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Rhizocephalan Barnacle *Briarosaccus hoegi* sp. nov. – a Parasite of the Stone Crab *Hapalogaster dentata* (De Haan, 1849) from Peter the Great Bay (Sea of Japan)

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Molecular and morphological methods are used to describe the rhizocephalan *Briarosaccus hoegi* sp. nov. from Russian waters of the Sea of Japan, parasitizing the stone crab *Hapalogaster dentata* (De Haan, 1849). *Briarosaccus hoegi* sp. nov. has minor differences by gross morphology from the closely related species *B. tenellus,* parasitizing *H. mertensii* in British Columbia and Alaska. *Briarosaccus hoegi* sp. nov. and *B. tenellus* are identified as distinct species by genetic markers. These two species have different hosts and different areas of distribution. Moreover, nauplii of *Briarossacus hoegi* sp. nov. have naupliar eyes not present in *B. tenellus* larvae. The presence/absence of larval eyes may be a clear character separating the two species. The prevalence of *Briaroaccus hoegi* sp. nov. on *H. dentata* in Peter the Great Bay is about 6%.

Key words: Rhizocephala, *Briarosaccus*, Lithodidae, DNA analysis, Histology, SEM, Peter the Great Bay

BACKGROUND

The degree of infestation of decapods by parasitic barnacles (Cirripedia: Rhizocephala) in Peter the Great Bay of the Sea of Japan is quite high. To date, 9 species of rhizocephalans belonging to 6 genera were known – *Peltogaster*, *Peltogasterella*, *Lernaeodiscus*, *Polyascus*, *Sacculina* and *Parasacculina* (Korn et al. 2004 2020a b 2021; Golubinskaya et al. 2021a b 2024). This is the first report of a rhizocephalan parasite of the genus *Briarosaccus* Boschma, 1930 (Rhizocephala: Peltogastridae) in the studied region.

Rhizocephalan barnacles of the genus *Briarosaccus* have been reported to parasitize a wide range of king crab species of the family Lithodidae. For a long time, all of these parasites have been assigned to a single species, *Briarosaccus callosus* Boschma, 1930, which had been assumed to have a global distribution. Recently, it was shown that *Briarosaccus* specimens parasitizing *Lithodes aequispinus* Benedict, 1895, *Paralithodes camtschaticus* (Tilesius, 1815) and *P. platypus* (Brandt, 1851) from Southeastern Alaska are morphologically distinct from *B. callosus*, and represent two separate species. The two new species, *Briarosaccus auratum* Noever, Olson & Glenner, 2016 and *B. regalis* Noever, Olson & Glenner, 2016, are cryptic by morphological characters and identified as distinct species by molecular markers. They occur sympatrically,

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with *B. auratum* only found on *L. aequispinus*, but *B. regalis* on both *Paralithodes* hosts (Noever et al. 2016).

Hapalogaster mertensii JF Brandt, 1950 (Anomura: Lithodidae) is not a commercially exploited crab. This species occurs from the intertidal to 55 m depth along the Pacific coast of North America from the Aleutian Islands to British Columbia (Walossek et al. 1996; GBIF Secretariat 2023). The rhizocephalan barnacle *Briarosaccus tenellus* Boschma, 1970 was described as a parasite of *H. mertensii* from Victoria (British Columbia) (Boschma 1970). Later, the larval development of *B. tenellus* from this host was also described (Walossek et al. 1996).

Hapalogaster dentata (De Haan, 1849), is widely distributed in Peter the Great Bay (Marin 2013), in Japan from Hokkaido to Kyushu, and also in the coastal waters of the Korean Peninsula (Goshima et al. 1995; GBIF Secretariat 2023). This species inhabits intertidal and subtidal cobble rocky shores. In Peter the Great Bay, *H. dentata* was infested by a rhizocephalan morphologically similar to *B. tenellus*. The aim of this study was to identify the parasite and host from this region using morphological and molecular methods, to compare it with the closely related *B. tenellus*, and to describe this rhizocephalan if it would turn out to be a new *Briarosaccus* species.

MATERIALS AND METHODS

Sampling

Specimens of the stone crab *Hapalogaster dentata* infested by the rhizocephalan were collected by SCUBA divers in Zhitkova and Sobol Coves, and also near Cape Vyatlina (Ussuriysky Bay, Peter the Great Bay, Sea of Japan), at a depth of 2–3 m (Fig. 1). The type material was fixed in 96% ethanol. The holotype and two paratypes of a new parasite on the host, and also two voucher specimens after DNA extraction were deposited at the Museum of the National Scientific Center of Marine Biology (MIMB), Vladivostok, Russia.

Since the description of *Briarosaccus tenellus* by Boschma (1970) is only superficial, we used six additional specimens of *Hapalogaster mertensii* infested by *B. tenellus* collected during low tide under rocks in Sitka (Alaska) for comparison of the two rhizocephalan species. One specimen was deposited at MIMB.

Molecular investigation of the externa

Samples fixed in 96% ethanol were subjected to molecular analysis. Both parasite and host sequences were obtained. Total genomic DNA was extracted from the externae and from the tissue of chelae using a chelating resin Chelex 100 (Bio-Rad) according to the protocol described by HwangBo et al. (2010). For the parasite, fragments of two mitochondrial – cytochrome *c* oxidase subunit I (*COI* mtDNA), large subunit ribosomal RNA (16S rDNA), and nuclear small-subunit ribosomal RNA (18S rDNA) gene markers were amplified and sequenced. For the host, fragments of *COI* mtDNA gene were sequenced.

PCR amplification was performed using a Tersus polimerase (Evrogen, Russia) and cycling parameters according to the manufacturer's protocol in 25 µl reactions. Primers and their annealing temperatures are shown in table 1. PCR products were checked for successful amplification and size conformity by electrophoresis in a 1.5% agarose gel using commercial DNA size standards. Amplification products were applied as templates for sequencing, using the same primers as the PCR and BrilliantDye ™Terminator Cycle Sequencing kit v3.1 (NimaGen, Nijmegen, The Netherlands) according to the manufacturer's protocol. Sequencing reaction products were purified by ethanol precipitation and analyzed on an ABI-3500 Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific Inc., Foster City, CA, USA). Sequences were verified by forward and reverse comparisons. Sequence editing and contig assembly were performed using SeqScape 2.5 (Applied Biosystems). Sections corresponding to the primer sequences at the 3' and 5' ends of both aligned gene sequences were eliminated before initiating the analyses. A BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 25 September 2023) was used to check new sequences against the database for possible contamination and sequence artifacts. The resulting sequences were submitted to the National Center for Biotechnology Information (GenBank, NCBI, https://www.ncbi.nlm.nih.gov/, accessed on 25 Sept. 2023) nucleotide database with the following accession numbers for *Briarosaccus* sp.: *COI*, OR466125–OR466127; 16S, OR469039–OR469041; 18S, OR469042–OR469044 (Table S1). The accession numbers for *Hapalogaster dentata*: *COI*, OR466128–OR466129.

To compare the obtained data and clarify the taxonomic position of *Briarosaccus* sp., the diversity of Peltogastridae family by the considered gene markers was used. Members of the family Peltogasterellidae were selected as an outgroup taxon for reconstructing the phylogenetic relationships. Sequences were downloaded from GenBank and compiled into a single file into MEGA v.11.0.8 (Tamura et al. 2021). We used only published data for sequence comparisons. Taxa included in the molecular analyses and their GenBank accession numbers are provided in table

S1. Sequences were aligned using MUSCLE (Edgar 2004) implemented in MEGA v.11.0.8 (Tamura et al. 2021). The quality of alignment was checked visually. Analyses were performed on the aligned DNA sequences. Two different methods for determining phylogenies were performed in this study: Bayesian inference (BI) and Maximum likelihood (ML). The best-fit model of nucleotide substitution for the data sets were identified using ModelFinder (Kalyaanamoorthy et al. 2017) on the IQ-TREE webserver (http://www.iqtree.org/, accessed on 25 September 2023) (Trifinopoulos et al. 2016). TIM+F+I+G4 model was selected as best for *COI*, TPM3u+F+I+G4 for 16S, and TNe+I+G4 for 18S. For Bayesian analysis, we used closest appropriate models. Bayesian trees were constructed using MrBayes 3.2.7a (Ronquist et al. 2012) implemented in CIPRES Science Gateway (http://www.phylo.org/, accessed on 25 September 2023) (Miller et al. 2010) with the following parameters: 10,000,000 generations, with four parallel chains and sample frequencies set to

