

## Article

# Morphological and Molecular Study of the Fish Parasitic Crustaceans *Cymothoa indica* and *Mothocya collettei* (Isopoda: Cymothoidae), with New Distribution Records

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**Abstract:** Cymothoidae are parasitic isopods that infest fishes inhabiting marine, brackish, and fresh-water environments. The systematics, distribution, and host specificity of cymothoids is often unknown. In this study, morphological observations and molecular analysis using the COI and 16S rRNA genes were conducted on a juvenile individual of *Cymothoa indica* infesting the wide-banded hardyhead silverside, *Atherinomorus lacunosus*, and an ovigerous female of *Mothocya collettei* infesting the hound needlefish, *Tylosurus crocodilus*, both collected from Okinawa Island, Japan. This is the first time that these two species of cymothoid have been collected in the Ryukyu Islands, and the juvenile *C. indica* in particular represents a new northern range limit for its species. That specimen was identified using molecular analysis and showed almost identical morphology to previously reported juveniles, but it differed in the numbers of robust setae on pereopods 6 and 7. The ovigerous female of *M. collettei* showed a high morphological similarity to *M. collettei* but had some morphological traits consistent with *Mothocya affinis*. DNA analysis indicated that these two nominal species of *Mothocya* are very closely related, and morphological and molecular studies using larger populations of both are needed.

**Keywords:** 16S rRNA; *Atherinomorus lacunosus*; COI; *Mothocya affinis*; Parasitic isopod; *Tylosurus crocodilus*



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## 1. Introduction

Cymothoidae Leach, 1818 (Crustacea: Isopoda) is a cosmopolitan family of parasitic isopods that includes more than 300 species in 42 genera [1]. It is one of the largest families of isopods [1,2] and inhabits all but the polar seas. Their hosts include diverse taxa of fishes inhabiting marine, brackish, and freshwater environments [3,4]. These parasites attach to their hosts at four attachment sites: the branchial cavity, buccal cavity, abdominal cavity, and body surface of fishes [4]. Cymothoids have five life-history stages: manca, juvenile, male, transitional (under sex change), and female. Free-swimming mancae search for and infest host fish. They grow into juveniles and adult males on the hosts, and then adult cymothoids change sex from male to female [5–7]. Cymothoids are mainly identified using the morphological characteristics of adult females. Thus, molecular analysis is almost the only way to identify manca, juvenile, and male specimens [8] and is often used for this purpose [9–11].

The wide intraspecific morphological variation in cymothoid isopods complicates their taxonomy, and in recent years there have been a series of revisions to the taxonomic system [4,12,13]. In Japan, two species of *Mothocya* Costa in Hope, 1851 (*Mothocya parvostis* Bruce, 1986 and *M. sajori* Bruce, 1986) that were long thought to be separate species have recently been shown to be synonyms [14]. Information on the distribution and host-specificity patterns of some cymothoids is also lacking.

In this study, new distribution records of *Cymothoa indica* Schioedte and Meinert, 1884 and *Mothocya collettei* Bruce, 1986 are presented, as well as a new host of *C. indica*, and a molecular analysis was performed to consider the taxonomy of these species.

## 2. Materials and Methods

### 2.1. Material for Morphological Examination

The host fish were collected using hand nets on 5 September 2022, at Tousoe fishing port, Yonabaru Town, Okinawa Island, Japan. The cymothoids were found in the buccal cavity of the wide-banded hardyhead silverside, *Atherinomorus lacunosus* (Forster, 1801), and in the branchial cavity of the hound needlefish, *Tylosurus crocodilus* (Péron and Lesueur, 1821). The two cymothoids were frozen and transported to the laboratory, then preserved in 99.5% ethanol. The hosts were identified based on [15].

The morphological descriptions were made with the aid of a SZX7 stereomicroscope and a BX50 light microscope (Olympus, Tokyo, Japan). The drawings were digitally inked using Illustrator (version 26.5) (Adobe, San Jose, CA, USA) and a DTC133 pen display (Wacom, Saitama, Japan) with reference to [16]. The measurements and terminologies essentially follow [17].

### 2.2. Molecular Analysis

The total DNA was extracted from the right pereopod 7 of the cymothoid specimen using the alkaline lysis method according to the recommended protocol for KOD FX Neo DNA polymerase (Toyobo, Osaka, Japan). The pereopods were mixed with 18 µL of NaOH (50 mM) and incubated at 95 °C for 10 min. Each tube containing 2 µL of Tris-HCl (1 M, pH 8.0) was extensively vortexed and centrifuged at 12,000 rpm for 5 min. The supernatant (DNA) was separated and frozen at −30 °C until use in the polymerase chain reaction (PCR).

