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Cover Page Footnote
We would like to express our sincere thanks to the captain and crews of TRV Toyoshio-maru, Hiroshima University, for their cooperation at sea. This study was partially supported by a grant-in-aid of the Japan Society of Promotion for Science awarded to SO (KAKEN 16K07825).

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Some Observations of Morphology and Behavior of a Hyperbenthic Misophrioid Copepod

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Abstract.—The locomotion, feeding, excretion, and oviposition of a member of the copepod family Misophriidae were observed based on a live specimen collected from a sandy bottom at a depth of 52 m off Nagannu Island, Okinawa, Japan. This species is related to *Arcticomisophria* Martínez Arbizu and Seifried, 1996 in the armature of leg 1, but the fifth leg is much more reduced. The combination of morphological characters strongly suggests that it represents an undescribed genus. The maxillipeds played a major role in attaching to the bottom and in crawling, while the antennae and mandibular palps were involved in slow swimming along the bottom. It fed on small-sized cultured phytoplankters, and excreted numerous fecal pellets. The female carried 4-5 eggs of 0.09 mm diameter that were loosely attached to the urosome. Nearly complete nuclear 18S and 28S rRNA gene sequences and a partial mitochondrial cytochrome *c* oxidase subunit 1 (CO1) gene sequence were obtained and are made available for future phylogenetic and systematic work.

The order Misophrioida is a compact podoplean order of the subclass Copepoda, and consists of three families accommodating 17 genera and 36 species (Walter and Boxshall 2017). The group exhibits numerous plesiomorphies in appendages, segmentation, and armature (Huys and Boxshall 1991; Boxshall and Halsey 2004). Misophrioids are exclusively distributed in deep/shallow hyperbenthic layers, the deep-sea, and anchialine caves, as free-living forms (Boxshall 1983; Boxshall and Iliffe 1986, 1987; Ohtsuka et al. 1992; Boxshall and Jaume 2000; Boxshall and Halsey 2004; Boxshall et al. 2014). Gurney (1933) established the order based on the body plan of adults and the peculiarly abbreviated life cycle, although Sars (1911) had formerly classified it as an aberrant family of another podoplean order, the Harpacticoida. Subsequently, Huys and Boxshall (1991) clearly recognized the Misophrioda as a robust taxon, although it is difficult to define by synapomorphies.

The biology and ecology of the Misophrioida have been poorly understood, mainly because of the difficulty of collection from their habitats, and their relatively low abundance

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in comparison with planktonic taxa such as the Calanoida and Cyclopoida. The only exception is the bathypelagic genus *Benthomisophria* Sars, 1909, in which the internal anatomy, post-naupliar development, specialized feeding organs, and feeding were well studied using deep-sea plankton samples (<4,000 m deep) (Boxshall and Roe 1980; Boxshall 1982, 1984). Sars (1911) observed the swimming behavior and the presence of an "ovisac" in the shallow-water hyperbenthic *Misophria pallida* Boeck, 1864. Gurney (1933) briefly described the naupliar and first copepodid stages of the species, and also confirmed the oviposition. The biological and ecological aspects of cavernicolous taxa remain unknown.

During our surveys on hyperbenthic copepods in subtropical regions of Japan, we found a live specimen of an undescribed misophrioid copepod (family Misophriidae) in a dredge sample collected from off Okinawa, southwestern Japan. The locomotion, feeding, excretion, and oviposition of the misophrioid were observed and are reported herein. In addition, we have succeeded in extracting and sequencing DNA of this misophrioid (Fig. 1), and the sequences were registered in the International Nucleotide Sequence Database Collaboration (INSDC) to be available for future phylogenetic and systematic work.

Materials and Methods

The live specimen of the undescribed taxon of the family Misophriidae (Fig. 1A) was collected from a depth of 52 m on sandy bottom off Nagannu Island, Okinawa (26°14.339′N, 127°32.280′E) during daytime (local time 13:52-14:17) on May 21, 2016. Sediment collections were carried out with a dredge (mouth 50 cm wide × 15 cm high; mesh size 5 mm) towed along the bottom twice by Hiroshima University's Training Research Vessel (TRV) *Toyoshio-maru*. Sediments were stirred up in sea-water, and the supernatant was filtered through a plankton net (mesh size 0.1 mm). The obtained specimen was kept in a lidded Tupperware container (200 mL) full of filtered sea-water at room temperature onboard and at ca. 20°C in the laboratory. It was adequately fed three species of cultured phytoplankters daily: diatom *Chaetoceros calcitrans* (Paulsen) Takano, 1968 (approximate cell size 6.8 μm × 4.8 μm); prasinophyte *Tetraselmis tetrathele* (West) Butcher, 1959 (14.8 μm × 9.2 μm); and eustigmatophyte *Nannochloropsis oculata* (Droop) Hibberd, 1981 (3.0 μm). Filtered sea-water was changed daily. Fecal pellets produced by the female were collected from the bottom of the Tupperware container with a fine pipette, fixed in 10% neutralized formalin-seawater, and measured.

