**Pontopolycope orientalis** sp. nov. (Crustacea: Ostracoda: Polycopidae), the First Report of a Living Species of the Genus from the Indo-Pacific Region

Hayato Tanaka¹*, Yusuke Kondo², and Susumu Ohtsuka²

¹Tokyo Sea Life Park, 6-2-3 Rinkai-cho, Edogawa-ku, Tokyo 134-8587, Japan. *Correspondence: E-mail: cladocopina@gmail.com (Tanaka)
²Takehara Station, Setouchi Field Science Center, Graduate School of Integrated Sciences for Life, Hiroshima University, 5-8-1 Minato-machi, Takehara, Hiroshima 725-0024, Japan. E-mail: ykondo@hiroshima-u.ac.jp (Kondo); ohtsuka@hiroshima-u.ac.jp (Ohtsuka)

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The present study describes *Pontopolycope orientalis* sp. nov. (Polycopidae), which was collected from the shallow sandy bottom off of Nagannu Island, Okinawa Prefecture, southwestern Japan. This new species is the first living representative of *Pontopolycope* from the Indo-Pacific region. We also provided a key to identifying five living congeners throughout the world’s oceans. Since *Pontopolycope* is defined by the morphological characteristics of the carapace, the following five fossil and subfossil (empty valve) species of *Polycope* (Polycopidae) are moved to *Pontopolycope*: *Pontopolycope luxuriosa* (Herrig, 1964) comb. nov., *P. sanctacatherinae* (Whatley and Downing, 1983) comb. nov., *P. krauseae* (Herrig, 1994) comb. nov., *P. proboscidea* (Herrig, 1994) comb. nov., and *P. isolata* (Whatley, Jones and Wouters, 2000) comb. nov. Finally, the nucleotide sequence data of three genes (18S rRNA, 28S rRNA, and mitochondrial cytochrome c oxidase subunit 1) of the new species are provided for future systematic and phylogenetic studies.

**Key words:** *Pontopolycope*, Myodocopa, New species, New combination, Southwestern Japan, Indo-Pacific, Molecular data.

**BACKGROUND**

Ostracods are small bivalved crustaceans that live in various aquatic environments including freshwater (e.g., Meisch 2000; Karanovic 2012; Külköylüoğlu et al. 2017; Diaz and Martens 2018) and marine habitats ranging from the supralittoral zone (Hiruta et al. 2007) to the hadal zone (Brandão et al. 2019; Yoo et al. 2019). A number of ostracods are also known to be symbiotic with invertebrates (Mestre et al. 2014; Tanaka and Arai 2017) and vertebrates (Tanaka and Hayashi 2019).

The polycopid ostracod genus *Pontopolycope* Chavtur, 1981 is characterized by a fully developed rostrum and concavity immediately ventral to the rostrum on the anterior margin of the carapace. This is a special characteristic of the family Polycopidae. To date, all four living congeners have been described from the Atlantic: *Pontopolycope rostrata* (Müller, 1894), type species; *P. dentata* (Brady, 1868); *P. mylax* Kornicker and Iliffe, 1992; and *P. storthynx* Kornicker, Iliffe and Harrison-Nelson, 2007.

Here, we describe the first living representative of the *Pontopolycope* species from the Indo-Pacific region and provide a key to identifying five living species in the genus. In addition, the present study examines all fossil species of *Polycope* in the available literature and suggests that some Mesozoic and Cenozoic fossil Polycopidae should be recategorized into...
Pontopolycope. Finally, the nucleotide sequence data of three genes (18S rRNA, 28S rRNA, and mitochondrial cytochrome c oxidase subunit 1) of the currently undescribed species are provided for future systematic and phylogenetic studies.