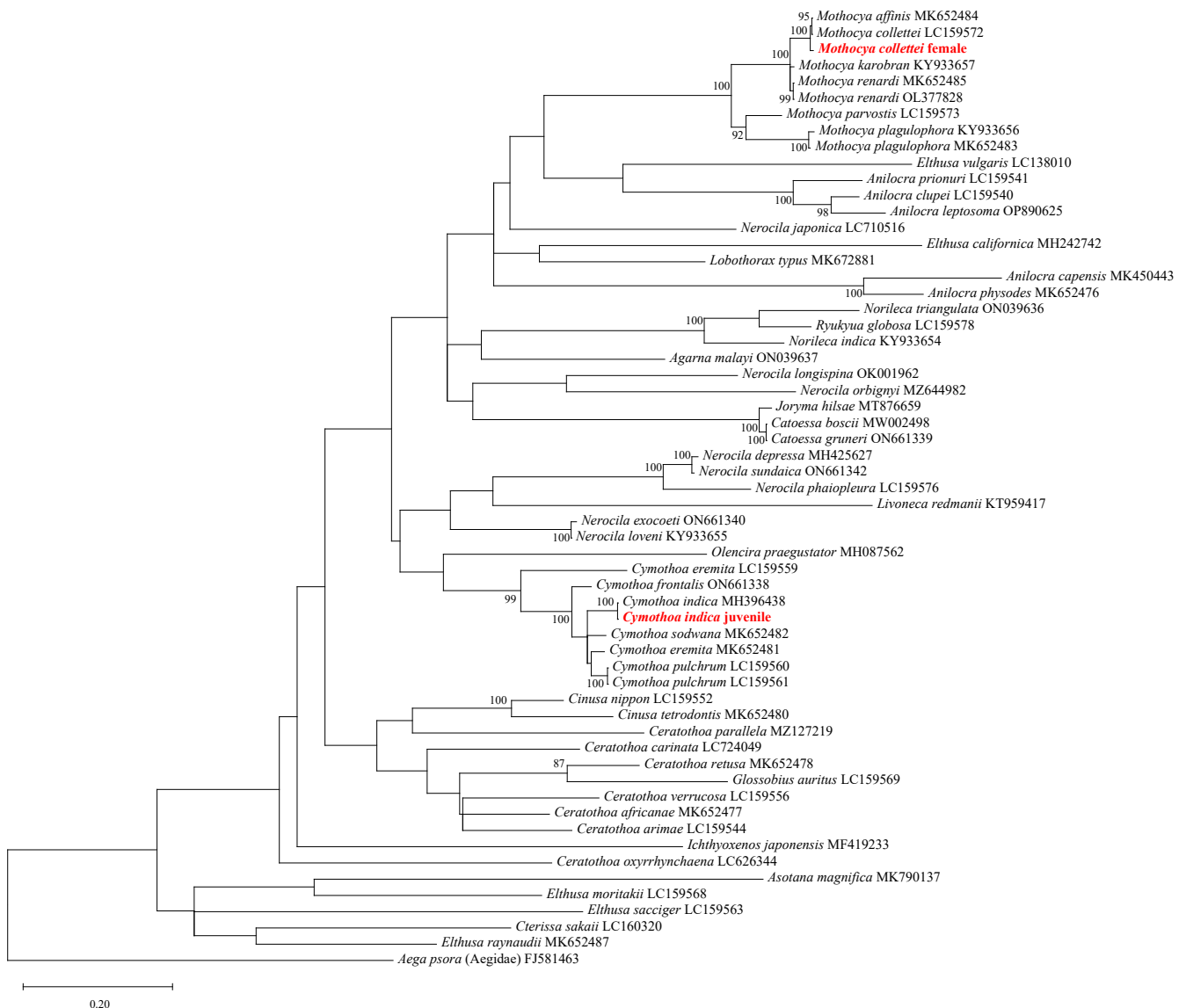
The partial cytochrome c oxidase subunit I (COI) gene sequences were amplified using the primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') [18], and the partial 16S rRNA sequences were amplified using the primers 16Sar (5'-CGCCTGTTTAAACAAAAACAT-3') and 16Sbr (5'-CCGGTCTGAACTCAGATCATGT-3') [19]. The total volume for each PCR was 8.1 µL, which was composed of 1 µL of DNA, 0.78 µL of ultrapure water, 4.06 µL of 2× PCR buffer, 1.62 µL of dNTP mix, 0.24 µL of each primer (10 µM solutions), and 0.16 µL of KOD FX Neo DNA polymerase (Toyobo, Osaka, Japan). The thermocycler profile of COI consisted of an initial denaturation at 94 °C for 2 min; 35 cycles of denaturation at 98 °C for 10 s, annealing at 45.5 °C for 30 s, and extension at 68 °C for 45 s; and a final extension at 68 °C for 7 min. The thermocycler profile of the 16S rRNA consisted of an initial denaturation at 94 °C for 2 min; 35 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and extension at 68 °C for 45 s; and a final extension at 68 °C for 7 min. The PCR products were sequenced with the dye terminator method using an ABI 3130xl genetic analyzer (Applied Biosystems, Foster City, CA, USA).

The sequences of 56 representative species of cymothoids for COI and 37 species for 16S rRNA and an outgroup species (*Aegidae* sp.) for both genes were downloaded from GenBank. The sequences were aligned using PLANK [20], implemented in MEGA 10 [21], trimmed, and collapsed into haplotypes. All sequences were deposited in GenBank (accession numbers: LC776736–LC776739). The maximum likelihood trees were generated using the GTR + G + I model for each of the COI (573 bp, including gaps) and 16S rRNA (682 bp, including gaps) sequences.

## 3. Results

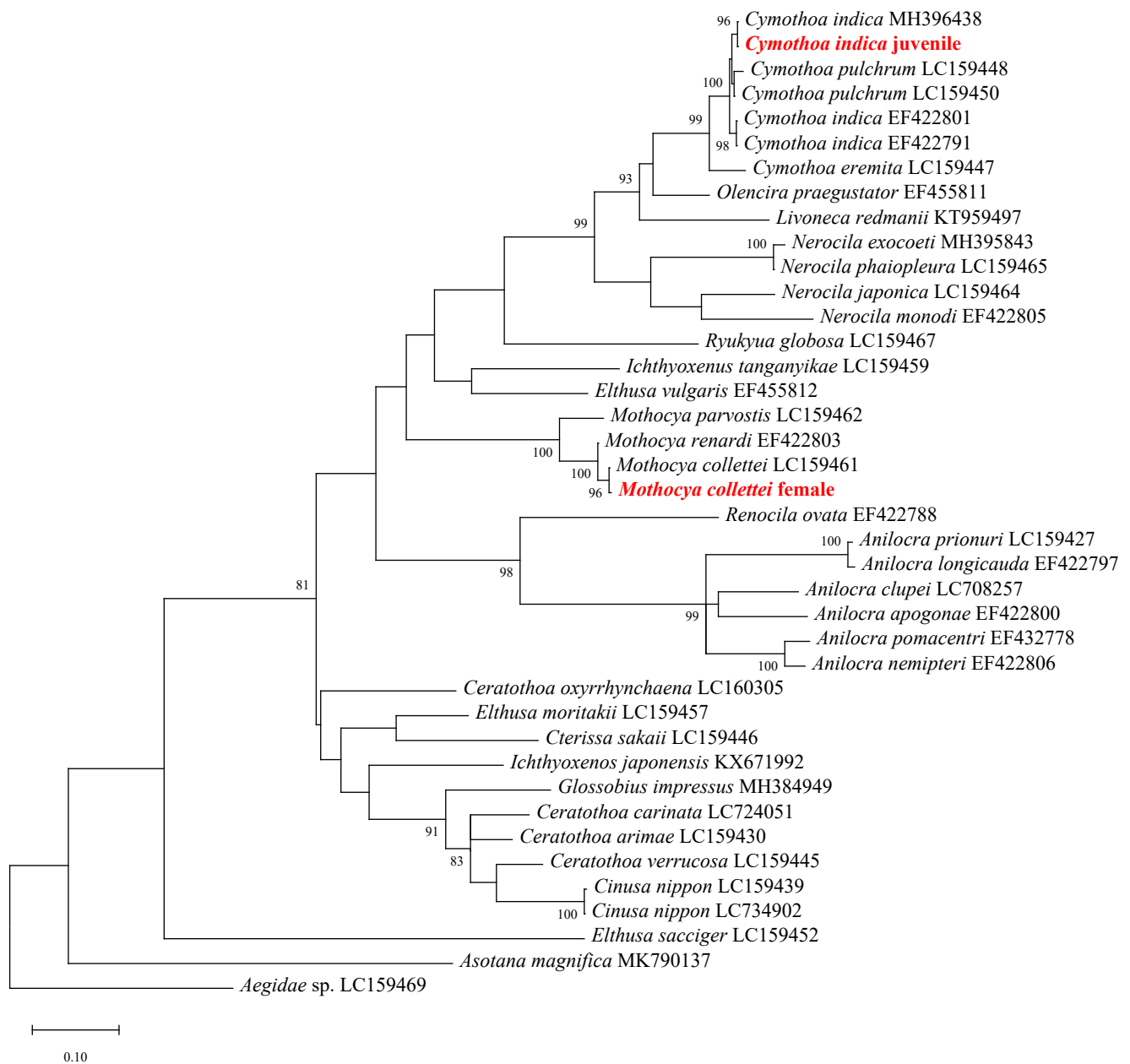
The cymothoid from *Atherinomorus lacunosus* was included in the clade of *Cymothoa* in the phylogenetic trees based on both the COI and 16S rRNA gene sequences (Figures 1 and 2). Furthermore, in the COI phylogenetic tree, it formed a clade with *C. indica* close to but distinct from the clade of *Cymothoa pulchrum* Lanchester, 1902. The

phylogenetic tree based on the 16S rRNA gene did not separate *C. indica* and *C. pulchrum*, and the clade formed by these two species did include the present individual.



