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Descriptions of two new species of *Rhizorhina* Hansen, 1892 (Copepoda: Siphonostomatoida: Nicothoidae) parasitic on tanaidacean crustaceans, with a note on their phylogenetic position

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Abstract Two new species of nicothoid copepod are described. *Rhizorhina ohtsukai* n. sp. found on a leptocheliid (*Leptochelia* sp.) collected at depths of 151–136 m in the North Pacific Ocean is most similar to *Rhizorhina aesthetes* Boxshall & Harrison, 1988 but can be distinguished by the possession of a narrower body with a rounded anterior end, and in the position of the gonopores. *Rhizorhina soyoae* n. sp. found on an apseudid (*Fageapseudes* sp.) collected at depths of 1,595–1,557 m in East China Sea most closely resembles *Rhizorhina tanaidaceae* Gotto, 1984 but differs in having a narrower body with a rounded anterior end. Partial nucleotide sequences of the cytochrome *c* oxidase subunit I (COI) gene were obtained from the two copepods in order to enable future barcoding. A phylogenetic reconstruction based on the 18S rRNA gene placed the copepods within the Siphonostomatoida Burmeister, 1835, with the nicothoid *Choniosphaera maenadis* (Bloch & Gallien, 1933), and separate from the *Rhizorhina* spp. clade, suggesting that the family Nicothoidae Dana, 1849 is not monophyletic.

This is the third report of copepods parasitic on tanaidacean crustaceans.

Introduction

Copepods in the family Nicothoidae Dana, 1849 are parasitic, and are classified in 22 genera (Boxshall & Halsey, 2004; Ohtsuka et al., 2005; Bamber & Boxshall, 2006; Boyko, 2009). Among these genera, females of species of *Rhizorhina* Hansen, 1892 and *Arhizorhina* Bamber & Boxshall, 2006 have the urosome incorporated into a swollen, globular body and lack mouthparts, but differ from each other in the shape of the mouth, or "stalk" (Bamber & Boxshall, 2006), penetrating the host body. The stalk is narrow and rod-shaped in *Rhizorhina* and distally widened and funnel shaped in *Arhizorhina* (see Bamber & Boxshall, 2006). To date, six species in *Rhizorhina* have been reported from representatives of four crustacean host groups: three on species of Isopoda Latreille, 1817, and one each on members of Leptostraca Claus, 1880, Amphipoda Latreille, 1816 and Tanaidacea Dana, 1849 (see Boxshall & Harrison, 1988). *Arhizorhina mekonicola* Bamber & Boxshall, 2006, the sole member of the genus, was collected from a tanaidacean host (Bamber & Boxshall, 2006).

During faunal surveys in the North Pacific Ocean and East China Sea in 2014, I obtained four specimens of *Rhizorhina* spp. parasitic on tanaidaceans; this is the third report of copepod parasites utilising Tanaidacea. These copepods turned out to represent two undescribed species, which are described herein. For both species, partial sequences of the cytochrome *c* oxidase subunit I (COI) gene were obtained to aid future DNA barcoding, and partial sequences of the 18S rRNA (18S) gene were generated and used to assess the phylogenetic position of the new species within the Siphonostomatoida Burmeister, 1835.

Materials and methods

Two infected tanaidaceans from the North Pacific Ocean were obtained with a biological dredge from the TR/V *Seisui-maru* (Mie University), at depths of 151–136 m; the hosts were *Leptochelia* sp. in the family Leptocheliidae Lang, 1973. An infected animal from the East China Sea was collected with a small plankton net attached inside a larger beam trawl (see figure 2B in Akiyama et al., 2008) from the R/V *Soyo-maru* (National Research Institute of Fishery Science), at depths of 1,595–1,557 m; the host was *Fageapseudes* sp. in the family Apseudidae Leach, 1814. The three tanaidaceans were fixed and preserved in 70% ethanol.

Host animals were dissected with chemically sharpened tungsten needles to detach the copepods. One egg-sac of each copepod species was placed in 99% ethanol for DNA extraction. Detached copepods in ethanol were mounted on a concavity slide (Plate for Blood Test; Sansyo Co., Ltd., Tokyo, Japan), covered with a coverslip, and photographed at different focal planes through an OLYMPUS BX51 light microscope. The serial images were assembled by using CombineZM (Hadley, 2008) and subsequently processed with Adobe Photoshop/Illustrator CS6. Measurements were made with ImageJ (Rasband, 2014). The copepods were then transferred into *c*.10% glycerin in 70% ethanol and mounted on a glass slide with a pair of coverslip spacers, and their holdfast and gonopore were observed with the BX51. Illustrations were prepared with Adobe Illustrator CS6 from draft line drawings made with a camera lucida and/or from digital micrograph images. After observation, the copepods were stored in 70% ethanol.

The terminology for the orientation and morphology of the tanaidaceans follows Larsen (2003). All measurements in the text are in micrometres, unless noted otherwise. Body length (BL) was measured from the base of the antennule to the tip of the pleotelson in the tanaidaceans, and from the anterior to posterior edges of the body in the copepods; body width (BW) was measured at the widest portion of the carapace (tanaidaceans) or body (copepods); body thickness (BT) was measured at the thickest portion of body in copepods.

Total DNA was extracted from an egg-sac by using a DNeasy Brood & Tissue Kit (Qiagen GmbH, Hilden, Germany). Table 1 lists the primers used for the polymerase chain reaction (PCR) amplification and cycle sequencing. PCR amplification conditions were 95°C for 1 min; 35 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 1 min (COI) or 2.5 min (18S); and 72°C for 7 min. All nucleotide sequences were determined by direct sequencing with a BigDye Terminator Kit ver. 3.1 and a 3130 or 3730 Genetic Analyzer (Life Technologies, California, USA). The 18S dataset comprising 62 terminal taxa (including the two species described herein) was aligned by using MAFFT version 7 (Katoh & Standley, 2013) with the "Auto" strategy ("L-INS-i" selected; Katoh et al., 2005); ambiguous sites were then removed by using trimAl (Capella-Gutiérrez et al., 2009) with the option "automated1". The aligned sequences were trimmed in MEGA 5.2 (Tamura et al., 2011) to a length of 1,637 nt, the shortest length among the sequences. The optimal substitution model was GTR + I + G, determined under the Akaike information criterion (Akaike, 1974) with jModelTest 2.1.4 (Darriba et al., 2012) and Kakusan4 version 4.0.2012.12.14 (Tanabe, 2011). No significant nucleotide compositional heterogeneity was detected (Chi-square test in Kakusan4: p = 1.00000). A maximum likelihood (ML) analysis was conducted in RAxML version 8.1.5 (Stamatakis, 2014), assisted with phylogears2 version 2.0.2013.10.22 (Tanabe, 2008), and nodal support values were obtained through ML analyses of 1,000 bootstrap pseudoreplicates (Felsenstein, 1985).

Family Nicothoidae Dana, 1849

Genus *Rhizorhina* Hansen, 1892

***Rhizorhina ohtsukai* n. sp.**

Type-host: *Leptochelia* sp. (Tanaidacea: Paratanaoidea: Leptocheliidae).