MATERIALS AND METHODS

Sediment samples were obtained from the sandy bottom at 52 m depth off the Nagannu Island, Okinawa Prefecture, southwestern Japan (26°14.339'N, 127°32.280'E) on May 21, 2016 (Fig. 1) by using a dredge (mouth 50 cm wide × 15 cm high; mesh size 5 mm) towed along the bottom twice by the **TRV Toyoshio-maru** (Hiroshima University). Sediments were stirred in seawater, and the supernatant was filtered with a small plankton net (mesh size 0.1 mm). All living ostracod specimens were extracted from the remaining deposits under a binocular stereomicroscope (SZ60, OLYMPUS Co., Ltd.). The collected specimens were fixed and preserved in 80% ethanol at room temperature for observation of morphological characteristics or 99.5% ethanol at -20°C for DNA extraction. The valves and soft parts were dissected with fine needles. The valves were preserved on a cardboard cell slide and the soft parts mounted in Neo-Shigaral, a gum-chloral medium (Shiga Konchu Fukyusha Co., Ltd.), on glass slides under the binocular stereomicroscope. These specimens were then observed and illustrated using a transmitted-light binocular microscope (BX53, OLYMPUS Co., Ltd.) with a differential interference contrast system and a camera lucida. The valves were washed with distilled water and gold-coated using an ion sputtering device (JFC-1100, JEOL Co., Ltd.). The materials were then observed using scanning electron microscopy (SEM) (JSM-6510LV, JEOL Co., Ltd.). The type series was deposited in the collection of the National Museum of Nature and Science, Tokyo (NSMT) under the prefix ‘NSMT-Cr’.

DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing were performed. Total DNA was extracted the paratype (NSMT-Cr 27378) following the method described by Tanaka and Ohtsuka (2016). Morphological voucher specimens were deposited into the NSMT. 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 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), 12.5 μl of 2X PCR buffer for KOD FX

**Fig. 1.** Map showing the sampling site. A, Japan; B, type locality of *Pontopolycope orientalis* sp. nov. (open circle).
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 and 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 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.

RESULTS

TAXONOMY

Subclass Myodocopa Sars, 1866
Order Halocyprida Dana, 1853
Suborder Cladocopina Sars, 1866
Family Polycopidae Sars, 1866
Genus Pontopolycope Chavtur, 1981

Pontopolycope orientalis sp. nov. Tanaka, Kondo and Ohtsuka (Figs. 2–6)
urn:lsid:zoobank.org:act:A73D0D0D-1BB3-4786-962A-2BCBEB009724

Type series: Holotype: adult male (NSMT-Cr 27373), right valve length 0.42 mm, height 0.34 mm, left valve length 0.44 mm, height 0.34 mm, soft parts mounted on one glass slide and valves preserved in one cardboard cell slide. Paratypes: 2 adult males (dissected on one slide and valves on SEM stab, NSMT-Cr 27374, 27375), 3 adult males (dissected on one slide and valves preserved in one cardboard cell slide, NSMT-Cr 27376 to 27378), 1 adult female (dissected on one slide and valves preserved in one cardboard cell slide, NSMT-Cr 27380 to 27382); same collecting data with holotype.

Type locality: The holotype specimen was collected from a sea bottom consisting of coarse coral sand grains off of Nagannu Island, Okinawa Prefecture, southwestern Japan (26°14.339’N, 127°32.280’E) at a depth of 52 m on May 21, 2016.

Diagnosis: Carapace circular in lateral view, with developed rostrum and concavity immediately ventral to rostrum of the anterior margin. Carapace surface covered with shallow pits. Carapace margin with serrations, along the anterior to posterior margin in left valve and anterior to ventral margin in right valve. Pore system consisting of shallow depression and a small dome with pore canal. Male copulatory organ consisting of a long pill-shaped tube.

Description: (Measurements: Table 2). Adult male. (Figs. 2A–D, G, H; 3; 4E, F, H; 5A–E; 6A, B).

