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A new *Sphaeronella* species (Copepoda: Siphonostomatoida: Nicothoidae) parasitic on *Euphilomedes* sp. (Ostracoda: Myodocopa: Philomedidae) from Hokkaido, Japan, with an 18S molecular phylogeny

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Abstract

With about 80 species, *Sphaeronella* is the most species-rich genus in the copepod family Nicothoidae. To date, 20 named *Sphaeronella* species have been reported as ectoparasites on ostracod crustaceans. Here we describe *Sphaeronella uyenoi* **sp. nov.** parasitic on the philomedid ostracod *Euphilomedes* sp. collected from Akkeshi Bay, Hokkaido, Japan, northwestern Pacific. *Sphaeronella uyenoi* most closely resembles *S. monothrix* (Bowman & Kornicker, 1967), parasitic on the cylindroleberidid *Parasterope pollex* Kornicker in Bowman & Kornicker in the northwestern Atlantic, but differs from the latter in having (1) the submedian skeleton containing paired, strongly chitinized, Λ-shaped areas and paired wide oblong holes bearing a strongly chitinized fringe posteriorly, and (2) maxillipedal segment 3 with antero-subdistal serration. We determined partial sequences for the cytochrome *c* oxidase subunit I (COI) and 18S rRNA (18S) genes for *S. uyenoi* and constructed an 18S-based phylogenetic tree of copepods. In our tree, Nicothoidae was not monophyletic, and *S. uyenoi* was the sister taxon to *Cancerilla* sp. in Cancerillidae (ectoparasites on brittle stars).

Introduction

Parasitic copepods in the family Nicothoidae Dana, 1849 utilize other crustaceans (Boxshall & Halsey, 2004). The family contains 22 genera and about 140 species (WoRMS, 2022), four of which have been reported from Japan (Ohtsuka et al., 2005; Wakabayashi et al., 2013; Kakui, 2016): *Choniomyzon inflatus* Wakabayashi, Otake, Tanaka, & Nagasawa, 2013; *Neomysidion rahotsu* Ohtsuka, Boxshall, & Harada, 2005; *Rhizorhina ohtsukai* Kakui, 2016; and *Rhizorhina soyoae* Kakui, 2016.

Sphaeronella Salensky, 1868, the most species-rich nicothoid genus, with 78 species (Bradford, 1975, 1980; Boxshall & Lincoln, 1983; Yoo & Lim, 1996; O'Reilly, 2001, 2002), is ectoparasitic on amphipods, isopods, cumaceans, or ostracods. Currently, 20 named species are known to be parasitic on ostracods (Table 1; Bradford, 1975, 1980; Yoo & Lim, 1996; O'Reilly, 2001), occupying the space inside the two-valved host carapace (cf. Bradford, 1975: fig. 1).

During light-trap sampling in Akkeshi Bay, Hokkaido, Japan, we found an undescribed *Sphaeronella* species inside the carapace of the myodocopan ostracod *Euphilomedes* sp. Here we describe this species as new, provide partial sequences for its cytochrome *c* oxidase subunit I (COI) and 18S rRNA (18S) genes to aid future DNA barcoding, and infer its phylogenetic position in Siphonostomatoida based on 18S data.

Materials and methods

We set a light trap (YF-500, Yamada Electric Industry, Chiba, Japan) at 19:30 on 1 July 2022 and collected ostracods that came to the light with a plankton net having 100 μm mesh openings between 20:00 and 21:00. Ostracods were anesthetized in a roughly 1:9 mixture of absolute ethanol in sea water, examined for parasite infection under a Nikon SMZ-10 stereomicroscope, and preserved in 70–100% ethanol.

To extract parasites, six infected ostracods were dissected with chemically sharpened needles under an SZX9 stereomicroscope. Four extracted parasites were transferred into a roughly 1:9 mixture of glycerin and absolute ethanol, kept at room temperature until the ethanol evaporated, mounted on glass slides in glycerin, and observed with an Olympus BX53 microscope. Illustrations were prepared with Adobe Illustrator CS6 from draft line drawings made with a camera lucida. Body length (BL) was measured from the anterior to posterior ends of the body, and head width (HW) and trunk width (TW) at the widest portion of the head and trunk, respectively. All measurements were obtained from individuals under a cover slip and are presented in the text in micrometers, unless noted otherwise. The specimens studied were deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Japan, under catalog numbers ICHUM8340–8347.

DNA was extracted from the whole body of two extracted parasites and the left antenna 2 of two

hosts by using a NucleoSpin Tissue XS Kit (Macherey-Nagel, Germany). PCR primers used for the amplification and cycle sequencing of COI were LCO1490 and HCO2198 (Folmer et al., 1994). Amplification primers for 18S were SR1 and SR12 (Nakayama et al., 1996); five primers (18S-b4F, 18S-b4R, 18S-b5F, 18S-a6R, and 18S-b8F; Kakui et al., 2011, 2021) were used in cycle sequencing. PCR amplification conditions for COI with TaKaRa Ex Taq DNA polymerase (TaKaRa Bio, Japan) and for 18S with KOD FX Neo (Toyobo, Japan) were as described by Okamoto & Kakui (2022). All nucleotide sequences were determined with a BigDye Terminator Kit ver. 3.1 and a 3730 DNA Analyzer (Life Technologies, USA). Fragments were concatenated by using MEGA7 (Kumar et al., 2016). The sequences we determined were deposited in the International Nucleotide Sequence Database (INSD) through the DNA Data Bank of Japan. Genetic distances among sequences were calculated with MEGA7.