Type-locality: Off Owase, Kumano Sea, Japan, North Pacific Ocean (34°09.228′N, 136°49.751′E to 34°09.513′N, 136°49.740′E), depths of 151–136 m.

Attachment site: The ventral side of the pereonite or pleonite.

Type-material: Holotype (ZIHU-5030; 1 vial): ovigerous female (BL = 333 μm; BW = 278 μm) attached to ventral side of pleonite 1 of female *Leptochelia* sp. (BL = 3.13 mm; BW = 0.43 mm), detached from host; TR/V *Seisui-maru*, Off Owase, Kumano Sea, Japan, North Pacific Ocean (34°09.228′N, 136°49.751′E to 34°09.513′N, 136°49.740′E), 151–136 m depth, biological dredge, 30.x.2014, collected by K. Kakui. Paratype (ZIHU-5031, 1 vial): 1 non-ovigerous female attached to ventral side of pereonite 3 of female *Leptochelia* sp. (BL = 3.04 mm; BW = 0.43 mm), not detached from host. Collection data as for the holotype.

Representative sequences: The COI (660 nt, 219 deduced amino acids) and 18S (1,760 nt) sequences were determined from the right egg-sac of the holotype specimen; the DNA Data Bank of Japan (DDBJ) accession numbers are LC054032 (COI) and LC054034 (18S).

Etymology: The species is named for Susumu Ohtsuka in recognition of his great contributions to the knowledge of copepod biology.

Description (Figs. 1–4)

Ovigerous female [Based on the holotype.] Body (Figs. 1–3, 4A) oval but slightly flattened dorsoventrally; anterior margin rounded, without swelling (*sensu* Boxshall & Harrison, 1988); posterior protrusion absent. Body 333 long, 278 wide, 235 thick (BL/BW = 1.20; BW/BT = 1.18). Rod-shaped stalk located in ventral, subanterior region of body (Fig. 3C), connecting branching holdfast (Fig. 4B). Gonopores opening on ventral surface; separation between left and right gonopores 170 (Fig. 3D), 0.61× BW. Gonopore surrounded by thicker region (Fig. 4C). Egg-sac prolate spheroidal (Fig. 3E), containing *c*.10 eggs, 80 in diameter.

Remarks

Rhizorhina ohtsukai n. sp. is the seventh species described in *Rhizorhina*, and the first copepod known to parasitise paratanaoid tanaidaceans. It most resembles *Rhizorhina aesthetes* Boxshall & Harrison, 1988 in having the body longer than wide and the gonopores opening on the ventral surface of the body. However, *Rhizorhina ohtsukai* n. sp. differs from *R. aesthetes* in the shape of anterior margin of the body (rounded in *R. ohtsukai* n. sp.; the anterior portion of the body, together with the rod-shaped stalk located in the anterior region of the body, protruding as a tapering anterior swelling in *R. aesthetes*), in having a narrower body (BL/BW 1.20 *vs* 1.08), and in the position of the gonopores (more laterally in *R. ohtsukai* n. sp., separated by 0.61× BW *vs* 0.47× BW in *R. aesthetes*). The host crustaceans also differ (a tanaidacean for *R. ohtsukai* n. sp. *vs* an isopod for *R. aesthetes*) (see Boxshall & Harrison, 1988). Differences among all congeners are summarised in Table 2.

***Rhizorhina soyoae* n. sp.**

Type-host: *Fageapseudes* sp. (Tanaidacea: Apseudoidea: Apseudidae).

Type-locality: East China Sea (27°30.458′N, 126°58.182′E to 27°30.797′N, 126°58.067′E), depths of 1,595–1,557 m.

Attachment site: The lateral or dorsal surface of the pereonite.

Type-material: Holotype (ZIHU-5032; 1 vial): ovigerous female (BL = 461 µm; BW = 455 µm) attached to right side of pereonite 3 of male *Fageapseudes* sp. (BL = 8.79 mm; BW = 0.74 mm), detached from host; R/V *Soyo-maru*, Stn kago-9, East China Sea (27°30.458′N, 126°58.182′E to 27°30.797′N, 126°58.067′E), 1,595–1,557 m depth, plankton net in beam trawl, 26.vii.2014, collected by K. Kakui. Paratype (ZIHU-5033, 1 vial): 1 non-ovigerous female attached to dorsal

surface of pereonite 5 of male *Fageapseudes* sp. (same host individual as for holotype), not detached from host. Collection data as for the holotype.

Representative sequences: The COI (658 nt, 219 deduced amino acids) and 18S (1,760 nt) sequences were determined from the left egg-sac of the holotype specimen; the DDBJ accession numbers are LC054033 (COI) and LC054035 (18S).

Etymology: The specific name is from R/V *Soyo-maru*, the vessel from which the type-specimens were collected.

Description (Figs. 5–8)

Ovigerous female [Based on the holotype.] Body (Figs. 5–8) roughly spherical, but widened posteriorly and slightly flattened dorsoventrally; anterior margin rounded, without swelling; posterior protrusion present. Body (including posterior protrusion) 461 long, 455 wide, 384 thick (BL/BW = 1.01; BW/BT = 1.18). Rod-shaped stalk located in ventral, subanterior region of body (cf., Fig. 6), connecting branching holdfast. Gonopores opening on slight posterior hump on posterior surface (Figs. 7E, 8B); separation between left and right gonopores 185 (Fig. 7E), 0.40× BW. Egg-sac nearly spherical (Fig. 7A, C), holding *c*.25 eggs, 90 in diameter.

Remarks

Rhizorhina soyoae n. sp. is the eighth species described in *Rhizorhina*. It closely resembles *Rhizorhina serolis* Green, 1959 and *Rhizorhina tanaidaceae* Gotto, 1984 in having a posterior protrusion between the gonopores. However, *Rhizorhina soyoae* n. sp. differs from *R. serolis* in body size (BL 461 µm *vs* 2,000 µm; BW 455 µm *vs* 2,500 µm) and in the shape of the anterior margin (rounded *vs* concave) and egg-sacs (roughly spherical *vs* prolate spheroidal). The hosts are also different (a tanaidacean for *R. soyoae* n. sp. and an isopod for *R. serolis*) (see Green,

1959).

Rhizorhina soyoae n. sp. is also very similar to *R. tanaidaceae* but differs from the latter in having a narrower body (BL/BW 1.01 *vs* 0.84) and in the shape of the anterior margin (rounded *vs* straight). The hosts for the two species are both in the family Apseudidae, but in different genera [*Fageapseudes* sp. for *R. soyoae* n. sp.; *Leviapseudes hanseni* (Lang, 1968) for *R. tanaidaceae*] (see Gotto, 1984). Differences among all congeners are summarised in Table 2.

Except for *R. soyoae* n. sp., all copepod parasites reported on Tanaidacea (including *R. ohtsukai* n. sp. described above) attach to the ventral surface of the females (e.g. Fig. 1). They might be harmful for the host reproduction, as previously suggested (Ohtsuka et al., 2007), because tanaidaceans mate facing each other ventrally, and females form a marsupium on the ventral surface to brood eggs. The two specimens of *R. soyoae* n. sp., however, attached on the lateral and dorsal surfaces of the male host (Fig. 5); while they may not affect the host's mating behaviour, they might negatively affect its growth and/or sexual maturation; their attachment induced a lesion on the host exoskeleton (Fig. 6, *arrowhead*). More infected tanaidaceans are needed to confirm whether the infection sites I observed for *R. soyoae* n. sp. are typical.

