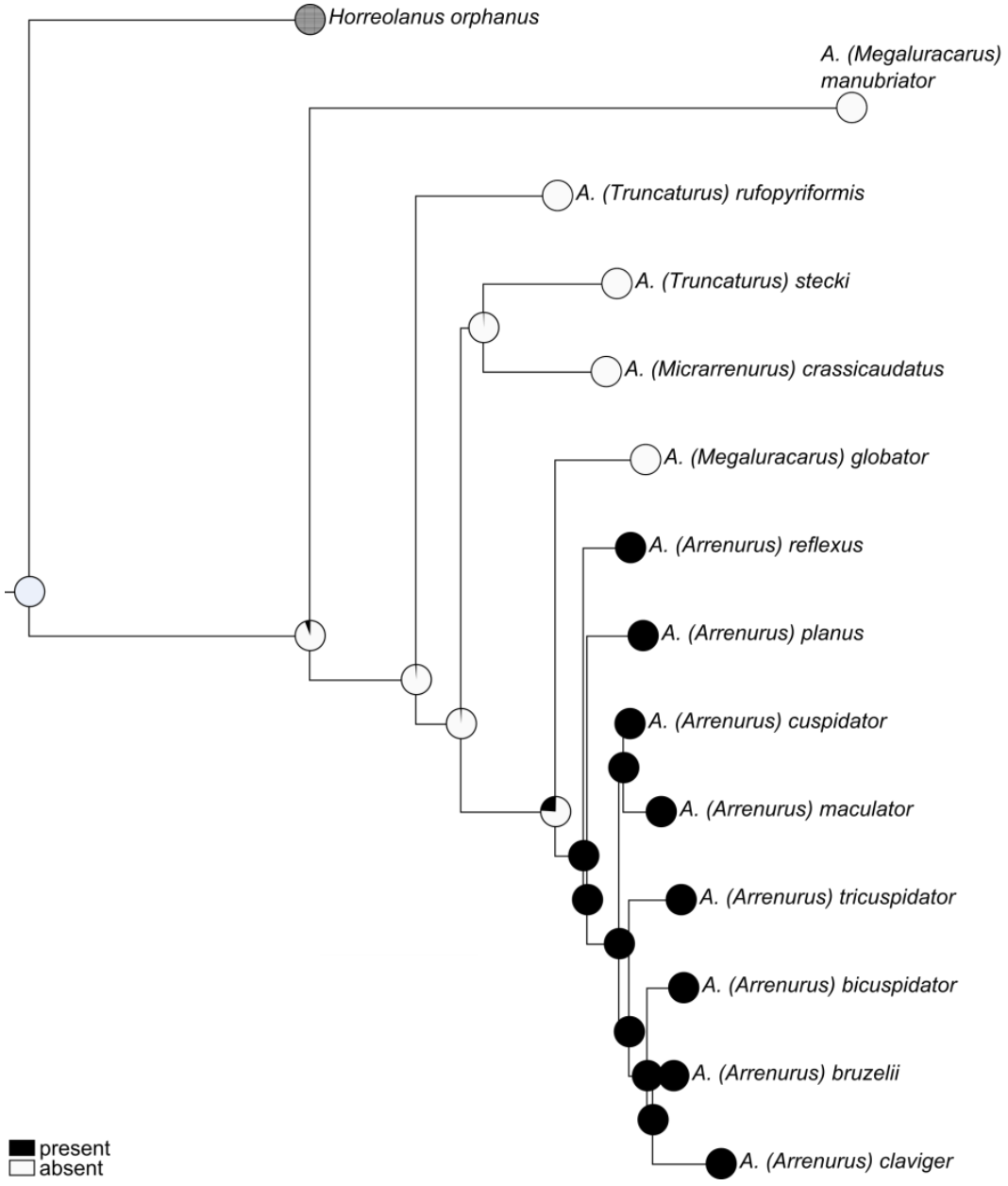

Figure 4.4.3.9. Results of the ancestral reconstruction analysis for character 'long periods of motionlessness when spermatophore deposition and collection are completed'. The analysis was reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model. With grey circle unknown character states are indicated.

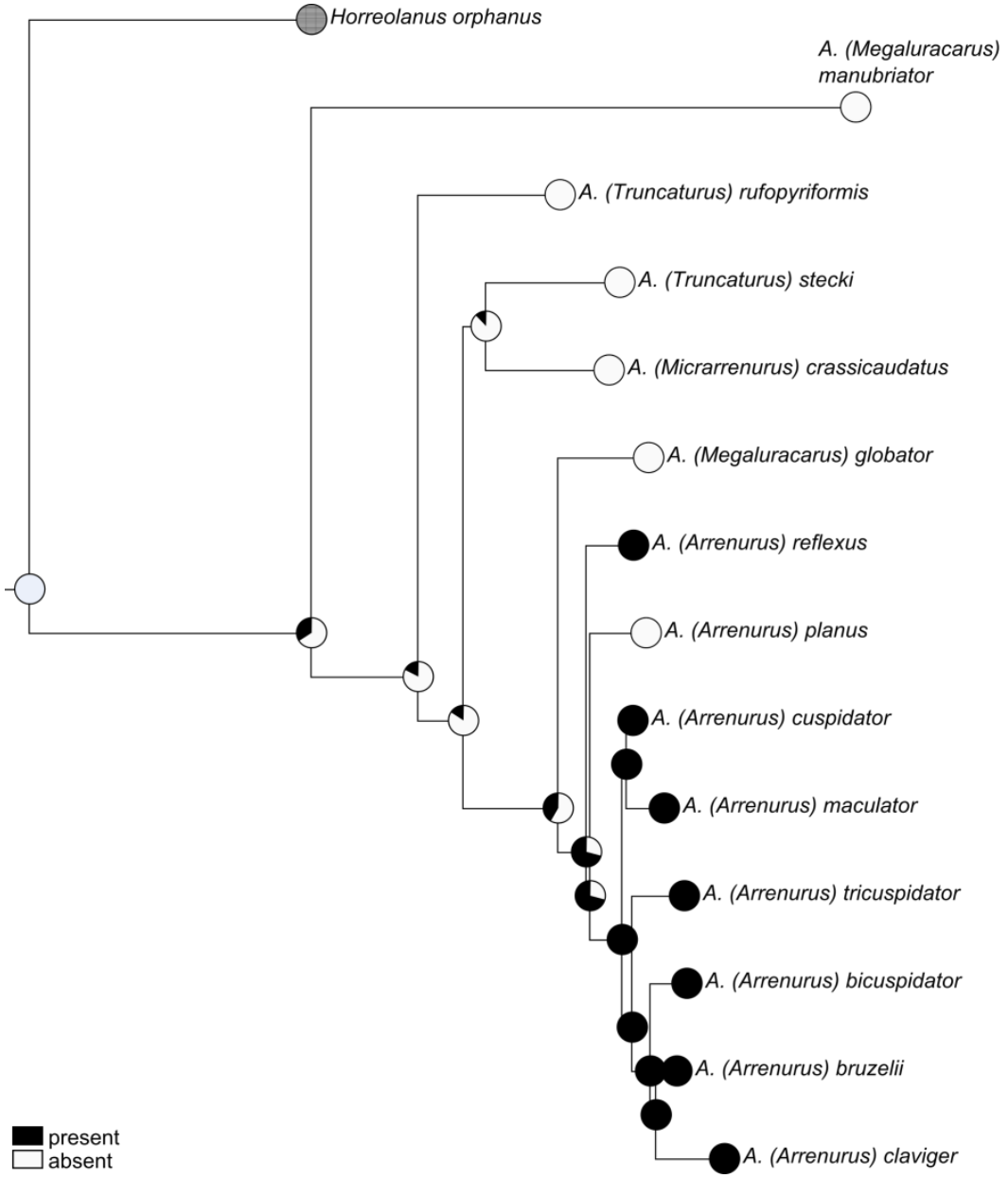

Figure 4.4.3.10. Results of the ancestral reconstruction analysis for character 'trembling third legs throughout mating by male'. The analysis was reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model. With grey circle unknown character states are indicated.

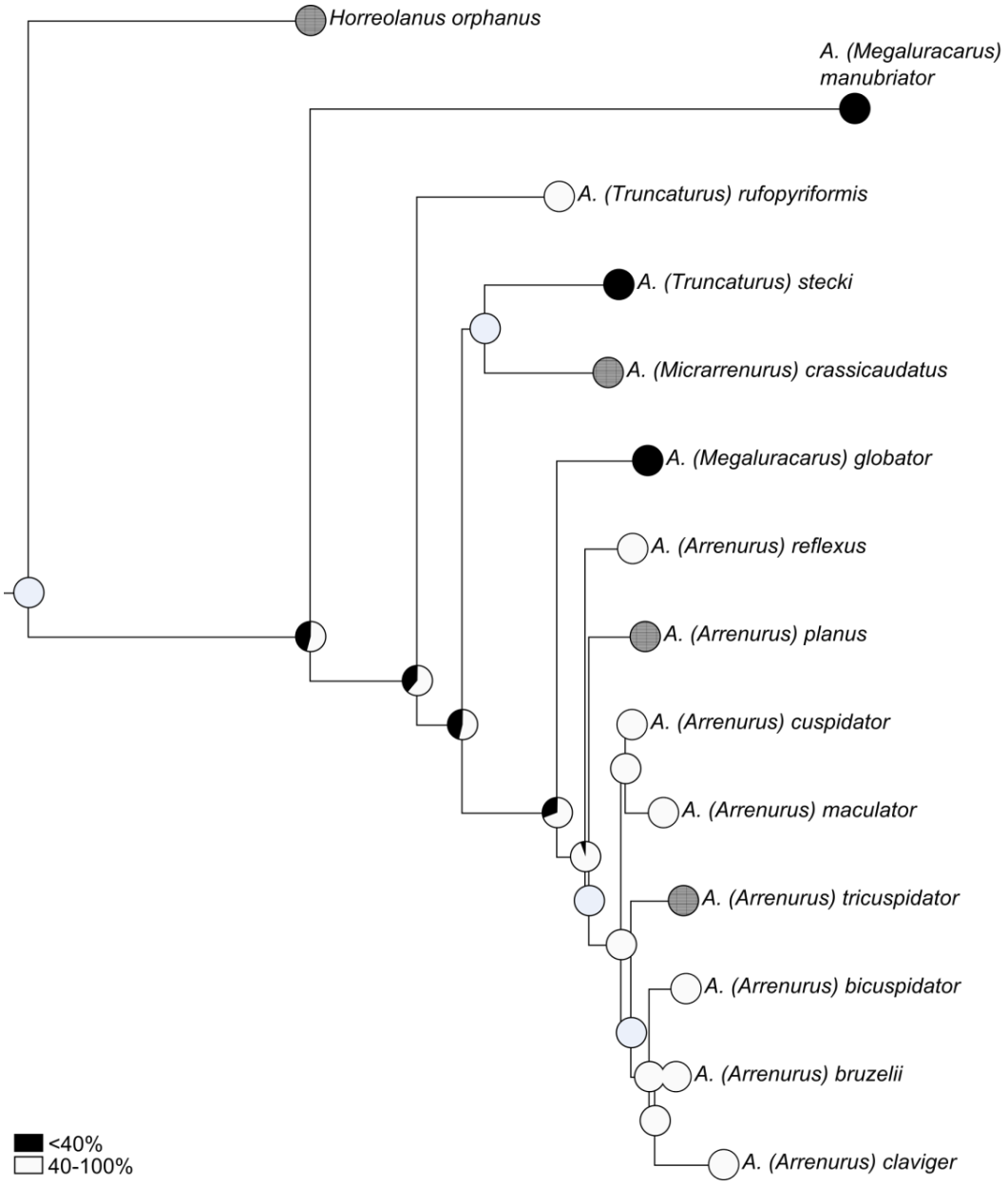

Figure 4.4.3.11. Results of the ancestral reconstruction analysis for character 'time spent in post-transfer behaviours (in %)'. The analysis was reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model. Grey circle: not applicable (*A. (Arr.) planus*, *A. (Mic.) crassicaudatus*); unknown character states (*Horreolanus orphanus*, *A. (Arr.) tricuspidator*).

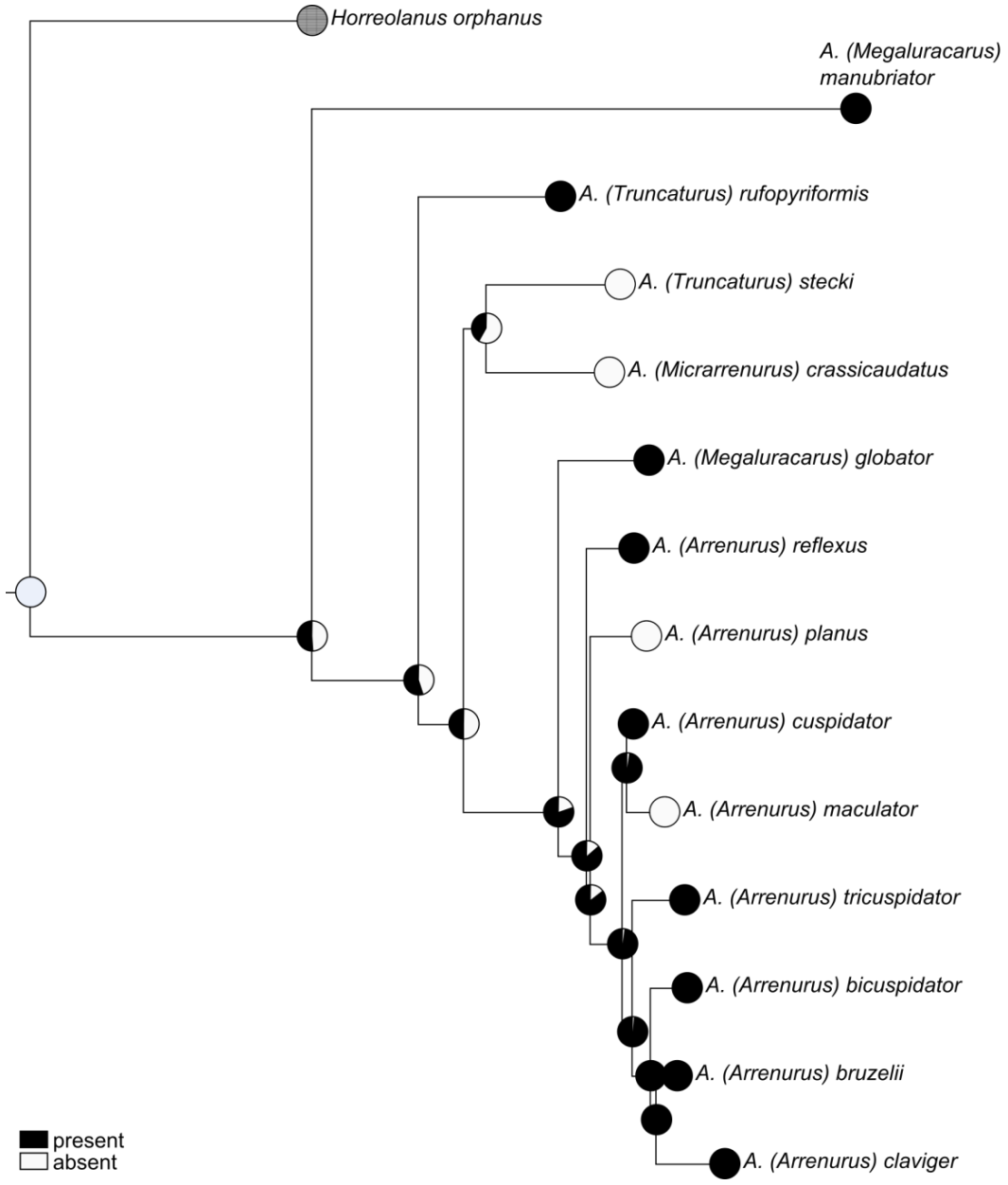

Figure 4.4.3.12. Results of the ancestral reconstruction analysis for character 'when courtship is completed female lies in a state of motionless rigidity'. The analysis was reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model. With grey circle unknown character states are indicated.

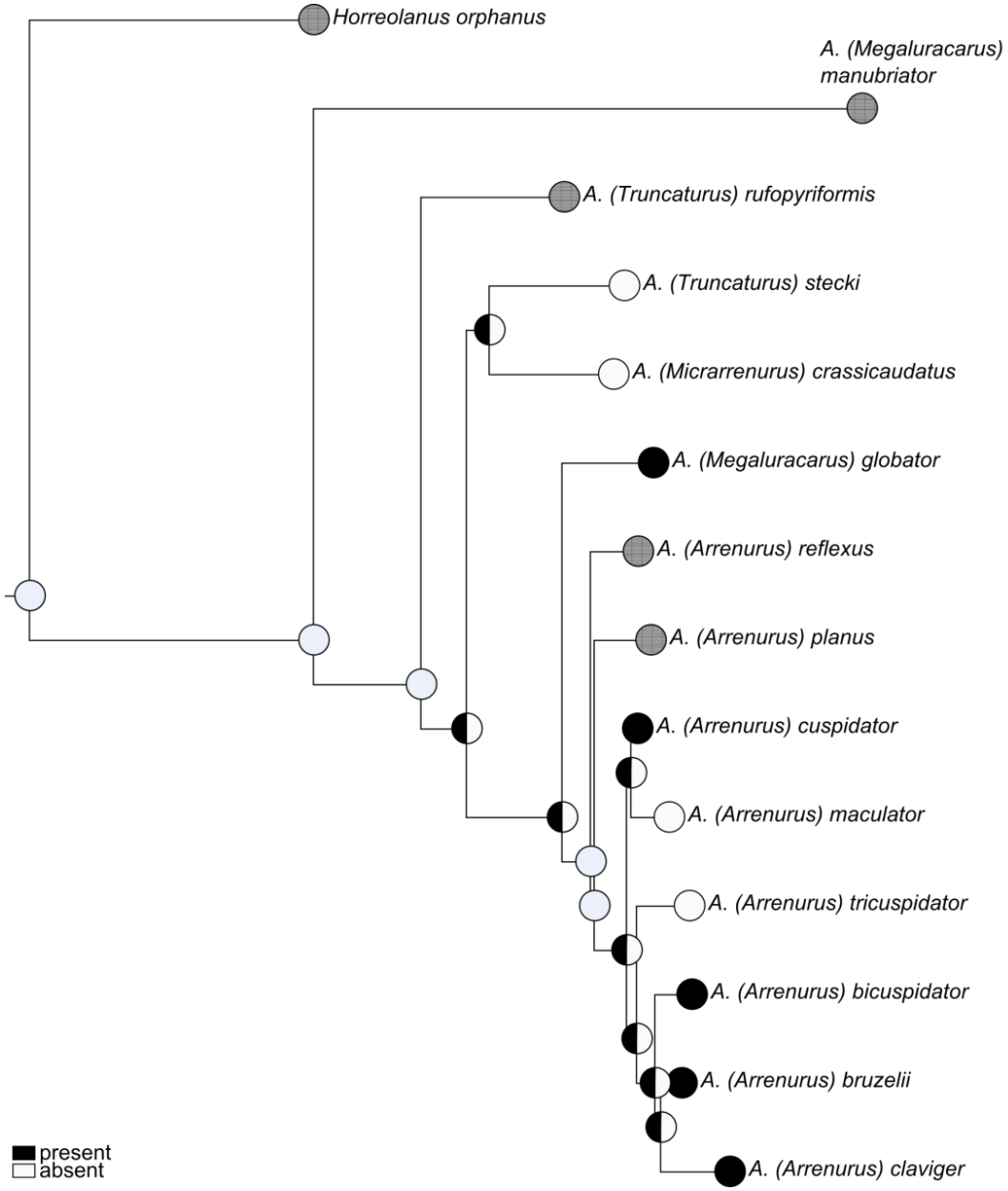

Figure 4.4.3.13. Results of the ancestral reconstruction analysis for character 'mate attendance'. The analysis was reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model. With grey circle unknown character states are indicated.

4. 5. Evolution of genitalia and grasping structures

Morphological features of males and females associated with mating were examined in 41 *Arrenurus* species from Europe and North America. The target morphological adaptations in males were the structure of the hindbody, which varied greatly among species, presence and structure of the intromittent organ (the petiole) and modifications of hind legs. In females, I considered only presence/absence of pigmented patches on the valves of the genital opening. The morphological characters and character states are presented in Table 3.2.5.2.

The spur on male's fourth legs appears to have arisen in the ancestral clade of *Arrenurus* and was subsequently lost at least twice (Fig. 4.5.1). The spur is present in two basal clades with *Megaluracarus* (e.g. *A. manubriator*, App. 8, F, H; *A. scutiliformis*, App. 11, G), in a clade containing *Arrenurus* s. str. (e.g. *A. bicuspidator*, App. 18, F, G; *A. magnicaudatus*, App. 28, E, F) and in its sister clade with *A. (Meg.) globator* (App. 7, F, G), *Arrenurus (Truncaturus)* sp3 (App. 3, F) and *A. (Tru.) truncatellus* (App. 4, C). The spur on IV-L is absent in *A. (Tru.) fontinalis* (App. 1, H), and in a clade composed of *Micruracarus* (e.g. App. 14, G) and *A. (Tru.) stecki* (App. 2, G). A spur was not found in any examined females (e.g. App. 2, F; App. 18, H).

The shape of cauda was a very variable character. The elongated and tubular cauda that is set off from the body appears to be ancestral in the genus *Arrenurus* (Fig. 4.5.2). This type of cauda appeared in two basally located clades that contain *Megaluracarus* (e.g. *A. intermedius*, App. 9, C). However, *A. (Meg.) globator* (App. 7, C, D) showed up in a clade with *A. (Tru.) truncatellus* (App. 4, A) and *Arrenurus (Truncaturus)* sp3 (App. 3, C) which have cauda developed as elongated and shallow concavity (but not set off from the body proper). Moreover, elongated cauda with shallow concavity and not set off from the body proper occurred also in *A. (Tru.) fontinalis* (App. 1, C, F) and in a clade consisted of *A. (Tru.) inexploratus* (App. 12, B, C), *A. (Tru.) stecki* (App. 2, C, D) and *A. (Tru.) fimbriatus*. The complex cauda with deep cleft appeared separately in *A. (Miu.) perforatus* and in a clade with *A. (Miu.) biscissus* and *A. (Miu.) sinuator* (e.g. *A. (Miu.) biscissus*, App. 14, C-F; *A. (Miu.) sinuator*, App. 15, C, D). The rudimentary hindbody with pygal lobes equipped with membranous sub-petiolar cavity under the petiole showed up in *A. (Mic.) albator* (App. 16, E-G) and *A. (Mic.) crassicaudatus* (App. 17, G-H) whose closest relatives were *A. (Miu.) biscissus* and *A. (Miu.) sinuator*. The shape of the hindbody in

females was invariant (see e.g. *A. (Tru.) fontinalis* App. 1, A; *A. (Meg.) globator* App. 7, B).

There were several characters associated with the male genital area and hindbody that seem to evolve together: well-developed sclerotized petiole with central piece, hyaline appendage, well-developed anterior dorsal humps and pygal lobes (see Figs. 4.5.3, 4.5.5, 4.5.6, 4.5.8, 4.5.12). The pattern of evolution of these structures in males is congruent with the pattern observed in the evolution of pigmented patches on the valves of the genital opening in females (Fig. 4.5.13). The petiole appears to have arisen in the ancestral clade of *Arrenurus* and was subsequently lost sevaral times (Fig. 4.5.11). In males of species from the clade with *Arrenurus* s.str. appeared a well developed sclerotized petiole with a central piece and an associated hyaline appendage (see e.g. *A. bicuspidator*, App. 18, E; Figs. 4.5.3, 4.5.4, 4.5.5, 4.5.6). However, there was a group of *Arrenurus* s.str. (*A. planus*, *A. pustulator* (App. 27, E, F), *A. maryellenae*, *A. magnicaudatus* (App. 28, C, D)) inside of this clade that have a petiole without central piece and hyaline appendage (Figs. 4.5.5, 4.5.6). The intromittent organ without central piece and hyaline appendage showed up also in *A. (Mic.) fimbriatus*, and in a clade consisted of *A. (Mic.) albator* and *A. (Mic.) crassicaudatus* (see e.g. *A. crassicaudatus*, App. 17, H; Figs. 4.5.5, 4.5.6). Furthermore, a small and partly membranous petiole is present in *A. (Miu.) biscissus* and *A. (Miu.) sinuator* (App. 14, I; App. 15, E, respectively; Figs. 4.5.3, 4.5.4). The petiole is rudimentary or absent in species from the subgenera *Megaluracarus* and *Truncaturus* (see e.g. *A. (Tru.) stecki*, App. 2, D; *A. (Meg.) globator*, App. 7, H; Figs. 4.5.3, 4.5.4, 4.5.5, 4.5.11). A rudimentary petiole is present also in *Micruracarus* (e.g. *A. (Miu.) inexploratus*, App. 12, B, C). Moreover, the angle of petiole in relation to the main axis of the body was considered (Fig. 4.5.7). The intromittent organ was directed parallel to the main axis in all petiolate species with the exception of *A. (Arr.) planus* (petiole at angle >180). The anterior dorsal humps of males are present in *Arrenurus* s.str. (except for *A. planus* and *A. pustulator*; see e.g. in *A. neumani*, App. 26, C, D) and in *A. (Mic.) fimbriatus* (see Figs. 4.5.8, 4.5.9). Similarly, the well developed pygal lobes of males were characteristic for *Arrenurus* s.str. (except for *A. planus*; but present i.a. in *A. affinis*, App. 25, A-C; Fig. 4.5.12). Pigmented patches on the valves of the female genital opening were the only external structure involved in reproduction that differentiated females in *Arrenurus*. They appeared predominantly in the clade with *Arrenurus* s.str. (except for *A. planus*; see e.g. *A. bicuspidator*, App. 18, B, Fig. 4.5.13), but also in females of *A. (Meg.) globator* (App. 7, A, B) and (weakly developed) in *A. (Tru.) fontinalis* (App. 1, B) (Fig. 4.5.13).

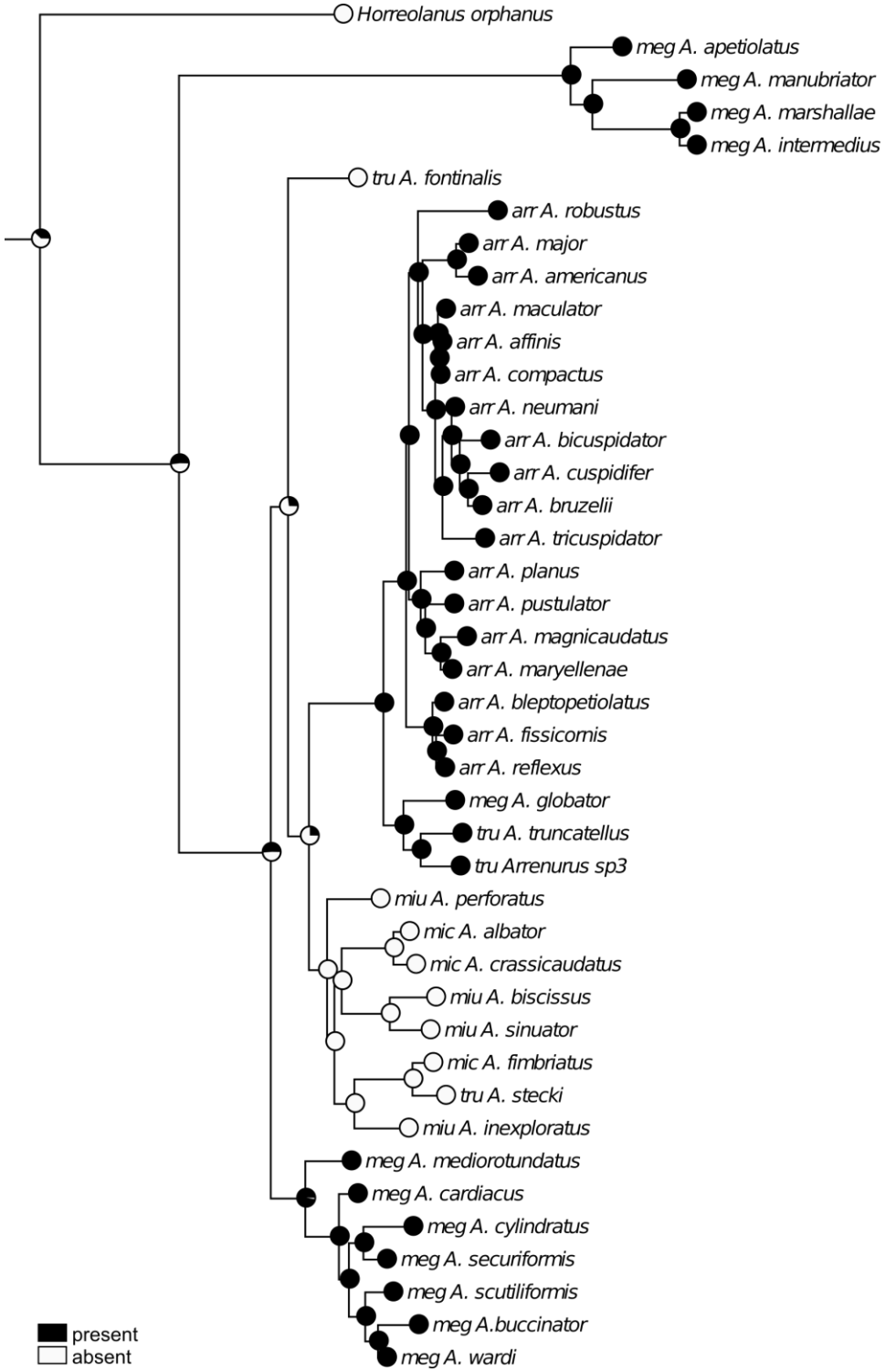

Figure 4.5.1. The evolution of the spur on leg IV in males from 41 species of *Arrenurus* reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model.

