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Ceratothoa arimae (Isopoda: Cymothoidae) Infesting Buccal Cavity of Largescale Blackfish, *Girella punctata* (Centrarchiformes: Kyphosidae), in Seto Inland Sea, Japan

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Abstract: The largescale blackfish, *Girella punctata* Gray, 1835, is important in the fishing industry and recreational fishing, and it is also cultured in East Asia. Cymothoidae (Crustacea: Isopoda) is a group of parasites that infest fish in marine, brackish, and freshwater environments. In this study, we report, for the first time, *Ceratothoa arimae* (Nunomura, 2001) (Cymothoidae) from the buccal cavity of *G. punctata* in the Seto Inland Sea, Japan. *Ceratothoa arimae* showed a prevalence of 29.4–66.7% in *G. punctata*. The morphology of the mancae of this species was also described in comparison with that of the adult female (ovigerous), transitional stage, and adult male. The manca of *Ceratothoa arimae* has more chromatophores than those of other *Ceratothoa* species from Japan, and is a candidate for a future taxonomic trait. This species may have a negative impact on cultured *G. punctata*, which would be important to determine in future studies. Currently, it is difficult to identify cymothoid mancae species based on their morphology, but the information provided in this study could be useful when combined with other methods developed in the future, such as molecular analysis.

Keywords: fish disease; manca; pathology; prevalence; wild fish

Key Contribution: The cymothoid parasite infesting the buccal cavity of the largescale blackfish, *Girella punctata*, is *Ceratothoa arimae*.

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1. Introduction

The largescale blackfish, *Girella punctata* Gray, 1835 (Centrarchiformes: Kyphosidae), is a coastal fish that inhabits the rocky reefs around Japan, Taiwan, and the East China Sea [1–3]. *Girella punctata* is economically important in coastal fisheries and popular among recreational anglers [1]. It is commercially cultured in Japan and South Korea [4]. It is important to collect information on the parasites infesting *G. punctata* because various parasites are a problem in aquaculture, as described below.

Parasites that damage cultured fish include cymothoids [5–9]. Cymothoidae Leach, 1818 (Crustacea: Isopoda), includes more than 360 species across 42 genera of broad distribution isopod parasites [10]. It is one of the largest families of isopods and can be found worldwide, with the exception of the polar oceans [11,12]. Their hosts encompass diverse taxa of fish species inhabiting marine, brackish, and freshwater environments [12,13]. These parasites adhere to their hosts at four distinct attachment sites: the branchial cavity,

buccal cavity, burrowed flesh, and body surface of fishes [12]. Cymothoids have six life stages: manca, juvenile, male, transitional, and female [12,14]. Free-swimming mancae develop into juveniles and adult males on their hosts, subsequently undergoing protandrous hermaphroditism from male to female [15–17]. Two species of cymothoid are known to parasitize *G. punctata*: *Anilocra prionuri* Williams and Bunkley-Williams, 1986 (infesting the body surface), and *Mothocya parvostis* Bruce, 1986 (infesting the branchial cavity) [18,19]. No cymothoids were reported to parasitize the buccal cavity of *G. punctata*.

Cymothoidae feed on the tissues or blood of fish and are thought to be dependent on their hosts for nutrition [20–22]. There have been reports of damage to the host body surface and a decrease in body fat caused by the parasitism of the externally attached *Nerocila phaiopleura* Bleeker, 1857 [23], and injuries caused by cymothoids can sometimes be the cause of infectious viral diseases [24]. It is also known to parasitize cultured fish [25–28], and there have been reports of increased mortality rates due to the presence of cymothoids [6].

In this study, we identified and described cymothoids collected in the buccal cavity of *G. punctata* from the Seto Inland Sea, Japan. In addition, we morphologically described the life cycle stages of this species and sequenced its cytochrome *c* oxidase subunit I (COI) and 16S rRNA genes.

2. Materials and Methods

2.1. Sample Collection and Morphological Observation

From December 2020 to June 2021, 44 *G. punctata* were collected from three sampling sites (Nakajima Island, Matsuyama, Ehime, Japan; Itsukishima Island, Kure, Hiroshima, Japan; and Ohkurokamishima Island, Etajima, Hiroshima, Japan) in the Seto Inland Sea by line angling (Supplementary File S1), and cymothoids were collected from the buccal cavity (mouth floor) of *G. punctata*. The cymothoids were transported to the laboratory on ice and fixed in 99.5% ethanol. The life stages of each were determined according to Aneesh et al. [14]. The prevalence was calculated as the number of infected fish with the parasite divided by the total number of fish examined at each site, expressed as a percentage when used descriptively and as a proportion in models. The sampling result from 10 May 2021, at Itsukushima Island, is not included in the calculation of prevalence because only infested fish were selectively collected (Table 1). The prevalence of *C. arimae* in *G. punctata* at each site was analyzed using a generalized linear model (GLM), with the numbers of infested–non-infested fish as the response variable and sampling locations as the explanatory variables. We used analysis of deviance to investigate whether the sampling site affected the prevalence. The statistical analyses were conducted using R (version 4.4.2) [29] and package EZR (Easy R) (version 1.68) [30].

Table 1. The number of samples and prevalence of *Ceratothoa arimae* (Nunomura, 2001) infesting *Girella punctata* Gray, 1835, collected from three locations in the Seto Inland Sea, Japan.

Date	Location	Number of Fish		Prevalence of				Total Prevalence (%)
		Total	Infested	Female (%)	Transitional (%)	Male (%)	Manca (%)	
10 December 2020	Nakajima Island	22	8	13.6	18.2	36.4	0.0	36.4
10 May 2021 *	Itsukishima Island	-	2	-	-	-	-	-
14 May 2021 19 May 2021	Itsukishima Island	12 5	3 2	17.6	11.8	29.4	0.0	29.4
6 June 2021	Ohkurokamishima Island	3	2	33.3		33.3	33.3	66.7

* On 10 May 2021, only the infested fish were selectively collected.

We observed three each of mancae, males, transitional stages, and ovigerous females, as follows. Cymothoid photographs were taken under a stereomicroscope SMZ18 (Nikon, Tokyo, Japan) using the real-time EDF function in NIS-Elements Documentation (version 5.30.00) (Nikon), and the photographs were combined using Photoshop 2025 (version 26.2.0) (Adobe, San Jose, CA, USA). Morphological descriptions were made using a stereomicroscope SMZ800 with a drawing tube, P-IDT, and an upright microscope ECLIPSE E200 with a drawing tube, Y-IDT (Nikon). The drawings were digitally rendered utilizing Illustrator 2024 (version 28.4.1) (Adobe) and a DTC133 pen display (Wacom, Saitama, Japan). The measurements and terminology largely follow Aneesh et al. [14] and Fujita [31]. The diagnoses were prepared in the DEscription Language for TAxonomy (DELTA), according to Coleman et al. [32]. The taxonomic descriptions were also shown in the DELTA format (Supplementary File S2). These individuals were deposited at the Seto Marine Biological Laboratory, Field Science Education and Research Center, Kyoto University (SMBL-V0829–V0840).

