Taxonomy and phylogeny of Anthuroidea (Crustacea: Isopoda) and its parasites

（ウミナナフシ類（甲殻亜門等脚目）および

その寄生虫の系統分類学的研究）

SHOKI SHIRAKI

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Department of Natural History Sciences, Graduate School of Science,

Hokkaido University, Sapporo, Hokkaido 060-0810, Japan

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2. Shiraki S, Shimomura M, Kakui K (2022) First report of Hyssuridae (Isopoda: Anthuroidea) from Japan, with the description of a new *Kupellonura* species. Nauplius 30: e2022023.
3. Shiraki S, Shimomura M, Kakui K (2022) A new neotenous genus and species, *Deltanthura palpus* gen. et sp. nov. (Isopoda: Anthuroidea: Paranthuridae) from Japan, with a revised key to the genera in Paranthuridae. Zoosystematics and Evolution 98: 109–115.
4. Shiraki S, Kakui K (2022) Observations on predation in *Paranthura japonica* Richardson, 1909 (Isopoda: Cymothoida: Paranthuridae). Zoological Science 39: 270–274.
5. Shiraki S, Kakui K (2024) Description of a new *Cruranthura* species from the Miyako Islands, Japan, with its DNA barcode (Isopoda: Anthuroidea: Paranthuridae). Species Diversity 29: 65–72.
6. Shiraki S, Kakui K (2024) Ontogenetic changes in pigmentation pattern in *Mesanthura miyakoensis* (Crustacea: Isopoda: Anthuridae). Zoological Science 41: 323–328.
7. Shiraki S, Kakui K (2024) Isopods on isopods: integrative taxonomy of Cabiropidae (Isopoda: Epicaridea: Cryptoniscoidea) parasitic on anthuroid isopods, with descriptions of a new genus and three new species from Japan. Invertebrate Systematics 38: IS24013.

8. Shiraki S, Yoshida R, Kakui K (2024) *Paranthura oriens* sp. nov. (Isopoda: Anthuroidea: Paranthuridae) from Tateyama, Chiba, Japan. Plankton and Benthos Research 19: 233–243.
9. Shiraki S, Kakui K (2025) Trematode metacercariae parasitic in the estuarine crustacean *Cyathura muromiensis* Nunomura, 1974 (Peracarida: Isopoda: Anthuroidea). Parasitology International 104: 102973.
10. Shiraki S, Kakui K (2025) Four species of *Mesanthura* (Crustacea: Isopoda: Anthuroidea) from Japan, including the description of a new species. Journal of the Marine Biological Association of the United Kingdom 105: e48.
11. Shiraki S, Kakui K (2025) First report of *Paranthura japonica* Richardson, 1909 (Crustacea: Isopoda: Paranthuridae) from Okinawa, Japan. Aquatic Animals 2025: AA2025-30. [in Japanese]
12. Shiraki S, Kakui K, Abatay M, Caneos W, Dahilog-Caralde M, Kajihara H, Tsuyuki A, Abato J (2025) A new anthurid *Amakusanthura camiguinensis* sp. n. (Crustacea: Isopoda: Anthuroidea) from Camiguin Island, Philippines. Journal of Natural History 59: 2175–2186.
13. Shiraki S, Kakui K, Along A, Calagui L, Calagui SI, Demetillo M, Kajihara H, Leones JA, Rojas-Salinas A, Sanchez-De los Reyes KA, Seronay R, Tsuyuki A, Abato J (2025) Taxonomic revision of *Sauranthura* (Isopoda: Anthuroidea: Anthuridae), with the description of *Sauranthura liangaensis* sp. nov. from Surigao del Sur, Philippines. Marine Biology Research 21: 206–220.

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Author: Shoki Shiraki

Affiliation: Department of Natural History Sciences, Graduate School of Science, Hokkaido University, Sapporo, Hokkaido 060-0810, Japan

Thesis committee:

Hiroshi Kajihara (Chair, Professor, Faculty of Science, Hokkaido University)

Kazuhiro Kogame (Vice chair, Professor, Faculty of Science, Hokkaido University)

Keiichi Kakui (Vice chair, Lecturer, Faculty of Science, Hokkaido University)

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CONTENTS

CHAPTER I: GENERAL INTRODUCTION

Anthuroidea Leach, 1814 (derived from the type-genus name *Anthura* Leach, 1814 combining Ancient Greek *anthos* [flower] and *oura* [tail], referring to its flower-shaped tailfan formed by uropods and telson), which is sometimes called as “wormpod” (e.g. Thomas Thorpe 2024), is a group of aquatic isopods typically 8–15 mm in length (Cadien and Brusca 1993). It is characterized by the following combination of characters: the elongate cylindrical body; the uropodal exopod attached to the uropodal peduncle proximally and dorsally; the tailfan; pereopodal coxae absent; and the maxilla 2 fused with the lower lip, forming a hypopharynx (Poore 2001).

Montagu (1808) described the first anthuroid species, *Oniscus gracilis* Montagu, 1808 from the United Kingdom. Leach (1814) then erected the genus *Anthura* and the family Anthuridae Leach, 1814 for this species. Monod (1922) put Anthuridae in the suborder Anthuridea Monod, 1922, later Brandt and Poore (2003) demoted the latter to the superfamily (Anthuroidea) in the suborder Cymothoida Wägele, 1989 which is the current parent taxon of Anthuroidea. However, recent phylogenomic analyses showed that Anthuroidea is a distinct clade from Cymothoida (Thomas Thorpe 2024; Barta et al. preprint). In the analysis by Barta et al. (preprint), although it includes only a single anthuroid species (*Accalathura gigantissima* Kussakin, 1967), Anthuroidea was placed sister to the clade containing Cymothooidea, Limnoriidea, Valvifera, and Sphaeromatidea.

Poore (2001) conducted a morphology-based systematic revision of this group and classified all anthuroids into six families: Antheluridae Poore & Lew Ton, 1988, Anthuridae, Expanathuridae Poore, 2001, Hyssuridae Wägele, 1981, Leptanthuridae Poore, 2001, and Paranthuridae Menzies & Glynn, 1968. These families fall into two major groups defined by the form of mouthparts: one has ‘biting’ mouthparts that contain

robust mandibles with incisor, lamina dentata, and molar and comprises the former four families; and the other has ‘piercing’ mouthparts that transform into a suction tube, containing the latter two (Poore 2001). As of the time I started my research, 62 genera and 618 species were included in Anthuroidea (Boyko et al. 2025).

To date, only three works conducted exhaustive phylogenetic analysis in Anthuroidea, all of which are based on morphological characters (Wägele 1981, 1989; Poore 2001). Wägele (1981) and Wägele (1989) showed almost the same relationships but Poore (2001) showed different result from them (cf. Fig. V-1 in Chapter V). Poore’s (2001) tree showed the monophyly of all the six families. On the other hand, in the trees of the other two studies (Wägele 1981, 1989), Antheluridae and Hyssuridae were monophyletic, but the remaining four families were not (when each genus was assigned to the six families; at that time, Expanathuridae and Leptanthuridae were not established yet). Current systematics is following Poore (2001), but future molecular phylogenetic analysis is needed to test the relationship. The use of molecular information is quite delayed in Anthuroidea and there has been no molecular phylogenetic analysis targeting Anthuroidea so far.

Most anthuroids are benthic and marine (Kensley 2001). They have been reported from the intertidal zone to the deep sea to up to 6500 m depth (Wolff 1956; Poore and Bruce 2012), but 75% of known species are from the subtidal zone (Poore and Bruce 2012). Some anthuroids have been discovered in non-marine environments such as brackish river mouths, freshwater rivers, and anchialine caves (e.g. *Cyathura* Norman & Stebbing, 1886 species, *Cruregens fontanus* Chilton, 1882, and *Curassanthura yucatanensis* Alvarez, Benitez, Iliffe & Villalobos, 2019, respectively; Poore 2001; Alvarez et al. 2019). Anthuroid isopods utilize various environments. They dwell on muddy/sandy bottoms, between sands (interstitial), under rocks, among thalli of

algae/seaweed, among coral rubble, or inside tubes formed by other organisms. Their high diversity has been observed in/around coral reefs (Kensley 2001). Some *Eisothistos* Haswell, 1884 species are known to invade and inhabit the tubes constructed by serpulid polychaetes (Knight-Jones and Knight-Jones 2002).

Knowledge on the feeding habits of anthuroids is quite limited, but they are considered generally to be predators (Poore and Bruce 2012). *Cyathura* species with biting-type mouthparts fed on various stuff such as polychaetes, detritus, diatoms, crustaceans, or dead fishes (Burbanck 1959; Burbanck and Burbanck 1979; Wägele 1981; Wägele et al. 1981; Horikoshi 2012). *Eisothistos* species, which possess biting-type mouthparts and invade the tubes of serpulid polychaetes, seem to feed on specific serpulids (Wägele 1981). Species with piercing-type mouthparts fed mainly on crustaceans (observed for *Paranthura* Bate & Westwood, 1866 and *Accalathura* Barnard, 1925; Wägele 1981, 1985; Shiraki and Kakui 2022; Matsushima et al. 2025). The prey-side perspective of anthuroids is also not well known. Fishes and birds have been reported as their predators so far (e.g. Burbanck 1959, 1963; Burbanck and Burbanck 1979; Santos et al. 2005).

Knowledge on the parasites of anthuroids is quite limited. Only three groups were known to parasitize anthuroids before my study: epicaridean isopods (Wägele 1979), rhizocephalan barnacles (Smith 1906), and trematodes (Villot 1879; Burbanck 1962; Reimer 1963; Schulenburg and Wägele 1998; Schulenburg et al. 1999; Jensen et al. 2004; Ferreira et al. 2005a, 2005b). As commensals, the copepod *Cithadius cyathurae* Bowman, 1972 is known to associate with *Cyathura polita* (Stimpson, 1855) (Bowman 1972). So far, parasitic anthuroids are unknown; as for *Eisothistos* species, although it is not a parasitism, they invade the tubes constructed by serpulid polychaetes, feed on the

polychaetes, and utilize the tube as their shelter (Wägele 1981; Knight-Jones and Knight-Jones 2002).

Anthuroids show pronounced sexual dimorphism. Males differ from females in having enlarged eyes, elongate and swollen antennulae bearing numerous aesthetascs, elongate pereopods, and an appendix masculina on pleopod 2 (Poore 2001). Gonopores are opened on pereonites 7 (males) or pereonite 5 (females) ventrally (Cadien and Brusca 1993). Even though the life history of most species is unsurveyed (Poore 2001), anthuroids are generally considered to be protogynous (Poore and Bruce 2012), i.e., females change sex and become terminal swimming males (or secondary males). In some species, the presence of primary males (males born as males) as well as secondary males has been suggested (e.g., *Cyathura muromiensis* Nunomura, 1974 and *Ptilanthura tenuis* Harger, 1878; Kensley 1996; Horikoshi 2012). Males are usually rare among populations (Poore 2001).

Chapter II deals with the taxonomy of Anthuroidea in Japan. Before this study, 39 species in ten genera and five families in Anthuroidea had been recorded from Japan, but it is undoubtful that the Japanese anthuroid fauna is underrated. To reveal it, I conducted exhaustive samplings around Japan. Here I establish one new genus (*Deltanthura* **gen. nov.**), describe eight new species (*Anthomuda iriomotensis* **sp. nov.**, *Malacanthura seisuiae* **sp. nov.**, *Mesanthura sol* **sp. nov.**, *Expanathura monile* **sp. nov.**, *Kupellonura tamago* **sp. nov.**, *Cruranthura viridis* **sp. nov.**, *Deltanthura palpus* **sp. nov.**, and *Paranthura oriens* **sp. nov.**), and extend the distribution ranges for four species (*Mesanthura cinctula* Nunomura, 2006, *Mesanthura miyakoensis* Nunomura, 1979, *Mesanthura nigrodorsalis* Nunomura, 1977, and *Paranthura japonica* Richardson, 1909), representing the first reports of Hyssuridae and six genera from Japan. Additionally, I examine the ontogenetic change in pigmentation pattern in *Me. miyakoensis* to address

the taxonomic utility of pigmentation pattern in *Mesanthura* taxonomy, and investigate the predation behavior in *P. japonica*. This study updates the faunal composition of Japanese anthuroids to 47 species in 16 genera and six families.

Chapter III deals with the taxonomy of Anthuroidea in the Philippines. Before this study, only two species in two genera and two families were reported in the country. I conducted samplings in Mindanao, southern Philippines, aiming to reveal the anthuroid fauna in the region. Here I describe two new species (*Amakusanthura camiguinensis* **sp. nov.** and *Sauranthura liangaensis* **sp. nov.**). This study updates the faunal composition of anthuroids in the Philippines to four species in four genera and two families.

Chapter IV deals with the parasites of anthuroids. Before my study, animals in only three taxa, i.e., Epicaridea (Crustacea: Isopoda), Rhizocephala (Crustacea: Thecostraca), and Trematoda (Platyhelminthes: Neodermata), are known to parasitize anthuroid isopods. Here I firstly report cabiropid epicarideans parasitic on anthuroids with the establishment of one new genus *Anthuroniscus* **gen. nov.** and the descriptions of three new species (*Anthuroniscus shimomurai* **sp. nov.**, *Anthuroniscus dentatus* **sp. nov.**, and *Anthuroniscus latus* **sp. nov.**); describe the second anthuroid-parasitic rhizocephalan *Duplorbis changeling* **sp. nov.**; and firstly report trematodes parasitic to *Cy. muromiensis*.

Chapter V deals with the molecular phylogeny of Anthuroidea. I determined partial sequences of the four gene markers (18S rRNA gene [18S], 28S rRNA gene [28S], 16S rRNA gene [16S], and cytochrome *c* oxidase subunit I gene [COI]) from 37 Japanese species representing at least 20 genera and all the current six families, and reconstructed the phylogenetic tree of Anthuroidea based on those markers. The resulting tree recovered five major clades: Clade 1 contained two paranthurid genera, *Colanthura* Richardson, 1902 and *Cruranthura* Thomson, 1946; Clade 2 contained the other paranthurids and leptanthurid *Accalathura*; Clade 3 contained the other leptanthurids; Clade 4 consisted of

all anthurids; and Clade 5 contained Antheluridae, Expanathuridae, and Hyssuridae. The result showed the monophyly of Anthuridae and Hyssuridae with moderate and full supports, respectively, but those of the remaining four families were not supported. Inter-familial relationship was not resolved in the analysis.

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CHAPTER II: TAXONOMIC STUDY OF ANTHUROIDEA IN JAPAN

INTRODUCTION

From Japan, 39 species in ten genera and five families had been recorded as of the time my never-ending research journey begun (Table II-1). In addition to those in Table II-1, '*Haliophasma* (?) sp.' was reported by Nunomura (1993), but later the same author mentioned that its generic affiliation must be reevaluated (Nunomura 2015); Saigusa (2001) reported *Haliophasma* sp. from Iriomote Island, but without any morphological information or specimen deposition; and one unidentified species of *Leptanthura* G. O. Sars, 1897 was reported by Kimura et al. (2019). Until the 1970s, there had been no anthuroid taxonomists in Japan, and all collected anthuroids were identified as *Paranthura japonica* Richardson, 1909, the first anthuroid species described from Japan (Nunomura and Shimomura 2012a). Since the middle of the 1970s, Noboru Nunomura has actively investigated anthuroid fauna and described 37 species as new from Japan (Nunomura and Shimomura 2012a–d; Nunomura 2013, 2014, 2015, 2016a, 2016b); the remaining two species were described by foreign taxonomists (Richardson 1909; Wägele 1984). Except for the four species collected from 200 m or deeper depth and *Ananthura ocellata* (Nunomura, 1992) lacking the sampling depth information, all named anthuroids reported from Japan are shallow water species, mainly collected from the intertidal or subtidal zones. Four deep-water species are *Accalathura ochotensis* Nunomura, 1976, *Ananthura rigida* Nunomura, 1976, *Apanthura longiunguis* Nunomura, 2014, and *Mesanthura cinctula* Nunomura, 2006, each of which was described from depths of 390 m in the Okhotsk Sea (Nunomura 1976a), 1008–1050 m in Suruga Bay (Nunomura 1976b), 277 m in Tosa Bay (Nunomura 2014), and 247–269 m in Sagami Bay (Nunomura

2006), respectively. High species diversity of genera *Cyathura* Norman & Stebbing, 1886 and *Paranthura* Bate & Westwood, 1866 is observed in Japan (seven and nine species, respectively), but it may be due to sampling bias. Still, many undiscovered anthuroid species must be harbored in Japan (Nunomura and Shimomura 2012a).

