

Abyssal cradle: flatworm egg capsules on a deep-sea sea spider, with an integrative redescription of *Ascorhynchus mariae* Turpaeva, 1971

(Pycnogonida: Ascorhynchidae and Platyhelminthes: Rhabdocoela)

Shogo Sekiguchi & Keiichi Kakui

Sekiguchi, S. & Kakui, K. 2026. Abyssal cradle: flatworm egg capsules on a deep-sea sea spider, with an integrative redescription of *Ascorhynchus mariae* Turpaeva, 1971 (Pycnogonida: Ascorhynchidae and Platyhelminthes: Rhabdocoela). Spixiana 49(1): 5–22.

We redescribe the deep-sea sea spider *Ascorhynchus mariae* Turpaeva, 1971 based on one male and one female collected at the depths of 3685–3893 m in the Japan Trench. This is the third and deepest record of this species globally and the first in Japanese waters, and expands its known distributional range from only the Kuril–Kamchatka Trench to the Japan Trench. We determined sequences for its 18S rRNA (18S) and cytochrome *c* oxidase subunit I (COI) genes. On the female specimen, we found flatworm egg capsules, which represents the first reported case of flatworms utilizing pycnogonids as a substrate for egg attachment. A molecular phylogenetic analysis based on 18S and 28S rRNA (28S) sequences placed the flatworm species firmly within the family Trigonostomidae (Rhabdocoela) of free-living flatworms, and as the sister taxon to a *Ptychopera* clade, with moderate branch support. This provides the second-deepest record for free-living flatworms.

Shogo Sekiguchi (corresponding author), Department of Natural History Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan; e-mail: podapodapantopoda@gmail.com

Keiichi Kakui, Department of Biological Sciences, Faculty of Science, Hokkaido University, Sapporo 060-0810, Japan

Introduction

Ascorhynchus Sars, 1877 is the most species-rich genus in the pycnogonid family Ascorhynchidae, with 78 valid species (Bamber 2002, 2013, Child 2002, Sabroux et al. 2022). *Ascorhynchus* sea spiders are widely distributed geographically and vertically, having been reported from all oceans and from depths of 0–6260 m (Müller 1993). To date, 11 species have been reported from Japanese waters (Miyazaki 2022).

Ascorhynchus mariae Turpaeva, 1971 is a deep-sea species originally described from six individuals collected at the depths of 3145–3250 m in the Kuril–Kamchatka Trench, off the southeastern coast of Simushir Island, Kuril Islands (Turpaeva 1971). It differs from congeners in having an apically bifid

ocular tubercle, each trunk segment with a postero-dorsal transverse setal row but without dorsomedian tubercles, and lateral processes without long setae on the anterior and posterior margins. Melzer et al. (2020) reported an additional specimen (sex not mentioned) collected at 3432 m depth near the type locality and provided an image of its habitus. The female morphology of this species remained undescribed.

Sea spiders can serve as sites of attachment or egg-laying substrates for various organisms (e.g., ciliophorans, hydrozoans, bryozoans, molluscs; Sekiguchi et al. 2025). The diversity of their epibiotic associations is relatively well documented (cf. Wambreuse et al. 2021), yet new associations continue to be discovered, such as the recent finding of a cystidean-

stage crinoid on a sea spider (Sekiguchi et al. 2025).

During a recent deep-sea faunal survey in the Japan Trench in 2023, we collected one male and one female *A. mariae* at depths of 3685–3893 m. This constitutes the third record of this species and the first from outside the Kuril–Kamchatka Trench. Here we redescribe this species based on these specimens and present first partial sequences of its 18S rRNA (18S) and cytochrome *c* oxidase subunit I (COI) genes. On the female sea spider, we found several oval structures, which were molecularly identified as flatworm egg capsules; this is the first example of flatworms utilizing pycnogonids as an egg-laying substrate. We describe the gross morphology of the flatworm capsules, present their partial 18S and 28S rRNA (28S) sequences, and present the results of a molecular phylogenetic analysis by which we attempted to identify them.

Materials and methods

The two pycnogonids (see “Material examined” in the description below for relevant collection information) collected during cruise KH-23-5 of R/V Hakuho-maru (Japan Agency for Marine–Earth Science and Technology; JAMSTEC) were fixed in 80% ethanol and then preserved in 99% ethanol. Appendages were detached from the trunk with needles. Body parts were observed

with a Nikon SMZ 1500 stereomicroscope under lateral UV illumination from a YF-990 UV light (Yamada Electric, Japan). Appendages were mounted in glycerin on a glass slide with paired spacers and observed with Olympus BX51 or BX53 compound microscopes. Internal structures in palps and ovigers were photographed at different focal planes with a BX53. The serial images were assembled by using Adobe Photoshop 2021. Illustrations were prepared with Adobe Illustrator 2019 and Photoshop 2019 from draft line drawings made with a drawing tube. Morphological terminology follows Child (1979), except that the term “article” is used instead of “segment” for all appendages (Hosoda & Kakui 2020). The cephalon, with proboscis, ocular tubercle, chelifore, palp, oviger, and leg 1, represents the first trunk segment; trunk segments 2–4 bear legs 2–4, respectively. The formula for the number of compound spines on the oviger follows Hosoda et al. (2018). Measurements (presented in millimeters) were made axially (dorsally for the body and chelifore; laterally for the proboscis and abdomen; medially for the palp and oviger; and posteriorly for legs). The body length of the pycnogonids was measured from the base of the chelifore to the posterior end of the fourth lateral process, and body width as the distance across the distal ends of the second lateral processes. The studied specimens have been deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Japan.

All five egg capsules and three empty capsule-like structures were photographed through an SMZ1500; three capsules were detached from the pycnogonid with

Table 1. List of PCR and cycle sequencing (CS) primers used in this study. P, pycnogonids; F, flatworm egg capsules.

Marker	Primer	Reaction	Target	Primer sequence	Source
18S	18SU	PCR	P	GCGAATGGCTCATTAATCAG	Nakamura et al. (2007)
	R9	PCR	P	GATCCTTCCGACGGTTACCTAC	Giribet et al. (1996)
	SR1	PCR	F	TACCTGGTTGATCCTGCCAG	Nakayama et al. (1996)
	SR12	PCR	F	CCTTCCGACGGTTCACCTAC	Nakayama et al. (1996)
	18S-b3F	CS	P & F	CCTGAGAAACGGCTACCAT	Kakui & Shimada (2017)
	18S-b3R	CS	F	ATGTGGTAGCCGTTTCTCAGG	Kakui & Shimada (2022)
	18S-b4F	CS	P & F	TGCGGTTAAAAAGCTCGTAGTTG	Kakui et al. (2011)
	18S-b4R	CS	P & F	TCCAACACGAGCTTTTAAACC	Kakui et al. (2011)
	18S-b5F	CS	P & F	GATCGAAGGCGATYAGATACC	Kakui et al. (2021)
	18S-b6F	CS	P & F	CCTGCGGCTTAATTTGACTC	Kakui et al. (2011)
	18S-a6R	CS	P & F	AACGGCCATGCACCAC	Kakui et al. (2011)
	18S-b8F	CS	P & F	GGTCTGTGATGCCCTTAGATG	Kakui et al. (2011)
28S	LSUD1F	PCR	F	ACCCGCTGAATTTAAGCATA	Lenaers et al. (1989)
	28S_a6R	PCR	F	AGCCGAGTGCTTGCTACTAC	Shiraki et al. (2025)
	300F	CS	F	CAAGTACCGTGAGGGAAAGTTG	Lockyer et al. (2003)
	300R	CS	F	CAACTTTCCTCACGGTACTTG	Lockyer et al. (2003)
	900F	CS	F	CCGTCTTGAACACCGGACCAAG	Lockyer et al. (2003)
	28S Rd4.2b	CS	F	CCTTGGTCCGTGTTTCAAGAC	Whiting (2002)
	28S_b5F	CS	F	TATCCGGTAAAGCGAATGATTAGA	Kakui & Tsuyuki (2024)
COI	LCO1490	PCR & CS	P	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)
	HCO2198	PCR & CS	P	TAAACTTCAGGTGACCAAAAAATCA	Folmer et al. (1994)

needles; one capsule was lost during observation; and the remaining capsule and the capsule-like structures were left on pycnogonids. One capsule in ethanol was mounted on a glass slide with paired spacers and observed and photographed at different focal planes with a BX51. The serial images were assembled by using Photoshop 2021.

Total DNA was extracted from soft tissue of the two pycnogonids and from two detached egg capsules by using a NucleoSpin Tissue XS Kit (Macherey-Nagel,

Germany). Table 1 lists the primers used for PCR and cycle sequencing, and literature. PCR amplification conditions for 18S, 28S, and COI with KOD ONE PCR Master Mix (Toyobo, Japan) were 45 cycles of 98°C for 10 s, 52°C (18S) or 56°C (28S, COI) for 5 s, and 68°C for 10 s (18S, 28S) or 1 s (COI). The nucleotide sequences were determined with a QuantumDye Terminator v3.1 Cycle Sequencing Kit (Quintara Biosciences, USA; for 18S and COI), a BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies, USA; for 28S), and a

Table 2. Rhabdoceol species included in the phylogenetic analysis in this study.

