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A new parasitic barnacle (Crustacea, Cirripedia, Rhizocephala, *Mycetomorpha*) from the abyssal zone in the northwestern Pacific

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Abstract

I describe the parasitic barnacle *Mycetomorpha abyssalis* **sp. nov.** from the crangonid shrimp *Sclerocrangon zenkevitchi* collected from 3893–3890 m depth off the eastern coast of Iwate, Japan, northwestern Pacific. This is the first *Mycetomorpha* rhizocephalan from the abyssal zone and the third species in *Mycetomorpha. Mycetomorpha abyssalis* **sp. nov.** differs from its congeners *M. vancouverensis* and *M. albatrossi* in (1) triangular shield lacking, (2) stalk one-quarter of length from posterior end of externa, (3) mantle opening clearly anterior to stalk, (4) different host genus, and (5) depth range much deeper. I determined partial sequences for the mitochondrial cytochrome c oxidase subunit I (COI) and 16S rRNA genes and nuclear 18S rRNA and 28S rRNA genes from *M. abyssalis* **sp. nov.** for future DNA barcoding and phylogeny. Kimura 2-parameter distances between *M. abyssalis* **sp. nov.** and *M. vancouverensis* were 21.2% (16S), 0.6% (18S), and 1.5% (28S).

Key Words

Caridea, deep sea, integrative taxonomy, mesoparasite, parasite, turbo taxonomy

Introduction

Mycetomorpha Potts, 1912, the sole genus in the rhizocephalan barnacle family Mycetomorphidae, contains the two species Mycetomorpha vancouverensis Potts, 1912 and Mycetomorpha albatrossi Høeg & Rybakov, 1996 (Høeg and Rybakov 1996). These utilize crangonid shrimps as hosts and have been reported only from the northern Pacific, at depths shallower than 300 m (Potts 1912; Butler 1955, 1980; Høeg and Rybakov 1996; Sloan et al. 2001; Wheeler and McIntosh 2021; Eibye-Jacobsen et al. 2024; GBIF 2024; Orrell and Informatics Office 2024) (Fig. 1). The two species differ in external morphology (Høeg and Rybakov 1996) and utilize host shrimps in different genera: Neocrangon communis (Rathbun, 1899) for M. vancouverensis; Metacrangon variabilis (Rathbun, 1902) and Metacrangon acclivis (Rathbun, 1902) for M. albatrossi. Molecular phylogenetic analyses (Høeg et al. 2020; Korn et al. 2020) have suggested that Mycetomorphidae is closely related to the family Peltogastridae. Here I describe a new *Mycetomorpha* species based on one individual parasitic on the crangonid *Sclerocrangon zenkevitchi* Birshtein & Vinogradov, 1953 from the abyssal zone in the Japan Trench, northwestern Pacific. This is the first abyssal record for *Mycetomorpha*. Additionally, I provide partial sequences for its cytochrome *c* oxidase subunit I (COI), 16S rRNA, 18S rRNA, and 28S rRNA genes for DNA barcoding and future phylogenetic analyses.

Methods

The host shrimp *Sclerocrangon zenkevitchi* (identified by Tomoyuki Komai; Natural History Museum and Institute, Chiba) was collected with a beam trawl on 29 September 2023 during cruise KH-23-5 of R/V *Hakuho-maru* (Japan Agency for Marine-Earth Science and Technology; JAMSTEC), at Station F2 (39°28.555'N, 143°47.347'E to 39°27.934'N, 143°47.240'E), depth 3893–3890 m. The fresh shrimp was photographed.

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Figure 1. Map showing the global distribution of *Mycetomorpha*. Bathymetric contour intervals are 1000 m, with thicker contour lines every 2000 m. The map and plots were generated with GMT6 software (Wessel et al. 2019) based on data publicly available from ETOPO1 (Amante and Eakins 2009; NOAA National Geophysical Data Center 2009).

Pleonite 1 bearing the parasite was removed from the body with scissors and placed in a petri dish. Soft tissue from host pleonite 1 was recovered, and fixed and preserved in 99% ethanol. The pleonite-1 exoskeleton penetrated by the parasite stalk was photographed. The parasite and surrounding pleonite-1 exoskeleton were removed and photographed. Some lobes of the parasite were detached, and fixed and preserved in 99% ethanol. The remaining portions of the host and parasite were fixed and preserved in 80% ethanol. The fixed parasite was observed with a Nikon SMZ1500 stereomicroscope; it was not sectioned, in order to retain the option for future non-destructive observation. The material examined in this study is deposited in the Natural History Museum and Institute, Chiba, Japan, catalogued under the acronym CBM-ZC.