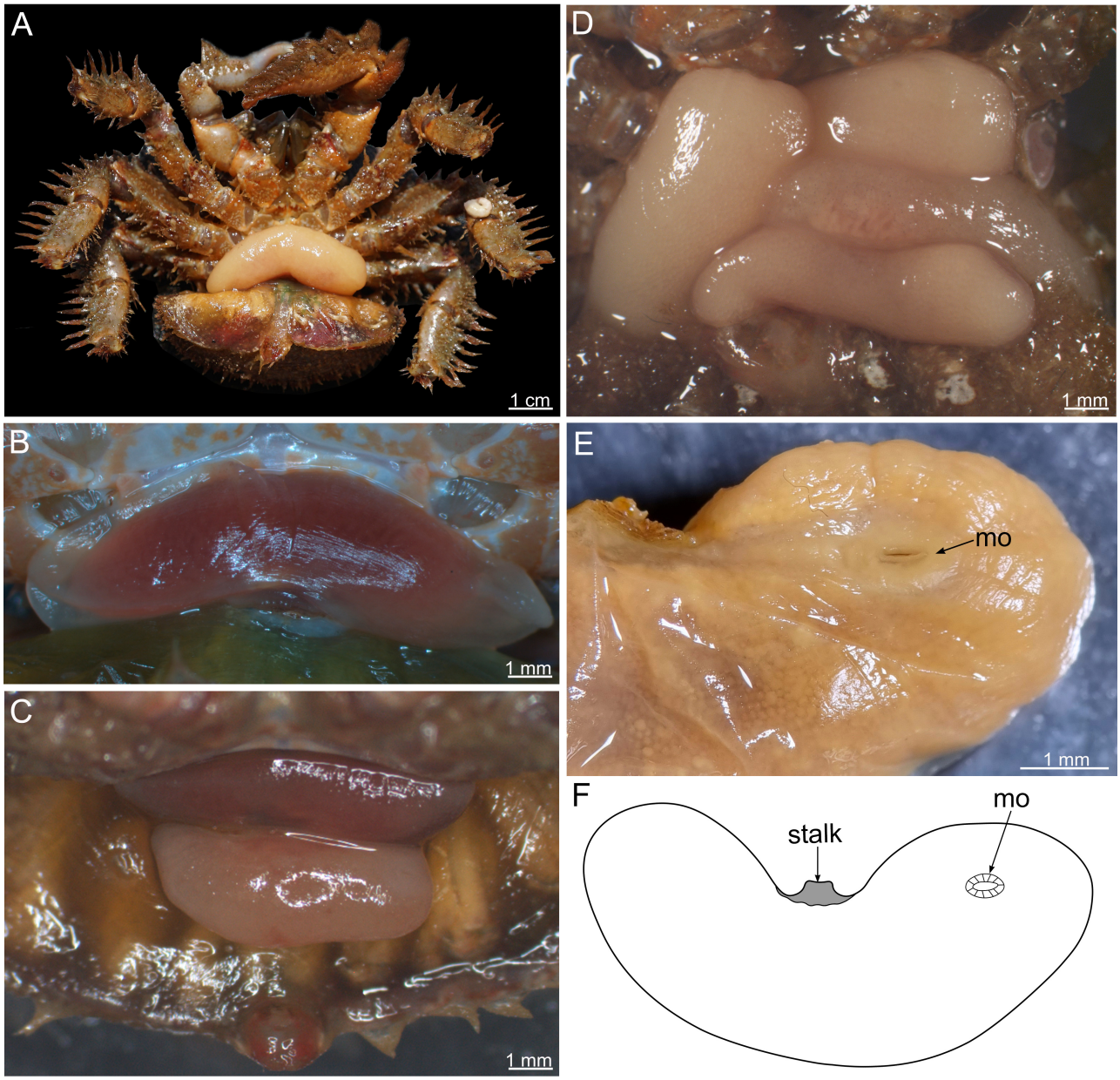

Fig. 1. Host crab, *Hapalogaster dentata*, infested by *Briarosaccus hoegi* sp. nov., with solitary mature externa (A), with solitary immature externa (B), with two externae (C), with four externae (D); anterior part of the externa with mantle opening (E); body outlines of the right side of the externa (F). mo, mantle opening.

500 in two separate runs. Based on the convergence of likelihood scores, 25% of the sampled trees were discarded as burn-in. The maximum likelihood tree was built using the online software IQ-TREE (Nguyen et al. 2015) on the IQ-TREE webserver (http://www.iqtree.org/) (Trifinopoulos et al. 2016). The tree topology was evaluated by ultrafast bootstrap approximation (UFBoot) (Minh et al. 2013; Hoang et al. 2018) for 1000 replications. Finally, the sequences of *COI* and 16S genes were combined using SequenceMatrix v.1.7.8 (Vaidya et al. 2011), and trees were constructed using the Bayesian (BI) method. According to the best-suggested scheme, the final supermatrix was divided into two datasets through the application of appropriate parameters of selected models. All results trees were visualized using FigTree v. 1.4.4 (Rambaut 2012). The pairwise genetic distances were calculated using MEGA v.11.0.8 (Tamura et al. 2021). Intra- and interspecific nucleotide variability of the analyzed species was based on the Kimura 2-parameter model (K2P) (Kimura 1980) for *COI* sequences, and uncorrected genetic distances were calculated for 16S and 18S gene sequences.

Morphological investigation of the externa

Infested specimens of *Hapalogaster dentata* were photographed alive. Sex of *H. dentata* and *H. mertensii* was identified based on the location of gonopores on the coxae of the third pereopods in females (McLaughlin 1980). The carapace width of both host crabs (including lateral spines) was measured. Rhizocephalan externae were removed from host crabs, photographed and drawn using a Zeiss Discovery v. 12 stereomicroscope. The length (distance between anterior and posterior ends) and height at the level of the stalk were measured. All measurements of hosts and parasites were made to the nearest 0.1 mm under an MBS-10 stereomicroscope.

The investigation of gross morphology was made under an MBS-10 stereomicroscope. Two externae of a new species (immature and mature) were detached from the host crabs and fixed in Bouin's solution, dehydrated through a gradient ethanol-xylene series and embedded in paraffin. Transverse sections, 6 µm thick, were stained with Ehrlich hematoxylin, examined with a Zeiss Axio Imager Z.2 light microscope furnished with a digital camera.

For SEM, the mantle cuticle of externae of *Briarosaccus* sp. and *B. tenellus*, was dehydrated in an alcohol series and acetone, critical point dried in CO_2, and sputtered with chromium. It was observed with a Zeiss Sigma 300 VP microscope.

The nauplii were obtained from mature externa of a new species with embryos at the last stage of development. The larvae photos were made with a Zeiss Axio Imager Z.2 light microscope furnished with a digital camera.

All morphological terminology and orientation of organs follows Øksnebjerg (2000).

RESULTS

Molecular analysis

Because the topology of phylogenetic trees based on *COI* and 16S rDNA sequences were nearly identical, we presented only one tree based on combined sequences of these two genes. Supplementary materials contain trees based on single gene sequences and trees based on combined data sets including all available sequences from NCBI involved in the current analysis. All phylogenetic trees demonstrated that the genus *Briarosaccus* was not monophyletic. This genus formed two separate clades. One of them included *B. regalis* and *B. auratum* which formed a single clade with *Peltogaster postica* Yoshida & Osawa, 2011 and

Table 1. Primers and their annealing temperatures used for PCR

Gene (Marker)	Primers	Sequences (5'–3')	Annealing temperature (°C)	Reference
COI	CO1F-ALT / CO1R-ALT	ACAAATCAYAARGAYATYGG / TTCAGGRTGNCCRAARAAYCA	47	Chen et al. 2011
16S	16SL3-Ven / 16SH1-Ven	GCAAYGAGAGTTGTRCTAAGGTAGC / ATAATCCAACATCGAGGTCGCAAA	52	Kappner and Bieler 2006
18S	1F / 5R	TACCTGGTTGATCCTGCCAGTAG / CTTGGCAAATGCTTTCGC	52	Giribet et al. 1996
	3F / 18Sbi	GTTCGATTCCGGAGAGGGA / CTAGAGTCTCGTTCGTTATCGG	52	Giribet et al. 1996 / Whiting et al. 1997
	a2.0 / 9R	ATGGTTGCAAAGCTGAAAC / GATCCTTCCGCAGGTTCACCTAC	52	Whiting et al. 1997 / Giribet et al. 1996

Peltogaster paguri Rathke, 1842 (BI = 1) (this clade included also *P. reticulata* Shiino, 1943 on *COI* tree) (Figs. 2, S1). *Briarosaccus tenellus* and *Briarosaccus hoegi* sp. nov. formed another clade with *P. lineata* Shiino, 1943 as a sister taxon (BI = 1).

