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Sumatrella chelonica gen. n., sp. n., a new remarkable genus and species from Indonesia, Sumatra (Acari, Uropodina, Oplitidae)

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Abstract

A new genus *Sumatrella* **gen. n.** is described and illustrated based on the new species *Sumatrella chelonica* **sp. n.** collected in Sumatra, Indonesia. The new genus belongs to the family Oplitidae based on its hypertrichous internal malae and the absence of strongly sclerotized structures on the dorsal shield. The new genus is closely related to the genus *Chelonuropoda* Sellnick, 1954 but the transverse furrow on ventral idiosoma close to coxae IV and the strongly sclerotized C-shaped dorsal line are missing in the new genus. These characters can be found in species of *Chelonuropoda*.

Keywords

South-East Asia, taxonomy, turtl mites

Introduction

The Uropodina mites are one of the well-characterized members of the soil mite fauna. They can be found with a high diversity in tropical regions (Lindquist et al. 2009), but currently only 10% of the known species are from this region (Vázquez and Klompen 2007).

The family Oplitidae (Lindquist et al. 2009, Beaulieu et al. 2011) is a very district group among the Uropodina, which possesses several specific characters. The internal malae is subdivided into several branches bearing pilose margins, the hypostomal setae are not situated in a longitudinal row, and the dorsal shield does not bear strongly sclerotized structures. Its members can often be found in ant nests (Mašán 2001). This family was previously treated as one genus, and divided into several species groups in previous systems (Wiśniewski and Hirschmann 1993). Recently it was elevated to family level [Lindquist et al. (2009), Beaulieu et al. (2011)] and is divided into further genera (e.g. Kontschán 2010, Kontschán and Starý 2012).

In 2014, some days were spent in the Arachnida collection of Natural History Museum in Geneva, where I found several specimens of a very unusual oplitid species, described here as a new genus and new species.

Material and methods

Specimens of this unusual species were cleared in lactic acid, investigated on half-covered deep slides and illustrations were made with the aid of a drawing tube. Photographs were taken with a Nikon CoolPix900 digital camera. All specimens are stored in ethanol and deposited in the Natural History Museum in Geneva (NHMG). All measurements are given in micrometres (μm).

Taxonomic

***Sumatrella* gen. n.**
http://zoobank.org/360C7C81-9BA6-4CEC-B92A-9F02B5E06768

Diagnosis. Idiosoma small, oval, posterior margin rounded and very convex. All part of marginal shield wide and fused anteriorly to dorsal shield. Dorsal and ventral setae smooth and needle-like. Genital shield of female octagonal, without sculptural pattern and anterior process. Dorsal and marginal shields neotrichous. Corniculi horn-like, internal malae with several long branches. Hypostomal setae h3 longer than others, h2 situated outside the longitudinal row h1–h4 and shorter than others. Tritosternum with narrow basis, laciniae divided into two short and two long pilose branches. Epistome hemispherical and marginally pilose. Leg I without claw, trochanters II-IV with a triangular process.

Type species. *Sumatrella chelonica* sp. n.

Etymology. The name of the new genus refers to the name of island where the specimens were collected. Gender feminine.

Systematic notes. On the basis of the shape of internal malae (divided into pilose branches), the absence of the T-shaped dorsal setae and the hypostomal setae h2 position lateral to row h1–h4, I refer this genus to Oplitidae. Recently several genera and

Table 1. The distinguishing characteristics between *Chelonuropoda* Sellnick, 1954 and *Sumatrella* gen. n.

	Chelonuropoda	***Sumatrella***
Length of idiosoma	1000<	600>
Width of marginal shield	only on anterior area	on all area
C-shaped strongly sclerotized dorsal lines	present	absent
Transverse furrow near coxae IV on ventral idiosoma	present	absent
Shape of female genital shield	linguliform	octagonal
Shape of peritreme	long, hook-like, mushroom-like or R-shaped	short, C-shaped
Epistome	triangular	hemispherical
Triangular process on trochanters of legs II–IV	absent	present
Claws on leg I	present	absent

species groups have been recognized in this family (Kontschán 2010, Kontschán and Starý 2012, Wiśniewski and Hirschmann 1993), but the new genus differs from the others on the basis of the very convex idiosoma, the octagonal genital shield and the wide marginal shield. Only the genus *Chelonuropoda* Sellnick, 1954 shares this combination of character states with the new genus (i.e. very convex idiosoma and wide marginal shield) but the former differs in several characters, the most important of which are summarized in Table 1.

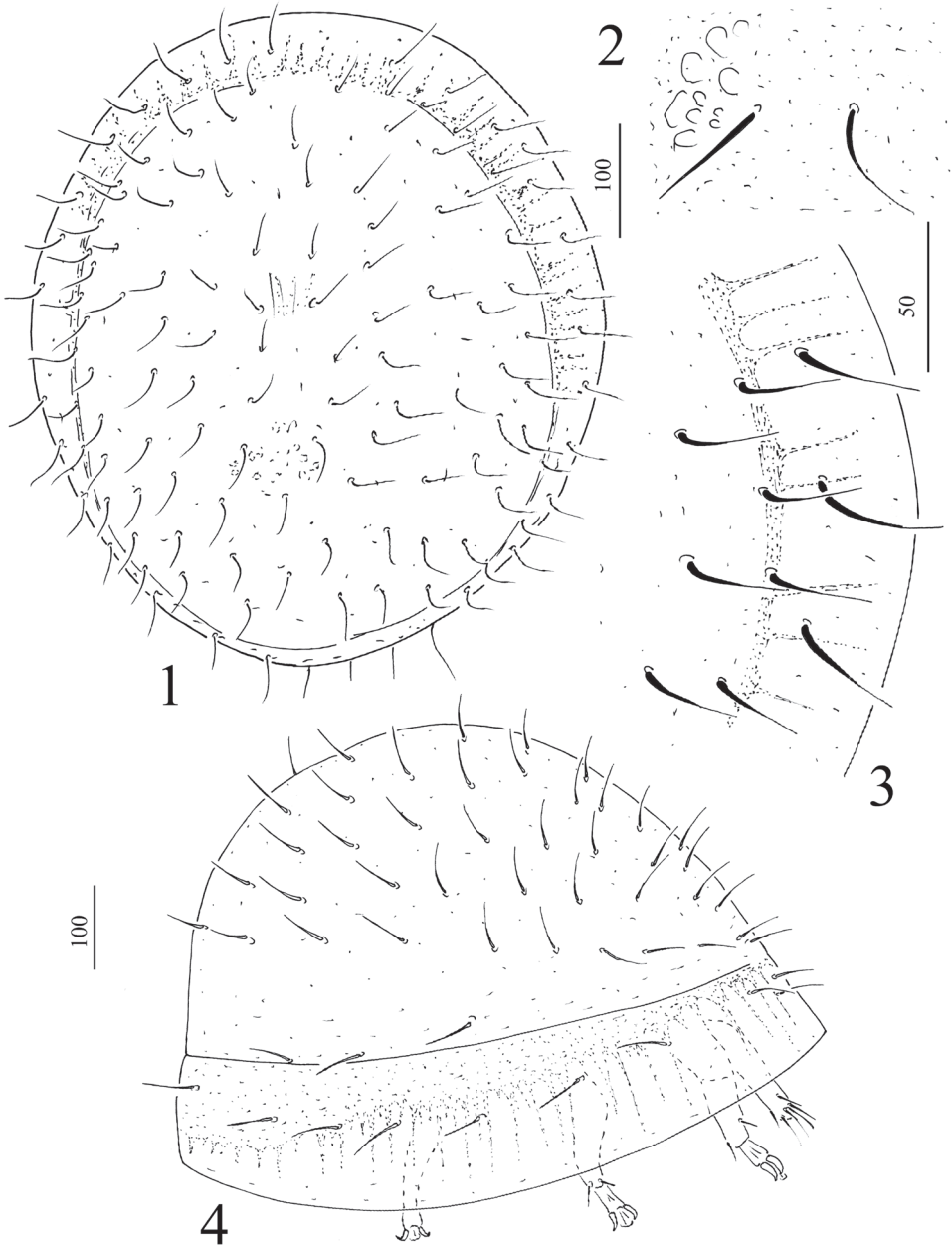
***Sumatrella chelonica* sp. n.**
http://zoobank.org/F7450850-E63B-4B07-B18F-346699ACF37F
Figs 1–22

Material examined. *Holotype*. Female. Indonesia, Sumatra, West Sumatra Province, primary forest at buttom of Haran Canyon, near Echo Point, N of Pavakumbah, 0°06'21"S, 100°39'50"E, 500m, 8.VI.2006. leg. A. Schulz. *Paratypes.* Three females from Indonesia, Sumatra, West Sumatra Province, primary forest at buttom of Haran Canyon, near Echo Point, N of Pavakumbah, 0°06'21"S, 100°39'50"E, 500m, 8.VI.2006. leg. A. Schulz and 7 females from Indonesia, Sumatra, West Sumatra Province, distributed primary forest near road Lubuksikaping Bonjol, *ca.* 10 km S of Lubuksikaping, 0°03'16"N, 100°12'33"E, 500m, 12.VI.2006. leg. A. Schulz.

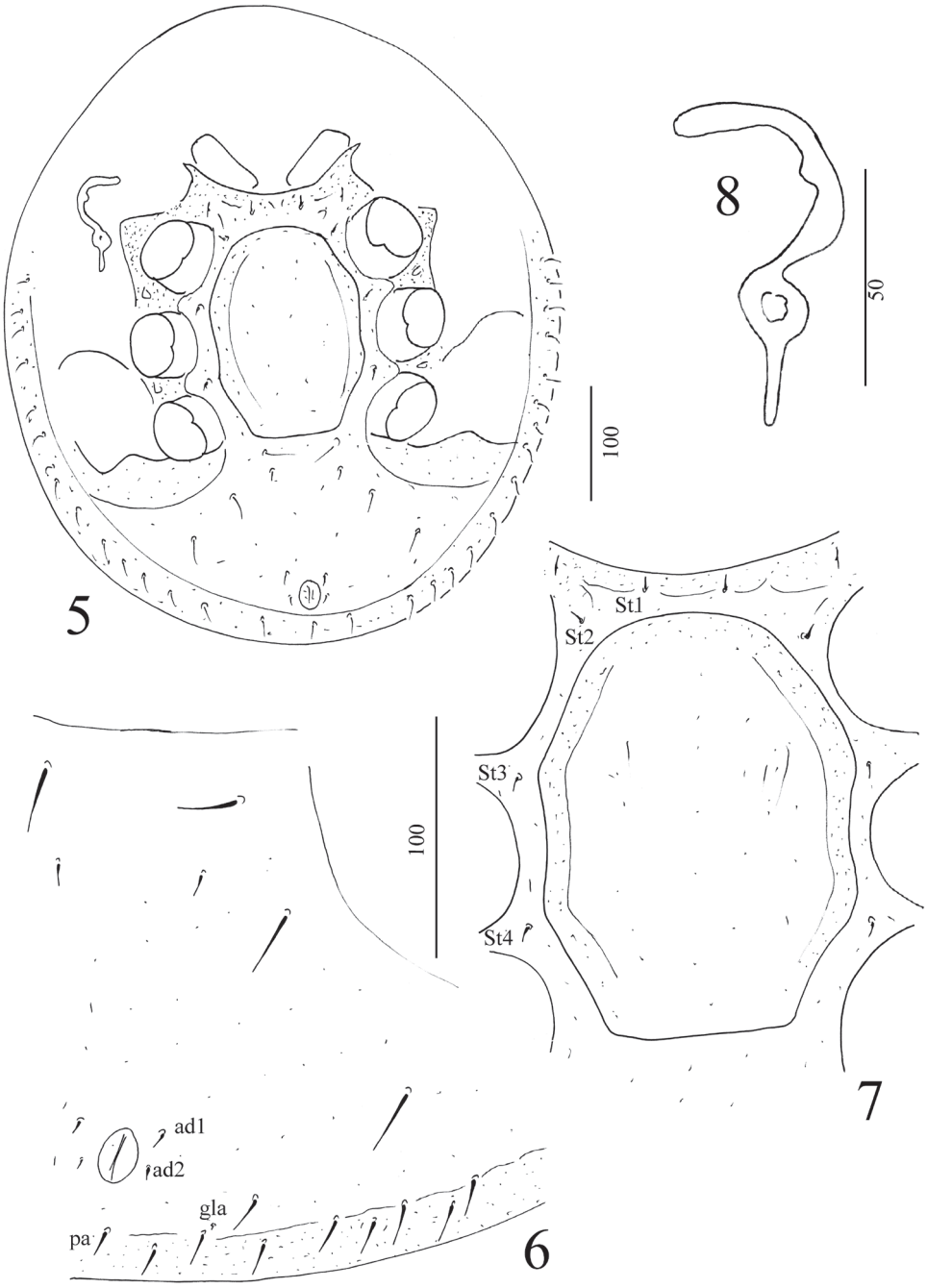
Diagnosis. As for the genus.

Description of the females. Length of idiosoma 560–580 μm, width 470–510 μm, height 560–570 μm (n=11). Shape oval, posterior margin rounded and dorsally extremely domed (Figs 18–19). Color reddish brown.

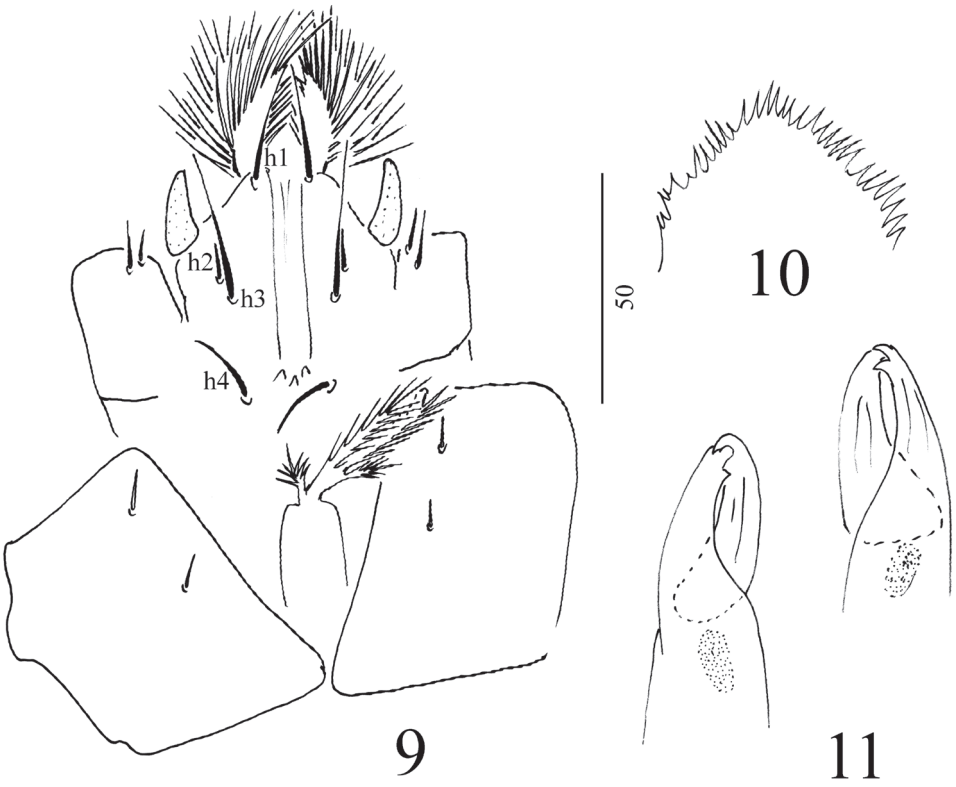
Dorsal idiosoma (Figure 1): Dorsal and marginal shields fused apically. Dorsal shield neotrichous, all dorsal setae smooth and needle like (*ca.* 32–44 μm) (Figure 2). Surface of dorsal shield smooth, only some muscle scars can be seen at level of coxae IV. Marginal shield very wide (Figure 4) with darker and spine–like patterns on inner margins, setae on marginal shield similar in shape and length to dorsal setae (Fig. 4).



Figures 1–4. *Sumatrella chelonica* gen. n., sp. n., female, holotype: **1** body in dorsal view **2** setae on dorsal shield **3** setae on marginal shield **4** body in lateral view. Scale bars in micrometers.

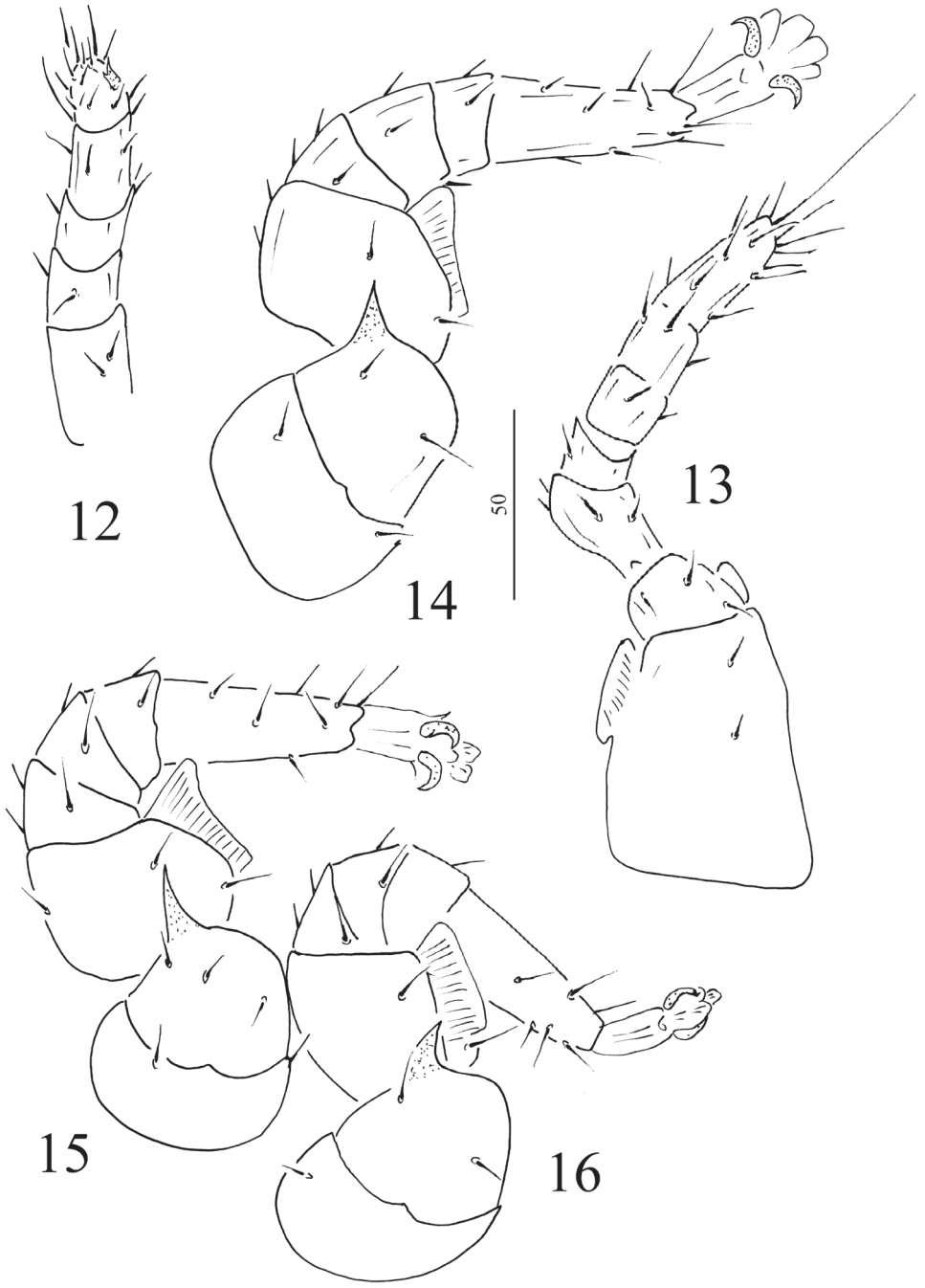


Figures 5–8. *Sumatrella chelonica* gen. n., sp. n., female, holotype: **5** body in ventral view **6** anal and ventral regions **7** intercoxal area **8** peritreme. Scale bars in micrometers.

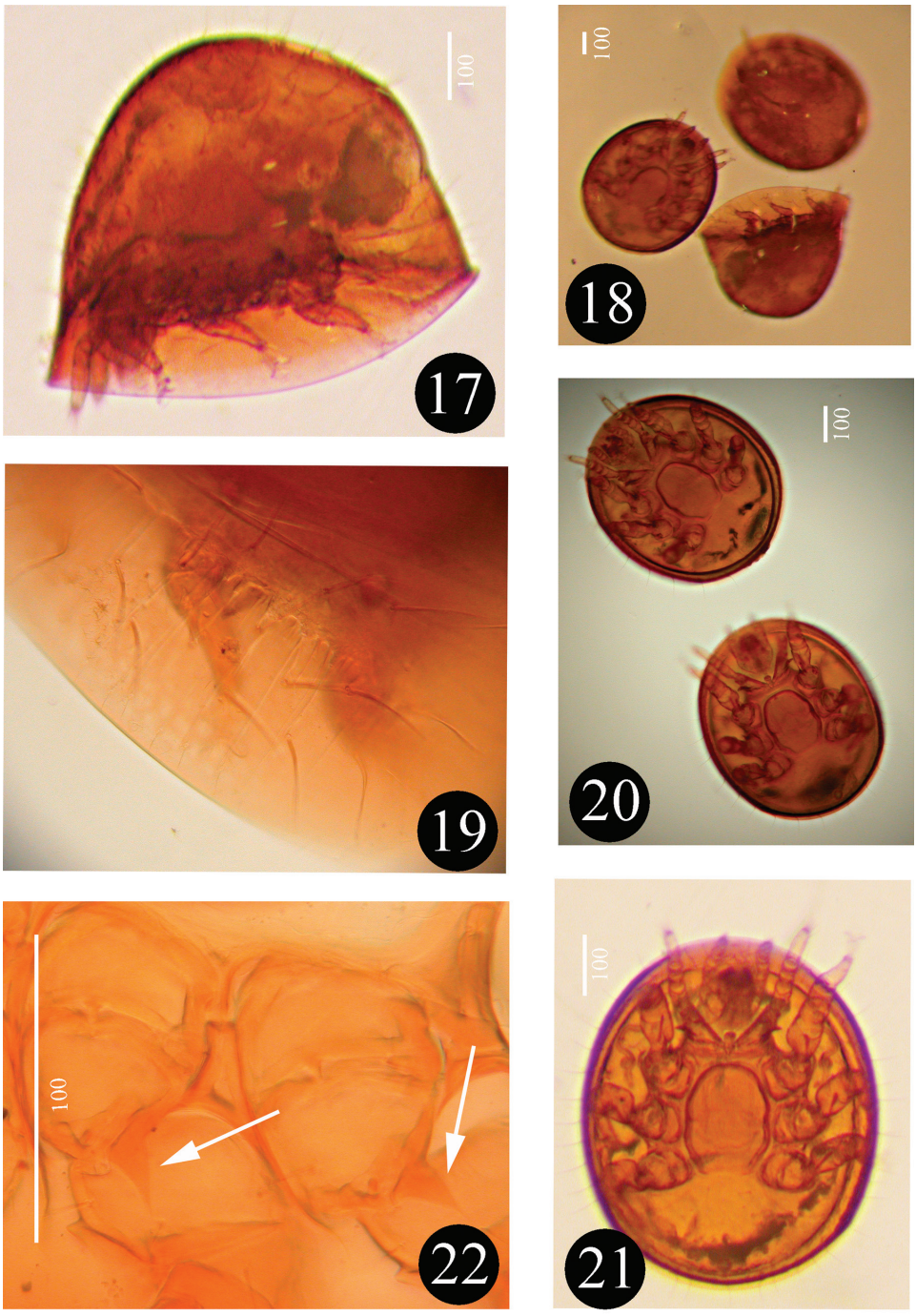


Figures 9–11. *Sumatrella chelonica* gen. n., sp. n., female, holotype: **9** ventral view of tritosternum, gnathosoma and coxae I **10** epistome **11** chelicerae. Scale bars in micrometers.

Ventral idiosoma (Figure 5): Tritosternum with narrow, quadrangular basis; laciniae with two short and two long pilose branches (Figure 9). Sternal shield without ornamentation, four pairs of sternal setae smooth, short (*ca.* 6–7 μm) and needle-like. St1 situated near anterior margin of sternal shield, St2 at level of anterior margin of genital shield, St3 at level of anterior margin of coxae III, St4 at level of posterior margin of coxae III. One pair of lyriform fissure situated close to anterior margin of sternal shield. Three pairs of longer (*ca.* 25–30 μm) and one pair of shorter (*ca.* 10–11 μm) ventral setae situated, all ventral setae smooth and needle-like. Two pairs of adanal setae short (*ca.* 5–6 μm) smooth and needle-like, postanal seta smooth, needle-like and long (*ca.* 14–15 μm). Second setae from postanal seta associated with a setae-like sensory organ. Margins of ventral idiosoma bearing numerous smooth and needle-like setae (*ca.* 14–17 μm). Surface of ventral shield without ornamentation (Figure 6). Genital shield octagonal, *ca.* 180 μm long and *ca.* 130 μm wide, without sculptural pattern and anterior process. Stigmata situated between coxae II and III. Prestigmatic part of peritremes C-shaped with a very short central branch, poststigmatic part short and straight (Figure 8). Pedofossae deep, their surface smooth, separated furrows for tarsi IV absent.



Figures 12–16. *Sumatrella chelonica* gen. n., sp. n., female, holotype: **12** ventral view of palp **13** ventral view of leg I **14** ventral view of leg II **15** ventral view of leg III **16** ventral view of leg IV. Scale bars in micrometers.



Figures 17–22. Photos about *Sumatrella chelonica* gen. n., sp. n., female: **17** body in lateral view in holotype **18** bodies in lateral, ventral and dorsal views in paratypes **19** marginal shield and setae in holotype **20** bodies in ventral view of paratypes **21** body in ventral view of holotype III **22** triangular processes on trochanters of legs II-III in holotype (arrows show the processes). Scale bars in micrometers.

Gnathosoma (Figure 9): Corniculi horn-like, internal malae longer than corniculi and divided into several pilose branches. All hypostomal setae smooth and needle-like, h1 (*ca.* 25–27 μm) situated near anterior margin of gnathosoma, h2 very short (*ca.* 9–10 μm) and situated close to h3 and placed lateral to h1-h4 row. Setae h3 long (*ca.* 33–35 μm), h4 shorter (*ca.* 16–17 μm). Three ventral denticles situated on central part of ventral gnathosoma at level of h4.

All setae on palp smooth and needle-like (Figure 12). Epistome hemispherical and marginally pilose (Figure 10), chelicerae with one teeth on movable and fixed digits, internal sclerotized node present (Figure 11).

Legs (Figures 15–18): Claws absent at the tip of the ambulacral prolongation of leg I. Flap-like prolongations placed on femora II–IV and an unusual triangular process situated on trochantes II-IV.

Male, nymph and larva are unknown.

Etymology. The name of the new species refers to the raised shape of the mite body which is reminiscent of a turtle.

Zoogeographical notes

Species of the probably closely related genus *Chelonuropoda* Sellnick, 1954 occur in South America and the Afrotropical region; a distribution pattern which has been named 'Amphiatlantic' (Kontschán and Starý 2012). Based on zoogeography, the genus *Chelonuropoda* must have originated during a geological period when Africa and South America were still connected to each other; i.e. prior to the Upper Cretaceous. The new genus occurring on Sumatra Island is not situated on a Gondwanan fragment. Therefore we can consider two hypotheses about its distribution: either Sumatra was colonized by the new genus by other dispersal means, or the similarities in morphology are the result of parallel evolution and are examples of homoplasy.

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The distribution and taxonomy of *Lissotriton* newts in Turkey (Amphibia, Salamandridae)

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Abstract

Two and perhaps three taxa of *Lissotriton* newt occur in Turkey. Their species status is controversial. The distribution of these taxa and the taxonomic status of each are reviewed and discussed. A database of 128 Turkish *Lissotriton* localities was compiled and species distribution models were constructed. We reiterate that the presence of *L.* (*v.*) *lantzi* in Turkey is disputed and needs confirmation. The range of *L.* (*v.*) *kosswigi* is restricted to north-western Anatolia – given the small global range of this Turkey endemic, a closer look at its conservation status is warranted. The distribution of *L. v. schmidtleri* covers western Asiatic and European Turkey. The findings support an allopatric distribution of the Turkish *Lissotriton* species. We reflect on the biological significance of previously reported morphological intermediates between *L.* (*v.*) *kosswigi* and *L. v. schmidtleri* in the light of the recent proposal to recognize *kosswigi* at the species level. The available data are in line with species status for *L.* (*v.*) *lantzi* and *L.* (*v.*) *kosswigi*. Although *L. v. schmidtleri* is a genetically diverged taxon as well, the extent of gene flow with parapatric European *Lissotriton* taxa is as yet unknown.

Keywords

Anatolia, Bosphorus, historical biogeography, *Lissotriton kosswigi*, *Lissotriton lantzi*, *Lissotriton vulgaris schmidtleri*, Smooth newt

Introduction

The Smooth newt *Lissotriton vulgaris* group (Amphibia: Salamandridae) is distributed in Europe and adjacent Asia (Schmidtler and Franzen 2004). The taxonomy of the group is a matter of dispute, with the inclusive taxa usually referred to as subspecies, although some of these have been occasionally regarded as specifically distinct (see Dubois and Raffaëlli 2009, Speybroeck et al. 2010). Based on the taxonomy of Babik et al. (2005) the *Lissotriton vulgaris* group consists of seven taxa, namely *ampelensis* (Fuhn 1951), *graecus* (Wolterstorff 1906), *kosswigi* (Freytag 1955), *lantzi* (Wolterstorff 1914), *meridionalis* (Boulenger 1882), *schmidtleri* (Raxworthy 1988) [following the rationale of Dubois (2007), Dubois and Raffaëlli (2009) make the case that the original name *schmidtleri* as in Raxworthy (1988) is correct, rather than the name *schmidtlerorum* introduced in Raxworthy (1990)], and the nominal species *vulgaris* (Linnaeus 1758).

In Turkey, two and perhaps three *Lissotriton* occur (Fig. 1; Schmidtler and Franzen 2004). The range of *lantzi* covers the Caucasus region and the taxon might occur in the extreme north-east of Turkey, near the border with Georgia (Schmidtler and Franzen 2004, Skorinov et al. 2014). The taxon *kosswigi* is restricted to north-western Anatolia (Schmidtler and Franzen 2004). The taxon *schmidtleri* was originally considered to be restricted to western Asiatic Turkey, but Raxworthy (1988, 1990) suggested it might extend into Europe. Genetic data have subsequently confirmed that this taxon's range encompasses European Turkey (Nadachowska and Babik 2009; Pabijan et al. 2014). The range of *schmidtleri* protrudes further into the Balkan Peninsula, but its range limit is as yet unclear; newts with mitochondrial DNA typical of *schmidtleri* have been recorded as far north-west as easternmost Greece and central Bulgaria (Pabijan et al. 2014). Previous records of *vulgaris* from Turkey reflect incomplete taxonomy and can be referred to the other taxa (cf. Dubois and Raffaëlli 2012, Olgun et al. 1999).

The *Lissotriton vulgaris* group comprises two main morphological types: one with a smooth crest and flappy feet and another with a ragged crest and limited fringing on the feet (Fig. 2). Distinguishing the taxa within the two main groups is less straightforward and this topic is beyond the scope of the present paper (we refer to Raxworthy (1990) and Schmidtler and Franzen (2004) for a detailed treatment). Relevant for the current paper is that *kosswigi* belongs to the 'smooth-crested with flappy feet' type and *schmidtleri* to the 'ragged-crested with limited feet-fringing' type and that morphological intergradation has been reported between these two taxa (e.g. Freytag 1955, 1957, Tabrizi 1980, Yılmaz 1983). In Fig. 2 typical males of *kosswigi* and *schmidtleri* are depicted. Next to the smooth crest and flappy feet, *kosswigi* possesses a tail filament and its crest starts at a more posterior position than in *schmidtleri*. Although *lantzi* belongs to the 'ragged-crested with limited feet-fringing' type as well, confusion with *schmidtleri* is ruled out based on geography.

An overview of the distribution of the Turkish *Lissotriton* taxa is provided by composing a database of localities and constructing species distribution models. The focus is mainly on the taxa *kosswigi* and *schmidtleri* and particularly the supposed genetic admixture between the two. Finally, we reflect on the as yet controversial proposal to treat the Turkish *Lissotriton* taxa as distinct species.

Material and methods

The distribution of *Lissotriton* in Turkey has been reviewed and a database compiled of localities based on: 1) the collection of the Zoology Laboratory of the Department of Biology at Science and Arts Faculty, Adnan Menderes University, 2) extensive personal field observations, and 3) a review of the literature (Bozkurt et al. in press, Çevik et al. 1997, Çiçek and Ayaz 2011, Demirsoy 1996, Eiselt 1966, Freytag 1955, Freytag 1957, Mulder 1995, Olgun et al. 1999, Raxworthy 1988, Schmidtler and Schmidtler 1967, Skorinov et al. 2014, Sparreboom and Arntzen 1987, Tabrizi 1980, Taşkın and Olgun 2003, Yılmaz 1983, 1989). In this paper we particularly focused on *kosswigi*, this being the rarest and most restricted taxon globally. The aim was not to be exhaustive for *schmidtleri*, which is common were not included, and widely distributed in western Turkey. Localities within one kilometre of one another and in such cases the locality with the most accurate information available was chosen. We particularly focused on records of presumed transitional forms between *kosswigi* and *schmidtleri* reported in the literature, considering their relevance in the taxonomic treatment of the different *Lissotriton* taxa occurring in Turkey.

For a species distribution modelling exercise for *lantzi* (and a comprehensive overview of the distribution of this taxon outside of Turkey) we refer to Skorinov et al. (2014). Species distribution models were constructed for *kosswigi* and *schmidtleri* using Maxent 3.3.3k (Phillips et al. 2006). For climate layers bioclimatic variables were used, at 2.5 arcminute resolution (c. 5 × 5 km) available from the WorldClim database 1.4 (Hijmans et al. 2005; http://www.worldclim.org). We trimmed these layers to an extent that broadly encompasses the distribution of the genus *Lissotriton*: the area between -15 and 65 degrees longitude and between 30 and 75 degrees latitude. Following Guisan and Thuiller (2005) and Peterson (2011) a subset considered to reflect physiological limitations of the study species (in this case seasonality) was selected while showing little multicollinearity (a Pearson's correlation of $r < 0.7$): bio10 = mean temperature of warmest quarter, bio11 = mean temperature of coldest quarter, bio15 = precipitation seasonality, bio16 = precipitation of wettest quarter, and bio17 = precipitation of driest quarter. To determine whether our species distribution model performs better than random expectation, we tested its AUC value against a null model based on 99 models for random localities (see Raes and ter Steege 2007 for details). Random point data were created with ENMTools 1.3 (Warren et al. 2010). To more thoroughly cover the range of environmental conditions experienced by *schmidtleri* the only four confirmed populations from outside the Turkish range (noted on Fig. 1; details in Pabijan et al. 2014) were included.

Results

A database of 128 distribution records of Turkish *Lissotriton* newts (49 *kosswigi*, 78 *schmidtleri* and one *lantzi*) is provided in Suppl. material 1. Fig. 1 shows these records

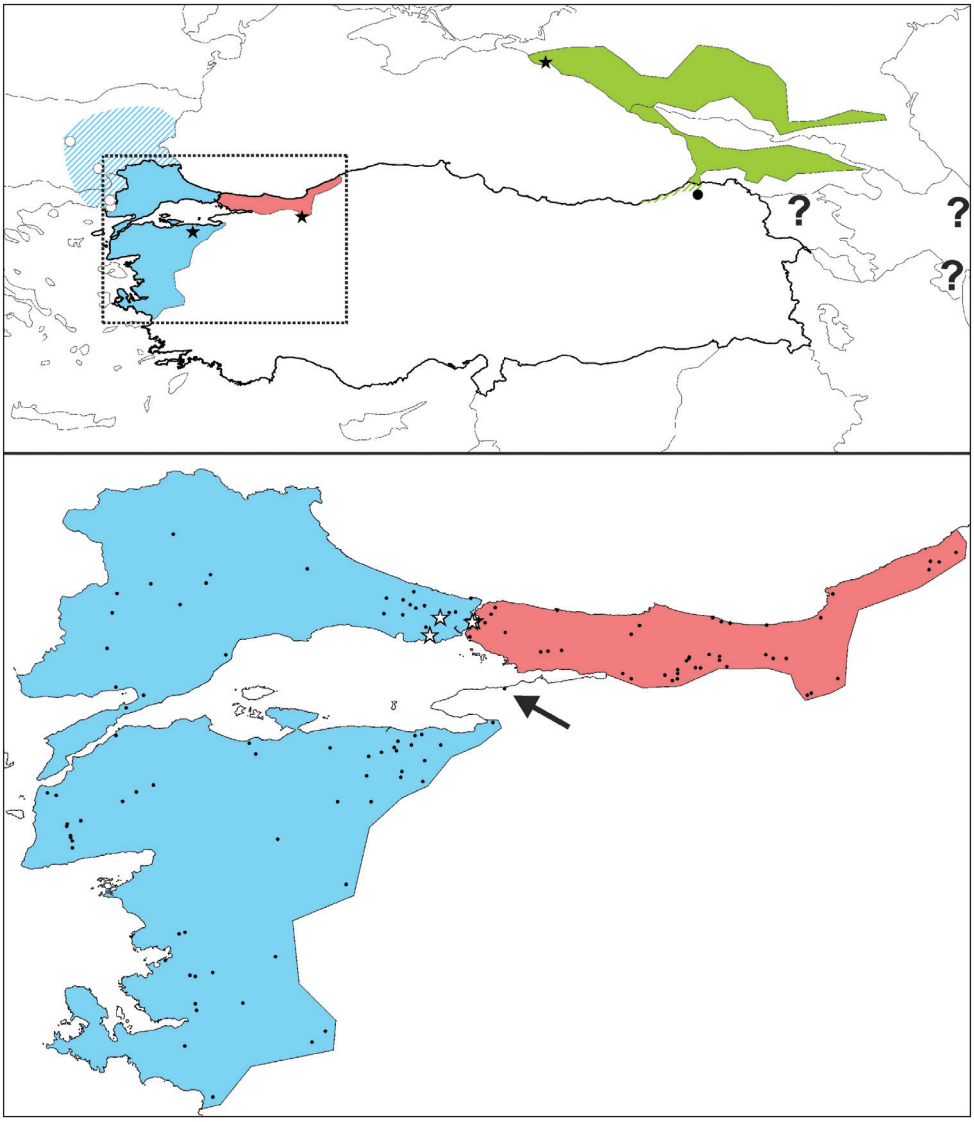

Figure 1. Map showing the distribution of the taxa of the *Lissotriton vulgaris* group that occur in Turkey. The inset shows the rough outlines of the ranges of *lantzi* (in green), *kosswigi* (in red) and *schmidtleri* (in blue). Type localities are marked with a black star. The blue hatched area reflects the unclear range of *schmidtleri* outside of Turkey (see discussion), with four confirmed records denoted with white dots. The green hatched area reflects the potential occurrence of *lantzi* in the extreme northeast of Turkey, with a black dot depicting the single historical record for Turkey (see discussion); question marks denote historical records in Armenia and Azerbaijan. The cut-out shows Turkish localities for *kosswigi* and *schmidtleri* as black dots. Localities supposedly showing intergradation between *kosswigi* and *schmidtleri* are marked with a white star. The arrow highlights a poorly documented locality attributed to *kosswigi* (see discussion). Details on Turkish localities are provided in Suppl. material 1.

plotted on a map. The map also shows the type localities of *kosswigi* and *schmidtleri*, as well as populations reported to contain morphological intermediates between the two taxa. Fig. 3 shows the species distribution models for *kosswigi* and *schmidtleri*. The AUC values of these models (0.991 for both *kosswigi* and *schmidtleri*) rank above the 99 AUC values based on random points, meaning our species distribution models perform significantly better than random expectation ($P < 0.05$).