**Figure 1.** Maximum likelihood tree based on the COI gene, indicating the *Cymothoa indica* and *Mothocya collettei* collected in this study in red (bold), along with cymothoid sequences downloaded from GenBank. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. Bootstrap supports lower than 80% are not shown.

In the COI phylogenetic tree, *Mothocya affinis* Hadfield, Bruce and Smit, 2015 and *M. collettei* formed one clade, which included the cymothoid obtained from *Tylosurus crocodilus*. In the 16S rRNA phylogenetic tree, for which no 16S rRNA sequence of *M. affinis* was available in GenBank, the present cymothoid female from *T. crocodilus* still formed a clade with *M. collettei*.



**Figure 2.** Maximum likelihood tree based on 16S rRNA gene, detected from the *Cymothoa indica* and *Mothocya collettei* collected in this study in red (bold), along with cymothoid sequences downloaded from GenBank. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. Bootstrap supports lower than 80% are not shown.

#### 4. Description

**Order:** Isopoda Latreille, 1816

**Superfamily:** Cymothoidea Leach, 1814

**Family:** Cymothoidae Leach, 1814

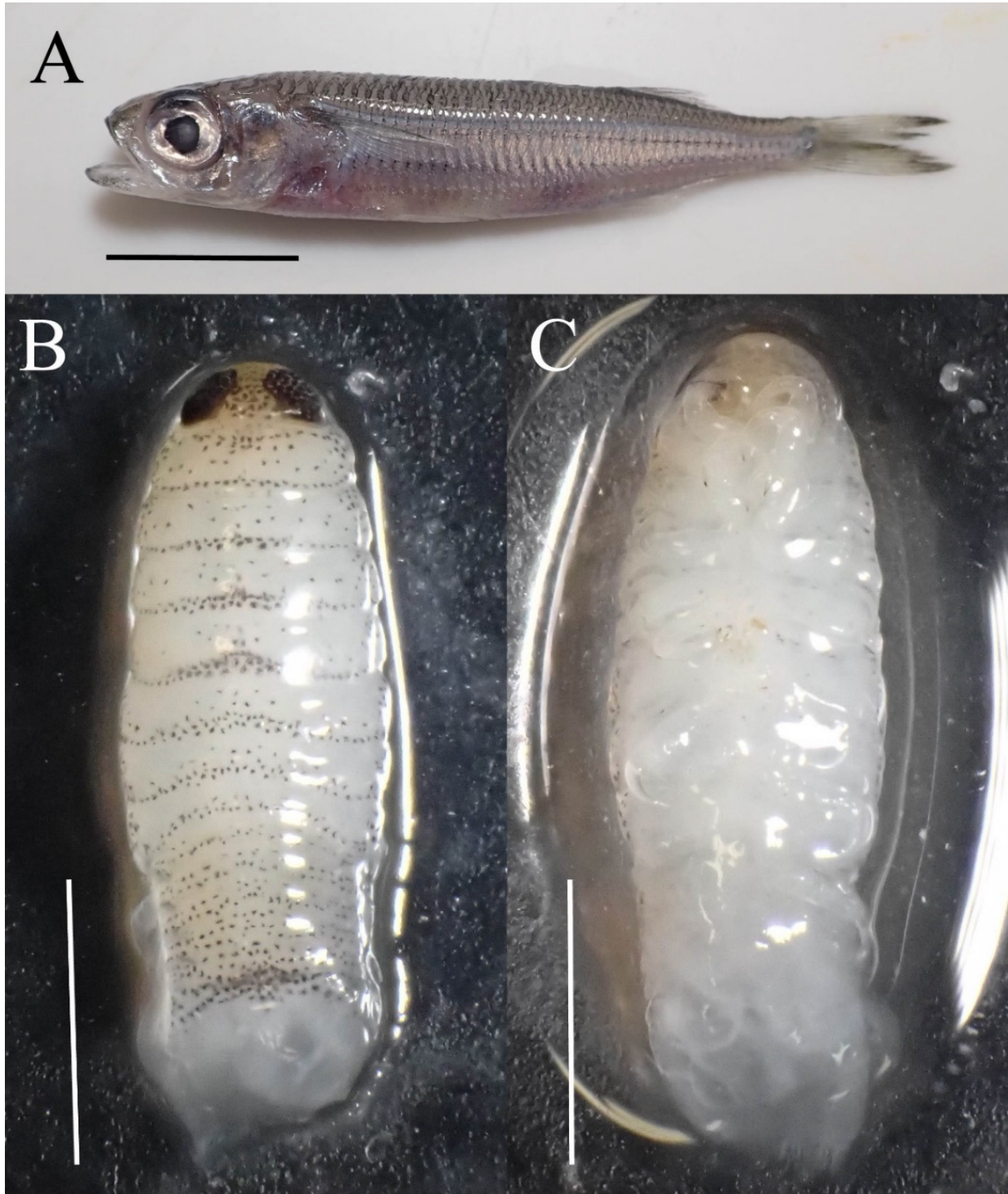
**Genus:** *Cymothoa* Fabricius, 1793

***Cymothoa indica* Schioedte and Meinert, 1884**

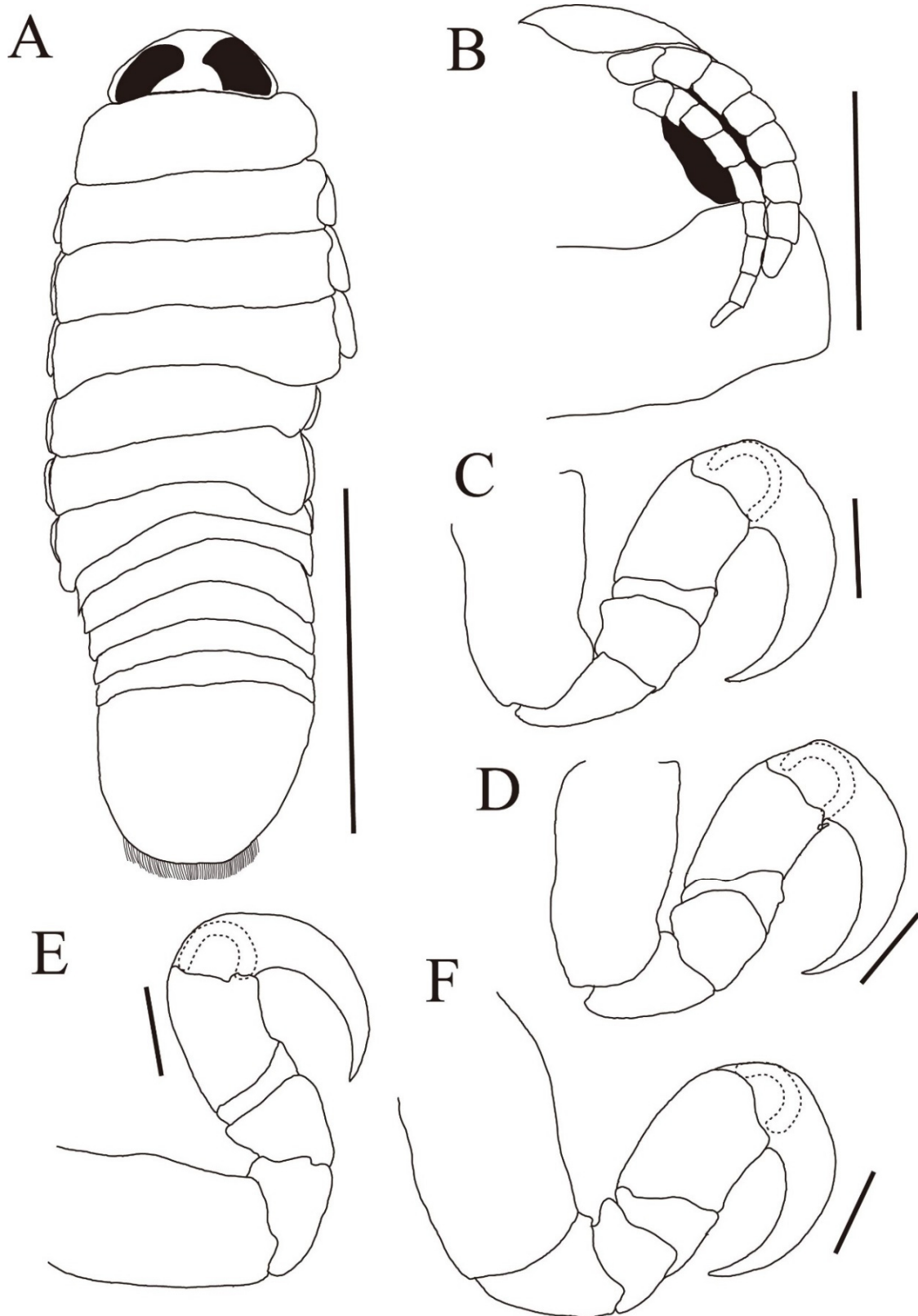
[New standard Japanese name: Minami-uonoe]

(Figures 3–5)

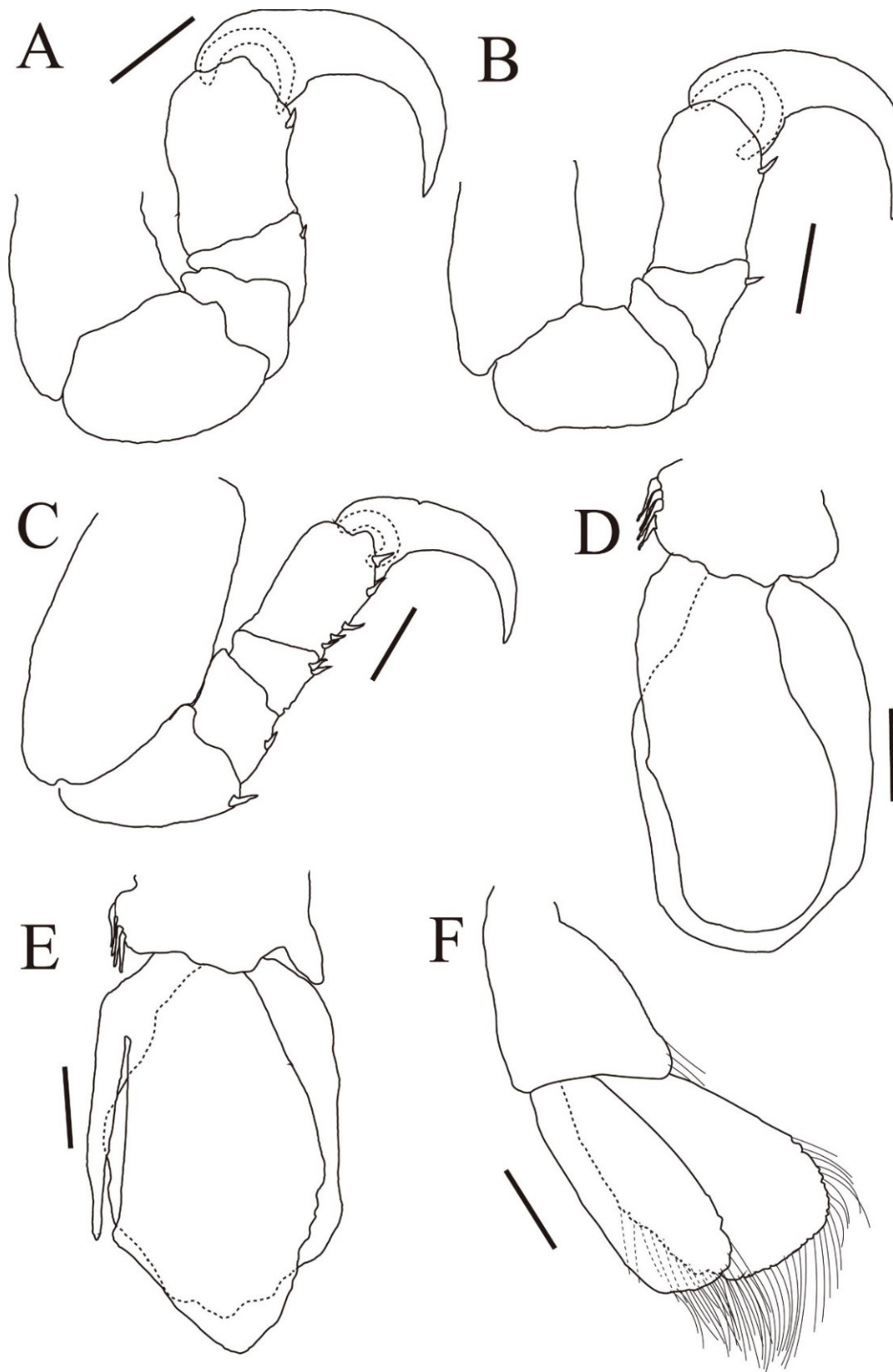
Material examined: one juvenile (BL: 8.2 mm) from Tousoe fishing port, Yonabaru Town, Okinawa Island, Japan, in buccal cavity of *Atherinomorus lacunosus* (SL: 74.3 mm), 5 September 2022.



**Figure 3.** (A), host fish, *Atherinomorus lacunosus* (74.3 mm, SL), (B), dorsal view of the juvenile of *Cymothoa indica* (8.2 mm, BL), (C), ventral view of the juvenile of *Cymothoa indica*. Scale bars: (A), 20 mm; (B,C), 3 mm.