The behavior of the live specimen was observed in a Costar cell plate and documented with a Sony Handycam HDR-CX550 video camera attached to an Olympus SZ-X7 dissecting microscope. The body and fecal pellets were measured with an Olympus DP-20 CCD camera attached to the same dissecting microscope. The misophrioid became moribund on June 6, 2016 (laboratory survival = 17 days), and was then fixed in 99.5% ethanol for genetic analysis.

After treatment for the genetic analysis described below, the specimen (exoskeleton only) was dissected and examined in lactophenol with a Nikon Optiphoto differential interference microscope. The urosome and appendages were mounted on two glass slides for morphological observation. The dissected specimen is deposited in the Kitakyushu Museum of Natural History and Human History, Japan (KMNH IvR 500959). Terminology used in descriptions follows Huys and Boxshall (1991).

Total DNA was extracted from the 99.5% ethanol preserved specimen using the DNeasy Blood and Tissue Kit (Qiagen, USA) following the manufacture's protocol with minor

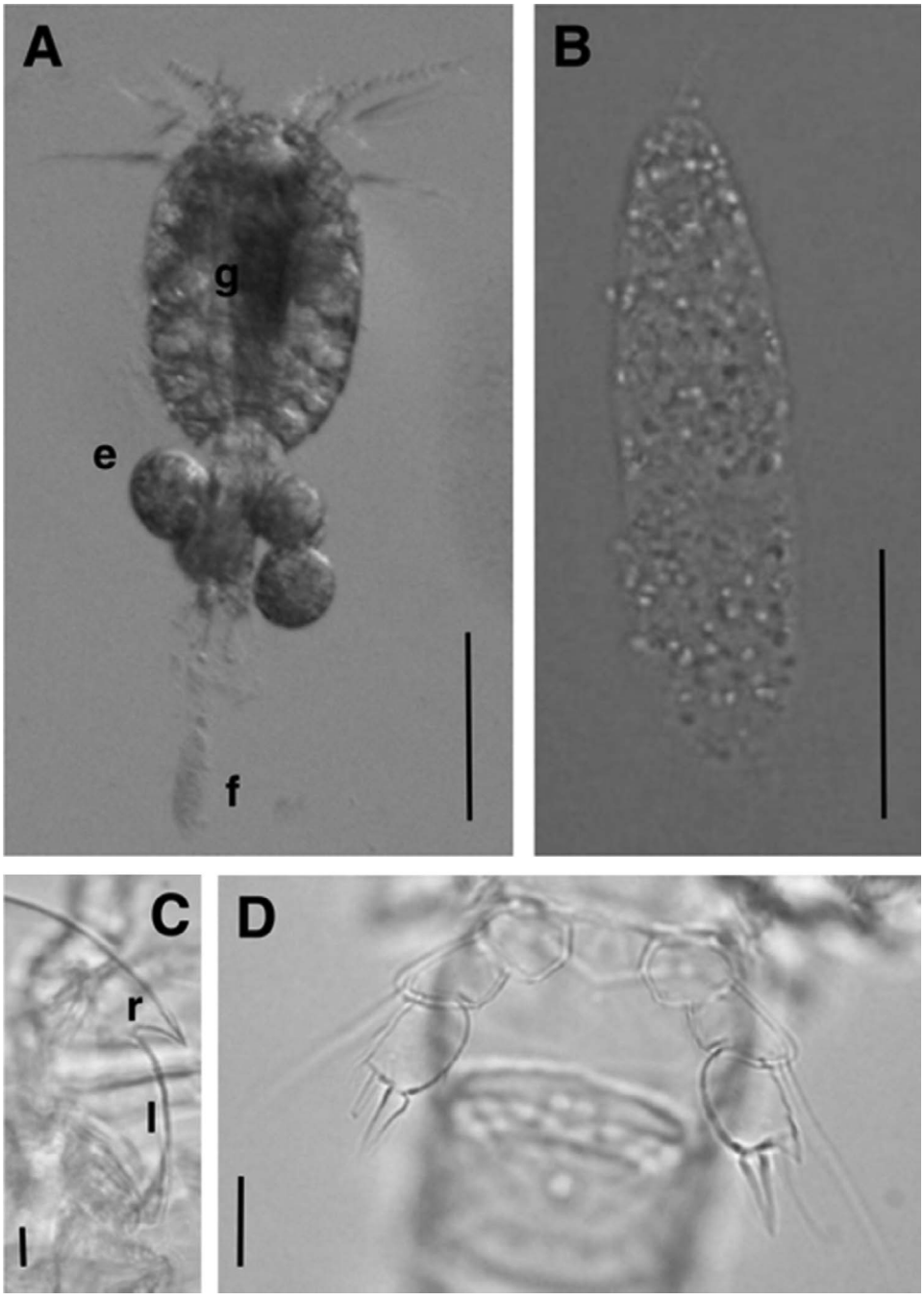

Fig. 1. Photographs of the ovigerous female of an undescribed genus of Misophriidae. (A) Habitus, dorsal view (e: egg mass, f: fecal pellet, g: gut full of cultured microalgae); (B) Fecal pellet fixed in 10% neutralized formalin-seawater; (C) Rostrum (r) and labrum (l), lateral view; (D) Leg 5, ventral view. Scale bars = 0.2 mm (A); 0.05 mm (B); 0.02 mm (C, D).