Discussion

Distribution

The taxon *lantzi* is widely distributed in the Caucasus region (Schmidtler and Franzen 2004, Skorinov et al. 2014). A species distribution modelling exercise (Skorinov et al. 2014) revealed that suitable environmental conditions protrude into the extreme north-east of Turkey, near the border with Georgia. However, the continued occurrence of *lantzi* in Turkey needs confirmation; there is only a single record, dating from the beginning of the twentieth century (Schmidtler and Franzen 2004, Skorinov et al. 2014). Intriguingly, there are also old reports of the Crested newt *Triturus karelinii* (Strauch 1870) from this part of Turkey (Wielstra et al. 2010). Just as *lantzi*, *T. karelinii* is widely distributed in the Caucasus and, although its occurrence in Turkey is suggested by species distribution modelling (Wielstra et al. 2013c), its actual presence requires further scrutiny. In any case, *lantzi* is allopatric from the other Turkish taxa: *Lissotriton* newts are absent from north-east Anatolia (Schmidtler and Franzen 2004; Fig. 1).

The distribution of the Turkey endemic *kosswigi* is restricted to north-western Anatolia (our exhaustive survey revealed 49 localities; Fig. 1). The species distribution model suggests that suitable environmental conditions extend further to the east along most of the Turkish Black Sea coast (Fig. 3). However, this area appears to be devoid of *Lissotriton* newts (Fig. 1). Over-prediction is a well-known problem in species distribution modelling (Elith et al. 2011). This could suggest that the climate layers used to create the species distribution model do not properly reflect the factors limiting the distribution of the species, but it could also suggest that not all suitable area could be colonized due to dispersal constraints.

The taxon *schmidtleri* occurs in the west of Asiatic Turkey and is now known to extend into Europe, across the marine corridor connecting the Aegean and Black Seas (Nadachowska and Babik 2009, Pabijan et al. 2014). The permeability of this apparent barrier can be ascribed to sea level fluctuations related to glacial cycles and the disjunct distribution pattern of *schmidtleri* is mirrored by the co-distributed crested newt species *T. ivanbureschi* Arntzen & Wielstra, 2013 in Wielstra et al. (2013a) (Wielstra and Arntzen 2012). Although the Balkan range of *schmidtleri* outside of Turkey is poorly understood, the taxon appears to occur well into Bulgaria (Pabijan et al. 2014). This is in conflict with the species distribution model (Fig. 3). It could be that mitochondrial DNA does not properly reflect the range of *schmidtleri* and overestimates

Figure 2. Example of the two morphological types comprising the *Lissotriton vulgaris* group of newts. Shown (not to scale) are a typical *kosswigi* male (**A**) and a typical *schmidtleri* male (**B**). Notice the shape of the doral fin (smooth in *kosswigi* and ragged in *schmidtleri*), the position where the dorsal fin starts (approximately above the forelimbs in *kosswigi* and at the back of the head in *schmidtleri*), the presence of a thread-like tail filament (found in *kosswigi* but not in *schmidtleri*) and the extensiveness of the fringing on the feet (with *kosswigi* having much more flappy feet than *schmidtleri*).

its occurrence in Bulgaria. However, we consider it more likely that, due to the lack of confirmed *schmidtleri* localities from Bulgaria (whereas the taxon might well be abundant there), the species distribution model underestimates the environmental space inhabited by *schmidtleri*.

The taxa *kosswigi* and *schmidtleri* currently appear allopatric. We have particularly surveyed the area for *Lissotriton* (pers. obs.) and no localities are known between the *schmidtleri* locality Gemlik (Olgun et al. 1999; locality 62 in Suppl. material 1) and *kosswigi* locality Yalova (Demirsoy 1996; locality 18 in Suppl. material 1). The Yalova locality lacks documentation and needs confirmation (note that the locality was not included in Schmidtler and Franzen 2004) and it is suggested that there is probably a larger distribution gap, with the next closest *kosswigi* locality from the perspective of *schmidtleri* being Kocaeli (museum record; locality 3 in Suppl. material 1). This apparent distribution gap disagrees with the species distribution models, which suggest suitable environmental conditions for both *kosswigi* and *schmidtleri* occur south of the Marmara Sea (Fig. 3).

Based on introgression of *schmidtleri* mitochondrial DNA into *kosswigi* (very similar to mitochondrial DNA found in *schmidtleri* today) it has been hypothesized that

kosswigi displaced *schmidtleri* on the Istanbul Peninsula as the waterway between the Black and Marmara Seas rerouted within the last 10,000 years (Nadachowska and Babik 2009, Wielstra et al. 2013b). Similarly, an as yet undescribed *Triturus* species was proposed to have displaced *T. ivanbureschi* in this region (Wielstra et al. 2013a, 2013b). The species distribution models suggest suitable environmental conditions here for both *kosswigi* and *schmidtleri* and hence do not provide further insight into how *kosswigi* was able to locally outcompete *schmidtleri* (Fig. 3).

Genetic admixture

In light of the current allopatric distribution pattern of *kosswigi* and *schmidtleri*, previous reports of transitional forms are curious. Following up on a possible intermediate specimen from Sapanca, Eiselt (1966) could only confirm the presence of pure *kosswigi* there. Freytag (1955) indicated that in a *Lissotriton* population from Kanlıca (locality 37 in Suppl. material 1), on the eastern side of the Bosphorus, some males showed characteristics of *schmidtleri*, namely the dorsal fin being ragged and starting at the back of the head and the lack of a tail filament (cf. Fig. 2). Tabrizi (1980) studied a larger sample of newts from populations throughout the range of *kosswigi*. He found that four out of 70 newts in Kanlıca showed a *schmidtleri*-like, relatively anterior starting position of the dorsal fins; all other newts were classified as typical *kosswigi*. Considering the biogeographical scenario outlined above, a relict *schmidtleri* population in the process of being replaced by *kosswigi* via genetic swamping is a possibility. A study on historical gene flow between the two taxa unfortunately did not include samples from the potentially admixed populations, but did suggest ancient gene flow from *schmidtleri* into *kosswigi* (Nadachowska and Babik 2009).

Furthermore, Freytag (1957) mentioned that in a *Lissotriton* population from Baltalimanı (locality 104 in Suppl. material 1), on the western side of the Bosphorus, some males shared similarities with *kosswigi*, in terms of possessing tail filaments and smooth dorsal fins that started relatively posteriorly (Fig. 2). Yılmaz (1983) studied a larger sample encompassing more populations from European Turkey. He noted newts with *kosswigi* characteristics at Habibler and Küçükçekmece (localities 121 and 125 in Suppl. material 1). Out of 80 studied newts, 20 had dorsal fins that began at the forelimbs rather than at the back of the head, 41 had smooth dorsal fins and 37 had tail filaments to varying degree (17 with 0–2 mm, 10 with 2–4 mm, 5 with 4–6 mm, and 5 with over 6 mm). Schmidtler and Franzen (2004) state that in *schmidtleri* males can show *kosswigi*-like characteristics, but do not provide further details. The presence of *kosswigi* west of the Bosphorus would not make sense in light of the biogeographical scenario outlined above, unless it could be proven that the Bosphorus on initial formation had a more westward position or formed only after *kosswigi* reached European Turkey. The rerouting of the marine connection between the Marmara and Black Seas is not yet fully understood and a matter of debate in the paleogeological literature (e.g. Nazik et al. 2011, 2012, Yaltırak et al. 2012). We suggest that historical

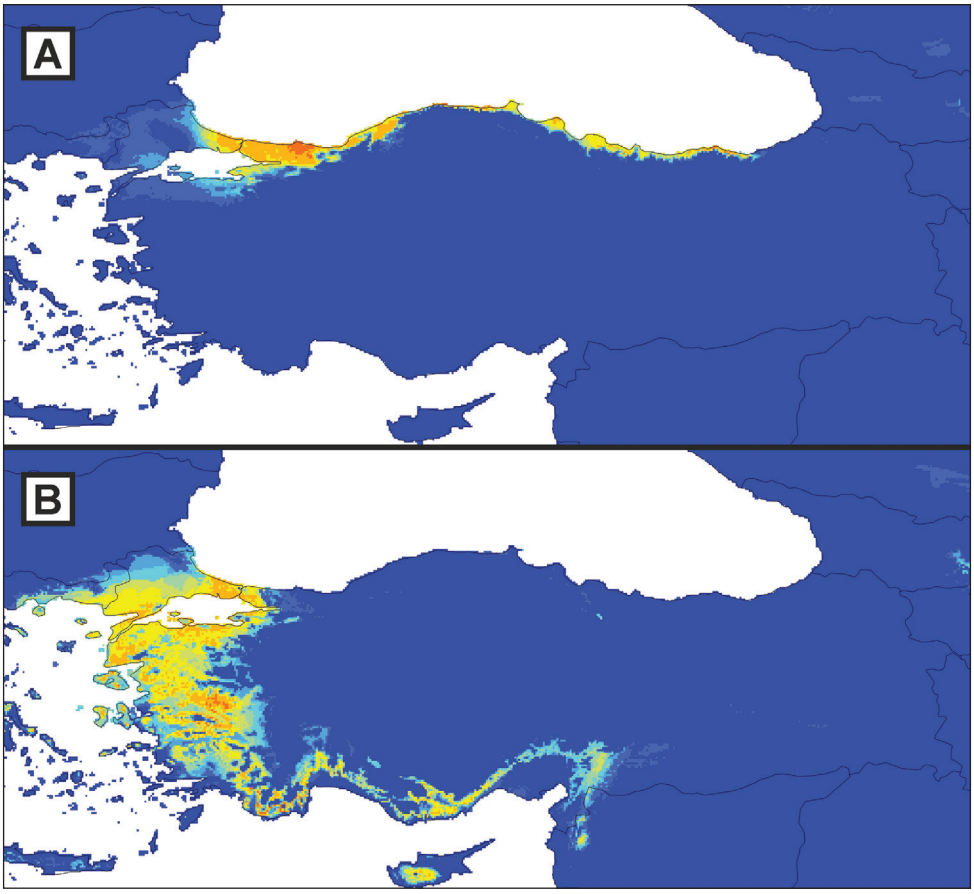

Figure 3. Species distribution models for two Turkish *Lissotriton* taxa. Shown are species distribution models for *kosswigi* (**A**) and *schmidtleri* (**B**). The maps depict predicted suitability, which ranges from 0 to 1, in ten equal intervals, with higher values expressed by warmer colours.

biogeographical patterns such as shown by *Lissotriton* (and *Triturus*) newts might assist paleogeological reconstruction.

Genetic data from the potentially admixed *kosswigi* and *schmidtleri* populations are as yet lacking, but would provide more insight in the matter. However, considering the expansion of the Istanbul agglomeration it should be taken into account that these populations might well have gone extinct. We conclude that potential *kosswigi-schmidtleri* admixture represents, at most, the remnants of a former contact zone. The main ranges of the two taxa are currently isolated in the region by the Bosphorus and hence the influence of potentially admixed populations on the genetic integrity of the two taxa can be expected to be negligible. In this light we make some remarks on the not (yet) generally accepted treatment of the Turkish *Lissotriton* taxa as distinct species (Dubois and Raffaëlli 2009, Frost 2014).

Taxonomy

Following the taxonomy of Babik et al. (2005), the *Lissotriton vulgaris* group consists of seven taxa, namely *ampelensis*, *graecus*, *kosswigi*, *lantzi*, *meridionalis*, *schmidtleri* and the nominal *vulgaris*. Four of these taxa, *graecus*, *kosswigi*, *lantzi* and *meridionalis*, are sometimes regarded as specifically distinct (Dubois and Raffaëlli 2009, Frost 2014). The split of *graecus* and *meridionalis* has been criticised (Speybroeck et al. 2010) as a misinterpretation of the phylogenetic position of the congener *L. montandoni* which, due to mitochondrial DNA introgression, is nested within the *L. vulgaris* group from the perspective of mitochondrial DNA (Babik et al. 2005, Zieliński et al. 2013). However, the taxa *kosswigi* and *lantzi* are genuinely genetically diverged for mitochondrial DNA (Babik et al. 2005).

Within the *Lissotriton vulgaris* group mitochondrial DNA suggests a basal split between *lantzi* and the rest (Babik et al. 2005). Although the distinction of *lantzi* from the perspective of the nuclear genome has as yet not been determined, the divergence in the mitochondrial genome and the at least currently disjunct distribution support a scenario of long-term disrupted gene flow with other *Lissotriton* newts.

The next split in the *Lissotriton vulgaris* group is between *kosswigi* and the remaining taxa (Babik et al. 2005). The distinction of *kosswigi* from its geographical neighbour *schmidtleri* has been supported in a study exploring gene flow based on eight nuclear DNA markers (Nadachowska and Babik 2009). Given that *kosswigi* is genetically distinct and currently allopatric from other *Lissotriton* taxa, its treatment at the species level seems justified. From the conservation perspective it is important whether this geographically restricted, Turkish endemic is treated as a 'unique species' or 'merely a subspecies'.

Although *schmidtleri* represents a distinct mitochondrial DNA clade as well, it is genetically nested within the European *Lissotriton* taxa (Babik et al. 2005, Pabijan et al. in prep.). The phylogeography of *Lissotriton* on the Balkan Peninsula is highly complex, with morphologically distinct subspecies being highly polyphyletic from the mitochondrial DNA perspective (Babik et al. 2005, Pabijan et al. in prep.). Furthermore, no doubt in part because of its turbulent taxonomical history, the morphological distinctiveness of *schmidtleri* is not well understood (Schmidtler and Franzen 2004). Hence, we refrain from making further comments on the taxonomic status of *schmidtleri* and rather await further research on nuclear gene flow between *schmidtleri* and the other *Lissotriton* taxa on the Balkan Peninsula.

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Supplementary material 1

Locality data for Turkish *Lissotriton* newts

Authors: Ben Wielstra, Emin Bozkurt, Kurtuluş Olgun

Data type: ZIP archive

Explanation note: The ZIP archive contains the locality database as an Excel file and a shape file.

ZooKeys 484: 25–52 (2015)
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RESEARCH ARTICLE

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The systematics of *Echinorhynchus* Zoega in Müller, 1776 (Acanthocephala, Echinorhynchidae) elucidated by nuclear and mitochondrial sequence data from eight European taxa

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Abstract

The acanthocephalan genus *Echinorhynchus* Zoega in Müller, 1776 (*sensu* Yamaguti 1963) is a large and widespread group of parasites of teleost fish and malacostracan crustaceans, distributed from the Arctic to the Antarctic in habitats ranging from freshwaters to the deep-sea. A total of 52 species are currently recognised based on the conventional morphological species concept; however, the true diversity in the genus is masked by cryptic speciation. The considerable diversity within *Echinorhynchus* is an argument for subdividing the genus if monophyletic groups with supporting morphological characters can be identified. With this objective in mind, partial sequences of two genes with different rates of evolution and patterns of inheritance (nuclear 28S rRNA and mitochondrial cytochrome c oxidase subunit I) were used to infer the phylogenetic relationships among eight taxa of *Echinorhynchus*. These included representatives of each of three genus group taxa proposed in a controversial revision of the genus based on cement gland pattern, namely *Echinorhynchus* (*sensu stricto*), *Metechinorhynchus* Petrochenko, 1956 and

Pseudoechinorhynchus Petrochenko, 1956. These groupings have previously been rejected by some authorities, because the diagnostic character is poorly defined; this study shows that *Echinorhynchus* (*sensu stricto*) and *Metechinorhynchus* are not natural, monophyletic groups. A revision of *Echinorhynchus* will require tandem molecular phylogenetic and morphological analyses of a larger sample of taxa, but this study has identified two morhological characters that might potentially be used to define new genera. The estimated phylogeny also provides insight into the zoogeographical history of *Echinorhynchus* spp. We postulate that the ancestral *Echinorhynchus* had a freshwater origin and the genus subsequently invaded the sea, probably several times. The freshwater taxa of the *E. bothniensis* Zdzitowiecki & Valtonen, 1987 clade may represent a reinvasion of freshwater by one or more ancestral marine species.

Keywords

Acanthocephala, *Echinorhynchus bothniensis*, *Echinorhynchus brayi*, *Echinorhynchus cinctulus*, *Echinorhynchus gadi*, *Echinorhynchus salmonis*, *Echinorhynchus truttae*, *Acanthocephalus lucii*, phylogeny, molecular phylogeny, taxonomy, parasite, systematics, zoogeography

Introduction

The acanthocephalan genus *Echinorhynchus* Zoega in Müller, 1776 (*sensu* Yamaguti 1963) is a large and widespread group of parasites of teleost fish and malacostracan crustaceans, distributed from the Arctic to the Antarctic in diverse aquatic environments, including mountain streams, rivers, lakes, estuaries, coastal marine waters and the deep-sea. Over the last 125 years the number of described taxa has steadily increased (Fig. 1), a trend which may well continue, since many, if not most potential hosts (particularly from the deep-sea) have yet to be surveyed for parasites. A total of 52 species of *Echinorhynchus* were recognised in the most recent classification of the Acanthocephala (Amin 2013); however, the morphological species concept used to define these taxa masks the true diversity in the genus. Allozyme electrophoresis has revealed cryptic speciation within the marine *E. gadi* Zoega in Müller, 1776 and the freshwater *E. bothniensis* Zdzitowiecki & Valtonen, 1987 (see Väinölä et al. 1994). It is reasonable to assume that other taxa may also comprise sibling species. In addition to demonstrating previously unrecognised diversity in *Echinorhynchus*, allozyme electrophoresis also showed marked genetic divergence between the species of the *E. gadi* complex and *E. salmonis* Müller, 1784 (genetic identity ≈ 0), suggesting that the genus represents "an evolutionary unit deeper and wider than genera in most other animal groups" (Väinölä et al. 1994).

Given the species diversity and genetic divergence within *Echinorhynchus,* it would be useful to split the genus if monophyletic groups with supporting morphological characters can be identified. Petrochenko (1956) attempted to revise this genus on the basis of cement gland pattern, which he considered to be a "fairly constant" taxonomic character. He amended *Echinorhynchus* (type-species: *E. gadi*) to include only those worms which have their cement glands situated along the mid-line like a "string of beads". At the same time, he erected two new genera, *Pseudoechinorhynchus* Petrochenko, 1956 (type-species: *P. clavula* (Dujardin, 1845)) for acanthocephalans displaying

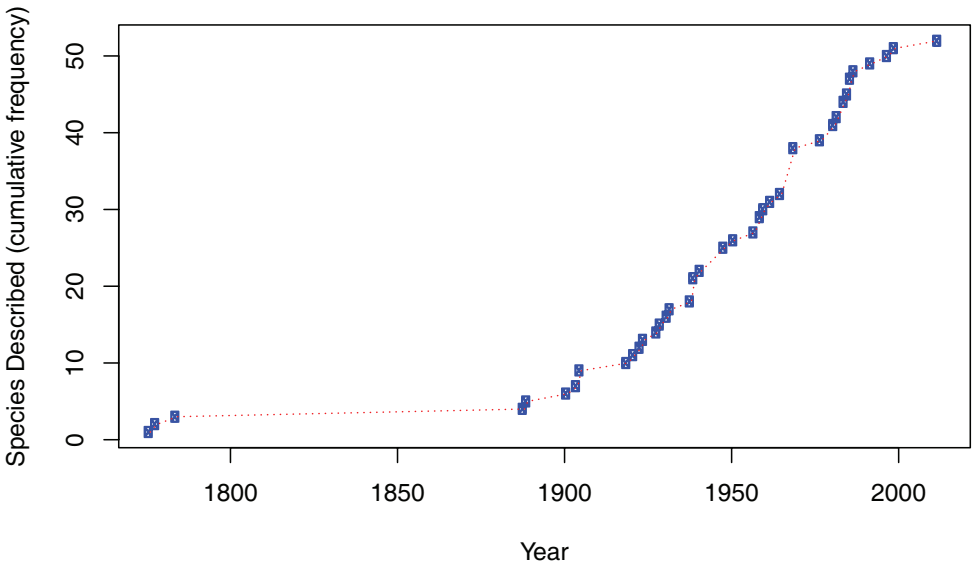

Figure 1. Historical record of species discovery in *Echinorhynchus*. Recognised diversity, as measured by the cumulative number of described taxa, plotted against time. Only species recognised by Amin (2013) are included.

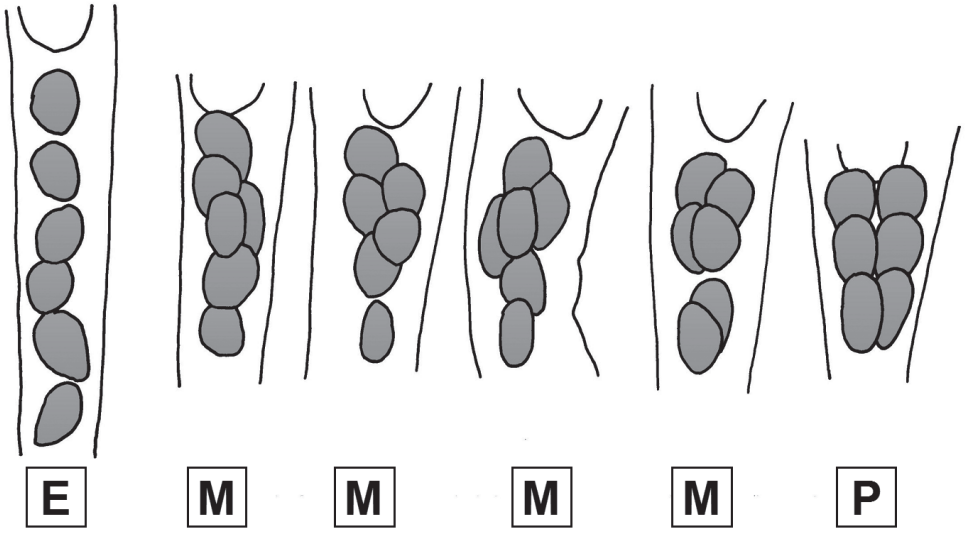

Figure 2. Cement gland arrangements of the genera recognised by Petrochenko (1956). E. *Echinorhynchus*. M. *Metechinorhynchus*. P. *Pseudoechinorhynchus*.

three regular pairs of cement glands and *Metechinorhynchus* Petrochenko, 1956 (type-species: *M. salmonis*) for worms having cement glands arranged in no definite pattern (Fig. 2). Petrochenko's three genera appeared to have the attractive property of being associated with the habitat of the acanthocephalan's hosts: species of *Echinorhynchus*

are parasites of marine fish, whereas species of *Metechinorhynchus* and *Pseudoechinorhynchus* were thought to be typically parasites of freshwater fish.

Golvan (1969) initially accepted Petrochenko's classification with only minor amendments. However, he later relegated *Pseudoechinorhynchus* and *Metechinorhynchus* to the status of subgenera of *Echinorhynchus* (*sensu lato*) (see Golvan 1994). Huffman and Kliever (1977) felt unable to place a new species of *Echinorhynchus* (*sensu lato*) in Petrochenko's system. Most specimens of *E. canyonensis* Huffman & Kliever, 1977 conformed to the diagnosis of *Metechinorhynchus*, but some displayed the moniliform cement gland pattern of *Echinorhynchus* (*sensu stricto*). Huffman and Kliever considered Petrochenko's genera ill-defined and *Metechinorhynchus* to be particularly ambiguous, a view shared by Amin and Redlin (1980), who found that male *E. salmonis* (type-species of *Metechinorhynchus*) frequently exhibited the evenly paired cement glands characteristic of *Pseudoechinorhynchus*. Both pairs of authors concurred with Yamaguti (1963) in regarding *Pseudoechinorhynchus* and *Metechinorhynchus* to be junior synonyms of *Echinorhynchus.* In this paper, *Echinorhynchus* will be used to refer to the broad concept of the genus *sensu* Yamaguti (1963), unless otherwise stated.

Although molecular systematics have revealed that species of *Echinorhynchus* show a degree of genetic divergence that would indicate a generic division, such a division would not produce taxa concordant with Petrochenko's system (Väinölä et al. 1994). If *E. bothniensis* was to be classified under Petrochenko's scheme, it would be placed in *Metechinorhynchus*, since males exhibit no definite cement gland pattern (Zdzitowiecki and Valtonen 1987). However, phylogenetic analysis of allozyme data indicated that *E. bothniensis* has a much closer affinity to the *E. gadi* (type-species of *Echinorhynchus* (*sensu stricto*)) complex than to *E. salmonis* (type-species of *Metechinorhynchus*), indicating that *Metechinorhynchus* would be paraphyletic.

A further problem for Petrochenko's classification is the taxonomic status of *P. clavula*, his type-species for *Pseudoechinorhynchus*. When Petrochenko published his classification, two morphologically distinct species were conflated under the specific binomen *Echinorhynchus clavula* Dujardin, 1845. Dujardin's original description did not include drawings and lacked sufficient detail for the taxon to be reliably identified by other workers. Subsequently, Lühe (1911) made a redescription of the species with figures, based on a collection of acanthocephalans which conformed to Dujardin's incomplete description, but were not in fact conspecific. Lühe's more detailed description became the reference for determining this taxon.

The incompatibility between *E. clavula* Dujardin and *E. clavula* Dujardin *sensu* Lühe (1911) became apparent when Grabda-Kazubska and Chubb (1968) compared acanthocephalans determined as *E. clavula* from the British Isles with those from Poland which fitted the description given by Lühe (1911). Both groups conformed to the diagnosis of the subfamily Echinorhynchinae Cobbold, 1879, but they differed from each other in a key generic character, the position of the nerve ganglion in the proboscis receptacle. In the acanthocephalans from the British Isles, the nerve ganglion was situated at the base of the proboscis receptacle, placing this group in the genus *Acanthocephalus* Koelreuther, 1771. However, in the Polish sample, the nerve ganglion

was situated mid-way along the proboscis receptacle, as is characteristic of species of *Echinorhynchus*. Through reference to Dujardin's unpublished drawings of *E. clavula*, which indicated a basal position for the nerve ganglion in the proboscis receptacle, Grabda-Kazubska and Chubb (1968) were able to conclude that the material from the British Isles conformed to the original concept of *E. clavula* and that the correct name of this taxon was *Acanthocephalus clavula* (Dujardin, 1845). These authors asserted that *E. clavula* Dujardin *sensu* Lühe should remain in the genus *Echinorhynchus* under the name of *E. borealis* von Linstow, 1901. However, since this latter name is pre-occupied, *E. borealis* von Linstow, 1901 is now considered a synonym of *E. cinctulus* Porta, 1905 (see Golvan 1994, Amin 2013). Petrochenko (1956) used Lühe's description of *E. clavula* in his classification and therefore *E. cinctulus* would be the type-species of *Pseudoechinorhynchus*, if this genus was to be recognised as a valid taxon.

Further attempts at revising *Echinorhynchus* should be underpinned by evidence of the phylogenetic relationships of its constituent taxa. To this end we have used sequences from two genes with different patterns of inheritance and different rates of evolutionary change (28S rRNA and cytochrome c oxidase subunit I) to reconstruct a phylogeny for nine populations of *Echinorhynchus*, representing eight distinct biological taxa (Table 1). In addition to resolving taxonomic problems, phylogenetic analyses of the relationships of *Echinorhynchus* species present the best means of understanding the zoogeography of the group.

Material and methods

Taxa sampled

Collection data for the samples are provided in Table 1. This section provides a description of the samples analyzed, summarized by nominal taxon. In order to gain insight into the zoogeography of *Echinorhynchus*, samples were selected to include taxa from a range of aquatic environments, including: both lotic and lentic freshwaters, coastal marine waters and the deep-sea. All three of Petrochenko's genera are represented in the material, including the type-species of each. Furthermore, the samples include four taxa of *Metechinorhynchus*, so that the apparent paraphyly of this taxon (Väinölä et al. 1994) can be tested. The samples also represent a range of different levels in the systematic hierarchy from conspecific populations to taxa displaying strong genetic divergence for a congeneric comparison, according to the allozyme study of Väinölä et al. (1994). Individual molecular markers are generally suitable for phylogeny reconstruction at a particular level in the systematic hierarchy (Avise 1994). Consequently, the current study aims to provide some indication of the phylogenetic resolution provided by 28S rRNA and COI genes in terms of acanthocephalan systematics, which should inform the planning of future phylogenetic studies on this group of helminths.

E. bothniensis Zdzitowiecki & Valtonen, 1987 is known from fresh- and brackish-water environments of Northern Fennoscandia. Based on molecular differences, it may

Table 1. Sample information.

Species	Host	Locality	Date collected	Genus *sensu* Petrochenko (1956)	Environment	GenBank # (28S rDNA / COI)	Voucher specimens
Acanthocephalus lucii (outgroup)	*Perca fluviatilis* (L.) (Percidae)	Lake, Bleasby, Nottinghamshire, UK	4/06/1997	*Acanthocephalus*	Freshwater	KM656148 / KP261016	BM(NH) 2002.2.4.284–292
E. bothniensis	*Osmerus eperlanus* (L.) (Osmeridae)	Lake Keitele, central Finland	10/10/1996	*Metechinorhynchus*	Freshwater	KM656146 / KP261018	BM(NH) 2002.2.4.102–122
E. 'bothniensis'	*Platichthys flesus* (L.) (Pleuronectidae) *Mysis segerstralei* Audzijonyte & Väinölä (Mysidae)*	Lake Pulmankijärvi, northern Finland	11/06/1990	*Echinorhynchus*	Freshwater	KM656143 / KP261019	NA
E. brayi	*Pachycara crassiceps* (Roule) (Zoarcidae)	Porcupine Seabight, 49°49.9'N, 13°08.2'W, depth 2,444 m	13/08/1997	*Metechinorhynchus*	Marine, deep-sea	KM656151 / KP261015	BM(NH) 1997.12.8.3 (holotype); BM(NH) 1997.12.8.4–28
E. cinctulus (= *E. borealis*)	*Lota lota* (L.) (Lotidae)	Kuopio, Finland	15/10/1996	*Pseudoechinorhynchus*	Freshwater	KM656142 / KP261014	BM(NH) 2002.2.4.123–131
E. gadi sp. I	*Gadus morhua* L. (Gadidae)	Baltic Sea, off Tvärminne, Hanko	21/10/1992	*Echinorhynchus*	Marine	KM656144 / KP261022	BM(NH) 2002.2.4.90–101
E. gadi sp. I	*G. morhua*	Mys Kartesh, Gulf of Kandalaksha, White Sea	31/08/1994–2/09/1994	*Echinorhynchus*	Marine	KM656150 / KP261021	NA
E. gadi sp. III	*G. morhua*	Mys Kartesh, Gulf of Kandalaksha, White Sea	31/08/1994–2/09/1994	*Echinorhynchus*	Marine	KM656149 / KP261020	NA
E. salmonis	*Coregonus lavaretus* (L.) (Salmonidae)	Bothnian Bay, Baltic Sea	27/08/1996	*Metechinorhynchus*	Freshwater	KM656145 / KP261017	BM(NH) 2002.2.4.132–226
E. truttae	*Salmo trutta* L. (Salmonidae)	Loch Walton Burn, River Carron catchment, central Scotland (National Grid Reference NS 668 865)	24/06/1996	*Metechinorhynchus*	Freshwater	KM656147 / KP261013	BM(NH) 2002.2.4.264–275

*Acanthocephalans from *P. flesus* and *M. segerstralei* were the source of the 28S rDNA and COI sequences, respectively.

be further subdivided into two allopatric taxa (Väinölä et al. 1994). One of them occurs in the Bothnian Bay of the Baltic Sea (type-locality) and Lake Keitele, central Finland, where it uses *Osmerus eperlanus* (L.) as a definitive host and *Mysis relicta* Lovén (= *M. relicta* sp. I *sensu* Väinölä 1986) as an intermediate host. The second one is found in Lake Pulmankijärvi, northern Finland, and was designated *E. 'bothniensis'* (Väinölä et al. 1994). The definitive hosts of *E. 'bothniensis'* include *Coregonus lavaretus* (L.), *Platichthys flesus* (L.) and *Salvelinus alpinus* (L.). *Mysis segerstralei* Audzijonytė and Väinölä 2005 (= *M. relicta* sp. III *sensu* Väinölä 1986) is the intermediate host (Väinölä et al. 1994). Usage of a mysid intermediate host is rare in members of *Echinorhynchus*, being reported for only one other species, the Nearctic *E. leidyi* Van Cleave, 1924 (Prychitko and Nero 1983, Wolff 1984); all other known life-cycles of *Echinorhynchus* spp. involve amphipod intermediate hosts. *E. bothniensis* and *E. 'bothniensis'* cannot be consistently distinguished by morphology alone (Wayland 2013), but the range of their cement gland patterns, like those of many other species in the genus, straddle the generic boundaries proposed by Petrochenko (1956). Most specimens of *E. bothniensis* conform to the diagnosis of *Metechinorhynchus*, whereas the majority of specimens of *E. 'bothniensis'* conform to the diagnosis of *Echinorhynchus* (*sensu stricto*).

E. brayi Wayland, Sommerville & Gibson, 1999 was described from *Pachycara crassiceps* (Roule) (Zoarcidae) collected from the Porcupine Seabight at a depth of 2,444 metres (Wayland et al. 1999). The samples used in this study were collected from the same host (infrapopulation) as the type-specimens. Similarities in morphology and common usage of a deep-sea zoarcid definitive host suggest a phylogenetic affinity to the Pacific *E. canyonensis* Huffman & Kliever, 1977. The intermediate host of *E. brayi* is not known, but may well be an amphipod, given that this crustacean order is both the typical intermediate host of *Echinorhynchus* spp. and an important part of the diet of *P. crassiceps*. Allozyme electrophoresis has previously shown that *E. brayi* is genetically divergent from the *E. gadi* complex, sharing not one allozyme at any of seven surveyed loci (Wayland et al. 2005). *E. brayi* displays the cement gland arrangement characteristic of *Metechinorhynchus* (Table 2).

As explained in the Introduction, *E. cinctulus* Porta, 1905 is the correct name for the type-species of Petrochenko's genus *Pseudoechinorhynchus* that has commonly been referred to as *E. borealis* Linstow. This species is found in fresh and oligohaline waters of the Palaearctic (Grabda-Kazubska and Ejsymont 1969). The burbot *Lota lota* (L.) (Lotidae) is the usual definitive host, but it has been found in a systematically diverse range of fishes (Grabda-Kazubska and Ejsymont 1969). Intermediate hosts of *E. cinctulus* are the amphipods: *Gammarus pulex* L. (see Nybelin 1923), *Pallaseopsis quadrispinosa* (G.O. Sars, 1867) (see Valtonen and Crompton 1990) and *Monoporeia affinis* (Lindström, 1855) (see Bauer 1953).

E. gadi Zoega in Müller, 1776, the type-species of *Echinorhynchus*, is the most frequently reported acanthocephalan from fish of the North Atlantic and North Pacific Oceans (Gibson 2001). The definitive host spectrum is broad, and numerous amphipod crustacean species have been reported as intermediate hosts (Marcogliese 1994). Using allozyme electrophoresis Väinölä et al. (1994) demonstrated that *E. gadi* from gadid fish

Table 2. Cement gland arrangement in male *Echinorhynchus* spp. Notation for cement gland pattern from Shostak et al. (1986): A, clumped, three even pairs; B, clumped, three staggered pairs; C, chain-like, two pairs and two singles; D, chain-like, one pair and four singles; E, chain-like, six singles. Only specimens with six cement glands were used. Data sources: *E. bothniensis, E. 'bothniensis'* and *E. truttae* (Wayland 2013); *E. brayi*, *E. gadi* and *E. salmonis* (Wayland 2002); *E. cinctulus* (Grabda-Kazubska and Ejsymont 1969).

Species	A	B	C	D	E
E. bothniensis	0	1 (5.3%)	4 (21.1%)	10 (52.6%)	4 (21.1%)
E. 'bothniensis'	0	0	0	4 (44.4%)	5 (55.6%)
E. brayi	1 (8%)	7 (54%)	3 (23%)	2 (15%)	0
E. cinctulus	218 (100%)	0	0	0	0
E. gadi	0	0	0	3 (8%)	34 (92%)
E. truttae	0	1 (3%)	16 (53%)	13 (43%)	0
E. salmonis	6 (37.5%)	10 (62.5%)	0	0	0

of the northeast Atlantic comprises at least three, partly sympatric, sibling species, designated species I-III. Species I was present in all regions sampled, namely the northern Baltic, North Sea and Norwegian Sea. Species II was found in the North Sea and species III in the Norwegian Sea. Subsequently, both species I and III were also identified in the Gulf of Kandalaksha, White Sea (Väinölä, unpubl.). In the present study, we analyze allozymically identified samples from the Baltic and White Sea populations of species I and the White Sea population of species III. A later allozyme study also detected two sympatric sibling species of *E. gadi* in gadid fish from the North Sea (termed species A and B) and further demonstrated that they could be distinguished on the basis of subtle differences in hook morphometrics (Wayland et al. 2005). Morphological similarity suggested that species A of Wayland et al. (2005) is probably conspecific with species I of Väinölä et al. (1994). A more recent study of *E. gadi* from Atlantic cod *Gadus morhua* L. did not find variation among eight North Atlantic and Arctic populations in the slowly evolving 18S rRNA sequence marker (Sobecka et al. 2011).

E. salmonis Müller, 1784 is the type-species of Petrochenko's (1956) genus *Metechinorhynchus*. This is a fresh and brackish water species distributed throughout much of the Holarctic. Salmoniform fishes are the usual definitive host of this parasite, but it can develop to sexual maturity in a systematically diverse range of fish hosts (Valtonen and Crompton 1990). The amphipod intermediate hosts include species of *Gammarus* Fabricius, 1775, *Pallaseopsis* Kamaltynov & Väinölä, 2002, *Monoporeia* Bousfield, 1989 and *Diporeia* Bousfield, 1989 (e.g. Valtonen 1980, Measures and Bossé 1993). The population from which the sample used in this study was taken was characterized morphologically by Wayland et al. (2004).

E. truttae Schrank, 1788 is another common parasite of salmonid fishes in northern Europe. In the original description of *E. bothniensis*, Zdzitowiecki and Valtonen (1987) distinguished their new taxon from *E. truttae* on the basis that it had a shorter proboscis and much longer eggs. A subsequent analysis of morphological variation in these taxa demonstrated that *E. truttae* cannot be distinguished from *E. bothniensis* or

E. 'bothniensis' on the basis of proboscis length, egg length or any other conventional morphological character (Wayland 2013). However, *E. truttae* can be discriminated from the *E. bothniensis* group using multivariate analysis of hook morphometrics (Wayland 2013), as applied by the Proboscis Profiler tool (Wayland 2010). The amphipod intermediate hosts include *Gammarus fossarum* Koch, 1836 (see Van Maren 1979) and *G. pulex* L. (see Lühe 1911). Petrochenko (1956) assigned *E. truttae* to *Metechinorhynchus*. The sample was taken from a population which has been studied morphologically (Wayland 2013).