2.2. DNA Sequencing

The COI and 16S rRNA sequences for the four individuals were determined to accumulate DNA information linked to morphological details. The COI and 16S rRNA sequences of a manca (SMBL-V0840), a male (SMBL-V0830), a transitional (SMBL-V0835), and an ovigerous female (SMBL-V0829) were sequenced. The host DNA attached to the surface of the sample was removed, according to Fujita and Nakano [33]. DNA was extracted from the pereopods utilizing an alkaline lysis method in accordance with the recommended protocol for KOD One PCR Master Mix (Toyobo, Osaka, Japan). The COI region was amplified using the primers jgLCO1490 (5′-TITCIACIAAYCAYAARGAYATTGG-3′) and jgHCO2198 (5′-TAIACYTCIGGRTGICCRAARAAYCA-3′) [34]. The 16S rRNA region was amplified using the primers 16Sar (5′-CGCCTGTTTAACAAAAACAT-3′) and 16Sbr (5′-CCGGTCTGAACTCAGATCATGT-3′) [35]. The total reaction volume for each PCR was 12.5 μL, comprising 1 μL of DNA, 4.5 μL of distilled water, 0.375 μL of each primer (10 μM solutions), and 6.25 μL of KOD One PCR Master Mix. The PCR conditions for COI were 35 cycles of 98.0 °C for 10 s, 45.0 °C for 5 s, and 68.0 °C for 1 s. The PCR conditions for 16S rRNA were 35 cycles of 98.0 °C for 10 s, 50.0 °C for 5 s, and 68.0 °C for 1 s. The PCR products were sent to Eurofins Genomics (Tokyo, Japan) sequencing services and sequenced using the dye terminator method. The sequences have been deposited in GenBank (accession numbers: PV235262–PV235265 and PV241151–PV241155).

The sequences were aligned using MUSCLE [36], implemented in MEGA 11 [37]. Pairwise genetic distances among these sequences, using the Kimura two-parameter (K2P) model [38], were calculated.

3. Results

3.1. Morphological Identification

The cymothoid specimens collected in this study (total of 48 individuals) were identified as *Ceratothoa arimae* (Nunomura, 2001), as follows.

3.2. Taxonomy

Order: Isopoda Latreille, 1816
Superfamily: Cymothooidea Leach, 1814
Family: Cymothoidae Leach, 1814
Genus: *Ceratothoa* Dana, 1852
Ceratothoa arimae (Nunomura, 2001)
[Japanese name: Takabe-noe]

(Figures 1–6, Supplementary File S2)

Glossobius arimae Nunomura, 2001: 29, Figures 1 and 2 [39].

Ceratothoa arimae—Martin, Bruce, and Nowak, 2015: 346–347 [40]—Kawanishi, Kohya, and Hata, 2019: graphical abstract [41].

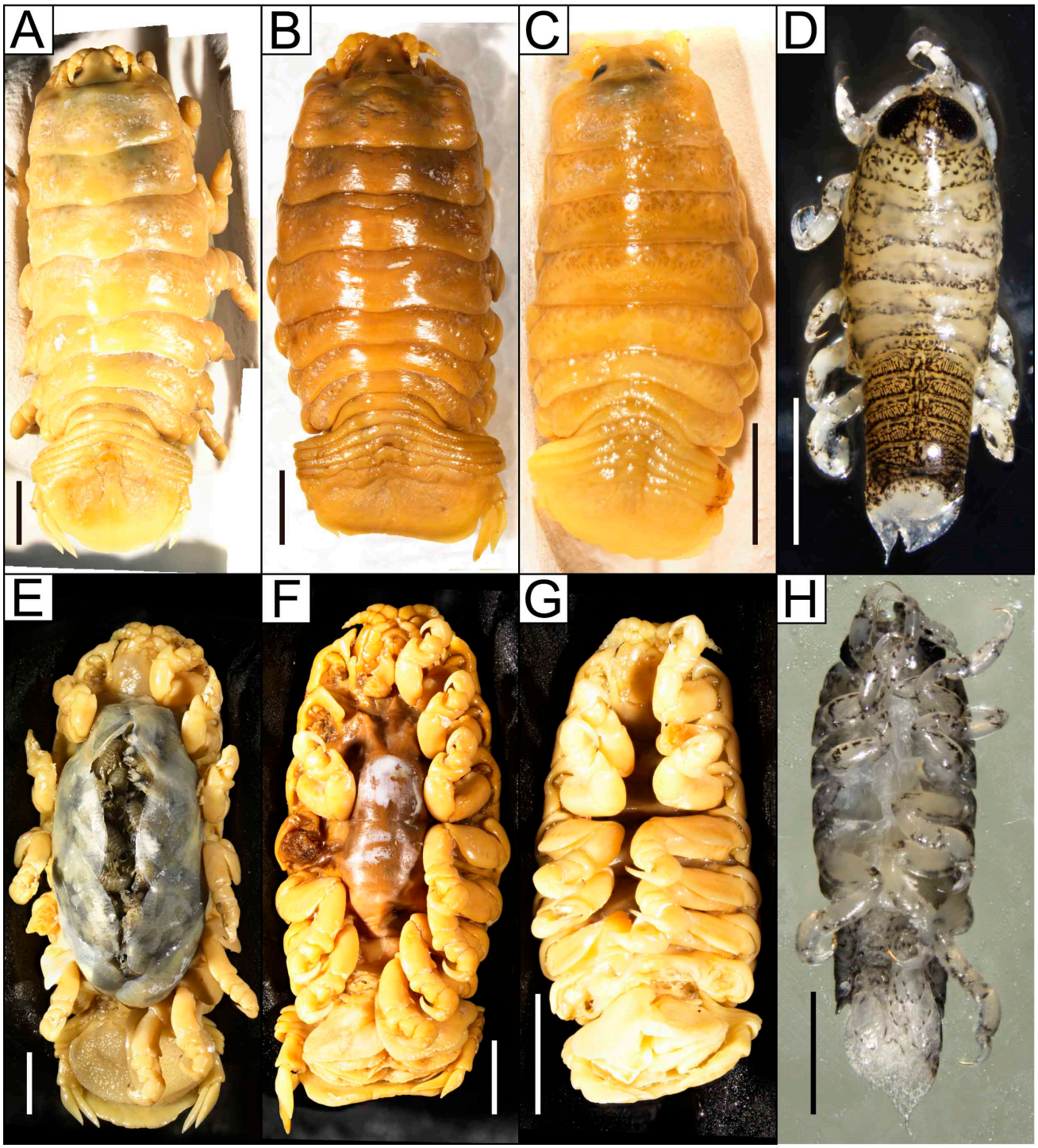

Figure 1. *Ceratothoa arimae* (Nunomura, 2001) collected from largescale blackfish, *Girella punctata* Gray, 1835. (**A**–**D**), dorsal view; (**E**–**H**), ventral view; (**A**,**E**), SMBL-V0829, ovigerous female (TL 41.5 mm); (**B**,**F**), SMBL-V0835, transitional (TL 34.7 mm); (**C**,**G**), SMBL-V0830, male (TL 20.6 mm); (**D**,**H**), SMBL-V0840, manca (TL 4.6 mm). Scale bars indicate: (**A**–**C**), (**E**–**G**), 5 mm; (**D**,**H**) 1 mm.