changes (elution volume = 100 μl). The whole body of the single specimen was put in a 1.5 ml microtube and protein was digested by a Proteinase K solution for 10 h at 56°C. After the protein digestion step, the chitinous exoskeleton was retrieved from the microtube and mounted as a morphological voucher specimen. Nearly complete sequences of nuclear 18S rRNA (18S) and 28S rRNA (28S) and mitochondrial cytochrome *c* oxidase subunit 1 (CO1) genes were PCR amplified. Primer sets for the PCR and Cycle sequencing (CS) reactions used in this study are shown in Table 1. The PCR reactions were performed using a T100 Thermal Cycler (Bio-Rad). The reaction solutions consisted of a 25 μl solution containing 0.5 μl KOD FX Neo (Toyobo, Japan), 12.5 μl of 2X PCR buffer for KOD FX Neo, 5 μl of dNTP mix, 1 μl of each primer (5 pmol), template DNA (2 μl for 18S and 1 μl for 28S and CO1), and 3 or 4 μl of sterilized distilled water. The PCR conditions consisted of an initial denaturation step at 95°C for 2 min, followed by 40 cycles of denaturation at 98°C for 10 s, annealing at 52°C (18S), 50°C (28S), or 45°C (CO1) for 30 s, extension at 68°C for 1 min 30 s (18S), 2 min (28S), or 1 min (CO1), and a final extension at 68°C for 5 min. The quantity and length of the PCR products were checked by 1% Agarose S (Nippon Gene, Japan) gel electrophoresis and stained with ethidium bromide. The products were purified for sequencing using a FastGene Gel/PCR Extraction Kit (Nippon Gene, Japan), according to the manufacture's protocol. Sequencing was performed by the Macrogen Japan Corp. (Tokyo) with the primer sets shown in Table 1. A homology search was performed by BLAST (Altschul et al. 1990, 1997) with blast program from the National Center for Biotechnology Information (NCBI, http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

Adult female. Body (Figs. 1A, 2A) cyclopiform, 0.52 mm in length, 0.23 mm in maximum width. Prosome (Figs. 1A, 2A) oval, about 1.5 times longer than wide, ca. 1.4 times as long as urosome; pediger 1 totally covered by carapace-like extension; pediger 4 posteriorly produced into paired lamellar expansions (Fig. 2B). Urosome (Fig. 2B) comprising pediger 5, genital double-somite, three free abdominal somites, and caudal rami; pediger 5 acutely produced posterolaterally; genital somite incompletely fused to first abdominal somite with suture clearly visible; gonopore covered by plate-like leg 6 with outer seta, small terminal spine, and minute subterminal prominence; copulatory pores paired, located at posterior one-third length; anal operculum weakly developed, fringed with spinular row dorsally.

Rostrum (Figs. 1C, 2A) forming triangular process, directed posteroventrally. Labrum (Figs. 1C, 2A) swollen ventrally. Antennule stretched out anterolaterally when alive (Fig. 1A) (terminal segments lost during DNA extraction). Antenna (Fig. 2C) consisting of coxa, basis, 3-segmented endopod, and 6-segmented exopod; coxa unarmed; basis with 2 setae distally; first endopod segment having 1 subterminal seta, second segment with 2 minute mid-lateral and 2 terminal setae, third segment with 6 setae distally; setal formula of exopod 0, 2, 1, 1, 1, 3 (based on suture lines on posterior surface). Mandible gnathobase (Fig. 2D) stout, bearing 8 teeth and 2 setae, with ventralmost tooth serrated terminally; palp (Fig. 2E) consisting of basis with 1 minute seta subdistally and patches of minute spinules; exopod incompletely 4-segmented, with setal formula 1, 1, 2, 2; endopod 2-segmented, with setal formula 2, 6. Maxilla (Fig. 2F) with praecoxal and coxal endites having 7, 3 and 3, 3 setae, respectively; basis fused to first endopod segment to form allobasis, with heavily chitinized process plus 3 setae; free endopod 3-segmented, with setal formula 2, 2, 4. Maxilliped basis (not figured) with 3 setae; endopod 5-segmented, with setal formula 2, 2, 2, 2, 4 (3 large + 1 small).