In order to root the phylogenetic trees, sequence data were also determined from *Acanthocephalus lucii* (Müller, 1776), another member of the subfamily Echinorhynchinae. *Acanthocephalus* and *Echinorhynchus* appear to be closely related genera discriminated on the basis of only one morphological character, the position of the nerve ganglion or "brain", which is situated at the base of the proboscis receptacle in *Acanthocephalus* but mid-way along the receptacle in *Echinorhynchus* (see Petrochenko 1956). Moreover, molecular phylogenies for the Acanthocephala demonstrate an affinity between these two genera (García-Varela and Nadler 2005, 2006). The principal definitive host of *A. lucii* is the perch *Perca fluviatilis* L. (see Brattey 1988) and its intermediate host is the isopod *Asellus aquaticus* L. (see Andryuk 1979, Brattey 1983). The cement glands of *A. lucii* are typically arranged in pairs (Petrochenko 1956).

Sample collection and DNA extraction

All acanthocephalans were washed in saline and then fixed in 90–100% alcohol immediately after collection, or alternatively frozen in liquid nitrogen and stored at -80 °C. Single specimens of each sample were used for the sequencing of each gene, but different individuals were analyzed for the different genes (in different laboratories). The anterior ends of the worms were removed before DNA extraction to avoid contamination of the samples with any host tissue attached to the proboscis. For the 28S analysis, individual acanthocephalans were washed in TE, ground in 150 µl TE (pH 8.0), 0.5% SDS, and digested overnight with the addition of 6 µl proteinase K (10 mg ml^{-1}) at 37 °C. DNA was phenol-chloroform extracted and precipitated for 15 minutes at -20 °C with 0.1 vol. sodium acetate, at pH 5.0, and 2.5 vols 100% ethanol. DNA pellets were washed in 70% ethanol, dried, resuspended in TE (pH 8.0) and stored at -20 °C. Spectrophotometry was used to estimate the concentration of nucleic acids. Alternatively, for the COI data set, the CTAB extraction protocol of Doyle and Dickson (1987) was used.

DNA amplification and sequencing

For most taxa, a c.1,600 base-pair segment of the 28S rRNA gene spanning variable regions D1 to D6 was amplified using the primers LSU5 (5′-TAGGTCGACCCGCTGAAYTTAAGCA-3) and LSUD6-3 (5′-GGAACCCTTCTCCACTTCAGTC-3′)

(Littlewood et al. 2000). For sequencing, these two amplification primers along with three internal primers were used (ECD2: 5′-CCTTGGTCCGTGTTTCAAGACGGG-3′, 900F: 5′-CCGTCTTGAAACACGGACCAAG-3′, LSU1200R: 5′-GCATAGTTCACCATCTTTCGG-3′). For a single species, *E. cinctulus*, the 1600-bp fragment could not be amplified in full, but a partial 750-bp fragment was obtained by amplification and sequencing with the LSU5 and ECD2 primers. Amplification was done in 50 µl PCR reactions containing 200 µM of each deoxynucleotide, 2 mM $MgCl_2$, 1 × reaction buffer (Perkin-Elmer, UK), 1 unit of *Taq* DNA polymerase (Amplitaq, Perkin-Elmer, UK), 10 pM of each primer and c.200 ng template DNA. Thermal cycling involved an initial denaturation of 95 °C for 5 minutes followed by 30 cycles of 94 °C/1 minute, 50 °C/1 minute and 72 °C/1 minute, and a final incubation at 72 °C/5 minutes. A minimum of two successful reactions were performed for each template. Amplified products were run on a 1% TAE agarose gel, cut out, pooled and purified using a QIAquick PCR Purification Kit (QIAGEN). Sequencing was performed with standard procedures on a 373 ABI automated sequencer with the ABI PRISM TM dye terminator cycle sequencing ready reaction kit (Perkin-Elmer, UK).The sequences were aligned using ClustalW (Thompson et al. 1994) with default weighting and gap penalties.

For analysis of a part of the mitochondrial COI gene, the universal "barcoding" primers of Folmer et al. (1994) were used for amplification and sequencing, following the procedures in Väinölä et al. (2001). The final COI alignment used for analyses was 585 bp long.

Phylogenetic analysis

The 28S rDNA and COI sequences were analyzed independently and also concatenated into a single dataset. Three methods of phylogenetic reconstruction were applied to each dataset: Bayesian inference (BI), maximum likelihood (ML) and maximum parsimony (MP). *A. lucii* was used as an outgroup in all analyses. For the phylogenetic reconstruction methods involving modelling of sequence evolution (BI and ML), the data-sets were partitioned to accommodate heterogeneity in patterns and rates of substitutions between genes and/or codon positions. The COI data-set was divided into three partitions, one for each codon position. The concatenated 28S rDNA and COI data-set was separated into four partitions, one for the 28S rDNA sequence and three for each of the codon positions in the COI sequence. The 28S rDNA data-set was not partitioned.

Mr Bayes version 3.2.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003) was used for BI, with the following settings: two simultaneous runs with four Markov chains (one cold and three heated) and one million MCMC generations, sampled every 500 generations and a temperature parameter of 0.1. To avoid the uncertainty of selecting the correct substitution model *a priori*, reversible jump MCMC was used to sample across all possible time-reversible rate matrices according to their posterior probability (Ronquist et al. 2012). For each run log likelihood was plotted

against number of generations and burn-in was assumed to have occurred when the curve reached a plateau. The number of generations (samples) discarded as burn-in were 10,000 (20), 30,000 (60) and 70,000 (40), for the 28S rDNA, COI and concatenated data-sets, respectively.

ML analysis was carried out using the genetic algorithm implemented in MetaPIGA 3.1 (Helaers and Milinkovitch 2010). The nucleotide substition model for each data-set was selected using the Bayesian Information Criterion (BIC). For the 28S rDNA data-set, the generalized time reversible (GTR) model (Tavaré 1986) with gamma distributed rate heterogeneity (four categories) was chosen. TN93 (Tamura and Nei 1993) and a gamma distribution with four rate categories was selected as the best model for the COI and concatenated 28S rDNA + COI data-sets (further details of model parameters in Suppl. material 2). Each analysis was run with a minimum of 100 and a maximum of 10,000 replicates and was stopped once the mean relative error among 10 consecutive consensus trees was less than 5%. Starting trees were generated by loose neighbour joining and were selected using the tournament algorithm.

MP analysis was performed using PAUP version 4.0b10 (Swofford 2003). Gaps in the 28S rDNA sequence alignments were treated as missing data. An exhaustive search was performed on each data-set and the frequency distribution of tree scores was determined. Bootstrap resampling (n = 10,000) was used with the branch and bound algorithm to quantify clade support.

Phylograms and other graphics were created using R (R Core Team 2014) and the APE package (Paradis et al. 2004).

Data resources

All sequence data have been submitted to GenBank; accession numbers are provided in Table 1. Additionally, the sequence alignment used in this study is provided in Suppl. material 1.

Results

Patterns of sequence divergence

The aligned partial 28S rDNA sequence data consisted of 1,607 nucleotide sites for all taxa except *E. cinctulus*, for which only the first 750 base pairs of the segment could be sequenced (Suppl. material 1). In comparisons among the *Echinorhynchus* sequences, 261 (16.2%) of the 1,607 sites were variable, and 133 of those (51%) were parsimony informative. Of the ingroup taxa, *E. salmonis* and *E. cinctulus* sequences were the most divergent, differing by 15% and 7%, respectively, from the remaining group of very closely related sequences, which only differed by less than 1% from each other.

Table 3. Observed sequence divergence (%) between pairs of echinorhynchid species for the 28S rDNA (below the diagonal) and COI sequence data (above the diagonal).

	1	2	3	4	5	6	7	8	9	10
1. *A. lucii*	—	36.1	33.3	34.5	32.8	34.2	34.4	34.0	34.0	34.7
2. *E. salmonis*	18.5	—	29.7	27.7	28.7	29.7	29.7	29.4	28.7	28.9
3. *E. cinctulus*	31.1	23.1	—	21.7	22.2	21.5	21.7	22.9	22.9	23.1
4. *E. brayi*	19.1	15.5	6.6	—	16.8	17.4	17.3	19.0	17.1	18.0
5. *E. truttae*	19.3	15.3	7.5	0.8	—	8.2	8.4	9.1	8.9	8.9
6. *E. gadi* sp. I (Baltic Sea)	19.2	15.4	7.1	0.5	0.3	—	0.2	7.2	6.5	6.3
7. *E. gadi* sp. I (White Sea)	19.2	15.4	7.1	0.5	0.3	0.0*	—	7.4	6.5	6.3
8. *E. gadi* sp. III	19.2	15.4	7.1	0.5	0.3	0.0*	0.0*	—	3.3	3.1
9. *E. bothniensis*	19.2	15.4	7.1	0.5	0.3	0.0*	0.0*	0.0*	—	1.5
10. *E. 'bothniensis'*	19.2	15.4	7.1	0.5	0.3	0.0*	0.0*	0.0*	0.0*	—

* sequences are identical

Five samples possessed identical 28S sequences: *E. gadi* sp. I (Baltic Sea), *E. gadi* sp. I (White Sea), *E. gadi* sp. III, *E. bothniensis* and *E. 'bothniensis'* (Table 3).

In the 585 base-pair alignment of the COI sequences, 249 (42.6%) of the nucleotide sites were variable within *Echinorhynchus*, of which 62 (24.9%) were at a first codon position, 23 (9.2%) at a second codon position and 164 (65.9%) at a third codon position (Suppl. material 1). Of the variable sites, 148 (59.4%) were parsimony informative. Uncorrected sequence divergence between pairs of *Echinorhynchus* sequences ranged from 0.2% (Baltic vs. White Sea sequences of *E. gadi* sp. I) to 29.7% (*E. salmonis* vs. *E. cinctulus* and *E. salmonis* vs. *E. gadi* sp. I) (Table 3). In pairwise comparisons of samples with relatively similar COI sequences (uncorrected sequence divergence < 20%), most substitutions were transitions (Suppl. materials 3, 4). However, in comparisons involving the more divergent *E. cinctulus*, *E. salmonis* and *A. lucii*, transitions were generally outnumbered by transversions, suggesting that multiple substitutions at some variable nucleotide sites have erased the record of previous transitions. Saturation occurs primarily at the fast evolving third codon position (Suppl. materials 3, 4).

Phylogenetic relationships

Since identical sequences were obtained from members of the *E. gadi* complex, *E. bothniensis* and *E. 'bothniensis'*, the 28S rDNA data-set could only be used to resolve the deeper branches in the phylogeny. BI identified a hierarchy of three clades, each with a maximal posterior probability (Fig. 3): ((((*E. gadi* complex + *E. bothniensis* complex, *E. truttae*), *E. brayi*), *E. cinctulus*), *E. salmonis*). The 50% consensus tree derived from the ML analysis had an identical topology to the BI tree and moderate bootstrap support for each of the three clades (74–99%). MP analysis yielded two most parsimonious trees (length = 488, consistency index (CI) = 0.957, retention index (RI) = 0.859), the

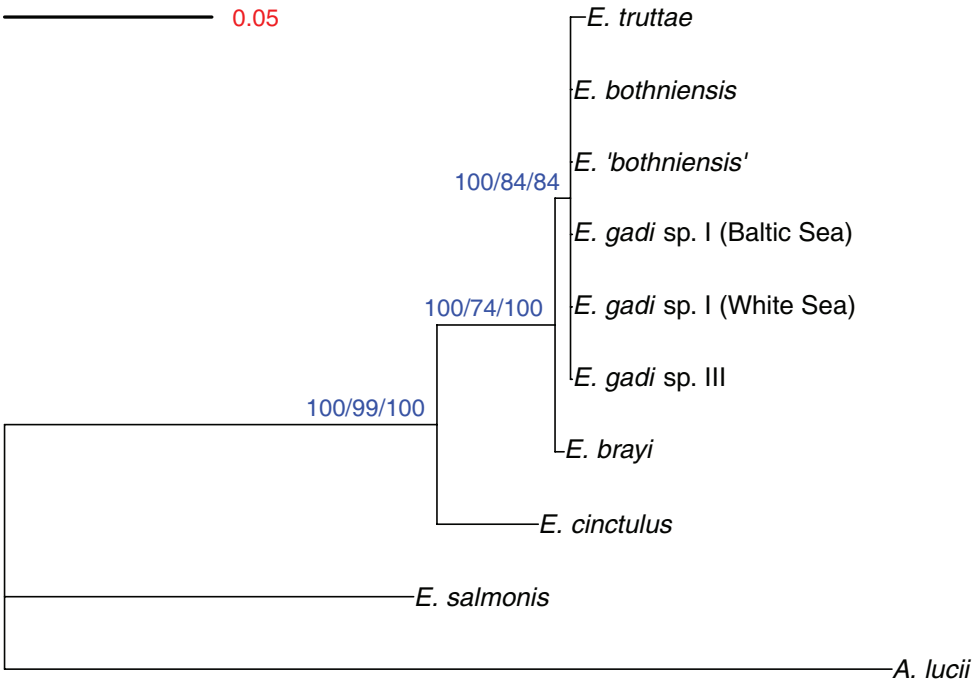

Figure 3. Phylogram estimated using Bayesian inference analysis of 28S rDNA sequence data. Numbers at nodes are clade support values (%) for each method of phylogeny reconstruction (BI/ML/MP). Tree is rooted on the outgroup *A. lucii*.

consensus cladogram for which also had an identical topology to the BI phylogram and provided strong bootstrap support (84–100%) for all three clades.

A fully resolved tree was recovered from the mitochondrial COI data-set (Fig. 4). The topology for the basal parts was identical to that resolved by the 28S data above. Within the remaining terminal cluster of very closely related taxa, the *E. gadi* sp. I sequences from the two regions grouped together and so did *E. bothniensis* + *E. 'bothniensis'*. *E. gadi* sp. III made a sister group to the *E. bothniensis* clade rather than to *E. gadi* sp. I. The BI analysis yielded high posterior probability values (92–100%) for all clades, except for the one comprising all *Echinorhynchus* spp. but *E. salmonis* (81%). The ML tree topology was identical to that from BI, but with a weaker clade support (50–95%).

MP analysis of the COI data-set produced a single most parsimonious tree, 542 steps long (CI = 0.795, RI = 0.615), which differed from the BI and ML phylograms at a single point, regarding the basal placement of *E. cinctulus* instead of *E. salmonis* (Fig. 5a). Strong bootstrap support (86–100%) was found for all clades, except for that defining the basal node and comprising all *Echinorhynchus* but *E. cinctulus*, which only had 66 % support. The conflict between the MP vs. the BI/ML trees appears to be the result of homoplasy at third codon positions. When MP analysis was repeated after eliminating the 3rd codon positions, a total of five most parsimonious

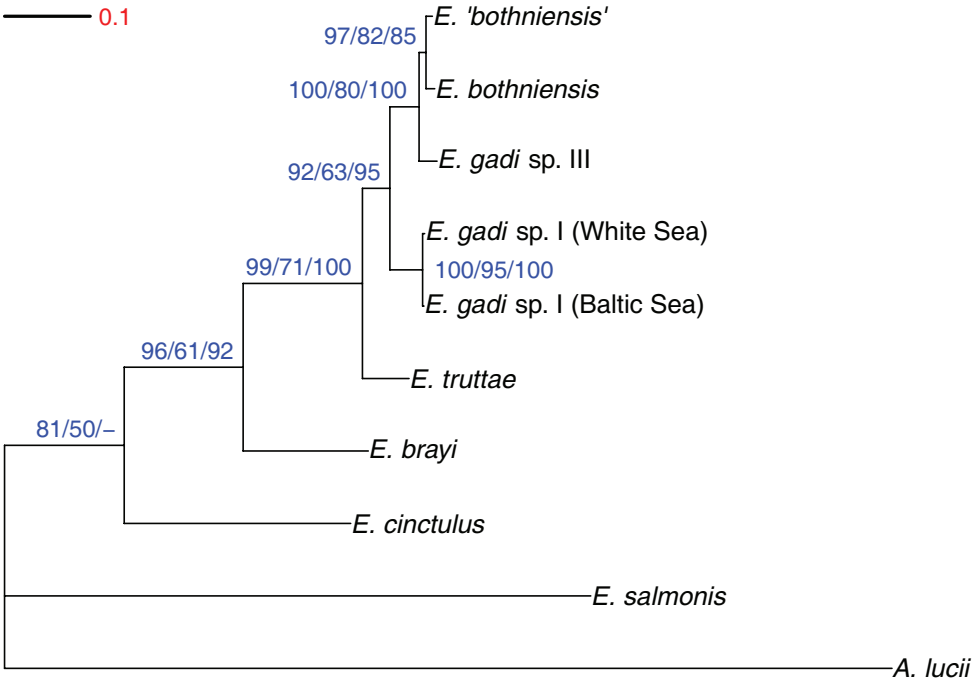

Figure 4. Phylogram estimated using Bayesian inference analysis of COI sequence data. Numbers at nodes are clade credibility values (%) for each method of phylogeny reconstruction (BI/ML/MP). Tree is rooted on the outgroup *A. lucii*.

trees (length = 177, CI = 0.932, RI = 0.786) were found. The consensus cladogram for these five trees (Fig. 5b) is concordant with the BI/ML tree for the full COI data-set. However, the relationships of the six most similar sequences were not fully resolved with the reduced 1st+2nd position data, which retained just 11 variable and only seven parsimony informative characters as regards information within the six-sequence cluster.

BI, ML and MP analysis of the combined data-sets all yielded the same phylogram, which was topologically identical to the BI/ML tree for the COI data-set and displayed similar support for most clades (Fig. 6). The most parsimonious tree (CI = 0.869; RI = 0.691) had a length of 1,033 steps.

Discussion

The following discussion is based on the fully resolved phylogeny recovered from the total molecular data. It is important to note that, whereas the deeper branches in the phylogeny are supported by sequence data from both genes, the interrelationships of the five most closely related species were resolved using the COI data-set alone.

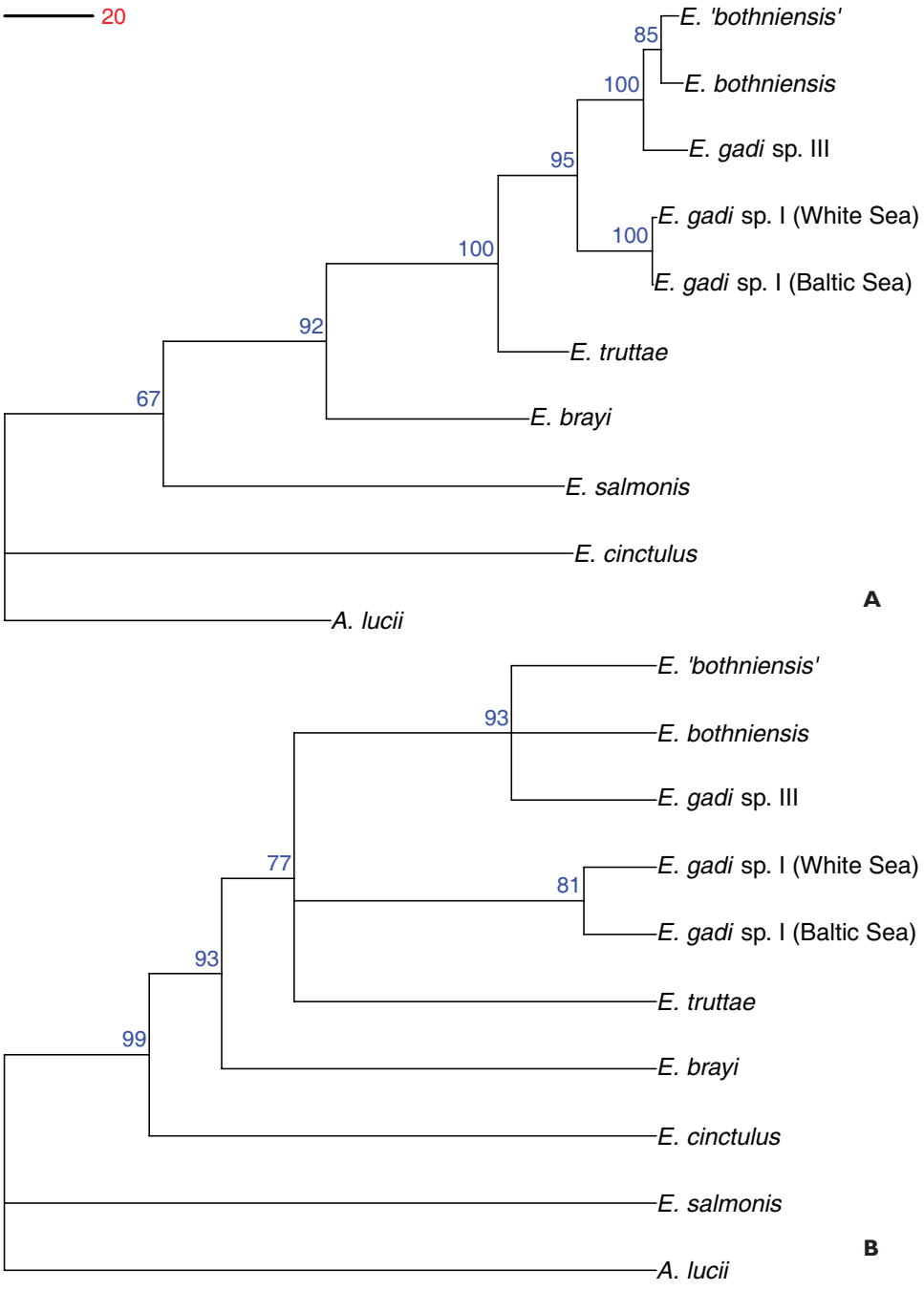

Figure 5. Phylogenetic relationships of *Echinorhynchus* spp. inferred from maximum parsimony analysis of COI data-set. Trees are rooted on the outgroup *A. lucii*. **A** Phylogram estimated using maximum parsimony analysis of COI sequence data. Numbers at nodes indicate bootstrap support (n = 10,000) **B** Consensus cladogram from maximum parsimony analysis of COI sequence data excluding third codon positions. Numbers at nodes indicate bootstrap support (n = 10,000).

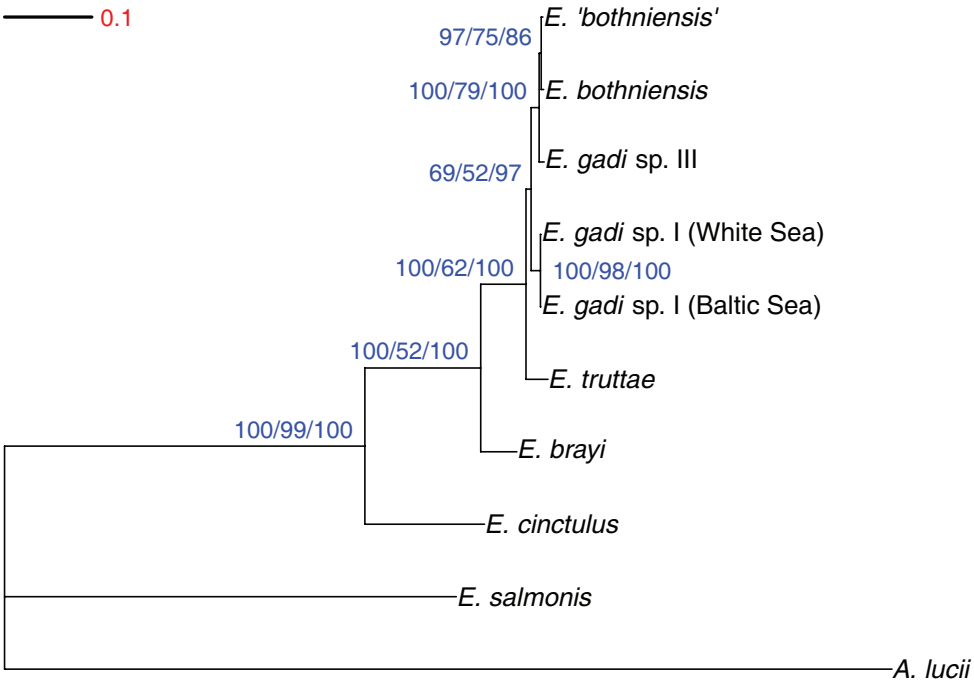

Figure 6. Phylogram estimated using Bayesian inference analysis of concatenated 28S rDNA and COI sequence data. Numbers at nodes are clade support values (%) for each method of phylogeny reconstruction (BI/ML/MP). Tree is rooted on the outgroup *A. lucii*.

Systematics

No support for Petrochenko's (1956) revision of *Echinorhynchus*, involving subdivision into three genera based on the cement gland pattern, is provided by the present study. The phylogeny derived from the total molecular data (Fig. 7) indicates that *Metechinorhynchus* (*sensu* Petrochenko 1956) may be a polyphyletic assemblage. Furthermore, *Echinorhynchus* (*sensu* Petrochenko 1956) would be paraphyletic, if evidence of cement gland differentiation in the *E. bothniensis* complex is deemed significant. Thus, this study supports the work of Väinölä et al. (1994), who rejected the hypothesis of monophyly of *Metechinorhynchus* on the basis of allozyme data from a more limited range of taxa. In view of the poor morphological definition of Petrochenko's genera and their incongruity with phylogenetic hypotheses from independent data-sets, we concur with other authors (Yamaguti 1963, Huffman and Kliever 1977, Amin and Redlin 1980, Amin 2013), who have recommended that the names *Metechinorhynchus* and *Pseudoechinorhynchus* should be designated junior synonyms of *Echinorhynchus*. Golvan (1994) relegated *Echinorhynchus* (*sensu* Petrochenko 1956), *Metechinorhynchus* and *Pseudoechinorhynchus* to the status of subgenera of *Echinorhynchus* (*sensu lato*). However, this scheme is subject to the same criticisms as Petrochenko's original classification and so should also be dismissed.

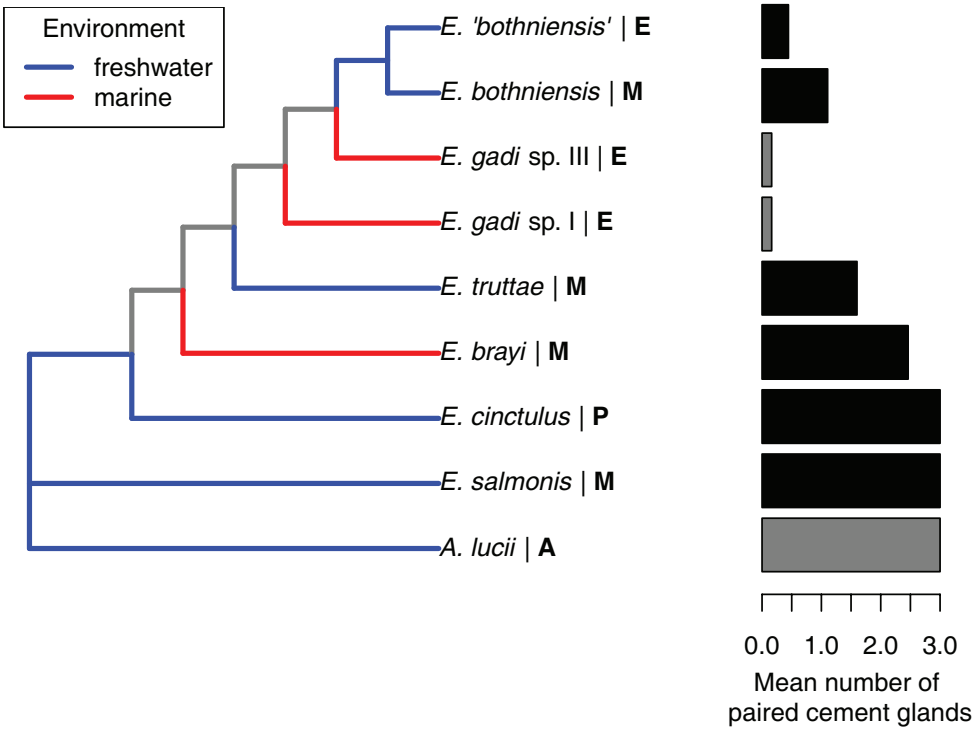

Figure 7. Aquatic environment (freshwater/marine) mapped on to the fully resolved phylogeny inferred from the concatenated 28S and COI sequences. Bold letter indicates genus according to Petrochenko's (1956) scheme: E, *Echinorhynchus*; M, *Metechinorhynchus*; P, *Pseudoechinorhynchus*. The bar chart shows the mean number of paired cement glands in each taxon. Data for *Echinorhynchus* spp. are from Table 2. Since the particular cement gland pattern exhibited by each of the species of the *E. gadi* group is not known, data from a collection of worms determined as *E. gadi* have been used for *E. gadi* spp. I & III (the bars for these species are shaded grey rather than black, to indicate a lower level of confidence in the data). Since *A. lucii* typically displays paired cement glands (Petrochenko 1956), the mean number of paired cement glands in this taxon was assumed to be approximately three (bar shaded grey to indicate approximation).

Cement gland arrangement displays continuous variation, from the pattern of three regular pairs through to the strictly moniliform pattern, with each *Echinorhynchus* species displaying a range of variation along this continuum (Table 2). The absence of discrete character states presents practical difficulties in using cement gland arrangement as a criterion of generic identity. To examine the presence of a phylogenetic signal in cement gland pattern, we used the average number of paired cement glands in each species as a summarizing variable, and plotted the variation of this character alongside the fully resolved tree (Fig. 7). Since cement gland patterns have not been determined for any of the electrophoretically identified species of the *E. gadi* complex, *E. gadi* spp. I and III were assumed to display the same cement gland pattern recorded from unidentified specimens of the *E. gadi* complex from gadid fishes (Wayland

2002). On the phylogeny comprising of six nested clades, an association between the clade identity and the average number of paired cement glands is evident, indicating that cement gland pattern conveys a phylogenetic signal, although the variability implies much homoplasy also. A more rigorous test of this morphological character will require accurate data for the species of the *E. gadi* complex. Notably, the species on the basal branches of the phylogeny (*E. salmonis* and *E. cinctulus*) displayed three pairs of cement glands, suggesting that this pattern is the plesiomorphic condition.

Further and more conclusive evidence that the ancestral cement gland arrangement is three regular pairs is available from both outgroup comparison and ontogeny. Firstly, outgroup comparison is based on the assumption that the character state found in related groups is the plesiomorphic condition (Watrous and Wheeler 1981). For the purposes of this comparison, genera in the same subfamily as *Echinorhynchus* have been chosen as outgroups. In the most recent classification of the Acanthocephala (Amin 2013), the Echinorhynchinae Cobbold, 1876 comprises six genera in addition to *Echinorhynchus*, namely *Acanthocephalus* Koelreuther, 1771, *Anuracanthorhynchus* Bursey, Vreibradic, Hatano & Rocha, 2006, *Brasacanthus* Thatcher, 2001, *Frilloechinorhynchus* Bhattacharya, 2007, *Pilum* Williams, 1976 and *Pseudoacanthocephalus* Petrochenko, 1956. *Acanthocephalus* and *Pseudoacanthocephalus* are diverse, containing 53 and 18 species respectively; the other four genera are monotypic. The majority of the species in these outgroup genera display regular pairs of cement glands, indicating that this is the plesiomorphic condition. Three regular pairs of cement glands are typical of the many species of *Acanthocephalus* and *Pseudoacanthocephalus*, whereas the monotypic *Pilum* is characterized by four regular pairs (Petrochenko 1956, Williams 1976). *Anuracanthorhynchus tritaxisentis* Bursey, Vreibradic, Hatano & Rocha, 2006 and *Brasacanthus sphoeroides* Thatcher, 2001, the type-species and sole representatives of their respective genera, have their cement glands arranged in parallel, a pattern not found in *Echinorhynchus* (see Thatcher 2001, Bursey et al. 2006). The only species in the outgroup to display its six cement glands in the moniliform pattern is *Frilloechinorhynchus meyeri* (Gupta & Naqvi, 1986) (see Bhattacharya 2007). Ontogenic evidence comes from a study of the embryology of *E. truttae*, in which the developing cement gland primordia were illustrated as three, approximately regular, pairs (see figure 7 and 8 of Awachie 1966); as an adult *E. truttae* never displays three regular pairs of cement glands (Table 2). Thus, the moniliform pattern represents a derived or apomorphic condition.

E. cinctulus and *E. salmonis* exhibit a relatively strong genetic divergence from each other and from the other taxa of the ingroup (Table 3). Each of these taxa also displays physical peculiarities not observed in other members of the ingroup. A study of the morphology of the reproductive system of *Echinorhynchus* spp. (Wayland 2002) revealed that female *E. salmonis* possess two vaginal sphincters, whereas all of the other taxa in the ingroup have a single vaginal sphincter (Fig. 8). Since the outgroup used in the current analysis, *Acanthocephalus lucii*, also has only a single vaginal sphincter, the double vaginal spincter may represent an apomorphy.

The acanthors of *E. cinctulus* display a unique pattern of hooks and spines which has not been observed in other species of *Echinorhynchus*, although relatively few taxa

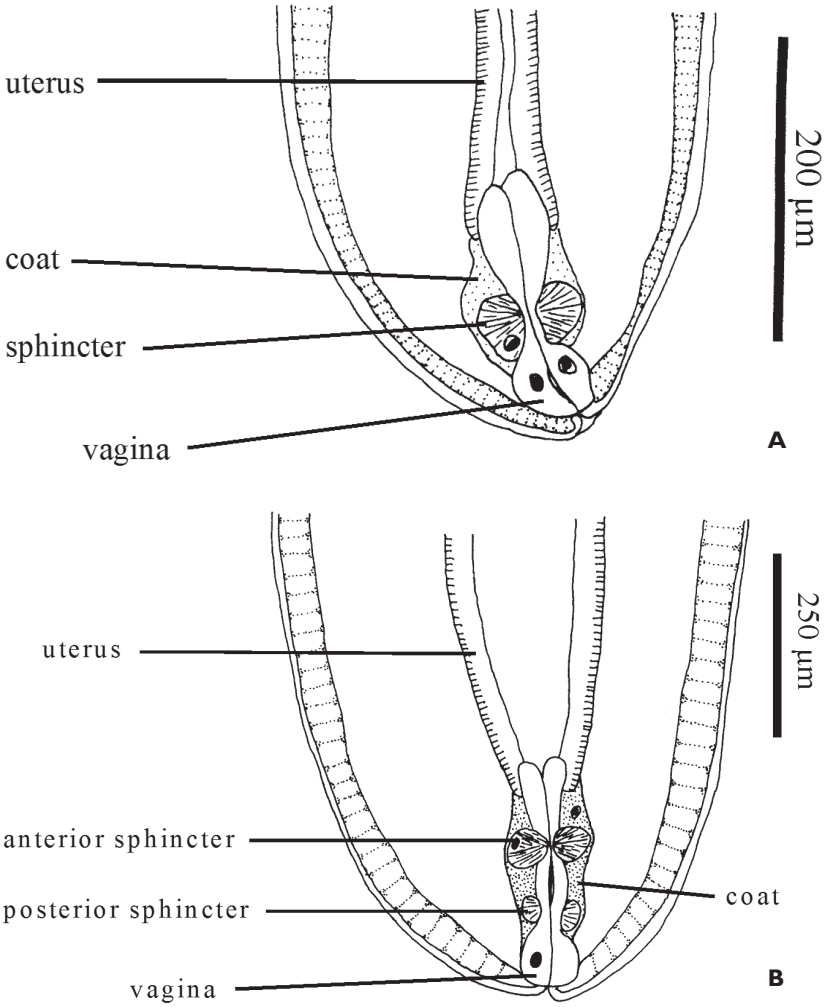

Figure 8. Structure of the vagina in *Echinorhynchus* spp. **A** *E. brayi*, a species with a single vaginal sphincter **B** *E. salmonis*, a species with two vaginal sphincters.

have been studied (Grabda-Kazubska 1964). The acanthors of *E. gadi* and *E. truttae* exhibit a well differentiated armature consisting of two large spade-like hooks and other smaller hooks on the rostellum plus small spines covering the rest of the body (Grabda-Kazubska 1964). Acanthors of *E. bothniensis*, *E. 'bothniensis'*, *E. brayi* and *E. salmonis* display a similar armature (Wayland 2002). In contrast, the relatively undifferentiated armature of the acanthors of *E. cinctulus* comprises small hooks on the rostellum and small spines covering the rest of the body (Grabda-Kazubska 1964, Grabda-Kazubska and Ejsymont 1969). The acanthors of the outgroup taxon, *A. lucii*, display a well differentiated, but asymmetrical, armature (Grabda-Kazubska 1964). While neither the type of acanthor armature nor the number of vaginal sphincters provide synapomor-

phies for clades identified in this study, these characters may yet prove to be useful in a revision of the genus.

Another taxonomic finding of the current study is paraphyly of the *E. gadi* group with respect to the monophyletic *E. bothniensis* group (Fig. 7). Thus, the current terminology is misleading, as it seems to imply that *E. gadi* and *E. bothniensis* are distinct groups (clades), when in fact *E. bothniensis* is a subgroup nested within the *E. gadi* species group. At this point, these informal taxonomic labels may however be maintained, as they convey biological information related to the habitat and host spectrum of the taxa. The *E. gadi* group parasitize fish and amphipods in the sea, whereas the *E. bothniensis* group infect fish and *Mysis* spp. in fresh and brackish waters.

One significant problem in the systematics of *Echinorhynchus*, which could not be addressed with the current data, is the monophyly of the genus. Further phylogenetic analyses incorporating a range of echinorhynchid acanthocephalans will be needed to resolve this issue. The relatively slowly evolving 28S rRNA gene, along with nuclear protein coding genes, should prove to be particularly useful in this respect.

Zoogeography

Since our phylogeny represents only a small proportion of the species in the genus, it is impossible to make any definitive claims about the zoogeography of this group of worms. However, the limited observations do suggest hypotheses that could be tested with additional data.

Echinorhynchus spp. are distributed from the Arctic (e.g. Shostak et al. 1986) to the Antarctic (e.g. Zdzitowiecki 1986), occurring in most aquatic environments, including mountain streams, rivers, lakes, estuaries, coastal marine waters and the deep-sea. They are found in both temperate and tropical regions (e.g. Machado Filho 1948). No other genus of acanthocephalans is known to display such an extensive geographical range. The genus may have had its origins in freshwater, because taxa displaying what is postulated to be the plesiomorphic cement gland arrangement (three regular pairs) occur almost exclusively in freshwater fishes, whereas the apomorphic condition (moniliform pattern) is generally only found in marine species. Transitional forms in the assumed transformation from regular pairing of cement glands to the moniliform pattern can be found in freshwater and the sea. Furthermore, of the six other genera of the subfamily Echinorhynchinae, four (including the species-rich *Acanthocephalus* and *Pseudoacanthocephalus*) are composed entirely of parasites of freshwater fish or amphibians (Petrochenko 1956, Yamaguti 1963, Williams 1976, Bursey et al. 2006). Basal positions in the molecular phylogeny for two of the freshwater species (*E. salmonis* and *E. cinctulus*) lend additional support to this hypothesis. However, implicit in this supposition is the unverified assumption of a monophyletic *Echinorhynchus*.

From this suggested freshwater origin and radiation, *Echinorhynchus* spp. have invaded the sea, most likely several times (Fig. 7). Various scenarios may have facilitated

the colonisation of marine hosts. Of particular relevance in this respect is the association of *Echinorhynchus* spp. with diadromous definitive hosts. Fish hosts of *Echinorhynchus* spp. which migrate between freshwaters and the sea include *Coregonus lavaretus* (L.), *Osmerus eperlanus* (L.), *Salmo salar* L. and *S. trutta* L. (see Kottelat 1997). Estuaries and other brackish environments, such as the Bothnian Bay, Baltic Sea, may provide further opportunities for parasite exchange between freshwater and marine fish. The Bothnian Bay has a very low salinity (less than 0.3%) and so its fish fauna is dominated by species of freshwater origin. Nevertheless, marine fishes, such as *Gadus morhua* L., occasionally enter this region, presumably following more saline currents from the main region of the Baltic Sea (Valtonen and Crompton 1990). Acanthocephalans display a relatively weak specificity towards their definitive hosts (Golvan 1957), a phenomenon favouring host-switching (García-Varela et al. 2013). The adoption of new definitive hosts would potentially allow *Echinorhynchus* spp. to invade new aquatic habitats and so be an important factor in geographical range extension. Moreover, gradual adaptation of species of freshwater origin to marine conditions (and *vice versa*) might take place in brackish environments, such as estuaries.

