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A new genus and species of octocoral with aragonite calcium-carbonate skeleton (Octocorallia, Helioporacea) from Okinawa, Japan

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Abstract

A new genus and species of octocoral with a calcium-carbonate skeleton, *Nanipora kamurai* **sp. n.**, is described from a shallow coral reef in Okinawa, Japan. Contrary to most octocorals, the skeleton is composed of crystalline aragonite as in blue coral *Heliopora*. The results of molecular phylogenetic analyses of sequences of mtMutS, COI, and ITS1-5.8s-ITS2-28S region suggest *Nanipora* **gen. n.** specimens should be included in order Helioporacea. Based on morphological results compared with other Helioporacea including the genus *Epiphaxum* (family Lithotelestidae), we establish the new genus *Nanipora* within Lithotelestidae. This is the first time that a close molecular phylogenetic relationship between *Heliopora* and a related genus within Helioporacea has been revealed.

Keywords

Aragonite skeleton, molecular phylogeny, new species, octocoral, relict species, taxonomy

Introduction

Octocorals (class Anthozoa, subclass Octocorallia) are sessile marine benthic organisms. Most octocorals support their body by sclerites in their tissue, or having a solid axial structure made of calcite calcium-carbonate or of protein, unlike scleractinians with a massive aragonite calcium-carbonate skeleton. The blue coral *Heliopora coerulea* (Pallas, 1766) (Helioporacea, Helioporidae) is especially peculiar as it is an octocoral with a massive aragonite calcium-carbonate skeleton similar to scleractinians.

Although *H. coerulea* was long considered to be the sole member of the order Helioporacea, Bayer and Muzik (1977) described an octocoral with aragonite skeleton from a specimen in the Barbados collection of J. B. Lewis as *Lithotelesto micropora* (Bayer & Muzik, 1977) and placed the species in the family Lithotelestidae established within Helioporacea. Bayer (1979) reclassified this species as *Epiphaxum micropora*, as Lonsdale (1850: 237–324) had previously described a very similar fossil octocoral as *Epiphaxum auloporoides* Lonsdale, 1850. In total, two fossil and three extant *Epiphaxum* species, two from the Caribbean and one from the western Indian Ocean (Madagascar) (Bayer 1992; Louzet and Molodtsova 2008), have been recorded. While fossils of *Epiphaxum* have been recovered sporadically but widely from Europe, from a wide range of geological ages (Lozouet and Molodtsova 2008), extant species' records are very rare and this genus remains enigmatic (Daly et al. 2007; McFadden et al. 2010).

In this study, we report on our examinations of unknown octocoral specimens with a calcium-carbonate skeleton from a shallow reef off Zamami Island, Okinawa, Japan. Morphology and structure of skeleton for these specimens were examined by using SEM and micro-CT. X-ray diffraction was used to determine the calcium-carbonate composition of the skeleton. Three molecular markers; mitochondrial mismatch repair protein (mtMutS), mitochondrial cytochrome c oxidase subunit 1 (COI), and the nuclear ribosomal gene complex of the 3' end of the 18S subunit, ITS-1, 5.8S subunit, ITS-2, and the 5' end of the 28S subunit (ITS1-5.8s-ITS2-28S) were sequenced to determine the phylogenetic placement of these specimens. Based on these specimens from Zamami Island, this octocoral is described as *Nanipora kamurai* gen. et sp. n. within the family Lithotelestidae.

Methods

Collection of specimens

Specimens were collected by snorkeling using a chisel and a hammer from Ama Beach, Zamami Island, Ryukyu Archipelago, Japan (26°23'N; 127°29'E) at a depth of 1 m in July 2012 (Suppl. material 1). Colonies were attached to the bottoms (=downward facing sides) of carbonate stones. Digital images were also taken in situ to record the appearance of living colonies. Specimens were fixed in 99% ethanol immediately after collection.

Morphological analyses

Digital images were utilized to examine the color and shape of living colonies and polyps (Fig. 1). Skeletons were soaked in household bleach containing sodium hypochlorite for 15–20 min, followed by rinsing with distilled water and air-drying, to remove soft tissues. Skeletal specimens were stuck to aluminum specimen mounts by carbon double-faced tape, then examined and imaged with a scanning electron microscope (SEM) VE-8800 (Keyence, Osaka, Japan). CT images of ethanol-preserved specimens were taken with micro CT (in vivo micro X-ray CT system R_mCT2, Rigaku, Tokyo, Japan) and examined with the DICOM imaging application OsiriX v. 5.9 32-bit (Rosset et al. 2004) to investigate the structure of the skeleton in a non-invasive manner. By using this application, areas of particular CT-value in organisms can be visualized: e.g. showing skeleton (high CT-value) and hiding soft tissue (low CT-value), or vice versa. To check for the presence of sclerites, small pieces of EtOH preserved colonies were dissolved with household bleach on a well-slide and examined with an optical microscope. Additionally, several polyps were dissolved on carbon double-faced tape. Deposits from washing solution of whole colonies were retrieved and carefully rinsed, followed by mounting on carbon double-faced tape. These specimens were examined by SEM. X-ray diffraction analyses were performed with an X-ray diffractometer RINT Ultima/PC (Rigaku, Tokyo, Japan) at the Instrumental Research Center, University of the Ryukyus, to examine the crystal forms of calcium carbonate (aragonite/calcite) of the skeleton. Skeletal specimens were smashed to powder, mounted on well-washed glass slides and analyzed using Cu Kα radiation (40 kV, 30 mA), scanning between a 2θ angle ranging from 25° to 37° at 0.02° steps. The calcite/aragonite component (%) of samples was determined by comparing with data of standard samples of 100% calcite/aragonite. For comparison, a sample skeleton of the blue coral *Heliopora coerulea* was prepared and analyzed in the same manner.

DNA extraction and PCR amplification

DNA was extracted from tentacles and anthocodial tissue of ethanol-preserved samples by a guanidine extraction protocol following Sinniger et al. (2009). PCR amplifications were performed using HotStarTaq DNA polymerase (Qiagen, Tokyo, Japan) according to the manufacturer's instructions. The mitochondrial mismatch repair protein (mtMutS) was amplified by semi-nested PCR following the procedure of McFadden et al. (2006), as no visible PCR product was yielded by a single PCR reaction. First, primers ND42599F (5'-GCCATTATGGTTAACTATTAC-3') (France and Hoover 2002) and Mut-3458R (5'-TSGAGCAAAAGCCACTCC-3') (Sánchez et al. 2003) were used to amplify the 5' end of mtMutS, and then a second PCR reaction was run using internal forward primer ND42625F (5'-TACGTG GYACAATTGCTG-3') (Lepard 2003) and reverse primer Mut-3458R. Both PCR reactions were performed under the following conditions: 15 min at 94 °C; 35 cycles of 1.5 min at 94 °C, 1.5 min at 58 °C, and 1 min at 72 °C; and a final extension of 5 min at 72 °C. Mitochondrial cytochrome oxidase subunit I (COI) was amplified by the primers COII-8068 (5'-CCATAACAG-GACTAGCAGCATC-3') (McFadden et al. 2004) and COI-OCTr (5'-ATCATAG-CATAGACCATACC-3') (France and Hoover 2002) under the following conditions:

5 min at 95 °C; 35 cycles of 1 min at 94 °C, 1 min at 40 °C, and 1.5 min at 72 °C; and then a final extension of 7 min at 72 °C. The nuclear ribosomal gene complex of the 3' end of the 18S subunit, internal transcribed spacer 1 (ITS-1), 5.8S subunit, ITS-2, and the 5' end of the 28S subunit was amplified by the primers 1s (5'-GGTACCCTTTGTACACACCGCCCGTCGCT-3') and 2ss (5'-GCTTTGGGCTGCAGTCCCAAGCAACCCGACTC-3') (Chen et al. 1996) under the following conditions: 15 min at 95 °C; 35 cycles of 0.5 min at 94 °C, 1 min at 52 °C, and 1.5 min at 72 °C; and a final extension of 5 min at 72 °C. Amplified products were visualized with 1.0% agarose gel electrophoresis. Positive PCR products were cleaned up by Exonuclease I and Shrimp Alkaline Phosphatase (Takara, Shiga, Japan) before sequencing.

Sequence analyses

Sequencing was performed by Fasmac (Kanagawa, Japan). Cycle sequencing was performed in both directions using the forward and reverse primers separately with BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) under reaction conditions according to the manufacturer's instructions. Reaction products were analyzed on an ABI PRISM 3700 DNA Analyzer (Applied Biosystems). The sequences were analyzed by 4Peaks Version 1.7.2 software (mekentosj.com, Amsterdam, Netherlands).

By using Se-AL v2.0a11 software (Rambaut 2002), the nucleotide sequences of mtMutS, COI, and ITS1-5.8s-ITS2-28S from specimens obtained in the present study were separately aligned with sequences of *Heliopora* and other octocoral species retrieved from GenBank (Suppl. material). The alignments were checked by eye and manually edited to remove any ambiguous sites (e.g. double peaks) before phylogenetic analyses. For each alignment, none or only one to two base pairs were edited in this manner. Consequently, three aligned data sets were generated: 1) 861 sites of 33 sequences (mtMutS), 2) 735 sites of 12 sequences (COI), and 3) 697 sites of 5 sequences (ITS1-5.8s-ITS2-28S). The alignment data are available on request from the corresponding author. Additional octocoral sequences retrieved from GenBank are shown in Supplementary Table 2.

Phylogenetic analyses

Maximum-likelihood (ML) analyses with PhyML Online (Guindon and Gascuel 2003) of these datasets were independently performed using input trees generated by BIONJ (Gascuel 1997) with the general time reversible (GTR) model. PhyML bootstrap trees (1000 replicates) were constructed using the same parameters as the individual ML trees. Genetic distances were calculated using Kimura's two-parameter model (Kimura 1980).

Bayesian trees were reconstructed by running the program MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) within the program Geneious version 8.0.4 (Restricted) created by Biomatters (available from http://www.geneious.com/). One cold and three heated Markov chain Monte Carlo (MCMC) chains with default-chain temperatures were run for 1,000,000 generations, sampling log-likelihoods (InLs), and trees at 1000-generation intervals (1,000 InLs and trees were saved during MCMC). The first 100,000 generations of all runs were discarded as "burn-in" for the dataset and remaining 900 trees were used to obtain posterior probabilities and branch-length estimates, respectively. Neighbor-joining (NJ) trees were also reconstructed by using CLC Free Workbench 4 software (CLCbio.com, Aarhus North, Denmark) (1000 replicates).

Systematics

Class ANTHOZOA Ehrenberg, 1831
Subclass OCTOCORALLIA Haeckel, 1866
Order HELIOPORACEA Bock, 1938

Family Lithotelestidae Bayer & Muzik, 1977

Type genus. *Lithotelesto* Bayer & Muzik, 1977 (junior synonym of *Epiphaxum* Lonsdale, 1850).

Diagnosis (after Bayer and Muzik 1977; Lozouet and Molodtsova 2008, revised)**.** Helioporacean octocoral with growth form of encrusting, stoloniferous or upright sparsely branched stems. Whole colony rigid with internal skeleton of crystalline aragonite. Polyps fully retractile. Sclerites may be present or absent; if present, capstans and crosses in form, composed of calcite.

Genus *Nanipora* gen. n.

http://zoobank.org/283000C2-355E-4302-A44F-94CB19202538

Type species. *N. kamurai* sp. n., here designated.

Diagnosis. Encrusting, partly stoloniferous colony with cylindrical calyces up to 5 mm tall, attached to hard substratum. Polyps monomorphic and retractile. Coenenchyme and calyces rigid with internal skeleton, not composed with fused sclerites but of unitary crystalline aragonite. Reticulate pattern on whole colony's surface, made of tiny pores on the surface. Top of the calyx serrated, with 16–20 indentations. Longitudinal cavities in the calicular wall, connecting to solenial canals in the base of the colony. Interior of calyx smooth, lacking septae. Surface of calyces occasionally wrinkled. Completely lacks sclerites. Living colonies ivory or pale brown. Skeleton colourless. Azooxanthellate.

Etymology. Named from the Japanese 'nani' plus latin 'pora': 'nani' means 'what is this?', as the genus is highly unusual in having an aragonitic skeleton; 'pora' is originally meaning of 'pore', name used for many anthozoan (especially scleractinian) species with porous skeleton. Gender is feminine.

Nanipora kamurai sp. n.

http://zoobank.org/98A4C103-57A8-469B-A157-81B088EDC717
Figs 1–11

Type material. Holotype: NSMT-Co1562, Ama Beach, Zamami, Okinawa, JAPAN (26°13.31'N; 127°17.28'E), 1 m depth, collected by Yu Miyazaki (Y.M.), 16 July 2012, fixed in 99% EtOH, deposited in National Museum of Nature and Science, Tokyo, Japan (NSMT). GenBank accession numbers: mtMutS, KP195280; mt COI, KP195281; ITS1-5.8s-ITS2-28S, KP195282; Paratype 1: Specimen number RMNH 41731. Ama Beach, Zamami, Okinawa, JAPAN (26°23'N; 127°29'E), 1 m depth, collected by Yu Miyazaki (Y.M.), 16 July 2012, fixed in 99% EtOH, deposited in Naturalis Biodiversity Center, Leiden, the Netherlands (RMNH). Paratype 2: USNM 1231377, Ama Beach, Zamami, Okinawa, JAPAN (26°23'N; 127°29'E), 1 m depth, collected by Yu Miyazaki (Y.M.), 16 July 2012, fixed in 99% EtOH, deposited in National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA (USNM); Other materials. Specimen number MISE-MY-120715. Ama Beach, Zamami, Okinawa, JAPAN (26°23'N; 127°29'E), 1 m depth, collected by Yu Miyazaki (Y.M.), 15 July 2012, fixed in 99% EtOH.

Description. The holotype colony is encrusting (Fig. 1A), attached to the bottom (=downward facing side) of carbonate stone of dimensions 80 × 50 × 50 mm. Colony occasionally with thin stolons (2–3 mm in width, less than 1 mm thick) growing over irregular surface (Fig. 1B, arrow). The polyps of holotype colony are completely withdrawn into calyces after fixation.

Tentacles are 3–4 mm long, with fine but distinct pinnules (Fig. 2A and B). Anthocodiae are fully retractile within calyces. Coenenchyme is thin (up to 3 mm, less than 1 mm in most portions). Both coenenchyme and calyces are rigid with internal skeletons.

Overall shape of the skeleton is virtually the same as the external shape of living colonies (Fig. 3). Calyces are cylindrical, up to 1 mm across; up to 5 mm in height, perforated by randomly distributed pores up to 50 μm in diameter (Fig. 4). The surface of the skeletal calyx is occasionally wrinkled (Fig. 4, 5). The top of the calyces are serrated, with usually 16, but as many as 20 indentations (Fig. 6). Inside of the calyces is simple and tubular, lacking any structures such as septae. Calicular walls are 0.08–0.1 mm thick at the apical end and gradually became thicker going down towards the proximal portion, where thicknesses reached up to 0.2 mm. In the calicular walls, from distal to proximal portions, 12–20 cavities up to 0.05 mm diameter pass through (Fig. 7). These cavities are often discontinuous, converged or branched.

The whole skeleton has a reticulate pattern on the surface (Fig. 3, 4). This pattern is made by numerous tiny pores (up to 5 μm in diameter, Fig. 5); darker parts with pores, and lighter parts without pores. The surface calcium carbonate of the darker portions is very thin like a sheet, compared to the part without pores (Fig. 8). Growing edges of colonies and tops of calyces tend to lack such calcium-carbonate sheets and therefore the surface of these regions has holes (up to 200 μm, Fig. 9A–D). In the cross sections of the coenenchymal skeleton, cavities 0.1–0.2 mm in diameter are observed (Fig. 10).

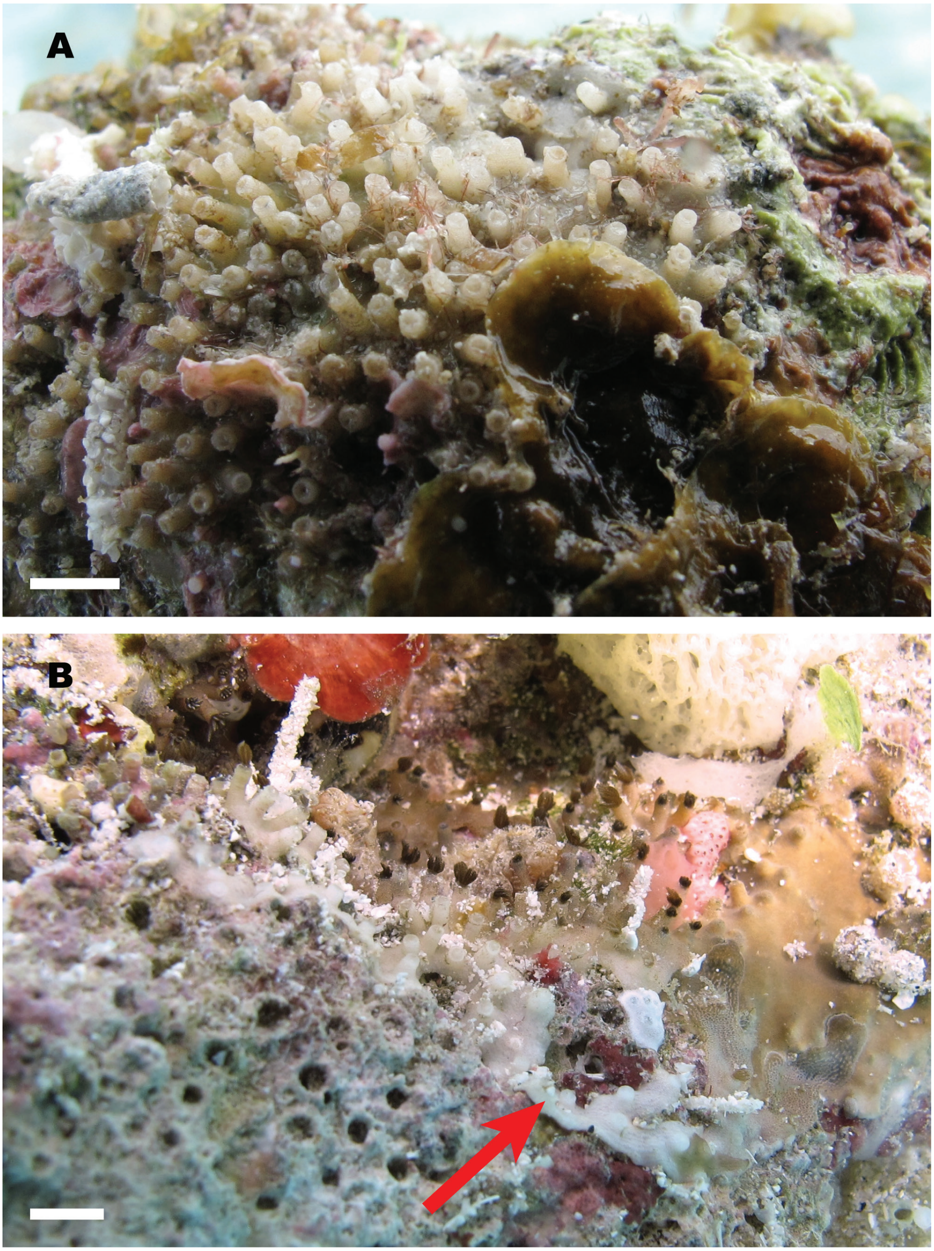

Figure 1. Living colony of *Nanipora kamurai* attached to the bottoms (=downward facing side) of calcium-carbonate stone at Ama Beach, Zamami Island, Okinawa, Japan, 16 July 2012. Scale bar: approximately 5 mm.

These cavities house solenia, connecting the gastric cavities of polyps and composing solenial network (Fig. 11, 3-dimensional CT images of soft tissue). Lacks sclerites. Azooxanthellate.

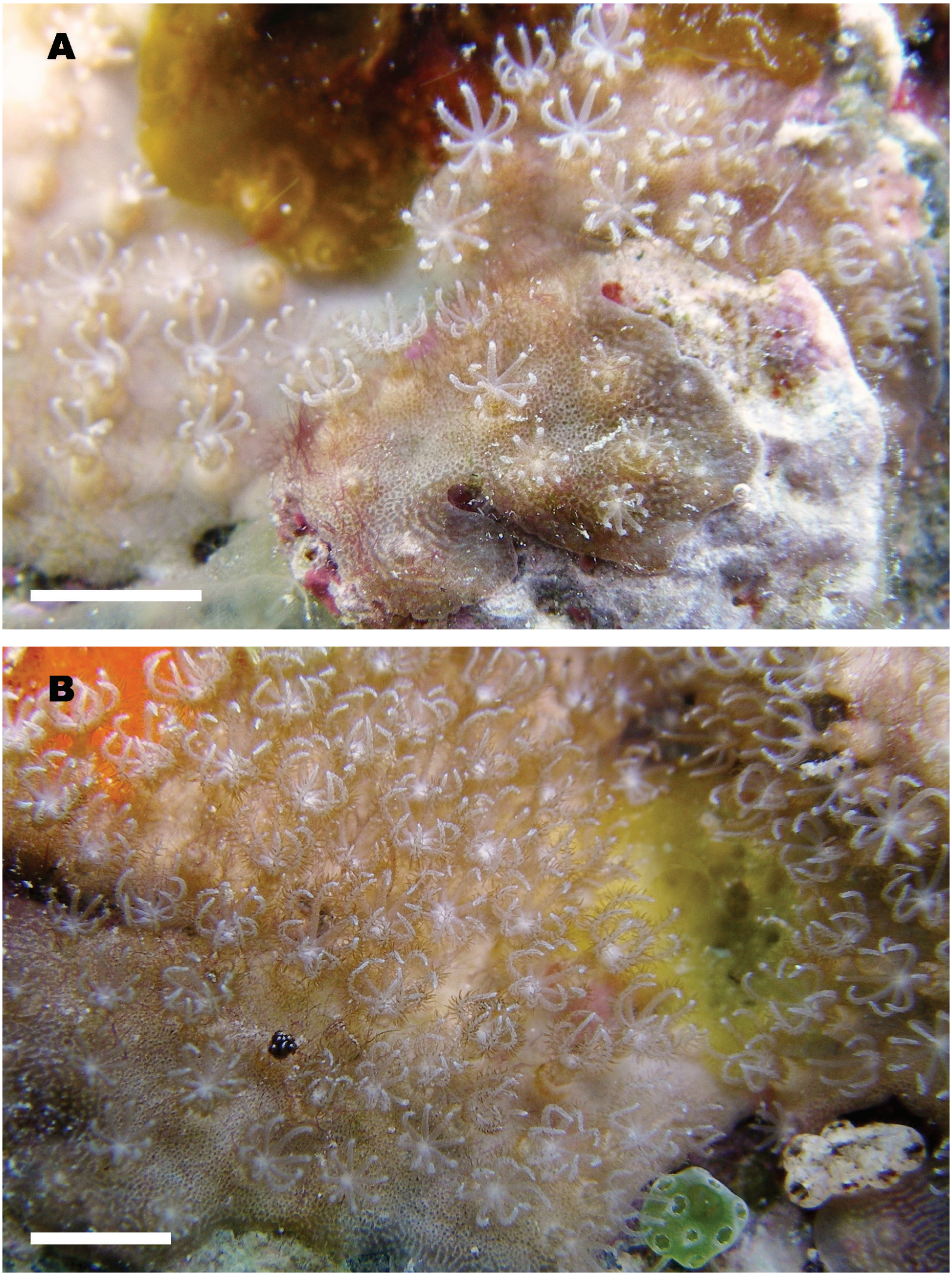

Figure 2. In situ colony of *Nanipora kamurai* with expanded polyps at Ama Beach, Zamami Island, Okinawa, Japan, 16 July 2012. **A** growing edge of the colony **B** middle portion of the colony. Scale bar: approximately 5 mm.

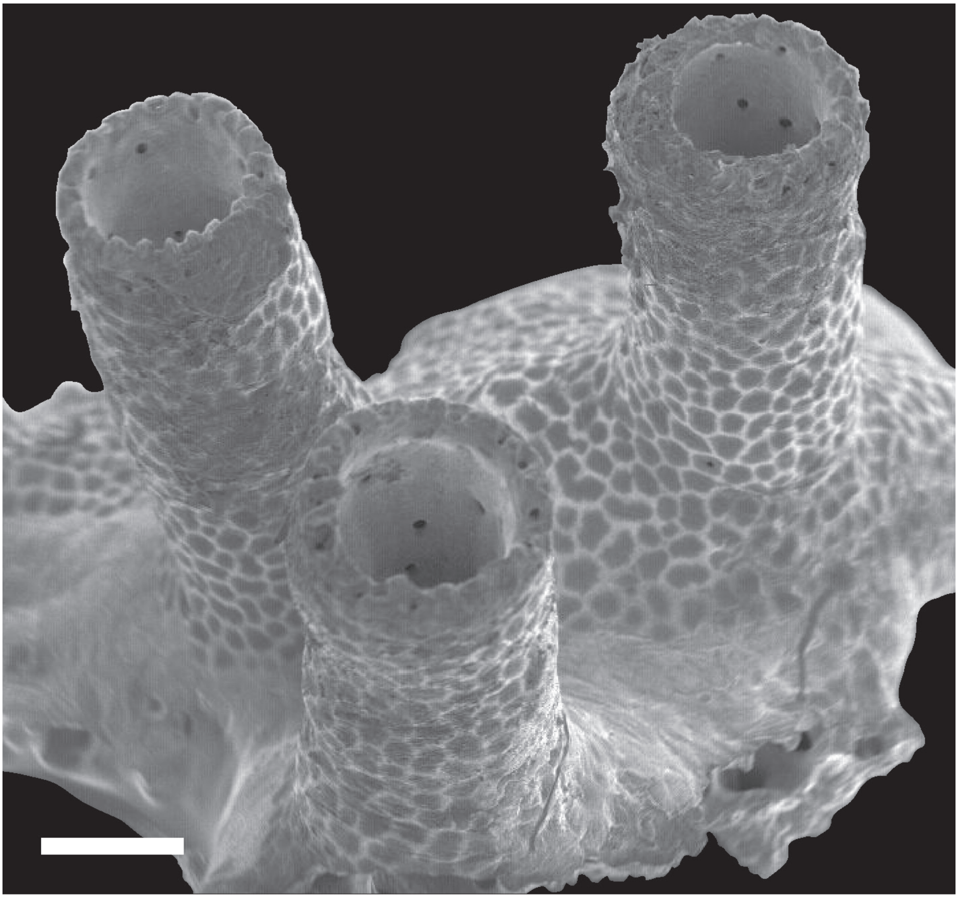

Figure 3. Scanning electro-microscope (SEM) image for skeleton of *Nanipora kamurai* colony. Scale bar: 0.5 mm.

Colour. Living colony is pale brown or ivory (Fig. 1A and B). Whole polyps are pale brown, but shrunken tentacles appear dark brown (Fig. 1B). Skeleton is colourless.

Etymology. Named after Hidefumi Kamura, a great jazz pianist who has continued playing classic style be-bop jazz from when Okinawa was under the rule of U.S. forces, and who can now be considered as a 'relict' classical be-bop jazz musician.

Habitat. *Nanipora kamurai* colonies are found on the bottoms (=downward facing sides) of carbonate stones on a sandy shallow beach at 1–1.5 m depths with very clear water. For now known only from Ama Beach, Zamami Island, Okinawa, Japan.

Comparison with other species. General morphology of *Nanipora kamurai* is quite similar to *Epiphaxum* Lonsdale, 1850. Unlike *Epiphaxum* species, presence of sclerites not observed by any means in any portion of specimen in this study. Secondary daughter calyces, such as seen in *Primnoa gracilis* Nielsen, 1925 (=*Epiphaxum auloporoides*) and Verrill's original drawing of *Lithotelesto micropora* Bayer & Muzik,

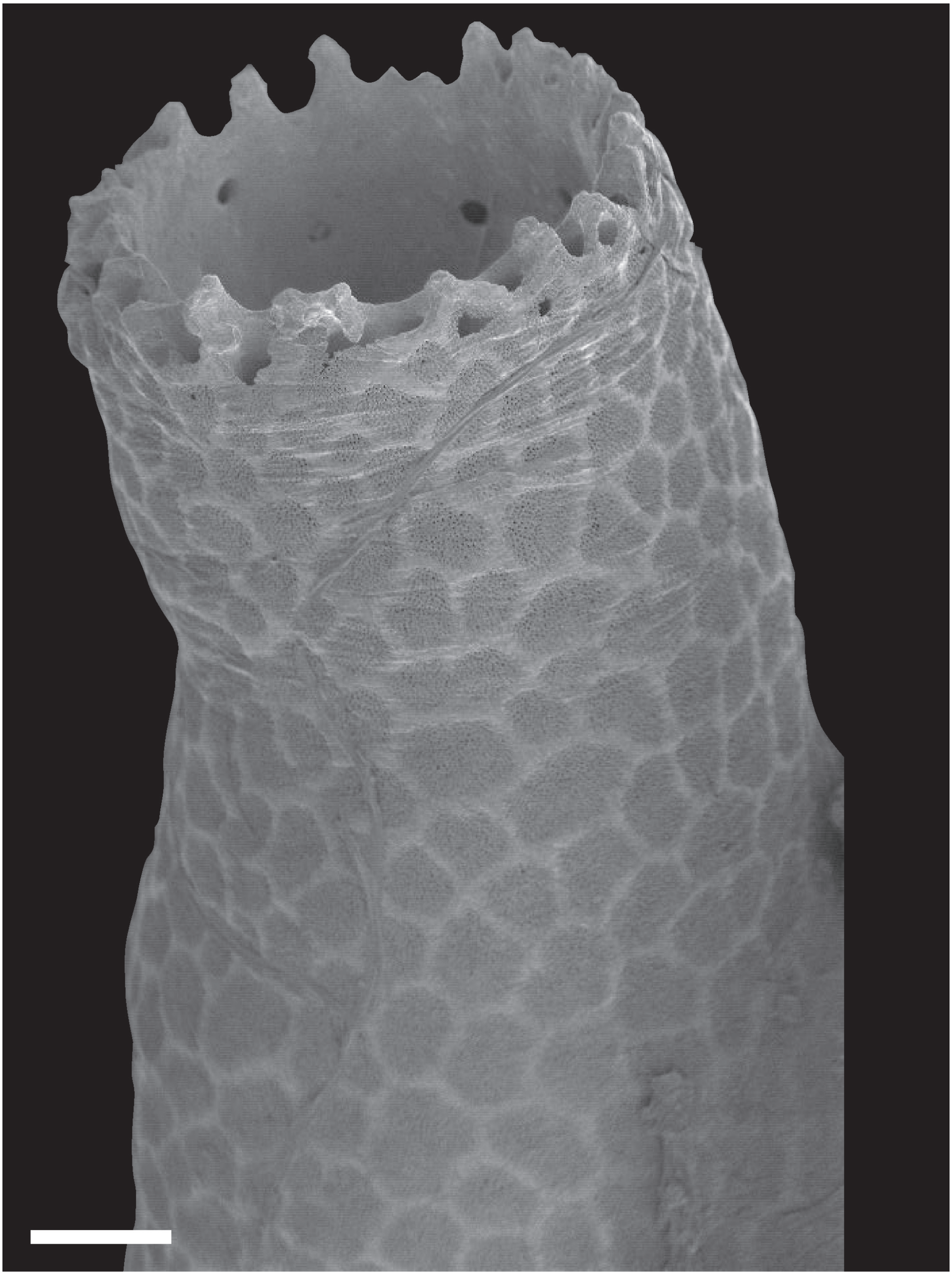

Figure 4. Calyx of *Nanipora kamurai*. Reticulate pattern and wrinkles are shown. Scale bar: 0.2 mm.

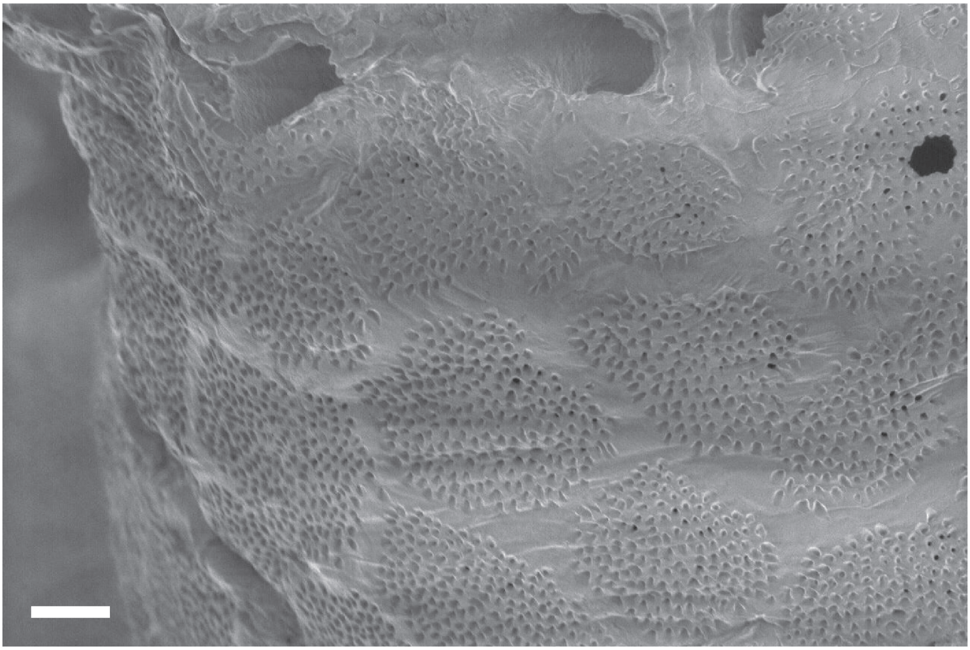

Figure 5. Surface of the calyx. Reticulate pattern made by numerous tiny pores. Scale bar: 0.04 mm.

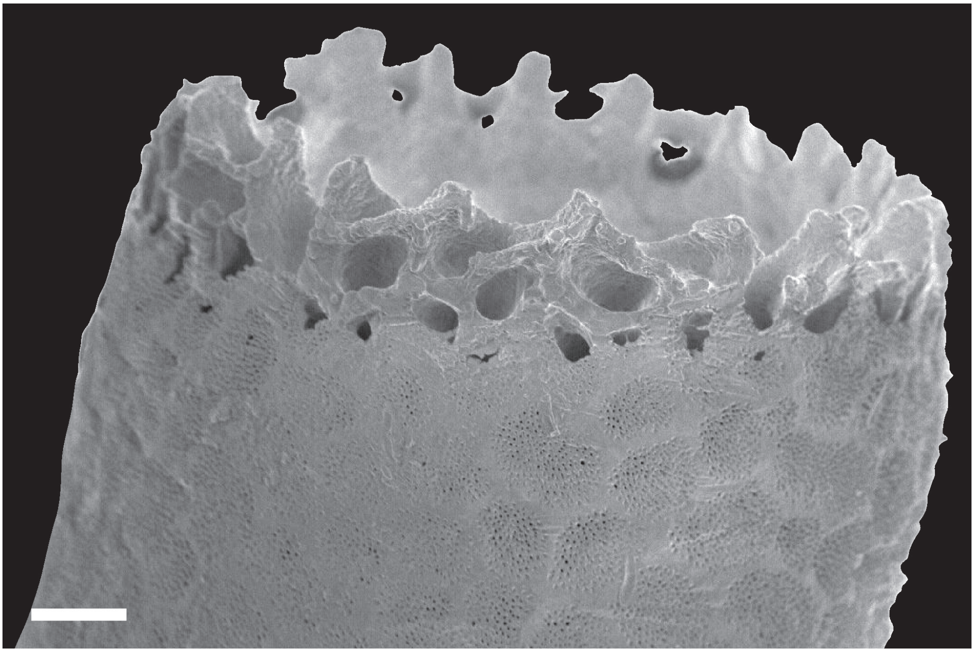

Figure 6. Indentations seen on the top of the calyx of *Nanipora kamurai*. Scale bar: 0.1 mm.

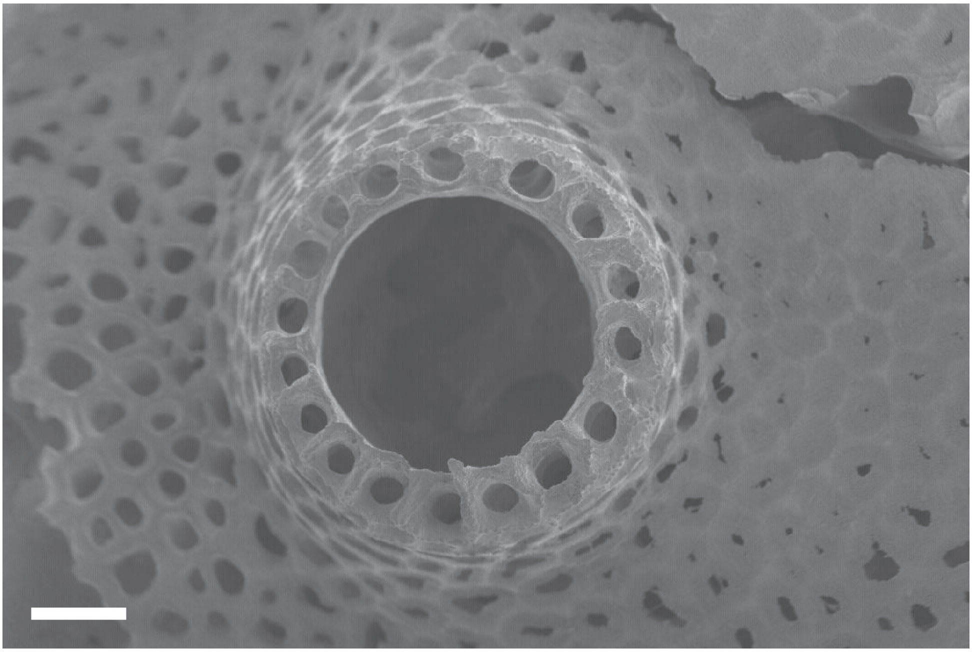

Figure 7. Calyx of *Nanipora kamurai* seen from above. Cavities extend in a longitudinal direction down through the calyx are shown. Scale bar: 0.2 mm.

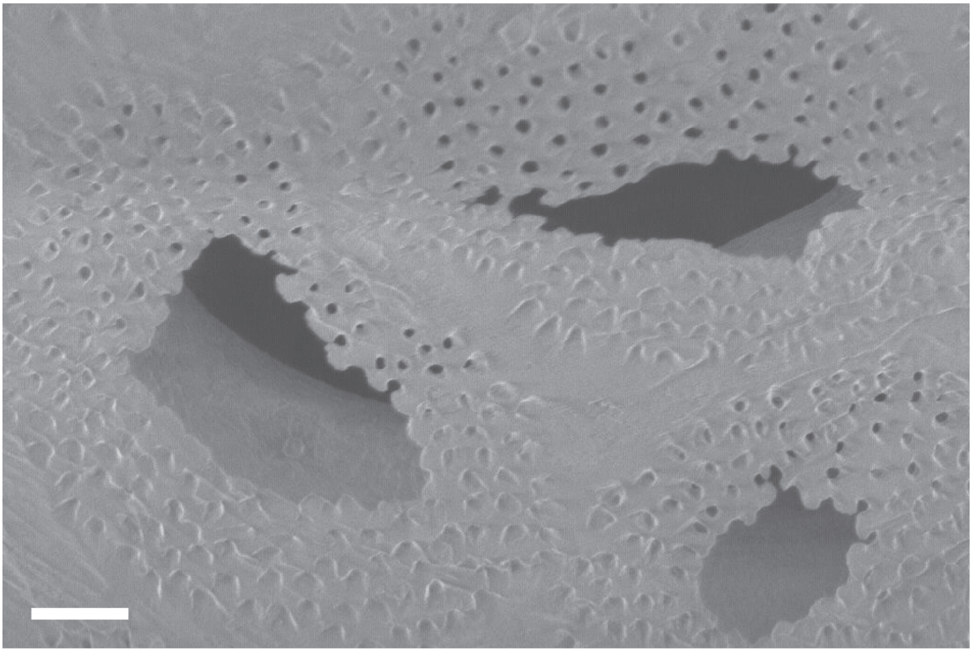

Figure 8. Surface calcium carbonate of the darker portions. Beneath, large holes (up to 200 μm) are shown. Scale bar: 0.02 mm.

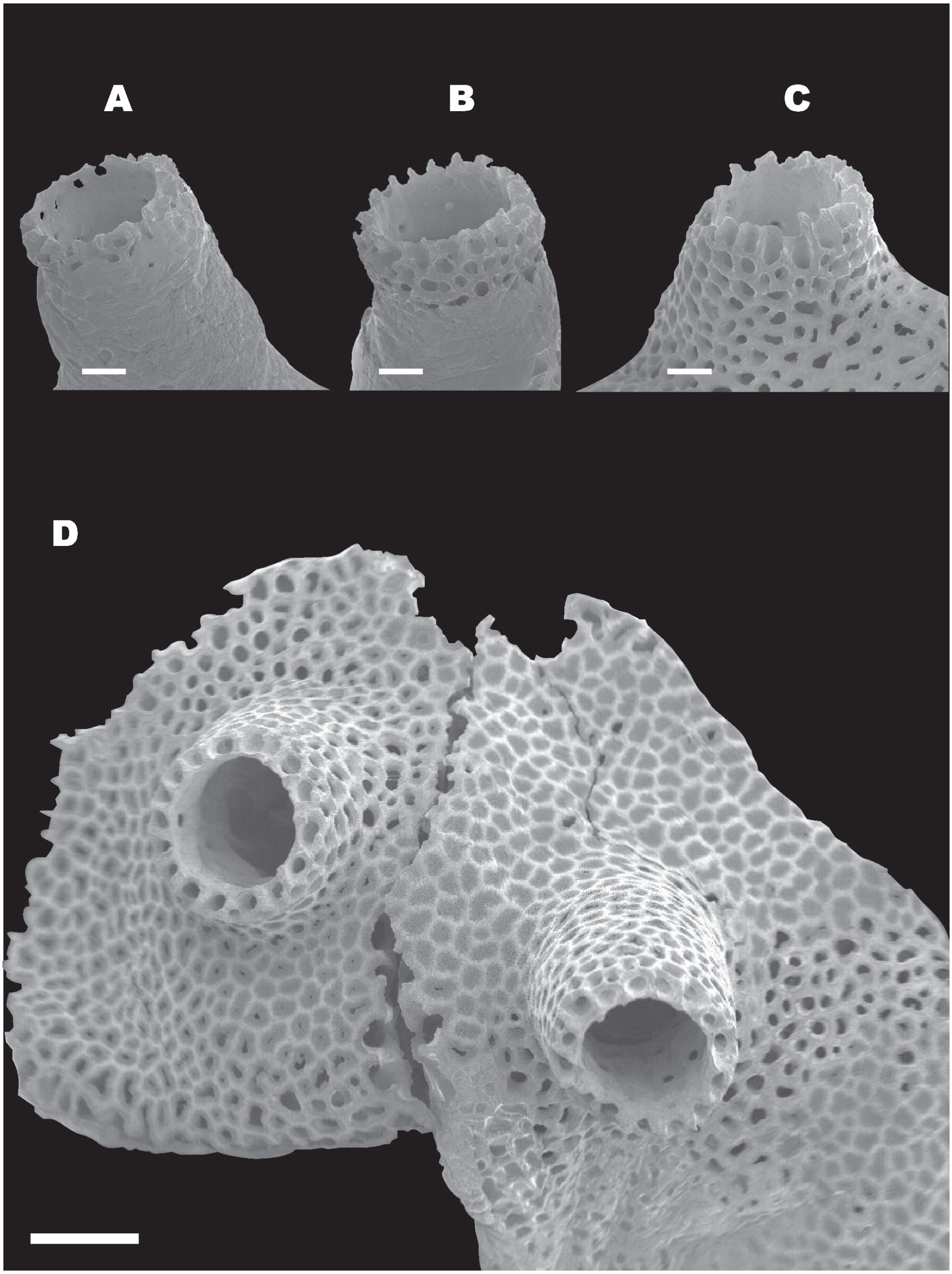

Figure 9. A–C Different surface calcium carbonate coverage of three calyces (**A>B>C**) **D** Skeleton of younger and marginal part of the colony, partly lacks surface cover. Scale bar: 0.2 mm (**A**), 0.5 mm (**B**).

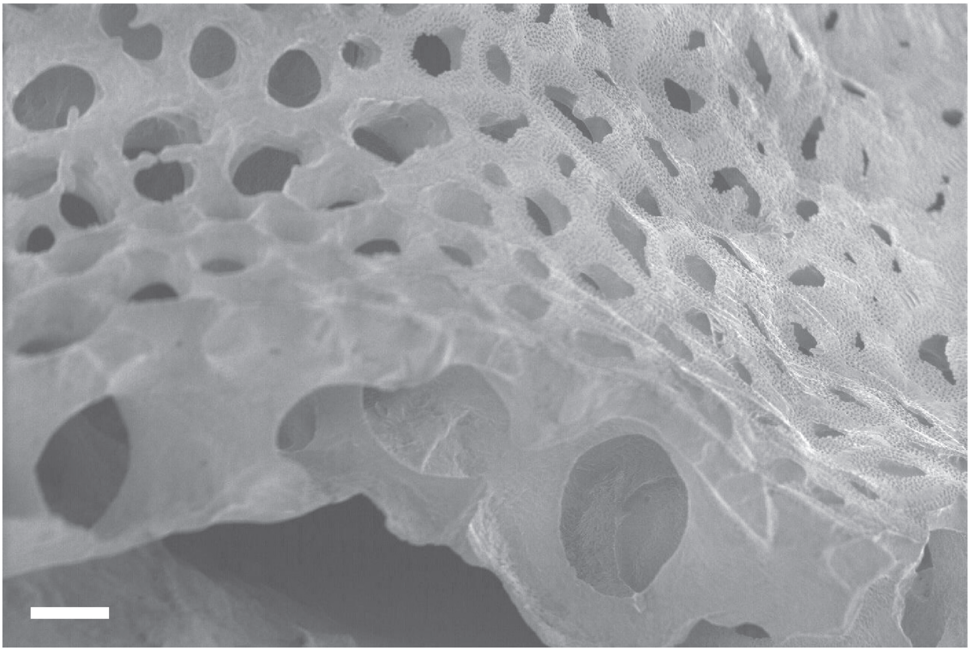

Figure 10. Cavities seen in cross sectioned coenenchymal skeleton. Scale bar: 0.1 mm.

1977 (=*Epiphaxum micropora*), are not observed. Pores perforating calicular walls of *N. kamurai* are distributed irregularly, not aligned in a line or in a row as seen in *Epiphaxum* species (Bayer and Muzik 1977; Bayer 1992; Lozouet and Molodtsova 2008). Neither an octagonal outline in the cross sections of calyces, such as seen in *E. breve* Bayer, 1992, nor sclerosepta as seen in *E. septifer*, are observed.

Skeleton. Examination of SEM images clearly showed the rigid skeleton of this species was not formed by fused sclerites as in *Tubipora*, but made of unitary calcium carbonate as in *Heliopora*. X-ray diffraction analyses revealed this skeleton was composed of 96% aragonite and 4% low-Mg calcite. Inclusion of traces of calcite may be contamination from calcareous algae attached to the surface of the colony. The skeleton of blue coral *Heliopora coerulea*, analyzed for comparison, was 100% aragonite.