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

Target gene	Primer name	Reaction	Sequence (5' to 3')	Direction	Source
18S rRNA	Euk18SF	PCR & CS	ACCTGGTTGATCCTGCCAG	Forward	Moon-van der Staay et al. (2000)
	Euk18SR	PCR & CS	TGATCCTTCYGCAGGTTCAC	Reverse	Moon-van der Staay et al. (2000)
	18SF2	CS	CCTGAGAAACGGCTRCCACAT	Forward	Yamaguchi and Endo (2003)
28S rRNA	28S-01	PCR & CS	GACTACCCCCTGAATTTAAGCAT	Forward	Kim et al. (2000)
	CS632	PCR & CS	CGATGAAGAACGCAGC	Forward	Schlötterer et al. (1994)
	28jj	PCR & CS	AGTAGGGTAAAACTAACCT	Reverse	Hillis and Dixon (1991)
	28S_18R	CS	CAGGCATAGTTCACCATCTTTC	Reverse	This study
	28S_24R	CS	ACATGGAACCCTTCTCCAC	Reverse	This study
	28S_32R	CS	AGAGCACTGGGCAGAATTC	Reverse	This study
	28S-42F	CS	GAGTTTGACTGGGGCGGTA	Forward	This study
COI	LCO1490	PCR & CS	GGTCAACAAATCATAAAGATATTGG	Forward	Folmer et al. (1994)
	HCO2198	PCR & CS	TAAACTTCAGGGTGACCAAAAAATCA	Reverse	Folmer et al. (1994)

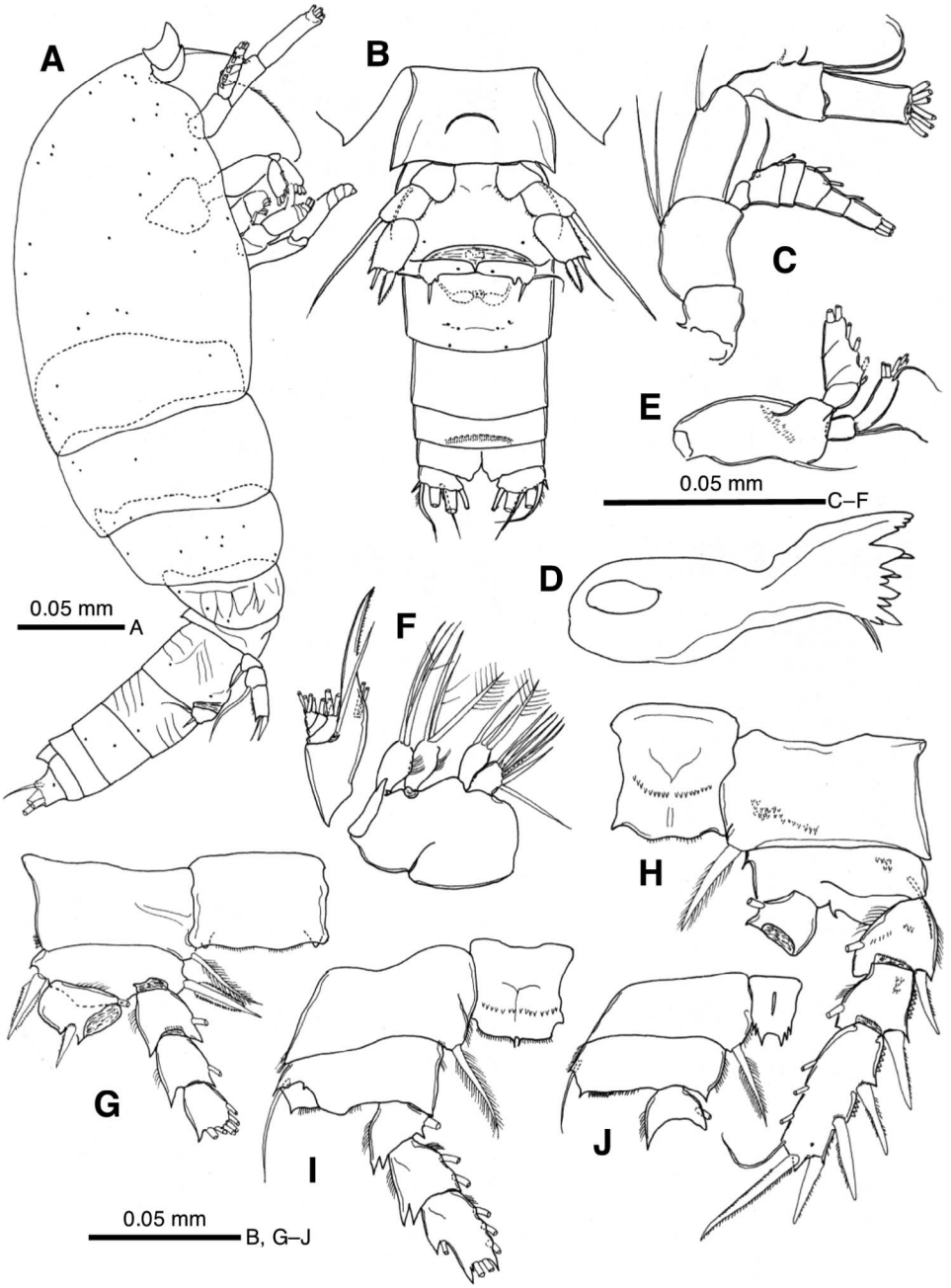

Fig. 2. Ovigerous female of an undescribed genus of Misophriidae. (A) Habitus, lateral view; (B) Prosomal end and urosome, ventral view; (C) Antenna; (D) Mandibular gnathobase; (E) Mandibular palp; (F) Maxilla; (G) Leg 1; (H) Leg 2; (I) Leg 3; (J) Leg 4.