The copepod 18S dataset for a phylogenetic analysis included one sequence we determined and 49 sequences greater than 1500 bp long from 48 siphonostomatoid species and one outgroup taxon (a misophrioid) taken from the INSD (Table 2). The sequences were aligned (1528 positions in the aligned dataset; see Supplementary Information) as described by Munakata et al. (2022); methods for selection of the optimal substitution model (GTR+F+R3), the maximum likelihood (ML) analysis, and drawing the tree were as described by Kakui & Shimada (2022).

Results

***Sphaeronella uyenoi* sp. nov.**

(Figs. 1, 2)

Diagnosis (females). Antennule 2-segmented, with segment-2 less than half as long as segment 1; antenna absent; maxillule with two branches; maxilliped 3-segmented, with segment 2 bearing distal spiniform seta and segment 3 bearing antero-subdistal serration; submedian skeleton posteriorly widely fan-shaped, with paired, Λ-shaped, strongly chitinized areas and paired wide oblong holes posteriorly; legs 1 and 2 present.

Type host. *Euphilomedes* sp. (Ostracoda: Myodocopa: Philomedidae).

Type locality. Around the pier of the Akkeshi Marine Station, Akkeshi Bay, Hokkaido, Japan, northwestern Pacific (43°1.272′ N, 144°50.202′ E), less than 5 m depth.

Attachment sites. Inside carapace.

Material examined. Holotype: female (ICHUM8340), BL 406, HW 138, TW 312, 1 vial containing extracted holotype and dissected male host. Paratypes: five females (ICHUM8341, BL 386, HW 135, TW 288; ICHUM8342, BL 377, HW 136, TW 305; ICHUM8343, BL 415, HW 136, TW 339; ICHUM8344; ICHUM8345; each comprising 1 slide and 1 vial containing dissected male host. Other material: five

females in five male hosts (ICHUM8346), 1 vial. Five non-infected male ostracods (ICHUM8347), 1 vial. All specimens were collected at the type locality on 1 July 2022.

Representative DNA sequences. Two COI (INSD accession numbers LC720687, LC720688; 641 bp long, encoding 213 amino acids) and two 18S (LC720685, LC720686; 1835 bp long) sequences were determined from two paratype females (ICHUM8344, 8345). Two COI (LC720683, LC720684; 625 bp long, encoding 208 amino acids) and two 18S (LC720681, LC720682; 1853 bp long) sequences were determined from two host ostracod males (ICHUM8344, 8345).

Etymology. The specific name is a noun in the genitive case, honoring Daisuke Uyeno, who has greatly contributed to the taxonomy of parasitic copepods.

New Japanese names. *Sphaeronella*: "Daruma-mijinko-zoku" (“daruma” is a hollow, round, Japanese traditional doll; “mijinko” is a general term for tiny crustaceans; “zoku” means genus; the body shape of *Sphaeronella* females resembles daruma). *Sphaeronella uyenoi*: "Gachapon-daruma-mijinko" (“gachapon” refers to vending-machine-dispensed capsule toys, analogous to a parasite in the capsule-like carapace of an ostracod).

Description

Female [based on the holotype unless noted otherwise]. Body (Figs. 1c, 2a) globular but slightly flattened dorsoventrally, with smooth surface; slightly tinted with orange (retained in ethanol; Fig. 1a, b). BL/HW 2.85; BL/TW 1.34. Head anterior, well defined from trunk; ventrolateral region smooth; anterior margin smooth, rounded. Caudal rami (Fig. 2b: ICHUM8341) represented by paired cylindrical segments, each with distal plumose seta. Antennule (Fig. 2c) 2-segmented; segment 1 with two mid-anterior, two anterodistal, one postero-subdistal, and three posterodistal setae and distal aesthetasc (Fig. 2c, shaded); aesthetasc slightly shorter than segment 1; segment 2 one-fourth as long as segment 1, with distal seta and distal bifurcate seta. Antenna absent. Mandible (Fig. 2d) with bifurcate tip. Maxillule (Fig. 2e) with two branches (anterior and lateral). Maxilla (Fig. 2f) 2-segmented; segment 1 robust, with row of spinules adjacent to articulation with segment 2; segment 2 tapering, curved, with several teeth in distal half. Maxilliped (Fig. 2g) 3-segmented, including distal claw (here called “segment 3”), with length ratio of segments 1–3 2.4:1.7:1.0; segment 1 with row of spinules adjacent to articulation with segment 2; segment 2 strongly constricted in middle region, with subproximal and distal rows of spinules and distal naked spine; segment 3 tapering, curved, with antero-subdistal serration. Submedian skeleton (Fig. 2d, shaded area) posteriorly widely fan-shaped (but median portion barely visible and may not exist), containing paired, Λ-shaped, strongly chitinized areas and paired wide oblong holes bearing strongly chitinized fringe posteriorly. Legs 1 and 2 (Fig. 2h, i) each 1-segmented, cylindrical, with distal seta. Genital area (Fig. 2a,

b) without ornamentation, border of plate not obvious; openings of oval seminal receptacles anterior to semicircular genital apertures.