Evidence of a re-invasion of freshwater by marine stock can also be found in the fully resolved phylogeny (Fig. 7). The clade comprising the freshwater taxa *E. bothniensis* and *E. 'bothniensis'* is nested within the clade for the species of the closely related, but marine, *E. gadi* group. Thus *E. bothniensis* and *E. 'bothniensis'* represent either: (1) the result of two independent invasions of freshwater from marine stock; or (2) the outcome of invasion of freshwater by a single lineage of marine origin, followed by divergence within fresh or brackish waters. The latter hypothesis seems more likely since the *E. bothniensis* group taxa are thought to have co-speciated with their intermediate hosts, i.e. freshwater/brackish species of the *Mysis relicta* species group (Väinölä et al. 1994). The definitive hosts of the *E. bothniensis* group include several diadromous species, such as *Salmo trutta*, *Osmerus eperlanus* and *Platichthys flesus* (see Valtonen and Crompton 1990). Such euryhaline species were probably instrumental in carrying the common ancestor from the sea into inland waters.

Final comments

This preliminary investigation of the phylogenetic relationships within *Echinorhynchus* (*sensu lato*) underscores the argument for rejecting Petrochenko's (1956) revision of the genus, by demonstrating that neither *Echinorhynchus* (*sensu* Petrochenko 1956) nor *Metechinorhynchus* represent natural monophyletic groups. Nevertheless, *Echinorhynchus* is a large and growing genus, and consequently its division into smaller units is desirable. A revision of this genus is beyond the scope of the current study and will require tandem molecular phylogenetic and morphological analyses of a much larger sample of taxa attributed to *Echinorhynchus* and to related genera. Such analyses would also provide additional insights into the factors determining the geographical distribution and host relationships of echinorhynchid acanthocephalans in general.

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Supplementary material 1

Aligned and concatenated partial sequences of COI and 28S rDNA

Authors: Matthew T. Wayland, Jouni K. Vainio, David I. Gibson, Elisabeth A. Herniou, D. Timothy J. Littlewood, Risto Väinölä

Data type: Nexus file

Explanation note: Aligned and concatenated partial sequences of COI and 28S rDNA in nexus format. Aligned partial sequences of COI and 28S rDNA from each acanthocephalan population have been concatenated. Gaps are indicated by '-'. The first 585 characters in each block correspond to COI and the remainder to 28S rDNA. The file contains data for all nine Echinorhynchus samples and the outgroup taxon, Acanthocephalus lucii. This nexus file was used in all phylogenetic analyses.

Supplementary material 2

Maximum likelihood model parameters

Authors: Matthew T. Wayland, Jouni K. Vainio, David I. Gibson, Elisabeth A. Herniou, D. Timothy J. Littlewood, Risto Väinölä

Data type: Adobe PDF file

Explanation note: Model parameters used in the maximum likelihood approach to phylogenetic reconstruction.

Supplementary material 3

Nucleotide substitutions

Authors: Matthew T. Wayland, Jouni K. Vainio, David I. Gibson, Elisabeth A. Herniou, D. Timothy J. Littlewood, Risto Väinölä

Data type: Comma-separated-value file of measurements

Explanation note: Substitutions of nucleotides (transitions/transversions) for 28S rDNA (below the diagonal) and COI sequence data (above the diagonal).

Supplementary material 4

Patterns of COI sequence variation

Authors: Matthew T. Wayland, Jouni K. Vainio, David I. Gibson, Elisabeth A. Herniou, D. Timothy J. Littlewood, Risto Väinölä

Data type: Adobe PDF file

Explanation note: Patterns of COI sequence variation. Graphs and discussion of patterns of nucleotide substitions in the COI data-set.

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RESEARCH ARTICLE

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Phylogenetic analysis of the sharpshooter genus *Subrasaca* Young, 1977 (Hemiptera, Cicadellidae, Cicadellini)

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Abstract

The South American sharpshooter genus *Subrasaca* comprises 14 species. Some species of this genus are quite common in the Brazilian Atlantic Rainforest. In this paper, a phylogenetic analysis of *Subrasaca*, based on a matrix of 20 terminal taxa and 72 morphological characters of the head, thorax, and male and female genitalia, is presented. The analysis yielded six equally most parsimonious trees (197 steps, CI = 0.6091, RI = 0.5722, and RC = 0.3486). The results suggest that *Subrasaca* is a monophyletic taxon, although the genus branch is not robust. The clade showing the highest bootstrap and Bremer scores is formed by species with longitudinal dark brown to black stripes on the forewings (*S. bimaculata*, *S. constricta*, *S. curvovittata*, and *S. flavolineata*), followed by *S. atronasa* + *S. austera*.

Keywords

Auchenorrhyncha, Cicadellinae, cladistics, Membracoidea, phylogeny

Introduction

The infraorder Cicadomorpha comprises three superfamilies, Cicadoidea (cicadas), Cercopoidea (spittlebugs or froghoppers), and Membracoidea (leafhoppers and treehoppers). According to Hamilton (1999), the monophyly of the Cicadomorpha is well-supported by morphological synapomorphies, including the presence of a complex filter chamber. Cryan (2005), based on molecular data (18S rDNA, 28S rDNA, and histone 3), also supports the monophyly of the Cicadomorpha and suggests the following relationships for the superfamilies: (Membracoidea (Cicadoidea, Cercopoidea)). Based both on morphological and molecular data, the monophyly of the Membracoidea is also well-supported (Evans 1963, Dietrich and Deitz 1993, Hamilton 1999, Cryan 2005). Synapomorphies of the Membracoidea include the enlarged, transverse metathoracic coxae and a pair of rod-shaped lateral apodemes associated with the scutellar suture (Dietrich and Deitz 1993).

The family Cicadellidae (leafhoppers), with over 21,000 described species placed in more than 120 family-group taxa (Oman et al. 1990, Hamilton 1999), includes many species of economic importance because they are vectors of pathogens of cultivated plants (Nielson 1985). According to the morphological phylogeny of Hamilton (1983) and the molecular phylogeny (28S rDNA) of Dietrich et al. (2001), Cicadellidae is a paraphyletic group because treehoppers (Aetalionidae and Membracidae) are derived from leafhoppers. Taxonomically, cicadellids can be distinguished from other membracoids by the mesanepisternum without a hooklike process, separated from the katepisternum by a suture, and hind tibia with setae of longitudinal rows usually large and conspicuous (Dietrich 2005). With over 2,000 known species and a cosmopolitan distribution, Cicadellinae (sharpshooters) is the third largest subfamily of the Cicadellidae (Mejdalani 1998, Takiya 2007, McKamey 2007). According to Young (1968, 1977, 1986), this subfamily is divided into two tribes, a cosmopolitan Cicadellini and a New World Proconiini. Sharpshooters feed on the low-nutrient xylem sap of vascular plants. Some species of this group are important vectors of xylem-borne phytopathogenic bacteria (Redak et al. 2004).

The genus *Subrasaca* Young, 1977 belongs to the Cicadellini. *Subrasaca* has records from Brazil and Argentina, as well as dubious records of *S. monacha* from Colombia (Young 1977, McKamey 2007, Silva et al. 2013b). Species records are mostly from the Atlantic Rainforest. *Subrasaca* comprises currently 14 species (Silva et al. 2013a,b): *S. atronasa* Young, 1977, *S. austera* Young, 1977, *S. bimaculata* Silva, Cavichioli & Mejdalani, 2013a, *S. constricta* Silva, Cavichioli & Mejdalani, 2013a, *S. curvovittata* (Stål, 1862), *S. diminuta* Silva, Cavichioli & Mejdalani, 2013b, *S. flavolineata* (Signoret, 1855), *S. flavoornata* (Stål, 1862), *S. ignicolor* (Signoret, 1854) (type species), *S. monacha* (Melichar, 1951), *S. nigriventris* (Signoret, 1855), *S. rachelae* Silva, Cavichioli & Mejdalani, 2013b, *S. rhienetta* (Signoret, 1854), and *S. rubra* Silva, Cavichioli & Mejdalani, 2013b.

Taxonomically, *Subrasaca* differs from other genera of the Cicadellini by the following combination of male genital characteristics (Silva et al. 2013b): (1) aedeagus usually short and dorsally expanded; (2) styles (parameres) with distinct preapical lobe; (3) paraphyses with two or four rami (except in *S. monacha*, with only one ramus); and

(4) subgenital plates connected to each other at base by a triangular membranous area, not extending posteriorly as far as pygofer apex. *Subrasaca* species are generally quite colorful and range in length from 4.8 to 7.7 mm. Young (1977: 445), based on overall similarity, included *Subrasaca* in his *Juliaca* group of genera, which also includes *Juliaca* Melichar, 1926, *Mesogonia* Melichar, 1926, *Rotigonalia* Young, 1977, *Geitogonalia* Young, 1977, *Plerogonalia* Young, 1977, *Scopogonalia* Young, 1977, *Cyclogonia* Melichar, 1926, *Beirneola* Young, 1977, and *Fusigonalia* Young, 1977.

Here we use morphological data of the head, thorax, male and female genitalia to investigate the phylogenetic relationships among the species of *Subrasaca*. Among our outgroups, we included four genera of the *Juliaca* group (*Cyclogonia*, *Juliaca*, *Geitogonalia*, and *Scopogonalia*).

Material and methods

Specimens for the study

Specimens of 12 of the 14 described species of *Subrasaca* were studied (*S. atronasa* and *S. monacha* were not obtained and thus coded based on Young 1977 and Wilson et al. 2009). The matrix includes 20 terminal taxa (14 *Subrasaca* species and six outgroups). The outgroups are four representatives of the *Juliaca* generic group [*Cyclogonia caeliguttata* Mejdalani & Nessimian, 1991, *Juliaca* sp., *Geitogonalia quatuordecimmaculata* (Taschenberg, 1884), *Scopogonalia subolivacea* (Stål, 1862)], *Versigonalia ruficauda* (Walker, 1851), and a member of the Proconiini, *Tretogonia cribrata* Melichar, 1926, which was employed for rooting the trees.

The studied specimens belong to the following institutions: Departamento de Entomologia, Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ, Rio de Janeiro); Coleção Entomológica Prof. José Alfredo P. Dutra, Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro (DZRJ, Rio de Janeiro); and Coleção de Entomologia Pe. Jesus S. Moure, Departamento de Zoologia, Setor de Ciências Biológicas, Universidade Federal do Paraná (DZUP, Curitiba). The number of specimens examined of each terminal taxon, their geographical distribution, and collections are listed in Table 1.

Techniques for preparation of specimens and terminology

The techniques for preparation of male and female genital structures follow Oman (1949) and Mejdalani (1998), respectively. The dissected parts are stored in small vials with glycerin, as suggested by Young and Beirne (1958). The first and second pair of valvulae of the ovipositor were mounted on temporary slides with glycerin. The descriptive terminology adopted herein follows mainly Young (1977), except for the facial areas of the head (Hamilton 1981, Mejdalani 1993, 1998) and the female genitalia (Nielson 1965, Hill 1970).

Table 1. Taxa included in the phylogenetic analysis of *Subrasaca* (in bold) and outgroups. The number of females and males examined, their distribution (Brazilian states), and collections are provided for each taxon.

Taxon	Females	Males	Distribution	Collection
Cyclogonia caeliguttata Mejdalani & Nessimian, 1991	2	2	RJ	MNRJ
Juliaca sp.	2	2	RJ	MNRJ
Geitogonalia quatuordecimmaculata (Taschenberg, 1884)	2	2	RJ	MNRJ
Scopogonalia subolivacea (Stål, 1862)	2	2	RJ, MG	MNRJ
Versigonalia ruficauda (Walker, 1851)	2	2	RJ	MNRJ
Tretogonia cribrata Melichar, 1926*	2	2	RJ	MNRJ
S. atronasa* Young, 1977*	–	–	–	–
***S. austera* Young, 1977**	2	1	SC	DZUP
***S. bimaculata* Silva et al., 2013**	19	26	MG, SP, PR	DZRJ, DZUP, MNRJ
***S. constricta* Silva et al., 2013**	2	3	BA	DZUP, MNRJ
***S. curvovittata* (Stål, 1862)**	11	6	RJ	DZRJ, DZUP, MNRJ
***S. diminuta* Silva et al., 2013**	8	6	SP, PR	DZUP, MNRJ
***S. flavolineata* (Signoret, 1855)**	9	15	RJ	DZRJ, DZUP, MNRJ
***S. flavoornata* (Stål, 1862)**	6	2	RJ	MNRJ
***S. ignicolor* (Signoret, 1854)**	22	14	RJ, SP	MNRJ
S. monacha* (Melichar, 1951)*	–	–	–	–
***S. nigriventris* (Signoret, 1855)**	10	10	RJ	MNRJ
***S. rachelae* Silva et al., 2013**	19	12	ES	DZRJ, DZUP, MNRJ
***S. rhienetta* (Signoret, 1854)**	3	3	RJ, SP	MNRJ
***S. rubra* Silva et al., 2013**	7	10	MG, RJ, SP	DZRJ, DZUP, MNRJ

Brazilian states: BA – Bahia; ES – Espírito Santo; MG – Minas Gerais; PR – Paraná; RJ – Rio de Janeiro; SC – Santa Catarina; SP – São Paulo. DZRJ – Departamento de Zoologia, Universidade Federal do Rio de Janeiro; DZUP – Departamento de Zoologia, Universidade Federal do Paraná; MNRJ – Museu Nacional, Universidade Federal do Rio de Janeiro; * root of the phylogenetic analysis; ** coded based on Young (1977) and Wilson et al. (2009).

Cladistic analysis

Morphological characters of the head, thorax, male and female genitalia were included in the unpolarized matrix (Nixon and Carpenter 1993), which was assembled using the Nexus Data Editor (Page 2001). Hypotheses of primary homology were proposed based on the topological identity of the structures (Pinna 1991). All characters were initially scored equal weights. Character states were scored as underscores (_) when inapplicable or as question marks (?) when unavailable. The *heuristic search algorithm*, as implemented in PAUP* 4.0 (Swofford 2002), was employed for searching the most parsimonious trees. The successive weighting procedure (Carpenter 1988, 1994) was based on the maximum rescaled consistency index (rc) of the characters (Farris 1969, 1989). The strict consensus method was employed for all original most parsimonious trees. Clade support was estimated by computing 10.000 bootstrap replicates (Felsen-

stein 1985) with heuristic search in PAUP* 4.0 and by decay indices (Bremer 1988, 1994) in TreeRot 3.0 (Sorenson and Franzosa 2007). Autapomorphic characters were included in the matrix, as suggested by Yeates (1992), but we provide consistency index (CI) values considering all characters as well as only the informative ones.

Results and discussion

The data matrix (Table 2) consists of 72 morphological characters, 35 of the external morphology, 25 of the male genitalia, and 12 of the female genitalia. Among these characters, 51 are binary and 21 are multistate, being 52 informative for the parsimony analysis. The characters, their states, and *ci* greater than 0.5 are listed below. Although many of the characters are based on color patterns, these are consistent intraspecifically in *Subrasaca*. Figures 1 (external morphology and male genitalia) and 2 (female genitalia) provide some examples of characters employed in the phylogenetic analysis.

Morphological characters of the phylogenetic analysis

External morphology and coloration

1. **Shape of anterior margin of crown, dorsal view:** (0) rounded (Fig. 1a), (1) pronounced with a triangular shape (Fig. 1c).
2. **Position of ocelli on crown:** (0) slightly anterior to imaginary line between anterior eye angles (Fig. 1c); (1) posterior to imaginary line between eye angles; (2) on imaginary line between eye angles.
3. **Color of face:** (0) black; (1) yellow; (2) light brown; (3) black with yellow central region enclosing black macula; (4) black with cream central macula and two orange maculae on anterior portion; (5) black with orange macula on posterior portion; (6) black with orange macula on anterior portion; (7) yellow with black Y-shaped macula; (8) yellow with black central portion enclosing yellow macula; (9) cream with black central portion and anterior region with orange macula; (A) yellow with brown streaks and median stripe; (B) black with orange lateral portions; (C) yellow with small black maculae on anterior portion. ci = 0.9.
4. **Macula or maculae, originated from face, limited to central portion of apex of crown:** (0) absent; (1) present (Fig. 1b).
5. **Color of macula or maculae, originated from face, limited to central portion of apex of crown:** (0) yellow; (1) brown with yellow (Fig. 1b); (2) orange; (3) brown. ci = 1.
6. **Maculae on lateroapical portions of crown, originated from face:** (0) absent; (1) present (Fig. 1a). ci = 0.5.
7. **Dark brown to black transversal band on anterior portion of crown:** (0) absent; (1) present (Fig. 1b). ci = 1.0.

Table 2. Data matrix for the phylogenetic analysis of *Subrasaca* (in bold) and outgroup taxa. (_) codes for inapplicable states, (?) for unavailable data, (A) for state 10, (B) for 11, and (C) for 12. Outgroup genera are *Cyclogonia*, *Juliaca*, *Geitogonalia*, *Scopogonalia*, *Versigonalia*, and *Tretogonia* (root).

Taxa	Characters 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9
C. caeliguttata	0 2 1 0 _ 0 0 1 2 0 1 0 0 0 _ 0 _ 0 1 2 0 5 0 3 0 _ _ 0 _ _ _ 0 _ 1 0 0 0 0 0
Juliaca sp.	1 1 1 0 _ 0 0 0 _ _ _ 1 1 0 _ 0 _ 1 0 _ _ 6 0 1 0 _ _ 0 _ _ _ 0 _ 1 0 0 0 0 1
G. quatuordecimmaculata	0 1 4 1 2 0 0 1 0 0 0 0 0 0 _ 0 _ 0 1 0 1 0 0 2 0 _ _ 0 _ _ _ 0 _ 1 1 0 0 0 0
S. subolivacea	1 2 C 0 _ 0 0 0 _ _ _ 0 0 0 _ 0 _ 0 0 _ _ 9 0 1 0 _ _ 0 _ _ _ 0 _ 1 0 0 1 1 0
V. ruficauda	0 2 B 0 _ 1 0 0 _ _ _ 0 1 0 _ 0 _ 0 0 _ _ 8 0 4 0 _ _ 1 0 2 0 0 _ 1 2 0 1 0 0
T. cribrata (root)	0 2 2 0 _ 0 0 0 _ _ _ 0 0 0 _ 0 _ 0 0 _ _ 5 0 3 0 _ _ 0 _ _ _ 0 _ 0 1 0 1 0 1
S. ignicolor	0 0 6 0 _ 0 0 1 0 1 0 0 0 0 _ 0 _ 0 1 0 0 0 0 2 0 _ _ 0 _ _ _ 0 _ 1 0 0 0 1 0
S. rhienetta	1 2 3 1 0 0 1 0 _ _ _ 1 1 0 _ 0 _ 1 0 _ _ 0 0 0 0 _ _ 0 _ _ _ 1 0 1 0 0 0 1 0
S. nigriventris	0 0 1 0 _ 1 0 1 0 1 1 0 0 0 _ 0 _ 0 1 0 2 0 0 0 0 _ _ 0 _ _ _ 0 _ 1 0 0 0 1 0
S. flavolineata	1 1 8 1 1 0 1 0 _ _ _ 1 1 0 _ 1 1 0 0 _ _ 2 0 1 1 0 0 1 0 0 0 0 _ 1 0 0 0 1 0
	1
S. monacha	0 0 2 0 _ 0 0 1 1 1 0 0 0 0 _ 0 _ 0 0 _ _ 5 0 3 0 _ _ 0 _ _ _ 0 _ 1 ? 0 0 ? 0
S. flavoornata	0 0 9 0 _ 0 0 1 0 1 0 0 0 0 _ 0 _ 0 1 0 3 0 0 0 0 _ _ 0 _ _ _ 0 _ 1 0 0 0 1 0
	3 1
S. curvovittata	1 1 7 1 0 0 1 0 _ _ _ 1 1 0 _ 1 1 0 0 _ _ 1 0 0 1 0 0 1 1 0 0 0 _ 1 0 0 0 1 0
	1
S. atronasa	1 0 ? 0 _ 0 0 1 2 1 0 0 0 1 0 0 _ 0 0 _ _ 5 1 1 0 _ _ 0 _ _ _ 1 1 1 0 0 0 1 0
S. austera	1 0 A 1 3 0 0 1 2 1 0 0 0 1 1 0 _ 0 0 _ _ 5 1 3 0 _ _ 0 _ _ _ 0 _ 1 0 0 0 1 0
	1
S. constricta	1 0 1 0 _ 0 1 0 _ _ _ 1 1 0 _ 1 0 0 0 _ _ 7 0 1 1 2 1 1 0 0 1 0 _ 1 0 1 0 1 0
S. bimaculata	1 1 6 1 0 0 1 0 _ _ _ 1 1 0 _ 1 1 0 0 _ _ 4 0 1 1 1 0 1 0 0 0 0 _ 1 0 0 0 1 0
	1 1
S. rachelae	0 0 1 0 _ 1 0 1 0 1 1 0 0 0 _ 0 _ 0 1 0 2 3 0 3 0 _ _ 0 _ _ _ 0 _ 1 0 0 0 1 0
S. diminuta	0 0 5 0 _ 0 0 1 0 1 0 0 0 0 _ 0 _ 0 1 0 0 0 0 0 0 _ _ 0 _ _ _ 0 _ 1 0 0 0 1 0
S. rubra	0 0 0 0 _ 0 0 0 _ _ _ 0 0 0 _ 0 _ 0 1 0 3 0 0 2 0 _ _ 0 _ _ _ 0 _ 1 1 0 0 1 0

8. Transverse band on middle portion of crown: (0) absent; (1) present. ci = 0.5.

9. Color of transverse band on middle portion of crown: (0) orange; (1) light brown; (2) whitish-yellow; (3) yellow. ci = 0.6.

10. Aspect of transverse band on middle portion of crown: (0) incomplete; (1) complete. ci = 1.0.

11. Lateral portions of median transverse band reaching posterior margin of crown: (0) absent; (1) present. ci = 0.5.

12. Dark brown to black transverse band on posterior portion of crown: (0) absent; (1) present (Fig. 1b). ci = 0.5.

13. Dark brown to black transverse band on anterior portion of pronotum: (0) absent; (1) present (Fig. 1b).

14. Whitish-yellow transverse band on middle portion of pronotum: (0) absent; (1) present. ci = 1.

Table 2. Continued.

Taxa	Characters 4 5 6 7 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2
C. caeliguttata	1 0 1 1 2 1 0 _ 1 0 _ _ 0 0 1 1 _ 0 0 _ 0 1 0 0 _ _ _ 0 4 1 1 0 0
Juliaca sp.	0 _ 1 1 1 1 0 _ 1 0 _ _ 0 0 1 1 _ 0 0 _ 0 3 0 0 _ _ _ 0 2 1 1 0 1
G. quatuordecimmaculata	1 1 1 0 2 1 0 _ 1 1 1 0 0 0 1 1 _ 0 0 _ 0 2 0 0 _ _ _ 0 3 0 1 0 0
S. subolivacea	0 _ 0 1 _ _ 0 _ 1 1 1 2 0 0 1 1 _ 0 0 _ 0 0 0 1 0 1 2 0 4 0 1 0 0
V. ruficauda	0 _ 1 1 1 1 0 _ 1 0 _ _ 0 1 0 _ _ _ _ _ _ 0 0 0 _ _ _ 0 4 0 1 1 2
T. cribrata (root)	1 0 3 1 1 1 0 _ 1 0 _ _ 0 1 0 _ _ _ _ _ _ 0 0 1 0 0 5 1 0 0 0 0 0
S. ignicolor	1 1 2 1 1 1 1 0 0 0 _ _ 0 0 1 1 _ 0 0 _ 0 0 0 1 1 1 2 0 4 0 0 0 0
S. rhienetta	1 0 2 1 1 1 1 1 0 0 _ _ 0 0 1 1 _ 0 0 _ 0 0 1 1 1 0 4 0 4 0 1 0 1
S. nigriventris	1 0 0 1 1 1 0 _ 1 1 1 2 0 0 1 1 _ 0 0 _ 0 0 1 1 1 0 0 0 4 0 0 0 0
S. flavolineata	1 1 1 1 1 1 1 0 0 0 _ _ 0 0 1 1 _ 0 1 1 0 0 1 1 1 1 1 0 4 1 0 0 0
S. monacha	1 0 2 1 1 1 0 _ 1 1 0 3 0 0 1 0 _ 0 0 _ 0 ? ? ? ? ? ? ? ? ? ? ? ?
S. flavoornata	1 0 1 1 0 1 0 _ 1 1 1 1 0 0 1 1 _ 0 0 1 1 1 0 1 1 1 4 1 4 0 1 0 0
S. curvovittata	1 0 1 1 1 1 1 0 0 0 _ _ 0 0 1 2 0 0 0 _ 0 0 1 1 1 1 1 4 1 6 1 0 0 0
S. atronasa	1 0 2 1 1 1 1 0 0 0 _ _ 0 0 1 1 _ 0 0 _ 0 ? ? ? ? ? ? ? ? ? ? ? ?
S. austera	1 0 2 1 1 1 1 0 0 0 _ _ 0 0 1 1 _ 0 0 _ 0 0 0 1 1 0 0 0 4 0 1 0 1
S. constricta	1 0 0 1 1 0 1 1 0 0 _ _ 0 0 1 1 _ 0 1 0 0 0 0 1 1 1 0 0 5 0 0 0 0
S. bimaculata	1 0 0 1 1 1 1 1 0 0 0 _ _ 0 0 1 2 1 0 0 _ 0 1 0 0 _ _ _ 0 1 1 0 0 0
	2
S. rachelae	1 0 0 1 1 0 1 0 0 0 _ _ 1 0 1 1 _ 0 0 _ 1 1 1 1 1 0 2 0 4 0 1 0 0
S. diminuta	1 0 2 1 1 1 1 0 0 0 _ _ 0 0 1 1 _ 1 0 _ 0 0 0 1 1 0 2 0 4 0 0 0 0
S. rubra	1 0 0 1 1 1 1 1 0 0 0 _ _ 0 0 1 1 _ 0 0 _ 0 0 0 1 1 1 3 1 4 0 1 1 1

15. Band on middle portion of pronotum with strong concavity: (0) absent; (1) present. ci = 1.

16. Dark brown to black transverse band located before base of pronotum (posterior margin): (0) absent; (1) present (Fig. 1b). ci = 1.

17. Aspect of dark brown to black transverse band located before base of pronotum (posterior margin): (0) narrow (Fig. 1c); (1) thick (Fig. 1b). ci = 1.

18. Dark brown to black transverse band at base of pronotum (posterior margin): (0) absent; (1) present. ci = 0.5.

19. Pair of maculae on central portion of pronotum: (0) absent; (1) present.

20. Color of pair of maculae on central portion of pronotum: (0) orange; (1) yellow; (2) blue. ci = 1.

21. Position of pair of yellow or orange maculae on pronotum: (0) strongly oblique and restricted to sides of pronotum; (1) strongly oblique and reaching central portion of pronotum; (2) moderately oblique (Fig. 1a); (3) not oblique. ci = 1.

22. Color of mesonotum: (0) entirely black; (1) black with yellow scutellum; (2) yellow with black T-shaped macula (Fig. 1b); (3) brown with brownish-yellow scutellum and two anterior maculae (Fig. 1a); (4) black with yellow scutellum and two anterior maculae; (5) light brown; (6) dark brown with yellow apex; (7) yellow (Fig. 1c); (8) black with irregular yellow maculae; (9) yellow with black maculae on lateroanterior portions. ci = 0.9.

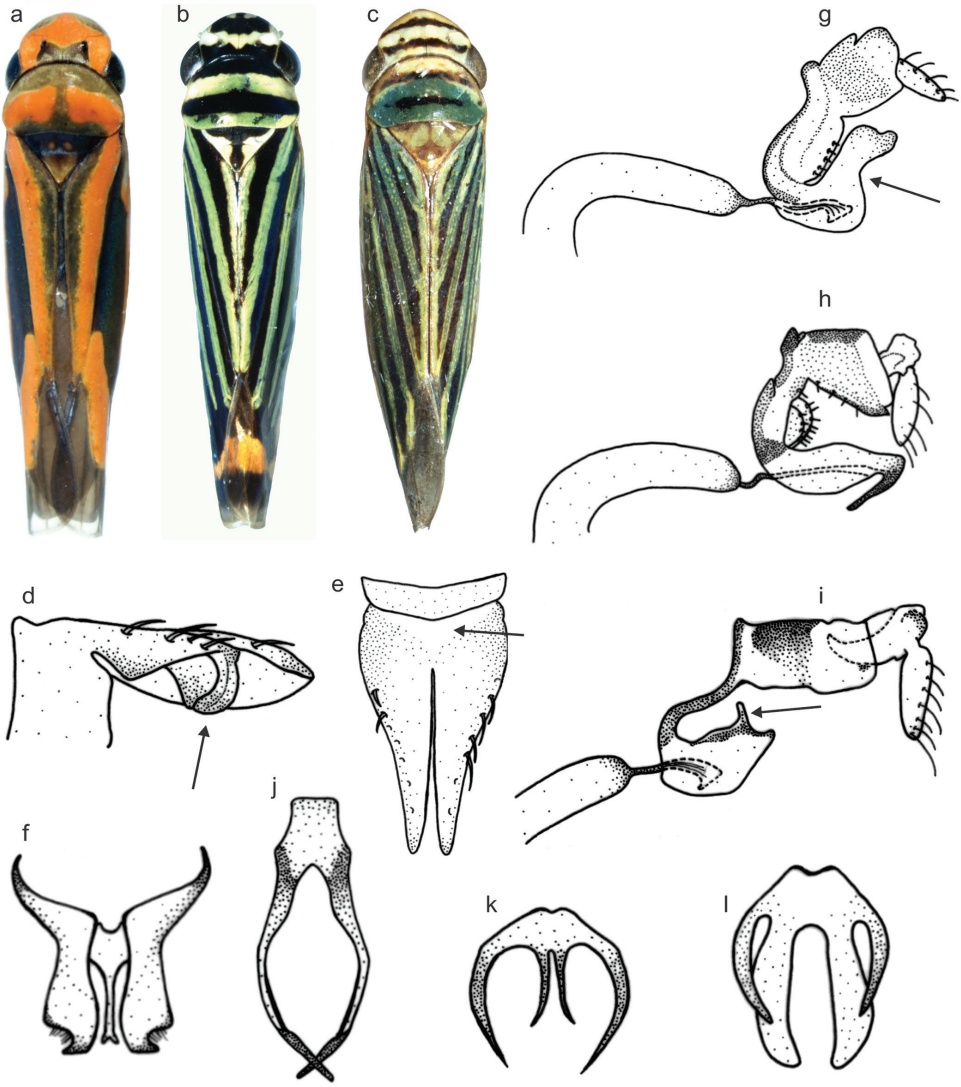

Figure 1. Examples of characters for the phylogenetic analysis of *Subrasaca* (external morphology and male genitalia). **a** body of *Subrasaca rachelae* (length 5.3 mm): rounded anterior margin of crown (character 1, state 0), maculae on lateroapical portions of crown (c6, s1), pair of moderately oblique maculae on pronotum (c21, s2) **b** *S. flavolineata* (length 5.4 mm): mesonotum with T-shaped macula (c22, s2), longitudinal stripes on forewings (c25, s1) **c** *S. constricta* (length 5.7 mm): pronounced anterior margin of crown (c1, s1) **d** pygofer lobe of *S. constricta*, dorsal view: dorsoapical process (c36, s1; arrowed) **e** subgenital plates of *S. bimaculata*: membranous basal area (c38, s1; arrowed) **f** *S. nigriventris*: styles with preapical lobe (c40, s1) and apex transversely truncate (c42, s0), stalk of connective clearly differentiated, not extending beyond apex of styles (c44, s1) **g** aedeagus of *S. constricta*: dorsal lobe (c46, s1) with constriction (c47, s1; arrowed) **h** aedeagus of *S. nigriventris*: shaft longer than high (c48, s1), pair of spiniform apical processes (c51, s2) **i** aedeagus of *S. rachelae*: pair of preapical processes (c52, s1; arrowed) **j** *S. rubra*: paraphyses with two rami (c55, s1) **k** *S. curvovittata*: paraphyses with four rami (c55, s2), inner rami small and narrow (c56, s0) **l** *S. bimaculata*: inner rami of paraphyses broader and larger than outer rami (c56, s1).

23. **Texture of forewings:** (0) coriaceous (Fig. 1a–c); (1) translucent. ci = 1.
24. **Color of basal portion of clavus, reaching apex of scutellum:** (0) black; (1) yellow (Fig. 1b, c); (2) orange; (3) light brown (Fig. 1a); (4) blue.
25. **Set of dark brown to black longitudinal stripes on forewings:** (0) absent; (1) present (Fig. 1b, c). ci = 1.
26. **Number of dark brown to black longitudinal stripes on forewings:** (0) four; (1) six; (2) eight. ci = 1.
27. **Position of longitudinal stripe on forewings:** (0) not along outer edge of inner apical cell; (1) along outer edge of inner apical cell. ci = 1.
28. **Transverse band on anteapical portion of forewings:** (0) absent; (1) present. ci = 0.5.
29. **Aspect of transverse band on anteapical portion of forewings:** (0) not connected to yellow longitudinal stripe; (1) connected to yellow longitudinal stripe. ci = 1.
30. **Color of transverse band on anteapical portion of forewings:** (0) yellow; (1) orange; (2) brown. ci = 1.
31. **Extension of transverse band on anteapical portion of forewings:** (0) reaching the four anteapical cells; (1) 1/2 of the width of wing, reaching a maximum of two anteapical cells. ci = 1.
32. **Transverse band of corium on region of apex of clavus:** (0) absent; (1) present. ci = 0.5.
33. **Color of transverse band of corium on apex of clavus:** (0) yellow; (1) whitish-yellow. ci = 1.
34. **Extension of hind legs at rest position:** (0) not reaching posterior margin of lateral lobe of pronotum; (1) reaching posterior margin of lateral lobe of pronotum. ci = 1.
35. **Color of legs:** (0) yellow; (1) brown; (2) red. ci = 0.5.

Male genitalia

36. **Dorsoapical process of pygofer:** (0) absent; (1) present (Fig. 1d). ci = 1.
37. **Ventroapical process of pygofer:** (0) absent; (1) present. ci = 0.5.
38. **Triangular membranous area uniting subgenital plates basally:** (0) absent; (1) present (Fig. 1e). ci = 0.5.
39. **Extension of subgenital plates in relation to pygofer:** (0) not extending beyond apex of pygofer; (1) extending beyond apex of pygofer. ci = 0.5.
40. **Preapical lobe of styles:** (0) absent; (1) present (Fig. 1f). ci = 0.5.
41. **Styles, length of portion posterior to preapical lobe:** (0) less than 1/3 of style length (Fig. 1f); (1) 1/3 of style length.
42. **Shape of apex of styles, dorsal view:** (0) transversely truncated (Fig. 1f); (1) obliquely truncated; (2) obtuse; (3) forked.
43. **Shape of connective:** (0) T-shaped; (1) Y-shaped (Fig. 1f). ci = 1.

44. **Aspect of stalk of connective:** (0) very short, not clearly differentiated; (1) clearly differentiated, not extending beyond apex of styles (Fig. 1f); (2) clearly differentiated, extending beyond apex of styles. ci = 1.
45. **Width of stalk of connective:** (0) similar to width of base of arms; (1) narrower than base of arms (Fig. 1f). ci = 0.5.
46. **Dorsal lobe of aedeagus:** (0) absent; (1) present (Fig. 1g).
47. **Constriction of dorsal lobe of aedeagus:** (0) absent; (1) present (Fig. 1g). ci = 0.5.
48. **Length of aedeagus:** (0) as long as high; (1) longer than high (Fig. 1h).
49. **Apical processes of aedeagus:** (0) absent; (1) present (Fig. 1h).
50. **Number of apical processes of aedeagus:** (0) one; (1) two. ci = 1.
51. **Shape of apical processes of aedeagus:** (0) pair of digitiform processes directed basally; (1) pair of small lobular processes; (2) pair of spiniform processes directed ventrally (Fig. 1h); (3) triangular projection directed ventrally. ci = 1.
52. **Pair of dorsally directed digitifom processes on preapical portion of aedeagus:** (0) absent; (1) present (Fig. 1i). ci = 1.
53. **Pair of basal processes of aedeagus:** (0) absent; (1) present. ci = 1.
54. **Paraphyses:** (0) absent; (1) present (Fig. 1j–l). ci = 1.
55. **Number of paraphyses rami:** (0) one; (1) two (Fig. 1j); (2) four (Fig. 1k, l). ci = 0.6.
56. **Aspect of inner rami of paraphyses with four rami:** (0) narrower and smaller than outer rami (Fig. 1k); (1) broader and larger than outer rami (Fig. 1l); (2) width and length similar to outer rami. ci = 1.
57. **Spiniform process of paraphyses:** (0) absent; (1) present. ci = 1.
58. **Process on median portion of rami of paraphyses:** (0) absent; (1) present. ci = 0.5.
59. **Number of processes on median portion of paraphyses rami:** (0) one on each ramus; (1) two on each ramus. ci = 1.
60. **Apical processes of paraphyses:** (0) absent; (1) present. ci = 0.5.

Female genitalia

61. **Aspect of median portion of posterior margin of sternite VII:** (0) concave (Fig. 2b); (1) convex (Fig. 2a); (2) concave with dentiform projection; (3) convex with short triangular projection. ci = 0.5.
62. **Distinctly sclerotized area on each side of anterior margin of sternite VII:** (0) absent; (1) present.
63. **Sclerites of "inner" sternite VIII:** (0) absent; (1) present (Fig. 2c–e).
64. **Number of sclerites of "inner" sternite VIII:** (0) one; (1) two (Fig. 2c–e). ci = 1.
65. **Texture of sclerites of "inner" sternite VIII:** (0) smooth (Fig. 2c); (1) punctuated (Fig. 2d, e).
66. **Shape of sclerites of "inner" sternite VIII:** (0) triangular; (1) somewhat quadrangular (Fig. 2e); (2) linear (Fig. 2c); (3) coniform; (4) oblique (Fig. 2d); (5) broad, narrowed posteriorly, and with apex located between bases of ovipositor valvulae I. ci = 0.7.

