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Taxonomy, genetic diversity, and phylogeny of the Antarctic mud dragon, *Polacanthoderes* (Kinorhyncha: Echinorhagata: Echinoderidae).

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Abstract

A new species *Polacanthoderes shiraseae* sp. nov. from three Antarctic regions (off Cape Darnley, off Totten Glacier, and in Lützow-Holm Bay) is described. In addition, type species of *Polacanthoderes*, *Polacanthoderes martinezi*, is redescribed. The new species is distinguished from *P. martinezi* by the presence of conspicuously thick lateroventral acicular spines on segments 8 and 9 and the presence of sublateral small acicular spines on segment 7. Both *P. martinezi* and *P. shiraseae* sp. nov. occasionally show intraspecific morphological variations in the position of some small acicular spines. The K2P genetic distances based on the mitochondrial cytochrome *c* oxidase subunit 1 gene sequences in *P. shiraseae* sp. nov. are 0–1.5 %, equivalent to those in

other echinoderid species. Phylogenetic analyses of *Polacanthoderes* based on the nuclear 18S rRNA and 28S rRNA gene sequences support the inclusion of the genus in Echinorhagata/Echinoderidae and suggest that the genus represents the most basal group of this order/family.

Keywords

meiofauna, new species, K2P distance, JARE, DESS

1. Introduction

The family Echinoderidae includes five genera (*Cephalorhyncha* Adrianov, 1999 in Adrianov and Malakhov, 1999, *Echinoderes* Claparède, 1863, *Fissuroderes* Neuhaus and Blasche, 2006, *Meristoderes* Herranz et al., 2012, and *Polacanthoderes* Sørensen, 2008a) and ca. 160 species, representing the most species-rich taxon in Kinorhyncha (Adrianov and Maiorova, 2020; Cepeda et al., 2020; Grzelak et al., 2021; Rucci et al., 2022; Sørensen et al., 2015, 2020, 2021; Yamasaki et al., 2020a, b). Echinoderid species have been recorded worldwide, from tropical to polar areas and from various environments such as intertidal flats, sandy beaches, sandy/muddy shallow bottoms, deep-sea floors, seamounts, and submarine caves (e.g., Grzelak et al., 2021; Neuhaus, 2013; Sørensen et al., 2016; Yamasaki, 2016; Yamasaki et al., 2019; Yıldız et al., 2017). The recent phylogenetic, total-evidence, and phylogenomic analyses supported the family to solely represent the order Echinorhagata; Echinoderidae/Echinorhagata, Kentrorhagata, and Xenosomata comprise one of the two kinorhynch classes, Cyclorhagida, and within this class Echinoderidae/Echinorhagata is a sister taxon of Kentrorhagata; together these clades represent the sister group of Xenosomata (Dal Zotto et al., 2013; Herranz et al., 2022; Sørensen et al., 2015; Yamasaki et al., 2013). While the relationships of higher kinorhynch taxa were revealed recently, those of lower taxa (e.g., below the family level) are still unclear. This is partly because some genera and species are known only from remote environments, making it difficult to resample and obtain the molecular data for analysis.

Polacanthoderes is one of the groups known only from a remote environment, Antarctica. Although kinorhynch surveys have been conducted in many parts of the world in recent years (e.g., Cepeda et al., 2019; Dal Zotto and Todaro, 2016; Grzelak and Sørensen, 2019; Sánchez et al., 2012; Sørensen et al., 2012), *Polacanthoderes* species has never been recorded since Sørensen (2008a) reported the type species *Polacanthoderes martinezi* Sørensen, 2008a from the deep floor (2,274–2,290 m depth) offshore the South Shetland Islands. All the collected specimens of *P. martinezi* were formalin-fixed and provided a great deal of morphological data. Because of the presence of unique morphological traits, e.g., the presence of a large number of acicular spines on segments 4–10, *P. martinezi* was established as a new genus and species in Echinoderidae. However, due to the difficulty of recollecting *P. martinezi*, molecular data on the genus and species has not been available to date, and only morphological data has been used in subsequent phylogenetic analyses (Sørensen, 2008b; Sørensen et al., 2015).

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Aside from the record of *P. martinezi*, there are only a very limited number of reports of Kinorhyncha from Antarctica, and most of them have been identified just as "Kinorhyncha sp." (e.g., Brannock et al., 2018; Ingels et al., 2006; Veit-Köhler et al., 2011). *Campyloderes vanhoeffeni* Zelinka, 1913 is the only kinorhynch identified to the species level other than *P. martinezi*. *Campyloderes vanhoeffeni* was first described based on the specimens collected at 385 m below the ice in the Antarctic Ocean during the Gauss expedition (the first German expedition to Antarctica): although the exact sampling coordinate is unknown, it is supposed to be around 66° S 90° E according to the shipping route record (Meinardus and Mecking, 1911; Zelinka, 1913). Subsequently, Neuhaus & Sørensen (2013) reported *Campyloderes* cf. *vanhoeffeni* at 411–801 m depth in the south of King George Island as well as at 2,893 m depth in the north of the South Shetland Islands. No kinorhynchs identified to the species level have been reported from Antarctica later on.

In this paper, we report a new species of *Polacanthoderes* from three regions in Antarctica collected during the 59th and the 61st Japanese Antarctic Research Expedition (JARE59 and JARE61, respectively). In addition to morphological data, we also succeeded in obtaining molecular data of several individuals of the new species. Using these data, the genetic diversity within the species as well as the phylogenetic position of *Polacanthoderes* within Kinorhyncha were investigated. We also report some morphological features of *P. martinezi* overlooked in the original description.

2. Material and methods

2.1. Sampling and morphological observation

During JARE59 and JARE61, sediment samples were obtained by a grab sampler at several stations off Cape Darnley, off Totten Glacier, and in Lützow-Holm Bay around Antarctica (Fig. 1; Table 1). Part of the sediment samples collected in JARE61 was fixed in 10% formalin immediately after sampling. The rest samples of JARE61 as well as all the samples of JARE59 have been kept frozen until the subsequent processing. In the laboratory, the samples were washed in a 32-µm sieve, and organisms were extracted from the sieved sediment using the floatation method with Ludox HS-40 (Giere, 2009; Higgins and Thiel, 1988). After sorting the extracted organisms under a stereomicroscope, kinorhynch specimens from the formalin-fixed sample were transferred to 70% EtOH, and those from the frozen sample were transferred to 99% EtOH or DESS solution (Yoder et al., 2006).

For light microscopic observation (LM), kinorhynch specimens were

transferred to dehydrated glycerine and individually mounted as glycerol-paraffin slides on Cobb aluminium frames or mounted in Fluoromount G® on H-S slides (Shirayama et al., 1993). Subsequently, they were observed with an Olympus BX53 microscope with differential interference contrast (DIC) and photographed with an Olympus DP74 camera. Measurements were made with Olympus cellSens software. A camera lucida equipped with the microscope was used to make drafts for line art illustrations. Final line illustrations were written out based on the drafts with Adobe Illustrator 2022.

Specimens for scanning electron microscopy (SEM) were transferred to dehydrate ethanol, immersed in 100% t-butyl alcohol, freeze-dried, mounted on aluminium stubs, sputter-coated with gold-palladium, and observed with a Hitachi SU3500 scanning electron microscope at Center for Advanced Instrumental and Educational Supports, Faculty of Agriculture, Kyushu University (Fukuoka, Japan).

The type specimens of *P. martinezi* for LM deposited at Museum für Naturkunde Berlin (Berlin, Germany), were observed with a Zeiss Axioskop 50 microscope equipped with a camera lucida. The line art illustrations were made with the same microscope by following the drawing method for the JARE specimens. Non-type specimens of *P. martinezi* for SEM, also deposited at the same museum were observed with a Zeiss EVO LS 10 scanning electron microscope. All observations of *P. martinezi* was done at the Museum für Naturkunde Berlin.

The type specimens of the JARE specimens have been deposited in the invertebrate collection of the Hokkaido University Museum, Hokkaido University, Sapporo, Japan (catalogue numbers ICHUM 8348–8404) and the Natural History Museum of Denmark, Copenhagen, Denmark (catalogue numbers NHMD 1174924–1174933).

The map was drawn with the Generic Mapping Tools (GMT) (Wessel et al., 2013), and subsequently modified with Adobe Illustrator 2022.

2.2. Molecular analyses

Total genomic DNA was extracted from 23 JARE specimens. Extractions were performed on single individuals with a DNeasy Tissue Kit (Qiagen, Tokyo), following the protocol of Yamasaki et al. (2013). After DNA extraction, the exoskeleton of each specimen was mounted for LM as described above. The exoskeletons of four specimens were lost during the DNA extraction. Parts of the nuclear 18S rRNA (18S) and 28S rRNA (28S) for a single individual, as well as a part of the mitochondrial cytochrome *c* oxidase subunit 1 (COI) for 21 individuals, were amplified by PCR with the primers

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3 listed in Table 2. All nucleotide sequences were determined by direct sequencing with a
4 BigDye Terminator Kit ver. 3.1 (Life Technologies, Co., USA) and a 3730 DNA
5 Analyzer (Life Technologies, Co., USA). Sequence fragments were assembled by using
6 MEGA X (Kumar et al., 2018). The assembled sequences were deposited in GenBank
7 under accession numbers LC721243 (18S), LC721244 (28S), and
8 LC721222–LC721242 (COI).
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12 The COI sequences were used for calculating the intraspecific genetic
13 variations by using MEGA X. After the alignment of the sequences, gap sites were
14 completely removed, resulting in a 541 bp dataset. Subsequently, the Kimura (1980)
15 2-parameter (K2P) genetic distances among and within populations were calculated.
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18 To detect the phylogenetic position of the genus *Polacanthoderes* within
19 Kinorhyncha, a maximum likelihood (ML) analysis and Bayesian inference (BI) were
20 conducted with the 18S and 28S sequences of kinorhynchs. The dataset was comprised
21 of 20 out of the 31 kinorhynch genera (including four out of the five echinoderid
22 genera) and two outgroup taxa (Priapulida and Nematoda) (Table 3). The remaining
23 echinoderid genus, *Fissuroderes*, was not included in the dataset because sequence data
24 of the genus has been unavailable so far. Sequences were aligned gene by gene using
25 MAFFT software (Kato and Toh, 2010). Ambiguous areas of alignments were
26 removed using trimAl software (Capella-Gutiérrez et al., 2009). Gap sites were also
27 removed except for the missing data regions. The resulting aligned sequences
28 comprised 1353 bp (18S) and 2750 bp (28S). Homogeneity of base frequencies was
29 tested gene by gene with a chi-square test in Kakusan4 (Tanabe, 2007), indicating $p >$
30 0.05 for each of 18S and 28S, i.e. the base composition was significantly homogeneous.
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33 The ML analysis was constructed with RAXML-NG integrated in raxmlGUI
34 2.0 (Edler et al., 2021) and the BI analysis was conducted in MrBayes 3.2.7a
35 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003; Ronquist et al.,
36 2012). In both analyses, the data were partitioned by gene. The substitution models for
37 each gene for ML were selected with raxmlGUI 2.0, and those for BI analyses were
38 selected with Kakusan4. For ML trees, nodal support was assessed through analyses of
39 1000 bootstrap replicates. For BI, Markov-chain Monte-Carlo searches were performed
40 with four chains, each of which was run for 1,000,000 generations, with trees sampled
41 every 100 generations. Stationarity was evaluated by monitoring likelihood values
42 graphically. The initial 10% of trees from each run were discarded as burn-in and the
43 remaining trees were used to construct majority-rule consensus trees and determine the
44 Bayesian posterior probability for each clade (Huelsenbeck and Ronquist 2001).
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3. Results and Discussion

3.1. Taxonomy

Class Cyclorhagida Zelinka, 1896 sensu Herranz et al. (2022)

Order Echinorhagata Sørensen et al., 2015

Family Echinoderidae Carus, 1885

Genus *Polacanthoderes* Sørensen, 2008a

3.1.1. Emended diagnosis for *Polacanthoderes*

Cuticle of first trunk segment forms complete ring; cuticle of segments 2–11 with midventral and lateroventral articulations resulting in one tergal and two sternal plates. Cuticular hairs and perforation sites absent on all segments. Primary pectinate fringe of ventral side on segment 1 with very large and conspicuous fringe tips; primary pectinate fringes of other areas on segment 1 and those on other segments with very small fringe tips. Middorsal acicular spines on segments 4–8; lateroventral acicular spines on segments 6–9; additional small acicular spines present in various positions on segments 4–10, at least in subdorsal position on segments 6 and 7, laterodorsal to midlateral position on segments 5–9, sublateral or lateral accessory position on segment 7, lateral accessory position on segments 6, 8, and 9, ventrolateral position on segments 8–10, and ventromedial position on segments 4–7. Tubes in midlateral position on segment 4; lateroventral position on segment 5; laterodorsal position on segment 10. Ventromedial setae present on segment 11. Females with ventromedial papillae on segment 6 and ventrolateral papillae on segment 7.

