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Source: Zoological Science, 33(6):659-666.

Published By: Zoological Society of Japan

URL: <http://www.bioone.org/doi/full/10.2108/zs160094>

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A New Species of Hyperbenthic Cyclopoid Copepod from Japan: First Record of the Genus *Cyclopicina* in the Indo-Pacific Region

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A new species of the cyclopinid genus *Cyclopicina*, *C. toyoshioae* sp. nov., was collected from the hyperbenthic layer off northern Kyushu Island, Japan; its description is based on two adult female specimens. This is the first record of the genus from the Indo-Pacific region. The new species can be distinguished from its two known congeners in: (1) the relatively short antennules which do not reach the posterior margin of the dorsal cephalothoracic shield; (2) the shape of seminal receptacles; (3) the segmentation and armature of the cephalothoracic appendages; (4) the shape of the basal protrusion between the rami of legs 1–4; (5) the presence of three outer spines on the third exopodal segment of leg 4; and (6) the presence or absence of outer setae on the free exopodal segment of leg 5. The genus *Cyclopicina* shows a wide but scattered distribution in hyperbenthic layers, from the continental shelves to deep-sea basins, in the northern hemisphere.

Key words: Copepoda, *Cyclopicina*, Cyclopoida, hyperbenthic, Indo-Pacific, taxonomy

INTRODUCTION

Studies of copepods from the hyperbenthic community (see Mees and Jones, 1997 for definition) have generated important insights into the evolutionary history of the Copepoda (Huys and Boxshall, 1991; Ohtsuka et al., 1994; Jaume and Boxshall, 2000; Bradford-Grieve, 2002, 2004). Copepods are rarely fossilized (Klompaker and Boxshall, 2016), so examination of the most basal copepod order, Platycopioidea, and the primitive calanoid superfamily Pseudocyclopoidea, which are both predominantly hyperbenthic or cavernicolous, have contributed significantly to our understanding of early copepod evolution (Boxshall and Halsey, 2004; Bradford-Grieve et al., 2014). However, the hyperbenthic community is inhabited by a mixture of basal and derived groups of copepods and it has been suggested that the latter have secondarily colonized this habitat from the water column (Ohtsuka et al., 1994, 2005; Soh, 1998; Bradford-Grieve, 2002, 2004). In comparison to the North Atlantic, there have been relatively few faunal surveys of hyperbenthic copepods in Japanese and neighboring waters. Our intensive investigations in shallow and deep near-bottom waters off Japan have revealed striking similarities in familial and generic composition between these two regions, suggesting a full Tethyan track distribution in at least some of these primitive taxa (Ohtsuka and Boxshall, 1994; Ohtsuka et al., 1992, 1996, 1998, 2005; Soh et al., 2013).

An undescribed species of the genus *Cyclopicina* Lindberg, 1953 was found in a near-bottom sample collected at a depth of 74 m during a research cruise off Kyushu, western Japan, in October 2015. The copepod exhibits highly plesiomorphic character states with regard to limb segmentation and armature among the cyclopoids including: a 26-segmented antennule in the female, which is the maximum number of antennular segments reported for the order Cyclopoida; the presence of an inner coxal seta on the antenna; the antennary exopod is represented by three setae; and legs 1–4 exhibit the full armature pattern as elucidated for the order by Huys and Boxshall (1990). Prior to the discovery reported here, the genus accommodated only two species, both known from the deep hyperbenthos: *C. longifurcata* (Scott, 1901) from the North Atlantic, and *C. sirenkoi* Martínez Arbizu, 1997 from the Laptev Sea (Huys and Boxshall, 1990; Martínez Arbizu, 1997). The present paper provides a description of this new cyclopoid copepod, together with remarks on the systematics and distribution of the genus.

MATERIALS AND METHODS

Copepods were sorted from a plankton sample collected from the zone just above the sandy bottom in coastal waters off northern Kyushu, Japan (33°53.479'N, 129°53.833'E to 33°53.379'N, 129°53.908'E), at a depth of 74 m using an originally-designed sledge net (see Ohtsuka et al., 1992). The sample was collected on 23 October 2015 and the gear was towed at a speed of 2 knots by TRV Toyoshio-maru, Hiroshima University. Samples were fixed with 99.5% ethanol immediately after collection. The DNA extraction was performed on the holotype specimen, using DNeasy Blood and Tissue Kit (Qiagen, USA) following the manufacturer's protocol, but extraction was unsuccessful. After the protein digestion step by pro-

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teinase K, the holotype chitinous body was retrieved from the microtube. One specimen was examined and dissected in lactophenol, and then mounted in CM-10 (Masters Company, Inc., USA). Drawings were carried out with the aid of a drawing tube attached to a

differential interference contrast microscope (Optiphot, Nikon). Morphological terminology follows Huys and Boxshall (1991). Types are deposited at the Kitakyushu Museum of Natural History and Human History (KMNH IvR).