Molecular phylogeny. The four specimens of *Nanipora kamurai* in this study had completely identical mtMutS, COI and ITS1-5.8s-ITS2-28S sequences. In the ML trees for mtMutS (Fig. 12) and COI (not shown) alignments, the sequences of *N. kamurai* in this study and *Heliopora coerulea* formed a strongly supported clade (ML=99% for mtMutS; ML=90% for COI) to the exclusion of all other octocoral sequences, and sequences of the new specimens formed a subclade clearly different from *Heliopora* (ML=100% in mtMutS, ML=100% in COI). p-distances between *N. kamurai* and *H. coerulea* were 0.053 (mtMutS) and 0.034 (COI), while distances between *N. kamurai* and other soft corals included in phylogenetic analyses were at least >0.061 (mtMutS) and >0.042 (COI). ITS1-5.8s-ITS2-28S sequences of the unknown octocoral and *H. coerulea* could be aligned together, but they could not be accurately aligned with any

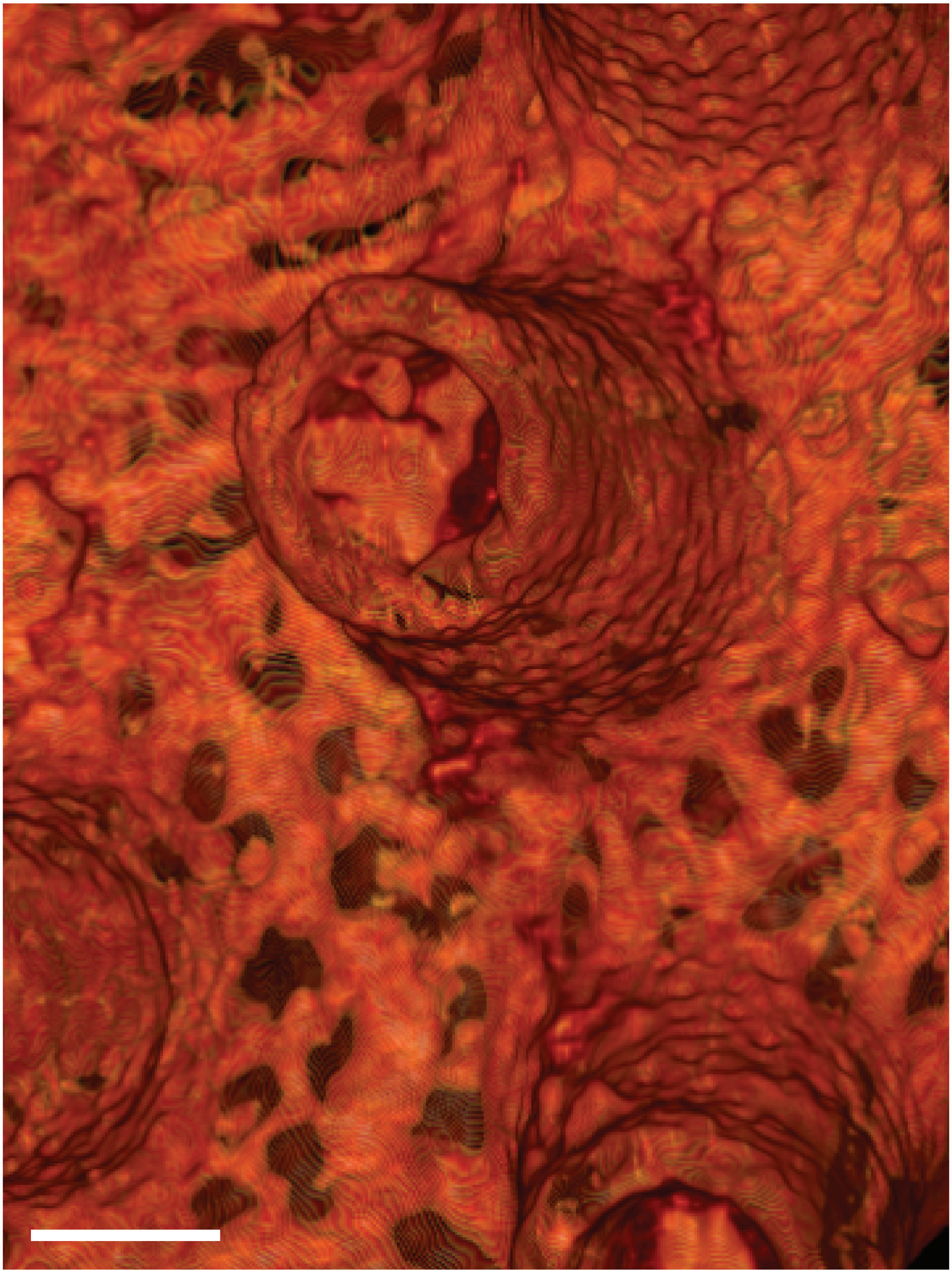

Figure 11. 3D CT image of soft tissue. Solenial tubes forming network are shown. Scale bar: 0.5 mm.

other known octocoral ITS1-5.8s-ITS2-28S sequences due to sequence divergence. The nuclear ITS1-5.8s-ITS2-28S region sequence of *N. kamurai* had 94 nucleotide differences from *H. coerulea* sequences over 697 nucleotides (=13.5% variation).

Remarks. The distribution of *Nanipora* gen. n. is currently known from only one site in Okinawa. Although the lack of reports may result from their tiny size and cryptic habitat, considering the sporadic distribution and extraordinary rarity of related *Epiphaxum* spp., *Nanipora kamurai* may also be a relict species surviving with a very limited distribution. This species is one of the few exceptional species with an aragonite calcium-carbonate skeleton among Octocorallia.

Common Japanese name. Zamami-ishi-hanagoke.

Discussion

Phylogenetic status of *Nanipora kamurai* in Helioporacea

Results of phylogenetic analyses apparently show that *Nanipora kamurai* is more closely related to *Heliopora coerulea* than to other groups of octocorals. The genetic distance between *Nanipora* and *Heliopora* as shown in branch lengths in the mtMutS tree (Fig. 12) is comparable to what is typically seen among different families of octocorals (e.g. McFadden et al. 2006) (see also Suppl. materials 3, 4). This is the first time the existence of an extant relative of *H. coerulea* has been confirmed using molecular phylogenetic analyses, as no phylogenetic research on *Epiphaxum* spp. has yet been conducted.

Taxonomic relationship between *Nanipora kamurai* and *Epiphaxum*

Lonsdale (1850), who described the fossil genus *Epiphaxum* from Chalk (Upper Cretaceous) in Sussex, United Kingdom, felt that *Epiphaxum* was not related to any extant family. According to Lozouet and Molodtsova (2008), *Epiphaxum* was later placed by Voigt (1958) into Clavulariidae, which had been defined by the presence of cylindrical anthosteles and connecting stolons. Finally, Bayer and Muzik (1977) placed this genus in the order Helioporacea by examining the type of calcium-carbonate present in skeletons.

The general colony shape of *N. kamurai* closely resembles encrusting and stoloniferous species of *Epiphaxum* (Bayer, 1992). As well, the basic structure of the skeleton (simple and tubular calyx, indentations on the top of the calyx) is common among *Nanipora* and *Epiphaxum* spp. However, longitudinal grooves on the surface of calyces' skeleton, common to every *Epiphaxum* spp. (see Bayer and Muzik 1977; Bayer 1992; Lozouet and Molodtsova 2008), were not observed in *N. kamurai*. Instead, the entire surface of the *Nanipora* skeleton is covered by a reticulate pattern (Fig. 4). The various descriptions of genus *Epiphaxum* mention possession of calcite calcium-carbonate sclerites (Bayer and Muzik 1977; Bayer 1992; Lozouet and Molodtsova 2008), although actually in some species of *Epiphaxum* the presence of sclerites has not been confirmed due to the absence of soft-tissue in the holotype specimen (*E. septifer* Bayer, 1992), or due to the type specimen being fossilized (*E. arbuscula* Bayer, 1992) (Bayer 1992;

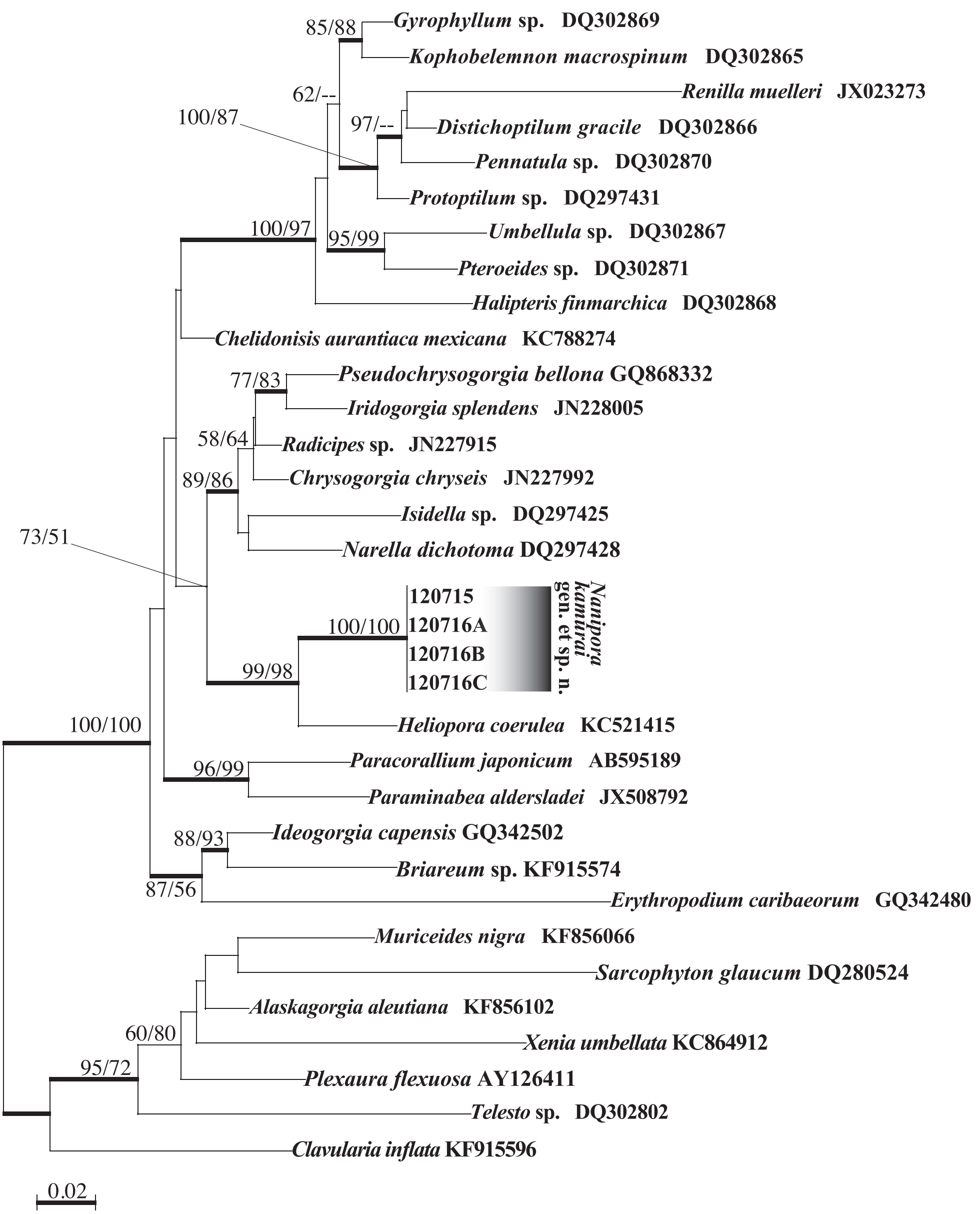

Figure 12. Maximum likelihood tree for mtMutS sequences. Values at branches represent ML and NJ bootstrap probabilities, respectively (>50%). Bold lines represent branches with very high support in Bayesian analyses (>0.95). Sequences without accession numbers were newly obtained in this study.

Lozouet and Molodtsova 2008). The stoloniferous colony is one of the simplest colony morphologies in Octocorallia and is found among many unrelated groups of octocorals, but many other morphological characters apparently suggest the close relatedness of *N. kamurai* and *Epiphaxum*. In particular, considering the complete lack of sclerites, placement of *N. kamurai* in a new genus inside Lithotelestidae is much more appropriate than a major modification of genus *Epiphaxum* and inclusion of *N. kamurai* within *Epiphaxum*. Molecular phylogenetic data for *Epiphaxum* spp. are necessary to construct the complete phylogeny of Helioporacea.

Taxonomic status of order Helioporacea

The blue coral *Heriopora coerulea*, the sole member of Helioporidae, has been considered to be extraordinarily distinct among octocorals. When Bayer (1981) reorganized the entire subclass Octocorallia into only three orders, one order was Helioporacea. However, McFadden et al. (2006) showed that *H. coerulea* sequences were placed within a large Calcaxonia–Pennatulacea clade. In this study, similar to McFadden et al. (2006), *H. coerulea* and *Nanipora kamurai* sequences fell into the same sub-major clade of Octocorallia. However, although no recent molecular study supports the phylogenetic distinctiveness of Helioporacea as an order within subclass Octocorallia, the order Helioporacea is still used. The taxonomic status of this order needs to be reexamined with careful morphological and molecular phylogenetic comparisons. The discovery and confirmation of the phylogenetic relationship of *N. kamurai* to Helioporacea in this study should contribute to this reassessment.

Geographic distribution and the origin of *Nanipora kamurai*

Extant *Heliopora coerulea* is restricted to the Indo-Pacific, and extant species of *Epiphaxum* are found from the Caribbean and the western Indian Ocean (Madagascar), while fossil species of both genera have been found sporadically but widely from Europe. So far, *Nanipora* has only been found in Zamami Island, Okinawa, Japan, although surveys are needed to confirm its exact distribution. As indicated in Lozouet and Molodtsova (2008), recent *H. coerulea* is considered to be a relict of fossil *Heliopora* species distributed throughout the Tethys Ocean. Considering the morphological characters of these genera, we can hypothesize that Helioporidae branched first from a common ancestor, subsequently followed by the division of *Epiphaxum* and *Nanipora*, although the geographical timing of this split is unknown. As pointed out by Lozouet and Molodtsova (2008), the discontinuous distribution of *Epiphaxum* across the Pacific and the Atlantic implies that at least *Epiphaxum* already existed before the closure of the Tethyan connection. Additionally, the morphological distinctiveness of *Nanipora* strongly supports the hypothesis that *Nanipora* and *Epiphaxum* radiated before the disjunction of the Atlantic and the Pacific, rather than *Nanipora* radiating from Indo-West Pacific *Epiphaxum*. Molecular phylogenetic analyses of Atlantic and Pacific *Epiphaxum* and corroborated studies with paleontology should help confirm the evolutionary history of this unique group.

Cryptic habitat of *Epiphaxum* and *Nanipora*

Detailed habitat information of extant species of *Epiphaxum* is unknown as all known living specimens were obtained by dredging or trawling (Bayer and Muzik 1977; Bayer 1992). Lozouet and Molodtsova (2008) indicted fossil species of *Epiphaxum* (*E. arbuscula*) were strongly related to submarine canyons, which also included fossil fauna found in muddy sea-floor at depths corresponding to the outer continental shelf or upper slope, submarine cave environments, and rocky circa-littoral cliffs. Considering this information together with the cryptic habitat of *Nanipora kamurai* (bottom side of carbonate stones), *Epiphaxum* and *Nanipora* appear to not reside in shallow well-lit subtropical and tropical environments like *Heliopora*, but instead in shaded or cryptic environments. Detailed surveys of such cryptic environments may lead to the discovery of other unknown *Epiphaxum* or *Nanipora* species.

Importance of cryptic fauna in Octocorallia

It is astonishing that a unique, relict species such as *Nanipora kamurai* was found from shallow waters. Generally, relict species are thought to be most commonly found in stable and undisturbed environments, such as abyssal waters, as demonstrated by the discovery of Coelacanthiformes in Africa and Indonesia (Smith 1939; Erdmann et al. 1998). Although not from abyssal depths, extant *Epiphaxum* spp. were also found from quite deep habitats (*E. micropora*: 50–400 m, *E. breve*: 183 m, *E. septifer*: 200–360 m). The discovery of *N. kamurai* in this study demonstrates the importance of the study of cryptic anthozoan fauna in shallow coral reef areas (see also Fujii and Reimer 2011), not only for a more correct understanding of coral reef biodiversity, but also to make progress in clarifying the phylogeny and taxonomy of octocorals.

Conclusions

The aragonite calcium-carbonate skeleton is considered to be a synapomorphy among Helioporacea, although only three genera including *Nanipora* are currently known from this order. Considering the close phylogenetic relationship between *Heliopora* and *Nanipora kamurai*, and the morphological affinity between *N. kamurai* and *Epiphaxum*, Bayer and Muzik's (1977) placement of *Epiphaxum* within Helioporacea based only on small surface structure and aragonite calcium-carbonate skeleton appears appropriate. In this study the phylogenetic position of *Nanipora* specimens was suggested by using a molecular phylogenetic approach. mtMutS works well in determining phylogenetic position of such unknown, unclassified species among subclass Octocorallia. Surveying cryptic environments and utilizing proper molecular markers with detailed morphological examinations are an effective way to reveal the true diversity of octocorals.

Acknowledgements

This manuscript was aided by a collaborative research agreement with Dr Masanori Nonaka (Churaumi Aquarium), and we deeply thank him for his kind and generous help. Dr Ryuji Asami (University of the Ryukyus) kindly performed X-ray diffraction analyses of calcium-carbonate. Dr Stephen Cairns (Smithsonian Institution) is thanked for sending precious and rare literature. Dr Katherine Muzik gave us advice on the literature of Bayer. Mr Martyn Low (Lee Kong Chian Natural History Museum, NUS, Singapore) is thanked for advice on the naming of new taxa. All specimens of *Heliopora coerulea* were legally collected under special permission from Okinawa Prefecture (permission number 26-38). The second author was funded by the International Research Hub Project for Climate Change and Coral Reef/Island Dynamics at the University of the Ryukyus, and by a Japan Society for the Promotion of Science 'Zuno-Junkan' grant entitled 'Studies on origin and maintenance of marine biodiversity and systematic conservation planning'. Comments from reviewers and the editor improved this manuscript.

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Supplementary material 1

Supplemental Table 1

Authors: Yu Miyazaki, James Davis Reimer

Data type: species data

Explanation note: List of *Nanipora kamurai* sp. n. specimens examined in this study. Collection information and GenBank accession numbers for corresponding sequences are shown.

Supplementary material 2

Supplemental Table 2

Authors: Yu Miyazaki, James Davis Reimer

Data type: molecular data

Explanation note: Outgroup sequences from GenBank used in molecular phylogenetic analyses.

Supplementary material 3

Supplemental Table 3

Authors: Yu Miyazaki, James Davis Reimer

Data type: molecular data

Explanation note: Genetic distances (p-distances) for mtMutS between *Nanipora kamurai* and *Heliopora coelurea* and for other species of octocorals included in mtMutS phylogeny (see Fig. 12 and Suppl. material 2).

Supplementary material 4

Supplemental Table 4

Authors: Yu Miyazaki, James Davis Reimer

Data type: molecular data

Explanation note: Genetic distances (p-distances) for mtMutS between *Nanipora kamurai* and *Heliopora coelurea*, and for other species of octocorals included in COI phylogeny (See Suppl. material 2).

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RESEARCH ARTICLE

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New data on two remarkable Antarctic species *Amblydorylaimus isokaryon* (Loof, 1975) Andrássy, 1998 and *Pararhyssocolpus paradoxus* (Loof, 1975), gen. n., comb. n. (Nematoda, Dorylaimida)

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Abstract

The taxonomic position of two antarctic dorylaimid species *Amblydorylaimus isokaryon* (Loof, 1975) Andrássy, 1998 and *Pararhyssocolpus paradoxus* (Loof, 1975), **gen. n.**, **comb. n.** are discussed on the basis of morphological, including SEM study, morphometric, postembryonic and sequence data of 18S rDNA and the D2-D3 expansion fragments of large subunit rDNA. The evolutionary trees inferred from 18S sequences show insufficient resolution to determine the assignment of the two species to particular families, moreover *P. paradoxus* **gen. n.**, **comb. n.** (=*Rhyssocolpus paradoxus*) previously regarded as a member of Nordiidae or Qudsianematidae, showed distant relationship both to *Rhyssocolpus vinciguerrae* and *Eudorylaimus* spp. The phylogram inferred from 28S sequences revealed that *A. isokaryon* is a member of a well-supported group comprised of several *Aporcelaimellus* spp., while, no close relationships could be revealed for the *P. paradoxus* **gen. n.**, **comb. n.** to any nematode genus. On the basis of molecular data and morphological characteristics, some taxonomic changes are proposed. *Amblydorylaimus isokaryon* is transferred from family Qudsianematidae to family Aporcelaimidae, and a new monotypic genus *Pararhyssocolpus* **gen. n.** is proposed, attributed to Pararhyssocolpidae **fam. n.** The diagnosis of the new family is provided together with emended diagnosis of the genera *Amblydorylaimus* and *Pararhyssocolpus* **gen. n.** Data concerning distribution of these endemic genera in the Antarctic region are also given.

Keywords
18S rDNA, D2-D3 28S rDNA, morphology, new geographic records, nomenclature, SEM, taxonomy

Introduction

Taxonomic studies on Antarctic nematodes are sparse and current knowledge about species distribution, biogeography and their relationship to the global fauna is still poor (Velasco-Castrillón and Stevens 2014). Almost all Antarctic nematode species have been recorded only from this region (Andrássy 1998a; Maslen and Convey 2006; Convey et al. 2008). This high degree of endemism is probably caused by the long-term isolation and harsh climate of the region (Convey et al. 2008; Nielsen et al. 2011), and indicates that they are glacial survivors rather than post-glacial colonists (Andrássy 1998a; Maslen and Convey 2006; Chown and Convey 2006).

Nineteen species of order Dorylaimida Pearse, 1942 have been recorded from this region which is approximately 0.7% of the known dorylaimid species; all of them being endemic. Regarding the genera distribution a single genus, namely *Amblydorylaimus* Andrássy, 1998 inhabiting Maritime Antarctic is considered endemic.

Molecular studies of free-living Dorylaimida members are increasing (Mullin et al. 2005; Holterman et al. 2006; 2008; Pedram et al. 2009; 2011; Álvarez-Ortega et al. 2013a, b, c; Nedelchev et al. 2014; Peña-Santiago et al. 2015). Nevertheless, Antarctic dorylaimids have received little attention in this respect with only one study of this widespread order in Antarctic. Velasco-Castrillón and Stevens (2014) analysed the morphological and molecular diversity of Antarctic nematodes using the mitochondrial cytochrome c oxidase subunit I (COI) gene.

Here we present data on the morphology, molecular taxonomy and distribution of two dorylaimid species with unclear taxonomic position occurring in the Maritime Antarctic.

Materials and methods

Samples were collected from Livingston, Nelson and King George Islands by Dr. N. Chipev, Dr R. Mecheva (IBER) and Dr R. Zidarova (Faculty of Biology, Sofia University St. Kliment Ohridski) during regular Bulgarian Antarctic Expeditions (1997–2013). Nematodes were extracted from soil and plant materials by using a Baerman funnel method for 48 or more hours of exposition, killed by gentle heat and fixed in 4% formalin.

For light-microscopy, specimens were processed in anhydrous glycerine by a Seinhorst method (1959) and mounted on permanent slides. Drawings were prepared using an Olympus BX 51 compound microscope with DIC and a drawing tube. Photographs were taken using an Axio Imager.M2 – Carl Zeiss compound microscope with a digital camera (ProgRes C7) and specialised software (CapturePro Software 2.8).

Measurements were made using an Olympus BX 41 light microscope with a drawing tube and digitizing tablet (CalComp Drawing Board III, GTCO CalCom Peripherals, Scottsdale, AZ, USA) and Digitrak 1.0f computer program (Philip Smith, John Hutton Institute, Dundee, UK).

Specimens used for SEM observations were rinsed in 0.1 M cacodylate buffer (twice for 10 min), post-fixed in 1% OsO_4 for 2 h, washed twice for 10 min in 0.1 M cacodylate buffer and dehydrated in an ethanol series (Mutafchiev et al. 2013), immersed in hexamethyldisilazane for 30 min and air dried. They were coated with gold in fine coater JEOL JFS 1200 and examined using a JEOL JSM 5510 microscope at 10 kV.

The location of pharyngeal gland nuclei is presented following Loof and Coomans (1970) and Andrássy (1998b).

DNA extraction, amplification and sequencing

Genomic DNA was extracted from one male and one female specimen of both species using a standard nematode digestion protocol (Holterman et al. 2006). The specimens used for DNA extraction, amplification and sequencing are from Nelson (*Amblydorylaimus isokaryon* (Loof, 1975) Andrássy, 1998) and King George (*Pararhyssocolpus paradoxus* gen. n., comb. n.) Islands. For further details, see Nedelchev et al. (2014). Identical sequences were obtained from both individuals of the same species. The sequences of both Antarctic species have been deposited in GenBank with the following accession numbers: for the 18S rDNA KM092519 (*A. isokaryon*) and KM092521 (*P. paradoxus* gen. n., comb. n.) and for the D2-D3 rDNA KM092520 (*A. isokaryon*) and KM092522 (*P. paradoxus* gen. n., comb. n.).

Sequence and phylogenetic analysis

A BLAST (Basic Local Alignment Search Tool) search at NCBI (National Center for Biotechnology Information) was performed using the obtained sequences as queries to confirm their nematode origin and to identify the most closely related nematode sequences. The sequences revealing highest similarity to newly obtained sequences were included in the phylogenetic analyses of both ribosomal gene regions (Griffiths et al. 2006; Holterman et al. 2006; Meldal et al. 2007; Lesaulnier et al. 2008; Pedram et al. 2010; Pedram et al. 2011; Álvarez-Ortega and Peña-Santiago 2012a; 2012b; Donn et al. 2012; Álvarez-Ortega et al. 2013; Nedelchev et al. 2014).

The Multiple Sequence Alignments (MSA) of both gene regions were performed using the Clustal Omega tool (Sievers et al. 2011) via the EBI webserver: http://www.ebi.ac.uk/Tools/msa/clustalw2/. Two datasets (big and small, consisting of 61 and 17 sequences, respectively) were analysed for 18S rDNA. Subsequently, the MSAs were manually optimised and trimmed using MEGA 6 (Tamura et al. 2013). Newly acquired sequence from another Antarctic species *Coomansus gerlachei* (de Man, 1904)

Jairajpuri & Khan, 1977 from Nelson Island (accession number KM092523) was used as outgroup species for the big 18S dataset. Otherwise, midpoint rooting was applied for other sequence datasets due to the uncertainties in species identification and non-monophyly of the families Aporcelaimidae Heyns, 1965, Qudsianematidae Jairajpuri, 1965 and Nordiidae Jairajpuri & Siddiqi, 1964, observed in other studies (Holterman et al. 2008). The best-fitting model (General Time Reversible model plus Gamma distribution rates (GTR + G)) of nucleotide substitution for both datasets was estimated using the Bayesian (BIC) and Aikaike Information Criteria (AIC) in MetaPIGA v3.1 (Helaers and Milinkovitch 2010). Subsequently, the phylogenetic reconstructions were performed using the Bayesian Inference (BI) algorithm implemented in MrBayes 3.2.2. (Huelsenbeck and Ronquist 2001; Ronquist et al. 2012). A total of 759 and 1616 positions in the final datasets were used for D2-D3 and 18S rDNA dataset, respectively. The Bayesian MCMC tree searches were run using default heating parameters for 2 000 000 generations with a sample frequency of 1000 generations. The first 25% of the chains discarded as burning and the remaining 75% trees kept to summarise the tree topology, branch lengths, and posterior probabilities (PP) of branch support. Convergence diagnostic values were calculated every 1000 generations with a predefined stop value equal to 0.01. A single strict consensus tree was visualised using FigTree v1.4.0 graphical viewer (http://tree.bio.ed.ac.uk/software/figtree/). Posterior probabilities values of ^{3}0.80 were considered as credible support values for nodes.

Taxonomy

Amblydorylaimus isokaryon (Loof, 1975) Andrássy, 1998

Figures 1–13, 24–26

= *Eudorylaimus isokaryon* Loof, 1975

Material examined. Twenty-two females, nineteen males and thirteen juveniles (J1-J4) collected from three islands from Maritime Antarctic (Table 1).

Measurements. See Table 2.

Description. *Female.* Body large, curved ventrad after fixation, especially in posterior end. Cuticle 2–4 µm thick at postlabial region, 4–6 µm at mid-body and on tail posterior to anus, three-layered, outer layer thin with fine and distinct transverse striation (especially well visible on SEM, annules 0.4–0.7 µm wide); intermediate layer also thin, refractive, especially on tail region; inner layer thicker than the others. Lateral chord occupying 20–27% of midbody diam. Lateral pores well perceptible, often conspicuous throughout entire body, 10–14 in number in neck region, dorsal pores 3–4, ventral pores along the whole body, 9–11 in neck region. Lip region angular, set off from the adjoining body by a constriction; 2–3 times as wide as high, about 23–32% of body diameter at neck base. Based on SEM photographs oral aperture dorsoventral, vestibulum hexagonal; labial and cephalic papillae prominent, labial papillae

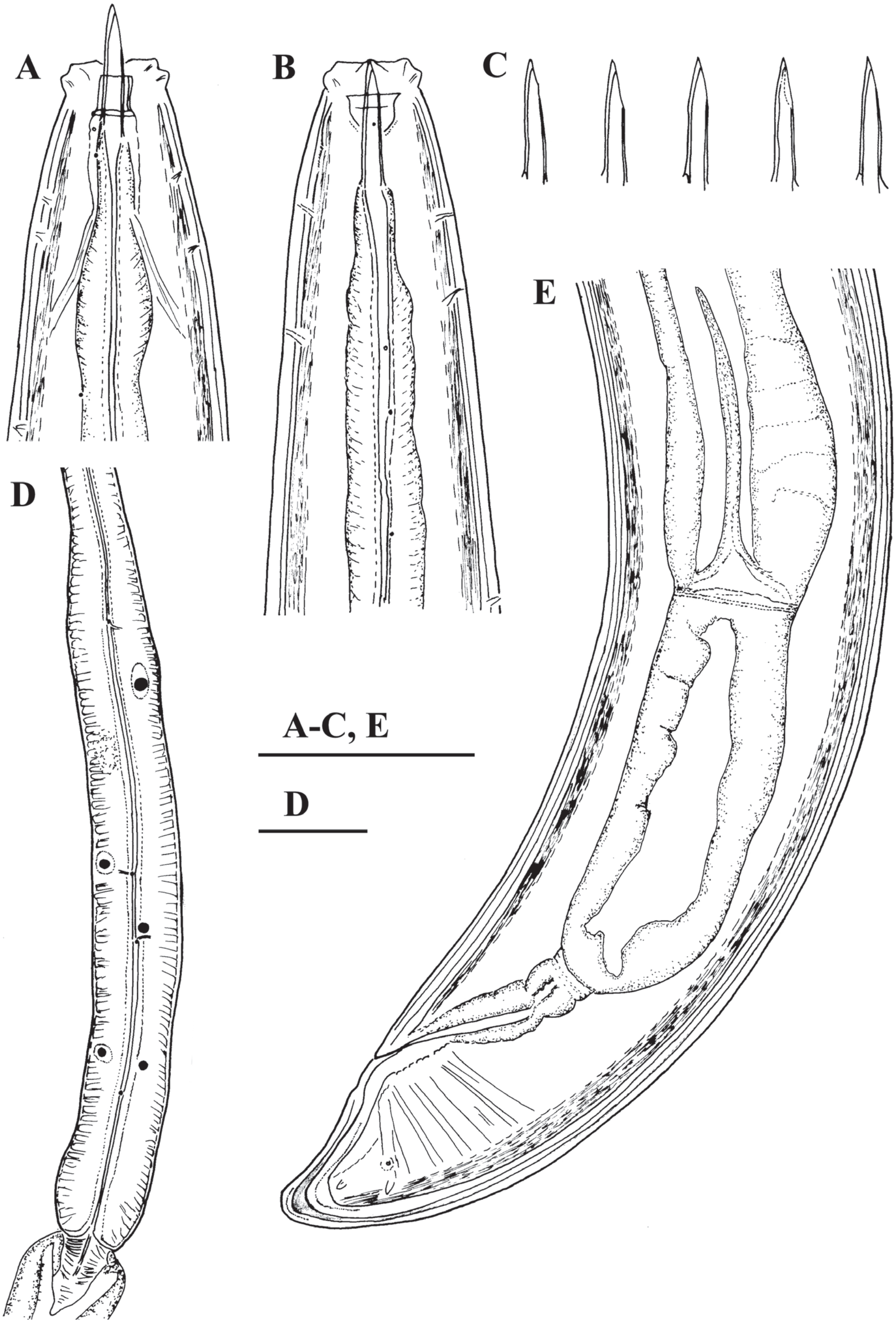

Figure 1. *Amblydorylaimus isokaryon* (Loof, 1975). *Female:* **A, B** Anterior ends (**A** NI; **B** LI, SDC) **C** Odontostyle variations **D** Pharyngeal expansion, pharyngeal glands, cardia (KGI) **E** Posterior end (NI). Scale bar: 50 µm (**A–E**).

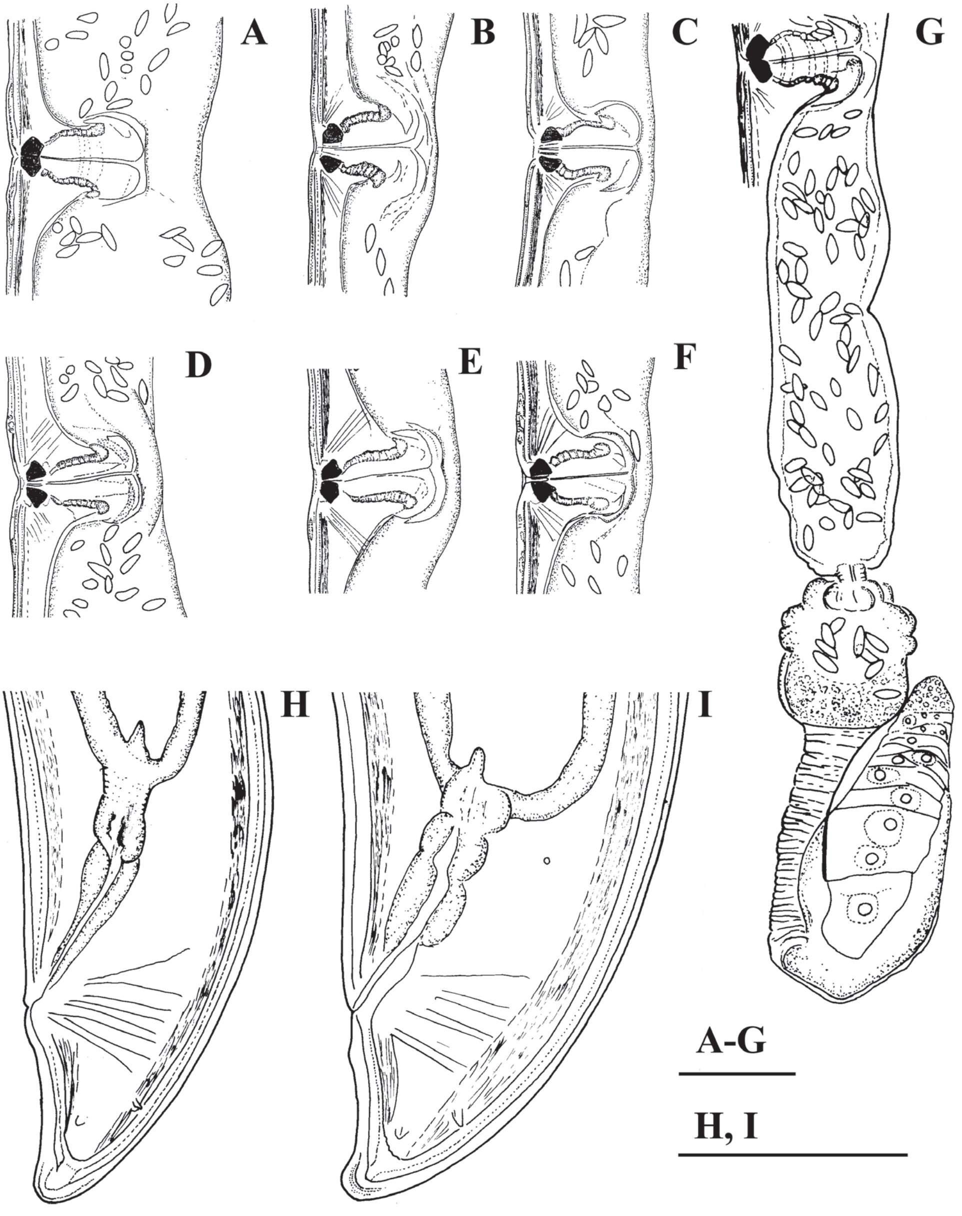

Figure 2. *Amblydorylaimus isokaryon* (Loof, 1975). *Female:* **A–F** Vulval region (**A** LI, SDC; **B–F** NI) **G** Posterior genital branch (LI, CDM) **H, I** Tail ends (NI). Scale bar: 50 μm (**A–I**).

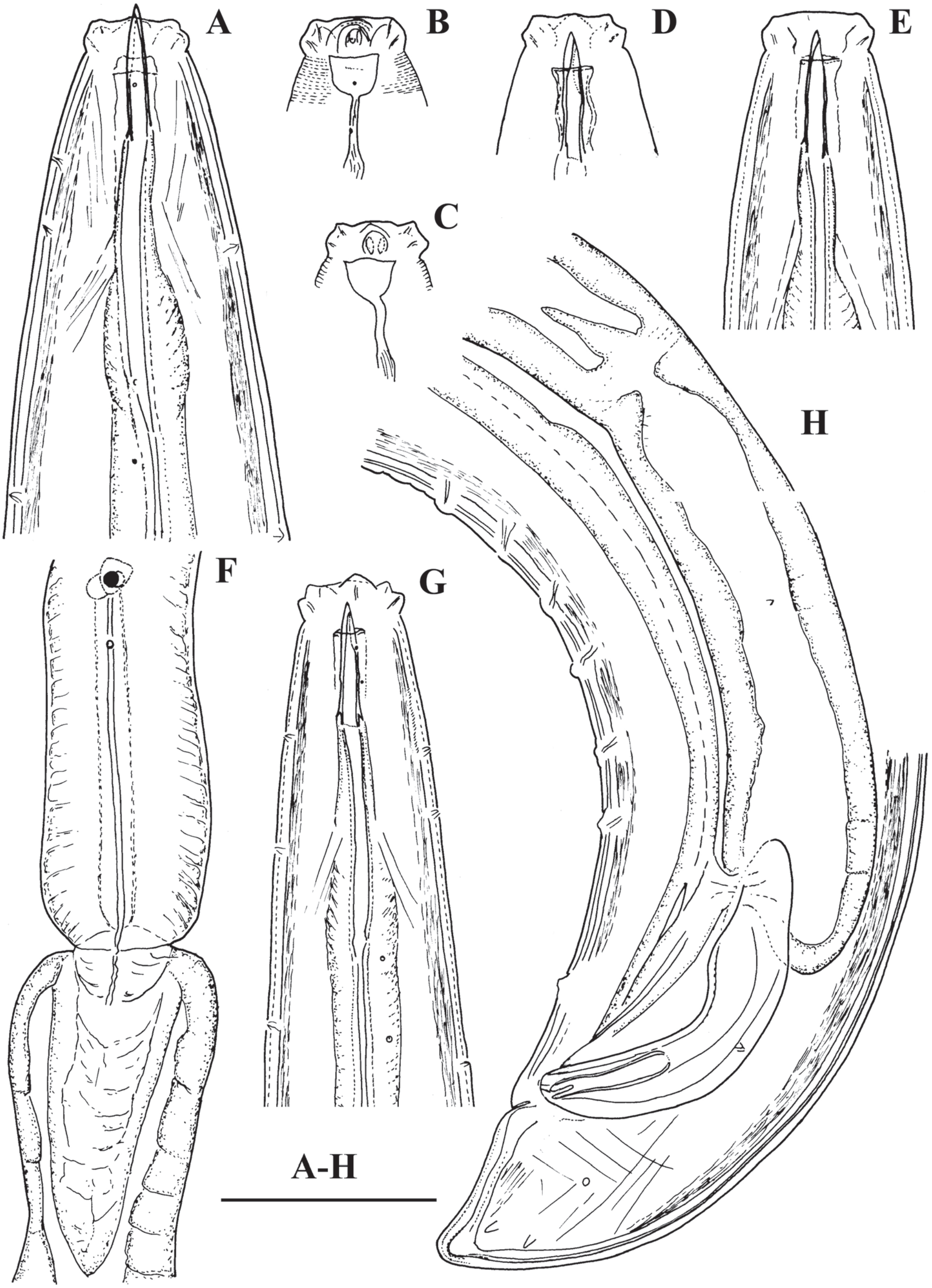

Figure 3. *Amblydorylaimus isokaryon* (Loof, 1975). *Male*: **A, D, E, G** Anterior end (**A** NI; **D, E, G** LI, HPPS) **B, C** Amphidial fovea (**B** NI; **C** LI, HPPS) **F** Posterior ventrosublateral glands, cardia (KGI) **H** Posterior end (NI). Scale bar: 50 μm (**A–H**).

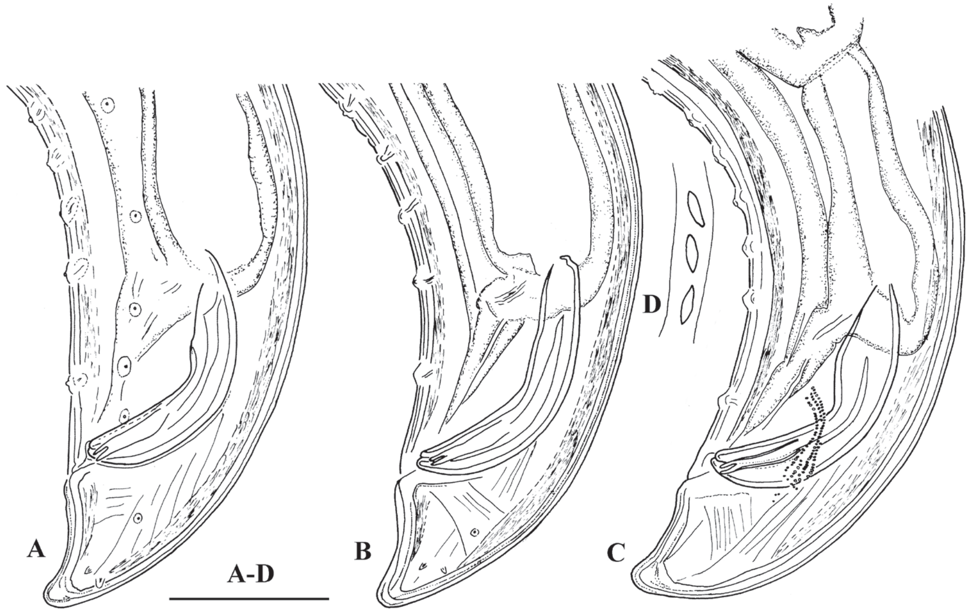

Figure 4. *Amblydorylaimus isokaryon* (Loof, 1975). *Male*: **A–C** Posterior ends (**A** LI, HPPS; **B** NI; **C** KGI) **D** Sperm in *ductus ejaculatoris* (**KGI**). Scale bar: 50 µm (**A–D**).

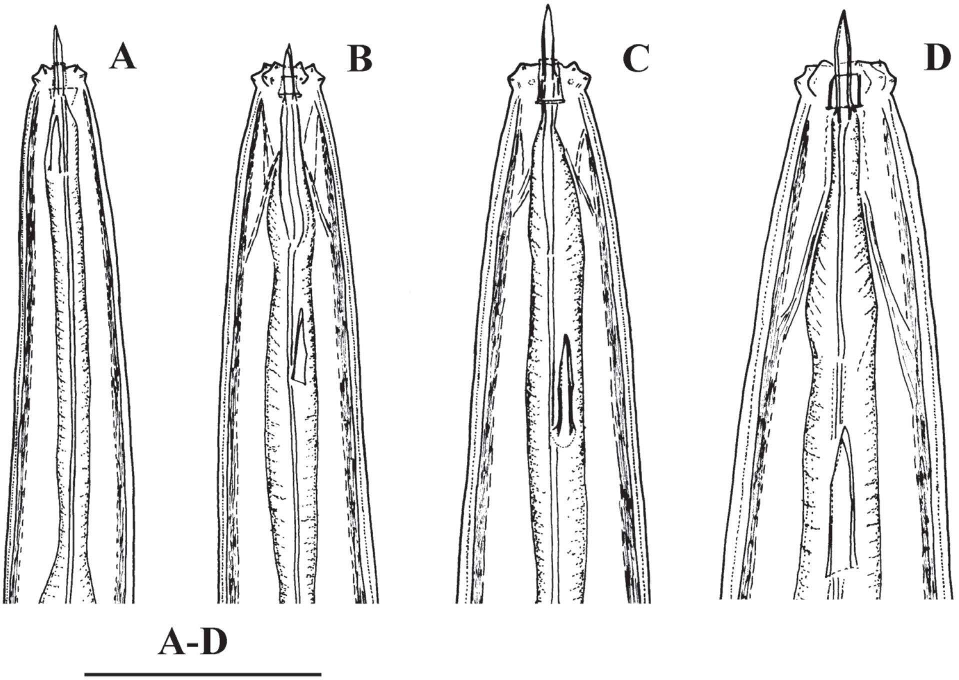

Figure 5. *Amblydorylaimus isokaryon* (Loof, 1975). *Juveniles*: **A–D** Lip region of J1-J4 (**NI**). Scale bar: 50 µm (**A–D**).

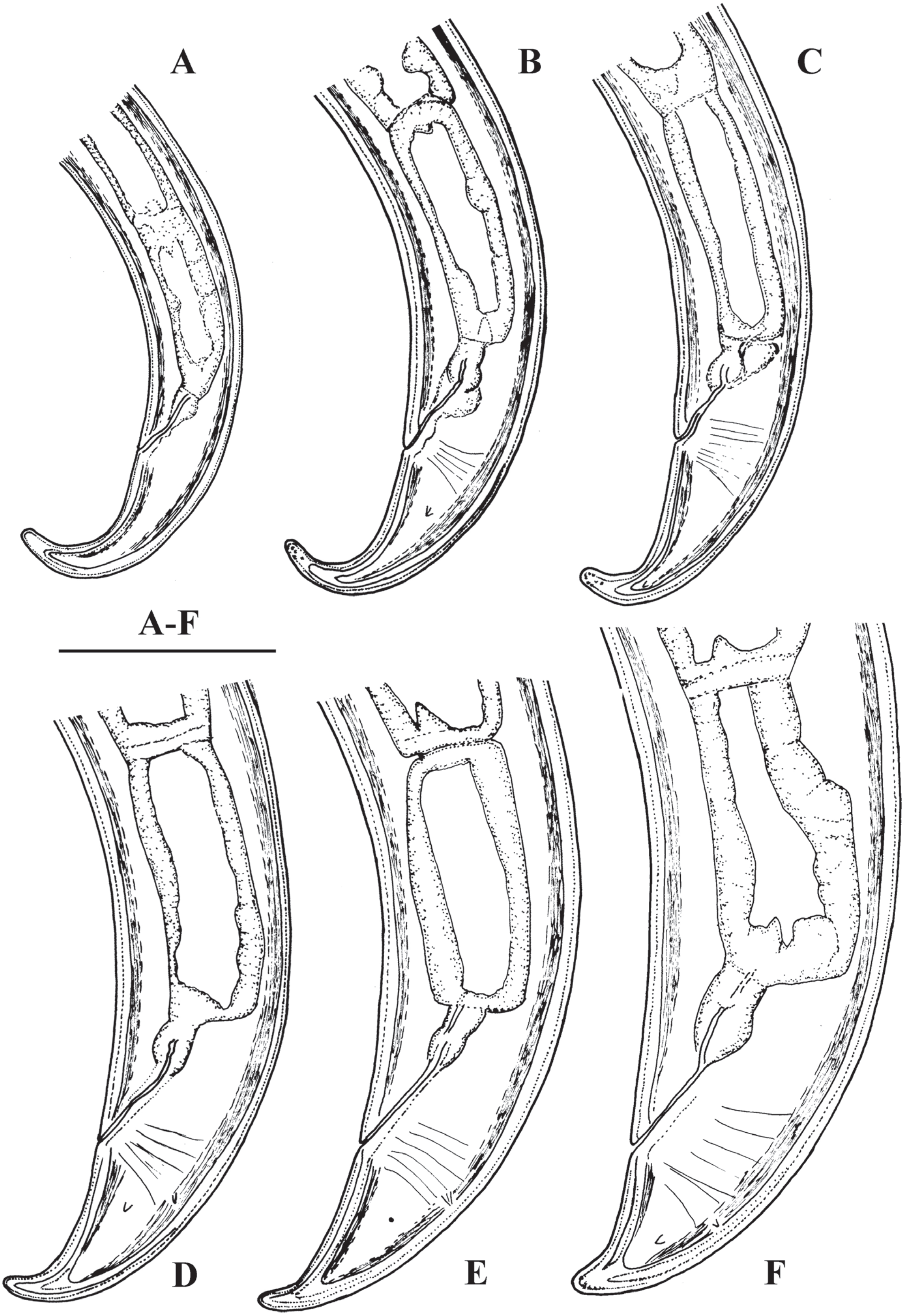

Figure 6. *Amblydorylaimus isokaryon* (Loof, 1975). *Juveniles*: **A–F** Tail ends (**NI**) **A** J1 **B, C** J2 **D, E** J3 **F** J4. Scale bar: 50 μm (**A–F**).

Figure 7. *Amblydorylaimus isokaryon* (Loof, 1975). *Female*: **A, B** Anterior ends (**A** NI; **B** LI, HPPS) **C–E** Amphidial fovea (**C** LI, HPPS; **D, E** NI) **F** Entire body (**NI**) **G, I–K** Vulval region (**G, I** NI; **J, K** LI, HPPS) **H** Cardia and dorsal cellular mass (marked by an arrow) (NI) **L–N** Tail ends (**L, M** NI; **N** LI, HPPS). Scale bars: 10 µm (**A–E, G–N**); 200 µm (**F**).