3.1.2. *Polacanthoderes martinezi* Sørensen, 2008a

(Figs. 2–4; Table 4)

Polacanthoderes martinezi Sørensen 2008a: 230e3–230e11, figs 1–5, tables 1 and 2.

3.1.2.1. Emended Diagnosis

Polacanthoderes with small lateral accessory acicular spines on segment 7; lateroventral acicular spines on segments 8 and 9 similar in width to those on segments 6 and 7.

3.1.2.2. Material examined

Holotype: Adult female, collected 14 February 2002 at north of South Shetland Islands (61°23.73'S, 58°50.26'W, 2274 m depth), mounted in Fluoromount G® (ZMB 11237). Allotype: Adult male, collected 14 February 2002 at north of South Shetland Islands (61°24.14'S, 58°51.15'W, 2290 m depth), mounted in Fluoromount G® (ZMB 11238a). Paratypes: Three adult females and one adult male, collected at the same time as holotype or allotype, mounted in Fluoromount G® (ZMB 11238b–d and 11238f). Non-types: three females and one male, collected at the same localities, mounted on aluminium stubs for SEM.

3.1.2.3. Redescription and remarks

Adult with head, neck, and eleven trunk segments (Figs. 2A–D and 3A–F). See table 1 in Sørensen (2008a) for measurements. Cuticular structures are summarized in Table 4. The distribution of cuticular structures generally follows the original description in Sørensen (2008a) (Figs. 2A–D, 3A–F, 4A–D), hence the following notes provide additional morphological data not mentioned in the original description.

Our observation confirmed the presence of all sensory spots and glandular cell outlets mentioned in Sørensen (2008a). In addition to the original description, the following previously unnoticed features were observed: the presence of subdorsal and laterodorsal sensory spots as well as ventromedial type-1 glandular cell outlets on segment 1 (Figs. 2A, B, and 3A–C); ventromedial sensory spots and middorsal type-1 glandular cell outlet on segment 2 (Fig. 2A, B); ventromedial sensory spots on segments 3 and 4 (Figs. 2B and 4A); paradorsal sensory spots on segments 6–8 (Fig. 2A); two pairs of subdorsal sensory spots on segment 9 (Figs. 2A and 3D); and subdorsal sensory spots on segments 10 and 11 (Figs. 2A, C, 3D). Segment 9 also possesses small-rounded sieve plates in the sublateral position (Fig. 2A, B). Females with ventromedial papillae on segment 6 and ventrolateral papillae on segment 7 (Figs. 2B, 3E, and 4C, D compared to Fig. 3F). We also confirmed the presence of long setae on segment 11 in both females and males (Fig. 2A–D).

Among the tubes and acicular spines on the trunk segments, the midlateral ones on segment 4 were referred to as “spines” in the original description. However, our observation confirmed they are thinner than the following small acicular spines and most likely not spines but tubes (Figs. 2A, 3C, 4A). The original description also mentioned the variable occurrence of some spines on segments 8 and 9. We additionally found the presence of subdorsal tubes on segment 2 only in one specimen (ZMB 11238a) (Fig. 3C); subdorsal small acicular spines or tubes on segment 5 in two

specimens (ZMB 11238a and a male SEM specimen); and the absence of midlateral tubes on segment 4 in one specimen (ZMB 11238d).

3.1.3. *Polacanthoderes shiraseae* Yamasaki, sp. nov.

(Figs. 5–9; Tables 5 and 6)

3.1.3.1. Diagnosis

Polacanthoderes with sublateral small acicular spines on segment 7; lateroventral acicular spines on segments 8 and 9 being conspicuously thicker than those on segments 6 and 7.

3.1.3.2. Etymology

The specific name “*shiraseae*” is a noun in the genitive case, named after the Japanese icebreaker *Shirase*, which was used for collecting the sediment sample containing *P. shiraseae* sp. nov.

3.1.3.3. Material examined

Holotype: Adult female (catalogue No. ICHUM 8348), collected 19 December 2019 from off Totten Glacier, Antarctica (66°47'31"S, 117°21'20"E, 691 m depth), mounted in Fluoromount G[®] on H-S slides. Paratypes: 30 adult males and 36 adult females (catalogue no. ICHUM 8349–8404 and NHMD 1174924–1174933), detailed sampling data shown in the table 1, all individually mounted in Fluoromount G[®] on H-S slides. Non-type: nine adult males and eight adult females, detailed sampling data shown in the table 1, mounted on aluminium stubs for SEM.

3.1.3.4. Type locality

Off Totten Glacier, Antarctica (66°47'31.20"S, 117°21'19.80"E), 691 m depth.

3.1.3.5. Description

Adult with head, neck, and eleven trunk segments (Figs. 5, 6A–D, and 7A). See Table 5 for measurements. Table 6 indicates the positions of cuticular structures.

Head consists of retractable mouth cone and introvert (Fig. 7B). Distal part of mouth cone with pharynx crown, three rings of inner oral styles, and one ring of nine outer oral styles. Each of ring-03 and ring-02 bears five inner oral styles, whereas details of inner oral styles in ring-01 not observable. Outer oral styles alternating in

length: slightly longer in odd sectors than in even sectors. Outer oral style in sector 6 missing. Introvert with seven rings of spinoscalids and one ring of trichoscalids. Ring 01 with ten primary spinoscalids composed of one basal sheath and one distal end piece. Each basal sheath with two overlapping fringes: proximal fringe composed of several short projections between two long and slightly thicker lateral ones; distal fringe with several long projections. Distal end pieces of primary spinoscalids longest within all spinoscalids. Rings 02 and 04 with 10 spinoscalids; rings 03 and 05 with 20 spinoscalids. Each spinoscalid of rings 02–05 with basal sheath and end piece. Rings 06 and 07 not examined in detail, but with at least seven and four spinoscalids, respectively. Six trichoscalids covered with long hairs on entire surface and each attached to trichoscalid plates. Number and arrangement of inner oral styles, outer oral styles, and spinoscalids summarized in Fig. 8.

Neck with 16 placids (Figs. 5A, B, 6A, B, and 8). Midventral placid wider than others, and others similar in width.

Trunk with eleven segments; segment 1 consists of complete cuticular ring; segments 2–11 consist of one tergal and two sternal plates (Figs. 5 and 6A–D). Thickened cuticle forms pachycyclus at anterior margin and along midventral and tergosternal articulations of segments 2–10 (Fig. 6). Cuticular hairs and perforation sites absent on all segments (Figs. 6, 7, and 9).

Segment 1 (Figs. 5A, B, 6A, B, and 7C, D) with type-1 glandular cell outlet in middorsal position. Additional pair of type-1 glandular cell outlets in ventromedial position. Sensory spots present subdorsally, laterodorsally, sublaterally, and ventromedially. Posterior edge of this and following nine segments with primary pectinate fringe. Fringe tips of primary pectinate fringe in ventromedial area on segment 1 conspicuously large, whereas those in other areas and on other segments small and recognizable only in SEM.

Segment 2 (Figs. 5A, B, 6A, B, and 7C, D) with type-1 glandular cell outlets in middorsal and ventromedial positions. Two pairs of sensory spots present laterodorsally. Additional sensory spots present middorsally and ventromedially.

Segment 3 (Figs. 5A, B, 6A, B, and 7C, D) with middorsal and ventromedial type-1 glandular cell outlets. Sensory spots present in subdorsal, laterodorsal, midlateral, and ventromedial positions.

Segment 4 (Figs. 5A, B, 6A, B, and 7C–F) with middorsal acicular spine. Small acicular spines present ventromedially and tubes present midlaterally. Nineteen out of 84 examined specimens showed irregular spine patterns on this segment: 17 specimens with additional pair of subdorsal small acicular spines (e.g., compare Fig. 7E

and Fig. 7F); one specimen with additional midlateral small acicular spine/tube only on left side; one specimen lacks midlateral tubes. Type-1 glandular cell outlets present in paradorsal and ventromedial positions. Sensory spots present subdorsally and ventromedially.

Segment 5 (Figs. 5A, B, 6A, B, 7D–F, and 9A) with middorsal acicular spine. Small acicular spines present in midlateral and ventromedial positions, and tubes present in lateroventral position. Twenty-four out of 84 examined specimens showed irregular spine patterns on this segment: 23 specimens possess additional pair of subdorsal small acicular spines (e.g., compare Fig. 7E and Fig. 7F); one specimen with an additional subdorsal small acicular spine only on left side. Type-1 glandular cell outlets present in paradorsal and ventromedial positions. Sensory spots present subdorsally, midlaterally, and ventromedially.

Segment 6 (Figs. 5A, B, 6A, B, E–G, 7E, F, and 9A, B) with middorsal and lateroventral acicular spines. Small acicular spines present in subdorsal, laterodorsal, lateral accessory, and ventromedial positions. Three out of 84 examined specimens showed irregular spine patterns on this segment: lateral accessory small acicular spines moved to sublateral position in 2 specimens; one specimen with an additional sublateral small acicular spine only on right side. Type-1 glandular cell outlets present in paradorsal and ventromedial positions. Type-2 glandular cell outlets present in midlateral position. Sensory spots present paradorsal, subdorsal, and ventromedial positions. Ventromedial papillae present in females.

Segment 7 (Figs. 5A, B, 6C–G, 7A, E, and 9A–C) with middorsal and lateroventral acicular spines. Small acicular spines present subdorsally, laterodorsally, sublaterally, and ventromedially. Eight out of 84 examined specimens showed irregular spine pattern on this segment: sublateral small acicular spines moved to lateral accessory position in four specimens, of which one specimen also lacks lateroventral acicular spine on left side; one specimen with a non-paired sublateral small acicular spines on right side and paired lateral accessory small acicular spines; one specimen lacks one laterodorsal small acicular spine on right side; two specimens with one additional lateral accessory small acicular spines only on one side. Type-1 glandular cell outlets present in paradorsal and ventromedial positions. Sensory spots present paradorsally, subdorsally, midlaterally, and ventromedially. Papillae present in ventrolateral position in females.

Segment 8 (Figs. 5A, B, 6C, D, G, 7A, and 9B, C, E) with middorsal and lateroventral acicular spines. Lateroventral acicular spines conspicuously thicker and longer than those on previous two segments. Small acicular spines present laterodorsal,

lateral accessory, and ventrolateral positions. One out of 84 examined specimens with one subdorsal small acicular spine only on right side. Type-1 glandular cell outlets present paradorsally and ventromedially. Type-2 glandular cell outlets present in midlateral position. Sensory spots present in paradorsal, subdorsal, midlateral, and ventrolateral positions.

Segment 9 (Figs. 5A, B, 6C, D, and 9C–F) with lateroventral acicular spines, which are thicker than those on segments 6 and 7, but slightly shorter than those on segment 8. Small acicular spines present in laterodorsal, lateral accessory, and ventrolateral positions. Type-1 glandular cell outlets present paradorsally and ventromedially. Two pairs of sensory spots present in subdorsal position, and pair of sensory spots present in laterodorsal and ventrolateral positions. Small rounded sieve plate present sublaterally.