Male and copepodid. Unknown.

Variation. Three female paratypes (ICHUM8341–8343) observed in addition to holotype showed all diagnostic character states seen in holotype. Ranges (mean with standard deviation in parentheses) of BL, HW, and TW were 386–415 (396±18), 135–138 (136±1), and 288–339 (311±21), respectively. Ranges of BL/HW and BL/TW were 2.77–3.05 and 1.22–1.34, respectively.

Genetic divergence and phylogenetic analysis

Two COI (641 bp) sequences from two paratypes differed by five nucleotide substitutions, and both the uncorrected p-distance and Kimura's (1980) 2 parameter (K2P) distance between them were 0.8%. Their 18S (1835 bp) sequences were identical.

In the ML tree (Fig. 3), monophyly is strongly supported for seven of the nine families with two or more genera included in our analysis (Caligidae, Dirivultidae, Eudactylinidae, Lernaeopodidae, Pandaridae, Pennellidae, and Sphyriidae), with 97–100% ultrafast bootstrap support (uBS). The other two families (Asterocheridae and Nicothoidae) are not monophyletic. In Asterocheridae, two species parasitic in cnidarians (*Orecturus* sp. and Asterocheidae sp. 1; Huys et al., 2006) form a fully supported clade, but this clade is not the sister taxon to *Asterocheres aesthetes*, parasitic in sponges (Ho, 1984). Three nicothoid genera form a scarcely supported clade (uBS = 40%), along with Cancerillidae, Nanaspididae, and Pontoeciellidae. The sister-group relationship between *Sphaeronella uyenoi* on ostracods (Nicothoidae) and *Cancerilla* sp. on ophiuroid echinoderms (Cancerillidae; Huys et al., 2006) is supported with 100% uBS. The branch length for *S. uyenoi* is relatively long.

Discussion

Morphological comparison

Sphaeronella uyenoi **sp. nov.** lacks an antenna, has a 2-branched maxillule, and has a 3-segmented maxilliped, which are shared with females of six congeners: *Sphaeronella anarthronis* Bradford, 1975; *Sphaeronella gottoi* O'Reilly, 2001; *Sphaeronella monothrix* (Bowman & Kornicker, 1967); *Sphaeronella parasteropeae* Bradford, 1975; *Sphaeronella philomedesi* Bradford, 1975; and *Sphaeronella spinosa* Bradford, 1980.

Sphaeronella uyenoi differs from *S. anarthronis*, *S. gottoi*, *S. philomedesi*, and *S. spinosa* in having the following character states (character state of the four latter in parentheses): the ventrolateral region of the head is smooth (uneven longitudinal spinule rows are present); antennular segment 2 is

one-fourth the length of segment 1 (subequal); legs 1 and 2 are present (both absent). *Sphaeronella philomedesi* lacks a distal naked spine on maxillipedal segment 2, but *S. uyenoi* has one. Their distribution and host species also differ: *S. uyenoi* is parasitic on *Euphilomedes* sp. from Japan, northwestern Pacific; *S. anarthronis* is parasitic on the philomedid *Anarthron dithrix* Kornicker from off the western coast of Chile, southeastern Pacific and on *Anarthron chilensis* (Hartmann) from off the southeastern coast of Argentina, southwestern Atlantic; *S. gottoi* is parasitic on the cylindroleberidid *Synasterope norvegica* (Sars) from the North Sea, northeastern Atlantic; *S. philomedesi* is parasitic on the philomedid *Philomedes subantarcticus* Kornicker from off the southeastern coast of Argentina, southwestern Atlantic; *S. spinosa* is parasitic on the rutidermatid *Rutiderma darbyi* Kornicker from off South Carolina, northwestern Atlantic (Bradford, 1975, 1980; O'Reilly, 2001).

Sphaeronella uyenoi differs from *S. parasteropeae* in having the following character states: the ventrolateral region of the head is smooth (has uneven longitudinal spinule rows in *S. parasteropeae*); the antennule is 2-segmented (1-segmented in *S. parasteropeae*); maxillipedal segment 3 has antero-subdistal serration (no serration in *S. parasteropeae*). *Sphaeronella parasteropeae* parasitizes the cylindroleberidid *Parasterope* sp. from off the eastern coast of Argentina, southwestern Atlantic (Bradford, 1975).