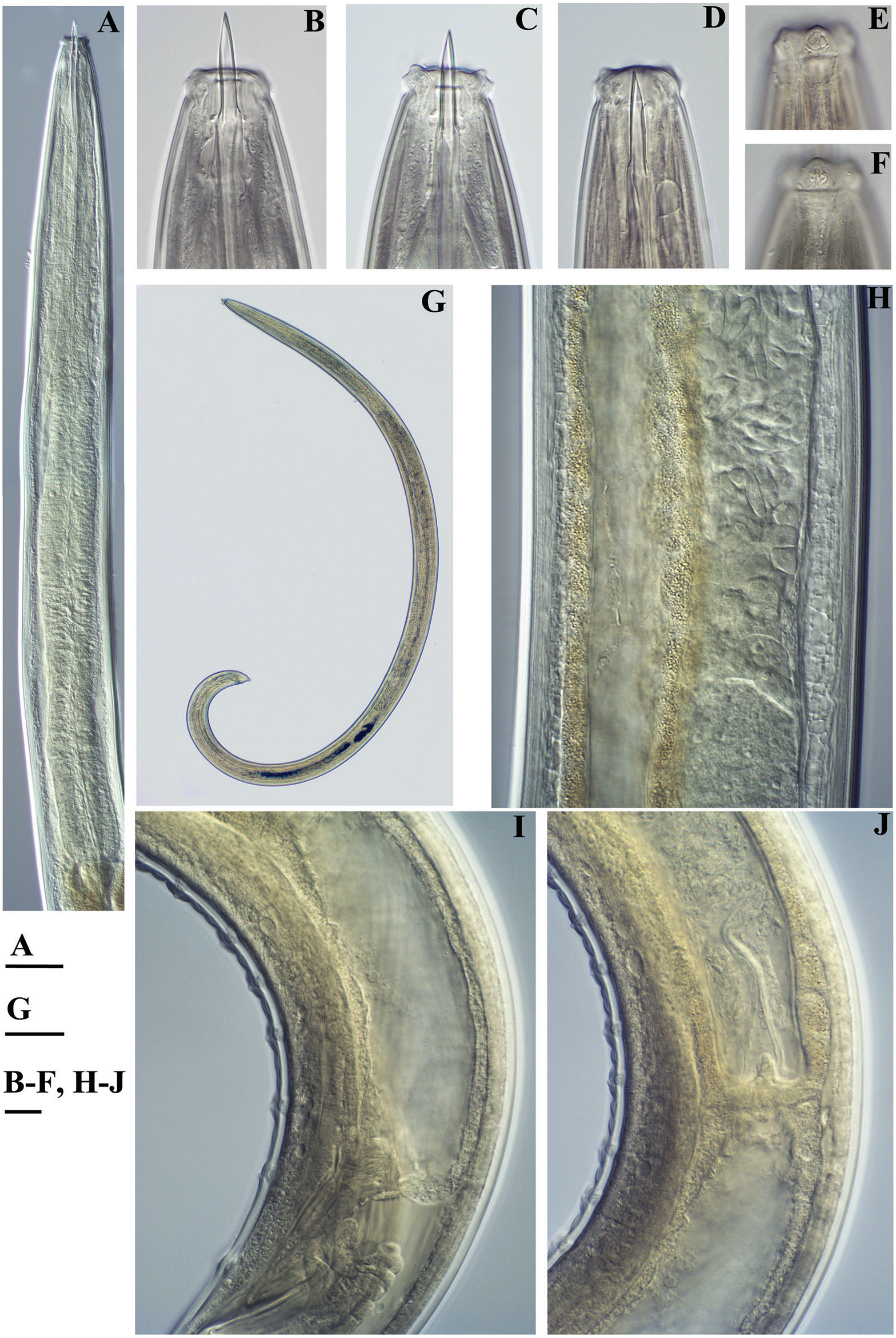

Figure 8. *Amblydorylaimus isokaryon* (Loof, 1975). *Male*: **A** Pharyngeal region (NI) **B–D** Lip region (**B, C** NI; **D** LI, HPPS) **E, F** Amphidial fovea (**E** LI, HPPS; **F** NI) **G** Entire body (NI) **H** Part of testis with sperm (NI) **I** Prerectum, rectum and ejaculatory glands (LI, HPPS) **J** Tongue-like projection (LI, HPPS). Scale bars: 50 µm (**A**); 10 µm (**B–F, H–J**); 200 µm (**G**).

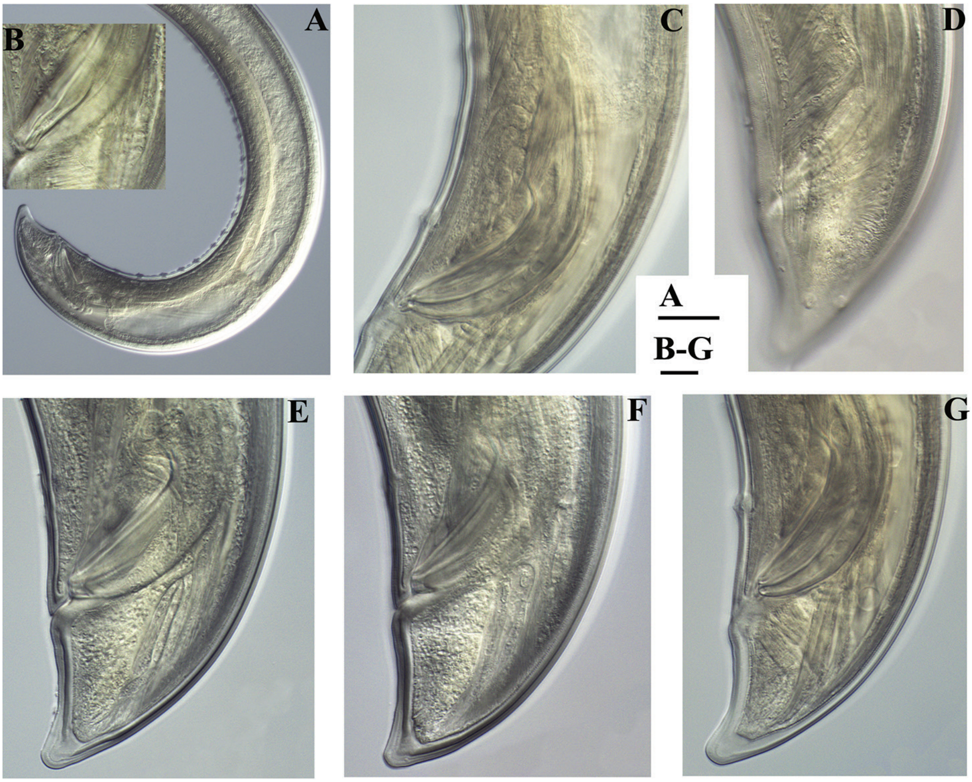

Figure 9. *Amblydorylaimus isokaryon* (Loof, 1975). *Male*: **A** Posterior end (LI, HPPS) **B** Lateral guiding piece (NI) **C** Spicula (LI, HPPS) **D** Tail end, caudal pores (NI) **E–G** Tail ends (**E, F** NI; **G** LI, HPPS). Scale bars: 50 µm (**A**); 10 µm (**B–G**).

mamilliform, surrounded by circular annules, cephalic papillae button-like, without such annules, perioral field slightly elevated. Amphidial fovea cup shaped its aperture 41–52% of lip region diam., fusus (sensillium pouch) at 29–32 µm from anterior end, small posterior pouches present which are not always discernable. Odontostyle long, weakly sclerotised, 7–9 times as long as wide, 1.2–1.4 times lip region diam., aperture occupying 1/3 to more than 1/2 of its length (30–53%), av. 2/5, depending on the position of the body (under SEM it is seen that the aperture reaches 12–14 µm which is about 2/5 of the odontostyle length), two edges of the slit do not overlap. Guiding sheath distinct, its anterior edge located at 11–13 µm (or 0.4–0.6 times lip region diam. to anterior end (in not protruded odontostyle)) and seems cuticulised stronger than the posterior edge which is located at the base of odontostyle. Odontophore rod like, 1.5–1.9 times long as odontostyle. Anterior region of pharynx enlarging gradually, basal expansion 350–450 µm, (321 µm in the specimen from King George Island), occupying 50–60% of total neck length. Anterior subventral nuclei equal in size and shape, slightly smaller than dorsal nucleus; dorsal gland nucleus 4.5–6 µm diam., first and second pair subventral gland nuclei 4–4.5 and 3–4 µm diam., respectively.

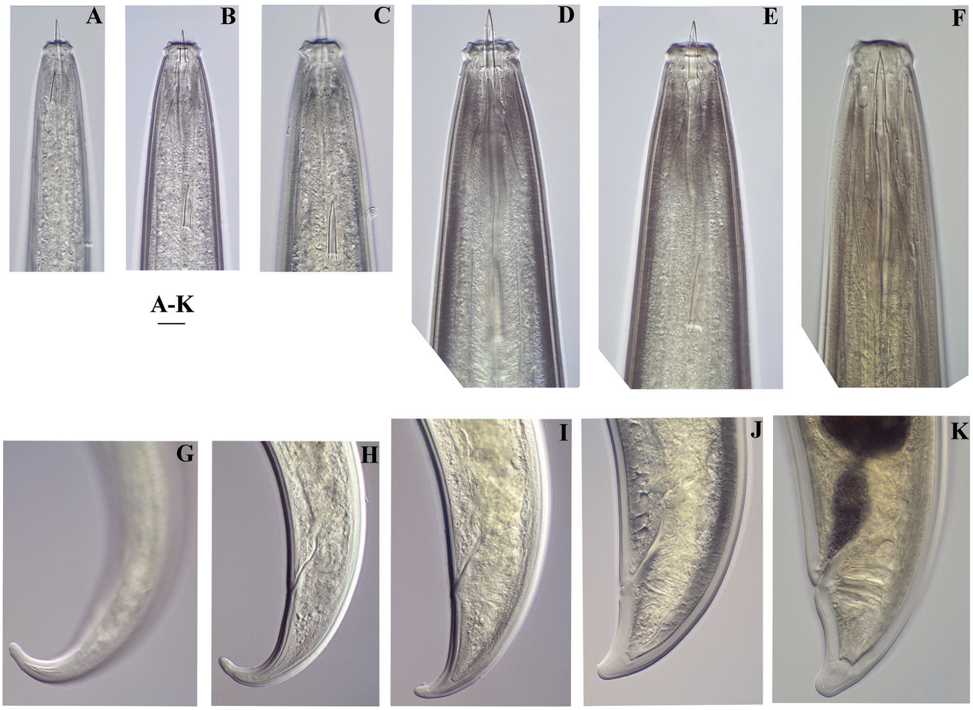

Figure 10. *Amblydorylaimus isokaryon* (Loof, 1975). **A–E, G–J** *Juveniles* (NI): **A–E** Lip region of **A** J1 **B** J2 **C** J3 **D, E** J4 **G–J** Tail ends J1-J4 **F, K** *Female* (LI, HPPS): **F** Anterior end **K** Tail end. Scale bar: 10 µm (**A–K**).

Table 1. Origin of the examined materials of *Pararhyssocolpus paradoxus* gen. n., comb. n. and *Amblydorylaimus isokaryon*.

Site description	Collection year	Abbreviation	Nematode species
King George Island (KGI)			
Fildes Peninsula Soil	2013	KGI1	*Pararhyssocolpus paradoxus*; *Amblydorylaimus isokaryon*
Livingston Island, Punta Hesperides (LI)			
Grass spot (*Deschampsia antarctica* E. Desv.), on a high rock, on the beach near Johnson Dock inlet.	1994	DA	*A. isokaryon*
A moss-grass (*D. antarctica-Polytrichum* sp.) community, on top of a small flat rock, on the beach near Johnson Dock inlet.	1994	DAP	*A. isokaryon*
A small moss tuft (*Sanionia* sp.), transect over a large rock.	1994	S	*P. paradoxus*
Moss *S. georgico-uncinata* (Müll. Hal.) and grass *D. antarctica.*	2001	HPPS	*A. isokaryon*
Grasses *Colobanthus quitensis* (Kunth) Bartl. and *D. antarctica*, moss.	2003	CDM	*P. paradoxus* *A. isokaryon*
Grasses *D. antarctica*, *C. quitensis.*	2003	SDC	*P. paradoxus*; *A. isokaryon*
Grass *D. antarctica*, moss.	2003	DM	*P. paradoxus*
Nelson Island (NI)			
Duthoit Point Moss	2013	M	*P. paradoxus*; *A. isokaryon*

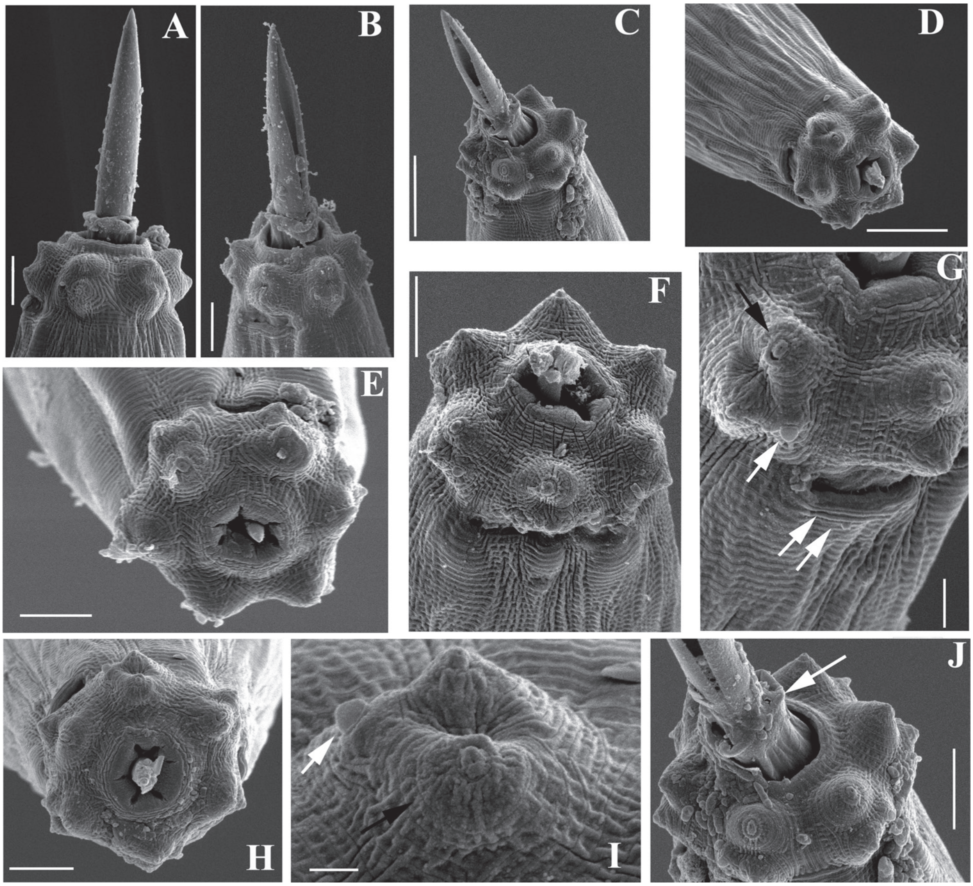

Figure 11. SEM micrographs. *Amblydorylaimus isokaryon* (Loof, 1975). *Female* (**NI**): **A** Lip region with protruded odontostyle **D, E** Lip region, in face view **G, I** Cephalic (marked by white arrow) and labial papillae (marked by black arrow), amphid aperture (marked by two arrows) *Male*: **B, C** Lip region, odontostyle aperture **F, H** Lip region **J** Cephalic and labial papillae, anterior edge of guiding sheath (marked by an arrow). Scale bars: 5 µm (**A, B, E, F, H, J**); 10 µm (**C, D**); 2 µm (**G**); 1 µm (**I**).

Location of pharyngeal glands and their orifices is presented in Table 3. Cardia rounded conoid, ending with sharply pointed tongue, variable in size and shape. In some specimens a dorsal cellular mass present at cardia level. The posterior end of the intestine with tongue-like structure of variable length. Prerectum 1.9–3.2 (1.5 times in specimen from King George Island), rectum 1.0–1.5 times anal body diam. long. Prerectum separated from intestine by a transverse muscular ring. Sphincter between rectum and prerectum well developed. Female genital system didelphic amphidelphic, both branches equally and well developed – anterior 510.1±86.5 (390–695.5) µm and posterior 527.4±67.9 (430–705.5) µm long, respectively (anterior 313 and posterior 347 µm long in specimen from King George Island). Ovaries short well developed, often not reaching sphincter level. Oviduct with well-developed *pars dilatata*, often

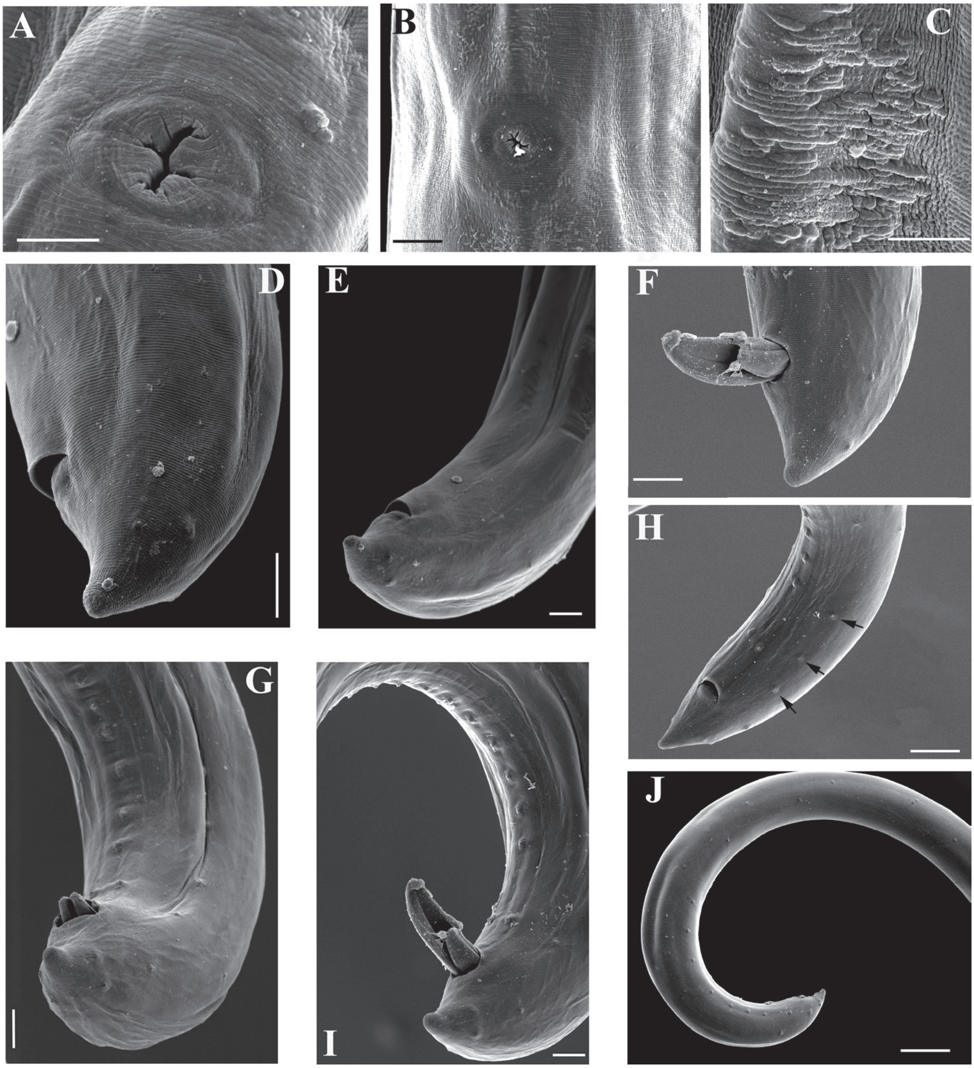

Figure 12. SEM micrographs. *Amblydorylaimus isokaryon* (Loof, 1975). **A–E** *Female*: **A, B** Vulval region (NI) **C** Irregularities around vulva (LI) **D, E** Tail ends (NI) **F–J** *Male* (NI): **F** Tail end with protruded spicules **G** Tail end (ventral view), spicules, ventromedian supplements **H** Posterior end, ventromedian supplements and lateral pores (marked by arrows) **I, J** Posterior ends. Scale bars: 5 µm (**A, C**); 10 µm (**B, D–H**); 50 µm (**J**); 20 µm (**I**).

containing sperm. Sphincter between *pars dilatata oviductus* and uterus small, well developed. Uterus long, anterior 232–435 µm, posterior 226–395 µm long, without differentiation, filled with sperm. Vulva longitudinal (based on SEM observations, Fig. 12A, B). Vagina extending inwards for 51–68% of body diam.; *pars proximalis* 36–44×15.5–23 µm, *pars refringens* with two trapezoidal sclerotisations, with combined width of 17.5–22 µm; *pars distalis* 6–10 µm long. Three females each with one uterine

Table 2. Morphometrics of *Amblydorylaimus isokaryon* (females and males). All measurements, unless indicated otherwise, are in µm (and in the form: mean±SD (range).

Locality	Nelson Island		Livingston Island									King George Island	
	M		SDC		HPPS		CDM		DA		DAP	KGI1	
Characters	♀(n=10)	♂ (n=10)	♀ (n=4)	♂	♀	♂	♀	♂	♀	♂	♂	♀	♂
L (mm)	2.85±0.25 (2.47–3.31)	2.88± 0.18 (2.58–3.24)	3.08±0.09 (3.00–3.20)	3.14, 3.23	3.00, 3.32	2.62, 3.00, 3.09	3.01, 3.02	2.76	2.63, 2.76, 2.89	2.96	2.76	2.13	2.65
a	29.2±2.6 (25.6–32.9)	31.6± 2.4 (28.5–34.8)	27.6±1.2 (26.6–29.3)	33.2, 29.6	35.3, 38.2	31.4, 34.1, 38.3	28, 27.3	30.3	27.3, 29.9, 29.6	35.2	36.7	26	34.2
b	4.1± 0.3 (3.7–4.4)	4.2± 0.2 (3.9–4.4)	4.4± 0.1 (4.3–4.5)	4.4, 4.6	4.6, 4.4	4.1, 4.1, 4.4	4.1, 4.3		3.6, 4.0, 4.0	4.2	4.3	3.8	4.8
c	70.6±7.4 (59.7–79.1)	66.6±5.1 (59.9–77.2)	73.7±2.1 (71.2–75.9)	74.8, 83.8	63.8, 77.7	62.0, 75.8, 69.2	73.4, 79.3	61.3	72.1, 57.6,79.3	64.0	65.6	68.6	62.4
c‘	0.9±0.1 (0.8–1.0)	0.9±0.1 (0.8–1.0)	1.0±0.1 (0.9–1.0)	1.0, 0.9	1.0, 1.0	0.9, 0.9, 1.0	0.9, 0.9	0.9	0.9, 1.0, 0.9	1.0	0.9	0.8	0.9
V %	52.2± 2.0 (50–56)		53.5± 1.3 (52–55)		54, 53		52, 52		54, 57, 58			53	
Lip region width	24.4±0.7 (23–25.5)	25.1±1.0 (23.5–27)	24.1±1.1 (23–25.5)	24, 26	25, 27	24.5, 24, 24	24, 24	25	25.5, 24, 26	25.5	23	24	26
Odontostyle	31.1±0.7 (30–32)	32.1±1.4 (30–35)	30.7±0.6 (30–31.5)	30, 30	31, 32	31, 30, 31	30, 30	29	30, 31, 32	30	29	29	29
Odontophore	51.9±2.0 (50–56)	52.03±3.4 (47.5–56)	53.4±1.3 (52–54)			-, 57, 51	54, -					46	
Pharynx	703.2±41.0 (632.5–756)	695.3±40.9 (650–765)	706.1±10.3 (692–716)	710, 707	652, 745	646, 732, 706.5	730, 700		737, 698, 715	697	648	566	550
Width at pharynx base	89.8± 8.4 (78–104)	87.3± 6.1 (75.5–94)	93.6±7.5 (83–99)	93, 98	81, 83.5	79, 83, 78	102, 105		87, 87, 94	83	73	75	73
Width at mid body	97.9±9.0 (85–110)	91.4±6.2 (79–101)	111.8±6.1 (103–115)	95, 109	85, 87	83, 88, 81	108, 111		96.5, 92, 98	84	75	82	77
Prerectum length	106.2±11.1 (92–131)	136.1±11.2 (120–149)	88.4±8.6 (82–101)	-, 154	110, 104.5	130, 154, -	100, 105		-, 117,131			58	

Locality	**Nelson Island**		**Livingston Island**									**King George Island**	
	M		**SDC**		**HPPS**		**CDM**		**DA**		**DAP**	**KGI1**	
Rectum length	52.6±3.6 (46–58)		49.4±1.8 (47–51)		55.5, 48		54, 48		56.5, 62, 52			52	
Tail	40.5±3.6 (35–47)	43.4±1.6 (41–46)	41.9±2.3 (40–45)	42, 39	47, 43	42, 40, 45	41, 38	45	36.5, 48, 36.5	46	42	31	42
Spicules		95.1±3.3 (89–100)		91, 96.5		93, 93.5, 93		86		94	88		93
Ventromedian supplements		12–16		15, 12		15, 14, 13		14		15	15		14

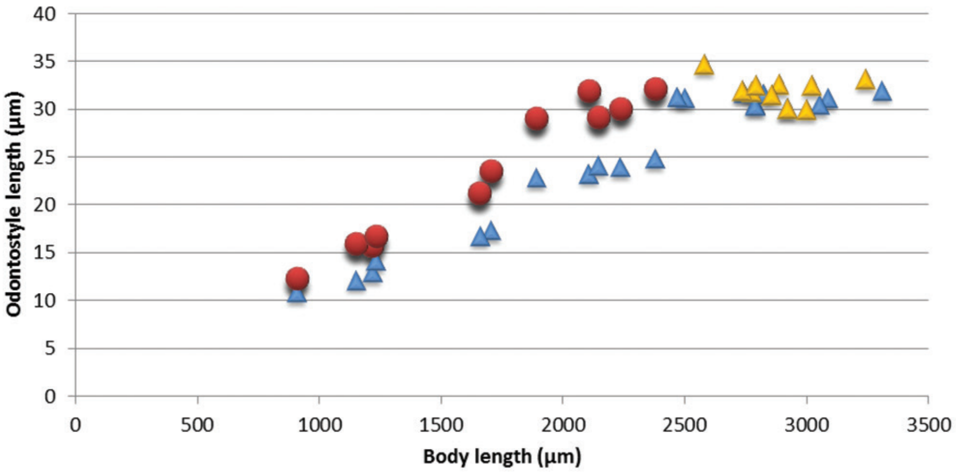

Figure 13. *Amblydorylaimus isokaryon* (Loof, 1975). Scatter plot of the functional (●) and replacement odontostyle (▲) in relation to the body length of the juvenile stages and adults: females (▲) and males (▲).

Table 3. Pharyngeal characters of *Amblydorylaimus isokaryon*. For abbreviations see Loof and Coomans (1970) and Andrássy (1998b).

Locality	Nelson Island				Livingston Island				King George Island	
		M			SDC		CDM		KGI1	
Characters	**n**	**female**	**n**	**male**	**female**	**male**	**female**	**male**	**female**	**male**
DO	4	51–53	2	45, 53		53	51		48	51
DN=D	7	54–57	6	54–57	58, 59	58	57, 55	60	54	56
Distance DO-DN %	4	3–5	2	5–10		5	6		6	9
S_1O_1	4	70–74	2	65, -		71	70		70	69
S_1O_2	3	75–77	2	71.5, 75		76	76		75	75
S_1N_1	7	69–72	6	69–72	72, 73		71, 70	73	69	70
S_1N_2	7	74.5–76	6	73–76	77		77	79	74	75
S_2O	4	87–88	2	86, 88.5		88	88		87	87
S_2N_1	7	84–85.5	6	83–86	87, 86	86	85, 85	87	84	
S_2N_2	7	84–87	6	84–86	87, 86	87	87, 85	87	85	84
AS_1	7	30–38	6	31–35	33, 35		33, 33	33	33	31
AS_2	7	42–45	6	40–45	45		46	47	43.5	43
PS_1	7	63–68	6	62–68	69, 66	68	66, 66	67	66	
PS_2	7	63–70	6	64–68	69,67	68	69, 68	67	67	64

egg, measuring 123–135×56–82.5 µm, in one female one egg located in *pars dilatata oviductus*, measuring 123×80 µm. Irregularities of body cuticle present around vulva, on SEM observation they appeared as additional cuticle masses. Tail short conoid, ventrally arcuate, with bluntly rounded tip, 1.3–1.7% of body length. Caudal pores two pairs, on SEM appeared papillae-like.

Male. General morphology similar to that of the female, except for the genital system. In one specimen the odontostyle aperture ventral. Arrangement of pharyngeal gland nuclei and their orifices is presented at Table 3. Genital system diorchic, with opposite testes: anterior 376.4±28.9 (339–418) µm and posterior 361.9±56.8 (279–442) µm long (n=6), (anterior 237 and posterior 255 µm in a specimen from King George Island), respectively. Spicules dorylaimoid, strongly curved ventrad and robust, their length about 1.7–2.3 times cloacal body diam. Ventromedian supplements preceded by one adcloacal pair of papillae, 12–16 in number, regularly spaced, with small cuticular folds between them, adcloacal pair located at 29–37.5 µm apart from cloacal opening (26 µm in specimen from King George Island). Sperm spindle shaped, measuring 11–13×3–4 µm. Lateral guiding pieces, cylindrical with bifurcate end, measuring 24–31×3–5 µm. Tail short conoid, ventrally arcuate, with obtusely rounded tip, two pairs of caudal pores.

Juveniles. Morphometrics obtained from juvenile specimens, and the relationship between the lengths of their functional and replacement odontostyles and body lengths, identified four juvenile stages (Figure 13). Tail in J1-J3 elongated conoid, ventrally arcuate with rounded terminus, in J4 as in females, c' decreasing during successive stages to female (Table 4, Figs 6, 10).

Sequence and phylogenetic analyses. The BLAST search using D2-D3 region sequence of *A. isokaryon* showed highest similarity (96%) to *Aporcelaimellus salicinus* Álvarez-Ortega, Subbotin & Peña-Santiago, 2013 (JX094341–42), while the 18S rDNA sequence was closest (99% similarity, 4–10 nucleotide differences of about 1700 bp) to several *Aporcelaimellus* spp., *Allodorylaimus andrassyi* (Meyl, 1955) Andrássy, 1986 and four sequences acquired during environmental studies of arable soil (Griffiths et al. 2006) and trembling aspen rhizosphere (Lesaulnier et al. 2008). Since the 18S rDNA phylogram based on bigger dataset (Figure 24) did not show clearly the evolutionary relationships of *A. isokaryon* a smaller dataset with the closest sequences was analysed (Figure 25). Although the very low 18S rDNA resolution this analysis yielded a tree with *A. isokaryon* being a part of well-resolved group of species assigned to three families: Dorylaimidae De Man, 1876 (*Labronema vulvapapillatum* (Meyl, 1954) Loof & Grootaert, 1981 and *Mesodorylaimus centrocercus* (de Man, 1880), Geraert, 1966, Qudsianematidae (*Ecumenicus* spp.) and Aporcelaimidae (includes mainly *Aporcelaimellus* spp. and *A. andrassyi* which probably is misidentified). Further, in the 28S rDNA-based phylogenetic tree *A. isokaryon* appeared a sister species of *A. salicinus*, again being a part of a well-supported group of several *Aporcelaimellus* spp. and *A. andrassyi* (Figure 26).

Discussion. The main morphological characters of the studied populations are very similar, only the specimen from King George Island differs by its shorter body, pharynx, pharyngeal expansion, anterior and posterior female genital branches, prerectum and tail (Table 2). Our materials generally agree well with the type specimens (Loof, 1975), although some differences occurred: the present specimens have broader lip region (length of odontostyle 1.2–1.3 *vs* 1.5 times longer than lip region diam., longer uterine eggs (123–135 *vs* 117–122 µm), and somewhat longer distance adcloa-

Table 4. Morphometrics of *Amblydorylaimus isokaryon* (juveniles). All measurements, unless indicated otherwise, are in µm (and in the form: mean±SD (range).

Locality	**Nelson Island**				**Livingston Island**	
	M				**DA**	**DAP**
Characters/Stages	J1	J2	J3	J4 (n=5)	J4	J4
L (mm)	0.91	1.22, 1.15, 1.23	1.70, 1.66	2.15±0.2 (1.89–2.38)	2.53	2.43
a	30.2	29.9, 26.5, 29.6	27.7, 28.6	27.9±2.1 (24.9–30.2)	34.5	36.2
b	3.5	3.6, 3.4, 3.4	3.9, 4.0	4.0±0.2 (3.8–4.4)	4.1	4.1
c	18.0	22.7, 21.7, 24.4	35.4, 34	54.8±4.3 (49.6–59.5)	57.8	65.6
c‘	2.5	2.4, 2.1, 2.1	1.6, 1.7	1.1±0.1 (1.1–1.2)	1.3	1.2
Lip region width	11	14, 14, 14	16.5, 16	19.9±1.4 (18–22)	19	20
Odontostyle	11	13, 12, 14	17, 17	23.9±0.8 (23–25)	22	24
Replacement odontostyle	12	16, 16, 17	24, 21	30.5±1.5 (29–32)	29	29
Pharynx	262.5	338, 342, 359	438, 420	538.0±41.3 (485.5–595)	612	592.5
Width at pharynx base		40.5, 40, 41	59, 52	71.4±5.4 (65–78)	68	65
Width at mid body	30	41, 43, 42	61, 58	77.2±7.2 (69.5–85)	73	67
Prerectum length		58.5, 59, -	65, 67	80.7±13.1 (68–97)	67	91
Rectum length		25, 24, 23.5	37, 29	41.1±1.4 (39–42)	45.5	43
Tail	50	53.5, 53, 50.5	48, 49	39.4±3.7 (35–43.5)	44	37
Genital primordium	17	-, 23, -	25, 25			

cal pair of papillae – cloaca (29–37.5 *vs* 26–29 µm) (Andrássy 1998a). Loof (1975) described the vulva as longitudinal but according to Andrássy (1998a) it is more or less a roundish pore, although he may not have observed females in ventral position. Our SEM studies confirm Loof's observations. Andrássy (1998a) reported that spermatozoids have an atypical shape for dorylaimids being rounded or potato-like, however our observations showed that their shape is spindle-like, similar to the drawings by Loof (1975). Further, the presence of a tongue-like projection between the intestine and prerectum not mentioned in the original description was observed. None of the above mentioned authors reported the cuticular irregularities around the vulva documented here both by LM and SEM.

This species was originally described as *Eudorylaimus isokaryon* by Loof (1975); later Andrássy (1998a) established a new genus, *Amblydorylaimus* to accommodate it on the basis of several morphological characters (amphidial fovea and odontostyle shape, equally sized mid-pharyngeal nuclei, atypical sperm shape, nipper-like adspicular pieces and unusual location of adcloacal pair of supplements). He described and illustrated *A. isokaryon* having a specific shape of odontostyle – resembling garden shears; the aperture appeared small. He suggested that this unusual shape was not caused by fixation artefacts as "other organellum of cuticular origin is clearly visible, without any deformation" and "all other *Eudorylaimus* species collected by Spaull (Loof, 1975) in his study trip do possess normally shaped, well preserved dorylaimid spear". Andrássy (1998a) suggested that it would be necessary to know if living specimens possessed this shape of odontostyle. We examined living specimens of this species, and did not find the peculiarities of the odontostyle shape observed by this author. In the original description, Loof (1975) did not mention this special feature of odontostyle and noted that the odontostyle aperture occupied one-third of its length. In our specimens the odontostyle is weakly sclerotised, regular with usual dorylaimid shape; the length of aperture longer, occupying 1/3–1/2 of the odontostyle. In earlier prepared slides, the odontostyle showed some irregularities similar to those described by Andrássy (1998a). The same author (1998a, 2009a) considered this genus as a member of family Qudsianematidae, but noted that it significantly differs from every genus of this family with its characteristic morphology. Molecular data based on 28S rDNA, however showed that this genus is a member of family Aporcelaimidae and not family Qudsianematidae. This conclusion is supported by our morphological evidences: large aperture of odontostyle (reaching almost ½ of odontostyle length), oral opening a dorso-ventral slit, cuticle thick with refractive layer, not fixed guiding ring etc. which confirm *Amblydorylaimus* fits better to the family Aporcelaimidae. Based on morphology and molecular data (28S) *Amblydorylaimus* is closely related to genus *Aporcelaimellus* Heyns, 1965 from which it can be differentiated by its longer and not robust odontostyle with shorter aperture (av. 2/5 *vs* more than 1/2 of odontostyle length), and not overlapping *vs* overlapping edges, lip region with radial *vs* bilateral symmetry (Álvarez-Ortega and Peña-Santiago 2013), vulva longitudinal *vs* transverse (except *A. macropunctatus* (Heyns, 1967) Jimenez-Guirado, 1994 distinguished by its longitudinal vulva), position of adcloacal pair of papillae in males (more distant from cloacal opening *vs* very close) and lateral guiding pieces bifurcate *vs* simple. Recently, Andrássy (2009b) proposed a new genus close to *Aporcelaimellus* and *Amblydorylaimus*, the genus *Aporcelinus* Andrássy, 2009. The latter genus differs from the genus *Amblydorylaimus* by the structure of cardia (with a small dorsal lobe), transverse vulva, eggshell wrinkled, ventromedian supplements small, irregularly spaced, without precloacal space, location of adcloacal pair and shape of tail (conoid tail with sharply pointed terminus). Vinciguerra et al. (2014) believed that the taxonomic position of *Aporcelinus* is ambiguous; they noted that this genus could also be assigned to family Qudsianematidae on the basis of its morphological features (odontostyle aperture length, simple guiding ring and thickness of cuticle, composed of two layers). Related to the cuticle structure, it should be mentioned that genus *Aporcelinus* has three layered cuticle with inner refractive layer, well visible on several photomicrographs (Figs 4E, 8A–C) presented by Vinciguerra et al. (2014). Further, the location of adcloacal pair of male ventromedian papillae (comparatively far from cloaca opening) in *A. isokaryon* shows some similarity to *Crassolabium persicum* Jabbari, Niknam, Vinciguerra, Moslehi, Abolafia & Peña-Santiago, 2012, but the latter species differs from it by the odontostyle structure (weakly sclerotised *vs* quite robust), not differentiated *vs* bipartite uterus, structure of *pars distalis* (without differentiation *vs* with two small sclerotisations close to the *pars refringens* in *C. persicum*) (Jabbari et al. 2012).

On the basis of morphological and molecular data, we propose the genus *Amblydorylaimus* to be transferred from family Qudsianematidae to the family Aporcelaimidae. It is worth mentioning that the latter family obviously is non monophyletic and we propose this taxonomic change on the base of the close relationships with the genus *Aporcelaimellus* now regarded as a member of family Aporcelaimidae.

Diagnosis (emended). *Amblydorylaimus*.

Aporcelaimidae. Aporcelaiminae. Body large, about 3 mm. Cuticle three-layered, outer layer thin with fine but distinct transverse striation. Lip region angular, offset from adjacent body by a constriction. Oral aperture dorso-ventral, hexagonal. Amphidial fovea caliciform with small posterior pouches. Odontostyle long, weakly sclerotised. Guiding sheath distinct, anterior and posterior edges moderately cuticularised. Odontophore rod like. Pharynx expanded in its posterior half. Nuclei distinct, dorsal nucleus fairly posterior in position, first subventral pair large and equal in size, posterior pair rather far from the end of pharyngeal expansion. Prerectum sharply separated from mid-intestine. Female genital system didelphic amphidelphic. Ovaries very short, uterus long without differentiation. Vulva longitudinal, cuticular irregularities present around it. *Pars refringens vaginae* well developed. The posterior end of the intestine with tongue-like structure. Sperm spindle shaped. Spicula dorylaimid, lateral guiding piece distally bifurcate. Ventromedian supplements numerous, regularly spaced, preceded by one adcloacal pair of papillae comparatively far from cloacal aperture. Tail similar in both sexes, short conoid, ventrally arcuate, with bluntly rounded tip. Tail in J1-J3 conoid elongated, in J4 as in female.

Distribution. *Amblydorylaimus* is an endemic genus of the maritime Antarctic. It has been reported from several islands (Intercurrence, Elephant, Galindez, Livingston and King George) (Loof 1975; Maslen 1979; Peneva et al. 2009; Kito 2009). The present finding from Nelson Island represents a new geographical record.

Pararhyssocolpus paradoxus **(Loof, 1975), gen. n., comb. n.**

Figures 14–26

=*Eudorylaimus paradoxus* Loof, 1975
=*Rhyssocolpus paradoxus* (Loof, 1975) Andrássy, 1986

Material examined. Eighteen females, seven males and ten juveniles (J1, J3, J4) collected from three islands in Maritime Antarctic (Table 1).

Measurements. See Table 5.

Description. *Female*. Habitus curved ventrad after fixation, more so in posterior body end. Cuticle smooth, when viewed under light microscope, 3–4 µm thick in postlabial region, 5–7 µm at mid-body and 4–7 µm on tail; consisting of three layers the inner one much ticker and refractive, not reaching the end of tail. Under SEM it is finely transversally striated (annules *ca* 0.6 µm wide). Lip region appears rounded, slightly offset by a depression, 2.3–3.4 times as broad as high, lips amalgamated, outer labial and cephalic papillae protruding above lip region contour. Under SEM inner labial papillae not elevated, close to each other and to oral aperture, outer labial and cephalic papillae below the margin of oral field. Oral aperture seems round hexagonal. Lateral pores well visible (13–14 in the pharyngeal region), the first four as two pairs at the anterior end, next more or less equally spaced. Cheilostom a truncate cone. Amphidial fovea funnel-shaped, opening at level of labial depression, its aperture about half of lip region diam. Odontostyle slender, with clear lumen, aperture subterminal, narrow (Figure 22 B) and indistinct as observed by LM in adults (Figures 18A–C, 19B, 21F); 8–12 times longer than wide, 0.9–1.0 lip region diam. long. Odontophore simple, 1.9–2.3 times odontostyle length long. Guiding ring double, situated at 0.7–0.8 times lip region diam. from anterior end. Nerve ring located at 151–178 µm from anterior end or 32–38% of total neck length. Pharynx consisting of slender but muscular anterior section enlarging gradually and "bibulbar" (Andrássy, 1986), basal expansion with somewhat narrower middle part, 206–231 µm long or 44–52% of total neck length (Figs 14A, 18D). Dorsal nucleus (DN) lying very close to anterior edge of pharyngeal expansion. One nucleus of anterior ventrosublateral pair of pharyngeal glands well visible, large, posterior pair of ventrosublateral nuclei slightly larger, nuclei located almost at one and the same level (pharyngeal characters presented in Table 6). Cardia conoid, measuring 28–39 × 14–19 µm, cell mass near cardia present in some specimens. The posterior end of the intestine with tongue-like projection. Prerectum short, 2–4 times, rectum 1.3–1.8 anal body diam. long. Distinct sphincter at prerectum and rectum junction. Genital system didelphic-amphidelphic, with both branches equally and well developed, anterior 450.5±21.3 (422–478) µm, posterior 463.8±37.9 (404–531) µm long, respectively. Ovaries usually large, oviduct consisting of a tubular part and well developed *pars dilatata*. Sphincter between oviduct and uterus moderately developed. Uterus long (anterior 220–307 µm, posterior 222.5–356 µm long, respectively), bipartite, consists of a wider proximal part followed by narrower distal part surrounded by large hyaline cells. Uteri contain sperm. Vagina extending inwards for 55–74% of body diameter, *pars proximalis* 35–50 × 22–30 µm, with straight walls, *pars refringens* (in lateral view) consisting of two massive trapezoidal separate sclerotised pieces with a combined width of 18–21 µm, *pars distalis* 8.5–12 µm long. Vulva a transverse slit; under SEM vulval lips spindle shaped, irregularities and ruptures of body cuticle present on both sides of vulva. Lateral vulval flaps absent. In two females uterine eggs observed, measuring 133–148 × 68.5–77 µm. Tail conical, ventrally arcuate, distal part offset, tip finger-like, sharply pointed. Three pairs of caudal pores.

Males. General morphology similar to that of female, except for the genital system. Arrangement of pharyngeal gland nuclei presented at Table 6. Genital system

Table 5. Morphometrics of *Pararhyssocolpus paradoxus* n.gen., n.comb. (adults, juveniles). All measurements, unless indicated otherwise, are in µm (and in the form: mean±SD (range).

Locality	**King George Island**		**Livingston Island**						**Nelson Island**	**King George Island**		
Characters	**KGI1**		**CDM**		**S**	**SDC**	**DM**		**M**	**KGI1**		
n	♀(n=8)	♂	♀	♂	♀	♀	♀ (n=4)	♂	♀	J1	J3	J4 (n=7)
L (mm)	2.42±0.1 (2.18–2.69)	2.28, 2.40, 2.24	2.61	2.61	2.07, 2.36, 1.69	2.41	2.7±0.1 (2.35–2.87)	2.16, 1.96, 2.59	2.71	0.87	1.39, 1.37	1.93±0.2 (1.69–2.11)
a	23.4±0.98 (22.2–24.9)	22.8, 26.5, 22.8	21.6	22.1	20.6, 19.6, 18.0	23.1	22.6±1.2 (21.3–23.6)	21.3, 20.2, 23.4	24.3	26.6	24.2, 25.0	27.0±3.0 (22.5–32.0)
b	5.2± 0.4 (4.8–5.9)	5.2, 5.2, 4.9	5.7	5.9	4.7, 5.7, 4.5	5.8	5.7± 0.4 (5.3–6.2)	4.9, 4.4, 6.0	5.8	3.7	4.1, 3.9	4.8± 0.4 (4.1–5.2)
c	34.9±4.4 (28–40.4)	45.7, 34.7, 39.4	36.9	39.8	31.6, 31.6, 30.1	36.2	37.4±2.1 (35.6–40)	32.6, 30, 39.2	40.9	10.1	18.3, 15.8	24.4±3.8 (20.8–31.9)
c‘	1.5±0.2 (1.4–1.8)	1.2,1.2, 1.2	1.4	1.0	1.5, 1.6, 1.5	1.5	1.5±0.02 (1.4–1.5)	1.2, 1.2, 1.3	1.4	3.8	2.5, 2.4	1.9±0.2 (1.5–2.2)
V %	46.6± 1.5 (44–49)	-	49	-	46.5, 47, 48	49	47.6± 2.2 (44–49)	-	47	-	-	-
Lip region width	20.7±0.9 (20–22.5)	20, 21.5, 21	22	22	21, 22, 21.5	20.3	21.4±0.5 (21–22)	22, 21, 21	22	10	15, 16	18.7±0.5 (18–20)
Odontostyle	19.8±0.6 (19–21)	19, 19, 20	20	20	20.5, 21, 19	21	20.5±0.5 (20–21)	20, 21, 19	20	10	14, 13	17±1.0 (16–18)
Replacement odontostyle	-	-	-	-		-	-	-	-	11	17, 16	19.9±0.9 (18–21)
Odontophore	41.1±1.9 (39–43)	40	45	-		44	43, 43	-, 45, 37	-	-	-	-
Anterior end guiding ring	16	-	15	15	14, 15, -	-	15	15, 15.5, -	-	-	-	-
Anterior end nerve ring	162.9±9.3 (151–177.5)	160, 162.5, 152.5	177.5	-	-	160	168.3±10.1 (154–178)	172, 176, 151	-	-	-	-
Pharynx	463.9±10.1 (456–487)	443, 461, 462.5	460	441	444, 414, 375	417	470.2±21.4 (446–494)	444, 447, 431	470	237	344, 356	405.4±12.6 (382–418)
Width at pharynx base	88.3± 6.8 (78–97)	88, 81, 84.5,	103	108	93, 99, 81	94.5	91.8± 7.9 (93–110.4)	92.5, 86, 94	97	-	-	-

Locality	**King George Island**		**Livingston Island**						**Nelson Island**	**King George Island**		
Characters	**KGI1**		**CDM**		**S**	**SDC**	**DM**		**M**	**KGI1**		
Width at mid body	103.7±9.8 (88.5–115)	100, 91, 98	121	118	101, 120.5, 94	104.5	118.8±11.9 (108–135)	102, 97, 111	112	33	57.5, 55	71.8±7.2 (65–84)
Prerectum length	136.7±20.2 (95–163)	171, 179, 172.5	164	-	105, 99, 97	191	163.7±23 (134–188)	-	137	-	93	127.2±10.8 (110–138)
Rectum length	73.1±4.8 (68–81)	-	67	-	65, 75, 62	-	74.1±6.4 (71–84)	-	75	23	39, 48.5	50.4±6.4 (43–61)
Tail	70.1±7.2 (61–80)	50, 69, 57	71	66	66, 75, 56	67	71.4±3.8 (66–74.5)	66, 65, 66	66	86	76, 87	80.1±11.4 (65–96)
Genital primordium	-	-	-	-	-	-	-	-	-	29.5	53	54.0±14.4 (43–74)
Spicules	-	104, 106, 104	-	106	-	-	-	106, 113, 112	-	-	-	-
Ventromedian supplements	-	25, 25, 27	-	24	-	-	-	24, 28, 26	-	-	-	-

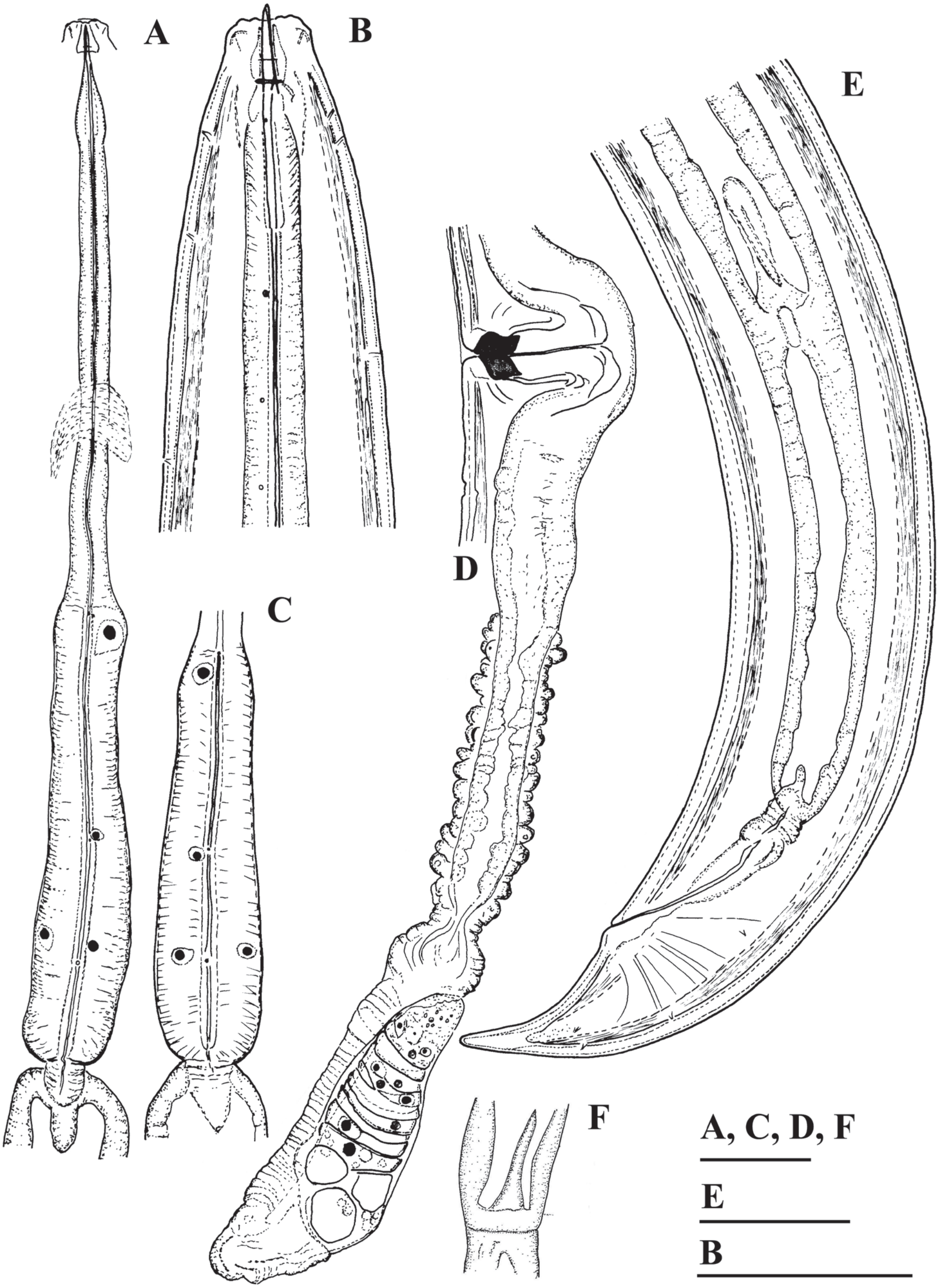

Figure 14. *Pararhyssocolpus paradoxus* (Loof, 1975), gen. n., comb. n. **A, B, D–F** *Female*: **A** Pharyngeal region (KGI) **B** Anterior region (KGI) **D** Posterior genital branch (LI, DM) **E** Posterior end (KGI) **F** Tongue like projection (KGI) *Male* (LI, DM): **C** Pharyngeal expansion. Scale bar: 50 µm (**A–F**).