The mean genetic distances between these two groups of *Briarosacus* was 31.1 ± 0.27% (mean ± standard deviation) for *COI* gene (intra- and interspecific distances for all included in current analysis taxa are showed in Table S2). Genetic distances between *B. regalis* and *B. auratum* was 11.5 ± 1.5%, between *B. tenellus* and *Briarosaccus hoegi* sp. nov. – 14.6 ± 0.2% (Table 2).

The mean genetic distances between two groups of *Briarosacus* was 20.1 ± 2.6% (mean ± standard deviation) for 16S gene (Table S3). Genetic distance between *B. regalis* and *B. auratum* was 2.9 ± 1.0%, and between *B. tenellus* and *Briarosaccus hoegi* sp. nov. was 4.4 ± 1.2% (Table 3).

The tree based on 18S rDNA sequences showed a similar topology with clustering of *Briarosaccus* species. But on this tree, a single clade with *B. auratum* included the species *Tortugaster boschmai* (Brinkman, 1936), *Septosaccus rodriguezii* (Fraisse, 1878), *Galatheascus striatus* Boschma, 1929, and *Peltogaster curvata* Kossmann, 1874 which were absent in the other

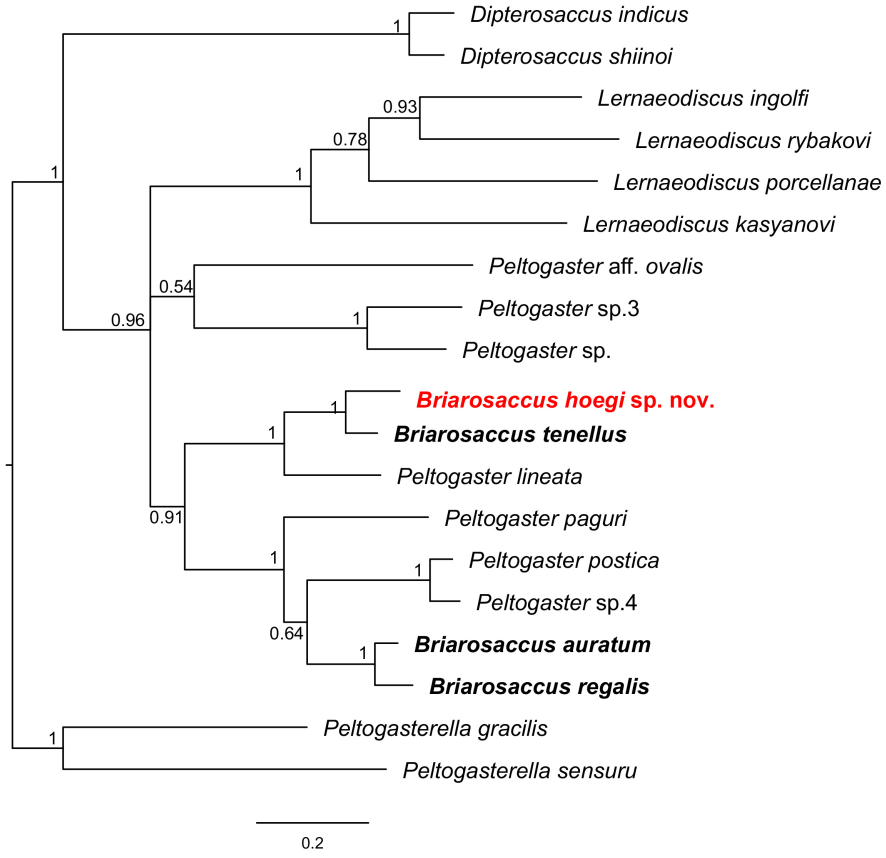

Fig. 2. Bayesian phylogenetic tree of the family Peltogastridae for combined molecular data (concatenated sequences from *COI* and 16S rRNA gene fragments). Numbers above or under the branches are Bayesian posterior probabilities.

Table 2. *COI* K2P genetic distances of the genus *Briarosaccus*

No	Species	between species (above the diagonal is the SD) 1	2	3	4	within species (mean ± SD)
1	*Briarosaccus regalis*		0.015	0.033	0.030	0.003 ± 0.001
2	*Briarosaccus auratum*	0.115		0.031	0.029	0.002 ± 0.001
3	*Briarosaccus tenellus*	0.328	0.323		0.020	-
4	*Briarosaccus hoegi* sp. nov.	0.299	0.311	0.146		0

analyses (Figs. 3, S6; Table S4).

Thus, *Briarosaccus hoegi* sp. nov. is identified as a distinct species by the genetic markers.

TAXONOMY

Infraclass Rhizocephala Müller, 1862
Family Peltogastridae Lilljeborg, 1861; amended by Høeg et al. (2020)
Family: Peltogastridae Lilljeborg, 1861
Genus: *Briarosaccus* Boschma, 1930

***Briarosaccus hoegi* sp. nov.**

(Figs. 4–9)

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Etymology: The new species is named in honor of Jens Thorvald Høeg, professor of the Department of Biology (University of Copenhagen, Denmark), who has spent most of his professional life investigating thoracican and rhizocephalan barnacles.

Material examined: *Holotype*: One specimen (21.4/9.0 mm, with embryos), on *Hapalogaster dentata*

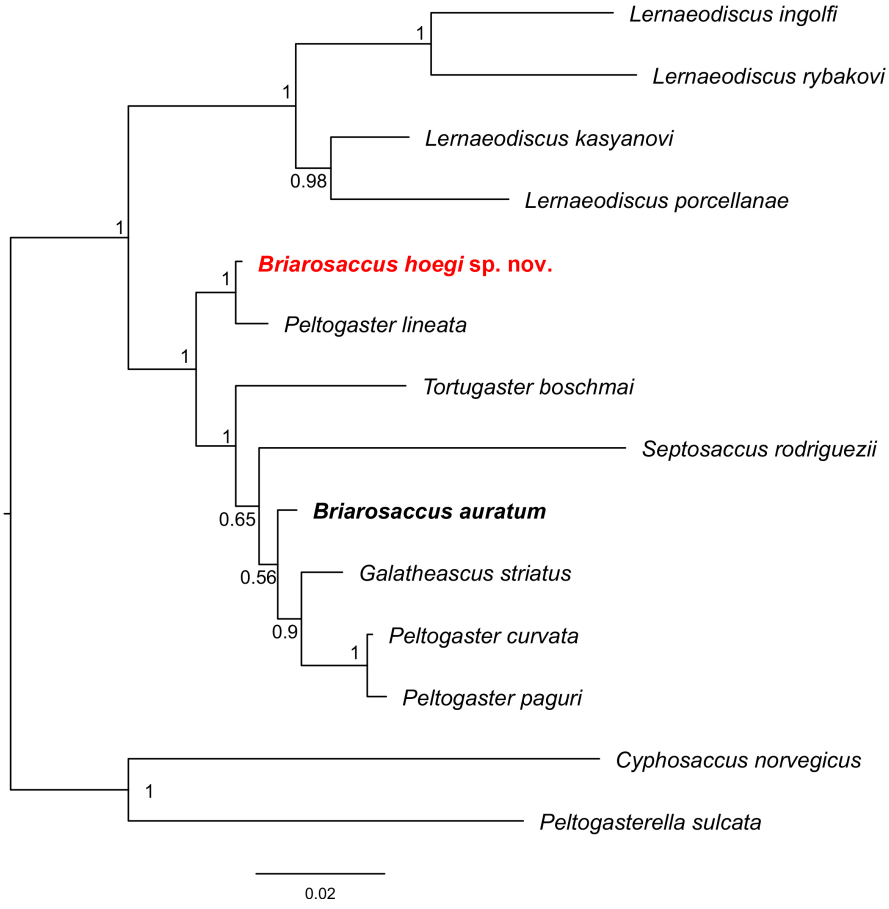

Fig. 3. Bayesian phylogenetic tree of the family Peltogastridae for 18S gene fragments. Numbers above or under the branches are Bayesian posterior probabilities.