Many segments of legs 1-4 lost during treatment for DNA extraction, but some diagnostic features confirmed. Leg 1 (Fig. 2G) with spiniform element on each side of basis; left endopod 3-segmented with single seta present on inner margin of second endopodal segment. Leg 2 (Fig. 2H) with middle spinular row on intercoxal sclerite; inner proximal margin of basis with tiny, acutely-pointed process; exopod 3-segmented, with setal formula I-1; I-1; III,I,4. Leg 3 (Fig. 2I) intercoxal sclerite with middle spinular row and 2 tiny, acutely-pointed processes on distal margin; endopod 3-segmented, with setal formula 0-1; 0-2; 1,2,3; outer distal corners of first and second endopodal segments each sharply produced into 2 processes. Leg 4 (Fig. 2J) with narrow intercoxal sclerite having 3 pairs of small acute processes distally.

Leg 5 (Figs. 1D, 2B) uniramous, symmetrical; protopod with single seta at outer distal corner; exopod 2-segmented, with first segment bearing long outer seta reaching beyond terminal seta on succeeding segment, and second segment having fine seta and spine distally (corresponding to elements "e" and "f", respectively, sensu Boxshall and Jaume (2000)) and triangular process on outer distal corner.

Two types of locomotory behavior were observed: (1) crawling over the bottom with frequent intermittent stops, and (2) free-swimming in a continuous and smooth manner across the water column. In the first locomotory type, the antennae and mandibular palps beat rapidly and legs 1-4 were held in an anteriorly-directed position as the copepod moved slowly along the bottom with the ventral side of the body down. The aforementioned head appendages ceased movement during intermittent stops. The maxillipeds did not firmly grasp the bottom during this behavior. This was confirmed by the observation that when it started climbing the vertical wall of a cell, it sometimes slowly fell without maxillipedal grasping, indicating a weak attachment. However, successful vertical climbs were also observed. Mean speed determined between two successive stops was 2.91 $\pm$ 0.71 (SD) mm per second (N = 17). Free swimming in the water column typically commenced suddenly, after a period of crawling along the bottom. After a short period of free swimming in the water column, the female returned to the bottom and resumed crawling motions.

The misophrioid copepod frequently remained stationary, clinging to the bottom using the maxillipeds and first legs. Attachment seemed to be mainly secured using the tips of the maxillipedal setae, although the anterodistal surface of leg 1 also touched the surface of the bottom supporting the copepod in position. While stationary, the antennules, antennae and mandibular palps were stretched out laterally, and were not involved in attachment. The body was flexed slightly ventrally at the prosome/urosome articulation. In this position, feeding seemed to take place, with the antennae and mandibular palps rapidly beating.

Feeding of the misophrioid copepod on cultured phytoplankters was indirectly confirmed by the excretion of numerous fecal pellets (Fig. 1B) and by its survival for 17 days in the laboratory. The fecal pellets were elongate, oval, 112.6-154.2 μm long and 32.7-38.7 μm wide (average $\pm$ standard deviation = 134.1 $\pm$ 15.8 μm, 34.8 $\pm$ 1.9 μm, N = 10). Pellet contents were tinged brownish green, indicating that the copepod had fed on the cultured phytoplankters. The approximate volume of fecal pellets produced was 8.5×10^4 μm^3, assuming they were uniformly oval in shape.

Oviposition took place twice during incubation. On the fifth day after collection, the first oviposition event was observed. The female produced 5 eggs carried on the urosome (Fig. 1A). The eggs seemed to be loosely attached to the copepod body, and were not enveloped by a sac-like structure, as seen in some calanoids (see Boxshall and Jaume 2000).

The eggs were ca. 0.09 mm in diameter (Fig. 1A). Two days later all the eggs were detached from the copepod. Hatching of the detached eggs was not confirmed, because they were lost during treatment. On the 10th day of incubation, the second oviposition event was recorded. The female produced 4 eggs. All the eggs had detached from the copepod two days after oviposition. Hatching was unsuccessful.

Three nucleotide sequences were obtained: 28S (Accession no. LC320121, 3496 bp); 18S (Accession no. LC320120, 1718 bp); and CO1 (Accession no. LC320122, 658 bp).