Phylogeny

The only previous molecular phylogeny examining the phylogenetic position of Nicothoidae in Siphonostomatoida placed Nicothoidae in a clade with Dirivultidae Humes & Dojiri, 1981, with weak branch support (Huys et al., 2007). The monophyly of the Nicothoidae, however, has not been tested because Huys et al. (2007) included only one nicothoid taxon, *Choniosphaera maenadis* (Bloch & Gallien, 1933). The phylogenetic analysis in the present study included species in two nicothoid genera, *Choniosphaera* Connolly, 1929 (*C. maenadis*) and *Rhizorhina* (*R. ohtsukai* n. sp. and *R. soyoae* n. sp.), allowing a weak test of nicothoid monophyly.

The present 18S dataset included two or more species each for nine of 18 ingroup families (Asterocheridae Giesbrecht, 1899, Caligidae Burmeister, 1835, Dirivultidae, Eudactylinidae C. B. Wilson, 1922, Hatchekiidae Kabata, 1979, Kroyeriidae Kabata, 1979, Larnaeopodidae Milne Edwards, 1840, Nicothoidae, and Pandaridae Milne Edwards, 1840) (see Table 3 for details). The ML tree (Fig. 9) strongly supported the monophyly of eight of these families, but did not recover Nicothoidae as monophyletic. The two species of *Rhizorhina* comprised a clade with 100% bootstrap support (BS), and this in turn formed a weakly supported (BS 35.8%) clade with Ecbathyriontidae Humes, 1987 and Diribultidae. The other nicothoid, *C. maenadis*, appeared as the sister group to the Asterocheridae, though with very weak nodal support (BS 14.8%). The relationships between *Rhizorhina* + Ecbathyriontidae + Diribultidae and *Choniosphaera* + Asterocheridae were unresolved.

Each of the two nicothoid genera exhibits a peculiar trait within the family Nicothoidae. Females of *Rhizorhina* spp. lack mouthparts but instead bear a “stalk” (Bamber & Boxshall, 2006), a trait shared only with species of *Arhizorhina*. *Choniosphaera* is one of two genera in which the offspring hatch as a nauplius (Wakabayashi et al., 2013). Boxshall & Lincoln (1983) placed *Rhizorhina* and *Choniosphaera* into two different species groups, the *Rhizorhina* and the *Nicothoe* group, respectively, among the three species groups they proposed in Nicothoidae (the third being the *Sphaeronella* group); the three groups were based mainly on the host taxon and character states for the antennules and maxillipeds, and “may represent natural groups” (see p. 899 in Boxshall & Lincoln, 1983). The separation of *Rhizorhina* and *Choniosphaera* in the present 18S tree possibly reflects the differences in morphology and developmental pattern mentioned above.

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Compliance with ethical standards

Conflict of interest The author declares that he has no conflict of interest.

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed.

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Figure legends

Fig. 1 *Rhizorhina ohtsukai* n. sp. parasitising female *Leptochelia* sp., fresh specimens. A, B, E, Holotype, ovigerous female, ventral (A) and left ventral (B, E) views of host; C, D, F, Paratype, non-ovigerous female, ventral (C) and left ventral (D, F) views of host. *Arrowheads* indicate *R. ohtsukai*. *Scale-bars*: 0.5 mm

Fig. 2 *Rhizorhina ohtsukai* n. sp. Holotype, penetrating ventral region of pleonite 1 of host (dissected), fixed specimen. *Abbreviations*: *p6*, pereonite 6; *pl1*, pleonite 1; *rs*, rod-shaped stalk. *Scale-bar*: 0.1 mm

Fig. 3 *Rhizorhina ohtsukai* n. sp. Holotype, fixed specimen. A–C, Habitus, dorsal (A), ventral (B), and left (C) views; D, Ventral view of posterior region, showing positions of gonopores (*arrowheads*); E, Right egg-sac. *Scale-bars*: 0.1 mm

Fig. 4 *Rhizorhina ohtsukai* n. sp. Holotype, line drawings from fixed specimen. A, Habitus, dorsal view (*arrow* indicates the rod-shaped stalk located in ventral, subanterior region of body); B, Rod-shaped stalk (*arrow*) and branching holdfast, ventral view; C, Left gonopore and surrounding wall-like structures, ventral view. *Abbreviations*: *an*, anterior; *po*, posterior; *es*, empty egg-sac. *Arrowhead* indicates region where possible copulatory pore is located.

Scale-bars: A, 0.1 mm; B, C, 0.05 mm

Fig. 5 *Rhizorhina soyoae* n. sp. parasitising male *Fageapseudes* sp., fresh specimens. A, B, Two copepods on host, habitus, dorsal and right views of host, respectively; C, D, Non-ovigerous (paratype) and ovigerous (holotype) females, respectively, right view of host. *Abbreviations*: *nf*, non-ovigerous female; *of*, ovigerous female. *Scale-bars*: 1 mm

Fig. 6 *Rhizorhina soyoae* n. sp. Holotype, with induced lesion on host exoskeleton (*arrowhead*), fixed specimen. *Abbreviation*: *rs*, rod-shaped stalk. *Scale-bar*: 0.1 mm

Fig. 7 *Rhizorhina soyoae* n. sp. Holotype, fixed specimen. A–C, Habitus, dorsal (A), ventral (B), and left posterior (C) views; D, E, Posterior region, dorsal (D) and ventral (E) views. Black *arrow* indicates rod-shaped stalk. Black *arrowheads* indicate structure that may be remnant of male cephalic shield. White *arrowheads* indicate gonopores. *Scale-bars*: 0.1 mm

Fig. 8 *Rhizorhina soyoae* n. sp. Holotype, line drawing of fixed specimen. A, Habitus, dorsal view, with *arrow* indicating rod-shaped stalk located in ventral, subanterior region of body; B, Left gonopore, ventral view. *Abbreviations*: *an*, anterior; *po*, posterior. *Arrowhead* indicates opening of gonopore. *Scale-bars*: 0.1 mm

Fig. 9 Tree from ML analysis of 18S sequences (1,637 nt), including sequences from *R. ohtsukai* n. sp. and *R. soyoae* n. sp. Clades containing more than two confamilial terminal taxa were collapsed (terminal triangles), except for those in the Dirivultidae and Nicothoidae. Numbers near nodes are bootstrap values in percent. Family names are indicated in capital letters.