Table 3. 16S rDNA uncorrected genetic distances of the genus *Briarosaccus*

No	Species	between species (above the diagonal is the SD) 1	2	3	4	within species (mean ± SD)
1	*Briarosaccus auratum*		0.026	0.026	0.010	0
2	*Briarosaccus hoegi* sp. nov.	0.218		0.012	0.024	0.005 ± 0.004
3	*Briarosaccus tenellus*	0.216	0.044		0.024	-
4	*Briarosaccus regalis*	0.029	0.193	0.192		0

(female, 19.2 mm width), depth 2–3 m, Zhitkova Cove (43°01'07.9"N, 131°55'49.1"E), 21.05.2023 (catalogue number 46887, MIMB).

Paratypes: One specimen (10.0/3.5 mm, without embryos), on *H. dentata* (female, 13.0 mm width), depth 2–3 m, Sobol Cove, 19.07.2023 (catalogue number 46888, MIMB); Two specimens (12.3/5.2 mm, without embryos and 11.0/6.2 mm, with embryos), on *H. dentata* (male, 19.0 mm width), depth 2–3 m, near the Cape Vyatlina, 3.06.2020 (catalogue number 46889, MIMB).

Voucher specimens: Two specimens (20.4/8.9 mm, with embryos and 18.1/6.6 mm, with embryos), on *H. dentata* (female, 18.1 mm width), depth 2–3 m, Zhitkova Cove, 14.05.2020 (catalogue number 46890, MIMB); One specimen (12.1/5.4 mm, with embryos), on *H. dentata* (female, 11.1 mm width), depth 2–3 m, Zhitkova Cove, 14.05.2020 (catalogue number 46891, MIMB).

Specimens for histology: One specimen (15.9/10.0 mm, with embryos), on *H. dentata* (male, 21.0 mm width), depth 2–3 m, near the Cape Vyatlina, 3.06.2020; One specimen (7.1/2.0 mm, without embryos), on *H. dentata* (male, 17.7 mm width), depth 2–3 m, near the Cape Vyatlina, 22.11.2022.

Specimens for SEM: One specimen (13/4.6 mm, without embryos), on *H. dentata* (female, 18.5 mm width), depth 2–3 m, Sobol Cove, 16.03.2023; One specimen (12.0/4.7 mm, without embryos), on *H. dentata* (female, 16.0 mm width), depth 2–3 m, Sobol Cove, 15.02.2023; One specimen (22.0/10.5 mm, with embryos), on *H. dentata* (female, 18.2 mm width), depth 2–3 m, Zhitkova Cove, 21.05.2020; One specimen (20.4/7.8 mm, with embryos), on *H. dentata* (female, 19.5 mm width), depth 2–3 m, Zhitkova Cove, 21.05.2020; One specimen (18.0/7.5 mm, with embryos), on *H. dentata* (male, 16.5 mm width), depth 2–3 m, Zhitkova Cove, 21.05.2020; One specimen (20.1/7.8 mm, with embryos), on *H. dentata* (female, 18.6 mm width), depth 2–3 m, Zhitkova Cove, 21.05.2020.

Briarosaccus tenellus on Hapalogaster mertensii: One specimen (11.5/4.3 mm, without embryos), on *H. mertensii* (female, 12.5 mm width), intertidal, Sitka (Alaska), 07.2012; One specimen (15.5/4.5 mm, without embryos), on *H. mertensii* (female, 17.2 mm width), intertidal, Sitka (Alaska), 07.2012; One specimen (8.5/2.5 mm, without embryos), on *H. mertensii* (female, 13.0 mm width), One specimen (10.6/2.8 mm, without embryos), on *H. mertensii* (female, 13.5 mm width), intertidal, Sitka (Alaska), 07.2012 (catalogue number 47652, MIMB); One specimen (10.3/3.8 mm, with embryos), on *H. mertensii* (female, 11.1 mm width), intertidal, Sitka (Alaska), 07.2012; One specimen (11.5/4.0 mm, with embryos), on *H. mertensii* (male, 16.2 mm width), intertidal, Sitka (Alaska), 07.2012.

Type locality: Zhitkova Cove (43°01'07.9"N, 131°55'49.1"E, Ussuriysky Bay, Peter the Great Bay, Sea of Japan).

Host: *Briarosaccus hoegi* sp. nov. was found on the crab *Hapalogaster dentata* (De Haan, 1849) (Anomura: Lithodidae). *H. dentata* differs from the related species *H. mertensii* by the seven spines along the lateral margin of the carapace and by the tubercles on the first pereopods (Makarov 1938). The morphological species identification of *H. dentata* was confirmed by molecular data (Fig. S8; Table S5).

The carapace width of the infested crabs ranged from 10.1 to 19.5 mm. The prevalence of infestation in the crab population reached 6.3%. Most crabs were singly infested, while some specimens of *H. dentata* carried two to four externae (Fig. 1A–D).

Distribution and bathymetrical range: We sampled the host crab *Hapalogaster dentata* in Peter the Great Bay at a depth of about 2–3 m. This species is also widely distributed in coastal intertidal and subtidal waters of Japan, from Hokkaido to Kyushu, and the Korean Peninsula (Goshima et al. 1995; Marin 2013). We have no data on the true geographical distribution and depth range of the new parasite.

Externa morphology: The externa of *Briarosaccus hoegi* sp. nov. is attached to the basal part of the soft ventral side of the host abdomen via a short stalk, which connects the externa with the internal trophic root system. Its long axis is at right angle to that of the host (Fig. 1). The externa varies from 5.3 to 22 mm in length and from 1.0 to 10.5 mm in height. The externa is elongated, cylindrical and slightly curved with the ventral outline being convex, and the dorsal outline concave. Anterior part is unilobed, slightly thicker than the posterior one and oriented to the left side of the host (Fig. 1A, E, F). Posterior end of the externa is rounded. The stalk is near the central part of the dorsal side. The chitinous shield around the stalk is fusiform, has growth rings, and covers from 1/4 to 1/3 of the externa. The mantle opening is placed in the anterior part on the right side of the externa facing the host. It is not elevated but slit-like and surrounded by lips (Figs. 1E, 8D).

The overall shape and color of *Briarosaccus hoegi* sp. nov. varies due to the stage of the reproductive cycle. The immature externa without embryos in the mantle cavity is reddish, the color of mature externa with embryos is white, yellow, pale, or light brown (before larval hatching) (Fig. 1A–D). The interna roots are green.

The mantle is thick – from 84 to 117 μm in a immature rhizocephalan and from 157 to 212 μm in a mature specimen (Fig. 4A, B). The muscle sphincter

surrounding the mantle opening is visible (Fig. 4C, D). A visceral sac extends dorsally along most of the externa (Fig. 4A). The ovary is composed of large numbers of developing oocytes, arranged in tubes (Fig. 5). The ventral part of the mantle cavity is densely filled with developing embryos. From mid-May to mid-July, all investigated externae were ovigerous. The colleteric glands represent short folded flattened tubes about of 0.5 mm in length located inside the visceral sac on the lateral sides of the ovary inside the shield level (Fig. 5C, D). The colleteric glands expand in the middle part, with a larger diameter ranging from 200 to 350 μm in immature externae and up to 800 μm in mature ones (Fig. 5C, D). Before opening into the mantle cavity, the colleteric gland bends and two tubes with a diameter of 100–150 μm are observed on the section.

Paired receptacles represent cylindrical tubes 1.8–3.5 mm in length located in the dorsal part of the visceral sac and parallel to the long axis of the externa (Fig. 6). Receptacles begin and end inside the shield level. Anterior tops of receptacles are blind. Anterior parts with a diameter of about 150 μm are narrow, slightly flattened and more or less straight, central parts with a diameter from 200 to 270 μm are slightly twisted