Discussion

The present specimen is assigned to an undescribed genus of the family Misophriidae based on the following features: (1) the carapace-like extension covering the first pedigerous somite; (2) the reduction in segmentation (6-segmented) and setation (0, 2, 1, 1, 1, 3) of the antennary exopod; and (3) the absence of an intercoxal sclerite on the fifth legs (see Boxshall and Jaume 2000). A close relationship between this species and the genus *Arcticomisophria* Martínez Arbizu and Seifried, 1996 is suggested by the presence of: (1) a spiniform outer element on the basis of leg 1; (2) a single inner seta on the second endopod segment of leg 1 (see Jaume and Boxshall 1997; Martínez Arbizu and Jaume 1999). *Arcticomisophria* comprises two species, *A. bathylaptevensis* Martínez Arbizu and Seifried, 1996 and *A. hispida* Jaume and Boxshall, 1997 which share the above-mentioned diagnostic features. However, leg 5 has more plesiomorphic segmentation and setation in *Arcticomisophria* than in the present misophriid specimen. Among genera of the Misophriidae, *Arcticomisophria* exhibits the most primitive state of leg 5 (ANCESTOR in Fig. 3). The fifth leg of the present undescribed genus is much more reduced than that of *Arcticomisophria* in both segmentation and setation (Fig. 3), and resembles to some extent those of species of other misophriid genera such as, *Misophria* Boeck, 1865, *Misophriella* Boxshall, 1983, *Misophriopsis* Boxshall, 1983 and *Stygomisophria* Ohtsuka, Huys, Boxshall and Itô, 1992, based on either one of these two characters: (1) no separation is present between the coxa and basis, resulting in an undivided protopod; or (2) the endopod is absent. Loss of the subdivision of the protopod seems to have occurred convergently in the Misophriidae. The present female specimen shares some apomorphic character states with some of these genera, but we consider that its formal taxonomic classification should be postponed pending the description of all the appendages from newly collected specimens of both sexes.

The locomotory behavior of a misophrioid copepod had been only briefly observed before, in a shallow-water hyperbenthic species *Misophria pallida* by Sars (1911). It was characterized by a combination of rapid rotation of the antennae and oral appendages (possibly the mandibular palps) and powerful strokes of the legs and urosome along the bottom (Sars 1911). In the misophrioid examined in the present study, a similar motion pattern was observed, but power strokes of the legs were not observed during the slow swimming behavior along the bottom.

The crawling behavior of the present specimen using the maxillipeds is similar to that reported for the siphonostomatoid *Aphotopontius mammillatus* Humes, 1987 (Heptner and Ivanenko 2002), although the latter lacks oral appendages for swimming. In *A. mammillatus*, Heptner and Ivanenko (2002) distinguished between crawling and walking by differences in the position of the maxillipeds. In the present misophrioid, only crawling was observed.

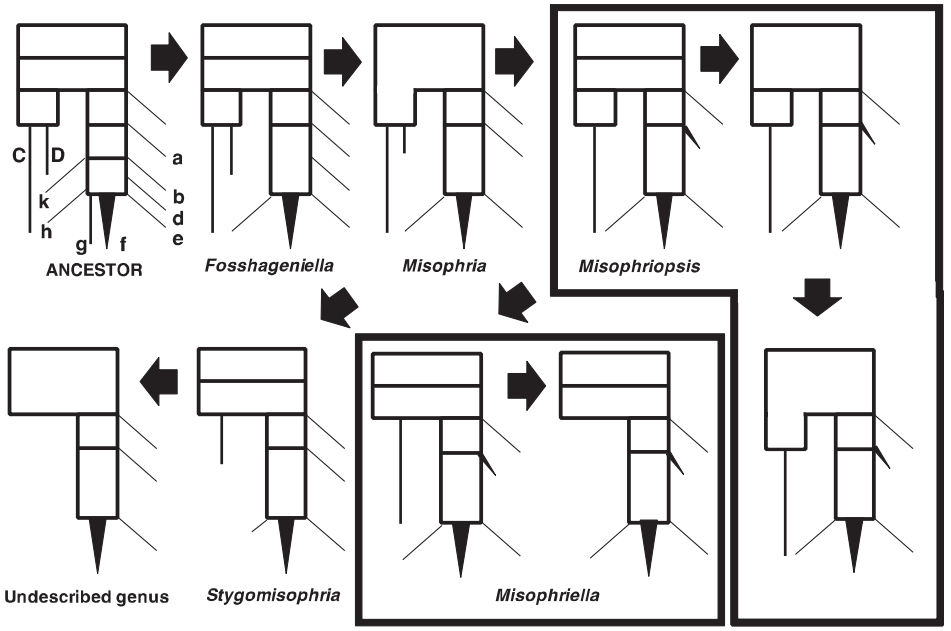

Fig. 3. Schematic illustrations showing segmentation and setation patterns of leg 5 of the adult female of some representatives of the family Misophriidae. Hypothetical ancestor based on *Arcticomisophria hispida* described by Jaume and Boxshall (1997), but elements e and f missing in their original description. Arrows indicate possible derivations of segmentation and setation (modified from Huys and Boxshall (1991)). Elements a, b, e, f, h, k, C, and D as identified by Boxshall and Jaume (2000).

The feeding habits of the deep-sea genus *Benthomisophria* were well studied by Boxshall and Roe (1980). Copepodids and adults of *Benthomisophria palliata* Sars, 1909 gorged on zooplankters such as small copepods, chaetognaths, cnidarians, and radiolarians, at depths of 2,500 m to 4,000 m (Boxshall and Roe 1980). Their prosomes occasionally became grossly expanded due to the volume of gut contents, suggesting an opportunistic feeding strategy of the copepod in the nutritionally poor deep ocean (Boxshall and Roe 1980). The gut content analysis of *Misophriella schminkei* Martínez Arbizu and Jaume, 1999 collected from hyperbenthic waters in Antarctica revealed that it may be a predator or a scavenger, feeding on cyclopoid copepods (Martínez Arbizu and Jaume 1999).