Sphaeronella uyenoi closely resembles *S. monothrix* and shares the following character states (1) antennular segment 2 is less than half as long as segment 1 and (2) legs 1 and 2 are present, but differs from the latter in having (i) the submedian skeleton with paired, strongly chitinized, Λ-shaped areas and paired wide oblong holes bearing a strongly chitinized fringe posteriorly (the submedian skeleton in *S. monothrix* has a paired, strongly chitinized, inverted β-shaped area and paired long oblong holes bearing a strongly chitinized fringe posteriorly) and (ii) maxillipedal segment 3 has antero-subdistal serration (no serration in *S. monothrix*). The host for *Sphaeronella monothrix* is not philomedid ostracods but the cylindroleberidid *Parasterope pollex* Kornicker, collected from Hadley Harbor, northwestern Atlantic (Bowman & Kornicker, 1967).

Sphaeronella hebe (Bowman and Kornicker, 1968) and *Sphaeronella homasteropeae* Bradford, 1975 were described based on copepodid-stage individuals and lack information on females. Both species use host ostracod genera different from that of *S. uyenoi* and were reported far from Japan: *S. hebe* was found on the philomedid *Pseudophilomedes ferulanus* Kornicker (phylogenetically different from *Euphilomedes* sp. at the subfamily level) collected from the North Carolina shelf, northwestern Atlantic (Bowman & Kornicker, 1968); *S. homasteropeae* was found on the cylindroleberidid *Homasterope micra* Kornicker (phylogenetically different from *Euphilomedes* sp. at the superfamily level) collected from off the western coast of Chile, southeastern Pacific (Bradford, 1975). Based on these differences in host species and distribution, we deemed *S. uyenoi* not to be conspecific with *S. hebe* or *S. homasteropeae*.

Around Hokkaido, Wakayama (2010) reported nicothoid copepods parasitic on two *Euphilomedes* species and suggested that they may represent two *Sphaeronella* species. This reference presented little information on the copepods' morphology, so it is unclear whether either of them is *S. uyenoi*.

Non-monophyly of Nicothoidae

Our 18S-based phylogeny suggested that, as indicated by Kakui (2016), Nicothoidae is not monophyletic. Branch support for the relationships among nicothoids and other families was low, but given that most families in our tree were strongly supported clades, the three nicothoid genera may differ at the family level. The genera *Choniosphaera*, *Rhizorhina*, and *Sphaeronella* included in our study respectively belong to the *Nicothoe*, *Rhizorhina*, and *Sphaeronella* groups proposed by Boxshall & Lincoln (1983). Our result concurs with Boxshall and Lincoln's (1983: p. 899) phylogenetic view that "The three groups of genera may represent natural groups," that is, they may be distinct, independent lineages in Siphonostomatoida.

Due to the long branch length for *S. uyenoi*, the phylogenetic position of this species in our tree should be treated with caution; if the position is correct, our tree indicates a close relationship between *Sphaeronella* in Nicothoidae and *Cancerilla* in Cancerillidae. The clade containing these two genera formed a moderately supported clade (uBS = 86%) with a sequence from *Nanaspis* in Nanaspididae. *Cancerilla* and *Nanaspis* copepods are ectoparasites on brittle stars and sea cucumbers (both in Echinodermata), respectively (Boxshall & Halsey, 2004), whereas *Sphaeronella* are ectoparasites on crustaceans. This relationship may imply that a host group shift from echinoderms to crustaceans has occurred.

At present, DNA sequences are not available for most nicothoid genera. It is thus too early to discuss how many families should be erected for nicothoids or whether a host-group shift from echinoderms to crustaceans has occurred. Genetic information from additional siphonostomatoid genera, including other nicothoids, will be necessary to clarify this issue.

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Supplementary Information

Supplementary files S1 and S2 Aligned 18S sequences used for the maximum-likelihood analysis. (zip format)

Declarations

Author contributions: KK made morphological observations on the copepods and conducted the molecular analysis; MM made morphological observations on the ostracods; KK and MM conceived and designed the study, wrote the manuscript, and read and approved the final draft.

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Consent: Not applicable.

Data availability: The raw data (sampling locality; sampling date; museum deposition numbers, INSD accession numbers, and the depository for specimens) are included in the manuscript.

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Figure legends

Fig. 1 *Sphaeronella uyenoi* **sp. nov.** (female) and its host *Euphilomedes* sp. (male), fixed specimens. a, male host individual containing a female *S. uyenoi* (ICHUM8340), left view; b, same, left valve of the host removed; *S. uyenoi* (ICHUM8340), ventral view. Arrowheads, *S. uyenoi*.

Fig. 2 *Sphaeronella uyenoi* **sp. nov.** a, c–i, holotype, female (ICHUM8340); b, paratype, female (ICHUM8341). a, body, ventral view; b, genital area, posteroventral view; c, right antennule (aesthetasc shaded); d, oral sucker and medial skeleton (shaded); e, right maxillule; f, right maxilla; g, right maxilliped; h, i, right legs 1 and 2.

Fig. 3 Maximum-likelihood (ML) tree based on 18S sequences (1528 positions). Values near nodes are ultrafast bootstrap values; values < 80% (except those for nicothoids) not shown. Black circles indicate 100% ultrafast bootstrap support. Family names are in gray font. Species names outlined in black are taxa in Nicothoidae. The scale bar indicates branch length in number of substitutions per site.