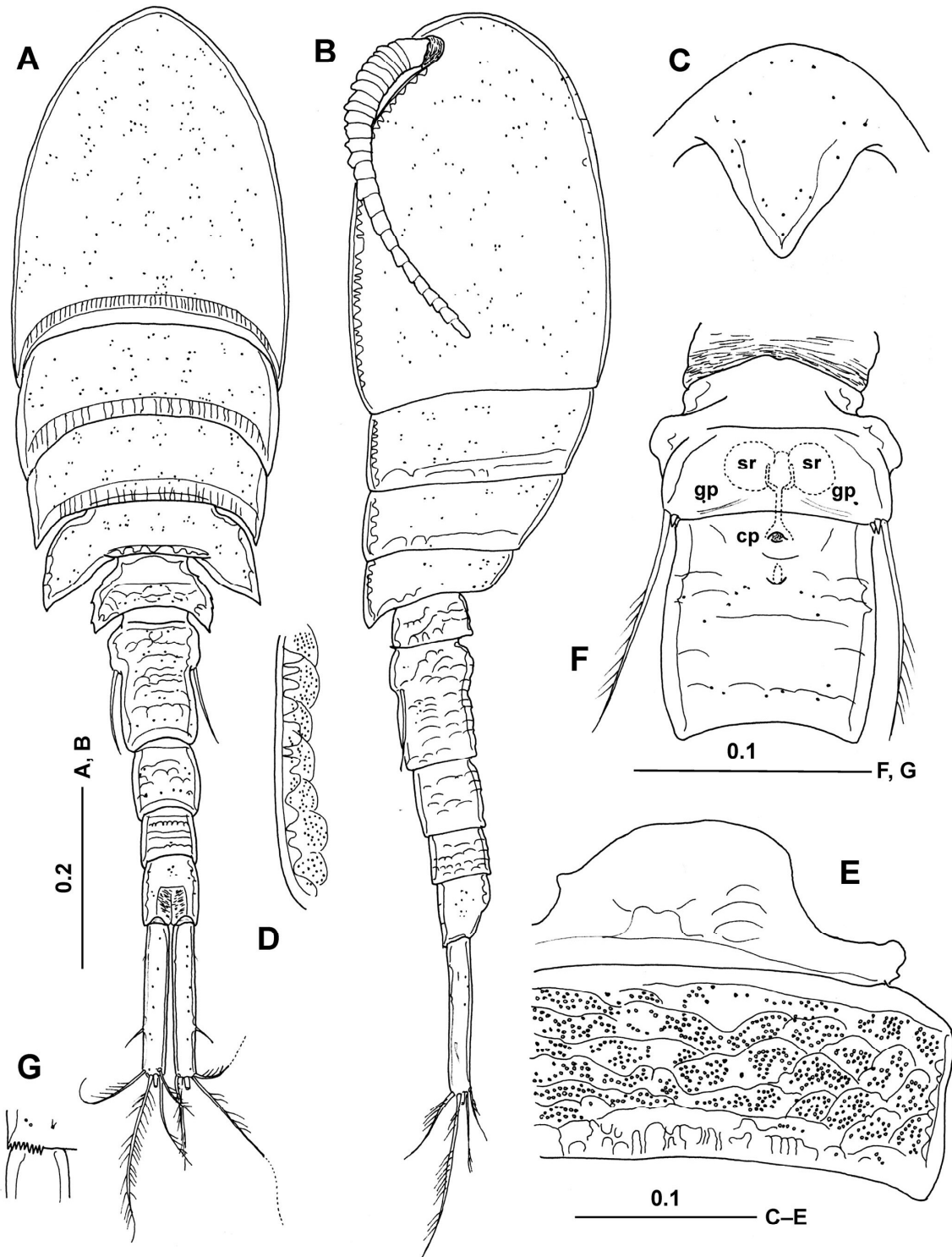


Fig. 1. *Cyclopicina toyoshioae* sp. nov., adult female, paratype (KMNH IvR 500882). (A) habitus, dorsal; (B) habitus, lateral; (C) rostrum, ventral; (D) lateral margin of dorsal cephalothoracic shield; (E) second pedigerous somite, dorsal; (F) genital double-somite, ventral; (G) distal corner of anal somite, ventral. Abbreviations: cp, copulatory pore; gp, gonopore; sr, seminal receptacle. Scales in mm.