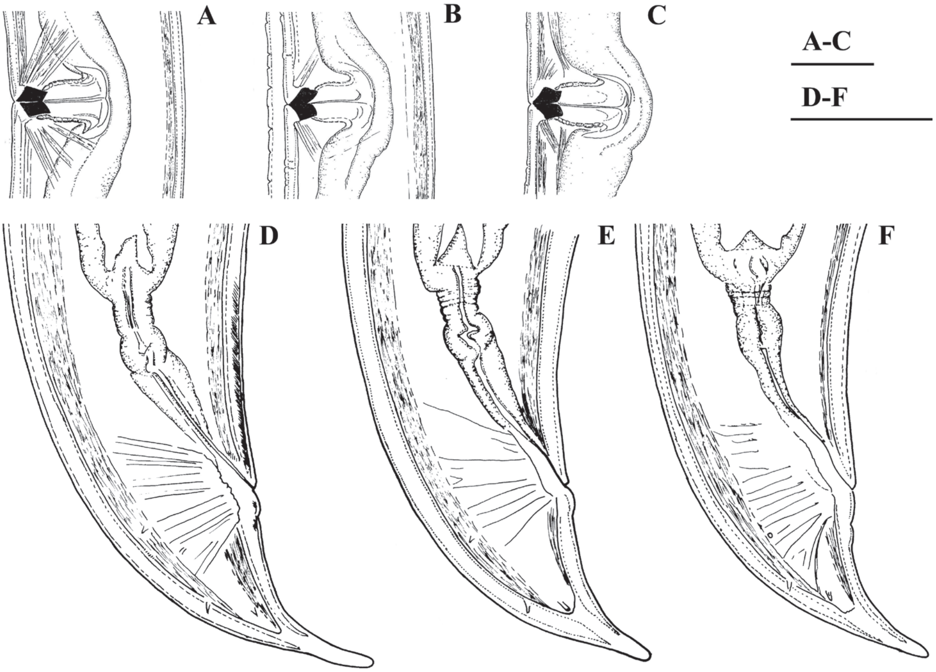

Figure 15. *Pararhyssocolpus paradoxus* (Loof, 1975) gen. n., comb. n. *Female*: **A–C** Vulval region (KGI) **D–F** Tail ends (**D, E** KGI; **F** LI, DM). Scale bar: 50 µm (**A–F**).

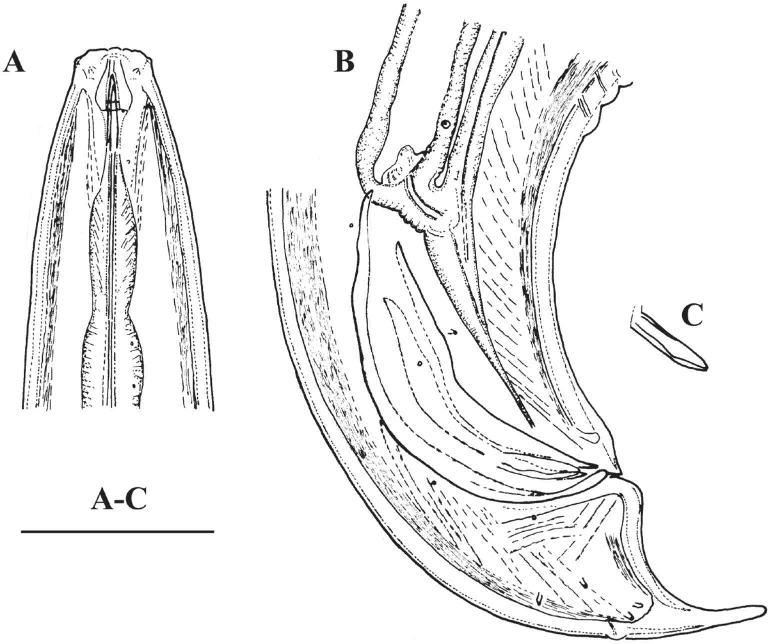

Figure 16. *Pararhyssocolpus paradoxus* (Loof, 1975), gen. n., comb. n. *Male*: **A** Anterior region (LI, DM) **B** Tail end (KGI) **C** Lateral guiding piece (KGI). Scale bar: 50 µm (**A–C**).

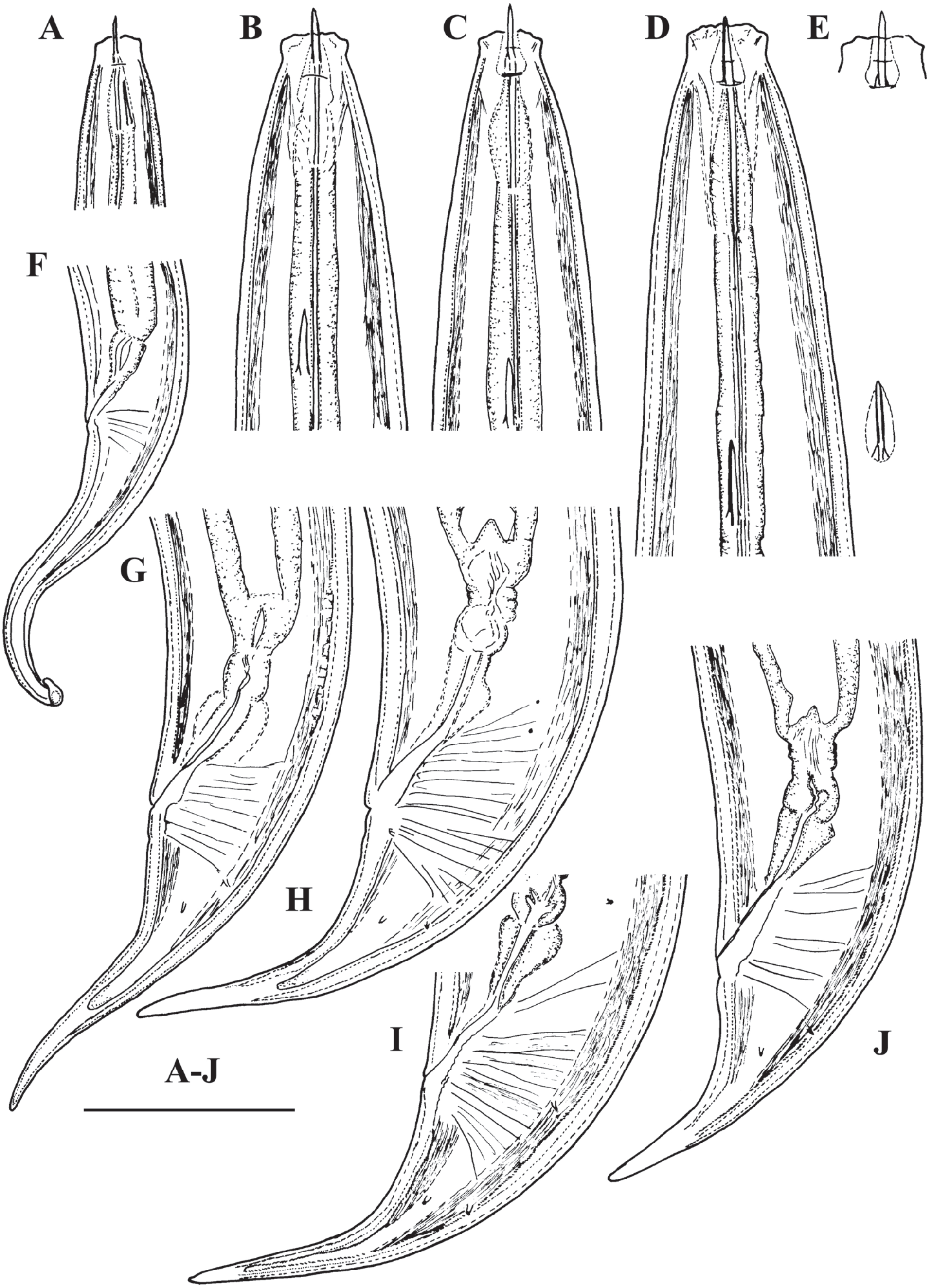

Figure 17. *Pararhyssocolpus paradoxus* (Loof, 1975), gen. n., comb. n. *Juveniles* (KGI): **A–E** Lip region of **A** J1 **B, C** J3 **D, E** J4 **F–J** Tail end of **F** J1 **G, H** J3 **I, J** J4. Scale bar: 50 μm (**A–J**).

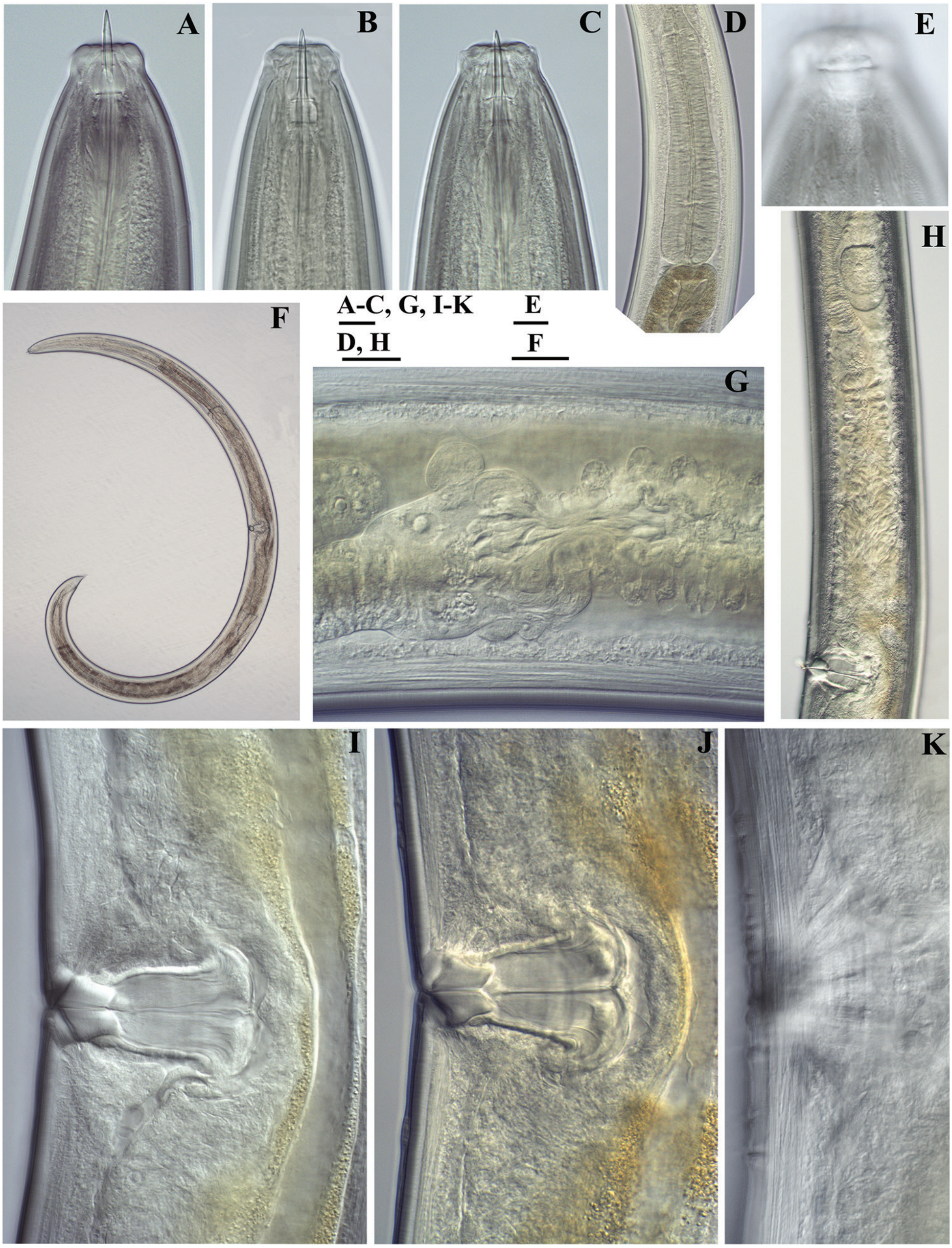

Figure 18. *Pararhyssocolpus paradoxus* (Loof, 1975), gen. n., comb. n. *Female*: **A–C** Anterior region (**A** KGI; **B** LI, DM; **C** NI) **D** Pharyngeal expansion, cardia (KGI) **E** Amphidial fovea (KGI) **F** Entire body (KGI) **G** Sphincter between uterus and *pars dilatata oviductus* (KGI) **H** Anterior genital branch (KGI) **I, J** Vulval region (KGI) **K** Irregularities around vulva (KGI). Scale bars: 10 µm (**A–C, G, I–K**); 50 µm (**D, H**); 6 µm (**E**); 200 µm (**F**).

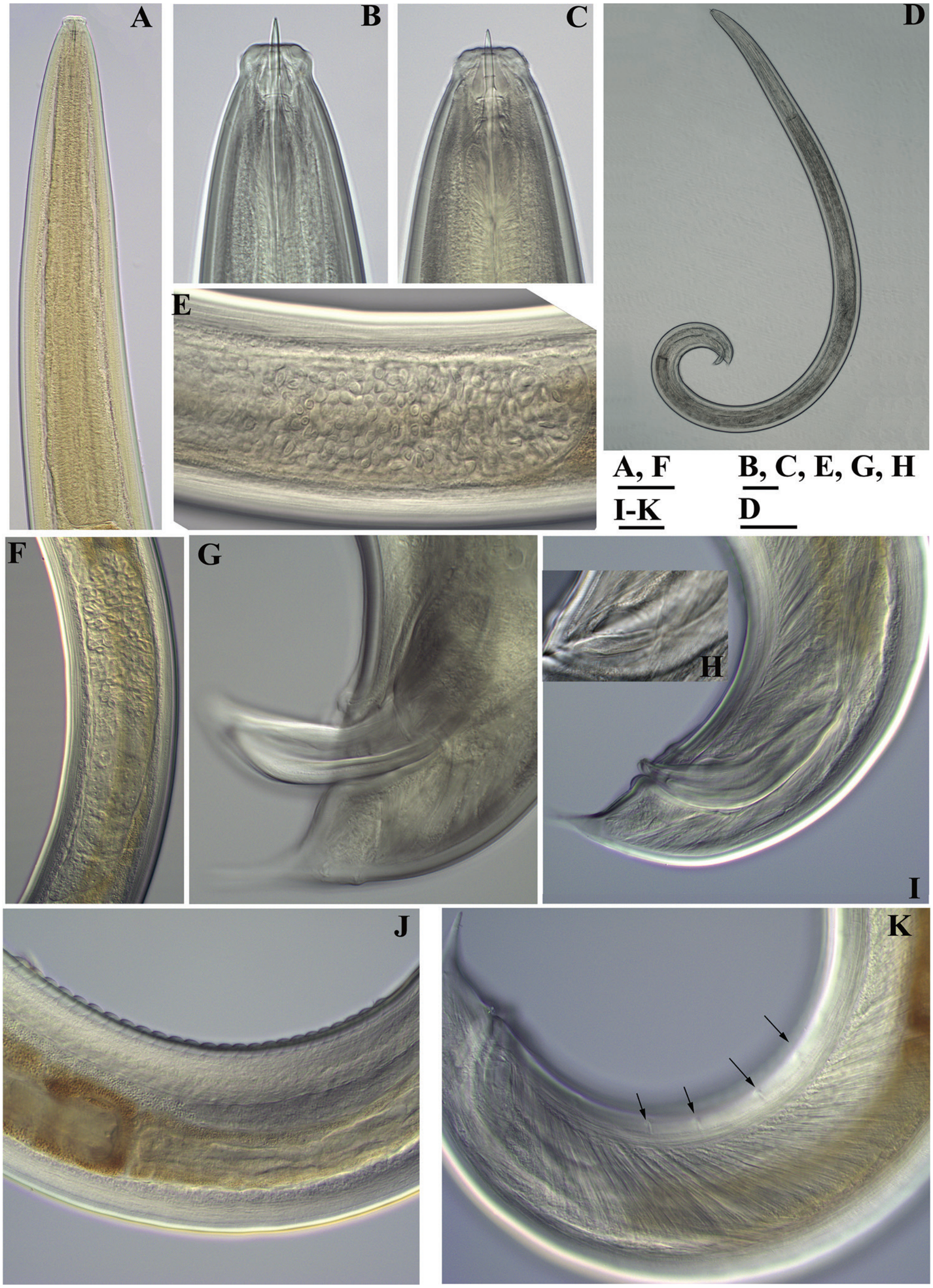

Figure 19. *Pararhyssocolpus paradoxus* (Loof, 1975), gen. n., comb. n. *Male*: **A** Pharyngeal region (LI, CDM) **B, C** Anterior end (**B** KGI; **C** LI, DM) **D** Entire body (KGI) **E** Sperm (KGI) **F** Posterior testis (KGI) **G, I** Spicules (KGI) **H** Lateral guiding piece (KGI) **J** Ventromedian supplements (KGI) **K** Subventral papillae marked by arrows (KGI). Scale bars: 50 µm (**A, F**); 10 µm (**B, C, E, G, H**); 200 µm (**D**); 20 µm (**I–K**).

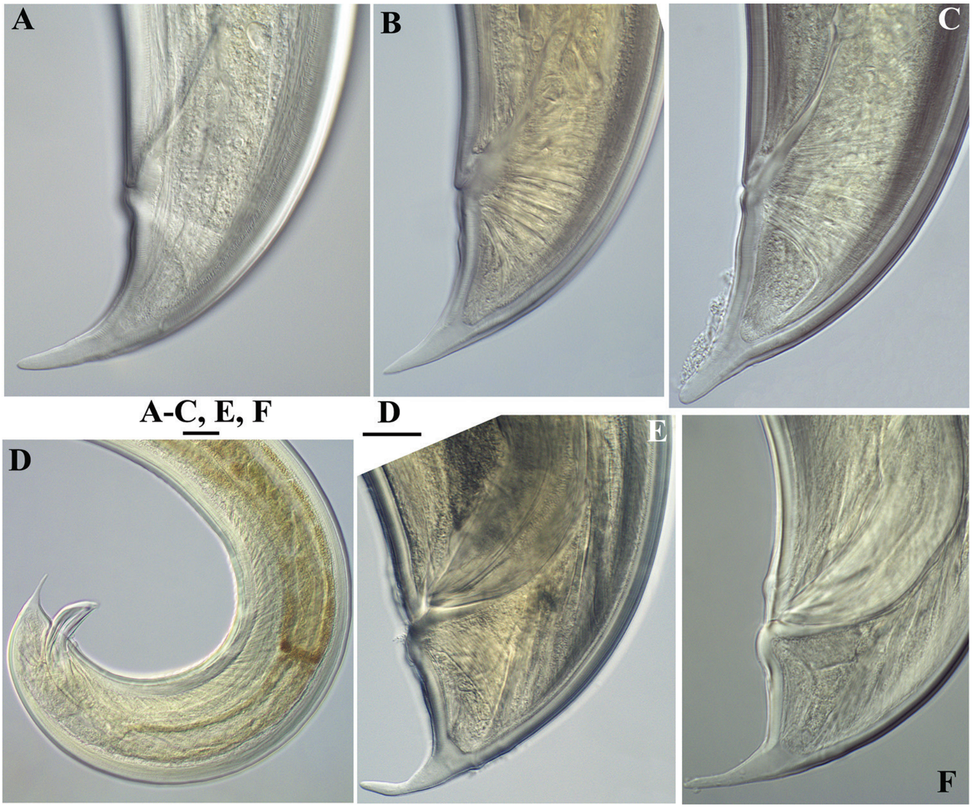

Figure 20. *Pararhyssocolpus paradoxus* (Loof, 1975), comb. n. **A–C** *Female:* **A–C** Tail ends (**A** KGI; **B** LI, DM; **C** NI). **D–F***Male:* **D** Posterior end (KGI) **E, F** Tail ends (**E** CDM, LI; **F** KGI). Scale bars: 10 μm (**A–C, E, F**); 50 μm (**D**).

Table 6. Pharyngeal characters of *Pararhyssocolpus paradoxus* gen. n., n.comb. For abbreviations see Loof and Coomans (1970) and Andrássy (1998b).

Locality	King George Island				Livingston Island			
Characters	KGI1				CDM		DM	
	n	female	n	male	female	male	female	male
DO	2	57, 59	2	54, 56				58
DN=D	8	56–60	3	55, 57, 55	60	57	55	56, 59, 59
Distance DO-DN %	2	1, 0.5	2	1, 1				1
S_1O	1	78	2	75, 77				78
S_1N_1	1	78						
S_1N_2	7	75–79	3	75, 76.5, 74			76	76, 77, 79
S_2O	4	88–91	2	88, 89				89
S_2N_1	7	86–89	3	86.5, 87, 87	88	88	87	87, 87, 88
S_2N_2	8	87–92	3	87, 87, 88	88	88	87	88, 87.5, 89
AS_1	1	48						
AS_2	5	43–49	3	45, 45, 42			46	46, 45, 48
PS_1	4	69–72	3	70, 70, 70	69	71	70	71, 68, 71
PS_2	6	70–80	3	71, 70, 73	70	72	70	73, 70, 73

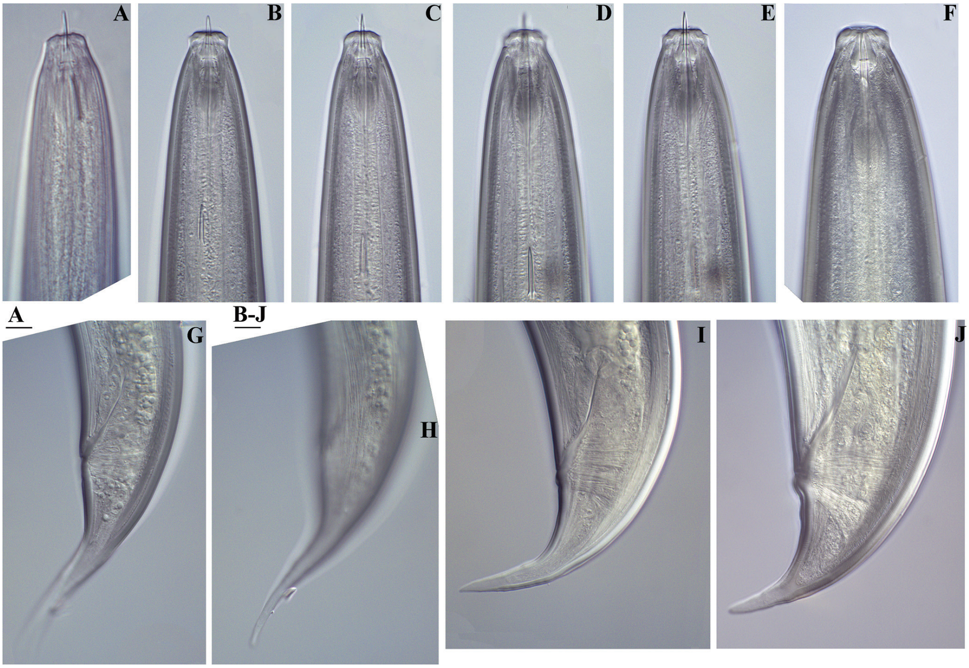

Figure 21. *Pararhyssocolpus paradoxus* (Loof, 1975), comb. n. **A–E, G–I** *Juveniles* (KGI): **A–E** Lip region of **A** J1 **B, C** J3 **D, E** J4 **G–I** Tail end of **G, H** J3 **I** J4. **F, J** *Female:* (KGI): **F** Lip region **J** Tail end. Scale bars: 6 µm (**A**); 10 µm (**B–J**).

diorchic, testes opposed, anterior 318–474 µm (n=3) and posterior 278–436 µm (n=2) long, respectively. Spicules dorylaimid, stout, 1.7–2.6 cloacal body diam. long. Lateral guiding piece with triangular distal part, 19–24 µm long. Sperm oval, measuring 5–9×3–4 µm. Ventromedian supplements contiguous, 24–28 in number, preceded by one adcloacal pair of papillae located at 9–16 µm distance from cloacal opening, out of spicules range; a series of well developed subventral spaced papillae (Jairajpuri and Ahmad 1992) in number 11–18 observed. Post-cloacal papilla present. Tail compared to that in female with narrower finger like tip. Three pairs of caudal pores.

Juveniles. Comparison of length of functional and replacement odontostyle and body length yielded in identification of three juvenile stages (second stage juvenile not found). The tail in J1 elongated, sigmoid, in J3 tail elongate with long hyaline extension, ventrally arcuate, sometimes slightly sigmoid, sharply tipped; in J4 ventrally arcuate with gradually tapering distal part, c’ decreases during successive stages to females (Table 5).

Sequence and phylogenetic analyses. The BLAST search using D2-D3 region sequence of *P. paradoxus* gen. n., comb. n. showed highest similarity (93%) to the sequences of several *Opisthodorylaimus sylphoides* (Williams, 1959) Carbonell & Coomans, 1985 clones and *Prodorylaimus* sp. (AY593008–10, EF207241, Holterman et al. 2008). The 18S rDNA sequence showed 99% similarity to several dorylaimid

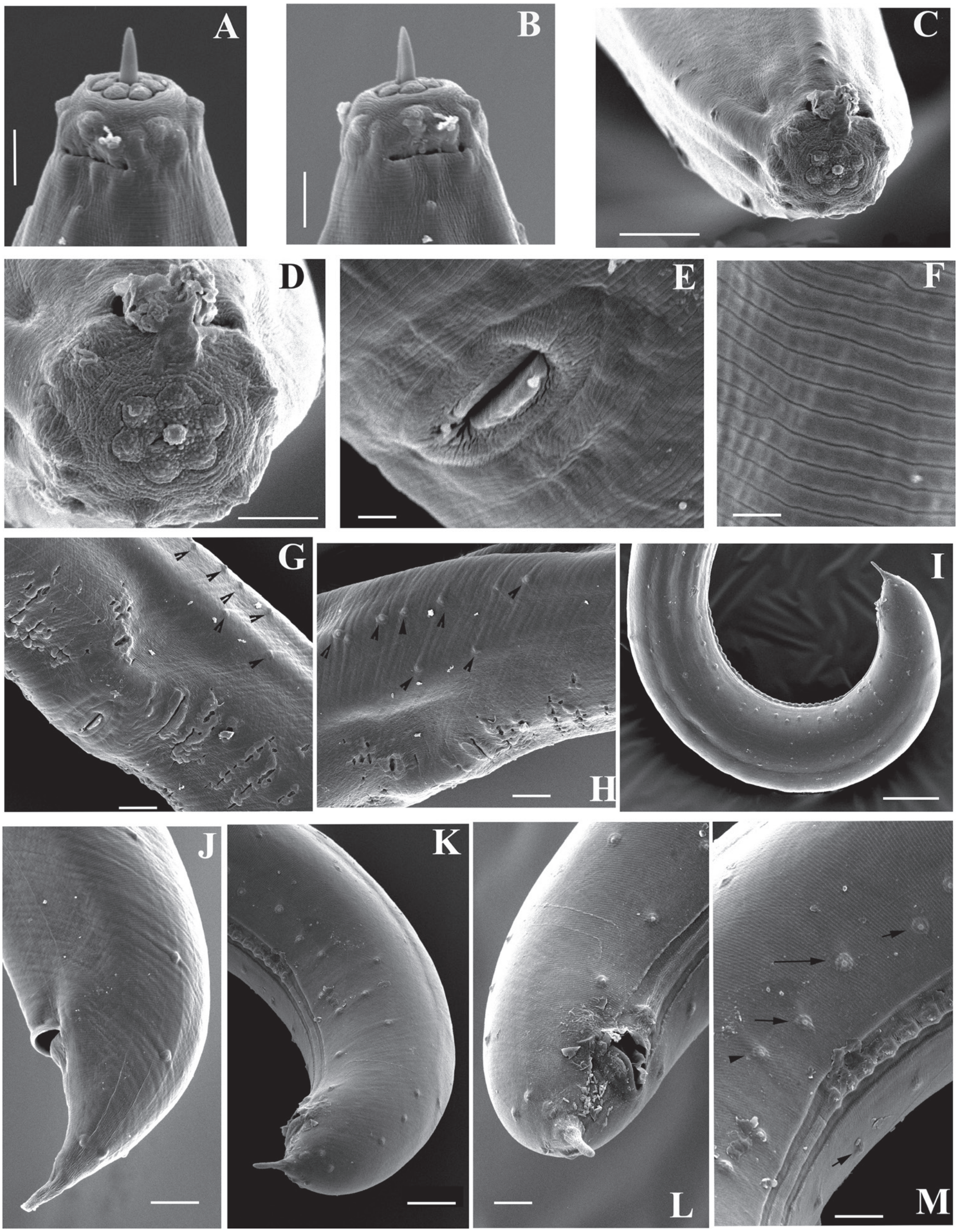

Figure 22. SEM micrographs. *Pararhyssocolpus paradoxus* (Loof, 1975), comb. n. **A–H, J** *Female*: **A–D** Lip region (**A** Sublateral view (NI); **B** Lateral view (NI); **C, D** (LI) In face view) **E** Vulval region (NI) **F** Cuticle striations (NI) **G**, **H** Vulval region, irregularities around vulva, lateral body pores marked by arrows (NI) **J** Tail end (LI) **I, K–M** Male (LI): **I** Posterior end, lateral view **K** Tail end **L** Cloaca **M** Ventromedian supplements and subventral papillae (marked by arrows). Scale bars: 5 µm(**A, B, D**); 10 µm (**C, G, H, J, L, M**); 50 µm (**I**); 20 µm (**K**); 2 µm (**E**); 1 µm (**F**).

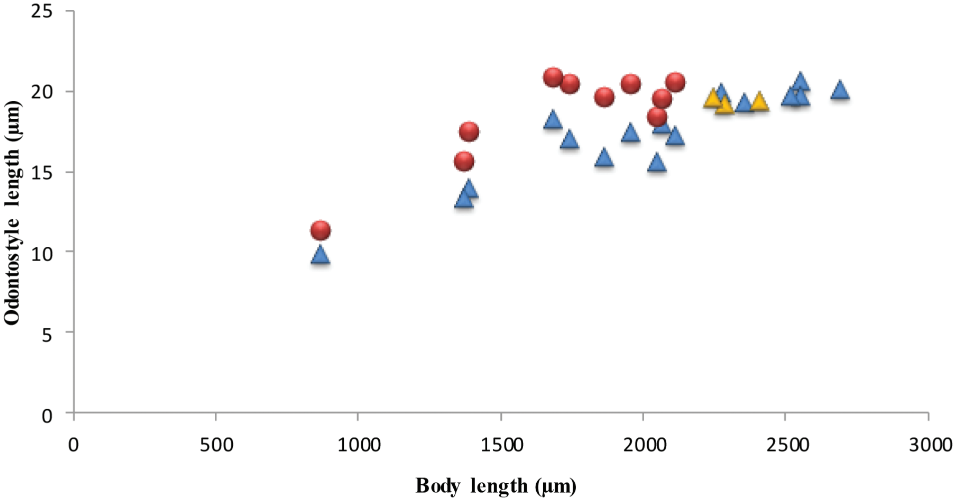

Figure 23. *Pararhyssocolpus paradoxus* (Loof, 1975), comb. n. Scatter plot of the functional (●) and replacement odontostyle (▲) in relation to the body length of the juvenile stages and adults: females (▲) and males (▲).

species belonging to different families including *A. isokaryon*, and various *Aporcelaimellus* spp. The hypothesis testing using closely and more distantly related 18S rDNA sequences (Figure 24) revealed distant relationship of *P. paradoxus* gen. n., comb. n. to the only available sequences of *Rhyssocolpus* Andrássy, 1971 (*R. vinciguerrae* Pedram, Pourjam, Robbins, Ye, Peña-Santiago, 2011, Figure 4) (fam. Nordiidae) and *Eudorylaimus* Andrássy, 1959 (two *Eudorylaimus* spp.) (fam. Qudsianematidae). The ambiguous position of both *P. paradoxus* gen. n., comb. n. and *A. isokaryon* could be a result of the low resolution of the SSU rDNA, non-monophyly of these four families and/or probably incorrect species identifications. The majority of the nematode sequences belonging to the superfamily Dorylaimoidea de Man, 1876 available at the GenBank have no morphological and metrical data and their identification is questionable.

In an additional analysis using the most closely related sequences performed in order to clarify the possible evolutionary relationships of *P. paradoxus* gen. n., comb. n. (Figure 25): it clustered into the same clade with *A. isokaryon* and some other species of the families Qudsianematidae, Dorylaimidae and Aporcelaimidae. Further, in the 28S rDNA-based phylogenetic tree *P. paradoxus* gen. n., comb. n. grouped with species belonging to different families (Figure 26) and no close relationships to any of them were revealed.

Discussion. The specimens examined generally agree well with data reported for this species, although some differences occurred: lip region offset by slight depression *vs* deep depression; vulva transverse *vs* "probably pore-like rather than transverse", smaller DN-DO distance (0.5–1 *vs* 1.6–3.4%) (Loof 1975). Further, the distinct sphincter at prerectum/rectum junction, tongue-like structure at the posterior end of intestine and subventral papillae in male were not mentioned in the original description.

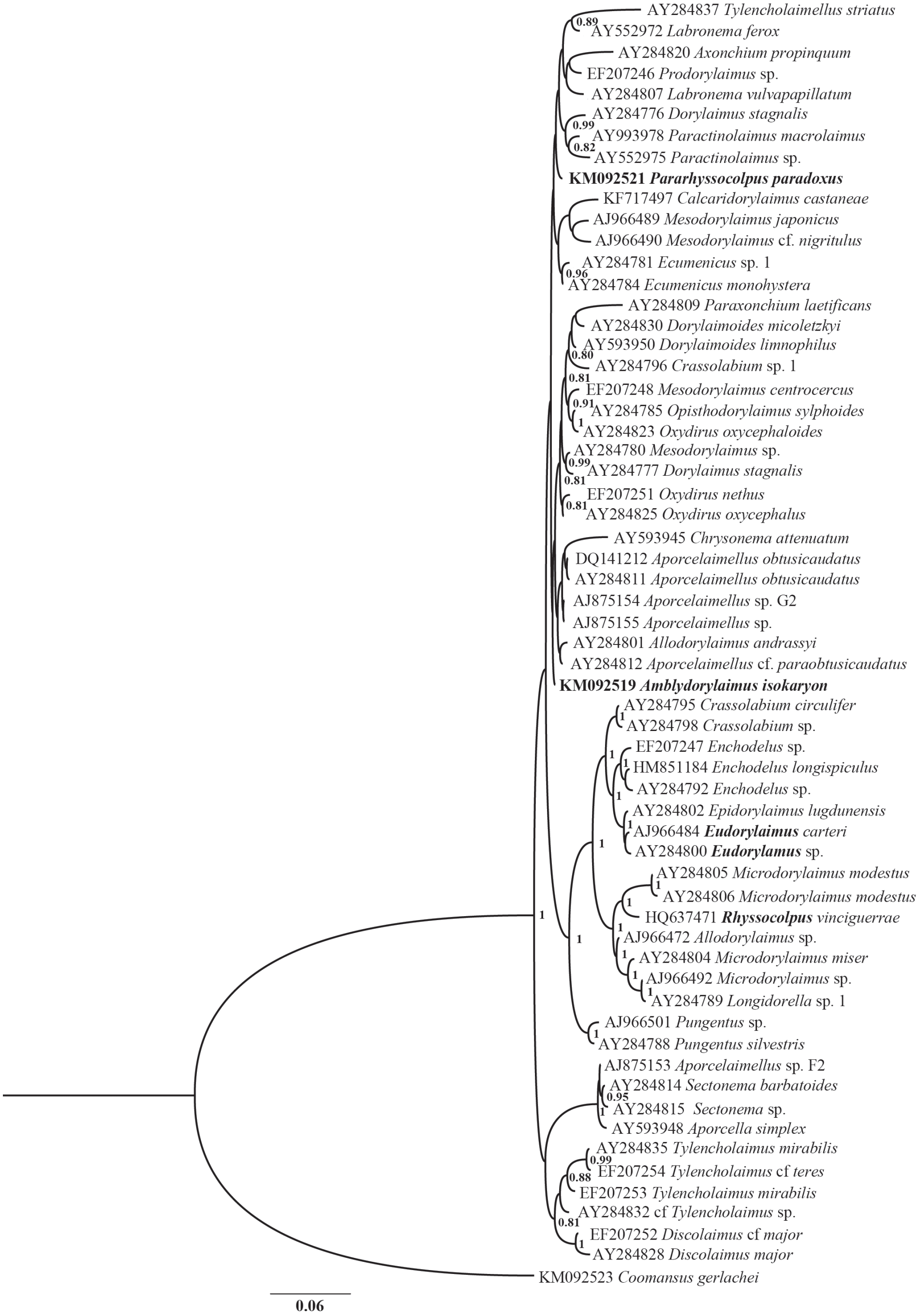

Figure 24. Hypothesis of the phylogenetic relationships of *Amblydorylaimus isokaryon* (Loof, 1975) and *Pararhyssocolpus paradoxus* (Loof, 1975), gen. n. comb. n. based on 18S rDNA (61 sequences) inferred from a Bayesian analysis using GTR+G model and *Coomansus gerlachei* (de Man, 1904) for rooting the tree. Posterior probabilities higher than 0.8 are presented.

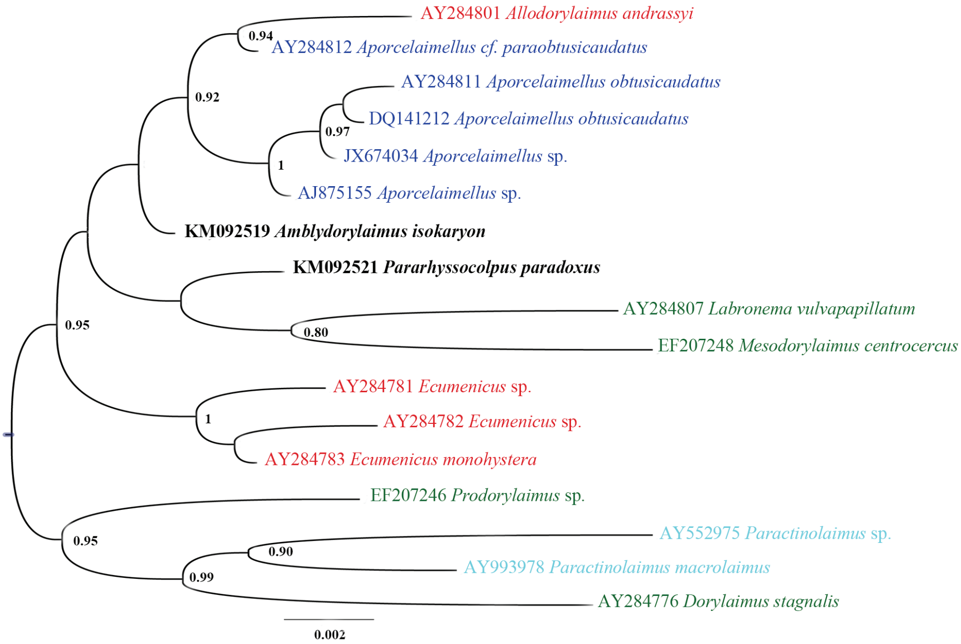

Figure 25. Hypothesis of the phylogenetic relationships of *Amblydorylaimus isokaryon* (Loof, 1975) and *Pararhyssocolpus paradoxus* (Loof, 1975), gen. n. comb. n. based on 18S rDNA of closest species (17 sequences) inferred from a Bayesian analysis using GTR+G model and midpoint rooting of the tree. Posterior probabilities higher than 0.8 are presented. Species coloured according the classification of Andrássy (2009a) and Peña-Santiago and Álvarez-Ortega (2014): dark blue – fam. Aporcelaimidae, light blue – fam. Actinolaimidae, green – fam. Dorylaimidae, red – fam. Qudsianematidae.

Originally this species was attributed to family Qudsianematidae. Loof (1975) placed it in *Eudorylaimus*, because of widened near the middle pharynx and numerous ventromedian supplements. Nevertheless, he reported that it showed several characters close to *Rhyssocolpus* (shape of lip region, short odontostyle, and wrinkled cuticle near vulva, although he regarded the last one a not generic rank character). Subsequently Andrássy (1986) included it in family Nordiidae (genus *Rhyssocolpus*) ignoring the characters in which this species differs from the other members of genus *Rhyssocolpus* e.g. the greater number of contiguous ventromedian supplements and specific shape of pharyngeal expansion. Again, Loof (1988) reported that many features of this species (numerous and contiguous supplements, pharyngeal expansion at about half pharynx length, DN lying at about 60% of pharynx, distinct first pair of ventrosublateral pharyngeal glands) conflicted with the diagnosis of *Rhyssocolpus* and continued to regard this Antarctic species as a member of *Eudorylaimus* (Qudsianematidae). Very recently, Peña-Santiago et al. (2015) provided a revised taxonomy of the genus *Rhyssocolpus* and proposed *R. paradoxus* be retained under *Eudorylaimus*. However, it differs from the latter genus by the arrangement of ventromedian supplements in males (contiguous *vs*

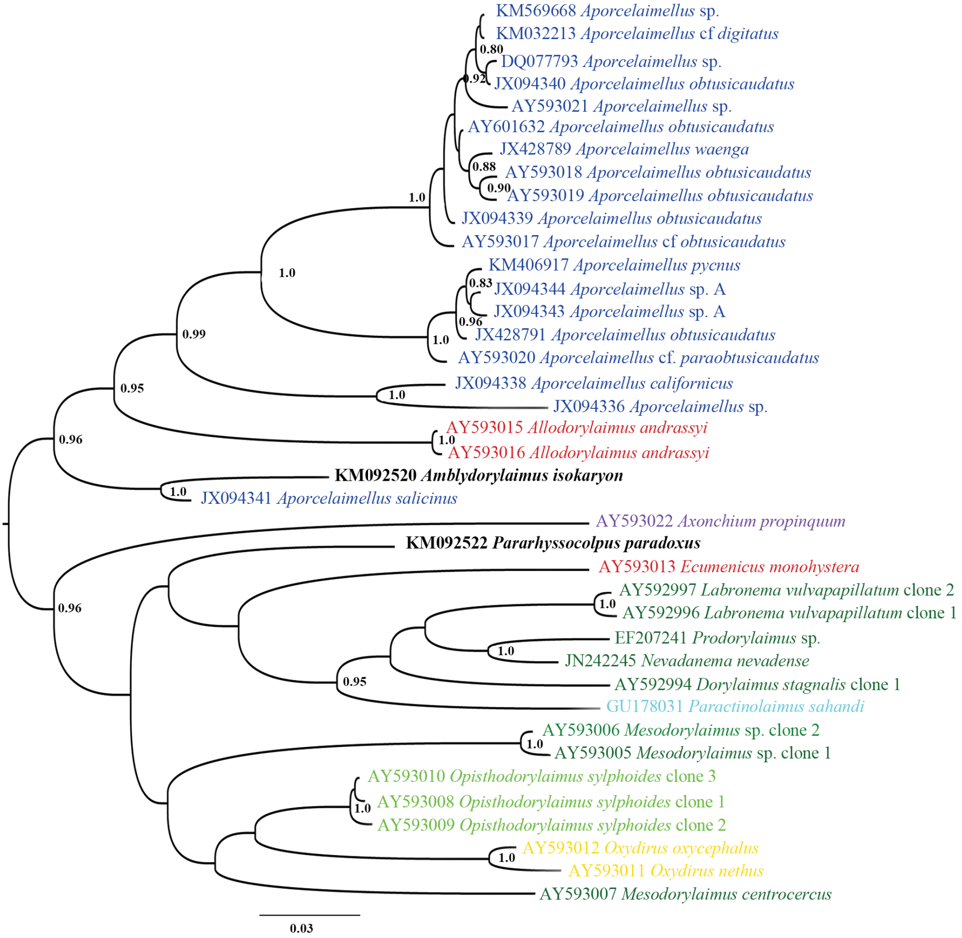

Figure 26. Hypothesis of the phylogenetic relationships of *Amblydorylaimus isokaryon* (Loof, 1975) and *Pararhyssocolpus paradoxus* (Loof, 1975), gen. n. comb. n. based on 28S rDNA D2-D3 inferred from a Bayesian analysis using GTR+G model and midpoint rooting of the tree. Posterior probabilities higher than 0.8 are presented. Species coloured according the classification of Andrássy (2009a) and Peña-Santiago and Álvarez-Ortega (2014): dark blue – fam. Aporcelaimidae, light blue – fam. Actinolaimidae, dark green – fam. Dorylaimidae, light green – fam. Thornenematidae, red – fam. Qudsianematidae, yellow – fam. Swangeriidae, violet – fam. Belondiridae. *For abbreviations of localities see Table 1

spaced), double *vs* single guiding ring, slender *vs* wider odontostyle and specific shape of pharyngeal expansion.

Recent molecular studies (Holterman et al. 2008; Pedram et al. 2011; Peña-Santiago et al. 2015) as well as our molecular data inferred from the analysis of 18S and D2-D3 expansion segments of the 28S rDNA, showed that this genus could not be assigned to any known Dorylaimoidea family.

With considering the differences discussed above, as well as molecular data, the herein studied species cannot be regarded either as a member of the genus *Rhyssocolpus* or the genus *Eudorylaimus* and their attributed families, consequently a new genus *Pararhyssocolpus* gen. n., and a new family Pararhyssocolpidae fam. n. are proposed to accommodate this species.

Family Pararhyssocolpidae fam. n.

http://zoobank.org/B2D2F40F-283F-41A6-A2C4-5DFD3461EB80

Diagnosis. Dorylaimoidea. Nematodes of a medium sized body, 2–3 mm. Cuticle dorylaimoid, finely transversally striated. Lip region rounded, inner labial papillae distinct, not elevated, amalgamated and close to oral aperture, outer labial and cephalic papillae below the margin of oral field. Odontostyle slender, shorter than or as long as labial diameter, with narrow aperture, indistinct under LM and clear lumen. Odontophore simple. Guiding ring double. Pharynx occupying about half total pharynx length. Female genital system didelphic-amphidelphic, uterus bipartite, *pars refringens vaginae* well developed, vulva transverse. Irregularities and ruptures of body cuticle around vulva present. Spicules dorylaimoid, lateral guiding piece simple. Ventromedian supplements contiguous, numerous. Tail similar in both sexes, conical, ventrally arcuate, distal part long, finger-like. First stage juvenile with long sigmoid tail.