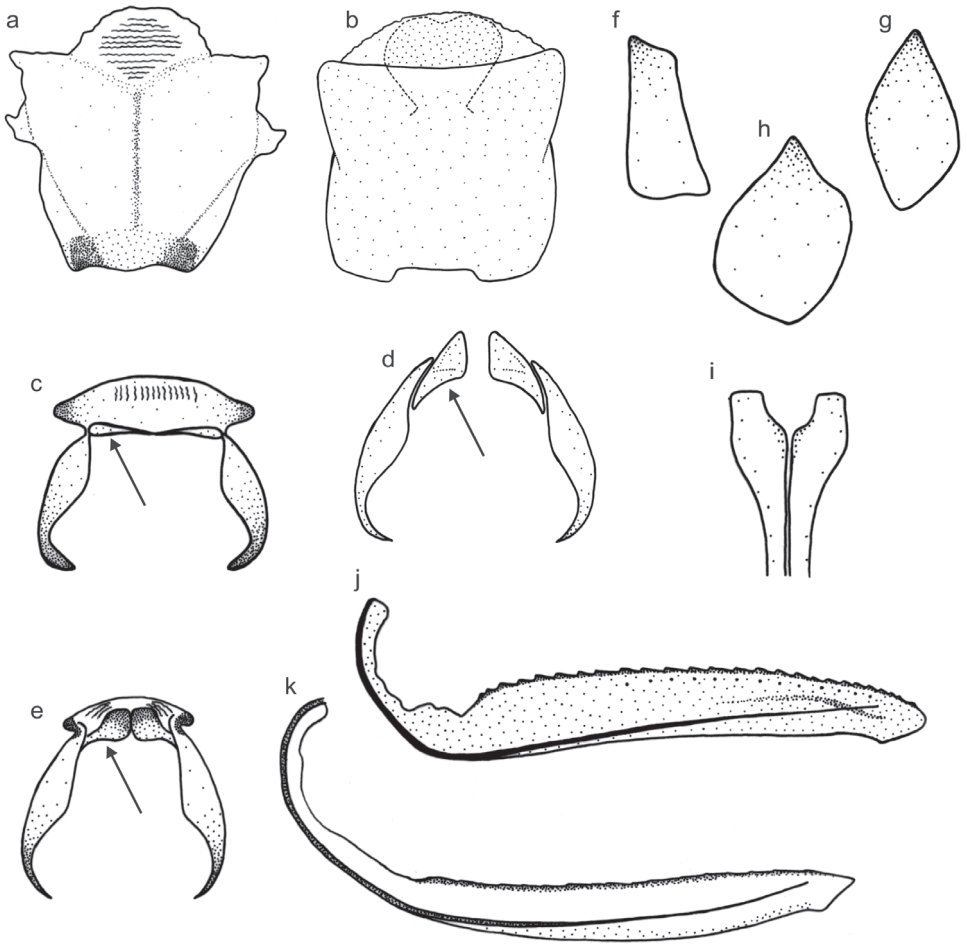

Figure 2. Examples of characters for the phylogenetic analysis of *Subrasaca* (female genitalia). **a** sternite VII of *S. bimaculata*: convex posterior margin at median portion (character 61, state 1) **b** sternite VII of *S. rubra*: concave posterior margin at median portion (c61, s0) **c** *S. rachelae*: smooth sclerites of "inner" sternite VIII (c65, s0) with linear aspect (c66, s2; arrowed) **d** *S. flavoornata*: sclerites of "inner" sternite VIII with oblique aspect (c66, s4; arrowed) **e** *S. flavolineata*: punctuated sclerites of "inner" sternite VIII (c65, s1; arrowed) **f** *Juliaca* sp.: valvifer I subrectangular (c68, s2) **g** *S. diminuta*: valvifer I ellipsoid (c68, s4) **h** *S. curvovittata*: valvifer I gutiform (c68, s6) **i** *S. rhienetta*: valvulae I with expanded base (c69, s0) **j** *S. constricta*: valvula II with obtuse apex (c70, s0) and convex dorsal margin (c72, s0) **k** *S. rubra*: valvula II with acute apex (c70, s1), linear and indistinct teeth (c71, s1), and rectilinear dorsal margin (c72, s1).

67. Sclerites of "inner" sternite VIII directed ventrally: (0) absent; (1) present.

68. Shape of valvifers I, lateral view: (0) quadrangular; (1) trapezoidal; (2) subrectangular (Fig. 2f); (3) gutiform with lobe on distal half of dorsal margin; (4) elliptical (Fig. 2g); (5) subtriangular; (6) gutiform, posteriorly expanded (Fig. 2h). ci = 1.

69. Aspect, in ventral view, of basal portion of valvulae I of ovipositor: (0) expanded (Fig. 2i); (1) continuous, without expansion.

70. **Aspect of apex of valvulae II of ovipositor:** (0) obtuse (Fig. 2j); (1) acute (Fig. 2k).
71. **Shape of teeth of valvulae II of ovipositor:** (0) triangular and distinct (Fig. 2j); (1) linear and indistinct (Fig. 2k). ci = 0.5.
72. **Aspect of dorsal margin of valvulae II of ovipositor:** (0) convex (Fig. 2j); (1) rectilinear (Fig. 2k); (2) concave (Young 1977: Fig. 881k).

Main aspects and discussion of the phylogenetic analysis

The analysis with equal weights resulted in six most parsimonious trees with length = 197, consistency index (CI) = 0.6091 (excluding uninformative characters = 0.5389), retention index (RI) = 0.5722, and rescaled consistency index (RC) = 0.3486. The trees differ from one another (1) in the position of *V. ruficauda* (outgroup), (2) positions of *S. rubra* and *S. flavoornata*, which appear as sister groups or not, and (3) positions of *S. rubra*, *S. flavoornata*, *S. nigriventris*, and *S. rachelae*. These four species formed a clade with *S. ignicolor* + *S. diminuta* in two trees. A strict consensus of the six trees is given in Fig. 3a.

The successive weighting analysis yielded one tree, which is also one of the six original trees, with length = 80, CI = 0.8249 (excluding uninformative characters = 0.7199), RI = 0.7641, and RC = 0.6303 (Figs 3b, 4). Thirty-two characters had maximum weight (= 1.0) and 40 had lower weights. Twenty characters were parsimony-uninformative. Figure 4 gives bootstrap estimates (when > 50%) and Bremer support indices for the clades recovered under equal weights. Apomorphies of this tree are given in Table 3.

The monophyly of *Subrasaca* was recovered in all most parsimonious trees (Fig. 3a). This clade, however, is not robust (bootstrap < 50%, Bremer = 0) (Fig. 4). Phylogenetically, *Subrasaca* can be tentatively defined by the following synapomorphic traits of its groundplan: (1) ocelli located slightly anterad of the imaginary line between the anterior angles of eyes (character 2, state 0; Fig. 1c), (2) complete transverse band on middle portion of crown (character 10, state 1), (3) triangular membranous area uniting subgenital plates basally (character 38, state 1; Fig. 1e), (4) obtuse shape of apex of styles in dorsal view (character 42, state 2), and (5) sclerites of female "inner" sternite VIII present (character 63, state 1; Fig. 2c–e).

Other phylogenetic (e.g., Felix and Mejdalani 2011) or purely taxonomic (e.g., Mejdalani et al. 2014) studies on the Cicadellini highlighted the need for more precise definitions of various genera of this tribe. In the introduction of his impressive monograph on the New World Cicadellini, Young (1977: 10) expressed his perception of this problem as follows: "The Cicadellini are an intricate group. Their morphology suggests rapid radiation and often shows small discontinuities compared with those found in many of the Proconiini." Small discontinuities are precisely what we have observed between *Subrasaca* and the genera here employed as outgroups. In any case, the cladistic analysis allowed us to propose a more objective definition of the genus.

Two clades appeared in all six most parsimonious trees and were fairly robust in the analysis (Fig. 4). The clade formed by the species with longitudinal dark brown

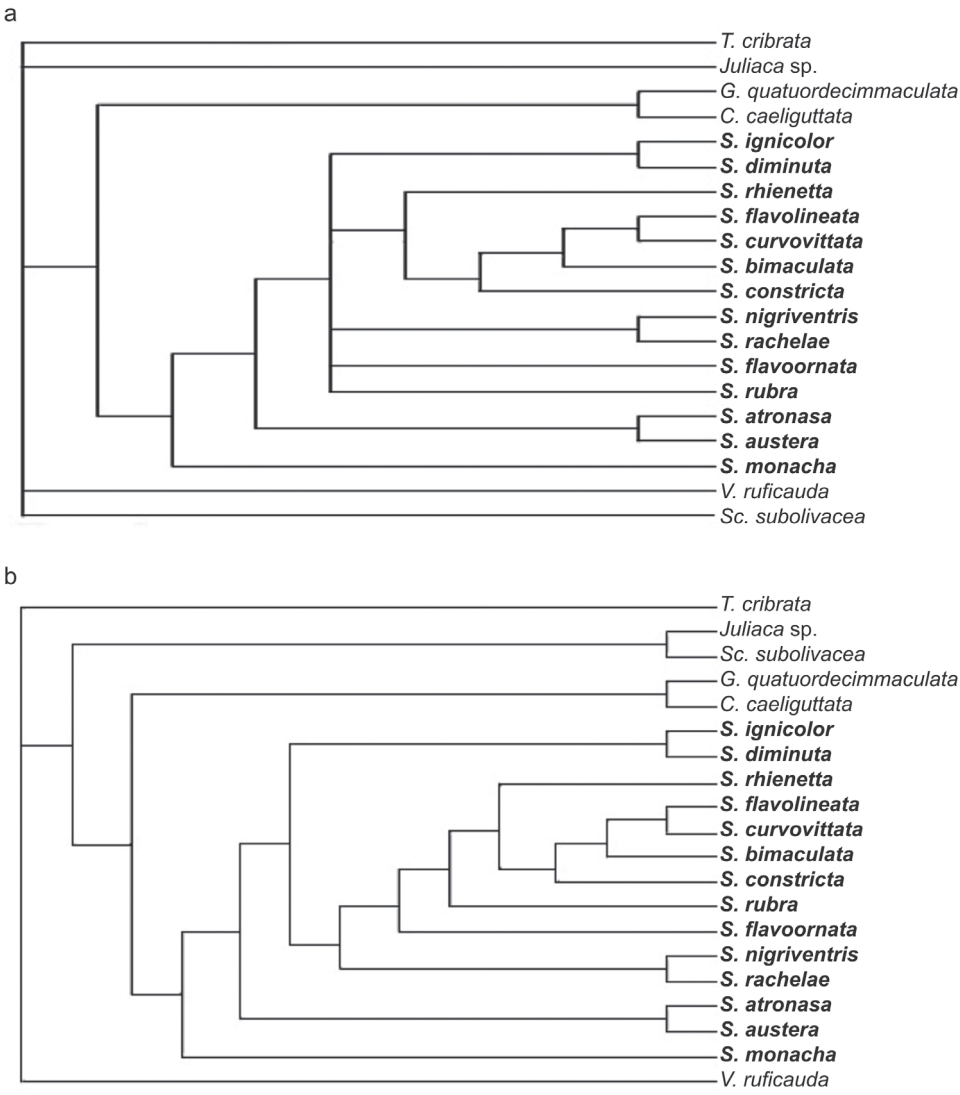

Figure 3. a Strict consensus of the six equally most parsimonious trees of the phylogenetic analysis of *Subrasaca* and outgroup taxa **b** Most parsimonious tree obtained with the successive weighting procedure; length = 80, consistency index = 0.8249 (excluding uninformative characters = 0.7199), retention index = 0.7641, rescaled consistency index = 0.6303. Outgroup genera are *Cyclogonia*, *Geitogonalia*, *Juliaca*, *Scopogonalia*, *Versigonalia*, and *Tretogonia* (root).

to black stripes on the forewings (*S. bimaculata*, *S. constricta*, *S. curvovittata*, and *S. flavolineata*) had the highest percentage of bootstrap (= 83%) and was supported by seven apomorphic conditions (Table 3, node 26), including the conspicuous set of dark brown to black longitudinal stripes on the forewings (character 25, state 1; Fig. 1b, c). The Bremer support of this clade was 3. It shows the following internal

Table 3. Apomorphy list for clades of Fig. 4 of the phylogenetic analysis of *Subrasaca* and outgroup taxa. Non-homoplastic characters are in bold.

Node or terminal taxon	Apomorphies
37	37(0), **53(0)**, **54(1)**
21	1(1), 22(6), 24(1), 65(1)
36	8(1), 40(1), **64(1)**
22	**5(2)**, 19(1), **44(2)**, **51(0)**, 61(1)
35 (*Subrasaca*)	2(0), **10(1)**, 38(1), 42(2), 63(1)
34	46(1), 48(0)
33	1(1), 3(A), **5(3)**, 9(2), **14(1)**, **23(1)**, **33(1)**, 66(0), 72(1)
32	19(1), 22(0), 24(0)
23	3(5), 70(0)
31	**21(2)**, 42(0)
30	6(1), 11(1), 62(1)
29	**21(3)**, **51(1)**, 65(1), 66(4), 67(1)
28	8(0), 72(1)
27	1(1), 4(1), **7(1)**, 12(1), 13(1), 19(0), 47(1), 67(0)
26	**16(1)**, 22(1), 24(1), **25(1)**, 28(1), 70(0), 72(0)
25	2(1), 3(8), **17(1)**, 47(0), 55(2), **59(1)**, 69(1)
24	42(1), 62(1)
Versigonalia ruficauda	3(B), 6(1), 13(1), 22(8), 24(4), 28(1), **30(2)**, 35(2), 71(1), 72(2)
Juliaca sp.	2(1), 12(1), 13(1), 18(1), 39(1), 61(3), **68(2)**, 69(1), 72(1)
Scopogonalia subolivacea	3(C), 22(9), 37(1), 38(1), 42(0), 49(1), 63(1)
G. quatuordecimmaculata	2(1), 3(4), 4(1), **21(1)**, 22(0), 24(2), 35(1), 41(1), **43(0)**, 49(1), 61(2), **68(3)**
Cyclogonia caeliguttata	9(2), 11(1), **20(2)**, 69(1)
Subrasaca monacha	3(2), 9(1), 49(1), **50(0)**, **51(3)**, 55(0)
Subrasaca atronasa	24(1), 32(1)
Subrasaca austera	4(1), **15(1)**
Subrasaca ignicolor	3(6), 24(2), 41(1), 65(1)
Subrasaca diminuta	**57(1)**
Subrasaca nigriventris	46(0), 48(1), 49(1), 66(0), 70(0)
Subrasaca rachelae	22(3), 24(3), 45(0), **52(1)**, 60(1), 61(1)
Subrasaca flavoornata	3(9), 42(1), **44(0)**, 46(0), 48(1), 49(1), 60(1), 61(1)
Subrasaca rubra	3(0), 24(2), 35(1), 66(3), 71(1)
Subrasaca rhienetta	2(2), 3(3), 18(1), 32(1), 42(2), 62(1), 65(0)
Subrasaca constricta	4(0), 22(7), **26(2)**, **27(1)**, **31(1)**, **36(1)**, 45(0), 58(1), 66(0), **68(5)**
Subrasaca bimaculata	22(4), **26(1)**, **56(1,2)**, 61(1), 63(0), **68(1)**
Subrasaca flavolineata	**5(1)**, 22(2), 41(1), 55(1), 58(1), 66(1)
Subrasaca curvovittata	3(7), **29(1)**, 67(1), **68(6)**

relationships in all trees (Fig. 3a): (*S. constricta* (*S. bimaculata* (*S. flavolineata*, *S. curvovittata*))). These four species were described in detail by Silva et al. (2013a). The group is distributed in the Atlantic Forest from northeastern (state of Bahia)

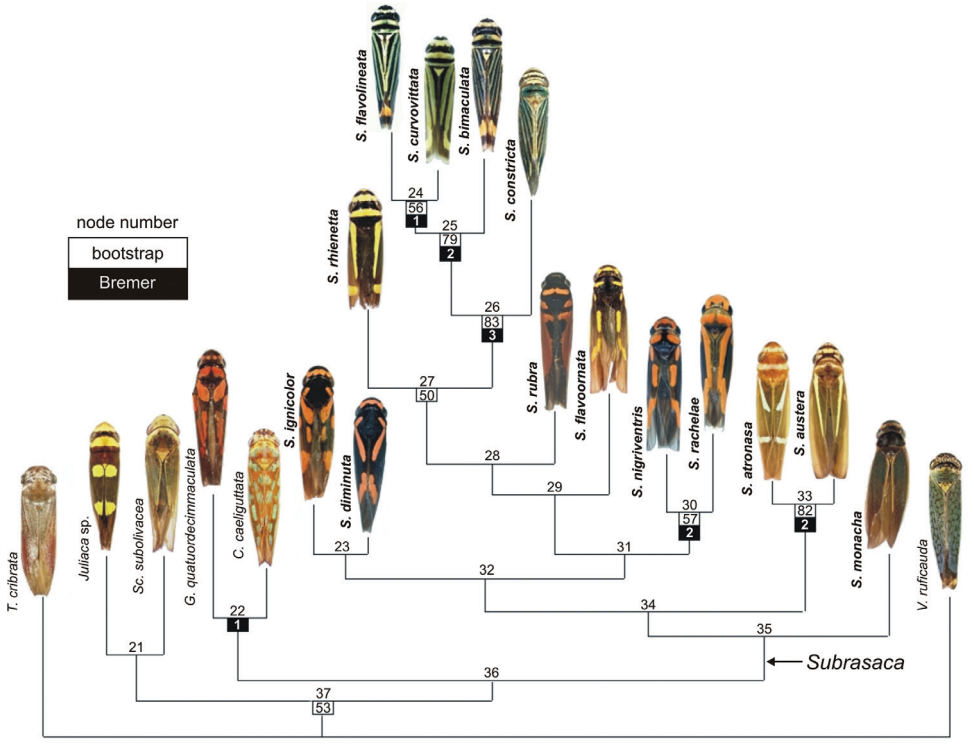

Figure 4. One of the most parsimonious trees of the phylogenetic analysis of *Subrasaca* and outgroup taxa; this is also the single tree obtained with the successive weighting procedure. Length = 197, consistency index = 0.6091 (excluding uninformative characters = 0.5389), retention index = 0.5722, rescaled consistency index = 0.3486. Species of *Subrasaca* in bold. Apomorphies are given in Table 3. Most sharpshooter images from Wilson et al. (2009).

to southern Brazil (state of Paraná). The second clade, formed by *S. atronasa* + *S. austera*, is supported by nine apomorphic conditions (Table 3, node 33), including a whitish-yellow transverse band on middle portion of pronotum (character 14, state 1; Fig. 4, node 33). This clade also had relatively high bootstrap (= 82%) and Bremer (= 2) scores. These two species, which are known only from the state of Santa Catarina (Zanol and de Menezes 1982) in southern Brazil (Atlantic Forest), were described by Young (1977), who considered them "very close" to each other (Young 1977: 479).

Although with low support scores, the clades formed by *S. ignicolor* + *S. diminuta* and *S. nigriventris* + *S. rachelae* were recovered in all most parsimonious trees (Fig. 4, nodes 23 and 30, respectively). Unlike the species with longitudinal dark brown to black stripes on the forewings (node 26), those with contrasting orange marks (*S. diminuta* + *S. ignicolor*, *S. nigriventris* + *S. rachelae*, and *S. rubra*) did not form a monophyletic group in any of the six most parsimonious trees.

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Three new species of *Epicephala* Meyrick (Lepidoptera, Gracillariidae) associated with *Phyllanthus microcarpus* (Benth.) (Phyllanthaceae)

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Abstract

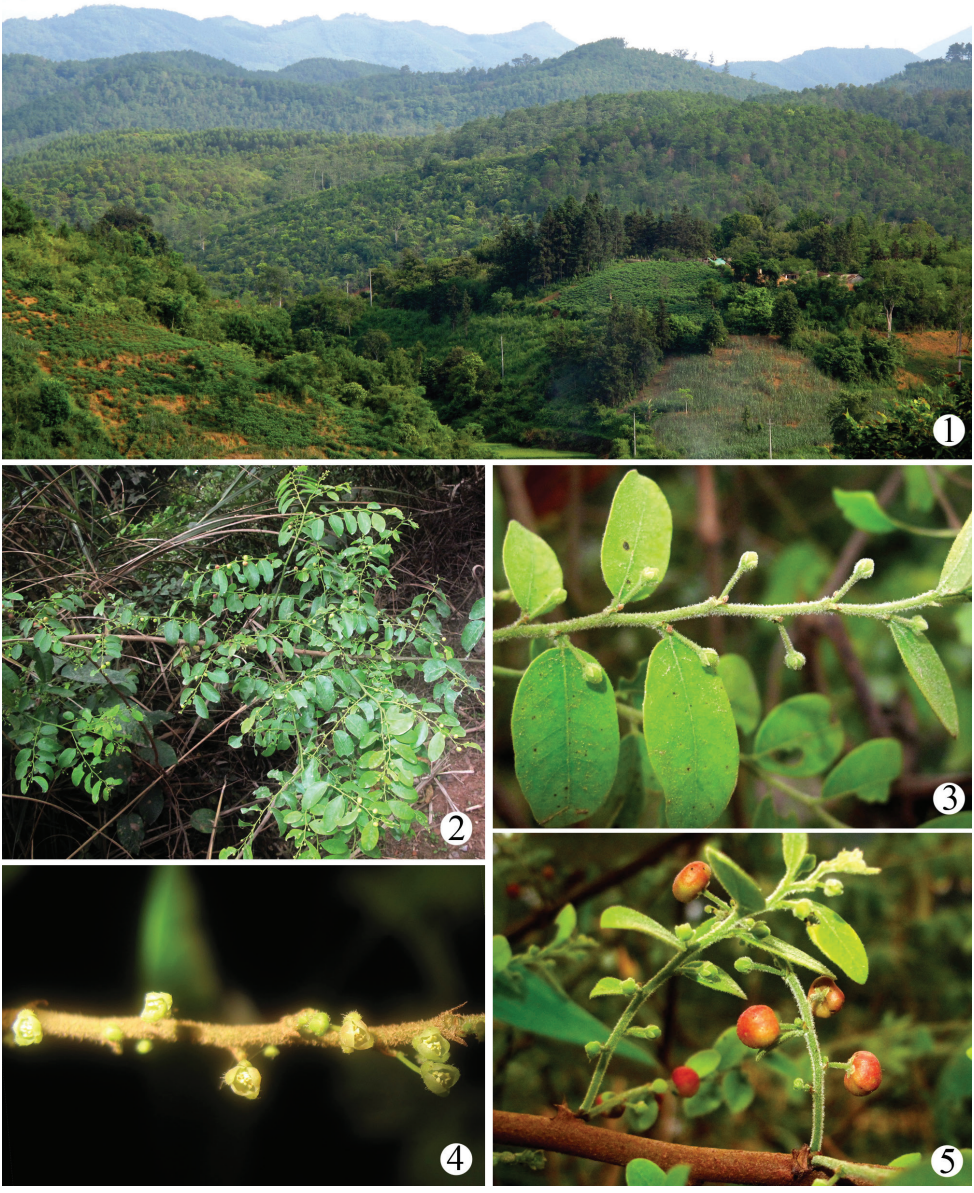
Three new species of *Epicephala* Meyrick, 1880 are described based on specimens reared from fruits of *Phyllanthus microcarpus* (Benth.): *Epicephala microcarpa* **sp. n.** and *E. laeviclada* **sp. n.** from Guangxi and Hainan, and *Epicephala tertiaria* **sp. n.** from Guangdong and Guangxi. Photographs of adults and illustrations of genital structures are provided.

Keywords

Lepidoptera, Gracillariidae, Phyllanthaceae, *Epicephala*, *Phyllanthus*, new species, China

Introduction

The genus *Epicephala* Meyrick, 1880 of the moth family Gracillariidae has been reported to have close coevolutionary relationships with the genera *Glochidion*, *Phyllanthus* and *Breynia* of the plant family Phyllanthaceae. *Epicephala* currently consists of 46 described species worldwide, mainly distributed in the Old World (Vári 1961; Kuznetzov 1979; Nielsen et al. 1996; De Prins and De Prins 2005, 2011; Zhang et al. 2012). In China, nine species have been recorded prior to this study (Zhang et al. 2012).



Figures 1–5. Habitats of *Phyllanthus microcarpus* (Benth.), the host plant of *Epicephala* species in Shaoping Forestry Centre, Pingxiang, Guangxi. **1** general habitat; **2–5** morphological features: **2** an individual tree **3** female flowers and leaves **4** male flowers **5** female flowers and fruits.

The present paper describes three new species based on specimens reared from the host-plant, *Phyllanthus microcarpus* (Benth.) (Figs 1–5) from Guangxi Zhuang Autonomous Region, Hainan and Guangdong provinces, while the authors were study-

ing their biology and coevolution with the host-plant (to be reported upon in different papers). *Phyllanthus microcarpus* was previously a synonym of *Phyllanthus reticulatus* Poir. until Luo et al. (2011) showed they are two different species, with differences in vegetative and floral characteristics, and different habitats and distribution.

Material and methods

The field study was conducted from 2011 to 2013 in Pingxiang, Guangxi Zhuang Autonomous Region, and from 2009 to 2014 in several nature reserves in Hainan Province, China. Specimens examined in this study were collected or reared from fruits of *Phyllanthus microcarpus* (Benth.). Genitalia dissection and mounting methods follow Li and Zheng (1996). Photos of the host-plants were taken in the field using a Canon PowerShot G10 digital camera. Photos of adult specimens were taken with a Leica M250A stereo microscope, and illustrations of the genitalia were prepared by using a Leica DM750 microscope, and refined in Photoshop®CS4 software.

The type specimens are deposited in the Insect Collection, College of Life Sciences, Nankai University, Tianjin, China and some paratypes are deposited in the Department of Entomology, Natural History Museum, London, UK (BMNH).

Description of new species

***Epicephala microcarpa* Li, sp. n.**
http://zoobank.org/F9726A27-9218-4780-BA70-36BFE564B432
Figs 6, 9, 12

Material examined. 237 males and 206 females, including all their genitalia preparations.

Holotype ♂ – **CHINA: Hainan Province:** Diaoluoshan, 18.xii.2012, reared from fruit of *Phyllanthus microcarpus* Poir. by Zhibo Wang, genitalia slide no. WZB14371.

Paratypes – **CHINA: Hainan Province:** 3♂, 1♀, Nanxi Forestry Station, Diaoluoshan, Lingshui County, 300 m, 9–15.viii.2008, under light trap, leg. Bingbing Hu and Li Zhang; 4♂, 4♀, Diaoluoshan, 12–29.iv.2008, 11.xi-10.xii.2009, reared from fruits of *Phyllanthus microcarpus* by Bingbing Hu, 12♂, 14♀, 18.xii.2012, reared from fruits of *Phyllanthus microcarpus* by Zhibo Wang; 11♂, 11♀, Tropical Botanical Garden, Danzhou, 30.xi-28.xii.2009, reared from fruits of *Phyllanthus microcarpus* by Bingbing Hu; 1♂, 3♀, Yinggeling Mountain Nature Reserves (19°01'N, 109°33'E), 450 m, 8–20.vi.2010, reared from *Phyllanthus microcarpus* by Bingbing Hu; 3♂, 1♀, Jianfenling, 24.vi.2010, leg. Bingbing Hu. **Guangxi Zhuang Autonomous Region:** 171♂, 203♀, Shaoping Forestry Centre (22°05'N, 106°54'E), 200 m, Pingxiang, 22.vii–12.viii.2011, 6.iv–28.vii.2012, 27.iii–22.vii.2013, reared from fruits of *Phyllanthus microcarpus* by Xiaofei Yang (2♂, 2♀, deposited in BMNH). **INDIA:** 1♂, label 1: Surat, Bombay, RM. 24.1.[19]29; label 2: *Epicephala vermiformis*, 1/2 Meyr.,

Figures 6–8. Adults of *Epicephala* spp. **6** *E. microcarpa* sp. n., paratype ♂ **7** *E. laeviclada* sp. n., holotype ♂ **8** *E. tertiaria* sp. n., paratype ♀ (Scale bars = 1.0 mm).

E. Meyrick det., in Meyrick Coll.; label 3: Meyrick Coll., B. M. 1938–390; label 4: B. M. Genitalia slide No. 32328, dissected by Houhun Li, deposited in the Natural History Museum, London (BMNH).

Diagnosis. This species is similar to *Epicephala exetastis* Meyrick, 1908 both in appearance by having similar densely compacted markings and in the genital structures. It can be separated from the latter in the female by the broad cone-shaped ovipositor, the inconspicuous lamella postvaginalis, the expanded antrum and ductus bursae, and the broad signa. In *E. exetastis* Meyrick, the ovipositor is slender, the lamella postvaginalis is conspicuous, the antrum and ductus bursae are narrow, and the signa are narrow in the female.

Description. Adult (Fig. 6). Forewing expanse 5.0–7.5 mm. Head white to pale yellowish brown, lateral sides with long black scales. Labial palpus black, inner surface of second and distal portion of third segments mixed with white scales. Antenna dark brown, with narrow greyish white rings, more distinct on dorsal surface. Thorax white. Tegula with basal half brown, distal half greyish white. Forewing greyish brown to dark brown, markings dense and compact; three pairs of white striae from both costal and dorsal 2/3,

1/2 and 3/4 extending obliquely outward to middle and end of cell as well as outside of cell, dorsal striae broader and clearer than costal striae; basal 1/6 of dorsum with broad white band; a narrow silvery-white fascia with metallic reflection from costal 5/6 to dorsum, arched outward medially; distal 1/6 ochre brown, with a central black dot edged by a short white streak or a dot near costa, with a white band along dorsum; cilia greyish white except black at base and apex, adjacent white from costal margin along termen to tornus, then grey along dorsal margin. Hindwing and cilia greyish brown. Abdomen dark brown.

Male genitalia (Fig. 9). Tegumen broadly elliptical, lateral sides narrow and sclerotized. Valva rectangular, longer than tegumen, nearly parallel dorso-ventrally, apex obliquely rounded, with long dense setae ventrally. Sacculus narrowed, elongate triangular, approximately 4/5 length of valva, tapered to sharp or truncate apex distally; densely with long setae ventrally. Transtilla slender, S-shaped, curved downward distally, acute apically. Vinculum broad, nearly U-shaped; saccus slender, nearly the same length as vinculum, apex rounded. Phallus straight, approximately 3/4 length of valva; cornuti formed by dense spinules grouped into two bundles.

Female genitalia (Fig. 12). Ovipositor broad, cone-shaped, constricted basally, dentate laterally, acute apically. Apophysis posterioris strong, 1.2 times longer than apophysis anterioris. Lamella postvaginalis broad and very short, unconspicuous. Antrum thick, strongly sclerotized, nearly as long as 8th abdominal segment. Ductus bursae broad, slightly longer than antrum, basal 2/3 sclerotized with wide longitudinal pleats; ductus seminalis expanded, arising from base of ductus bursae. Corpus bursae oval, shorter than ductus bursae, medially with pair of large signa, apex of signum with two teeth.

Host-plant. Phyllanthaceae: *Phyllanthus microcarpus* (Benth.). The larva feeds on seeds in the fruit.

Distribution. China (Guangxi and Hainan), India (Bombay).

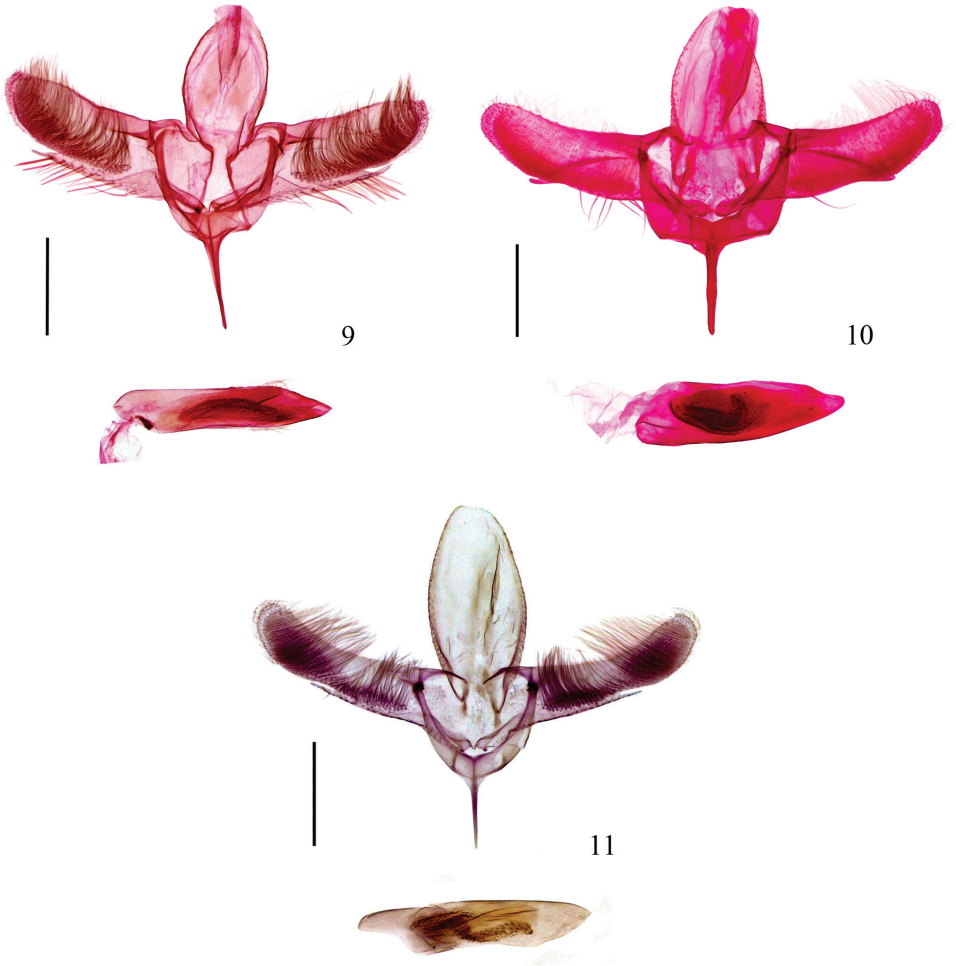
Etymology. This new species is named after its host-plant *Phyllanthus microcarpus* (Benth.).

Remarks. One specimen of the new species collected in India was determinated as *Epicephala vermiformis* Meyrick, 1936 by Meyrick himself. However, this specimen is quite different from the two Indonesian syntypes of *E. vermiformis* by having a distinctly narrower forewing (Natural History Museum, London, examined). Moreover, the host-plant of *E. vermiformis* is *Cajanus cajan* (L.) (Fabaceae) (De Prins and De Prins 2014), while all species in the genus are host-specific.

***Epicephala laeviclada* Li, sp. n.**
http://zoobank.org/CE54CF03-7092-4998-834D-5AF25EDDC43C
Figs 7, 10, 13

Material examined. 10 males and 5 females, including all their genitalia preparations.

Holotype ♂ – **CHINA: Guangxi Zhuang Autonomous Region:** Shaoping Forestry Centre (22°05'N, 106°54'E), 200 m, Pingxiang, 20.vi.2012, reared from fruit of *Phyllanthus microcarpus* (former identification *P. reticulatus* var. *glaber*) by Xiaofei Yang, genitalia slide no. YXF14198.



Figures 9–11. Male genitalia of *Epicephala* spp. **9** *E. microcarpa* sp. n., paratype, genitalia slide No. WZB14371 **10** *E. laeviclada* sp. n., paratype, genitalia slide No. YXF14282 **11** *E. tertiaria* sp. n., paratype, genitalia slide No. ZJ10021 (Scale bars = 0.2 mm).

Paratypes – **CHINA: Guangxi Zhuang Autonomous Region:** 9♂, 4♀, same locality and host-plant as holotype, 26.vii.2011, 26.iv–24.vi.2012, 27.iii–14.iv.2013 collected under light or reared from fruits of host-plant. **Hainan Province:** 1♀, Tropical Botanical Garden, Danzhou, 30.xi.2009, reared from fruits of *Phyllanthus microcarpus* (Benth.) by Bingbing Hu.

Diagnosis. This species is similar to *Epicephala microcarpa* sp. n. in appearance, but can be separated from the latter by the compacted sacculus with bluntly rounded apex that connects with a ridge in the inner surface of the valva, the stout bullet-like phallus with cornuti composed of spinules that are grouped into one bundle in the male; the cone-shaped lamella postvaginalis is conspicuous, and the corpus bursae with only one small signum in the female. In *E. microcarpa* sp. n., the sacculus is narrower and longer, and its apex is usually sharp and lacks a sclerotized ridge in the inner

surface of the valva, and the straight phallus has cornuti composed of spinules that are grouped into two bundles in the male; the broad and very short lamella postvaginalis is unconspicuous, and the corpus bursae has a pair of large signa in the female.

Description. Adult (Fig. 7). Forewing expanse 5.0–7.5 mm. Head white to greyish brown, lateral sides with long black scales. Labial palpus black, inner surface greyish white to black, basal 1/3 of second and both ends of third segments greyish white. Antenna dark brown, scape with long and narrow scales, flagellum with narrow greyish rings. Thorax white to greyish brown. Tegula brown, apically greyish white. Forewing brown to dark brown; three white striae from costal 1/4, 1/3 and 2/5 extending obliquely outward to 1/3 width of forewing; dorsum with broad white band along basal 1/3, serrated on upper edge, distally with a stria extending obliquely outward to middle of cell, with a small triangular white spot and an obliquely outward stria at middle and before 5/6, respectively; a narrow silvery-white fascia with metallic reflection from costal 5/6 to dorsum; distal 1/6 ochreous, with a central black spot edged by a white dot near costa and a white band along dorsum; cilia greyish white except black at basal margin and apex. Hindwing and cilia greyish brown. Abdomen dark brown.

Male genitalia (Fig. 10). Tegumen elongate elliptical, lateral sides narrow and sclerotized. Valva rectangular, somewhat longer than tegumen, nearly parallel dorsoventrally, costal margin gently curved, apex rounded. Sacculus narrowed, compact, elongate triangular, approximately 2/3 length of valva, apex bluntly rounded and connected with sclerotized ridge obliquely arched to base of vinculum; sparsely with long setae ventrally. Transtilla S-shaped, stout basally, curved downward distally, acute apically. Vinculum short and broad, somewhat rectangular; saccus slender, nearly the same length as vinculum, apex bluntly rounded. Phallus stout, bullet-like, approximately 3/4 length of valva; cornuti composed of dense spinules grouped into a bundle.

Female genitalia (Fig. 13). Ovipositor broad, cone-shaped, dentate laterally, acute apically. Apophysis posterioris strong, 1.2 times longer than apophysis anterioris. Lamella postvaginalis situated at base of antrum medially, short cone-shaped, approximately 2/5 width of antrum, same length with width. Antrum thick, heavily sclerotized, slightly longer than 8th abdominal segment. Ductus bursae membranous, broadly expanded, as long as antrum; ductus seminalis expanded, arising from base of ductus bursae. Corpus bursae oval, shorter than ductus bursae, medially with a small semilunar signum.

Host-plant. Phyllanthaceae: *Phyllanthus microcarpus* (Benth.). The larva feeds on seeds in the fruit.

Distribution. China (Guangxi and Hainan).

Etymology. The specific name is derived from the Latin *laevis* (smooth) and *cladus* (branch), in reference to individuals of the host-plant, *Phyllanthus microcarpus* (Benth.), having glabrous branches.

Remarks. The host-plant, *Phyllanthus microcarpus* (Benth.), has glabrous and pubescent forms that were formerly identified as the varieties *P. reticulatus* var. *glaber* (glabrous) and *P. reticulatus* var. *reticulatus* (pubescent). However, *P. reticulatus* also has such forms, and other characters are needed to separate the two plant species. The larva of *Epicephala laeviclada* sp. n. has only been found on the glabrous plants.