In contrast, the misophrioid examined in the present study survived only on cultured phytoplankters. The size of the phytoplankters was more than 3 μm, implying that the misophrioid employs suspension feeding, as observed in small particle-feeding calanoids (e.g., Paffenhöfer et al. 1982). The volume of fecal pellets produced by the adult female feeding on small cultured phytoplankters was ca. 8.5×10^4 μm^3, which is larger than those of *Oithona* spp. (of nearly equal prosome length), but within the range exhibited by small calanoids such as *Paracalanus* Boeck, 1865 and *Pseudocalanus* Boeck, 1872 (Uye and Kaname 1994; Mauchline 1998). This might not be the only feeding mode of the misophrioid since the heavily chitinized claw present on the maxillary basis (Fig. 2F) suggests "chopsticks mode" carnivory (Boxshall 1985) could occur as well.

Gurney's (1933) brief observations on the oviposition and developmental stages of *M. pallida* were of pivotal importance in understanding the biology of misophrioids. Adult

females of *M. pallida* carry two to four eggs loosely attached to the genital double-somite (Sars 1911; Gurney 1933). The female of the undescribed misophrioid also carried four to five eggs on its genital double-somite. Retention of eggs by adult females is quite frequent among podoplean copepods (Huys and Boxshall 1991). The unique characteristic of these misophrioids is the low number of relatively large-sized eggs in each clutch. Egg size has never been reported for misophrioids, but can be measured at about 0.09 mm in diameter based on an illustration of *M. pallida* by Sars (1911, Plate I). In the present specimen, the egg diameter was very similar to that of *M. pallida*. The interval between two consecutive clutches was four days in the present study, at around 20°C. All eggs were detached from the adult soon after oviposition, but it is uncertain whether this is normal in the wild.

The hatching naupliar stage was briefly illustrated by Gurney (1933). It was non-feeding, lacking masticatory blades on the mandible (Gurney 1933). The naupliar stage metamorphosed directly into the first copepodid stage, indicating a considerably abbreviated life cycle for this misophrioid (Gurney 1933). Such a naupliar abbreviation is commonly seen in siphonostomatoid copepods such the Caligidae and Nicothidae, in which zero to two naupliar stages were found (Ohtsuka et al. 2005, 2007, 2009; Venmathi Maran et al. 2013; Otake et al. 2016). The number of copepodid stages was the typical six in *B. palliata* (Boxshall and Roe 1980).

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Literature Cited

Altschul, S.F., W. Gish, W. Miller, E.W. Myers, and D.J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol., 215:403–410.

——, T.L. Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D.J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res., 25:3389–3402.

Boxshall, G.A. 1982. On the anatomy of the misophrioid copepods, with special reference to *Benthomisophria palliata* Sars. Philos. Trans. Royal Soc. Lond. B, Biol. Sci., 297:125–181.

——. 1983. Three new genera of misophrioid copepods from the near-bottom plankton community in the North Atlantic Ocean. Bull. Br. Mus. Nat. Hist. (Zool.), 44:103–124.

——. 1984. The functional morphology of *Benthomisophria palliata* Sars, with a consideration of the evolution of the Misophrioida. Crustaceana, Suppl. 7:32–46.

——. 1985. The comparative anatomy of two copepods, a predatory calanoid and a particle feeding mormonilloid. Philos. Trans. Royal Soc. Lond. B, Biol. Sci., 311:303–377.

——, and S.H. Halsey. 2004. An introduction to copepod diversity. The Ray Society, London, 966 pp.

——, and T.M. Iliffe. 1986. New cave-dwelling misophrioids (Crustacea: Copepoda) from Bermuda. Sarsia, 71:55–64.

——, and ——. 1987. Three new genera and five new species of misophrioid copepods (Crustacea) from anchialine caves on Indo-West Pacific and North Atlantic Islands. Zool. J. Linn. Soc., 91:223–252.

——, and D. Jaume. 2000. Discoveries of cave misophrioids (Crustacea: Copepoda) shed new light on the origin of anchialine faunas. Zool. Anz., 239:1–19.

——, and H.S. Roe. 1980. The life history and ecology of the aberrant bathypelagic genus *Benthomisophria* Sars, 1901 (Copepoda, Misophrioida). Bull. Br. Mus. Nat. Hist. D., 38:9–41.

——, S. Zylinski, D. Jaume, T.M. Iliffe, and E. Suárez-Morales. 2014. A new genus of speleophriid copepod (Copepoda: Misophrioida) from a cenote in the Yucatan, Mexico with a phylogenetic analysis at the species level. Zootaxa, 3821:321–336.

Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol., 3:294–299.

Gurney, R. 1933. Notes on some Copepoda from Plymouth. J. Mar. Biol. Assoc. U. K. (N. Ser.), 19:299–304.

Heptner, M.V., and V.N. Ivanenko. 2002. Copepoda (Crustacea) of hydrothermal ecosystems of the world ocean. Arthropoda Sel., 11:117–134.