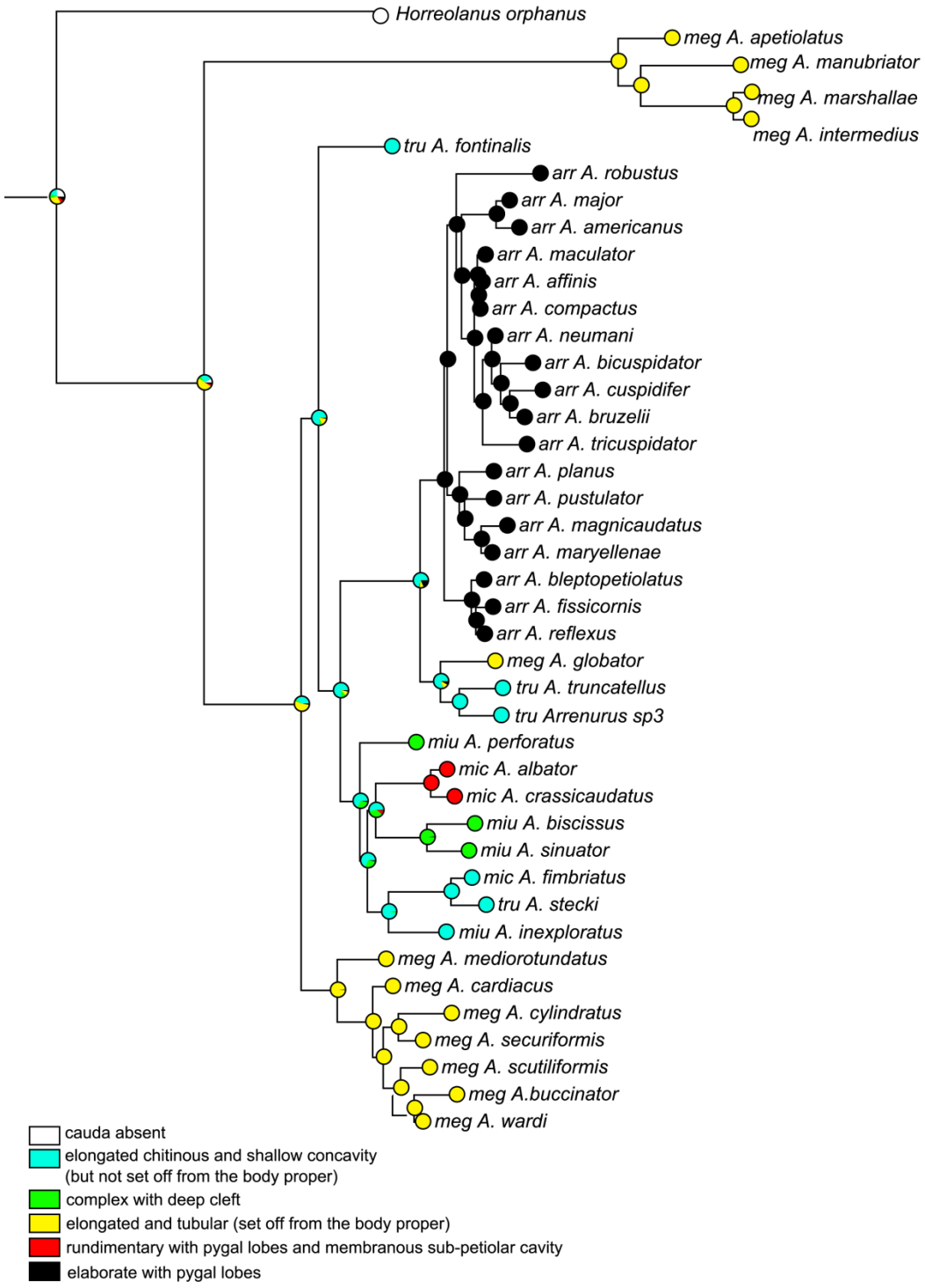

Figure 4.5.2. The evolution of the shape of the cauda in males from 41 species of *Arrenurus* reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model.

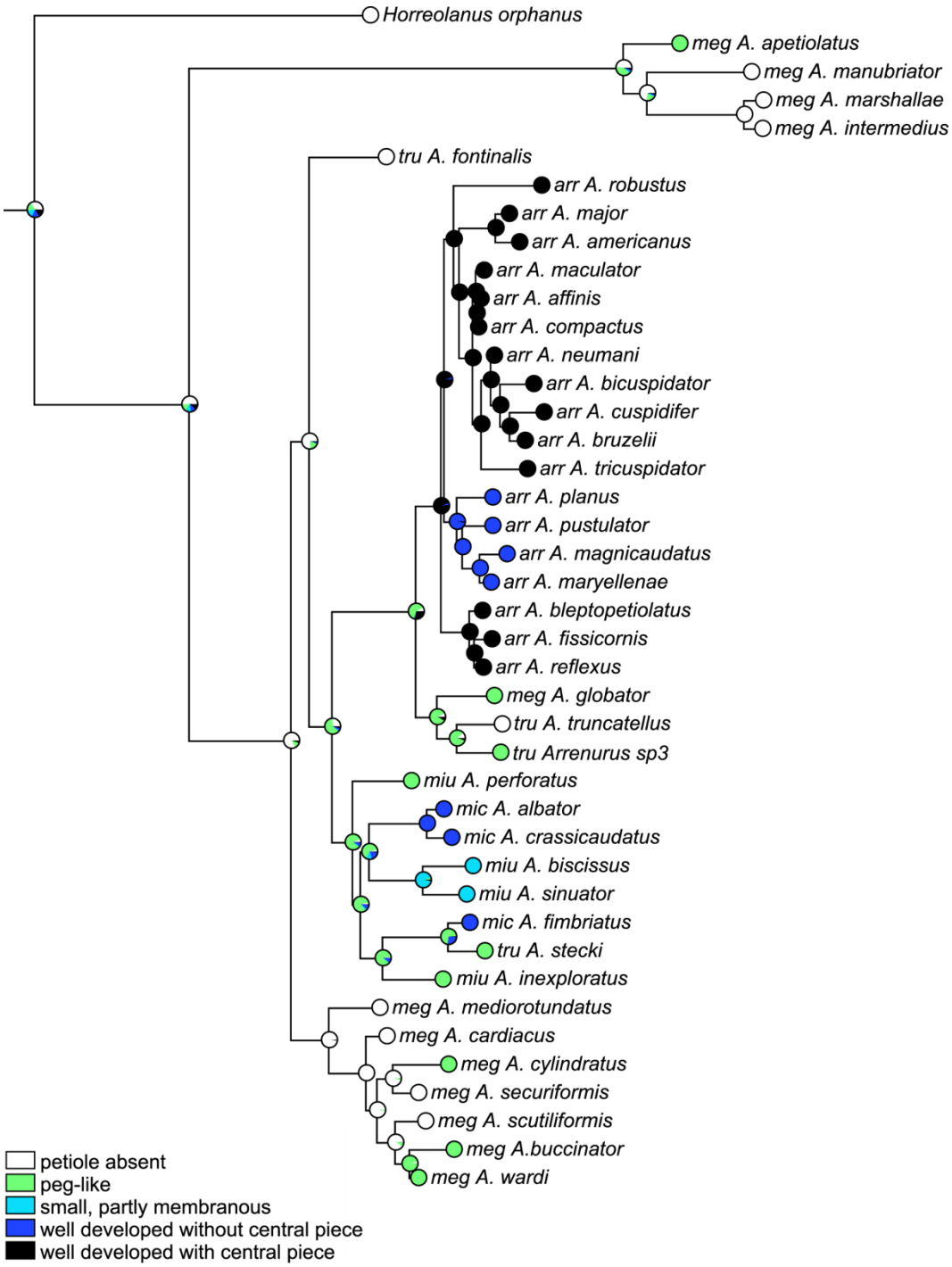

Figure 4.5.3. The evolution of the shape of the petiole (if present) in males from 41 species of *Arrenurus* reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model.

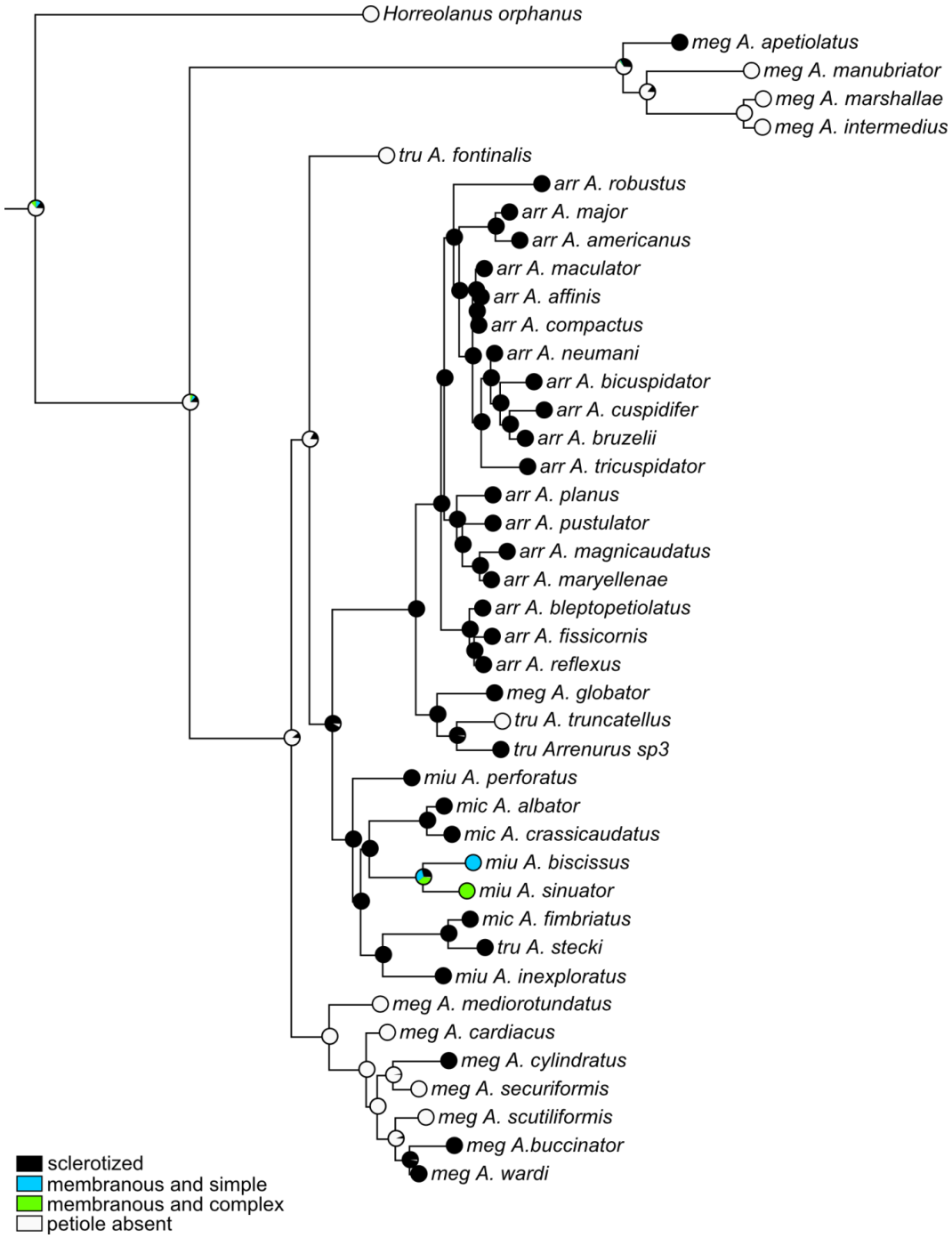

Figure 4.5.4. The evolution of the texture of the petiole in males from 41 species of *Arrenurus* reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model.

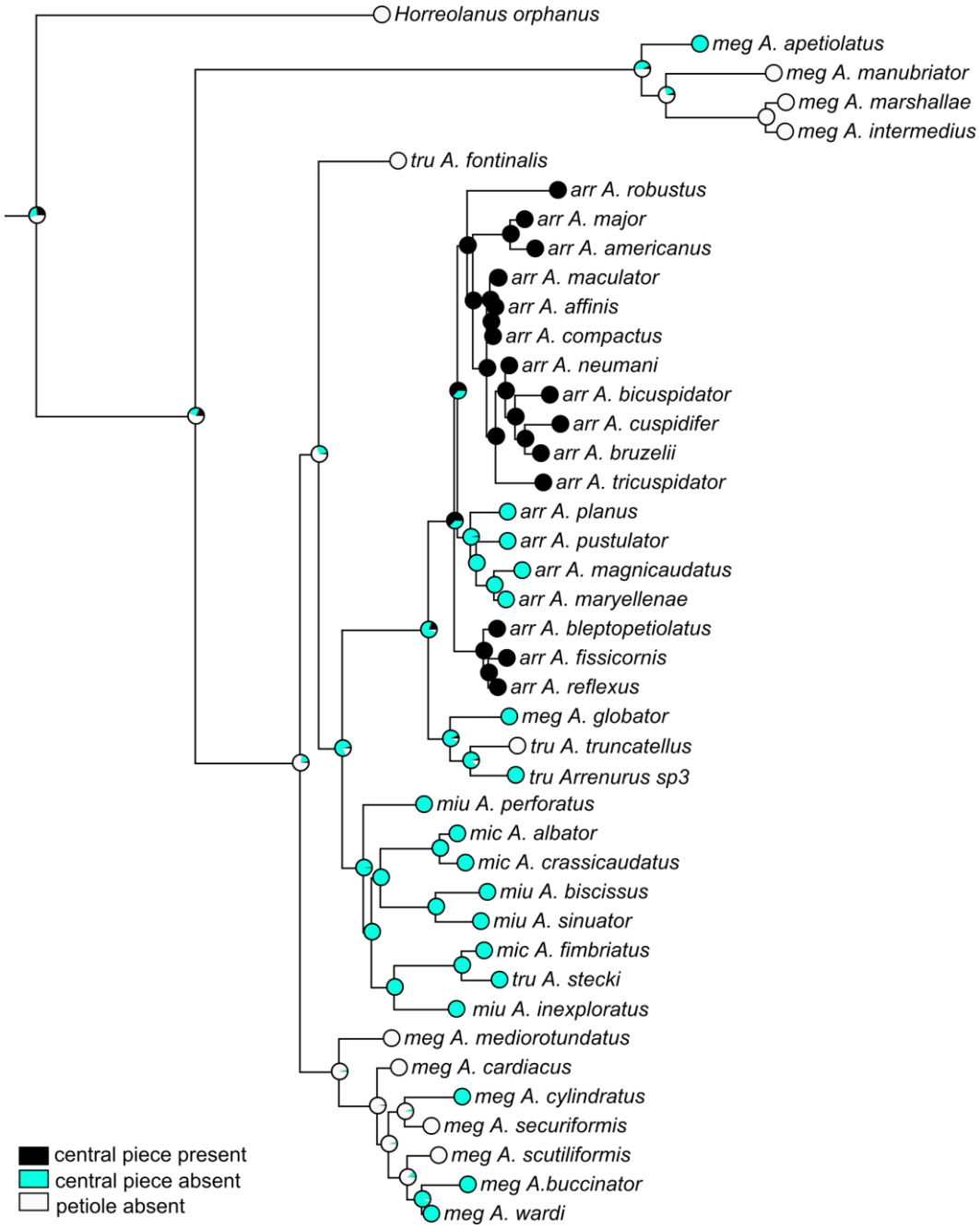

Figure 4.5.5. The evolution of the central piece of the petiole in males from 41 species of *Arrenurus* reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model.

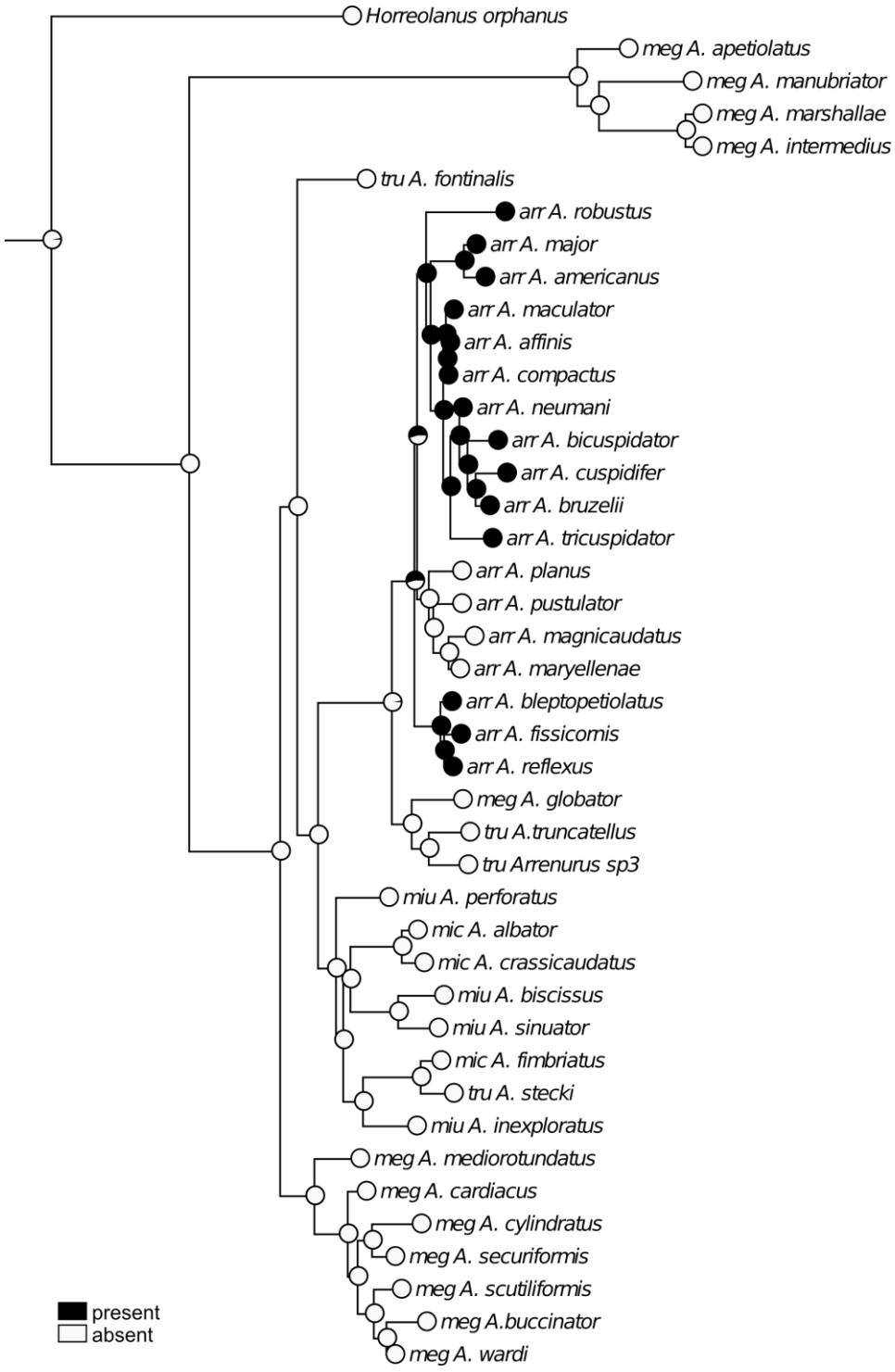

Figure 4.5.6. The evolution of the hyaline appendage in males from 41 species of *Arrenurus* reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model.

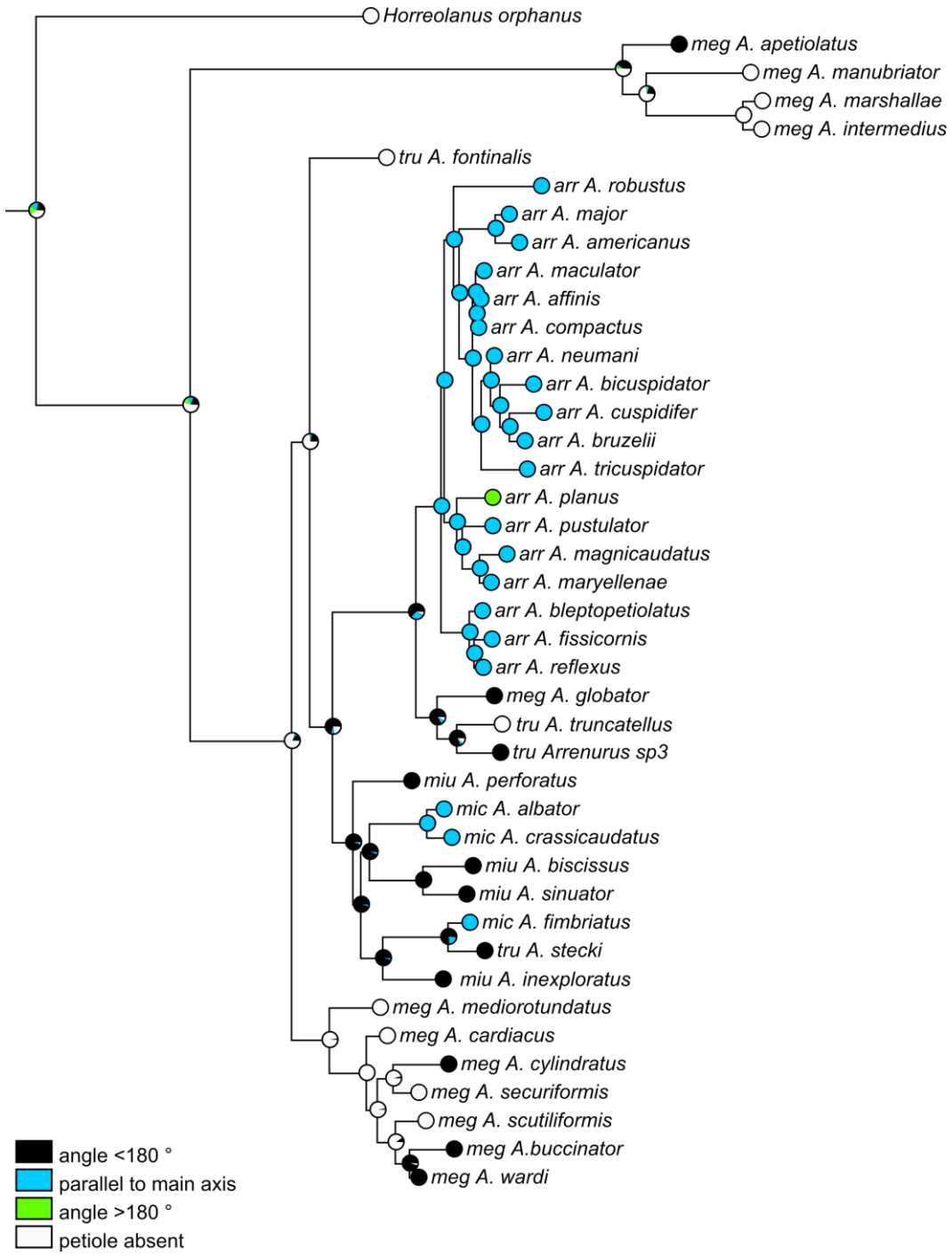

Figure 4.5.7. The evolution of the angle of the petiole (if present) in relation to the main axis of the body in males from 41 species of *Arrenurus* reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model.

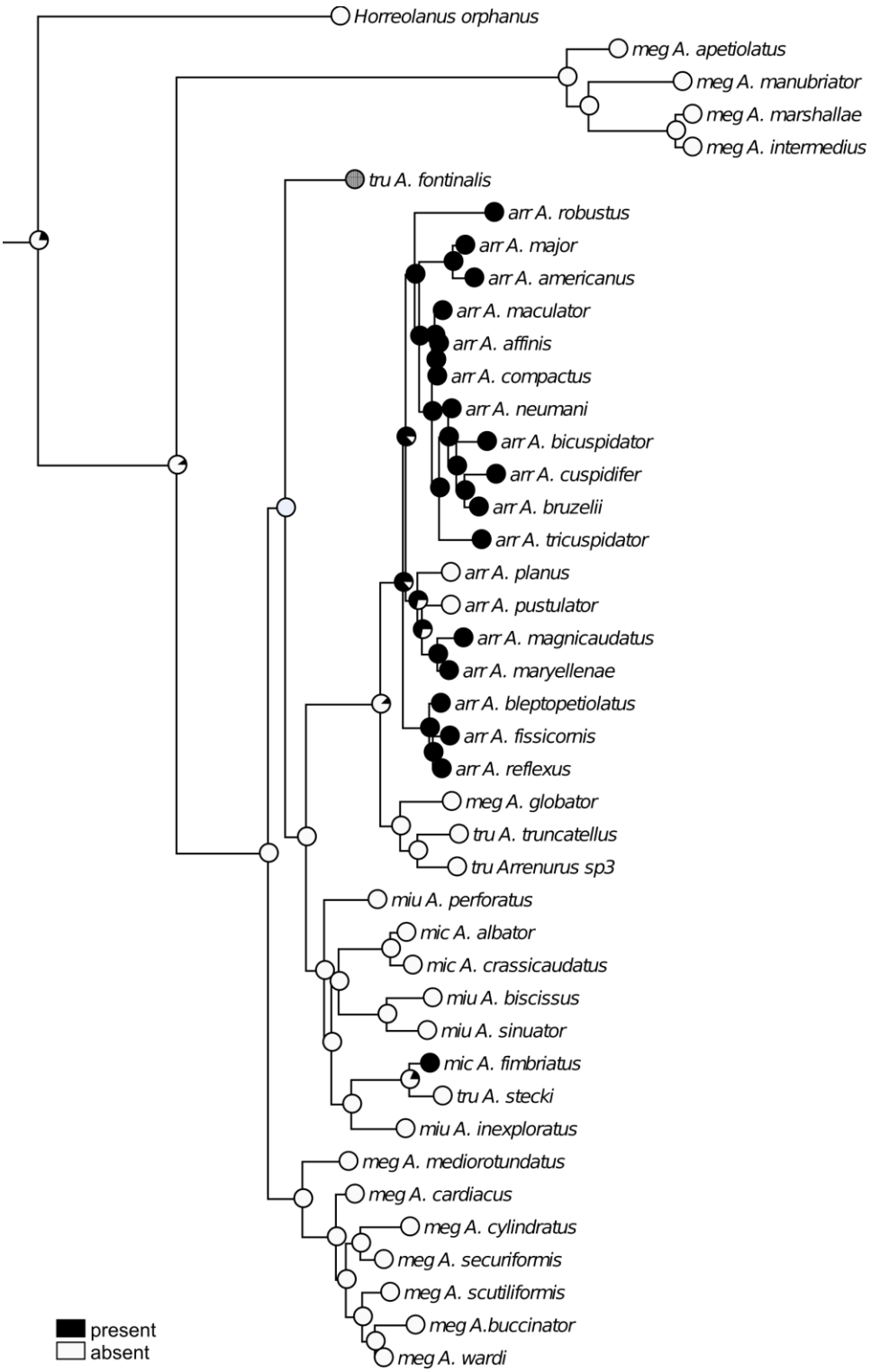

Figure 4.5.8. The evolution of the anterior dorsal humps in males from 41 species of *Arrenurus* reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model. With grey circle unknown character states are indicated.

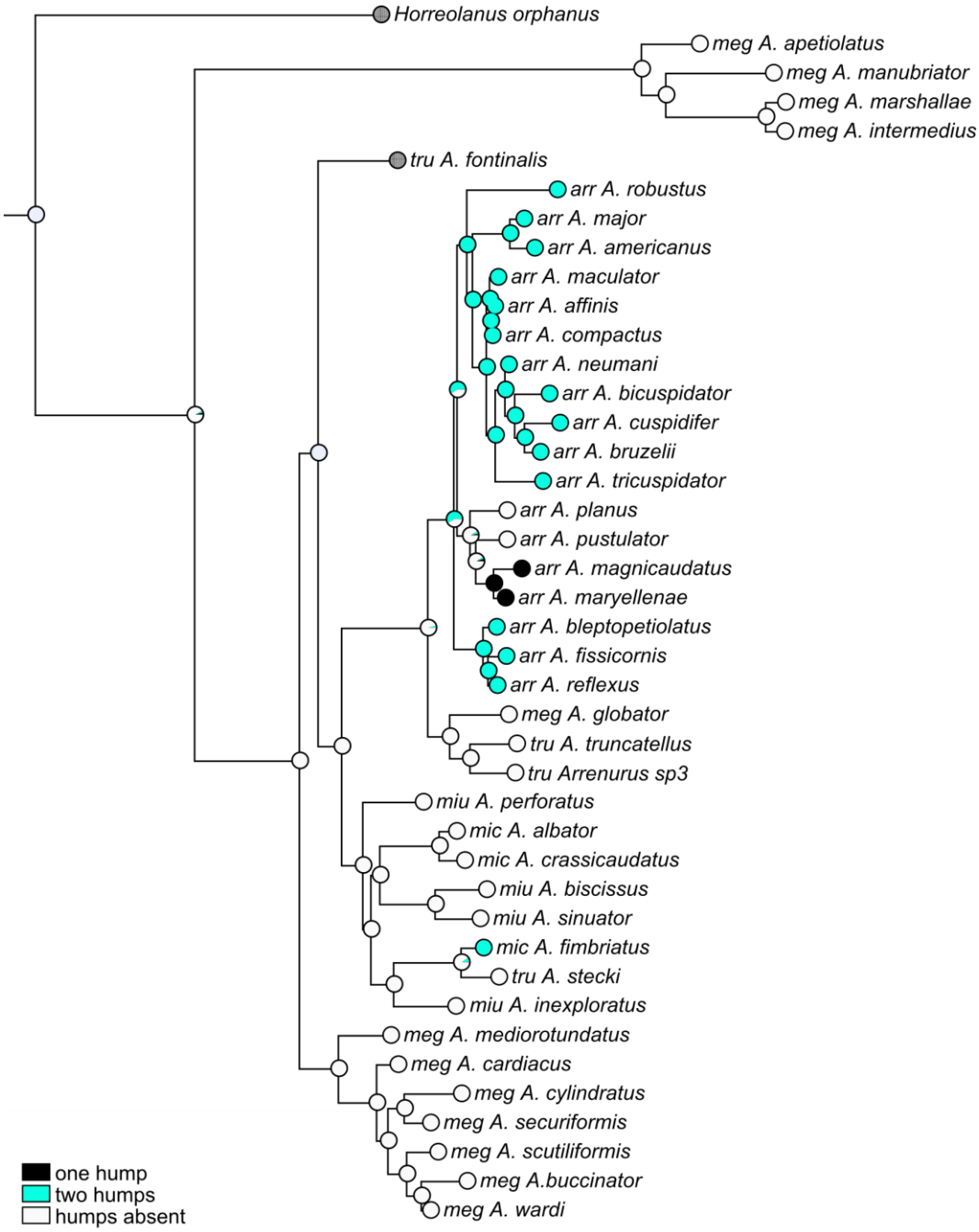

Figure 4.5.9. The evolution of the number of anterior dorsal humps (if present) in males from 41 species of *Arrenurus* reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model. With grey circle unknown character states are indicated.