**Figure 4.** *Cymothoa indica*, juvenile (8.2 mm BL) infesting the buccal cavity of *Atherinomorus lacunosus* collected from the Tousoe fishing port, Yonabaru Town, Okinawa Island, Japan; (A), dorsal view; (B), ventral view of cephalon; (C–F), pereopods 1–4. Some pilosity and surface sculpturing were omitted. Scale bars, (A), 3 mm; (B), 1 mm; (C–F), 0.3 mm.



**Figure 5.** *Cymothoa indica*, juvenile (8.2 mm BL) infesting the buccal cavity of *Atherinomorus lacunosus* collected from the Tousoe fishing port, Yonabaru Town, Okinawa Island, Japan; (A–C), pereopods 5–7; (D,E), pleopods 1, 2; (F), uropod. Some pilosity and surface sculpturing were omitted. Scale bars, 0.3 mm.

Description of juvenile (Figures 4 and 5): The body is elliptical, 2.9 times as long as greatest width, widest at pereonite 4, narrowest at pleonite 5, dorsal surfaces convex. The cephalon is 1.7 times wider than long, semi-oval, anterior margin rounded. The eyes are oval with distinct margins, the long axis of each eye is 1.3 times the length of cephalon, the short axis is 0.2 times the maximum width of the cephalon. The anterior border of pereonite 1 is straight medially, slightly curved laterally. Coxae 2–7 are narrow, slightly visible or invisible in dorsal view. Pereonite 1 is the longest, pereonite 7 is the shortest; the posterior margins of all pereonites are smooth and slightly curved laterally, and that of pereonite 7 is slightly recessed. The pleon constitutes 20% of the total length, 0.6 times as wide as greatest body width, with all pleonites visible in dorsal view. The pleotelson is 0.8 times as long as it is wide and 1.1 times as long as the pleon, with a fringe of short posterior marginal setae.

The antennula have eight articles, extending beyond the midlength of pereonite 1. The antenna has nine articles.

Pereopod 1 has a basis 1.6 times as long as the greatest width, an ischium 0.6 times as long as the basis, a merus 0.5 times as long as the ischium, a carpus 0.3 times as long as the merus, a propodus 5.6 times as long as the carpus, and a dactylus 1.8 times as long as the propodus. Pereopod 2 has a basis 1.9 times as long as the greatest width, an ischium 0.4 times as long as the basis, a merus 0.9 times as long as the ischium, a carpus 0.6 times as long as the merus, a propodus 2.1 times as long as the carpus with one robust seta on the inferior margin, and a dactylus 1.6 times as long as the propodus. Pereopods 3–4 are similar to pereopod 1. Pereopod 5 has a basis 1.3 times as long as the greatest width, an ischium 0.8 times as long as the basis, a merus 0.4 times as long as the ischium, a carpus 1.8 times as long as the merus with one robust seta on the inferior margin, a propodus 1.7 times as long as the carpus with one robust seta on the inferior margin, and a dactylus 1.2 times as long as the propodus. Pereopod 6 is similar to pereopod 5. Pereopod 7 has a basis 1.8 times as long as the greatest width, an ischium 0.5 times as long as the basis with one robust seta on the inferior margin, a merus 0.5 times as long as the ischium with one robust seta on the inferior margin, a carpus 0.9 times as long as the merus with two robust setae on the inferior margin, a propodus 2.0 times as long as the carpus with five robust setae on the inferior margin, and a dactylus 1.2 times as long as the propodus.

The pleopods are all lamellar with smooth surfaces. The peduncle of pleopod 1 is 1.6 times as wide as long, and its medial margin has four coupling hooks; the endopod is elliptical, 1.9 times as long as it is wide; the exopod is as long as the endopod, elliptical with an almost straight lateral margin, and is 1.5 times as long as it is wide. Pleopod 2 is similar to pleopod 1, and its endopod has an appendix masculina.

The uropodal peduncle is triangular, 1.4 times as long as it is wide, 0.9 times as long as exopod, with a distal corner with three setae; the rami are both of equal length, extending beyond the posterior margin of the pleotelson. The endopod is oval, 2.4 times as long as the greatest width, with the apical and the distal half or less of the medial and lateral margins having short marginal setae. The exopod is semi-triangular, 1.5 times as long as the greatest width, with the apical margins bearing short setae.

Coloration: the body is pearl yellow in both the fresh and ethanol-preserved conditions.

Hosts: in this study, only *Atherinomorus lacunosus*, although *C. indica* infests a wide range of fish species (see [22]).

Distribution: besides the present individuals from Okinawa Island, Japan, this species is known from the southeastern Pacific, Indian Ocean, and Red Sea [23,24].

Remarks: This individual was identified as *C. indica* based on molecular analysis. It was judged to be a juvenile because of the marginal setae on the uropods and the posterior margin of the pleotelson. However, since it does not have marginal setae on the pleopods, it is in a stage near to an adult male [25]. The morphology of juveniles of *C. indica* reported by [26] is almost identical to that of our specimen except that the numbers of robust setae of pereopods 6 and 7 were, respectively, 6 and 6 in [26] versus 2 and 9 herein.