Segment 10 (Figs. 5, 6C, D, and 9E, F) with laterodorsal tubes and ventrolateral small acicular spines. Two type-1 glandular cell outlets present in middorsal position, and one pair in ventromedial position. Sensory spots present subdorsally and ventrolaterally.

Segment 11 (Figs. 5, 6C, D, H, and 9E, F) with lateral terminal spines. Males with three pairs of penile spines: most dorsal and most ventral penile spines quite slender and long, whereas middle penile spines much thicker and short. Females with lateral terminal accessory spines. Two type-1 glandular cell outlets present middorsally, and sensory spots present subdorsally. Surface of ventromedial area of sternal plates furry. Sternal plates triangle. Posterior edge of tergal plate protruding subdorsally forming pointed tergal extensions. Thin and long setae present next to tergal extensions in both males and females.

3.1.3.6. Remarks

Polacanthoderes shiraseae sp. nov. is similar to *P. martinezi* in many characters: the presence of (1) very strong and conspicuous pectinate fringe in the ventral side on segment 1, (2) middorsal acicular spines on segments 4–8, (3) lateroventral acicular spines on segments 6–9, (4) small acicular spines present at least in subdorsal on segments 6 and 7, laterodorsal on segments 5–9, lateral accessory on segments 6, 8, and 9, ventrolateral on segments 8–10, and ventromedial on segments 4–7, (5) tubes in midlateral on segment 4, lateroventral on segment 5, and laterodorsal on segment 10, (6) type-1 glandular cell outlets in middorsal on segments 1–3, 10, and 11, paradorsal on segments 4–9, ventromedial on segments 1–10, (7) type-2 glandular

cell outlets in midlateral on segments 6 and 8, and (8) ventromedial setae on segment 11, and (9) females with ventromedial papillae on segment 6 and ventrolateral papillae on segment 7 (Sørensen et al. 2008a). Furthermore, both of the two species sometimes possess irregular small acicular spines, especially on segments 4–8.

Although the two *Polacanthoderes* species share many characters, they can be distinguished clearly in the shapes of lateroventral acicular spines: the lateroventral acicular spines on segments 8 and 9 are conspicuously thicker than those on segments 6 and 7 in *P. shiraseae*, whereas those on segments 6–9 are all similar in width in *P. martinezi*. In addition, *P. shiraseae* can be distinguished from *P. martinezi* in the position of the small acicular spines on segment 7: the spines are in the sublateral position in the former, whereas they are in the lateral accessory position in the latter species. Although the spine position may be irregularly moved from sublateral to lateral accessory position in *P. shiraseae*, such a mutation seldom occurs (less than 10% of the examined specimens of *P. shiraseae* have small acicular spines in the lateral accessory position in segment 7). Furthermore, even if the spine position on segment 7 is inapplicable for some specimens, the width of the lateroventral acicular spines on segments 8 and 9 can be used for the identification of the species.

3.2. Genetic diversity within and among three populations of *Polacanthoderes*

The K2P genetic distances for the COI sequence showed distances within *P. shiraseae* are 0–1.5% (Table 7), which are comparable to the previously studied intraspecific K2P distances for other two echinoderid species (0–2.1% in *Echinoderes sensibilis*, 0–1.7% in *Echinoderes songae*) (Yamasaki et al., 2014). It should be noted that seven (six from off Cape Darnley and one from off Totten Glacier) out of the sequenced 21 individuals showed an irregular pattern in small acicular spines, but no great differences in K2P distances have not observed between them and “regular-spine pattern” individuals. Hence, the molecular data support such differences in the pattern of small acicular spines do not represent interspecific differences but intraspecific ones.

While the K2P values within and among the “off Totten Glacier” and “off Cape Darnley” populations are low (0–0.6%), those within the “Lützow-Holm Bay” as well as between “Lützow-Holm Bay” and the other two populations are relatively high (0.6–1.5%). This may suggest the presence of dispersal barrier(s) between Lützow-Holm Bay and Cape Darnley, and no barriers between Cape Darnley and Totten Glacier. Further studies with additional populations and study sites will provide more details on the mechanisms of the population connectivity of the species around

Antarctica.

3.3. Phylogenetic position of *Polacanthoderes*

The BI and ML analyses produced trees of very similar topology, hence we show the ML tree with nodal support (ML bootstrap values (BS) and BI posterior probability (PP)) in the Fig. 10. The resulted topology is mostly congruent with those of the previous phylogenetic and phylogenomic studies of kinorhynchs (Dal Zotto et al., 2013; Herranz et al., 2022; Sørensen et al., 2015; Yamasaki et al., 2013). The only remarkable difference among the new and previous results is the position of the clade *Paracentrophyes* + *Franciscideridae* (*Franciscideres* and *Gracilideres*): it is represented as the sister clade of *Pycnophyiidae* in the analyses based on the 18S and 28S gene sequences (Yamasaki et al. 2013; Sørensen et al. 2015; this study), whereas it composes the *Anomoirhaga* clade together with *Dracoderes* and *Cateria* in the phylogenomic analyses (Herranz et al. 2022). Aside from the difference between this and previous analyses found in the *Allomalorhagida* clade, the relationships of cyclorhagids (*Echinorhagata*, *Kentrorhagata*, and *Xenosomata*) are consistent throughout all analyses.

In our results, the *Echinorhagata*/*Echinoderidae* clade including *Polacanthoderes*, *Cephalorhyncha*, *Echinoderes*, and *Meristoderes* was detected with high support values (nodal support bootstrap value/posterior probability = 100/1.00). In this clade, *Polacanthoderes* branches off most basally, representing the sister taxon to a clade with the remaining three genera. The clade *Cephalorhyncha* + *Echinoderes* + *Meristoderes* was also supported with high values (99/1.00), however, the monophyly of any of the three genera was not supported.

The phylogenetic position of *Polacanthoderes* detected by this study does not conflict with the previous analyses based on morphology (Sørensen, 2008b) or the total evidence analysis (Sørensen et al., 2015) but indicates its position more clearly. Both of the previous studies suggested the inclusion of *Polacanthoderes* in the family *Echinoderidae*, which is strongly supported by the current results. Sørensen (2008b) also suggested that *Polacanthoderes* is the most basal taxon of the family, being the sister taxon of the clade consisting of three echinoderid genera (*Cephalorhyncha*, *Echinoderes*, and *Fissuroides*). In the total-evidence analysis in Sørensen et al. (2015), *Polacanthoderes* composed a clade together with *Fissuroides*, but the root of the family appeared as an unresolved polytomy and failed to clarify the relationships between the *Polacanthoderes* + *Fissuroides* clade and the remaining three echinoderid genera. Our new results support that *Polacanthoderes* is one of the most basal taxa of

Echinoderidae, and *Cephalorhyncha*, *Echinoderes*, and *Meristoderes* are more closely related to each other than to *Polacanthoderes*. Because our analyses lack *Fissuroderes* data, the phylogenetic position of *Fissuroderes* remains unclear. Nevertheless, considering the phylogenetic position of *Polacanthoderes*, there is no doubt that the genus is one of the key taxa to detect the evolution of Echinoderidae. Further analysis with broader taxon sampling, including *Fissuroderes* and various *Echinoderes* species, will provide a more detailed understanding of the phylogenetic relationships within the family Echinoderidae and their evolutionary history in the group.

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Figure captions

Figure 1. Map of Antarctica indicating the type localities of *P. martinezi* and the sampling stations of *P. shiraseae* in Lützow-Holm Bay, off Cape Darnley, and off Totten Glacier.

Figure 2. *Polacanthoderes martinezi*, camera lucida drawings. A, B, holotype female (ZMB 11237), segments 1–11, dorsal view (A) and ventral view (B); C, D, allotype male (ZMB 11238a), segments 10 and 11, dorsal view (C) and ventral view (D). Abbreviations: (ac), acicular spine; gco1/2, type-1/2 glandular cell outlet; LA, lateral accessory; LD, laterodorsal; ltas, lateral terminal accessory spine; lts, lateral terminal spine; LV, lateroventral; MD, middorsal; mdp, middorsal placid; ML, midlateral; mvp, midventral placid; pa, papilla; pe, penile spine; (sac), small acicular spine; SD, subdorsal; se, seta; si, sieve plate; ss, sensory spots; (tu), tube; VL, ventrolateral; VM, ventromedial. Digits in the abbreviations (except for gco1/2) indicate the corresponding segment number.

Figure 3. *Polacanthoderes martinezi*, DIC photomicrographs. A, B, D, E, the holotypic female (ZMB 11237), head, neck, and segments 1–5, dorsal view (A) and ventral view (B), segments 6–11, dorsal view (D) and ventral view (E); C, the allotype male (ZMB 11238a), neck and segments 1–6, dorsal view; F, paratype male (ZMB 11238d), segments 7–11, ventral view. Black arrows, white arrowheads, and black arrowheads indicate sensory spot, type-1 glandular cell outlet, and type-2 glandular cell outlet, respectively. Abbreviations: (ac), acicular spine; LA, lateral accessory; LD, laterodorsal; LV, lateroventral; MD, middorsal; ML, midlateral; pa, papilla; pe, penile spine; (sac), small acicular spine; SD, subdorsal; (tu), tube; VL, ventrolateral; VM, ventromedial. Digits in the abbreviations indicate the corresponding segment number.

Figure 4. *Polacanthoderes martinezi*, scanning electron micrographs of females. A, segments 2–5, lateroventral view; B, segments 5–7, lateral view; C, segments 6–8, lateroventral view; D, segment 7, lateroventral view. Black arrows and a black arrowhead indicate sensory spot and type-2 glandular cell outlet, respectively. Abbreviations: (ac), acicular spine; LA, lateral accessory; LD, laterodorsal; LV, lateroventral; ML, midlateral; pa, papilla; (sac), small acicular spine; SD, subdorsal; (tu), tube; VM, ventromedial; VL, ventrolateral. Digits in abbreviations indicate the corresponding segment number.

Figure 5. *Polacanthoderes shiraseae* sp. nov., camera lucida drawings. A, B, holotype female (ICHUM 8348), segments 1–11, dorsal view (A) and ventral view (B); C, D, paratype male (ICHUM 8371), segments 10 and 11, dorsal view (C) and ventral view (D). Abbreviations: (ac), acicular spine; gco1/2, type-1/2 glandular cell outlet; LA, lateral accessory; LD, laterodorsal; ltas, lateral terminal accessory spine; lts, lateral terminal spine; LV, lateroventral; MD, middorsal; mdp, middorsal placid; ML, midlateral; mvp, midventral placid; pa, papilla; pe, penile spine; (sac), small acicular spine; SD, subdorsal; se, seta; si, sieve plate; SL, sublateral; ss, sensory spots; (tu), tube; VL, ventrolateral; VM, ventromedial. Digits in the abbreviations (except for gco1/2) indicate the corresponding segment number.

Figure 6. *Polacanthoderes shiraseae* sp. nov., DIC photomicrographs. A–F, the holotypic female (ICHUM 8348), neck and segments 1–6, dorsal view (A) and ventral view (B), segments 7–11, dorsal view (C) and ventral view (D), segments 6 and 7, ventral view, focused at cuticle surface (E) and focused at deeper layer (F); G and H, paratype male (ICHUM 8371), segments 6–8, ventral view (G), segments 10 and 11, dorsal view (H). Black arrows, white arrowheads, and black arrowheads indicate sensory spot, type-1 glandular cell outlet, and type-2 glandular cell outlet, respectively. Abbreviations: (ac), acicular spine; LA, lateral accessory; LD, laterodorsal; ltas, lateral terminal accessory spine; lts, lateral terminal spine; LV, lateroventral; MD, middorsal; ML, midlateral; pa, papilla; pe, penile spine; (sac), small acicular spine; SD, subdorsal; si, sieve plate; SL, sublateral; (tu), tube; VL, ventrolateral; VM, ventromedial. Digits in the abbreviations indicate the corresponding segment number.