In this chapter, I establish one new genus, describe eight new species, and extend the distribution ranges for four species, representing the first reports of Hyssuridae Wägele, 1981 and six genera from Japan. Additionally, I examine the ontogenetic change in pigmentation pattern in *Me. miyakoensis* to address the taxonomic utility of pigmentation pattern in *Mesanthura* Barnard, 1914 taxonomy, and investigate the predation behavior in *P. japonica*. This study updates the Japanese anthuroid fauna to 47 species in 16 genera and six families.

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Chapter II-I. *Anthomuda iriomotensis* sp. nov. (Antheluridae) from Iriomote Island

INTRODUCTION

Antheluridae Poore & Lew Ton, 1988 is a small anthuroid family with 19 species (Kensley and Schotte 2000; Poore 2001; Negoescu 2005; Shiraki et al. 2022) and characterized by having biting mouthparts, free pleonites 1–5, telson with single statocyst, and operculiform pleopod 1 (Poore 2001). This family includes seven nominal genera now; Poore and Lew Ton (1988) synonymized four of them (i.e., *Austranthura* Kussakin, 1967, *Bathura* Schultz, 1966, and *Valoranthura* Kensley, 1978 with *Ananthura* Norman & Stebbing, 1886; *Diaphoranthura* Kensley, 1980 with *Anthomuda* Schultz, 1979), Antheluridae thus currently comprises three valid genera, namely *Ananthura*, *Anthelura* Barnard, 1925, and *Anthomuda*. Species of *Anthomuda* inhabit shallow waters while those of the other two genera are reported from deep waters (Poore 2001).

Anthomuda is distinguished from the other two genera by having (1) the flat telson, not domed, (2) the telson 2.5 times as long as wide, (3) the telson and the uropodal rami apically rounded, and (4) the carpus of pereopod 4–7 about as long as wide (Poore 2001). *Anthomuda* includes eight valid species, and each species is morphologically very similar to the others (Poore and Lew Ton 1988). *Anthomuda stenotelson* Schultz, 1979, the type species of *Anthomuda*, is synonymized with *Anthomuda affinis* (Richardson, 1902) (Kensley 1994).

To date, two anthelurid species belonging to *Ananthura* have been reported from Japan (Table II-1). Nunomura (1976) described *Ananthura rigida* Nunomura, 1976 from Suruga Bay at a depth of 1008–1050 m, and later reported also from Sagami Bay at a

depth of 135–150 m (Nunomura 2006). Nunomura (1992) described *Ananthura ocellata* (Nunomura, 1992) from Yaeyama Islands, Okinawa. There have been no reports of *Anthelura* and *Anthomuda* from Japan until now.

In this chapter, I describe a new *Anthomuda* species collected from Iriomote Island. This is the first report of *Anthomuda* from Japan.

MATERIALS AND METHODS

A single specimen was collected from the subtidal zone of Iriomote Island, Japan on 12 August 2016 and fixed and preserved in ethanol. It was dissected with chemically sharpened tungsten wire needles under a Nikon SMZ 1500 microscope. Appendages were mounted on glass slides in glycerin and observed with an Olympus BX51 microscope; after observation, preparations were sealed with Canada balsam. Illustrations were prepared with Adobe Illustrator CC from draft line drawings made with a camera lucida (for appendages) or from sequential photographs (for habitus). Body length was measured from the tip of the anterolateral lobe of the head to the tip of telson, and body width was measured at the widest portion of pereonite 4. I treated the specimen as a female lacking oostegites as it has complete pereopod 7 but lacks oostegites or appendix masculina. The material examined is deposited in the Shoki Shiraki's Anthuroid Collection (SSAC).

TAXONOMY

Family Antheluridae Poore & Lew Ton, 1988

Genus *Anthomuda* Schultz, 1979

Anthomuda iriomotensis sp. nov.

(Figs II-I-1–II-I-4)

Diagnosis. Body with reddish brown reticulate pattern of pigmentation dorsally. Antenna with seven flagellar articles. Article 3 of mandibular palp with four spiniform setae. Article 4 of maxillipedal palp with six simple setae. Propodus of pereopod 7 with two spiniform setae.

Etymology. The specific name is an adjective, derived from the type locality.

Material examined. Holotype: female lacking oostegites (SSAC-01; 14 slides and 1 vial), body length 4.64 mm, among coral rubble, 21 m depth, Iriomote Island, Japan, collected by Daisuke Uyeno, 12 August 2016.

Description of female holotype. Body slender (Figs II-I-1, II-I-2A, B), length 12.02 times width, with reddish brown reticulate pattern of pigmentation dorsally. Head (Fig. II-I-2A) length 1.02 times head width, with several lateral simple setae and dorsolateral small eyes; dark band-like pigmentation between eyes and faint pigmentation in posterior half; rostrum not overreaching anterolateral lobes. Pereonites 1–7 (Fig. II-I-2A) with several lateral simple setae, with length ratio 1.53:1.48:1.37:1.61:1.71:1.44:0.90 based on head length. Pleonites 1–6 (Fig. II-I-2A) free, with length ratio 1.00:0.89:0.98:1.01:1.51:1.23; combined length of pleonites 1–6 0.11 times body length; pleonites 1–4 with several lateral simple setae; pleonites 4 and 5 with row of posterolateral setae; posterior margin of pereonite 6 concave. Telson (Fig. II-I-4J) narrow,

apically rounded, with length 2.67 times width, slightly convex ventrally, with single statocyst and four apical long and 21 short simple setae.

Antennula (Fig. II-I-2C) with three peduncular articles and four flagellar articles. Peduncular article 1 longest, with five plumose sensory setae and outer simple seta; article 2 with three plumose sensory setae and simple seta; article 3 with five distal simple setae. Flagellar article 1 with plumose sensory seta; article 2 naked; article 3 with two aesthetascs and simple seta; article 4 with aesthetasc and five simple setae.

Antenna (Fig. II-I-2D) with five peduncular article and seven flagellar articles. Peduncular article 1 with inner row of setules; article 2 with ventrodistal projection reaching distal edge of article 3 and four simple setae; article 3 with two inner distal simple setae; article 4 with four distal simple setae; article 5 with four distal plumose sensory setae and six inner simple setae. Flagellar articles 1–7 each with numerous simple setae.

Mandible (Fig. II-I-2E) with triarticulate palp. Palp article 1 with distal simple seta; article 2 longest, with distal simple seta; article 3 with four spiniform setae. Incisor with three cusps. Lamina dentata with nine denticulations. Molar rounded.

Maxilliped (Fig. II-I-2F) with 4-articulate palp. Palp article 1 naked; article 2 with inner simple seta; article 3 with four distal simple setae; article 4 with six simple setae. Endite overreaching palp article 1, with four simple setae. Epipod oval.

Pereopod 1 (Fig. II-I-3A) subchelate. Basis with two dorsal plumose sensory setae and four simple setae. Ischium with one dorsal and one ventrodistal simple setae. Merus with three dorsal and five ventral simple setae. Carpus triangular, with five ventral simple setae. Propodus with two dorsodistal, three outer, and 17 ventral simple setae. Dactylus with ventrodistal spiniform seta and one ventral, two ventrodistal, and four inner distal simple setae. Unguis about three-quarters dactylus length, naked.

Pereopod 2 (Fig. II-I-3B) almost as wide as pereopod 1. Basis with two dorsal plumose sensory setae and two dorsal and two ventral simple setae. Ischium with one dorsal and four ventral simple setae. Merus with three dorsal and six ventral simple setae. Carpus triangular, with ventrodistal spiniform seta with sensory bristle sensu Negoescu (1994) and four ventral simple setae. Propodus with two ventral spiniform setae with sensory bristle, dorsal plumose sensory seta, and five dorsal, three dorsodistal, and eight ventral simple setae. Dactylus with ventrodistal spiniform seta and five distal and two outer dorsal simple setae. Unguis about two-thirds dactylus length.

Pereopod 3 (Fig. II-I-3C) similar to pereopod 2 except in number of plumose sensory setae of basis and simple setae.

Pereopod 4 (Fig. II-I-3D) slightly narrower than pereopods 1–3. Basis with three dorsal plumose sensory setae and two dorsal and three ventral simple setae. Ischium with one dorsal and five ventral simple setae. Merus with two dorsal and seven ventral simple setae. Carpus trapezoidal, with two ventral spiniform setae with sensory bristle, dorsal plumose sensory seta, and one dorsal and four ventral simple setae. Propodus with two ventral spiniform setae with sensory bristle, dorsodistal plumose sensory seta, and six dorsal and seven ventral simple setae. Dactylus with ventrodistal spiniform seta and one ventral, six ventrodistal, and three outer dorsal simple setae. Unguis about half dactylus length, naked.

Pereopods 5 and 6 (Fig. II-I-3E, F) similar to pereopod 4 except in number of plumose sensory setae and simple setae.

Pereopod 7 (Fig. II-I-3G) with basis, ischium, merus, carpus, dactylus, and unguis similar to those of pereopod 4, except in number of plumose sensory setae and simple setae. Propodus with four dorsodistal trifurcate spiniform setae, two ventral spiniform setae with sensory bristle, and four dorsal and seven ventral simple setae.

Pleopod 1 (Fig. II-I-4A) protopod with three inner short spiniform setae, outer plumose seta, and inner simple seta. Exopod operculiform, 2.03 times as long as wide, with 23 distal plumose setae and 23 simple setae on ventral surface. Endopod with four distal plumose setae.

Pleopods 2–5 (Fig. II-I-4B–G) similar to each other. Protopods with two inner spiniform setae and outer plumose or simple seta. Exopods with eight distal plumose setae and two (exopods 2 and 3) or one (exopods 4 and 5) outer plumose setae. Endopods with six (endopods 2, 3, and 5) or five (endopod 4) distal plumose setae.

Uropod (Fig. II-I-4H, I) protopod triangular prism shaped, with 16 outer and two ventral plumose setae and one dorsal and four outer simple setae. Endopod (Fig. II-I-4H) oval, with eight plumose sensory setae and 43 simple setae. Exopod (Fig. II-I-4I) 1.87 times longer than wide, with six plumose setae, 57 simple setae, and four setae (tip broken).

Remarks. *Anthomuda iriomotensis* **sp. nov.** differs from *A. affinis* and *Anthomuda hapla* (Kensley, 1980) by the number of simple setae in article 4 of maxillipedal palp (six in *A. iriomotensis* **sp. nov.** vs. five in *A. affinis* and *A. hapla*) and spiniform setae in propodus of pereopod 7 (two in *A. iriomotensis* **sp. nov.** vs. three in *A. affinis* vs. one in *A. hapla*) (Kensley 1980, 1994). *Anthomuda iriomotensis* **sp. nov.** differs from *Anthomuda chorizema* Poore & Lew Ton, 1988 and *Anthomuda cracens* (Kensley, 1980) by the number of simple setae in article 4 of maxillipedal palp (six in *A. iriomotensis* **sp. nov.** vs. five in *A. chorizema* and *A. cracens*) and spiniform setae in article 3 of mandibular palp (four in *A. iriomotensis* **sp. nov.** vs. five in *A. chorizema* and *A. cracens*) (Kensley 1980; Poore and Lew Ton 1988). *Anthomuda iriomotensis* **sp. nov.** is distinguished from *Anthomuda alba* Müller, 1990 by the presence of dorsal

pigmentations on the body (no pigmentations in *A. alba*; Müller 1990a). *Anthomuda iriomotensis* **sp. nov.** differs from *Anthomuda hovea* Poore & Lew Ton, 1988, *Anthomuda poorei* Müller, 1990, and *Anthomuda quadrilineata* Kensley & Schotte, 2000 by the number of flagellar articles of antenna (seven in *A. iriomotensis* **sp. nov.** vs. five in *A. hovea*, six in *A. poorei*, and four in *A. quadrilineata*) (Poore and Lew Ton 1988; Müller 1990b; Kensley and Schotte 2000).

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Chapter II-II. *Malacanthura seisuiae* sp. nov. (Anthuridae) from the Kumanonada Sea

INTRODUCTION

Malacanthura Barnard, 1925 is one of the 26 anthurid genera and is characterized in having (1) the pleon without dorsal sutures, (2) the telson without longitudinal ridge, (3) the maxillipedal palp with three articles in dissimilar length, (4) the pereopods 2–7 bearing few short setae, (5) the carpi of pereopods 4–7 rectangular, and (6) the uropodal exopod leaf-like, not cylindrical (Poore 2001; Chew et al. 2014). Although only five species are contained in it (Boyko et al. 2025), this genus is one of the most taxonomically confused anthurid genera as its members have been placed arbitrarily in *Malacanthura* or *Haliophasma* Haswell, 1881 (Poore 2001).

In this chapter, I describe a new *Malacanthura* species based on specimens collected from the Kumanonada Sea off the southern coast of Mie Prefecture, Japan. I also determined partial sequences of the cytochrome *c* oxidase subunit I (COI) gene from the new species.

MATERIALS AND METHODS

Two anthurid individuals, one of which is parasitized by one rhizocephalan barnacle (see Chapter IV-II for the rhizocephalan), were collected from the Kumanonada Sea off the southern coast of Mie Prefecture, Japan, by means of biological dredge during research cruise (Cruise 2423) of the TRV *Seisui-maru* (Mie University, Japan) on 31 October and 1 November 2024 (Fig. II-II-1). After photographing, non-infected individual was fixed

and preserved in 99% ethanol. Infected individual was fixed in Bouin's solution (picric acid [trinitrophenol] : paraformaldehyde : acetic acid = 15 : 5 : 1) for about one week, after photographing and removing some pereopods which was preserved in 99% ethanol; then transferred to 70% ethanol. It was then dissected, and the appendages were mounted on slides, observed with an Olympus BX53 microscope, drawn, and sealed with Canada balsam, mostly as described in Shiraki et al. (2021). Body length was measured from the tip of the anterolateral lobe of the head to the tip of the telson, and body width at the widest portion of pereonite 5. Telson length was measured from the tip to the narrowest point. Measurements were made axially from digital images by using Adobe Illustrator CC or ImageJ (Schneider et al. 2012). The ontogenetic stage of each specimen was determined according to Shiraki and Kakui (2024). The material examined is deposited in the Shoki Shiraki's Anthuroid Collection (SSAC).

Total DNA was extracted from a pereopod using a NucleoSpin Tissue XS Kit (Macherey–Nagel, Düren, Germany). For the COI gene, primers LCO-1490 and HCO-2198 (Folmer et al. 1994) were used for PCR amplification and cycle sequencing. Amplification conditions with TaKaRa Ex Taq DNA polymerase (TaKaRa Bio, Japan) were 94°C for 1 min; 35 cycles of 98°C for 10 s, 42°C for 30 s, and 72°C for 50 s; and 72°C for 2 min. All nucleotide sequences were determined by direct sequencing with a BigDye Terminator Cycle Sequencing Kit ver. 3.1 and a 3730 Genetic Analyzer (Life Technologies, Carlsbad, USA).