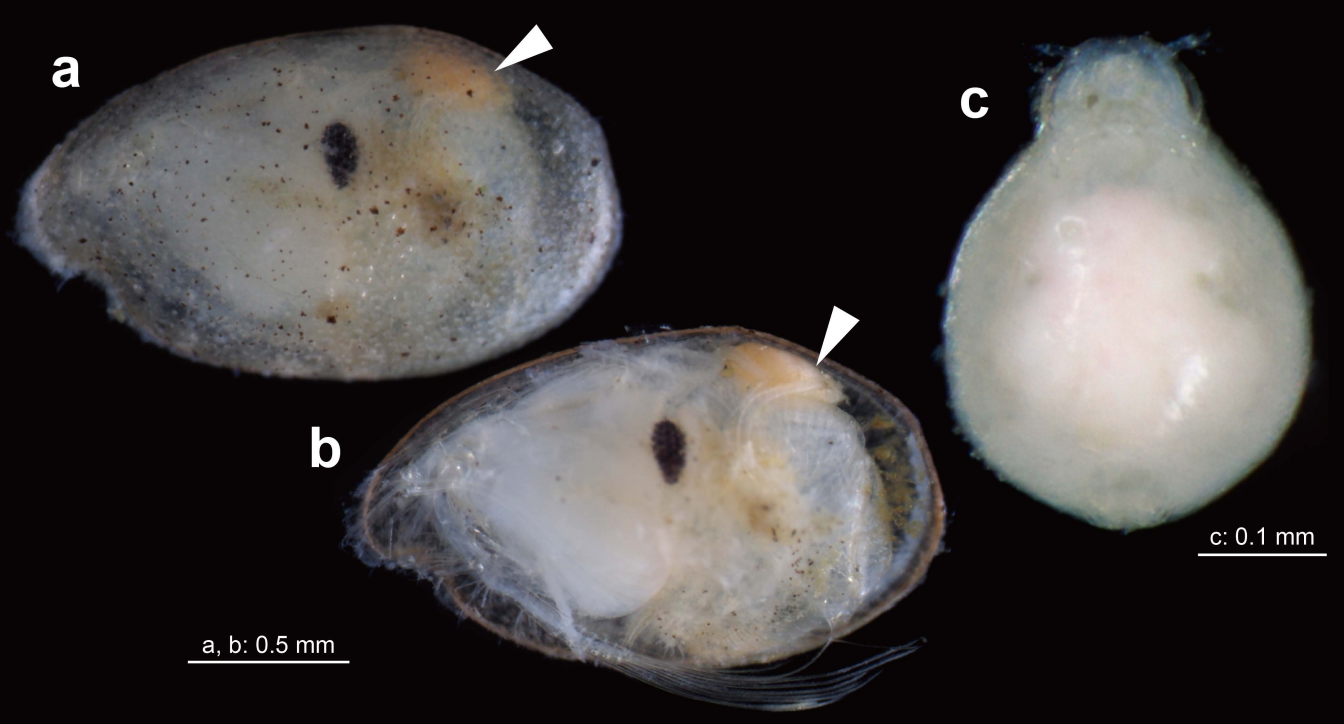
Table 1 Information on *Sphaeronella* copepods utilizing ostracod hosts. Ostracods: bold, Philomedidae; [1], Cylindroleberididae; [2], Cypridinidae; [3], Sarciellidae; [4], Rutidermatidae.

Species	Host	Ocean	Depth (m)	Reference
S. anarthronis Bradford, 1975	***Anarthron dithrix* Kornicker,**	SE Pacific,	44–135	Bradford (1975)
	***Anarthron chilensis* (Hartmann)**	SW Atlantic		
S. antarctica Bradford, 1975	*Skogsbergiella macrothrix* Kornicker[1]	SW Atlantic	3590–3777?	Bradford (1975)
S. dikrothrix (Kornicker & Bowman, 1969)	*Metavargula ampla* Kornicker[2]	SE Pacific	1927–1997	Kornicker & Bowman (1969)
S. doloriae Bradford, 1975	*Doloria pectinata* Skogsberg[2]	SE Pacific	21.3–101	Bradford (1975)
S. gottoi O'Reilly, 2001	*Synasterope norvegica* (Sars)[1]	NE Atlantic	51	O'Reilly (2001)
S. hebe (Bowman & Kornicker, 1968)	***Pseudophilomedes ferulanus* Kornicker**	NW Atlantic	85–165	Bowman & Kornicker (1968)
S. homasteropeae Bradford, 1975	*Homasterope micra* Kornicker[1]	SE Pacific	112	Bradford (1975)
S. kornickeri Bradford, 1975	***Philomedes assimilis* Brady**	Antarctic	36–146	Bradford (1975)
S. metavargulae Bradford, 1975	*Metavargula adinothrix* Kornicker[2]	SW Atlantic	2452	Bradford (1975)
S. monothrix (Bowman & Kornicker, 1967)	*Parasterope pollex* Kornicker[1]	NW Atlantic	1–2	Bowman & Kornicker (1967)
S. parasteropeae Bradford, 1975	*Parasterope* sp.[1]	SW Atlantic	1475	Bradford (1975)
S. philomedesi Bradford, 1975	***Philomedes subantarctica* Kornicker**	SW Atlantic	424–428	Bradford (1975)
S. rugosidoloriae Bradford, 1975	*Rugosidoloria serrata* Kornicker[2]	SW Atlantic	44	Bradford (1975)
S. sarsiellae Bradford, 1975	*Sarsiella lunata* Kornicker[3]	(Off South Africa)	179	Bradford (1975)
S. scleroconchae Bradford, 1975	***Scleroconcha gallardoi* Kornicker**	SW Atlantic	1180	Bradford (1975)
S. siphonostrae Bradford, 1975	*Siphonostra hallex* Kornicker[2]	SW Atlantic	44	Bradford (1975)
S. skogsbergiellae Bradford, 1975	*Skogsbergiella macrothrix* Kornicker[1],	SE Pacific,	10–1146	Bradford (1975)
	Skogsbergiella scotti Kornicker[1]	SW Atlantic, Indian		
S. spinosa Bradford, 1980	*Rutiderma darbyi* Kornicker[4]	NW Atlantic	26–43	Bradford (1980)
S. squamosa Yoo & Lim, 1996	*Codonocera* sp.[2]	NW Pacific	unknown	Yoo & Lim (1996)
S. synasteropeae Bradford, 1975	*Synasterope dimorpha* (Hartmann)[1]	SE Pacific	2657–2470	Bradford (1975)
S. uyenoi sp. nov.	***Euphilomedes* sp.**	NW Pacific	< 5	This study