Taxon name	Accession no.		Source
	18S	28S	
Family Adenorhynchidae (outgroup)			
<i>Cilionema hawaiiensis</i>	KC529428	KC529556	Van Steenkiste et al. (2013)
<i>Coronhelmis lutheri</i>	KC529426	KC529554	Van Steenkiste et al. (2013)
<i>Coronhelmis multispinosus</i>	KC529427	KC529555	Van Steenkiste et al. (2013)
<i>Litucivis serpens</i>	AY775758	KC529552	18S: Willems et al. (2006); 28S: Van Steenkiste et al. (2013)
<i>Tvaerminnea karlingi</i>	MF321755	MF321764	Van Steenkiste & Leander (2018)
Family Trigonostomidae			
<i>Beklemisheviella angustior</i>	KC529412	KC529538	Van Steenkiste et al. (2013)
<i>Beklemisheviella contorta</i>	KC529413	KC529539	Van Steenkiste et al. (2013)
<i>Ceratopera</i> sp.	KC529421	KC529548	Van Steenkiste et al. (2013)
<i>Ceratopera axi</i>	MF321746	MF321756	Van Steenkiste & Leander (2018)
<i>Ceratopera complicata</i>	MF321747	MF321757	Van Steenkiste & Leander (2018)
<i>Ceratopera gracilis</i>	KC529422	KC529549	Van Steenkiste et al. (2013)
<i>Ceratopera pacifica</i>	MF321748	MF321758	Van Steenkiste & Leander (2018)
<i>Ceratopera pilifera</i>	MF321749	MF321759	Van Steenkiste & Leander (2018)
<i>Parapharyngiella</i> sp.	KC529405	KC529531	Van Steenkiste et al. (2013)
<i>Parapharyngiella</i> sp.	OR490856	OR490872	Van Steenkiste et al. (2023)
<i>Parapharyngiella</i> sp.	OR490857	OR490873	Van Steenkiste et al. (2023)
<i>Proxenetes bilioi</i>	KC529407	KC529533	Van Steenkiste et al. (2013)
<i>Proxenetes fasciger</i>	KC529408	KC529534	Van Steenkiste et al. (2013)
<i>Proxenetes karlingi</i>	KC529409	KC529535	Van Steenkiste et al. (2013)
<i>Proxenetes puccinellicola</i>	KC529411	KC529537	Van Steenkiste et al. (2013)
<i>Proxenetes quinquespinosus</i>	KC529406	KC529532	Van Steenkiste et al. (2013)
<i>Proxenetes simplex</i>	KC529410	KC529536	Van Steenkiste et al. (2013)
<i>Ptychopera</i> sp.	KC529420	KC529547	Van Steenkiste et al. (2013)
<i>Ptychopera japonica</i>	MF321751	MF321760	Van Steenkiste & Leander (2018)
<i>Ptychopera unicornis</i>	MF321752	MF321761	Van Steenkiste & Leander (2018)
<i>Trigonostomum armatum</i>	KC529419	KC529545	Van Steenkiste et al. (2013)
<i>Trigonostomum armatum</i>	OR608004	OR616258	Diez et al. (2023)
<i>Trigonostomum franki</i>	KC529416	KC529542	Van Steenkiste et al. (2013)
<i>Trigonostomum penicillatum</i>	KC529414	KC529540	Van Steenkiste et al. (2013)
<i>Trigonostomum setigerum</i>	KC529418	KC529544	Van Steenkiste et al. (2013)
<i>Trigonostomum sinensis</i>	MN316548	MN316544	Hu et al. (2019)
<i>Trigonostomum sinensis</i>	MN316549	MN316545	Hu et al. (2019)
<i>Trigonostomum tillicum</i>	MF321753	MF321762	Van Steenkiste & Leander (2018)
<i>Trigonostomum tori</i>	MF321754	MF321763	Van Steenkiste & Leander (2018)
<i>Trigonostomum venenosum</i>	KC529417	KC529543	Van Steenkiste et al. (2013)
<i>Trigonostomum watsoni</i>	KC529415	KC529541	Van Steenkiste et al. (2013)
Flatworm egg capsule on sea spider	LC909289	LC909288	This study

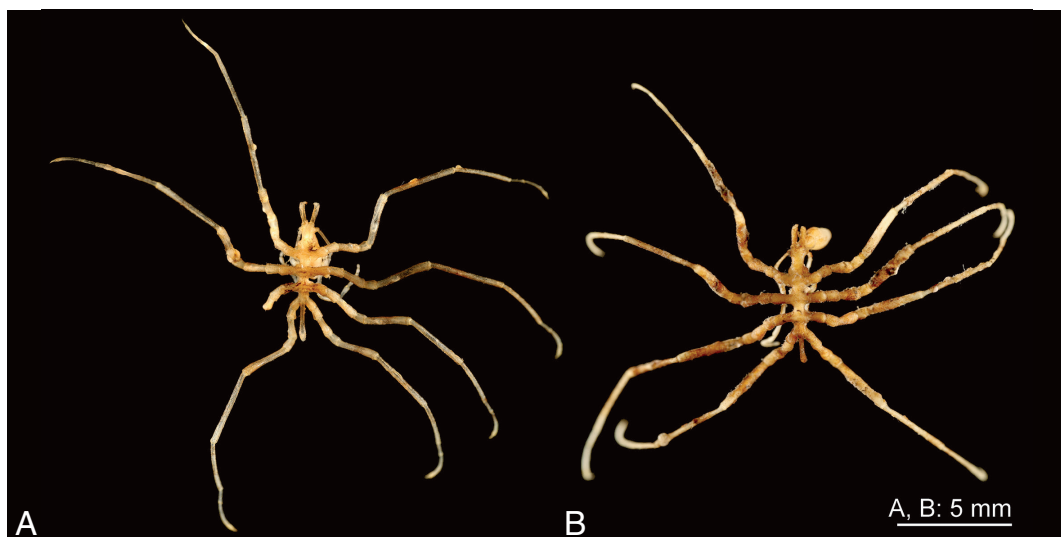


Fig. 1. *Ascorhynchus mariae* Turpaeva, 1971, ethanol-fixed specimens, dorsal view. **A.** Male (ICHUM9132). **B.** Female (ICHUM9133).

3730 DNA Analyzer (Life Technologies). Amplicons were concatenated by using GeneStudio Professional Edition ver. 2.2.0.0. (GeneStudio, USA). The determined sequences were deposited in the International Nucleotide Sequence Database (INSD) through the DNA Data Bank of Japan (DDBJ) under numbers LC909288–LC909293. Similarity searches of the INSD for sequences most similar to those we determined were conducted with BLAST (Altschul et al. 1990).

The BLAST result for our flatworm 18S sequence indicated that the egg capsules belonged to family Trigonostomidae Graff, 1905, a group of free-living rhabdocoels. We thus prepared a combined 18S+28S dataset that included sequences from 32 trigonostomid and five adenorhynchid (outgroup) taxa (Table 2) for a maximum likelihood (ML) analysis. The 18S and 28S datasets were independently aligned as described by Kakui & Tsuyuki (2024). The final aligned 18S and 28S datasets were 1665 and 1476 positions long, respectively, and deposited in figshare repository (Sekiguchi & Kakui 2026). The methods for selecting Q mixture model for the datasets (MIX{GTR,TPM3,K3P}+I+G4 for 18S; MIX{GTR,TPM3,TN,F81,TPM2u}+I+R3 for 28S), the ML analysis, and tree drawing were as described by Katsuragawa et al. (2025). Nodal support was estimated by analysing 1000 pseudoreplicates for both Shimodaira–Hasegawa-like approximate likelihood ratio tests (SH-aLRT; Guindon et al. 2010) and ultrafast bootstraps (UFBoot) as a function of IQ-TREE.

We calculated pairwise Kimura (1980) 2-parameter (K2P) distances and p-distances separately for the 18S and 28S sequences from our flatworm egg capsule and species of *Ptychopera* Den Hartog, 1964. For the 18S analysis, our sequence and those from three *Ptychopera* species used in a phylogenetic analysis by Van Steenkiste et al. (2013) and Van Steenkiste & Leander (2018) (see Table 2), and *P. westbladi* (Luther, 1943) from Belgium (AY775770, Willems et al. 2006) were realigned as described above, and distances were calculated using MEGA7 (Kumar et al. 2016). For the 28S analysis, the distances between our sequence and those from the three *Ptychopera* species in the phylogenetic analysis and *P. westbladi* from the Netherlands (KC529546, Van Steenkiste et al. 2013) were calculated in the same manner.

Results

Taxonomy

Family Ascorhynchidae Hoek, 1881

Genus *Ascorhynchus* Sars, 1877

Ascorhynchus mariae Turpaeva, 1971

[New Japanese name:

Hebijita-tokkuri-umigumo]

Figs 1–8

Ascorhynchus mariae Turpaeva, 1971: pp. 280, 281, fig. 4.

Ascorhynchus mariae: Melzer et al. (2020), pp. 269, 270, fig. 1A; Dudnik & Kremenetskaia (2025), pp. 5, 13, fig. 3e.

Material examined: One male (ICHUM9132), one jar, Station F2 (39°28.555' N, 143°47.347' E to 39°27.934' N, 143°47.240' E), 3890–3893 m depth, coll. 29 September 2023; one female (ICHUM9133), one jar, Station F1 (39°27.885' N, 143°45.623' E to 39°27.285' N,

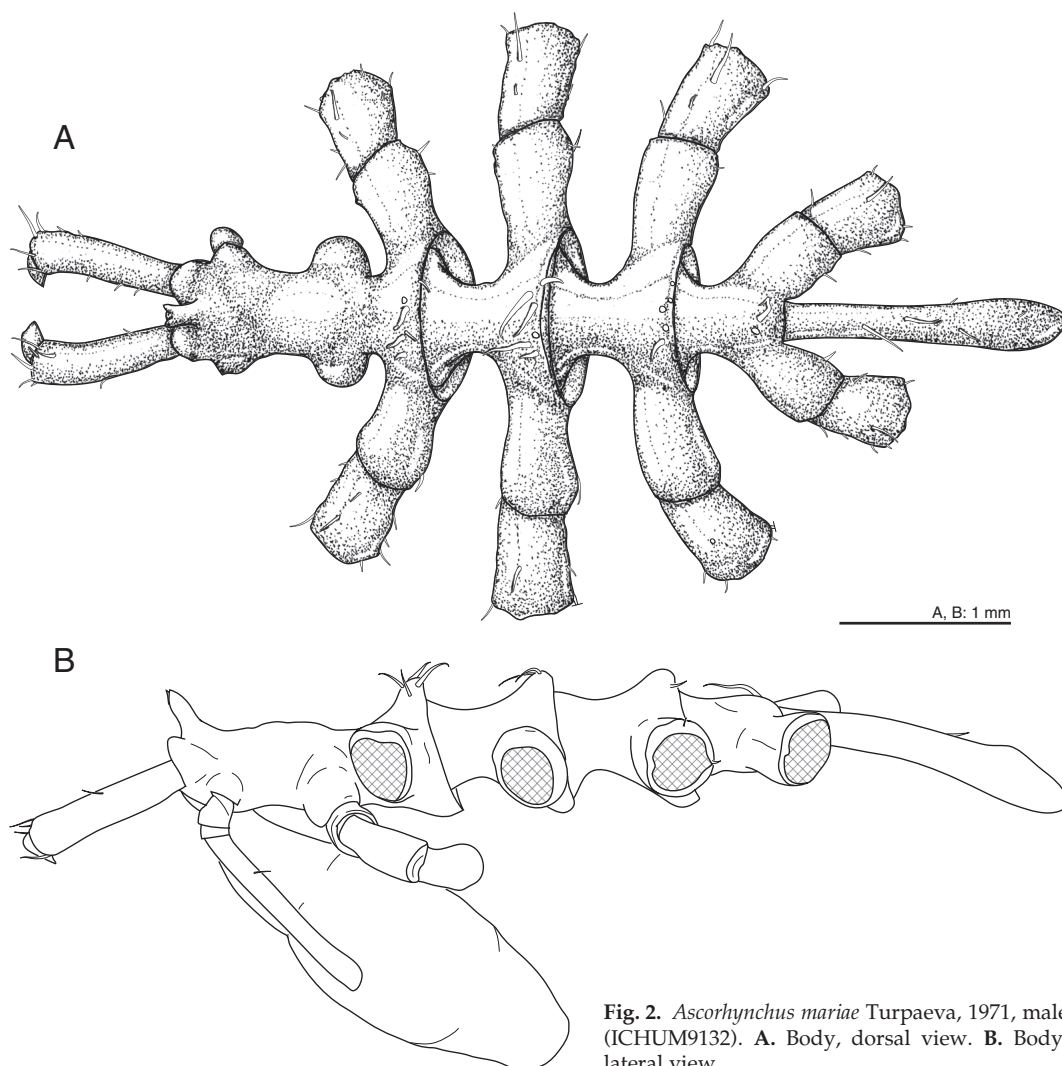


Fig. 2. *Ascorhynchus mariae* Turpaeva, 1971, male (ICHUM9132). **A.** Body, dorsal view. **B.** Body, lateral view.