TAXONOMY

Anthuridae Leach, 1814

Genus *Malacanthura* Barnard, 1925

***Malacanthura seisuiae* sp. nov.**

[New Japanese name: Akame-uminanafushi]

(Figs II-II-2–II-II-5)

Diagnosis. Body without pigmentation. Eyes relatively large, 0.36 times head length. Maxillipedal palp with three articles, article 2 not exceeding article 3, article 3 rounded. Maxillipedal endite absent. Carpus of pereopod 7 short, less than twice longer than wide. Uropodal endopod longer than width. Uropodal exopod sinuate.

Etymology. The specific name is a noun in the genitive case, which is derived from TRV *Seisui-maru*, the vessel from which the species was collected.

Material examined. Holotype: female lacking oostegites (SSAC-02, 13 slides and 1 vial), body length 10.37 mm; 743 m depth, the Kumanonada Sea (34°02′47.4″N 136°53′31.2″E), off the southern coast of Mie Prefecture, Japan, 31 October 2024. Paratype: ovigerous female (SSAC-03, 1 vial), body length 9.84 mm, infected by a rhizocephalan (see Chapter IV-II); 758–759 m depth, the Kumanonada Sea (34°02'24.6"N 136°53'24.6"E to 34°02'24.6"N 136°53'25.2"E), off the southern coast of Mie Prefecture, Japan, 1 November 2024.

COI sequence. 652 bp, from the holotype specimen; 652 bp from the paratype specimen. They differ by two nucleotides.

Description of female lacking oostegites based on holotype. Body (Figs II-II-2,

II-II-3a–d) slender, length 14.06 times body width, whitish, without dorsal pigmentation. Head (Fig. II-II-3a) length 1.28 times head width, with some dimples; rostrum not protruding anterolateral lobes; dorsolateral eyes (Fig. II-II-2) reddish-brown when fresh and preserved in ethanol, relatively large, 0.36 times head length. Pereonites 1–7 (Fig. II-II-3a–d) with length ratio 1.05 : 1.54 : 1.38 : 1.59 : 1.73 : 1.43 : 0.89 relative to head length. Pleonites 1–5 (Fig. II-II-3c, d) fused, with shallow lateral articulation, 0.07 times body length. Pleonite 6 (Fig. II-II-3c, d) free from pleonites 1–5, not fused dorsally to telson. Telson (Fig. II-II-5h) elliptical, length 2.22 times width, with two apical plumose setae and 34 surface and eight apical simple setae; with two statocysts anteriorly.

Antennula (Fig. II-II-3e) with three peduncular and four flagellar articles. Peduncular article 1 longest, with two plumose sensory setae; article 2 with four distal plumose sensory setae and two simple setae; article 3 with distal plumose sensory seta and six simple setae. Flagellar article 1 naked; article 2 with aesthetasc and two simple setae; article 3 with two aesthetascs and four simple setae.

Antenna (Fig. II-II-3f) with five peduncular and five flagellar articles. Peduncular article 1 with outer simple seta; article 2 with inner simple seta, two distal setae (tip broken), and fine setae on posterior region; article 3 with three simple setae and distal seta (tip broken); article 4 with two distal plumose sensory setae, five simple setae, and distal seta (tip broken); article 5 with four distal plumose sensory setae, nine simple setae, and three setae (tip broken). Flagellar articles 1–5 with numerous distal simple setae.

Mandible (Fig. II-II-3g) with tri-articulate palp. Palp article 1 with distal simple seta; article 2 longest, with outer membrane-like process and simple seta; article 3 with six spiniform setae. Incisor with three cusps. Lamina dentata with four denticles. Molar process rounded.

Maxilla (Fig. II-II-3h) bent inward, with one large (tip broken) and five small

distal teeth.

Maxilliped (Fig. II-II-3i) with tri-articulate palp. Palp article 1 with inner simple seta; article 2 with three inner and one outer simple setae; article 3 slightly wider than long, with five plumose setae. Endite lacking. Epipod oval.

Pereopod 1 (Fig. II-II-4a) subchelate. Basis with dorsal plumose sensory seta, four simple setae, and three setae (tip broken). Ischium with one dorsal and four ventral simple setae. Merus with two dorsal, one mid-outer, and one ventral simple setae. Carpus triangular, with five ventral simple setae and ventral seta (tip broken). Propodus oval, with nine outer, one inner, and two ventrodistal simple setae. Palm weakly stepped, with seven simple setae. Dactylus with ventrodistal spiniform seta, three distal plumose setae, and one ventral and three ventrodistal simple setae. Unguis about four-fifths as long as dactylus, naked.

Pereopod 2 (Fig. II-II-4b) narrower than pereopod 1. Basis with two dorsal plumose sensory setae and six simple setae. Ischium with two dorsal and five ventral simple setae. Merus with three dorsal and five ventral simple setae. Carpus triangular, with five ventrodistal simple setae. Propodus weakly wider proximally, with ventral transparent membranous process, ventrodistal spiniform seta with sensory bristle sensu Negoescu (1994), dorsodistal plumose sensory seta, and four dorsal and eleven ventral simple setae. Dactylus with ventrodistal spiniform seta, three distal plumose setae, and five distal simple setae. Unguis about two-fifths as long as dactylus, naked.

Pereopod 3 (Fig. II-II-4c) similar to pereopod 2 except in numbers of plumose sensory setae on basis, plumose setae on dactylus, and simple setae.

Pereopod 4 (Fig. II-II-4d) slightly narrower than pereopods 2 and 3. Basis with three dorsal plumose sensory setae, one dorsal and two ventral simple setae, and three setae (tip broken). Ischium with three dorsal and three ventral simple setae. Merus with

two dorsal and four ventral simple setae. Carpus trapezoidal, less than twice longer than wide, with ventrodistal spiniform seta, dorsal plumose sensory seta, and one dorsal and six ventral simple setae. Propodus parallel at dorsal and ventral margins, with ventral transparent membranous processes, ventrodistal spiniform seta with sensory bristle, dorsodistal plumose sensory seta, and four dorsal and eight ventral simple setae. Dactylus with ventrodistal spiniform seta, three distal plumose setae, and one ventral, two ventrodistal, and three outer simple setae. Unguis about one-third as long as dactylus, naked.

Pereopods 5–7 (Fig. II-II-4e–g) similar to pereopod 4, except for numbers of spiniform setae, plumose sensory setae, plumose setae, and simple setae. Propodus of pereopod 7 with 16 ventral membrane-like processes and three ventrodistal spiniform setae with sensory bristle. Dactylus of pereopod 7 with seven ventral membrane-like processes.

Pleopod 1 (Fig. II-II-5a) protopod with two inner hooks, outer plumose seta, and distal simple seta. Exopod operculiform, with 33 plumose setae and 20 short simple setae on surface. Endopod shorter than exopod, 0.86 times as long as exopod, with eight plumose setae.

Pleopod 2 (Fig. II-II-5b) protopod with inner simple seta. Exopod with slit in middle, eleven distal and one short outer plumose setae, and 17 simple setae. Endopod with eight plumose setae.

Pleopods 3–5 (Fig. II-II-5c–e) similar to pleopod 2 except in number of plumose setae and simple setae.

Uropodal (Fig. II-II-5f, g) protopod shaped like triangular prism, with two plumose setae and seven simple setae. Exopod (Fig. II-II-5f) sinuous, dorsodistally moderately concave (angle 120°), 1.77 times longer than wide, with 40 plumose setae and

34 simple setae. Endopod (Fig. II-II-5g) rounded, length 1.34 times width, with five plumose sensory setae, eight plumose setae, and numerous simple setae.

Remarks. As the two genera *Malacanthura* and *Haliophasma* are similar to each other and taxonomically confused (Poore 2001), I compared my new species with all the species in the two genera. Among the species of the two genera, my species is similar to *Malacanthura linguicauda* (Barnard, 1920) and *Haliophasma coronicauda* Barnard, 1925 in sharing (1) the body without pigmentation, (2) large eyes (the length about one-third the head length), (3) tri-articulate maxillipedal palp, (4) article 2 of maxillipedal palp not exceeding the article 3, (5) the carpus of pereopod 7 short (less than twice longer than wide), (6) sinuate uropodal exopod, (7) the uropodal endopod longer than width (Barnard 1920, 1925). However, my species differs from the former species in the shape of article 3 of the maxillipedal palp (rounded in my species and *M. linguicauda* vs. triangular in *H. coronicauda*) and from the latter in lacking the maxillipedal endite (present in *M. linguicauda*; absent in *H. coronicauda* as well).

I tentatively placed my new species in the genus *Malacanthura* in having tri-articulate maxillipedal palp. The number of articles of the maxillipedal palp is three in *Malacanthura* whereas two in *Haliophasma* (Poore 2001). Like mentioned by Poore (2001), further work is needed to reliably define the two genera morphologically.

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Chapter II-III. Ontogenetic changes in pigmentation pattern in *Mesanthura miyakoensis* Nunomura, 1979 (Anthuridae)

INTRODUCTION

The moderately diverse anthurid genus *Mesanthura* Barnard, 1914, is common at shallow depths in tropical and temperate seas (Müller 1993) and currently contains 52 valid species (Boyko et al. 2025). Species in *Mesanthura* have a species-specific dark pigmentation pattern on the body (Poore 2001) and are distinguished mainly by differences in the dorsal pattern in females (Wägele 1984a). Other interspecific morphological differences are minimal (Müller 1993).

This taxonomic focus on pigmentation has potentially led to ambiguity or misidentification. Anthurids undergo direct development and are generally protogynous (Poore and Bruce 2012), meaning that after release from the mother's marsupium (brood pouch), young *Mesanthura* isopods likely first develop as females and then change sex to become males. In some species where only males were known, differences in pigmentation pattern were used to distinguish those males from the females of putative congeners (e.g. Wägele 1984b). However, this approach assumed that no ontogenetic changes in pigmentation pattern occurred (cf. Poore and Bruce 2012) as the females developed, or after they developed into males.

To my knowledge, no previous study has systematically investigated ontogenetic changes in pigmentation pattern in *Mesanthura*. Some studies (e.g. Müller 1993) have reported the sexes to have different patterns, whereas others (e.g. Pires 1981) have reported the same pattern. Only in *Mesanthura protei* Kensley, 1980, polymorphism of female pigmentation patterns has been reported (Kensley and Poore 1982; Chew et al.

2015). None of these previous studies molecularly confirmed whether their studied animals were conspecific; it is possible that ones they identified as the same species (i.e. males and females; polymorphic females) were not, in fact, conspecific.

In this study, I examined ontogenetic changes in the pigmentation pattern in *Mesanthura miyakoensis* Nunomura, 1979 to address (1) how the pattern differs between males and females; (2) in what sex and at what ontogenetic stages the color pattern is taxonomically informative; and (3) whether storage in ethanol affects the color pattern. Through rearing observations, I was able to examine the time at which pigmentation first appears in brooded mancae, and to obtain pigmentation data for the first free-living instar stage and two stages of males. Finally, I used DNA sequences to assess conspecificity for a male and a female with markedly different pigmentation patterns.

MATERIALS AND METHODS

Animals, staging, and measurement. Twenty-six isopods were collected from intertidal coral rubble at Tatsukushi (32°47'09.3"N 132°51'22.2"E), Kochi, Shikoku, Japan, on 13 June 2021 and 26 July 2021, and from 1 m depth at the same locality on 10 October 2022. All individuals were photographed alive to record pigmentation patterns, 15 of which (seven mancae, two post-mancae, five females lacking oostegites, and one subadult male) were then fixed and preserved in 70–99% ethanol; the others were used for rearing experiments (see below). I categorized individuals into one of six ontogenetic stages: (1) **manca**, without pereopod 7; (2) **post-manca**, with incomplete pereopod 7; (3) **female lacking oostegites** (components of the marsupium), with complete pereopod 7 and slender antennulae but without oostegites; (4) **ovigerous female**, with complete pereopod 7, slender antennulae, and oostegites; (5) **subadult male**, with enlarged eyes

and swollen antennulae lacking aesthetascs; and (6) **adult male**, with enlarged eyes and swollen antennulae covered with numerous aesthetascs. Ovigerous females brood eggs and hatchlings in a marsupium and release mancae. Head length (from tip of rostrum to posterior end of head) was measured from digital images by using ImageJ (Schneider et al. 2012).

Verification of male–female conspecificity with 16S rRNA sequences. To pair a conspecific male and female molecularly, I determined partial mitochondrial 16S rRNA (16S) sequences from three specimens, namely, one subadult male (similar pigmentation pattern as Fig. II-III-4A) collected on 13 June 2021, and one post-manca (similar pigmentation pattern as Fig. II-III-2C) and one ovigerous female (Fig. II-III-2E) collected on 10 October 2022. DNA was extracted from a pereopod of each specimen by using a NucleoSpin Tissue XS Kit (Macherey-Nagel, Germany). Primers Copepod16S_F and Copepod16S_R (Kakui et al. 2023) were used for PCR amplification and cycle sequencing; amplification conditions with KOD One (Toyobo, Japan) were as described by Kakui et al. (2023). Nucleotide sequences were determined with a BigDye Terminator Cycle Sequencing Kit ver. 3.1 and a 3730 DNA Analyzer (Life Technologies, USA). The sequences obtained were deposited in the International Nucleotide Sequence Database (INSD) through the DNA Data Bank of Japan, under accession numbers LC787697 (subadult male), LC787698 (post-manca), and LC795631 (ovigerous female).

Rearing. Eleven individuals (four mancae, three post-mancae, two females lacking oostegites, one ovigerous female, and one subadult male) collected from intertidal coral rubble at Tatsukushi (32°47'09.3"N 132°51'22.2"E), Kochi, Shikoku, Japan on 10 October 2022 were maintained in several small aquaria filled with seawater from the

sampling locality at room temperature and under ambient light. The ovigerous female released seven mancae during the study period, and these were maintained individually, as above. Reared individuals were intermittently observed and photographed to record dorsal pigmentation patterns, and were fixed and preserved in 70–99% ethanol when they died. Potential food organisms (several polychaetes, several tanaidaceans, a nematode, and pieces of algae) collected with the *Mesanthura* individuals on 10 October 2022 were offered occasionally, but I did not observe the *Mesanthura* individuals feeding on any of them.

RESULTS AND DISCUSSION

Verification of male–female conspecificity with 16S rRNA sequences. The genetic distances among the 16S sequences of three specimens were 0.2–0.9% (p-distance), much lower than the 3–10% "gray zone" (Riehl et al. 2018) in which distances can represent either interspecific or intraspecific variation in various isopods. This confirmed that my females and males were conspecific, and I treated the individuals used in this study were all same species.

Early ontogeny and pigmentation of mancae in the marsupium. Ten eggs were observed in the marsupium of a female on 10 October 2022 (Figs. II-III-1A, II-III-2E). The female released seven mancae: two on 6 November 2022 (this release may have been induced through pipetting the female), one between 6 and 11 November, and four on 11 November. This ovigerous female lost the marsupium through one molt; the anterior and posterior halves of her exuvia were retrieved on 22 November.

The embryos on 10 October were oblong and enclosed with chorion, suggesting

they had been laid into the marsupium a few days earlier (cf. Mercer et al. 2008); if this was correct, then the brooding period (from a few days before 10 October to 6 November) of this individual was about one month. Three embryos may have not hatched, though I do not know their fate.