Pararhyssocolpus gen. n.

http://zoobank.org/95A47B1D-D7A7-4379-ABC9-EDF1A227AED1

Diagnosis. With characters of the family.

Relationships. On the basis of main characters, this genus/family appears close to family Nordiidae, Qudsianematidae (subfamily Qudsianematinae Jairajpuri, 1965) and Dorylaimidae. The new family differs from the first family in pharynx widening at the middle of neck *vs* pharynx widening behind the middle of the neck, the pharyngeal expansion shape (somewhat "bibulbar", with narrower middle part *vs* cylindrical), ventromedian supplements contiguous *vs* mostly spaced (except *Lenonchium* Siddiqi, 1965, it differs from the new family by its longer and filiform tail). From subfamily Qudsianematinae, Pararhyssocolpidae fam. n. can be differentiated by its double *vs* single guiding ring and labial papillae arrangement (small *vs* larger distance between inner labial papillae), indistinct *vs* distinct aperture of odontostyle. Also, the new family differs from fam. Dorylaimidae in odontostyle aperture (indistinct *vs* distinct) and especially in its characteristic postembryonic development pattern – J1 with long tail, c' decreasing in successive stages and adults caused by the increasing of anal diameter rather than shortening of tail, adults with similar tail shape - conical with distal third much narrower, finger-like *vs* one or more juvenile stages bearing long (filiform or conical elongated) caudal region, adults with similar (either long or short and rounded, never conical) or dissimilar (long in females, short and rounded, exceptionally conical, in males) tail (Peña-Santiago and Álvarez-Ortega 2014). Recent studies based on molecular data (Holterman et al. 2008; Pedram et al. 2011; Álvarez-Ortega et al. 2013a, c; Peña-Santiago et al. 2015) show that the current classification of superfamily Dorylaimoidea is questionable with most families being not monophyletic taxa, as some of the genera are closer to members of other families. Further integrative studies are needed to clarify its phylogeny and systematics and to understand which characters are homologous and which are the results of convergent or parallel evolution (Vinciguerra et al. 2014).

Distribution. This species (genus, family) is endemic in Maritime Antarctic, having been recorded from many islands: Signy (Loof 1975; Maslen 1979; 1981), Coronation, Elephant, Intercurrence, Galindez, Blaiklock, Limpet (Loof 1975; Maslen 1979), Guébriant (Maslen 1979), Adelaide (Maslen and Convey 2006; Newsham et al. 2006), Anchorage, Leonie (Maslen and Convey 2006), Livingston (Peneva et al. 2009), Francis (Velasco-Castrillón and Stevens 2014) and King George Islands (Kito 2009; Russell et al. 2014). This is the first report of the species from Nelson Island.

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In the shadow of a megalopolis, a new *Flexamia* from a threatened grass species in the New Jersey Pine Barrens (Hemiptera, Cicadellidae, Deltocephalinae, Paralimnini)

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Abstract

A previously unknown species of the North American leafhopper genus *Flexamia, F. whitcombi* **sp. n.**, is described from pinebarren smokegrass (*Muhlenbergia torreyana* (Schult.) Hitchc.), a state-listed threatened grass in the New Jersey Pine Barrens. The *serrata* species group, to which it belongs, is redefined and a key to the species of the group is provided. This is the first reported insect association with *M. torreyana*.

Keywords

Hemiptera, Cicadellidae, Deltocephalinae, leafhopper, *Flexamia*, new species, *Muhlenbergia torreyana*, New Jersey Pine Barrens

Introduction

Flexamia is a charismatic North American leafhopper genus of specialist grass feeders with the species typically feeding upon a single, widespread host species. The hosts they spend their lives on include some of the iconic grasses of the American prairie, rangelands, and deserts, and *Flexamia* are often described as prairie or grassland leafhoppers. By these standards, the new species described herein is an outlier, occurring in the most densely populated state in the US where its host *Muhlenbergia torreyana* (Schult.)

Hitchc. grows, though neither New Jersey nor the Pine Barrens—also known as the New Jersey Pinelands—are usually thought of in a grassland context.

Known colloquially as pinebarren smokegrass, Torrey's dropseed, Torrey's muhly, and New Jersey muhly, *Muhlenbergia torreyana* is a native perennial C4 Chloridoid grass, considered either to be a southern coastal plains species at or near the northern edge of its range in New Jersey (Caiazza and Fairbrothers 1980; McAvoy and Wilson 2014), or a species with centers of distribution in both the New Jersey Pine Barrens and the coastal plain of the Carolinas (Sorrie et al. 1997). *M. torreyana* is rare further south (ibid), with only 13 populations in North Carolina (North Carolina Natural Heritage Program 2015) and "a few" occurrences in Tennessee (NatureServe 2015). Apparently extirpated in parts of its former range including New York (Zaremba and LaMont 1993), *M. torreyana* is locally abundant in wet meadows and seasonally flooded depressions in the Pine Barrens (Fig. 1) and its status is listed as threatened in New Jersey (Special Plants of New Jersey 2013). This is the first report of a leafhopper—or any other insect—using this plant as a host and represents a departure for a member of the species group *serrata* which were previously known only from *Muhlenbergia richardsonis* (Trin.) Rydb. (Whitcomb and Hicks 1988, Bess and Hamilton 1999).

Because of the similarity of its specific epithet, it is also occasionally confused with *Muhlenbergia torreyi* (Kunth) Hitchc. ex Bush, a species known as ring muhly from the southwestern USA.

Materials and methods

Thirty-five specimens (excluding juveniles) belonging to the new taxon were collected on the host plants by sweeping in two localities. Dissection, measurements, and photographs were completed with the use of a stereo microscope with a digital camera attachment. Genitalia were prepared following techniques found in Oman (1949) and Young and Beirne (1958). Photographs were processed with Helicon Focus photo-stacking software and post-processed using the 5th generation of the standard image-editing software. Micrographs of the aedeagus were acquired using a JEOL JSM-6480 Scanning Electron Microscope.

Systematics

The *serrata* species group of the genus *Flexamia* was erected by Whitcomb and Hicks (1988) for *F. serrata* Beamer & Tuthill based on the morphology the aedeagal apex, specifically the presence of the pair of dorsal processes. To accommodate *F. huroni* Bess & Hamilton, in which the paired processes were described as apicolateral or lateral terminal, not dorsal, the *serrata* group is redefined here as having 5 or more unbranched processes on the aedeagal apex.

***Flexamia whitcombi* sp. n.**

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Description. Length of male 3.5–3.8 mm, length of female 3.7–4.2 mm; head with length of crown ca. 1.5 times interocular width and ca. 0.68 times transocular width. Base color of dried specimens (Figs 2 & 3) usually stramineous, occasionally ivory above, venter entirely or partially fuscous often lighter caudally, occasionally merely stramineous; crown and pronotum without well-defined fuscous spots or stripes except for pair of dark spots at crown apex. Fore-wing typical of the genus, a few scattered small irregular fuscous markings present, veins slightly paler, apex of the abdomen usually exposed on female specimens.

Face (Fig. 4) coloration varies but typically heavily pigmented apically and laterally, paler medially, 5–6 fine pale transverse lines between eyes not meeting medially, apex of clypellus dark.

Male genitalia. Pygofer (Fig. 5), with posterior lobe truncate apically, caudoventral margin heavily sclerotized, angled and terminating ventrally in a rounded process bearing fine denticulation. Subgenital plates short, extending about 2/3 length of pygofer (Fig. 6), apices relatively blunt, rounded. Connective fused to the aedeagus, keel extending dorsad slightly less than half the height of the dorsal apodeme (Fig. 7). Styles typical of the genus. Aedeagus symetrical, straight in ventral aspect (Fig. 8); in lateral aspect (Fig. 7) shaft long, recurved, tapering evenly before expanding apically, apex (Fig. 9) with 5 processes: on the caudoventral surface, extending laterally and curved ventrad, one pair of short, stout divergent processes with blunt apices, also on the caudoventral surface a longer acute unpaired process extending basad, curved ventrad and bearing the gonopore (Fig. 9C) in the form of a slit extending from the apex of the aedeagal shaft ending subapically on the unpaired process; on the dorsal surface a pair of long spine-like processes which usually cross over the shaft of the aedeagus (Fig. 9A, B). Both apex of shaft and pygofer process occasionally visible in undissected specimens.

Female. Posterior margin of abdominal sternum VII (Fig. 10) typical of the genus, shallowly concave on either side of the slightly notched and embrowned median convex lobe; ovipositor with bases of first valvulae as in Figure 11.

Material examined. Holotype male, USA: NEW JERSEY: Atlantic Co., Mullica Twp., Batso Fireline Rd., 8.5km SE of Atsion, 27 July 2012 ALHicks, ca. 25 ft asl, 39.6798°N, 74.6705°W. Swept from *Muhlenbergia torreyana*. 6 male and 11 female paratypes, same data.

7 male and 3 female paratypes, NEW JERSEY: Burlington Co., Washington Twp., 10.1 km SE of Atsion, Batso Lk. Rd., 27 July 2012 ALHicks, ca. 20 ft asl, 39.6667°N, 74.6501°W.

Holotype and paratypes in the entomology collection of the University of Colorado Museum of Natural History (UCMC); paratypes in the collection of the United States National Museum of Natural History (USNM).

Figure 1. A stand of *Muhlenbergia torreyana* in the Pine Barrens. Photo courtesy of Uli Lorimer of the Brooklyn Botanic Garden.

Figure 2. Habitus, male, lateral aspect.

Etymology. The specific epithet honors an extraordinary mentor, colleague and friend, the late Dr. Robert Whitcomb, who made, among many other accomplishments, major contributions to leafhopper taxonomy and ecology.

Diagnosis. *F. whitcombi* is included in the *serrata* species group by characters of the male genitalia but easily distinguished from the related *F. serrata* and *huroni* by

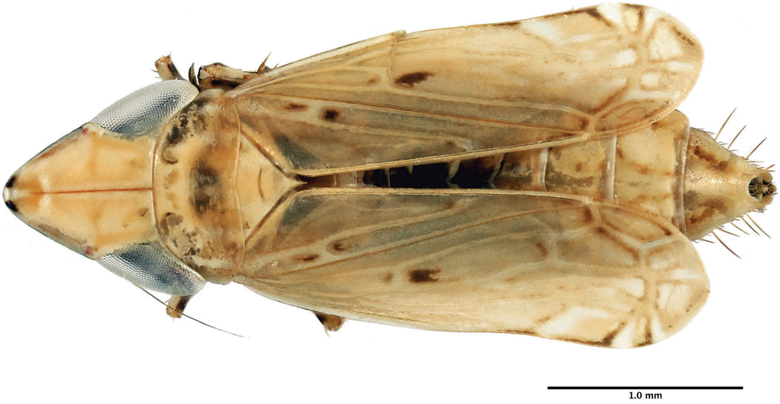

Figure 3. Habitus, female, dorsal aspect.

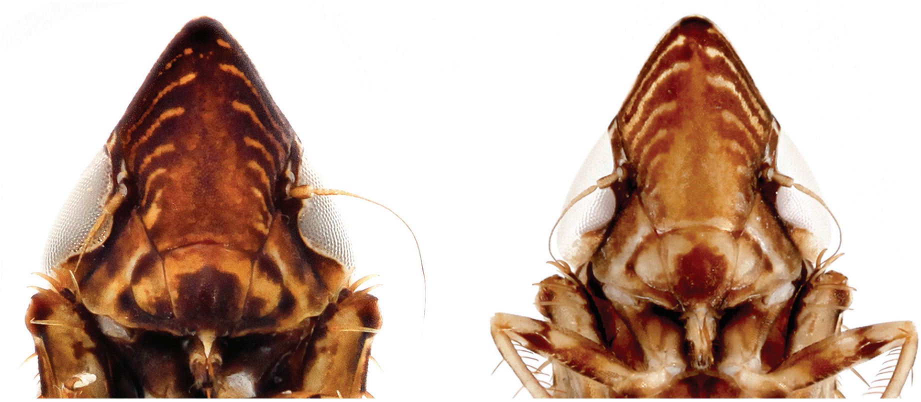

Figure 4. Face, ventral aspect, showing variation in amount of pigmentation.

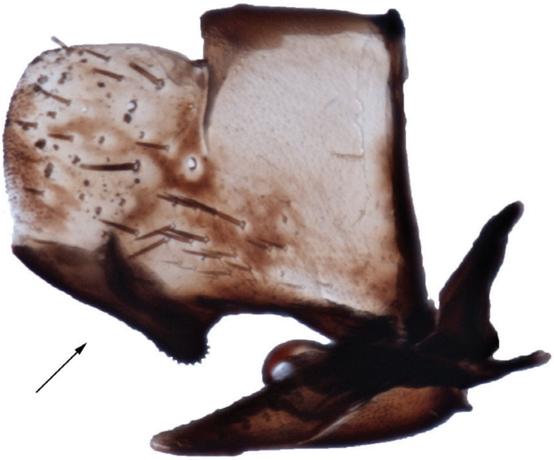

Figure 5. Pygofer and subgenital plates, male, lateral aspect. Note heavily sclerotized caudoventral margin (arrow) and length of subgenital plates relative to pygofer.

Figure 6. Subgenital plates, ventral aspect, outlined for clarity.

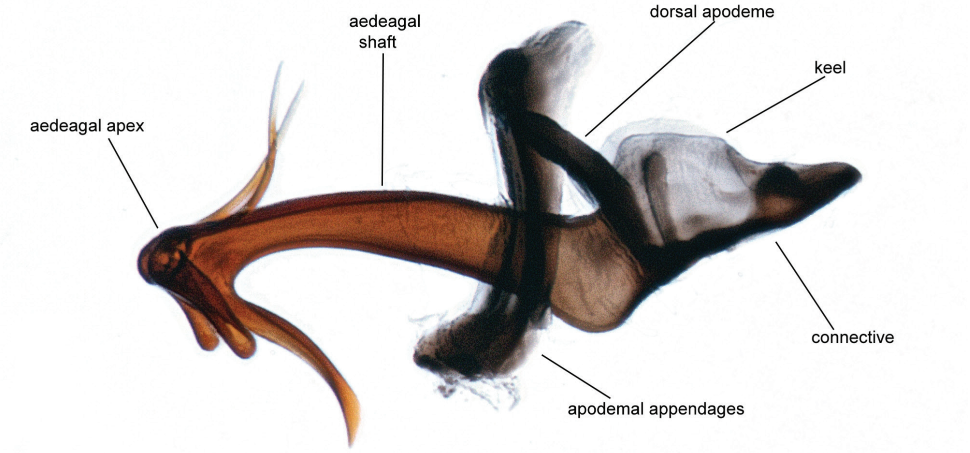

Figure 7. Aedeagus, connective, lateral aspect.

Figure 8. Aedeagus, connective, ventral aspect.

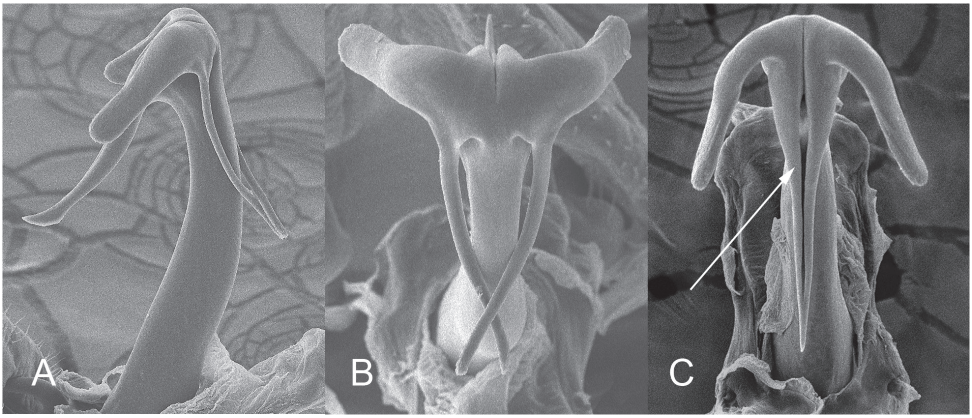

Figure 9. SEMs of the aedeagal apex, from left to right, lateral, dorsal, and caudoventral aspects, the latter illustrating the position of the gonopore on the ventral unpaired process.

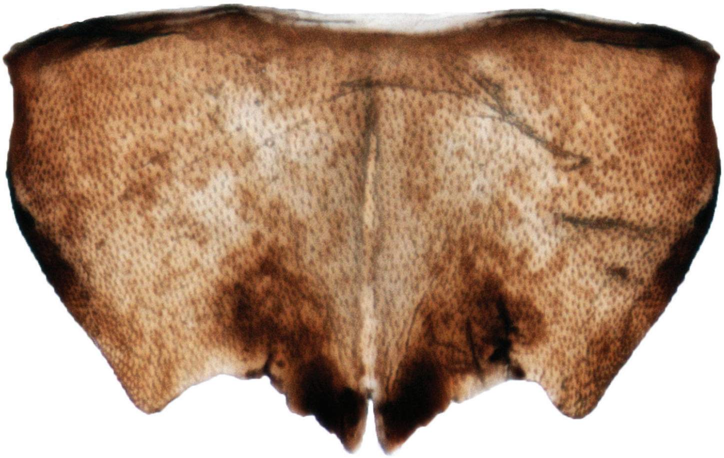

Figure 10. Female 7th sternite. Commonly illustrated for this genus but only occasionally useful to separate species.

its habitus (dark below and stramineous above). In addition, males can be separated from all other species by the denticulate ventral pygofer process and by the apex of the aedeagus, females by the bases of the valvulae (Fig. 11). Because of its habitus and distribution, it is likely to be mistaken for other eastern species like *F. sandersi* (Osborn) but readily differentiated from this and all other *prairiana* group species by the short, blunt subgenital plates (Fig. 6).

Figure 11. Female, bases of valvulae. Within *Flexamia* these structures provide a means for specific identification of female specimens.

Discussion

Osborn and Ball (1898) first associated *Flexamia* with *Muhlenbergia* in the description of *Flexamia imputans* in 1898. The recent transfer (Peterson et al. 2010) of *Redfieldia flexuosa* (Thurb.) Vasey to *Muhlenbergia* as *M. ammophila* and this publication brings the total to twelve of 45 *Flexamia* species documented using *Muhlenbergia* as their host. All of the known host species are found in 2 of the 5 subgenera and sections of *Muhlenbergia*—*Pseudosporobolus* and *Muhlenbergia*. (Peterson et al. 2010; Paul Peterson, pers. comm. 2014).

While *M. torreyana* may be characterized as a relatively overlooked part of the eastern US flora, *Flexamia* is, thanks largely to the efforts of the late Bob Whitcomb, a very well-studied non-vector genus of North American Cicadellidae. As of this publication, host-plant associations (at least to genus) are known for 39 of the 45 valid species. Online range maps and photographs (Leafhopper Distribution Maps; Bugguide) exist for a number of species, there is a recent revision (Whitcomb and Hicks 1988), phylogeny (Dietrich et al. 1999), and recent new species description (Bess and Hamilton 1999).

The relative abundance of *M. torreyana* in the Pine Barrens suggests that it is the epicenter of a host with a very limited distribution (Fig. 12). But to date, as no attempt has been made to look for *F. whitcombi* in Tennessee and the coastal plain of North Carolina where *M. torreyana* occurs, the extent of its range is unclear. The Pine Bar-

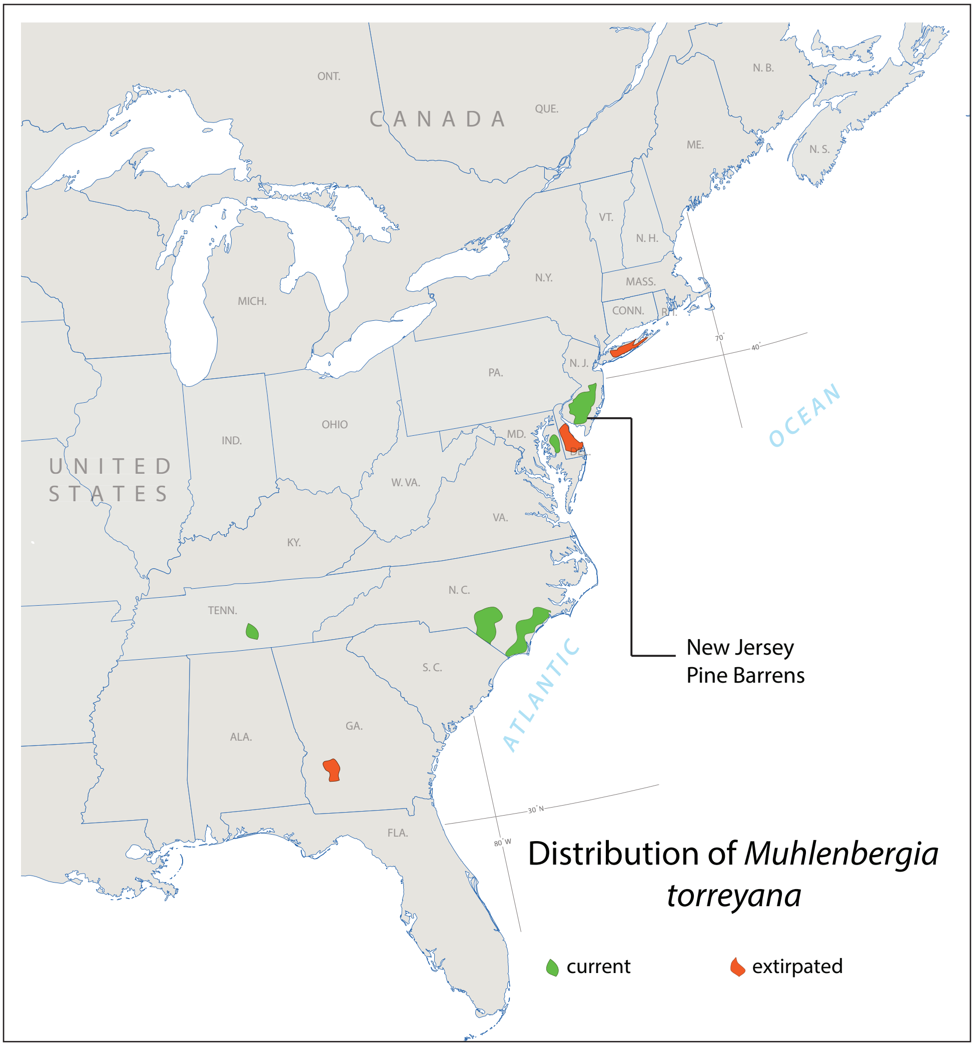

Figure 12. Distribution of *Muhlenbergia torreyana* and location of the Pine Barrens. Modified from data found on the Grass Manual on the Web and BONAP's North American Plant Atlas.

rens are already suffering the effects of a warming climate, as evidenced by the recent irruption there of the Southern Pine Beetle (Gillis 2013). Should the effects of climate change or other anthropomorphic pressures cause the local extinction of the host (as has apparently already occurred elsewhere in its range), there will be little opportunity for the survival of this *Flexamia*. But that might be said of most species described today (Dirzo et al. 2014, Kolbert 2014). The description of any new species may serve as a catalyst for additional research, and this will be best accomplished while the species still can be found in nature—something that can no longer taken for granted. To delay the publication of a species description until the time of a genus revision is to deny the pace of change in the natural world in the 21st century and may consign said new species to a future status of "known from a single collection", or, "presumed extinct, life history unknown".

Key to the males of the *serrata* species group

1	Caudoventral margin of pygofer distinctly thickened, embrowned and terminating ventrally as a denticulate knob; on *M. torreyana*	***whitcombi*** **sp. n.**
–	Caudoventral margin of pygofer not particularly embrowned or thickened, ventral margin lacking process; on *M. richardsonis*	**2**
2	Apical portion of aedeagus with 5 processes	***huroni***
–	Apical portion of aedeagus with 6 processes	***serrata***

Acknowledgments

Dr. Gerry Moore of the Natural Resources Conservation Service in Greensboro, NC kindly led me to the host plant in the field. His assistance greatly facilitated the discovery of this species. The photos of *M. torreyana* posted online by Uli Lorimer of the Brooklyn Botanic Garden were the catalyst for this investigation. Both Gerry Moore and Uli Lorimer shared their time and botanical knowledge of the Pinelands ecosystem. Laura Robinson, Botanist at the NC Natural Heritage Program, provided information about pinebarren smokegrass populations in North Carolina. Virginia Scott and Deane Bowers, Collection Manager and Curator respectively of the Entomology collection at the University of Colorado, provided lab space; Dr. David Stock graciously provided access to the electron microscope. My thanks to two anonymous reviewers for their helpful critiques of the manuscript. Publication of this article was funded by the University of Colorado Boulder Libraries Open Access Fund.

Dedicated to my father, Robert L. Hicks 1922–2012.

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RESEARCH ARTICLE

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Tumidusternus, a new genus of Aspidimerini from China (Coleoptera, Coccinellidae)

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Abstract

Tumidusternus **gen. n.**, along with *T. fujianensis* **sp. n.** (Coleoptera, Coccinellidae, Aspidimerini) from China is described and illustrated. A key to the tribe Aspidimerini is given.

Keywords

Coleoptera, Coccinellidae, Aspidimerini, *Tumidusternus*, new genus, new species, China

Introduction

Aspidimerini was erected by Weise (1900) with two genera: *Aspidimerus* Mulsant, 1850 and *Cryptogonus* Mulsant, 1850. Kapur (1948) revised this tribe and erected two new genera: *Pseudaspidimerus* and *Acarinus*. Huo et al. (2015) added another genus *Trigonocarinatus*. This tribe can be characterized by: (1) legs with trochanters and femora extremely broad and flattened, forming together subrectangular or oval clubs; tibiae and tarsi can be completely hidden under the trochanter-femur club for protection; tibiae flattened, tarsi 3-segmented, tarsal claws bifid; (2) Antenna very short, 8 or 9 segmented, hidden in antennal grooves, partially visible in ventral view; scape large, transversely oval, pedicel smaller and subtriangular, antennal club fusiform or clavated;

(3) Abdomen with 6 ventrites in both sexes; ventrite 1 distinctly longer than ventrite 2, with hind margin arcuate posteriorly.

During our recent study on Aspidimerini, an unusual species was discovered, which prosternum is extremely tumid, highly raised above the ventral surface. A further comparison of more characters (e.g. prosternum, mentum, antenna and legs) with other genera of Aspidimerini revealed that this species is distinctive. Hence, a new genus, *Tumidusternus* gen. n., is here proposed to accommodate this unusual species.

Material and methods

All studied materials were collected from China. Type specimens designated in the present paper are deposited in the Department of Entomology, South China Agriculture University, Guangzhou and the Institute of Zoology (IOZ), Chinese Academy of Science, Beijing.

External morphology was observed with a stereomicroscope (SteREO Discovery V20, Zeiss). Measurements were made using an ocular micrometer attached to the stereomicroscope as follows: (TL) total length, from apical margin of clypeus to apex of elytra; (TW) total width, across both elytra at widest part; (TH) total height, through the highest point of elytra to metaventrite; (HW) head width, including eyes; (PL) pronotal length, from the middle of anterior margin to the base of pronotum; (PW) pronotal width at widest part; (EL) elytral length, along the suture, from the apex to the base including the scutellum; (EW) elytral width, across both elytra at widest part; (ID) interocular distance, nearest distance between two eyes. Male and female genitalia were dissected, cleared in 10% solution of NaOH by boiling for several minutes, and examined with an Olympus BX51 compound microscope. Images were photographed with digital cameras (AxioCam HRc and Coolsnap-Procf & CRI Micro*Color). The software AxioVision Rel. 4.8 and Image-Pro Plus 5.1 were used to capture images from both cameras, images were cleaned up and laid out in plates with Adobe Photoshop CS5. Morphological terms follow Ślipiński (2007) and Ślipiński and Tomaszewska (2010).

Taxonomy

Tumidusternus **Huo & Ren, gen. n.**

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Figures 1–2

Type species. *Tumidusternus fujianensis* Huo & Ren, sp. n.

Diagnosis. *Tumidusternus* can be easily distinguished from other genera of Aspidimerini by middle part of prosternum extremely tumid, highly raised above the ventral surface (Fig. 1a–d) and anterior margin of mentum triangularly emarginate

(Fig. 1g). While in *Acarinus* and *Cryptogonus*, middle part of prosternum is flat and anterior margin of mentum has a small, rectangular notch at middle; in *Aspidimerus*, middle part of prosternum is evenly convex, but not tumid and anterior margin of mentum is truncate, without a notch or an emargination; in *Pseudaspidimerus*, only area of the prosternum between the parallel carinae lies at a higher level than the lateral parts outside the carinae, and anterior margin of mentum possesses a small, triangular emargination at middle.

Description. Body small (TL: 2.73–2.95 mm), oblong oval (TL/TW=1.25–1.29) and moderately convex, dorsum finely punctate and pubescent.

Head. Head transverse, brown. Eyes large, rounded and finely faceted, with sparse interfacetal setae. Clypeus widely emarginate, partially covering labrum. Antenna (Fig. 1h) very short, 9-segmented, with sparse long setae at inner side, hidden in antennal grooves, invisible from above and partially visible from below. Scape large, transversely oval, 1.5 times as wide as long, pedicel smaller and subtriangular, Antennal club fusiform, distinctly longer than width of scape, terminal antennomere acutely conical, as long as penultimate one. Maxilla (Fig. 1f) with cardo and stipes subtriangular. Maxillary palp 3-segmented, always hidden under the cardo and stipes for protection, terminal palpomere securiform. Mentum (Fig. 1g) subtrapezoidal, with anterior margin widely triangularly emarginate and posterior margin slightly incurved, partially covering labium. Labial palp (Fig. 1g) 2-segmented, basal palpomere gradually thicker to apex, apex 2 times as wide as base of terminal palpomere. Terminal palpomere cylindrical, slightly tapering apically, rounded at apex. Mandible (Fig. 1e) broad with apex bifid and basal tooth pointed. Labrum transverse (Fig. 1i), 2.0–2.5 times as long as wide, covered with long and sparse setae.

Prothorax. Prothorax convex and transverse, anterior margin deeply emarginate, lateral margins arcuate with anterior corners rounded and posterior corners nearly orthogonal. Prosternum T-shaped with middle part extremely tumid, highly raised above ventral surface of the body, with surface coarsely punctate and densely pubescent (Fig. 1a–d). Each side folded down constituting a prosternal fold (Fig. 1c). Anterior margin of prosternum with broad border well visible in front view (Fig. 1d). Procoxal cavity distinctly transverse, longitudinal diameter shorter than prosternum in front of coxae. Prosternal process broad, width equal to length of prosternum in front of coxae, with apex rounded. Carinae parallel along 3/4 length of prosternal process then confluent with the tumid part of prosternum (Fig. 1c).

Prerothorax. Mesoventrite (Fig. 1j) with mesoventral process 0.5 times as long as longitudinal mesocoxal diameter; anterior margin widely emarginate and concave at middle to receive prosternal process; mesoventral process as broad as mesocoxal diameter; meso-metaventral junction slightly arcuate anteriorly. Metaventral postcoxal lines joined medially, recurved and complete laterally. Discrimen long but incomplete. Metendosternite stalk 0.5 times as long as broad, tendons separated by less than width of stalk and situated on laminae (Fig. 1l). Scutellum small, subtriangular, black. Elytra moderately convex. Humeral calli weakly visible. Elytral epipleuron incomplete, gradually narrowing from base to 3/5 of elytral length, with clearly delimited cavities to accommodate apices of mid and hind femora (Fig. 2d). Wings well developed. Legs with trochanters and femora extremely broad and flattened, forming together trochanter-femur clubs. Front leg with trochanter-femur club very broad, inner margin partially straight (Fig. 1m); mid and hind leg with trochanter-femur club oval, inner margin arcuate (Fig. 1n–o). Tibia and tarsus can be completely hidden under the trochanter-femur club for protection. Tibia flattened, outer margins with groove for receiving the folded tarsus, tarsi 3-segmented, tarsal claws bifid.

Abdomen. Abdomen with 6 ventrites in both sexes. Ventrite 1 distinctly longer than ventrite 2, at middle 4–5 times as long as ventrite 2 and laterally slightly longer than ventrite 2. Ventrite 2 short at middle, 0.5 times length of lateral margin. Ventrites 3–4 with margins straight, equal in length. Ventrite 5–6 longer than 3–4. Ventrite 6 weakly emarginate apically in male (Fig. 1k) and rounded in female. Postcoxal lines deep but not merging with hind margin of ventrite, laterally incomplete.

Etymology. The generic name is derived from Latin *tumidus* and *sternum*, referring to its tumid prosternum. Gender masculine.

Tumidusternus fujianensis Huo & Ren, sp. n.

http://zoobank.org/1361BBDF-EBC3-45DD-8C06-BCD425C94C81
Figure 2

Types. Holotype: 1♂, **China: Fujian Prov.**: Xiangxi Village, Nanjing County, [24°31.07'N, 117°17.08'E], ca255m, 18.VIII.2012, Huo LZ et al. leg.

Paratypes (8): 1♂2♀, with same data as holotype; **Guangdong Prov.**: 1♂, Hengshitang Town, Yingde City, [24°18.51'N, 113°22.60'E], ca185m, 5.X.2004, Ren SX et al. leg; 1♂, Shimentai Village, Yingde City, [24°25.38'N, 113°18.14'E], ca420m, 29.X.2004, Wang XM leg; 1♂ (deposited in IOZ), Guangdong Ruyuan Grand Canyon, Ruyuan County, [24°31.22'N, 113°07.38'E], ca650m, 23.IX.2005, Wang XM leg; 1♂1♀, Yinna Mountain, Meizhou City, [24°24.16'N, 116°23.40'E], ca450m, 11.X.2014, Wang XM et al. leg.

Diagnosis. This species can be easily distinguished from all known Aspidimerini species by its tumid prosternum.

Description. TL: 2.73–2.95 mm, TW: 2.11–2.35 mm, TH: 1.35–1.46 mm, TL/TW: 1.25–1.29; PL/PW: 0.49–0.52; EL/EW: 1.02–1.06, HW/PW: 0.59–0.62; PW/EW: 0.72–0.73. ID/HW: 0.49–0.50.

Body oblong oval, densely covered with short, silver white pubescence (Fig. 2a–c). Pronotum black with anterior margin and anterior corners yellowish brown (Fig. 2b). Scutellum black. Elytra black with apical 1/3 yellowish brown (Fig. 2a, c). Underside black except prothoracic hypomeron yellowish brown, prosternum, legs and lateral, and posterior margins of abdomen reddish brown (Fig. 2d).

Punctures on frons coarse and very dense, 0.2–0.5 diameters apart; on elytra and pronotum sparse, 1.0–3.0 diameters apart; on metaventrite densely distributed, 0.5–3.0 diameters apart. Ventral surface with short, dense, silver pubescence.

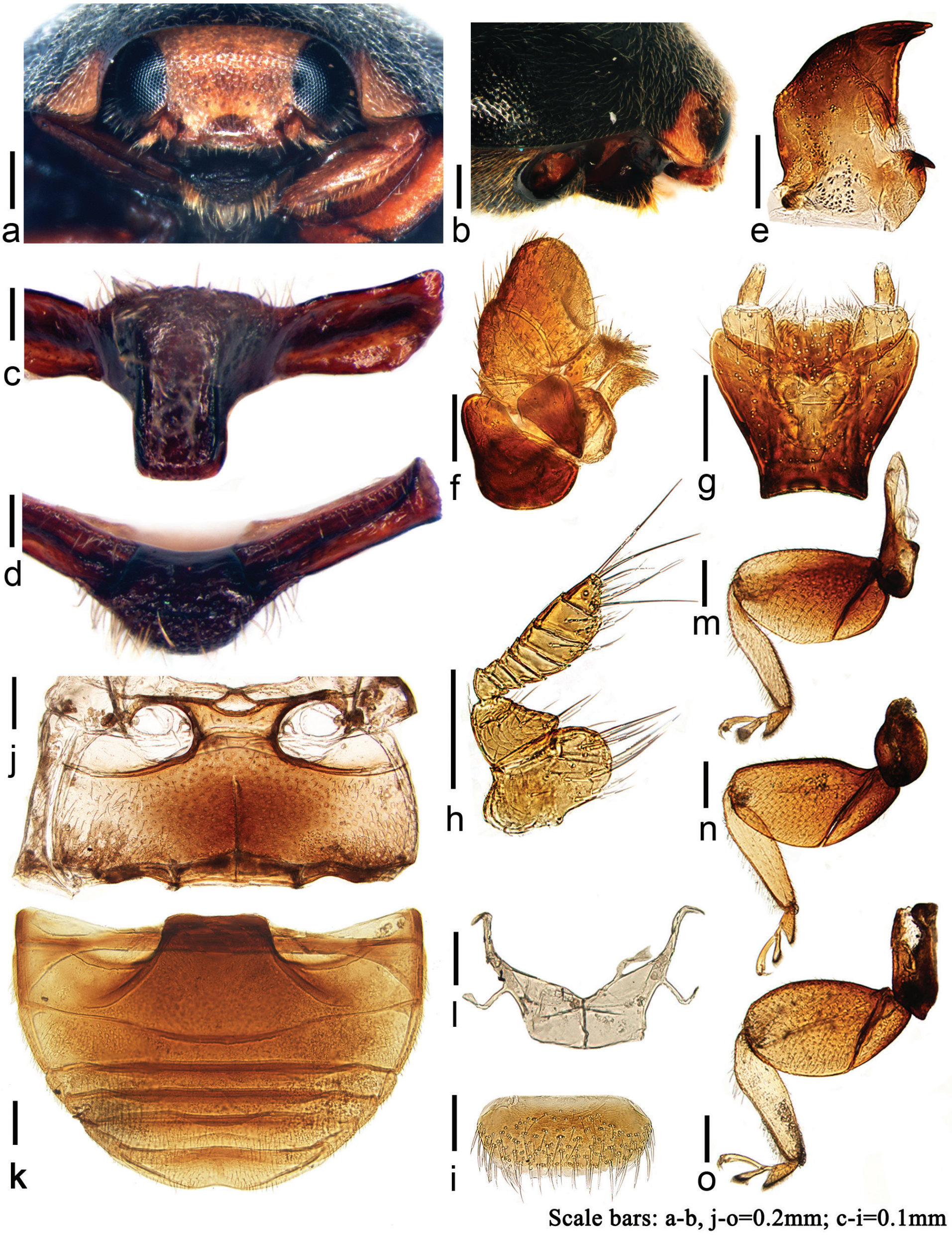

Figure 1. *Tumidusternus fujianensis* Huo & Ren, sp. n. **a** frontal view **b** anterior half of body, lateral view **c** prosternum **d** prosternum, anterior view **e** mandible **f** maxilla **g** labium **h** antenna **i** labrum **j** mesoventrite and metaventrite **k** abdomen, male **l** metendosternite **m** front leg **n** mid leg **o** hind leg.

Male genitalia. Penis slender, curved at basal half, apex pointed (Fig. 2f). Penis capsule with inner arm slightly longer and thinner than outer one. Tegminal strut as long as main part of the tegmen. Parameres 2 times of phallobase length and 1.5 times

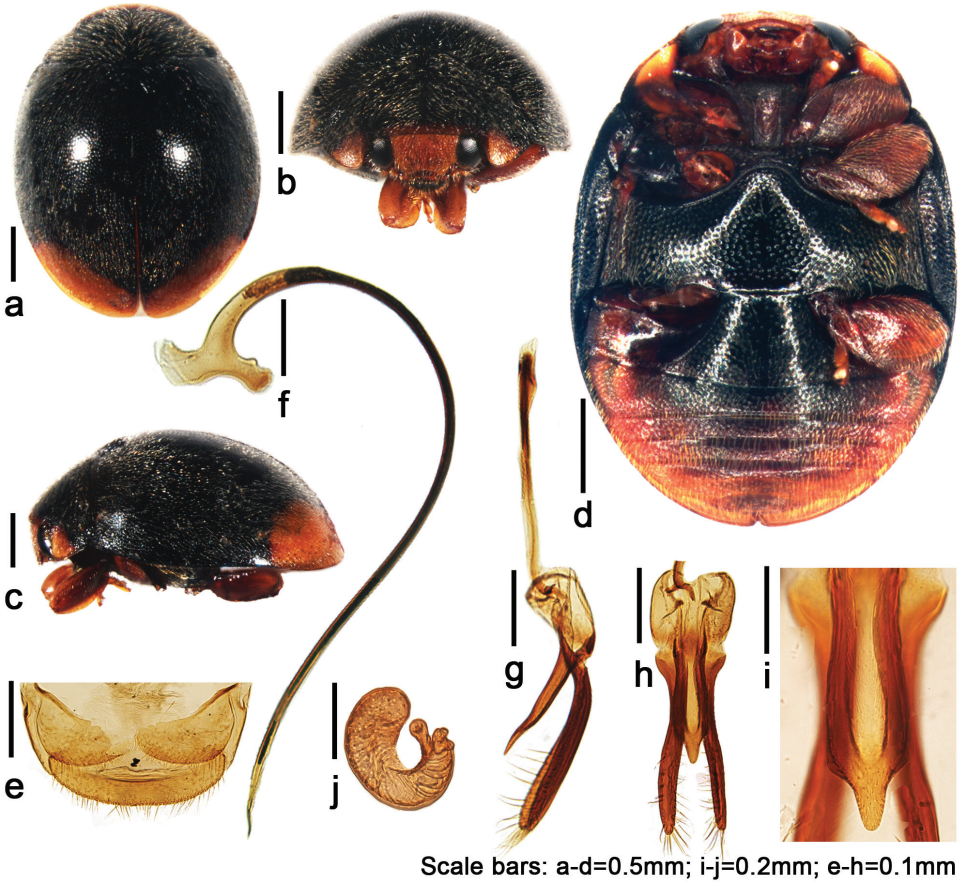

Figure 2. *Tumidusternus fujianensis* Huo & Ren, sp. n. **a** dorsal view **b** anterior view **c** lateral view **d** ventral view **e** ovipositor **f** penis **g** lateral view of tegmen **h** ventral view of tegmen **i** ventral view of penis guide **j** spermatheca.

of penis guide length, apical half with long sparse setae (Fig. 2g–h). Penis guide 4 times as long as wide, parallel for basal 3/4, then convergent to rounded apex (Fig. 2i).

Female genitalia. Coxites subtriangular (Fig. 2e), with dense, short terminal setae. Spermatheca curved, C-shaped, with distinct ramus and short nodulus (Fig. 2j).

Etymology. The specific name refers to the holotype locality, Fujian, China.

Distribution. China (Fujian, Guangdong).

Key to the genera of *Aspidimerini*

1 Prosternal carinae absent .... ***Acarinus* Kapur, 1948**
– Prosternal carinae distinct **2**
2 Prosternal fold absent .... ***Trigonocarinatus* Huo & Ren, 2015**
– Prosternal fold distinct **3**

3 Antennal club fusiform, terminal antennomere conical, as long as penultimate antennomere; trochanter-femur club of front leg extremely broad with inner margin straight at mid length **4**

– Antennal club clavate, terminal antennomere rounded, distinctly shorter than penultimate one, trochanter-femur club of front leg moderately broad with inner margin curved.. **5**

4 Middle part of prosternum extremely tumid, highly raised above the ventral surface of the body............................. ***Tumidusternus* Huo & Ren, gen. n.**

– Middle part of prosternum not tumid, only the rectangular area enclosed by carinae lies at higher level than lateral parts outside carinae.......................... .... ***Pseudaspidimerus* Kapur, 1948**

5 Middle part of prosternum evenly convex, carinae widely divergent anteriorly, the area between them convex and widening anteriorly to form a chinband; trochanter-femur club with inner margin angulate at middle .... ***Aspidimerus* Mulsant, 1850**

– Middle part of prosternum flat, carinae varying in outline; trochanter-femur club with inner margin perfectly arcuate.......... ***Cryptogonus* Mulsant, 1850**

Acknowledgements

The research was supported by the National Natural Science Foundation of China (2006FY120100, 2008FY210500) and Science and Technology Program of Guangzhou, China (151800033).

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RESEARCH ARTICLE

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Contribution to the genus *Xanthocorus* Miyatake (Coleoptera, Coccinellidae, Chilocorini)

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Abstract

The genus *Xanthocorus* Miyatake, 1970 consists of three species from China, including two new species described here: *X. nigrosuturalis* **sp. n.** and *X. mucronatus* **sp. n.** A key to identification of species is given. Diagnoses, detailed descriptions, illustrations, and distributions are provided.

Keywords

Coleoptera, Coccinellidae, *Xanthocorus*, new species, China

Introduction

Xanthocorus Miyatake, 1970 is a small genus within the tribe Chilocorini which currently consists of 26 genera (Łączyński and Tomaszewska 2012). Initially Miyatake (1970) treated *Xanthocorus* as the subgenus of *Exochomus*, with *Exochomus* (*Xanthocorus*) *nigromarginatus* Miyatake, 1970 as the type species. Kovář (1997) comprehensively revised the genera *Brumus* Mulsant and *Exochomus* Redtenbacher, and considered *Xanthocorus* Miyatake as a distinct genus. Kovář's (1997) generic concept of *Xanthocorus* was accepted by Fürsch (2007) and is followed in the present paper.

The genus *Xanthocorus* was monotypic until recently. It was only recorded from China (Miyatake 1970; Pang and Mao 1979; Pang et al. 2004; Ren et al. 2009) and without any new species added in recent decades. In this paper two new species of *Xanthocorus* from China are described bringing to three, the number of known species in the genus. A diagnosis of the genus and a key to its known species are also given.

Material and methods

Specimens examined in this study were collected in China. Type specimens designated in the present paper are deposited at the Department of Entomology, South China Agriculture University (SCAU), Guangzhou, and the Institute of Zoology (IOZ), Chinese Academy of Science, Beijing.

External morphology was observed with a dissecting stereoscope (SteREO Discovery V20). The following measurements were made with an ocular micrometer: TL–total length, length from apical margin of clypeus to apex of elytra; TW–total width, width across both elytra at widest part; TH–total height, from the highest part of the beetle to elytral outer margins; HW–head width in frontal view, head widest part; PL–pronotal length from the middle of anterior margin to the base of pronotum; PW–pronotal width at widest part; EL–elytral length, from the apex to the base including the scutellum; EW–elytral width, equal in TW. Morphological terms of the Coccinellidae follow Ślipiński (2007) and Ślipiński and Tomaszewska (2010).

Male and female genitalia were dissected, cleared in 10% solution of NaOH by boiling for several minutes, and examined with an Olympus BX51 microscope. Photographs of the morphological characters of the genitalia were generated with digital cameras (AxioCam HRc and Coolsnap-Procf & CRI Micro*Color), attached to microscopes using AxioVision Rel. 4.8 and Image-Pro Plus 6.0 to capture images, and photographs were cleaned up and laid out in plates with Adobe Photoshop CS 8.0.

Taxonomy

Xanthocorus Miyatake, 1970

Exochomus (*Xanthocorus*) Miyatake, 1970: 312. Type species: *Exochomus* (*Xanthocorus*) *nigromarginatus* Miyatake, 1970, by original designation. Validated by Kovář 1997: 24.

Diagnosis. The genus *Xanthocorus* can be distinguished from other genera of the tribe Chilocorini by the following combination of characters: antenna composed of 10 antennomeres, relatively slender, terminal antennomere very small and inserted in antennomere 9 (Fig. 1a); pronotal basal margin without bordering line; prosternal process narrow, without carinae (Fig. 1e); elytral epipleura distinctly oblique and without foveae;

abdominal postcoxal lines semicircular, incomplete laterally (Figs 2d, 3d, 4d); front tibiae without apical spurs, mid and hind tibiae with two apical spurs (Figs 1h, 1i); tarsal claw with basal tooth (Fig. 1j).

Description. Body broadly oval to almost circular in outline, moderately convex. Dorsum glabrous.

Head relatively large, 0.50–0.58 times pronotal width, covered with short, greyish pubescence; antenna composed of 10 antennomeres, relatively slender, scape and pedicel stout, of the same width, and pedicel distinctly shorter than scape, antennomeres 3–8 gradually broadening, antennomere 9 slightly narrower than 8, and terminal antennomere short, partially embedded in antennomere 9 (Fig. 1a). Mandible unidentate, prostheca distinct, lateral margin of mandible strongly curved (Fig. 1b). Terminal maxillary palpomere broadening apically, apical margin strongly obliquely truncate (Fig. 1d). Terminal labial palpomere stout with rounded apex (Fig. 1c).

Prothorax descending anteriorly. Base of pronotum and elytra not contiguous all along their length. Basal margin of pronotum without bordering line. Prosternum T-shaped, in front of coxae distinctly longer than basal width of prosternal process. Prosternal process narrow, parallel sided, without carinae (Fig. 1e). Mesoventrite approximately trapezoidal, mesal surface with emarginate fossa for receiving apex of prosternal process. Mesoventral process narrow; meso-metaventral process narrow, junction arcuate anteriorly, with visible suture (Fig. 1f). Metendosternite stalk as long as broad (Fig. 1g). Scutellum small,triangular. Elytra distinctly wider than pronotum at base, surface finely or coarsely punctate. Elytral epipleura distinctly oblique and without foveae. Abdomen with five ventrites in female and six ventrites in male. Abdominal postcoxal lines recurved, incomplete laterally. Front tibiae without apical spurs, mid and hind tibiae with two apical spurs (Fig. 1h, 1i); tarsal claw with basal tooth (Fig. 1j).