Carapace (Figs. 2A–D, G, H; 3): Circular in lateral view, with developed rostrum and concavity immediately ventral to rostrum of anterior margin. Posterior end situated slightly near the ventral side. Surface covered with shallow pits (Fig. 2A, B). Along anterior to posterior margin in left valve and anterior to ventral margin in right valve with serrations (Figs. 2C, D; 3). Pore system consisting of a shallow depression and a small dome with pore canal (Fig. 2G). Adductor muscle scar consisting of three closely spaced scars (Figs. 2C, D, H; 3). Marginal infold with groove around internal marginal zone of postero-dorsal, anterior and ventral margins in right valve (Fig. 2C) and corresponding ridge in left valve (Fig. 2D). Hinge

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

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer name</th>
<th>Reaction</th>
<th>Sequence (5’ to 3’)</th>
<th>Direction</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>18S rRNA</td>
<td>Euk18SF</td>
<td>PCR &amp; CS</td>
<td>ACCTGGTTGATCCTGCCAG</td>
<td>Forward</td>
<td>Moon-van der Staay et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>Euk18SR</td>
<td>PCR &amp; CS</td>
<td>TGATCCTCCYGCAGGTTCA</td>
<td>Reverse</td>
<td>Moon-van der Staay et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>18SF2</td>
<td>CS</td>
<td>CCTGAGAAACGGCTRCCACAT</td>
<td>Forward</td>
<td>Yamaguchi and Endo (2003)</td>
</tr>
<tr>
<td>28S rRNA</td>
<td>28S-01</td>
<td>PCR &amp; CS</td>
<td>GACTACCCCCTGAATTTAAGCAT</td>
<td>Forward</td>
<td>Kim et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>28S-18F</td>
<td>CS</td>
<td>GAAAGATGGTTGAACTATGCGT</td>
<td>Forward</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>28S-24F</td>
<td>CS</td>
<td>GTTGGAGAAGGTTTTCATGT</td>
<td>Forward</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>28S-18R</td>
<td>CS</td>
<td>CAGGCATAGGTGCACCATCTTTC</td>
<td>Reverse</td>
<td>Ohtsuka et al. (2018)</td>
</tr>
<tr>
<td></td>
<td>28S-32R</td>
<td>CS</td>
<td>AGACGACTGGGCAAGAATTC</td>
<td>Reverse</td>
<td>Ohtsuka et al. (2018)</td>
</tr>
<tr>
<td>CO1</td>
<td>LCO1490</td>
<td>PCR &amp; CS</td>
<td>GGTCAACAAATCATAAGATGG</td>
<td>Forward</td>
<td>Folmer et al. (1994)</td>
</tr>
<tr>
<td></td>
<td>HCO2198</td>
<td>PCR &amp; CS</td>
<td>TAAACTTCAGGGTGACCAAAATCA</td>
<td>Reverse</td>
<td>Folmer et al. (1994)</td>
</tr>
</tbody>
</table>

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structure consisted of simple groove in right valve (Fig. 2C), and of corresponding bar in left valve (Fig. 2D).

**Bellonci organ** (Fig. 4A): Two setulous seta.

**Upper lip** (Fig. 4B): Semicircular in lateral view, with fine setae on surface.

**Antennula** (Fig. 4C): Uniramous, four podomeres. First podomere with setulae on dorsal margin and lateral surface. Second podomere about four-fifths the length of first podomere, with one setulous annulated seta near dorso-proximal end, one simple seta on ventro-distal end, and setulae on dorsal and ventral middle margins and at dorso-distal end. Third podomere about one-fourth the length of first podomere, with one setulous seta at dorsal end and three setae consisting of one setulous annulated seta and two setae with curved tip on ventro-distal margin. Fourth podomere small, with four long setulous annulated setae.

**Antenna** (Fig. 4E, F): Biramous, with exopodite and endopodite consisting of nine and three podomeres, respectively. Basis triangular and tapering distally. Exopodite (Fig. 4F): first podomere about half lengths of basis; Second podomere about one-fifth the length of first podomere; podomere lengths decreasing in size from second to eighth, each podomere with one setulous annulated long seta, respectively; ninth podomere very small, with one long annulated, one medium annulated and one short simple setae at distal end. Endopodite (Fig. 4E): first podomere same length as first podomere of exopodite; second podomere about one-third the length of first podomere, with three simple setae along dorsal margin, one clavate process at proximal middle end, and five setulous annulated setae at distal end. Third podomere one-fifth the length of first podomere, with one dorsal outgrowth, and four setulous annulated setae at distal end.