TAXONOMY

Cyclopicina toyoshioae sp. nov.
(Figs. 1–4)

Types examined. Holotype: adult female, whole specimen, (KMNH IvR 500881), body length (from the anterior end of cephalothorax to the posterior end of caudal rami) 0.94 mm. Paratype, adult female, dissected and mounted on

seven slides (KMNH IvR 500882), body length 0.92 mm.

Type locality. Both type specimens collected from hyperbenthic layer above sandy bottom at a depth of 74 m off northern Kyushu, Japan (33°53.479'N, 129°53.833'E to 33°53.379'N, 129°53.908'E), on 23 October 2015.

Description. Female (paratype). Body (Fig. 1A, B) cyclopiform, 0.92 mm long, excluding caudal setae. Prosome about 1.2 times longer than urosome, excluding caudal setae; comprising cephalothorax completely incorpo-

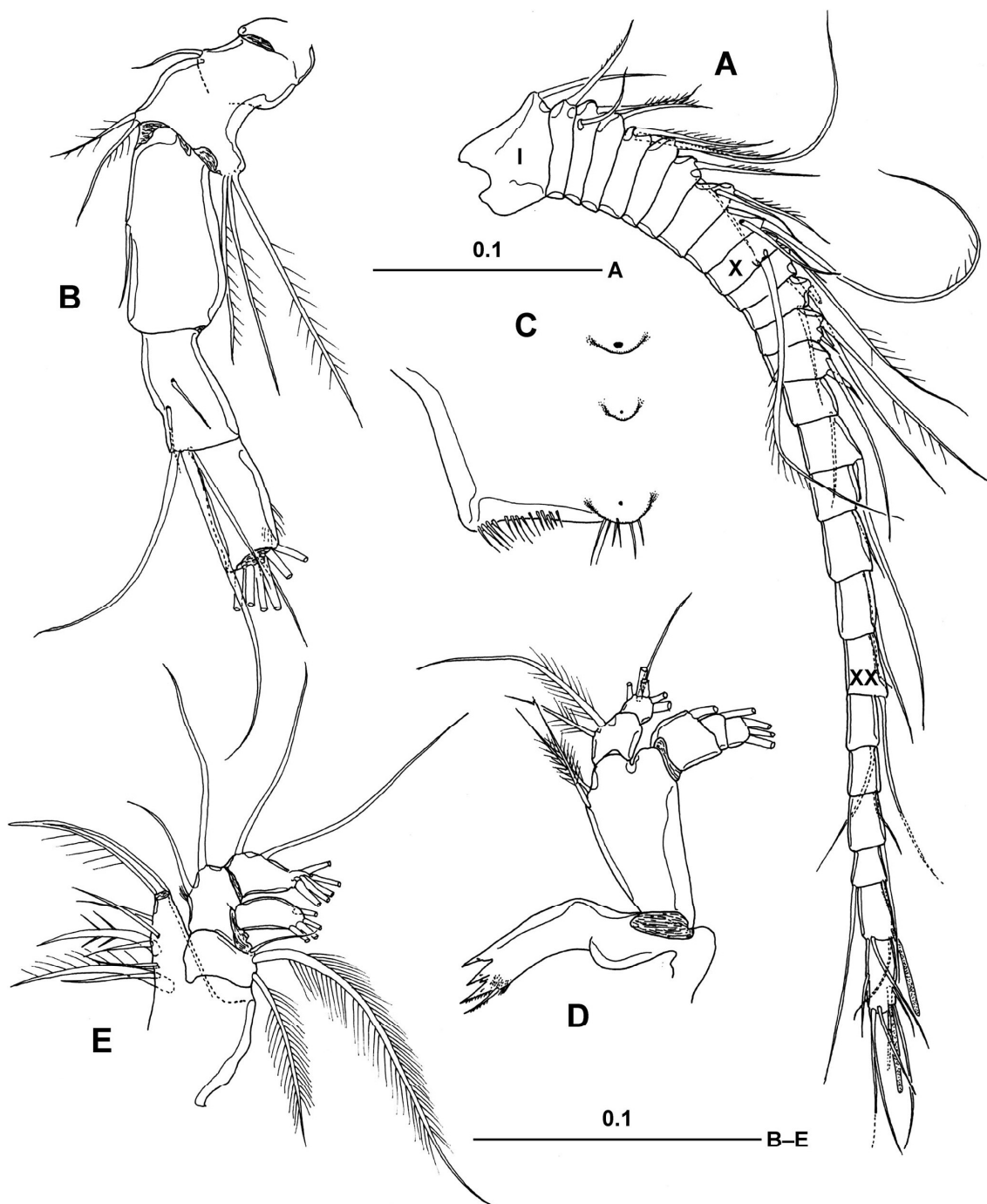


Fig. 2. *Cyclopicina toyoshioae* sp. nov., adult female, paratype (KMNH IvR 500882). (A) antennule; (B) antenna; (C) labrum; (D) mandible; (E) maxillule. Scales in mm.

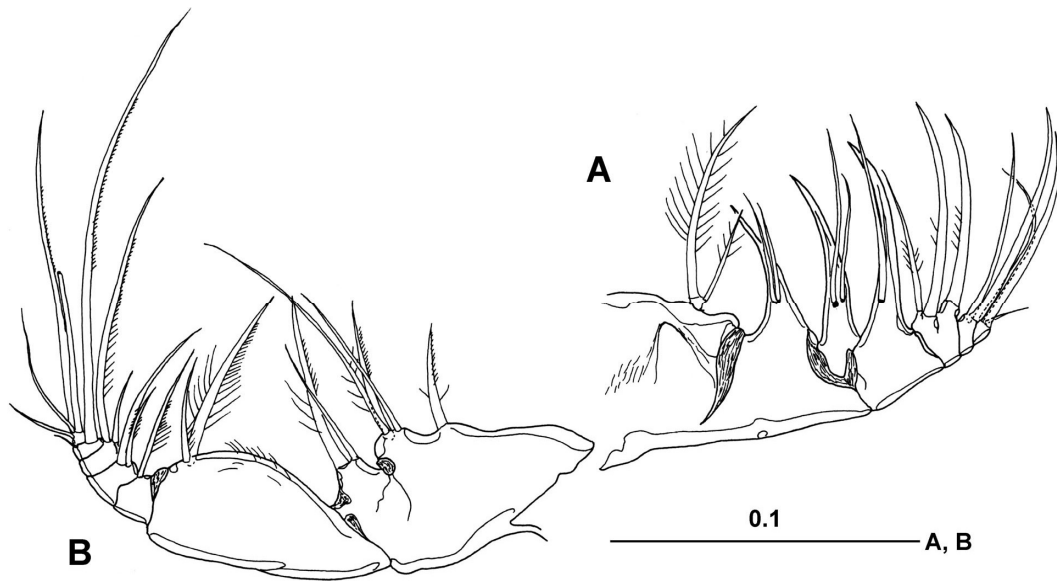


Fig. 3. *Cyclopicina toyoshioae* sp. nov., adult female, paratype (KMNH IvR 500882). (A) maxilla; (B) maxilliped. Scales in mm.

rating first pedigerous somite, plus three free pedigerous somites with well-developed tergites. No carapace-like expansion (as seen in *Cyclopicina sirenkoi*) (Fig. 1A, B, E). Surface of dorsal cephalothoracic shield and tergites of free pedigers (Fig. 1D, E) entirely ornamented with small, shallow depressions, sensory