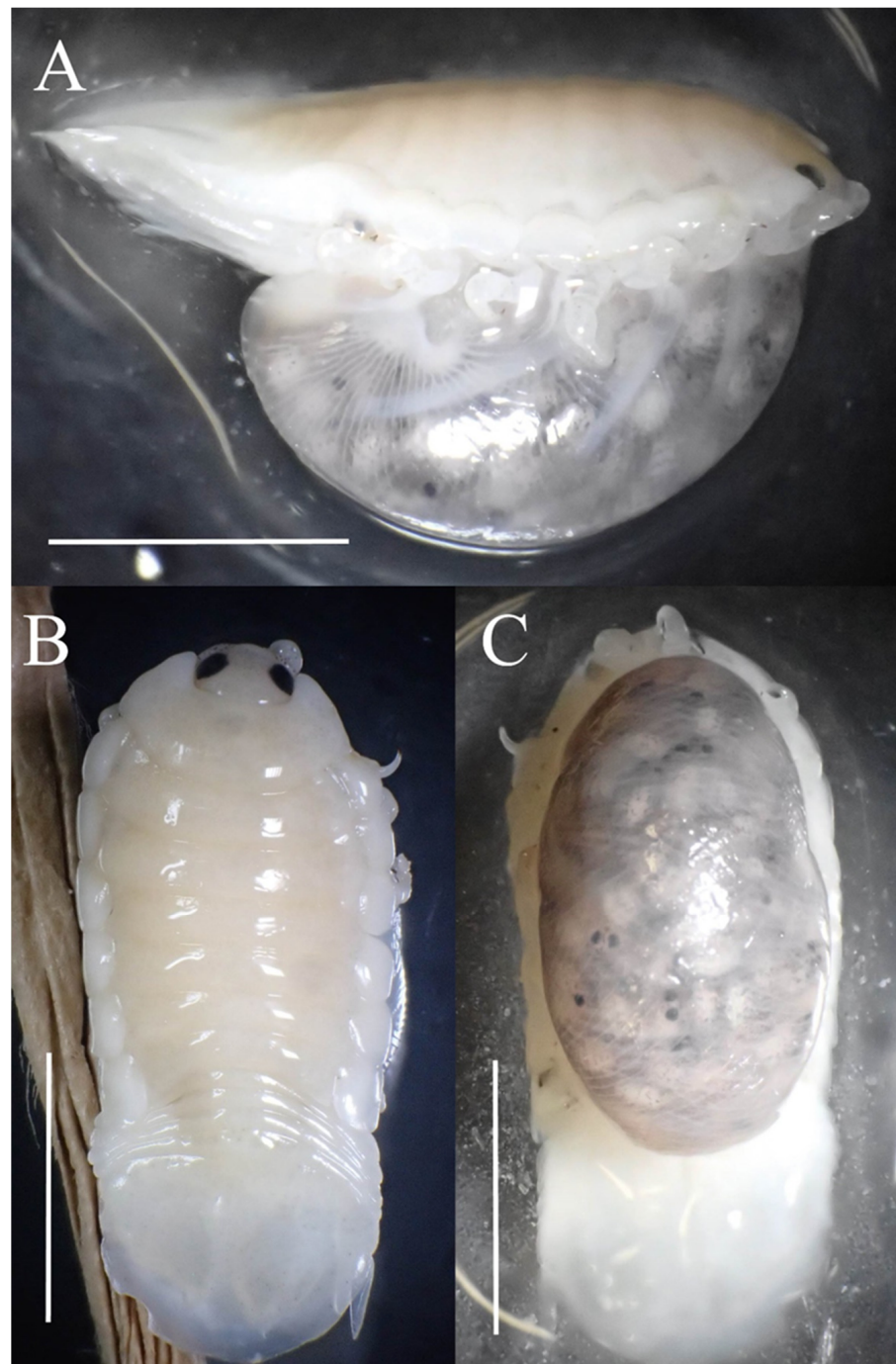
**Genus:** *Mothocya* Costa in Hope, 1851.

***Mothocya collettei* Bruce, 1986.**

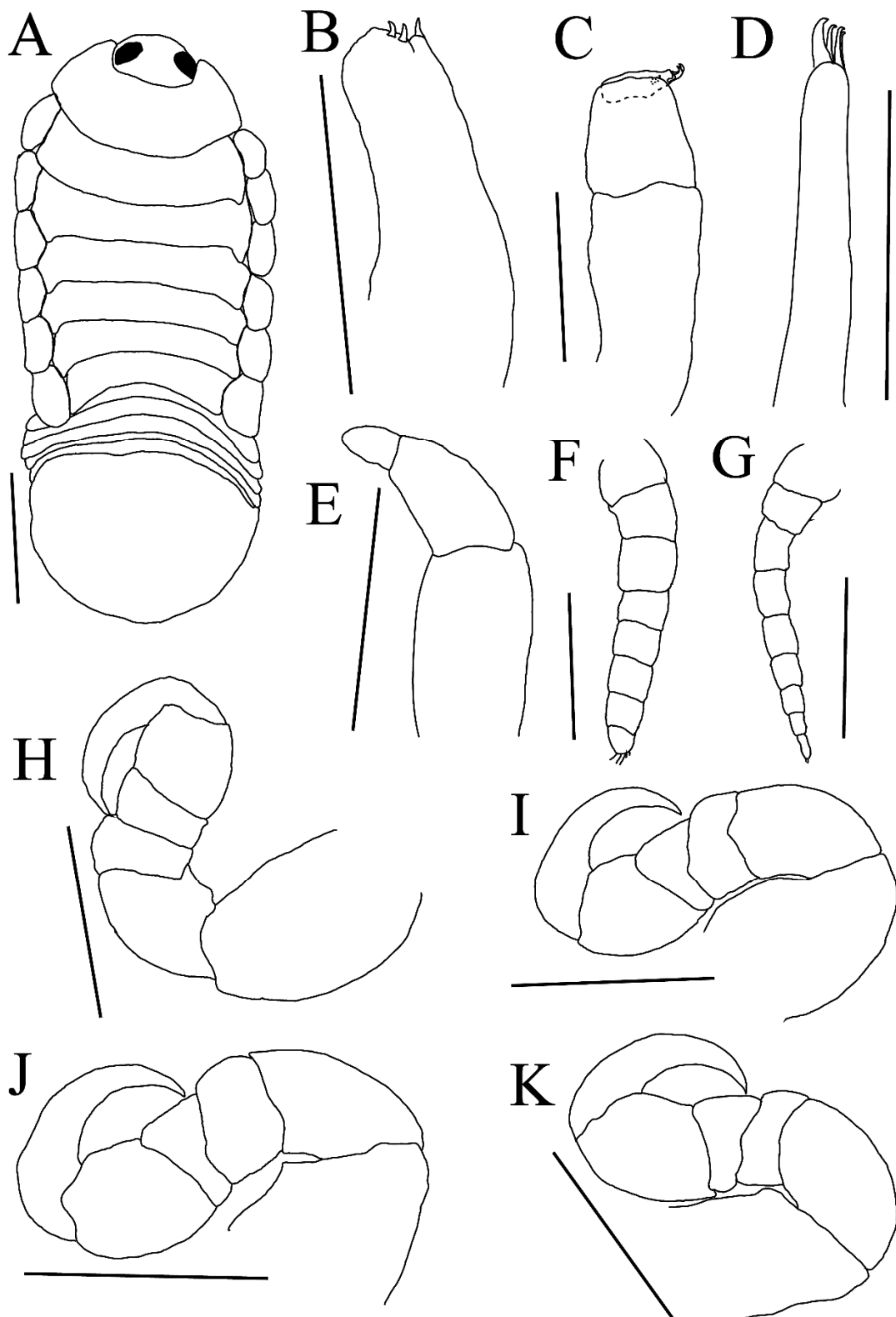
[New standard Japanese name: Okizayori-eranushi].

(Figures 6–8).