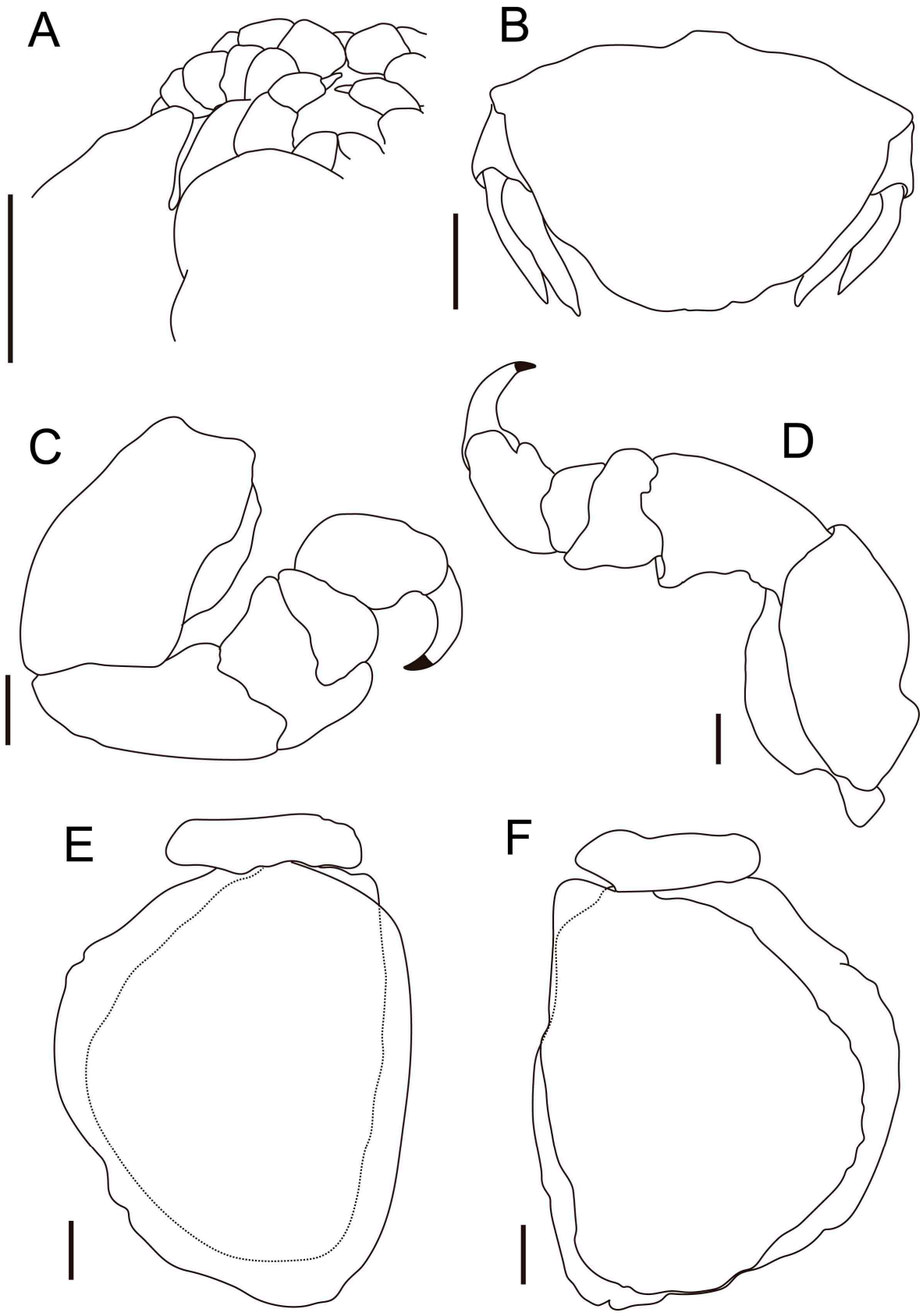

Figure 2. Ovigerous female (TL 41.5 mm) of *Ceratothoa arimae* (Nunomura, 2001) collected from largescale blackfish, *Girella punctata* Gray, 1835, SMBL-V0829. (**A**), ventral view of cephalon; (**B**), pleotelson and uropods; (**C**), pereopod 1; (**D**), pereopod 7; (**E**), pleopod 1; (**F**), pleopod 2. Scale bars indicate: (**A**,**B**), 3 mm; (**C**–**F**), 1 mm.

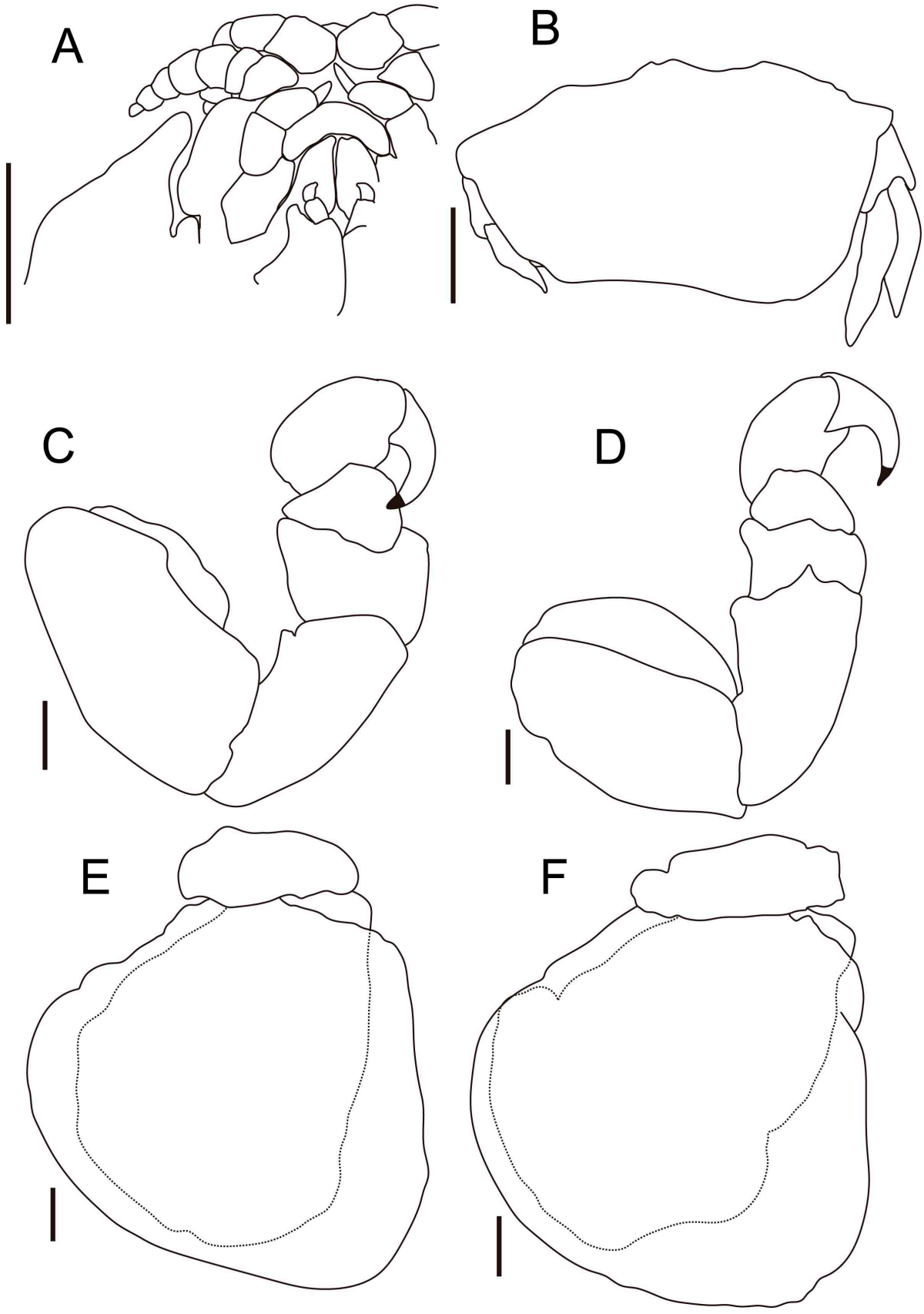

Figure 3. Transitional (TL 34.7 mm) of *Ceratothoa arimae* (Nunomura, 2001) collected from largescale blackfish, *Girella punctata* Gray, 1835, SMBL-V0835. (**A**), ventral view of cephalon; (**B**), pleotelson and uropods; (**C**), pereopod 1; (**D**), pereopod 7; (**E**), pleopod 1; (**F**), pleopod 2. Scale bars indicate: (**A**,**B**), 3 mm; (**C**–**F**), 1 mm.