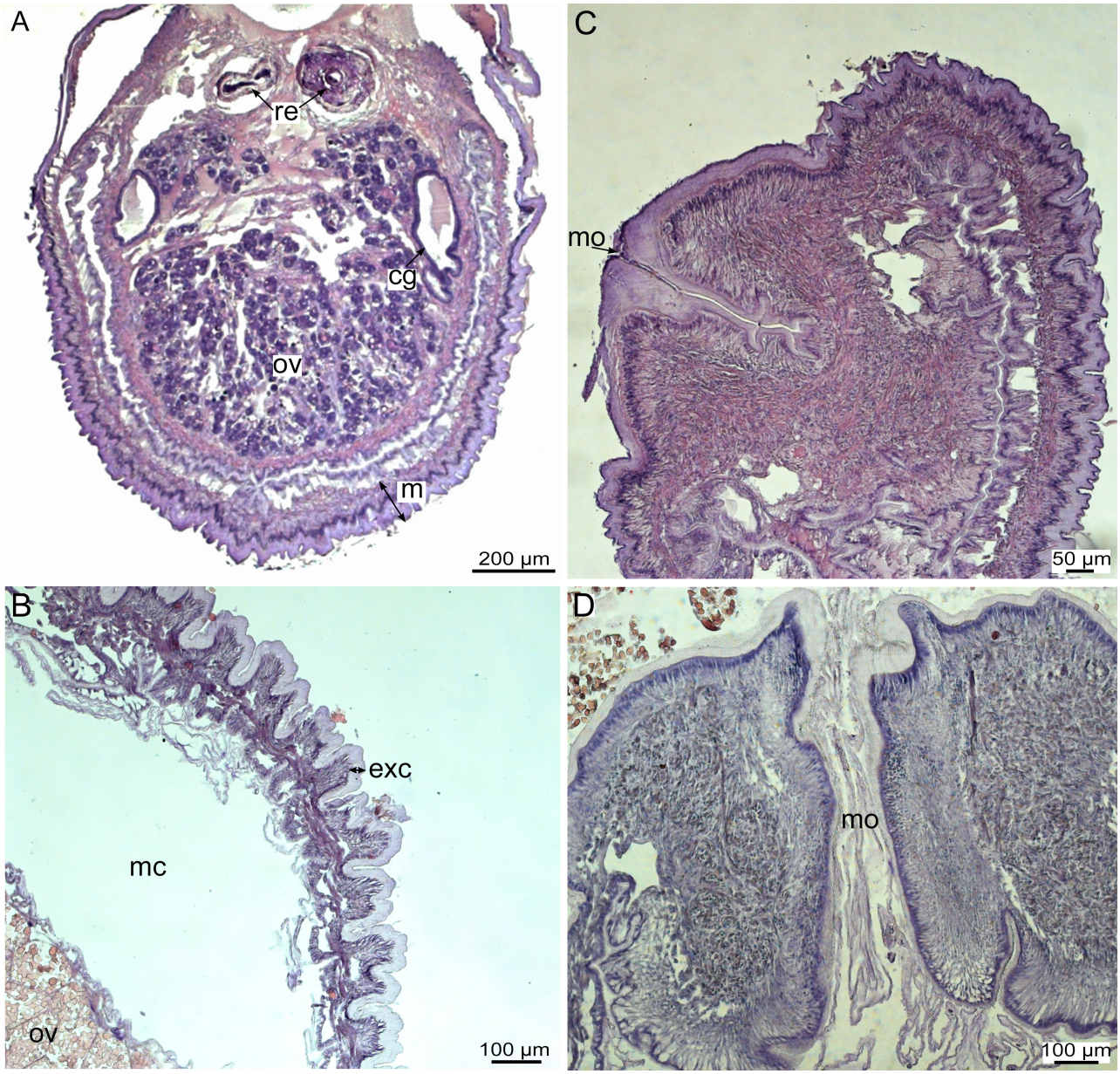

Fig. 4. Histology of the externa of *Briarosaccus hoegi* sp. nov. Transverse section of the whole externa (A); mantle (B); mantle opening (C, D). cg, colleteric gland; exc, external cuticle with "collagen fibers"; mc, mantle cavity; mo, mantle opening; ov, ovary; re, receptacles.

(Fig. 7A, D). Receptacles gradually pass into receptacle ducts with a diameter of 80–100 µm in immature externae and up to 200 µm in mature ones (Fig. 7B, C, E, F). The receptacle ducts are coiled in immature externae and almost straight in mature ones. They open on the lateral surfaces of the visceral sac.

Mantle cuticle: The width of *Briarosaccus hoegi* sp. nov. external (outer) cuticle with "collagen fibers" is 25–50 µm. It is smooth, without papillae or excrescences (Fig. 4B) and covered with longitudinal grooves (Fig. 8B). A dorsal strip of transversal grooves from the mantle opening to the stalk is visible (Fig. 8A). In SEM, this strip and area around the mantle opening is cellular (Fig. 8C). The mantle opening is densely covered with numerous spines (hairs) of 8–10 µm in length (Fig. 8D–F). Moreover, rounded depressions are rarely scattered on the outer cuticular surface (Fig. 8G).

The internal (inner) cuticle is wrinkled and covered with sparse hairs (finger-like processes) of 1.5–3.0 µm in length which may be united in groups with a common base (Fig. 8H). Retinacula of 10–20 µm in length, densely barbed, single or united into groups of 2–4 are rarely found (Fig. 8I, J).

Larvae: Nauplii of *Briarosaccus hoegi* sp. nov. have a reticulated collar and also a distinct naupliar eye demonstrating positive phototaxis (Fig. 9).

Briarosaccus tenellus from Sitka (Alaska)

The carapace width of the infested *Hapalogaster mertensii* ranges from 11.1 to 17.2 mm. All crabs are singly infested. The externa of *Briarosaccus tenellus*

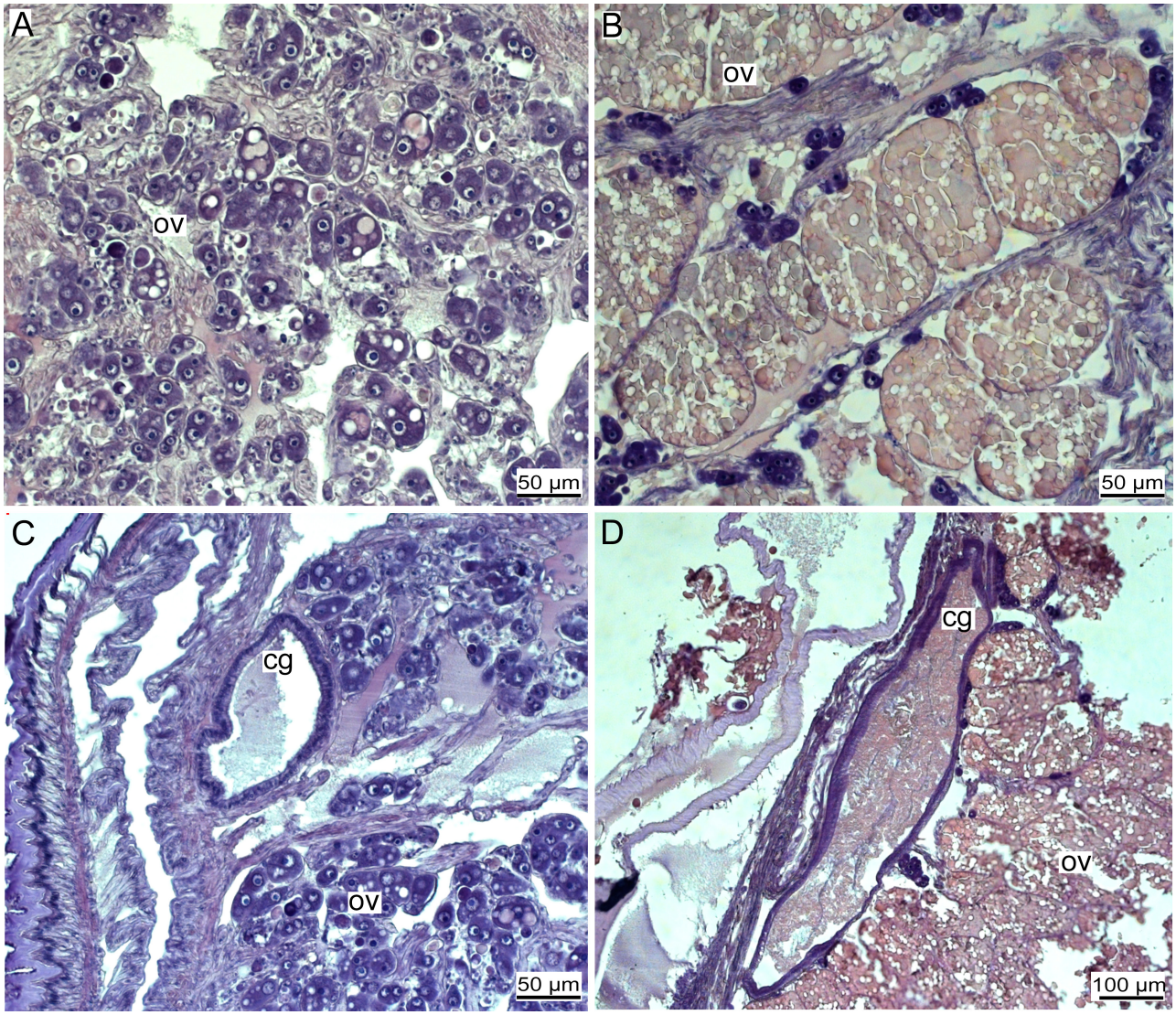

Fig. 5. Female reproductive organs of *Briarosaccus hoegi* sp. nov., transverse section. Ovary of the immature specimen (A); ovary of the mature specimen (B); colleteric gland of the immature externa (C); colleteric gland of the mature externa (D). cg, colleteric gland; ov, ovary.