143°45.397'E), 3685–3693 m depth, coll. 27 September 2023. Both were collected off the eastern coast of Iwate, Japan, northwestern Pacific, beam trawl, by Keiichi Kakui.

Description of male specimen

Body (Figs 1A, 2A,B) elongate, fully segmented; cephalon and trunk segments 2–4 each with posterodorsal transverse row of setae. Lateral processes separated by 1.1 (between 1–2), 1.2 (between 2–3), and 0.9 (between 3–4) times their basal diameter; each process about 1.5 times as long as basal diameter, with several small setae on posterodistal margin, except for left third lateral process. Cephalon as

long as trunk segments 2–3 combined, gently swollen dorsally at mid-length of neck. Ocular tubercle (Fig. 2A,B) in anterodorsal region of cephalon, apically bifid, slightly inclined anteriorly, without eyes. Proboscis (Fig. 2B) inflated, widest in middle, blunt distally, with two slight constrictions; slightly longer than cephalon and trunk segment 2 combined; mouth triradiate. Abdomen elongate, extending posteriorly and slightly ventrally, length subequal to trunk segments 3–4 combined; base narrow, gently widening posteriorly; several dorsal and lateral setae present.

Chelifore (Fig. 3A) with single scape and chela. Scape with several short setae sparsely arranged and long dorsodistal setae. Chela tiny, vestigial, extended

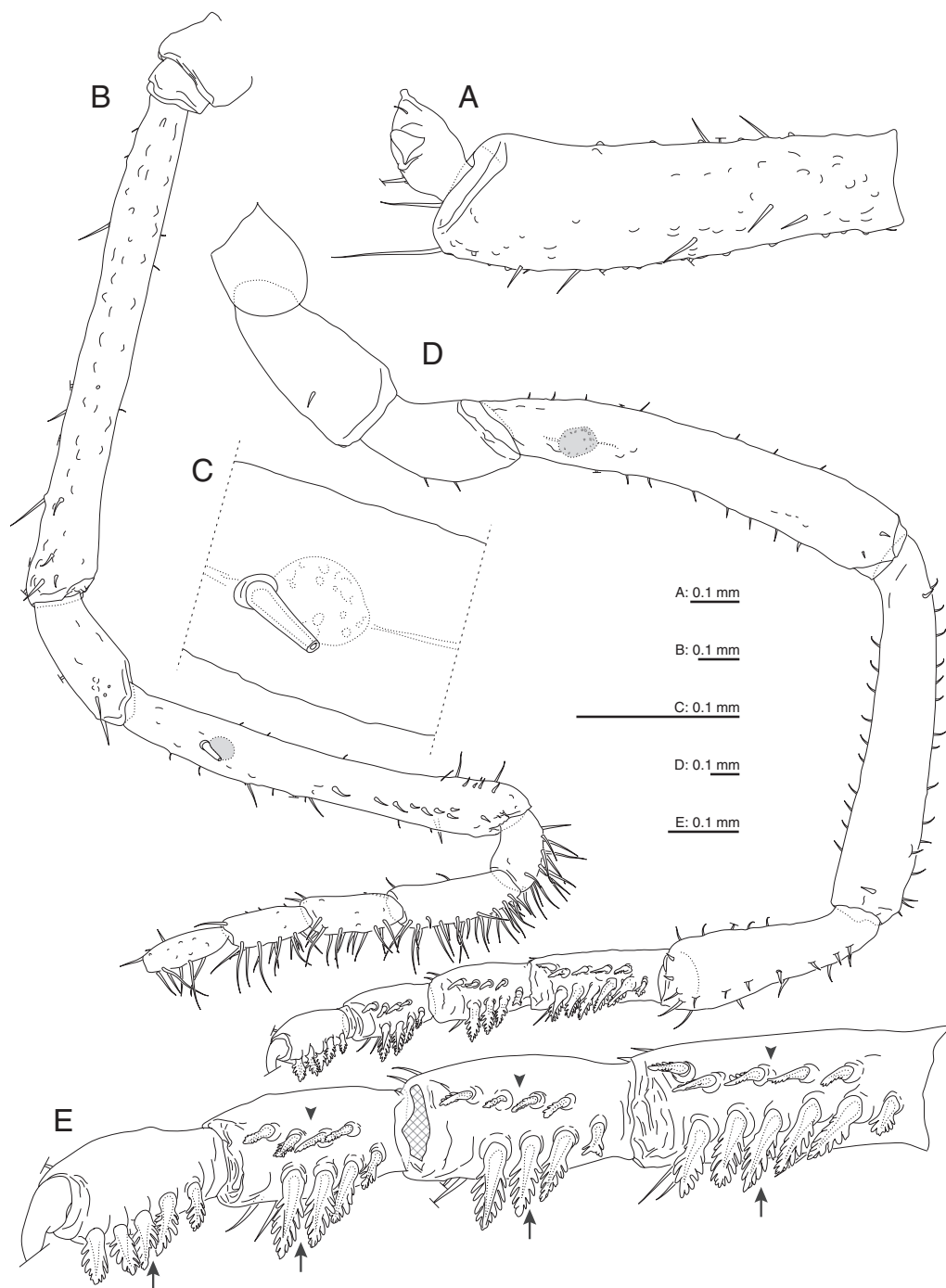


Fig. 3. *Ascorhynchus mariae* Turpaeva, 1971, male (ICHUM9132). **A.** Right chelifore, ventral view. **B.** Right palp, medial view. **C.** Right palp, internal organ in article 5, medial view. **D.** Right oviger, medial view. **E.** Right oviger, distal four articles, medial view. B, D, shaded areas, internal organs; C, enlargement showing spine and internal structure of organ; E, black arrowheads, dorsal row of compound spines; black arrows, ventral row of compound spines; broken area indicated by hatching.

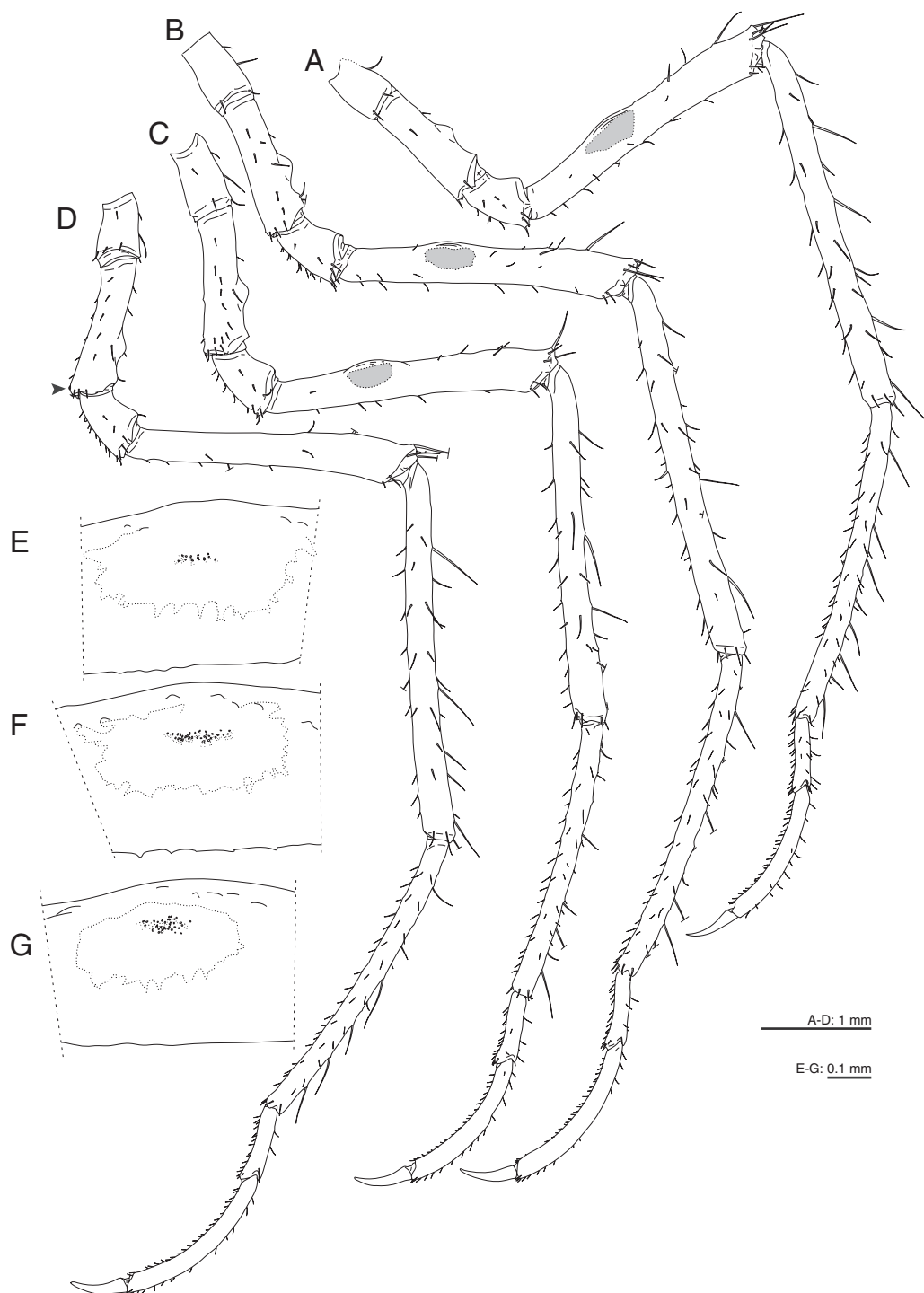


Fig. 4. *Ascorhynchus mariae* Turpaeva, 1971, male (ICHUM9132). **A-D.** Right legs 1-4, posterior view. **E-G.** Cement gland and pores, right legs 1-3, posterior view. **A-C,** shaded areas, cement glands; **D,** arrowhead, position of genital pore; **E-G,** enlargements showing internal structure of cement glands.