Key to species of the genus *Xanthocorus* from China

1 Body broadly oval, elytra yellow or brownish yellow, suture black **2**
– Body almost circular, elytra yellow with only lateral margins black (Fig. 2a).....
....***X. nigromarginatus***
2 Elytra yellow, with lateral and anterior margins, and suture black (Fig. 3a), penis with blunt and bifurcate apex (Fig. 3f)............***X. nigrosuturalis* sp. n.**
– Elytra brownish yellow, only elytral suture black (Fig.4a), penis with pointed apex (Fig. 4f) ....***X. mucronatus* sp. n.**

***Xanthocorus nigromarginatus* (Miyatake, 1970)**

Figs 1a–j, 2a–i, 5

Exochomus (*Xanthocorus*) *nigromarginatus* Miyatake, 1970: 312; Pang and Mao 1979: 76; Cao et al. 1992: 152; Pang et al. 2004: 316; Ren et al. 2009: 136.

Xanthocorus nigromarginatus: Kovář, 1997: 24.

Diagnosis. This species can be distinguished from other species of *Xanthocorus* by the following combination of characters: body almost circular, pronotum black with anterior angles and anterior margin yellow; elytra yellow, only elytral lateral margins of elytra black (Fig. 2a–c); apex of penis bifurcate, penis guide slightly asymmetrical, parameres stout (Fig. 2e–h).

Description. TL: 5.53–5.92 mm, TW: 5.11–5.30 mm, TH: 2.80–2.88 mm, TL/TW: 1.08–1.12, PL/PW: 0.49–0.50, EL/EW: 0.97–0.99.

Body almost circular, moderately convex. Head yellow with black vertex in male, entirely black in female. Mouthparts and antennae brown, sparsely covered with short, greyish pubescence. Pronotum black, only anterior angles and anterior margin yellow. Scutellum black. Elytra yellow, only elytral bead black (Fig. 2a–c). Underside black except inner part of elytral epipleura yellow and abdominal ventrites brownish black, sparsely covered with short, greyish pubescence.

Head relatively large, 0.50 times pronotal width, punctation on frons large and densely distributed, 0.5–1.5 diameters apart, surface polished between punctation. Eyes approximately oval, densely faceted, interocular distance 0.54 times head width (Fig. 2c). Pronotum 0.54 times elytral width, pronotal punctation large and densely distributed, smaller than those on head, 1.0–2.0 diameters apart, surface polished between punctation. Punctation on elytra moderately large and densely distributed, 1.0–2.0 diameters apart, similar to those on pronotum. Prosternal process narrow with sides parallel. Posterior margin of abdominal ventrite 5 and 6 slightly emarginate medially in male (Fig. 2d).

Male genitalia: penis slender, penis capsule with short outer and inner arms, apex of penis bifurcate with membranous appendage (Fig. 2e–f). Tegmen stout with penis guide slightly asymmetrical in ventral view and widest at base with sides parallel from base to 1/2 length, then gradually converging to blunt apex in lateral view. Parameres stout, distinctly longer than penis guide, densely covered with long setae at inner surfaces and apices with group of long setae in lateral view (Fig. 2g–h).

Female genitalia: coxites distinctly elongate, approximately triangular (Fig. 2i).

Material examined. China: Jiangxi Prov: 2 males and 1 female, Luofu village, Jinggangshan County, [26°65.41'N; 114°22.49'E], ca 763m, 18.ix.2004, Wang XM leg; 1 female, Jingzhu Mountain, Jinggangshan County, [26°37.95'N; 114°08.98'E], ca 1142m, 22.ix.2004, Wang XM leg.

Distribution. China (Gansu, Zhejiang, Jiangxi, Fujian, Yunnan) (Fig. 5).

Remarks. The Chinese specimens of this species studied were identical with the illustrations and descriptions of adult and male genitalia given by Miyatake (1970).

Xanthocorus nigrosuturalis Li & Ren, sp. n.

http://zoobank.org/720BAC11-200D-408E-8D30-B68B2FD92B95
Figs 3a–j, 5

Diagnosis. This species resembles *Xanthocorus nigromarginatus*, but can be distinguished from it by having slightly elongate body; black pronotum with only anterior

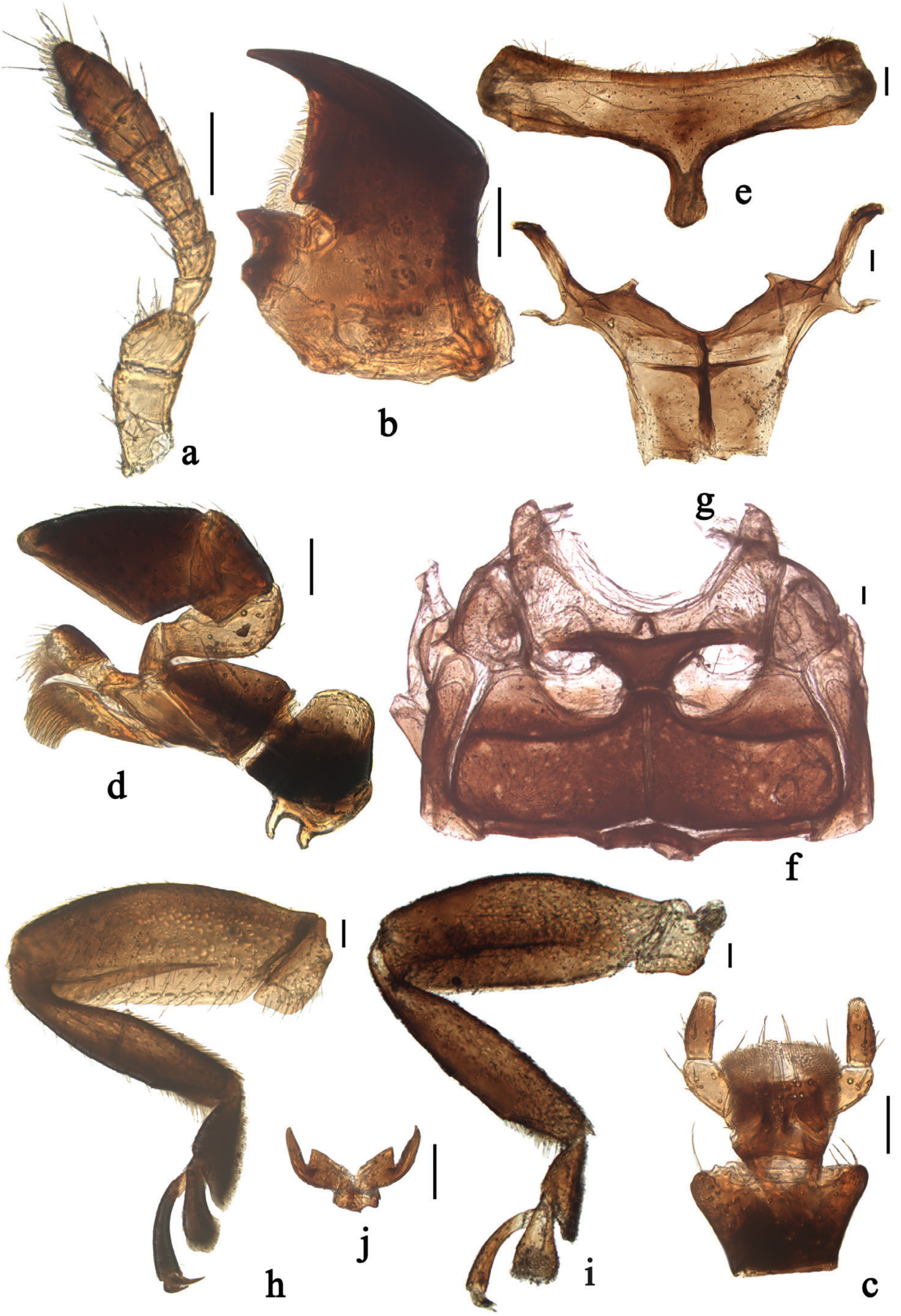

Figure 1. *Xanthocorus nigromarginatus* (Miyatake, 1970). **a** antenna **b** mandible **c** labium **d** maxilla **e** prosternum **f** meso- and metaventrite **g** metendosternite **h** front leg **i** hind leg **j** tarsal claws. Scale bars: 0.1 mm.

angles yellow; elytra yellow with black elytral lateral margin, anterior margin and suture (Fig. 3a–c); slender parameres (Fig. 3g). In *X. nigromarginatus*, body almost circular, pronotum black except anterior angles and anterior margin yellow; elytra yellow, only elytral lateral margin black (Fig. 2a–c); parameres stout (Fig. 2g).

Description. TL: 4.16–5.79 mm, TW: 3.35–4.81 mm, TH: 1.83–2.77 mm, TL/TW: 1.20–1.24, PL/PW: 0.47–0.51, EL/EW: 1.04–1.05.

Body broadly oval, moderately convex. Head and mouthparts black. Antenna brownish yellow, sparsely covered with short, greyish pubescence. Pronotum black with anterior angles yellow. Scutellum black. Elytra yellow with black elytral bead, anterior margin and suture (Fig. 3a–c). Underside black except inner margins of elytral epipleura yellow. Abdominal ventrites brown, sparsely covered with short, greyish pubescence.

Head relatively large, 0.56 times pronotal width, punctation on frons large and densely distributed, 1.0–1.5 diameters apart, surface polished between punctation. Eyes approximately oval, densely faceted, interocular distance 0.51 times head width (Fig. 3c). Pronotum 0.55 times elytral width, pronotal punctation large and moderately densely distributed, smaller than those on head, 2.0–3.0 diameters apart, surface polished between punctation. Punctation on elytra moderately large and moderately densely distributed, 2.0–3.0 diameters apart, similar to those on pronotum. Prosternal process narrow with parallel sided. Posterior margin of male abdominal ventrite 5 distinctly emarginate and ventrite 6 slightly emarginate medially (Fig. 3d).

Male genitalia: penis slender, penis capsule with short outer and inner arms. Apex of penis bifurcate with membranous appendage (Fig. 3e–f). Tegmen stout with penis guide distinctly asymmetrical in ventral view and with sides parallel from base to 2/3 length, then abruptly converging to blunt apex in lateral view. Parameres slender, strongly constricted at base, expanded toward apex, distinctly longer than penis guide, densely covered with long setae at inner surfaces and apices with group of long setae in lateral view (Fig. 3g–h).

Female genitalia: coxites distinctly elongate, approximately triangular (Fig. 3i). Spermatheca C-shaped, cornu without appendage (Fig. 3j).

Types. Holotype. male, China: Shannxi Prov: Daguping and Xihe Conservation Station, Foping National Nature Reserve, No. SCAU (E) 11475, [33°35.57'N; 107°50.25'E], ca 1428m, 22.vii.2009, Wang XM leg (SCAU). Paratypes. 1 male and 1 female with same data as holotype (1 female in SCAU, 1 male in IOZ); Sichuan Prov: 7 females, Mamize village, Leibo County, [28°25.02'N; 103°36.13'E], ca 2600m, 19–20.ix.2007, Liang JB leg (SCAU); 2 females, Dafengding National Nature Reserve, Meigu County, [28°60.77'N; 103°23.72'E], ca 2400m, 21.ix.2007, Chen XS leg (SCAU); 2 males and 1 female, Liziping National Nature Reserve, Shimian County, [29°09.89'N; 102°33.84'E], ca 2000m, 26–27.ix.2007, Chen XS leg (2 males in SCAU, 1 female in IOZ). Hubei Prov: 1 male and 1 female, Banqiao Conservation Station, Shennongjia National Nature Reserve, [31°45.90'N; 110°37.02'E], ca 1170m, 21–24.vii.2007, Wang XM leg (SCAU); 4 females, Guanmenshan Scenic Spot, Shennongjia National Nature Reserve, [31°42.94'N; 110°36.53'E], ca 1260m, 2.viiiI.2007, Chen XS leg (SCAU).

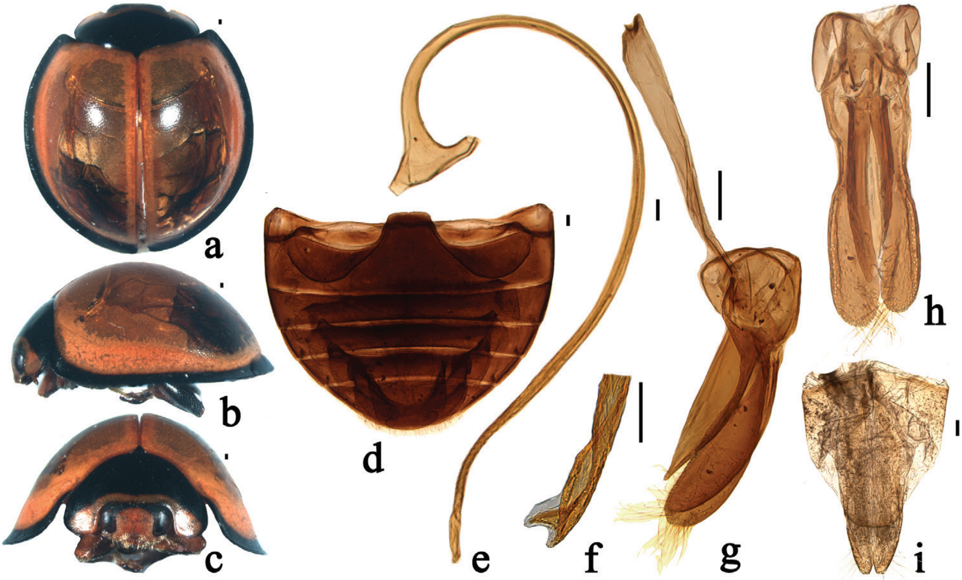

Figure 2. *Xanthocorus nigromarginatus* (Miyatake, 1970). **a** dorsal view **b** lateral view **c** anterior view **d** abdomen **e** penis **f** apex of penis **g** tegmen, lateral view **h** tegmen, ventral view **i** female genitalia: ovipositor. Scale bars: 0.1 mm.

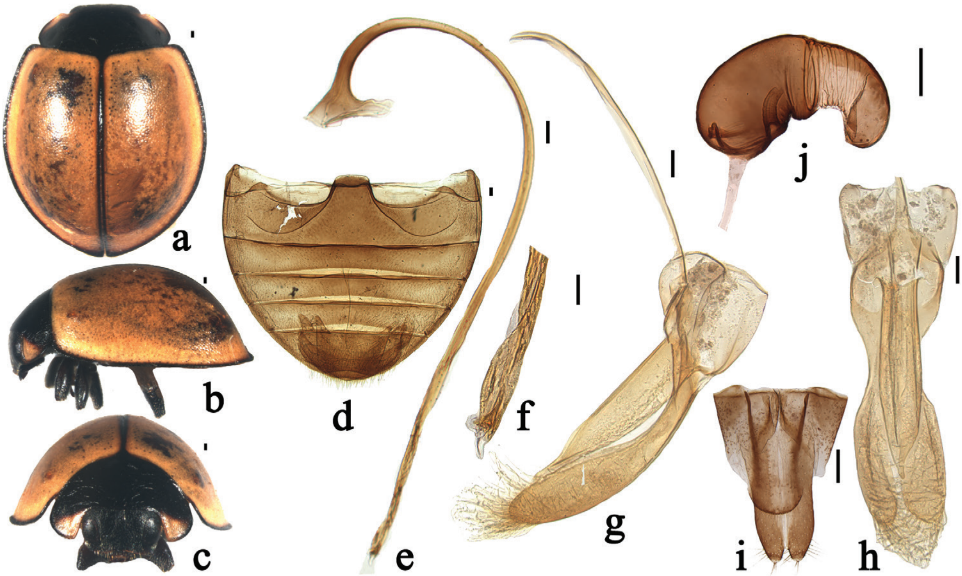

Figure 3. *Xanthocorus nigrosuturalis* sp. n. **a** dorsal view **b** lateral view **c** anterior view **d** abdomen **e** penis **f** apex of penis **g** tegmen, lateral view **h** tegmen, ventral view **i–j** female genitalia: **i** ovipositor **j** spermatheca. Scale bars: 0.1 mm.

Distribution. China (Hubei, Sichuan, Shannxi) (Fig. 5).

Etymology. The species name is derived from Latin and refers to the black elytral suture.

Xanthocorus mucronatus Li & Ren, sp. n.

http://zoobank.org/190749C1-140A-438F-877B-2011BCCB6D7D

Figs 4a–h, 5

Diagnosis. This species is similar to *Xanthocorus nigrosuturalis* sp. n., but can be distinguished from it by having lateral margins of elytra yellow (Fig. 4a) and apex of penis pointed (Fig. 4f). In *X. nigrosuturalis* sp. n., elytral lateral margin is black (Fig. 3a) and penis has blunt and bifurcate apex (Fig. 3f).

Description. TL: 3.75 mm, TW: 2.98 mm, TH: 1.62 mm, TL/TW: 1.27, PL/PW: 0.49, EL/EW: 1.01.

Body broadly oval, moderately convex. Head black except sides of clypeus yellow. Mouthparts black. Antenna brown, sparsely covered with short, greyish pubescence. Pronotum black with anterior angles yellow. Scutellum black. Elytra yellow with black suture (Fig. 4a–c). Underside black except elytral epipleura yellow, sparsely covered with short, greyish pubescence.

Head relatively large, 0.58 times pronotal width, punctation on frons fine and moderately densely distributed, 2.0–3.0 diameters apart, surface polished between punctation. Eyes approximately oval, densely faceted, interocular distance 0.55 times head width (Fig. 4c). Pronotum 0.55 times elytral width, pronotal punctation fine and sparsely distributed, smaller than those on head, 3.0–4.0 diameters apart, surface polished between punctation. Punctation on elytra moderately fine and sparsely distributed, 2.0–3.0 diameters apart, similar to those on pronotum. Prosternal process narrow with sides parallel. Posterior margin of abdominal ventrite 5 and 6 distinctly emarginated medially in male (Fig. 4d).

Male genitalia: penis slender, penis capsule with short outer and inner arms. Apex of penis strongly narrow with membranous appendage (Fig. 4e–f). Tegmen stout with penis guide with parallel sided from base to 1/2 length, then gradually converging to blunt apex in ventral view; in lateral view, penis guide widest at base, gradually constricted to apex. Parameres strongly constricted at base and expanded toward apex, longer than penis guide, densely covered with long setae at the inner sides and distal end with a group of long setae in lateral view (Fig. 4g–h).

Female genitalia: unknown.

Types. Holotype. male, **China: Yunnan Prov:** Shangri-La, No. 20051215051, [27°90.22'N; 99°63.37'E], ca 3450m, 3.ix.2005, Wang XM leg (SCAU).

Distribution. China (Yunnan) (Fig. 5).

Etymology. The species name is derived from Latin and refers to the pointed apex of penis.

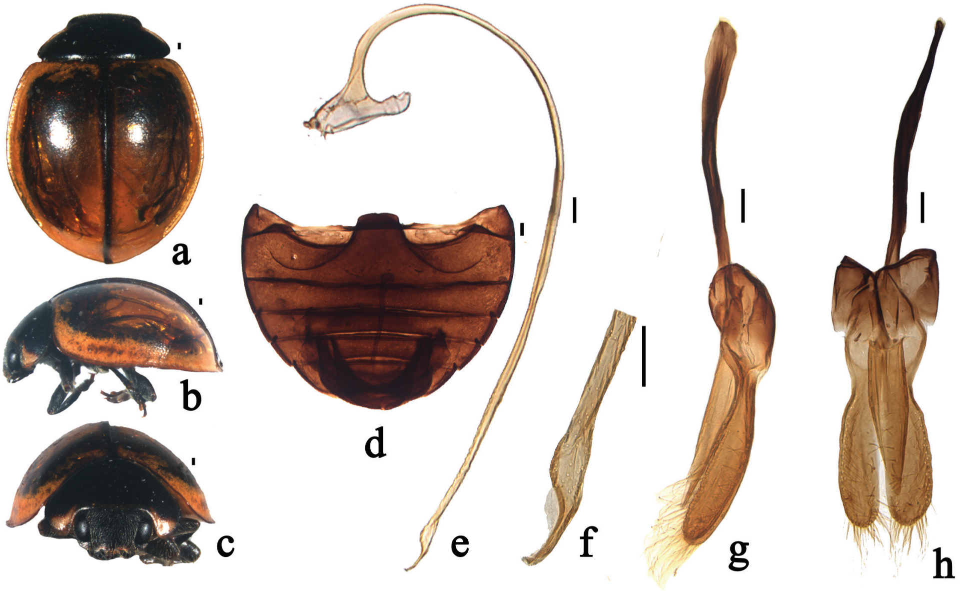

Figure 4. *Xanthocorus mucronatus* sp. n. **a** dorsal view **b** lateral view **c** anterior view **d** abdomen **e** penis **f** apex of penis **g** tegmen, lateral view **h** tegmen, ventral view. Scale bars: 0.1 mm.

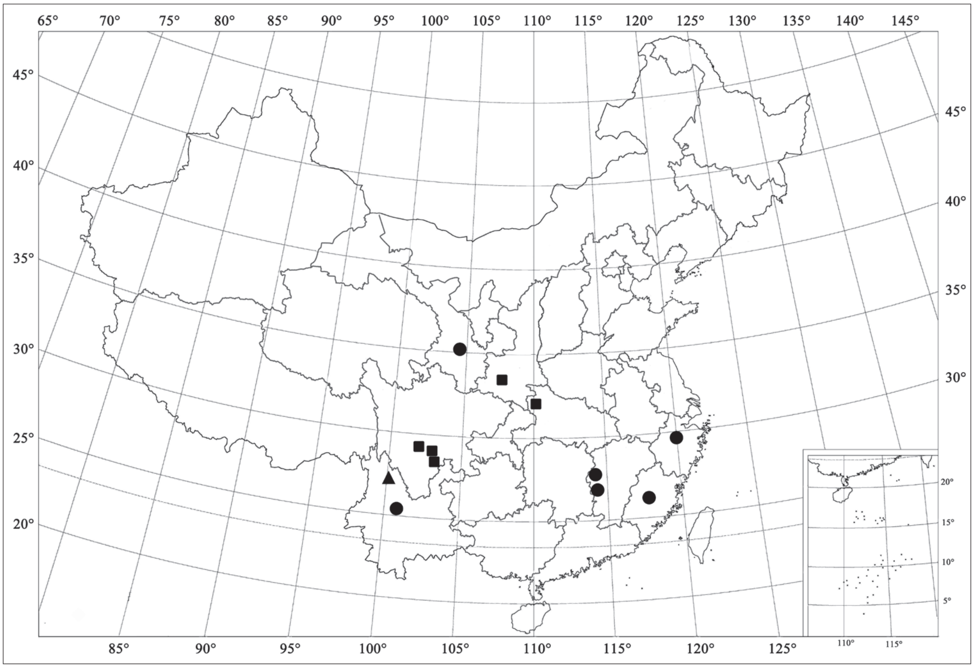

Figure 5. Distribution map. (●) *Xanthocorus nigromarginatus* (Miyatake, 1970); (■) *Xanthocorus nigrosuturalis* sp. n.; (▲) *Xanthocorus mucronatus* sp. n.

Acknowledgements

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A review of the omicrine genera *Omicrogiton*, *Mircogioton* and *Peratogonus* of China (Coleoptera, Hydrophilidae, Sphaeridiinae)

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Abstract

The Chinese species of the genera *Omicrogiton* Orchymont, 1919, *Peratogonus* Sharp, 1884 and *Mircogioton* Orchymont, 1937 are reviewed, diagnosed and keyed. *Mircogioton* and *Omicrogiton* are reported for the first time from China, *Peratogonus* for the first time for mainland China. Five species are recognized: *Omicrogiton coomani* Balfour-Browne, 1939 (Guangdong, Hongkong), *O. hainanensis* **sp. n.** (Hainan), *O. roberti* **sp. n.** (Hainan), *Mircogioton coomani* Orchymont, 1937 (Yunnan), and *Peratogonus reversus* Sharp, 1884 (Guangdong, Jiangxi, Taiwan). Lectotype of *Omicrogiton coomani* is designated. *Mircogioton cognitus* (Malcolm, 1981), **syn. n.** is considered a junior subjective synonym of *M. coomani* Orchymont, 1939. Species of *Mircogioton* and *Omicrogiton* inhabit decaying banana trunks, whereas *Peratogonus reversus* was always collected from moist forest leaf litter.

Keywords

Hydrophilidae, Sphaeridiinae, Omicrini, *Omicrogiton*, *Mircogioton*, *Peratogonus*, new species, new synonym, new record, Oriental region, China

Introduction

A total of 15 genera and 104 species of the tribe Omicrini Smetana, 1975 have been described world-wide (Hansen 1999; Short and Fikáček 2011, 2013). Eleven of these genera may be found in the Oriental Region: *Oreomicrus* Malcolm, 1980, *Tylomicrus* Schödl, 1995, *Nannomicrus* Bameul, 1991, *Litrosurus* Orchymont, 1925, *Stanmalcolmia* Bameul, 1993, and *Mircogioton* Orchymont, 1937 are endemic to the Oriental Region, *Peratogonus* Sharp, 1884 and *Noteropagus* Orchymont, 1919 are principally Oriental but also reach the Palaearctic or Pacific Regions, respectively. *Paromicrus* Scott, 1913 and *Psalitrus* Orchymont, 1919 occur in the Oriental and Afrotropical Regions (Africa), and *Aculomicrus* Smetana, 1990 that occurs in the Neotropic Region reaches the Oriental Region only in the Malay Archipelago (and this Bornean species may actually belong to a different genus: Fikáček 2010). Only two species of the tribe were so far recorded from China, in both cases from Taiwan: *Peratogonus reversus* Sharp, 1884 by Knisch (1921) and *Psalitrus sauteri* Orchymont, 1929 by Orchymont (1929). Not a single species of the tribe was so far recorded from the mainland China.

Since 2009, a lot of material of the tribe Omicrini was collected by us or our colleagues in various parts of Southern China, confirming that at least six omicrine genera occur in mainland China and/or Taiwan: *Psalitrus*, *Noteropagus*, *Paromicrus*, *Peratogonus*, *Omicrogiton* and *Mircogioton*. The latter three genera are revised in this contribution, in which we are providing diagnoses, identification keys and biology data of five species, of which two are described as new.

Material and methods

Male genitalia were dissected in a portion of specimens of each species. In specimens deposited in SYSU, dissected genitalia was transferred to a drop of absolute alcohol for removing membranes after 8-10 hours in 10% KOH at room temperature, and subsequently mounted into a drop of glycerine on a piece of transparent plastic slide attached below the respective specimens. In specimens deposited in NMPC and in the holotype of *O. hainanensis* sp. n., the dissected male genitalia were mounted into a drop of alcohol-soluble Euparal resin on a piece of glass attached below the respective specimens. Specimens from BMNH were dissected by R. B. Angus, the genitalia were placed without any additional treatment into a water-soluble dimethyl hydantoin formaldehyde resin on the same card as the beetle. Male genitalia and morphological characters were examined using a Nikon SMZ800 compound microscope. Genitalia photographs were taken using a Zeiss Axioskop 40 or Olympus BX41 compound microscopes and combined with AutoMontage or Helicon Focus software, respectively. Photographs of habitus and external morphology were taken using a Leica M205C stereomicroscope and combined with AutoMontage software.

Detailed descriptions of the tribe Omicrini and the genera treated in this study were provided by Hansen (1991). Morphological terminology largely follows Hansen (1991) and Komarek (2004), classification follows Short and Fikáček (2013).

Examined specimens are deposited in the following collections:

AFCD	Agriculture, Fisheries and Conservation Department, Hong Kong;
BMNH	Natural History Museum, London;
IRSN	Institute Royal de Sciences naturelles, Brussels, Belgium;
IZCAS	Chinese Academy of Sciences, Institute of Zoology, Beijing, China;
NMPC	National Museum, Prague, Czech Republic;
MNHG	Museum d'Histoire naturelle, Genève, Switzerland;
SYSU	Entomological Collection of Sun Yat-sun University, Guangzhou, China.

For comparative reasons, we have examined also the following material of *Omicrogiton* species not occurring in China:

Omicrogiton gomyi Bameul, 1986: Holotype: male (MNHG): La Réunion / Takamaka 26-I-78 / chemin du Barrage / tamisage souche très humide / Y Gomy // male symbol // HOLOTYPE // Omicrogiton / gomyi n. sp. / HOLOTYPE / F. BAMEUL det. 1985. The specimen is dissected and its genitalia were probably mounted in a drop of dimethyl hydantoin formaldehyde resin, which is still present on a piece of transparent plastic below the specimen. However, we failed to find any genitalia in this drop – either they were never placed there, or they became completely transparent due to the long-term effect of dimethyl hydantoin formaldehyde resin. We were therefore not able to compare the genital morphology of this species with that of *O. hainanensis* sp. n., as originally planned. New material from Reunion Island is necessary to perform this detailed comparison.

Omicrogiton insularis Orchymont, 1919: Syntype: 1 female (IRSN): Engamo / Bua-Bua V.-VI. / Modigliani 1891 // Coll. A. d'Orchymont // Para- / type // A. d'Orchymont det / Omicrogiton / insularis Orch. / Cotype. Additional specimens: 1 male (NMPC): Sarawak, Kapit distr., Sebong, Baleh riv., 6-21.iii.1994, Sv. Bílý lgt.; 1 male, 2 females, 1 unsexed specimen (NMPC): Solomon Islands, Guadalcanal, Mt. Austine – Barana vill. env. (gardens, in rotten *Musa*), 9°28.0'S 159°58.4'E, 280 m, 23.xi.–8.xii.2013, Jiří Hájek lgt.

Key to Chinese Omicrini

The following key allows to identify all genera of the tribe Omicrini occurring in China based on our published and unpublished data, and all species of the genus *Omicrogiton* based on the revision performed in this paper. The generic key is adapted from that of Hansen (1991).

1 Antenna with 8 antennomeres, antennal club loosely segmented. Mesoventral plate slightly wider than long, subpentagonal, contacting metaventral process .... ***Psalitrus* Orchymont, 1919**
(more species known from China, to be revised by the authors)

– Antenna with 9 antennomeres, antennal club compact. Mesoventral plate either wider than long, longer than wide, or distinctly isolated from metaventrite **2**

2 First ventrite not carinate medially. Mesoventral plate narrowly elongate.... **3**

– First ventrite carinate medially. Mesoventral plate broadly pentagonal........ **4**

3 Mesoventral plate fused with metaventral process, forming a common meso-metaventral keel. First metatarsomere much longer than second metatarsomere (best seen in dorsal view)................ ***Mircogioton* Orchymont, 1937**
(one species known from China: *M. coomani* Orchymont, 1937)

– Mesoventral plate not contacting metaventrite, separated from the latter by a broad gap. First metatarsomere only a little longer than second metatarsomere (best seen in dorsal view)............... ***Omicrogiton* Orchymont, 1919**

a Pronotum with fine mesh-like microsculpture on interstices (best seen with spot light and using the light diffuser). Adeagus as in Figs 17–18, with parameres lacking the S-shaped sclerite and median lobe narrow apically .... ***O. coomani* Balfour-Browne, 1939**

– Pronotum without microsculpture on interstices. Parameres with or without S-shaped sclerite.......... **b**

b Aedeagus elongate. Phallobase very short. Paramere without S-shaped strongly sclerotized part, narrow and nearly straight. Median lobe very wide apically, with very large gonopore (Fig. 16) .... ***O. roberti* sp. n.**

– Aedeagus robust and wide. Phallobase only slightly shorter than parameres. Parameres with strongly sclerotized S-shaped sclerite. Median lobe narrow apically, with small subapical gonopore (Fig. 15) .... ***O. hainanensis* sp. n.**

4 Mesocoxae widely separated; mesoventral plate much wider than long, widely contacting metaventrite. Prothorax with antennal grooves **5**

– Mesocoxae rather narrowly separated; mesoventral plate in form a narrowly carinate elevation, only narrowly contacting mesoventrite. Prothorax without antennal grooves .... ***Paromicrus* Scott, 1913**
(two species from Taiwan available in our material)

5 Size 2.0–2.2 mm, highly convex beetles. Elytral series deeply impressed especially sublaterally. Epipleuron wide anteriorly, then becoming extremely narrow, seemingly absent in posterior third of elytra. Pronotum with a transverse series of slightly coarser punctures along posterior margin............................ .... ***Peratogonus* Sharp, 1884**
(single species occurring in China, *P. reversus* Sharp, 1884)

– Size 1.2–1.7 mm, at most moderately convex beetles. Elytral series not distinctly impressed. Epipleuron gradually narrowing posteriad, well developed in the posterior third of elytra. Pronotum without distinct transverse row of slightly larger punctures along posterior margin ....***Noteropagus* Orchymont, 1919**
(multiple species occur in China, a taxonomic revision is needed)

Species-level taxonomy

Omicrogiton coomani Balfour-Browne, 1939

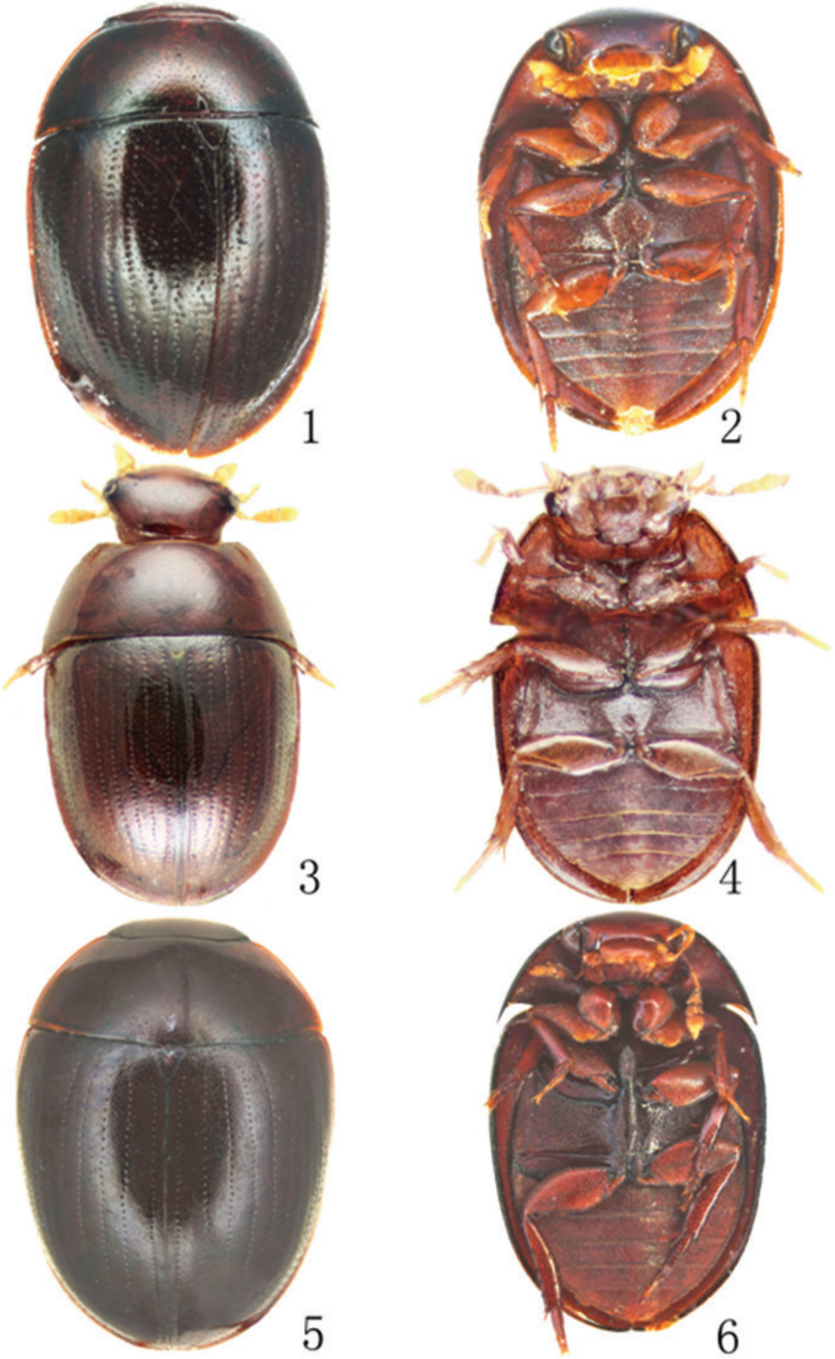
Figs 3–4, 17–18, 23–24

Omicrogiton coomani Balfour-Browne, 1939: 471.

Type material examined. Lectotype (hereby designated): dissected male (BMNH): "LACTHO / Tonkin. / de Cooman // Andrewes / Bequest / B. M. 1922-22 // Omicrogiton / coomani Paratypes! / J. Balfour-Browne det. // A. d'Orchymont det. / Omicrogiton / insularis / d'Orchymont // Coll. d'Orchym.". Paralectotypes: 1 unsexed spec. (BMNH): same data as the lectotype [this specimen was originally pinned on the same pin as the lectotype, and is now moved to the seperate pin to whoch copies of the aforementioned label are attached]; 1 unsexed specimen (BMNH): " LACTHO / Tonkin. / de Cooman // C. G. Champion / Brit. Mus. / 1925-42 // A. d'Orchymont det. / Omicrogiton / insularis / d'Orchymont // PARATYPE // Omicrogiton / coomani Type! / J. Balfour-Browne det."; 1 unsexed specimen (BMNH): " LACTHO / Tonkin. / de Cooman // C. G. Champion / Brit. Mus. / 1925-42 // A. d'Orchymont det. / Omicrogiton / insularis / d'Orchymont // Omicrogiton / coomani Paratype! / J. Balfour-Browne det.".

Additional material examined. CHINA: Guangdong: 3 males, 4 females, 87 unsexed spec. (SYSU): Zhaoqing, Heishiding Natural Reserve, 4–6.x.2013, Fenglong Jia, Ye Jia, Bingjie Chen, Renchao Lin et Weilin Xu leg.; 2 males, 2 females, 15 unsexed spec. (SYSU): Fengkai, Heishiding Natrual Reserve, 179 m, 12.v.2011, Song Keqing leg.; 138 spec. (SYSU): Fengkai, Heishiding Natural Reserve, 20.xi.2010, Fenglong Jia leg.; 88 spec. (SYSU): Fengkai, He'erkou, in decaying banana trunks, 14.viii.2010, Fenglong Jia leg.; 14 spec. (SYSU): Fengkai, Heishiding Natural Reserve, 2.v.2011, Fenglong Jia leg.; 2 males, 1 female, 44 spec. (SYSU): Fengkai, Guangling village, 9.x.2010, Yan Mei, Lijun Yang, Yali Yu leg.; 1 male (SYSU): Fengkai, Yanshuitian, 8.x.2010, Yan Mei et Lijun Yang leg. 1 male, 11 unsexed specimens (NMPC): W of Qixing, Heishiding nature reserve, rotting trunks of banana along the dried-up stream in the primary lowland forest, 190-260 m a.s.l., 23°27.9'N 111°54.3'E, 1.–3.v.2011, Fikáček & Hájek leg. **Hainan:** 1 male (NMPC): Limushan Mts., 19°9.1–9.2'N 109°45–46'N, along the road, 550–750 m a.s.l., rotting banana trunks at the stream in secondary forest, 5.v.2011, Fikáček & Zhao leg. **Hong Kong**: 2 males, 3 females, 25 unsexed spec (AFCD): Hong Kong, Wutongzhai, 27.ix.2013, F.L. Jia, Yingming Lee & Eric Chen leg. **VIETNAM:** 2 males (SYSU): Tonkin, Hoa-Binh, leg. A. de Cooman, with labels "Omicrogiton insularis d'Orchym." [handwritten] and "En-121415 [En-121416, respectively], Sun Yat-sen University, Biomuseum" [transcript from Chinese]; 1 male, 1 female (IZCAS): Tonkin, Hoa-Binh, leg. A. de Cooman.

Diagnosis. Body length 1.9–2.1 mm, width 1.2–1.3 mm. Head and elytra brown; scapus ca. 3.5× as long as antennomeres 2–5 combined. Interstices of pronotum with fine mesh-like microsculpture; prosternum strongly tectiform. Phallobase ca. half as



Figures 1–6. General habitus of Chinese *Omicrogiton* Orchymont, 1919 and *Mircogioton* Orchymont, 1937 **1–2** *O. roberti* sp. n. in dorsal and ventral view **3** Lectotype of *O. coomani* Balfour-Browne, 1939, dorsal view **4** Paralectotype of *O. coomani*, ventral view **5–6** *M. coomani* Orchymont, 1937, dorsal and ventral view.

long as parameres, without distinct manubrium; paramere without strongly sclerotized S-shaped portion, rather wide throughout, weakly sinuate on outer margin, apex semicircular; median lobe slightly narrower than paramere, wide basally, then abruptly narrowing and rather narrow in apical half, apex narrowly rounded, gonopore small, apical.

Differential diagnosis. *Omicrogiton coomani* differs from all other species of the genus except of *O. cheesmanae* from New Hebrides by the presence of the fine microsculpture on pronotal interstices. It may be also easily distinguished from all other species of the genus by the morphology of the aedeagus, which lacks the strongly sclerotized S-shaped sclerite of the paramere, and has narrow median lobe with small apical gonopore.

Remark. When Balfour-Browne (1939) checked the material of *Omicrogiton insularis* Orchymont, 1919 deposited in BMNH, he found that the specimens from "Tonkin (Lac Tho)" (i.e. Lac Tho in the Hoa Binh Province in northern Vietnam) differ from those from Engano Island near Sumatra (type locality of *O. insularis*) and described them under the name *O. coomani*. Since the diagnosis of *O. coomani* was very short and it was only presented in the discussion concerning another species of the genus from New Hebrides, the species remained virtually unknown and unrecognized in the collections. The aedeagus of the dissected type specimen of *O. coomani* (Fig. 17) really differs from that of *O. insularis* and we can therefore confirm that *O. coomani* is a separate species.

Within this study, we are showing that *O. coomani* may co-occur syntopically with other species of the genus, from which it may be distinguished by male genitalia only. To fix the identity of the species and prevent any future confusion, we are hence designating here the only dissected syntype specimen as the lectotype of *O. coomani*. Aedeagus of this specimen is shown in Fig. 17.

Biology. All examined specimens were found in the decaying banana trunks, typically in still standing trunk bases which are decaying after the apical part of the plant was cut or broken.

Distribution. China (Guangdong, Hong Kong), northern Vietnam. New for China.

Omicrogiton hainanensis sp. n.

http://zoobank.org/5FAF1857-A193-4186-A8CF-1CE9260EB15E
Fig. 15

Type material. Holotype: male (SYSU): CHINA: Hainan Isl.: Limushan Mts., 19°9.1–9.2'N, 109°45-46'N, along the road, 550–750 m a.s.l., rotting banana trunks at the stream in secondary forest, 5.v.2011, Fikáček & Zhao lgt. Paratypes: 2 males (SYSU, NMPC): same locality as the holotype; 2 males (NMPC): China: Hainan Isl.: Jianfengling Mts., Tiachi Lake env., Bishu villa, rotting banana trunk at the bank of a drying-up stream in the primary forest above the hotel area, 18°44.7'N 108°50.7'E, 950 m a.s.l., 9–11.v.2011, Fikáček, Kubeček & Li lgt.

Diagnosis. Body length 1.9–2.0 mm. Head and elytra brown; scapus ca. 3.5× as long as antennomeres 2–5 combined. Interstices of pronotum without microsculpture;

prosternum weakly tectiform. Phallobase ca. as long as parameres, wide anteriorly, with wide rounded manubrium; paramere with strongly sclerotized S-shaped portion and membranous mesal and apical portions, sclerotized parts of left and right paramere forming very obtuse angle basally; median lobe narrower than phallobase and paramere, rather wide basally, gradually narrowing towards apex, apex rather widely rounded, gonopore subapical (Fig. 15).

Description. ***Form and Color.*** Body oval, weakly convex, length 1.9–2.0 mm, width 1.2 mm. Head, pronotum and elytra brown; lateral margin of elytra paler than disc; labrum, maxillary palpomeres and antennomeres 1-6 reddish brown, antennal club of antennae slightly paler; ventral surface brown, legs reddish brown.

Head. Clypeus with fine punctures, interstices without microsculpture; lateral deflexed extensions not defined from clypeal disc by ridge; anterior margin with narrow marginal bead laterally. Frontoclypeal suture undetectable. Frons with sparser and coarser punctures than on clypeus, interstices without microsculpture. Eyes small, clearly protruding, interocular distance ca. 5× as wide as one eye in dorsal view. Labrum exposed, sinuate on anterior margin. Mentum dull, densely granulate, without punctures, ca. 2× as wide as long, not depressed anteromedially, anterior margin slightly protruding medially. Submentum declined below the level of mentum. Antenna with 9 antennomeres, scapus ca. 3.5× as long as antennomeres 2–5 combined, club compact, last club antennomere the widest. Maxillary palpomere 2 moderately swollen, palpomere 4 almost symmetrical, widest at midlength, equal in length to palpomere 2, longer than palpomere 3.

Thorax. Pronotum ca. 2.6× as wide as long. Pronotal punctation similar to that on frons, interstices without microsculpture. Lateral margins with strong bead overlapping to anterior margin, posterior margin of pronotum without bead. Prosternum weakly tectiform; antennal grooves absent. Mesoventrite strongly and abruptly raised medially to form a narrow longitudinal lamina not reaching metaventral process posteriorly; cavities for reception of procoxae absent. Metaventrite weakly convex, without glabrous median portion, with weak posteromedial depression on elevated portion. Elytra widely explanate laterally, with 10 series of large punctures, series 1–5 almost reaching base, series 6–10 abbreviated anteriorly; interval punctures very fine but distinct, similar to those on pronotum, interstices without microsculpture; humeral bulge absent; lateral margin of elytron finely serrate; epipleuron wide throughout. Profemur glabrous, anterior margin angulate near base, with a large basal depression with golden pubescence, tibial groove sharply defined. Mesofemur with sparse and coarse punctures on anterior half, each puncture with a short seta; posterior half glabrous, with fine longitudinal sculpture. Metafemur with fine longitudinal sculpture and scattered fine punctures. Tibiae flat, meso- and metatibiae with long and stout spines along outer face and 1 or 2 pairs of spines on apical half of inner face; metatibial long spur ca. as long as first metatarsomere. First metatarsomere almost as long as metatarsomeres 2–3 combined.

Abdomen. Abdomen with five ventrites; first ventrite not longer than ventrites 2–5 each; first ventrite without median longitudinal carina, fifth ventrite narrowly rounded, not emarginate apically.

Male genitalia. Phallobase ca. as long as parameres, wide anteriorly, with wide rounded manubrium; paramere with strongly sclerotized S-shaped portion and membranous mesal and apical portions, membranous apex of paramere widely rounded; sclerotized parts of left and right paramere forming very obtuse angle basally; median lobe narrower than phallobase and paramere, wide basally, gradually narrowing apicad, apex rather widely rounded, gonopore subapical (Fig. 15).

Differential diagnosis. *Omicrogiton hainanensis* sp. n. belongs to the species with strongly sclerotized S-shaped portion of the paramere, together with the Oriental *O. insularis* Orchymont, 1919 and *O. gomyi* Bameul, 1986 from the Reunion Island (Bameul 1986). It differs from *O. insularis* by the much wider aedeagus (aedeagus is generally very narrow in *O. insularis* (Fig. 19), much wider parameres with bases of strongly sclerotized parts forming a very obtuse angle (parameres are narrow and bases of sclerotized portions form acute angle in *O. insularis*) and wide median lobe with subapical gonopore (median lobe is extremely narrow apically and the gonopore is situated at midlength in *O. insularis*). The aedeagus of *O. gomyi* is similar to that of *O. hainanensis* in the proportions (i.e., it is wide and robust in both species), but *O. gomyi* easily differs by wide median lobe with apical gonopore. *Omicrogiton coomani* and *O. roberti*, differ from all above species including *O. hainanensis* in parameres lacking the strongly sclerotized S-shaped portion, and *O. coomani* and *O. cheesmanae* may be distinguished from other species including *O. hainanensis* by the pronotum with fine mesh-like microsculpture.

Etymology. The species name is patronymic, referring to the Hainan Island where this species is commonly collected.

Biology. All type specimens were collected in decaying banana trunks in primary or secondary rainforests. On the type locality, the specimens of this species were collected in the same banana trunk as two other *Omicrogiton* species occurring in Hainan (i.e. *O. roberti* sp. n. and *O. coomani*), which indicates that multiple species may occur syntopically in this genus. For this reason, we excluded females from the type series of this species.

Distribution. China (Hainan).

Omicrogiton roberti sp. n.

http://zoobank.org/960DC7E9-66F4-423B-AC47-0349093381C9
Figs 1, 2, 16

Type material. Holotype: male (SYSU): CHINA: Hainan isl., Limushan Mts., Limu temple, 5.v.2011, 19°9.1–9.2'N, 109°45–46'E, 550–750 m; along the road, rotting banana trunks at the stream in secondary forest, Fikáček & Zhao lgt.