The terms for orientation (anterior, posterior, left, right, dorsal, ventral) used herein for the parasite's externa correspond to those for the host ("dorsal" herein corresponds to the "upper" or "stalk side" in Høeg and Rybakov 1996). Externa length was measured from the anterior to posterior ends, lobes excluded; externa width was measured at the widest portion, lobes excluded. The carapace length (cl) of the host was measured from the orbital margin to the midpoint of the posterodorsal margin of the carapace.

Total DNA was extracted from several lobes of the parasite and from pleonal muscle of the host shrimp by using a NucleoSpin Tissue XS Kit (Macherey-Nagel, Germany). For the COI gene, primers LCO1490 and HCO2198 (Folmer et al. 1994) were used for PCR amplification and cycle sequencing (CS). For the 16S gene, primers 16sar-L and 16sbr-H (Palumbi et al. 2002) were used for amplification and CS. For the 18S gene, primers SR1 and SR12 (Nakayama et al. 1996) were used for amplification, and primers SR3, 18S-b3F, 18S-b4F, 18S-b4R, 18S-a4R, 18S-b5F, 18S-b6F, 18S-a6R, and 18S-b8F (Nakayama et al. 1996; Kakui et al. 2011, 2021; Kakui and Shimada 2017, 2022; Kakui and Hiruta 2022) for CS. For the 28S gene, primers 300F and L1642 (Lockyer et al. 2003) were used for amplification, and primers 28S-Rd4.2b, 300F, 900F, and U1148 (Whiting 2002; Lockyer et al. 2003) for CS. Amplification conditions for COI and 16S with TaKa-Ra Ex Taq DNA polymerase (TaKaRa Bio, Japan) were

94 °C for 1 min; 35 cycles of 98 °C for 10 s, 50 °C (COI) or 42 °C (16S) for 30 s, and 72 °C for 50 s; and 72 °C for 2 min. Conditions for 18S and 28S with KOD FX Neo (Toyobo, Japan) were 94 °C for 2 min; 45 cycles of 98 °C for 10 s, 65 °C (18S) or 52 °C (28S) for 30 s, and 68 °C for 75 s; and 68 °C for 3 min. All nucleotide sequences were determined with a BigDye Terminator Kit ver. 3.1 and 3730 DNA Analyzer (Life Technologies, USA). Fragments were concatenated by using MEGA7 (Kumar et al. 2016). The sequences determined in this study were deposited in the International Nucleotide Sequence Database (INSD) through the DNA Data Bank of Japan (DDBJ).

The 16S, 18S, and 28S sequences from the new species were aligned individually with homologs from *M. vancouverensis* (16S, 534 bp, MH974513; 18S, 1757 bp, MH974514; 28S, 682 bp, MH974515; Høeg et al. 2019) by using MUSCLE (Edgar 2004) and trimmed to the shortest length between them (16S, 494 bp; 18S, 1757 bp; 28S, 683 bp) after alignment. Kimura's (1980) 2-parameter (K2P) distance between the two species was calculated with MEGA7 for each gene.

Taxonomy

Family Mycetomorphidae Høeg & Rybakov, 1992

New Japanese name: ミノフクロムシ科 (Mino-fukuromushi-ka)

Genus Mycetomorpha Potts, 1912

New Japanese name: ミノフクロムシ属 (Mino-fukuromushi-zoku)

Mycetomorpha abyssalis sp. nov.

https://zoobank.org/9C0FBC4F-5779-4100-BDC9-8D585C1A4160 Figs 2-4

New Japanese name: $\forall \uparrow \forall \land \forall \land \forall \land \forall \land \forall \land \forall$ (Meifu-no-mino-fukuromushi)

Etymology. The specific name *abyssalis* (Latin: abyssal) is an adjective referring to the collection of this species from an abyssal depth.

Type host. Sclerocrangon zenkevitchi Birshtein & Vinogradov, 1953 (Decapoda: Caridea: Crangonidae).

Attachment site. Pleonite 1 sternite.

Type locality. Off the eastern coast of Iwate, Japan, northwestern Pacific $(39^{\circ}28.555'N, 143^{\circ}47.347'E \text{ to } 39^{\circ}27.934'N, 143^{\circ}47.240'E)$, depth 3893–3890 m.

Material examined. Holotype, female (CBM-ZC 17789), one vial, ex. *S. zenkevitchi* (cl 26.7 mm; CBM-ZC 17788), collected on 29 September 2023 at the type

locality, R/V *Hakuho-maru* cruise KH-23-5, coll. by Keiichi Kakui.

Representative DNA sequences. One sequence each was determined from the holotype (CBM-ZC 17789) for COI (INSD accession number LC799150; 637 bp, encoding 212 amino acids), 16S (LC799151; 490 bp), 18S (LC799152; 1826 bp), and 28S (LC799153; 1169 bp).