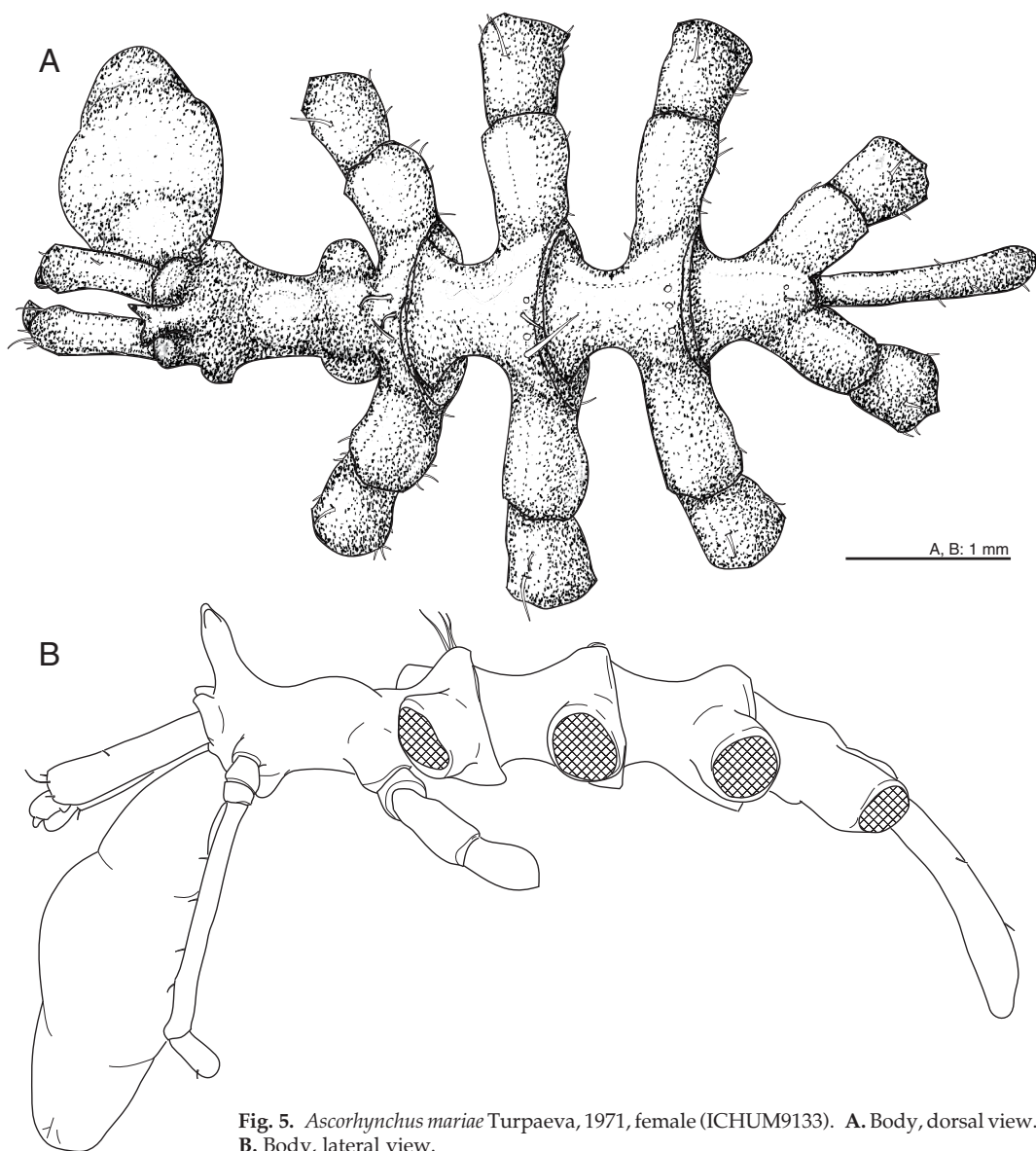


Fig. 5. *Ascorhynchus mariae* Turpaeva, 1971, female (ICHUM9133). **A.** Body, dorsal view. **B.** Body, lateral view.

ventrally; palm with lateral tubercle at base of movable finger and long lateral seta; immovable finger with short seta.

Palp (Figs 3B, C, 8A) with 10 articles. Articles 1 and 2 short, shorter than their diameters. Article 3 with sparsely arranged short setae, several anterior long setae, and three anterodistal setae. Article 4 twice as long as wide, with long setae. Article 5 with sparsely arranged short setae, several long lateroventral setae; several medial short setae between mid-length and distal end, and several dorsodistal

setae; large medial spine and internal spherical structure one-fifth of article 5 length from proximal end (Fig. 3B, shaded area; Figs 3C, 8A). Article 6 arched anteriorly, with numerous ectal long setae. Articles 7–10 with numerous setae and several middle and dorsodistal setae.

Oviger (Figs 3D, E, 8C) with 10 articles and terminal spine. Article 1 naked, length equal to width. Article 2 with short medial seta. Article 3 with several short ectal setae. Articles 4 and 5 with sparsely arranged short ectal and endal setae, and several

short mediobasal setae; article 4 with proximomedial internal spherical structure (Fig. 3D, shaded area; Fig. 8C). Article 6 with three rows of setae, and several short and long distal setae. Strigilis (= articles 7–10) with one dorsal (articles 7–9) and one ventral (articles 7–10) rows of endal compound spines on each article; spine formula 5:4:4:0 (dorsal rows, Fig. 3E, black arrowheads) and 5:4:4:5 (ventral rows, Fig. 3E, black arrows); ventral compound spines larger than dorsal ones; proximal compound spine in ventral row smallest on each article; ventral compound spines with 4–6 broad lateral serrations per side; dorsal compound spines with tiny serrations. Terminal spine moderately curved.

Leg 1 (Fig. 4A, E) coxae 1–3 with several posterior and ventrodorsal setae; coxa 1 with long dorsal setae; coxa 2 with dorsal tubercle at mid-length. Femur with sparsely arranged setae and distal long setae; swollen around cement gland; cement gland at mid-length (Fig. 4A, shaded area; Fig. 4E); cement gland pores tiny, opening posterodorsally. Tibia 1 longest, with dorsal row of long setae, ventral row of short, sparsely arranged setae, and distal setae. Tibia 2 with dorsal row of long setae, sparsely arranged short setae, and distal short setae. Tarsus slightly shorter than half propodus length, with sparsely arranged short setae and distal short setae. Propodus with dorsal and ventral rows of short setae. Claw well curved, four times as long as its basal diameter. Auxiliary claws absent.

Legs 2–4 (Fig. 4B–D, F, G) similar to leg 1, except leg 4 lacking cement gland (Fig. 4D).

Genital pore opening ventrally near distal end of coxa 2 of leg 4 (Fig. 4D, arrowhead).

Measurements of male specimen (mm). Body length 3.73; width 2.39. Proboscis length 2.55. Proboscis width at base, first constriction, middle portion, second constriction, tip: 0.30, 0.66, 0.91, 0.75, 0.37. Cephalon length 1.56. Minimum width of neck 0.48. Width at anterior expansion of cephalon 0.59. Ocular tubercle height 0.31. Abdomen length 1.84; width at base 0.22. Chelifore scape length 0.88. Length of palpal articles 1–10: 0.13, 0.08, 1.27, 0.30, 0.99, 0.15, 0.28, 0.23, 0.20, 0.22. Length of oviger articles 1–10 and terminal claw: 0.32, 0.51, 0.37, 1.43, 1.22, 0.72, 0.47, 0.36, 0.26, 0.26, 0.10. Length of leg articles (from coxa 1 to propodus) and claw: 0.63, 1.02, 0.45, 2.49, 3.37, 2.92, 0.59, 1.36, 0.46 (leg 1); 0.70, 1.16, 0.50, 2.65, 3.61, 3.09, 0.63, 1.51, 0.56 (leg 2); 0.71, 1.23, 0.41, 2.46, 3.21, 2.51, 0.55, 1.37, 0.49 (leg 3); 0.60, 1.16, 0.41, 2.47, 3.45, 2.86, 0.60, 1.49, 0.52 (leg 4).

Description of female specimen

Trunk (Figs 1B, 5A, B, 6A) similar to that in male, except lateral processes separated by 0.8 (between 1–2), 1.0 (between 2–3), and 1.1 (between 3–4) times their basal diameter, and ocular tubercle larger than in male.

Chelifore (Fig. 6A, B) similar to that in male, except chela bearing two long lateral setae on palm but no seta on immovable finger.

Palp (Figs 6C–E, 8B) similar to that in male, except all articles proportionally longer than in male; internal structure oval (Fig. 6C, shaded area; Fig. 6D; Fig. 8B); articles 7–9 without dorsodistal setae; article 9 with several medial long setae; right article 10 with several dorsal and medial setae; left article 10 (Fig. 6E) 0.44 in length, longer than that of right, weakly bent at mid-length, with terminal claw.

Oviger (Figs 6F, G, 8D) similar to that in male, except article 1 with several short setae; articles 2 and 3 with several short endal setae; article 4 without ectal setae; internal structure oval (Fig. 6F, shaded area; Fig. 8D); article 6 without dorsal setae; compound spine formula 4:6:4:0 (dorsal rows, Fig. 6G, black arrowheads) and 6:5:4:5 (ventral rows; Fig. 6G, black arrows); article 8 with irregularly positioned compound spine (asterisk); ventral compound spines with 3–6 broad lateral serrations per side; dorsal and irregularly positioned compound spines with tiny serrations.

Legs 1–4 (Fig. 7A–D) similar to those in male, except for femur, which is gently swollen from proximal end to mid-length and gently tapering from mid-length to distal end. Oocytes observed (shaded areas in Fig. 7A–D).

Genital pore opening ventrodorsally near distal end of coxa 2 on legs 1–4 (arrowheads in Fig. 7A–D).

Measurements of female specimen (mm). Body length 3.94; width 2.41. Proboscis length 2.48. Proboscis width at base, first constriction, middle portion, second constriction, tip: 0.29, 0.63, 0.98, 0.74, 0.38. Cephalon length 1.50. Minimum width of neck 0.46. Width of anterior expansion of cephalon 0.65. Ocular tubercle height 0.51. Abdomen length 1.74; width at base 0.19. Chelifore scape length 0.99. Length of right palpal articles 1–10: 0.13, 0.11, 1.42, 0.36, 1.06, 0.18, 0.41, 0.31, 0.28, 0.27. Length of oviger articles 1–10 and terminal claw: 0.30, 0.51, 0.39, 1.32, 1.10, 0.71, 0.46, 0.36, 0.32, 0.32, 0.15. Length of leg articles (from coxa 1 to propodus) and claw: 0.61, 1.01, 0.39, 2.82, 3.55, 3.04, 0.70, 1.39, 0.39 (leg 1); 0.58, 1.08, 0.43, 2.98, 3.76, 3.23, 0.74, 1.48, 0.46 (leg 2); 0.51, 1.14, 0.37, 2.75, 3.51, 2.77, 0.56, 1.25, 0.39 (leg 3); 0.51, 1.11, 0.38, 2.85, 3.68, 3.05, 0.69, 1.65, 0.48 (leg 4).