**Maxillula** (Fig. 5A–D): Precoxal seta (Fig. 5A, B) with eight annulated plumose setae. Coxa (Fig. 5A, C) with eight plumose setae on ventral side. Basis (Fig. 5A) rectangular with dorso-proximally hump in lateral view, with one plumose and one plumose annulated setae on ventral margin. First podomere of endopodite bare. Second podomere with one plumose seta on ventral margin. Third podomere with two setulous and one annulated setae at ventral margin, and two setulous setae at dorso-distal end. Fourth podomere with one setulous annulated seta, three annulated stouter setae with spines on middle part and setae on distal part. Exopodite (Fig. 5D) with setae along dorsal margin, and 10 annulated setae at distal end.

**Fifth limb** (Fig. 5E): Coxa bearing branchial plate (epipodite) with 13 long plumose setae, and two setulous short setae on dorso-lateral area and two setulous short setae at dorso-distal end. Basis with three plumose and two plumose setae on dorsal and ventral margin, respectively. Endopodite consisting of two podomeres. First podomere with one setulous stout seta. Second podomere cylindrical tapering proximally, with one plumose stout seta. Exopodite with four plumose setae.

**Uropod** (Fig. 6A, B): Each lamella with eight claws. Anterior-most claw small, with a row of fine setae on posterior margin. Anterior second to eighth claws with a row of setae on anterior and posterior margins, respectively.

**Male copulatory organ and posterior body** (Fig. 6A): Male copulatory organ arising from outer surface of body on left side of terminal trunk segment as a long pillar-shaped tube. A row of stout small setae on ventral margin. One spinous process (telson) at terminal end.

**Adult female** (Figs. 2E, F; 4D, G; 6C): Bellonci organ, mandibula, maxillula, fifth limbs, and upper lip similar to those of adult male.

**Carapace** (Fig. 2E, F): Carapace length and height slightly larger than adult male (Table 2). Carapace length and height slightly larger than adult male (Table 2).

**Antenna** (Fig. 4D): Only third and fourth podomeres different from those of adult male. Third podomere about one-fourth the length of first podomere, two annulated plumose setae at distal end.

Table 2. Dimension of valves of *Pontopolycope orientalis* sp. nov. from the type locality

<table>
<thead>
<tr>
<th></th>
<th>Length (mm)</th>
<th>Height (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Observed range</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right valve</td>
<td>0.41</td>
<td>0.39–0.43</td>
</tr>
<tr>
<td>Left valve</td>
<td>0.42</td>
<td>0.39–0.44</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right valve</td>
<td>0.46</td>
<td>0.44–0.49</td>
</tr>
<tr>
<td>Left valve</td>
<td>0.46</td>
<td>0.45–0.47</td>
</tr>
</tbody>
</table>

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Fig. 2. *Pontopolypode orientalis* sp. nov., SEM images. A, B, G, paratype male (NSMT-Cr 27374); C, D, H, paratype male (NSMT-Cr 27375); E, F, paratype female (NSMT-Cr 27379). A, E, external lateral view of right valve; B, F, external lateral view of left valve; C, internal lateral view of right valve; D, internal lateral view of left valve; G, external surface of left valve; H, adductor muscle scar, internal view of right valve. Scale bars: A–F =100 μm; G = 15 μm; H = 30 μm.
with one setulous seta at dorsal end. Fourth podomere small, with five long setulous annulated setae.

Antenna (Fig. 4G): Only second and third podomeres of endopodite are different from those of adult male. Second podomere with one annulated seta on dorsal margin and six annulated setae at distal end. Third podomere one-sixth the length of first podomere with four annulated setae at distal end.

Uropod (Fig. 6C). Each lamella with eight claws.

DNA sequences: Three nucleotide sequences were obtained from the paratype (NSMT-Cr 27378): 28S (Accession no. LC528590, 3217 bp), 18S (Accession no. LC528589, 1794 bp), and CO1 (Accession no. LC528833, 660 bp).