Table 2 Copepod species included in the 18S analysis in this study. INSD, International Nucleotide Sequence Database.

Higher taxon name	OTU name	Accession #	Reference
Misophrioida			
Misophriidae	*Misophriopsis okinawensis* Ohtsuka et al., 1992	JF781532	Huys et al. (2012)
Siphonostomatoida			
Asterocheridae	*Asterocheres aesthetes* Ho, 1984	KR048771	INSD
	Orecturus sp.	AY627017	Huys et al. (2006)
	Asterocheidae sp. 1	AY627018	Huys et al. (2006)
Caligidae	*Alebion* sp.	FJ447442	Dippenaar (2009)
	Caligus fugu (Yamaguti, 1936)	KC569364	Freeman et al. (2013)
	Caligus uniartus (Ho et al., 2004)	KC569363	Freeman et al. (2013)
	Gloiopotes watsoni Kirtisinghe, 1934	AY627019	Huys et al. (2006)
	Lepeophtheirus hippoglossi (Krøyer, 1837)	DQ538503	Huys et al. (2007)
	Lepeophtheirus salmonis (Krøyer, 1837)	AF208263	Huys et al. (2007)
	Paralebion elongatus C. B. Wilson, 1911	FJ447441	Dippenaar (2009)
Cancerillidae	*Cancerilla* sp.	AY627021	Huys et al. (2006)
Dichelesthiidae	*Anthosoma crissum* (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)
Hatschekiidae	*Hatschekia pagrosomi* Yamaguti, 1939	AY627026	Huys et al. (2006)
	Hatschekia sp.	DQ538507	Huys et al. (2007)
Kroyeriidae	*Kroyeria longicauda* Cressey,	FJ447427	Dippenaar (2009)