pits, and secretory pores; lateral margins provided with linear array of conspicuous pits and associated pores. Rostrum (Fig. 1C) produced posteroventrally into triangular process. Labrum (Fig. 2C) truncate along posterior margin, furnished with medial rounded prominence midway and row of fine setules on each side. Pedigers 2 and 3 bluntly produced at posterolateral corners; deep constriction present between pedigers 3 and 4; posterolateral corners of pediger 4 produced into acutely pointed wing-like expansions.

Urosome consisting of pediger 5, genital double-somite, and three free abdominal somites including anal somite bearing paired caudal rami; length ratios of urosomites 1: 2.5: 1.3: 1: 1.4. Pediger 5 produced posterolaterally into acute processes, viewed dorsally. Genital double-somite (Fig. 1F) longest, expanded laterally in anterior third; gonopores paired, covered with common operculum comprising fused sixth legs, each represented by one long seta and two minute spines; single copulatory pore located in mid-line just posterior to level of sixth legs; seminal receptacles paired, bulbous. Anal somite with anus dorsally; ornamented with serrations at insertion of caudal rami (Fig. 1G). Caudal rami symmetrical, longer than genital double-somite, and about 6.9 times longer than wide; seta I minute, located at anterior one-sixth, seta II at posterior one-third, seta VII located posterodorsally, about one-third as long as seta IV.