Figure 7. *Polacanthoderes shiraseae* sp. nov., scanning electron micrographs of females (A–E) and a male (F). A, overview from lateroventral side; B, head (right side); C, segments 1–4, lateral view; D, segments 1–5, ventral view; E, segments 4–7, lateral view; F, segments 4–6, laterodorsal view. Black arrows and black arrowhead indicate sensory spot and type-2 glandular cell outlet, respectively. Abbreviations: (ac), acicular spine; LA, lateral accessory; LD, laterodorsal; LV, lateroventral; MD, middorsal; ML, midlateral; oos, outer oral style; phc, pharynx crown; psp, primary spinoscalid; (sac), small acicular spine; SD, subdorsal; sec, sector number; SL, sublateral; sps, spinoscalid followed by the corresponding ring number; (tu), tube; VM, ventromedial. Digits after abbreviations (except for sec and sps) indicate the corresponding segment number.

Figure 8. Polar-coordinate diagram of mouth cone, introvert, and placids in *Polacanthoderes shiraseae* sp. nov. Grey area and heavy line arcs show mouth cone and placids, respectively. The table lists the arrangement of styles and scalids by sector.

Figure 9. *Polacanthoderes shiraseae* sp. nov., scanning electron micrographs of females (A, C–E) and a male (B and F). A, segments 5–7, ventral view; B, segments 6–8, ventral view; C, segments 7–9, lateral view; D, close-up of sieve plate on segment 9; E, segments 8–11, laterodorsal view; F, segments 9–11, ventral view. Black arrows and black arrowhead indicate sensory spot and type-2 glandular cell outlet, respectively. Abbreviations: (ac), acicular spine; LA, lateral accessory; LD, laterodorsal; ltas, lateral terminal accessory spine; lts, lateral terminal spine; LV, lateroventral; MD, middorsal; pa, papilla; pe, penile spine; (sac), small acicular spine; SD, subdorsal; se, seta; si, sieve plate; SL, sublateral; (tu), tube; VL, ventrolateral; VM, ventromedial. Digits after abbreviations indicate the corresponding segment number.

Figure 10. Maximum-likelihood tree of Kinorhyncha based on 18S (1353 bp) + 28S (2750 bp) dataset. Numbers near nodes are the maximum-likelihood bootstrap (BS) and Bayesian posterior probability (PP) values, respectively; asterisks indicate the node with the full support values (BS=100% and PP=1.00); BS values lower than 50% indicate as polytomy; PP values lower than 0.95 are indicated by dashes. The scale bar indicates branch length in substitutions per site.

Table 1. Data on sampling stations, type status, and catalogue numbers of *Polacanthoderes martinezi* and extraction and the specimens of which exoskeleton was lost during the DNA extraction.

Species	Locality	Sampling station	Sampling date
<i>Polacanthoderes martinezi</i>	north of South Shetland Islands	PS105-2/6	14 Feb. 2002
<i>Polacanthoderes shiraseae</i> sp. nov.	off Cape Darnley	JARE59-CD	26 Feb. 2018
	off Totten Glacier	JARE61-15	17 Dec. 2019
		JARE61-17	18 Dec. 2019
		JARE61-18	18 Dec. 2019
		JARE61-X23	19 Dec. 2019
	Lützow-Holm Bay	JARE61-25	19 Dec. 2019
		JARE61-26	19 Dec. 2019
		JARE61-108	05 Mar. 2020
		JARE61-109	05 Mar. 2020
		JARE61-LH2a	31 Jan. 2020
		JARE61-LH3a	31 Jan. 2020
		JARE61-LH5a	30 Jan. 2020

P. shiraseae sp. nov. "(ex)" and "lost" in the mc

Depth (m)	Latitude	Longitude	Mounting
2290	61°23'44"S	58°50'16"W	LM
			LM
			SEM
205	67°40'38"S	68°55'22"E	LM
			LM (ex)
			lost
			SEM
691	66°47'31"S	117°21'20"E	LM
			SEM
608	66°45'44"S	117°44'28"E	LM
			LM (ex)
523	66°45'31"S	118° 3'38"E	LM
			SEM
487	66°13'43"S	119°59'17"E	LM
			LM
			LM (ex)
627	66°37'26"S	119°20'26"E	LM
693	66°29'44"S	119°33'41"E	LM
309	66°29'23"S	120°47'4"E	LM
431	66°22'53"S	120°40'7"E	LM
310	68°42'10"S	38°30'58"E	LM
264	68°28'20"S	38°29'1"E	LM
219	68°51'57"S	38°53'26"E	LM
			LM (ex)
			lost
			SEM

Counting column indicate the specimens of which exoskeleton was mounted after DNA

Type status and catalogue number

1♀, holotype (ZMB 11237)
2♂, 4♀, paratypes (ZMB 11238a-f)
1♂, 3♀, nontypes (ZMB 11238g-n)
1♂, 3♀, paratypes (ICHUM 8349–8352)
5♂, 6♀, paratypes (ICHUM 8386–8396)
2 individuals
4♂, 1♀, nontypes
2♂, 2♀, paratypes (ICHUM 8353–8356); 1♂, 1♀, paratypes (NHMD 1174924, 1174925)
1♂, 4♀, nontypes
2♂, paratypes (ICHUM 8357–8358)
1♂, paratype (ICHUM 8404)
1♂, 2♀, paratypes (ICHUM 8359–8361); 1♂, 1♀, paratypes (NHMD 1174926, 1174927)
2♂, 1♀, nontypes
1♀, holotype (ICHUM 8348)
2♂, 4♀, paratypes (ICHUM 8362–8367); 1♂, 1♀, paratypes (NHMD 1174928, 1174929)
2♂, 2♀, paratypes (ICHUM 8397–8400)
2♂, 1♀, paratypes (ICHUM 8368–8370)
1♂, paratype (ICHUM 8371)
4♀, paratypes (ICHUM 8372–8375)
2♂, paratypes (ICHUM 8376–8377); 1♂, 1♀, paratypes (NHMD 1174930, 1174931)
1♂, 3♀, paratypes (ICHUM 8378–8381)
1♂, paratype (ICHUM 8382)
2♂, 1♀, paratypes (ICHUM 8383–8385); 1♂, 1♀, paratypes (NHMD 1174932, 1174933)
3♀, paratypes (ICHUM 8401–8403)
2 individuals
2♂, 2♀, nontypes

Table 2. List of PCR and cycle sequencing (CS) primers used in this s

Gene	Primer name	Reaction PCR
18S rRNA (18S)	F1	PCR & CS
	R9	PCR & CS
	F2	CS
	F3	CS
	F4	CS
	R6	CS
	R7	CS
	R8	CS
28S rRNA (28S)	28S-01	PCR & CS
	28Sr	PCR & CS
	28Sf	PCR & CS
	28S-3KR	PCR & CS
	28S-2KF	PCR & CS
	28jj-3′	PCR & CS
	28S-n05R	CS
	28SR-01	CS
	28S-15R	CS
	28S-3KF	CS
	28v-5′	CS
	28S-42F	CS
cytochrome <i>c</i> oxidase subunit 1 (COI)	LCO	PCR & CS
	HCO	PCR & CS
	Ki_COIF	PCR & CS
	Ki_COIR	PCR & CS
	Ec_COIF	PCR & CS
	Ec_COIR	PCR & CS
	Ec_COIpF	CS

tudy.

Primer sequence (in 5'–3' direction)	Direction	Source
TACCTGGTTGATCCTGCCAG	Forward	Yamaguchi and Endo (2003)
GATCCTTCCGCAGGTTACCTAC	Reverse	Yamaguchi and Endo (2003)
CCTGAGAAACGGCTRCCACAT	Forward	Yamaguchi and Endo (2003)
GYGRTCAGATACCRCCSTAGTT	Forward	Yamaguchi and Endo (2003)
GGTCTGTGATGCCCTYAGATGT	Forward	Yamaguchi and Endo (2003)
TYTCTCRKGCTBCCTCTCC	Reverse	Yamaguchi and Endo (2003)
GYYARAAGTAGGGCGGTATCTG	Reverse	Yamaguchi and Endo (2003)
ACATCTRAGGGCATCACAGACC	Reverse	Yamaguchi and Endo (2003)
GACTACCCCTGAATTTAAGCAT	Forward	Kim et al. (2000)
ACACACTCCTTAGCGGA	Reverse	Luan et al. (2005)
TGGGACCCGAAAGATGGTG	Forward	Luan et al. (2005)
CCAATCCTTTTCCCGAAGTT	Reverse	Yamasaki et al. (2013)
TTGGAATCCGCTAAGGAGTG	Forward	Yamasaki et al. (2013)
AGTAGGGTAAAGTAACTAACCT	Reverse	Palumbi (1996)
CTCACGGTACTTGTTCGCTAT	Reverse	Yamasaki et al. (2013)
GACTCCTTGGTCCGTGTTTCAAG	Reverse	Kim et al. (2000)
CGATTAGTCTTTCGCCCCTA	Reverse	Yamasaki et al. (2013)
AGGTGAACAGCCTCTAGTCG	Forward	Yamasaki et al. (2013)
AAGGTAGCCAAATGCCTCATC	Forward	Palumbi (1996)
GAGTTTGACTGGGGCGGTA	Forward	Yamasaki et al. (2013)
GGTCAACAAATCATAAAGATATTGG	Forward	Folmer et al. (1994)
TAAACTTCAGGGTGACCAAAAAATCA	Reverse	Folmer et al. (1994)
GGACTGCTTATAGGGTTATTATTCG	Forward	Sánchez et al. (2016)
CCCCCTCCTCTAACCTCATAAAA	Reverse	Sánchez et al. (2016)
GGGTTTTGATCTCGTCGTTG	Forward	This study
CGATGCCATAAATGAAATACATC	Reverse	This study
TTTTAATTTTACCTGGGTTTGG	Forward	This study

Table 3. List of taxa included in the phylogenetic analyses.