***Epicephala tertiaria* Li, sp. n.**
http://zoobank.org/ECB0B192-9905-44D7-BE46-520BBB219537
Figs 8, 11, 14

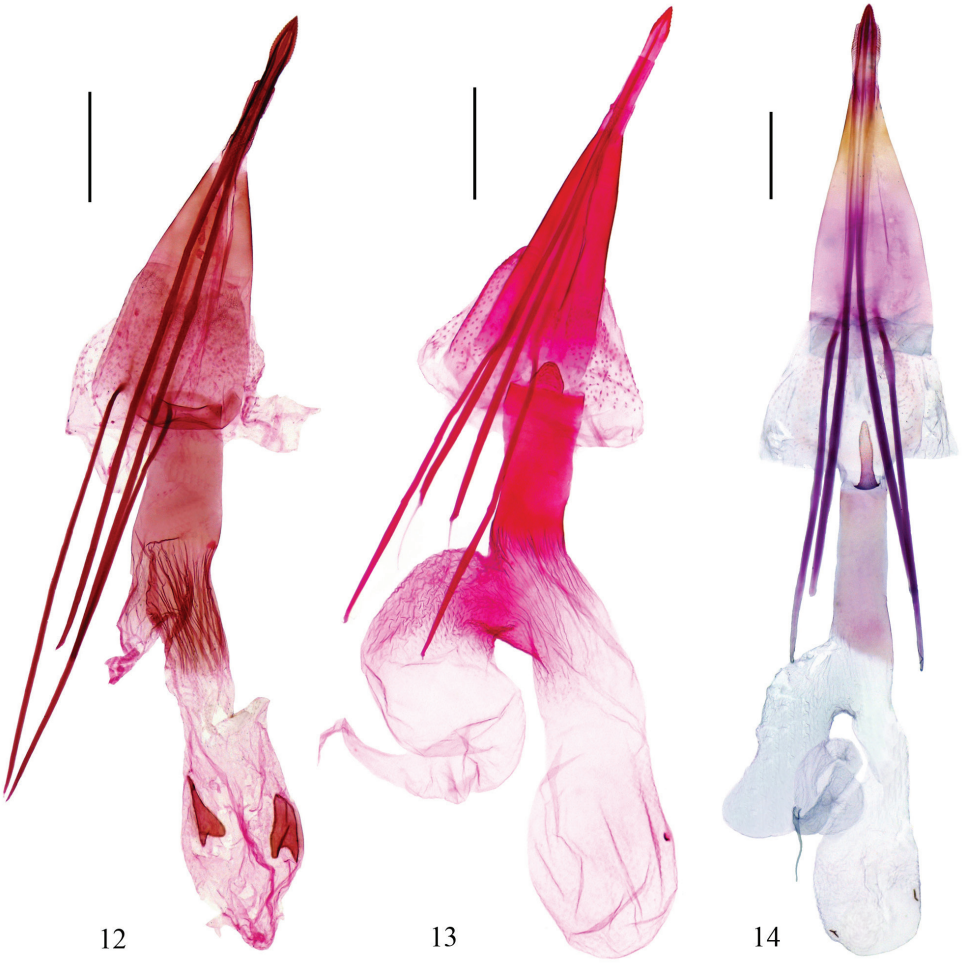
Material examined. 21 males and 14 females, including all their genitalia preparations.

Holotype ♂ – **CHINA: Guangdong Province:** South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, (23°11'N, 113°22'E), 210 m, 22.ii.2006, reared from fruit of *Phyllanthus microcarpus* (former identification *P. reticulatus* var. *glaber*) by Houhun Li, genitalia slide no. YXF14039.

Paratypes – **CHINA: Guangdong Province:** 11♂, 7♀, same data as holotype; 7♂, 4♀, same locality and host-plant, vii-viii.2006, collected mature larvae by Shixiao Luo and reared to adults by Houhun Li. **Guangxi Zhuang Autonomous Region:** 2♀, Shaoping Forestry Centre (22°05'N, 106°54'E), 200 m, Pingxiang, 24, 29.vi.2012, reared from fruit of *Phyllanthus microcarpus* (former identification *Phyllanthus reticulatus* var. *reticulatus*) by Xiaofei Yang; 2♂, same locality, 27.iii, 10.iv.2013, reared from fruit of *Phyllanthus microcarpus* by Xiaofei Yang, whether the plants are glabrous or pubescent was not recorded.

Diagnosis. This species is similar to *Epicephala microcarpa* sp. n. in both appearance and genitalia, but can be separated from the latter by distal 1/6 of the forewing having a broad white band along costa; the narrower valva as long as the tegumen and rounded at apex, and the narrower and shorter sacculus approximately 2/3 length of the valva in the male; the ovipositor not constricted basally, the lamella postvaginalis digitated, the ductus bursae membranous, and the smaller corpus bursae with very minute signa in the female. In *E. microcarpa* sp. n., the forewing has a short white streak or a dot near costa in distal 1/6; the valva is broader and longer than the tegumen and its apex is oblique, the sacculus is somewhat broader and approximately 4/5 length of the valva in the male; the ovipositor is constricted at base, the lamella postvaginalis is unconspicuous, basal 2/3 of the ductus bursae is sclerotized and densely covered with longitudinal wide pleats, and the signa are large in the female.

Description. Adult (Fig. 8). Forewing expanse 6.0–8.5 mm. Head cream white, with dark brown laterally. Labial palpus black, inner surface and outer ventral margin of second segment white, inner surface of third segment white to greyish brown. Antenna dark brown, with narrow greyish white rings. Thorax white. Tegula and forewing brown to dark brown; forewing with three pairs of white striae from both costal and dorsal 1/4, 2/3 and 3/4 extending obliquely outward to middle and end of cell as well as outside of cell respectively, costal striae narrow, inconsecutive and usually indistinct, dorsal striae broad and clear, latter two striae inconsecutive; dorsum with a broad white band along basal 1/3; a narrow silvery-white fascia bearing bluish metallic reflection from costal 5/6 to dorsum, arched outward medially; distal 1/6 ochreous, with a central black dot near fascia at 5/6, with broad white band along costa and dorsum; cilia along termen to tornus pale grey except black at base and ochre brown at apex. Hindwing and cilia pale grey. Abdomen greyish brown.



Figures 12–14. Female genitalia of *Epicephala* spp. **12** *E. microcarpa* sp. n., paratype, genitalia slide No. YXF14026 **13** *E. laeviclada* sp. n., paratype, genitalia slide No. YXF14060 **14** *E. tertiaria* sp. n., paratype, genitalia slide No. ZJ10028 (Scales = 0.2 mm).

Male genitalia (Fig. 11). Tegumen broadly elliptical, lateral sides narrow and sclerotized. Valva narrowed, rectangular, as long as tegumen, slightly narrowed medially, gently curved upward, apex rounded, with long dense setae ventrally. Sacculus narrowed, approximately 2/3 length of valva, tapered to sharp apex. Transtilla slender, curved downward, acute apically. Vinculum broad, nearly U-shaped; saccus slender, shorter than vinculum, apex acute. Phallus broad, straight, approximately 3/4 length of valva; cornuti composed of dense spinules.

Female genitalia (Fig. 14). Ovipositor broad, cone-shaped, dentate laterally, acute apically. Apophysis posterioris strong, 1.2 times longer than apophysis anterioris. Lamella postvaginalis digitated, arising from base of antrum medially, 1/4 length of apophysis anterioris, apex rounded. Antrum developed, cylindrical, straight, longer than

8th abdominal segment. Ductus bursae narrow, membranous, shorter than antrum; ductus seminalis expanded, broader than ductus bursae, arising from base of ductus bursae. Corpus bursae oval, small, as long as ductus bursae; paired signa placed anteriorly, small, short linear.

Host-plant. Phyllanthaceae: *Phyllanthus microcarpus* (Benth.). The larva feeds on seeds in the fruit.

Distribution. China (Guangdong and Guangxi).

Etymology. The specific name is derived from the Latin *tertiarius* (third), indicating that this is the third species reared from the host-plant *Phyllanthus microcarpus* (Benth.).

Remarks. The larvae were reared from glabrous individuals of *Phyllanthus microcarpus* in Guangzhou, Guangdong Province, and from pubescent individuals of *P. microcarpus* in Pingxiang, Guangxi Zhuang Autonomous Region. Both glabrous and pubescent forms are now treated as one species (Luo et al. 2011). This interesting phenomenon may have some significance in the coevolution between the *Epicephala* moths and the Phyllanthaceae plants.

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RESEARCH ARTICLE

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A new genus and species of tettigarctid cicada from the early Miocene of New Zealand: *Paratettigarcta zealandica* (Hemiptera, Auchenorrhyncha, Tettigarctidae)

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Abstract

A new genus and species of primitive cicada (Hemiptera: Tettigarctidae) is described from the early Miocene of southern New Zealand. *Paratettigarcta zealandica* **gen.** et **sp. n.** is the first cicada (Cicadoidea) fossil from New Zealand and exhibits wing venation patterns typical for the subfamily Tettigarctinae. It differs from other fossil taxa and the extant genus *Tettigarcta* in the early divergence of CuA2 from the nodal line in the forewing, its parallel-sided subcostal cell, the early bifurcation of vein M and long apical cells of the hindwing, and in wing pigmentation patterns.

Keywords

Cicadoidea, Tettigarctidae, Miocene, New Zealand, Hindon Maar, Otago

Introduction

Tettigarctidae (hairy cicadas) is the sister-group to singing cicadas (Cicadidae) from which they are distinguished by various morphological characters such as the greatly expanded pronotum (concealing much of the mesonotum), timbals present in both

sexes, a completely developed forewing venation with the radial sector arising near the wing base and veins 1A and 2A separated, and a well-developed, conspicuous nodal line on the forewing (Evans 1941, Moulds 2005). Lacking tympanal auditory organs and possessing only rudimentary timbals, Tettigarctidae are not capable of producing the characteristic sound of singing cicadas – their acoustic signals are instead substrate-transmitted (Claridge et al. 1999). While singing cicadas are known since the Paleocene (Cooper 1941) and comprise about 2,000 extant species on all continents (except Antarctica), Tettigarctidae is a mainly Mesozoic radiation that is represented by just two extant species of *Tettigarcta* White, 1845: *T. crinita* Distant, 1883 in southeast Australia and *T. tomentosa* White, 1845 in Tasmania (Shcherbakov 2009, Moulds 2012).

The fossil record of family Tettigarctidae (summarised in Shcherbakov 2009 and Moulds in prep.) includes 19 extinct genera in subfamilies Cicadoprosbolinae Bekker-Migdisova and Tettigarctinae Distant mainly from terminal Triassic to Upper Cretaceous strata in the Northern Hemisphere. There are three Paleogene records, one from the Paleocene of Menat, France (*Meuniera haupti* Piton, 1940) and the Eocene of Scotland (*Eotettigarcta scotica* Zeuner, 1944) and Germany (Tettigarctidae gen. et sp. indet.; Wappler 2003), the latter representing the youngest fossil record of Tettigarctidae to date. The only Southern Hemisphere fossils of Tettigarctidae are *Architettix compacta* Hamilton, 1990 and *Tettagalma striata* Menon, 2005 from the Lower Cretaceous (Aptian) Santana Formation in Brazil, with *Magrebarcta* [*Liassotettigarcta*] *africana* Nel, Zarbout, Barale & Philippe, 1998 from the Lower Cretaceous (Aptian) in Tunisia complementing the meagre record from Gondwana.

Here we describe *Paratettigarcta zealandica* gen. et sp. n. as the first cicada fossil from New Zealand and, as it is of early Miocene age, the youngest fossil record of Tettigarctidae. This new genus and species comes from a newly discovered paleontological site at Hindon Maar in southern New Zealand and a brief discussion is presented of the depositional setting and the age of the locality as it is currently known.

Locality and age

Hindon Maar is a new paleontological site in Otago, South Island, New Zealand, ~10 km N of Outram, near Dunedin (45°45.62'S; 170°15.88'E; Fig. 1). Hindon Maar is located in the southern part of the Waipiata Volcanic Field, which comprises about 150 volcanic remnants of maar-diatremes, scoria cones, plugs, dikes and lava flows (Coombs et al. 2008). The fossil site is situated on private farmland within a shallow, semi-circular topographic depression (500×800 m in diameter) cut into regional metamorphic basement (Otago Schist of Jurassic age). The topographic basin coincides with an aeromagnetic high that is likely to indicate the presence of volcanic material at some depth below surface (data from Glass Earth Gold/Otago Regional Council; released in 2011). Bedded volcanoclastic rocks exposed at the margin of the basin are presumably the remnants of a largely eroded tephra rim; these have yet to be studied and mapped in detail (pers. obs.).

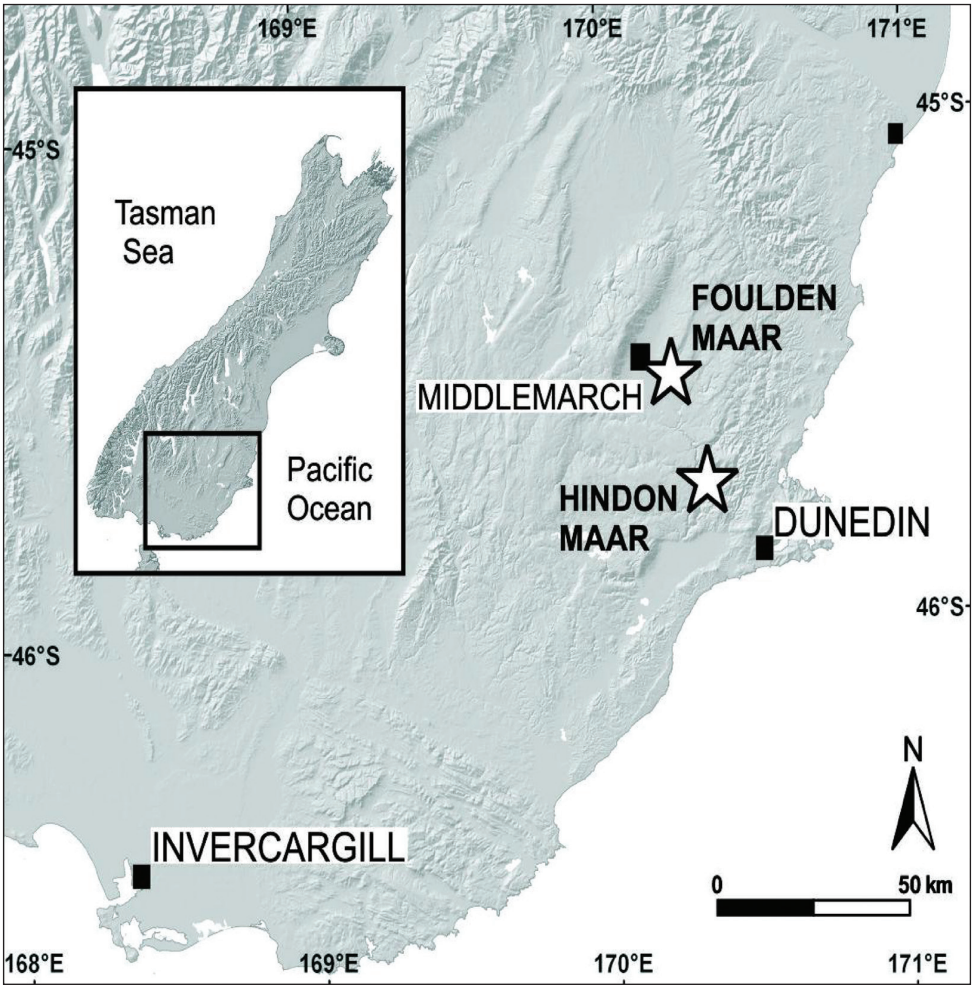

Figure 1. Map showing the position of the new fossil locality at Hindon Maar on the South Island of New Zealand (see text for explanation).

Youngson (1993) reported a 15 m thick sequence of coarse-grained schistose siliciclastics overlain by diatomaceous and carbonaceous laminites from a nearby site, and proposed deposition in a maar lake. Temporary excavations by the Geology Department, University of Otago, at two sites within the basin in early 2014 encountered two lacustrine facies associations beneath 1–2 m of Quaternary loess and alluvium. One facies represents a >3 m thick, thinly (? seasonally) laminated, fossiliferous diatomite interbedded with graded or massive diatomaceous mass-flow beds, which is very similar to that described from the earliest Miocene Foulden Maar fossil Lagerstätte, 26 km NNW of Hindon (Lindqvist and Lee 2009). The second facies is a >2.5 m thick, dark-brown, thickly laminated, carbonaceous mudstone containing cm-thick intervals of thinly laminated intervals. Both facies types are clearly of

lacustrine origin, as indicated by siliceous limnic microfossils such as pennate diatoms, siliceous spicules of freshwater sponges (*Spongilla* sp.), and chrysophycean cysts. The thickly laminated mudstone in which the cicada fossil was found contains a well-preserved, diverse terrestrial biota, including spores/pollen, leaves, flowers, fruits, seeds and cones from ferns, conifers and several angiosperm families (JM Bannister pers. comm. 2014, DC Mildenhall pers. comm. 2014), numerous coprolites (presumably from waterbirds), juvenile and adult galaxiid fish, and insects of the orders Hemiptera, Coleoptera, Hymenoptera and Trichoptera (pers. obs.). Preserved leaf and insect cuticles and soft tissue of fish, as well as the laminated biogenic sediment showing no signs of bioturbation all suggest deposition in a deep, presumably anoxic lacustrine environment.

The circular topographic basin, coinciding with an aeromagnetic high and situated in a monogenetic volcanic field, as well as the bedded pyroclastic deposits at the basin margin are all features typically associated with partly eroded maar-diatreme volcanoes, while the fine-grained laminated, biogenic sediment argues for (but is not restricted to) deposition in a maar-lake. The geological and paleontological evidence that is currently available thus suggests that Hindon Maar is a maar-type fossil lagerstätte that may contribute significantly to our understanding of Neogene Australasian biodiversity in the future.

The early Miocene age of Hindon Maar is based on palynology of the lacustrine sediments and on radiometric ages previously published for Waipiata volcanic rocks. K-Ar and $^{40}Ar/^{39}Ar$ ages provided by Coombs et al. (2008) indicate that Waipiata volcanism lasted from 24.8±0.6 to 8.9±0.9 Ma, which represents the time range in which the Hindon Maar very likely erupted. The palynological assemblage in the lacustrine diatomite at Hindon indicates an early Miocene age (Aquitanian/Burdigalian; 23.03–15.97 Ma) corresponding to New Zealand stages Otaian to Altonian (DC Mildenhall in Youngson 1993), with a maximum age close to the Duntroonian–Waitakian boundary, as indicated by the presence of *Coprosma* pollen (DC Mildenhall pers. comm. 2014). The fossil biota from Hindon Maar might therefore be coeval with or slightly younger than that of the Foulden Maar fossil lagerstätte, which has been dated at 23 Ma (Lindqvist and Lee 2009).

Material and methods

The studied fossil comprises overlapping fragments of a hind and forewing preserved as part and counterpart (Fig. 3A, B). The venation in basal parts of both wings, and that of the distal part of the forewing, is not preserved; the wing outline is decipherable for the distal margin of the hindwing only. Forewing and hindwing have mostly separated when the sediment was split open; as a consequence, venation of the hindwing is mainly visible on the part (together with faint traces of forewing venation; Fig. 3A) and venation of the forewing is mainly visible on the counterpart (Fig. 3B). The insect body and its appendages are not preserved.

Photomicrographs were taken with a Canon T3 camera attached to a Nikon SMZ1000 stereomicroscope. Wetting the specimen with ethanol accentuated the visibility of venation patterns and outlines of the wings. Photomicrographs taken at several depths of field were stacked using Photoshop CS5.1 software (Adobe Systems Inc.). Our terminology of wing venation and cells follows that of Moulds (2005) (see Fig. 1). The specimen is stored in a refrigerator (in order to prevent desiccation of the mudstone matrix) in the Geology Department, University of Otago under catalogue number OU45476.

Systematic paleontology

Family Tettigarctidae Distant, 1905
Subfamily Tettigarctinae Distant, 1905

***Paratettigarcta* gen. n.**
http://zoobank.org/9AD59A6E-DDE2-4ED7-AC62-BB88EE02C988

Type species. *Paratettigarcta zealandica* new species, designated herein (Figs 3, 4). No other species are currently included in the genus.

Diagnosis. *Paratettigarcta* is most similar in hindwing venation to that of *Eotettigarcta* Zeuner, 1944 from the Paleocene of the United Kingdom (*Eotettigarcta* is known only from a partial hindwing) but differs in its more parallel-sided subcostal cell (the most anterior of the distal cells) where RA lies parallel to Sc for most of its length rather than gradually diverging, and in the branching of vein M where M_1 branches before M_3 (after in *Eotettigarcta*). There are also similarities in the forewing of *Paratettigarcta* with extant *Tettigarcta* from which *Paratettigarcta* differs in the early divergence of CuA_2 from the nodal line in the forewing (late divergence in *Tettigarcta*). The hindwing of *Paratettigarcta* is quite different from that of *Tettigarcta*, especially in the apical cells that are much longer than those of *Tettigarcta*, in particular the anterior most cell (subcostal cell) that is wide and extended far beyond crossvein r (narrow and only a little extended beyond r in *Tettigarcta*). Further, *Paratettigarcta* has pigmented wing patterns not unlike those present in *Eotettigarcta* (and some other fossil Tettigarctidae) but such patterns are absent in extant *Tettigarcta*.

Description. Forewing veins R and M branched close to base of forewing so that ulnar cells u1-u3 and medial cell are long and narrow; vein CuA strongly bowed before branching. Nodal line clearly defined and departing the extremity of vein CuA_2. Crossvein r-m nearly straight, steeply angled to RP and M_1; m gently bowed, almost perpendicular to M_2 and M_3; m-cu strongly bowed, meeting M_4 nearly perpendicularly and meeting CuA_1 at a steep angle. Hindwing apical cells tending long and narrow, a1 almost as long as a2 so that crossvein r meets RA within its proximal quarter; Sc and RA wide apart, almost as wide as width of apical cell 1.

Etymology. The genus name is a combination of *para* (Latin from Greek, meaning "near") and the extant genus-group name *Tettigarcta*.

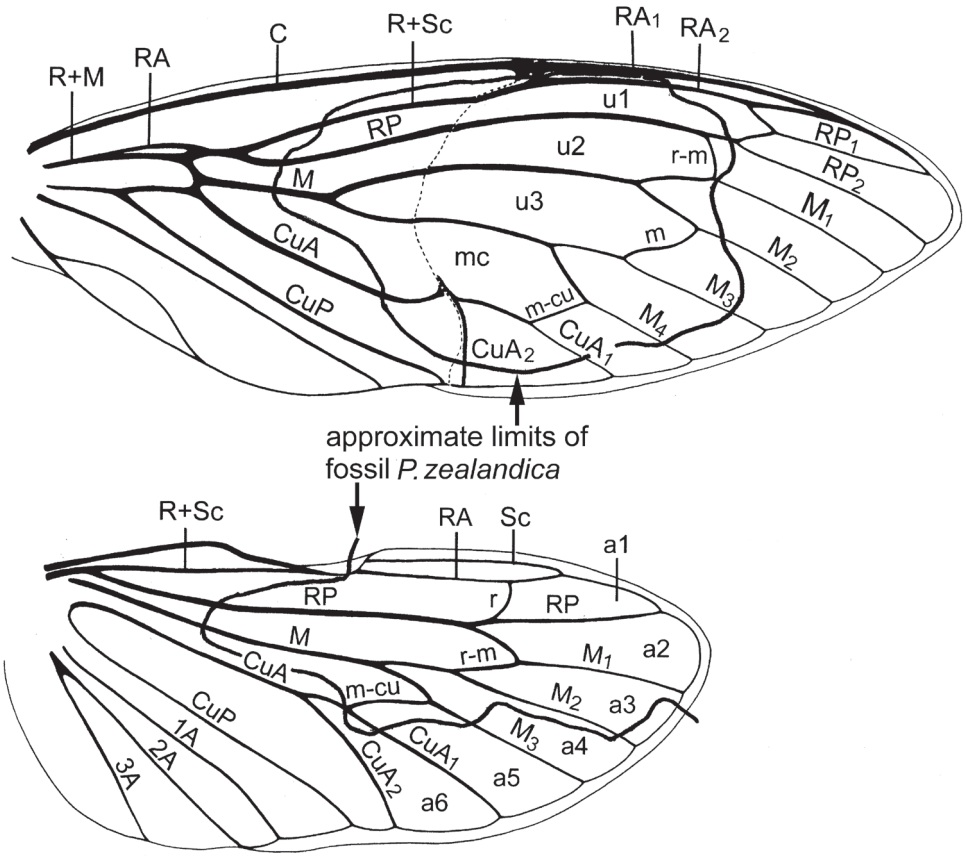

Figure 2. *Tettigarcta crinita* Distant, fore and hindwings; areas of the wings preserved in the new fossil species are indicated (modified from Moulds 2005). **A** anal vein **a** apical cell **C** costal vein **CuA** cubitus anterior vein **CuP** cubitus posterior vein **M** median vein **m** medial crossvein **mc** medial cell **m-cu** mediocubital crossvein **nl** nodal line **R** radius **r** radial crossvein **RA** radius anterior **r-m** radiomedial crossvein **RP** radius posterior **Sc** subcostal vein **u** ulnar cell.

***Paratettigarcta zealandica* sp. n.**
http://zoobank.org/2EB7CDEB-D467-4F04-9432-378176CB74D6
Figs 3, 4

Diagnosis. *Paratettigarcta zealandica* sp. n. differs from other Tettigarctidae by the attributes discussed in the generic diagnosis above. In particular the forewing of *Paratettigarcta zealandica*, that is remarkably similar to extant *T. crinita* Distant, 1883 (Fig. 2) and *T. tomentosa* White, 1845 (the only described species of *Tettigarcta*), differs as follows: (a) forewing crossvein m gently bowed and almost perpendicular to M_2 and M_3 rather than steeply angled and broadly 'S'-shaped; (b) forewing crossvein m-cu strongly bowed rather than nearly straight; (c) hindwing apical cells long and narrow, much longer than those of extant *Tettigarcta* (compare Figs 2 and 4).

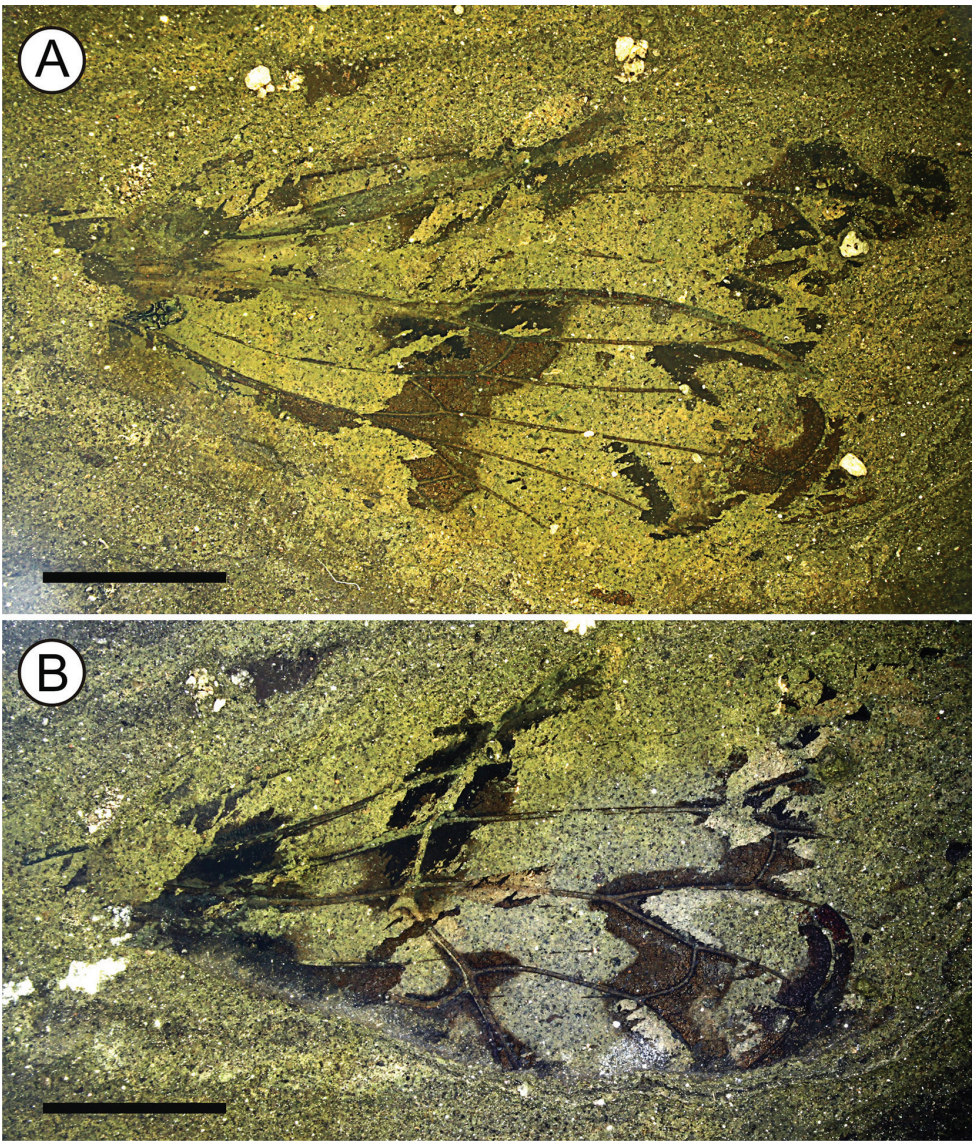

Figure 3. Photomicrographs of *Paratettigarcta zealandica* gen. et sp. n., holotype OU45476, fore and hindwing **A** part and **B** counterpart (mirror inverted), photographed under ethanol. Scale bar: 5 mm.

Description. Holotype. *Forewing* similar to extant *Tettigarcta* in size, shape and venation (compare Figs 2, 4). Impression 24.3 mm maximum length by 10.2 mm maximum width. Bearing dark pigmented transverse bands, one near the wing base, one following the nodal line between costa and M_{3+4}, one following the bases of apical cells, and one along apical margin. Nodal line strongly defined; CuA strongly bowed before forking at nodal line; crossvein r-m nearly straight and angled to both RP and M_1; crossvein m gently bowed, nearly perpendicular to M_2 and M_3; cross-

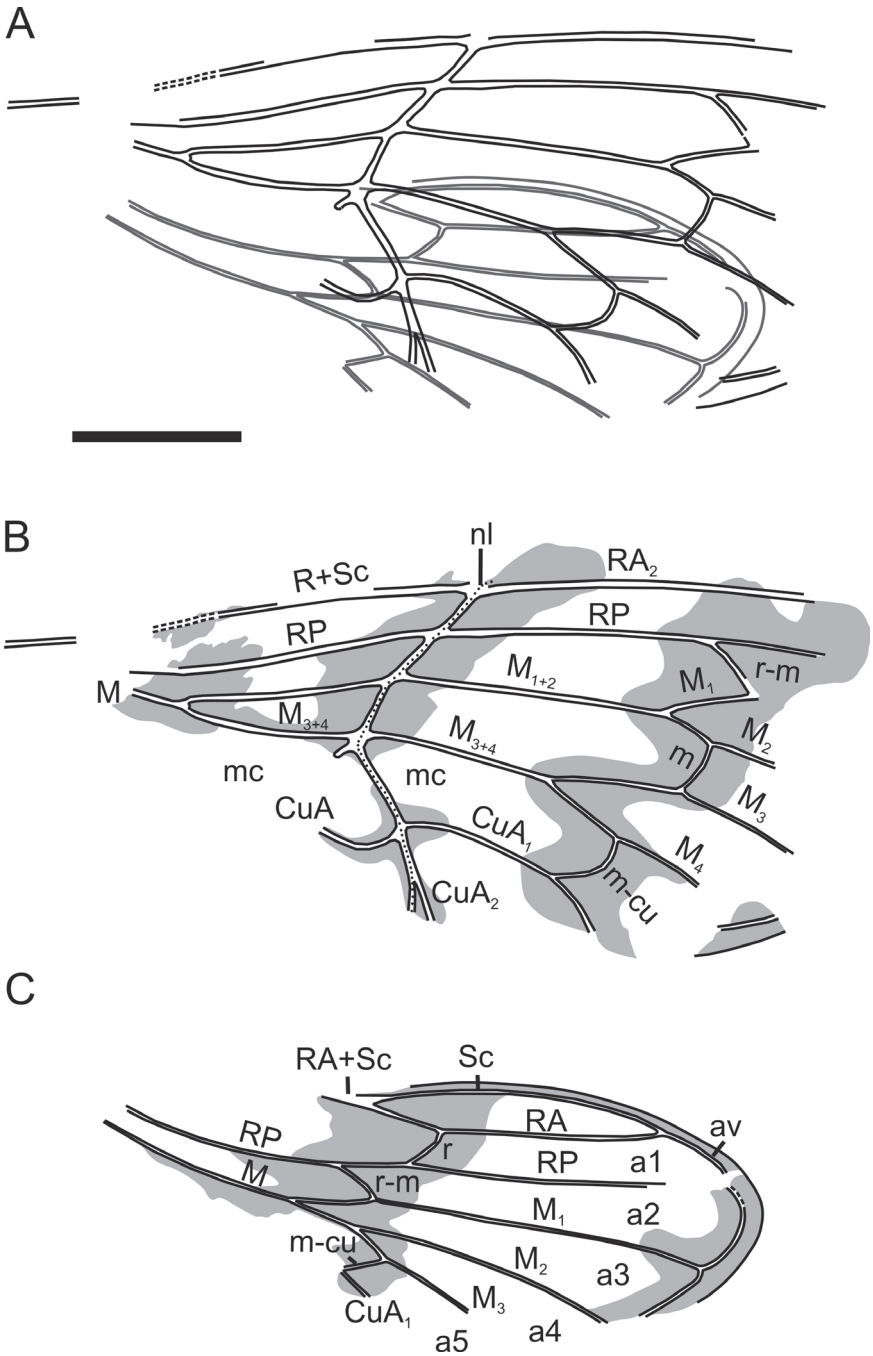

Figure 4. Line drawings of *Paratettigarcta zealandica* gen. et sp. n., holotype OU45476 **A** overlapping fore and hindwing as preserved **B** forewing and **C** hindwing, pigmented areas shown as preserved. **A** apical cell **av** ambient vein **C** costal vein **CuA** cubitus anterior vein **CuP** cubitus posterior vein **M** median vein; medial crossvein **mc** medial cell **m-cu** mediocubital crossvein **nl** nodal line **R** radius **r** radial crossvein **RA** radius anterior **r-m** radiomedial crossvein **RP** radius posterior **Sc** subcostal vein. Scale bar: 5 mm.

vein m-cu strongly bowed, meeting M_4 nearly perpendicularly, meeting CuA_1 at a steep angle. *Hindwing* impression 19.5 mm maximum length by 7.3 mm maximum width. Bearing dark bands, one from near coupling lobe to at least crossvein m-cu, and a dark streak behind M stem. Marginal membrane well developed but not exceedingly broad. Crossvein r nearly straight and angled to both RA and RP; crossvein r-m nearly straight and steeply angled to both RP and M; crossvein m-cu straight and angled to both M_3 and CuA_1.

Type-specimen. Holotype OU45476, hind and forewing from lacustrine mudstones at Hindon Maar (early Miocene; I44/f0392 in the New Zealand Fossil Record File), Waipiata Volcanic Field, 10 km N of Outram, Otago, southern New Zealand; deposited in the Department of Geology, University of Otago.

Etymology. The species name refers to New Zealand, where this species was distributed in the Miocene.

Comments. *Paratettigarcta zealandica* gen. et sp. n. appears closest to *Eotettigarcta scotica* based on the hindwing venation (the latter known only from a partial hindwing). In particular Sc and RA are widely separated, and it is likely that the apical cells are of similar length with a1 being almost as long as a2. If that is so then *Paratettigarcta* is best placed in the tribe Protabanini of the subfamily Tettigarctinae, family Tettigarctidae, following the classification of Shcherbakov (2009). At around 23–16 Ma this would make *P. zealandica* the youngest known Tettigarctidae fossil, the next youngest at 44 Ma being an undescribed Tettigarctinae tentatively placed in the tribe Tettigarctini (Wappler 2003, Shcherbakov 2009).

The forewing venation of *P. zealandica* shows a clear affinity with that of extant species of *Tettigarcta* of which there are only two closely related species, *T. crinita* and *T. tomentosa* (Moulds 1990). The overall branching of veins and therefore cell proportions are remarkably similar (compare Figs 2, 4). This confirms the placement of *P. zealandica* in the subfamily Tettigarctinae and similar dark wing patterns are found in some extinct genera of this subfamily (e.g. *Liassocicada* Bode, 1953).

Discussion

The discovery of a *Paratettigarcta zealandica* at Hindon Maar in southern New Zealand documents the presence of family Tettigarctidae in Australasia in the early Miocene. It thus partially fills the spatial and temporal gap that existed between the next youngest Tettigarctidae fossil from the mid-Eocene of Germany (Wappler 2003), tentatively placed into Tettigarctini (Shcherbakov 2009), and the two surviving members of this relict family in southeastern Australia and Tasmania. Extant cicadas of New Zealand comprise 34 endemic species in five genera (all placed in family Cicadidae, tribe Cicadettini), which occur in a wide range of habitats from lowland coastal areas to alpine zones (Larivière et al. 2010). Molecular phylogenetic studies suggested that the extant fauna is the result of two relatively recent (~12 Ma) transoceanic dispersal events from Australia and New Caledonia and subsequent

divergence related to the Southern Alps orogeny and glaciations within the last 5 Ma (Arensburger et al. 2004). *P. zealandica* described herein is the first cicada fossil from New Zealand and, although not in family Cicadidae, indicates the presence of cicadas (Cicadoidea) in New Zealand prior to the more recent incursions and radiations that formed the modern fauna. It establishes the relict family Tettigarctidae in southern New Zealand in the early Miocene and documents the extinction of this hemipteran family in New Zealand since then.

By the early Miocene, New Zealand had been an isolated island landmass for at least 57 My, following separation from Australia in the Late Cretaceous. Two biogeographical scenarios can consequently be hypothesised to explain the occurrence of Tettigarctidae at Hindon Maar: (1) colonization of New Zealand via trans-oceanic dispersal of members of this family in or before the early Miocene, for example from Australia or New Caledonia, as proposed for the Cicadidae (Arensburger et al. 2004), or (2) a vicariance origin in which *P. zealandica* evolved from a Gondwanan lineage that had been present in New Zealand since it separated from neighbouring landmasses. The validity of either hypothesis can only be tested by additional finds of Tettigarctidae fossils in the future.

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On the desmitracheate "micronetine" genus *Nippononeta* Eskov, 1992 (Araneae, Linyphiidae)

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Abstract

The desmitracheate system in a "micronetine" genus *Nippononeta* Eskov, 1992 is recognized for the first time in the present study. This makes the subfamilial placement of this genus problematic. A morphological study was conducted for *N. kurilensis* Eskov, 1992 (the type species of *Nippononeta*) and *N. coreana* (Paik, 1991). Characters of genitalia and tracheal system, as well as some somatic characters were studied in detail by using scanning electronic microscopy (SEM), and compared with those of *Agyneta*. Updated descriptions of the genus *Nippononeta* and its two species are presented. Putative synapomorphies for *Nippononeta* and *Agyneta* are provided, as well as some putative synapomorphies shared by the two genera. The results imply that both scaped epigynum and desmitracheate tracheal system are probably homoplastic. The placement of *Nippononeta* and *Agyneta* within Linyphiidae need to be resolved in future studies.

Keywords

Taxonomy, desmitracheate, scaped epigynum, genital morphology, "micronetine"

Introduction

Linyphiidae Blackwall, 1859 is the second largest spider family, including over 4,500 species (World Spider Catalog 2014). The genital characters are species-specific in linyphiids, and provide rich information for species identification, taxonomy, and phy-

logenetic reconstruction (e.g. Hormiga 2000; Miller and Hormiga 2004; Hormiga and Scharff 2005; Tu and Hormiga 2011). Seven linyphiid subfamilies are recognized (Tanasevitch 2014); however, the results of phylogenetic analysis based on molecular data show that most of them are not monophyletic (Arnedo et al. 2009).