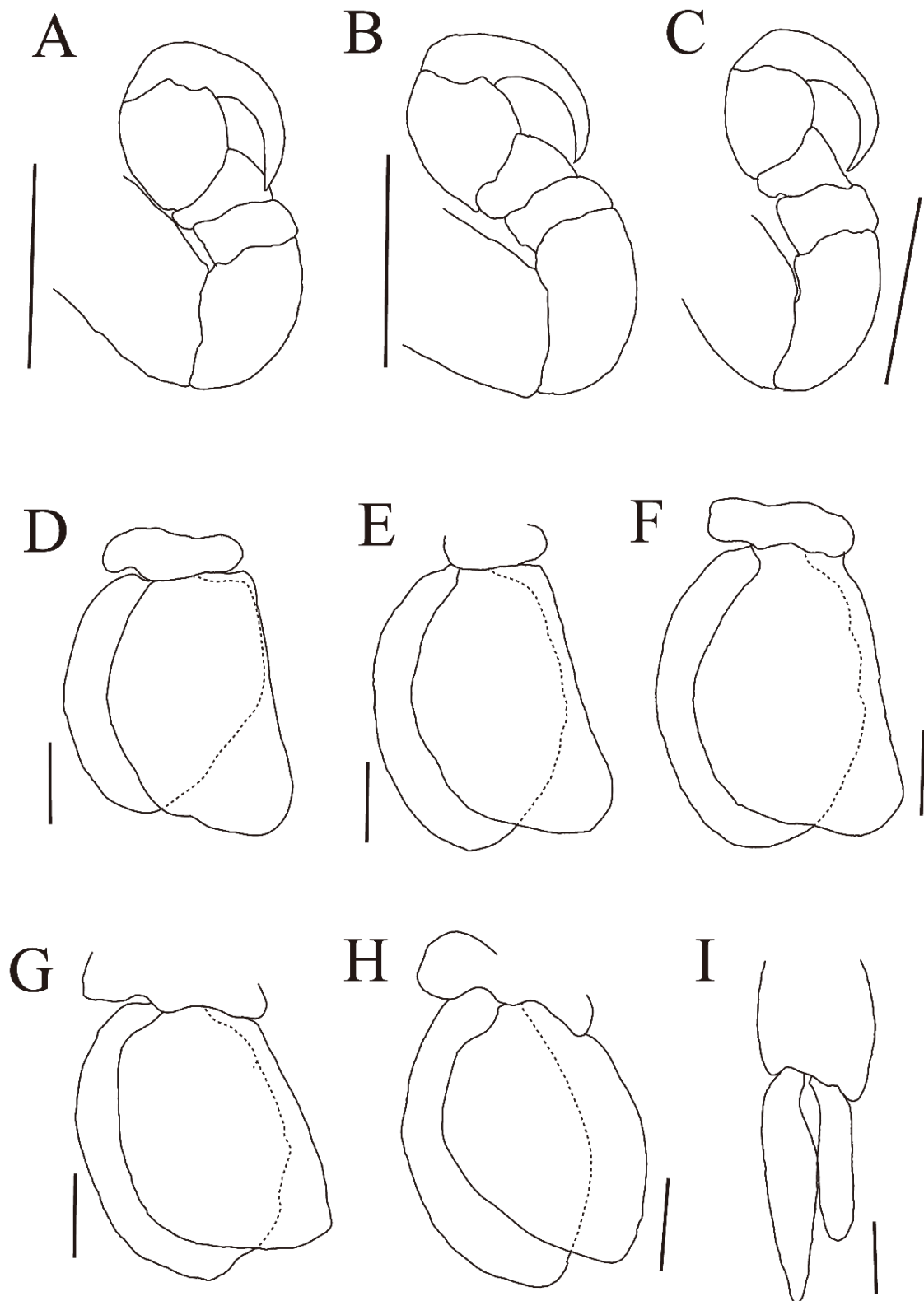
Material examined: one ovigerous female (BL: 13.5 mm), from Tousoe fishing port, Yonabaru Town, Okinawa Island, Japan, in a branchial cavity of *Tylosurus crocodilus*, 5 September 2022.



**Figure 6.** Ovigerous female of *Mothocya collettei* (13.5 mm, SL) infesting branchial cavity of *Tylosurus crocodilus* collected from the Tousoe fishing port, Yonabaru Town, Okinawa Island, Japan; (A), lateral view; (B), dorsal view; (C), ventral view. Scale bars, 5 mm.



**Figure 7.** *Mothocya collettei*, ovigerous female (13.5 mm, SL) infesting branchial cavity of *Tylosurus crocodilus* collected from the Tousoe fishing port, Yonabaru Town, Okinawa Island, Japan; (A), dorsal view; (B), maxilla; (C), maxilliped; (D), maxillula; (E), tip of mandible; (F), antennula; (G), antenna; (H–K), pereopods 1–4. Some pilosity and surface sculpturing were omitted. Scale bars, (A), 3 mm; (B–G), 0.5 mm; (H–K), 1 mm.



**Figure 8.** *Mothocya collettei*, ovigerous female (13.5 mm, SL) infesting the branchial cavity of *Tylosurus crocodilus* collected from the Tousoe fishing port, Yonabaru Town, Okinawa Island, Japan; (A–C), pereopods 5–7; (D–H), pleopods 1–5; (I), uropod. Some pilosity and surface sculpturing were omitted. Scale bars, (A–C), 1 mm; (D–H), 1 mm; (I), 0.5 mm.

Description of female (Figures 7 and 8): The body is elliptical, 2.3 times as long as the greatest width, widest at pereonite 3, narrowest at pereonite 7, with convex dorsal surfaces. The cephalon is 1.7 times wider than long, semi-oval, moderately immersed in pereonite 1. The eyes are oval with distinct margins, with the long axis of each eye 0.6 times the length of the cephalon and the short axis 0.2 times the maximum width of the cephalon. The anterior border of pereonite 1 is straight medially, medially curving laterally. Coxae 2–7 are slightly visible in dorsal view, strongly expressed laterally. Pereonite 1 is the longest; pereonite 6 is the shortest, the posterior margins of all pereonites are smooth and slightly curved laterally, and that of pereonite 7 has a slightly recessed posterior margin. The pleon constitutes 10% of the total length, and the pleon is 1.1 times as wide as the greatest pereon width, with the pleonites all visible in dorsal view. The pleotelson is semi-oval, 0.7 times longer than wide, and 2.5 times as long as the pleon.

The antennula have eight articles, extending beyond the midlength of the cephalon. The antenna has eight articles, reaching the posterior margin of pereonite 1. The mandible has a molar process; palp article 1 is the longest, 1.9 times as long as it is wide; article 2 is 0.6 times as long as article 1 and 1.5 times as long as it is wide; article 3 is the shortest, 0.4 times as long as article 2, 1.5 times as long as it is wide, and 0.3 times as wide as article 1. The maxillula is simple, with four terminal robust setae. The maxilla lateral lobe has two robust setae; the mesial lobe has one robust seta. Maxilliped article 3 has four robust setae.

Pereopod 1 has a basis 1.5 times as long as the greatest width and an ischium 0.6 times as long as the basis, a merus 0.4 times as long as it is wide and 0.3 times as long as ischium, a carpus 0.4 times as long as it is wide, a propodus 0.9 times as long as it is wide, and a dactylus 1.7 times as long as the propodus. Pereopod 2 has a basis 1.2 times as long as the greatest width, an ischium 0.8 times as long as the basis and 1.6 times as long as it is wide, a propodus 1.5 times as long as it is wide and 0.8 times as long as the ischium, and a dactylus 1.8 times as long as the propodus and 1.1 times as long as it is wide. Pereopod 3 has a basis 1.5 times as long as it is wide, an ischium 0.5 times as long as the basis and 0.8 times as long as it is wide, a carpus 1.8 times as long as it is wide, a propodus 0.9 times as long as it is wide and 1.3 times as long as the ischium, and a dactylus as long as the propodus and 2.9 times as long as it is wide. Pereopods 4–6 are similar to 3. Pereopod 7 has a basis 1.4 times as long as its greatest width, an ischium 0.9 times as long as the basis and 1.9 times as long as it is wide, a merus 0.6 times as long as it is wide and 0.4 times as long as the ischium, a carpus 0.7 times as long as it is wide and 0.3 times as long as the ischium, a propodus 1.1 times as long as it is wide and 0.7 times as long as the ischium, and a dactylus 1.3 times as long as the propodus.