Dorsal pigmentation began to be evident in the brooded mancae on 28 October (Fig. II-III-1D), 9 days before the first of them were released, and gradually became more intense (Fig. II-III-1). A pigmentation pattern similar to that of released mancae was observed on 31 October, 6 days before the first mancae were released (Figs II-III-1E, II-III-5A).

Ontogenetic changes in pigmentation pattern after release of mancae from marsupium. I observed the pigmentation pattern for 26 wild-caught individuals (11 mancae, five post-mancae, seven females lacking oostegites, one ovigerous female, and two subadult males), seven mancae released from the ovigerous female, the same female after molting and becoming a female lacking oostegites, and one adult male derived from one of the two subadult males that molted.

Mancae and females

Two patterns of dark, reddish-brown pigmentation (Fig. II-III-2A, B) were evident in mancae. In Type A (Figs II-III-2A, II-III-5A) observed in seven wild-caught mancae and all reared mancae in their first instar after release from the marsupium, there was a relatively broad pigmented area in the anterior half of the head, and a narrower band-like pigmented area on each pereonite and fused pleonites 1–5.

The Type B (Fig. II-III-2B) pattern observed in four wild-caught mancae was quite similar to that seen in post-mancae (Fig. II-III-2C) and females lacking oostegites

(Fig. II-III-2D). Type B individuals (head length 0.246–0.264 mm; average 0.255 mm; n = 4) were larger than Type A individuals (head length 0.211–0.244 mm; average 0.231 mm; n = 14), suggesting that the manca stage comprised at least two instars (Fig. II-III-3). I did not observe a molt by a first instar manca, and it remains unknown exactly when the pigmentation pattern changes from Type A to Type B. Furthermore, I cannot rule out that the manca stage may contain more than three instars.

The pigmentation pattern from Type B mancae through females lacking oostegites was as follows. (1) In the head, a relatively narrow band of dark brown pigmentation loci (nearly solid, Fig. II-III-2B, C; as rows of dots, Fig. II-III-2D) occupying the anterior half. (2) In pereonite 1, a transverse band of pigmentation (irregular, Fig. II-III-2B; rectangular, Fig. II-III-2C; rounded rectangular, Fig. II-III-2D) occupying less than half the pereonite length, located mid-pereonite. (3) In pereonites 2 and 3, a rounded-square (convex anteriorly, straight posteriorly) zone of pigmentation with dots inside, occupying more than half of each pereonite, located mid-pereonite. (4) In pereonites 4–6, a roughly square (convex anteriorly, straight or convex posteriorly) zone of pigmentation occupying approximately half the segment, located mid-pereonite, with an elliptical, non-pigmented "window" along the midline. (5) In pereonite 7, similarly to pereonite 1, a narrower, transversely subrectangular zone of pigmentation occupying roughly half the pereonite length, more or less mid-pereonite or closer to the anterior end. (6) In fused pleonites 1–5, a dome-shaped zone of pigmentation with a weakly (Fig. II-III-2B) to strongly (Fig. II-III-2C, D) convex anterior margin and straight posterior margin, half or less the segment length, located mid-pleon.

The pigmentation pattern in ovigerous females (Fig. II-III-2E) appeared superficially different from those in Fig. II-III-2B–D, in that many of the pigmentation zones had a moderately (P3, P7, fused pleonites 1–5) to greatly (P4–P6) enlarged non-

pigmented "window" along the midline. Nonetheless, the general shapes of the pigmentation zones and their relative sizes and positions persisted until females became ovigerous. Enlargements of the non-pigmented median area may reflect allometric changes within the pigmentation fields as females increase in size and become ovigerous; taking into account differences in the scale bars in Fig. II-III-2, pereonites 4 and 5 in the ovigerous female (Fig. II-III-2E) were roughly twice as wide as in the female lacking oostegites (Fig. II-III-2D) and proportionally even wider than in Type B mancae (Fig. II-III-2B) and post-mancae (Fig. II-III-2C).

In addition to the dark, reddish-brown pigmentation, yellow pigmentation was observed in all individuals except released Type A mancae. Yellow pigmentation occurred mainly at the anterior edge of pereonite 1, the posterior edge of each pereonite, and the base of the tailfan (Fig. II-III-2). Some individuals partially lacked the yellow pigmentation (e.g. an individual without yellow pigmentation at the posterior edge of pereonite 7; Fig. II-III-2B).

Males

Subadult males (Fig. II-III-4A) had medial strands of pigmentation on the head. Small, paired, diagonal or comma-shaped patches of pigmentation were evident anterolaterally on pereonites 1–3 and posterolaterally on pereonites 4–7. On fused pleonites 1–5, three complete, narrow transverse bands of pigmentation were evident posteriorly. Yellow pigmentation was seen in subadult males, as in females, but gradually faded after the molt to adult males and eventually disappeared (Fig. II-III-4B–E). It is unclear whether the pigmentation pattern of subadult males changes gradually within the instar.

Immediately after the molt to adult (Fig. II-III-4B), males retained the subadult

pattern, though many of the pigmentation loci were slightly enlarged and more intense. With age following the molt (Fig. II-III-4C–E), and no further molting, the pigmentation loci became increasingly large, extending posteriorly (P1–P3) or anteriorly (P4–P7) along each side of the segment; the posterior half of the head developed an X-shaped pattern (Fig. II-III-4C) and eventually became entirely diffusely pigmented (Fig. II-III-4D, E). The male in Fig. II-II-5E, 27 days after the molt to adult, shows a heavily pigmented head, three nearly complete, narrow transverse pigmented bands in fused pleonites 1–5, and dark pigmentation diffused throughout the whole body. This male died on 3 December 2022, 51 days after the molt to adult.

Impact of ethanol fixation and preservation on pigmentation pattern. In various crustacean groups, pigmentation and color are known to fade after long-term preservation in ethanol (Nagasawa and Mizuno 2022). I thus examined how fixation and preservation in ethanol affected the pigmentation pattern in *M. miyakoensis*.

The same dark pigmentation as seen in the living state was maintained in most specimens preserved in ethanol for nearly one year (e.g. compare Fig. II-III-5D with Fig. II-III-5E–G). The only exceptions were two mancae released on 6 November 2022 (Fig. II-III-5A); their pigmentation bands became more gappy and dotted after fixation (Fig. II-III-5B, C) though they retained their general sizes and shapes. I interpret this to mean that discrete pigmentation loci maintained their pigmentation, whereas pigment diffusing into tissues surrounding these loci was dissolved by the fixative. Yellow pigmentation disappeared in all ethanol-fixed individuals (Fig. II-III-5E–G vs Fig. II-III-5D).

My observations on the effects of fixative extended only over about 13.5 months, whereas specimens in museums remain in fixative for decades or even centuries. From my limited observations, I can conclude only that pigmentation patterns (in females)

remain relatively intact and taxonomically informative for as long as they remain visible.

My observations showed that (1) ethanol fixation and preservation may cause artifactual pigmentation pattern variation, at least in mancae and (2) some colors disappear after ethanol fixation (yellow in this study).

Taxonomic utility of pigmentation patterns in *Mesanthura* taxonomy. With regard to the taxonomic utility of pigmentation patterns in *Mesanthura* taxonomy, I can summarize the following conclusions from my study of *M. miyakoensis*.

1) The pigmentation pattern in males differs from that in mancae and females. Subadult and adult males (separated by a molt) show a similar pattern. As adult males age, pigmentation loci enlarge until the whole body in living animals appears reddish brown. The pigmentation patterns are not as discrete as in females, and this, coupled with the diffuse pigmentation in older individuals, reduces the taxonomic utility of pigmentation in males.

2) The pigmentation pattern in first-instar mancae differs from that in putative second (and subsequent?) instar mancae. Segmental pigmentation zones are quite similar from putative second-instar mancae to females lacking oostegites, making the overall pigmentation pattern at these stages a useful taxonomic character.

3) The pattern of dark-brown pigmentation seen from putative second-instar mancae to females lacking oostegites was well preserved for up to 410 days (~13.7 months) in ethanol, suggesting that as long as a reasonable amount of pigment remains, the pigmentation pattern can be compared across species for preserved individuals in these stages.

For taxonomic comparisons among *Mesanthura*, the pigmentation pattern should be observed in living individuals where possible, and additionally in multiple ethanol-

fixed female individuals, at the post-manca or later stages.

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Chapter II-IV. Four *Mesanthura* species (Anthuridae) including *M. sol* sp. nov.

INTRODUCTION

With 52 valid species (Boyko et al. 2025), *Mesanthura* Barnard, 1914 in the isopod family Anthuridae is characterized by having a body with conspicuous dorsal pigmentation (the chief character; Poore 2001), the tri-articulate maxillipedal palp with the terminal article at least one-third as long as the penultimate article, the pereopod 1 with stepped palm and the carpus not fused with the propodus, the upper margin on the carpus of pereopods 4–7 shorter than the lower margin, the pereopods 4–7 with ventrodistal spiniform seta, and the leaf-like uropodal exopod (Poore 2001; Chew et al. 2014). Within species, the pigmentation pattern is roughly similar in females across ontogenetic stages from post-manca to ovigerous (Shiraki and Kakui 2024) and is the primary taxonomic character (Wägele 1984a). Although some studies have used the male pigmentation pattern in species discrimination, the pattern in males generally differs from that in females (Müller 1993); this was confirmed with molecular evidence in *Mesanthura miyakoensis* Nunomura, 1979 (Shiraki and Kakui 2024). *Mesanthura* isopods commonly occur in shallow waters in tropical or temperate seas (Müller 1993).

To date, five *Mesanthura* species have been reported from Japan (Fig. II-IV-1). *Mesanthura nigrodorsalis* Nunomura, 1977, with females almost completely pigmented, was described from shallow water at Amakusa, Kumamoto (Nunomura 1977). *Mesanthura miyakoensis*, with females having a roughly square zone of black pigmentation dorsally on each pereonite, was described from a depth of 3 m off Miyako Island, Okinawa (Nunomura 1979) and was subsequently reported from Tatsukushi,

Kochi (Shiraki and Kakui 2024); in addition, Nunomura (1992) reported *M*. sp. aff. *miyakoensis* from Kuroshima, Yaeyama Islands, Okinawa. *Mesanthura atrata* Nunomura, 1985, for which information on female pigmentation is lacking, was described from a single male from a rocky shore at a depth of 1 m in Toyama (Nunomura 1985). *Mesanthura cinctula* Nunomura, 2006, in which females have a dark square zone dorsally on each pereonite, was described from depth of 247–269 m in Sagami Bay (Nunomura 2006). Finally, *Mesanthura saikaiensis* Nunomura, 2016, for which the original description did not mention the female pigmentation pattern in detail, was described from the subtidal zone in Shijiki Bay, Nagasaki (Nunomura 2016); an illustration in Nunomura (2016) shows the female to have a single large pigmented patch on pereonite 1 and a pair of patches on each of pereonites 2–7.

Here I describe a new species of *Mesanthura* having orange pigmentation, collected from coral rubble at 10–15 m depth on the north reef of Irabu Island, Miyako Islands, Okinawa, Japan and at 1 m depth of Onna Village, Okinawa main island, Okinawa, and present a description of *M*. *miyakoensis* based on specimens collected from Tatsukushi, Kochi, Shikoku, Japan. I also provide the first records for *M*. *cinctula* and *M*. *nigrodorsalis* after their original descriptions. For four of the species mentioned above, I present partial sequences of the 16S rRNA gene (16S) and a phylogenetic tree based on them.

MATERIALS AND METHODS

Mesanthura individuals were collected around Japan from 2018 to 2023: intertidally by hand, from subtidally by free or SCUBA diving, and from deeper water by biological dredge (Fig. II-IV-1; Table IV-1). Animals were photographed alive to record the

pigmentation pattern and then fixed and preserved in 70–99% ethanol. The ontogenetic stage of each specimen was determined according to Shiraki and Kakui (2024). Some specimens were dissected, mounted on glass slides, and drawn as described in Shiraki et al. (2021). Appendages were mounted on slides, observed with an Olympus BX53 microscope, and sealed with Canada balsam. Body length was measured from the tip of the anterolateral lobe of the head to the tip of the telson, and body width at the widest portion of pereonite 5. Telson length was measured from the posterior tip to the narrowest point in the anterior half. Measurements were made axially from digital images by using Adobe Illustrator CC or ImageJ (Schneider et al. 2012). The specimens examined are deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Japan.

DNA was extracted from a pereopod of selected specimens by using a NucleoSpin Tissue XS Kit (Macherey-Nagel, Germany). Primers Copepod16S_F and Copepod16S_R (Kakui et al. 2023) were used for PCR amplification and cycle sequencing. Amplification conditions for 16S from *M. cinctula* with TaKaRa Ex Taq DNA polymerase (TaKaRa Bio, Japan) were 94°C for 1 min; 35 cycles of 98°C for 10 s, 45°C for 30 s, and 72°C for 50 s; and 72°C for 2 min, and those for 16S from the remaining three species (*M. miyakoensis*, *M. nigrodorsalis*, and my new *Mesanthura* species) with KOD One (Toyobo, Japan) were as described by Kakui et al. (2023). Nucleotide sequences were determined with a BigDye Terminator Cycle Sequencing Kit ver. 3.1 and a 3730 DNA Analyzer (Life Technologies, USA). Sequences obtained were deposited in the International Nucleotide Sequence Database (INSD) through the DNA Data Bank of Japan (DDBJ).

My 16S dataset for phylogenetic analysis (Table II-IV-1) comprised eight new sequences from four *Mesanthura* species, three sequences from *M. miyakoensis*

(accession numbers LC787697, LC787698, LC795631 determined in Chapter II-III; Shiraki and Kakui 2024), and one sequence from the paranthurid *Paranthura japonica* Richardson, 1909 as an outgroup taxon (accession number GQ302694; unpublished). Sequences ranged in length from 421 to 439 bp. The sequences were aligned by using the MAFFT online platform version 7 (Katoh and Standley 2013; Katoh et al. 2019) with the "Q-INS-i" strategy (Katoh and Toh 2008), and ambiguous sites were removed with Gblocks version 0.91b (Castresana 2000); the final alignment was 396 bp long. The optimal phylogenetic tree was determined with the maximum likelihood (ML) method implemented in IQtree version 2.1.2 (Minh et al. 2020), with nodal support values obtained through 1000 Shimodaira-Hasegawa-like approximate likelihood ratio tests (SH-aLRT; Guindon et al. 2010) and analysis of 1000 ultrafast bootstrap (UFBoot; Hoang et al. 2018) pseudoreplicates. Clades with SH-aLRT ≥ 80 and UFBoot ≥ 95 were considered to be well supported. The optimal substitution model determined under the corrected AIC (Akaike information criterion) option in ModelFinder (Kalyaanamoorthy et al. 2017) was TIM2 + F + G4. The resulting phylogenetic tree was drawn with FigTree software version 1.4.4 (https://github.com/rambaut/figtree/releases/tag/v1.4.4) and edited with Adobe Illustrator CC. P-distances and Kimura (1980) 2-parameter (K2P) distances among the eleven aligned *Mesanthura* sequences were calculated with MEGA7 (Kumar et al. 2016).

TAXONOMY

Family Anthuridae Leach, 1814

Genus *Mesanthura* Barnard, 1914

Remarks. *Mesanthura atrata* and *Mesanthura javensis* Wägele, 1984 were described from only a single male specimen each and are distinguished from congeners mainly by differences in pigmentation pattern (Wägele 1984b; Nunomura 1985). As species in *Mesanthura* show sexual dimorphism in pigmentation pattern, the male pattern in one species cannot be compared to the female pattern in other species (e.g. Shiraki and Kakui 2024; this study). Morphological information of their females that are molecularly linked to the males are needed to fix their taxonomic status.