	1970		
	Kroyeria sphyrnae Rangnekar, 1957	FJ447426	Dippenaar (2009)
Lernaeopodidae	*Clavella adunca* (Strøm, 1762)	AY627028	Huys et al. (2006)
	Clavellopsis sp.	HM545893	INSD
	Parabrachiella bispinosa (Nordmann, 1832)	AY627027	Huys et al. (2006)
	Lernaeopodidae sp.	KR048774	INSD
Nanaspididae	*Nanaspis tonsa* Humes & Cressey, 1959	AY627029	Huys et al. (2006)
Nicothoidae	*Choniosphaera maenadis* (Bloch & Gallien, 1933)	DQ538509	Huys et al. (2007)
	Rhizorhina ohtsukai Kakui, 2016	LC054034	Kakui (2016)
	Rhizorhina soyoae Kakui, 2016	LC054035	Kakui (2016)
	Sphaeronella uyenoi sp. nov.	LC720685	This study
Pandaridae	*Achtheinus oblongus* C. B. Wilson, 1908	FJ447452	Dippenaar (2009)
	Dinemoura latifolia (Steenstrup & Lütken, 1891)	DQ538501	Huys et al. (2007)
	Nesippus crypturus Heller, 1865	FJ447444	Dippenaar (2009)
	Nesippus orientalis Heller, 1865	FJ447445	Dippenaar (2009)
	Pandarus smithii Rathbun, 1886	DQ538502	Huys et al. (2007)
	Pannosus japonicus (Shiino, 1960)	FJ447450	Dippenaar (2009)
	Perissopus dentatus Steenstrup & Lütken, 1861	FJ447453	Dippenaar (2009)
	Phyllothyreus cornutus (Milne Edwards, 1840)	FJ447449	Dippenaar (2009)
	Pseudopandarus longus (Gnanamuthu, 1951)	FJ447451	Dippenaar (2009)
Pennellidae	*Haemobaphes pannosus* Kabata, 1979	KR048773	INSD
	Lernaeocera branchialis (Linnaeus, 1767)	AY627030	Huys et al. (2006)
Pontoeciellidae	*Pontoeciella abyssicola* (T. Scott, 1893)	AY627031	Huys et al. (2006)
Sphyriidae	*Lophoura edwardsi* Kölliker, 1853	MW242661	Galil et al. (2021)