Table 1 List of PCR and cycle sequencing (CS) primers used in this study

Marker	Primer	Sequence (5' to 3')	Reaction	Source
COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	PCR & CS	Folmer et al. (1994)
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	PCR & CS	Folmer et al. (1994)
18S	SR1	TACCTGGTTGATCCTGCCAG	PCR	Nakayama et al. (1996)
	SR8	GGATTGACAGATTGAGAGCT	CS	Nakayama et al. (1996)
	SR9	AACTAAGAACGGCCATGCAC	CS	Nakayama et al. (1996)
	SR10	AGGTCTGTGATGCCCTTAGA	CS	Nakayama et al. (1996)
	SR12	CCTTCCGCAGGTTCACCTAC	PCR & CS	Nakayama et al. (1996)
	EU929R	TTGGCAAATGCTTTCGC	CS	Puitika et al. (2007)
	18S554f	AAGTCTGGTGCCAGCAGCGCG	CS	Maraun et al. (2009)
	18S614r	TCCAACTACGAGCTTTTTAACC	CS	Maraun et al. (2009)

Table 2 Comparison of key characters for females of species of *Rhizorhina*, with information on hosts

Species	BL	BW	BL/BW	Body shape	Anterior margin of body	Posterior protrusion	Gonopores		Egg-sac shape	Host
							Position	Separation		
R. ohtsukai n. sp.	333	278	1.20	subspherical	rounded	absent	ventral	170	spheroidal	Tanaidacea
R. soyoae n. sp.	461	455	1.01	subspherical	rounded	present	posterior	185	almost spherical	Tanaidacea
R. aesthetes Boxshall & Harrison, 1988	304	282	1.08	subspherical	with swelling	absent	ventral	132	–	Isopoda
R. ampeliscae Hansen, 1892	960	1,050	0.91	subspherical	with swelling	absent	posterior	187	almost spherical	Amphipoda
R. hystrix Boxshall & Harrison, 1988	788	859	0.92	subspherical	with swelling	absent	posterior	353	–	Isopoda
R. leptostracae Gotto, 1984	521	552	0.94	discoid	concaved	absent	posterior	205	spherical	Leptostraca
R. serolis Green, 1959	2,000	2,500	0.80	subrectangular	concaved	present	posterior	1,200	spheroidal	Isopoda
R. tanaidaceae Gotto, 1984	500	594	0.84	subrectangular	straight	present	posterior	258	–	Tanaidacea

Abbreviations: BL, body length; BW, body width

Table 3 Copepod species included in the 18S analysis in this study

Family	Taxon	GenBank accession no.	Reference
Asterocheridae	Asterocheidae sp. 1	AY627018	Huys et al. (2006)
	Orecturus sp.	AY627017	Huys et al. (2006)
Caligidae	*Gloiopotes watsoni* Kirtisinghe, 1934	AY627019	Huys et al. (2006)
	Lepeophtheirus salmonis (Krøyer, 1837)	AF208263	Huys et al. (2007)
	Lepeophtheirus hippoglossi (Krøyer, 1837)	DQ538503	Huys et al. (2007)
	Lepeophtheirus hospitalis Fraser, 1920	DQ123831	Jones et al. (2006)
	Lepeophtheirus natalensis Kensley & Grindley, 1973	FJ447440	Dippenaar (2009)
	Lepeophtheirus parvicruris Fraser, 1920	DQ123830	Jones et al. (2006)
	Caligus clemensi Oarker & Margolis, 1964	DQ123833	Jones et al. (2006)
	Caligus elongatus Nordmann, 1932	AY627020	Huys et al. (2006)
	Caligus fugu (Yamaguti, 1936)	KC569364	Freeman et al. (2013)
	Caligus uniartus (Ho et al., 2004)	KC569363	Freeman et al. (2013)
	Paralebion elongatus C. B. Wilson, 1911	FJ447441	Dippenaar (2009)
	Alebion sp.	FJ447442	Dippenaar (2009)
Cancerillidae	Cancerillidae sp.	AY627021	Huys et al. (2006)
Dichelesthiidae	*Anthosoma crassum* (Abildgaard, 1794)	FJ447459	Dippenaar (2009)
Dinopontiidae	*Stenopontius* sp.	AY627022	Huys et al. (2006)
Dirivultidae	*Aphotopontius mammilatus* Humes, 1987	DQ538508	Huys et al. (2007)
	Ceuthoecetes sp.	DQ538506	Huys et al. (2007)
	Rhogobius contractus Humes, 1987	AY627023	Huys et al. (2006)
Dissonidae	*Dissonus manteri* Kabata, 1966	DQ538500	Huys et al. (2007)
Ecbathyriontidae	*Ecbathyrion prolixicauda* Humes, 1987	AY627024	Huys et al. (2006)
Eudactylinidae	*Eudactylina pusilla* Cressey, 1967	FJ447439	Dippenaar (2009)
	Eudactylinodes niger (C. B. Wilson, 1905)	FJ447438	Dippenaar (2009)
	Nemesis lamna Risso, 1826	FJ447431	Dippenaar (2009)
	Nemesis sp. 3	FJ447435	Dippenaar (2009)
	Nemesis sp. 4	FJ447436	Dippenaar (2009)
Hatschekiidae	*Hatschekia pagrosomi* Yamaguti, 1939	AY627026	Huys et al. (2006)
	Hatschekia sp.	DQ538507	Huys et al. (2007)
Kroyeriidae	*Kroyeria* sp.	DQ538499	Huys et al. (2007)
	Kroyeria longicauda Cressey, 1970	FJ447427	Dippenaar (2009)
	Kroyeria sphyrnae Rangnekar, 1957	FJ447426	Dippenaar (2009)
	Kroyeria papillipes C. B. Wilson, 1932	FJ447425	Dippenaar (2009)
	Kroyeria dispar C. B. Wilson, 1935	FJ447424	Dippenaar (2009)
Lernaeopodidae	*Parabrachiella bispinosa* (Nordmann, 1832)	AY627027	Huys et al. (2006)

	Clavella adunca (Strøm, 1762)	AY627028	Huys et al. (2006)
	Clavellopsis sp.	HM545893	unpublished
Nanaspididae	*Nanaspis tonsa* Humes & Cressey, 1959	AY627029	Huys et al. (2006)
Nicothoidae	*Rhizorhina ohtsukai* n. sp.	LC054034	This study
	Rhizorhina soyoae n. sp.	LC054035	This study
	Choniosphaera maenadis (Bloch & Gallien, 1933)	DQ538509	Huys et al. (2007)
Pandaridae	*Dinemoura latifolia* (Steenstrup & Lütken, 1891)	DQ538501	Huys et al. (2007)
	Pandarus smithii Rathbun, 1886	DQ538502	Huys et al. (2007)
	Perissopus dentatus Steenstrup & Lütken, 1861	FJ447453	Dippenaar (2009)
	Achtheinus oblongus C. B. Wilson, 1908	FJ447452	Dippenaar (2009)
	Pseudopandarus longus (Gnanamuthu, 1951)	FJ447451	Dippenaar (2009)
	Pannosus japonicus (Shiino, 1960)	FJ447450	Dippenaar (2009)
	Phyllothyreus cornutus (Milne Edwards, 1840)	FJ447449	Dippenaar (2009)
	Nesippus orientalis Heller, 1865	FJ447445	Dippenaar (2009)
	Nesippus crypturus Heller, 1865	FJ447444	Dippenaar (2009)
	Nesippus vespa Cressey, 1964	FJ447443	Dippenaar (2009)
Pennellidae	*Lernaeocera branchialis* (Linnaeus, 1767)	AY627030	Huys et al. (2006)
Pontoeciellidae	*Pontoeciella abyssicola* (T. Scott, 1893)	AY627031	Huys et al. (2006)
Sphyriidae	*Paeon elongatus* C. B. Wilson, 1932	FJ447460	Dippenaar (2009)
Outgroup			
Cyclopidae	*Apocyclops royi* (Lindberg, 1940)	AY626997	Huys et al. (2006)
	Euryte sp.	AY626996	Huys et al. (2006)
Mytilicolidae	*Trochicola entericus* Dollfus, 1914	AY627006	Huys et al. (2006)
	Pectinophilus ornatus Nagasawa et al., 1988	AY627032	Huys et al. (2006)
Anthessiidae	*Anthessius* sp.	AY627002	Huys et al. (2006)
Synapticolidae	*Scambicornus* sp.	AY627011	Huys et al. (2006)
Ectinosomatidae	*Bradya* sp.	AY627016	Huys et al. (2006)
Canthocamptidae	*Bryocamptus pygmaeus* (G. O. Sars, 1863)	AY627015	Huys et al. (2006)