Antennule (Figs. 1B, 2A) 26-segmented, but not reaching beyond posterior end of dorsal cephalothoracic shield. Armature elements as follows: I-1, II-1, III-2, IV-1, V-2, VI-1, VII-2, VIII-2, IX-2, X-2, XI-2, XII-2 (one missing in Fig. 2A), XIII-2, XIV-2, XV-1, XVI-1, XVII-1, XVIII-1, XIX-1, XX-1, XXI-1, XXII-1, XXIII-1, XXIV-1+1, XXV-1+1+ae, XXVI-XXVIII-7+ae; longest setae present on segments IX, X and XVI.

Antenna (Fig. 2B) biramous, with vestigial exopod; coxa and basis incompletely fused with suture line marking plane of fusion; coxa with minute inner seta; basis armed with two equal setae at inner distal corner; endopod 3-segmented, first segment with one fine seta at mid-length, second segment bearing two short setae mid-way and three long setae distally, third segment with one short and six long setae; exopod represented by three setae on small knob at outer distal corner of basis.

Mandible (Fig. 2D) with three large and one minute monocuspid teeth, plus one spinulose seta on coxal gnathobase; basis with two distal setae on inner margin; endopod 2-segmented, first segment with two unequal setae, second segment with one short and five long setae; exopod 3-segmented, first two segments each bearing one inner seta, compound terminal segment with three setae.

Maxillule (Fig. 2E) with tapering praecoxal arthrite bearing three large, spinulose and six slender setae; coxal epipodite represented by one small and two large setae on outer margin; coxal endite reduced, unarmed; basal endite with two slender setae; endopod 1-segmented, bearing two inner and five terminal setae; exopod 1-segmented with four distal setae.

Maxilla (Fig. 3A) with praecoxa and coxa fused to form syncoxa; proximal endite bearing two spinulose setae of unequal length; second and third endites each with two slender setae and stout claw; basis distinct, furnished with one slender seta and stout claw; endopod 3-segmented, with armature of three, two and three setae.

Maxilliped (Fig. 3B) 7-segmented; syncoxa with three endites armed with one, three, and two setae, respectively; basis elongate, convex inner margin bearing two unequal spinulose setae distally; free endopod 5-segmented, first to fifth segments bearing one, two, two, one plus one, and four setae, respectively.

Legs 1 to 4 (Fig. 4A–F) biramous with 3-segmented rami, each pair joined by intercoxal sclerite: seta and spine formula shown in Table 1; basis with protrusion between