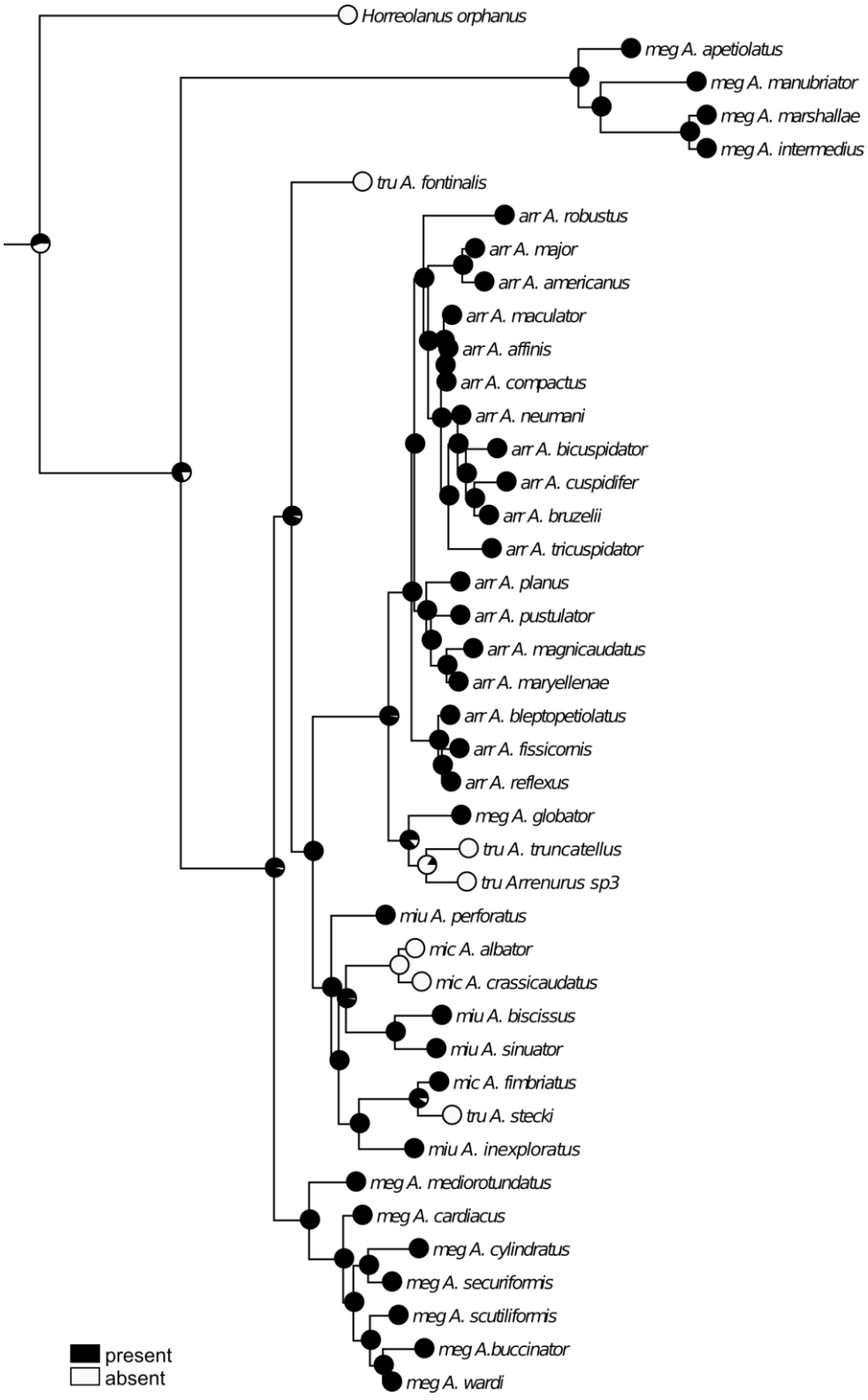

Figure 4.5.10. The evolution of the humps in the posterior part of the cauda in males from 41 species of *Arrenurus* reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model.

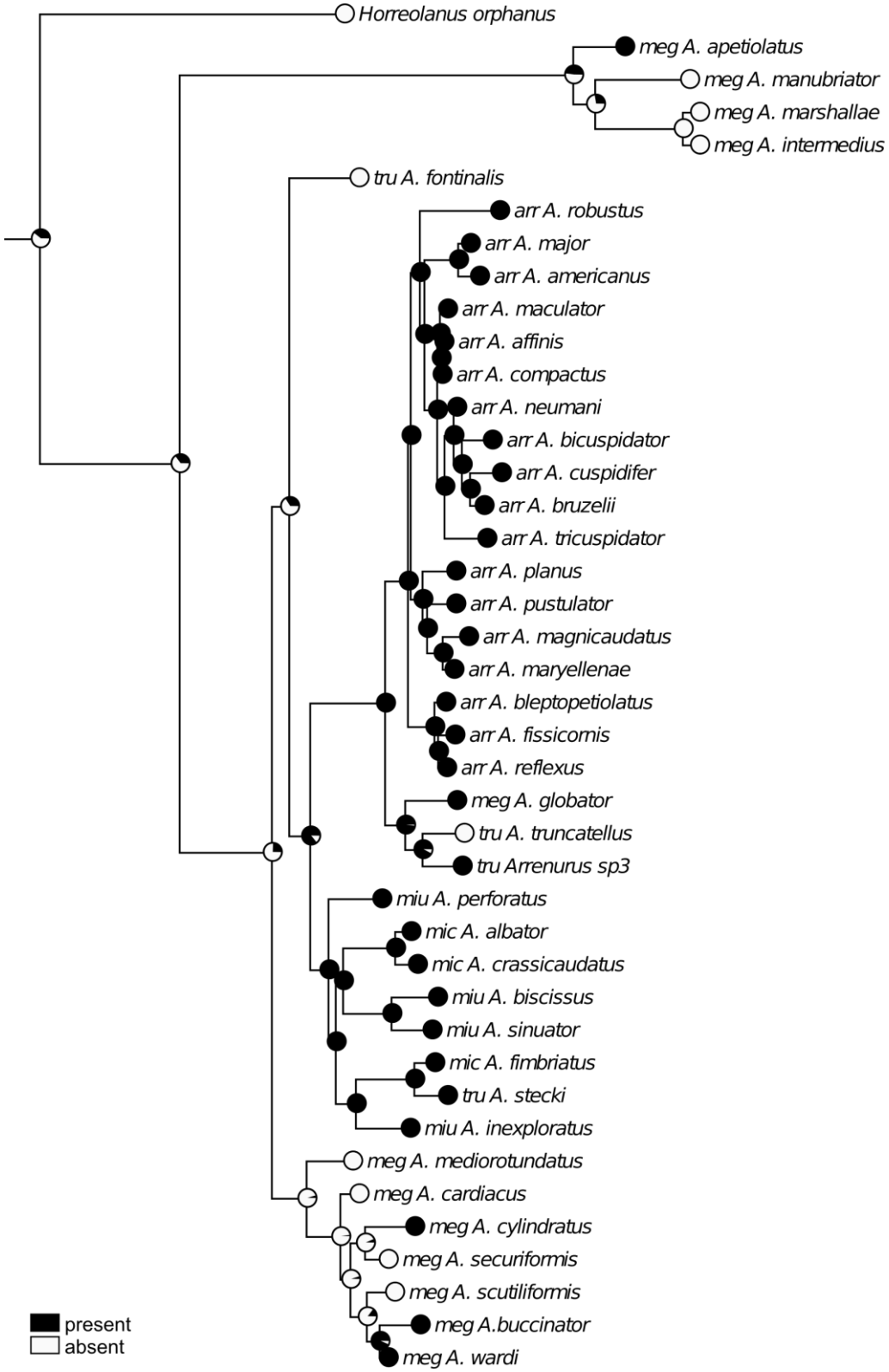

Figure 4.5.11. The evolution of the petiole in males from 41 species of *Arrenurus* reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model.

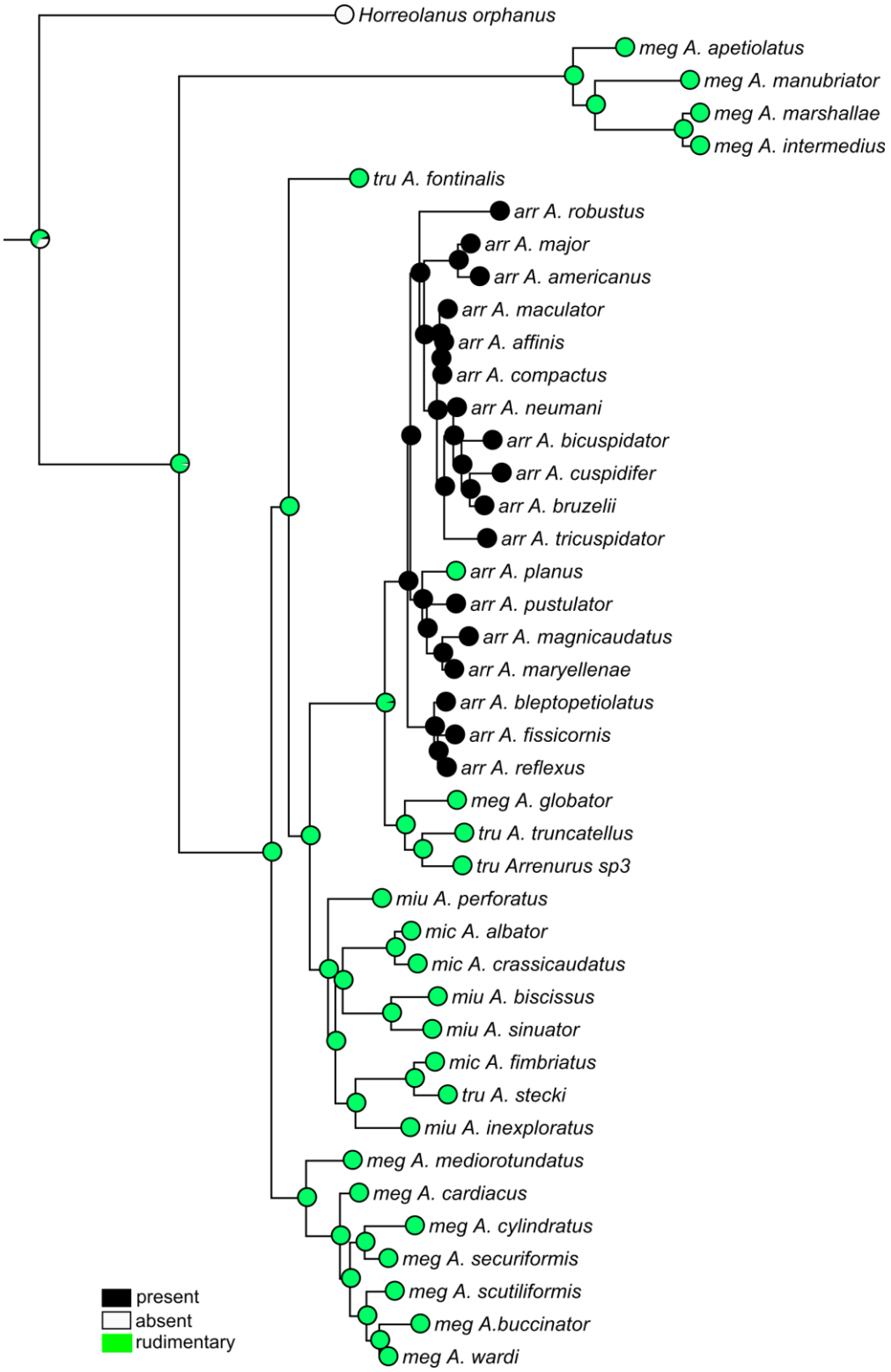

Figure 4.5.12. The evolution of the pygal lobes in males from 41 species of *Arrenurus* reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model.

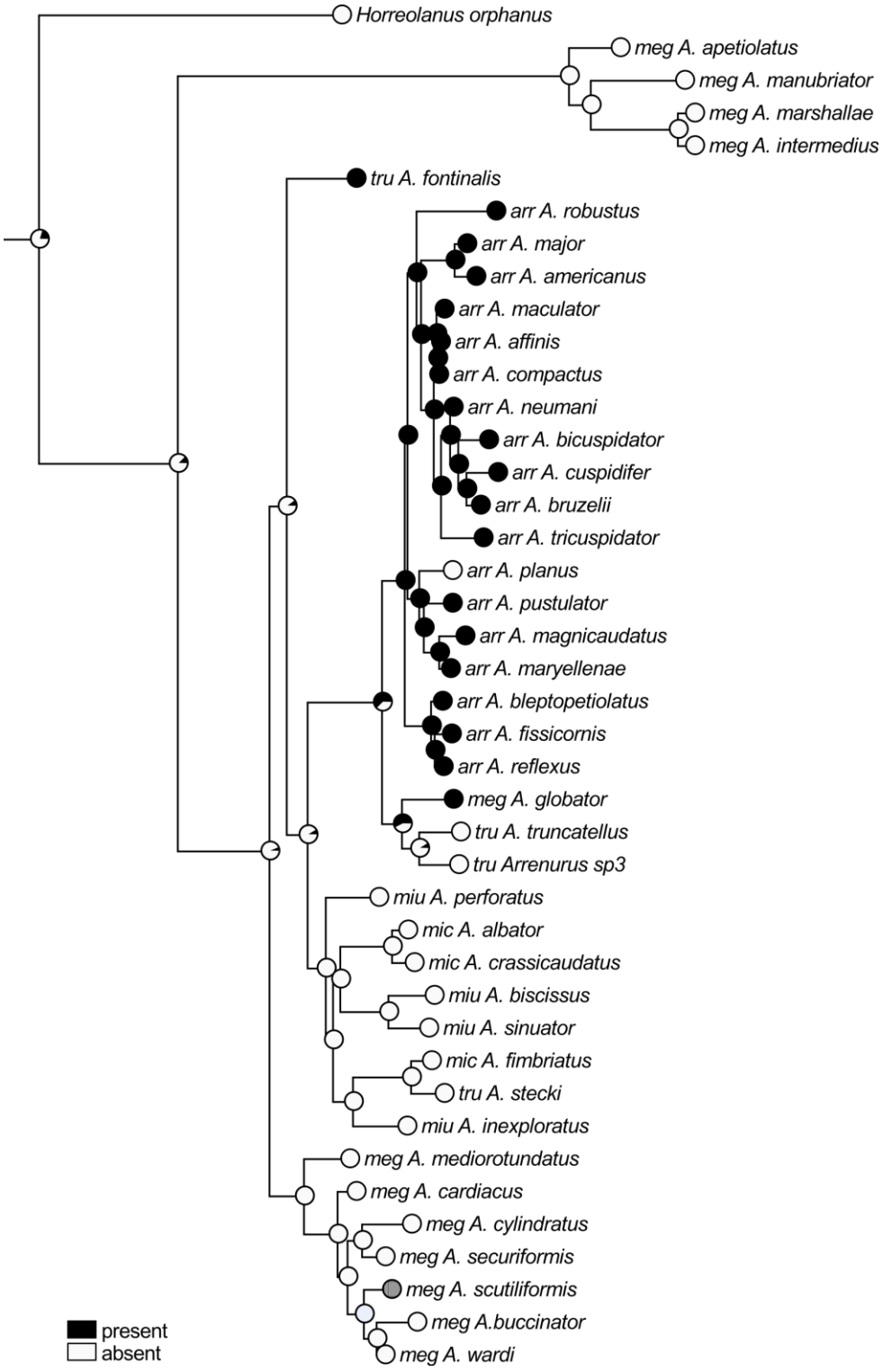

Figure 4.5.13. The evolution of the presence of pigmented patches on the valves of the female genital opening in 41 species of *Arrenurus* reconstructed on the ML tree (COI + D2 28S rDNA) with the Likelihood Markov k-state 1 parameter model. With grey circle unknown character states are indicated.

5. Discussion

5. 1. Inferred phylogeny and species boundaries

Cook (1974) felt that the subgeneric classification of *Arrenurus* is artificial. Jin and Wiles (1997) came to the same conclusion based on a cladistic analysis of the petiole and hindbody. They showed that there are several sets of parallel changes in male cauda and petiole based on analysis of *Arrenurus* s. str., *Megaluracarus*, *Micruracarus* and *Truncaturus* from China. Moreover, Zawal (2008) presented the phylogeny of European *Arrenurus* relying on larval morphology rather than male morphology, and based on it postulated changes in systematics of subgenera *Micruracarus* and *Truncaturus*, and transferring *A. (Arr.) nobilis* from *Arrenurus* s. str. to *Micruracarus*.

The results of molecular phylogeny reconstruction and mapping of morphological characters show that subgeneric categories of the genus *Arrenurus* should be in most part redefined. There are significant inconsistencies between the inferred phylogeny of *Arrenurus* from Europe and North America and the current subgeneric classification. The only monophyletic subgenus proved to be *Arrenurus* s. str., and the subgenera *Megaluracarus*, *Micrarrenurus*, *Micruracarus* and *Truncaturus* are polyphyletic (Fig. 4.1.1). Most notably, there is a subset of species from the New World from the subgenus *Megaluracarus* which form a well supported sister clade to the clade containing all other European and North American *Arrenurus* (Fig. 4.1.1).

The subgenus *Megaluracarus* is represented by two early derivative clades (A, C) and by *A. (Meg.) globator* (clade E, for SEM micrographs see App. 7), which surprisingly appears in a sister clade to *Arrenurus* s. str. (Fig. 4.1.1). Males of *Megaluracarus* have cauda distinctly elongated and set off from the body proper, fourth legs are equipped with spur, and petiole is absent or peg-like (if present) (Fig. 4.5.1, Fig. 4.5.2, Fig. 4.5.3). There are differences in host spectra between the *Megaluracarus* from clades A and C, and *A. (Meg.) globator*. *Arrenurus (Meg.) globator* larvae parasitize Diptera, Odonata and very rarely Coleoptera (Böttger and Martin, 2003), whereas other *Megaluracarus* only parasitize Diptera (Zawal, 2008; Bruce Smith, pers. comm.). Although in the phylogeny based on larval morphology proposed by Zawal (2008) species of *Megaluracarus* in most part appear in different places than in the phylogeny inferred in this study (*A. (Meg.) mediorotundatus* clusters with *Micruracarus* and *Truncaturus*; *A. (Meg.) buccinator*, *A. (Meg.) cylindratus* and *A. (Meg.) securiformis* cluster with *Micrarrenurus* and

Micruracarus), *Arrenurus (Meg.) globator* also appears in a sister clade to *Arrenurus* s. str. (compare Fig. 4.1.1; Zawal, 2008). It seems that male morphological structures associated with reproduction do not reflect phylogenetic relationships of *Megaluracarus*. Since the New World *Megaluracarus* species form a distinct and strongly supported clade, based solely on molecular characters, they should be raised to the subgenus or even genus level. The *Megaluracarus* grouped in the clade C could be treated as a separate subgenus. Moreover, based on molecular characters and possessing spur on hind leg, *A. (Meg.) globator* may be combined with *A. (Truncaturus)* sp3 (for SEM micrographs see App. 3) and *A. (Tru.) truncatellus* (for SEM micrographs see App. 4) that have males with spur on IV-L (in contrast to other studied *Truncaturus*) and raised to subgenus level.

The subgenus *Arrenurus* s. str. is represented by the clade F on the phylogenetic tree (Fig. 4.1.1). Hence, this is the only monophyletic subgenus in examined *Arrenurus*. There are several clusters within clade F which group species represented by similar morphotypes. The clade with most distinct *Arrenurus* s. str. contains *A. planus*, *A. pustulator* (for SEM micrographs see App. 27), *A. magnicaudatus* (for SEM micrographs see App. 28) and *A. maryellenae*. Males of these species have elaborated cauda with pygal lobes and large hump on male's back (exception, *A. planus*) (Fig. 4.5.2, Fig. 4.5.12), and lack central piece of petiole and a hyaline appendage (Fig. 4.5.5, Fig. 4.5.6). In sum, the clade F contains species with males that have spur on IV-L, well developed petiole, and elaborated, well developed pygal lobes (Fig. 4.5.1, Fig. 4.5.3, Fig. 4.5.12), and females that are characterized by the presence of pigmented patches on genital valves (except for *A. planus*, Fig. 4.5.13). Moreover, all *Arrenurus* s. str. parasitize exclusively Odonata (Cook, 1974). The only species which differs in morphology (and also in mating behaviour) from other members of this subgenus is *A. planus*. *Arrenurus planus* resembles in morphology (petiole without central piece, short cauda with weakly developed pygal lobes, Fig. 4.5.3, Fig. 4.5.12) and mating behaviour (sperm transfer via legs, Fig. 4.4.3.2) *A. (Mic.) crassicaudatus* (for SEM micrographs see App. 17), which, in turn, clusters with *Micruracarus* (see clades D, F, Fig. 4.1.1). However, DNA sequences from the nuclear genome should be applied to confirm the phylogenetic position of *A. planus*. It seems that male epigamic traits (and possessing odonates as hosts) used to define the type subgenus do reflect phylogenetic position of *Arrenurus* s. str. Therefore, the *Arrenurus* s. str. should be retained as a separate subgenus.

The *Micrarrenurus* subgenus groups several species primarily assigned to the subgenus *Arrenurus* s. str. which were later transferred by Cassagne-Méjean (1966) to a

new subgenus *Micrarrenurus*. These mites are represented in this study by *A. albator* (for SEM micrographs see App. 16), *A. crassicaudatus* and *A. fimbriatus*. The first two species have males lacking a spur on IV-L (Fig. 4.5.1), but possess a membranous sub-petiolar cavity, very short cauda with pygal lobes (Fig. 4.5.2), and well developed petiole without a central piece (Fig. 4.5.3). In the reconstructed phylogeny *A. albator* and *A. crassicaudatus* form a separate clade, and *A. fimbriatus* groups with *Micruracarus* and *Truncaturus* characterized by elongated hindbody with shallow concavity (Fig. 4.1.1, Fig. 4.5.2). In a phylogeny built based on morphology of larvae, *A. (Mic.) albator* and *A.(Mic.) crassicaudatus* also cluster together (Zawal, 2008). However, *Arrenurus (Mic.) fimbriatus* groups in the analysis of Zawal (2008) with *Megaluracarus*. I suggest that *Micrarrenurus* species that have males characterized by membranous sub-petiolar cavity (*A. albator*, *A. crassicaudatus*) could be grouped with *Micruracarus* with complex cauda with medial cleft. Furthermore, *Arrenurus fimbriatus* could be grouped with *Micruracarus* and *Truncaturus* species that have males possessing slightly elongated, but not set off from the body proper cauda with shallow concavity (and lack spur on IV-L).

The *Micruracarus* species occur in the clade D in Fig. 4.1.1. However, this clade consists of *Micrarrenurus*, *Micruracarus* and *Truncaturus*, and is a mixture of species that lack spur on IV-L (Fig. 4.5.1) and parasitize Diptera (Zawal, 2008; B. P. Smith, pers. comm.). The *Micruracarus* with males that are characterized by elaborated and deep medial cleft and dorsal furrow confined to the dorsum (Fig. 4.5.2), and have short and often partly membranous petiole (Fig. 4.5.3, Fig. 4.5.4) cluster with *Micrarrenurus* equipped with membranous sub-petiolar cavity (*A. (Mic.) albator*, *A.(Mic.) crassicaudatus*). The *Micruracarus* which have males with slightly elongated cauda that is not set off from the body proper and lack spur on IV-L cluster together with morphologically similar *Truncaturus* (*A. stecki*, for SEM micrographs see App. 2) and *Micrarrenurus* (*A. fimbriatus*) (clade D, Fig. 4.1.1, Fig. 4.5.1, Fig. 4.5.2). The results obtained in this study are supported by the analysis conducted based on larval morphology (Zawal, 2008), where species with a medial cleft that lack spur on IV-L (*A. (Miu.) perforatus*, *A. (Miu.) biscissus* (SEM, App. 14) and *A. (Miu.) sinuator* (SEM, App. 15)) cluster together, and *A. (Tru.) stecki* with slightly elongated cauda (no spur on IV-L) groups with morphologically similar *A. (Miu.) inexploratus* (for SEM micrographs see App. 12). Therefore, I suggest that perhaps it would be wise to raise the two above mentioned subclades within clade D (Fig. 4.1.1) to the subgenus level.

The subgenus *Truncaturus* consists of species that appear in three different places on the phylogenetic tree (Fig. 4.1.1). These mites with males characterized by unmodified and slightly elongated cauda and lack of petiole (if present, peg-like) differ in whether the male possesses a spur on IV-L (Fig. 4.5.1, Fig. 4.5.2, Fig. 4.5.3). In analysis of Zawal (2008) *A. (Tru.) truncatellus* (spur on IV-L present, for SEM micrographs see App. 4) occurs in a large clade with *Arrenurus* s. str. (spur on IV-L present) and *A. (Meg.) globator* (spur on IV-L present), and *A. (Tru.) stecki* (spur on IV-L absent) clusters with *Micruracarus* (spur on IV-L absent) which corresponds with results obtained in this study (Fig. 4.1.1). Based on this, I suggest that *Truncaturus* species with males equipped with spur on IV-L may be grouped with *A. (Meg.) globator* in a separate subgenus. The remaining species may be grouped with *Micruracarus* and *Micrarrenurus* (*A. (Tru.) stecki*). Although *A. (Meg.) fontinalis* differs in morphology from *Megaluracarus* species (luck of spur, cauda not set off from the body proper), based solely on molecular data, this species could be combined with *Megaluracarus* (Fig. 4.2.2 for support; for SEM micrographs see App. 1).

In most cases, molecular analyses support the placement of individuals into named species or putative species based on morphology. I found that 35 of 52 named and putative species are clades and show genetic distances to sister clades that meet requirements of a rule of thumb for recognizing new species (Fig. 4.1.1) (Hebert et al., 2004). This rule states that the COI distances between *a priori* identified species are expected to be at least 10 x the intracluster variation. In addition, the network analysis supported 33 of the above mentioned 35 named and putative species (a single haplotype of *A. (Arr.) bruzelii* and *A. (Miu.) setiger* were disconnected, Fig. 4.2.5; for SEM micrographs of *A. bruzelii* see App. 20). There are species groups within the subgenus *Megaluracarus* and *Arrenurus* s. str. in which several species and color variants show genetic differentiation typical for intraspecific variation. The molecular data show that *A. (Meg.) megalurus* and red and blue populations of *A. (Meg.) intermedius* form a single variable species (Fig. 4.1.1, Fig. 4.2.3, Fig. 4.2.5 (Network 3); for SEM micrographs of *A. intermedius* see App. 9). The species in the clade with *A. intermedius* and *A. megalurus* are represented by short branches in phylogenetic analysis which may indicate rapid and divergent evolution (this study and Bruce Smith, pers. inf.). Since clear sexual dimorphism occurs in these species (invariable females vs. males with very elongated cauda and leg spurs) it is possible that sexual selection explains this pattern. Similarly, a low differentiation in mitochondrial marker does not allow us to separate *A. (Arr.) americanus* (red and green forms) from

morphologically distinct *A. (Arr.) mucronatus* (Fig. 4.1.1, Fig. 4.2.3, Fig. 4.2.5 (Network 6)). Moreover, there are two forms of *A. (Meg.) apetiolatus* that differ in body coloration, but do not show an 'interspecific' degree of genetic differentiation (Fig. 4.1.1, for SEM micrographs of *A. apetiolatus* see App. 10). Members of the genus *Arrenurus* often have distinctive color patterns of various shades of yellow, green, blue, red or orange (Viets, 1936). These patterns probably have the adaptive value and can be interpreted as camouflage (Smith et al., 2009). The blue *A. (Meg.) apetiolatus* was collected in the slightly greenish waters of Lake Opinicon (Ontario) and the red *A. (Meg.) apetiolatus* in brownish, acidic Hebert's Bog (Ontario). Moreover, the greenish *A. (Meg.) intermedius* came from Lake Opinicon and red *A. (Meg.) intermedius* specimens from the clear and relatively oligotrophic East Pit Lake west of Edmonton, Alberta. I suggests that when other morphological differences are lacking, body coloration alone is not a good character for species delimitation in *Arrenurus*, since these strikingly different color variants were not strongly genetically differentiated; instead, the variation in color patterns is likely population-level adaptation to different habitats. Moreover, I found that the clade containing *A.* (*Meg.*) *manubriator* consists of two subclades represented by populations from distinct regions and habitat types (blue individuals, Ontario; reddish individuals, Texas) (Fig. 4.1.1, for SEM micrographs of *A. manubriator* see App. 8). As I have shown, these populations have originated probably through disruptive selection, because random coalescence (genetic drift) did not prove to be responsible for this pattern. Furthermore, both standing- and running-water populations of *A.(Meg.) manubriator* usually lack parasitic larvae. Populations without larval parasitism diverge over even very short geographical distances when compared to populations that retain parasitic associations (Bohonak et al., 2004). Hence, these populations are presumably at an early stage of differentiation which is also supported by network analysis (for a within species genetic distance see Fig. 4.2.3; Network 4 in Fig. 4.2.4).