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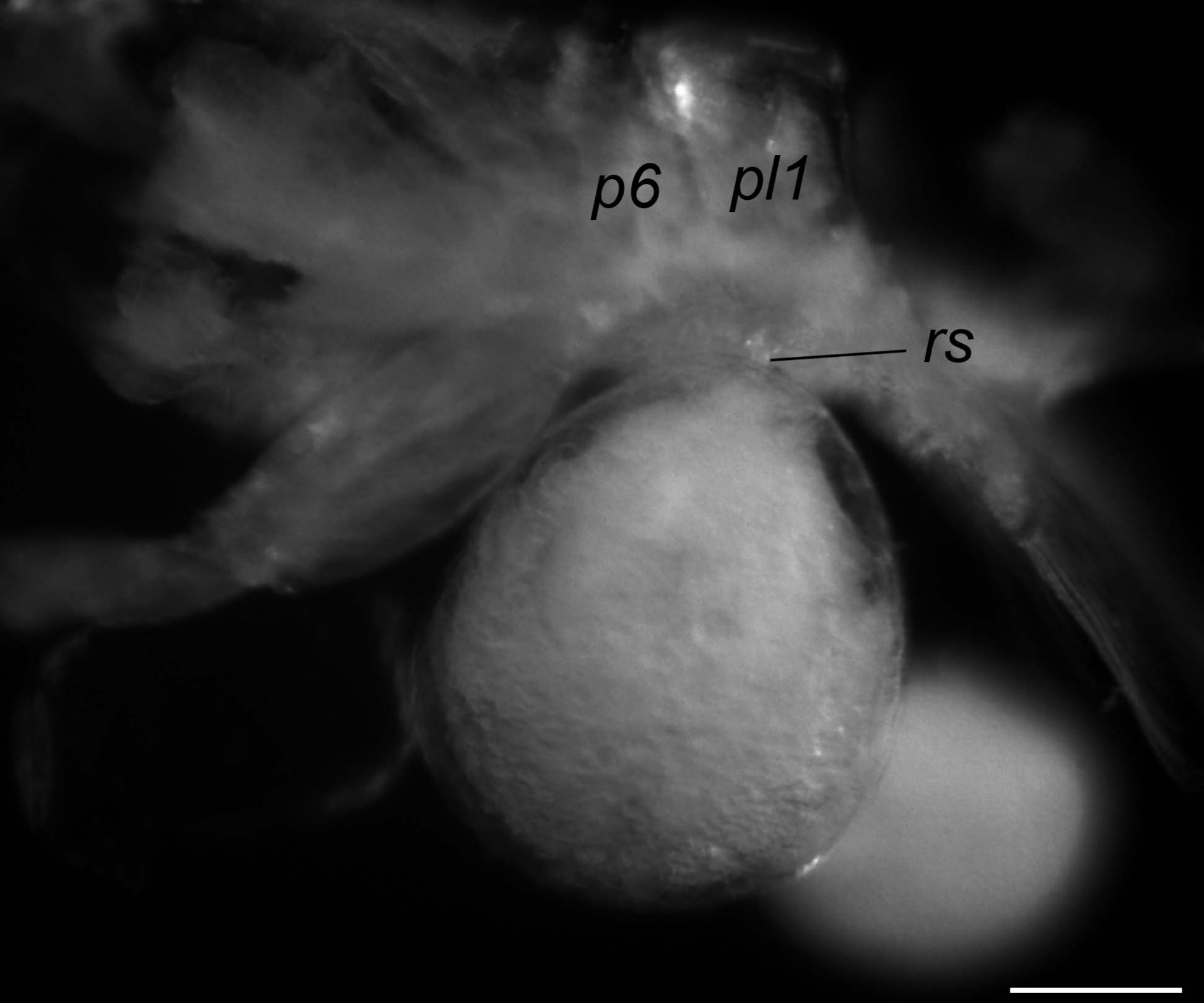
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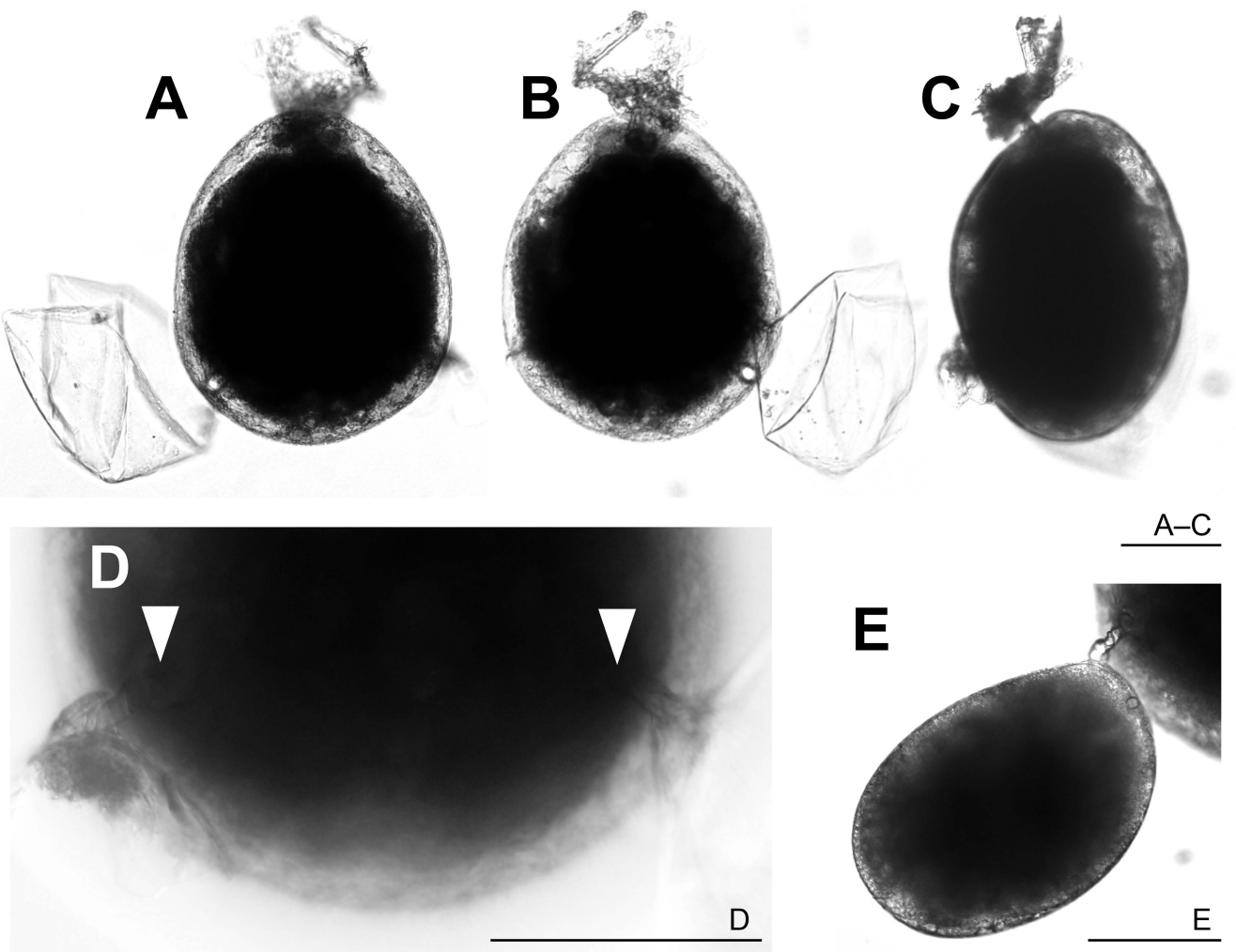
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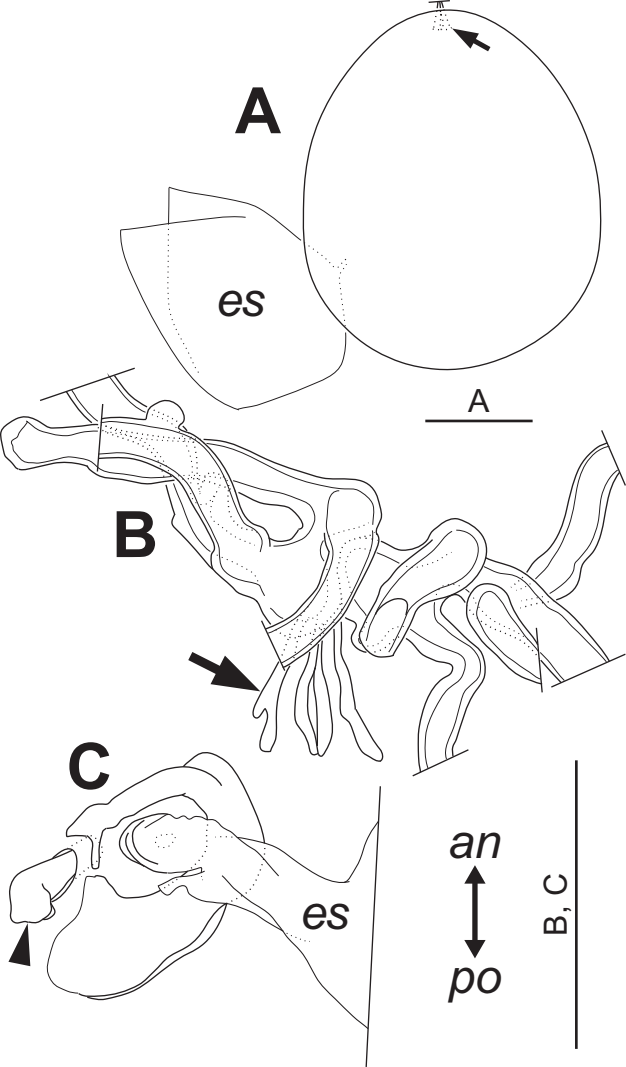
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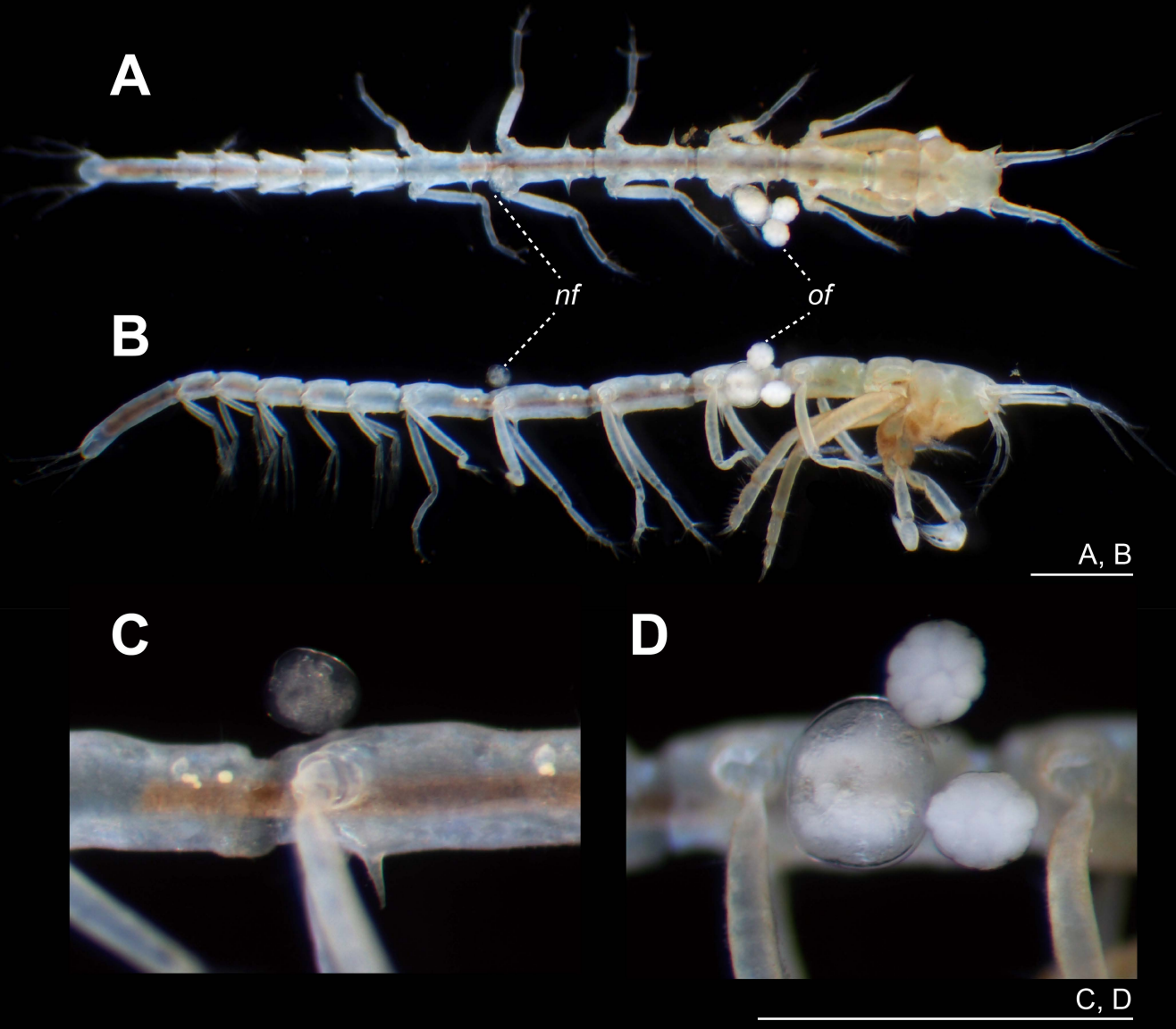