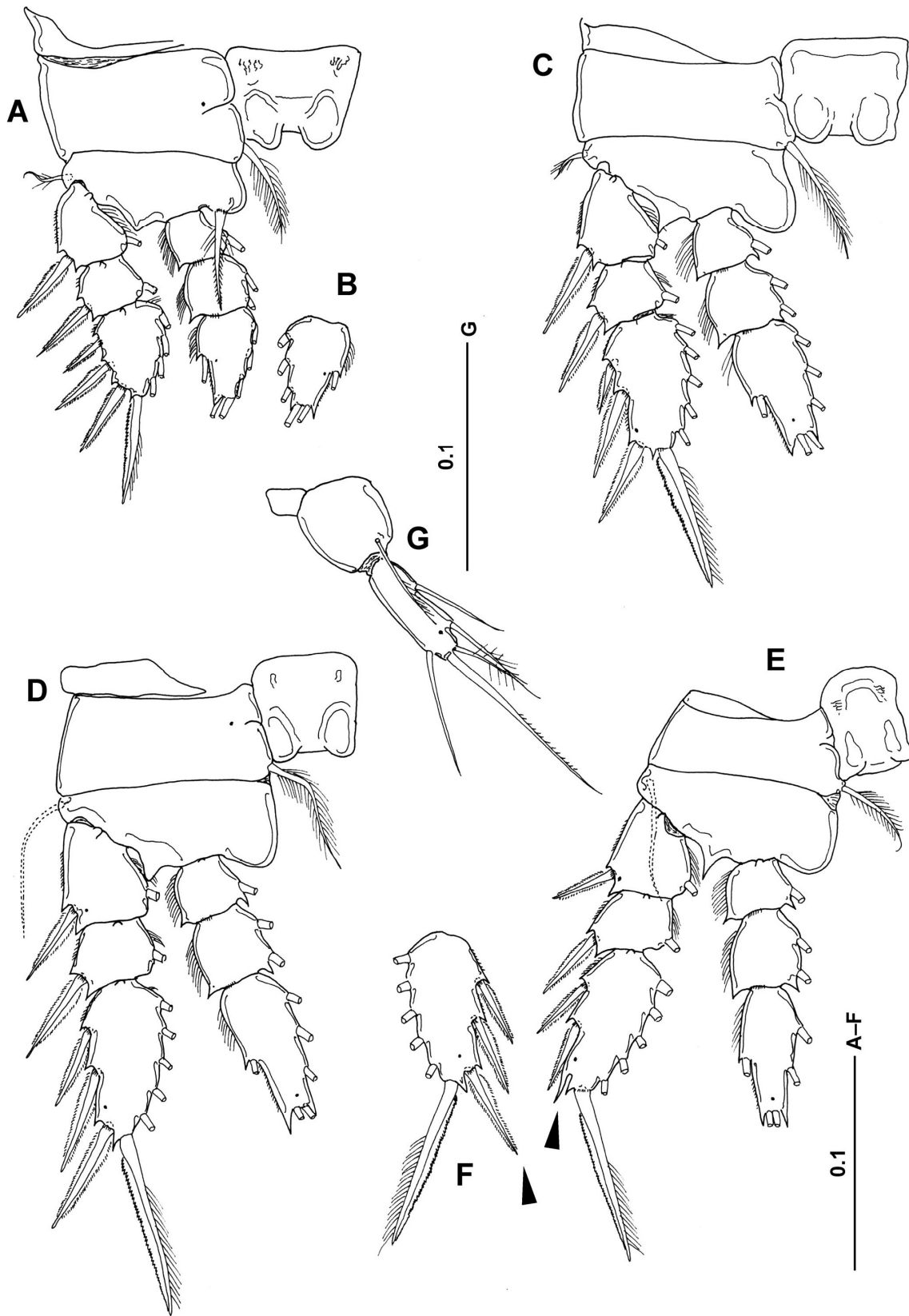


Fig. 4. *Cyclopicina toyoshioae* sp. nov., adult female, paratype (KMNH IvR 500882). (A) leg 1, right, anterior; (B) third endopodal segment of leg 1, left, anterior; (C) leg 2, anterior; (D) leg 3, anterior; (E) leg 4, right, anterior; (F) third exopodal segment of leg 4, left, anterior; (G) leg 5, posterior. Arrowheads indicating counterparts. Scales in mm.

Table 1. Seta and spine formula of legs 1–4. Roman and Arabic numerals indicating spine and seta, respectively.

	Coxa	Basis	Endopod	Exopod
Leg 1	0-1	1-1	0-1; 0-2; 1, 2, 3	I-1; I-1; III, I, 4
Leg 2	0-1	1-0	0-1; 0-2; 1, 2, 3	I-1; I-1; III, I, 5
Leg 3	0-1	1-0	0-1; 0-2; 1, 2, 3	I-1; I-1; III, I, 5
Leg 4	0-1	1-0	0-1; 0-2; 1, 2, 2	I-1; I-1; III, I, 5

rami with blunt tip in leg 1, but acutely pointed tip in legs 2–4. Leg 1 (Fig. 4A, B) with stout spiniform seta at inner corner of basis, reaching distal margin of second endopodal segment. Leg 2 (Fig. 4C) with inner basal process swollen. Leg 3 with outer basal seta missing in paratype (Fig. 4D) but present in holotype. Leg 4 (Fig. 4E) aberrant on right, with terminal outer process rather than spine, but armed with three outer spines on left counterpart (arrowheads in Fig. 4E, F) (as in holotype).

Leg 5 (Fig. 4G) 2-segmented, with distinct intercoxal sclerite connecting protopods; protopod with outer distal plumose seta; free exopodal segment bearing two outer and three terminal setae. Sixth legs (Fig. 1F) represented by common plate bearing, on each side, pair of two minute spinules and long seta reaching posterior margin of genital double-somite

Male. Unknown.