Class	Order	Family	Genus and species	Accession n 18S rRNA	
Allomalorhagida	Anomoirhaga	Dracoderidae	<i>Dracoderes abei</i>	AB738350	
			<i>Dracoderes nidhug</i>	LC007040	
			<i>Dracoderes snufkini</i>	LC032113	
		Franciscideridae	<i>Gracilideres mawatarii</i>	AB738378	
			Neocentrophyidae	<i>Paracentrophyes anurus</i>	AB738368
		<i>Paracentrophyes quadridentatus</i>		LC007051	
		<i>incertae sedis</i>	Pycnophyidae	<i>Cristaphyes yushini</i>	AB738370
				<i>Fujuriphyes ponticus</i>	LC081128
				<i>Fujuriphyes rugosus</i>	LC081127
				<i>Pycnophyes oshoroensis</i>	AB738372
				<i>Pycnophyes robustus</i>	LC007053
				<i>Pycnophyes tubuliferus</i>	LC081130
				<i>Pycnophyes zelinkaei</i>	LC007055
				<i>Setaphyes dentatus</i>	LC007052
				<i>Setaphyes flaveolatus</i>	LC081129
Cyclorhagida	Echinorhagata	Echinoderidae	<i>Cephalorhyncha</i> sp.	AB738352	
			<i>Echinoderes dujardinii</i>	LC007044	
			<i>Echinoderes hwiizaa</i>	AB899167	
			<i>Echinoderes komatsui</i>	AB899164	
			<i>Echinoderes microaperturus</i>	LC007046	
			<i>Echinoderes ohtsukai</i>	LC096961	
			<i>Echinoderes rex</i>	LC081126	
			<i>Echinoderes sensibilis</i>	LC007047	
			<i>Meristoderes macracanthus</i>	LC007049	
			<i>Meristoderes</i> sp.	LC007050	
			<i>Polacanthoderes shiraseae</i> sp. nov.	LC721243	
	Kentrorhagata	Centroderidae	<i>Centroderes</i> sp.	LC008445	
			<i>Condyloderes</i> sp. 1	LC007038	
			<i>Condyloderes</i> sp. 2	LC007039	
		Semnoderidae	<i>Antygomonas</i> sp. 1	AB738340	
			<i>Antygomonas</i> sp. 2	AB738342	
			<i>Semnoderes armiger</i> (Italian population)	LC007056	
			<i>Semnoderes armiger</i> (Norwegian population)	LC007057	
			<i>Sphenoderes poseidon</i>	AB738364	
			<i>Triodontoderes anulap</i>	LC007058	
		Zelinkaderidae	<i>Zelinkaderes</i> sp.	AB738366	
			<i>incertae sedis</i>	<i>Tubulideres seminoli</i>	LC007059
		Xenosomata	Campyloderidae	<i>Campyloderes</i> sp. 1	AB738344
				<i>Campyloderes</i> sp. 2	AB738346
OUTGROUP (Priapulida)			<i>Priapulius caudatus</i>	X87984	
OUTGROUP (Nematoda)			<i>Xiphinema rivesi</i>	HM921344	

umber	Reference
28S rRNA	
AB738351	Yamasaki et al. (2013)
LC007064	Sørensen et al. (2015)
LC032114	Yamasaki (2015)
AB738378	Yamasaki et al. (2013)
AB738368	Yamasaki et al. (2013)
LC007076–77	Sørensen et al. (2015)
AB738371	Yamasaki et al. (2013)
LC081133	Sánchez et al. (2016)
LC081132	Sánchez et al. (2016)
AB738373	Yamasaki et al. (2013)
LC007070	Sørensen et al. (2015)
LC081135	Sánchez et al. (2016)
LC007071	Sørensen et al. (2015)
LC007069	Sørensen et al. (2015)
LC081134	Sánchez et al. (2016)
AB738353	Yamasaki et al. (2013)
LC007065	Sørensen et al. (2015)
AB899168	Yamasaki and Fujimoto (2014)
AB899165	Yamasaki and Fujimoto (2014)
LC007066	Sørensen et al. (2015)
LC096962	Randsø et al. (2019)
LC081131	Sánchez et al. (2016)
LC032121	Sørensen et al. (2015); Yamasaki (2015)
LC007067	Sørensen et al. (2015)
LC007068	Sørensen et al. (2015)
LC721244	This study
LC008446	Sørensen et al. (2015)
LC007062	Sørensen et al. (2015)
LC007063	Sørensen et al. (2015)
AB738341	Yamasaki et al. (2013)
AB738343	Yamasaki et al. (2013)
LC007072	Sørensen et al. (2015)
LC007073	Sørensen et al. (2015)
AB738365	Yamasaki et al. (2013)
LC007074	Sørensen et al. (2015)
AB738367	Yamasaki et al. (2013)
LC007075	Sørensen et al. (2015)
AB738345	Yamasaki et al. (2013)
AB738347	Yamasaki et al. (2013)
AY210840	Winnepeninckx et al. (1995); Mallatt et al. (2004)
Ay210845	Gutiérrez-Gutiérrez et al. (2011); Mallatt et al. (2004)

Table 4. Summary of locations of cuticular structures and spines in *Polacanthoderes martine*. Characters indicate the characters not reported in the original description in Sørensen (2008a). Characters were usually absent, but appear in some specimens. Asterisks indicate the character moved its position. Abbreviations: ac, acicular spine; gco1/2, type-1/2 glandular cell outlet; L, accessory; LD, laterodorsal; ltas, lateral terminal accessory spine; lts, lateral terminal spine; LMD, middorsal; ML, midlateral; PD, paradorsal; pe, penile spine; sac, smaller acicular spine; se, seta; si, sieve plate; SL, sublateral; ss, sensory spot; tu, tube; VL, ventrolateral; VM, ventro-

Position segment	MD	PD	SD	LD	ML	SL	LA	LV	VL
1	gco1		<u>ss</u>	<u>ss</u>		ss			
2	<u>gco1</u> , ss		(<u>tu</u>)	ss, ss					
3	gco1		ss	ss	ss				
4	ac	gco1	ss		<u>tu</u> *				
5	ac	gco1	(<u>sac or tu</u>), ss		sac, ss			<u>tu</u>	
6	ac	gco1, <u>ss</u>	sac, ss	sac	gco2		sac	ac	
7	ac	gco1, <u>ss</u>	sac, ss	sac	ss		sac	ac	<u>pa</u> (♀)
8	ac	gco1, <u>ss</u>	(sac), ss	sac*	gco2		sac	ac	sac, ss
9		gco1	<u>ss, ss</u>	sac*		<u>si</u>	sac	ac	sac*, ss
10	gco1, gco1		<u>ss</u>	<u>tu</u>					sac
11	gco1, gco1		<u>ss</u>			pe×3 (♂), ltas (♀)		lts	

zi . Underlined
. Parenthetic
r rarely absent or
.A, lateral
.V, lateroventral;
SD, subdorsal;
omedial; (♀),

VM

gcol, ss
gcol, ss
gcol, ss
sac, gcol, ss
sac, gcol, ss
sac, gcol, pa (♀)
sac, gcol, ss
gcol
gcol
gcol
se

Table 5. Measurements for adult *Polacanthoderes shiraseae* sp. nov. (in micrometers). Columns N and SD indicate size and standard deviation, respectively. Numbers in the Character column indicate the corresponding segment. Abbreviations: (ac), acicular spine; LA, length of lateral accessory character; LD, length of laterodorsal character of lateral terminal accessory spine; lts, length of lateral terminal spine; LV, length of lateroventral character; M, middorsal character; MSW, maximum sternal width; pe, length of penile spine; S, segment length; (sac), small SD, length of subdorsal character; SL, length of sublateral character; SW, standard width; TL, trunk length; (tu) length of ventromedial character.

Character	Holotype	Range	N	Mean	SD	Character	Holotype	Range	N
TL	453	369–512	65	454.7	23.63	MD6 (ac)	98	90–116	65
MSW-7	80	72–89	54	80.6	4.02	SD6 (sac)	20	17–28	67
MSW/TL	17.69%	16.33%–20.30%	54	17.80%	0.93%	LD6 (sac)	22	16–30	67
SW-10	68	61–73	55	66.9	2.91	LA6 (sac)	15	12–20	67
SW/TL	14.99%	13.12%–17.32%	55	14.78%	0.87%	LV6 (ac)	28	21–30	67
S1	50	48–57	62	51.9	1.87	VM6 (sac)	14	12–22	67
S2	32	27–42	62	33.0	2.34	MD7 (ac)	118	104–131	61
S3	36	30–44	62	36.2	2.55	SD7 (sac)	20	18–29	67
S4	40	33–47	62	40.0	2.75	LD7 (sac)	23	18–29	67
S5	40	36–47	62	41.3	2.3	SL7 (sac)	20	16–24	67
S6	46	41–51	62	45.9	2.4	LV7 (ac)	31	23–32	67
S7	49	44–57	62	49.6	2.47	VM7 (sac)	15	13–19	67
S8	57	53–62	63	57.3	2.2	MD8 (ac)	150	131–167	63
S9	58	49–68	63	57.8	3.11	LD8 (sac)	20	15–26	67
S10	76	65–81	63	73.7	3.69	LA8 (sac)	18	14–24	67
S11	48	37–52	63	45.3	2.6	LV8 (ac)	45	40–52	67
MD4 (ac)	61	51–71	66	61.9	4.72	VL8 (sac)	14	13–22	67
SD4 (sac)	n.a.	16–25	14	22.2	2.33	LD9 (sac)	23	16–26	67
LD4 (tu)	17	12–20	64	16.4	1.64	LA9 (sac)	17	13–22	67
VM4 (sac)	12	10–17	67	12.6	1.42	LV9 (ac)	41	31–51	67
MD5 (ac)	81	51–92	64	79.7	6.72	VL9 (sac)	14	13–22	67
SD5 (sac)	n.a.	18–27	18	22.4	2.38	LD10 (tu)	14	11–19	66
LD5 (sac)	22	14–24	67	20.7	2.08	VL10 (sac)	15	13–22	67
LV5 (tu)	14	12–19	67	15.5	1.67	lts	231	202–265	65
VM5 (sac)	12	11–22	67	13.7	1.76	lts/TL	51.10%	45.01%–60.02%	63
						ltas	82	73–102	37
						ltas/TL	18.02%	15.88%–22.26%	36
						pe	n.a.	88–106	30
						pe/TL	n.a.	18.77%–25.92%	29

licate sample
t.
ter; ltas, length
lD, length of
acicular spine;
) , tube; VM,

Mean	SD
100.6	5.85
23.6	2.52
21.8	2.22
15.8	1.69
25.6	2.15
15.0	1.63
117.1	6.39
24.2	2.37
23.1	1.81
20.9	1.7
28.0	2.16
16.6	1.36
148.2	7.27
21.2	1.96
18.7	1.89
45.0	2.92
16.9	1.8
21.0	1.63
17.3	1.64
40.1	3.44
16.9	1.81
14.7	1.91
16.7	2.07
235.9	13.88
51.95%	3.57%
85.2	6.18
18.52%	1.37%
96.1	4.66
21.56%	1.45%

Table 6. Summary of locations of cuticular structures and spines in *Polacanthoderes shiras*. Parenthetic characters indicate the features usually absent, but found in some specimens. As character rarely absent in one or both sides, moved to adjacent position, or additional one present. Abbreviations: ac, acicular spine; gco1/2, type-1/2 glandular cell outlet; LA, lateral accessory spine; lts, lateral terminal spine; LV, lateroventral; MD, midlateral; PD, paradorsal; pe, penile spine; sac, smaller acicular spine; SD, subdorsal; se, sensory spine; SL, sublateral; ss, sensory spot; tu, tube; VL, ventrolateral; VM, ventromedial; (♀), character rarely absent in one or both sides, moved to adjacent position, or additional one present.

Position segment	MD	PD	SD	LD	ML	SL	LA	LV	VL
1	gco1		ss	ss		ss			
2	gco1, ss			ss, ss					
3	gco1		ss	ss	ss				
4	ac	gco1	(sac), ss		tu*				
5	ac	gco1	(sac), ss		sac, ss			tu	
6	ac	gco1, ss	sac, ss	sac	gco2		sac*	ac	
7	ac	gco1, ss	sac, ss	sac*	ss	sac*		ac	pa (♀)
8	ac	gco1, ss	(sac), ss	sac	gco2, ss		sac	ac	sac, ss
9		gco1	ss, ss	sac, ss		si	sac	ac	sac, ss
10	gco1, gco1		ss	tu					sac, ss
11	gco1, gco1		ss				pe×3 (♂), lts (♀)	lts	

aeae sp. nov.
 asterisks indicate the
 present in one side.
 ry; LD, laterodorsal;
 dorsal; ML,
 seta; si, sieve plate;
 or found in female;