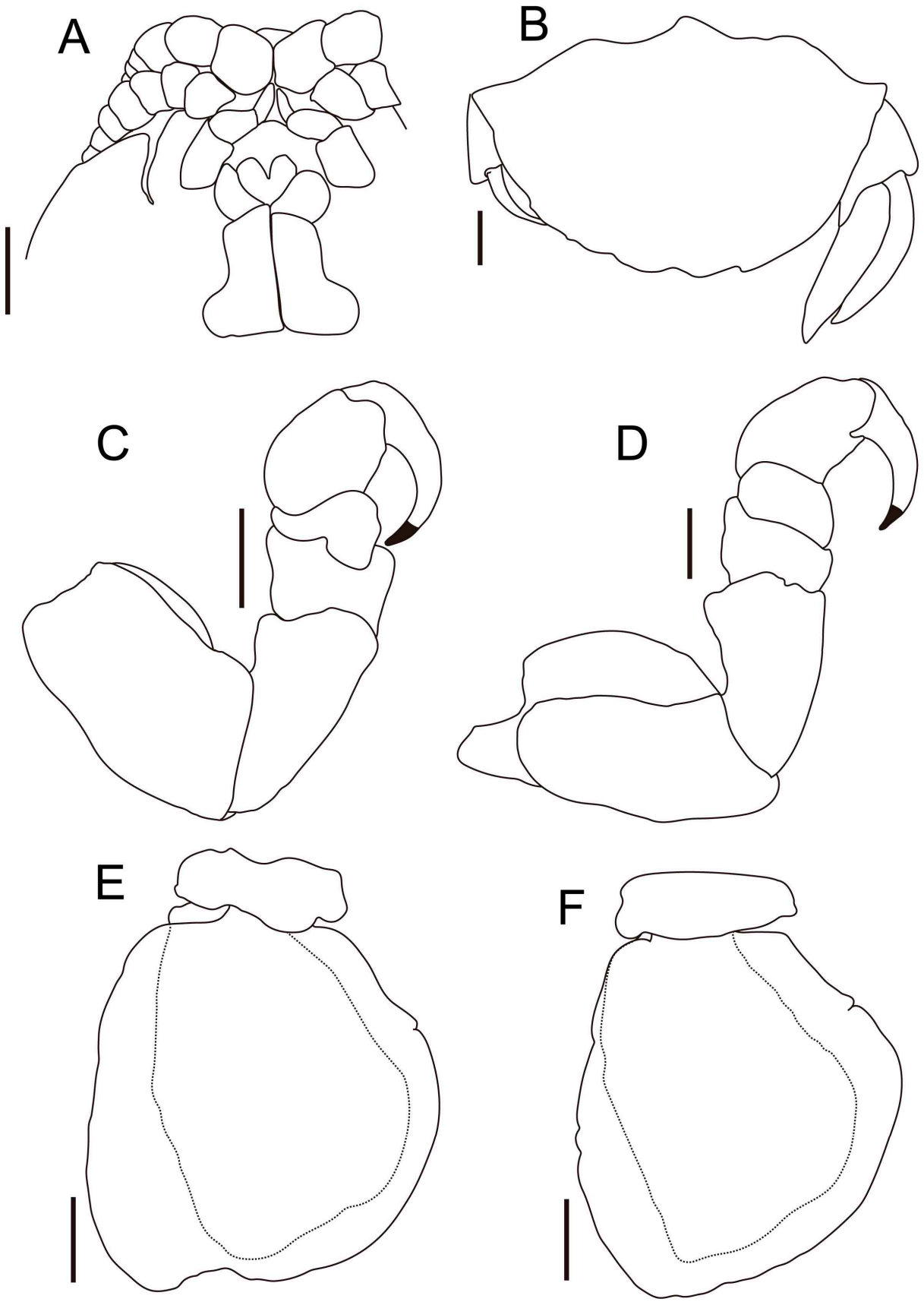

Figure 4. Male (TL 20.6 mm) of *Ceratothoa arimae* (Nunomura, 2001) collected from largescale blackfish, *Girella punctata* Gray, 1835, SMBL-V0830. (**A**), ventral view of cephalon; (**B**), pleotelson and uropods; (**C**), pereopod 1; (**D**), pereopod 7; (**E**), pleopod 1; (**F**), pleopod 2. Scale bars indicate 1 mm.