Etymology: The specific name orientalis (Latin, meaning in the east) refers to the first record of the living species of the genus Pontopolycope in the Indo-Pacific region.

**DISCUSSION**

The present study is the first to report a living species of the genus Pontopolycope in the Indo-Pacific region. All previous congeners were found in the North Atlantic and Mediterranean Sea (Fig. 7), namely Pontopolycope rostrata from between lime (calcareous) algae in the Gulf of Naples (Fig. 7B); P. strothyx, from Ocean Blue Holes of the Bahamas (Fig. 7C); P. mylax from the anchialine caves of Jamaica (Fig. 7D); and P. dentata, from off the coast of Shetland (Fig. 7E).

Pontopolycope orientalis sp. nov. can be easily distinguished from Pontopolycope rostrata and P. strothyx by the structure of the carapace surface: the new species has no posterior projections, while the latter two species have four posterior projections. The ornamentation of carapace surface of the new species differs from that of P. mylax: shallow punctations in the former versus reticulations, and surface within reticulate with pebbly texture in the latter. The carapace and appendage morphologies of the new species resemble those of P. dentata. Pontopolycope dentata was originally described by carapace morphology alone (Brady 1868), until Müller (1894) described appendages of this species based on the materials collected from sand between the roots of sea grasses in the Gulf of Naples. Pontopolycope orientalis sp. nov. and P. dentata can be distinguished based on the following features: (1) the rostral tip of the carapace is obtuse rather than sharp; (2) the mandibular exopodite possess one thin broad setulous seta versus one pillar-shaped seta and one simple seta; (3) setae on the ventral margin of male copulatory organ is stout rather than fine.

In general, the carapace of Polycopidae is heavily calcified and well preserved as fossils up to the Carboniferous (Moore 1961). However, due to the presence of a relatively simple valve, fossil polycopid ostracods tend to be classified as the genus Polycop (type genus of the family Polycopidae). From the literature survey carried out in the present study, we established that 114 species have been described as Polycop among the 121 known fossil species of Polycopidae. Genus-level classification of polycopids is very difficult without knowing the anatomy of soft parts. This is due to the fact that the carapaces of some genera closely resemble one another, even though their soft parts are clearly different (e.g., Polycopetta Chavtur, 1981 and Polycopissa Chavtur, 1981). In contrast,

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**Fig. 3.** Pontopolycope orientalis sp. nov., holotype male (NSMT-Cr 27373). A, external lateral view of the right valve; B, external lateral view of the left valve. Scale bar = 100 μm.
Fig. 4. *Pontopolycope orientalis* sp. nov. A–C, E, F, H, holotype male (NSMT-Cr 27373); D, G, paratype female (NSMT-Cr 27379). A, Bellonci organ; B, upper lip; C, antennula; D, distal part of antennula; E, antenna except exopodite; F, exopodite of antenna; G, endopodite of antenna; H, mandibula. Scale bar = 50 μm. Abbreviations: cp, clavate process; do, dorsal outgrowth.
*Pontopolycope* is special in the family with respect to their developed rostrum and concavity just ventral to the rostrum along the anterior margin of the carapace. Considering this morphological uniqueness, some fossil and subfossil (empty valve) polycopid species could be classified as *Pontopolycope* due to their similarities with respect to these features. Although no appendage is described, the following five *Polycope* species can most likely be reclassified as *Pontopolycope*:

- *Pontopolycope luxuriosa* (Herrig, 1964) comb. nov. from Rügen Island (Late Cretaceous, Late Maastrichtian, Fig. 7F);
- *Pontopolycope sanctacatherinae* (Whatley and Downing, 1983) comb. nov. from south-east Australia (Middle Miocene, Langhian, Fig. 7G);
- *Pontopolycope orientalis* sp. nov., holotype male (NSMT-Cr 27373). A, maxillula; B, precoxa of maxillula; C, coxa of maxillula; D, exopodite of maxillula; E, fifth limb. Scale bar = 50 μm. Abbreviations: ba, basis; cx, coxa; en, endopodite; ex, exopodite; pc, precoxa.
krauseae (Herrig, 1994) comb. nov. from Rügen Island (Late Cretaceous, Late Maastrichtian, Fig. 7H); Pontopolycope proboscidea (Herrig, 1994) comb. nov. from Bornholm Island (Late Cretaceous, Late Maastrichtian, Fig. 7I); and Pontopolycope isolata (Whatley, Jones and Wouters, 2000) comb. nov. from Easter Island (Holocene, Fig. 7J). In addition, some Polycope species in open nomenclature could be assigned to Pontopolycope. There are: Polycope sp. B sensu Cronin (1983) from the bathyal seafloor of Florida-Hatteras Slope; Polycope sp. 2 sensu Bergue et al. (2006) from the bathyal seafloor of Santos Basin,

**Fig. 6.** Pontopolycope orientalis sp. nov. A, B, holotype male (NSMT-Cr 27373); C, paratype female (NSMT-Cr 27379). A, left lateral view except right uropodal lamella; B, right uropodal lamella; C, left uropodal lamella. Scale bar = 50 μm.
Brazil; *Polycope* sp. A and *Polycope* sp. B sensu Titterton and Whatley (2006) from the shallow seafloor of Honiara Bay, the Solomon Islands. *Polycope moenia* Joy and Clark, 1977 described as having empty carapace collected from the deep sea floor of the Arctic Ocean was classified as *Pontopolycope* by Chavtur (1983). However, the present study excludes *Polycope moenia* from the *Pontopolycope* group because this species lacks concavity along the anterior margin of the carapace and has more elliptic shaped carapace in the lateral view.

The distribution of living and fossil species of *Pontopolycope* seems to imply a Tethyan origin. From the type locality of *Pontopolycope orientalis* sp. nov. (off Nagannu Island), some copepod species have been reported as Tethyan relicts (Ohtsuka and Boxshall 1994; Ohtsuka et al. 1998). More extensive studies on living and fossil species of *Pontopolycope* in the world’s oceans will enable us to reveal the natural history of this genus.

**Key to living species of *Pontopolycope* Chavtur, 1981**

1. Posterior projections present on carapace surface .................. 2

   - Posterior projections absent on carapace surface .................. 3

2. Reticulations on carapace surface fine; a long seta absent on dorsal side of basis of mandible ...........................................

   - Reticulations on carapace surface coarse; a long seta present on dorsal side of basis of mandible .................................

   - *Pontopolycope rostrata* (Müller, 1894)

3. Carapace surface reticulated ...........................................

   - Carapace surface punctuated ...........................................

4. Tip of rostrum of carapace is sharp; the mandibular exopodite possess one pillar-shaped seta and one simple seta; setae on the ventral margin of male copulatory organ is fine ........................................

   - Tip of rostrum of carapace is obtuse; the mandibular exopodite possess one thin broad setulous seta; setae on the ventral margin of male copulatory organ is stout ........................................

   - *Pontopolycope orientalis* sp. nov.

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Acknowledgments: This work and new species name have been registered with ZooBank under urn:lsid:zoobank.org:pub:1F460398-080D-47D0-BD0D-19D4EA205BD9. We thank the captain and crew of the TRV Toyosho-maru, Hiroshima University, for their cooperation at sea. We also thank the plankton laboratory members at Takehara Station, Setouchi Field Science Center, Graduate School of Integrated Sciences for Life, Hiroshima University for their assistance in collecting specimens. This study was funded by the JSPS KAKENHI Grant Numbers JP263700 (HT).

Authors’ contributions: HT collected and processed the samples, conceived the study, and drafted the manuscript. YK and SO assisted with sample collection. All authors read and approved the final manuscript.

Competing interests: The authors declare that they have no conflict of interest.

Availability of data and materials: Sequences generated in the study have been deposited into the DNA Data Bank of Japan (DDBJ) database (accession numbers in manuscript).

Consent for publication: Not applicable.

Ethics approval consent to participate: Not applicable.

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