Classical taxonomy of Linyphiidae is often confusing because of the characters overlapping among groups. The currently accepted seven subfamilies are based on different characters. For example, the epigynum furnished with a scape carrying copulatory grooves and openings (referred to as "scaped epigynum" below) was used as the main diagnostic feature for Micronetinae Hull, 1920 (Millidge 1984; Saaristo and Tanasevitch 1996), and the desmitracheate tracheal system, in which the median trunks are extensively branched and extend into the prosoma, was used as the main diagnostic feature for Erigoninae (Blest 1976; Millidge 1984). However, both scaped epigynum and desmitracheate system can be also found in the "micronetine" genera: *Tennesseellum* Petrunkevitch, 1925, *Agyneta* Hull, 1911 (including *Meioneta* Hull, 1920, now a junior synonym of *Agyneta*), and *Anibontes* Chamberlin 1924 (Millidge 1986; Hormiga 1994; Dupérré 2013). At the same time, neither of these two characters is found in some erigonines: *Asthenargus* Simon & Fage, 1922, *Gongylidiellum* Simon, 1884, and *Ostearius* Hull, 1911 (Hormiga 2000; Miller and Hormiga 2004). This makes the subfamilial placement of abovelisted genera problematic. A recent phylogenetic study of Linyphiidae based on molecular and morphological data (Arnedo et al. 2009) indicated that the "micronetines", as a paraphyletic group, together with the erigonines form the "micronetines+erigonines" clade. The morphology data suggested a single origin of the desmitracheate system, as a synapomorphy for Erigoninae, while the total evidence favored double origins.

Micronetinae is a large subfamily, which currently includes 1212 species placed in 91 genera (Tanasevitch 2014). After it was redefined by Saaristo and Tanasevitch (1996), Micronetinae has been extensively revised at genus level based on genital characters (e.g. Saaristo and Tanasevitch 2002a, b; Saaristo and Marusik 2004; Saaristo et al. 2006; Tu et al. 2006; Tu and Li 2006; Dupérré 2013; Sun et al. 2014). Comparative studies show that a series of transitions exist among the forms of scaped epigynum (Tu and Hormiga 2010). Five characters were proposed to accommodate interspecific variation in tracheal anatomy (Miller and Hormiga 2004; Arnedo et al. 2009). These imply a more complex pattern of the relationships among "micronetines" and erigonines than previously suggested. Accordingly, analyzing more groups that possess transitional characters between the typical "micronetine" and erigonine versions may help us infer the character evolution and resolve the phylogenetic relationships among linyphiid groups.

In the present study, we report another "micronetine" genus *Nippononeta* Eskov, 1992, having a scaped epigynum, but also a desmitracheate system. The genus *Nippononeta* was separated from *Agyneta*, the senior synonym of *Meioneta* (Eskov, 1992). We conducted a morphological study of *Nippononeta*. Characters of the genitalia and tracheal system, as well as some somatic characters, were documented with SEM images

for *N. kurilensis* Eskov, 1992 (the type species of *Nippononeta*) and *N. coreana* (Paik, 1991), and compared with those of *Agyneta*, as well as those of Erigoninae. Putative synapomorphies were proposed for *Nippononeta* and *Agyneta*, which need to be tested in future studies.

Materials and methods

Specimens were examined and measured using a Leica M205A stereomicroscope. Male palps and epigyna were examined after they were dissected from the body. Left structures (e.g. palps, legs, etc.) were depicted. Embolic divisions were excised by breaking the membranous column which connects the suprategulum and the radix. Male palps and epigyna were cleared in methyl salicylate. Scanning electron microscopy (SEM) images were taken under a Hitachi S-3400N scanning electron microscope at the China Agricultural University. For SEM examination, the specimens were prepared following Álvarez-Padilla and Hormiga (2008). SEM images of the embolic division taken from the right palp were mirrored to match those taken from the left palp. All examined specimens are deposited in the College of Life Sciences, Capital Normal University, China (CNU). Terminology of genital and somatic characters follows Tu and Hormiga (2010) and Hormiga (2000) respectively. Anatomical abbreviations used in the text and figures are:

Somatic morphology

AC aciniform gland spigot(s)
AG aggregate gland spigot(s)
ALS anterior lateral spinneret
CY cylindrical gland spigot(s)
FL flagelliform gland spigot
MAP major ampullate gland spigot
mAP minor ampullate gland spigot
PI piriform gland spigot(s)
PLS posterior lateral spinneret
PMS posterior median spinneret

Male palp
ARP anterior radical process
AX apex of embolus
CRL cymbial retrolateral lobe
DTA distal tibial apophysis
E embolus
EBT embolus basal tooth(teeth)

EC embolus column
EM embolic membrane
EP embolus proper
LC lamella characteristica
P paracymbium
PF proximal cymbial fold
PH pit hook
PHS pit hook sclerite
PTP proximal tibial process
R radix
RTP retrolateral tibial process
SPT suprategulum
T tegulum
TA terminal apophysis
TH thumb of embolus

Epigynum
CG copulatory groove
DP dorsal plate
LL lateral lobe on sacpe
SC scape
ST stretcher
TDF transversal dorsal fold of epigynum
TP turning point of copulatory groove
VP ventral plate

Taxonomy

Linyphiidae Blackwall, 1859

***Nippononeta* Eskov, 1992**

Composition. The genus includes 24 species; the type species is *Nippononeta kurilensis* Eskov, 1992.

Diagnosis (updated). *Nippononeta* species are similar to *Agyneta* in many genital characters and the desmitracheate system, but differ in the presence of a dorsal pattern on the abdomen, which is absent in most *Agyneta*. Male palps of *Nippononeta* can be distinguished from *Agyneta* by the conical elevation on the cymbium absent in the former (Fig. 1A), present in the latter; the presence of proximal cymbial fold (Fig. 1D) and the spine-like embolus thumb (Fig. 1G) in *Nippononeta*, absent in *Agyneta*. The scaped epigynum in *Nippononeta* can be distinguished by its narrowed epigynal basal part covered by a transversal fold, the well developed stretcher and remnant lateral

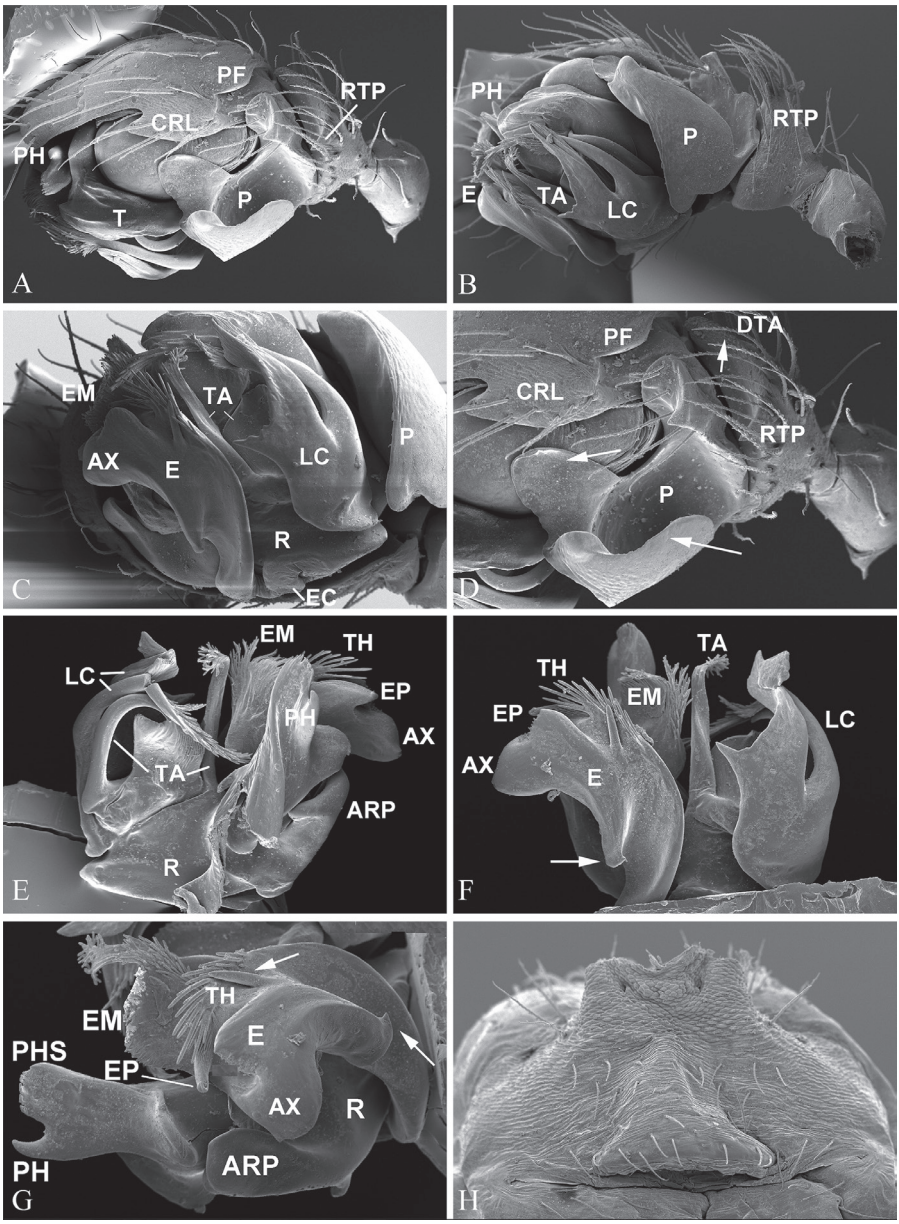

Figure 1. *Nippononeta kurilensis*. **A–G** male palp **A** retrolateral **B** ventral **C** anteroventral **D** detail of A, arrows indicate the serrated surface of DTA (upper), median branch of paracymbium (left) and outer margin fold continue with distal arm (lower) **E–G** embolic division **E** dorsal **F** ventral, arrow indicates basal hook of embolus **G** embolus, ventral, upper arrow indicates the last strongest spine of thumb; lower arrow indicates basal hook of embolus **H** anterior part of male abdomen, ventral, shows epiandrous gland spigots absent. ARP anterior radical process; AX apex of embolus; CRL cymbial retrolateral lobe; DTA distal tibial apophysis; E embolus; EC embolus column; EM embolic membrane; EP embolus proper; LC lamella characteristica; P paracymbium; PF proximal cymbial fold; PH pit hook; PHS pit hook sclerite; R radix; RTP retrolateral tibial process; T tegulum; TA terminal apophysis; TH thumb of embolus.

lobes (Fig. 2A–B), while in *Agyneta* the epigynal basal part is normal, the stretcher usually small or absent, but the lateral lobes are well developed bearing lateral pockets and copulatory openings (Tu and Hormiga 2010: fig. 6a).

Description (updated). Chelicerae of normal size, with narrowed fang base and stronger stridulatory ridges in the male than in the female (Fig. 3C–F). Female palpal claw absent (Fig. 2F). Tracheal system: Median trunks wider in diameter than lateral pair, highly branched and extending into prosoma (Fig. 2E); tracheoles with taenidia. Lateral pair and median trunks arising independently from spiracular atrium. Epiandrous gland spigots absent in the male (Fig. 1H). Spinnerets: PLS in females having the mesal cylindrical gland spigot base enlarged (Fig. 2G), the triplet formed by one flagelliform and two aggregate gland spigots present in males PLS (Fig. 2H). For other somatic characters and measurements see Eskov (1992).

Male palp (Fig. 1A–G). Tibia short, with serrated distal apophysis and pointed retrolateral process, sometimes with additional proximal process. Cymbium with small retrolateral lobe and proximal fold above paracymbial base. Paracymbium U-shaped, with median branch arising from inner margin. Distal suprategular apophysis modified into pit hook and hook sclerite. Embolic membrane large, with many papillae. Embolic division: radix boat shaped, connected to embolus by S-shaped membranous column. Embolus with pointed proper and serrated area, thumb modified into many spine-like projections, large apex and basal apophysis. Lamella characteristica usually splitting into two or three branches, at least one of them ribbon like with thread projections distally. Terminal apophysis composed by one large basal sclerite and one or two branches with papillae apex.

Epigynum (Figs 2A–D, 5). Epigynal basal part narrowed, covered by transversal fold formed by the tegument connecting to dorsal side of epigynum. Median plate absent and epigynal cavity dorsally opened. Scape sigmoid folded with well developed stretcher furnished with a pit; lateral lobes remnant; copulatory openings covered by folded scape.

Distribution. China, Japan, Korea, Russia.

Nippononeta kurilensis Eskov, 1992

N. kurilensis Eskov, 1992: 159, f. 27–30 (Dmf); Ono et al. 2009: 318, f. 899–902 (mf).

Material examined. 1♂ and 1♀ (CNU), Russia, Sakhalin Island, near Novoalexandrovsk, 11 Sept. 1992, A. Basarukin leg.

Diagnosis. The male of *N. kurilensis* is distinguished from *N. coreana* and all other *Nippononeta* species by: 1) the absence of proximal tibial process (Fig. 1A, D), present in the latter (Fig. 4A–B); 2) the outer margin fold of the U-shaped paracymbium is continued with the distal arm in *N. kurilensis* (Fig. 1A, D), only a small pointed tooth in *N. coreana* (Fig. 4A–B); 3) the anterior sclerites of lamella characteristica with smooth margin in *N. kurilensis* (Fig. 1F), while that in

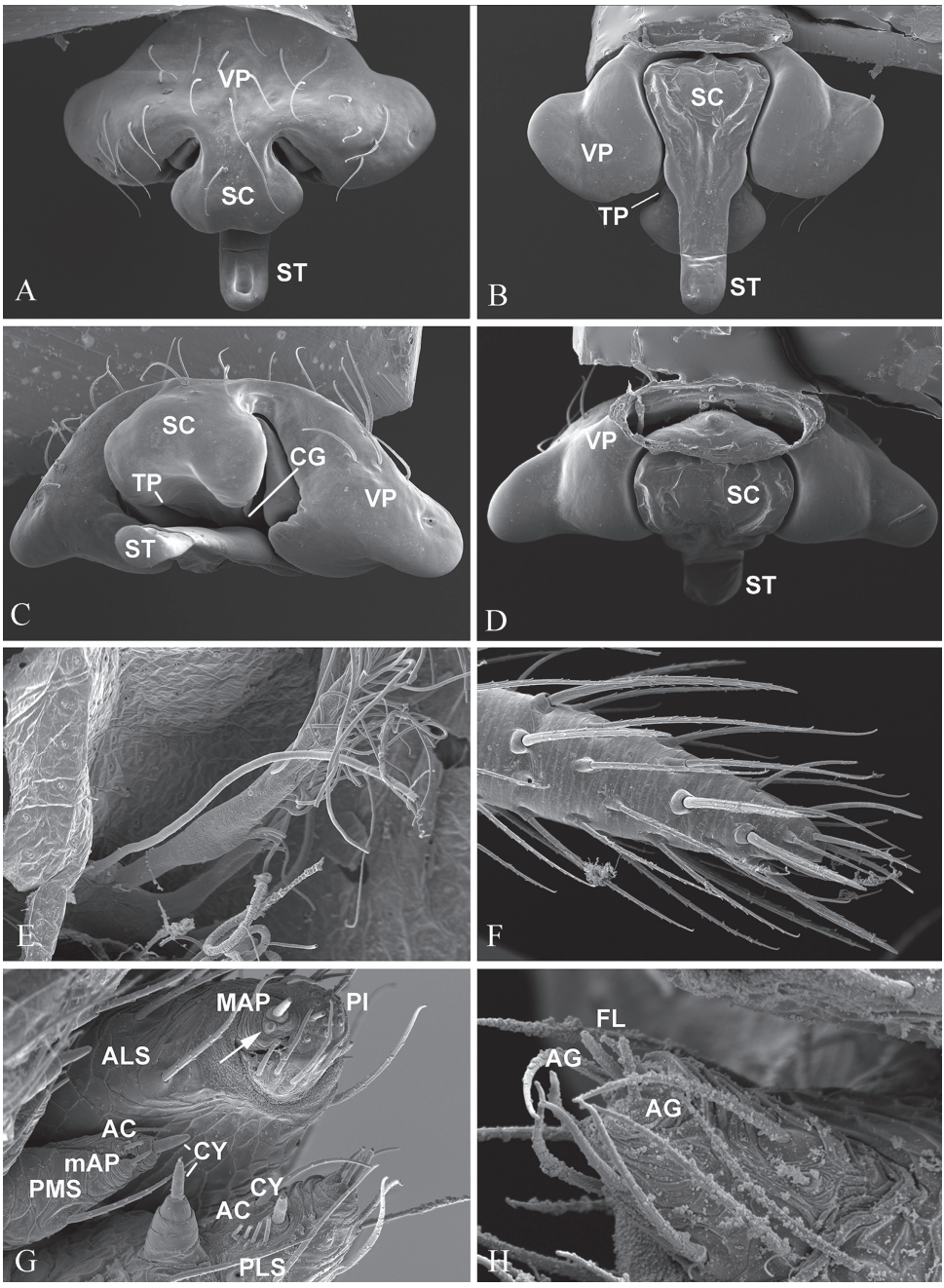

Figure 2. *Nippononeta kurilensis*. **A–D** epigynum. **A** ventral **B** dorsal **C** caudal **D** anterior **E** tracheal system, with soft tissue digested **F** female palp **G** female spinneret spigots, arrow indicates MPA nubbin on ALS **H** male PLS spigots. AC aciniform gland spigots; AG aggregate gland spigots; ALS anterior lateral spinneret; CG copulatory groove; CY cylindrical gland spigots; FL flagelliform gland spigot; MAP major ampullate gland spigot; mAP minor ampullate gland spigot; PI piriform gland spigots; PLS posterior lateral spinneret; PMS posterior median spinneret; SC scape; ST stretcher; TP turning point; VP ventral plate.

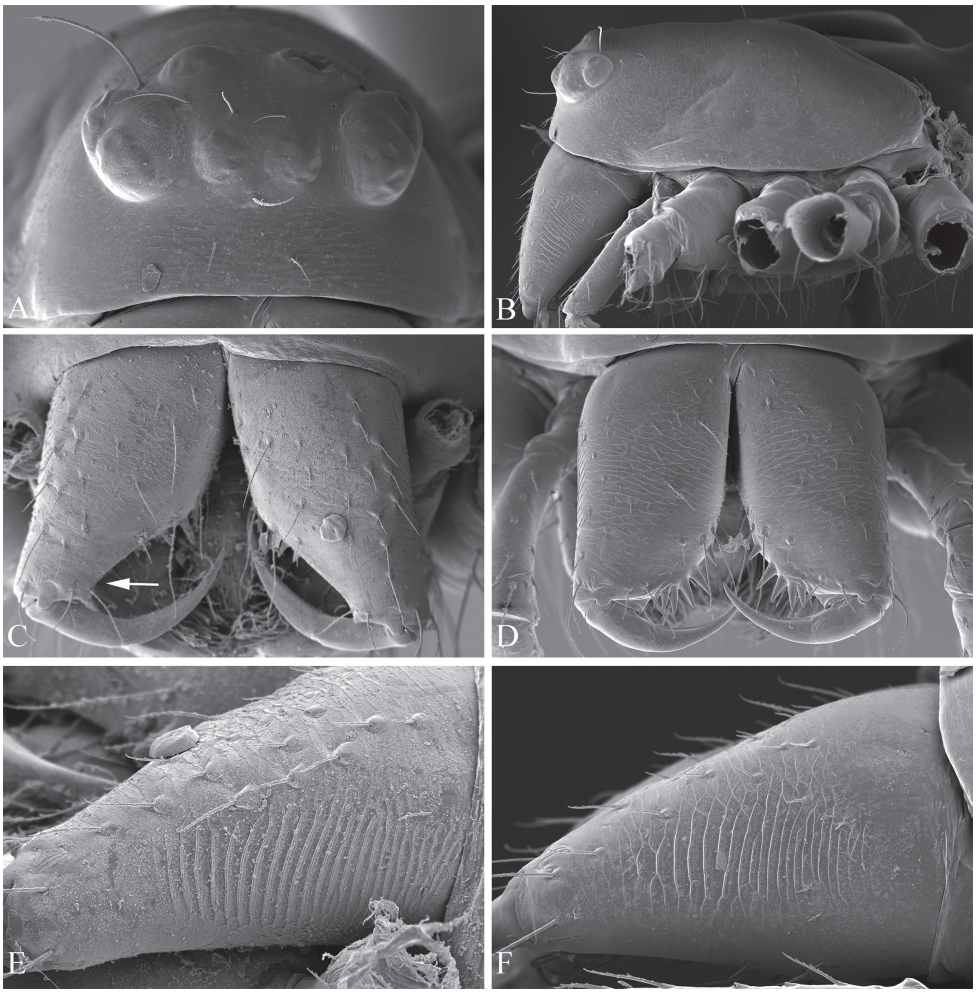

Figure 3. *Nippononeta kurilensis*. **A–B** female prosoma. **A** frontal **B** lateral (**C–F**) chelicera **C** male, frontal, arrow indicates narrowed fang base **D** female, frontal **E** male, ectal **F** female, ectal.

N. coreana serrated (Fig. 4D), and 4) the single embolus basal tooth hook-shaped in *N. kurilensis* (Fig. 1F), whereas in *N. coreana* the embolus has three basal teeth, one of them spine-like (Fig. 4E). The female can be distinguished by the appearance of epigynum: diamond shaped in *N. kurilensis* (Fig. 2A), T-shaped in *N. coreana* (Fig. 5).

Description. Somatic and genital characters as in the genus description.

Distribution. Russia (Sakhalin, South Kuril Islands) and Japan (Hokkaido).

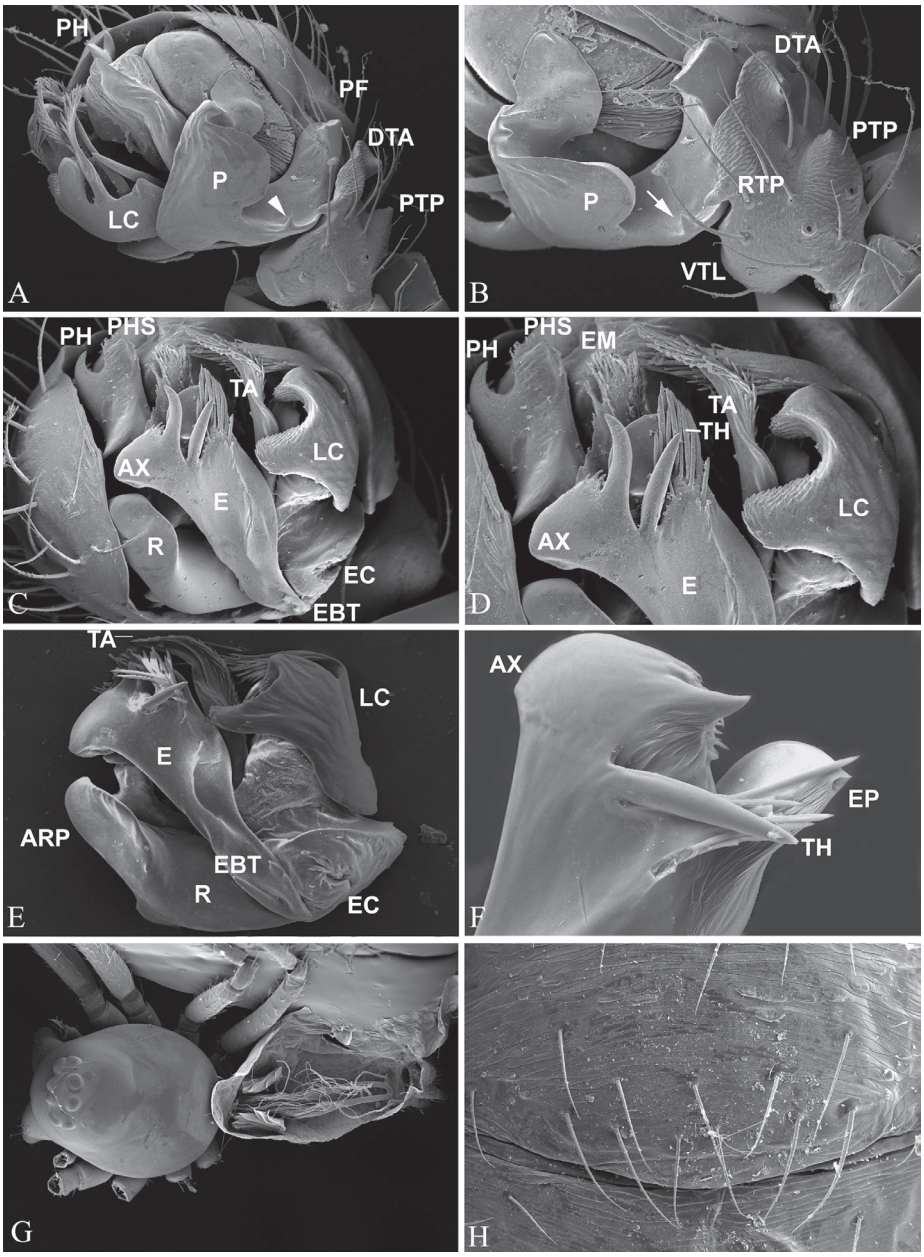

Figure 4. *Nippononeta coreana*. **A–F** male palp. **A** retrolateral, arrow indicates outer margin tooth **B** detail of A, arrow indicates outer margin tooth **C** ventral **D** detail of C **E** embolic division, ventral **F** embolus, dorsal **G** male body with soft tissues digested, shows tracheal system **H** anterior part of male abdomen, ventral, shows epiandrous gland spigots absent. ARP anterior radical process; AX apex of embolus; DTA distal tibial apophysis; E embolus; EBT embolus basal teeth; EC embolus column; EM embolic membrane; EP embolus proper; LC lamella characteristica; P paracymbium; PF proximal cymbial fold; PH pit hook; PHS pit hook sclerite; PTP proximal tibial process; R radix; RTP retrolateral tibial process; TA terminal apophysis; TH thumb of embolus.

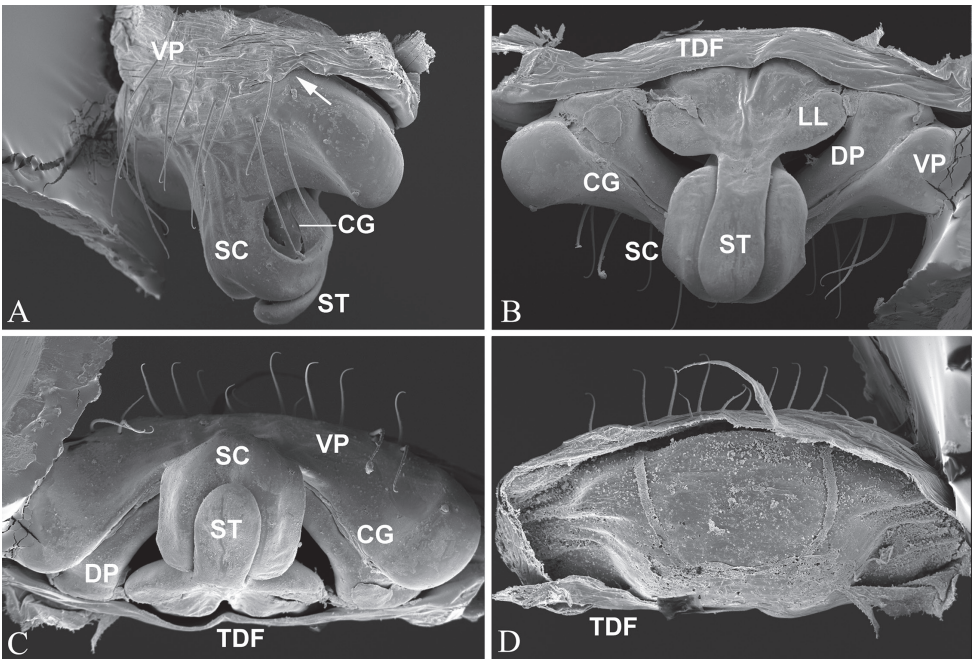

Figure 5. *Nippononeta coreana*, epigynum. **A** ventrolateral **B** dorsal **C** caudal **D** anterior, with soft tissues digested. CG copulatory groove; DP dorsal plate; LL lateral lobe on sacpe; SC scape; ST stretcher; TDF transversal dorsal fold of epigynum; VP ventral plate.

Nippononeta coreana (Paik, 1991)

Macrargus coreanus Paik, 1991: 2, f. 30-38 (Df).
Nippononeta coreana: Eskov 1992: 159 (Tf from *Macrargus*); Li et al. 1996: 10, f. 1–7 (f, Dm); Song et al. 1999: 199, f. 113A–C (mf); Yin et al. 2012: 539, f. 256a–e (mf).

Material examined. 2♂ and 2♀ (CNU), China, Sichuan Province, Tianquan County, Mt. Erlangshan Natural Forest Park, 8 July 2004, L. Tu leg.

Diagnosis. See the diagnosis of *N. kurilensis*.

Description. Other genital characters see the description by Paik (1991).

Distribution. China (Guangxi, Hunan, Hubei, Jilin, Sichuan), Korea.

Discussion

The putative synapomorphies based on genital characters suggest that the four desmitracheate "micronetine" genera: *Nippononeta*, *Agyneta*, *Tennesseellum*, and *Anibontes* have a common ancestor. Eskov (1992) erected the genus *Nippononeta* to allocate those species which were not consistent with several generic diagnoses of

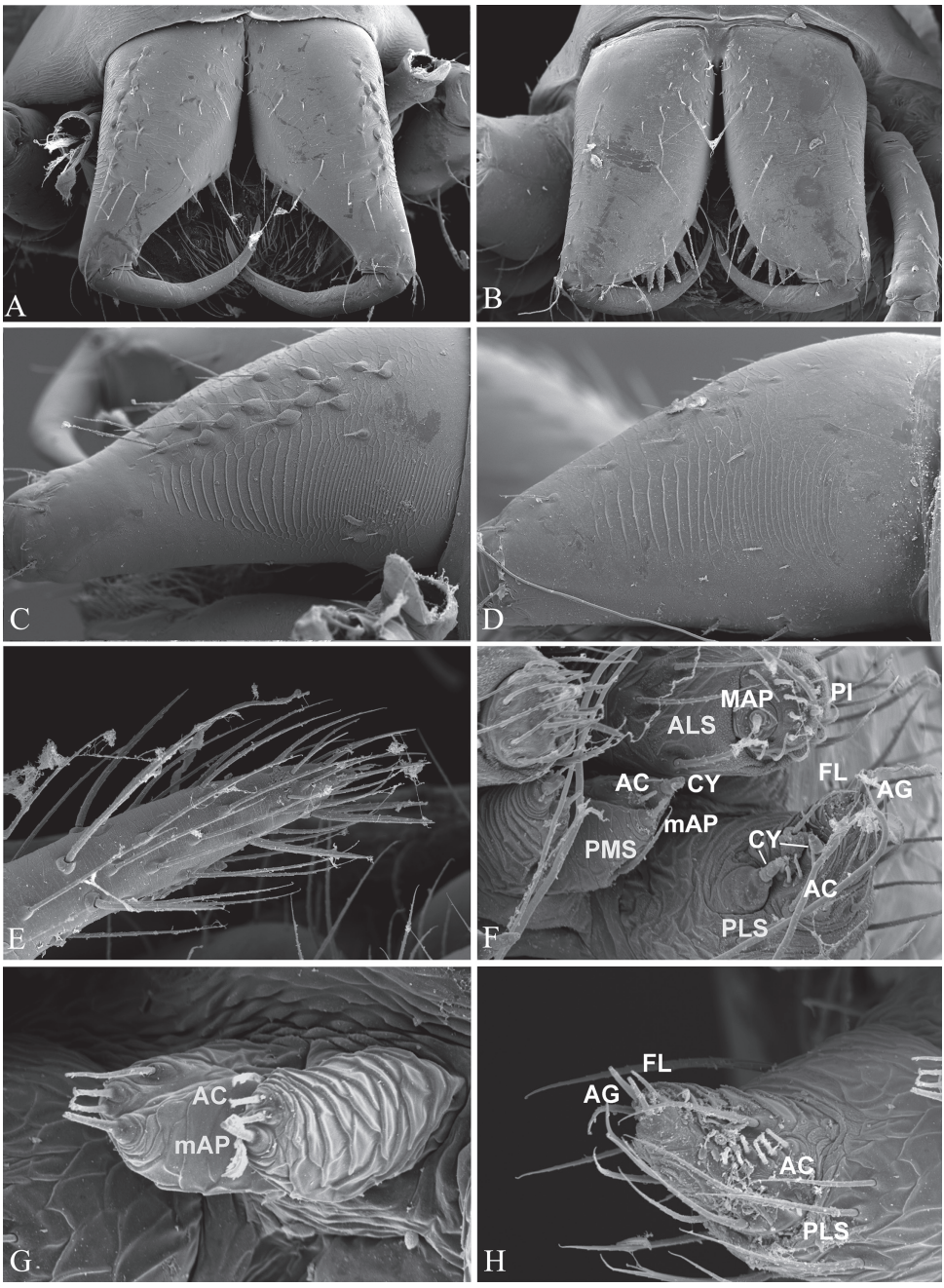

Figure 6. *Nippononeta coreana*. **A–D** chelicerae **A** male, front **B** female, front **C** male, ectal **D** female, ectal **E** female palp **F–G** spinnerets **F** female **G** male, PMS spigots **H** male PLS spigots. AC aciniform gland spigots; AG aggregate gland spigots; ALS anterior lateral spinneret; CY cylindrical gland spigots; FL flagelliform gland spigot; MAP major ampullate gland spigot; mAP minor ampullate gland spigot; PI piriform gland spigots; PLS posterior lateral spinneret; PMS posterior median spinneret.

Meioneta (a junior synonym of *Agyneta*). Although *Nippononeta* and *Agyneta* have a desmitracheate system, the genital characters of both genera are of a typical "micronetine" type: male palpal embolic division has well developed lamella characteristica and terminal apophysis, and females have a scaped epigynum. At the same time, in most Erigoninae, lamella characteristica and terminal apophysis are secondarily lost (Hormiga 2000), and a scaped epigynum has never been documented. There are several putative synapomorphies based on genital anatomy in both genera. For *Nippononeta*, putative synapomorphies include: the embolus thumb modified into many spine-like projections (Fig. 1G), the presence of proximal cymbial fold (Fig. 1A), and the narrowed epigynal basal part covered by the transversal dorsal fold (Fig. 2B). For *Agyneta*, putative synapomorphies include: the presence of conical cymbial elevation, and the scaped epigynum with the stretcher remnant or absent, but a pair of well developed lateral lobes bearing lateral pockets and copulatory openings (Tu and Hormiga 2010: fig. 6a). Further, putative synapomorphies for (*Nippononeta* + *Agyneta*) clade are: the serrated distal tibial apophysis (Fig. 1D) and the absence of median plate (Fig. 2B). According to Dupérré (2013), the genitalia of *Tennesseellum* and *Anibontes* have the exact same configuration as that of *Agyneta*, only different in small details. Therefore, based on genital synapomorphies, *Nippononeta* should form the sister clade to the group including the other three "micronetine" genera (*Agyneta*, *Tennesseellum*, and *Anibontes*).

The results of phylogenetic analyses support the single origin of desmitracheate system (Arnedo et al. 2009; Hormiga 2000; Miller and Hormiga 2004). Four types of tracheal anatomy have been recognized in Linyphiidae: desmitracheate, haplotracheate, and two intermediate types (Miller and Hormiga 2004). Based on the examination for 121 linyphiids belonging to 98 genera, Blest (1976) found the desmitracheate system in 85 erigonines belonging to 65 genera. This tracheal system is addressed as an erigonine type, although there are eight erigonine species with the simple (haplotracheate) type. At the same time, 19 "micronetine" species belonging to 16 genera, and all other linyphiids examined by Blest (1976) had a haplotracheate system. Later studies found that the typical "micronetine" genera *Agyneta*, *Tennesseellum*, *Anibontes* (Millidge 1986; Hormiga 1994; Dupérré 2013) and *Nippononeta* also have a desmitracheate system. Although a phylogenetic analysis of linyphiids rendered "micronetines" paraphyletic, the morphological partition suggests that the desmitracheate system in linyphiids has a single origin (Arnedo et al. 2009), with secondary reduction to the haplotracheate system within erigonines (Miller and Hormiga 2004).

Comparative studies on the tracheal morphology suggest that the transformation between haplotracheate and desmitracheate systems is not a result of a single morphological change. A total of five characters were proposed to allocate interspecific variation of tracheal anatomy in Linyphiidae (Miller and Hormiga 2004; Arnedo et al. 2009). Further, variation of the desmitracheate system was documented: the median trunk tracheoles can either have taenidia or not; the lateral tracheae can arise either independently from the spiracular atrium (Figs 2E, 4G,

see also Millidge 1986: fig. 12) or from the basal part of the median trunk (Hormiga 1994: fig. 18; Dupérré 2013: figs 31–33); and the lateral tracheae can either be branching or non-branching (Millidge 1986: figs 1, 12; Hormiga 1994: fig. 18; Dupérré 2013: figs 31–33). The presence of taenidia in *Nippononeta* and *Agyneta* makes their tracheal characters similar to those observed in deeper clades within erigonines, e.g. genera *Hilaira*, *Drepanotylus*, and *Leptorhoptrum* (Blest 1976: plate 1b). At the same time, it makes *Nippononeta* and *Agyneta* different from the 'distal erigonines' clade, which includes generas having simple type genitalia, desmitracheate system, and the tracheoles without taenidia, e.g. *Erigone*, *Oedothorax*, and *Gonatium* (Hormiga 2000, Arnedo et al. 2009). The branching lateral tracheae, reported from some distal erigonines, e.g. *Erigone* (Millidge 1986: fig. 5; Hormiga 1994: fig. 18C), can also be found in *Tennesseellum* (Millidge 1986: fig. 1; Hormiga 1994: fig. 17). Furthermore, two intermediate types are found in several distantly related groups: *Helophora* ("micronetine"), *Allomengea* (linyphiine), *Solenysa* ("ipaine"), and some erigonines (Millidge 1986; Hormiga 1994; Miller and Hormiga 2004; Tu and Hormiga 2011). Some of them represent intermediate steps of the evolution from the haplotracheate system to the desmitracheate one, while some are results of reversals (Hormiga 2000; Miller and Hormiga 2004). Accordingly, multiple pathways are possible for the evolution of desmitracheate system. These hypotheses need to be tested with denser sampling in future studies.

Furthermore, we find that, in addition to the desmitracheate system, some confirmed synapomorphies of erigonines (Hormiga 2000; Arnedo et al. 2009) such as the presence of triplet of spigots in male PLS (Fig. 2H), the absence of epiandrous gland spigots in males (Fig. 1H), and the absence of palpal claw in females (Fig. 2F) are shared not only with *Nippononeta* and *Agyneta*, but also with some haplotracheate "micronetines", e.g. *Microneta viaria* (Arnedo et al. 2009), *Macrargus rufus*, *Maro sublestus*, *Oreonetides vaginatus* and *Ryojius* sp. (Tu, unpublished data). This is consistent with the results of phylogenetic analysis demonsrating that "micronetines" are a paraphyletic group (Arnedo et al. 2009). The placement of *Nippononeta* and *Agyneta* within Linyphiidae and their relationships with other "micronetines" and erigonines need to be resolved in future studies.