Although the two molecular markers applied for species delimitation proved to be in most cases consistent in distinguishing species, I identified very low differences in mitochondrial sequences in contrast to the pattern observed in nuclear sequences among some morphologically defined *Arrenurus* s. str. - *A. affinis*, *A. bicuspidator*, *A. compactus*, *A. cuspidator* and *A. neumani*. (Fig. 4.2.1, Fig. 4.2.2; for genetic distances see Fig. 4.2.3; for SEM micrographs see App. 18, App. 22, App. 23, App. 25, App. 26). The above mentioned species inhabit acidic water bodies in peatlands and often occur in sympatry (Cichocka, 1998). Moreover, they are reported from different geographical locations

(Poland, Austria, Germany, the Netherlands). Therefore, I assume that the apparent cyto-nuclear discordance in this species group is the result of past and probably ongoing introgression of mitochondrial DNA. Nevertheless, other factors may cause a low diversification in mitochondrial marker, such as genome rearrangements or influence of endosymbionts (e.g. *Wolbachia*) that can function as sex ratio distorters (Bachtrog et. al, 2006). Because of the identified incongruence between information inferred from COI and D2 sequences, molecularly based species delimitation in *Arrenurus* should not be based only on a single molecular marker, but ideally on representatives of mitochondrial and nuclear genome. In sum, understanding of species boundaries in *Arrenurus* should rely on careful interpretation of male morphology (including morphology of palps, also in females), barcode sequences, and life-history traits.

5. 2. Species recognition and reproductive isolation

Pheromone communication is common among various mite taxa. Sonenshine (1985) mentions chemical communication in spider mites, astigmatid mites and ticks. There are also observations which may indicate the use of sex pheromones in terrestrial and aquatic parasitengonine mites (e.g. Witte 1984, Proctor, 1992b). Males of various species of water mites (e.g. *Limnesia undulate* (Müller, 1776), Limnesiidae) begin producing spermatophores when placed in water where conspecific females were maintained (Proctor, 1992b). Furthermore, leg fanning over spermatophores by *Neumania* spp. (Unionicolidae) males was considered to serve as dispersing their pheromones (Proctor 1991, 1992a). Communication via sex pheromones among *Arrenurus* species was examined by Smith and Hagman (2002), and Smith and Florentino (2004). Smith and Hagman (2002) showed that extraction of a male-attractant stimulus of *A. (Meg.) manubriator* females from water is feasible. They noticed that female-conditioned water elicited behavioural responses in conspecific adult males. In the study of Smith and Florentino (2004), widespread communication by sex pheromones was proved based on responses of males of several species of *Arrenurus*, *Megaluracarus* and *Truncaturus*. In these experiments males of *Arrenurus* reacted to water conditioned by conspecific females (and in a few cases by heterospecific females) with arrestant behavior, fanning of fourth legs and readiness posture, which are normally displayed in the pre-pairing stage of mating (Proctor and Wilkinson, 2001).

In the experiments conducted here, males responded in most cases positively to water conditioned with conspecific females (Fig. 4.3.1.1). Moreover, I noted strong interspecific interactions between males and females from the same, but also from different subgenera (Fig. 4.3.1.1, Fig. 4.3.2.1). These results correspond with observations of North-American *Arrenurus* (Smith and Florentino, 2004). It is likely that in cohesive species groups the same or similar sex pheromones elicit heterospecific male responses. However, though Smith and Florentino (2004) detected cross-attractancy among closely related species, heterospecific responses between the members of different subgenera were not reported.

It seems that long-lasting pheromones are produced by *A. (Meg.) globator* females. The effects of the conspecific cues did not wear off after 24 hours after adding them in to the microaquaria with males. The males of *A. (Meg.) globator* responded strongly with arrestant posture and leg fanning to pure water added in to containers with males on the next day. I suggest that the pheromonal cue is detected by and induces mating readiness in males, and stimuli such as water movements can elicit arrestant posture or leg fanning. I observed that males of *A. (Meg.) globator* often reacted with excitement (by ready position) whenever they detected water vibrations caused even by ostracods when conspecific female was present in the same container. Böttger (1962) and Proctor and Smith (1994) observed similar phenomena in *A. (Meg.) globator* and *A. (Meg.) manubriator*, respectively. Furthermore, the use of primer pheromones is possible at least in certain *Arrenurus*. Since males of *A. (Arr.) bicuspidator* failed to respond to treatment with water conditioned by their own females, a repeat of the test was conducted the next after. In the second day there were noticed positive male responses to conspecific cues, what can be explained by the presence of primer pheromones (Tab. 4.3.1.1). In addition, males of *A. (Arr.) bicuspidator* were indifferent to cues of their own females in the experiment, in which cross-attractancy among *A. (Arr.) bicuspidator*, *A. (Arr.) neumani*, *A. (Arr.) compactus* and *A. (Arr.) cuspidator* was examined (Tab. 4.3.2.1). The phenomenon of delayed response between stimulus and final physiological or behavioral result has been observed in some insects (Howard and Blomquist, 2005). Nevertheless, more replications of the treatment with responses of *A. (Arr.) bicuspidator* to conspecific cues should be conducted before one can make conclusions about the presence of primer pheromones.

Species of *Arrenurus* usually inhabit small standing-water bodies rich in plants, and it is common for many species to occur in sympatry (Mitchell, 1964). Taking into account evidence for cross-attraction among different species of *Arrenurus* in laboratory conditions

(Smith and Florentino, 2004), hybridization is potentially possible among closely related forms. The species used in the experiments conducted in this study frequently share the same water bodies and microhabitats within them (Smit and Van der Hammen, 2000; Więcek et al., in prep.). Therefore, the cross-attractancy between closely related *A. (Arr.) tricuspidator* (for SEM micrographs see App. 19) and *A. (Arr.) bicuspidator* (Fig. 4.3.1.1), and among *A. (Arr.) bicuspidator*, *A. (Arr.) compactus*, *A. (Arr.) cuspidator* and *A. (Arr.) neumani* (Fig. 4.3.2.1) observed in this study could result in hybridization. Sexual selection hypothesis for hybridization explains the occurrence of hybrids with mitochondrial genome of the 'mother species' (Wirtz, 1999). It is well known that the sex that invests more in mating (most often females) is also the most choosy (Trivers, 1972). In turn, costs of reproduction are relatively low for the members of the less investing sex (usually males), thus they tend to mate more frequently than females. When conspecific stimuli are lacking, females can respond to stimuli from individuals of the other species which is more abundant in a given habitat. Individuals of a species that just immigrated to a particular water reservoir are assumed to search for mates. In such situations allospecific males will be rejected by females of the common species (the resident species) which already inhabits the pond. Nevertheless, females of the rare species (the immigrant species) that fail to find a conspecific mate become less discriminating over time and start to mate with males of the more common species. In result, members of the immigrant species produce hybrid offspring with maternally inherited mitochondrial genome. Therefore, the less common from the two parental species should be the 'mother species' (Wirtz, 1999). In the most simple case where one hybridization event took place in the past, only one type of mitochondrial DNA occurs in two or more hybrid species. Simultaneously, nuclear DNA of the immigrant species will be 'diluted' through long-term backcrossing (Wilson and Bernatchez, 1998; Wirtz, 1999). However, it is possible that sexual conflict underlies the historical hybridization events among *A. (Arr.) affinis*, *A. (Arr.) bicuspidator*, *A. (Arr.) compactus*, *A. (Arr.) cuspidator* and *A. (Arr.) neumani*. These species with mito-nuclear discordance are members of the subgenus *Arrenurus* s. str., in which males evolved a well developed sclerotized petiole that is introduced into the female reproductive tract during copulation. In addition, males possess a spur on fourth segment of IV-L and produce sticky secretion which glue female on male's back. These adaptations apparently serve to circumvent female choice and are designed to force females to take up male's sperm. This could lead to interspecific forced copulations, and finally to reciprocal hybridization.

5. 3. Evolution of mating behaviour and genitalia

The phylogenetic relationships among analyzed *Arrenurus* species indicate that a subset of behaviours displayed by apetiolate *Megaluracarus* with elongated cauda and a spur on IV-L appear to be ancestral (clades A, C, Fig.4.1.1). *Arrenurus* s. str. species whose males are equipped with well developed petiole with central piece, and share a subset of mating behaviours, seem to appear in a more recently evolved clade (clade F, Fig. 4.1.1). There are similarities in displayed behaviour among species from the same clades, but in a few cases there has apparently been convergent evolution of morphology or behaviour associated with mating. The behaviour of *Arrenurus* species is presented in a defined phylogenetic context for the first time.

5. 3. 1. Behavioural events

Behaviour of sperm transfer of *A. (Meg.) globator* resembles that of *A. (Meg.) manubriator* despite the fact that both species do not group in a monophyletic clade (Fig. 4.1.1). However, males of *A. globator* and of *A. manubriator* represent the same morphotype: IV-L are equipped with spur, hind body is elongated and tubular, and petiole is reduced (Fig. 4.5.1, Fig. 4.5.2, Fig. 4.5.3; for SEM micrographs see App. 7, App. 8). Males of both species display readiness posture and present their hind back to female in pre-pairing stage of courtship (Fig. 4.4.3.3, see also Lundblad, 1929; Proctor and Smith, 1994). Moreover, I have noticed that males of *A. globator* touch females with their first and second legs in the first stages of mating (Fig. 4.4.3.4). Proctor and Smith (1994) state that this behaviour is exercised by females of *A. manubriator* that touch males with palps and forelegs prior to mounting. Baker (1996) found chemosensory sensilla on the palpi, tarsi and tibiae of I and II L in *Arrenurus (Miu.) acutus* that may be involved in the perception of chemical cues produced by sexual partners. In addition, he did not find significant differences between males and females in the number, distribution and morphology of sensilla on palpi and legs. Furthermore, in *A. globator* females may take the active part in mounting hind backs of males. This was also suggested for *A. manubriator* females (Proctor and Smith, 1994; however, Proctor and Wilkinson, 2001 stated that this behaviour was misinterpreted). A female may be also grasped by a male with his fourth legs when she passess by him. In some cases, a male was not interested in mating when a female actively climbed his cauda, even though they were both apparently in a good

condition and the male mated with other females shortly thereafter. This could indicate that male mate choice, which is a widespread phenomenon in animals, underlies this behaviour (Edward and Chapman, 2011). Spermatophore deposition and collection are very similar in *A. globator* and *A. manubriator*. Furthermore, males of both species exhibit slow lateral waving, sharply jerk their backs upwards and jerk cauda vigorously side to side (Fig. 4.4.3.5, Fig. 4.4.3.6). Separation is achieved in a similar way in *A. globator* and *A. manubriator*. However, males of the first species can swim vigorously around or use their hind legs to detach the female, and in the second species separation is achieved by violent shaking of male cauda or by grabbing substratum by female (Proctor and Smith, 1994). Once separation is completed, a female of *A. globator* enters a state of motionless rigidity and lies on the well bottom (Fig. 4.4.3.12). Simultaneously, a male walks around a female, touches her with his first and second legs and crooks fourth legs over his back (mate attendance, Fig. 4.4.3.13). In general, females resist these mating attempts, but in one case the same pair mated twice in a row. It is possible that males may try to prevent female remating by engaging in mating after sperm has been transferred as it was observed in *A. manubriator* (Proctor, 2002).

Arrenurus (Tru.) stecki was the only species from the subgenus *Truncaturus* whose full courtship sequence I observed. Mating of *A. (Tru.) rufopyriformis* was described by Proctor and Wilkinson (2001). Interestingly, despite similarities in morphology (simple and slightly elongated hind body, no pygal lobes, reduced petiole) these species appear to represent distantly related clades (Fig. 4.1.1; for SEM micrographs of *A. stecki* see App. 2). *Arrenurus stecki* groups with *Micruracarus* and *Micrarrenurus*, including *A.* (*Mic.*) *crassicaudatus.* In turn, *Arrenurus rufopyriformis* groups with *Megaluracarus* (B. P. Smith, pers. comm.). Mating of both species has several similarities, but sperm transfer behaviour of *A. stecki* seems to be simplified in comparison with *A. rufopyriformis*. Interestingly, males of *A. stecki* do not have spur on IV-L and nevertheless crook hind legs and held them flat over their backs (see also Lundblad, 1929; Fig. 4.4.3.3, Fig. 4.5.1). The ready position occurs also in *A. rufopyriformis*, a species whose fourth legs are equipped with spur (Proctor and Wilkinson, 2001; Fig. 4.4.3.3). I have noted that there are many unsuccessful mating attempts in *A. stecki* in comparison to *A. (Meg.) globator*. Moreover, once attachment is achieved, both sexes of *A. stecki* separate repeatedly. This could be caused by lack of grasping structure on hind legs in males of *A. stecki*. Spermatophore deposition and collection are very similar in *A. stecki* and *A. rufopyriformis* (see also Lundblad, 1929; Proctor and Wilkinson, 2001). Furthermore, males of both species jerk

their cauda up, shift from leg to leg with female on their backs and stroke fourth legs along sides of female's body (stroking, Lundblad, 1929; Fig. 4.4.3.5, Fig. 4.4.3.7). The vigorous jerking of the cauda from side to side displayed by *A. (Meg.) globator* and *A. (Meg.) manubriator* do not occur in examined *Truncaturus* (Fig. 4.4.3.6). Separation appears to be achieved in *A. stecki* and *A. rufopyriformis* in a different manner. Males of *A. stecki* presumably achieve separation by sharp vertical jerking or fast swimming (see Lundblad, 1929), and in *A. rufopyriformis* females grab substrate or twist their body, or males push them up with fourth legs.

The most consistent mating occurs in the monophyletic *Arrenurus* s. str. Males of *A. (Arr.) bicuspidator* (for SEM micrographs see App. 18), *A. (Arr.) tricuspidator* (App. 19), *A.(Arr.) bruzelii* (App. 20), *A. (Arr.) claviger* (App. 21), *A. (Arr.) cuspidator* (App. 23) and *A. (Arr.) maculator* (App. 24) move fourth legs in a rotary motion or held them crooked over his back when displaying position of readiness in the pre-pairing stage (Fig. 4.4.3.3). In these species males present cauda to females and grasp them with spurs on fourth legs. However, there are differences between above mentioned species in behaviours displayed prior to spermatophore deposition. Both sexes of *A. tricuspidator* direct towards their ventral sides and wrestle, but no spermatophore transfer takes place during this behaviour (in contrary to *A. (Mic.) crassicaudatus*). In *A. claviger* males walk or swim with attached female and sharply jerk cauda upwards repeatedly. This behaviour was observed in apetiolate species with simple or elongated cauda - *A. (Tru.) stecki* and *A. (Meg.) globator* (and also in *A. (Meg.) manubriator*, *A. (Tru.) rufopyriformis*; Proctor and Wilkinson, 2001; see Fig. 4.4.3.5). Moreover, I have observed that both males and females can take the active part in climbing on male's cauda in *Arrenurus* s. str. It seems that in most cases males present cauda and actively grasp females using hind legs. This was the case in *A. claviger* and *A. cuspidator* (and *A. sp. nr. reflexus*). In *A. bicuspidator*, *A. bruzelii* and *A. tricuspidator* both sexes can take the active part in achieving mating position. In *A. maculator* a female willingly climbed on male's cauda. Spermatophore deposition and collection are very similar in *A. bicuspidator*, *A. bruzelii*, *A. claviger*, *A. cuspidator*, *A. maculator*, *A. tricuspidator* and *A. valdiviensis* (Böttger, 1965) and in *A. sp. nr. reflexus* (Proctor and Wilkinson, 2001). Males lift their back end (presumably drawing out a spermatophore) and subsequently lean forward, gathering sperm on the petiole. This is accompanied by slight rocking of the male's cauda. However, there are several behavioural events that occur in both *Arrenurus* s. str. and in other more distantly related species. The sideways leaning where a male shifts from leg to leg with female on his back

appears in species equipped with elaborate cauda and well developed petiole (*A. (Arr.) cuspidator*, *A. (Arr.) maculator*), and in species with slightly elongated cauda and reduced petiole (*A. (Tru.) stecki*, *A. (Tru.) rufopyriformis*) (Fig. 4.4.3.7, see also Proctor and Wilkinson, 2001). The vigorous sideways jerking of male's hind back with glued female is displayed by *A. (Arr.) bicuspidator*, *A. (Arr.) bruzelii*, *A. (Arr.) cuspidator*, *A. (Arr.) maculator*, *A. (Arr.) sp. nr. reflexus* (Proctor and Wilkinson, 2001), *A. (Meg.) manubriator* (Proctor and Wilkinson, 2001) and *A. (Arr.) globator* (Fig. 4.4.3.6). Nevertheless, there are several behaviours displayed after spermatophore deposition and sperm translocation that are characteristic for *Arrenurus* s. str. Firstly, long periods of motionlessness occur in mating of all studied *Arrenurus* s. str. (Fig. 4.4.3.9). Böttger (1965) and Proctor and Wilkinson (2001) report long periods of motionlessness for *A. (Arr.) valdiviensis* and *A. (Arr.) sp. nr. reflexus*, respectively. This behaviour in *Arrenurus* s.str. is usually accompanied by vibration of the third legs of the male against the female's sides (Fig. 4.4.3.10). However, the function of long periods of motionlessness and trembling third legs by males in post-transfer stage of mating is not clear.

Arrenurus (Arr.) planus differs in courtship display from the other *Arrenurus* s. str. (see also Proctor and Wilkinson, 2001). This species does not deposit spermatophores on the substratum, but transfers sperm with the use of legs and inserted petiole (Fig. 4.4.3.2). In the pre-pairing stage ready position is not displayed (Fig. 4.4.3.3). Similarly, vertical and side jerking, sideways leaning and trembling third legs throughout mating do not occur (Fig. 4.4.3.5, Fig. 4.4.3.6, Fig. 4.4.3.7, Fig. 4.4.3.10). However, mating of *A. planus* resembles strongly that of *A. (Mic.) crassicaudatus*. The two species do not cluster together (Fig. 4.1.1). *Arrenurus planus* groups with other *Arrenurus* s. str., and *A. (Mic.) crassicaudatus* groups with *Micruracarus*, *Truncaturus* (*A. stecki*) and other *Micrarrenurus* (*A. albator*) (Fig. 4.1.1). *Arrenurus planus* and *A. (Mic.) crassicaudatus* exhibit several similarities in external reproductive morphology (see App. 17 for SEM micrographs of *A. crassicaudatus*). Males of *A. planus* and *A. crassicaudatus* have well developed petiole without central piece and short cauda with rudimentary pygal lobes (Fig. 4.5.3, Fig. 4.5.12). Moreover, there is strong sexual dimorphism in body size in *A. crassicaudatus* and *A. planus* (males are smaller). In the pre-pairing stage of mating, males of *A. crassicaudatus* move their fourth legs in a rotary motion that is accompanied by sudden changes in walking direction. The sudden changes of walking direction were reported also for males of *A. (Tru.) stecki*. Males of *A. crassicaudatus* and *A. planus* wrestle in the pre-pairing stage of mating climbing over and around female's body. In *A.*

crassicaudatus both sexes turn towards ventral sides of their bodies. The male touches the female's venter and gnathosoma with his palpi repeatedly, and she appears to touch his back end with palpi and first legs. The male brushes his venter with his first, second and third legs, and female manipulates her first, second and third legs presumably to transfer sperm into her genital tract. Similar behaviour was observed in *A. planus* where male brushes his venter with forelegs presumably transferring sperm from his genital opening on to petiole, and female appears to push sperm with her fourth legs in to her genital opening (Proctor and Wilkinson, 2001). Mating position in both species is similar. Male is attached under the standing female facing in the opposite direction as her. The female drags the male around, interspersed with periods of motionlessness (Fig. 4.4.3.8, Fig. 4.4.3.9). The details of the use of petiole in *A. crassicaudatus* were not observed. However, it is possible the petiole in males of *Micrarrenurus* and *Micruracarus* is shaped to open the valves of the female genital opening, but not to anchor a male to a female and prolong postcopulatory association (in contrast to *Arrenurus* s. str.). It is also possible that the short and sometimes partly membranous petiole of *Micrarrenurus* and *Micruracarus* functions as a stimulatory organ (see Eberhardt, 1985; for SEM micrographs of the petiole see App. 14-17). In addition, the courtship display of *A. crassicaudatus* seems to resemble courtship of *A. (Miu.) forpicatus* (Lundblad, 1929). In both species male and female vigorously swim around the well and crash repeatedly with ventral sides of their bodies. Lundblad (1929) states that male and female of *A. forpicatus* touch with palpi and legs when being turned towards ventral sides of their bodies. Moreover, similarly to males of *A. crassicaudatus*, males of *A. forpicatus* do not show ready position. However, further observations of mating of *Micrarrenurus* and closely related *Micruracarus* species should be conducted to draw conclusions about mating characteristics.

5. 3. 2. Sexual selection and sexual conflict

Female choice is hypothesized to be the dominant force of selection in *Arrenurus* species that lack a well developed intromittent organ (*Truncaturus*, *Megaluracarus*) (Proctor and Smith, 1994). In these species females are assumed to control sperm uptake since males have no obvious morphological devices to circumvent female choice. In turn, sexual conflict is postulated to be a stronger aspect of sexual selection in species with males that have well developed petioles (*Arrenurus* s. str., petiolate *Micruracarus*) (Proctor and Smith, 1994; Proctor and Wilkinson, 2001). Male intromittent organs have been

recognized to anchor males to females and to function as devices that prolong mating and prevent or delay female remating (Eberhardt, 1985). Males of both apetiolate and petiolate species of *Arrenurus* seem to have adaptations that enable circumvention of female choice in the first stages of mating. Males of studied *Arrenurus* show leg fanning, and in *A. (Arr.) bicuspidator* males also vibrate 4-6 segments of hind legs when displaying ready position. These behaviours may serve in dispersing sex pheromones that attract females. Baker (1996) conducted behavioural test regarding perception of females of *A. (Miu.) acutus* to conspecific males, in which he demonstrated that females can detect males at a short distance. Moreover, males of *Arrenurus* s.str., *A. (Meg.) manubriator*, *Arrenurus (Meg.) globator* and *A. (Tru.) rufopyriformis* have a spur on fourth segment of IV-L which functions as a grasping structure. Females are grasped with fourth legs and glued to male's hind back by an adhesive produced by caudal glands of males. This contact with male's hind body can release in females some enigmatic behaviours. One female of *A. (Meg.) globator* after a contact with male's cauda behaved like she was attracted to the male because she crawled on to his cauda repeatedly, and then attempted to escape after a while. In contrast, unsuccessfully glued females of *A. (Arr.) bicuspidator* which prematurely separated from male's cauda did not want to continue courtship and mount this particular male again. In *A. (Arr.) bicuspidator* and *A. (Arr.) claviger* females that detached as a result of not successful gluing could not keep balance and lied in a state of motionless rigidity on the well bottom. Similarly, females may lie on the well bottom in a state of motionless rigidity after separation of the sexes in the last stage of mating, which is often accompanied by mate attendance behaviour (see Fig. 4.4.3.12, Fig. 4.4.3.13). It is possible that glands on the male cauda produce secretions which can be detected by female (Lundblad, 1929). These secretions may manipulate female's behaviour by attracting them or causing a state of motionless rigidity, or both depending on the dose. However, the state of motionless rigidity may be interpreted as resistance to male's harassment (Arnqvist and Rowe, 2005). This behaviour is displayed after unsuccessful gluing in the pre-pairing stage, and in last stages of mating since separation is achieved. In these situations males try to put their hind back under female, and even touch and move her with forelegs. In a few species of robber flies of the genus *Efferia* females grabbed by males ceases to move, and males lose interest in mating and release the female (Dennis and Lavigne, 1976). Males that are more successful in grasping and gluing during struggling with females are likely to achieve more mating. I observed that females which were unsuccessfully glued by males refused to mate with these particular males. Perhaps there is selection for males that

produce an adhesive of sufficient amount and quality that enables them to overpower females when struggling. This could be an explanation for development of exaggerated male hind body in both species which lack an intromittent organ (*Megaluracarus*), and in petiolate species (*Arrenurus* s. str.). This is supported by studies of anatomy of *Arrenurus*. Lundblad (1929) described enormous glands inside of male's cauda in *A. (Meg.) mediorotundatus* and in *A. (Meg.) globator*.

Duration of post-deposition courtship differs between species in which males are equipped with well developed petiole, and species whose males have rudimentary petiole or entirely lack this structure. It appears unlikely that optimal durations of mating for males and females overlap, therefore, conflicts over mating durations between the sexes are predictable (Arnqvist and Rowe, 2005). Prolonged association following sperm transfer occur in several invertebrate taxa (e.g. Vahed et al., 2014). Radwan and Siva-Jothy (1996) showed that a male of *Rhizoglyphus robini* (Acaridae) increases fertilisation of his eggs by prolonging attachment to a female. The differently modified intromittent organs of males of different invertebrate species have been postulated to attach a male to a female and prolong mating (Eberhard, 1985). Long postcopulatory stage of mating is often hypothesized to function as reducing the likelihood of a female remating with other males (mate guarding) (Proctor and Wilkinson, 2001). During this stage of mating males can affect female's metabolism through sperm displacement, stimulation of female oviposition or decreasing production of pheromones that attract males (Arnqvist and Rowe, 2005). In petiolate *Arrenurus* s. str., the prolonged post-deposition stage is accompanied with long periods of almost complete motionlessness, during which males vigorously tremble their third legs (Fig. 4.4.3.9, Fig. 4.4.3.10). It is possible that males ensure fertilization of eggs with their own sperm by prolonging this stage of mating (Arnqvist and Rowe, 2005). The trembling third legs may help in achieving advantage in sperm competition for instance by affecting sperm transport in the female reproductive tract. Postcopulatory processes in females are of a great importance to the male's reproductive interests (Eberhardt, 1985). Jackson (1980) states that in the salticid spider *Phidippus johnsoni* longer copulations result in higher probability of oviposition and lower probability of remating of females. *Arrenurus* species that lack well developed petiole (*A. (Meg.) manubriator, A. (Meg.) globator, A. (Tru.) stecki;* Fig. 4.5.3) do not show prolonged post-transfer associations (Tab. 4.4.1.1, exception: *A. (Tru.) rufopyroformis*, Proctor and Wilkinson, 2001). Since in these species females are assumed to control sperm uptake, the post-deposition courtship displayed by males may be designed to encourage females to take up sperm into genital

tract (see also Proctor and Smith, 1994). It is also possible that males that lack modified intromittent organ do 'force' females to insert their sperm in to female's genital tract by for instance vigorous and monotonous movements displayed during mating. These males may manipulate female behaviour by putting them in a state of motionless rigidity, and thus reduce female resistance. Although no morphological counteradaptations to male coercion were found, I assume that behavioural resistance of females is common in *Arrenurus* (see also Proctor and Wilkinson, 2001). The apparent unwillingness of females to mate (expressed in struggling with males) was observed in first stages of mating. After mounting male's cauda females of *Arrenurus* are restless and attempt to clasp male's body and flail their legs. This was observed in species from the subgenus *Arrenurus* s. str., *A. (Meg.) globator* and *A. (Tru.) stecki*. Moreover, females attempted to set free from male's hind body throughout mating. Males of both petiolate and apetiolate species exhibit during pairing behaviours which function remains unclear. It is possible that vigorously displayed behaviours - jerking up the cauda, side jerking, tapping female's body with hind legs - are designed to cause a state of motionless rigidity in females what was proposed also for *A. (Meg.) manubriator* (Proctor and Smith, 1994) and *A. (Meg.) globator* (Lundblad, 1929). Clearly, further ethological observations of more species should be conducted to test assumptions about the role of behaviours displayed during mating. In particular studies regarding internal morphology and physiological adaptations of females, and examination of content of male seminal fluids would enable understanding processes that underlie biology of mating in *Arrenurus*.

Sexual selection and sexual conflict promote evolutionary divergence and speciation through initiating and stimulating rapid and divergent evolution of sexually dimorphic characters (Arnqvist et al., 2000; Arnqvist and Rowe, 2005). The coevolutionary processes of the sexes in allopatric populations may be an engine of different adaptations, and thus promote speciation. Sexual conflict may be especially potent in driving evolution of persistence by males and resistance by females because it involves substantial direct costs (Arnqvist and Rowe, 2005; Bergsten and Miller, 2007). Arnqvist et al. (2000) state that postmating sexual conflict is an important engine of speciation in insects. They showed that clades with species experiencing sexual conflict are more species-rich in comparison to clades, in which no conflict over reproductive outcome was observed. If sexual conflict is an engine of evolutionary divergence in *Arrenurus* s. str. then elevated speciation rates should be observed in the clades where sexual conflict is suspected to occur. Indeed, the well defined *Arrenurus* s. str. (Cook, 1974; monophyly confirmed in this study, Fig. 4.1.1),

in which males have morphological (well developed petiole with central piece, exaggerated hind body, grasping structure) and behavioural (prolonging post-transfer stage of mating) adaptations that appear able to circumvent female choice, comprises about 300 species. This subgenus is next to the *Megaluracarus* (about 300 species; polyphyletic in Fig. 4.1.1) the most species-rich subgenus of the genus *Arrenurus* (the genus *Arrenurus*, about 950 species; http://bug.tamu.edu/research/collection/hallan/Acari/_Family/Arrenuridae.txt). In addition, branch length estimates indicate the recent history of the splits in the examined species of *Arrenurus* s. str. (Fig. 4.1.1). In contrast to exaggerated morphological structures (and adaptive behaviours) found in *Arrenurus* s. str., the unmodified male morphotype resembling females is found predominantly in the subgenus *Truncaturus*. This subgenus contains 'only' 54 extant species worldwide that appear to be distantly related (see Fig. 4.1.1). However, the influence of other factors like ecology and geographic distribution of species has to be considered in drawing final conclusions about the main driving force of evolution in the genus *Arrenurus*.