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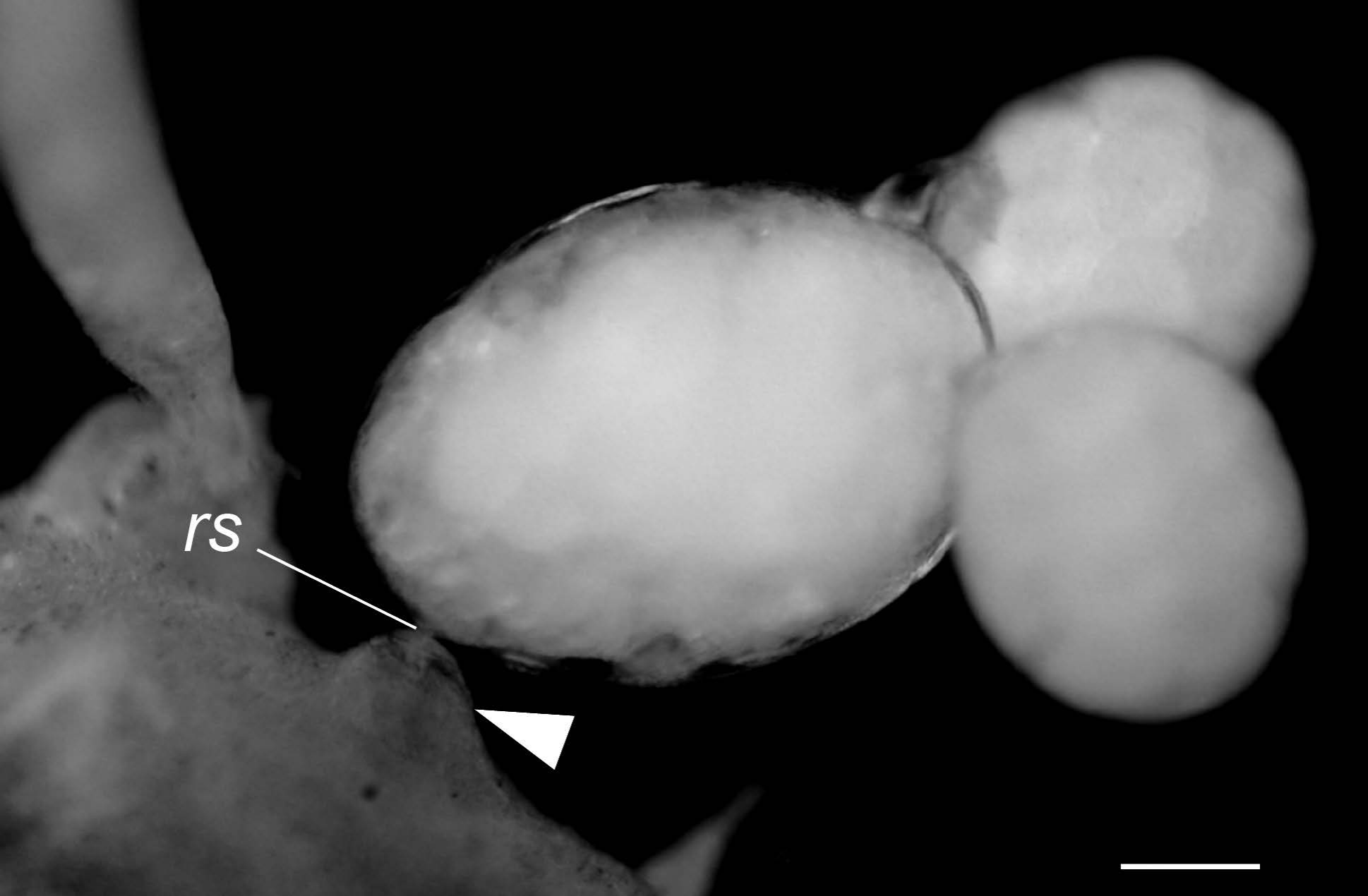
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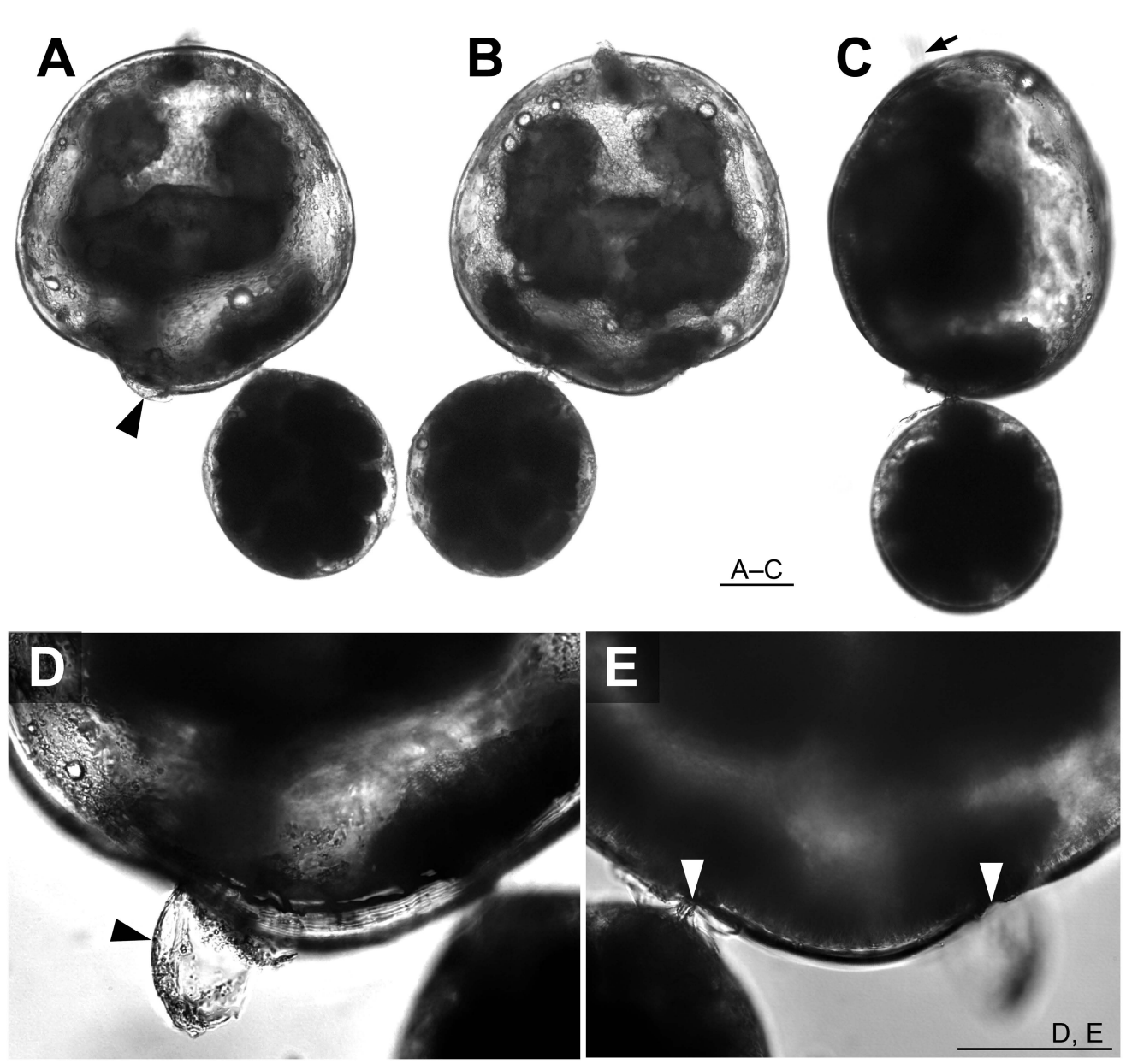
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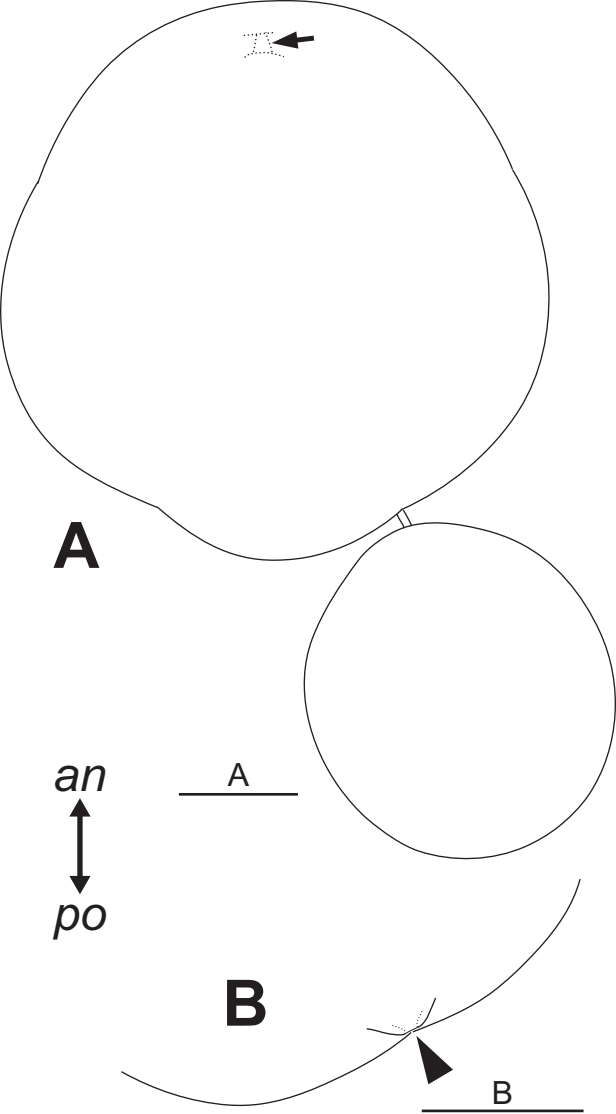