Among the five *Mesanthura* species known from Japan, *M. saikaiensis* may not be a true member of the genus because it shows the following characters which contradict Poore's (2001) generic diagnosis: short article 3 of maxillipedal palp; pereopod-1 palm not stepped; and the carpus of pereopods 4–7 without ventrodistal spiniform seta. In this study, however, I refrain from transferring the species to another genus because I did not observe *M. saikaiensis* specimens in this study.

Mesanthura cinctula Nunomura, 2006

[Japanese name: Futairo-uminanafushi]

(Fig. II-IV-2)

Mesanthura cinctula Nunomura, 2006, p. 14, fig. 4.

Material examined. Non-type: female lacking oostegites (ICHUM8940, 1 vial), body length 10.78 mm, sandy mud bottom, 321–224 m depth off Jogashima (35°07'13.0"N 139°33'46.0"E to 35°07'35.9"N 139°33'07.7"E), Kanagawa, Japan, collected by Hisanori Kohtsuka, 23 May 2018; manca (ICHUM8941, 1 vial), body length 5.71 mm, 109–144 m depth off Jogashima (35°08'30.3"N 139°32'56.9"E to 35°08'34.3"N

139°32'43.1"E), Kanagawa, Japan, collected by Keiichi Kakui, 22 February 2019, LC849816 (16S).

Distribution. *Mesanthura cinctula* is known from Sagami Bay, Japan, at depths of 109–321 m (Nunomura, 2006; this study) (Fig. II-IV-1).

Remarks. I assigned my specimens to *M. cinctula* based on (1) the square or rectangular pigmentation zone occupying half or more of each pereonite and located posteriorly in pereonites 4–6 and (2) the pleonites 1–5 dorsally fused but laterally articulated (Fig. II-IV-2A–C, F; Nunomura 2006). In the original description, the pigmentation zones on *M. cinctula* were simply described as "dark" (Nunomura 2006: p. 14), with no mention of the hue. I observed the pigmentation to be reddish orange in both fresh and ethanol-preserved specimens. This species is unique in its depth of occurrence (200 m or deeper); all other *Mesanthura* species are known only from much shallower depths (Poore 2001).

Mesanthura miyakoensis **Nunomura, 1979**

[Japanese name: Moyou-uminanafushi]

(Figs II-IV-3–II-IV-7)

Mesanthura miyakoensis Nunomura, 1979, p. 31, figs 1, 2; Shiraki and Kakui 2024, p. 3, figs 2, 3, 5, 6.

Mesanthura sp. (*aff miyakoensis*, Nunomura, 1979): Nunomura 1992, p. 53, fig. 4.

Material examined. Non-type: post-manca (ICHUM8942, 1 vial), body length

2.75 mm, coral rubble, 10–15 m depth, north reef of Irabu Island (24°51'55.5"N 125°08'39.6"E), Miyako Islands, Okinawa, Japan, collected by Shoki Shiraki, 24 April 2022, LC849817 (16S); post-manca (ICHUM8943, 1 vial), body length 3.21 mm, same collection data as for ICHUM8942; female lacking oostegites (ICHUM8944, 10 slides and 1 vial), body length 3.76 mm, coral rubble, intertidal zone, Tatsukushi (32°47'09.3"N 132°51'22.2"E), Kochi, Shikoku, Japan, collected by Yuki Oya, 26 July 2021; subadult male (ICHUM8945, 6 slides and 1 vial), body length 3.79 mm, same locality as for ICHUM8944, collected by Yuki Oya, 13 June 2021, LC787697 (16S; Shiraki and Kakui 2024); female lacking oostegites (see the footnote for Table II-IV-1) (ICHUM8946, 1 vial), body length 4.59 mm, coral rubble, 1 m depth, same locality as for ICHUM8944, collected by Shoki Shiraki, 10 October 2022, LC795631 (16S; Shiraki and Kakui 2024); post-manca (ICHUM8947, 1 vial), body length 2.92 mm, same collection data as for ICHUM8946, LC787698 (16S; Shiraki and Kakui 2024).

Description of female lacking oostegites based on ICHUM8944. Body (Figs II-IV-3A, II-IV-4A–D) length 12.49 times body width, slender. Dark brown pigmentation pattern as shown in Figs II-IV-4A–D, II-IV-6D, F. Head (Fig. II-IV-4A) length 1.21 times head width, with several lateral simple setae; rostrum protruding, slightly shorter than anterolateral lobes; eyes dorsolateral, relatively large, length 0.19 times head length. Pereonites 1–7 (Fig. II-IV-4A–D) with length ratio 1.17 : 1.15 : 1.05 : 1.21 : 1.49 : 1.32 : 0.84 : 0.90 relative to head length; each pereonite with several lateral simple setae. Pleonites 1–5 (Fig. II-IV-4C, D) completely fused, 0.08 times body length, with several simple setae. Pleonite 6 free from pleonites 1–5, fused dorsally to telson. Telson (Fig. II-IV-6F) linguliform, length 2.11 times width; with two indentations in apical edge comprising three protrusions, two posterior plumose sensory setae, one pair of posterior

short, 28 dorsal short, and two pairs of posterior long simple setae, patches of dark brown pigmentation, and two statocysts anterioly. Pigmentation pattern as described by Shiraki and Kakui (2024).

Antennula (Fig. II-IV-4E) with three peduncular articles and three flagellar articles. Peduncular article 1 longest, with plumose sensory seta, two simple setae, and seta (tip broken); article 2 with two plumose sensory setae; article 3 with four simple setae. Flagellar article 1 with plumose sensory seta; article 2 naked; article 3 with three aesthetascs and six simple setae.

Antenna (Fig. II-IV-4F, G) with five peduncular articles and five flagellar articles. Peduncular article 1 with outer simple seta; article 2 with one outer and two distal simple setae, and inner seta (tip broken); article 3 with three inner simple setae; article 4 with two distal plumose sensory setae, four simple setae, and two inner setae (each tip broken); article 5 with four distal plumose sensory setae and eleven simple setae. Flagellar articles 1–5 with numerous distal simple setae.

Mandible (Fig. II-IV-4H) with tri-articulate palp. Palp article 1 with distal simple seta; article 2 longest, without simple setae; article 3 with six spiniform setae. Incisor with three-toothed cusp and two-toothed cusp. Lamina dentata with three denticles. Molar process rounded.

Maxilla (Fig. II-IV-4I) bent inward, with six distal teeth.

Maxilliped (Fig. II-IV-4J) with tri-articulate palp. Palp article 1 with two inner simple setae; article 2 with three distal simple setae; article 3 nearly as long as broad, 0.63 times as long as article 2, with two inner plumose setae and three inner distal simple setae. Endite lacking. Epipod oval.

Pereopod 1 (Fig. II-IV-5A) subchelate. Basis with one dorsal and one ventrodistal simple setae. Ischium with ventrodistal simple seta. Merus with two dorsal and two

ventral simple setae. Carpus triangular, with three membranous processes and four ventral simple setae. Propodus oval, with one dorsodistal and one outer simple setae. Palm with middle rounded process, several transparent membranous processes, and nine simple setae. Dactylus with ventral transparent membranous process and one ventral, three ventrodistal, and four inner simple setae. Unguis as long as dactylus, naked.

Pereopod 2 (Fig. II-IV-5B) narrower than pereopod 1. Basis with three dorsal plumose sensory setae, and two dorsal and one ventrodistal simple setae. Ischium with two dorsal simple setae. Merus with two dorsal and four ventral simple setae. Carpus triangular, narrower than merus, with three ventral simple setae. Propodus rectangular, with ventrodistal transparent membranous process, ventrodistal spiniform seta with sensory bristle sensu Negoescu (1994), dorsal plumose sensory seta, and two dorsodistal and six ventral simple setae. Dactylus with ventrodistal spiniform seta, two ventrodistal plumose sensory setae, and one ventral, two ventrodistal, and three inner simple setae. Unguis about half length of dactylus.

Pereopod 3 (Fig. II-IV-5C) similar to pereopod 2 except in number of plumose sensory setae and simple setae.

Pereopod 4 (Fig. II-IV-5D) nearly as narrow as pereopods 2 and 3. Basis with four dorsal plumose sensory setae and five simple setae. Ischium with one dorsal and two ventral simple setae. Merus with two dorsal and two ventral simple setae. Carpus trapezoidal, with ventrodistal spiniform seta having sensory bristle, dorsal plumose sensory seta, and one dorsal and two ventral simple setae. Propodus rectangular, with ventrodistal spiniform seta having sensory bristle, dorsodistal plumose sensory seta, and two dorsodistal and five ventral simple setae. Dactylus with ventrodistal spiniform seta and one mid-ventral, four ventrodistal, and four outer simple setae. Unguis about half length of dactylus, naked.

Pereopods 5–7 (Fig. II-IV-5E–G) similar to pereopod 4, except in numbers of spiniform setae, plumose sensory setae on basis, and simple setae. Propodus of pereopod 7 with two ventrodistal trifurcate spiniform setae in addition to ventrodistal spiniform seta with sensory bristle.

Pleopod 1 (Fig. II-IV-6A) protopod with three inner spiniform setae. Exopod operculiform, with twelve plumose setae and seven short simple setae on surface near distal margin. Endopod shorter than exopod, with three plumose setae.

Pleopods 2 and 3 (Fig. II-IV-6B, C) with protopod bearing one and two simple setae, respectively. Exopods with six distal plumose setae and two short outer simple setae. Endopods with four plumose setae.

Uropodal (Fig. II-IV-6D, E) protopod shaped like triangular prism, with network of stellate patches of dark brown pigmentation and eight plumose setae. Endopod (Fig. II-IV-6D) rounded, pigmented similarly to protopod, with two distal plumose sensory setae, five plumose setae, and 31 simple setae. Exopod (Fig. II-IV-6E) finely sinuate along dorsodistal margin, with 13 plumose sensory setae, 12 simple setae, and four setae (each tip broken).

Description of subadult male ICHUM8945. Body (Figs II-IV-3C, II-IV-7A) slender. Head length 1.59 times head width; rostrum overlapping anterolateral lobes; dorsolateral eyes large, 0.35 times as long as head. Pleonites 1–5 (Fig. II-IV-7A) completely fused, 0.10 times body length. Pleonite 6 free from pleonites 1–5, fused dorsally to telson. Telson linguliform, similar to that in females, with two indentations in apical edge comprising three protrusions; with two statocysts anteriorly. Pigmentation pattern as described by Shiraki and Kakui (2024).

Antennula (Fig. II-IV-7B) swollen, with three peduncular articles and eleven

flagellar articles. Peduncular articles 1–3 naked. Flagellar article 1 with three inner simple setae; articles 2–10 naked; article 11 with four distal simple setae.

Antenna similar to that in females, with five peduncular articles and five flagellar articles.

Mandible (Fig. II-IV-7C) with tri-articulate palp. Palp article 1 naked; article 2 longest, with zero (right) or one (left) simple seta; article 3 with six spiniform setae on both mandibles. Incisor, lamina dentata, and molar process reduced.

Maxilliped (Fig. II-IV-7D) similar to that in females, with tri-articulate palp. Palp article 1 with inner simple seta; article 2 with three distal simple setae; article 3 nearly as long as broad, with two inner plumose setae and two inner simple setae. Endite lacking.

Pereopod 1 (Fig. II-IV-7E) subchelate, slightly slenderer than and similar to that in females. Basis with one dorsal and two ventral simple setae. Ischium with one dorsal and one ventral simple setae. Merus with two dorsal and two ventral simple setae. Carpus triangular, with four ventral simple setae. Propodus oval, with inner distal spiniform seta and one dorsal, two dorsodistal, and one inner simple setae. Palm with middle weak rounded process and ten simple setae. Dactylus with one ventral, two ventrodisal, and five inner distal simple setae. Unguis slightly shorter than dactylus, naked.

Pereopods 3 and 7 slightly slenderer than and similar to those in females.

Pleopod 1 similar to that in females. Exopod operculiform, with 19 (right) or 17 (left) plumose setae. Endopod shorter than exopod, with six (right) or five (left) plumose setae.

Pleopod-2 (Fig. II-IV-7F) exopod with six distal plumose setae and outer simple seta. Endopod with four plumose setae and appendix masculina. Appendix masculina 0.83 times as long as endopod, slightly curved inward.

Uropodal exopod and endopod similar to those in females.

Distribution. *Mesanthura miyakoensis* is widely distributed in southwestern Japan (Fig. II-IV-1). It has been reported from Tatsukushi, Kochi, at a depth of 1 m (Shiraki and Kakui 2024; this study); Miyako Island, Miyako Islands, Okinawa, at a depth of 3 m (type locality; Nunomura 1979); Irabu Island, Miyako Islands, Okinawa, at a depth of 10–15 m (this study); and Kuroshima, Yaeyama Islands, Okinawa, depth unknown (Nunomura 1992).

Remarks. I identified non-male (i.e., post-manca to ovigerous female) specimens from Irabu and Tatsukushi as *M. miyakoensis* by their having a pigmentation pattern (Figs II-IV-3A, B, II-IV-4A–D) identical to that described by Nunomura (1979). However, some differences in morphological characters are evident between my description and the original description (character state of the latter in parentheses). My specimens have the pereopod-2 carpus narrower than the merus (as wide as the merus), one spiniform seta on the pereopod-3 propodus (two), a rounded uropodal endopod (weakly tapering; this apparent difference might be due to having drawn the uropod at a different angle), and two pairs of long simple setae on the posterior edge of the telson (one pair).

The pigmentation pattern in *M. miyakoensis* males differs from that in conspecific non-males (Shiraki and Kakui 2024). Other sexually dimorphic characters in this species are typical for *Mesanthura*: males have larger eyes; longer, swollen antennulae; longer pereopods; an appendix masculina; and a longer pleon.

I concluded that *Mesanthura* sp. aff. *miyakoensis* sensu Nunomura (1992) is conspecific with *M. miyakoensis*. Although Nunomura (1992) listed four morphological differences between the two, I judged none of these to be interspecific differences: (1) The eyes (= dark pigmentation of the ommatidia) being scattered or not is likely an effect

of fixation in alcohol; pigmentation has been reported to shrink after ethanol fixation (e.g. Shiraki and Kakui 2024). (2,3) Differences in the number of articles in the antennule and antenna; these numbers can vary among conspecific specimens in anthuroids (e.g. Shiraki et al. 2021: table 1). (4) The longer pereopodal merus in *M*. sp. aff. *miyakoensis*: only pereopod-1 merus looks longer in *M*. sp. aff. *miyakoensis* than in *M. miyakoensis* sensu stricto, but by measuring from the illustrations, the length ratio of the merus relative to the carpus was almost same in *M*. sp. aff. *miyakoensis* and *M. miyakoensis* sensu stricto, both 0.79 (Nunomura 1979, 1992).

Mesanthura nigrodorsalis **Nunomura, 1977**

[Japanese name: Seguro-uminanafushi]

(Fig. II-IV-8)

Mesanthura nigrodorsalis Nunomura, 1977, p. 77, figs 5, 6.

Material examined. Non-type: female lacking oostegites (ICHUM8948, 1 vial), body length 6.74 mm, seaweeds, 0.2 m depth, Nagamatsu Beach (34°19'51.4"N 135°09'10.4"E), Osaka, Japan, collected by Shoki Shiraki, 10 September 2022; female lacking oostegites (ICHUM8949, 1 vial), body length 3.91 mm, calcareous algae, 1 m depth, Araihama (35°09'37.4"N 139°36'38.9"E), Kanagawa, Japan, collected by Hisanori Kohtsuka and Aoi Tsuyuki, 25 July 2021, LC849812 (16S); female lacking oostegites (ICHUM8950, 1 vial), body length 5.19 mm, seaweeds, 1 m depth, Jogashima (35°08′11.4″N 139°36′39.3″E), Kanagawa, Japan, collected by Shoki Shiraki, 12 July 2022, LC849811 (16S).