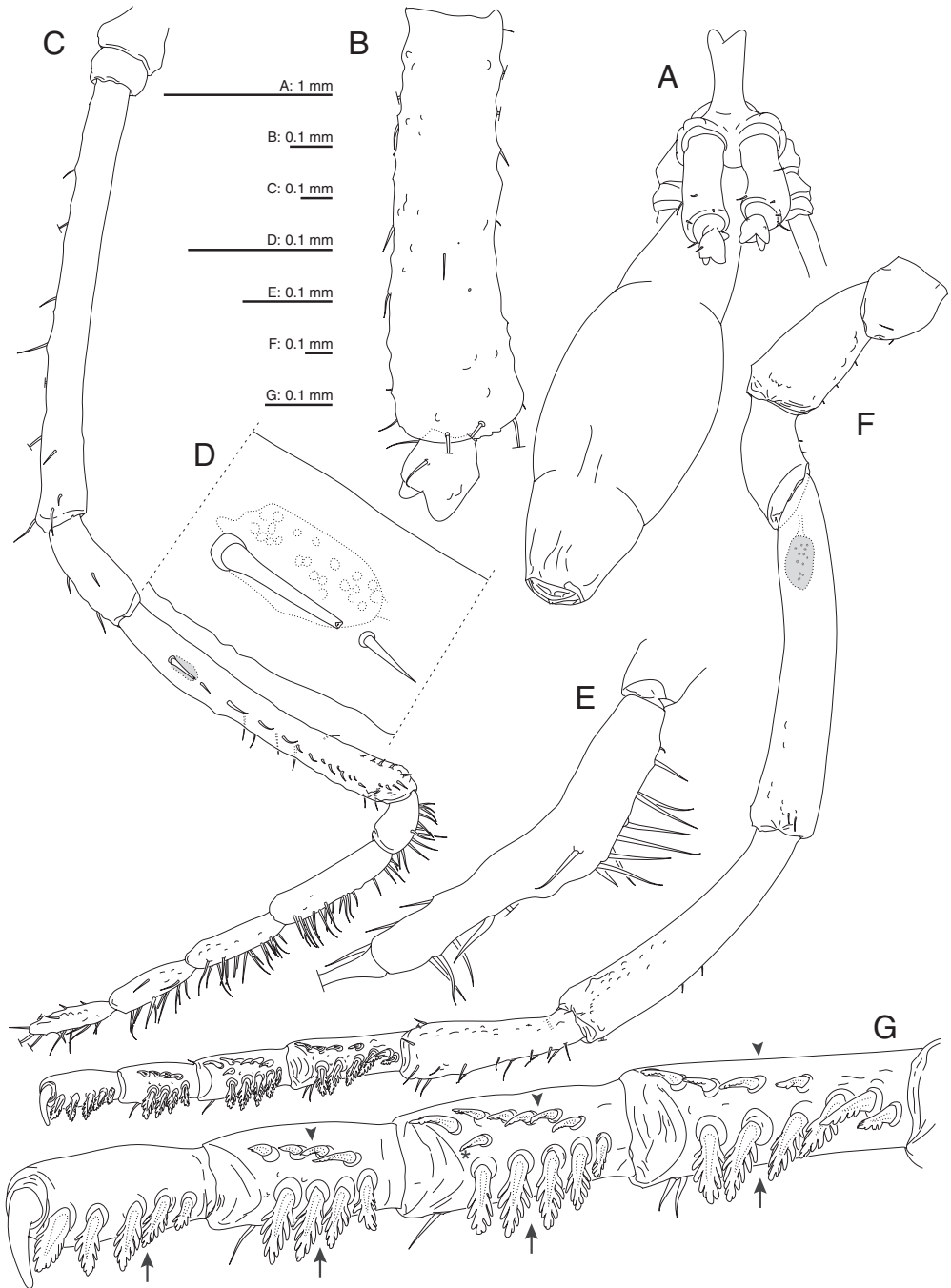


Fig. 6. *Ascorhynchus mariae* Turpaeva, 1971, female (ICHUM9133). **A.** Cephalon segment, anterior view. **B.** Right chelifore, dorsal view. **C.** Right palp, medial view. **D.** Right palp, internal organ of article 5, medial view. **E.** Left palp, distal article, anteromedial view. **F.** Right oviger, medial view. **G.** Right oviger, distal four articles, medial view. C, F, shaded areas, internal organs; D, enlargement showing spines and internal structure of organ; G, black arrowheads, dorsal row of compound spines; black arrows, ventral row of compound spines; asterisk, irregularly positioned compound spine.

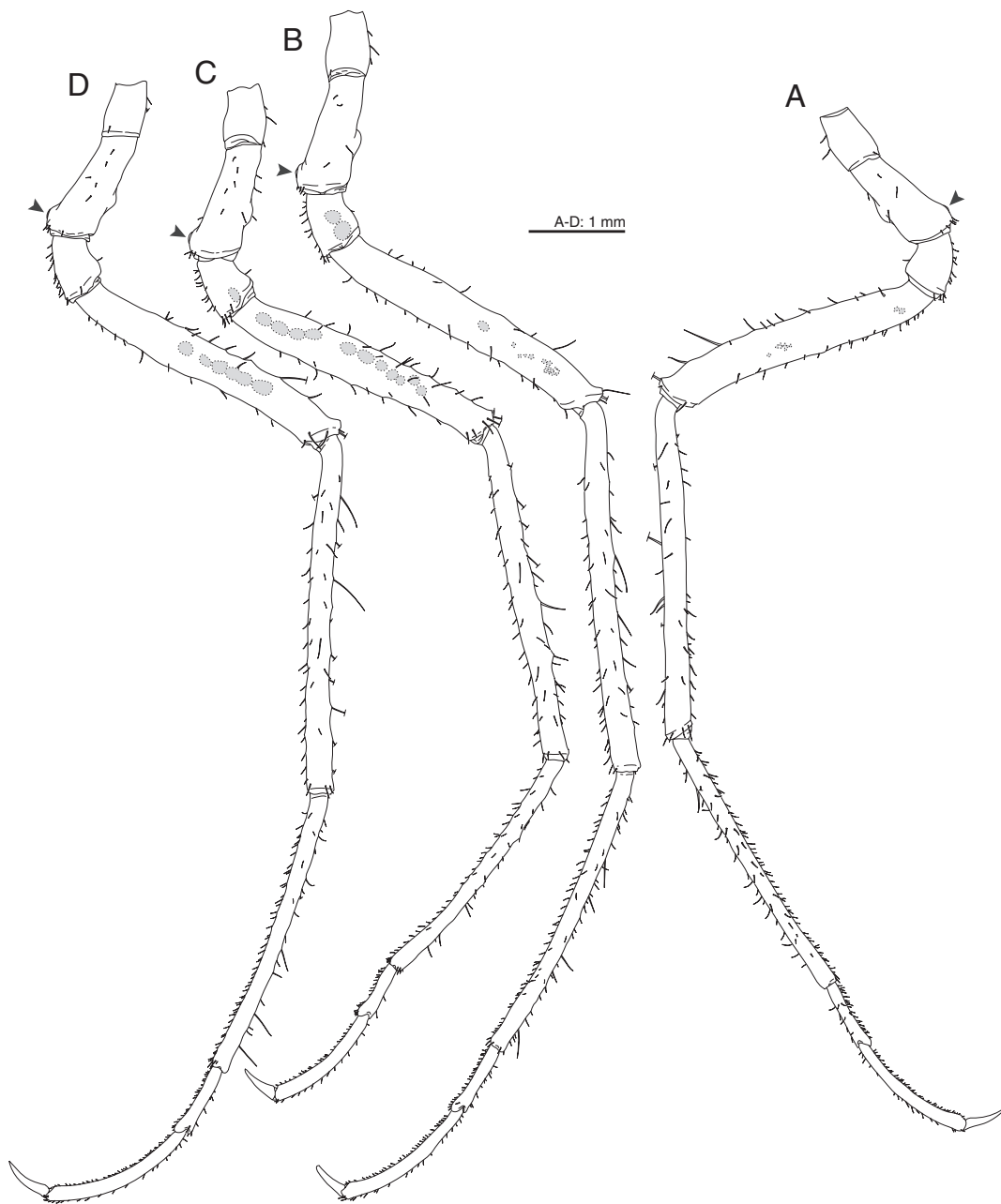


Fig. 7. *Ascorhynchus mariae* Turpaeva, 1971, female (ICHUM9133). A–D. Left leg 1, right legs 2–4, posterior views (shaded areas, oocytes; arrowheads, position of genital pore).

Genetic information. We determined partial 18S sequences (1701 bp; LC909291, LC909292) from the male and female specimens; the two sequences were identical. The sequence in the INSD most similar to ours, as determined by a BLAST search,

was from *Bathyzetes* sp. collected in New Caledonia (22°59.470' S, 167°42.770' E) (OQ065596, identity score 99 %, query cover 100 %; Sabroux et al. 2023). We determined a COI sequence (658 bp, encoding 219 amino acids; LC909293) from the female. The

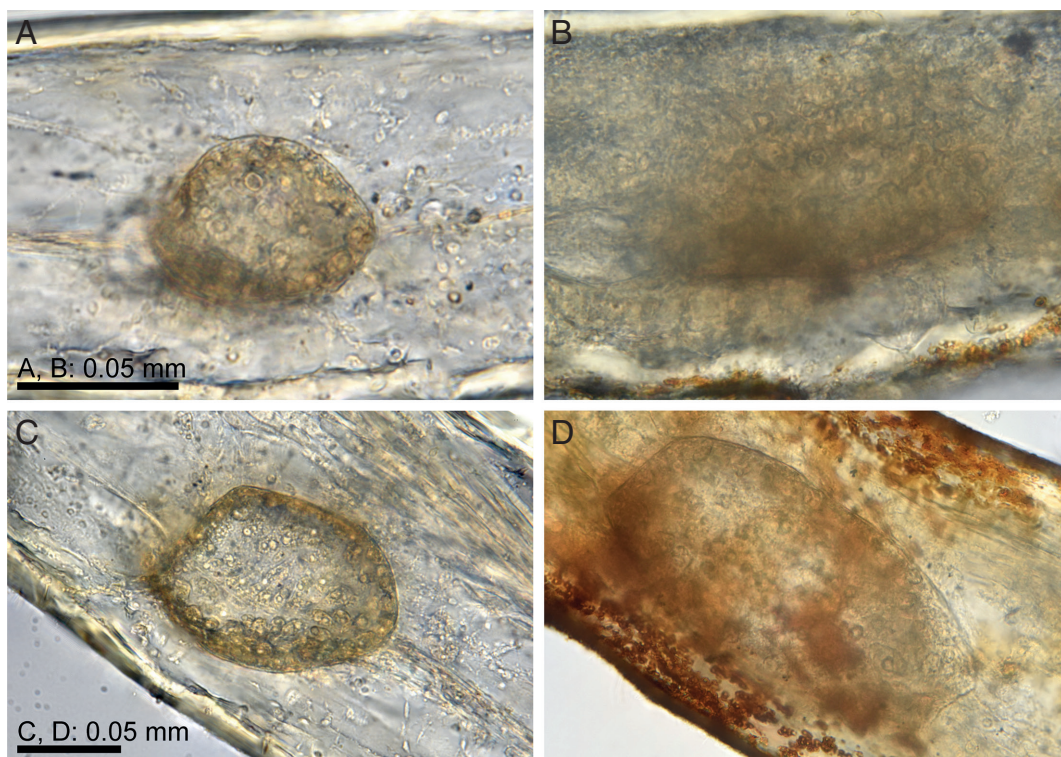


Fig. 8. *Ascorhynchus mariae* Turpaeva, 1971, male (ICHUM9132) (A,C); female (ICHUM9133) (B,D); both were ethanol fixed specimens. **A,B.** Internal organ, medial view, in article 5 of right palp. **C,D.** Internal organ, medial view, in article 4 of right oviger.

COI sequence in the INSD most similar to ours was from *Ascorhynchus hippos* Turpaeva, 1994 collected at 4469 m depth, off the south coast of Simushir Island (46°07.800' N, 152°10.300' E) (MW916507, identity score 90 %, query cover 92 %; Wolf et al. 2022).