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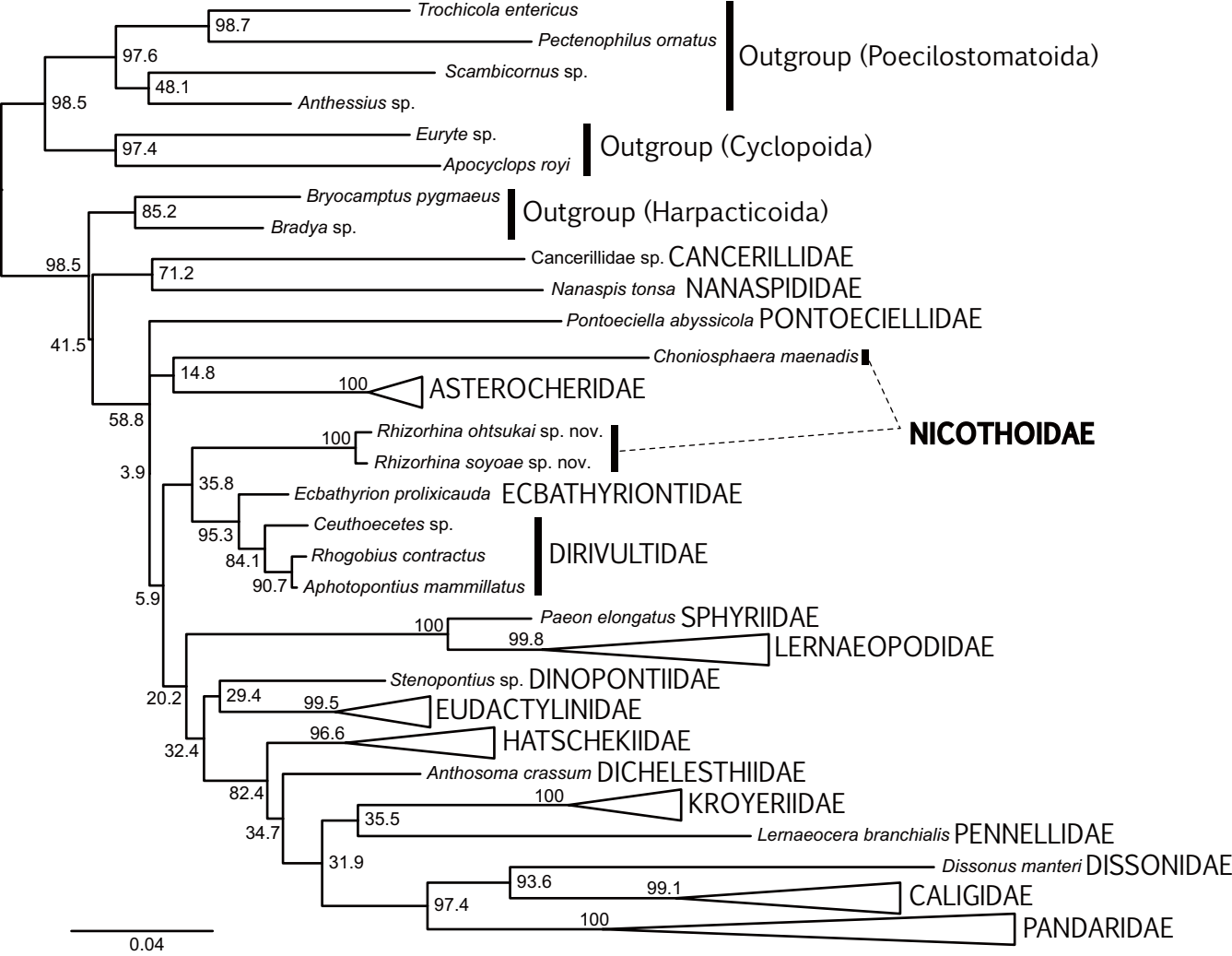
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Euryte sp.
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Apocyclops royi
Outgroup (Cyclopoida)
Bryocamptus pygmaeus
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Bradya sp.
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Pontoeciella abyssicola PONTOECIELLIDAE
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ASTEROCHERIDAE
58.8
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Rhizorhina soyoae sp. nov.
NICOTHOIDAE
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Ecbathyrion prolixicauda ECBATHYRIONTIDAE
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