VM

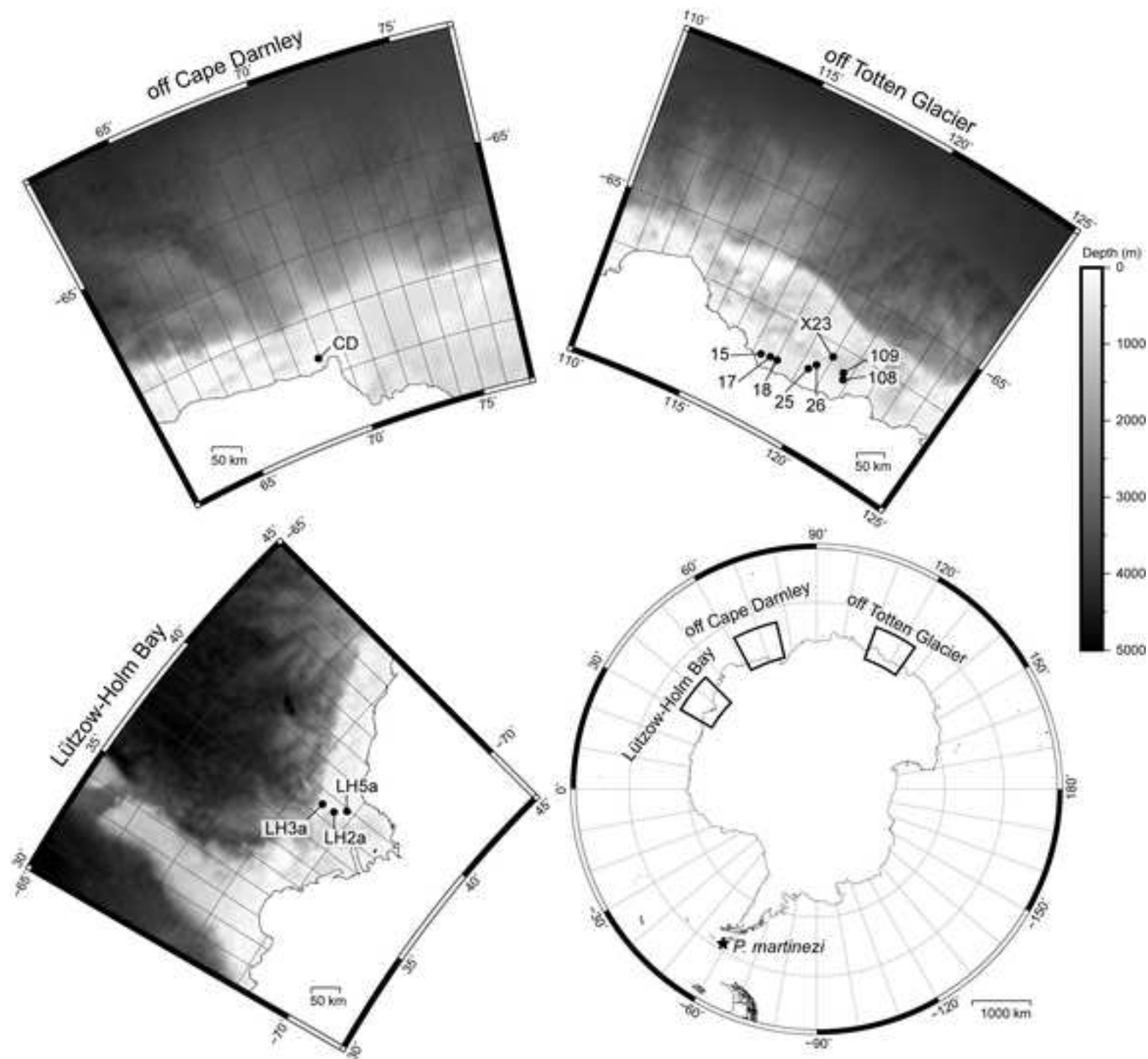
gcol, ss
 gcol, ss
 gcol, ss
 sac, gcol, ss
 sac, gcol, ss
 sac, gcol, ss, pa (♀)
 sac, gcol, ss
 gcol
 gcol
 gcol
 se

Table 7. K2P distances (percent) based on 541 bp of COI sequences within and among the three populations of *Polacanthoderes shiraseae* sp. nov. Parenthetic number after the locality name indicates the number of the sequenced individuals for each population.

	off Cape Darnley	off Totten Glacier	Lützow-Holm Bay
off Cape Darnley (12)	0–0.6		
off Totten Glacier (5)	0–0.6	0–0.4	
Lützow-Holm Bay (4)	0.6–1.3	0.6–1.5	0.6–1.5

Figure 1

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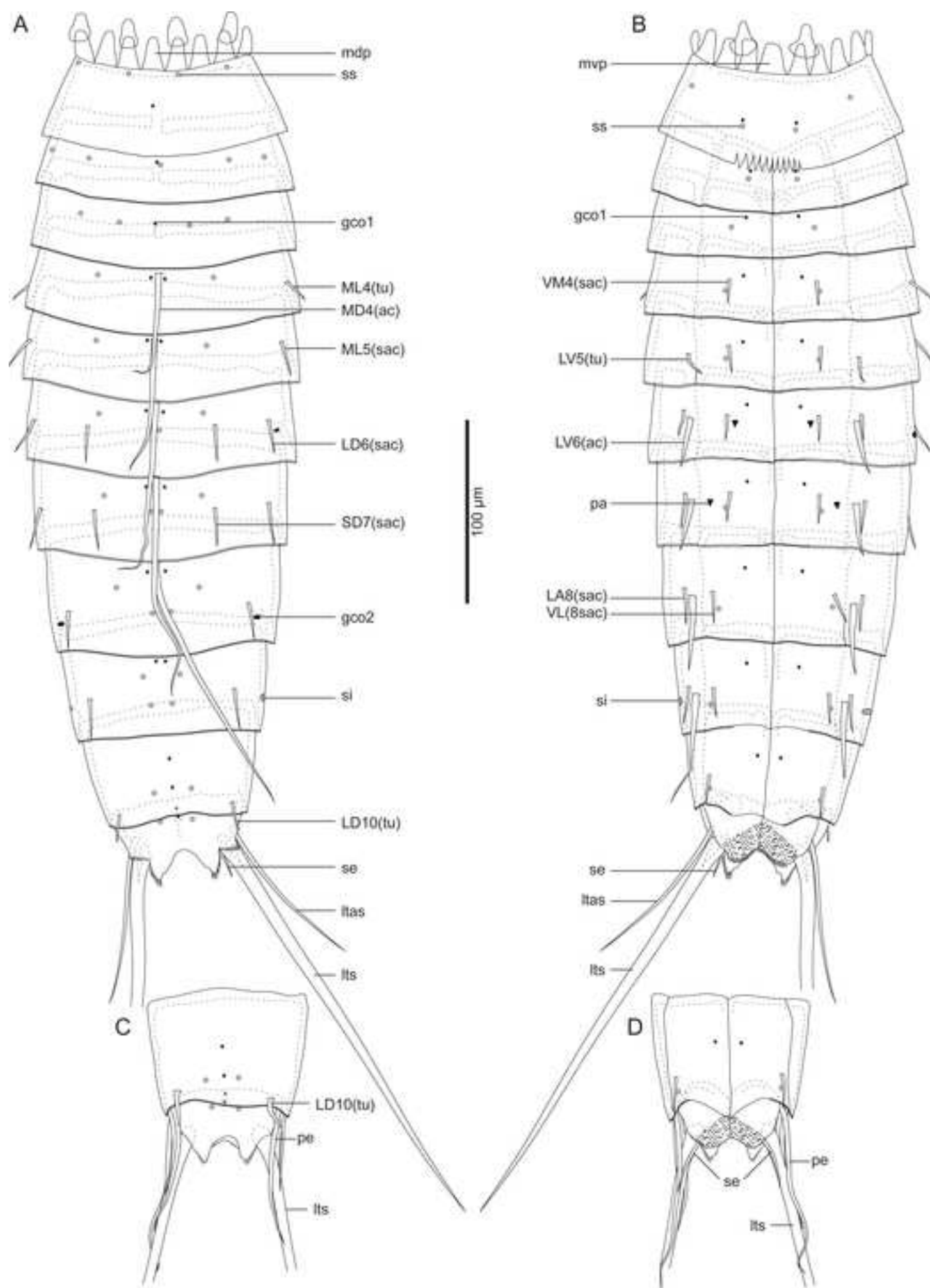
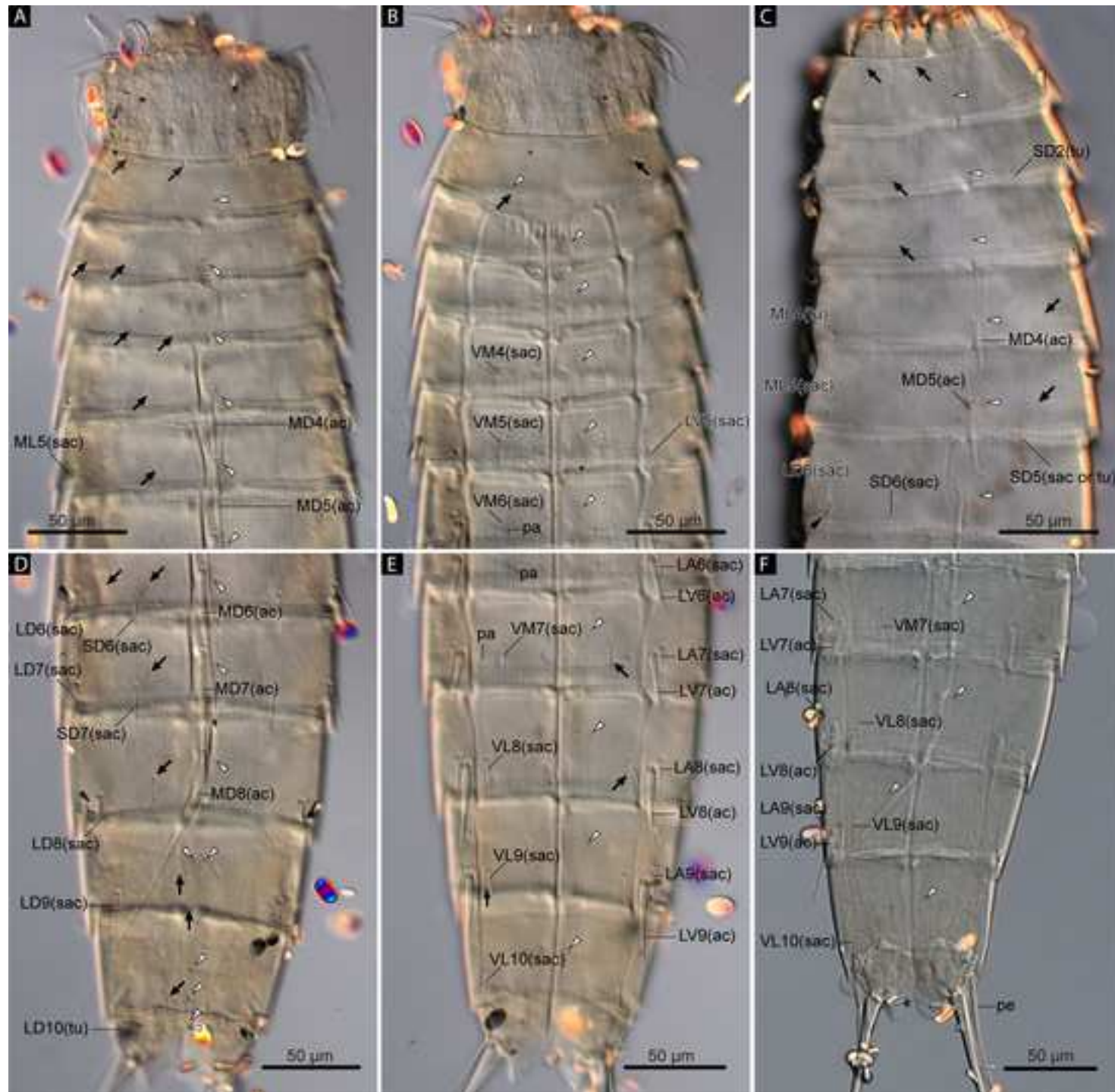