Figure 2. Mycetomorpha abyssalis sp. nov., holotype, attached to the host, Sclerocrangon zenkevitchi Birshtein & Vinogradov, 1953, fresh specimen. Scale bar: 10 mm.



Figure 3. *Mycetomorpha abyssalis* sp. nov., holotype, fresh specimen. A. Habitus, parasitic on the host, ventral view; B, C. Habitus, ventral (B) and dorsal (C) views; ant – anterior; lef – left; man – mantle opening; rig – right; pos – posterior; sta – stalk; tub – tubular lobe. Scale bars: 10 mm.



Figure 4. *Mycetomorpha abyssalis* sp. nov., holotype, showing stalk penetrating the host pleonite-1 sternite (soft tissue in pleonite 1 removed), anterodorsal view; no triangular shield observed. **ant** – anterior; **pos** – posterior; **sta** – stalk. Scale bar: 10 mm.

One sequence each was determined from the host (CBM-ZC 17788) for COI (LC799154; 658 bp, encoding 219 amino acids) and 18S (LC799155; 1846 bp).

Description of female holotype. Externa (Figs 2, 3) 16.6 mm in length, thinner than broad, a little over twice as long as maximum width (8.1 mm), rounded at ends, pale yellow (faded in ethanol, slightly yellowish); except dorsal and anteroventral regions, externa surface covered with short lobes; filled with developing embryos. Root system not observed. Stalk short, cylindrical, at one-quarter of length from posterior end of externa. Triangular shield lacking (Figs 3C, 4). Anterior, middle, and ventral lobes short, and ovoid or digitiform; posterior lobes short and branched. Tubular lobe anterior to stalk, at one-fifth of length from anterior end of externa, arising from right margin of externa, with mantle opening at tip; mantle opening anterior to stalk.

Distribution. Presently known only from the type locality.

Discussion

Mycetomorpha abyssalis sp. nov. is the third species described in this genus. The three congeners are morphologically similar to one another, but *M. abyssalis* sp. nov. differs from the others in (1) lacking a triangular shield (present in *M. vancouverensis*), (2) the location of the stalk at one-quarter the length from the posterior end of the externa (one-third in *M. albatrossi*), and (3) the mantle opening clearly anterior to the stalk (to the right of the stalk in *M. vancouverensis*; slightly anterior to the stalk in *M. albatrossi*) (Potts 1912; Høeg and Rybakov 1996). However, because the shape of the externa can vary ontogenetically (e.g., the size of externa, the distribution and

size of lobes; cf. Høeg and Rybakov 1996: fig. 1), these morphological differences should be treated with caution.

The genus of host shrimps is different among three species: M. vancouverensis, M. albatrossi, and M. abyssalis sp. nov. utilize the crangonid genera Neocrangon, Metacrangon, and Sclerocrangon, respectively. The depth range of 3893-3890 m recorded for M. abyssalis sp. nov. is far deeper than for the others (240 m or shallower for M. vancouverensis; 291 m or shallower for M. albatrossi; Høeg and Rybakov 1996; Wheeler and McIntosh 2021). The known depth range for S. zenkevitchi (2995–4070 m; Komai and Komatsu 2009) does not overlap those for N. communis (16-1537 m; Komai and Komatsu 2009), M. variabilis (92-1271 m; Komai 2012), and M. acclivis (146.3-486.5 m; Rathbun 1902). These differences in host group and vertical distribution of parasites and hosts support the conclusion that the specimen from Japan is not conspecific with either M. vancouverensis or M. albatrossi.

I determined COI, 16S, 18S, and 28S sequences of M. abyssalis sp. nov., and sequences for the last three genes were available for M. vancouverensis. K2P distances between two species were 21.2% (16S), 0.6% (18S), and 1.5% (28S). Noever et al. (2016) found K2P distances for 16S between two Briarosaccus species (Rhizocephala, Peltogastridae) in the range of 4.3-4.6%, suggesting the above difference in 16S may correspond to interspecific variation. In a BLAST search (Altschul et al. 1990), the COI sequence most similar to my sequence was from the insect Rhodopsalta cruentata (Fabricius, 1775) (MZ470333; identity score 73.85%, query cover 100%; Bator et al. 2021), a misleading result likely due to the lack of any congeneric COI sequences in INSD (cf. Kakui and Hiruta 2022). A BLAST search with the "Organism" option set to "Rhizocephala" selected Sacculina granifera Boschma, 1973 (DQ059779; identity score 72.64%, query cover 98%; Gurney et al. 2006) as the most similar sequence, but again the identity score was low. If congeneric sequences become available, COI sequences, which seem to evolve faster than 16S sequences (cf. Noever et al. 2016; Jung et al. 2021), will likely be a useful tool for Mycetomorpha taxonomy.

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