Hillis, D.M., and M.T. Dixon. 1991. Ribosomal DNA: Molecular evolution and phylogenetic inference. Quart. Rev. Biol., 66:411–453.

Huys, R., and G.A. Boxshall. 1991. Copepod evolution. The Ray Society, London, 468 pp.

Jaume, D., and G.A. Boxshall. 1997. A new genus and two new species of misophrioid copepods (Crustacea) from the North Atlantic hyperbenthos. Sarsia, 82:39–54.

Kim, C.-G., H.-Z. Zhou, Y. Imura, O. Tominaga, Z.-H. Su, and S. Osawa. 2000. Pattern of morphological diversification in the *Leptocarabus* ground beetles (Coleoptera: Carabidae) as deduced from mitochondrial ND5 gene and 28S rDNA sequences. Mol. Biol. Evol., 17:137–145.

Martínez Arbizu, P., and D. Jaume. 1999. New hyperbenthic species of *Misophriopsis* and *Misophriella*, first record of misophrioid copepods (Crustacea) from Antarctic waters. Helgol. Mar. Res., 53:102–117.

Mauchline, J. 1998. The biology of calanoid copepods. Adv. Mar. Biol., 33:1–710.

Moon-van der Staay, S.Y., G.W.M. van der Staay, L. Guillou, D. Vaulot, H. Claustre, and L.K. Medlin. 2000. Abundance and diversity of prymnesiophytes in the picoplankton community from the equatorial Pacific Ocean inferred from 18S rDNA sequences. Limnol. Oceano., 45: 98–109.

Ohtsuka, S., G.A. Boxshall, and S. Harada. 2005. A new genus and species of nicothoid copepod (Crustacea: Copepoda: Siphonostomatoida) parasitic on the mysids *Siriella okadai* Ii from off Japan. Syst. Parasitol., 43:65–81.

——, S. Harada, M. Shimomura, G.A. Boxshall, R. Yoshizaki, D. Ueno, Y. Nitta, S. Iwasaki, H. Okawachi, and T. Sakakihara. 2007. Temporal partitioning: dynamics of alternating occupancy of a host microhabitat by two different crustacean parasites. Mar. Ecol. Prog. Ser., 348:261–272.

——, R. Huys, G.A. Boxshall, and T. Itô. 1992. *Misophriopsis okinawensis* sp. nov. (Crustacea: Copepoda) from hyperbenthic waters off Okinawa, South Japan, with definitions of related genera *Misophria* Boeck, 1864 and *Stygomisophria* gen. nov. Zool. Sci., 9:859–874.

——, I. Takami, B.A. Venmathi Maran, K. Ogawa, T. Shimono, Y. Fujita, M. Asasakawa, and G.A. Boxshall. 2009. Developmental stages and growth of *Pseudocaligus fugu* Yamaguti, 1936 (Copepoda: Siphonostomatoida: Caligidae) host-specific to Puffer. J. Nat. Hist., 43:1779–1804.

Otake, S., K. Wakabayashi, Y. Tanaka, and K. Nagasawa. 2016. Life-cycle of *Choniomyzon inflatus* Wakabayashi, Otake, Tanaka & Nagasawa, 2013 (Copepoda: Siphonostomatoida: Nicothoidae) with the morphological descriptions of the nauplius, copepodid and adult male. Syst. Parasitol., 93:145–157.

Paffenhöfer, G.A., J.R. Strickler, and M. Alcaraz. 1982. Suspension-feeding by herbivorous calanoid copepods: a cinematographic study. Mar. Biol., 67:193–199.

Sars, G.O. 1911. Copepoda (Harpacticoida). An account of the Crustacea of Norway, with short descriptions of and figures of all species, Vol. 5. Bergen Museum, Bergen, 449 pp., 284 plates.

Schlötterer, C., M.T. Hauser, A. van Haeseler, and D. Tautz. 1994. Comparative evolutionary analysis of rDNA ITS regions in *Drosophila*. Mol. Biol. Evol., 11:513–522.

Uye, S.I., and K. Kaname. 1994. Relations between fecal pellet volume and body size for major zooplankters of the Inland Sea of Japan. J. Oceanogr., 50:43–49.

Venmathi Maran, B.A., S.Y. Moon, S. Ohtsuka, S.Y. Oh, H.Y. Soh, J.G. Myoung, A. Iglikowska, and G.A. Boxshall. 2013. The caligid life cycle: new evidence from *Lepeophtheirus elegans* reconciles the cycles of *Caligus* and *Lepeophtheirus* (Copepoda: Caligidae). Parasite, 20:15.

Walter, T.C., and G.A. Boxshall. 2017. World of Copepods database. Misophrioida. Accessed through: World Register of Marine Species at: http://marinespecies.org/aphia.php?p=taxdetails&id=22597 (accessed on September 11, 2017).

Yamaguchi, S., and K. Endo. 2003. Molecular phylogeny of Ostracoda (Crustacea) inferred from 18S ribosomal DNA sequences: Implication for its origin and diversification. Mar. Biol., 143:23–38.