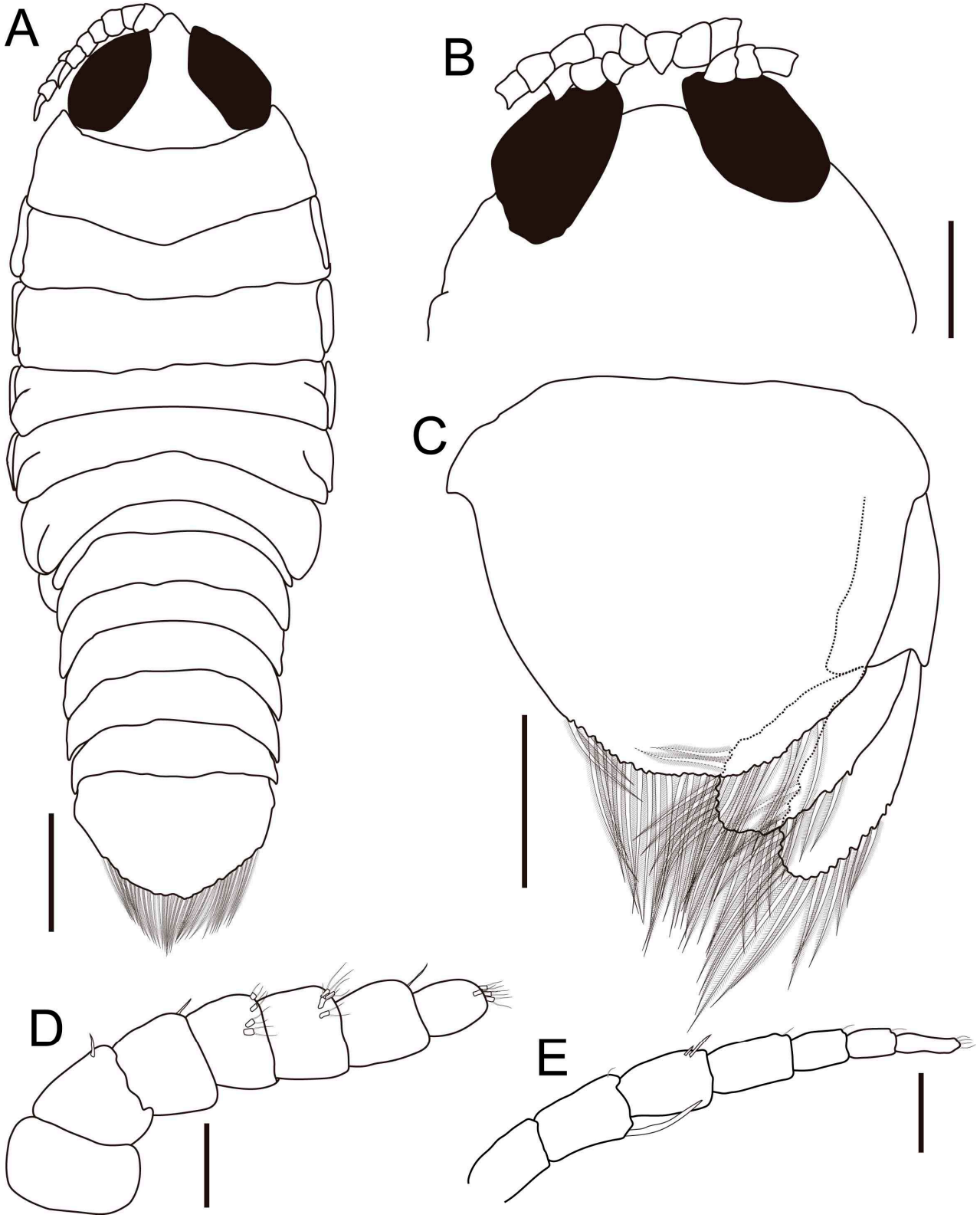

Figure 5. Manca (TL 4.6 mm) of *Ceratothoa arimae* (Nunomura, 2001) collected from largescale blackfish, *Girella punctata* Gray, 1835, SMBL-V0840. (**A**), dorsal view; (**B**), ventral view of cephalon; (**C**), pleotelson and uropods; (**D**), antennula; (**E**), antenna. Scale bars indicate: (**A**), 0.5 mm; (**B**,**C**), 0.3 mm; (**D**,**E**), 0.1 mm.

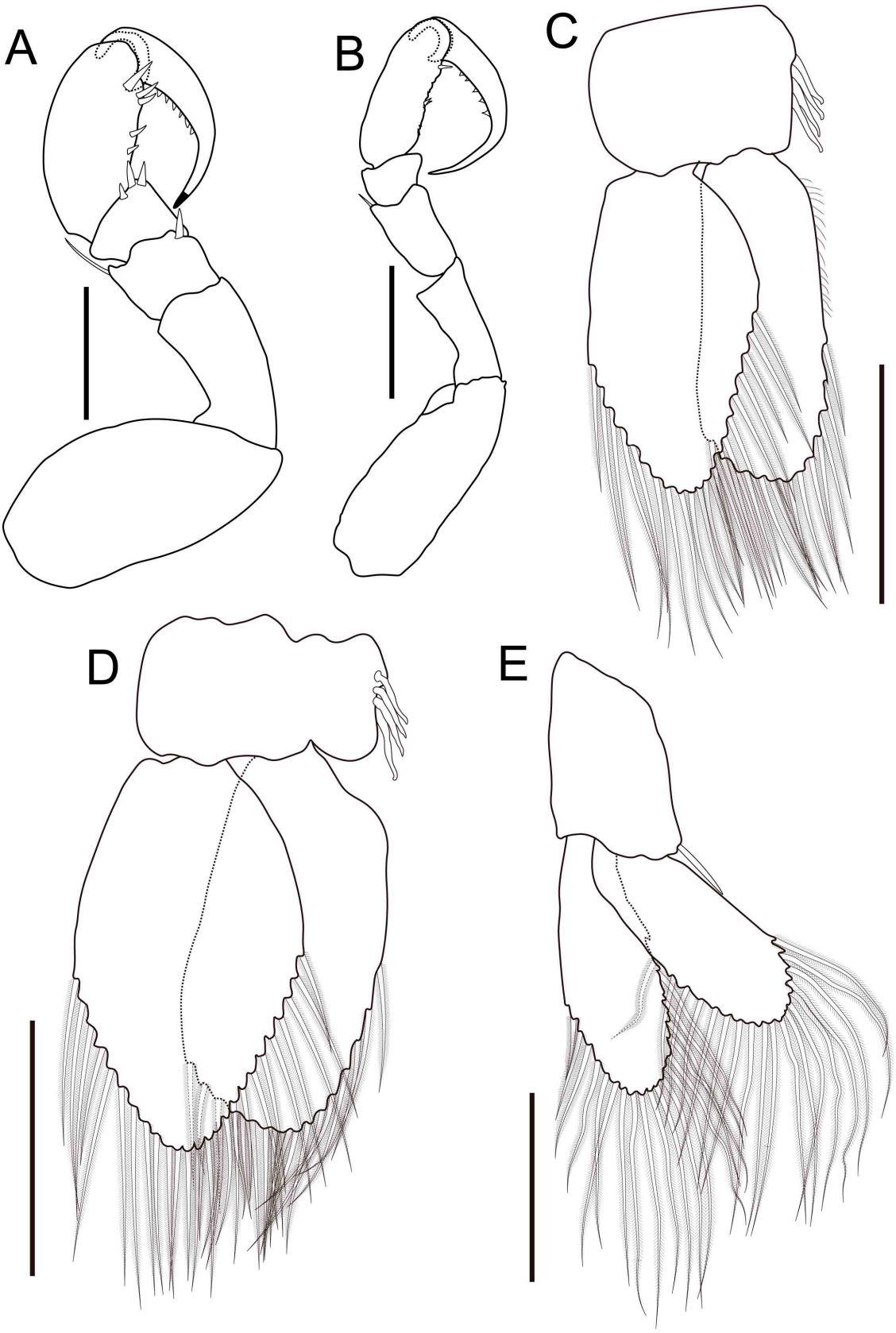

Figure 6. Manca (TL 4.6 mm) of *Ceratothoa arimae* (Nunomura, 2001) collected from largescale blackfish, *Girella punctata* Gray, 1835, SMBL-V0840. (**A**), pereopod 1; (**B**), pereopod 6; (**C**), pleopod 1; (**D**), pleopod 2; (**E**), uropod. Scale bars indicate 0.3 mm.

Material examined: Line angling, 20 Dec. 2020, coast of Nakajima Island, Matsuyama, Ehime, Seto Inland Sea, Japan (33°57′35.6″ N, 132°35′20.0″ E): ovigerous female (TL 41.51 mm), SMBL-V0829 (GenBank: PV235263, PV241152), buccal cavity (mouth floor) of *Girella punctata* Gray, 1835 (SL 267.3 mm); male (TL 20.6 mm), SMBL-V0830 (PV235264, PV241153), buccal cavity (mouth floor) of *Girella punctata* Gray, 1835 (SL 267.3 mm); two mancae (TL 5.19 mm, 5.20 mm), SMBL-V0831–V0832, in brood pouch of SMBL-V0829; transitional (TL 23.39 mm), SMBL-V0833, buccal cavity (mouth floor) of *Girella punctata* Gray, 1835 (SL 211.1 mm); transitional (TL 21.78 mm), SMBL-V0834, buccal cavity (mouth floor) of *Girella punctata* Gray, 1835 (SL 208.6 mm). Line angling, 20 10 May 2021, coast of Itsukishima Island, Kure, Hiroshima, Seto Inland Sea, Japan (34°07′04.3″ N, 132°47′29.8″ E): transitional (TL 34.74 mm), SMBL-V0835 (PV235265, PV241155), buccal cavity (mouth floor) of *Girella punctata* Gray, 1835 (SL 270.0 mm); ovigerous female (TL 38.76 mm), SMBL-V0836, buccal cavity (mouth floor) of *Girella punctata* Gray, 1835 (SL 331.0 mm); male (TL 17.64 mm), SMBL-V0837, buccal cavity (mouth floor) of *Girella punctata* Gray, 1835 (SL 331.0 mm). Line angling, 19 May 2021, coast of Itsukishima Island, Kure, Hiroshima, Seto Inland Sea, Japan (34°07′04.3″ N, 132°47′29.8″ E): ovigerous female (TL 23.14 mm), SMBL-V0838, buccal cavity (mouth floor) of *Girella punctata* Gray, 1835 (SL 171.3 mm); male (TL 10.96 mm), SMBL-V0839, buccal cavity (mouth floor) of *Girella punctata* Gray, 1835 (SL 171.3 mm). Line angling, 6 Jun. 2020, coast of Ohkurokamishima Island, Etajima, Hiroshima, Seto Inland Sea, Japan (34°09′40.7″ N, 132°23′55.9″ E): manca (TL 4.63 mm), SMBL-V0840 (PV241154), buccal cavity (mouth floor) of *Girella punctata* Gray, 1835 (SL 239.3 mm).