Flatworm egg capsules and other epibionts.

Flatworm egg capsules (Fig. 9A–G) were attached via a stalk to the lateral surface of trunk segment 2 (Fig. 9B); dorsal surface of right leg 1, coxa 2 (Fig. 9C); anterodorsal surface of left leg 2, coxa 1 (Fig. 9D); and medial surface of chelifore scapes (Fig. 9E–G). The capsules were oval, 0.17 mm long and 0.09 mm wide (n=1), yellowish in ethanol; a white mass (embryo(s)?) was observed inside. A stalk (black arrowhead in Fig. 9F) was 0.03 mm long (n=1).

In addition, three empty capsule-like structures (Fig. 10) were found: one was attached to the female, on the right anterodorsal surface of trunk segment 3 (Fig. 10A); and the other two were attached to the male, on the left anteroventral surface of trunk segment 2 (Fig. 10B) and the posterodorsal surface

of trunk segment 4 near the basal part of abdomen (Fig. 10C), respectively. Aside from these structures, we observed foraminiferans and ciliophorans as epibionts on the pycnogonids.

We determined partial 18S (1786 bp; accession no. LC909289) and 28S (1634 bp; LC909288) sequences for the egg capsule attached to right leg 1, coxa 2 of the female sea spider; the amplification of the COI sequences failed. The most similar 18S sequence in the INSD was from *Ceratopora axi* (Riedl, 1954) collected in the lower intertidal zone, Vancouver Island, British Columbia, Canada (48°24.200' N, 123°21.050' W) (MF321746, identity score 95 %, query cover 100 %; Van Steenkiste & Leander 2018). The most similar 28S sequence was from *Ceratopora complicata* Van Steenkiste & Leander, 2018 collected in the lower intertidal zone, Vancouver Island, British Columbia, Canada (48°24.200' N, 123°21.050' W) (MF321757, identity score 89 %, query cover 100 %; Van Steenkiste & Leander 2018). The partial 18S (1786 bp; LC909290) sequence we determined for the egg capsule attached to left leg 2, coxa 1, was identical to LC909289 above.

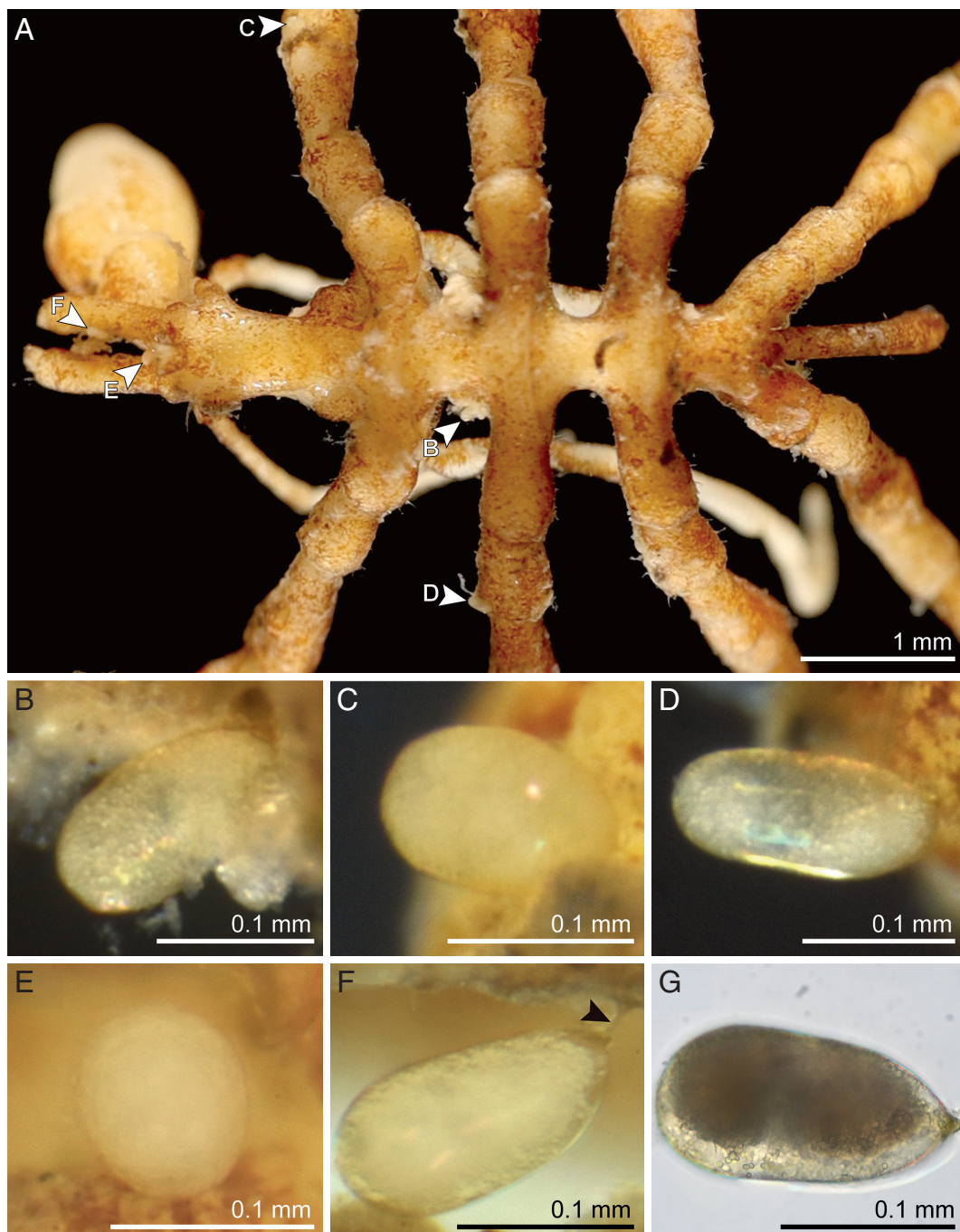


Fig. 9. Flatworm egg capsules on female *Ascorhynchus mariae* Turpaeva, 1971 (ICHUM9133). **A.** Egg-capsule attachment positions (white arrowheads) on sea spider, dorsal view, ethanol fixed specimen. **B–E.** Egg capsules attached to lateral surface of trunk segment 2, dorsal surface of right leg 1 coxa 2, anterodorsal surface of left leg 2 coxa 1, and medial surface of left chelifore scape, respectively (stereomicroscopic images). **F.** Egg capsule attached to medial surface of right chelifore scape (stereomicroscopic image), black arrowhead, stalk. **G.** Same, detached from sea spider (compound microscopic image).

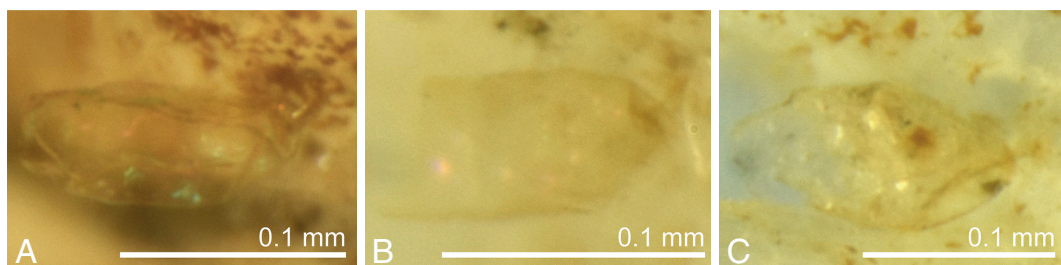


Fig. 10. Empty capsule-like structures on *Ascorhynchus mariae* Turpaeva, 1971 female (ICHUM9133) (A) and male (ICHUM9132) (B,C). A–C. Empty capsule-like structures attached to right anterodorsal surface of trunk segment 3, left anteroventral surface of trunk segment 2, and posterodorsal surface of trunk segment 4 near basal part of abdomen.

In the 18S+28S ML tree (Fig. 11), Trigonostomidae comprises *Parapharyngiella* Willems, Artois, Vermin, Backeljau & Schockaert, 2005 and a highly supported clade (SH-aLRT/UFBot = 99.6%/100%) containing the remaining trigonostomids. Within the latter, *Trigonostomum* Schmidt, 1852 is the sister group to a moderately supported clade (88.3%/95%) containing the remaining taxa, within which our flatworm species forms a moderately supported clade with *Ptychopera* (93.8%/79%), as the sister taxon to the latter.

The ranges in genetic distance for 18S between our flatworm egg capsule and four *Ptychopera* species were 5.9–6.8% (K2P) and 5.7–6.5% (p-distance) (Table 3); those for 28S were 14.8–18.2% (K2P) and 13.3–16.1% (p-distance) (Table 4). The ranges in genetic distance for 18S among the four *Ptychopera* species were 1.0–4.1% (K2P) and 1.0–3.9% (p-distance) (Table 3); those for 28S were 4.9–14.6% (K2P) and 4.7–13.2% (p-distance) (Table 4). The genetic distances among four *Ptychopera* species were lower than those between the four species and our flatworm.

Discussion

Our pycnogonids have an apically bifid ocular tubercle, which also occurs in *Ascorhynchus comatus* Child, 1992 and *A. laterospinus* Hedgpeth & Child,

1971. We identified our specimens as *A. mariae*, which differs from the latter two species in having a posterodorsal transverse setal row but lacking dorsomedian tubercles on each trunk segment, and in lacking long setae on the anterior and posterior margins of the lateral processes. *Ascorhynchus mariae* was previously known only from depths of 3145–3432 m in the Kuril–Kamchatka Trench. This study extended its known depth range (3685–3893 m) and showed that it also occurs in the Japan Trench.

The morphology of our male concurred well with the original description based on six individuals (Turpaeva 1971). Because Turpaeva (1971) illustrated only the body and a few appendages, we provided illustrations for the body and all appendages for both male and female individuals. We saw no marked sexual dimorphism, but found a sexual difference in the number of legs bearing the genital pore: only leg 4 in the male but legs 1–4 in the female.

Claw length did not differ among legs 1–4 or between the sexes in *A. mariae*, unlike congeners that have the leg-1 claw shorter or lacking, i.e., *A. armatus* (Wilson, 1881); *A. auchenicus* (Slater, 1879); *A. bacescui* Stock, 1975a; *A. cooki* Child, 1987; *A. cryptopygius* Ortmann, 1890; *A. dietheus* Child, 2002; *A. glaberimus* Schimkewitsch, 1913; *A. japonicus* Ives, 1891; *A. levissimus* Loman, 1908; *A. minutus* Hoek, 1881; *A. ovicoxa* Stock, 1975b; *A. pararmatus* Stock, 1975b; *A. parvituberculatus* Stock, 1953; *A. quartogibbus* Bam-

Table 3. Pairwise genetic distances (as percentages) for the 18S rRNA gene among the flatworm egg capsule in this study and four *Ptychopera* species, with K2P distances below the diagonal and p-distances above.