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RESEARCH ARTICLE

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Description of a new soft scale insect of the genus *Pulvinaria* Targioni Tozzetti (Hemiptera, Coccoidea, Coccidae) from Bogota, Colombia

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Abstract

A new soft scale (Hemiptera: Coccoidea: Coccidae) species, *Pulvinaria caballeroramosae* Tanaka & Kondo, **sp. n.**, is described from specimens collected on twigs of *Ficus soatensis* Dugand (Moraceae) in Bogota, Colombia. The new species resembles *P. drymiswinteri* Kondo & Gullan, described from Chile on *Drimys winteri* J.R. Forst. & G. Forst. (Winteraceae), but differs in the distribution of preopercular pores on the dorsum, the presence of dorsal tubular ducts, dorsal microducts, and reticulation on the anal plates; and in its feeding habits, i.e., *P. caballeroramosae* feeds on the twigs whereas *P. drymiswinteri* feeds on the leaves of its host. A key to the Colombian species of *Pulvinaria* Targioni Tozzetti is provided.

Keywords

Coccid, *Ficus soatensis*, soft scale insect, insect pest, urban pest

Introduction

With the exception of Argentina (Granara de Willink 1999), Brazil (Hempel 1900), Colombia (Mosquera 1979, 1984; Kondo 2001, 2010a, b, 2011, 2013, Kondo and Hodgson 2013, Kondo and Williams 2004, Walker 1852) and Chile (Kondo and Gullan 2010), the soft scale insect (Hemiptera: Coccoidea: Coccidae) fauna of most

countries in South America remains much under explored and studied. Some important earlier taxonomic works on soft scale insects of Colombia include those by Mosquera (1979, 1984) who contributed to the understanding of the genus *Ceroplastes* in that country (Kondo 2001). There are also species lists that include soft scale insects on some fruit crops in Colombia, namely avocado (Kondo et al. 2011), citrus (Kondo et al. 2012), mango (Kondo 2009, Kondo-Rodriguez 2010) and soursop (Kondo 2008). According to the scale insect database ScaleNet (Ben-Dov et al. 2014), the family Coccidae in Colombia is composed of 41 species distributed in 17 genera, of which 13 species (32%) are only known from Colombia, namely *Akermes colombiensis* Kondo & Williams, *Bombacoccus aguacatae* Kondo, *Ceroplastes boyacensis* Mosquera, *C. cundinamarcensis* Mosquera, *C. martinae* Mosquera, *C. mosquerai* Ben-Dov, *C. ocreus* Mosquera, *C. trochezi* Mosquera, *Coccus caudatus* Walker, *Cryptostigma philwardi* Kondo, *Foldilecanium multisetosum* Kondo, *Hemilecanium guanabana* Kondo & Hodgson and *Neotoumeyella caliensis* Kondo & Williams.

A few years ago, the second author of the present paper was informed by Mrs. Andrea Amalia Ramos-Portilla of a species of *Pulvinaria* causing damage to street trees in Bogota. Outbreaks of this *Pulvinaria* species have been known for quite some time in the capital city of Colombia where it is undoubtedly considered an urban pest. Herein we describe and illustrate this undescribed pest species of *Pulvinaria* based on adult female specimens. A key to the species of Colombian *Pulvinaria* is also presented.

Materials and methods

In the past, the genus *Pulvinaria* had been split into several genera, e.g. *Chloropulvinaria* (Borchsenius 1952), *Eupulvinaria* (Borchsenius 1953) and *Saccharipulvinaria* (Tao et al. 1983). However, these genera have been rarely accepted by current taxonomists (e.g., Williams and Watson 1990), and taxonomy of the tribe Pulvinariini (*Pulvinaria* and related genera) is in great need of further study (Tanaka 2012). We therefore treat the genus *Pulvinaria* in the broad sense here.

The scale insect samples were collected by the second author on 5 September 2014 from street trees of *Ficus soatensis* in the city of Bogota, Colombia with the help of Mrs. Andrea Amalia Ramos Portilla. The slide-mounting method followed Tanaka (2014). The morphology of the mounted specimens was examined under a phase-contrast light microscope (Olympus BH2-PH).

The description was based on multiple slide-mounted specimens. The terminology used to describe the adult female followed that of Kondo and Hodgson (2013), who avoided using the term "pregenital disc-pores" or "perivular pores" because in some soft scale species, these multilocular pores are not restricted to the pregenital or perivulvar region, and they can be present throughout the mid-areas of the venter; thus using the term "pregenital" or "perivulvar" is misleading. The term "multilocular pore" is therefore used herein for all the pores with multiple loculi, with the exception of spiracular pores.

The type specimens are deposited in the Colección Taxonómica Nacional "Luis María Murillo", Corpoica, C.I. Tibaitatá, Mosquera, Cundinamarca, Colombia (CTNI), the Museo Entomológico Facultad de Agronomía, Universidad Nacional de Colombia, Sede Bogotá, Bogotá, Cundinamarca, Colombia (UNAB), the National Museum of Natural History Entomological Collection, Washington, D.C., U.S.A. (USNM: Coccoidea collection held at USDA, Beltsville, Maryland), and the Tottori Prefectural Museum, Tottori, Japan (TRPM).

Taxonomy

Genus *Pulvinaria* Targioni Tozzetti, 1866: 146.

Type species. *Coccus vitis* Linnaeus, 1758: 456. By original designation and monotypy.

The new species described below is a typical member of the tribe Pulvinariini and the subfamily Coccinae, based on the definition of the tribe Pulvinariini presented by Hodgson (1994). The present species keys out to the genus *Pulvinaria* in Hodgson's keys to subfamilies, tribes and genera of Coccidae (Hodgson 1994) and fits into his *Pulvinaria*-group, in which tubular ducts are scarce or absent on the head. However, here we treat the genus in the broad sense, as explained in the Materials and methods section.

Key to Colombian species of the genus *Pulvinaria*

1 Most marginal setae with bifid, frayed, fimbriate, or finely split apices **2**

– Most marginal setae with sharply or rather bluntly pointed apices **3**

2 Ventral tubular ducts in submarginal area of head frequent and broadly distributed. Multilocular pores mainly each with 9–11 loculi. Marginal setae usually strongly fimbriate; setal collar of most setae narrower than setal tip. Spiracles of mature specimens usually surrounded by a strongly sclerotized crescentic plate ..... ***psidii***

– Ventral tubular ducts in submarginal area of head scarce or absent except in area near margin. Multilocular pores mainly each with 6–7 loculi. Marginal setae usually slightly to moderately fimbriate. Spiracles of mature specimens not surrounded by a strongly sclerotized crescentic plate ..... ***urbicola***

3 Submarginal area of head and thorax with ventral tubular ducts numerous and widespread. Dorsal setae lanceolate, each seta with a marked constriction at base. Body shape usually conspicuously elongate oval **4**

– Submarginal (and marginal) area of head and thorax without ventral tubular ducts. Multilocular pores mainly each with five loculi. Dorsal setae spiniform, each seta without a marked constriction at base. Body shape oval rather than elongate ..... ***caballeroramosae* sp. n.**

4 Multilocular pores absent medially on thorax. Ventral tubular ducts present medially on thorax between mesothoracic and metathoracic coxae..... ***iceryi***

– Multilocular pores present medially on thorax between mesothoracic and metathoracic coxae. Ventral tubular ducts absent medially on thorax..... ***elongata***

Notes. Morphological character states used for separating *P. iceryi* from *P. elongata* were taken from Mamet (1958). Character states of *P. urbicola* and *P. psidii* were taken from Williams and Watson (1990) and based also on the first author's personal observations of slide-mounted specimens collected in Japan.

Pulvinaria caballeroramosae **Tanaka & Kondo, sp. n.**
http://zoobank.org/BF0B0A32-D4E2-4952-8DD9-0A8C9569B774
Figures 1–2

Proposed common names. Spanish: Escama blanda algodonosa del caucho sabanero; English: Sabanero fig cottony scale.

Type series. Holotype, adult female. Colombia, Cundinamarca, Bogotá, D.C. Barrio Salitre, Carrera 68B, con Av. La Esperanza, Esquina Noroccidental, 05.xi.2014, coll. T. Kondo & Andrea Amalia Ramos Portilla, ex branches of *Ficus soatensis* Dugand (Moraceae), 1♀ (UNAB). Paratypes, same data as holotype, 11 ♀♀ (3 at UNAB, 3 CTNI, 3 USNM and 2 at TRPM).

Unmounted material (Figure 1A, B, C). Adult female in life oval, convex, 2.2–4.5 mm long, 1.9–3.8 mm wide, 0.9–2.0 mm tall, covered by a thin layer of glassy wax (Figure 1A). Body greenish brown to yellowish brown, especially around body margin, mid dorsum lighter in color, yellowish to ochre, usually with a dark mid-dorsal longitudinal line from head margin to just anterior to anal plates (Figure 1A, B). Anal plates conspicuous, reddish brown; area around anal plates generally smooth and yellowish (Figure 1A, B). Dorsal derm warty in appearance (except around anal plates), with round yellowish tubercles, especially on mid dorsum, tubercles fewer and smaller around margins and submargins; often with a pair of particularly large (two or more times wider than the anal plates) round submedial tubercles on mid dorsum, located diagonally from anal plates (Figure 1A, B). Ovisac long, four or more times the length of the adult female, produced in a straight or curved line, strongly adhered to substrate, eggs generally exposed and clearly visible through the fibrous ovisac; eggs orange, purplish or ochre in color (Figure 1C).

Slide-mounted specimens (n=12). Body oval, 2.5–4.6 mm long, 2.1–3.5 mm wide, margin with very shallow indentation at each stigmatic cleft; anal cleft about 1/5–1/8 body length.

Dorsum: Derm membranous, dermal areolation not developed. Dorsal setae spiniform, frequent, scattered over entire dorsum, each 9–15 μm long with well-developed basal socket. Preopercular pores circular, each diameter 3–7 μm, rather well-sclerotized and convex, extending broadly on medial area from area just anterior to anal plates

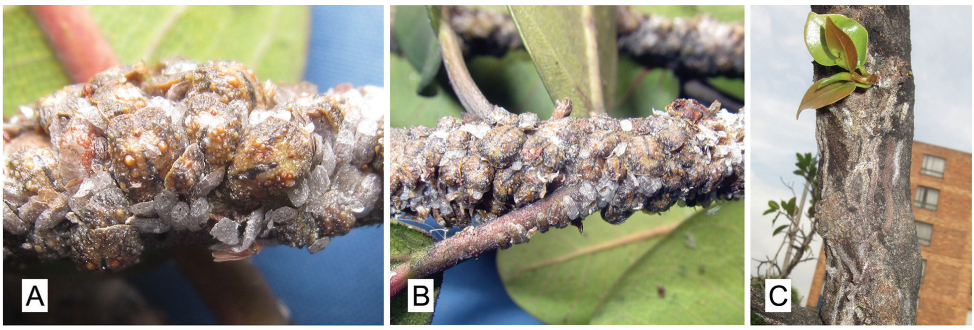

Figure 1. *Pulvinaria caballeroramosae* Tanaka & Kondo, sp. n.: **A** Adult females, male puparia and an alate adult male (on lower surface of twig) **B** Infestation on twig **C** Conspicuous long ovisacs on trunk of a young tree, *Ficus soatensis*. Bogota, Colombia.

forward to about mesothorax, but usually scarce anteriorly. Only a few tubular ducts present, situated anterior to anal plates, where they are intermixed with preopercular pores; sometimes ducts also present marginally on head and thorax. Dorsal microducts frequent throughout. Simple pores present, mostly distributed evenly. Dorsal tubercles absent. Anal plates together quadrate; each plate with posterior margin slightly convex and anterior margin slightly concave, with 3–4 (usually 3) fine apical setae; each plate 223–258 μm long, 128–166 μm wide, with supporting bar and reticulation on area near lateral angle. Ano-genital fold with four or five pairs of setae along anterior margin and one to three pairs laterally. Anal ring bearing about 10–12 setae. Eyespots present near margin.

Margin: Marginal setae with well-developed basal sockets and usually slightly blunt but rarely with simple, pointed apices; length of each seta 17–79 μm; with 4–12 setae present between anterior and posterior stigmatic clefts. Stigmatic clefts shallow or absent, each with 1–4 (usually 3) stigmatic spines, central spine longest, 50–103 μm long, about three to eight times as long as lateral spines.

Venter: Derm membranous. Multilocular pores each 5–9 μm wide, with 3–8 loculi (mainly 5), present around genital opening, on mediolateral areas of all abdominal segments, mesothorax, metathorax and head; a small group also present lateral to each coxa. Spiracular pores each 4.0–7.0 μm wide, with 3–6 loculi (mainly 5), present in rather narrow bands 1–5 pores wide between margin and each spiracle; anterior bands each with 25–47 pores, posterior bands each with 32–49 pores. Ventral microducts scattered evenly throughout, each about 2.0–3.0 μm wide. Preantennal pore not detected. Ventral tubular ducts of three types: type I with large outer ductule, flower-shaped well-developed terminal gland and stout inner ductule, present in medial area of thorax, the anterior two to four abdominal segments, and in inner submarginal band from area posterior to vulvar region near anal folds forwards to area just posterior to metathoracic spiracular pore band; type II tubular ducts each with rather small outer ductule, narrower inner ductule, shallow cup-shaped invagination and well-developed terminal gland, occurring in medial area of posterior abdominal segments; and type

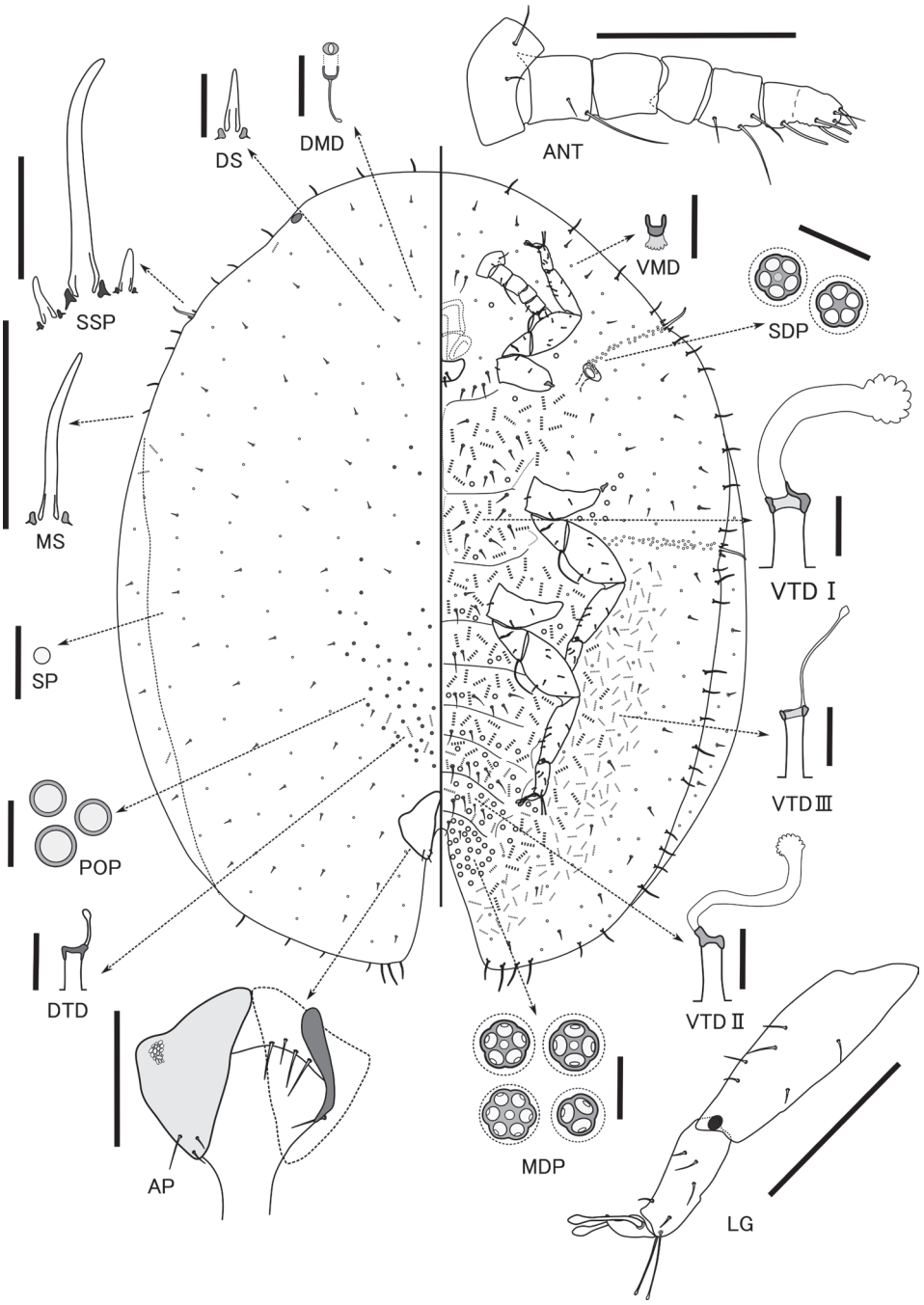

Figure 2. *Pulvinaria caballeroramosae* Tanaka & Kondo, sp. n., adult female. **ANT** antenna **AP** anal plates **DMD** dorsal microduct **DS** dorsal seta **LG** leg **MS** marginal seta **MP** multilocular pores **POP** pre-opercular pores **SP** simple pore **SDP** spiracular pores **SSP** stigmatic spines **VMD** ventral microduct **VTD** ventral tubular ducts of types I–III. Scales: 200 μm for **ANT**, **LG** ; 100 μm for **AP** ; 50 μm for **MS**, **SSP**; 10 μm for others.

III ducts similar to type II, but with a short, filamentous inner ductule and very small terminal gland, present in submarginal band from area posterior to vulvar region near anal folds forwards to area posterior to metathoracic spiracular pore band, intermixed with type I ducts in inner submarginal area. Ventral tubular ducts of all types absent marginally and submarginally from head to anterior thoracic segments and from the outer submarginal to marginal areas of posterior thorax and abdomen. Ventral submarginal setae short and fine, distributed evenly; other ventral setae relatively long and present in medial area of thorax, between antennae and in transverse rows of abdominal segments. Spiracles normal, rather large; width of each peritreme: anterior 90–117 μm, posterior 103–132 μm. Legs well developed and stout, each with a tibiotarsal articulation and an articulatory sclerosis; claws without denticles; both claw digitules rather broad and slightly shorter than thin tarsal digitules, as shown in Figure 2. Hind trochanter + femur 390–483 μm long, hind tibia 256–325 μm long, and hind tarsus 132–177 μm long. Antennae rather reduced, total length 302–404 μm; each with 5–7 segments, usually 6 or 7. Labium 110–170 μm wide.

Etymology. The species is named after Dr. Andrea Amalia Ramos Portilla and Mr. Alejandro Caballero who originally discovered this soft scale species on the streets of Bogota, Colombia.

Biology. The insects were found on the trunk, branches and twigs of the host. Adult males and puparia were commonly intermixed with the females (Figure 1A). *Pulvinaria caballeroramosae* sp. n. is commonly found in large numbers on *Ficus soatensis* (Figure 1A, B), a common street tree in Bogota, often causing dieback of twigs and branches and in severe cases, dieback of the entire tree. The females produce long ovisacs that are conspicuous on the infested twigs and branches (Figure 1C). No natural enemies, parasitoids or predators of *P. caballeroramosae* sp. n. were observed in the present study.

Host plant. Moraceae: *Ficus soatensis*.

Discussion

This species is considered to be close to *Pulvinaria drymiswinteri* Kondo & Gullan based on the distribution pattern of the ventral tubular ducts, tendency for reduction of the antennae and by the way it produces its ovisac, which is strongly adhered to the surface with the eggs exposed and visible through the fibrous ovisac. However, *P. caballeroramosae* is easily distinguishable from *P. drymiswinteri* by the following combination of features (character states of *P. drymiswinteri* in parenthesis): (1) dorsal tubular ducts present (absent); (2), dorsal microducts present (absent); (3) small reticulations on anal plates present (absent), (4) band of preopercular pores broadening anteriorly (not broadening anteriorly, present in a narrow band); and (5) multilocular pores mainly each with five loculi (multilocular pores mainly each with 5–8 loculi).

In the Neotropical region, 27 species of *Pulvinaria* have been recorded (Ben-Dov et al. 2014) of which five are considered to be invasive species in South America

(Kondo and Gullan 2010). *Pulvinaria caballeroramosae* sp. n. is considered an urban pest in Bogota, Colombia, because of the damage it causes to *Ficus soatensis* street trees. *Pulvinaria caballeroramosae* sp. n. appears to be an endemic species since it has only been found on a native host, *Ficus soatensis* (Moraceae) in Bogota, Colombia and has not been reported from elsewhere. Furthermore, the second author also examined other fig species while collecting *P. caballeroramosae* sp. n., i.e., a less frequent street tree, *Ficus elastica* Roxb. ex Hornem. and *F. benjamina* L. (a common ornamental). These *Ficus* spp. were not infested by *P. caballeroramosae* despite being in the proximity of infested trees, suggesting that this new *Pulvinaria* species is monophagous. However, further studies are needed in order to determine the host range of this new species of *Pulvinaria*.

Elucidating the taxonomic position of *P. caballeroramosae* sp. n. was out of the scope of our study. A comprehensive phylogenetic analysis of the genus *Pulvinaria* of the Neotropical region is needed, using morphological and molecular data, and characters from other instars and males.

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Morphological and molecular study of Symphyla from Colombia

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Abstract

The symphylans are a poorly studied group. In Colombia the number of symphylan species is unknown with only *Scutigerella immaculata* (Symphyla: Scutigerellidae) being reported previously. The aim of this research was to collect and identify the symphylan pests of flower crops in Colombia. Morphological descriptions showed that our specimens shared more than one of the characters that define different genera within Scutigerellidae. The *COI* barcode haplotype showed interspecific level genetic divergence with *S. causeyae* (at least 23%) and *Hanseniella* sp. (22%). Furthermore, our Colombian symphylans shared the same *COI* haplotype as some Symphyla found in Cameroon indicating a wide geographical distribution of this taxon. Our results suggest the presence of a new genus or subgenus in the class Symphyla.

Keywords

Scutigerella immaculata, Colombia, *COI* barcode, ITS2, morphology

Introduction

The symphylans (Arthropoda: Symphyla) are ancestral arthropods dating back to the early Silurian approximately 430 million years ago (Edgecombe 2004, Shear and Edgecombe 2010). Symphylans are a phylogenetic enigma within arthropods as they

have been proposed as sister taxa to different groups (Domínguez 2009). Symphyla is comprised of two families: Scutigerellidae (five genera and approximately 128 species) and Scolopendrellidae (nine genera and approximately 73 species) (Domínguez 2009). Symphylan species are morphologically determined mainly based on the chaetotaxy of the head, antennae size and shape of the scuta margins (Domínguez 1992, Edwards 1959a, b, Scheller 1961).

Only two genera in the family Scutigerellidae are considered to be pests in a wide range of crops: *Scutigerella* Ryder, 1882 and *Hanseniella* Bagnal, 1913 (Michelbacher 1938). *Scutigerella immaculata* Newport, 1845 is the only reported symphylan in Colombia where it is regarded as a pest of pineapple (Agredo 1988) and flower crops (Duran 1982, Navarro and Gaviria 2001). However, in these reports the authors did not describe how they identified *S. immaculata*. Questions are raised regarding the presence of *S. immaculata* in tropical Colombia. Domínguez (2009) only reports *Scutigerella* genus in northern temperate zones. In northern Brazil, bordering Colombia and Peru, de Morais and da Silva (2009) report the presence of *Hanseniella* and *Symphylella* (Scolopendrellidae). The distribution of the family Scutigerellidae is: *Scutigerella* mainly in northern temperate zones; *Hanseniella* in tropical and warm temperate zones; *Millotellina* in Africa, Madagascar, Réunion, Sri Lanka, New Guinea and Australia; *Scolopendrelloides* in South-East Asia and Australia; and *Scopoliella* in North America only (Domínguez 2009).

Mitochondrial DNA *Cytochrome Oxidase I* (*COI*) barcode region (Hebert et al. 2003, Smith et al. 2005) and the ribosomal nuclear Internal Transcribed Spacer 2 (ITS2) are used as molecular markers for arthropod species identification (Hebert et al. 2003, Ruiz et al. 2005, Wiemers et al. 2009). Barcoding is a fast and accurate method for species delimitation using the Kimura Two-Parameter model (K2P) (Padial and De la Riva 2007). There are few reports using these molecular makers in symphylans (Mallatt et al. 2004, Podsiadlowski et al. 2007, Spelda et al. 2011, Stoev et al. 2010, 2013) and none characterising Colombian symphylans.

Symphylan pests in Colombia are commonly identified as *S. immaculata* by the presence of a single morphological feature, a U-shape groove in the scuta of the last abdominal segment. The aim of this study was to capture symphylans in two departments of Colombia and describe these using multiple morphological characters and molecular markers.

Methods

Symphylan collection and examination

Symphylans were collected from two flower companies: Flores Esmeralda S.A.S C.I. in Antioquia (6°1'0"N, 75°25'0"W, 2180 m.a.s.l.) and Flexport and CIA.S.A.C.I. in Cundinamarca (4°45'4.10"N, 74°13'30.87"W, 2548 m.a.s.l.). Symphylan collection used a modified method of Umble et al. (2006); beet slices instead of potato baits covered with

black plastic to block the passage of light were set overnight for 12 hours on flowerbeds. The next morning, the symphylans were collected from the beets and soil around the baits and transported in Petri dishes – 20 individuals per dish, each dish 9 cm in diameter, containing 17 g of soil (previously sterilized at 121 °C) and beet as a food source – to the Bio-control and Microbiology Laboratory (BIOMA), University of Antioquia, Medellín, Colombia. Symphylans were identified by morphology (N = 30) using the descriptions and keys of Domínguez (2009, 2010), Halliday (2004), and Naumann and Scheller (1977). A total of 15 specimens from Antioquia (N = 10) and Cundinamarca (N = 5) were imaged using the Scanning Electron Microscope (SEM, Hitachi S-510) methodology of A. Acevedo (unpublished). In short, specimens were first fixed in 2% glutaraldehyde and then subsequently fixed in 1% osmium tetraxide. Each sample was dehydrated in up to 100% ethanol, critical-point dried and sputter coated with gold. Vouchers specimens are stored in BIOMA laboratory, University of Antioquia.

Molecular characterisation

DNAs of ten symphylans from Antioquia were extracted using DNeasy Blood and Tissue Kit (QIAgen®, USA). The *COI* barcode region was amplified by polymerase chain reaction (PCR) using the primers developed by Folmer et al. (1994) and following the protocol of Ruiz et al. (2010). The rDNA ITS2 PCR was carried out using the primers of Collins and Paskewitz (1996) following the protocol of Linton et al. (2001).

Bi-directional sequencing used the Big Dye Terminator Kit® on an ABI3730 automated sequencer (PE Applied BioSystems, Warrington, England). Raw sequence chromatograms were edited using Sequencher™ v. 4.8 (Genes Codes Corporation, Ann Arbor, MI), aligned automatically in MAFFT v. 7 (ITS2) (Katoh et al. 2002) or manually (*COI*) using MacClade v. 4.06 (Maddison and Maddison 2003). Sequence similarities were compared with those available (October 14, 2014) in GenBank using Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and Barcoding of Life Data Systems (BOLD Systems) (http://www.barcodinglife.com/).

Results

A total of 210 symphylans were collected from Antioquia (N = 180) and Cundinamarca (N = 30) and some were used for morphological and molecular studies.

Morphology

Morphometrics from the SEM images of 15 symphylans showed the following characters. **Size:** average symphylan 3.9 mm (range 2.9–4.75 mm excluding antennae). **Head:** somewhat heart-shaped, central rod had a knob before arriving to

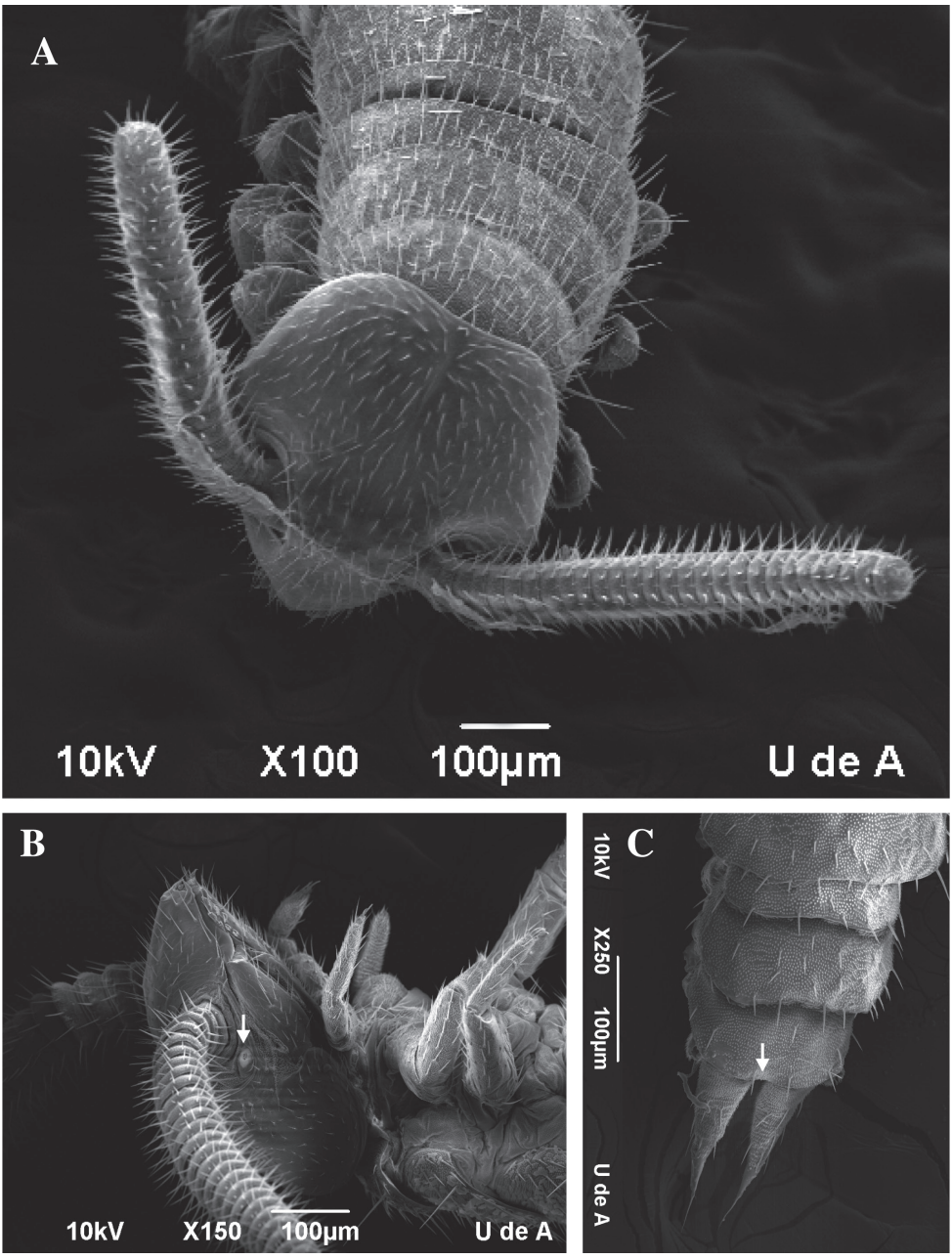

Figure 1. Colombian symphylan. **A** Heart-shaped head and antennae **B** Tömosvary organ (arrow) **C** Last scuta margin with a U-shaped groove (arrow).

the posterior point of the head. Tömosvary organ was clearly defined with a hole in the centre (Figure 1A, B). **Antennae:** between 22 and 31 segments covered with setae (Figure 1A). **Abdomen:** scutes with pubescent cuticles, convex anterior tergites and

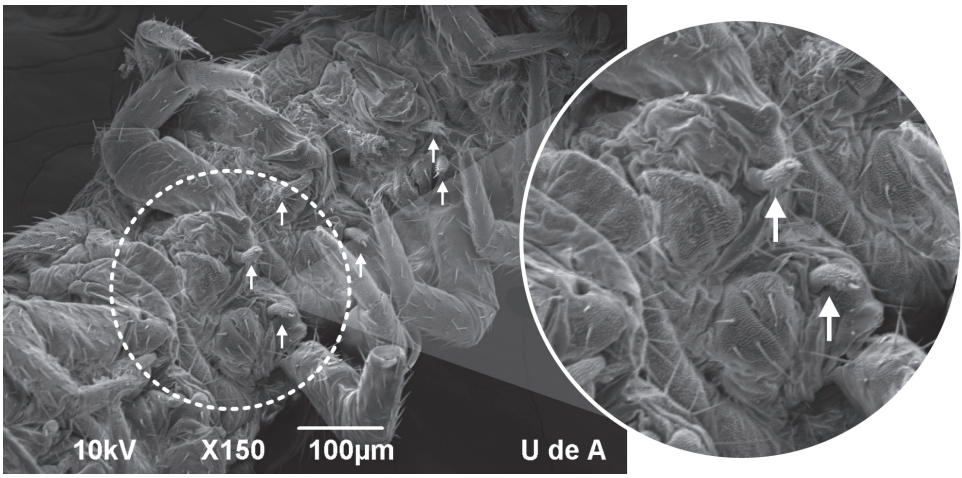

Figure 2. Ventral view of a Colombian symphylan. Presence of sternal appendages behind coxal sacs (arrows).

Table 1. Morphological characters of the genera belonging to the family Scutigerellidae. Colombian symphylans share more than one of the characters that define known genera within Scutigerellidae as described by Domínguez (2009, 2010), Halliday (2004), and Naumann and Scheller (1977).

Genus	Head	Cuticle of the scutes	Anterior tergites	Abdomen: U-shape groove in the last scuta	Legs: sternal appendages behind coxal sacs	Biogeographical distribution
Scutigerella	Heart-shaped	Pubescent	Convex	Present	Absent	Subcosmopolitan, mainly in the northern temperate zones
Hanseniella	Rounded	Glabrous	Not convex	Absent	Absent	Subcosmopolitan, mainly Neotropical and warm temperate zones
Millotellina	Longer than broad	Pubescent	Not convex	Absent	Present	Africa, Madagascar, Reunión, Sri Lanka, New Guinea and Australia
Scopoliella	Rounded	Pubescent	Convex	Absent	Absent	North America
Scolopendrelloides	Heart-shaped	Glabrous	Not convex	Absent	Absent	South-East Asia and Australia
Our specimens	Heart-shaped	Pubescent	Not convex	Present	Present	Colombia

last scuta margins with a U-shaped groove covered with thin dorsal setae, and long ventral and lateral setae (Figure 1C). **Legs:** presence of sternal appendages behind the 3rd to 9th coxal sacs (Figure 2) (Table 1).

Molecular analysis

Two out of ten symphylans captured from Antioquia were successfully characterised at *COI* (658 bp) and ITS2 (358 bp) and both specimens shared the same unique

haplotypes for each marker. An open reading frame was read for *COI* indicating the sequence likely represented a functional protein-coding gene not a pseudogene. GenBank sequence accession numbers: KP696390-91 (*COI*) and KP696392-93 (ITS2).

A comparison of our *COI* symphylan haplotype with sequences deposited in GenBank showed low homology with: *S. causeyae* (77%, query cover 99%, GenBank DQ666065) and *Hanseniella* n. sp. (78%, query cover 92%, GenBank AF370839). Using BOLD Systems database, 100% sequence homology was found with six specimens from Cameroon, described as Phylum Arthropoda, class Symphyla, status private, 77% homology with *Scutigerella* sp. (N = 2) from Bavaria (status private), 77% with *S. causeyae* (N = 2) source locality unknown (status private) and 76% with *S. causeyae* from Austria, Salzburg (status private).

The ITS2 haplotype characterised from our symphylans showed low homology with a sequence of *Scutigerella* sp. (95%, query cover 62%, GenBank DQ666184) and *Hanseniella* sp. (91%, query cover 70%, GenBank AY210821). The ITS2 haplotype could not be compared using BOLD Systems as this database does not collect sequences for this molecular marker.

Discussion

The taxonomy of the class Symphyla is unclear, a consequence of few published studies: two morphological keys for European (Edwards 1959a, b, Domínguez 2010) and one key for Neotropical (Scheller and Adis 1996) species. There are no published morphological descriptions or keys for Colombian Symphyla, therefore the exact number of genera and species is unknown. The only symphylan recorded in Colombia is *S. immaculata* (Agredo et al. 1988, Peña 1998, Corredor 1999), however, this species lacks formal morphological description and both the type specimen and the type locality (London, United Kingdom) have been destroyed and no redescription has been made (Scheller pers. comm.).

Our Colombian symphylans showed genus-level morphological ambiguity (Table 1). We observed a U-shaped groove in the anterior most scuta the character identifying *Scutigerella* (Halliday 2004), but paired sternal appendages behind the 3rd to 9th coxal sacs of the legs (Figure 2) that are unique to *Millotellina* (Naumann and Scheller 1977). Naumann and Scheller (1977) describe the sternal appendages in two subgenera of *Millotellina*, *Millotellina* with unpaired appendages between legs 5 and 10 and *Diplomillotellina* with pairs between legs 5 and 9. However, our symphylans presented paired appendages between legs 3 and 9, which could suggest the existence of a new subgenus within *Millotellina*.

According to Hebert et al. (2003) the threshold of genetic divergence for species delimitation is 3%. However, recent studies have shown that there is no single universal threshold for species' delimitation using the barcode region, which can differ according to the group studied (Rach et al. 2008). For example, Ruiz et al. (2010, 2013) reported in mosquitoes of South America a lower interspecific threshold between 2 and 2.5%. To our knowledge only three papers have used *COI* bardcoding within the subphylum Myriapoda, to which class Symphyla belongs. Spelda et al. (2011) showed for class Chilopoda

a mean interspecific genetic distance of 18.3%: range 12.0% between congeneric species to 25% between genera or families. Stoev et al. also for class Chilopoda showed mean interspecific genetic distances between 5 (2010) and 12 (2013) species of *Eupolybothrus* genus that ranged between 16.1–24.0% and 10.7–24.5%, respectively.

Our Colombian Symphyla *COI* haplotype showed genetic divergence with sequences of *S. causeyae* of at least 23% and *Hanseniella* n. sp. of 22%, similar to the congeneric ranges observed by Spelda et al. (2011) and Stoev et al. (2010, 2013). Unfortunately there are no published sequences of *S. immaculata* or a formal description of this species. As our specimens showed a mixture of morphological characters of *S. immaculata* and *Millotellina* genus, which has never before been reported in the literature, we speculate that Colombian symphylans belong to a new taxon. It is therefore necessary that a formal redescription of *S. immaculata* be published before the taxonomic status of these Colombian symphylans can be made.

It is interesting that our *COI* barcode shared the same haplotype as six Symphyla specimens found in Cameroon. This demonstrates that this taxon is not restricted to South America, it has a wide geographical distribution and therefore can be a widespread agricultural pest. We have two hypotheses to explain this taxon's distribution: 1. That the specimens found in Colombia are a "tramp species", which was introduced inadvertently by human commerce from Africa to the Americas or vice versa. 2. This taxon is native to Colombia, but due to the lack of specialists on this group along with the lack of morphological keys, this taxon has remained unrecognised.

Conclusion

We demonstrate for class Symphyla that the parallel use of DNA barcoding with morphological descriptions can contribute to the taxonomic resolution of this understudied group. Our specimens presented not only the morphological characters of the only symphylan species reported in Colombia, *S. immaculata*, but also the character identifying species within *Millotellina* genus whose distribution has not been recorded in the Americas (Table 1). Furthermore, we showed the same Symphyla *COI* haplotype in both South America and Africa. This research highlights the need for further studies of morphology and molecular phylogenies that include type material to determine the worldwide taxonomic status of class Symphyla.

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