varies from 8.5 to 15.5 mm in length and from 2.5 to 4.5 mm in height. It is attached to the basal part of the soft ventral side of the host abdomen via a short stalk (Fig. 10A). Its long axis is at right angles to that of the host. The mature externa is elongated, cylindrical and slightly curved with the ventral outline being convex, and the dorsal outline concave. Anterior part of the externa is slightly bilobed and somewhat thicker than posterior one (Fig. 10B). It is oriented to the left side of the host. The opposite, posterior end of the externa is rounded. The stalk occurs near the central part of the dorsal side. The chitinous shield around the stalk is fusiform, has growth rings, and covers from 1/4 to 1/3 of the externa. The mantle opening is placed in the anterior part on the right side of the externa facing the host. It is not elevated, slit-like and surrounded by lips (Fig. 10B, G).

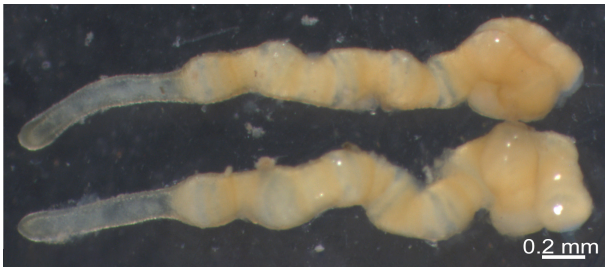

Fig. 6. Isolated receptacles of *Briarosaccus hoegi* sp. nov., light microscopy.

The external cuticle is smooth, without papillae or excrescences (Fig. 10E). It is covered with longitudinal grooves. A dorsal strip of transversal grooves from the mantle opening to the stalk is visible (Fig. 10D). In SEM, this strip and area around the mantle opening is cellular (Fig. 10F). The mantle opening is densely covered with numerous spines of 8–10 μm in length (Fig. 10H).

The internal cuticle is wrinkled and covered with sparse hairs of 4–8 μm in length which may be united in groups with a common base (Fig. 10I). Retinacula of 10–20 μm in length, densely barbed, single or united into groups of 2–4 are rarely found (Fig. 10J).

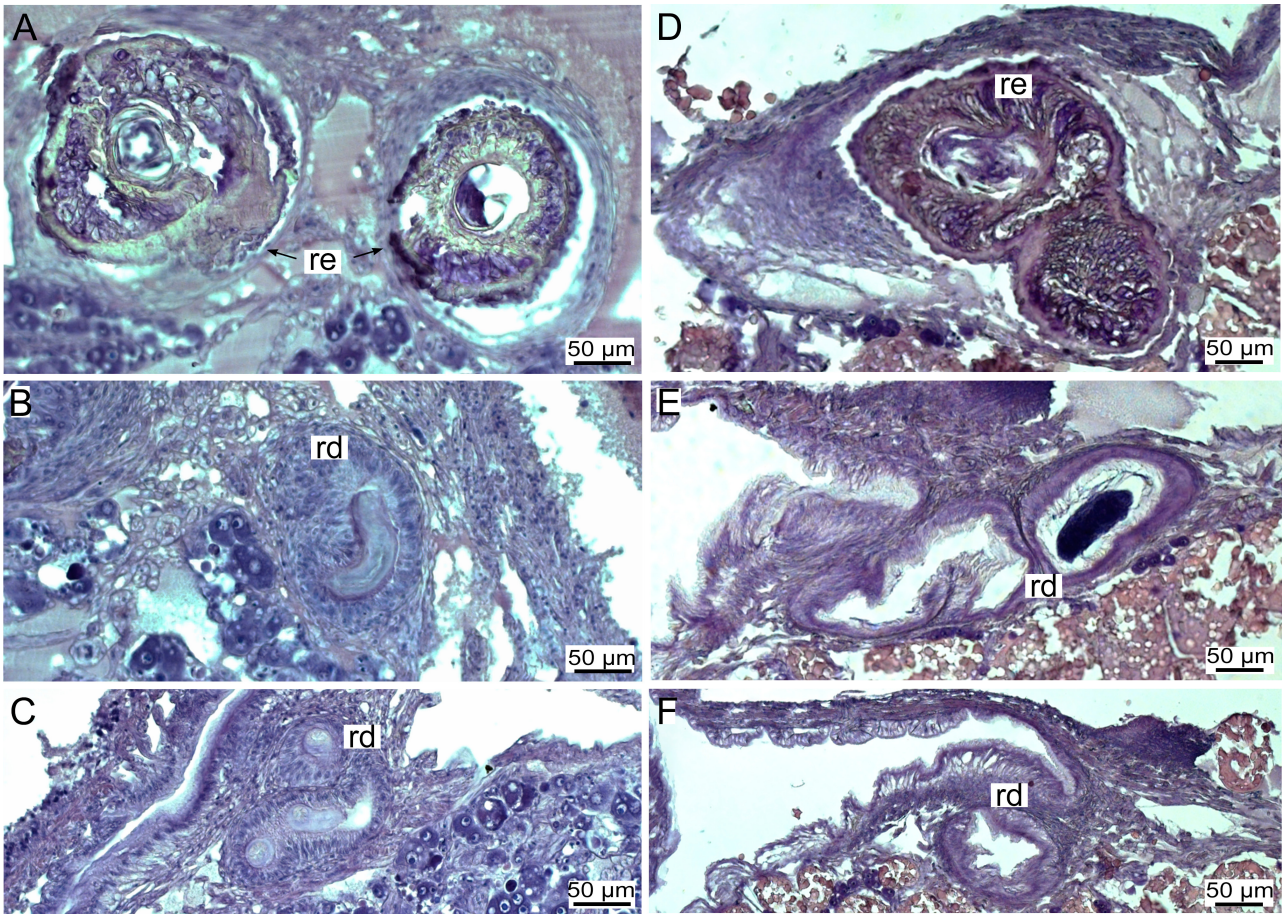

Fig. 7. Male reproductive organs of *Briarosaccus hoegi* sp. nov., transverse section. Receptacles (A, D); receptacle ducts (B, C, E, F). rd, receptacle duct; re, receptacles.