6. Conclusions

- The taxonomic status of 35 of 52 named and putative species was supported based on results obtained from DNA barcodes. Although external reproductive morphology of males (modifications of the hindbody, petiole and fourth legs) proved to be suitable in characterizing species, they do not consistently reflect phylogenetic relationships in the genus *Arrenurus*.

- The analysis of DNA sequences of colour variants of *A. (Arrenurus) americanus*, *A. (Megaluracarus) intermedius*, *A. (Megaluracarus) apetiolatus* and *A. (Megaluracarus) manubriator* show that body coloration itself is not decisive in distinguishing species.

- In morphospecies *A. (Arrenurus) americanus*, *A. (Americanus) mucronatus*, *A. (Megaluracarus) intermedius* and *A. (Megaluracarus) megalurus* were found higher rates of evolution of morphological structures than mitochondrial and nuclear DNA sequences.

- Mitochondrial transfer events are postulated for several morphospecies from the subgenus *Arrenurus* s. str. (*A. affinis*, *A. bicuspidator*, *A. compactus*, *A. cuspidator*, *A. neumani*). Since cross-attraction of pheromones was found in these naturally co-occuring

morphospecies, and forced copulations of heterospecifics are possible because of possessing by males a sclarotisized intromittent organ, past and ongoing hybridization is assumed to occur.

- A subset of the New World *Megaluracarus* species is sister to the remaining European and North American *Arrenurus*. Based on the species examined, the only monophyletic subgenus appears to be *Arrenurus* s. str., whereas subgenera *Megaluracarus*, *Micrarrenurus*, *Micruracarus* and *Truncaturus* are polyphyletic.

- Reconstructed molecular phylogeny of *Arrenurus* confirmed that morphology of the larval stage is a good predictor of phylogenetic relationships.

- Morphological adaptations to mating in males (modifications of cauda, petiole and hind legs) and pigmented patches on female genital valves evolved convergently or were lost in different evolutionary lineages.

- Male morphotype with elongated cauda that is set off from the body proper is ancestral in *Arrenurus*, whereas well developed pygal lobes, petiole (often with central piece) and hyaline appendage associated with male hindbody are derived structures; spur on hind legs appeared in most early derivative clade and was subsequently lost several times.

- Conspecific behavioural responses of males to sex pheromones were found in studied *Arrenurus*. The cues of heterospecific females elicited positive responses in males of *A. (Arrenurus) tricuspidator* and *A. (Micruracarus) biscissus*. In species with incongruence between mitochondrial and nuclear DNA sequences (*A. (Arrenurus) neumani* and *A. (Arrenurus) bicuspidator*) I observed stronger responses of males to heterospecific cues than to conspecific cues.

- In male behavioural responses to pheromonal cues two peaks occurred: the first and strongest peak was explained by responses to conspecific cues. The second and weaker peak reflected responses to sex pheromones of more distantly related species. In turn, the

male reactions among species with postulated mitochondrial transfer events (*A. (Arrenurus) bicuspidator*, *A. (Arrenurus) compactus*, *A. (Arrenurus) cuspidator*, *A. (Arrenurus) neumani*) formed a curve with a peak indicating stronger responses to heterospecific cues than to conspecific cues.

- In *A. (Micrarrenurus) crassicaudatus* an unusual form of sperm transfer (sperm transferred via legs) resembling behaviour found in *A. (Arrenurus) planus* was observed.

- In the first stages of mating males of different species take the active part in attempting to overcome female choice by grasping them and gluing to the hindbody. However, females can also take the active part in climbing on the male hindbody without assistance of males. It was found that males can show resistance to mating attempts of females that climb male hindbody and display fanning of hind legs.

- Species with males that have truncate and umodified hindbody and lack petiole (peg-like, if present, and presumably does not function as an intromittent organ) spend less time in mating than species with males characterized by elongated and set off from the body proper hindbody (petiole present or peg-like), and species with males with elaborated hindbody equipped with humps, pygal lobes, bumps and protrusions (petiole well developed).

- Species with well developed petiole spend significantly more time in post-spermatophore deposition stage of mating than species with males that lack petiole (if present, peg-like and presumably does not function as an intromittent organ). The prolonged postcopulatory associations are accompanied by long periods of almost complete motionlessness and trembling third legs by males that are assumed to facilitate receiving sperm.

- Females are postulated to resist male attempts to repeat already completed copulation (touching females with forelegs, fanning hind legs, showing readiness posture) by displaying a state of motionless rigidity.

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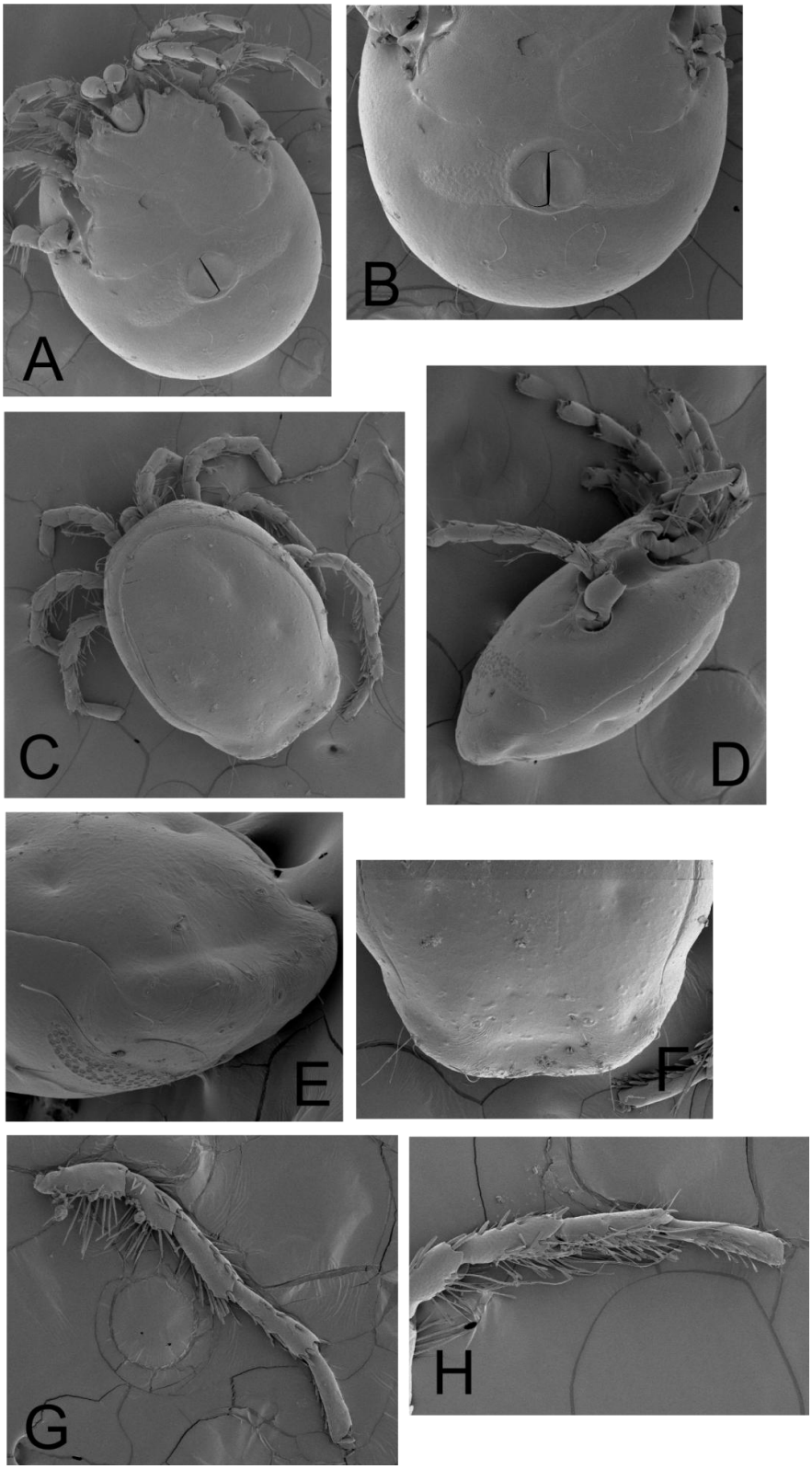
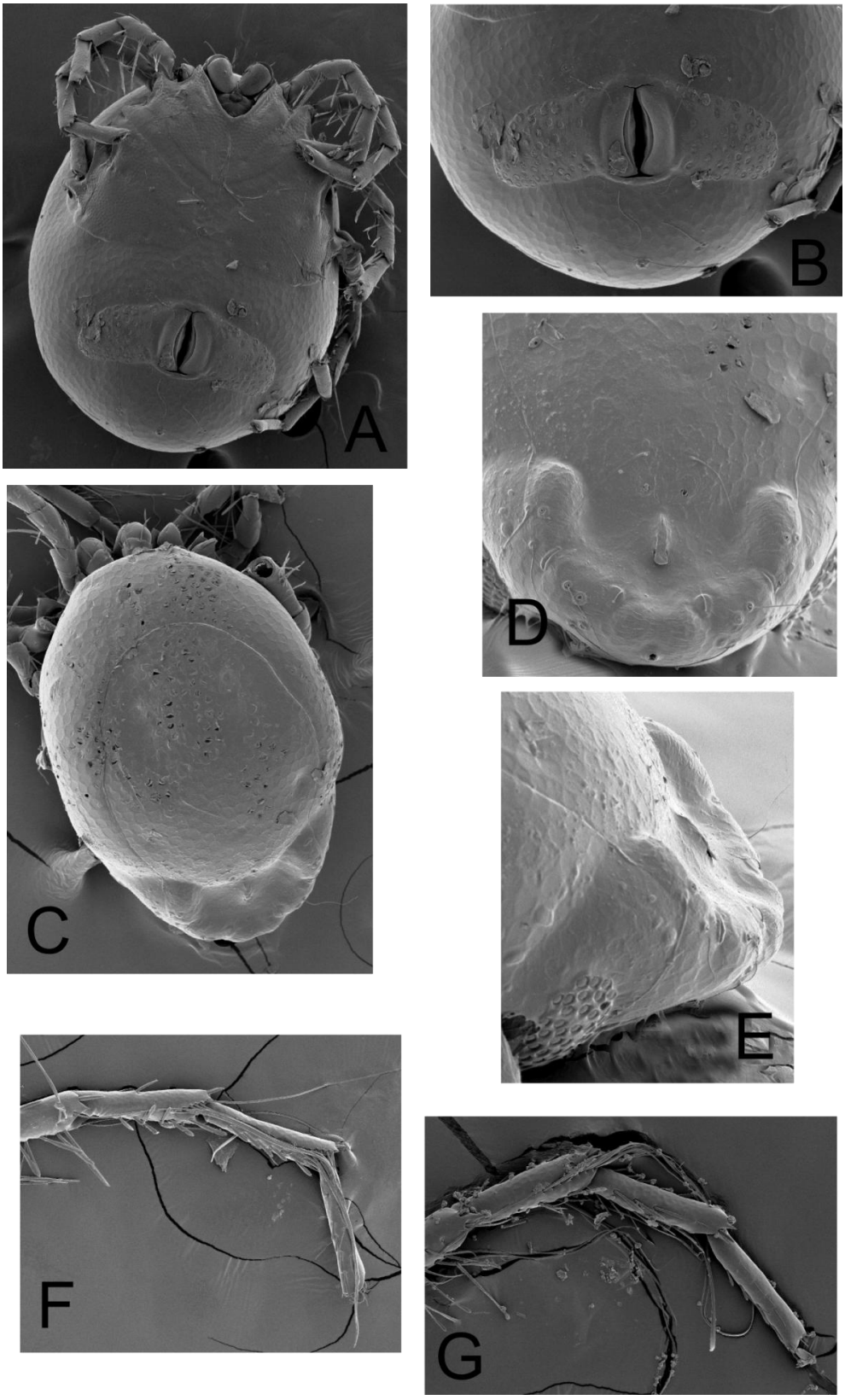
APPENDIX

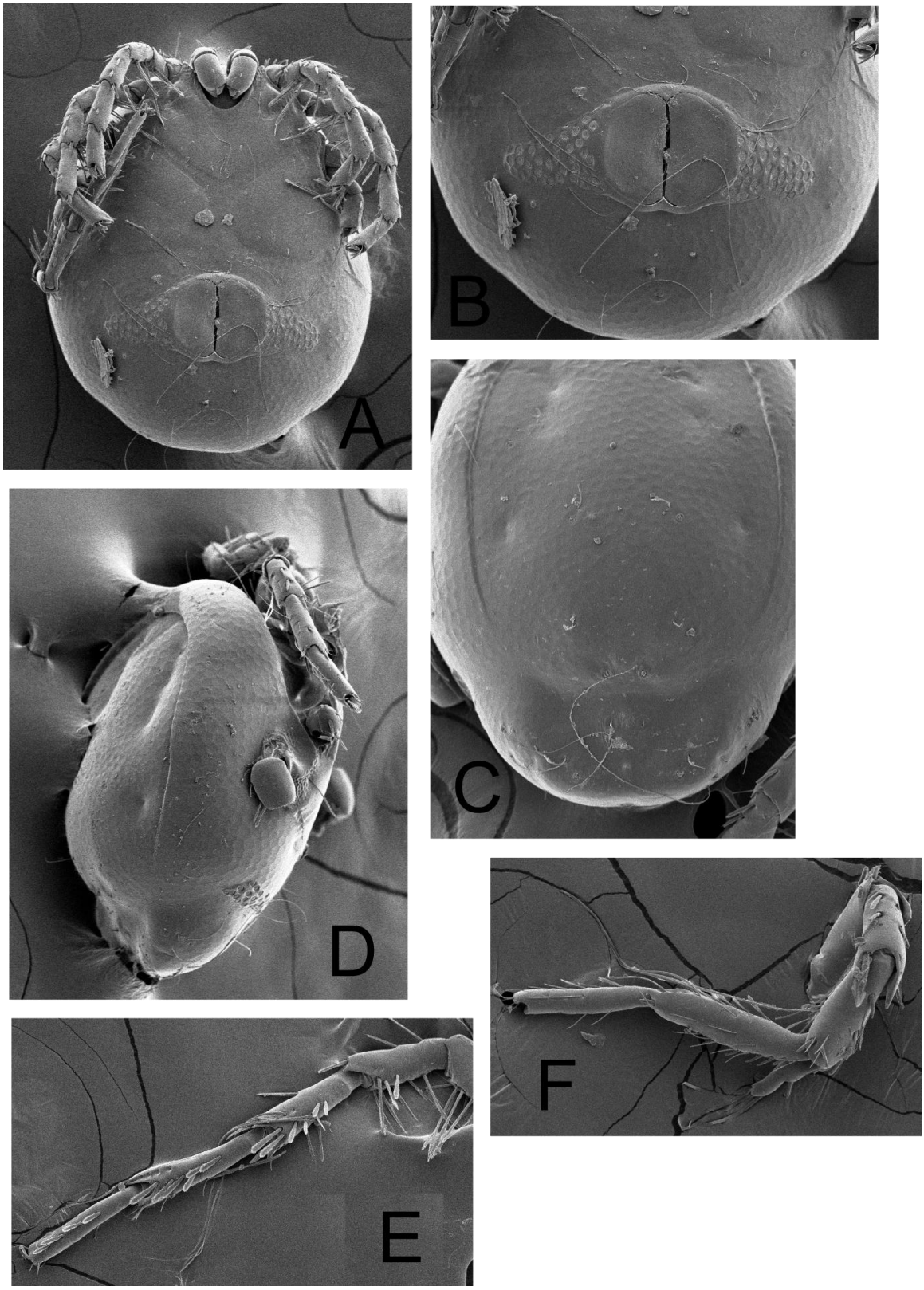
The appendix contains scanning electron micrographs of *Arrenurus* species that were taken with the use of a JEOL field emission scanning electron microscope (SEM) in the Department of Earth and Atmospheric Sciences (University of Alberta), and in the Faculty of Biology (Adam Mickiewicz University).



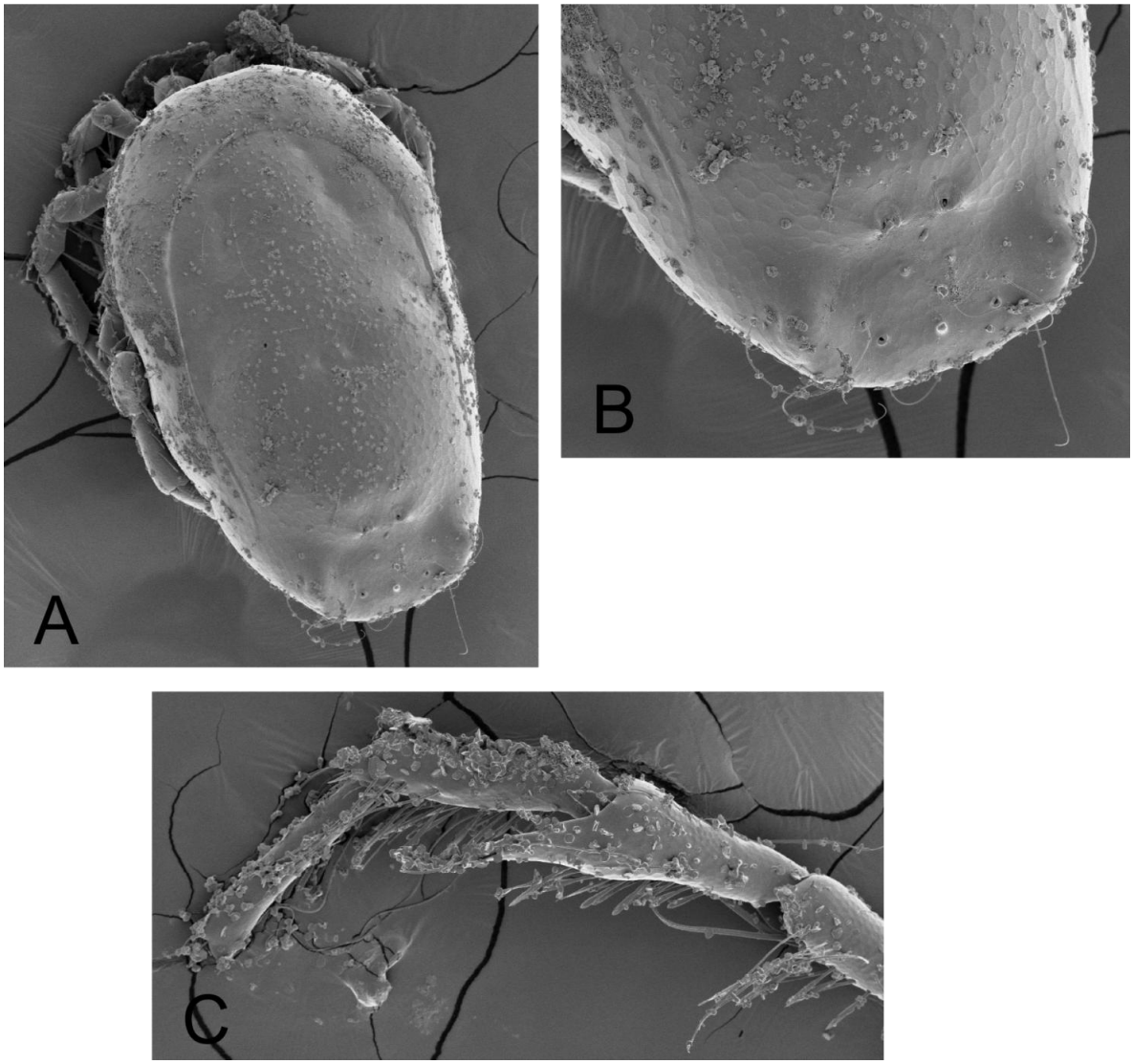
App. 1. Scanning electron micrographs of adult *A. (Tru.) fontinalis*; A. female, ventral view; B. female, ventral view, genital area; C. male, dorsal view; D. male, lateral view; E. male, cauda, dorso-lateral view; F. male, cauda, dorsal view; G. female, IV-L; H. male, IV-L.



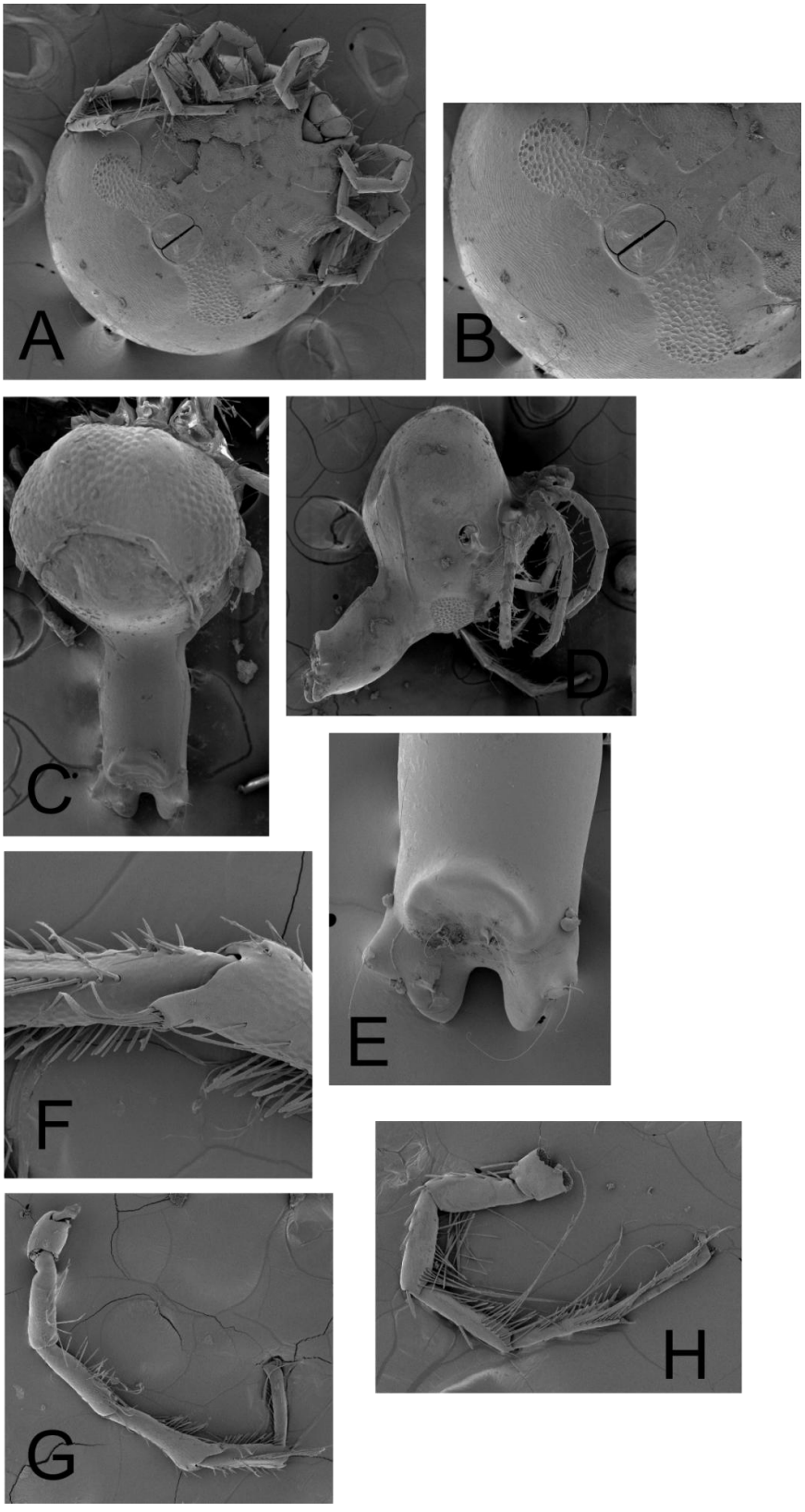
App. 2. Scanning electron micrographs of adult *A. (Tru.) stecki*; A. female, ventral view; B. female, ventral view, genital area; C. male, dorsal view; D. male, cauda, dorsal view; E. male, cauda, dorso-lateral view; F. female, IV-L; G. male, IV-L.



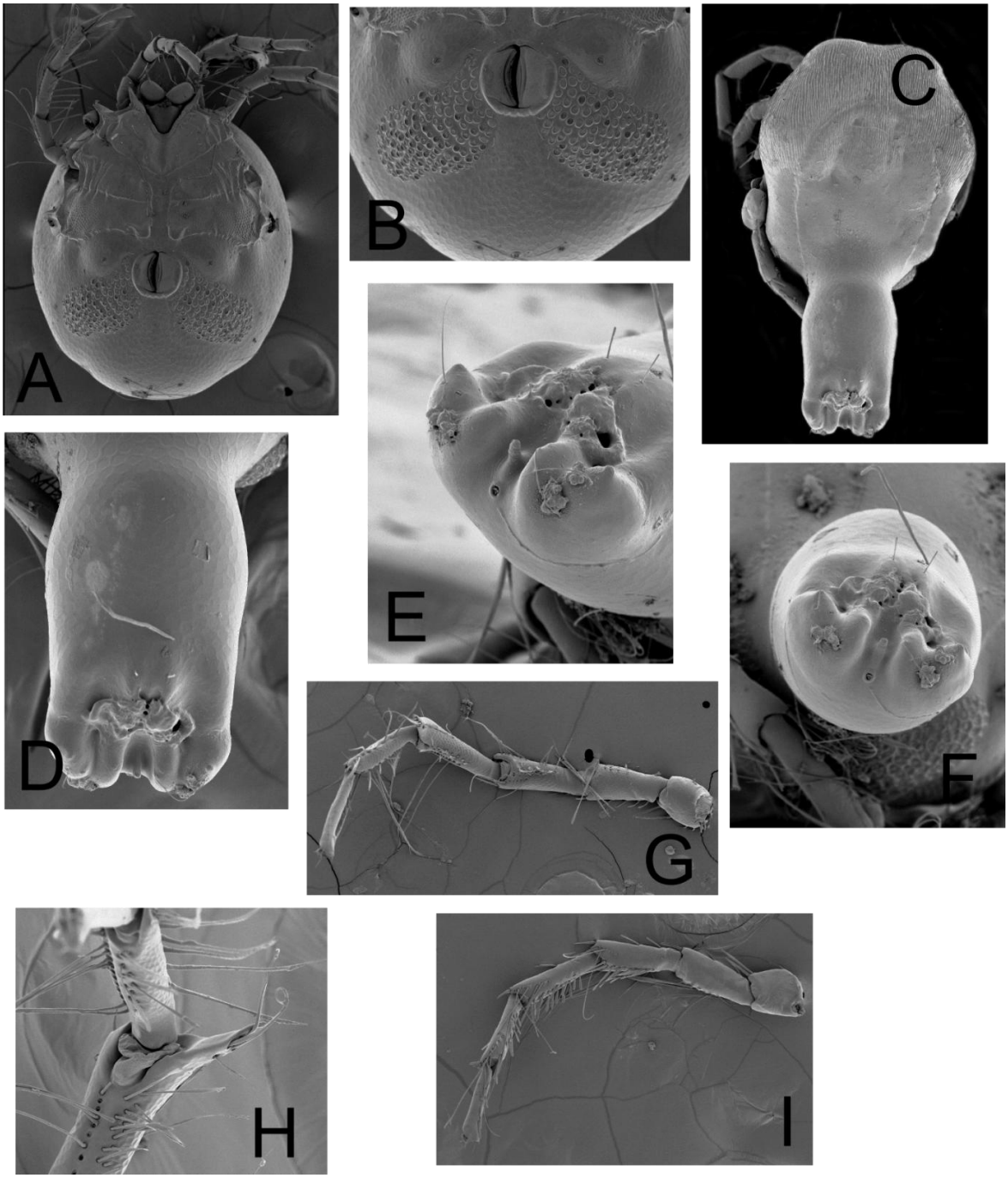
App. 3. Scanning electron micrographs of adult *Arrenurus (Tru.)* sp3; A. female, ventral view; B. female, ventral view, genital area; C. male, dorsal view; D. male, lateral view; E. female, IV-L; F. male, IV-L with spur on fourth segment.