Figure 3



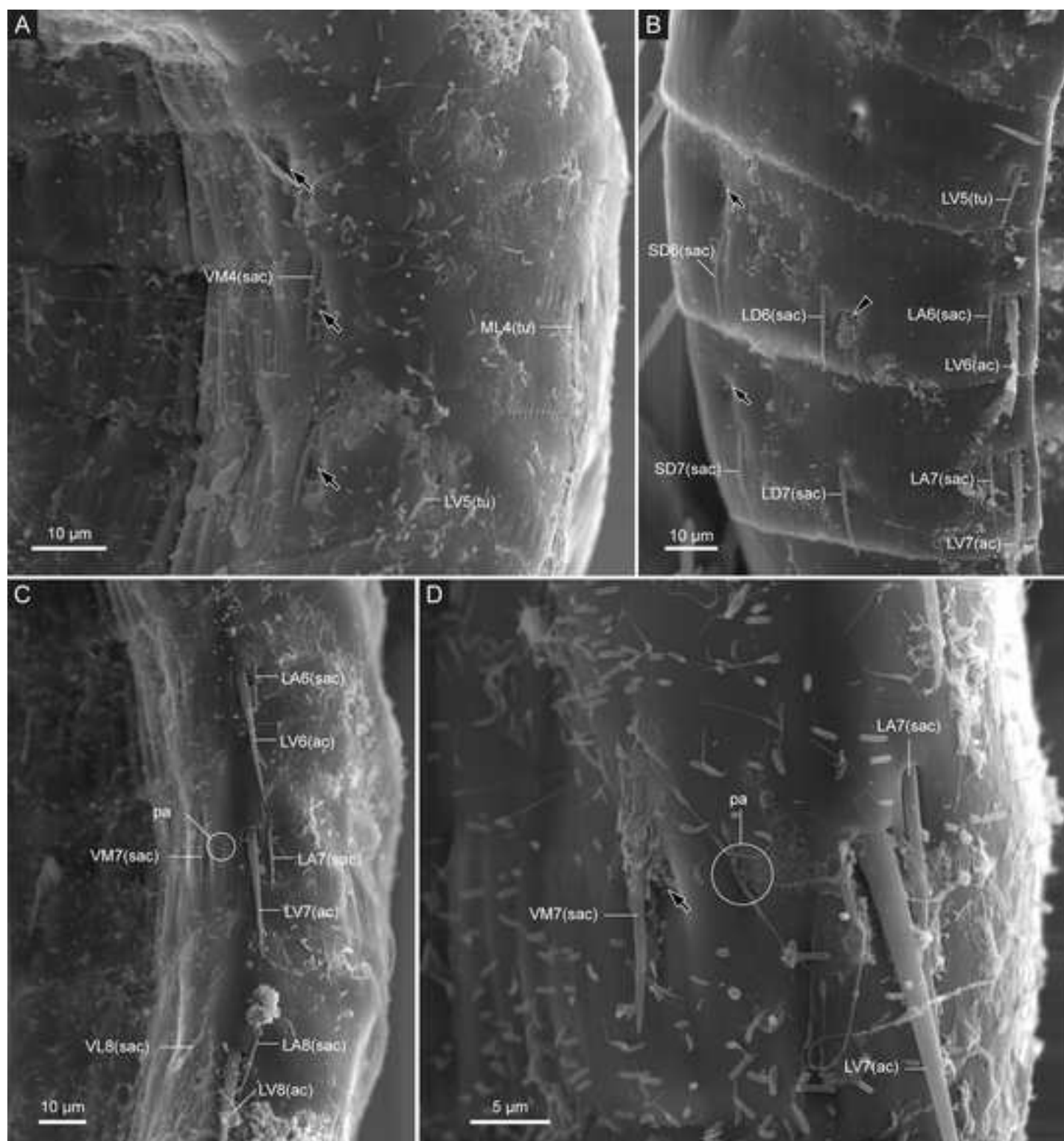
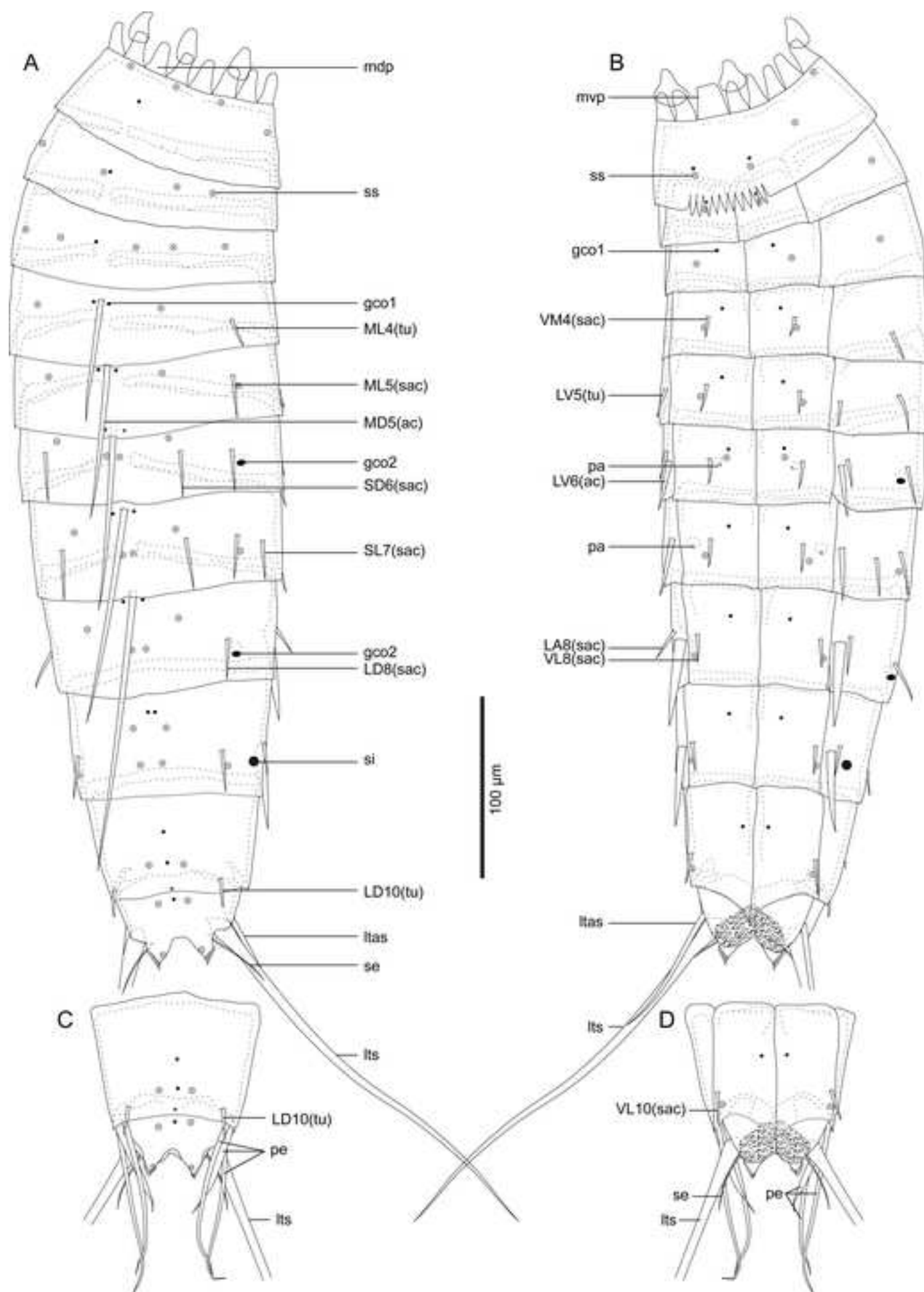
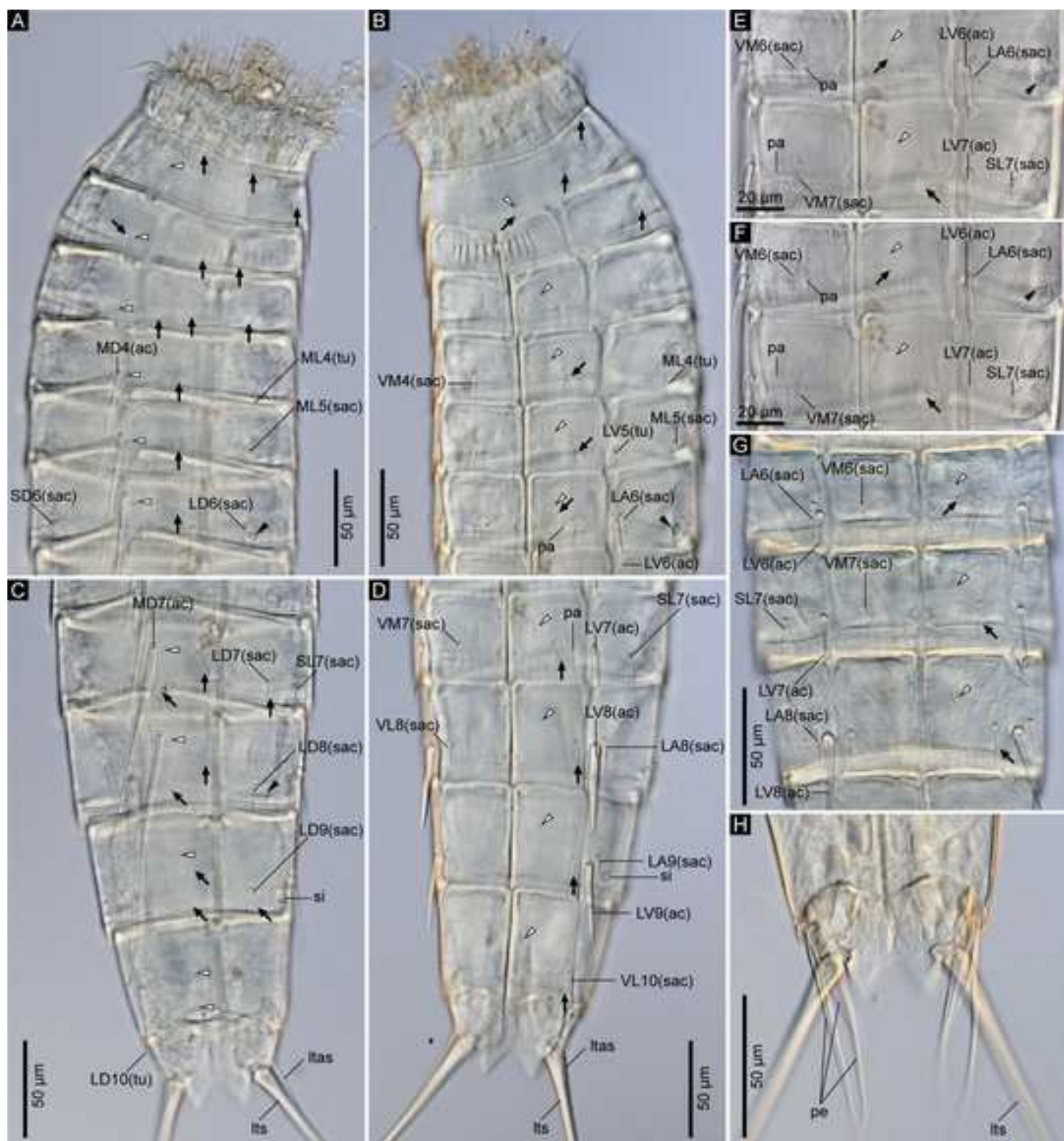
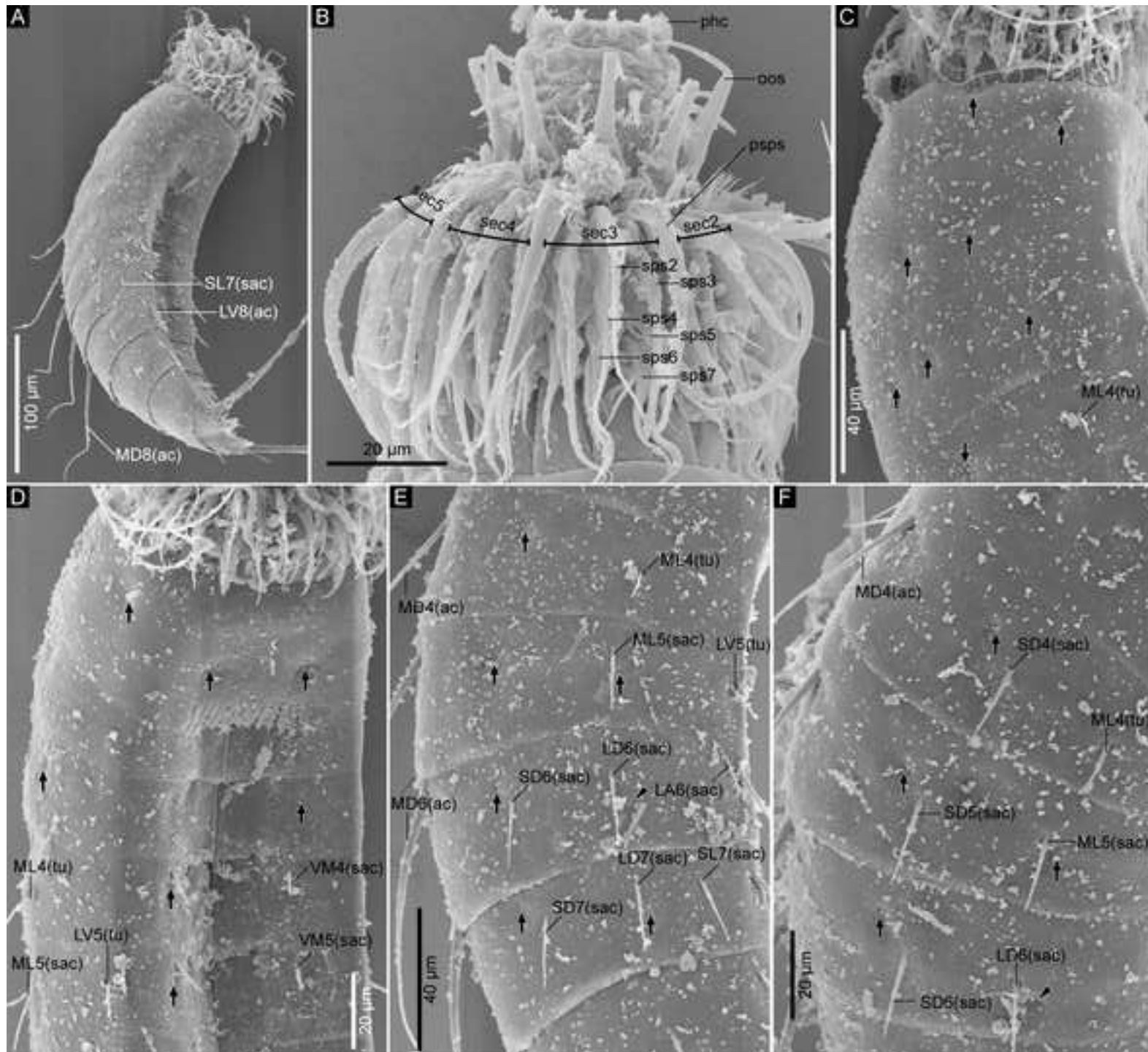