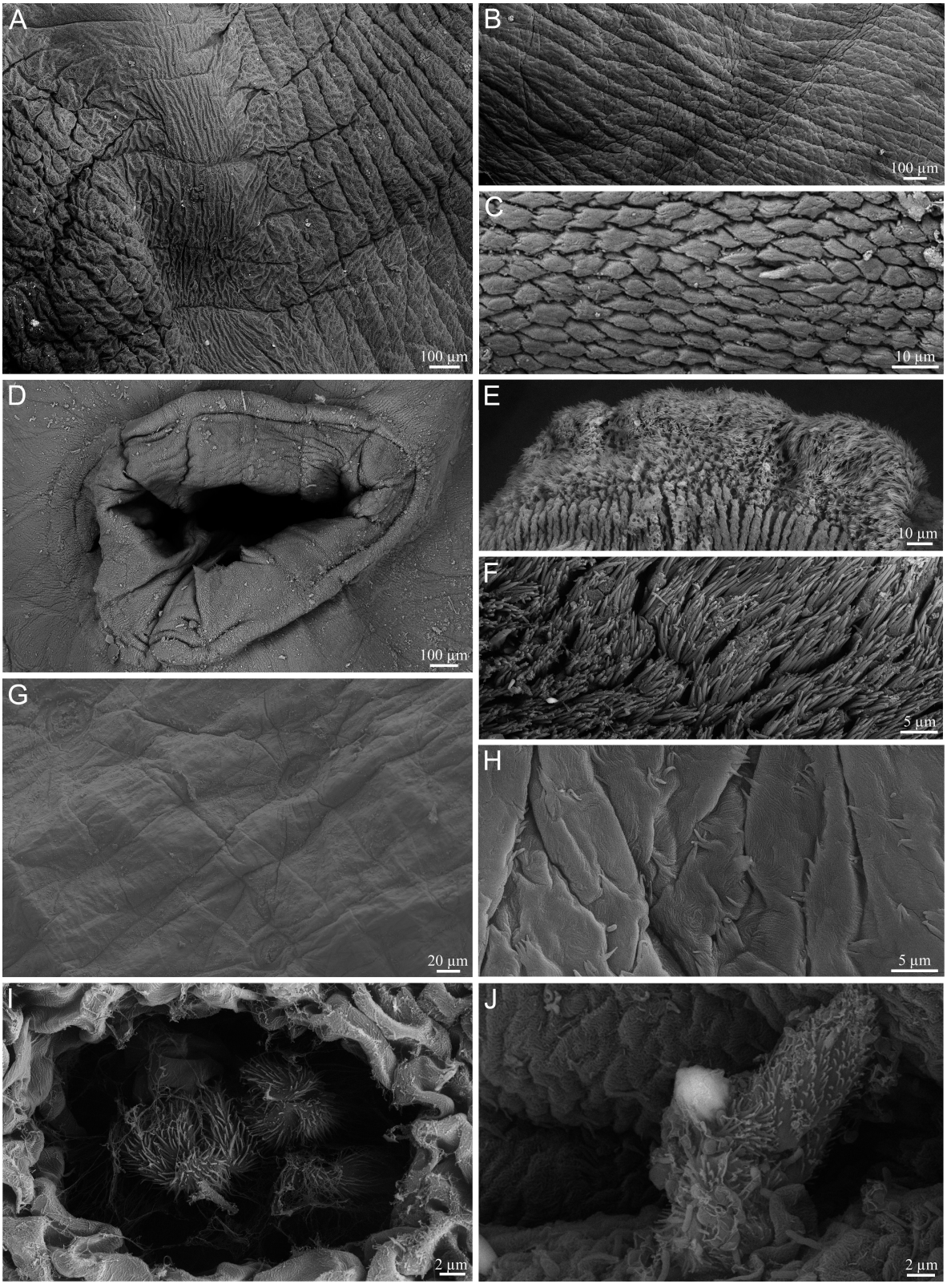

Fig. 8. SEM showing cuticle structure of the externa of *Briarosaccus hoegi* sp. nov. A strip of transversal grooves from the mantle opening to the stalk (A); longitudinal striation on the external cuticle (B); cellular surface around the mantle opening (C); mantle opening (D); numerous spines (hairs) densely covering mantle opening (E, F); depressions on the external cuticle (G); internal cuticle covered with hairs (finger-like processes) (H); retinacula (I, J).

DISCUSSION

To date, four species in the genus *Briarosaccus* have been described. *Briarosaccus hoegi* sp. nov. infesting the stone crab *Hapalogaster dentata* in Russian waters is the fifth representative of this genus. The main morphological features of *Briarosaccus hoegi* sp. nov. match all the characters of the genus *Briarosaccus* summarized by Boschma (1930). According to Boschma (1930), *Briarosaccus* differs from *Peltogaster* Rathke 1842 (type genus of the family Peltogastridae) by its comparatively narrow mesentery. For the comparison of congeneric species, we used the descriptions made by Boschma (1930 1962 1970), Haynes and Boschma (1969), Noever et al. (2016), and also a new specimens of *B. tenellus* from Alaska.

Externae of *Briarosaccus hoegi* sp. nov. differ from those of *Briarosaccus callosus*, *B. auratum*, and *B. regalis* by their size: anterior-posterior length was from 7 to 81 mm in *B. auratum* and *B. regalis*, and even 98 mm in *B. callosus*, but only from 5.3 to 22 mm in *Briarosaccus hoegi* sp. nov. The external surface is covered by small papillae in *B. callosus*, *B. auratum*, and *B. regalis*, but is smooth in *Briarosaccus hoegi* sp. nov. The mantle openings of *B. auratum* and *B. regalis* are placed on the anterior pole and elevated on the short tube, while it is shifted to the right side of the externa and not elevated in *Briarosaccus hoegi* sp. nov. as well as in *B. callosus.* Receptacles of *B. callosus* are described as straight tubes, while they are more or less tortuous in *Briarosaccus hoegi* sp. nov., as well as in *B. auratum* and *B. regalis*.

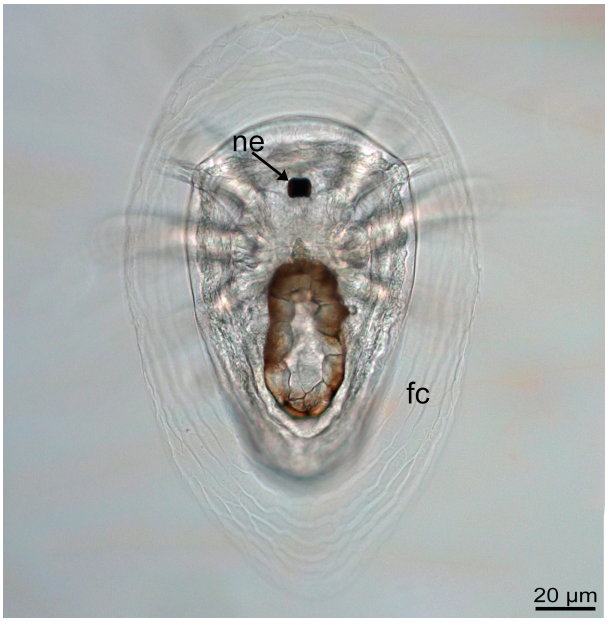

Fig. 9. Nauplius II of *Briarosaccus hoegi* sp. nov. ne, naupliar eye; fc, flotation collar.

Briarosaccus callosus was previously described as a parasite of *Neolithodes agassizii* (Smith, 1882) (see Boschma 1930), *Lithodes santolla* (Molina, 1782), *L. aequispinus*, *Paralomis granulosa* (Hombron & Jacquinot, 1846) (see Boschma 1962), *L. couesi* Benedict, 1895 (see Boschma 1970), and *Paralithodes camtschaticus* (see Haynes and Boschma 1969). *Briarosaccus tenellus* was found on *Hapalogaster mertensii* (see Boschma 1970). Later, two additional species of *Briarosaccus* were found on three king crab hosts in the Southeastern Alaska. Morphological comparison with the type specimen of *B. callosus* revealed that they are not conspecific. While the new species are morphologically different from *B. callosus*, they are indistinguishable from each other by morphology alone and were identified as distinct species using mitochondrial DNA and host specificity. *Briarosaccus auratum* was only found on *Lithodes aequispinus*, while *B. regalis* was found on *Paralithodes camtschaticus* and *Paralithodes platypus* (see Noever et al. 2016).

A similar situation is observed with two related species, *Briarosaccus tenellus* and *Briarosaccus hoegi* sp. nov. These rhizocephalans have different hosts which are not sympatric species: *H. mertensii* inhabits north America, while *H. dentata* is restricted to the Asian coastal waters, most likely with no overlap in their distribution ranges. *B. tenellus* and *Briarosaccus hoegi* sp. nov. are identified as distinct species by genetic markers. Genetic distance between *B. regalis* and *B. auratum* was 11.5 ± 1.5%, and between *B. tenellus* and *Briarosaccus hoegi* sp. nov. was 14.6 ± 0.2%. Despite these genetic differences, the gross morphology and external sizes species are remarkably similar. The externa varies from 5.3 to 22 mm in length and from 1.0 to 10.5 mm in height in *Briarosaccus hoegi* sp. nov., but from 9 to 17 mm in length and from 3 to 8 mm in height in *B. tenellus* from British Columbia (Boschma 1970), and from 8.5 to 15.5 mm in length and from 2.5 to 4.5 mm in height in *B. tenellus* from Alaska (our data). The overall shape of both parasites is similar including the shape and position of the mantle opening. The only difference found is a slightly bilobed anterior margin of the externa in *B. tenellus* and a more unilobed one in *Briarosaccus hoegi* sp. nov. A bilobed anterior margin, with the mantle opening inserted between the two lobes, is a characteristic for the externa of the genus *Peltogaster* (Yoshida and Naruse 2016; Korn et al. 2020a; Golubinskaya et al. 2021a). The arrangement and structure of reproductive organs in the visceral sac of *Briarosaccus hoegi* sp. nov. is similar to those described in *B. tenellus* by Boschma (1970).