Distribution. *Mesanthura nigrodorsalis* is known in the middle Japan (Fig. II-IV-1): from Araihama and Jogashima, Kanagawa, at a depth of 1 m (this study); Nagamatsu Kaigan, Osaka, at a depth of 0.2 m (this study); and Amakusa, Kumamoto, in shallow water (type locality; Nunomura 1977).

Remarks. I assigned my females lacking oostegites to *M. nigrodorsalis* due to their body entirely chestnut-colored dorsally (Fig. II-IV-8; Nunomura 1977), which likely acts as concealing coloration, as I collected *M. nigrodorsalis* from brown (*Sargassum* sp.) and calcareous algae that were similar in color. Another dark-bodied species, *Mesanthura nigra* Müller, 1993, was also reported from *Sargassum* sp. (Müller 1993).

***Mesanthura sol* sp. nov.**

[New Japanese name: Taiyo-uminanafushi]

(Figs II-IV-9–II-IV-14)

Material examined. Holotype: ovigerous female (ICHUM8951, 14 slides and 1 vial), body length 5.53 mm, coral rubble, 10–15 m depth, north reef of Irabu Island (24°51'55.5"N 125°08'39.6"E), Miyako Islands, Okinawa, Japan, collected by Shoki Shiraki, 24 April 2022, LC849814 (16S). Paratypes: all from coral rubble, 1 m depth, Onna Village (26°29'57.4"N 127°50'30.8"E), Okinawa main island, Okinawa, Japan, collected by Shoki Shiraki, 23 April 2023; female lacking oostegites (ICHUM8952, 1 vial), body length 5.39 mm, LC849810 (16S); female lacking oostegites (ICHUM8953, 1 vial), body length 4.21 mm; adult male (ICHUM8954, 1 vial), body length 6.09 mm, LC849815 (16S); adult male (ICHUM8955, 6 slides and 1 vial), body length 4.54 mm, LC849813 (16S).

Etymology. The specific name is a noun in apposition derived from the Latin noun *sōl* (sun) referring to the orange color of the species.

Diagnosis. *Female.* Head with hollowed-out rectangular zone of pigmentation composed of distinct patches of orange. Pereonites and fused pleonites with distinct patches of orange pigmentation along margins, with non-pigmented elliptical "window" located mid-segment and occupying almost half zone of each segment. Telson 2.28 times as long as wide. Uropodal exopod oval, sinuous along dorsodistal margin. *Male.* Telson with two indentations at apical edge. Uropodal exopod tapering.

Description of holotype ovigerous female. Body (Figs II-IV-9A, B, II-IV-10A–C) length 12.52 times body width, slender. Head (Fig. II-IV-10A) length 1.31 times head width, with hollowed-out rectangular pattern composed of distinct patches of orange pigmentation; rostrum protruding, as long as anterolateral lobes; dorsolateral eyes relatively large, 0.20 times head length. Pereonites 1–7 (Fig. II-IV-10A–C) with length ratio 1.48 : 1.32 : 1.19 : 1.51 : 1.47 : 1.33 : 0.94 relative to head length; each pereonite with several lateral simple setae, distinct patches of orange pigmentation along the margin of each pereonite, with pigmentation lacking in oval-shaped "window" in middle; yellow pigmentation along anterior margin of pereonite 1 and posterior part of other pereonites evident only in living animals. Pleonites 1–5 (Fig. II-IV-10B, C) completely fused, 0.08 times body length, with trapezoidal pattern of distinct patches of orange pigmentation but without pigmentation in oval-shaped "window" in the middle. Pleonite 6 (Fig. II-IV-10B) free from pleonites 1–5, fused dorsally to telson, with yellow pigmentation along posterior margin only in living animal. Telson (Fig. II-IV-12H) linguliform, length 2.28

times width, with two posterior plumose setae and 36 simple setae; pigmentation weak; with two statocysts anteriorly.

Antennula (Fig. II-IV-10D) with three peduncular and two flagellar articles. Peduncular article 1 longest, with three outer plumose sensory setae; article 2 with four distal plumose sensory setae and outer seta (tip broken); article 3 slightly shorter than article 1, with outer distal plumose sensory seta and four outer distal simple setae. Flagellar article 1 naked; article 2 with three distal aesthetascs and seven distal simple setae.

Antenna (Fig. II-IV-10E) with five peduncular and four flagellar articles. Peduncular article 1 triangular, with two outer simple setae; article 2 with one outer and two distal simple setae; article 3 with two inner simple setae and inner seta (tip broken); article 4 with two distal plumose sensory setae and one inner and four distal simple setae; article 5 with four distal plumose sensory setae, nine distal simple setae, and inner seta (tip broken). Flagellar articles 1–4 with numerous distal simple setae.

Mandible (Fig. II-IV-10F) with tri-articulate palp. Palp article 1 with distal simple seta; article 2 longest, with two simple setae; article 3 slightly shorter than article 1, with nine spiniform setae. Incisor with two three-toothed cusps. Lamina dentata with five denticles. Molar process weakly rounded.

Maxilla (Fig. II-IV-10G) bent inward, with six distal teeth.

Maxilliped (Fig. II-IV-10H) with tri-articulate palp. Palp article 1 with inner simple seta; article 2 with four inner and one outer simple setae; article 3 nearly as long as broad, length 0.68 times that of article 2, with two inner plumose setae and two inner simple setae. Endite lacking. Epipod oval.

Pereopod 1 (Fig. II-IV-11A) subchelate. Basis with three dorsal plumose sensory setae and four simple setae. Ischium with one dorsodistal and two ventral simple setae

and ventrodistal seta (tip broken). Merus with one dorsal and two ventral simple setae. Carpus triangular, with one inner, one outer, and three distal simple setae, and two setae (each tip broken). Propodus oval, with two dorsal, two dorsodistal, one inner, and 13 outer simple setae. Palm denticulate, with middle rounded process bearing seven simple setae and three distal simple setae. Dactylus with one ventral, five ventrodistal, and three inner simple setae. Unguis as long as dactylus, naked.

Pereopod 2 (Fig. II-IV-11B) narrower than pereopod 1. Basis with dorsal plumose sensory seta, two dorsal and two ventral simple setae, and two setae (each tip broken). Ischium with two dorsal and three ventral simple setae. Merus with two dorsal and three ventral simple setae and ventral seta (tip broken). Carpus triangular, with six simple setae. Propodus rectangular, with ventro-subdistal transparent membranous process, ventrodistal spiniform seta with sensory bristle sensu Negoescu (1994), dorsal plumose sensory seta, and four dorsal and ten ventral simple setae. Dactylus with ventrodistal spiniform seta and one ventral, five ventrodistal, and five inner simple setae. Unguis about half as long as dactylus.

Pereopod 3 (Fig. II-IV-11C) similar to pereopod 2 except in numbers of plumose sensory setae on basis and simple setae.

Pereopod 4 (Fig. II-IV-11D) slightly narrower than pereopods 2 and 3. Basis with four dorsal plumose sensory setae and seven simple setae. Ischium with two dorsal and three ventral simple setae. Merus with two dorsal and four ventral simple setae. Carpus trapezoidal, with ventral triangular transparent membranous process, ventrodistal spiniform seta with sensory bristle, dorsal plumose sensory seta, and one dorsal and five ventral simple setae. Propodus slightly narrower than pereopods 2 and 3, with six ventral triangular transparent membranous processes, ventrodistal spiniform seta with sensory bristle, dorsodistal plumose sensory seta, and five dorsal and 10 ventral simple setae.

Dactylus with ventrodistal spiniform seta and one mid-ventral, four ventrodistal, and five outer simple setae. Unguis about two-fifths as long as dactylus.

Pereopods 5–7 (Fig. II-IV-11E–G) similar to pereopod 4, except for numbers of triangular transparent membranous processes, spiniform setae, plumose sensory setae, and simple setae. Propodus of pereopod 7 with two ventrodistal spiniform setae with sensory bristle.

Pleopod 1 (Fig. II-IV-12A) protopod with outer spiniform seta. Exopod operculiform, with 20 plumose setae and ten simple setae on surface near distal margin. Endopod slightly shorter than exopod, with five plumose setae.

Pleopod 2 (Fig. II-IV-12B) protopod with two inner spiniform setae and outer simple seta. Exopod with seven distal and one short outer plumose setae, and outer simple seta. Endopod with five plumose setae.

Pleopod 3 (Fig. II-IV-12C) protopod with outer plumose sensory seta and two inner simple setae. Exopod with slight slit in the middle, with eight distal and one short outer plumose setae. Endopod with five plumose setae.

Pleopod 4 (Fig. II-IV-12D) protopod with outer plumose sensory seta. Exopod with slit in middle, with seven plumose setae and two outer and one proximal simple setae. Endopod with five plumose setae.

Pleopod 5 (Fig. II-IV-12E) protopod with outer plumose sensory seta and two inner simple setae. Exopod with slit in middle, with seven plumose setae (or eight, one possibly lost by damage to exopod) and outer simple seta. Endopod with three plumose setae.

Uropodal (Fig. II-IV-12F, G) protopod shaped like triangular prism, with seven plumose setae and two simple setae. Exopod (Fig. II-IV-12F) oval, sinuous along dorsodistal margin, with 21 plumose setae, 35 simple setae, and six setae (each tip broken).

Endopod (Fig. II-IV-12G) rounded, length 1.41 times width, with four plumose sensory setae, eight plumose setae, and numerous simple setae.

Description of adult male ICHUM8955. Body (Figs II-IV-9F, II-IV-13A–E) slender, length 14.28 times width. Head (Fig. II-IV-13A) length 1.70 times head width, entirely pigmented reddish brown dorsally; rostrum overlapping anterolateral lobes; dorsolateral eyes large, 0.33 times as long as head. Pereonites 1–7 (Fig. II-IV-13A–D) with length ratio 0.84 : 0.91 : 0.92 : 1.04 : 1.10 : 0.89 : 0.68 relative to head length; each pereonite with brown pigmentation around margin but lacking pigmentation in oval-shaped "window" in center. Pleonites 1–5 (Fig. II-IV-13D) completely fused, 0.10 times body length, with segment in posterior half of pleon outlined with marginal pigmentation, leaving paired, circular or oval unpigmented "windows" medially and laterally. Pleonite 6 (Fig. II-IV-13D) free from pleonites 1–5, fused dorsally to telson. Telson (Fig. II-IV-14I) linguliform, length 2.19 times width, with two indentations in apical edge comprising three protrusions, with two posterior plumose setae, 41 simple setae, and two setae (each tip broken); pigmented primarily in anterior half; with two statocysts anteriorly.

Antennula (Fig. II-IV-13F) swollen, with three peduncular and ten flagellar articles. Peduncular article 1 naked; article 2 with two plumose sensory setae; article 3 with three inner distal simple setae. Flagellar articles 1–10 with numerous aesthetascs on distal edge.

Antenna (Fig. II-IV-13G) with five peduncular and five flagellar articles. Peduncular article 1 triangular, naked; article 2 with two simple setae; article 3 with three simple setae; article 4 with two distal plumose sensory setae and 12 simple setae; article 5 with three distal plumose sensory setae and 13 simple setae. Flagellar articles 1–5 with numerous distal simple setae; article 5 tiny.

Mandible (Fig. II-IV-13H, I) with tri-articulate palp. Palp article 1 with distal simple seta; article 2 longest, with simple seta; article 3 naked (right) or with seven spiniform setae (left). Incisor, lamina dentata, and molar process reduced.

Maxilliped (Fig. II-IV-13J) with tri-articulate palp. Palp article 1 with inner simple seta; article 2 with two inner and one outer simple setae; article 3 nearly as long as broad, length 0.65 times that of article 2, with three inner plumose and two inner simple setae. Endite lacking. Epipod oval.

Pereopod 1 (Fig. II-IV-14A) subchelate, slenderer than in females. Basis with three plumose sensory setae and two simple setae. Ischium with four simple setae. Merus with two dorsal and two ventral simple setae. Carpus triangular, with five simple setae. Propodus narrow, with 27 inner spiniform setae and four dorsal, two dorsodistal, and three outer simple setae. Palm denticulate, with slight process in middle, with three outer distal and five ventral simple setae. Dactylus with ventral membranous process, ventrodistal spiniform seta, and one ventral and eight distal simple setae. Unguis as long as dactylus, naked.

Pereopod 2 (Fig. II-IV-14B) narrower than pereopod 1. Basis with two dorsal plumose sensory setae, two dorsal and three ventral simple setae, and seta (tip broken). Ischium with one dorsal and three ventral simple setae. Merus with two dorsal and five ventral simple setae. Carpus triangular, with four simple setae. Propodus rectangular, with ventrodistal spiniform seta with sensory bristle, dorsal plumose sensory seta, and two dorsal and eight ventral simple setae. Dactylus with ventral membranous process, ventrodistal spiniform seta, and nine simple setae. Unguis about half length of dactylus.

Pereopod 7 (Fig. II-IV-14C) slightly narrower than pereopod 2. Basis with five dorsal plumose sensory setae and five simple setae. Ischium with one dorsal and three ventral simple setae. Merus with ventral plumose seta, and two dorsodistal and two

ventral simple setae. Carpus trapezoidal, with two ventral triangular transparent membranous processes, ventrodistal spiniform seta with sensory bristle, dorsal plumose sensory seta, and one dorsal and three ventral simple setae. Propodus slightly narrower than pereopod 2, with three ventral triangular transparent membranous processes, three ventrodistal spiniform setae with sensory bristle, dorsodistal plumose sensory seta, and four dorsal and four ventral simple setae. Dactylus with ventral membranous process, ventrodistal spiniform seta, and eight distal simple setae. Unguis about two-fifths as long as dactylus.

Pleopod 1 (Fig. II-IV-14D) protopod with four inner spiniform setae and outer plumose seta. Exopod operculiform, with 15 plumose and seven simple setae. Endopod with six plumose setae and distal simple seta.

Pleopod 2 (Fig. II-IV-14E) protopod with two inner spiniform setae and outer simple seta. Exopod with nine distal and one short outer plumose setae, and outer simple seta. Endopod with three plumose setae and appendix masculina bearing six small triangular processes. Appendix masculina (Fig. II-IV-14E, F) 1.04 times as long as endopod, slightly curved inward.

Uropodal (Fig. II-IV-14G, H) protopod triangular-prism-shaped, with three plumose setae. Exopod (Fig. II-IV-14G) narrower than in female, tapering, not sinuous along dorsodistal margin, with 15 plumose and 21 simple setae. Endopod (Fig. II-IV-14H) rounded, 1.81 times as long as wide, with five plumose sensory setae, four plumose setae, and numerous simple setae.

Distribution. *Mesanthura sol* **sp. nov.** is known from Onna Village, Okinawa main island, Okinawa, Japan, at a depth of 1 m, and Irabu Island, Miyako Islands, Okinawa, Japan, at a depth of 10–15 m (type locality; this study).

Remarks. I determined the 16S sequences (LC849810, LC849813–LC849815) for two adult males and one female lacking oostegites from Onna Village and one ovigerous female from Irabu. The three sequences from Onna specimens were identical but were 3.2% divergent (p-distance) from the sequence from Irabu. Based on the low p-distance and the identical pigmentation pattern between Onna and Irabu females, I concluded that my males and females from the two sites are conspecific.