Diagnosis. Body length 2.1 mm. Head and elytra black or dark brown; pronotum paler than head and elytra. Scapus ca. 2.5× as long as antennomeres 2–5 combined. Prosternum strongly tectiform, with low longitudinal carina medially. Phallobase much shorter than parameres, with thin and long basal manubrium; paramere without distinct S-shaped more sclerotized portion, narrow, weakly curved on outer margin, rounded apically; median lobe much broader than paramere, bottle-shaped, widest at basal third, strongly narrowing ca. at midlength, apex broadly truncate; gonopore large, situated subapically (Fig. 16).

Description. ***Form and Color.*** Body oval, weakly convex (Fig. 1), length 2.1 mm, width 1.35 mm. Head and elytra dark brown; pronotum brown; lateral margin of elytra paler than disc; labrum, maxillary palpomeres and antennomeres 1–6 reddish brown, antennal club paler; ventral surface brown, legs reddish brown.

Head. Clypeus with rather densely arranged fine punctures, interstices without microsculpture; lateral deflexed extensions not defined by ridge. Anterior margin of clypeus with narrow bead laterally. Frontoclypeal suture undetectable. Frons with sparser and coarser punctures than on clypeus, without microsculpture on interstices. Eyes small, clearly protruding, interocular distance ca. 7× as wide as one eye in dorsal view. Labrum exposed, sinuate on anterior margin. Mentum densely granulated, without punctures, ca. 2× as wide as long, not depressed anteromedially, anterior margin slightly protruding medially. Submentum below the level of mentum. Antenna with 9 antennomeres, scapus ca. 2.5× as long as antennomeres 2–5 combined, club compact, last club antennomere the widest. Maxillary palpomere 2 moderately swollen, palpomere 4 almost symmetrical, widest in middle, equal to palpomere 2 in length, longer than palpomere 3.

Thorax. Pronotum ca. 2.6× as wide as long; pronotal punctation similar to that on frons, interstices without microsculpture. Lateral margins with strong bead overlapping to anterior margin, posterior margin of pronotum without bead. Prosternum strongly tectiform, antennal grooves absent. Mesoventrite strongly and abruptly raised medially to form a narrow longitudinal lamina not contacting metaventral process posteriorly, cavities for reception of procoxae absent. Metaventrite weakly convex, with a small glabrous portion, with a posteromedial depression on elevated portion. Elytra widely explanate laterally, with 10 series of large punctures, series 1–5 almost reaching base, series 6–10 abbreviated anteriorly; interval punctures very fine but distinct, similar to on pronotum; interstices without microsculpture. Humeral bulge absent, lateral margin of elytron very finely serrate; epipleuron wide throughout.Profemur glabrous, anterior margin angulate near base, with a large basal depression with golden pubescence, tibial groove sharply defined. Mesofemur with sparse and strong punctures on anterior half, each puncture with a short seta; posterior half glabrous, with fine longitudinal sculpture. Metafemur with fine longitudinal sculpture and scattered fine punctures. Tibiae flat, meso- and metatibiae with long and stout spines along outer face and 1 or 2 pairs of spines on apical half of inner face; metatibial long spur longer than first tarsomere. First metatarsomere almost as long as metatarsomeres 2–3 combined.

Abdomen. Abdomen with five ventrites; first ventrite not longer than ventrites 2–5, without median longitudinal carina; fifth ventrite rounded, not emarginate apically.

Male genitalia. Phallobase much shorter than parameres, with a thin and long basal manubrium. Paramere without S-shaped strongly sclerotized portion, narrow throughout, weakly curved on outer margin, rounded apically. Median lobe much broader than paramere, bottle-shaped, widest in basal third, strongly narrowed ca. at midlength, apex broadly truncate; gonopore large, situated subapically (Fig. 16).

Different diagnosis. *Omicrogiton roberti* is similar to *O. coomani* Balfour-Browne, 1939 in the aedeagus without the strongly sclerotized S-shaped portion of the paramere. It differs from *O. coomani* by the morphology of the aedeagus (median lobe very wide with very large subapical gonopore and very short phallobase in *O. roberti*, rather narrow and with small apical gonopore and rather long phallobase in *O. coomani*) and by the pronotal interstices without fine mesh-like microsculpture (with fine mesh-like microsculpture in *O. coomani*).

Etymology. The species is named after Dr. Robert Bagrie Angus, a British specialist on the Helophoridae, who helped us a lot with this study.

Biology. The holotype was collected in a decaying banana trunk together with specimens of *O. hainanensis* and *O. coomani*.

Distribution. China (Hainan).

Mircogioton coomani Orchymont, 1937

Figs 5–6, 14, 20

Mircogioton coomani Orchymont, 1937: 464.
Ischyromicrus cognitus Malcolm, 1981: 267. **New synonym.**
Mircogioton cognitus (Malcolm); Hansen 1991: 226

Material examined. CHINA, Yunnan: 2 males, 6 unsexed spec. (SYSU, NMPC): Laiyanghe, Xinzhai Cun, 1487 m, 22.631°N, 101.132°E, 21.v.2011, Song Keqing lgt.; 1 male, 2 unsexed spec (SYSU): Mandian Nabanhe Conv., 11.i.2004, Li & Tang lgt.; 1 spec. (NMPC): Laiyanghe, Yutang village, in decaying banana trunk, 22.v.2011, Keqing Song lgt. **VIETNAM:** 1 male (IZCAS): Tonkin, Hoa-Binh, leg. A. de Cooman.

Diagnosis. Body length 3.2–3.4 mm, width 2.2 mm. Dorsal surface dark brown, ventral surface brown to dark brown. Labrum weakly bisinuate on anterior margin, not distinctlx projecting anteriad. Scapus ca. 2.2× as long as antennomeres 2–5 combined, slightly shorter than antennal club. Head, pronotum and elytra with similar sparse and fine punctation, interstices without fine microsculpture; elytra with 10 series of punctures, series 6–7 abbreviated anteriorly. Prosternum strongly tectiform, sharp anteriorly. Mesoventral elevation much longer than wide, with distinct longitudinal groove medially, posteriorly fused with metaventral process, not projecting posteriad into a process overlapping metaventrite. Metaventrite with a longitudinal glabrous elevated band medially, forming together with mesoventral plate a joint meso-metaventral elevation. Phallobase ca. 0.3× as long as paramere; paramere rather wide throughout, outer margin slightly concave subapically, apex semicircular. Median lobe slightly narrower than paramere, lateral margin almost parallel, apex narrowly rounded, gonopore of moderate size, subapical (Fig. 20).

Differential diagnosis. *Mircogioton coomani* differs from *Mircogioton spinosus* Bameul, 1993, *M. seriatus* Hebauer, 2006 and *M. irregularis* Hebauer, 2006 in mesoventrite fused with metaventral process (in contrast, mesoventrite is projecting into a long process overlapping metaventrite in the latter three species). It differs from *M. grandis* Bameul, 1993 and *M. julieae* (Malcolm, 1981) by the anterior margin of the labrum bisinuate (in contrast, labrum is simply concave on anterior margin in the latter two species). From *M. julieae* it also differs by apically broad paramere and apex of median lobe not distinctly narrowed. From *M. grandis* it may be also distinguished by smaller body size (up to 3.5 mm, in comparison with 3.8 mm in *M. grandis*).

Remark. This species was described by d'Orchymont (1937) based on a single female collected by A. de Cooman in "Tonkin, Hoa Binh". The senior author examined one male collected by the same collector and bearing the same label data. Except of clearly being a part of the same material from which *M. coomani* was collected, the examined specimen agrees in all details with the original description. We therefore consider it represents *M. coomani* although we have not checked the female holotype.

Malcolm (1981) described *Ischyromicrus cognitus* Malcolm, 1981 based on a female from upper Mekong (later transferred to *Mircogioton* by Hansen (1991)). The species was redescribed by Bameul (1993). The characters described by Malcolm (1981) and Bameul (1993) are identical with the specimens of *M. coomani* in our hands. The type locality of *M. cognitus* is situated in northeastern Laos not far from the border with China rather than in Vietnam as supposed by Malcolm (1981) (Bameul 1993, Hansen 1999). Bameul (1981) moreover noticed that "in the description of *M. cognitus*, no characters really differ from those given by d'Orchymont (1937) in his description of *M. coomani*" and supposed that *M. cognitus* is synonym of *M. coomani*. We are following this opinion here and consider *M. cognitus* as a junior subjective synonym of *M. coomani*.

Biology. The recently collected specimens examined here were found in decaying banana trunk (K.-Q. Song and L. Tang, pers. comm.).

Distribution. China (Yunnan), northern Laos. New genus and species for China.

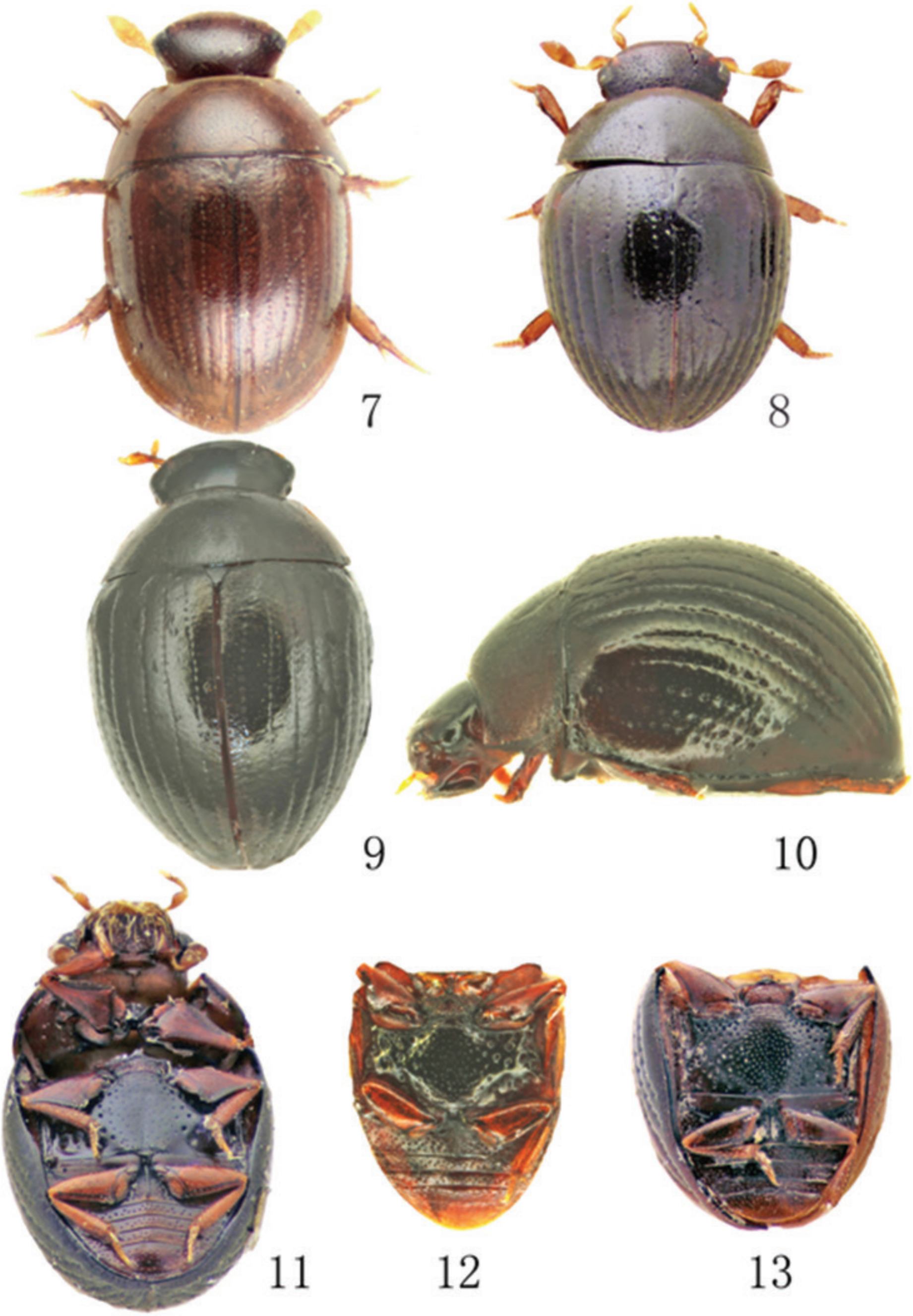
***Peratogonus reversus* Sharp, 1884**

Figs 8–12, 21–22

Peratogonus reversus Sharp, 1884: 461.

Type material examined. Syntypes: 2 specimens on one card (BMNH): "Peratogonus reversus Type DS Nagasaki 14.4.81 // Japan G. Lewis Sharp coll. 1905 – 313"; 1 upside down spec. (BMNH): "Peratogonus reversus Sharp // Japan G. Lewis // 30.3.81".

Additional material examined. JAPAN: 1 male (BMNH): "Japan, Kobe. Mayasan 14.vi.29 JEA Lewis. Nr 1530"; 1 male (NMPC): Kanagawa Pref., Manazuru Peninsula, 4.xi.2006, P. Jaloszynski lgt.; 1 spec. (NMPC): Chiba Pref., Kôzaki shrine, Kôzaki-machi, 15.x.2001, P. Jaloszynski lgt. **CHINA: Guangdong:** 1 male (SYSU):



Figures 7–13. Morphology of *Omicrogiton* Orchymont, 1919 and *Peratogonus* Sharp, 1884 **7** Dorsal habitus of *O. insularis* Orchymont, 1919 **8–11** Habitus of *P. reversus* Sharp, 1884 (**8** syntype from Japan, dorsal view **9** specimen from China, dorsal view **10** specimen from China, lateral view **11** syntype from Japan, ventral view) **12–13** Comparison of metaventral punctation of *Peratogonus* (**12** *P. reversus* **13** *P. grandis* Bameul, 1994).

Conghua, Liuxihe forest park, 16.v.2012, Tong Xiaoli leg. (in Chinese); 1 male (SYSU): Fengkai, Heishiding Natural Reserve, 23°27.9'N 111°54.3'E, 190–260 m, Fenglong Jia leg.; 1 male (NMPC): W of Qixing, Heishiding nature reserve, sifting of moist leaf litter in the dried-up streambeds and along the streams in the primary lowland forest, 190–260 m a.s.l., 23°27.9'N, 111°54.3'E, 1.–3.v.2011, Fikáček & Hájek lgt. **Jiangxi:** 1 male (NMPC): Jinggangshan Mts., Xiangzhou (forested valley S of the village), cut and decaying tops of bamboo trunks at side of a trail in the secondary forest and among the fields, 26°35.5'N, 114°16.0'E, 374 m, 26.iv.2011, Fikáček & Hájek lgt. **Taiwan:** 1 male, 9 spec. (NMPC, SYSU): Maoli County, Nanjhuang Twnsh., S-Nanjhuang Rd. 124, km 3 + forest road, forest compost, 26.x.2010, S. Vít lgt.

Diagnosis. Body length 2.1 mm, width 1.5 mm, strongly convex. Head and pronotum with fine microsculpture between punctures. Elytra with 10 striae, striae 1–5 reaching elytral base, striae 6–10 abbreviated anteriorly, not reaching base; elytral intervals with distinct fine punctures, without microsculpture between punctures. Prosternum steeply raised in middle to form a triangular medially carinate tablet. Mesoventrite flat medially, widely fused with metaventrite. Metaventrite laterally with much coarser and stronger punctures than on its median portion. Aedeagus (Figs 21–22) with phallobase ca. as long as paramere, tube-like; paramere broad basally, gradually narrowing towards apex; median lobe slender, parallel-sided, with very long basal struts, gonopore indistrinct, apex slightly emarginate.

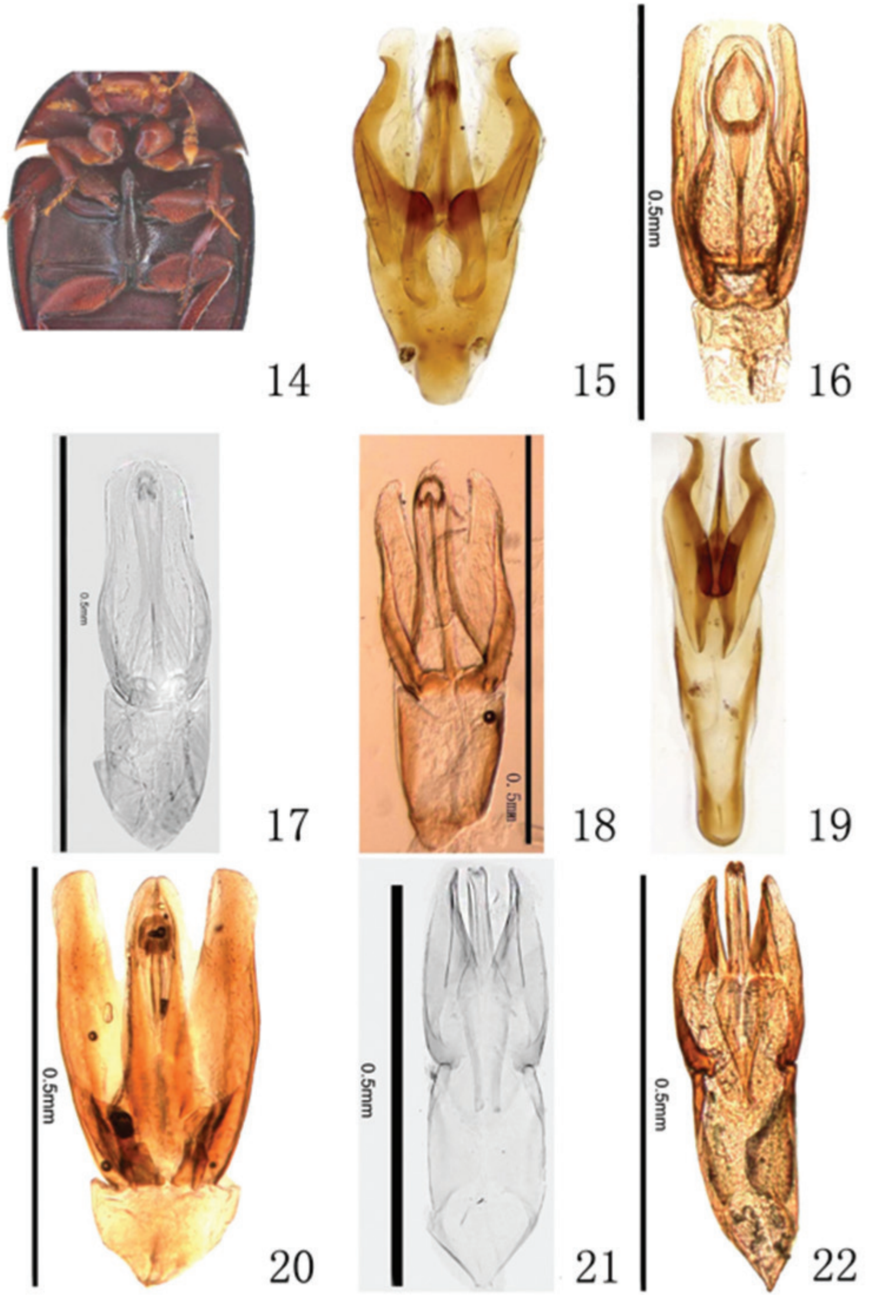
Differential diagnosis. This species can be easily distinguished from *P. grandis* Malcolm, 1981 occurring in India (Sikkim) by punctures on the lateral portion of the metaventrite much deeper and larger than medially. From *P. corporaali* Orchymont, 1926 occurring in Indonesia (Java), it may be distinguished by pronotum with distinct microsculpture between punctures, elytra with striae 6–10 not reaching elytral base (stria 8 almost reaching base, striae 9–10 reaching base in *P. corporaali*), elytral intervals with distinct punctures, flat mesoventral plate, and metaventrite with coaser and sparser punctures medially.

Remark. This species was firstly described from Nagasaki, Kyushu in southern Japan by Sharp (1884). It was subsequently reported from Taiwan by Knisch (1921). The comparison of the specimens from Taiwan and southern continental China revealed that they are identical with those from Japan.

When Malcolm (1981) described *Peratogonus grandis* Malcolm, 1981, he diagnosed it from *P. reversus* by the different body size (2.21×1.64 mm in *P. grandis* versus 1.72×1.31 in *P. reversus*) and by the shallower and smaller punctures on lateral portion of the metaventrite. The material examined by us revealed that the specimen of *P. reversus* examined by Malcolm (1981) was smaller than its individuals usually are (i.e. body length 1.9–2.2 mm). Therefore, the body size can not be used as a reliable character to distinguish the two species, in contrast to the punctation of the metaventrite, which seems to be a reliable character to distinguish the two species.

Biology. Most specimens examined here were found by sifting forest leaf litter.

Distribution. China (Guangdong, Jiangxi, Taiwan), Japan (Honshu, Kyushu). New genus and species for mailand China.



Figures 14–22. Ventral morphology (14) and morphology of aedeagus (15–22). **14** detail of meso- and metaventrite of *Mircogioton coomani* Orchymont, 1937 **15** *Omicrogiton hainanensis* sp. n., holotype **16** *O. roberti* sp. n., holotype **17–18** *O. coomani* Balfour-Browne, 1939 (**17** lectotype; **18** non-type specimen from China) **19** *O. insularis* Orchymont, 1919, specimen from Sarawak **20** *Mircogioton coomani* Orchymont, 1937, Chinese specimen **21–22** *Peratogonus reversus* Sharp, 1884 (**21** syntype **22** specimen from China).

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24

Figures 23–24. Examples of habitats of Chinese *Omicrogiton* Orchymont, 1919.

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We are indebted to Dr. Robert B Angus who checked and dissected types of *Omicrogiton coomani* and *Peratogonus reversus* and specimens of *O. insularis* deposited in BMNH and took their photographs for us. We are very grateful to Dr. Liang Tang (Shanghai Normal University, China) and Dr. Ke-qing Song (Chinese Academy of Sciences, Institute of Zoology, Beijing, China) for their donation to specimens of *Mircogioton coomani* to SYSU. This study was supported by the National Natural Science Foundation of China awarded to F.-L. Jia (grant no. 31272266) and to Sh.-X. Yu (grant no. J1210074), and by the Ministry of Culture of the Czech Republic (DKRVO 2014/13, National Museum, 00023272) to M. Fikáček.

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RESEARCH ARTICLE

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Two new species of *Paramesosciophilodes* (Diptera, Nematocera, Mesosciophilidae) from the Middle Jurassic of China

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Abstract

Two new species, *Paramesosciophilodes bellus* **sp. n.** and *Paramesosciophilodes rarissima* **sp. n.**, from the Jiulongshan Formation at Daohugou Village, Inner Mongolia, China, are described in the extinct family Mesosciophilidae. Altogether seven genera with 21 species of mesosciophilids have been described from the Jurassic of Siberia and Kazakhstan, the Lower Cretaceous of Transbaikalia, and the Middle Jurassic of Inner Mongolia. An emended generic diagnosis of *Paramesosciophilodes* and a list of known taxa of mesosciophilids are provided.

Keywords

Paramesosciophilodes, Daohugou, Inner Mongolia, China

Introduction

Mesosciophilidae is one of the extinct dipteran families of the suborder Nematocera. Rohdendorf (1946) described a species, *Mesosciophila venosa*, which was assigned to a new subfamily, Mesosciophilinae, within the family Allactoneuridae, along with Fungivoritinae. Later he implicitly synonymized Mesosciophilinae with Fungivoritidae and excluded *Allactoneura* DeMejere, 1907 from the family (Rohdendorf 1957, 1962). Kovalev (1985) elevated Mesosciophilinae to family level; and synonymized Fungivoritidae under the First Reviser Rule. Blagoderov (1993) erected the genus *Mesosciophilopsis* with three species within the family Mesosciophilidae, and also revised the diagnosis of the Mesosciophilidae. Two important generic characters of *Mesosciophilina* Kovalev, 1985, reported from the Middle Jurassic, are cell r distinctly large, longer than 1/6 of wing length, and r-m significantly shorter than bRs, which are regarded as “obvious ancestral characters” (Kovalev 1985). On the other hand, the generic features of *Mesosciophilopsis* Blagoderov, 1993, described from the Early Cretaceous, are cell r distinctly small, shorter than 1/6 of wing length, and r-m significantly longer than bRs, which are regarded as “derived characters” (Blagoderov 1993, Zhang 2002). Zhang (2007) established a monotypic genus *Paramesosciophilodes* for his new species, *P. ningchengensis*, and described another species within the genus *Mesosciophila*. The generic diagnosis of *Paramesosciophilodes* includes cell r 0.16–0.18 times as long as wing length, bRs markedly shorter than r-m and R_{4+5} is strongly arched near its midlength. Later, Zhang (2008) assigned three new species to three genera, including *Paramesosciophilodes eximia* Zhang, 2008, and reviewed all the records of mesosciophilids. Li and Ren (2009) described two species of *Jurasciophila* from the late Middle Jurassic Jiulongshan Formation of Daohugou in southeastern Inner Mongolia, China. Species of *Jurasciophila* Li & Ren, 2009 have cell r small, shorter than 1/6 of wing length, and r-m significantly shorter than bRs, which are regarded as “transitional characters” (Li and Ren 2009). Wang et al., in 2012, assigned two species respectively to *Mesosciophila* and *Paramesosciophilodes* of Mesosciophilidae (Wang et al. 2012). Shi et al. recently described a new genus with two new species, *Similsciophila singularis* and *S. sinuate*, from the late Middle Jurassic of Jiulongshan Formation (Shi et al. 2014). To date, 7 genera and 19 species of mesosciophilids have been described from the Jurassic of Siberia and Kazakhstan, the Lower Cretaceous of Transbaikalia, and the Middle Jurassic of Inner Mongolia, which are summarized in Table 1. In addition, an emended generic diagnosis of *Paramesosciophilodes*, based on the new findings, is provided.

There have been many transfers and corrections regarding species belonging to the Mesosciophilidae. *Eoboletina gracilis* Rohdendorf, 1946 from the Upper Jurassic of Kazakhstan might belong to the family Mesosciophilidae (Blagoderov 1993). The Mongolian genus *Mesosciophilites* Kovalev, 1985 of the Lower Cretaceous should be transferred to the Mycetophilidae (Blagoderov 1993). The Australian species *Pseudalysiinia fragmenta* Jell & Duncan, 1986 should be transferred to an unnamed genus of Mesosciophilidae rather than to the extant genus of *Pseudalysiinia* Tonnoir, 1929 of Mycetophilidae (Blagoderov 1993), and we agree with this change. The genus *Sciophilites* Kovalev, 1990 from the Lower Cretaceous of Transbaikalia might belong to either the Mesosciophilidae or to the Mycetophilidae (Blagoderov 1993). *Sinosciophila meileyingziensis* Hong, 1992 from the Lower Cretaceous of Kezuo has been transferred to the Sciophilidae (Zhang 2008), but it might be a representative of Mesosciophilidae. The other three species *Liaoxifungivora simplicis* Hong, 1992, *Atalosciophila yanensis* Ren, Lu, Guo & Ji, 1995 and *Huaxiasciophilites jingxiensis* Zhang, Hong & Li, 2001 from the Lower Cretaceous of China might belong to the family Mycetophilidae,

Table 1. A list of the described fossil Mesosciophilidae.

Genus	Species	Locality	Age
Mesosciophila	*Mesosciophila venosa* Rohdendorf, 1946	Karatau, Chimkent Oblast, Kazakhstan	Karabastau Fm., J_3
	Mesosciophila eucalla Zhang, 2007	Daohugou, Ningcheng, Inner Mongolia, China	Jiulongshan Fm., J_2
	Mesosciophila abstracta Zhang, 2008	Daohugou, Ningcheng, Inner Mongolia, China	Jiulongshan Fm., J_2
	Mesosciophila sigmoidea Wang, Zhao & Ren, 2012	Daohugou, Ningcheng, Inner Mongolia, China	Jiulongshan Fm., J_2
Mesosciophilodes	*Mesosciophilodes augustipennis* Rohdendorf, 1946	Karatau, Chimkent Oblast, Kazakhstan	Karabastau Fm., J_3
	Mesosciophilodes similis Rohdendorf, 1964	Karatau, Chimkent Oblast, Kazakhstan	Karabastau Fm., J_3
	Mesosciophilodes synchrona Zhang, 2008	Daohugou, Ningcheng, Inner Mongolia, China	Jiulongshan Fm., J_2
Mesosciophilina	*Mesosciophilina bolshakovi* Kovalev, 1985	Siberia, Russia	Itat Fm., J_2
	Mesosciophilina irinae Kovalev, 1985	Siberia, Russia	Itat Fm., J_2
Mesosciophilopsis	*Mesosciophilopsis curtus* Blagoderov, 1993	Baissa, Buryat, Yeravnenskiy, Transbaikalia	Zaza Fm., K_1
	Mesosciophilopsis expletus Blagoderov, 1993	Baissa, Buryat, Yeravnenskiy, Transbaikalia	Zaza Fm., K_1
	Mesosciophilopsis minor Blagoderov, 1993	Baissa, Buryat, Yeravnenskiy, Transbaikalia	Zaza Fm., K_1
Paramesosciophilodes	*Paramesosciophilodes ningchengensis* Zhang, 2007	Daohugou, Ningcheng, Inner Mongolia, China	Jiulongshan Fm., J_2
	Paramesosciophilodes eximia Zhang, 2008	Daohugou, Ningcheng, Inner Mongolia, China	Jiulongshan Fm., J_2
	Paramesosciophilodes aequus Wang, Zhao & Ren, 2012	Daohugou, Ningcheng, Inner Mongolia, China	Jiulongshan Fm., J_2
	Paramesosciophilodes bellus Gao, Shi, Shih & Ren, sp. n.	Daohugou, Ningcheng, Inner Mongolia, China	Jiulongshan Fm., J_2
	Paramesosciophilodes rarissima Gao, Shi, Shih & Ren, sp. n.	Daohugou, Ningcheng, Inner Mongolia, China	Jiulongshan Fm., J_2
Jurasciophila	*Jurasciophila curvula* Li & Ren, 2009	Daohugou, Ningcheng, Inner Mongolia, China	Jiulongshan Fm., J_2
	Jurasciophila lepida Li & Ren, 2009	Daohugou, Ningcheng, Inner Mongolia, China	Jiulongshan Fm., J_2
Similsciophila	*Similsciophila singularis* Shi, Shih & Ren, 2014	Daohugou, Ningcheng, Inner Mongolia, China	Jiulongshan Fm., J_2
	Similsciophila sinuate Shi, Shih & Ren, 2014	Daohugou, Ningcheng, Inner Mongolia, China	Jiulongshan Fm., J_2

(Notes: J_2-Middle Jurassic, J_3-Late Jurassic, K_1-Early Cretaceous)

rather than to its previous assignment to the family of Pleciofungivoridae or the family Mesosciophilidae (Zhang 2007).

Here, based on a combination of unique wing venational characters of two recently collected specimens, we describe *Paramesosciophilodes bellus* sp. n. and *Parame-*

sosciophilodes rarissima sp. n. These specimens with bodies and complete wings were collected from the late Middle Jurassic Jiulongshan Formation of Daohugou Village in the Ningcheng County, Chifeng City, southeastern Inner Mongolia, China. Many well-preserved fossil insects have been described from this locality recently (Ren et al. 2010, 2012), such as dipterans, neuropterans, orthopterans, heteropterans, etc. (Zhang et al. 2008, 2011; Wang et al. 2010; Gu et al. 2012; Yao et al. 2012).

Materials and methods

This study is based on two specimens housed in the Key Lab of Insect Evolution & Environmental Changes, Capital Normal University, Beijing, China (Curator: Dong Ren). The specimens were examined under a LEICA MZ12.5 dissecting microscope. The photos of fossils were taken with a Nikon SMZ1000 stereo microscope. Line drawings were prepared with the aid of CorelDraw 12 graphic software. The method of calculating the ratio of cell r length vs. wing length is as follows: the length of cell r is the length along R_1, while the length of wing is the length from wing base to wing apex. Wing venation nomenclature follows that of Wootton and Ennos (1989) and Shcherbakov et al. (1995): bRs or dRs = section of R_{4+5} basal or distal to r-m, respectively; bM_{1+2} or dM_{1+2} = section of M_{1+2} basal or distal to r-m, respectively.

Systematic paleontology

Order Diptera Linnaeus, 1758
Suborder Nematocera Latreille, 1825
Family Mesosciophilidae Rohdendorf, 1946

Genus *Paramesosciophilodes* Zhang, 2007

Type species. *Paramesosciophilodes ningchengensis* Zhang, 2007.

Included species. Type species; *Paramesosciophilodes eximia* Zhang, 2008; *Paramesosciophilodes aequus* Wang, Zhao & Ren, 2012; *Paramesosciophilodes bellus* Gao, Shi, Shih & Ren sp. n., *Paramesosciophilodes rarissima* Gao, Shi, Shih & Ren, sp. n.

Emended diagnosis. Medium (sized mesosciophilid gnats. Body (including legs) covered with long, dense pubescence. Mesonotum convex. Scutellum sharp, clearly projecting. Wing, Sc_1 elongate, slightly shorter than one-half of wing length (0.43–0.47 times as long as wing length); Sc_2 situated distinctly basad to Rs origin, arising near midway between h to Sc_1 ending; bRs shorter than r-m; R_1 slightly curved; both R_1 and R_{4+5} divergent terminally; Rs furcated distad or at level of fork of M_{1+2}; R_{2+3} oblique and curved; R_{4+5} arched near its midlength; cell r 0.16–0.19 times as long as wing length; stem of M not developed; M_{1+2} furcated slightly distad, or basad, to level of Sc_1 ending. Tibiae and tarsi with sparse, short setae.

Paramesosciophilodes bellus **Gao, Shi, Shih & Ren, sp. n.**

http://zoobank.org/8FEE85B5-4556-40CA-8B60-B8309F5B1504
Figs 1, 2

Etymology. The specific name is from the Latin of *bellus*, meaning beautiful and delicate, for the well-preserved and beautiful specimen.

Material. Holotype No. CNU-DIP-NN2013631 p/c, part and counterpart. A well-preserved insect with complete body and two wings but poorly preserved halter, without head, in dorsoventral aspect.

Locality and horizon. Daohugou Village, Shantou Township, Ningcheng County, Inner Mongolia, China, Jiulongshan Formation, late Middle Jurassic.

Diagnosis. The Sc_1 ending proximad of the midlength of cell r; bRs 0.7 times of the length of r-m; R_{4+5} strongly curved; M_{1+2} forking basad of forking of Rs, and distad of the level of Sc_1 ending; CuA strongly arched, reaching the posterior margin of the wing markedly basad of Rs forking to R_{2+3} and R_{4+5}.

Description of holotype. Medium-sized mesosciophilid with dark body, adult male, in dorsal aspects (Figs 1 and 2A). Wings out-spread, length 5.4 mm, width 2.0 mm. Body length 7.2 mm. Head and antennae not preserved. Thorax convex, length 2.0 mm, width 1.3 mm. Scutellum clearly projecting. Abdomen thin, subcylindrical, length 5.2 mm, width 1.7 mm, approx. 2.6 times as long as head and thorax combined, with eight abdominal segments, first four segments gradually widened distally, last four segments gradually narrowed terminally. Partially preserved male genitalia relatively small, distinctly narrower than eighth abdominal segment. Halters poorly preserved. Legs relatively thin and long, femora clearly thicker in the middle; femora, tibiae and tarsi with two rows of sparse and short setae. Hind leg length 6.3 mm (femur 1.7 mm, tibia 2.4 mm, tarsus 2.2 mm).

Wings membranous, oblong, darker in color in costal area, moderately wide (length 2.7 times of width), and not reaching the apex of abdomen at rest (Fig. 2). C strong, ending beyond wing apex, at which R_{4+5} ending. Sc_1 relatively long, approx. 0.4 times

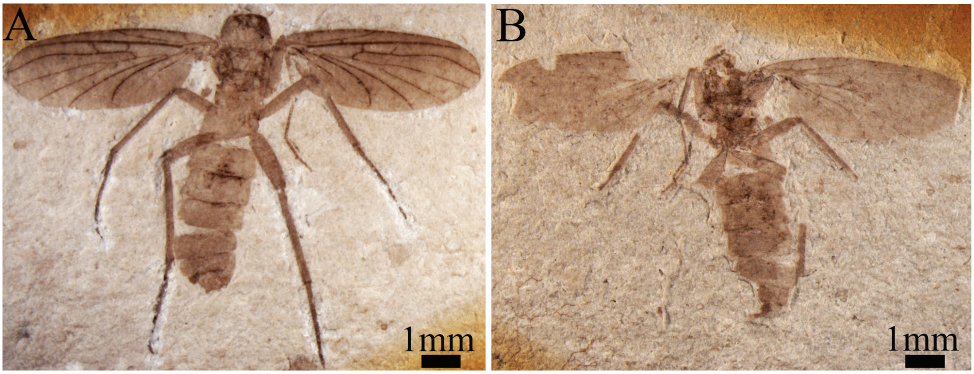

Figure 1. *Paramesosciophilodes bellus* sp. n., holotype, Photographs of habitus (dorsoventral aspect): **A** part No. CNU-DIP-NN2013631 p **B** counterpart CNU-DIP-NN2013631 c.

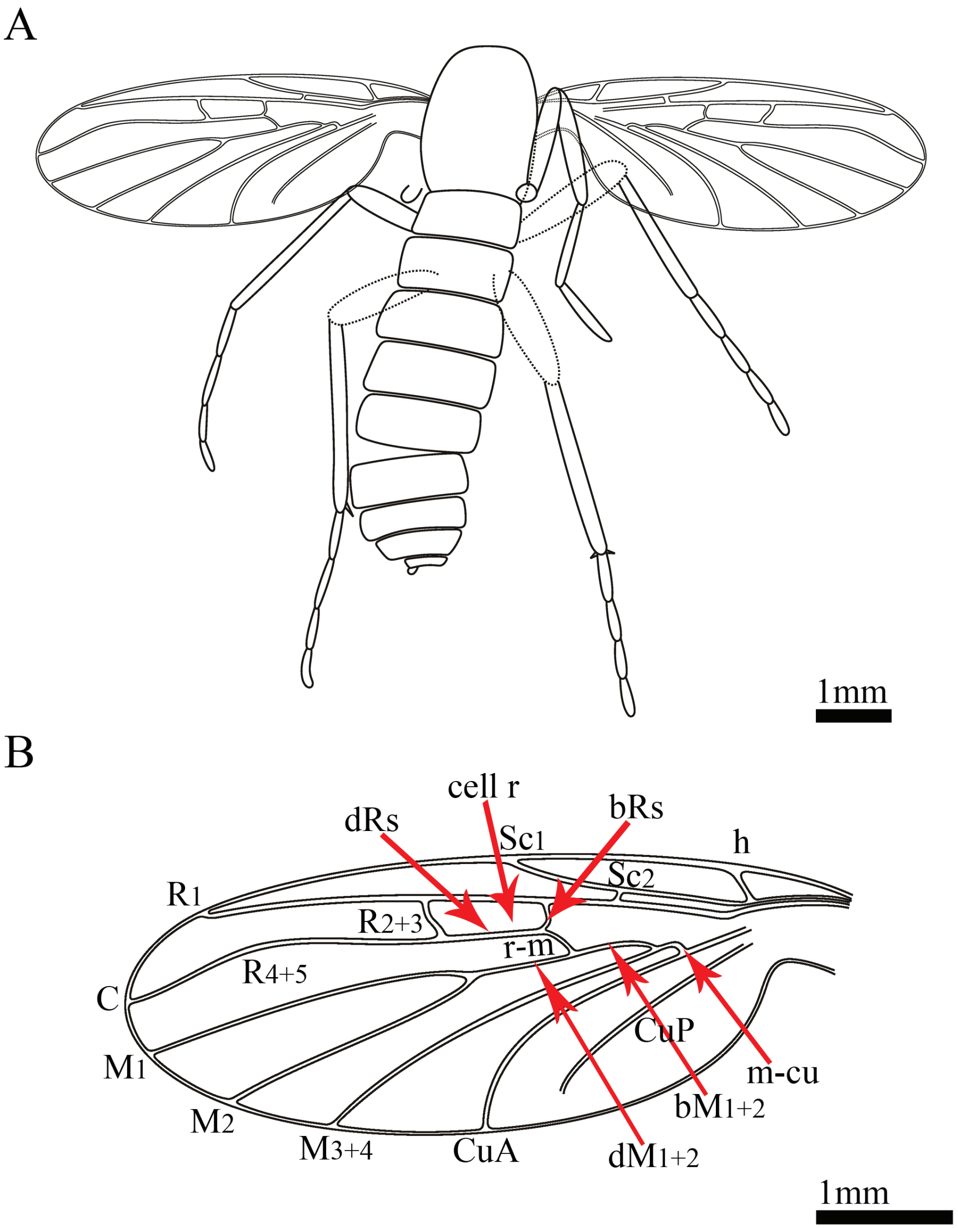

Figure 2. *Paramesosciophilodes bellus* sp. n., Line drawings of holotype: **A** part **B** wing venation.

the length of wing, ending far distad of the intersection of bRs and r-m. Humeral vein distinct and oblique. Sc_2 well developed, starting in front of bRs. Cell r distinctly small (0.89 mm), approx. 0.165 times the wing length (5.4 mm). Section of R from Sc_2 to bRs origin approx. 2.4 times as long as bRs. R forking into three branches: R_1, R_{2+3} and R_{4+5}. R_1 and R_{4+5} somewhat divergent terminally; R_{2+3} and R_{4+5} arched. Forking of Rs distad of the level of M forking. Rs strong, arising from beyond the basal one-third of length of wing, bRs+dRs nearly 0.4 times the R_{4+5}. Rs forking to R_{2+3} and R_{4+5} distad of forking of M_{1+2}. Section bRs 0.7 times the r-m. R_1 slightly curved, relatively long (nearly 0.5 times the length of wing), slightly deflected after junction with R_{2+3}; R_{2+3} slightly curved, shifted toward wing base, beyond the level of M_{1+2} forking. Vein R_{4+5} strongly arched near its midway, almost parallel with R_1, but slightly oblique at apex. Stem of M completely reduced basad of crossvein m-cu, with only a short segment distad of m-cu. Stem of M forking into M_{1+2} and $M_{3+4.}$ M_{1+2} forking into M_1 and M_2 near R_{2+3} level. M_1 arched anteriorly, M_2 nearly straight. Crossvein r-m short, curved, slightly oblique, shorter than bRs, nearly perpendicular to M_{1+2}, almost parallel to R_{2+3}, intersected at M_{1+2}, forking to bM_{1+2} and dM_{1+2}. bM_{1+2} approx. 6.6 times as long as m-cu. dM_{1+2} approx. as long as bM_{1+2}, and longer than r-m. CuA running parallel close to M_{3+4} basally. CuA reaching the posterior margin of wing at approx. the same level of M_{1+2} forking to M_1 and M_2. CuP short, slightly curved at its midway, not reaching the posterior margin of wing.

Remarks. *Paramesosciophilodes bellus* sp. n. resembles most closely *P. ningchengensis*, but can be distinguished from the latter in having Sc_1 ending at C proximad of the miglength of cell r (vs. at the miglength of cell r for *P. ningchengensis*) and CuA reaching the posterior margin of the wing markedly basad of Rs forking to R_{2+3} and R_{4+5} (vs. slightly basad of Rs forking to R_{2+3} and R_{4+5}).

This new species is differentiated from *P. ningchengensis*, *P. eximia*, *P. aequus*, and *P. rarissima* sp. n. based on a combination of characters listed in Table 2.

Paramesosciophilodes rarissima Gao, Shi, Shih & Ren, sp. n.

http://zoobank.org/2DC54917-79F7-4919-8B03-3BDA9BAF3B00
Figs 3, 4

Etymology. The specific name is from the Latin word of *rarissimus*, meaning rare.

Material. Holotype No. CNU-DIP-NN2013145 p/c, part and counterpart. A well-preserved insect with complete body with two wings, without head and halters, in dorsoventral aspect.

Locality and horizon. Daohugou Village, Shantou Township, Ningcheng County, Inner Mongolia, China, Jiulongshan Formation, late Middle Jurassic.

Diagnosis. Sc_1 ending near the midlength of cell r; bRs 0.8 times the r-m; R_{4+5} strongly curved; M_{1+2} forking basad of R_{2+3} level and distad of level of Sc_1 ending at C; CuA strongly arched, reaching the posterior margin of the wing at the level of intersection of Rs forking to R_{2+3} and R_{4+5}.

Description of holotype. Medium-sized mesosciophilid gnats, in dorsal aspect (Figs 3 and 4A). Body length (without head and part of thorax) 7.2 mm as preserved. Legs covered with long, dense pubescence. Head, antennae, and halters not preserved. Thorax length 1.8 mm, width 1.5 mm. Mesonotum convex. Scutellum sharp, clearly projecting. Wings membranous, oblong, length 5.0 mm, width 2.2

Table 2. Comparison of seven key characters of five species of *Paramesosciophilodes*.

	P. ningchengensis	***P. eximia***	***P. aequus***	***P. bellus* sp. n.**	***P. rarissima* sp. n.**
Length ratio of cell r and the wing	0.167 (left wing) 0.180 (right wing)	0.183 (left wing) 0.172 (right wing)	0.22 as described. But, the missing wing base was not included in wing length measurement.	0.165	0.184
Length of Sc_1	46–47 % of the wing length	46% of the wing length	24% of the wing length as described. But, the missing wing base was not included in wing length measurement.	46–47% of the wing length	43% of the wing length
Sc_1 ending at C	at the midlength of cell r	distad of midlength of cell r	proximad of midlength of cell r	proximad of the midlength of cell r	near the midlength of cell r
bRs vs r-m	0.6–0.7 times of length of r-m	0.5 times of the length of r-m	0.9 times of the length r-m	0.7 times of the length of r-m	0.8 times of the length of r-m
R_{4+5}	slightly curved	slightly curved	strongly curved	strongly curved	strongly curved
The position of base of M_{1+2} forking vs the forking of Rs	M_{1+2} forking distinctly basad of forking of Rs	M_{1+2} forking almost at level of forking of Rs	M_{1+2} forking basad of the forking of Rs	M_{1+2} forking basad of forking of Rs,	M_{1+2} forking basad of forking of Rs
The position of base of M_{1+2} forking vs the level of Sc_1 ending	M_{1+2} forking basad or distad of the level of Sc_1 ending	M_{1+2} forking at the level of Sc_1 ending	M_{1+2} forking distad of the level of the Sc_1 ending	M_{1+2} forking slightly distad of the level of Sc_1 ending	M_{1+2} forking slightly distad of the level of Sc_1 ending
CuA shape	CuA strongly arched	CuA smoothly arched	CuA smoothly arched	CuA strongly arched	CuA smoothly arched
CuA ending at the posterior margin vs. Rs forking to R_{2+3} and R_{4+5}	CuA ending slightly basad of Rs forking to R_{2+3} and R_{4+5}	CuA ending slightly distad of Rs forking to R_{2+3} and R_{4+5}	CuA ending slightly distad of Rs forking to R_{2+3} and R_{4+5}	CuA ending markedly basad of Rs forking to R_{2+3} and R_{4+5}	CuA ending slightly distad of Rs forking to R_{2+3} and R_{4+5}

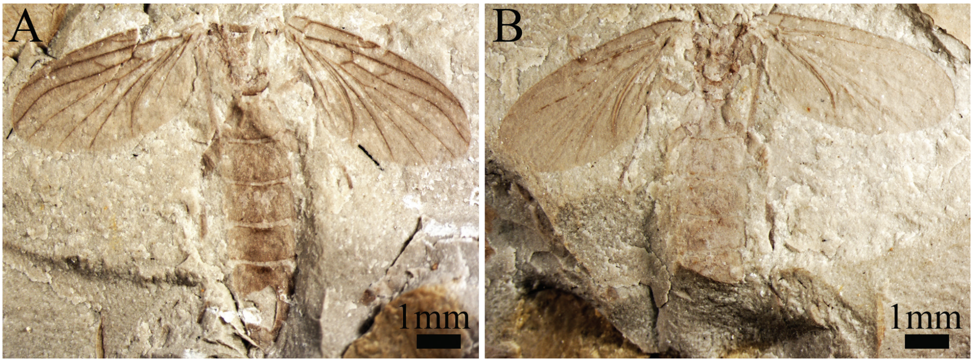

Figure 3. *Paramesosciophilodes rarissima* sp. n., holotype, Photographs of habitus (dorsoventral aspect): **A** No. CNU-DIP-NN2013145 p **B** No. CNU-DIP-NN2013145 c.

mm, darker in color in costal area, length 2.3 times width, and not reaching the apex of abdomen at rest. Abdomen thin, subcylindrical, length 5.4 mm, width 1.5 mm, with first five segments gradually widened distally, other segments gradually narrowed terminally. Legs poorly preserved, femora thicker in the middle, covered with numerous setae.