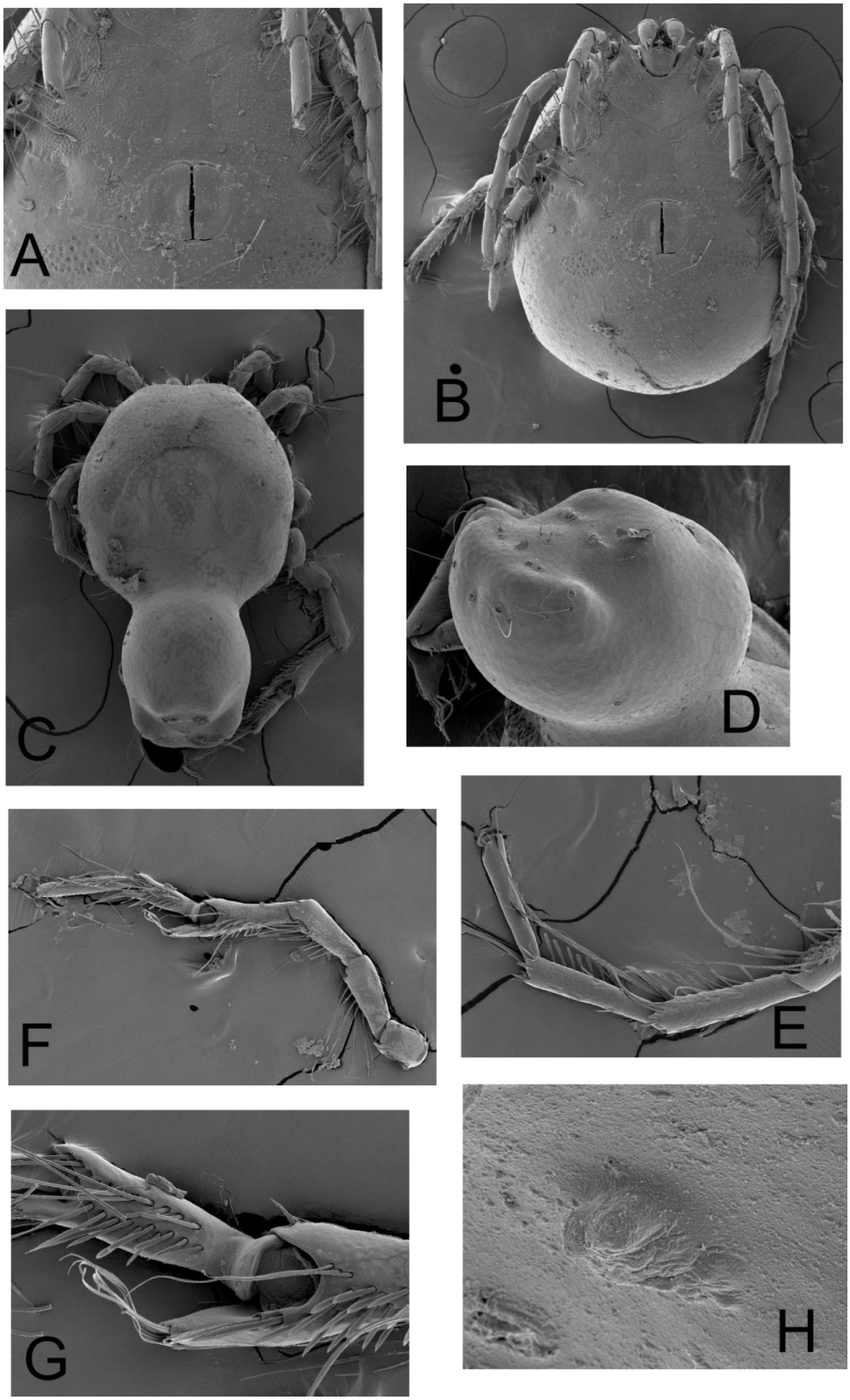
App. 4. Scanning electron micrographs of adult *A. (Tru.) truncatellus*; A. male, dorsal view; B. male, cauda, dorsal view; C. male, spur on fourth leg segment of IV-L.



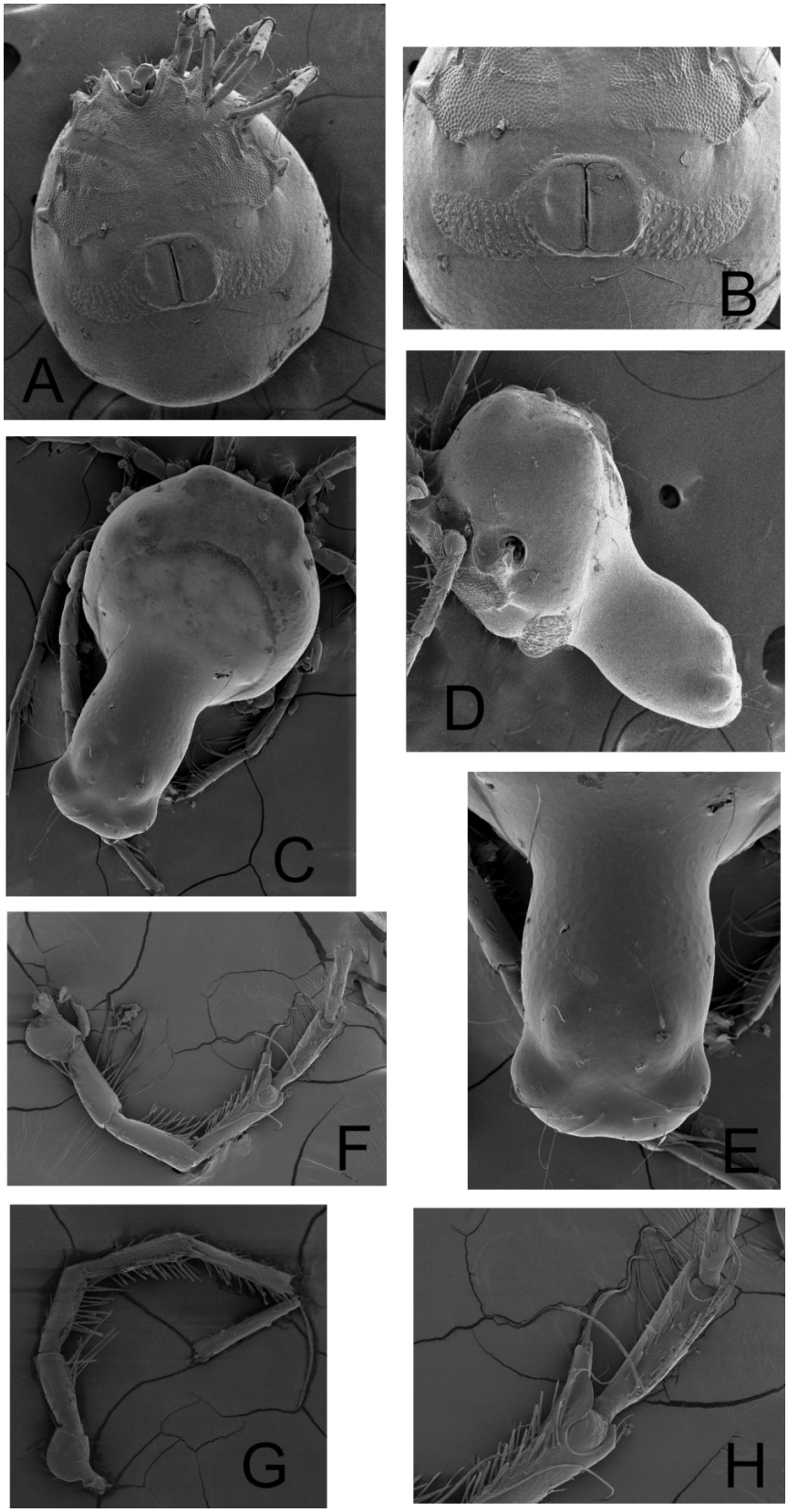
App. 5. Scanning electron micrographs of adult *A. (Meg.) cardiacus*; A. female, ventral view; B. female, ventral view, genital area; C. male, dorsal view; D. male, lateral view; E. male, cauda, dorsal view; F. male, spur on fourth leg segment of IV-L; G. male, IV-L, H. female, IV-L.



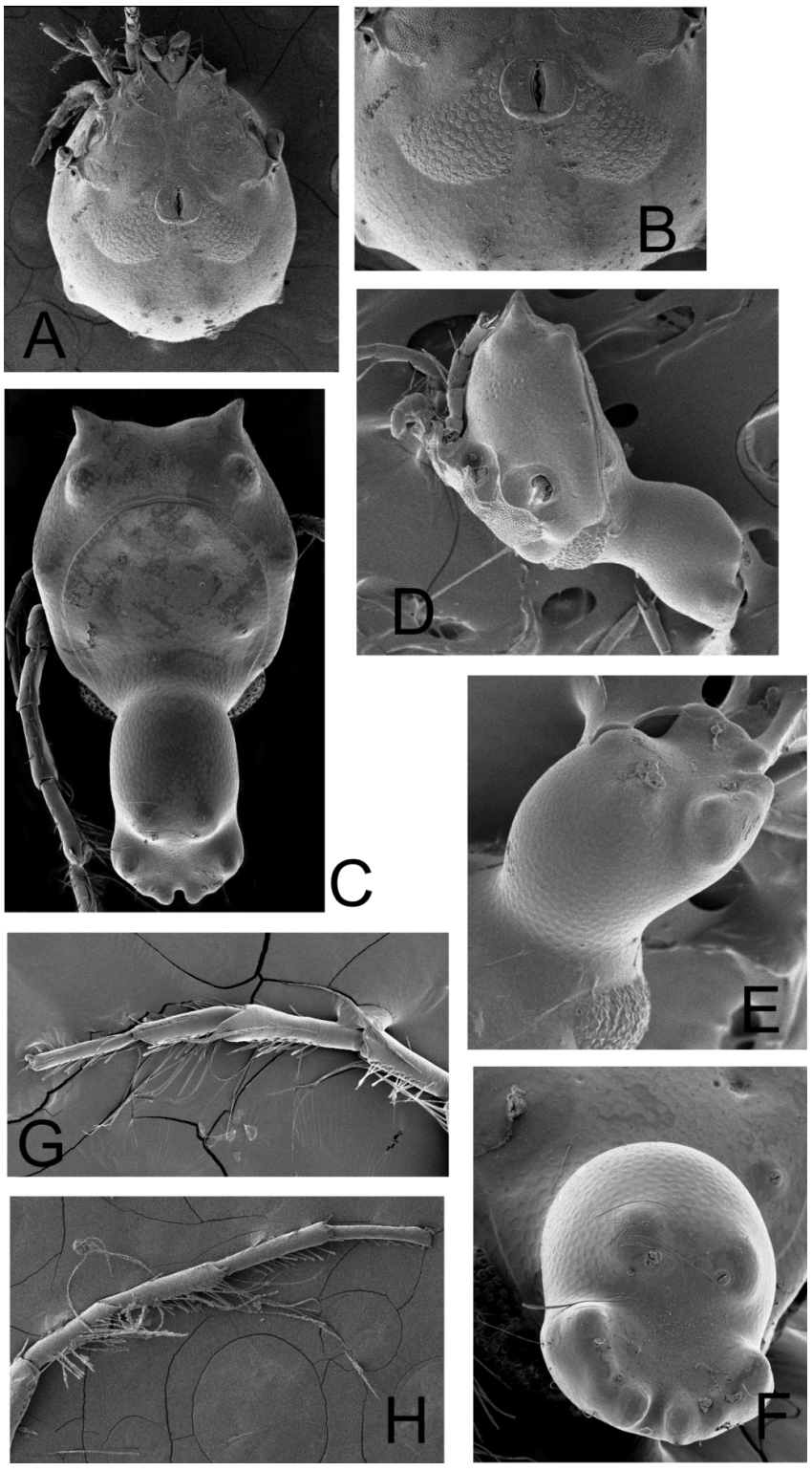
App. 6. Scanning electron micrographs of adult *A. (Meg.) wardi*; A. female, ventral view; B. female, ventral view, genital area; C. male, dorsal view; D. male, cauda, dorsal view; E. male, cauda, ventro-lateral view; F. male, cauda, posterior view; G. male, IV-L; H. male, spur on fourth leg segment of IV-L; I. female, IV-L.



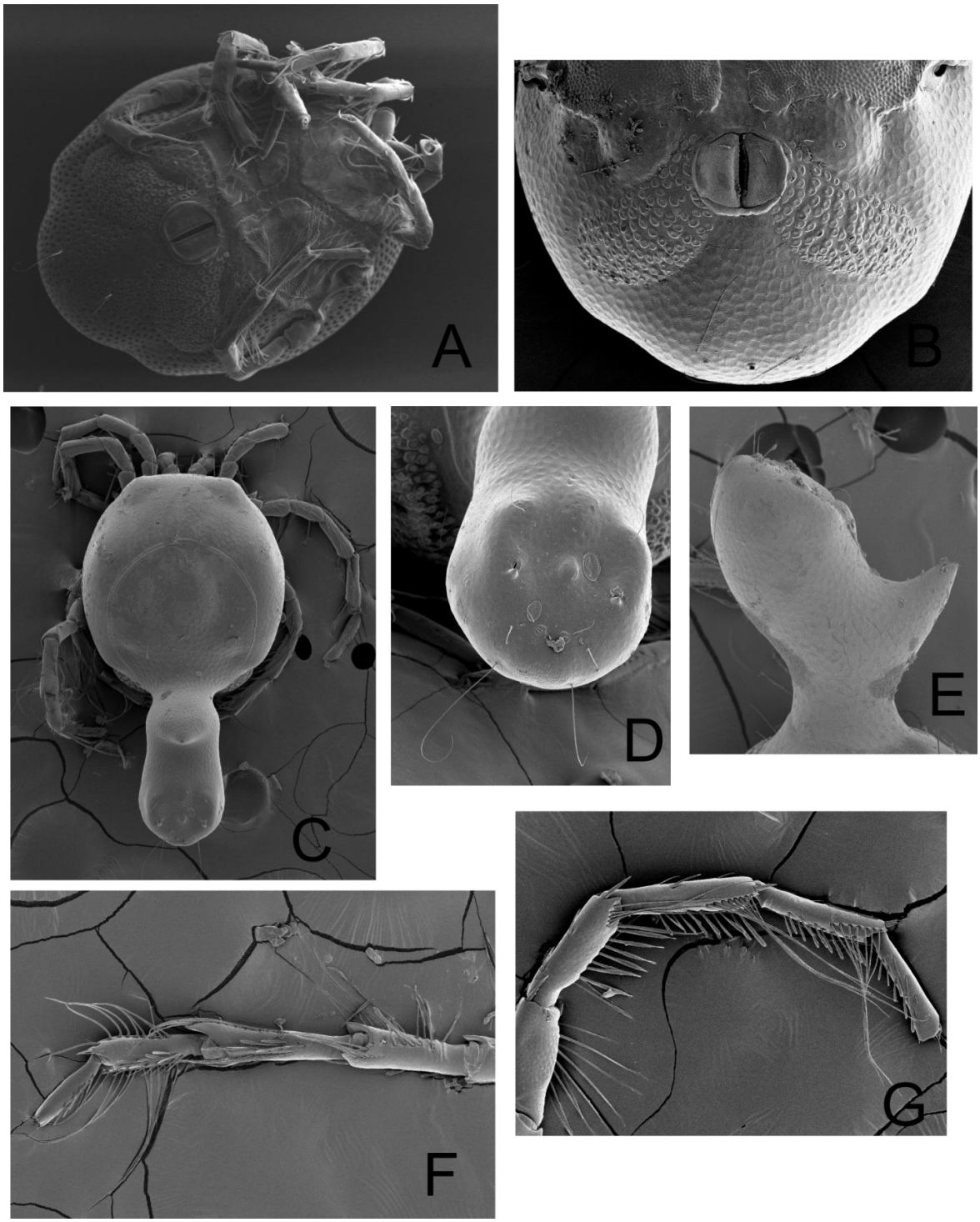
App. 7. Scanning electron micrographs of adult *A. (Meg.) globator*; A. female, ventral view, genital area; B. female, ventral view; C. male, dorsal view; D. male, cauda, lateral view; E. female, IV-L; F. male, IV-L; G. male, spur on fourth leg segment of IV-L; H. male, peg-like petiole.



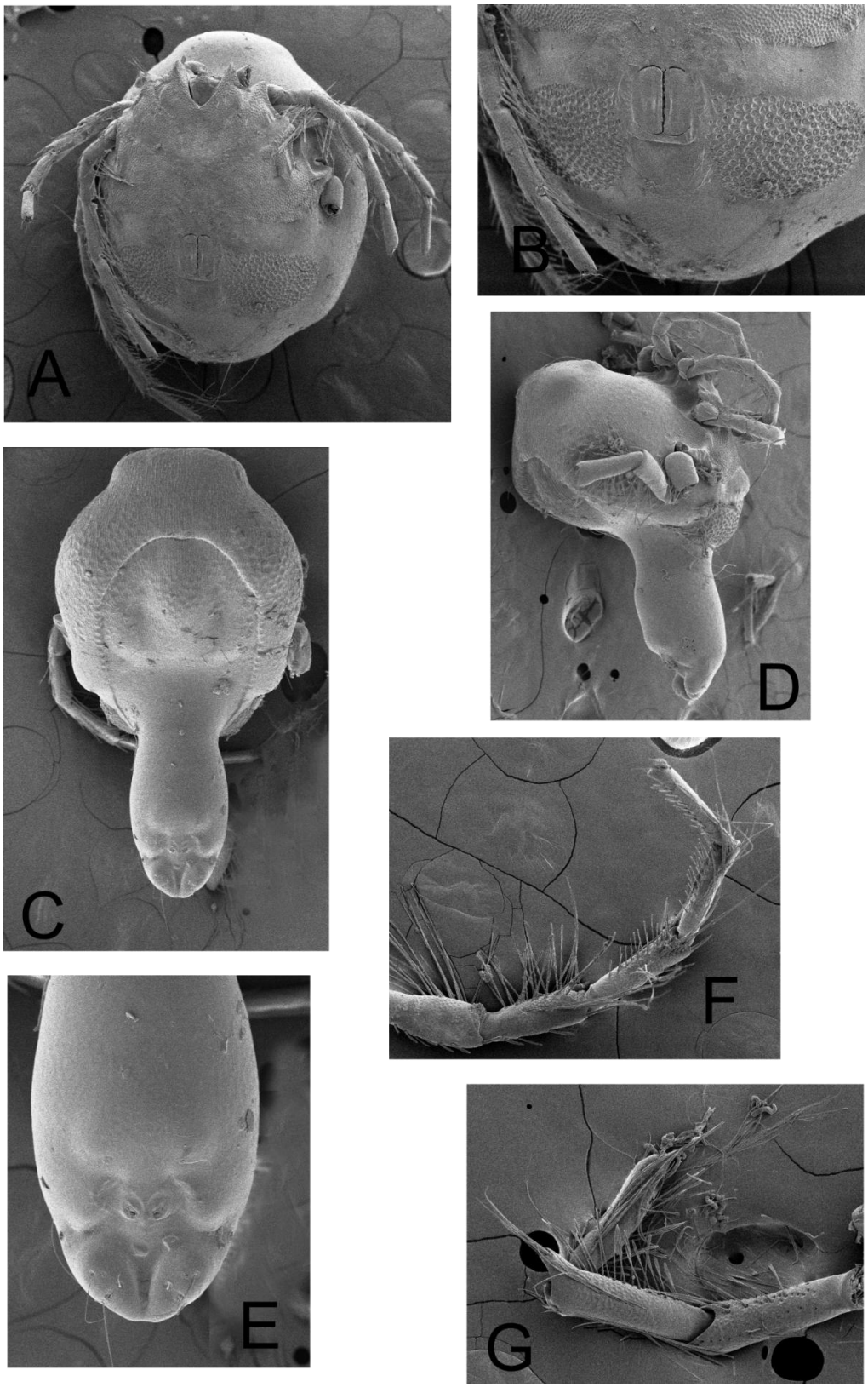
App. 8. Scanning electron micrographs of adult *A. (Meg.) manubriator*; A. female, ventral view; B. female, ventral view, genital area; C. male, dorsal view; D. male, lateral view; E. male, cauda, dorsal view; F. male, IV-L; G. female, IV-L; H. male, spur on fourth leg segment of IV-L.



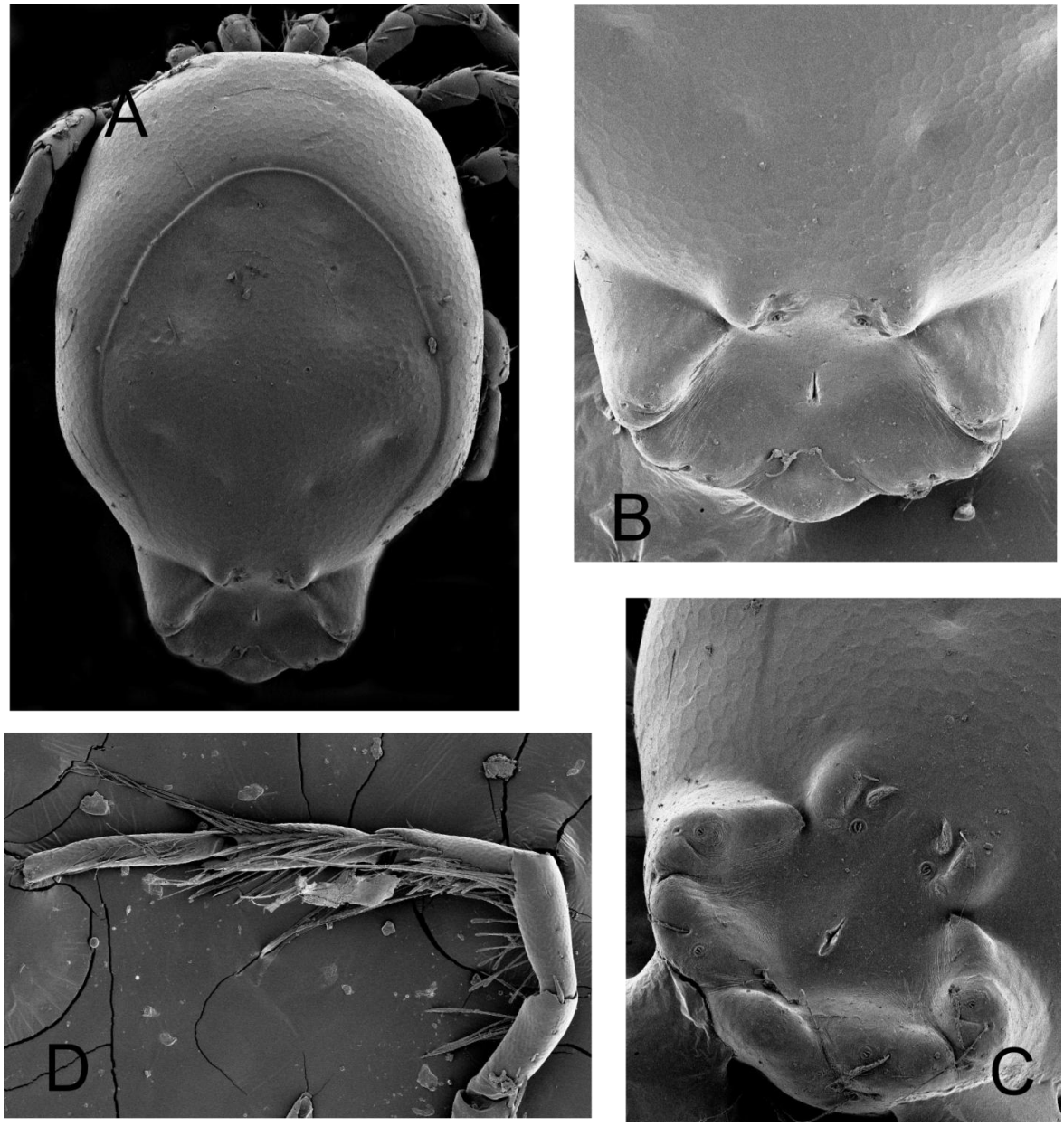
App. 9. Scanning electron micrographs of adult *A. (Meg.) intermedius*; A. female, ventral view; B. female, ventral view, genital area; C. male, dorsal view; D. male, lateral view; E. male, cauda, dorso-lateral view; F. male, cauda, dorsal view; G. male, IV-L with spur on fourth leg segment; H. female, IV-L.



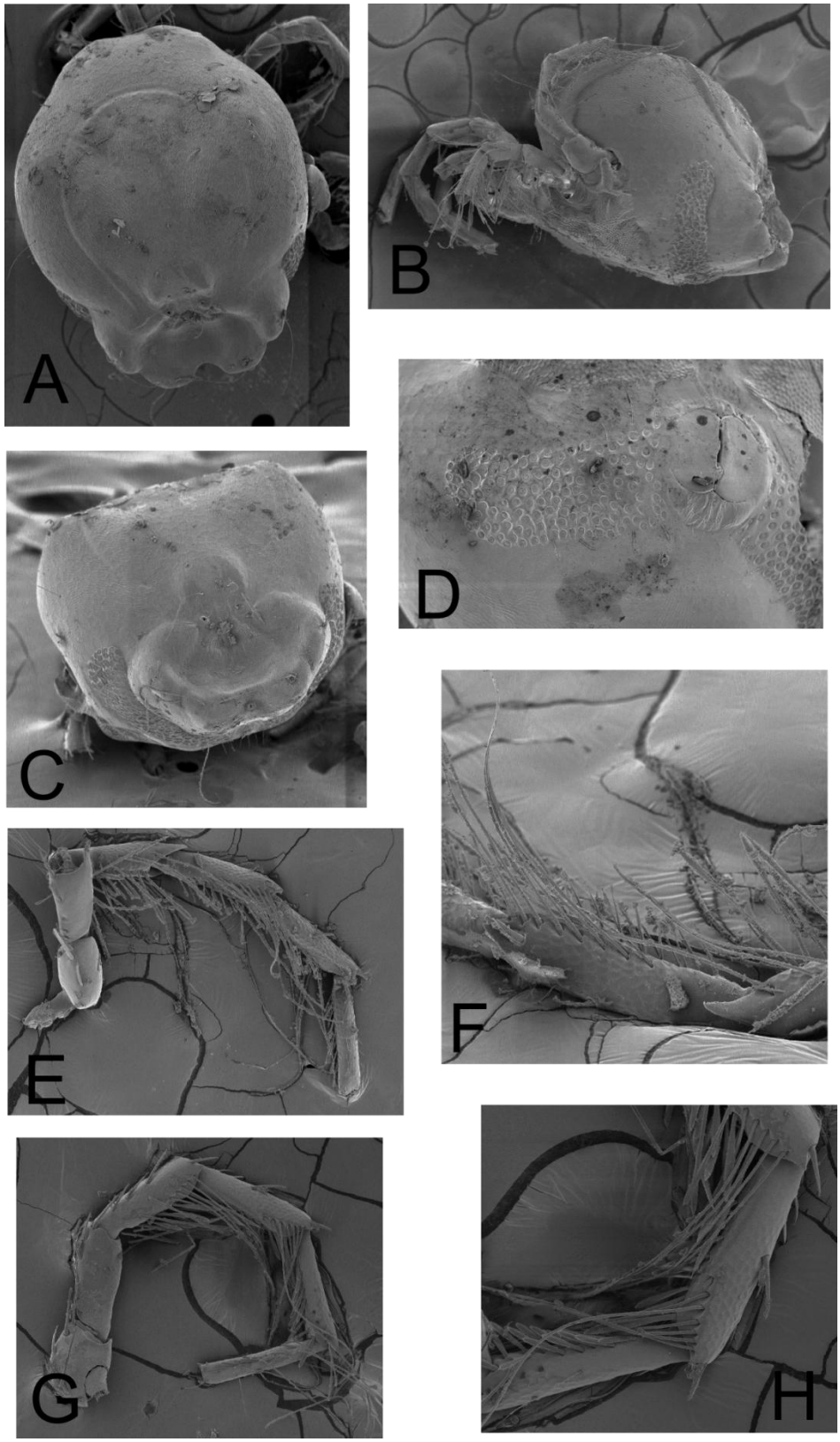
App. 10. Scanning electron micrographs of adult *A. (Meg.) apetiolatus*; A. female, ventral view; B. female, ventral view, genital area; C. male, dorsal view; D. male, cauda, dorsal view; E. male, cauda, lateral view; F. male, IV-L with spur on fourth segment; G. female, IV-L.