In *M. sol* **sp. nov.**, the pigmentation pattern is identical between ovigerous females (Figs II-IV-9A, B, II-IV-10A, B) and females lacking oostegites (Fig. II-IV-9C, D). The pigmentation pattern in males and females is similar for the pereonites, but differs in the head and pleon. Males have the head entirely pigmented (Figs II-IV-9E, F, II-IV-13A), and a rectangular zone of pigmentation occupying the posterior half of the pleon and showing median and laterally paired unpigmented "windows" (Figs II-IV-9E, F, II-IV-13D), while females have only the anterior two-thirds of the head pigmented (Figs II-IV-9A–D, II-IV-10A), and only a large, elliptical non-pigmented "window" medially (Figs II-IV-9A–D, II-V-10B). Yellow pigmentation was evident along anterior margin of pereonite 1 and posterior part of the other pereonites and pleonite 6 in females but absent in males. It should be noted that, in *M. miyakoensis*, the yellow pigmentation is present in adult males just after the molt but gradually faded and disappeared eventually (Shiraki and Kakui 2024: fig. 5B–E).

The female pigmentation pattern differentiates *M. sol* **sp. nov.** from all the other *Mesanthura* species for which the female pattern is known. The female pattern is unknown for *Mesanthura crucis* (Barnard, 1925); Barnard (1925) did not describe it, and Kensley (1987: p. 114) mentioned only that the female syntypes had "lost all pigmentation." However, female *M. sol* **sp. nov.** differs from female *M. crucis* in the

shape of the telson (length 2.28 times width in *M. sol* **sp. nov.**; 1.62 times in *M. crucis*) and the uropodal exopod (sinuous along dorsodistal margin; not sinuous) (Kensley 1987).

Males in *M. sol* **sp. nov.** differ from the two species *M. atrata* and *M. javensis* in which only a male is known: from *M. atrata* in the shape of the uropodal exopod (tapering in *M. sol* **sp. nov.**; rounded in *M. atrata*) and from *M. javensis* in the number of indentations at the apical edge of the telson (two in *M. sol* **sp. nov.**; one in *M. javensis*) (Wägele 1984b; Nunomura 1985).

In addition to the pigmentation pattern, the following characters were sexually dimorphic in *M. sol* **sp. nov.**: males have larger eyes; swollen and longer antennulae; longer pereopods; an appendix masculina, a longer pleon; reduced incisor, lamina dentata, and molar process (toothed incisor, denticulate lamina dentata, and weakly rounded molar process in females); an oval pereopod-1 propodus with the palm lacking spiniform setae (narrow propodus with the palm bearing 27 spiniform setae in females); and oval uropodal exopods with a sinuous dorsodistal margin (narrower and tapering exopods with a smooth dorsodistal margin in females).

Molecular phylogeny. In my phylogenetic tree (Fig. II-IV-15), *M. sol* **sp. nov.** forms a clade with *M. miyakoensis*, with high nodal support (SH-aLRT = 95.6% and UFBoot = 95%). The relationship between *M. cinctula* and the other taxa included is unclear due to low support values.

Genetic distances (Table II-IV-2) among the four species were 16.7–30.8% (p-distance) and 19.5–40.2% (K2P). These high values suggest that 16S may be evolving too rapidly for reliable inference of interspecific relationships in *Mesanthura*; sequencing more slowly evolving genes such as 18S rRNA or 28S rRNA may be necessary. Intraspecific distances were much lower, ranging from 0.0–5.1% (p-distance) or 0.0–

5.4% (K2P), and were lower within a single locality (0.2–0.7% for *M. miyakoensis* at Tatsukushi; 0.0% for *M. sol* **sp. nov.** at Onna) than between localities (4.4–5.1% p-distance and 4.6–5.4% K2P for *M. miyakoensis*; both distances 3.2% for *M. sol* **sp. nov.**). These inter-population values fall within the "gray zone" (Riehl et al. 2018) of 3–10% in p-distance, which can encompass both intraspecific and interspecific variations in Isopoda.

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Chapter II-V. *Expanathura monile* sp. nov. (Expanathuridae) from Iriomote Island

INTRODUCTION

The genus *Expanathura* Wägele, 1981 is one of seven genera in the family Expanathuridae Poore, 2001. It differs from other, confamilial genera in having (1) pleonite 6 marked off dorsally from the telson, (2) the mandibular palp with three articles, (3) the maxillipedal palp with five articles, and (4) pereopod 1 subchelate, with the propodus broader than that of pereopod 2 (Poore 2001).

So far, six *Expanathura* species have been reported from the tropics and subtropics (Fig. II-V-1), including Australia, Malaysia, and islands in the Indian Ocean and South Pacific (Kensley 1976, 1979, 1980; Poore and Kensley 1981; Kensley and Poore 1982; Müller 1990, 1993; Negoescu 1999; Negoescu and Brandt 2001; Poore and Lew Ton 2002; Keable and Reid 2015; Chew et al. 2018). Poore (2001) proposed two groups in *Expanathura*. One contains *Expanathura amstelodami* (Kensley, 1976), *Expanathura ardea* (Poore & Kensley, 1981), and *Expanathura mooreae* Müller, 1993, which have a tapering telson, short pleopod 1 endopod, and narrow uropodal rami. The other contains *Expanathura collaris* (Kensley, 1979), *Expanathura macronesia* (Kensley, 1980), and *Expanathura haddae* (Kensley & Poore, 1982), which have a broad telson, a moderately shortened pleopod 1 endopod, and very broad uropodal rami. In addition to these species, Poore and Lew Ton (2002) reported two unnamed species, *Expanathura* sp. 1 and *E.* sp. 2, whose morphology was incompletely described, noting that both resembled members in the second group.

Here I describe a new *Expanathura* species collected from Iriomote Island,

Okinawa, Japan, northwestern Pacific; provide a key to seven species in *Expanathura*; and briefly discuss the male polymorphism observed in this species.

MATERIALS AND METHODS

Specimens were collected from coral rubble at 14 m depth, on the north reef of Sabasaki (24°21.88′N, 123°42.79′E), Iriomote Island, Okinawa, southwestern Japan, on 10 August 2016, and fixed and preserved in 80% ethanol. Some specimens were dissected with chemically sharpened tungsten wire needles under a Nikon SMZ 1500 microscope. Appendages were mounted on glass slides in glycerin and observed with an Olympus BX51 microscope; after observation, preparations were sealed with Canada balsam. Illustrations were prepared with Adobe Illustrator CC from draft line drawings made with a camera lucida or from sequential photographs of specimens. Body length was measured from the tip of the anterolateral lobe of the head to the tip of telson, and body width at the widest portion of pereonite 1. The length and width of the head and length and width of the eye were also measured. Measurements were made axially from digital images by using ImageJ (Schneider et al. 2012). Non-manca individuals without oostegites or appendix masculina were treated as females lacking oostegites. Material examined is deposited in the collections of the Seto Marine Biological Laboratory (SMBL-V0629–V0636).

TAXONOMY

Family Expanathuridae Poore, 2001

Genus *Expanathura* Wägele, 1981

[New Japanese name: Obiro-uminanafushi-zoku]

***Expanathura monile* sp. nov.**

[New Japanese name: Kubikazari-uminanafushi]

(Figs II-V-2–II-V-8)

Diagnosis. Head with dorsal V-shaped band of brown pigmentation (in females). Telson oval, 1.56–1.63 (females) or 1.50–1.63 (males) times longer than width, with lateral setae subequal in length, all short. Antennular flagellum with three or four articles in females, seven articles in males. Antennal peduncular article 2 with outer triangular projection; flagellum with three or four articles. Uropodal endopod longer than wide; exopod very broad, with acute-triangular projection (apex angle about 90° in females; about 70° in males).

Etymology. The specific name is a singular noun in the nominative case, from the Latin *monile* (necklace), referring to the V-shaped band on the head.

Material examined. Holotype: ovigerous female after spawning mancae (SMBL-V0629; 14 slides and 1 vial), coral rubble, 14 m depth, north reef of Sabasaki (24˚21.88′N, 123˚42.79′E), Iriomote Island, Okinawa, southwestern Japan, collected by Michitaka Shimomura, 10 August 2016. Allotype: male (SMBL-V0630; 7 slides and 1 vial), collection data as for holotype. Paratypes: ovigerous female after spawning mancae (SMBL-V0631; 13 slides and 1 vial); male (SMBL-V0632; 5 slides and 1 vial); females lacking oostegites (SMBL-V0633; 10 slides and 1 vial); females lacking oostegites (SMBL-V0634; 10 slides and 1 vial); females lacking oostegites (SMBL-V0635; 10

slides and 1 vial); females lacking oostegites (SMBL-V0636; 9 slides and 1 vial); collection data for all paratypes as for holotype.

Measurements (body length, body width, head length, eye length, eye width; in mm): SMBL-V0629 (2.33, 0.27, 0.26, 0.03, 0.02); SMBL-V0630 (2.15, 0.23, 0.24, 0.12, 0.08); SMBL-V0631 (2.18, 0.24, 0.26, 0.04, 0.02); SMBL-V0632 (2.21, 0.23, 0.23, 0.08, 0.06); SMBL-V0633 (2.20, 0.23, 0.28, 0.03, 0.02); SMBL-V0634 (2.05, 0.24, 0.23, 0.03, 0.02); SMBL-V0635 (1.50, 0.19, 0.20, 0.03, 0.01); SMBL-V0636 (1.66, 0.22, 0.17, 0.03, 0.02)

Description of female based on holotype and paratype SMBL-V0631. Body length (Figs II-V-2A, B, II-V-3A–D, I) 8.67 times body width, relatively slender. Head length (Figs II-V-2G, H, II-V-3A) 1.01 times head width, with several lateral simple setae and dorsal V-shaped band of brown pigmentation; rostrum barely overreaching anterolateral lobes; dorsolateral eyes small, with eye-length/head-length 0.13. Pereonites 1–7 (Fig. II-V-3A–D) with length ratio 1.00:0.97:1.06:1.14:1.19:0.81:0.43; each pereonite with several lateral simple setae; pereonite 7 overlapping pleonite 1. Pleonites 1–6 (Fig. II-V-3D) distinct, with length ratio 1.00:0.83:0.95:1.18:2.18:4.48; combined length of pleonites 1–6 0.14 times body length; pleonite 5 with several dorsolateral simple setae. Telson (Fig. II-V-6I) broad, with length (tip of the telson to narrowest point) 1.56 times telson width, evenly rounded, slightly convex ventrally; posterior half with serrated margin; posterior region with one short and one or two long, paired simple setae; posterolateral region with several short simple setae (all subequal in length); lateral region with short simple seta; statocyst absent.

Antennula (Fig. II-V-4A) with three peduncular articles and four flagellar

articles. Peduncle article 1 as long as combined length of succeeding six articles, with inner serration, one outer distal and one inner distal plumose sensory setae, inner distal simple seta, and outer middle seta (tip broken); article 2 with two outer distal plumose sensory setae, one middorsal and one inner distal simple setae, and outer distal seta (tip broken); article 3 with three distal simple setae. Flagellum article 1 with distal plumose sensory seta; article 2 naked; article 3 with two distal simple setae; article 4 with three distal simple setae and two distal aesthetascs.

Antenna (Fig. II-V-4B) with five peduncular articles and three flagellar articles. Peduncular article 1 with outer and inner distal serration and outer simple seta; article 2 with outer and inner serration, outer lamellar projection, and one outer distal and one inner distal simple setae; article 3 with two inner distal simple setae; article 4 with two distal plumose sensory setae and three distal simple setae; article 5 with three distal plumose sensory setae and seven distal simple setae. Flagellar articles 1–3 with two, seven, and one distal simple setae, respectively; article 3 with distal lamellar expansion.

Mandibles (Fig. II-V-4D, E) with triarticulate palp. Palp article 1 naked; article 2 longest, with distal spiniform seta and distal simple seta; article 3 as long as article 1, with row of setules and four distal spiniform setae. Incisor with three (right) or four (left) cusps. Lamina dentata with fine denticulation. Molar absent.

Maxilla (Fig. II-V-4F) slender, with one strong and four smaller distal teeth. Endite with distal simple seta.

Maxilliped (Fig. II-V-4G) with 5-articulate palp. Palp articles 1 and 2 naked; article 3 longest, with distal spiniform seta and two distal simple setae; article 4 with distal setulate projection and two simple setae; article 5 with four distal simple setae. Endite barely overreaching distal margin of palp article 3, narrow, with one middle and two distal simple setae. Epipod lost during dissection.

Pereopod 1 (Fig. II-V-5A) subchelate, robust. Basis with two dorsodistal simple setae. Ischium naked. Merus trapezoidal, with ventral small, round process and two dorsodistal and three ventral simple setae. Carpus triangular, with ventrodistal blunt-triangular process, and three ventrodistal and one inner ventro-subdistal simple setae. Propodus broad, with two dorsodistal simple setae. Palm with inner distal spiniform seta and one subproximal, one mid-outer, two mid-inner, and three distal simple setae; thin, transparent margin composed of proximal large triangular process, subproximal multilobed process, and wide denticulate process in distal half. Dactylus with two distal, four inner subdistal, and one mid-ventral simple setae. Unguis length nearly half dactylus length, naked.

Pereopod 2 (Fig. II-V-5B) subchelate, narrower than pereopod 1. Basis with one dorsal and two ventral simple setae. Ischium with one dorsal and one ventral simple setae. Merus trapezoidal, with ventral small, round process and three dorsodistal and five ventral simple setae. Carpus triangular, with ventral triangular process and one ventro-subproximal and four ventrodistal simple setae. Propodus broad, with two dorsodistal simple setae. Palm with one middle and two distal short spiniform setae and one subproximal, one inner subproximal, two mid-outer, two mid-inner, and three distal simple setae; thin, transparent margin similar to that of pereopod 1. Dactylus and unguis similar to those in pereopod 1.

Pereopod 3 (Fig. II-V-5C) subchelate. Basis, ischium, merus, carpus, dactylus, and unguis similar to those in pereopod 2, except in number of simple setae. Propodus narrow, with two dorsodistal simple setae. Palm with two distal short spiniform setae and two mid-outer, two ventral, and three distal simple setae; thin, transparent margin restricted in distal half, consisting of three triangular processes.

Pereopod 4 (Fig. II-V-5D) with basis, ischium, and merus similar to those of

pereopod 3, except for number of simple setae and no ventral process on merus. Carpus trapezoidal, with ventral spiniform seta, dorsodistal plumose sensory seta, and one dorsodistal and two ventral simple setae. Propodus with one mid-ventral and one ventrodistal spiniform setae, one dorsodistal plumose sensory seta, and two dorsodistal, two mid-inner, and two ventrodistal simple setae. Dactylus with two distal, four inner subdistal, and one mid-ventral simple setae. Unguis nearly half as long as dactylus.

Pereopods 5 and 6 (Fig. II-V-5E, F) similar to pereopod 4, except in number of simple setae.

Pereopod 7 (Fig. II-V-5G, g1) with basis, ischium, merus, carpus, dactylus, and unguis similar to those of pereopod 4, except in number of simple setae. Propodus with two outer dorsodistal three-pronged spiniform setae (Fig. II-V-5g1), one mid-ventral and three ventrodistal spiniform setae, and two dorsodistal and two ventrodistal simple setae.

Pleopod 1 (Fig. II-V-6A) protopod lacking inner hooks. Exopod operculiform, 1.46 times as long as wide, 3.52 times wider than endopod, with 13 plumose setae. Endopod moderately shortened, longer than half exopod length, with four distal plumose setae.

Pleopods 2–5 (Fig. II-V-6B–E) similar. Protopod with one outer and one inner simple setae. Exopod with nine or ten plumose setae. Endopod length subequal to exopod length, with four plumose setae.