Description of ovigerous female (Figure 2): Body rectangular, 2.6 times as long as greatest width, widest at pereonite 4, narrowest at pleonite 1, with convex dorsal surfaces.

Cephalon 1.7 times wider than long, triangle, slightly immersed in pereonite 1. Eyes oval, with distinct margins, with long axis of each eye 0.4 times length of cephalon, and short axis 0.1 times maximum width of cephalon. Anterior border of pereonite 1 medially protrudes forward. Coxae almost visible in dorsal view; pereon longest at pereonite 1, shortest at pereonite 7, cuticle at both sides of pereonite thick, posterior margins of pereonites 1–5 smooth and slightly curved laterally, that of pereonite 6 and 7 slightly recessed. Pleonites 1–5 0.1 times as long as total length, 0.8 times as wide as greatest body width, with all pleonites visible in dorsal view. Pleotelson 0.6 times as long as wide, 1.6 times as long as pleonites 1–5.

Antennula 7 articles, extending beyond midlength of cephalon. Antenna 7–9 articles, extending beyond posterior of cephalon.

Pereopod 1 without robust seta and teeth. Pereopod 7 without robust seta and teeth.

Pleopods all lamellar with smooth surfaces. Pleopod 1 penduncle 0.3 times as wide as long; endopod rounded triangular, 1.4 times as long as wide, without swimming seta; exopod semicircular, 1.2 times as long as wide, 1.1 times as long as endopod, without swimming seta. Pleopod 2 peduncle 0.4 times as wide as long; endopod semicircular, 1.2 times as long as wide; exopod semicircular, 1.3 times as long as endopod, as long as wide.

Uropodal peduncle triangular, 1.4 times as long as wide, 0.6 times as long as exopod; endopod rami as long as that of exopod; extending beyond posterior margin of pleotelson; endopod pointed, 4.5 times as long as greatest width; exopod pointed, 4.4 times as long as greatest width.

Description of transitional (Figure 3): Body rectangular, 2.3 times as long as greatest width, widest at pereonite 3 or pereonite 4, narrowest at pleonite 1, with convex dorsal surfaces.

Cephalon 1.8 times wider than long, triangle, slightly immersed in pereonite 1. Eyes oval, with distinct margins, with long axis of each eye 0.5 times length of cephalon, and

short axis 0.1 times maximum width of cephalon. Anterior border of pereonite 1 medially protrudes forward. Coxae almost visible in dorsal view; pereon longest at pereonite 1, shortest at pereonite 7; posterior margins of pereonites 1–5 smooth and slightly curved laterally, that of pereonite 6 and 7 slightly recessed. Pleonites 1–5 0.1 times as long as total length, 0.9 times as wide as greatest body width, with all pleonites visible in dorsal view. Pleotelson 0.4 times as long as wide, 1.1 times as long as pleonites 1–5.

Antennula 5–7 articles, extending beyond midlength of cephalon. Antenna 7–8 articles, extending beyond posterior of cephalon.

Pereopod 1 basis 2 times as long as greatest width; ischium 0.6 times as long as basis; merus 0.5 times as long as ischium; carpus 0.9 times as long as merus; propodus 1.6 times as long as carpus; dactylus 0.7 times as long as propodus, without teeth. Pereopod 7 basis 1.2 times as long as greatest width; ischium 0.8 times as long as basis; merus 0.3 times as long as ischium; carpus 0.8 times as long as merus; propodus 2.4 times as long as carpus; dactylus 0.8 times as long as propodus, without teeth.

Pleopods all lamellar with smooth surfaces. Pleopod 1 penduncle 0.4 times as wide as long; endopod rounded triangular, 1.3 times as long as wide, without swimming seta; exopod quadrilateral, 0.9 times as long as wide, as long as endopod, without swimming seta. Pleopod 2 peduncle 0.4 times as wide as long; endopod semicircular, 1.1 times as long as wide; exopod rounded triangular, as long as endopod, 1.1 times as long as wide.

Uropodal peduncle triangular, as long as wide, 0.4 times as long as exopod; endopod rami 1.1 times as long as that of exopod; extending beyond posterior margin of pleotelson; endopod pointed, 4.4 times as long as greatest width; exopod pointed, 3.5 times as long as greatest width.

Description of male (Figure 4): Body rectangular, 2.2 times as long as greatest width, widest at pereonite 5, narrowest at pereonite 7, with smooth dorsal surfaces.

Cephalon 2.9 times wider than long, triangle, not immersed in pereonite 1. Eyes oval, with distinct margins, with long axis of each eye 0.8 times length of cephalon, and short axis 0.1 times maximum width of cephalon. Anterior border of pereonite 1 straight. Coxae almost visible in dorsal view; pereon longest at pereonite 1, shortest at pereonite 7, posterior margins of pereonites 1–5 smooth and slightly curved laterally, that of pereonite 6 and 7 slightly recessed. Pleonites 1–5 0.3 times as long as total length, 0.9 times as wide as greatest body width, with all pleonites visible in dorsal view. Pleotelson 0.4 times as long as wide, 1.2 times as long as pleonites 1–5.

Antennula 7 articles, extending beyond midlength of cephalon. Antenna 7–8 articles, extending beyond posterior of cephalon.

Pereopod 1 basis 1.7 times as long as greatest width; ischium 0.7 times as long as basis; merus 0.4 times as long as ischium; carpus 0.9 times as long as merus; propodus 1.7 times as long as carpus; dactylus 1.1 times as long as propodus, without teeth. Pereopod 7 basis 1.3 times as long as greatest width; ischium 0.7 times as long as basis; merus 0.4 times as long as ischium; carpus 0.8 times as long as merus; propodus 2.3 times as long as carpus; dactylus 0.9 times as long as propodus, without teeth.

Pleopods all lamellar with smooth surfaces. Pleopod 1 penduncle 0.4 times as wide as long; endopod rounded triangular, 1.4 times as long as wide, without swimming seta; exopod quadrilateral, as long as wide, 1.1 times as long as endopod, without swimming seta. Pleopod 2 peduncle 0.4 times as wide as long; endopod rounded triangular, 1.4 times as long as wide; exopod rounded triangular, as long as endopod, 1.1 times as long as wide.

Uropodal peduncle triangular, as long as wide, 0.5 times as long as exopod; endopod rami 0.9 times as long as that of exopod; extending beyond posterior margin of pleotelson; endopod pointed, 3.6 times as long as greatest width; exopod pointed, 4.3 times as long as greatest width.

Juvenile: Unknown

Description of manca (Figures 5 and 6): Body elliptical, 2.7 times as long as greatest width, widest at pereonite 5, narrowest at pleotelson, with smooth dorsal surfaces.