Variability. Asymmetry was observed in the legs of the paratypic female. The third exopodal segment of leg 4 bears three outer spines on the left side, but only two spines and an acutely pointed process on the right side (Fig. 4E). The terminal spine on the left side (Fig. 4F) seems to have been replaced by a process on the right side. A normal spine was also found in this position in the holotype. In addition, a minute sharply-pointed prominence on the inner distal corner of the third endopodal segment of leg 1 is lacking on the right side (Fig. 3A, B).

Remarks. The genus *Cyclopicina* has thus far accommodated only two species, *C. longifurcata* (Scott, 1901) and *C. sirenkoi* Martínez Arbizu, 1997. The type species of *Cyclopicina*, *C. longifurcata* was redescribed by Huys and Boxshall (1990) based on a female collected from the deep hyperbenthic layer in the North Atlantic at a depth of 2,980 m. The new species is easily distinguished from *C. longifurcata* by: (1) the body length being 0.92–0.94 mm, with prosome: urosome length ratio of 1.2 (1.01 mm, and 1.4 in *C. longifurcata*); (2) the anterior third of the genital double-somite, viewed ventrally, is laterally expanded (not expanded); (3) the seminal receptacles are less than half the width of the genital double-somite (nearly the same width); (4) the caudal ramus is 6.9 times longer than wide (7.7); (5) the antennule does not reach the posterior margin of the cephalothorax (reaching to middle of pediger 2); (6) the distal exopodal segment of the antenna has seven setae terminally (six setae); (7) the second seta on the maxillillary coxal exite longest (the first longest); (8) relative lengths and fusion of elements in the maxilla; (9) the inner basal spiniform seta of leg 1 reaching the posterior end of the second endopodal segment (half the segment); (10) the presence of an outer basal seta on leg 1 (absence); (11) the inner basal corner of leg 2 is extremely swollen (moderately swollen); (12) the presence of three outer spines on the third exopo-

dal segment of leg 4 (only 2); (13) the protopod of leg 5 as long as wide (longer than wide); and (14) the outer protopodal seta of leg 5 is short, not reaching beyond the endopod (beyond).

The second species, *C. sirenkoi*, was described in detail by Martínez Arbizu (1997) enabling a full comparison with the new species. In contrast to the new species and *C. longifurcata*, *C. sirenkoi* bears a carapace-like expansion from the posterior margin of the dorsal cephalothoracic shield which encloses the first pedigerous somite. There are many other differences in body form and in the segmentation/setation of the appendages between the new species and *C. sirenkoi*, which justify the establishment of the new taxon, including: (1) the body length is 0.92–0.94 mm (0.82 mm in *C. sirenkoi*); (2) the prosome: urosome length ratio is 1.2 (1.4); (3) the seminal receptacles are bulbous (inverted U-shaped); (4) the antennule is short, not reaching the posterior margin of the cephalothorax (beyond); (5) antennular segments III, XIII and XIV each have two setae (only one); (6) the antennary endopod is represented by three setae (two setae); (7) the mandibular basis and second endopod segment have two and six setae, respectively (one and five); (8) the mandibular exopod is 3-segmented (5-segmented); (9) the proximal two setae on the coxal exite of the maxillules are unequal in length (almost equal); (10) the maxilla bears armature elements of 2, process+2, process+2, process+1, 3, 2, 3 (2, 2, 2, process+2, 1, 2, 1); (11) the maxillipedal syncoxa and second to 4th endopodal segments having six, two, two and two setae, respectively (five, one, one, one); (12) the first and second endopodal segments of leg 1 have one and two setae, respectively (zero and one); (13) the first exopodal segments of legs 2–4 each have one inner seta (none); and (14) the terminal exopodal segment of leg 4 has three outer spines (two spines). *Cyclopicina sirenkoi* generally exhibits derived character states with reduced setation of the cephalic appendages and legs 1–4 in comparison with both other congeners, however, the mandibular exopod expresses more segments than in the other species.

The presence or absence of carapace-like expansion was well discussed by Martínez Arbizu (1997). The possession may be related to opportunistic gorging, as suggested for the deep-sea planktonic misophrioid *Benthomisophria* by Boxshall and Roe (1980) and Boxshall (1982).

Etymology. The new specific name *toyoshioae* is derived from TRV Toyoshio-maru, Hiroshima University; research carried from the vessel has contributed greatly to advances in marine biology.