The peduncles of all pleopods are short. Pleopod 1 has a peduncle 2.6 times as wide as it is long; an exopod 1.2 times as long as it is wide, distally rounded, with a convex mesial margin; an endopod 1.2 times as long as the exopod, 1.6 times as long as it is wide, with a weakly convex lateral margin and concave mesial margin. Pleopod 2 has a peduncle 2.4 times as wide as it is long; an exopod 1.4 times as long as it is wide, distally rounded, with a convex lateral margin and convex mesial margin; an endopod as long as the exopod, 1.5 times as long as it is wide, distally broadly rounded, with a weakly convex lateral margin and concave mesial margin. Pleopods 3–4 are similar to 2. Pleopod 5 has an exopod 1.3 times as long as it is wide, distally rounded, with a convex lateral margin and a convex mesial margin; an endopod 0.9 times as long as the exopod, 1.3 times as long as it is wide, distally broadly rounded, with a weakly convex lateral margin.

The uropod is more than half the length of the pleotelson, the peduncles are 0.7 times longer than the rami, and the apices are broadly rounded. The endopod is apically rounded, 4.5 times as long as the greatest width, with straight lateral and mesial margins. The exopod is 1.5 times longer than the endopod and 4.5 times as long as the greatest width, apically rounded, with straight lateral and mesial margins.

**Coloration:** The body coloration in the fresh condition is white and pearl yellow in the ethanol-preserved condition. The mancae in the brood pouch are gray in both the fresh and ethanol-preserved condition.

**Hosts:** In this study, the host of *M. collettei* was *Tylosurus crocodilus*. The known hosts are listed in [27].

**Distribution:** This study is the first record of *M. collettei* from the Ryukyu Islands. *Mothocya collettei* was previously reported to occur in the Indo-West Pacific, including Kenya, Thailand, Singapore (type locality), north-eastern Australia, Papua New Guinea, Philippines, Yap Island, Tubuai Island, Tahiti, Hawaii, India, and Pakistan [12,28,29]. In Japan, this species was reported from the Miyazaki and Mie prefectures [30,31].

**Remarks:** *Mothocya collettei* and *M. affinis* are morphologically similar, although *M. collettei* is distinguished from *M. affinis* by its larger body size, different host [*M. affinis* are only known from the tropical halfbeak, *Hyporamphus affinis* (Günther, 1866)], more symmetrical body shape, two robust setae on the maxilla lateral lobe and one robust seta on the maxilla mesial lobe (2 and 2, respectively, in *M. affinis*) [32]. Four recurved setae are on maxilliped article 3 (3 in *M. affinis*); the shape of the coxae is wider and shorter than in *M. affinis*, with longer uropods and a wider pleotelson. The present individual is 13.5 mm, infests *T. crocodilus*, has a weakly twisted body shape, two robust setae on the maxilla lateral lobe and two on the maxilla mesial lobe, four recurved setae on maxilliped article 3, wider coxae, shorter uropods, and a rounder pleotelson. All of the characteristics except the maxilla, uropods, and pleotelson are consistent with *M. collettei*, so we identified this individual as *M. collettei* based on the combination of the characteristics.

## 5. Discussion

In Japan, 40 cymothoid species in 14 genera have been reported [3,14,30,31,33–36], and 7 nominal species were previously recorded from the Ryukyu Islands [37,38]. *Cymothoa indica* is distributed in the Indo-West Pacific, but our specimen represents both the first record of this species from Japan and a new northern distributional limit for the species. Since it is often difficult to identify cymothoids other than adult females via morphology [8], our specimen had to be identified via molecular analysis. The COI tree shows that the juvenile in this study formed a single clade with *C. indica*, but the 16S rRNA shows that *Cymothoa pulchrum* and *C. indica* could not be distinguished using 16S rRNA. This is similar to the phylogenetic tree in [30] and suggests that COI may be more useful than 16S rRNA for species identification in this genus.

As was noted above, *Cymothoa indica* has low host specificity. In this study, we confirmed for the first time its parasitism of *Atherinomorus lacunosus*. Although this is the first record of this isopod from any fish of the order Atheriniformes, the isopod involved was a juvenile, so it is unclear whether *A. lacunosus* acts as a final or accidental host for *C. indica*. As an example of another cymothoid parasitic on an atheriniform fish, *M. parvostis* infests the cobaltcap silverside *Hypoatherina tsurugae* (Jordan and Starks, 1901) as an optional intermediate host [39]. In any case, the relationship between *A. lacunosus* and *C. indica* needs to be confirmed with a larger number of specimens.

*Mothocya collettei* is distributed in the Indo-West Pacific. This study is the first record from the Ryukyu Islands [37] and the third record from Japan. It has previously been collected off Kadogawa, Miyazaki Prefecture and off Watarai, Mie Prefecture [30,31].

The morphology of *M. collettei* is highly variable, and Bruce (1986) noted that specimens from the Central Pacific (Tubuai Island, French Polynesia) and Magnetic Island, Australia have an extremely broad pereon and may be a distinct species. In addition, it is noteworthy that *M. collettei* and *M. affinis* are morphologically similar despite being distinguished by host preferences as well as some morphological differences [32]. The present specimen was identified as *M. collettei* because it matched some morphological characteristics of this species. In both the COI and 16S rRNA molecular phylogenetic trees, the individual collected in this study formed a clade with *M. collettei* data from GenBank, but our individual also showed a high similarity to *M. affinis* in its COI gene. The morpho-

logical similarity of *M. collettei* and *M. affinis* suggests the possibility of misidentification of the original specimens that provided the sequences in GenBank [40], but the registrant of the sequence for *M. affinis* included the first author of the original description, so misidentification seems unlikely. *Mothocya collettei* and *M. affinis* need to be re-examined both morphologically and by means of molecular genetics using a large number of specimens collected from various locations.

In a previous study based on the 16S rRNA data [11], *Cinusa nippon* Nagasawa, 2021 was included in the clade of *Ceratothoa* Dana, 1852, and the possibility that *Cinusa* Schioedte and Meinert, 1884 are synonymous with *Ceratothoa* cannot be excluded. In the present study, similar results were obtained not only in the 16S rRNA phylogenetic tree, but also in the COI phylogenetic tree, in which two species of *Cinusa* were included in the *Ceratothoa* clade. In addition, the 16S rRNA and COI trees both show that *Elthusa* Schioedte and Meinert, 1884 are polyphyletic, as in previous studies [11,30]. Clearly, there are additional genera and species in the Cymothoidae that require taxonomic re-examination using both morphology and DNA.

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