Cephalon 1.8 times wider than long, semi-oval, with pointed rostrum, not immersed in pereonite 1. Eyes oval, with distinct margins, with long axis of each eye 0.9 times length of cephalon, and short axis 0.3 times maximum width of cephalon. Anterior border of pereonite 1 straight. Coxae almost visible in dorsal view; pereon longest at pereonite 1, shortest at pereonite 7, posterior margins of pereonites 1–5 smooth and slightly curved laterally, that of pereonite 6 and 7 slightly recessed. Pleonites 1–5 0.3 times as long as total length, 0.7 times as wide as greatest body width, with all pleonites visible in dorsal view. Pleotelson 0.6 times as long as wide, 0.5 times as long as pleonites 1–5, with long swimming setae posterior margins.

Antennula 7 articles, extending beyond midlength of cephalon. Antenna 7–8 articles, extending beyond anterior border of pereonite 1.

Pereopod 1 basis 2.7 times as long as greatest width; ischium 0.5 times as long as basis; merus 0.8 times as long as ischium, with 1 robust seta on superior distal angle; carpus 0.5 times as long as merus; propodus 3.6 times as long as carpus, with 1–3 robust setae on inferior margin; dactylus 0.9 times as long as propodus, with tooth. Pereopod 6 basis 1.9 times as long as greatest width; ischium 0.6 times as long as basis; merus 0.4 times as long as ischium, with 1 robust seta on superior distal angle, 1 robust seta on inferior margin; carpus 0.9 times as long as merus, with 2–3 robust setae on inferior margin; propodus 3 times as long as carpus, with 3–5 robust setae on inferior margin; dactylus 0.9 times as long as propodus, with tooth. Lack of pereopod 7.

Pleopods all lamellar with smooth surfaces. Pleopod 1 penduncle 0.7 times as wide as long, medial margin with 4 coupling hooks, and no plumose seta; endopod elliptical, 2.6 times as long as wide, posterior margin with long swimming setae; exopod quadrilateral, 1.8 times as long as wide, as long as endopod, posterior margin with long swimming setae. Pleopod 2 peduncle 0.6 times as wide as long, medial margin with 4 coupling hooks, and no plumose seta; endopod elliptical, 2 times as long as wide, without appendix masculina, posterior margin with long swimming setae; exopod quadrilateral, as long as endopod, 1.7 times as long as wide, posterior margin with long swimming setae.

Uropodal peduncle triangular, 1.8 times as long as wide, 0.8 times as long as exopod, distal corner with 1 seta; endopod rami 0.8 times as long as that of exopod; extending beyond posterior margin of pleotelson; endopod oval, 2.4 times as long as greatest width, with apical and distal half or less of medial and lateral margins with swimming setae; exopod quadrilateral, 2.7 times as long as greatest width, with apical and distal half or less of medial and lateral margins with swimming setae.

Coloration: The manca is dark gray, and the male, transitional, and female are pearl yellow in preserved ethanol.

Hosts: *Girella punctata* (new host). Previously, *C. arimae* had only been known in yellowstriped butterfish, *Labracoglossa argenteiventris* Peters, 1866 (type host) [39,41]. The prevalence of *C. arimae* in *G. punctata* in the Seto Inland Sea was 36.4–67% (Table 1). No significant differences in prevalence were observed among the three sampling locations in this study (GLM, analysis of deviance, $p = 0.73$).

Distribution: The species is known only from the coast of Japan [42]. Previously, *C. arimae* had only been found in the Tokyo Prefecture (Tokyo Bay and Izu Islands) [39,41]; however, in this study, *C. arimae* was collected from the Seto Inland Sea.

DNA sequences: COI: accession numbers PV235262–PV235265; 16S rRNA: accession numbers PV241151–PV241155. The K2P genetic distances among these sequences were as follows: COI: 0.00–0.63% and 16S rRNA: 0.00–0.46%.

Remarks: *Ceratothoa arimae* was described by Nunomura [39] as *Glossobius arimae* Nunomura, 2001, but was moved to *Ceratothoa* by Martin et al. [40]. *Ceratothoa arimae* is distinguished from other species in *Ceratothoa* by its relatively elongated and rectangular body shape, small eyes, cephalon medially buried in pereonite 1, and rounded posterior margin of the pleotelson. This species is similar to *Ceratothoa imbricata* (Fabricius, 1775) and *Ceratothoa springbok* Hadfield and Smit, 2020. *Ceratothoa arimae* is distinguished from *C. springbok* by its thick cuticle on both sides of the pleonites (uniform in *C. springbok*). *Ceratothoa arimae* is distinguished from *C. imbricata* by the lighter color of the posterior margin of the pereonite (darker color in *C. imbricata*).

The manca was distinguished from the male by (1) elliptical body (rectangular in male), (2) body narrowest at pleotelson (pereonite 7 in male), (3) cephalon semi oval (triangle in male), (4) bigger eyes, (5) pleotelson, pleopods, and uropods with swimming setae (no swimming seta in male), (6) without pereopod 7 (with pereopod 7 in male), (7) pereopods with robust setae (without robust seta in male), (8) pereopods dactylus with tooth, (9) pleopods 1 and 2 peduncles with 4 coupling hooks (without coupling hook in male), (10) pleopod 1 endopod elliptical (rounded triangular in male), (11) pleopod 2 endopod elliptical (rounded triangular in male), (12) pleopod 2 exopod quadrilateral (rounded triangular in male), (13) uropodal peduncle distal corner with robust seta (no seta in male), and (14) uropods rami are wide (pointed in male). The male was distinguished from the transitional by (1) body narrowest at pereonite 7 (pleonite 1 in transitional), (2) body with smooth dorsal surfaces (convex dorsal surfaces in transitional), (3) cephalon not immersed in pereonite 1 (slightly immersed in pereonite 1 in transitional), and (4) pleopod 2 endopod rounded triangular (semicircular in transitional). The transitional was distinguished from the female by (1) without oostegite (with oostegites in females), (2) pleopod 1 exopod quadrilateral (semicircular in female), and (3) pleopod 2 exopod rounded triangular (semicircular in female).

For many species in *Ceratothoa*, no morphological information is available, except for adults (Table 2). Of the seven species of *Ceratothoa* reported in Japan [13], only the dorsal view of the manca of *Ceratothoa carinata* (Bianconi, 1869) and *Ceratothoa verrucosa* (Schioedte and Meinert, 1883) were provided [43]. *Ceratotho arimae* mancae observed in this study have more chromatophores than those of the other two species in Fujita et al. [43], but whether this is a taxonomic trait or not is unknown.

Table 2. List of the presence or absence of the morphological information on mancae and juveniles of *Ceratothoa*.