	Egg capsule	<i>P. japonica</i>	<i>Ptychopera</i> sp.	<i>P. unicornis</i>	<i>P. westbladi</i>
Egg capsule LC909289	–	6.5	6.0	5.7	6.1
<i>P. japonica</i> MF321751	6.8	–	3.9	2.8	3.6
<i>Ptychopera</i> sp. KC529420	6.3	4.1	–	3.4	3.1
<i>P. unicornis</i> MF321752	5.9	2.9	3.5	–	1.0
<i>P. westbladi</i> AY775770	6.4	3.7	3.2	1.0	–

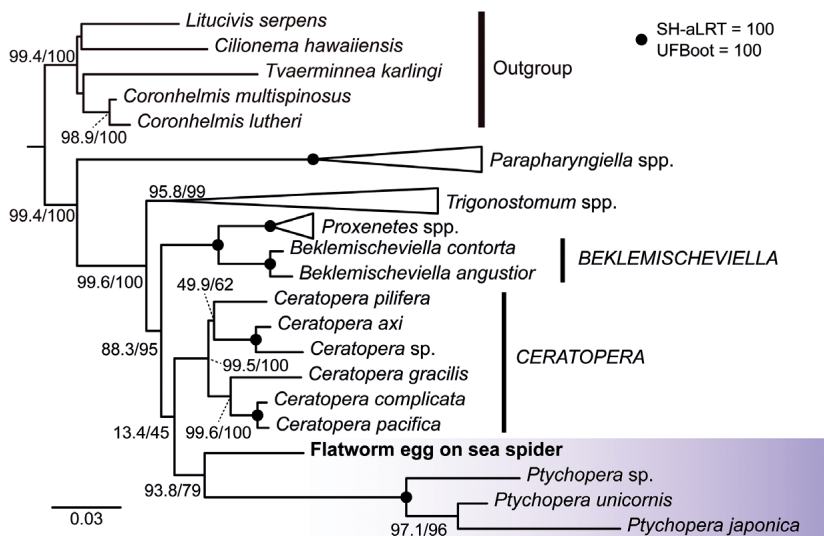


Fig. 11. Maximum-likelihood tree for trigonostomid rhabdocoels based on the 18S+28S dataset (3141 positions). Numbers near nodes are nodal support values as percentages, with values from the Shimodaira-Hasegawa-like approximate likelihood ratio test (SH-aLRT) left of the slash and ultrafast bootstrap (UFBoot) to the right. For nodal support among the outgroup taxa, only values of SH-aLRT $\geq 60\%$ and UFBoot $\geq 60\%$ are shown. The scale at the bottom indicates branch length in substitutions per site.

ber, 2013; *A. ramipes* (Böhm, 1879); *A. stocki* Hong & Kim, 1987; and *A. utinomii* Nakamura & Child, 1982 (Böhm 1879, Stock 1953, 1954, 1975a, 1975b, Nakamura & Child 1982, 1983, 1990, Child 1987, 2002, Hong & Kim 1987, Nakamura 1987, Bamber 2013). *Ascorhynchus mariae* differs from *A. breviscapus* Stock, 1968, which has a longer claw on leg 1 (Stock 1968).

We observed internal spherical/oval structures in palpal article 5 and ovigeral article 4 of the male and female *A. mariae* specimens (Figs 3B–D, 6C, D, F, 8A–D). Similar structures have previously been illustrated in several congeners (e.g., Child 1987, Nakamura 1987, Turpaeva 1994), but no possible functions were proposed. Future detailed anatomical observation is needed to elucidate their functions.

In our female, article 10 of the left and right palps differed morphologically. The one on the left (Fig. 6E) was longer than that on the right, weakly

bent at mid-length, and had a terminal claw. To our knowledge, the left and right articles in other *Ascorhynchus* species are similar to the right one in our female; the left one was likely abnormal.

We report the first case of flatworms utilizing sea spiders as an egg-laying substrate. We identified the flatworm molecularly as a member of Trigonostomidae (Fig. 11). In our phylogenetic tree, it formed a moderately supported clade with *Ptychopera*, but large genetic distances were observed between them (Tables 3, 4; Fig. 11). *Ptychopera* trigonostomids have been collected from the supralittoral to sublittoral zones (maximum depth 15 m), occurring in salt marshes, brackish pools, sandy or muddy sediments, and beds of mussels, seagrass, or algae (Beklemishev 1927, Den Hartog 1966, Ax 1971, 2008, Ehlers & Ax 1974, Karling 1974, Ax & Armonies 1990, Van Steenkiste & Leander 2018). The INSD currently lacks

Table 4. Pairwise genetic distances (as percentages) for the 28S rRNA gene among the flatworm egg capsule in this study and four *Ptychopera* species, with K2P distances below the diagonal and p-distances above.

	Egg capsule	<i>P. japonica</i>	<i>Ptychopera</i> sp.	<i>P. unicornis</i>	<i>P. westbladi</i>
Egg capsule LC909288	–	16.1	14.6	13.3	13.6
<i>P. japonica</i> MF321760	18.2	–	13.2	10.2	12.3
<i>Ptychopera</i> sp. KC529547	16.4	14.6	–	9.2	10.5
<i>P. unicornis</i> MF321761	14.8	11.0	9.8	–	4.7
<i>P. westbladi</i> KC529546	15.2	13.5	11.4	4.9	–

available sequences from six trigonostomid genera (*Brederveldia* Van der Velde & Van de Winkel, 1975; *Cryptostopera* Ehlers & Ax, 1974; *Feanora* De Clerck & Schockaert, 1995; *Lutheriella* Den Hartog, 1966; *Mahurubia* Willems, Artois, Vermin, Backeljau & Schockaert, 2005; *Petaliella* Ehlers, 1974), all of which have been reported from the intertidal to sublittoral zones (maximum depth: 1.5 m), occurring in salt marshes, mangrove areas, and sandy sediments (Den Hartog 1966, Ehlers 1974, Ehlers & Ax 1974, Van der Velde & Van de Winkel 1975, De Clerck & Schockaert 1995, Willems et al. 2005). The habitats of the above seven genera thus differ greatly from that of our species, and we concluded that our species could probably represent an undescribed genus. However, molecular data from genera not yet sampled is necessary to confirm this.

There are few previous reports of deep-sea, free-living flatworms, because their delicate bodies are easily damaged during sampling. Prior to this study, specimen-based records of free-living flatworms from depths greater than 3000 m were restricted to two species, the polyclad *Oligocladus voightae* Quiroga, Bolaños & Litvaitis, 2006, from 3232 m in the Escanaba Trough, northeastern Pacific (Quiroga et al. 2006) and the triclad *Maricola* sp. from 6176–6200 m in the Kuril–Kamchatka Trench, northwestern Pacific (Kakui & Tsuyuki 2024). This study represents the second deepest record for free-living flatworms.

Acknowledgements

We thank Shigeaki Kojima and Yasunori Kano for the opportunity to join R/V Hakuho-maru cruise KH-23-5, conducted as a part of the project “Comprehensive study of diversity and evolutionary mechanisms of deep-sea animals in trenches in the northwestern Pacific” supported by KAKENHI grant JP19H00999 from the Japan Society for the Promotion of Science (JSPS); Captain Naoto Sakai and the crew of R/V Hakuho-maru, technicians from Marine Work Japan and MOL Marine & Engineering, and researchers aboard for support in collecting; Yoshinobu Matsushima, for help with morphological observations; Kentaro Ageishi and Rintaro Ono for help with molecular work; and Matthew H. Dick for reviewing the manuscript and editing the English. This study was supported in part by the Atmosphere and Ocean Research Institute, The University of Tokyo, and KAKENHI grants JP19K06800, JP22H02681, and JP23K23944 from the JSPS.

References

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403–410.
- Ax, P. 1971. Zur Systematik und Phylogenie der Trigonostominae (Turbellaria, Neorhabdocoela). *Mikrofauna des Meeresbodens* 4: 1–84.
- Ax, P. 2008. Plathelminthes aus Brackgewässern der Nordhalbkugel. 696 pp., Stuttgart (Franz Steiner Verlag).
- Ax, P. & Armonies, W. 1990. Brackish water Plathelminthes from Alaska as evidence for the existence of a boreal brackish water community with circumpolar distribution. *Microfauna Marina* 6: 7–109.
- Bamber, R. N. 2002. Bathypelagic pycnogonids (Arthropoda, Pycnogonida) from the Discovery deep-sea cruises. *Journal of Natural History* 36: 715–727.
- Bamber, R. N. 2013. Deep-water Pycnogonida from recent cruises to Papua New Guinea and Melanesia, with an appendix of new records from Polynesia and descriptions of five new species. *Zoosystema* 35: 195–214.
- Beklemishev, V. 1927. Über die Turbellarienfauna der Bucht von Odessa und der in dieselbe mündenden Quellen. *Izvestiya Biologicheskogo Nauchno-Issledovatel'skogo Instituta i Biologicheskoy Stantsii pri Permskom Gosudarstvennom Universitete* 5: 177–207, pl. 1.
- Böhm, R. 1879. Über zwei neue von Herrn Dr. Hilgendorf in Japan gesammelte Pycnogoniden. *Sitzungsberichte der Gesellschaft Naturforschender Freunde zu Berlin* 4: 53–60.
- Child, C. A. 1979. Shallow-water Pycnogonida of the Isthmus of Panama and the coasts of Middle America. *Smithsonian Contributions to Zoology* 293: i–v, 1–86.
- Child, C. A. 1987. New and little known Pycnogonida from Antarctic and Subantarctic waters. *Proceedings of the Biological Society of Washington* 100: 902–916.
- Child, C. A. 2002. Some Pycnogonida from the Eastern (Hasa) District of Saudi Arabia. *Journal of Natural History* 36: 1805–1821.
- De Clerck, G. G. & Schockaert, E. R. 1995. Two peculiar new genera of Typhloplanoida from the Western Indian Ocean. *Hydrobiologia* 305: 3–9.
- Den Hartog, C. 1966. A preliminary revision of the *Proxenetes* group (Trigonostomidae, Turbellaria): supplement. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Series C* 69: 557–570.
- Diez, Y. L., Monnens, M., Wuyts, A., Brendonck, L., Reygel, P., Schmidt-Rhaesa, A. & Artois, T. 2023. Taxonomy and phylogeny of Dalytyphloplanida Willems et al., 2006 (Platyhelminthes: Rhabdocoela), with the description of a new family, a new genus, and sixteen new species from Cuba and Panama. *Organisms Diversity & Evolution* 23: 631–681.
- Dudnik, Z. & Kremenetskaia, A. 2025. Pycnogonida collection of the Shirshov Institute of Oceanology, Russian Academy of Sciences. *Biodiversity Data Journal* 13: e142496.