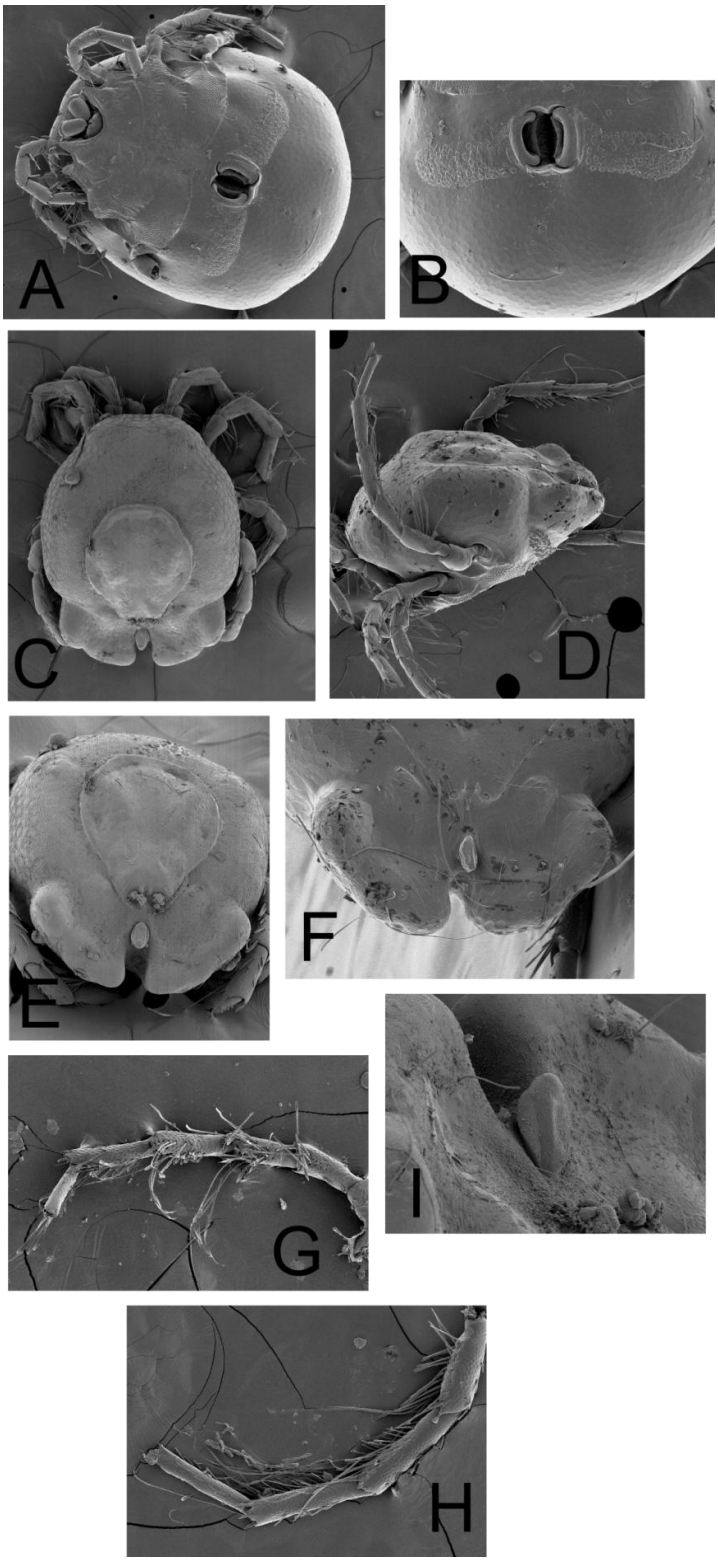
App. 11. Scanning electron micrographs of adult *A. (Meg.) scutiliformis*; A. female, ventral view; B. female, ventral view, genital area; C. male, dorsal view; D. male, lateral view; E. male, cauda, dorsal view; F. female, IV-L; G. male, IV-L with spur on fourth segment (fifth segment is missing).



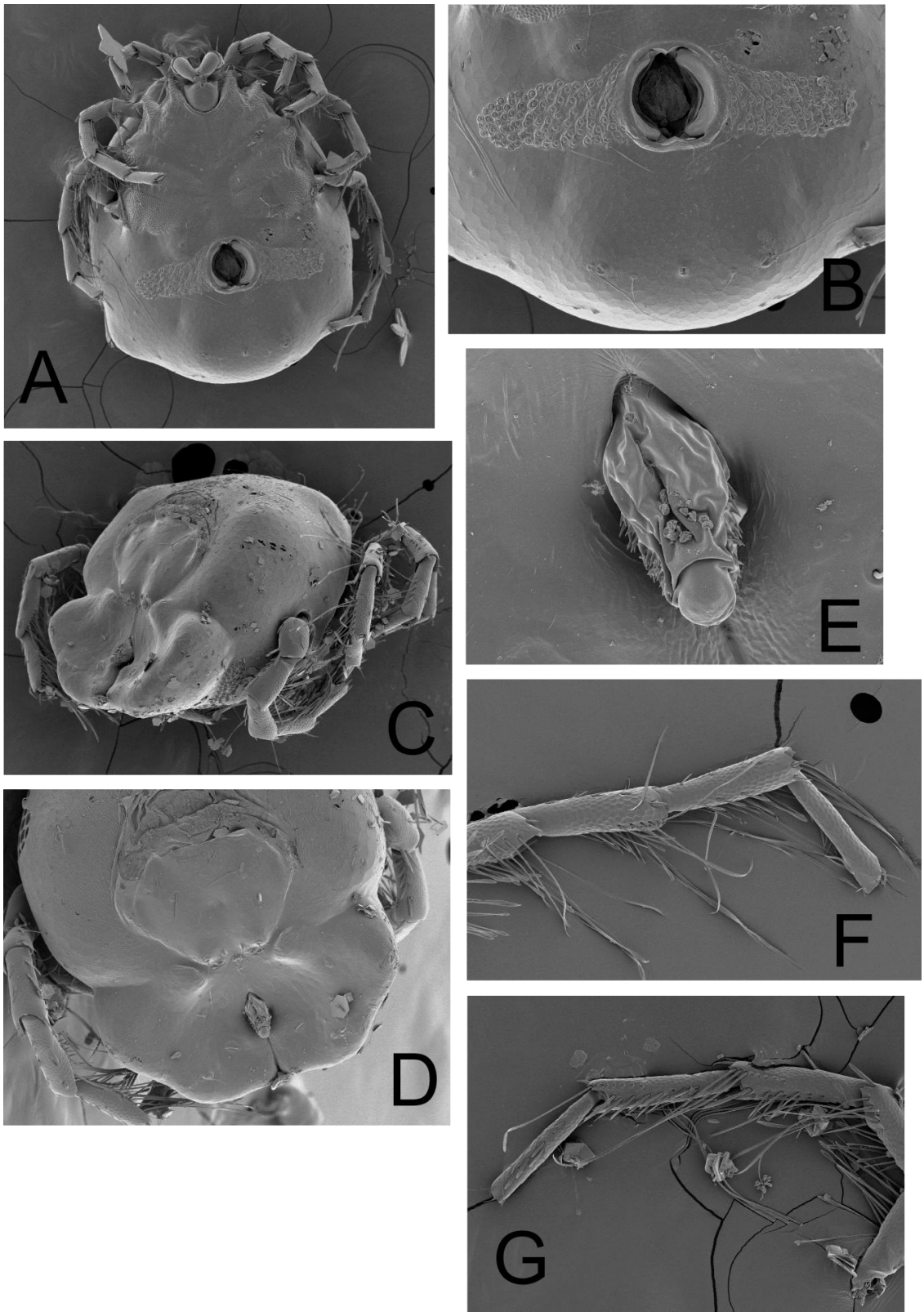
App. 12. Scanning electron micrographs of adult *A. (Miu.) inexploratus*; A. male, dorsal view; B, C. male, cauda, dorsal view; D. male, IV-L.



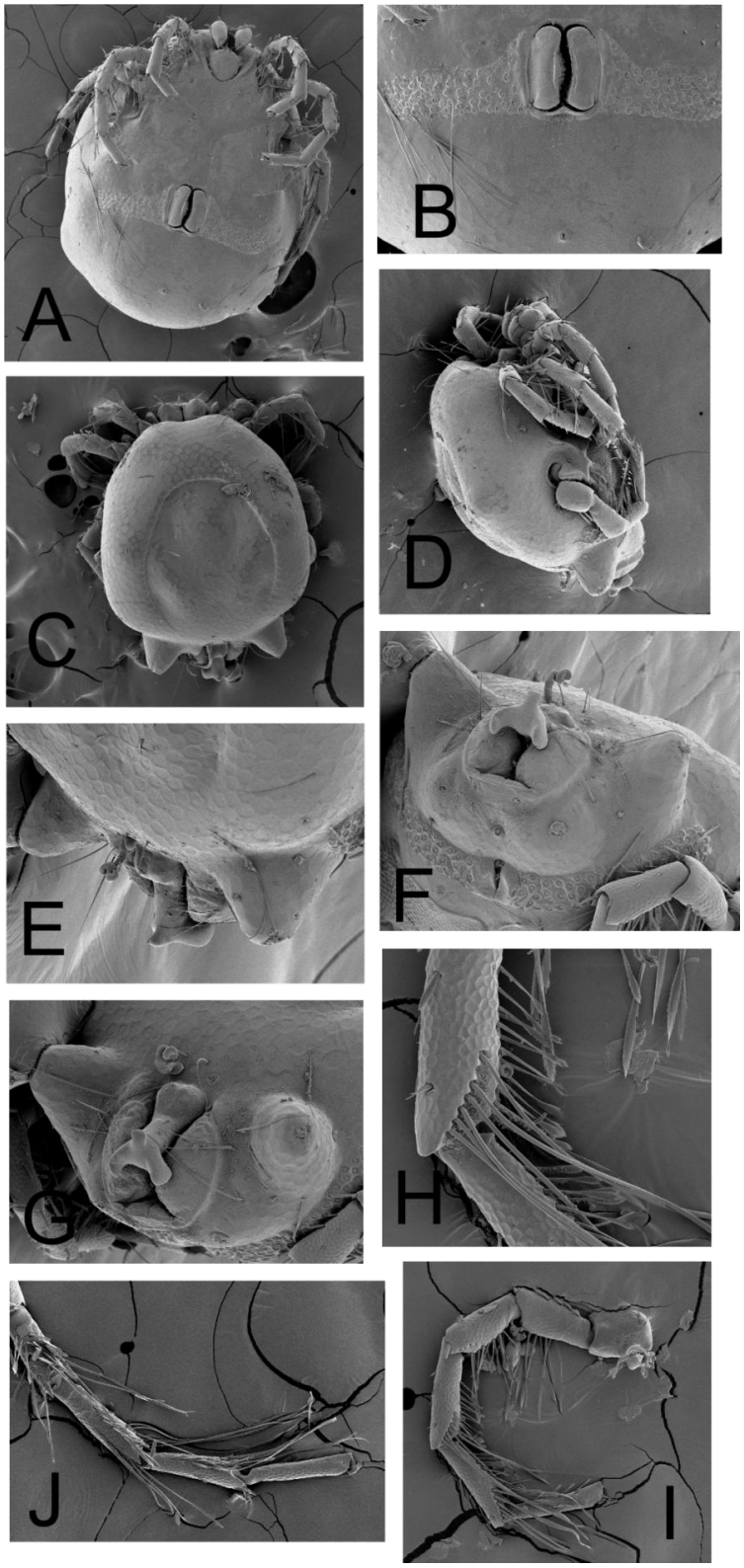
App. 13. Scanning electron micrographs of adult *A. (Miu.) tyrelii*; A. male, dorsal view; B. male, lateral view; C. male, posterior view; D. female, ventral view, genital area; E. male, IV-L; F. male, IV-L, fourth segment without spur; G. female, IV-L; H. female, IV-L, fourth segment.



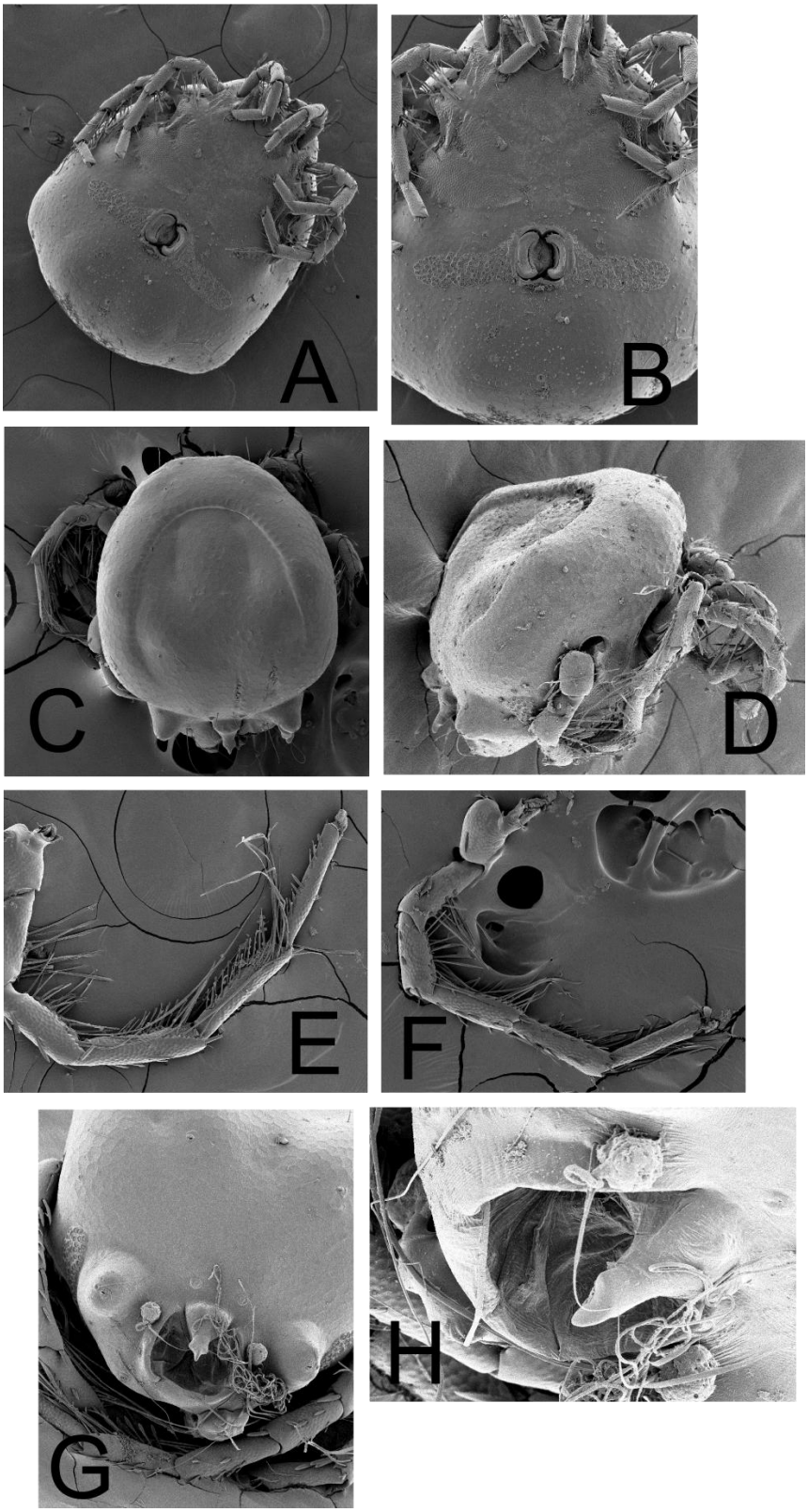
App. 14. Scanning electron micrographs of adult *A. (Miu.) biscissus*; A. female, ventral view; B. female, ventral view, genital area; C. male, dorsal view; D. male, lateral view; E. male, posterior view; F. male, cauda, dorsal view; G. male, IV-L without spur on fourth segment; H. female, IV-L; I. male, petiole.



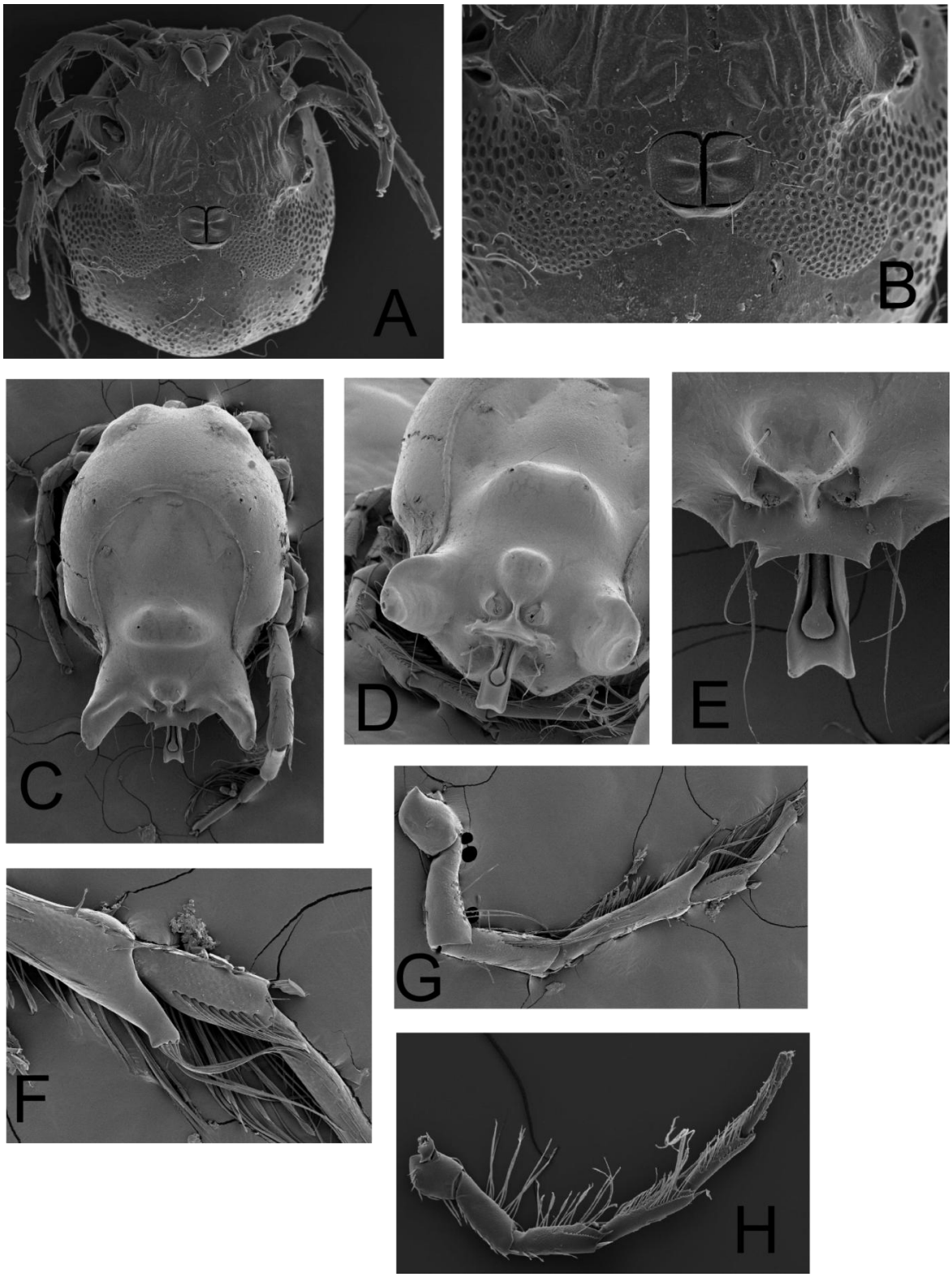
App. 15. Scanning electron micrographs of adult *A. (Miu.) sinuator*; A. female, ventral view; B. female, ventral view, genital area; C. male, dorso-lateral view; D. male, cauda, dorsal view; E. male, petiole; F. male, IV-L without spur on fourth segment; G. female, IV-L.



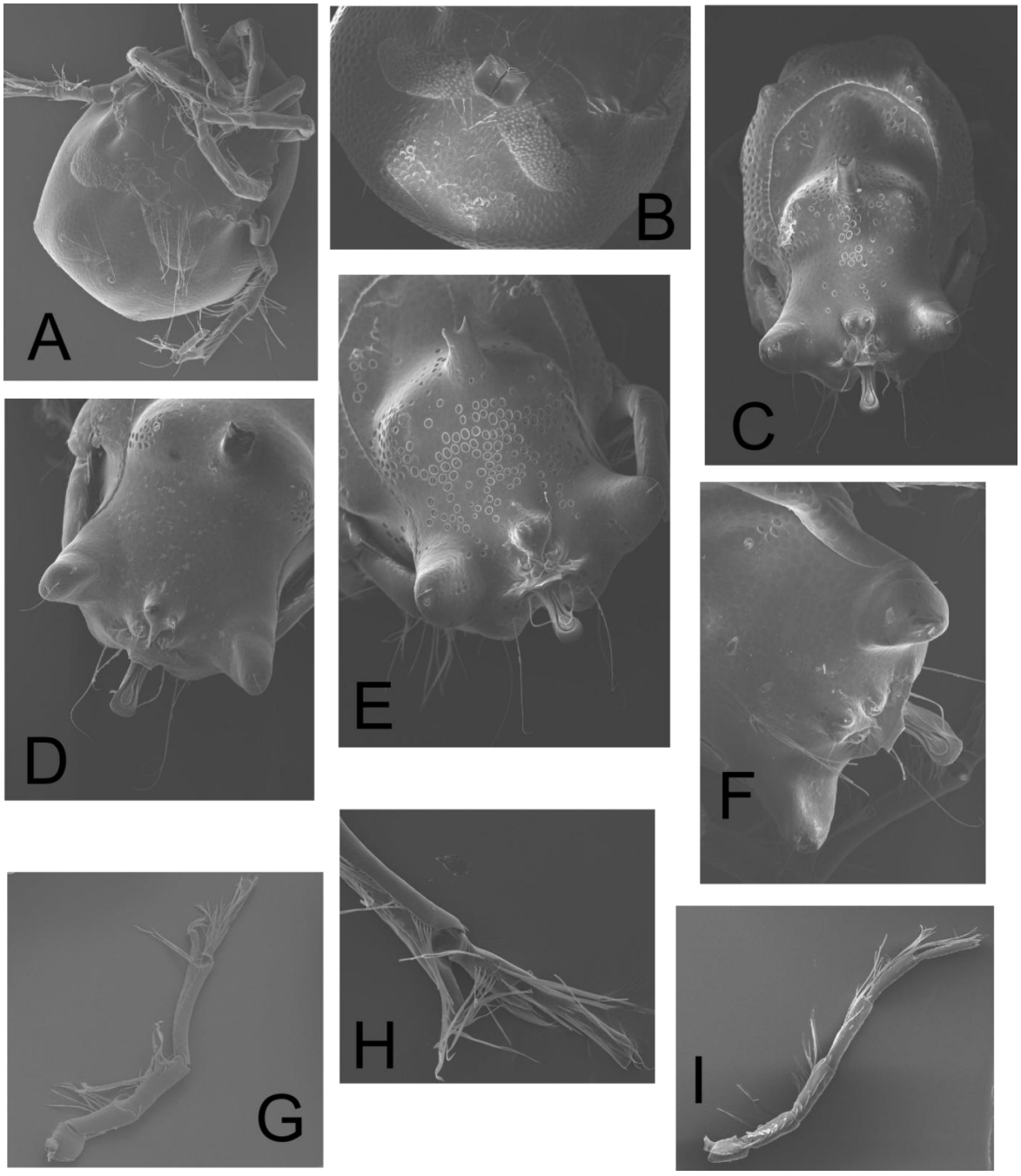
App. 16. Scanning electron micrographs of adult *A. (Mic.) albator*; A. female, ventral view; B. female, ventral view, genital area; C. male, dorsal view; D. male, lateral view; E. male, cauda, dorsal view; F. male, cauda, membranous sub-petiolar cavity with petiole, ventral view; G. male, cauda (entire petiole visible); H, I. male, IV-L without spur on fourth segment; J. female, IV-L.



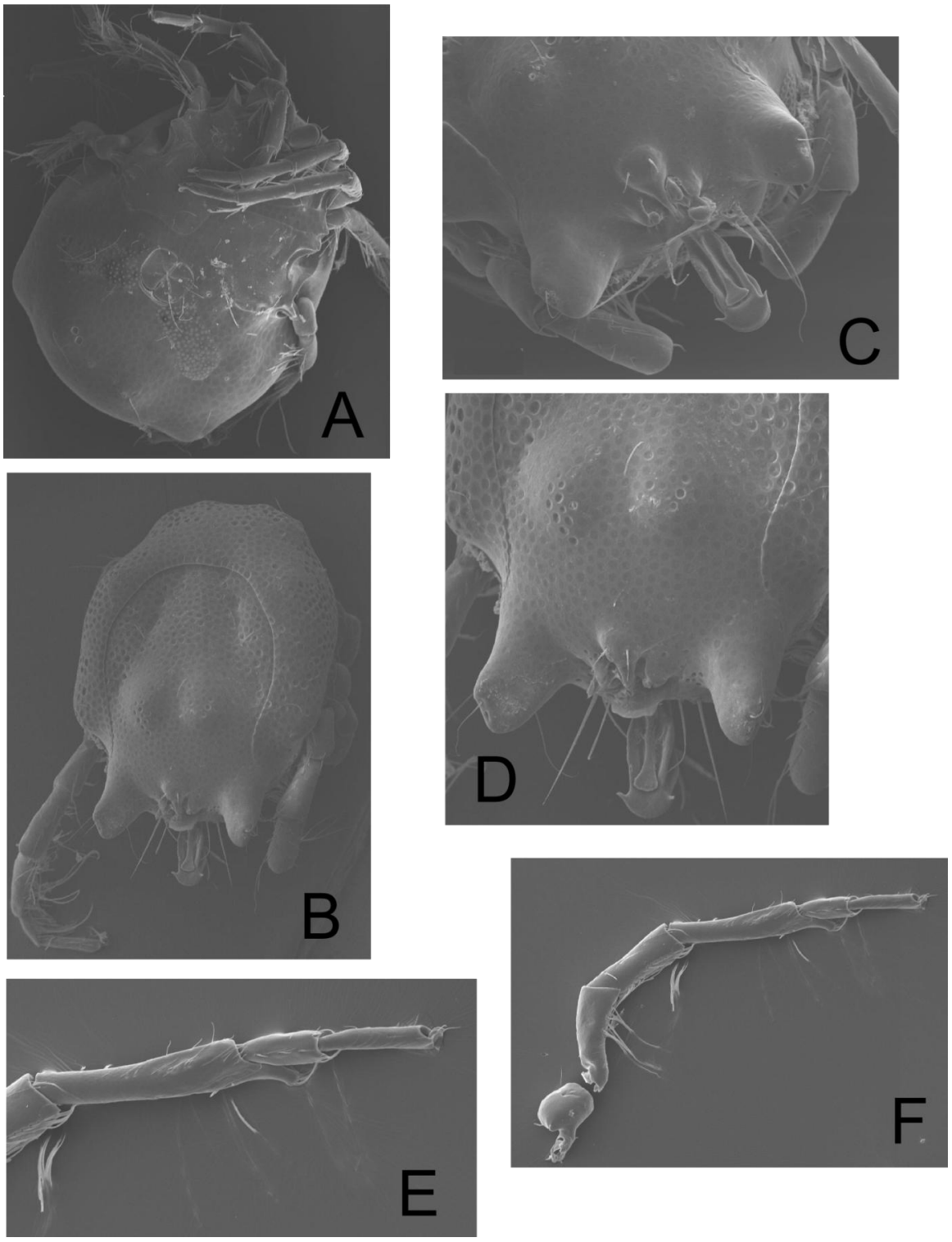
App. 17. Scanning electron micrographs of adult *A. (Mic.) crassicaudatus*; A. female, ventral view; B. female, ventral view, genital area; C. male, dorsal view; D. male, lateral view; E. female, IV-L; F. male, IV-L without spur on fourth segment; G. male, cauda, dorsal view; H. male, cauda, membranous sub-petiolar cavity with petiole.



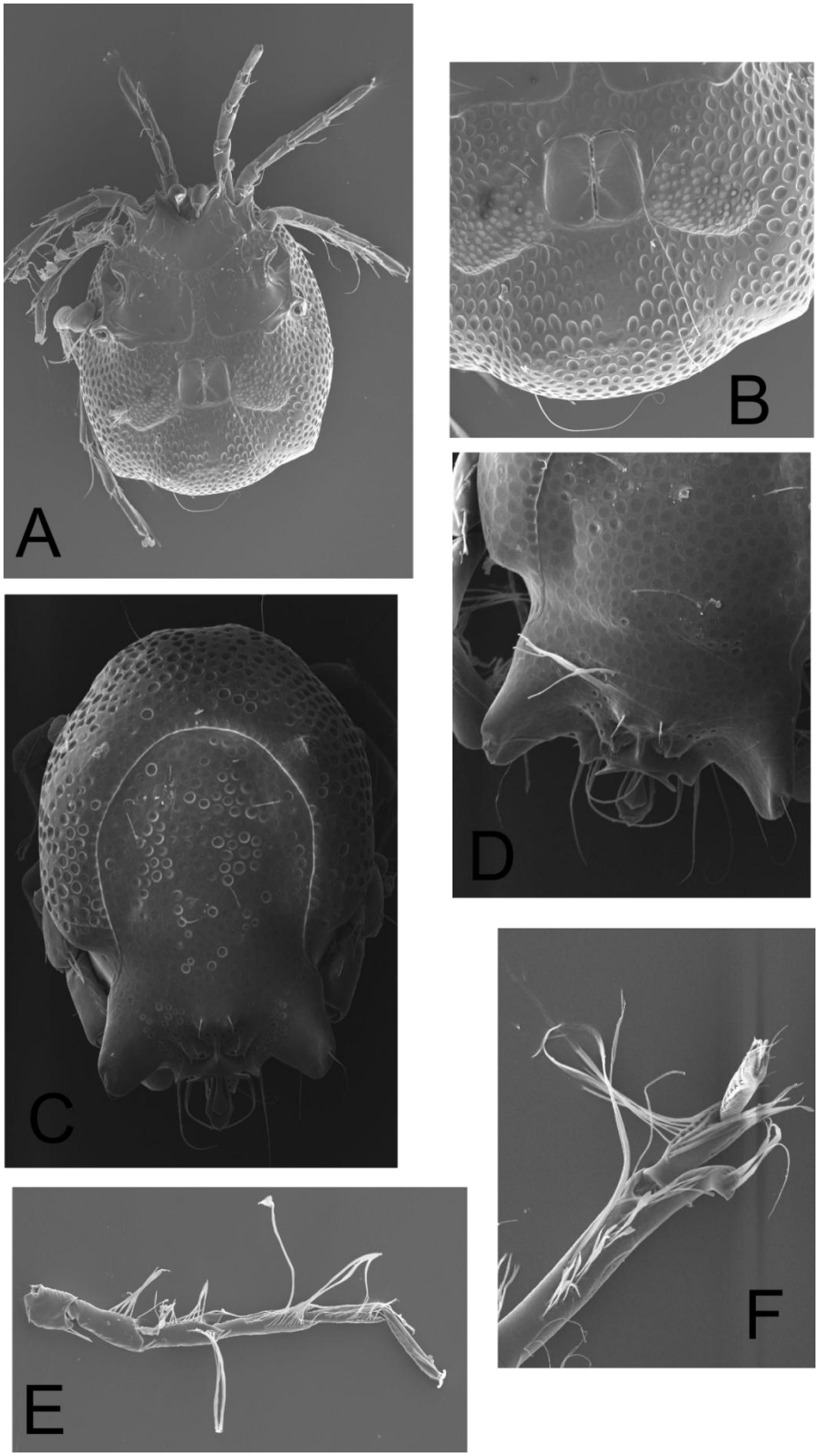
App. 18. Scanning electron micrographs of adult *A. (Arr.) bicuspidator*; A. female, ventral view; B. female, ventral view, genital area; C. male, dorsal view; D. male, cauda, dorsal view; E. male, petiole with central piece; F. male, spur on fourth leg segment of IV-L; G. male, IV-L; H. female, IV-L.