	Manca	**Juvenile**	**References**
Ceratothoa africanae Hadfield, Bruce, and Smit, 2014	-	-	
Ceratothoa angulata (Richardson, 1910)	-	-	
Ceratothoa arimae (Nunomura, 2001)	This study	-	
Ceratothoa banksii (Leach, 1818)	○	-	Schioedte and Meinert [44]
Ceratothoa barracuda Martin, Bruce, and Nowak, 2015	-	-	
Ceratothoa capri (Trilles, 1964)	-	-	
Ceratothoa carinata (Bianconi, 1869)	○ *	-	Fujita et al. [43]
Ceratothoa collaris Schiödte and Meinert, 1883	-	-	
Ceratothoa famosa Hadfield, Bruce, and Smit, 2014	-	-	
Ceratothoa gilberti (Richardson, 1904)	-	-	
Ceratothoa globulus Martin, Bruce, and Nowak, 2015	-	-	
Ceratothoa gobii Schiödte and Meinert, 1883	-	-	
Ceratothoa guttata (Richardson, 1910)	-	-	
Ceratothoa imbricata (Fabricius, 1775)	-	-	
Ceratothoa italica Schiödte and Meinert, 1883	○	-	Schioedte and Meinert [44]

Table 2. *Cont.*

	Manca	Juvenile	References
Ceratothoa marisrubri Trilles, Colorni, and Golani, 1999	-	-	
Ceratothoa oestroides (Risso, 1827)	○	-	Schioedte and Meinert [44] Mladineo [45]
Ceratothoa oxyrrhynchaena Koelbel, 1878	-	○	Saito et al. [46]
Ceratothoa parallela (Otto, 1828)	○	-	Schioedte and Meinert [44] Papapanagiotou and Trilles [47]
Ceratothoa retusa (Schioedte and Meinert, 1883)	-	-	
Ceratothoa springbok Hadfield and Smit, 2020	-	-	
Ceratothoa steindachneri Koelbel, 1878	-	-	
Ceratothoa toyamaensis (Nunomura, 1993)	-	-	
Ceratothoa trigonocephala (Leach, 1818)	○	-	Schioedte and Meinert [44]
Ceratothoa usacarangis (Avdeev, 1979)	-	-	
Ceratothoa verrucosa (Schioedte and Meinert, 1883)	○ *	○ *	Fujita et al. [43]

○ indicates that there is morphological information. * only the overall view.

4. Discussion

Ceratothoa arimae was previously thought to be exclusively parasitic on *L. argenteiventris*, but this study reports on its infestation of *G. punctata*. *Ceratothoa arimae* is the third species in the Cymothoidae that infests *G. punctata*, and is the only species in the Cymothoidae family that infests the buccal cavity. This is the second species of *Ceratothoa* that parasitizes the genus *Girella*.

In fish aquaculture farms, the number of hosts per unit area is high; therefore, it is easy for parasitic infections to spread [48]. To date, no confirmed cases of cymothoid parasitizing *G. punctata* have been reported in aquaculture. However, in marine aquaculture, it is possible that the manca released from wild fish will enter through the mesh of the fish preserve and parasitize cultured fish. In addition, in the case of aquaculture using wild juvenile fish, there is a possibility that the collected juveniles may already be parasitized. There were cases of sudden infection spreading to cultured fish (such as the case of *M. parvostis* parasitizing Japanese amberjack, *Seriola quinqueradiata* Temminck and Schlegel, 1845, in Japan [25,49]). Therefore, it is necessary to take measures against *C. arimae* infection in *G. punctata* aquaculture. To control parasites in cultured fish, it is necessary to understand their life cycle [48].

In this study, the K2P genetic distance among individuals in the COI and 16S rRNA sequences was less than the maximum value of intraspecific distance in *Ceratothoa* detected in Fujita et al. [50] (COI: 2.59%, 16S rRNA: 1.22%). Therefore, we determined that these individuals, including the manca, were of the same species. Similarly, molecular species identification is often used to identify species in individuals other than adult females [46]. Hata et al. [51] analyzed the COI and 16S rRNA regions of 29 species in 15 genera in Cymothoidae and examined the phylogenetic relationships of the Cymothoidae. They have increased the molecular information on Cymothoidae. However, because they did not provide any basis for identifying the cymothoid species, there are some instances of incorrect species labels [52,53]. Problems caused by nucleotide sequences registered with incorrect species labels have been pointed out in molecular species identification [54]. In order to solve this problem, nucleotide sequences linked to morphological information on adult females are necessary, and the sequences obtained in this study will be useful for future research.

In the life cycle of many species of Cymothoidae, except for adult females, they do not have taxonomic traits, and it is difficult to identify species by morphology. A system for identifying species based on key morphological traits that differ from those in adults

is necessary for mancae and juveniles. In recent years, morphological information has been accumulated through the examination of the morphology of mancae and juveniles identified by molecular data [52], but most species lack this information. In this study, we described, for the first time, the morphology of *C. arimae* mancae. To establish a method for identifying species, it is necessary to accumulate morphological and molecular data, and the data obtained in this study are useful.

It would be effective to investigate whether there are any *C. arimae* mancae in the vicinity of the aquaculture farm. It is known that the cymothoid species mancae can be collected using a light trap [43], so it should be possible to use a light trap to investigate whether mancae are present. At present, there is a lack of morphological information on *Ceratothoa* manca, other than *C. arimae*; therefore, it is necessary to identify the species of the manca collected by light trapping using molecular species identification. In the future, the accumulation of morphological information in other species of mancae will make it possible to identify species based on their morphology, which will be useful for control.

Although *G. punctata* is thought to be exposed to many fishermen and anglers, cymothoids that parasitize the buccal cavity have not been reported until now. In contrast, the prevalence of *C. arimae* was 29.4–66.7% in *G. punctata* in the Seto Inland Sea. Thus, there may be large regional differences in the prevalence of *C. arimae* in *G. punctata*. The prevalence of *M. parvostis* in Japanese halfbeak, *Hyporhamphus sajori* (Temminck and Schlegel, 1846) also differed among sampling sites in the Seto Inland Sea [9]. In this study, we were unable to detect significant differences in prevalence among sampling sites because we only had information on the collection sites where infested fish were found. Furthermore, data on the prevalence of *C. arimae* in *L. argenteiventris* (type host) includes only one case on the coast of Nii-jima Island, part of the Izu Islands, Tokyo, in July, 2013 (34.8%) [41]. *Labracoglossa argenteiventris* also inhabits the Seto Inland Sea [55]; however, it is unknown whether *C. arimae* parasitizes it there. By comparing the distribution and prevalence of *C. arimae* in *L. argenteiventris* and *G. punctata* in various locations in Japan, we may be able to use this as a resource for studying host switching in Cymothoidae.

5. Conclusions

Knowledge regarding the parasites in *G. punctata*, a commercially significant and cultivated species, is of considerable importance from the perspective of fisheries science. In this study, we report, for the first time, that *C. arimae*, previously thought to parasitize only *L. argenteiventris*, also parasitizes the buccal cavity of *G. punctata*. In addition, we provided a DNA barcode based on morphological identification, as well as the first description of the morphology of a *C. arimae* manca. There might be regional differences in the host selection of *C. arimae*, and this species is expected to be used as research material to investigate host switching in Cymothoidae.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes10030126/s1. Supplementary File S1: list of samples of *Ceratothoa arimae* (Nunomura, 2001) collected from the buccal cavity of largescale blackfish, *Girella punctata* Gray, 1835. Supplementary File S2: DELTA format file describing *Ceratothoa arimae* (Nunomura, 2001) collected from the buccal cavity of largescale blackfish, *Girella punctata* Gray, 1835.

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Institutional Review Board Statement: Ethical review and approval were waived following Notice (No. 71) of the Japanese Ministry of Education, Culture, Sports, Science, and Technology, as well as Regulations on Animal Experimentation at Kyoto University, because the research target species were invertebrates, and the fish were collected in accordance with the Fishery Act of Japan.

Data Availability Statement: The DNA sequences in this study were deposited in NCBI GenBank (accession numbers: PV235262–PV235265, PV241151–PV241155). The specimens used in this study are deposited in the Seto Marine Biological Laboratory (SMBL), Field Science Education and Research Center, Kyoto University (SMBL-V0829–V0840). Details of the sample are shown in Supplementary File S1. Taxonomic data is contained in Supplementary File S2.

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