- Ehlers, U. 1974. Interstitielle Typhloplanoida (Turbellaria) aus dem Litoral der Nordseeinsel Sylt. Mikrofauna des Meeresbodens 49: 1–102.
- Ehlers, U. & Ax, P. 1974. Interstitielle Fauna von Galapagos. VIII. Trigonostominae (Turbellaria, Typhloplanoida). Mikrofauna des Meeresbodens 30: 1–33.
- Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.
- Giribet, G., Carranza, S., Baguña, J., Riutort, M. & Ribera, C. 1996. First molecular evidence for the existence of a Tardigrada + Arthropoda clade. Molecular Biology and Evolution 13: 76–84.
- Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology 59: 307–321.
- Hong, J.-S. & Kim, I.-H. 1987. Korean pycnogonids chiefly based on the collections of the Korea Ocean Research and Development Institute. The Korean Journal of Systematic Zoology 3: 137–164.
- Hosoda, Y. & Kakui, K. 2020. A new species of *Pantopietta* (Pycnogonida: Austrordecidae) from the North Pacific, with a note on the palp articulation. Marine Biology Research 16: 390–395.
- Hosoda, Y., Tomioka, S. & Kakui, K. 2018. First record of *Hemichela nanhaiensis* (Pycnogonida: Ammotheidae) from Japanese waters, with the first description of females. Species Diversity 23: 215–218.
- Hu, X.-Z., Chen, Y.-S., Zhong, L.-H., Xie, Y.-H., Feng, W.-T., Zhang, Y. & Wang, A.-T. 2019. Two new species of Rhabdocoela (Polycystididae and Trigonostomidae) from China. Zootaxa 4695: 351–366.
- Kakui, K., Fukuchi, J. & Shimada, D. 2021. First report of marine horsehair worms (Nematomorpha: *Nec-tonema*) parasitic in isopod crustaceans. Parasitology Research 120: 2357–2362.
- Kakui, K., Katoh, T., Hiruta, S. F., Kobayashi, N. & Kajihara, H. 2011. Molecular systematics of Tanaidacea (Crustacea: Peracarida) based on 18S sequence data, with an amendment of suborder/superfamily-level classification. Zoological Science 28: 749–757.
- Kakui, K. & Shimada, D. 2017. A new species of *Tanaopsis* (Crustacea: Tanaidacea) from Japan, with remarks on the functions of serial ridges and grooves on the appendages. Zootaxa 4282: 324–336.
- Kakui, K. & Shimada, D. 2022. Dive into the sea: first molecular phylogenetic evidence of host expansion from terrestrial/freshwater to marine organisms in Mermithidae (Nematoda: Mermithida). Journal of Helminthology 96: 1–7.
- Kakui, K. & Tsuyuki, A. 2024. Flatworm cocoons in the abyss: same plan under pressure. Biology Letters 20: 20230506.
- Karling, T. G. 1974. Turbellarian fauna of the Baltic Proper: identification, ecology and biogeography. Fauna Fennica 27: 1–101.
- Katsuragawa, Y., Shiraki, S., Shimomura, M. & Kakui, K. 2025. A new bopyrid species (Crustacea: Isopoda: Epicaridea: Bopyroidea) parasitic on a squat lobster from Japan, with molecular phylogenies for Epicaridea. Marine Biodiversity 55: 118.
- Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–120.
- Kumar, S., Stecher, G. & Tamura, K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874.
- Lenaers, G., Maroteaux, L., Michot, B. & Herzog, M. 1989. Dinoflagellates in evolution. A molecular phylogenetic analysis of large subunit ribosomal RNA. Journal of Molecular Evolution 29: 40–51.
- Lockyer, A. E., Olson, P. D. & Littlewood, D. T. J. 2003. Utility of complete large and small subunit rRNA genes in resolving the phylogeny of the Neodermata (Platyhelminthes): implications and a review of the cercomer theory. Biological Journal of the Linnean Society 78: 155–171.
- Melzer, R. R., Hübner, J. & Dietz, L. 2020. Deep-water Pycnogonida collected by the SOKHOBIO and KURAMBIO campaigns along the NW Pacific. Pp. 267–276 in: Saeedi, H. & Brandt, A. (eds). Biogeographic atlas of the deep NW Pacific fauna. Sofia (Pensoft).
- Miyazaki, K. 2022. A taxonomic list of sea spiders (Arthropoda: Pycnogonida) from Japanese waters. Zoological Science 39: 16–40.
- Müller, H.-G. 1993. World catalogue and bibliography of the recent Pycnogonida. 110 pp., Wetzlar, Germany (Wissenschaftlicher Verlag).
- Nakamura, K. 1987. The sea spiders of Sagami Bay. i-x, 40 pls, 43 (en), 35 (ja) pp., Tokyo (Biological Laboratory, Imperial Household).
- Nakamura, K. & Child, C. A. 1982. Three new species of Pycnogonida from Sagami Bay, Japan. Proceedings of the Biological Society of Washington 95: 282–291.
- Nakamura, K. & Child, C. A. 1983. Shallow-water Pycnogonida from the Izu Peninsula, Japan. Smithsonian Contributions to Zoology 386: i-v, 1–71.
- Nakamura, K. & Child, C. A. 1990. Pycnogonida of the western Pacific islands, VII. On some rare species from the Flores Sea, Indonesia. Proceedings of the Biological Society of Washington 103: 304–310.
- Nakamura, K., Suzuki, N., Kano, Y., Namatame, T. & Kosaku, A. 2007. 18S rRNA phylogeny of sea spiders with emphasis on the position of Rhynchothoracidae. Marine Biology 153: 213–223.
- Nakayama, T., Watanabe, S., Mitsui, K., Uchida, H. & Inouye, I. 1996. The phylogenetic relationship between the Chlamydomonadales and Chlorococcales inferred from 18SrDNA sequence data. Phycological Research 44: 47–55.
- Quiroga, S. Y., Bolaños, D. M. & Litvaitis, M. K. 2006. First description of deep-sea polyclad flatworms from the North Pacific: *Anocellidus* n. gen. *profundus* n. sp. (Anocellidae, n. fam.) and *Oligocladus voightae* n. sp. (Euryleptidae). Zootaxa 1317: 1–19.

- Sabroux, R., Corbari, L. & Hassanin, A. 2023. Phylogeny of sea spiders (Arthropoda: Pycnogonida) inferred from mitochondrial genome and 18S ribosomal RNA gene sequences. *Molecular Phylogenetics and Evolution* 182: 107726.
- Sabroux, R., Hassanin, A. & Corbari, L. 2022. Sea spiders (Arthropoda: Pycnogonida) collected during the Madibenthos Expedition from Martinique shallow waters. *European Journal of Taxonomy* 851: 1–141.
- Sekiguchi, S. & Kakui, K. 2026. Supplementary materials from “Abyssal cradle: flatworm egg capsules on a deep-sea sea spider, with an integrative redescription of *Ascorhynchus mariae* Turpaeva, 1971”. figshare. <https://doi.org/10.6084/m9.figshare.31157608>
- Sekiguchi, S., Kohtsuka, H. & Kakui, K. 2025. First record of the deep-sea sea spider *Bathypallenopsis californica* (Arthropoda: Pycnogonida) from the northwestern Pacific, with a note on an attached crinoid. *Zoological Science* 42: 335–341.
- Shiraki, S., Kakui, K., Along, A., Calagui, L., Calagui, S. I., Demetillo, M., Kajihara, H., Leones, J. A., Rojas-Salinas, A., Reyes, K. A. S.-D. L., Seronay, R., Tsuyuki, A. & Abato, J. 2025. Taxonomic revision of *Sauranthura* (Isopoda: Anthuroidea: Anthuridae), with the description of *Sauranthura liangaensis* sp. nov. from Surigao del Sur, Philippines. *Marine Biology Research* 21: 206–220.
- Stock, J. H. 1953. Contribution to the knowledge of the pycnogonid fauna of the East Indian Archipelago. *Temminckia* 9: 276–313.
- Stock, J. H. 1954. Pycnogonida from Indo-West-Pacific, Australian, and New-Zealand waters. *Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening i Kjøbenhavn* 116: 1–168.
- Stock, J. H. 1968. Pycnogonida collected by the Galathea and Anton Bruun in the Indian and Pacific Oceans. *Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening i Kjøbenhavn* 131: 7–65.
- Stock, J. H. 1975a. Infralittoral Pycnogonida from Tanzania. *Travaux du Muséum d'Histoire Naturelle 'Grigore Antipa'* 16: 127–134.
- Stock, J. H. 1975b. Pycnogonida from the continental shelf, slope and deep sea of the tropical Atlantic and East Pacific. *Bulletin of Marine Science* 24: 957–1092.
- Turpaeva, E. P. 1971. The deep-water Pantopoda collected in the Kuril–Kamchatka Trench. *Trudy Instituta Okeanologii P. P. Shirshov* 92: 274–291.
- Turpaeva, E. P. 1994. Sea spiders (Pycnogonida) from the North Pacific Basin. *Trudy Instituta Okeanologii P. P. Shirshov* 131: 126–138.
- Van der Velde, G. & Van de Winkel, J. L. M. W. 1975. *Brederveldia bidentata* gen. n., sp. n., a new species of the family Trigonostomidae (Turbellaria, Neorhabdocoela) from the Netherlands. *Bulletin Zoologisch Museum Universiteit van Amsterdam* 4: 191–196.
- Van Steenkiste, N. W. L. & Leander, B. S. 2018. Molecular phylogeny of trigonostomine turbellarians (Platyhelminthes: Rhabdocoela: Trigonostomidae), including four new species from the Northeast Pacific Ocean. *Zoological Journal of the Linnean Society* 182: 237–257.
- Van Steenkiste, N. W. L., Tessens, B., Willems, W., Backeljau, T., Jondelius, U. & Artois, T. 2013. A comprehensive molecular phylogeny of Dalytyphloplanida (Platyhelminthes: Rhabdocoela) reveals multiple escapes from the marine environment and origins of symbiotic relationships. *PLoS ONE* 8: e59917.
- Van Steenkiste, N. W. L., Wakeman, K. C., Söderström, B. & Leander, B. S. 2023. Patterns of host–parasite associations between marine meiofaunal flatworms (Platyhelminthes) and rhytidocystids (Apicomplexa). *Scientific Reports* 13: 21050.
- Wambreuse, N., Hamel, J.-F. & Mercier, A. 2021. Epibiotic associations with the deep-sea pycnogonid *Nymphon hirtipes* Bell, 1855 as a source of biodiversity. *Deep-Sea Research Part I: Oceanographic Research Papers* 178: 103660.
- Whiting, M. F. 2002. Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera. *Zoologica Scripta* 31: 93–104.
- Willems, W., Artois, T., Vermin, W., Backeljau, T. & Schockaert, E. 2005. ‘Typhloplanoida’ (Platyhelminthes: Rhabdocoela) from the Indian Ocean, with the description of six new taxa. *Journal of Natural History* 39: 1561–1582.
- Willems, W. R., Wallberg, A., Jondelius, U., Littlewood, D. T. J., Backeljau, T., Schockaert, E. R. & Artois, T. J. 2006. Filling a gap in the phylogeny of flatworms: relationships within the Rhabdocoela (Platyhelminthes), inferred from 18S ribosomal DNA sequences. *Zoologica Scripta* 35: 1–17.
- Wolf, L., Melzer, R. R. & Lehmann, T. 2022. Rediscovery after 25 years – first photographic documentation and DNA barcoding of the deep-sea pycnogonid species *Ascorhynchus hippos* Turpaeva, 1994 (Chelicerata, Pycnogonida, Ascorhynchidae) from the Kuril–Kamchatka Trench. *Zoosystematics and Evolution* 98: 257–262.