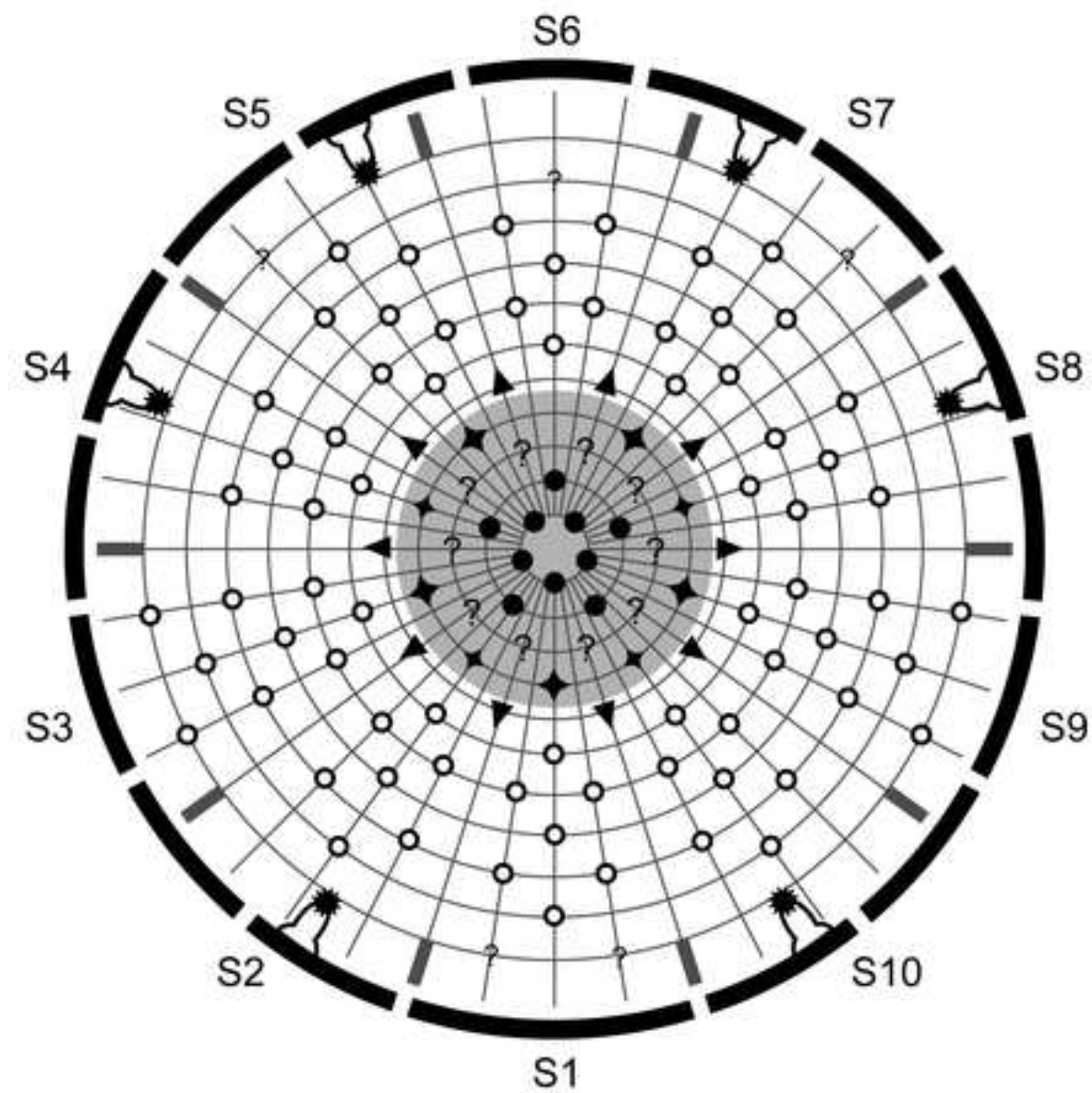
Figure 5

[Click here to access/download;Figure;Fig 5 P shiraseae_R1.tif](#)









Scalid and style arrangement

Ring/Section		1	2	3	4	5	6	7	8	9	10	Total
-03 inner oral styles	●	1	0	1	0	1	0	1	0	1	0	5
-02 inner oral styles	●	0	1	0	1	0	1	0	1	0	1	5
-01 inner oral styles	?	?	?	?	?	?	?	?	?	?	?	?
00 outer oral styles	◆	1	1	1	1	1	0	1	1	1	1	9
01 primary spinoscalids	▼	1	1	1	1	1	1	1	1	1	1	10
02 spinoscalids	○	1	1	1	1	1	1	1	1	1	1	10
03 spinoscalids	○	2	2	2	2	2	2	2	2	2	2	20
04 spinoscalids	○	1	1	1	1	1	1	1	1	1	1	10
05 spinoscalids	○	2	2	2	2	2	2	2	2	2	2	20
06 spinoscalids	○	1	1	1	?	1	?	1	?	1	1	≥7
07 spinoscalids	○	?	0	2	0	?	?	?	0	2	0	≥4
trichoscalids	✱	0	1	0	1	1	0	1	1	0	1	6

Figure 9

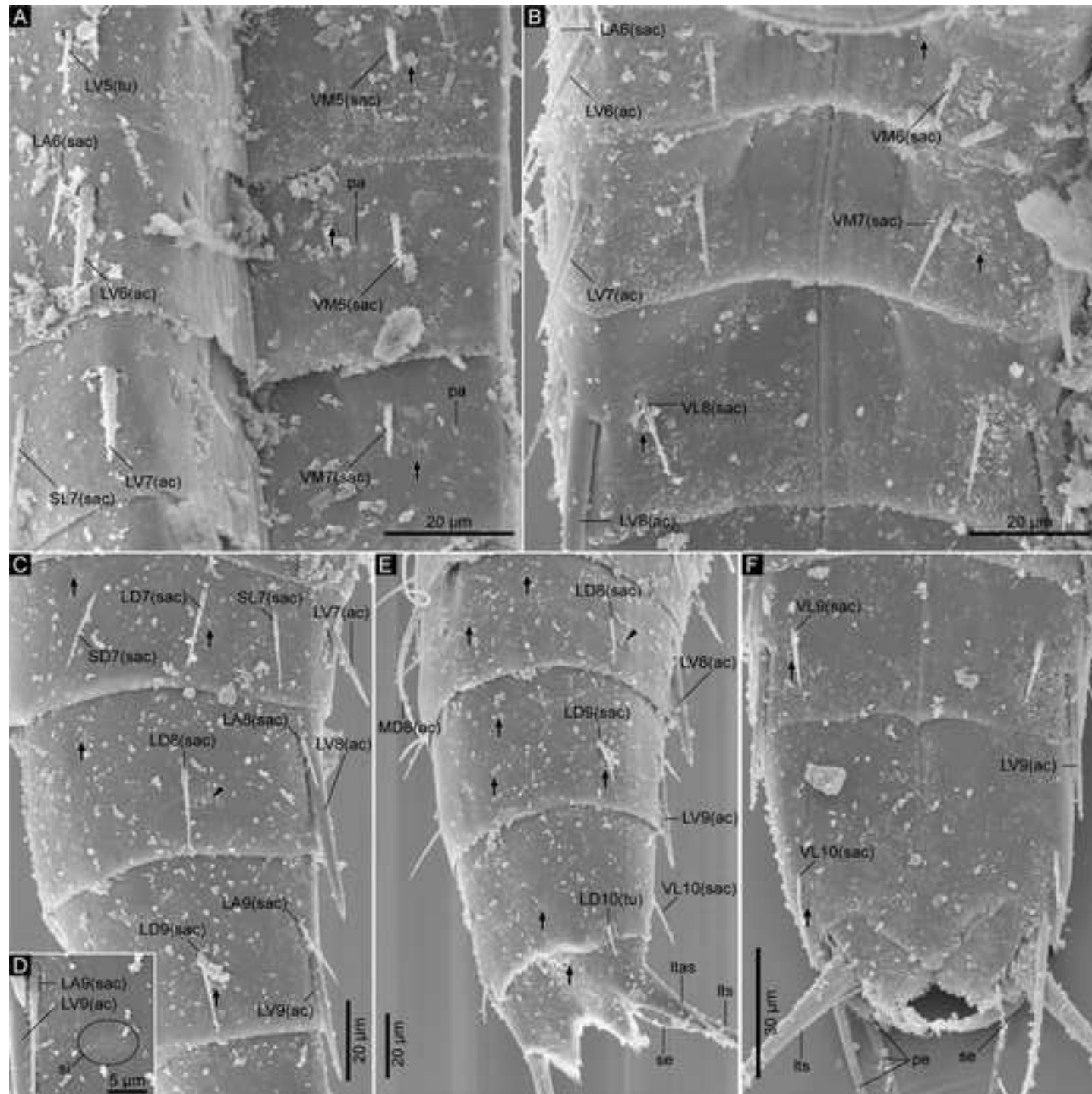


Figure 10

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