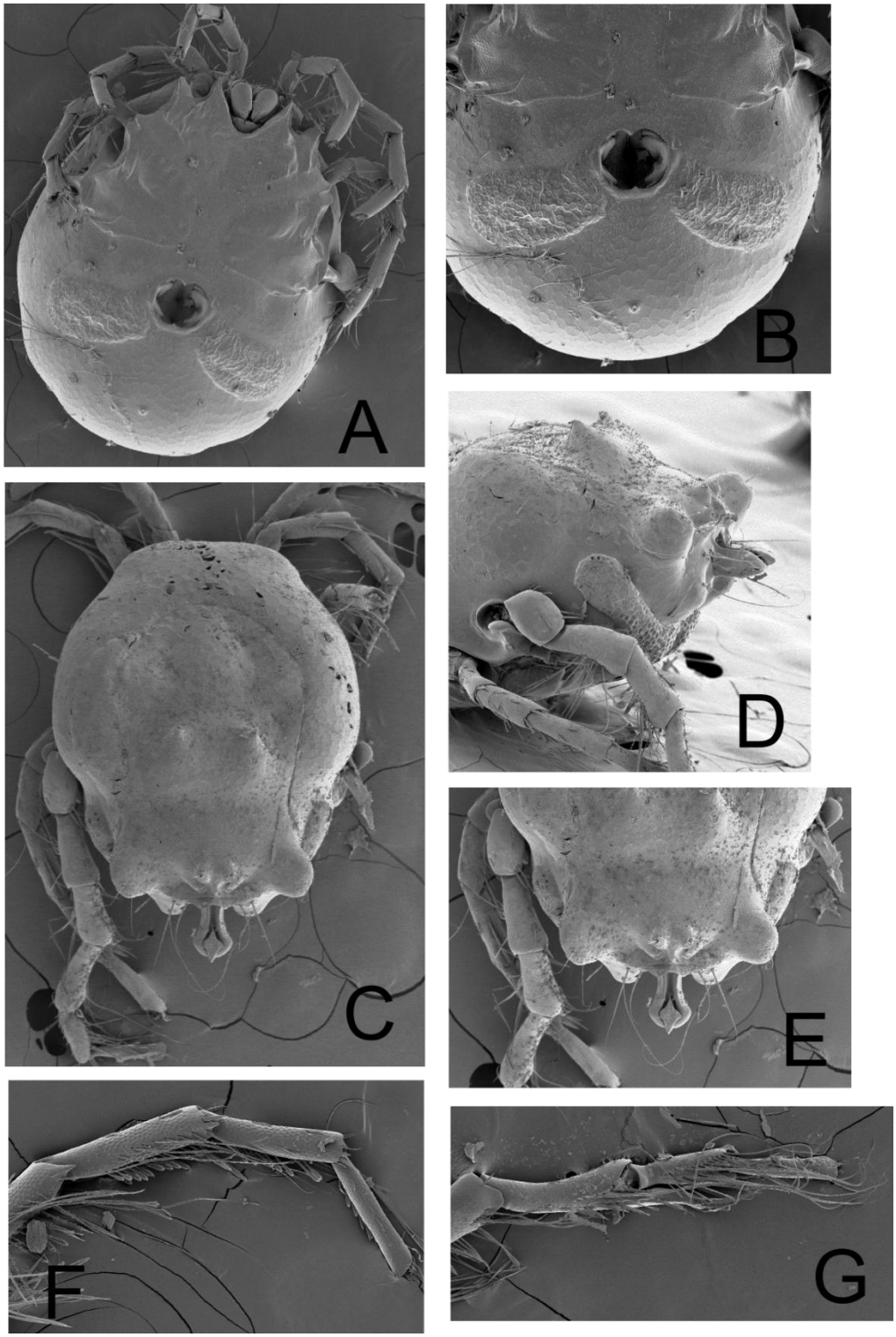
App. 19. Scanning electron micrographs of adult *A. (Arr.) tricuspidator*; A. female, ventral view; B. female, ventral view, genital area; C. male, dorsal view; D, E, F. male, cauda with petiole with central piece; G. male, IV-L; H. male, spur on fourth leg segment of IV-L; I. female, IV-L.



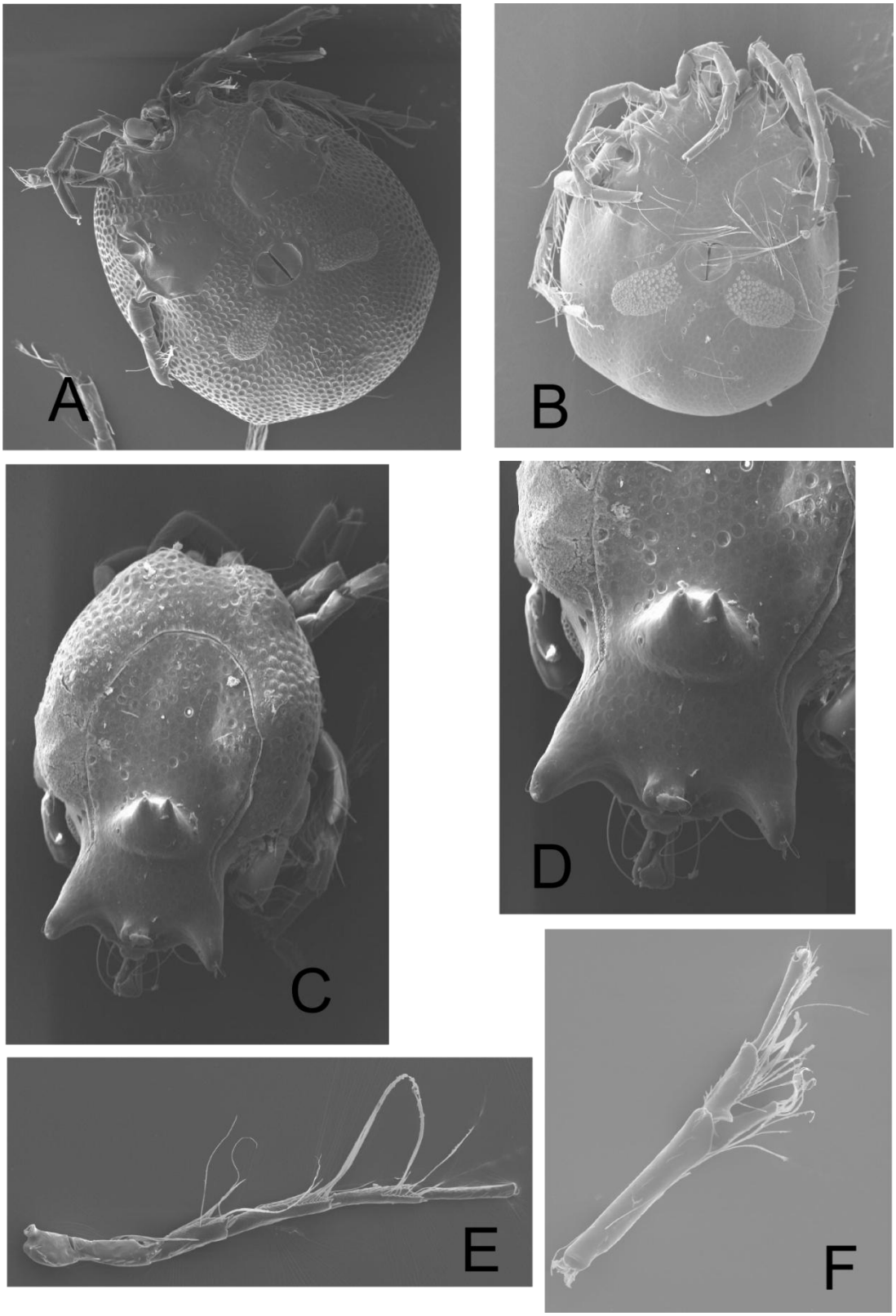
App. 20. Scanning electron micrographs of adult *A. (Arr.) bruzelii*; A. female, ventral view; B. male, dorsal view; C. male, cauda, petiole with central piece well visible, dorsal view; D. male, cauda, dorsal view; E. male, spur on fourth leg segment of IV-L; F. male, IV-L.



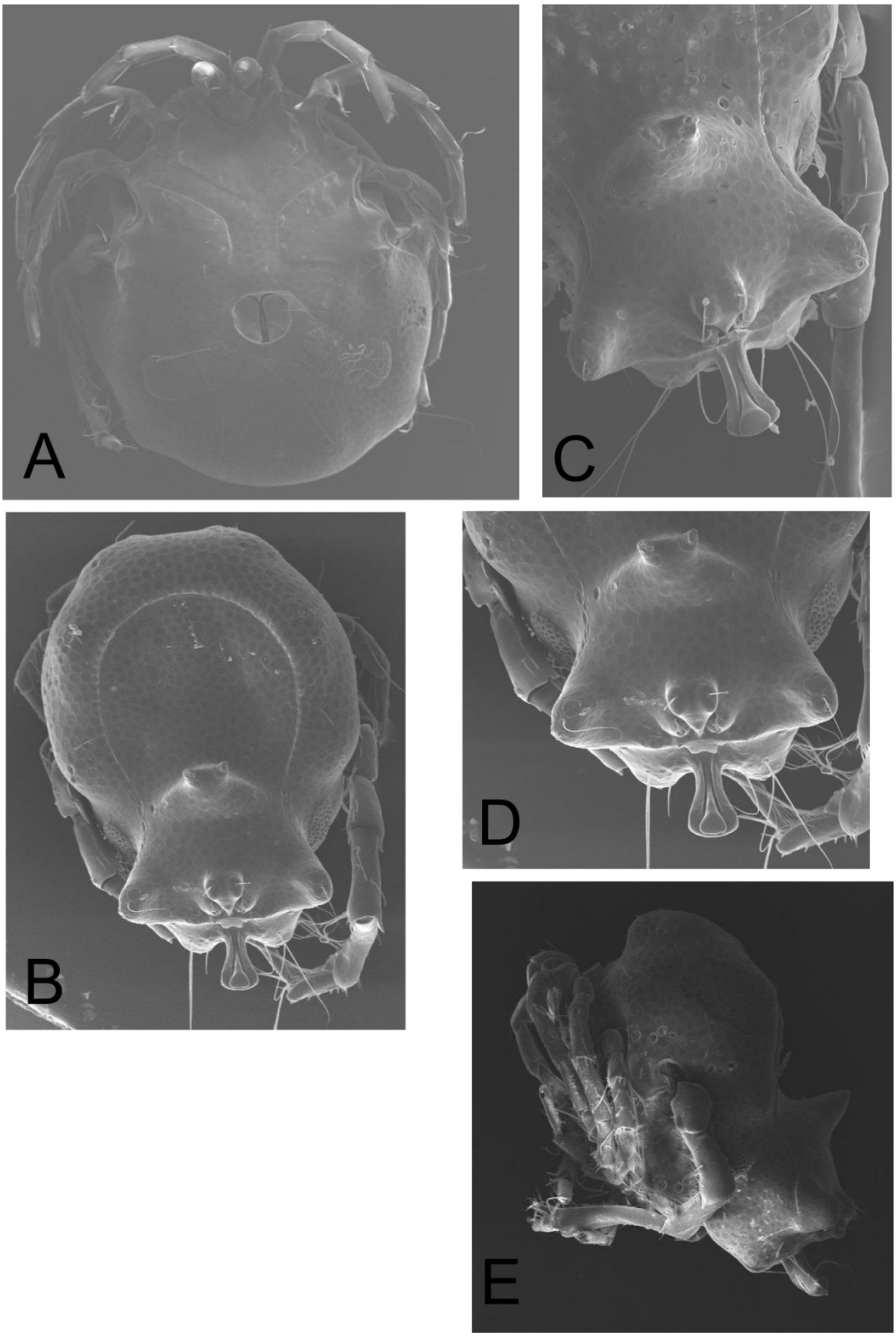
App. 21. Scanning electron micrographs of adult *A. (Arr.) claviger*; A. female, ventral view; B. female, ventral view, genital area; C. male, dorsal view; D. male, cauda (petiole with central piece well visible), dorsal view; E. female IV-L; F. male, spur on fourth leg segment of IV-L.



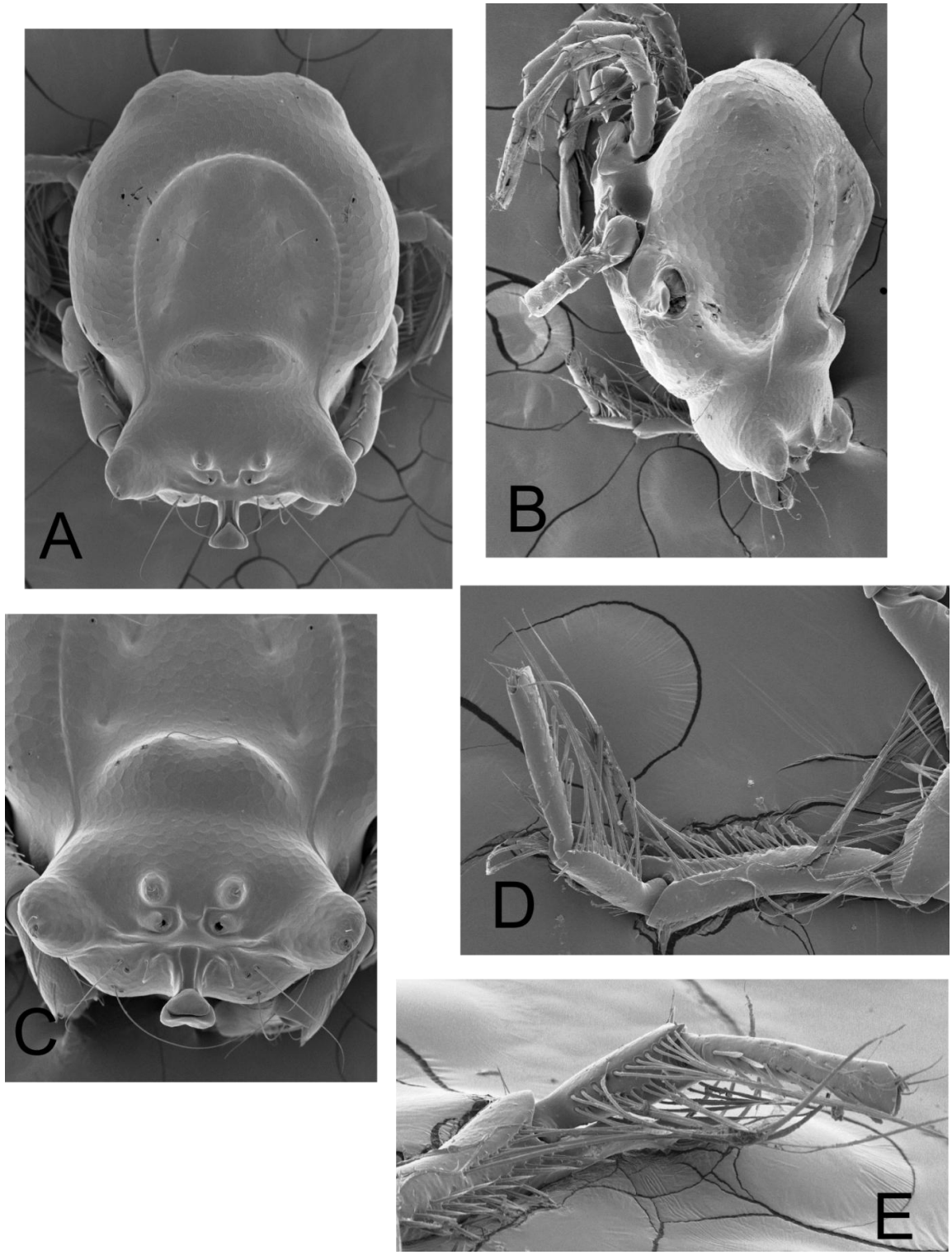
App. 22. Scanning electron micrographs of adult *A. (Arr.) compactus*; A. female, ventral view; B. female, ventral view, genital area; C. male, dorsal view; D. male, cauda, lateral view; E. male, cauda with petiole equipped with central piece; F. female, IV-L; G. male, IV-L with spur on fourth leg segment.



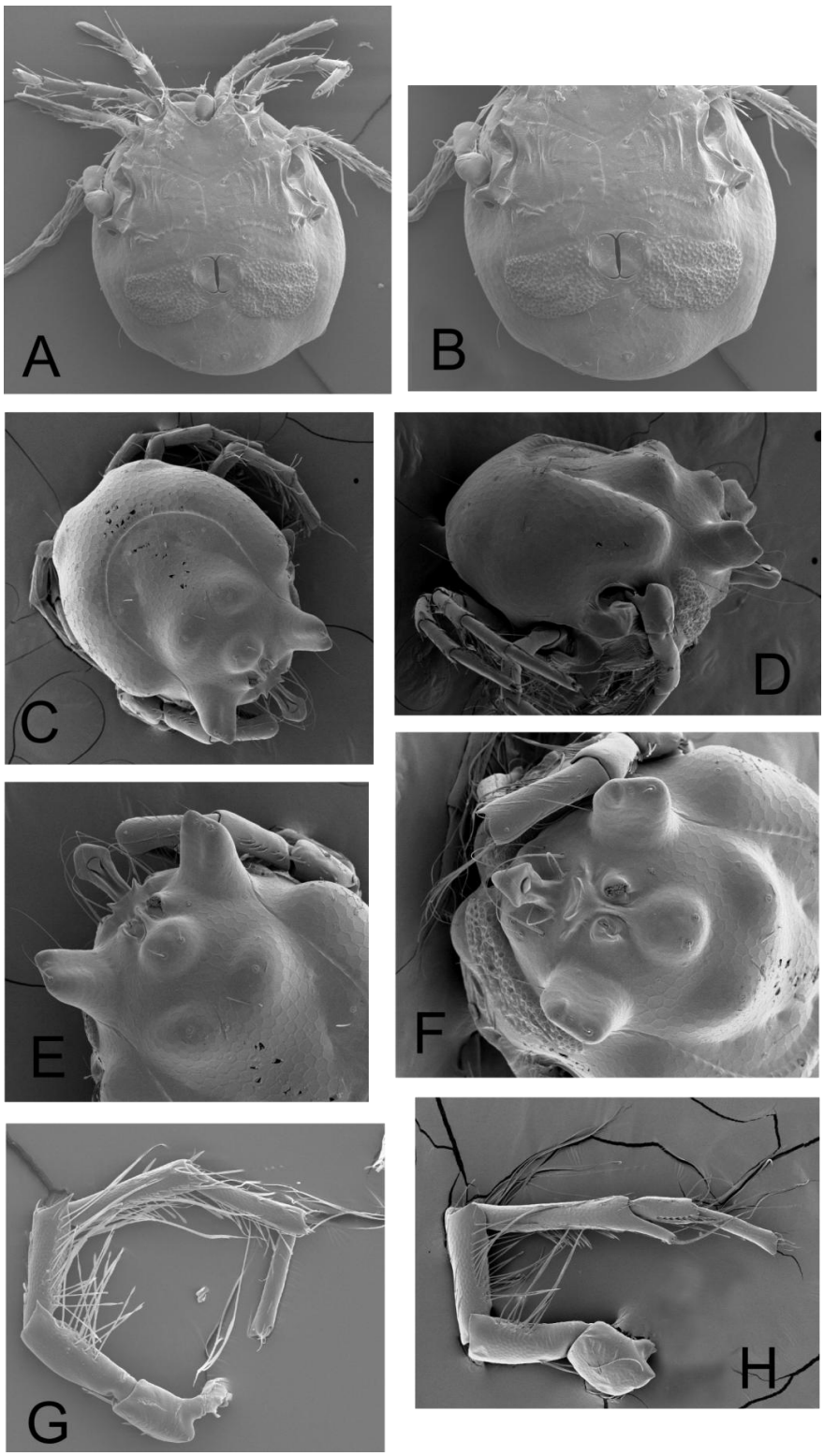
App. 23. Scanning electron micrographs of adult *A. (Arr.) cuspidator*; A, B. morphologically variable females, ventral view; C. male, dorsal view; D. male, cauda with well developed petiole, dorsal view; E. female, IV-L; F. male, spur on fourth leg segment of IV-L.



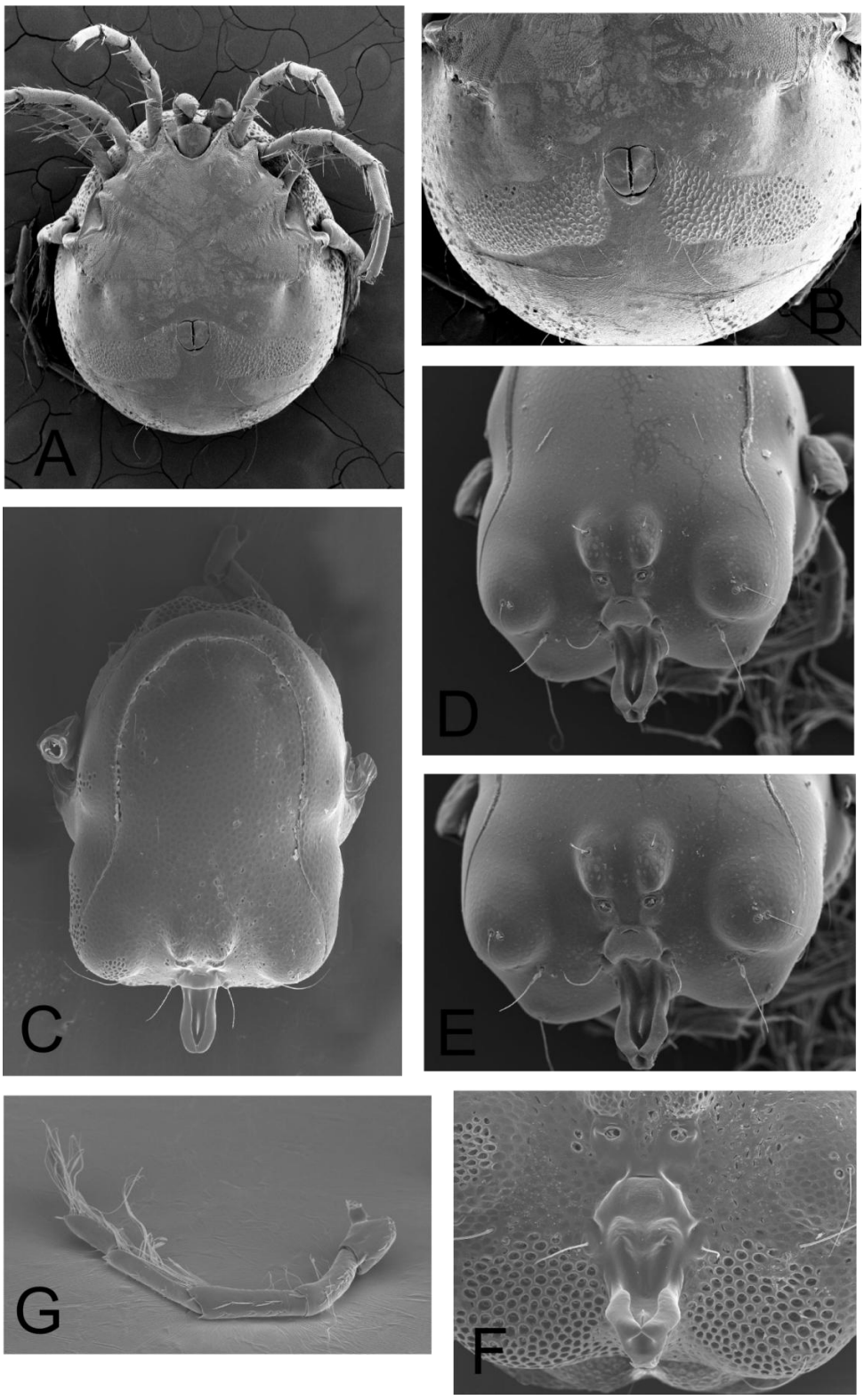
App. 24. Scanning electron micrographs of adult *A. (Arr.) maculator*; A. female, ventral view; B. male, dorsal view; C, D. male, cauda with petiole equipped with central piece, dorsal view; E. male, lateral view.



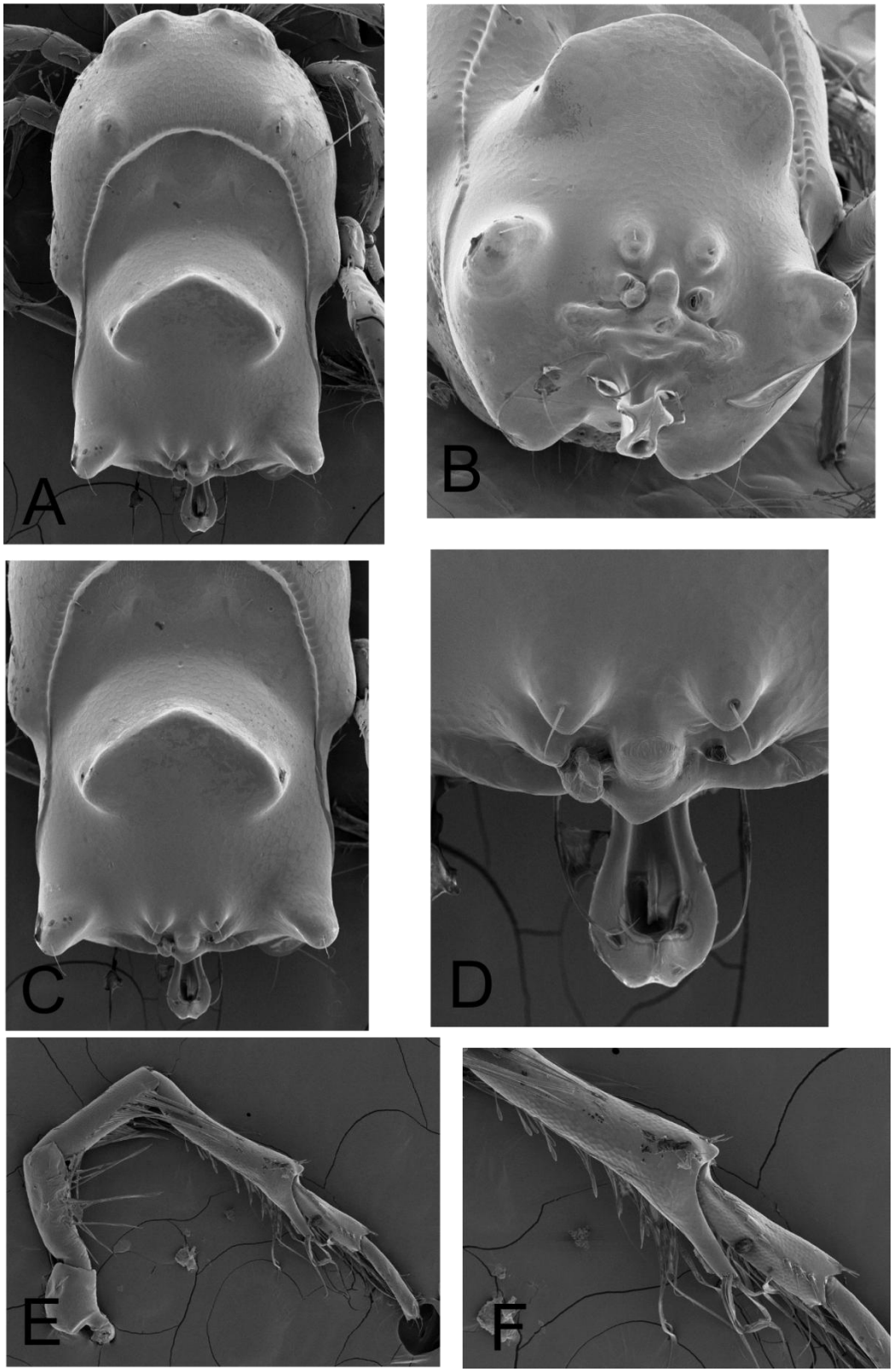
App. 25. Scanning electron micrographs of adult *A. (Arr.) affinis*; A. male, dorsal view; B. male, lateral view; C. male, cauda with petiole equipped with central piece, dorsal view; D, E. male, IV-L with spur on fourth leg segment.



App. 26. Scanning electron micrographs of adult *A. (Arr.) neumani*; A. female, ventral view; B. female, ventral view, genital area; C. male, dorsal view; D. male, lateral view; E. male, cauda with petiole equipped with central piece, dorsal view; F. male, cauda, posterior view; G. female, IV-L; H. male, IV-L with spur on fourth leg segment.



App. 27. Scanning electron micrographs of adult *A. (Arr.) pustulator*; A. female, ventral view; B. female, ventral view, genital area (pigmented patches on genital valves visible); C. male, dorsal view; D, E. male, cauda with petiole, posterior view; F. male, petiole without central piece; G. male, IV-L (spur on fourth leg segment not visible).



App. 28. Scanning electron micrographs of adult *A. (Arr.) magnicaudatus*; A. male, dorsal view; B. male, cauda with petiole, posterior view; C. male, cauda, dorsal view; D. petiole without central piece; E, F. male, IV-L with spur on fourth leg segment