DISCUSSION

Systematics

The genus *Cyclopicina* was retained in the family Cyclopinidae by Boxshall and Halsey (2004), but they recognized that this family was probably paraphyletic. In a series of papers Martínez Arbizu (2000a, b, 2001a, b, 2006) grouped the genera formerly considered as members of the Cyclopinidae into a set of monophyletic lineages to which he gave familial status: Cyclopettidae Martínez Arbizu, 2000, Giseliniidae Martínez Arbizu, 2000, Hemicyclopinidae Martínez Arbizu, 2001, Psammocyclopinidae Martínez Arbizu, 2001, and Schminkepinellidae Martínez Arbizu, 2006. As Boxshall

and Jaume (2012) pointed out, there has been no parsimony based testing of this phylogenetic re-arrangement of the former Cyclopinidae. Karanovic (2008) briefly addressed this topic but concluded that the characters used by Martínez Arbizu to support these new families were unreliable, and he continued to use the Cyclopinidae *sensu lato* to encompass all these lineages.

Subsequently there have been indications that the new families are gaining acceptance. Boxshall and Jaume (2012), in describing a new species of *Paracyclopinia* Smirnov, 1935 from Indonesia, allocated the genus to the family Cyclopettidae, and Zagami and Brugnano (2013) while establishing a replacement name, *Costanzoia* Zagami and Brugnano, 2013 for the preoccupied *Ginesia* Jaume and Boxshall, 1997, placed this genus in the family Smirnovipinidae, but this family name is nomenclaturally unavailable (Article 8.6; International Commission on Zoological Nomenclature [ICZN], 1999). It has been cited on various websites (e.g., www.lucioesce.net/copepods/), but has never been formally made available. Pesce (2016) listed the name Smirnovipinidae on the above-mentioned website, which he attributes to Martínez Arbizu (in press) in one place, but to Martínez Arbizu (1997) in another. The name was adopted by several others and was listed on the World Register of Marine Species [WoRMS] (2016), but it has never been published in print despite its appearance prior to the amendment of the Code to allow the registration of new names by electronic publication under certain conditions (ICZN, 2012a, b).

The discovery of the third species of *Cyclopicina* does not shed any new light on this wider systematic context, as *Cyclopicina* belongs to the core group of genera that are retained in the family Cyclopinidae. As pointed out by Boxshall and Jaume (2012) a comprehensive phylogenetic analysis is needed to test the validity of these families. However, our efforts to obtain molecular sequence data from the holotype of the new species have been unsuccessful. The presence of three outer spines on the third exopodal segment of leg 4 is significant, because the family Cyclopinidae including this genus was previously diagnosed by the presence of a maximum of two outer spines on the counterpart (Boxshall and Halsey, 2004). This state was confirmed in the holotype and on one side of the paratype of the new species; it represents the most plesiomorphic state among the Cyclopoida, shared with the genus *Limnoithona* Burckhardt, 1913 (Boxshall and Halsey, 2004), which is tentatively assigned to the family Cyclopettidae (Abiahy et al., 2006).

Distribution

Cyclopicina toyoshioae sp. nov. was collected from a hyperbenthic layer at a depth of 74 m off North Kyushu Island, Japan. Water temperature and salinity at depths of 71–76 m near the type locality were 20.2–20.7°C and 34.4, respectively (present study). In contrast, the original type specimen of *C. longifurcata* was found in the stomach of a predatory haddock caught at depths of 50–100 m in the northern North Sea (Scott, 1901). The second report of this species was from 2,980 m in the North Atlantic (Huys and Boxshall, 1990), where it seemed to be an inhabitant of the hyperbenthic layer. *Cyclopicina sirenkoi* was also discov-

ered from a hyperbenthic layer at a depth of 3,233 m in the Laptev Sea, the Arctic Ocean (Martínez Arbizu, 1997). Based on the morphology of pedigerous somites and legs 1–4, *Cyclopicina* can be regarded as a swimming-crawling form rather than a typical swimming type, because of the possession of relatively wide coxa and basis of legs 1–4 (Heptner and Ivanenko, 2002). Such features are also in accord with an inferred near-bottom habitat. Based on available data, the genus seems to be an inhabitant of hyperbenthic layers from the continental shelf to the deep-sea in the northern hemisphere.

ACKNOWLEDGMENTS

We express our sincere thanks to the captain and crew of TRV Toyoshio-maru, Hiroshima University and students in our laboratory for their cooperation at sea. This study was partially supported by grant-in-aids from the Japan Society of Promotion of Science, awarded to SO (KAKENHI No. 16K07825).

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(Received May 26, 2016 / Accepted August 10, 2016)