	Paeon elongatus C. B. Wilson, 1932	FJ447460	Dippenaar (2009)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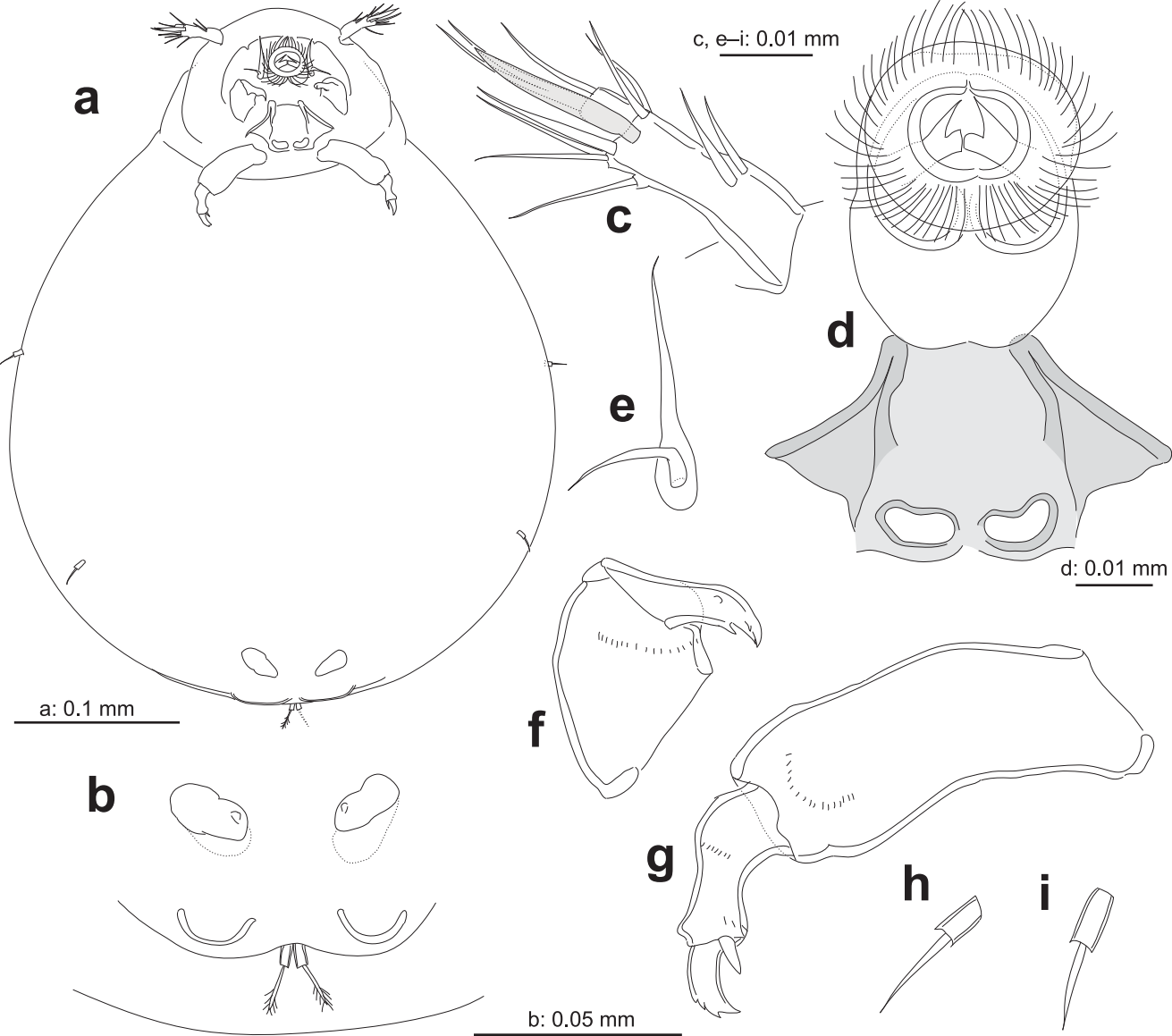
c: 0.1 mm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b
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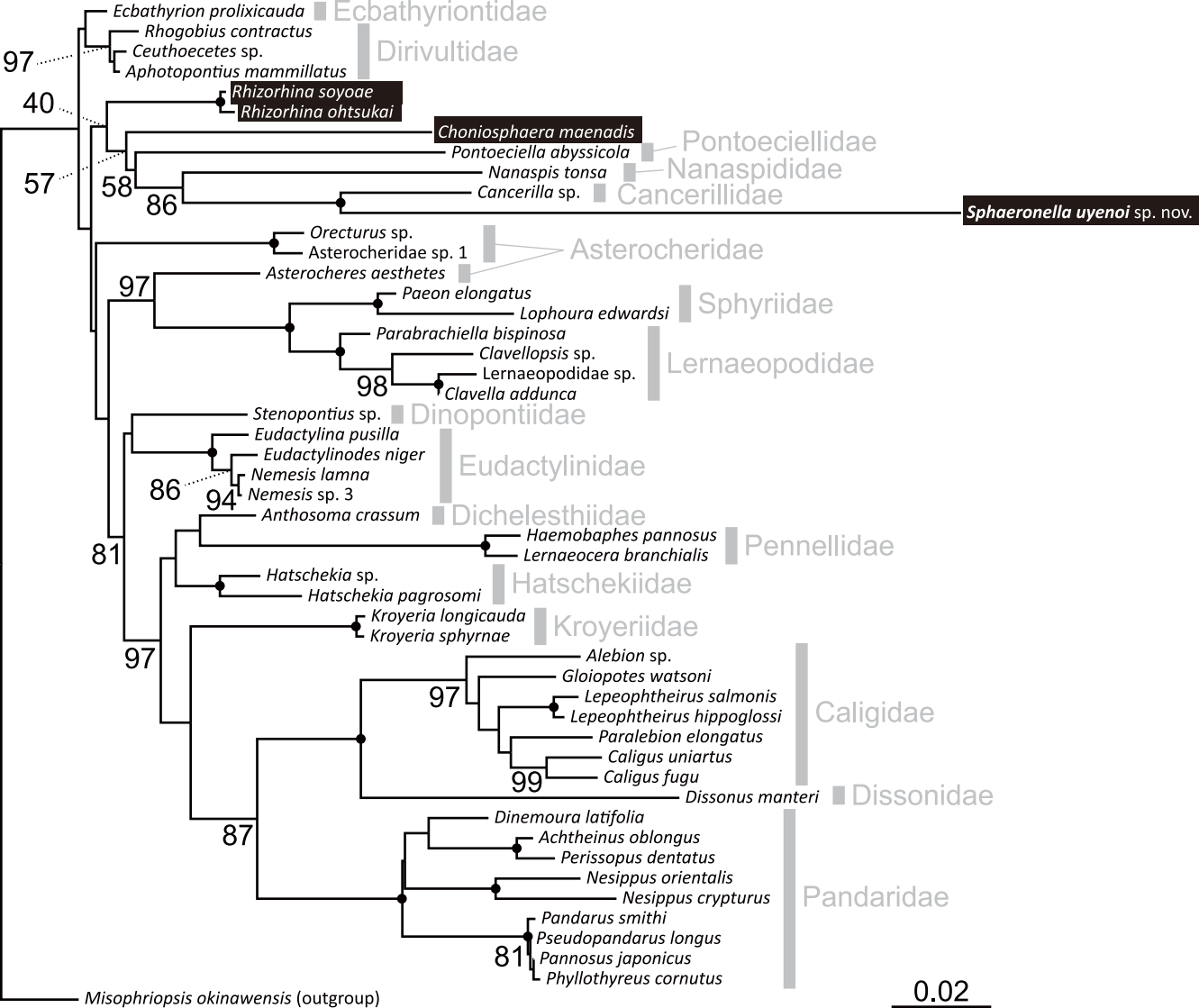
c
d
d: 0.01 mm
e
f
g
h
i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Rhogobius contractus
Ceuthoecetes sp.
Aphotopontius mammillatus
Ecbathyriontidae
Dirivultidae
97
40
Rhizorhina soyoae
Rhizorhina ohtsukai
Choniosphaera maenadis
Pontoeciella abyssicola
Pontoeciellidae
Nanaspis tonsa
Nanaspididae
57
58
Cancerilla sp.
Cancerillidae
86
Sphaeronella uyenoi sp. nov.
Orecturus sp.
Asterocheridae sp. 1
Asterocheridae
Asterocheres aesthetes
97
Paeon elongatus
Lophoura edwardsi
Sphyriidae
Parabrachiella bispinosa
Clavellopsis sp.
Lernaeopodidae sp.
Lernaeopodidae
98
Clavella addunca
Stenopontius sp.
Dinopontiidae
Eudactylina pusilla
Eudactylinodes niger
Eudactylinidae
86
Nemesis lamna
94
Nemesis sp. 3
Anthosoma crassum
Dichelesthiidae
Haemobaphes pannosus
Lernaeocera branchialis
Pennellidae
81
Hatschekia sp.
Hatschekia pagrosomi
Hatschekiidae
Kroyeria longicauda
Kroyeria sphyrnae
Kroyeriidae
97
Alebion sp.
Gloiopotes watsoni
97
Lepeophtheirus salmonis
Lepeophtheirus hippoglossi
Caligidae
Paralebion elongatus
Caligus uniartus
99
Caligus fugu
Dissonus manteri
Dissonidae
87
Dinemoura latifolia
Achtheinus oblongus
Perissopus dentatus
Nesippus orientalis
Nesippus crypturus
Pandaridae
Pandarus smithi
Pseudopandarus longus
81
Pannosus japonicus
Phyllothyreus cornutus
Misophriopsis okinawensis (outgroup)
0.02