Boschma (1970) noted that the external cuticle

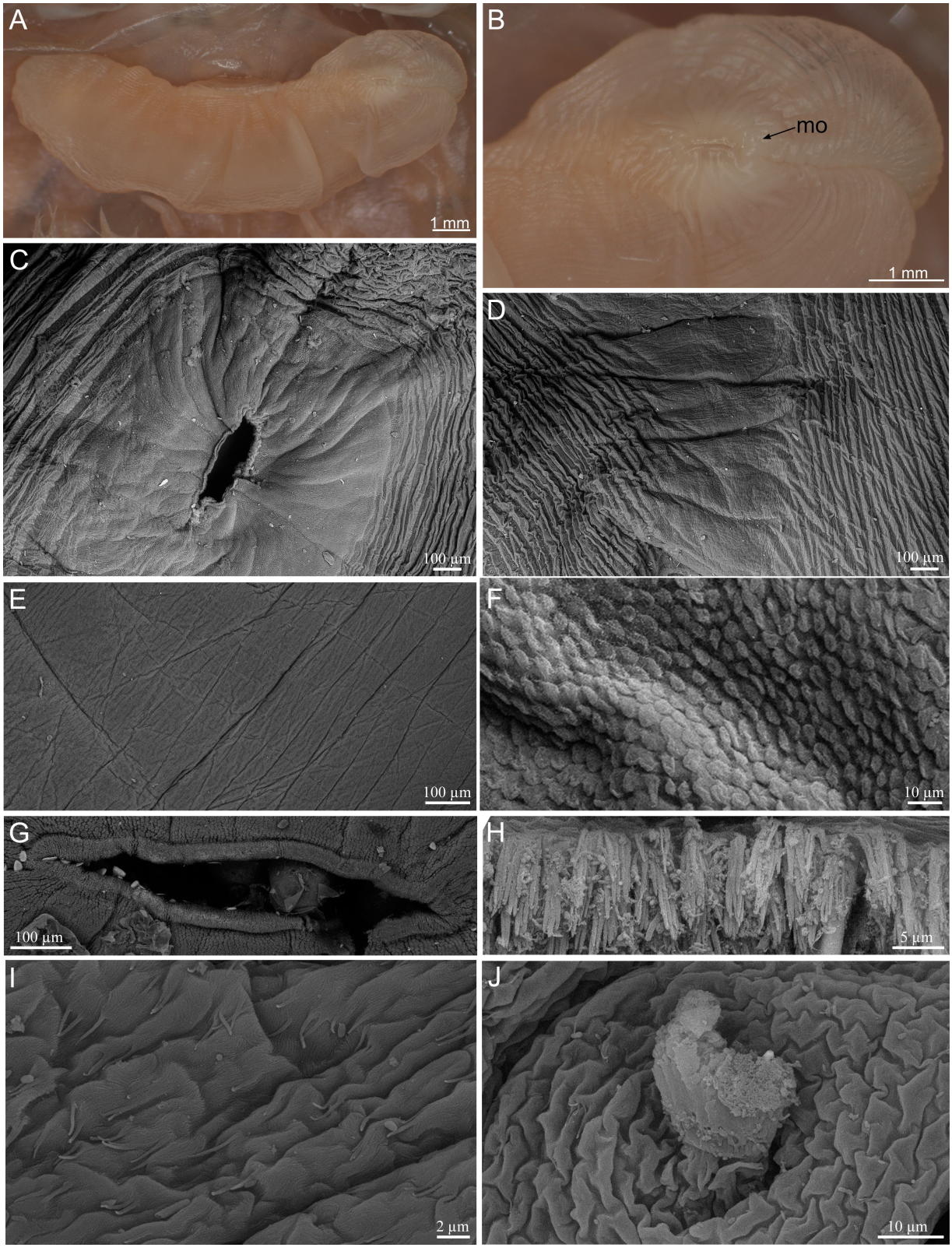

Fig. 10. SEM showing cuticle structure of the externa of *Briarosaccus tenellus.* Whole externa (A); slightly bilobed anterior part (B); mantle opening (C, G); a strip of transversal grooves from the mantle opening to the stalk (D); longitudinal striation on the external cuticle (E); cellular surface around the mantle opening (F); numerous spines (hairs) densely covering mantle opening (H); internal cuticle covered with hairs (finger-like processes) (I); retinaculum (J). mo, mantle opening.

of *B. tenellus* from British Columbia is very thin (from 9 to 18 μm), while in *Briarosaccus hoegi* sp. nov. it is considerably thicker (from 25 to 50 μm). Ultrastructure of the cuticle of these two species is very similar. The external cuticle is smooth with longitudinal striation and a dorsal strip with transversal grooves. The internal cuticle is wrinkled and covered by sparse hairs. Boschma (1970) did not find retinacula in *B. tenellus*. However, our investigation showed that the internal cuticles of *B. tenellus* from Alaska and *Briarosaccus hoegi* sp. nov. are covered by single or grouped barbed retinacula.

Previously, nauplii of *Briarosaccus* species with reticulated collars were thought to lack naupliar eyes (Hawkes et al. 1985; Walossek et al. 1996). It is surprising that nauplii and cyprids of *Briarosaccus hoegi* sp. nov. have naupliar eyes, while the larvae of *B. tenellus* probably lack them (Walossek et al. 1996). If so, it is a clear character distinguishing the two species.

CONCLUSIONS

Briarosaccus hoegi sp. nov. and *B. tenellus* are identified as distinct species using genetic markers. These two species have different hosts and areas of distribution. Adult parasites exhibit only subtle differences in their gross morphology. However, a detailed examination of the larval stages is likely to reveal distinct morphological variations between the two species.

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Authors' contributions: OMK performed the histology, wrote the manuscript; DDG performed SEM and all illustrations; SNS performed the molecular analysis; CN and HG sampled and processed the material from Alaska. All authors approved the final manuscript.

Competing interests: All authors declare that they have no competing interests.

Availability of data and materials: DNA sequences generated in the study were deposited into the National Center for Biotechnology Information (NCBI). Specimens of both parasites and hosts were deposited into the Museum of the A.V. Zhirmunsky National Scientific Center of Marine Biology, Vladivostok, Russia (MIMB) and the Museum of the University of Bergen, Bergen, Norway. Detailed molecular analysis is included in the supplemental material.

Consent for publication: All the authors consent to the publication of this manuscript.

Ethics approval consent to participate: All applicable international, national, and institutional guidelines for use of animals were followed by the authors.

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Supplementary materials

Table S1. GenBank accession details for all data used in phylogenetic analyses. (download)

Table S2. *COI* sequence pairwise distances for the species of the family Peltogastridae. Above the diagonal is the SD. (download)

Table S3. 16S sequence pairwise distances for the species of the family Peltogastridae. (download)

Table S4. 18S sequence pairwise distances for the species of the family Peltogastridae. (download)

Table S5. *COI* sequence pairwise distances for the species of the subfamily Hapalogastrinae (Lithodidae). (download)

Fig. S1. Bayesian phylogenetic tree of the family Peltogastridae for *COI* gene fragments. Number above or under the branches are Bayesian posterior probabilities. Blue color highlighted differences between BI and ML phylogenetic reconstruction. (download)

Fig. S2. Maximum likelihood tree of the family Peltogastridae for *COI* gene fragments. Number above or under the branches are bootstrap values. Blue color highlighted differences between BI and ML phylogenetic reconstruction. (download)

Fig. S3. Bayesian phylogenetic tree of the family Peltogastridae for 16S rRNA gene fragments. Number above or under the branches are Bayesian posterior probabilities. Red color highlighted differences between BI and ML phylogenetic reconstruction. (download)

Fig. S4. Maximum likelihood tree of the family Peltogastridae for 16S gene fragments. Number above or under the branches are bootstrap values. Red color highlighted differences between BI and ML phylogenetic reconstruction. (download)

Fig. S5. Bayesian phylogenetic trees of the family Peltogastridae for combined molecular data (concatenated sequences from *COI* and 16S rRNA gene fragments). Number above or under the branches are Bayesian posterior probabilities. (download)

Fig. S6. Bayesian phylogenetic tree of the family Peltogastridae for 18S rRNA gene fragments. Number above or under the branches are Bayesian posterior probabilities. Green color highlighted differences between BI and ML phylogenetic reconstruction. (download)

Fig. S7. Maximum likelihood tree of the family Peltogastridae for 18S gene fragments. Number above or under the branches are bootstrap values. Green color highlighted differences between BI and ML phylogenetic reconstruction. (download)

Fig. S8. Bayesian phylogenetic tree and genetic distances for the subfamily Hapalogastrinae (Lithodidae) for *COI* gene fragments. Number above or under the branches on phylogenetic tree are Bayesian posterior probabilities. Data highlighted in red were obtained in current research. (download)