C strong, ending beyond wing apex, at which R_{4+5} ending (Fig. 4). Sc converging with C before the level of R_{4+5}. Sc_1 elongate, slightly shorter than one-half of wing length (0.43–0.47 times the wing length), and ending far distad of the intersection of bRs and r-m. Vein h distinct and oblique. Sc_2 developed well, starting in front of Rs, situated distinctly basal to Rs origin, arising beyond midway between h to Sc_1 ending. Cell r relatively large (0.92 mm), approx. 0.18 times the wing length (5.0 mm). The section of R from Sc_2 to Rs origin approx. 0.7 times the section bRs. R forking to R_1 and Rs, then Rs to R_{2+3} and R_{4+5}. Both R_1 and R_{4+5} somewhat divergent terminally; R_{2+3} and R_{4+5} arched. Rs usually strong, arising from basal one-half of length of wing, forking to R_{2+3} and R_{4+5} beyond the forking of M_{1+2}. Section bRs 0.8 times the r-m. R_1 slightly curved, relatively long, nearly 0.5 times the wing. Both R_1 and R_{4+5} divergent terminally. R_{2+3} curved, beyond the level of M_1 and M_2 forking. R_{4+5} strongly arched near its midlength. Stem of M, basad to crossvein m-cu completely reduced, with only a short segment distal to m-cu. Stem of M forking into M_{1+2} and M_{3+4}. M_{1+2} forking into M_1 and M_2 basad of R_{2+3} level and distad to level of Sc_1 ending at C. M_1 arched cephalad. M_2 nearly straight. Crossvein r-m short, curved, slightly oblique, shorter than bRs, r-m intersecting M_{1+2} and dividing M_{1+2} into bM_{1+2} and dM_{1+2}. Section bM_{1+2} approx. 4.3 times the crossvein m-cu. Section dM_{1+2} approx. 1.2 times the section bM_{1+2}, and longer than r-m. CuA running parallel and close to M_{3+4} basally. CuP short, slightly curved midway, reaching the posterior margin of wing at the same level as Sc_1 ending at C.

Remarks. *Paramesosciophilodes rarissima* sp. n. is distinguished from all other species of *Paramesosciophilodes* based on a combination of characters listed in Table 2.

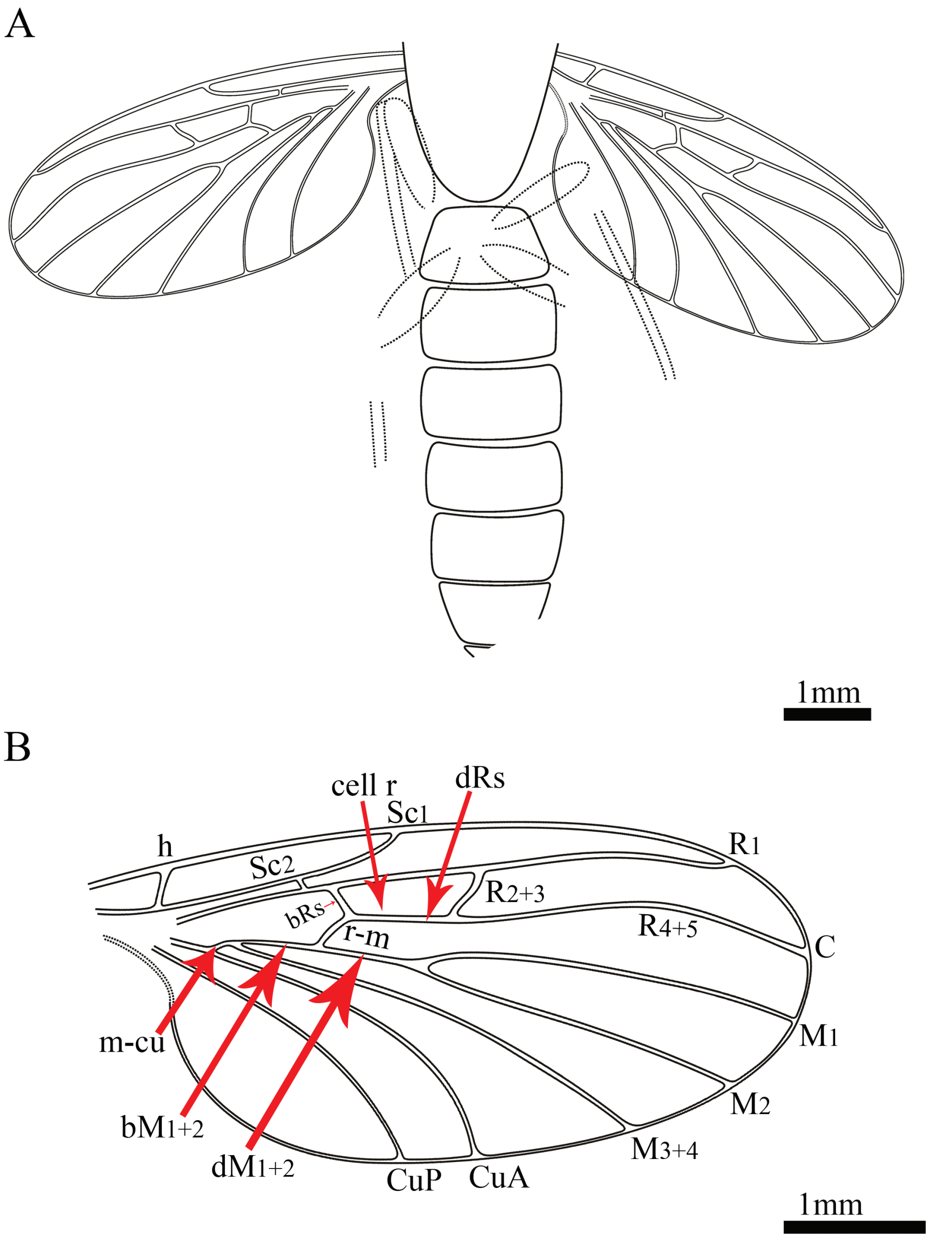

Figure 4. *Paramesosciophilodes rarissima* sp. n., Line drawings of holotype: **A** part **B** wing venation.

Discussion

As shown in Table 1, a total of 7 genera and 21 species of mesosciophilids have been reported from various localities in the Jurassic of Siberia and Kazakhstan, Lower Cretaceous of Transbaikalia, and Middle Jurassic of Inner Mongolia. One genus with 2 species was described in the Middle Jurassic Itat Formation, Siberia; 6 genera with 13 species were reported from the Middle Jurassic Jiulongshan Formation of Daohugou, Inner Mongolia, China; 2 genera with 3 species were described from the Late Jurassic Karabastau Formation in Kazakhstan; and one genus with 3 species was documented from the Early Cretaceous Zaza Formation at Baissa, Transbaikalia.

The data show that the known earliest mesosciophilids have been reported from the Middle Jurassic, while the latest ones are described from the Early Cretaceous. It seems that mesosciophilids became less diverse in the Early Cretaceous, and were possibly replaced by Mycetophilidae (Blagoderov 1993), which is supported by Zhang's data, who listed all the mesosciophilids and mycetophilids from Daohugou, and compared them with other faunas (Zhang 2002).

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Helminths of three species of opossums (Mammalia, Didelphidae) from Mexico

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Abstract

From August 2011 to November 2013, 68 opossums (8 *Didelphis* sp., 40 *Didelphis virginiana*, 15 *Didelphis marsupialis*, and 5 *Philander opossum*) were collected in 18 localities from 12 Mexican states. A total of 12,188 helminths representing 21 taxa were identified (6 trematodes, 2 cestodes, 3 acanthocephalans and 10 nematodes). Sixty-six new locality records, 9 new host records, and one species, the trematode *Brachylaima didelphus*, is added to the composition of the helminth fauna of the opossums in Mexico. These data, in conjunction with previous records, bring the number of taxa parasitizing the Mexican terrestrial marsupials to 41. Among these species, we recognized a group of helminths typical of didelphids in other parts of the Americas. This group is constituted by the trematode *Rhopalias coronatus*, the acanthocephalan *Oligacanthorhynchus microcephalus* and the nematodes *Cruzia tentaculata*, *Gnathostoma turgidum*, and *Turgida turgida*. In general, the helminth fauna of each didelphid species showed a stable taxonomic composition with respect to previously sampled sites. This situation suggests that the rate of accumulation of helminth species in the inventory of these 3 species of terrestrial marsupials in the Neotropical portion of Mexico is decreasing; however, new samplings in the Nearctic portion of this country will probably increase the richness of the helminthological inventory of this group of mammals.

Keywords

Didelphidae, *Didelphis virginiana*, *Didelphis marsupialis*, *Philander opossum*, parasites

Introduction

Less than 25% of the 525 species of mammals distributed in Mexico have been examined for helminth parasites (García-Prieto et al. 2012). To date, 336 nominal taxa of helminths have been recorded in mammals, 26 associated with 3 species of terrestrial opossums (Virginia opossum, *Didelphis virginiana* Kerr, the common opossum *Didelphis marsupialis* Linnaeus, and the Gray four-eyed opossum *Philander opossum* Linnaeus) from this country. However, the knowledge of the helminth richness associated with this host group is incomplete due to the wide distribution of these mammals in Mexico. *Didelphis marsupialis* occurs from Tamaulipas State and west San Luis Potosí until the Yucatán peninsula. *Didelphis virginiana* inhabits almost all of Mexico, except for the central Plateau and Baja California peninsula. *Philander opossum* occurs from south Tamaulipas State along the Gulf of Mexico coast and Chiapas State (Arcangeli-Álvarez 2010, Cervantes et al. 2010). The main objective of this work is to present new records of helminth species parasitizing these 3 species of opossums in Mexico and to compare the finding to previous records.

Materials and methods

From August 2011 to November 2013, 68 opossums (8 *Didelphis* sp., 40 *D. virginiana*, 15 *D. marsupialis*, and 5 *P. opossum*) were collected in 18 localities from 12 Mexican states (Table 1), under the collecting permit FAUT 0057 issued by the Secretaría del Medio Ambiente y Recursos Naturales (SEMARNAT), Mexico. Mammals were shot by local hunters or caught with Tomahawk traps and then killed with intraperitoneal sodium pentobarbital overdose. Opossums were dissected within the following 4 h. and all organs were examined under a stereomicroscope. Helminths were placed in Petri dishes with 0.85% saline solution. Platyhelminths and nematodes were fixed with hot 4% formalin and preserved in 70% ethanol; acanthocephalans were chilled in distilled water for 10–12 h. Once the proboscis was everted, they were preserved in 70% ethanol. Platyhelminths and acanthocephalans were stained with Mayer's paracarmin, cleared with methyl salicilate, and mounted in Canada balsam. Nematodes were cleared using Amman's lactophenol and temporarily mounted for morphological study (Lamothe-Argumedo 1997). Voucher specimens of all helminth species were deposited at Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City.

Results

A total of 12,188 helminths representing 21 taxa were identified in the 68 opossums collected from 18 localities within 12 states of Mexico (Figure 1). Six trematode, 2 cestode, 3 acanthocephalan, and 10 nematode species were collected. Below, we present

Table 1. Sampling sites for opossum species analyzed in this study.

State	Locality[†]/collection date (month/year)	Geographic coordinates	Sample size/host species	Altitude (easl)
Campeche	Escárcega[1] 07/2012	18°37'00"N; 90°43'13”W	3/*D. virginiana*; 1/*D. marsupialis*	82
Chiapas	Agua Fría[2] 06/2012; 03/2013	16°15'26"N; 93°53'55"W	5/*Didelphis* sp.; 1/*D. virginiana*	60
	Finca Brasil[3] 06/2012	15°05'41"N; 92°13'45"W	2/*Didelphis* sp.; 3/*D. virginiana*; 2/*D. marsupialis*	463
Colima	Coquimatlán[4] 09/2012	19°10'28"N; 103°50'39”W	6/*D. virginiana*	550
Distrito Federal	Pedregal de San Ángel[5] 02/2014	19°19'14"N; 99°12'33"W	2/*D. virginiana*	2268
Guanajuato	Rincón de Martínez[6] 02/2013	20°19'44"N; 101°34'42”W	2/*D. virginiana*	1730
Hidalgo	Tianguistengo[7] 03/2014	19°10'50"N; 99°28'06"W	2/*D. virginiana*	2620
Morelos	Tepoztlán[8] 08/2014	19°00'07"N; 99°06'00"W	1/*D. virginiana*	1700
Oaxaca	Cerro del Tepezcuintle[9] 08/2013	18°15'28”N; 96°24'00"W	2/*D. virginiana*	87
Puebla	Coapan[10] 08/2014	18°25'42"N; 97°24'30"W	1/*Didelphis* sp.; 1/*D. virginiana*	1648
	Zapotitlán Salinas[11] 08/2014	18º19'45"N; 97°28'30"W	1/*D. virginiana*	2240
Tabasco	Teapa[12] 06-07/2013	17°33'59"N; 92°57'00”W	2/*D. virginiana* 1/*D. marsupialis*	72
	Villahermosa[13] 01/2012	17°34'17”N; 92°57'09”W	3/*D. virginiana*	10
Veracruz	Tlacotalpan[14] 02/2012	18°37'40”N; 95°40'40”W	2/*D. virginiana*; 8/*D. marsupialis*; 3/*P. opossum*	10
	Los Tuxtlas[14] 08/2011; 03/2012	18°34'21”N; 95°04'30”W	3/*D. virginiana*; 3/*D. marsupialis* 2/*P. opossum*	300
Yucatán	Mérida[15] 11/2013	20°58'04"N; 89°37'18"W	5/*D. virginiana*	16
	Tzucacab[16] 11/2013	20°00'58"N; 89°01'12"W	1/*D. marsupialis*	36

[†]The superscript numbers indicate the position of the localities in the Figure 1.

a checklist of the helminth species recorded, indicating the site of infection, current records with State and locality where the hosts were collected, host species, CNHE accession numbers, and previous records from Mexico.

Parasite-Host list

† New locality record; ‡ New record for Mexico; * New host in Mexico.

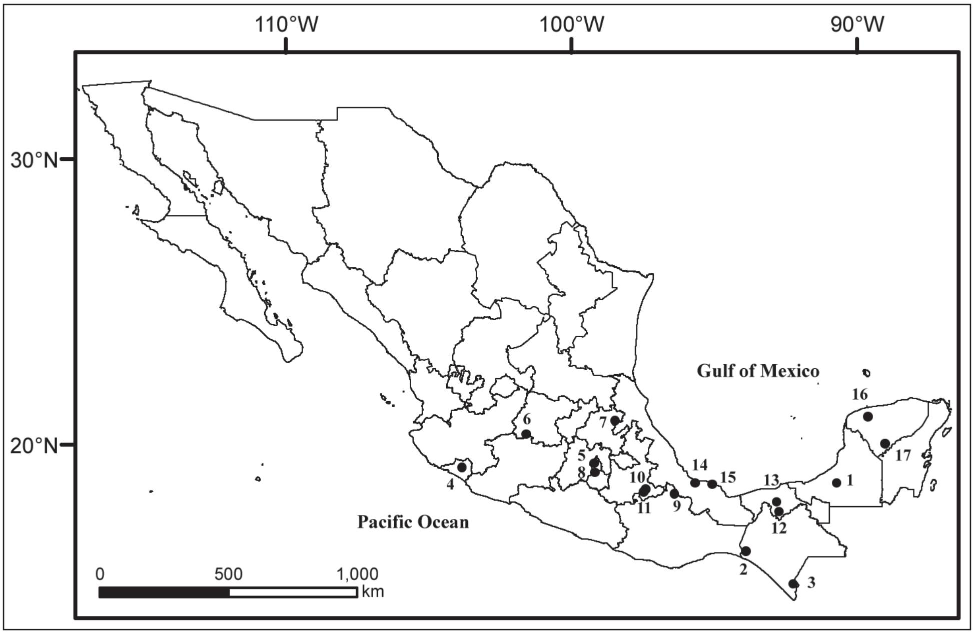

Figure 1. Map of Mexico showing the sampled localities in the present study.

Phylum Platyhelminthes Gegenbaur, 1859
Class Trematoda Rudolphi, 1808
Family Opisthorchiidae Braun, 1901

Amphimerus caudalitestis Caballero, Grocott & Zerecero, 1952

Site of infection. Gall-bladder.

Present records. VERACRUZ: Los Tuxtlas: *Didelphis marsupialis**, *Didelphis virginiana**.

Specimens deposited. CNHE 9481–2.

Previous records in Mexico. VERACRUZ: Los Tuxtlas: *Philander opossum* (Cañeda-Guzmán 1997).

Remarks. These specimens belong to *A. caudalitestis* due to the position of the reproductive organs and the separation of the vitelline glands in two fields lying anterior and posterior to the ovary. Furthermore, the uterus has a zig-zag shape, occupying intercecal extension and the S-shape of the excretory vesicle, sinuous between both testes (Caballero et al. 1952).

Brachylaima didelphus Premvati & Bair, 1979‡

Site of infection. Intestine.

Present records. CAMPECHE: Escárcega†: *Didelphis virginiana**.

Specimens deposited. CNHE 9483–4.

Remarks. The specific identification of this material follows Premvati and Blair (1979) and is based on the disposition of the vitellaria which extending from pharynx to posterior end.

Family Phaneropsolidae Mehra, 1935

Philandrophilus magnacirrus Thatcher, 1970

Site of infection. Gall-bladder.

Present records. Los Tuxtlas: *Didelphis marsupialis**, *Philander opossum*.

Specimens deposited. CNHE 9485–6.

Previous records in Mexico. VERACRUZ: Los Tuxtlas: *Philander opossum* (Cañeda-Guzmán 1997).

Remarks. In accordance with Thatcher (1970) this species is characterised by having body flattened and pyriform, covered with small spines. Cirrus and cirrus sac large. Parasites in gall-bladder of marsupials.

Family Rhopaliidae Looss, 1899

Rhopalias caballeroi Kifune & Uyema, 1982

Site of infection. Intestine.

Present records. VERACRUZ: Tlacotalpan†: *Philander opossum**.

Specimens deposited. CNHE 9487.

Previous records in Mexico. VERACRUZ: Los Tuxtlas: *Didelphis* sp. (Haverkost and Gardner 2008).

Remarks. *Rhopalias caballeroi* is distinguished by the absence of oral and flanking spines, and because it has between 4 and 11 spines visible within tentacle sacs (Haverkost and Gardner 2008).

Rhopalias coronatus (Rudolphi, 1819)

Site of infection. Intestine.

Present records. CHIAPAS: Agua Fría†: *Didelphis marsupialis*, *Didelphis virginiana*, *Didelphis* sp., *Philander opossum*; Finca Brasil†: *Didelphis marsupialis*, *Didelphis virginiana*, *Philander opossum*. OAXACA: Cerro del Tepezcuintle†, San Miguel Soyaltepec†: *Didelphis virginiana*. TABASCO: Cunduacán†: *Didelphis virginiana*; Grutas de Coconá†, Teapa†: *Didelphis marsupialis*. VERACRUZ: Los Tuxtlas: *Didelphis mar-*

supialis, *Didelphis virginiana*, *Philander opossum*; Tlacotalpan[†]: *Didelphis marsupialis*, *Didelphis* sp. YUCATÁN: Mérida[†]: *Didelphis marsupialis*.

Specimens deposited. CNHE 9488–9504.

Previous records in Mexico. CHIAPAS: Motozintla: *Didelphis* sp. (Caballero et al. 1944). NUEVO LEÓN: Colonia Country La Silla, Huinala, Los Lirios: *Didelphis marsupialis* (Romero 1981). OAXACA: Cuicatlán: *Didelphis* sp. (Pérez-Ponce de León et al. 2007). QUINTANA ROO: Rancho La Ceiba: *Didelphis marsupialis* (Kingston and Tai 1968). VERACRUZ: Los Tuxtlas: *Didelphis marsupialis*, *Didelphis virginiana*, *Philander opossum* (Cañeda-Guzmán 1997), *Didelphis* sp. (Haverkost and Gardner 2008); Alvarado: *Didelphis virginiana* (Monet-Mendoza et al. 2005).

Remarks. The diagnostic traits of this species are: flanking and oral spines present. Between 3 and 11 spines visible within tentacle sacs, which extend far beyond the posterior margin of the pharynx (Haverkost and Gardner 2008).

Rhopalias macracanthus Chandler, 1932

Site of infection. Intestine.

Present records. VERACRUZ: Los Tuxtlas: *Didelphis marsupialis*, *Didelphis* sp., *Didelphis virginiana*; Tlacotalpan[†]: *Didelphis marsupialis*, *Philander opossum*.

Specimens deposited. CNHE 9505–9.

Previous records in Mexico. COLIMA: Comala: *Didelphis marsupialis* (Lamothe-Argumedo 1978); La Esperanza: *Didelphis marsupialis* (Miyazaki et al. 1980). CHIAPAS: Jaltenango: *Didelphis* sp. (Caballero 1946); Motozintla: *Didelphis* sp. (Caballero et al. 1944); Pueblo Nuevo (Pérez-Ponce de León et al. 2007). OAXACA: Carretera Temascal-Tuxtepec: *Didelphis virginiana* (Monet-Mendoza et al. 2005). QUINTANA ROO: Rancho La Ceiba: *Didelphis marsupialis* (Kingston and Tai 1968). VERACRUZ: Alvarado: *Didelphis virginiana* (Monet-Mendoza et al. 2005); Los Tuxtlas: *Didelphis marsupialis*, *Didelphis virginiana*, *Philander opossum* (Cañeda-Guzmán 1997), *Didelphis* sp. (Haverkost and Gardner 2008).

Remarks. This species was identified by having tentacle sacs that do not extend beyond the posterior margin of the pharynx and by having only flanking spines (Haverkost and Gardner 2008).

Class Eucestoda Southwell, 1930

Family Anoplocephalidae Cholodkovsky, 1902

Mathevotaenia sp.

Site of infection. Intestine.

Present records. COLIMA: Colima: *Didelphis virginiana*.

Specimens deposited. CNHE 9514.

Previous records in Mexico. CHIAPAS: Lagos de Colón: *Didelphis virginiana* (Monet-Mendoza et al. 2005). COLIMA: Colima: *Didelphis virginiana* (García-Prieto et al. 2012).

Remarks. This material represents a new species which will be described separately.

Family Proteocephalidae La Rue, 1911

Thaumasioscolex didelphidis Cañeda-Guzmán, de Chambrier & Scholz, 2001

Site of infection. Intestine.

Present records. CHIAPAS: Finca Brasil†: *Didelphis virginiana**, *Didelphis marsupialis.*

Specimens deposited. CNHE 9528.

Previous records in Mexico. VERACRUZ: Los Tuxtlas: *Didelphis marsupialis* (Cañeda-Guzmán et al. 2001).

Remarks. In accordance with Cañeda-Guzmán et al. (2001), *T. didelphidis* is distinguished by the morphology of the scolex that is formed by 4 well separated lobes each containing 1 noncircular sucker opening laterally inside the exterolateral cavity, a large-sized body and by the shape of gravid proglottids that are inversely craspedote, among others.

Phylum Acanthocephala (Rudolphi, 1808)
Family Oligacanthorhynchidae Southwell & Macfie, 1925

Oligacanthorhynchus microcephalus (Rudolphi, 1819) Schmidt, 1972

Site of infection. Intestine.

Present records. HIDALGO: Tianguistengo†: *Didelphis virginiana*.

Specimens deposited. CNHE 9510.

Previous records in Mexico. CAMPECHE: Escárcega: *Didelphis marsupialis, Didelphis virginiana* (López-Caballero et al. 2015). COLIMA: Tecomán: *Didelphis virginiana* (García-Prieto et al. 2010). CHIAPAS: Agua Fría: *Didelphis marsupialis, Didelphis virginiana, Philander opossum* (López-Caballero et al. 2015); Cascadas de Agua Azul: *Didelphis virginiana* (Prado-Ancona 1993); Finca Brasil: *Didelphis marsupialis, Didelphis virginiana, Philander opossum* (López-Caballero et al. 2015). MICHOACÁN: Agua Blanca: *Didelphis virginiana* (Prado-Ancona 1993). GUANAJUATO: Rincón de Martínez: *Didelphis virginiana* (López-Caballero et al. 2015). MORELOS: Progreso: *Didelphis virginiana* (García-Prieto et al. 2010). OAXACA: Soyaltepec: *Didelphis virginiana* (López-Caballero et al. 2015); Temascal: *Didelphis virginiana* (García-Varela et al. 2000). TABASCO: Cunduacán: *Didelphis virginiana* (López-Caballero et al. 2015); Ranchería el Boquerón: *Didelphis marsupialis* (García-Prieto et al. 2010); Río Oxolotán: *Philander opossum* (Prado-Ancona 1993). VERACRUZ: Los

Tuxtlas: *Didelphis marsupialis*, *Didelphis virginiana*, *Philander opossum* (Prado-Ancona 1993; Cañeda-Guzmán 1997); Tlacotalpan: *Didelphis virginiana* (López-Caballero et al. 2015). YUCATÁN: Mérida: *Didelphis marsupialis*, *Didelphis virginiana* (López-Caballero et al. 2015).

Remarks. With the exception of records made by López-Caballero et al. (2015) all other previous records were listed as *Oligacanthorhynchus tortuosa*, but this species is a junior synonym of *Oligacanthorhynchus microcephalus* (Richardson et al. 2014). The hook and cement gland number (36 and 8, respectively), as well as the eggs size (0.83-0.110 X 0.38-0.50) are considered as diagnostic traits of this species by López-Caballero et al. (2015).

Oncicola luehei (Travassos, 1917) Schmidt, 1972

Site of infection. Intestine.

Present records. VERACRUZ: Los Tuxtlas: *Didelphis marsupialis**.

Specimens deposited. CNHE 9511–12.

Previous records in Mexico. VERACRUZ: Los Tuxtlas: *Didelphis virginiana* (Prado-Ancona 1993; Cañeda-Guzmán 1997).

Remarks. These specimens belong to *O. luehei* because the dimensions of the proboscis, the number of hooks (36), as well as its size and arrangement fits to the morphology mentioned by Machado (1950).

Family Plagiorhynchidae Golvan, 1960

Porrorchis nickoli Salgado-Maldonado & Cruz-Reyes, 2002

Site of infection. Intestine.

Present records. VERACRUZ: Los Tuxtlas: *Didelphis virginiana*.

Specimens deposited. CNHE 9513.

Previous records in Mexico. CHIAPAS: Cascadas de Agua Azul: *Didelphis virginiana* (Salgado-Maldonado and Cruz-Reyes 2002). TABASCO: Río Oxolotán: *Philander opossum* (Salgado-Maldonado and Cruz-Reyes 2002). VERACRUZ: Lago de Catemaco, Sontecomapan: *Didelphis virginiana* (Salgado-Maldonado and Cruz-Reyes 2002); Martínez de la Torre: *Didelphis marsupialis* (Salgado-Maldonado and Cruz-Reyes 2002); Los Tuxtlas: *Didelphis marsupialis*, *Didelphis virginiana*, *Philander opossum* (Salgado-Maldonado and Cruz-Reyes 2002).

Remarks. According to Salgado-Maldonado and Cruz-Reyes (2002), three characteristics diagnosed this acanthocephalan species: (1) a smaller proboscis, (2) the armature of proboscis bearing few rows and few hooks per row compared with other species, and (3) the male reproductive system occupying only the posterior half of trunk.

Phylum Nematoda Rudolphi, 1808
Family Metastrongylidae Leiper, 1912

Didelphostrongylus hayesi Prestwood, 1976

Site of infection. Lungs.

Present records. DISTRITO FEDERAL: Pedregal de San Ángel†: *Didelphis virginiana*; GUANAJUATO: Irapuato†: *Didelphis virginiana*†. HIDALGO: Tianguistengo†: *Didelphis virginiana*. MORELOS: Tepoztlán†: *Didelphis virginiana*.

Specimens deposited. CNHE 8969, 9024, 9554–9556, 9562.

Previous records in Mexico. COLIMA: ND: *Didelphis virginiana* (García-Márquez et al. 2012). GUERRERO: Laguna de Tres Palos, Taxco: *Didelphis virginiana* (Monet-Mendoza et al. 2005). OAXACA: Temascal: *Didelphis virginiana* (Monet-Mendoza et al. 2005).

Remarks. Our material was identified following Prestwood (1976); this species is characterised because the oral opening is surrounded by lips, the morphology and size of the spicules and the number and arrangement of bursal rays.

Family Aspidoderidae Skrjabin & Schikhobalova, 1947

Aspidodera raillieti Travassos, 1913

Site of infection. Intestine.

Present record. TABASCO: Villahermosa†: *Didelphis virginiana**. VERACRUZ: Los Tuxtlas†: *Didelphis virginiana*, *Philander opossum**.

Specimens deposited. CNHE 8971–3.

Previous records in Mexico. CHIAPAS: Motozintla: *Didelphis* sp. (Caballero and Zerecero 1944).

Remarks. These specimens were identified based on Jiménez-Ruiz et al. (2006) and compared with further description of the species made by Chagas-Moutinho et al. (2014). *Aspidodera raillieti* can be distinguished because the cephalic cordons exceed the level of the oral vestibule and touch the base of cephalic cap, as well as by having a digitiform projection on the left ventrolateral oral lip.

Family Kathlanidae Lane, 1914

Cruzia tentaculata (Rudolphi, 1819) Travassos, 1917

Site of infection. Caecum.

Present records. CAMPECHE: Escárcega†: *Didelphis marsupialis*, *Didelphis virginiana*. CHIAPAS: Arriaga†: *Didelphis* sp., *Didelphis virginiana*; Tapachula†: *Didelphis* sp., *Didelphis marsupialis*, *Didelphis virginiana*. COLIMA: Colima†: *Didelphis virginiana*. DISTRITO FEDERAL: Pedregal de San Ángel†: *Didelphis virginiana*. GUANAJUATO: Irapuato†: *Didelphis virginiana*. HIDALGO: Tianguistengo†: *Didelphis virginiana*. MORELOS: Tepoztlán†: *Didelphis virginiana*. OAXACA: Soyaltepec†: *Didelphis virginiana*. PUEBLA: Carretera Coapan-Huajuapan de León†: *Didelphis* sp.; Coapan†: *Didelphis virginiana*; Zapotitlán Salinas†: *Didelphis virginiana*. TABASCO: Teapa†: *Didelphis marsupialis*; Villahermosa†: *Didelphis virginiana*. VERACRUZ: Los Tuxtlas: *Didelphis marsupialis*, *Didelphis virginiana*, *Philander opossum*; Tlacotalpan†: *Didelphis marsupialis*, *Didelphis virginiana*, *Philander opossum*. YUCATÁN: Mérida†: *Didelphis virginiana*; Tzucacab†: *Didelphis marsupialis*.

Specimens deposited. CNHE 8999, 9000–17, 9533–9540, 9557, 9563.

Previous records in Mexico. CHIAPAS: Motozintla: *Didelphis* sp. (Caballero and Zerecero 1944); Jaltenango: *Didelphis marsupialis* (Caballero 1958). COLIMA: Comala: *Didelphis marsupialis* (García-Prieto et al. 2012); La Esperanza: *Didelphis marsupialis* (Miyazaki et al. 1980); ND: *Didelphis virginiana* (Lamothe-Argumedo et al. 1981). DISTRITO FEDERAL: ND: *Didelphis* sp. (Caballero 1937); Chapultepec: *Didelphis marsupialis* (Gutiérrez-Fuster 1966). ESTADO DE MÉXICO: ND: *Didelphis* sp. (García-Prieto et al. 2012). HIDALGO: Tasquillo: *Didelphis* sp. (Caballero 1937). JALISCO: Chamela: *Didelphis marsupialis* (García-Prieto et al. 2012). MORELOS: Reserva Estatal Sierra de Monte Negro: *Didelphis virginiana* (Slava-Araujo 2005). NUEVO LEÓN: San Nicolás de los Garza: *Didelphis virginiana* (García-Prieto et al. 2012). VERACRUZ: Los Tuxtlas: *Didelphis marsupialis*, *Didelphis virginiana*, *Philander opossum* (Cañeda-Guzmán 1997); ND: *Didelphis marsupialis* (Flores-Barroeta 1957).

Remarks. We identify these nematodes according to the re-description made by Adnet et al. (2009), who established the number of caudal papillae (ten pairs of button-like papillae, symmetrically ventro-laterally located), as well as the single median papilla at the anterior cloacal lip and four pairs of post-cloacal papillae, as diagnostic traits of this species.

Family Gnathostomatidae Railliet, 1895

Gnathostoma turgidum Stossich, 1902

Site of infection. Stomach (adult; larvae); liver (sub-adult).

Present records. CHIAPAS: Arriaga†: *Didelphis* sp. COLIMA: Colima†: *Didelphis virginiana*. OAXACA: Soyaltepec†: *Didelphis virginiana*. TABASCO: Teapa†: *Didelphis marsupialis*. VERACRUZ: Tlacotalpan: *Didelphis virginiana*.

Specimens deposited. CNHE 8979–86, 9548–9549.

Previous records in Mexico. CHIAPAS: Jaltenengo: *Didelphis marsupialis* (Caballero 1958). COLIMA: Laguna de Amela: *Didelphis virginiana* (García-Márquez 2005). GUERRERO: Laguna de Tres Palos: *Didelphis virginiana* (Monet-Mendoza et al. 2005). JALISCO: Carretera Juntas-Palmas (Puerto Vallarta): *Didelphis* *virginiana* (Monet-Mendoza et al. 2005); Chamela: *Didelphis virginiana* (see Lamothe-Argumedo et al. 1998). MORELOS: Valle de Amilcingo: *Didelphis virginiana* (Mosqueda-Cabrera 2003). OAXACA: Temascal: *Philander opossum* (Almeyda-Artigas et al. 2010), *Didelphis marsupialis* (Almeyda-Artigas et al. 2000, Oceguera-Figueroa 2002, Mosqueda-Cabrera 2003), *Didelphis virginiana* (Lamothe-Argumedo et al. 1998, Almeyda-Artigas et al. 2000, Mosqueda-Cabrera 2003). SINALOA: Tecualilla: *Didelphis virginiana* (Nawa et al. 2009, Díaz-Camacho et al. 2009). TABASCO: Rancho Mendoza Llergo: *Didelphis marsupialis* (León-Règagnon et al. 2005); Jardín Botánico de la UJAT, Oriente Segunda Sección, Ranchería El Limón, Ranchería Emiliano Zapata, Ranchería José María Pino Suárez, Ranchería La Palma: *Didelphis marsupialis* (Gallegos-Torres 2003). VERACRUZ: Laguna Los Vila, Laguna Novillera: *Didelphis virginiana* (León-Règagnon et al. 2005); Tlacotalpan: *Didelphis virginiana* (Almeyda-Artigas et al. 2000, Pérez-Álvarez et al. 2008), *Didelphis marsupialis* (Pérez-Álvarez et al. 2008).

Remarks. The presence of numerous points on the posterior end of cuticular spines at esophagus-intestine junction level, the body size, and the lack of spines in the posterior region of body, constitutes the diagnostic traits of this species in accordance with Bertoni-Ruiz et al. (2011).

Family Gongylonematidae Hall, 1916

Gongylonema sp.

Site of infection. Stomach.

Present records. CHIAPAS: Tapachula†: *Didelphis virginiana*.

Specimens deposited. CNHE 8970.

Remarks. Two species of the genus *Gongylonema* are distributed in Mexican didelphids: *Gongylonema mexicanum* (in Chiapas and Veracruz) and *Gongylonema pulchrum* (in Chiapas) (García-Prieto et al. 2012). The specific identification of our specimen was not possible because we collected only one female.

Family Physalopteridae Railliet, 1893

Turgida turgida Rudolphi, 1819

Site of infection. Stomach.

Present records. CAMPECHE: Escárcega†: *Didelphis marsupialis*, *Didelphis virginiana*. CHIAPAS: Arriaga†: *Didelphis* sp.; Tapachula†: *Didelphis* sp.; *Didelphis marsupialis*. COLIMA: Colima: *Didelphis virginiana*. DISTRITO FEDERAL: Pedregal de San Ángel: *Didelphis virginiana*. GUANAJUATO: Irapuato†: *Didelphis virginiana*. HIDALGO: Tianguistengo†: *Didelphis virginiana*. OAXACA: Soyaltepec†: *Didelphis* *virginiana*. PUEBLA: Coapan†: *Didelphis virginiana*. TABASCO: Teapa: *Didelphis marsupialis*; Villahermosa: *Didelphis virginiana*. VERACRUZ: Los Tuxtlas: *Didelphis marsupialis, Didelphis virginiana*; Tlacotalpan†: *Didelphis marsupialis*, *Didelphis virginiana*, *Philander opossum*.

Specimens deposited. CNHE 9018–23, 9025–36, 9541–9543.

Previous records in Mexico. CHIAPAS: Motozintla: *Didelphis* sp. (Caballero and Zerecero 1944, 388); Tonalá: *Philander opossum* (García-Prieto et al. 2012). COLIMA: Colima: *Didelphis virginiana* (Monet-Mendoza et al. 2005); Comala: *Didelphis virginiana* (Monet-Mendoza et al. 2005), *Didelphis marsupialis* (García-Prieto et al. 2012); Dos Amates: *Didelphis virginiana* (Monet-Mendoza et al. 2005); La Esperanza: *Didelphis marsupialis* (Miyazaki et al. 1980); Madrid: *Didelphis marsupialis* (Miyazaki et al. 1980), *Didelphis virginiana* (Monet-Mendoza et al. 2005); ND: *Didelphis virginiana* (Lamothe et al. 1981). DISTRITO FEDERAL: ND: *Didelphis* sp. (Caballero 1937), *Didelphis marsupialis* (Monsivais-Aguilar 1958); Pedregal de San Ángel: *Didelphis virginiana* (Pacheco-Coronel 2010); Chapultepec: *Didelphis marsupialis* (Gutiérrez-Fuster 1966). ESTADO DE MÉXICO: ND: *Didelphis* sp. (García-Prieto et al. 2012): Tequesquinahuac: *Didelphis virginiana* (Monet-Mendoza et al. 2005). GUERRERO: Carretera Coyuquilla-Zihuatanejo, Coyuquilla: *Didelphis virginiana* (Monet-Mendoza et al. 2005); Carretera Aeropuerto-Ixtapa: *Didelphis virginiana* (García-Prieto et al. 2012); Taxco El Viejo: *Didelphis virginiana* (Monet-Mendoza et al. 2005). HIDALGO: Tasquillo: *Didelphis* sp. (Caballero 1937). JALISCO: Chamela: *Didelphis marsupialis* (García-Prieto et al. 2012). MICHOACÁN: El Hortigal: *Didelphis virginiana* (Monet-Mendoza et al. 2005). MORELOS: Reserva Estatal Sierra de Monte Negro: *Didelphis virginiana* (Eslava-Araujo 2005). NAYARIT: Peñitas: *Didelphis virginiana* (Monet-Mendoza et al. 2005). NUEVO LEÓN: Marín, Monterrey: *Didelphis marsupialis* (García-Prieto et al. 2012). OAXACA: Dominguillo: *Didelphis marsupialis* (see Monet-Mendoza et al. 2005); Nizanda: *Didelphis virginiana* (Monet-Mendoza et al. 2005). VERACRUZ: Los Tuxtlas: *Didelphis marsupialis, Didelphis virginiana, Philander opossum* (Cañeda-Guzmán 1997); Medellín: *Didelphis marsupialis* (Caballero-Deloya 1969).

Remarks. These specimens were identified based on the re-description of this species (Matey et al. 2001). Its diagnostic traits are: the presence of 2 spongelike areas on the inner side of each pseudolabia, and the number of caudal papillae (22).

Family Trichuridae Railliet, 1915

Trichuris didelphis Babero, 1960

Site of infection. Caecum.

Present records. CAMPECHE: Escárcega†: *Didelphis virginiana*. CHIAPAS: Arriaga†: *Didelphis* sp. COLIMA: Colima†: *Didelphis virginiana*. HIDALGO: Tianguistengo†: *Didelphis virginiana*. MORELOS: Tepoztlán†: *Didelphis virginiana*. YUCATÁN: Mérida†: *Didelphis virginiana*; Tzucacab†: *Didelphis marsupialis*.

Specimens deposited. CNHE 8974–78, 9550–9553.

Previous records in Mexico. VERACRUZ: Los Tuxtlas: *Didelphis marsupialis*, *Didelphis virginiana*, *Philander opossum* (Cañeda-Guzmán 1997).

Remarks. Our material was identified based on the original description (Babero 1960). This species is characterised by the size of the spicule (0.47–0.6 mm), by having a spiny sheath, by the size of mature eggs (0.068 × 0.032 mm) and the posterior position of the vulva.

Capillariinae gen sp.

Site of infection. Lungs.

Present records. CAMPECHE: Escárcega: *Didelphis marsupialis**, *Didelphis virginiana**

Specimens deposited. CNHE 9031–2.

Remarks. Identification was not possible because only eggs were obtained.

Family Viannaiidae Neveu-Lemaire, 1944

Viannaia viannai Travassos, 1914

Site of infection. Intestine.

Present records. CAMPECHE: Escárcega†: *Didelphis virginiana*. CHIAPAS: Arriaga†: *Didelphis virginiana*, *Didelphis marsupialis**. COLIMA: Colima†: *Didelphis virginiana*. OAXACA: Soyaltepec†: *Didelphis virginiana*. PUEBLA: Coapan†: *Didelphis virginiana*. TABASCO: Teapa†: *Didelphis marsupialis*; Villahermosa†: *Didelphis virginiana*. VERACRUZ: San Andrés Tuxtla†: *Didelphis virginiana*, *Didelphis marsupialis*; Tlacotalpan†: *Didelphis marsupialis*, *Philander opossum**.

Specimens deposited. CNHE 8988–98; 9025–30, 9544–9547.

Previous records in Mexico. GUERRERO: Taxco El Viejo: *Didelphis virginiana* (Monet-Mendoza et al. 2005).

Remarks. Our specimens were identified following Guerrero (1985). The synlophe of *Viannaia viannai* at mid-body has 3 ventral ridges orientated to left, short spicules (0.133-0.141 mm) and bursal ray arrangement 2-1-2 type.

Travassostrongylus sp.

Site of infection. Intestine (Adult).

Present records. CHIAPAS: Arriaga†: *Didelphis* sp.

Specimens deposited. CNHE 8987.

Remarks. To date, 12 species of the genus *Travassostrongylus* have been described, all parasitizing New World marsupials; *Travassostrongylus orloffi* Travassos, 1935 is the only species of this genus recorded in Mexico as parasite of *Didelphis marsupialis*; however, the finding of only 8 females make species identification difficult, because taxonomy of this group is based on male characteristics (Scheibel et al. 2014).

Discussion

As a result of this study, we reported 66 new locality records, 9 new host records, and added one species to the composition of the helminth fauna of the opossums in Mexico: the trematode *B. didelphus* parasitizing *D. virginiana*, which had not been recorded in this country (see García-Prieto et al. 2012). A total of 21 helminth taxa were obtained from the 3 opossums species analyzed (6 trematodes, 2 cestodes, 10 nematodes and 3 acanthocephalans), all in adult stage, with exception of the larvae of *G. turgidum* collected during their migration through the liver of the hosts. The richest helminth fauna among the 3 host species was recorded in *D. virginiana*, (parasitized by 17 species), followed by *D. marsupialis* (11 species) and *P. opossum* (8 species). The digestive tract had the highest number of helminth species (12 intestinals, 2 in gall-bladder, 2 in caeca, and 3 in stomach); only 2 of the 21 taxa, *D. hayesi* and Capillariinae gen. sp. were found in another site of infection (lungs). The geographic distribution of the helminth species was heterogeneous. The nematode *C. tentaculata* was the only species found in all localities. Other helminth species were collected from 7 (*T. didelphis*), 8 (*R. coronatus*) and 9 (*V. viannai*) localities; however, most taxa (12) were found in only one locality.

These data bring the number of taxa parasitizing *D. virginiana*, *D. marsupialis*, and *P. opossum* to 37, 21 and 20, respectively (García-Prieto et al. 2012). In this work we sampled in 9 previously unstudied localities; nevertheless, 47.2%, 52.4% and 40% of the taxa collected were reported previously from the Virginia opossum, Black-eared opossum and Gray four-eyed opossum, respectively. These species are typical of didelphids in other parts of the Americas (see Alden 1995; Corrêa Gomes et al. 2003; Haverskot and Gardner 2008; Bertoni-Ruiz et al. 2011; Richardson et al. 2014), conforming a group basically represented by the trematode *R. coronatus*, the acanthocephalan *O. microcephalus* and the nematodes *C. tentaculata*, *G. turgidum*, and *T. turgida*; these species have been recorded associated to any of the three opossum species in 7,10,15, 9, and 17 Mexican states, respectively. In states where the 3 host species are distributed sympatrically, *O. microcephalus* and *R. coronatus* are the species more frequently shared between them. On the other hand, the most restricted geographic areas are presented by the trematodes *A. caudalitestis*, *B. didelphus*, and *P. magnacirrus,* the acanthocephalan *O. luehei*, and the nematodes *Gongylonema* sp., and *Travassostrongylus* sp., which are present exclusively in one locality. In total, the records of this group of mammals come from 20 of the 32 states of the Mexican Republic; however, the geographic information is asymmetrical, because most of the samplings were made in the state of Veracruz (13 sites). Other states, as Campeche and Quintana Roo, have been sampled once. Moreover, most of the species that have been found parasitizing these didelphid species represent point locality records in only one study about its parasites cover states or regions, particularly Los Tuxtlas, Veracruz. However, the host's collections were made along 13 years, in different year season and with a very distinct sample size (see Cañeda-Guzmán 1997).

Considering only the 27 nominal helminth species recorded to date, the 3 host species shared 12 worm species along the sampled sites in Mexico; 8 were exclusively found in *D. virginiana*, and 2 are specialist to *P. opossum*. The cestode *T. didelphidis* and the acanthocephalan *O. luehei* are shared by the 2 species of the genus *Didelphis* but not by *P. opossum*; the Virginia opossum and the Grey four-eyed opossum shared the digenean *D. proloba* and the nematode *A. raillieti*, whereas *D. marsupialis* and *P. opossum* shared only *P. magnacirrus*. The helminth fauna of these hosts throughout their range is composed by one group of 20 specialist species, and by *P. mexicanus*, *O. microcephalus*, *O. luehei*, *P. gethi*, *A. raillieti*, *D. longispiculata*, and *T. minuta* that act as generalist species. Accidental species have not been reported in any of the samples carried out to date in Mexico. At a local scale, both phenomena had been also observed in marsupials of French Guiana (Jiménez et al. 2011; Byles et al. 2013).

The structuring factor of the helminth fauna in the three didelphid species is the diet; most of the helminth species infect these host species through ingestion of eggs, larvae or intermediate hosts. Fifteen of the 27 named helminth species have indirect patterns of transmission (*T. didelphidis*, *B. didelphus*, *B. virginiana*, *D. proloba*, *A. caudalitestis*, *P. mexicanus*, *O. microcephalus*, *P. gethi*, *O. luehei*, *P. nickoli*, *G. turgidum*, *T. turgida*, *G. mexicanum*, *D. longispiculata*, and *D. hayesi*), five are transmitted directly by eggs ingestion (*A. raillieti*, *C. americana*, *C. tentaculata*, *T. didelphis*, *T. minuta*) and for *P. magnacirrus*, *R. baculifer*, *R. coronatus*, *R. macracanthus*, *R. caballeroi*, *V. didelphis* and *V. viannai*, the life cycle is unknown (Table 2). This result is in agreement with the generalist lifestyles and diets of the three species of opossums (Krause and Krause 2006), that exposed them to the same parasite species; local differences in composition and abundance of helminth species could be related to local availability of parasites (or their intermediate hosts), as well as to the compatibility among host and helminth species, as has been showed by Cañeda-Guzmán (1997) and Jiménez et al. (2011).

The data obtained in this study came from 68 opossums collected from 18 localities (nine not previously sampled for helminths); however, the helminth fauna of each didelphid species showed a stable taxonomic composition with respect to previously sampled sites. Only one species of trematode not previously found in this group of hosts in the country was added to their parasitological record as results of our samples. In spite of the reduced scope of our samplings, this situation suggests that the rate of accumulation of helminth species in the inventory of the 3 species of terrestrial marsupials distributed in the Neotropical portion of Mexico included in this study is decreasing; however, new samplings in the Nearctic portion of this country will probably increase the richness of the helminthological inventory of this group of mammals.

Table 2. Life cycles of the helminth species collected in the present study.

Phylum	Taxa	Cycle/ Intermediate host	Reference
Platyhelminthes Trematoda	*Amphimerus* spp.	Heteroxenous/fish	Yamaguti (1975)‡
	Brachylaima spp.	Heteroxenous/snail	Yamaguti (1975)‡
	Philandrophilus magnacirrus	Unknown	
	Rhopalias spp.	Unknown	
Cestoda	*Thaumasioscolex didelphidis*	Heteroxenous/crustaceans	Scholz (1999)‡
Acanthocephala	*Oligacanthorhynchus microcephalus*	Heteroxenous/millipede	Richardson (2006)
	Oncicola luehei	Heteroxenous/insects, crustaceans	Kennedy (2006)
	Porrorchis nickoli	Heteroxenous/insects, crustaceans	Kennedy (2006)
Nematoda	*Aspidodera raillieti*	Monoxenous/eggs ingestion	Jiménez et al. (2011)
	Cruzia sp.	Monoxenous/eggs ingestion	Anderson (2000)
	Didelphostrongylus hayesi	Heteroxenous/snails	Prestwood (1976)
	Gnathostoma sp.	Heteroxenous/copepods	Kifune et al. (2004)
	Gongylonema sp.	Heteroxenous/insects	Anderson (2000)
	Turgida turgida	Heteroxenous/insects	Anderson (2000)
	Trichuris spp.	Monoxenous/eggs ingestion	Anderson (2000)
	Viannaia spp.	Unknown	

‡Particular life cycle unknown; data obtained at supra-specific level.

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