Uropod (Fig. II-V-6F–H) with trigonal prismatic protopod bearing two ventrodistal simple setae and 12 outer plumose setae (three broken). Endopod (Fig. II-V-6F, G) oval, 1.49 times longer than width, with two plumose sensory setae and 20 simple setae. Exopod (Fig. II-V-6H) broad; outer half with one proximal and two distal simple setae; inner half with acute-triangular projection (apex angle about 90°), bearing 10 simple setae.

Description of male, based on allotype. Body (Figs II-V-2C, D, II-V-3E–H, J) more slender than in females, 9.27 times body width. Head length (Figs II-V-2I, II-V-3E) 1.04 times head width, with faint, amorphous brown area dorsally; rostrum barely overreaching anterolateral lobes; dorsolateral eyes much larger than in female, with eye-length/head-length 0.48. Pereonites 1–7 (Fig. II-V-3E–H) with length ratio 1.00:1.00:0.97:0.88:0.95:0.81:0.42; pereonite 7 not overlapping pleonite 1. Pleonites 1–6 (Fig. II-V-3H) distinct, with length ratio 1.00:0.75:0.78:0.82:1.14:2.40; combined length of pleonites 1–6 0.16 times body length; pleonite 5 without dorsolateral setae. Telson (Fig. II-V-8D) broad, with length 1.63 times telson width, evenly rounded, with serration on margin of posterior half; posterior region with three short setae and one or two long, paired simple setae; posterolateral region with several short simple setae subequal in length; dorso-posterolateral region with simple seta; lateral region with short, simple seta; statocyst absent.

Antennula (Fig. II-V-7A) with three peduncular articles and seven flagellar articles. Peduncle article 1 longest, with two outer distal plumose sensory setae, inner distal simple seta, and inner distal seta (tip broken); article 2 with two outer distal plumose sensory setae and inner distal simple seta; article 3 with two inner distal simple setae. Flagellum article 1 wider than succeeding articles, with several rows of aesthetascs in distal half; articles 2 and 6 each with two distal aesthetascs; articles 3–7 with one, three, zero, one, and two distal simple setae, respectively.

Antenna (Fig. II-V-7B) with five peduncular articles and four flagellar articles. Peduncle article 1 naked; articles 2 and 3 similar to those in female; article 4 with distal plumose sensory seta and five distal simple setae; article 5 with distal plumose sensory seta and seven distal simple setae. Flagellum articles 1–4 with two, six, seven, and four

distal simple setae, respectively.

Maxilliped (Fig. II-V-7C) with 5-articulate palp. Palp articles 1 and 2 naked; article 3 longest, with two distal simple setae; article 4 with three distal simple setae; article 5 with two distal simple setae. Endite reaching distal margin of palp article 2, naked. Epipod rounded, naked.

Pereopods 1, 2, 7 (Fig. II-V-7D–F) similar to those in female but more slender; differing in number of simple and spiniform setae on articles; no ventral processes on merus and carpus of pereopod 2.

Pleopod 1 (Fig. II-V-8A) with protopod bearing four inner hooks. Exopod operculiform but narrower than in female, 1.81 times as long as wide, 3.28 times wider than endopod, with 13 plumose setae. Endopod moderately shortened, longer than half exopod length, with five plumose setae.

Pleopod 2 (Fig. II-V-8B) with protopod bearing two inner hooks. Exopod with middle articulation-like constriction and nine plumose setae. Endopod with five plumose setae and appendix masculina overreaching distal margin of endopod.

Uropodal exopod (Fig. II-V-8C) broad but narrower than in female, with apex angle of projection about 70°.

Variation. In addition to the holotype and allotype specimens, four females lacking oostegites, one female with a fully developed marsupium, and one male were dissected, and their appendages were observed. All specimens shared the following characters: (1) the telson was oval in shape (1.56–1.63 (females) or 1.50–1.63 (males) times longer than width,); (2) antennal peduncular article 2 bore an outer projection; (3) the uropodal exopod was broad and had an acute triangular projection; and (4) the lateral

setae on the telson were subequal in length, short. All females had a V-shaped dorsal pigmentation pattern on the head (Fig. II-V-2G, H).

The following characters were sexually dimorphic in: (1) the number of articles in the antennular flagellum (three or four in females; seven in males); (2) the number of plumose setae on the pleopod 1 endopod (2–4 in females; five in males); and (3) the dorsal pigmentation pattern of head (V-shaped band in females; amorphous in males). In addition, males had larger eyes (eye-length/head-length = 0.34–0.48, eye-width/head-length = 0.25–0.32 in males; eye-length/head-length = 0.10–0.16, eye-width/head-length = 0.06–0.12 in females), smaller mouthparts, a longer pereopod relative to body length, and an appendix masculina on pleopod 2.

Two males bore rows of aesthetascs on article 1 of the antennular flagellum. Eye size differed between these two males: eye-length/head-length was 0.48 in the allotype (Figs II-V-2I, II-V-3E) but 0.34 in the paratype (Figs II-V-2J, II-V-3K).

DISCUSSION

Expanathura monile **sp. nov**. is the fourth named species in the second group sensu Poore (2001). One of three species in the second group, *E. collaris*, was originally described from Fiji and the Cook Islands, with the holotype and allotype from Fiji (Kensley 1979). Several papers subsequently reported this species from a broad area (Fig. II-V-1), with some redescriptions based on specimens from localities other than the type locality (Müller 1993; Negoescu and Brandt 2001; Poore and Lew Ton 2002; Keable and Reid 2015; Chew et al. 2018). Because *Expanathura* isopods show direct development and are benthic, inhabiting coral rubble, their dispersal capability is likely low, and it is quite possible that individuals collected far from the type locality are not *E. collaris*. For a

morphological comparison with *E. collaris*, I thus considered only specimens from Fiji, described by Kensley (1979) and Negoescu (1999).

Female *Expanathura monile* **sp. nov.** differs from females of congeners in the second group in the combination of six character states presented in Table II-V-1. There is little information on male morphology in *Expanathura*, and no males of *E. haddae* have been reported. Males of *E. monile* **sp. nov.**, *E. collaris*, and *E. macronesia* differ in the number of articles in the antennular flagellum (seven, six, and five, respectively; Kensley 1979, 1980), but I cannot rule out that one or more articles were overlooked in *E. collaris* and *E. macronesia*, as the distal articles are tiny.

Isopods of the superfamily Anthuroidea are generally protogynous (Poore and Bruce 2012); females change sex and become a terminal swimming male having enlarged eyes, an elongate pleon, swollen or elongate antennulae bearing numerous aesthetascs, reduced mouthparts, and elongate pereopods. While there is no direct evidence of sex change in *Expanathura*, one female *E. monile* **sp. nov.** having a fully developed marsupium bore an inner distal projection similar to an appendix masculina on both the right and left endopods of pleopod 2 (Fig. II-V-8E, F), as has been reported in immature males of paranthurid anthuroids (Negoescu 1999; Frutos et al. 2011), suggesting that species in *Expanathura* may be protogynous.

I observed a difference in eye size between two males (the allotype and one paratype) of *E. monile* **sp. nov.** Both individuals bore rows of aesthetascs on the antennular flagellum and thus were both in the “male” stage rather than the “submale” stage (cf. Poore and Lew Ton 2002). The allotype male was slightly shorter in body length than the paratype male (2.15 mm and 2.21 mm, respectively), but had larger eyes (eye-length/head-length = 0.48 and 0.34, respectively). The eye size in both males was larger than in females (eye-length/head-length = 0.10–0.16). These numbers suggest that the

allotype individual showed a higher degree of masculinization than the paratype. Other hypotheses could also explain the differences between the two males, such as that *E. monile* is diandric, with the allotype male being a primary male (i.e., a male born as a male). Additional studies, including rearing experiments, are needed to resolve this issue.

Key to the species in female *Expanathura* (modified from Poore and Lew Ton 2002)

1. Uropodal exopod lanceolate; telson tapering; pleopod 1 endopod much shorter than exopod … 2 (first group)
- Uropodal exopod broad; telson spoon-shaped; pleopod 1 endopod barely shorter than exopod … 4 (second group)
2. Telson less than twice as long as wide; pleopod-1 endopod ca. one-half length of exopod … *E. amstelodami* (Kensley, 1976)
- Telson more than twice as long as wide; pleopod-1 endopod ca. one-third length of exopod … 3
3. Uropodal exopod 3.5 times as long as wide; pleopod 1 endopod >3 times as long as wide … *E. mooreae* Müller, 1993
- Uropodal exopod 2.5 times as long as wide; pleopod 1 endopod twice as long as wide … *E. ardea* (Poore & Kensley, 1981)
4. Uropodal exopod with obtuse-triangular projection (apex angle about 120°) … 5
- Uropodal exopod with acute-triangular projection (apex angle about 90°) … 6
5. Telson with several short and 1 long posterolateral setae; telson oval … *E. collaris* (Kensley, 1979)
- Telson with posterolateral setae all short; telson truncate … *E. haddae* (Kensley & Poore, 1982)
6. Antennal peduncular article 2 without outer triangular projection … *E. macronesia*

(Kensley, 1980)

- Antennal peduncular article 2 with outer triangular projection … *E. monile* **sp. nov.**

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Chapter II-VI. *Kupellonura tamago* sp. nov. (Hyssuridae) from the Boso Peninsula

INTRODUCTION

Species in the anthuroid isopod family Hyssuridae Wägele, 1981 are distinguished from members in the other families by having (1) elongate pleonites 1–5, (2) similar, non-operculiform pleopods 1–5, and (3) the carpi of pereopods 2–3 strongly produced ventrodistally (Poore 2001). This family currently comprises 41 species in six genera: *Belura* Poore & Lew Ton, 1988 (2 species); *Galziniella* Müller, 1991 (1 species); *Hyssura* Norman & Stebbing, 1886 (7 species); *Kupellonura* Barnard, 1925 (18 species); *Neohyssura* Amar, 1953 (6 species); and *Xenanthura* Barnard, 1925 (7 species) (Poore and Bruce 2012; Annisaqois and Wägele 2021).

Kupellonura is the most species-rich hyssurid genus and differs from the other five genera by combining the following characters: (1) the uropodal exopod longer than wide, (2) the uropodal exopod and telson without cuticular spine, (3) the carpi of pereopods 4–7 trapezoidal, with 1 marginal spiniform seta, and (4) the pleopod 1 endopod longer and broader than the exopod (Poore 2001). At present, *Kupellonura* species have been reported from the intertidal zone to 2829 m depth, from all marine ecoregions except the Arctic, Temperate Northern Pacific, Tropical Eastern Pacific, and Temperate South America (Poore 2001; Poore and Bruce 2012; Annisaqois and Wägele, 2021; cf. Spalding et al. 2007) (but see the Remarks section for *Kupellonura* below).

Here I describe a new species of *Kupellonura* collected in the North Pacific off the southern coast of the Boso Peninsula, Japan, at a depth of 445–407 m. This is the first record of Hyssuridae not only for Japan but also for the Temperate Northern Pacific

ecoregion. In addition, I transfer *Kupellonura flexibilis* (Pasternak, 1982) to the genus *Anthelura* Norman & Stebbing, 1886 in Antheluridae Poore & Lew Ton, 1988.

MATERIALS AND METHODS

A specimen was collected in 2003 off the southern coast of Boso Peninsula, Japan, with a biological dredge having a 1 m opening, during cruise KT03-17 of RV *Tansei-maru*, and fixed and preserved in 70% ethanol. The methods used for dissection, preparation of slides, light microscopy, and drawing were as described in Shiraki et al. (2021). Body length was measured from the tip of the anterolateral lobe of the head to the tip of telson, and body width at the widest portion of pereonite 7. Measurements were made axially from digital images by using ImageJ (Schneider et al. 2012). I treated non-manca individuals without oostegites or appendix masculina as females lacking oostegites. The material examined is deposited in the collections of the Seto Marine Biological Laboratory (SMBL), Wakayama, Japan.

TAXONOMY

Family Hyssuridae Wägele, 1981a

Genus *Kupellonura* Barnard, 1925

Remarks. *Kupellonura flexibilis* was described from the Mediterranean as *Ananthura flexibilis* Pasternak, 1982. Negoescu (1984) provisionally transferred it to *Kupellonura*, but noted that this species does not show some of the features diagnostic for *Kupellonura*; its short pleonites 1–5 and the operculiform pleopod 1 contradict even

the familial diagnosis of Hyssuridae. Here I transfer this species to *Anthelura* in Antheluridae as *Anthelura flexibilis* (Pasternak, 1982) **comb. nov.**, based on the following features: (1) pleonites 1–5 free, (2) broad maxillipedal palp, (3) pereopods 4–7 carpi much longer than wide, and (4) pereopod 1 merus broader than the propodus (cf. Poore 2001). This species differs from the sole congener *Anthelura elongata* Norman & Stebbing, 1886 in the number of articles in the antennal flagellum, which are eight in *A. flexibilis* but seven in *A. elongata* (Pasternak 1982; Negoescu 1984; Poore 2001). This changes the deepest record for *Kupellonura* to 1141 m (Negoescu 2006), reported for *Kupellonura cryosi* Negoescu, 2006.

Species composition. Eighteen species: *Kupellonura afareaitu* Müller, 1991; *K. biriwa* Poore & Lew Ton, 1988; *K. capensis* (Kensley, 1975); *K. caudoserrata* Negoescu, 1994; *K. cryosi*; *K. currawan* Poore & Lew Ton, 1988; *K. formosa* (Menzies & Frankenberg, 1966); *K. gidgee* Poore & Lew Ton, 1988; *K. imswe* (Kensley, 1982); *K. indonesica* Annisaqois & Wägele, 2021; *K. macaroni* Annisaqois & Wägele, 2021; *K. marrongie* Poore & Lew Ton, 1988; *K. mediterranea* Barnard, 1925 (type species); *K. proberti* Wägele, 1985; *K. racovitzai* George & Negoescu, 1985; *K. serritelson* Wägele, 1981; *K. tamago* **sp. nov.**; and *K. werawera* Poore & Lew Ton, 1988.

***Kupellonura tamago* sp. nov.**

[New Japanese name: Tamago-haranaga-uminanafushi]

(Figs II-VI-1–II-VI-4)

Diagnosis. Eyes present. Telson margins smooth. Length of antennular flagellum article 2, 2.50 times width. Margins of uropodal endopod smooth, without serrations.

Length of uropodal exopod 1.47 times width, oval, widest at proximal third; inner margin smooth, without serration or crenulation; outer margin not concave.

Etymology. The specific name is derived from the Japanese noun *tamago* ("egg"), referring to the oval shape of uropodal exopod.

Material examined. Holotype: female lacking oostegites (SMBL-V0638, 14 slides and 1 vial), body length 6.79 mm, body width 0.44 mm, head length 0.38 mm, head width 0.38 mm, eye length 0.04 mm, 34°57.091'N 140°07.774'E to 34°57.404'N 140°07.626'E, Stn. KG-4, off the southern coast of the Boso Peninsula, Japan, Northern Pacific, 445–407 m depth, collected by Michitaka Shimomura, 17 November 2003.

Description of female holotype. Body length (Figs II-VI-1, II-VI-2A–E) 15.31 times body width, slender, without dorsal pigmentation in fixed specimen. Head length (Fig. II-VI-2A) 1.00 times head width; rostrum protruding as much as anterolateral lobes; eyes dorsolateral, small; length ratio of eye to head, 0.04. Pereonites 1–7 (Fig. II-VI-2A–C) with length ratio 1.00:1.22:1.25:1.65:1.74:1.74:1.57. Pleonites 1–6 (Fig. II-VI-2D) articulated, with length ratio 1.00:1.04:0.99:0.93:1.08:0.93; combined length pleonites 1–6, 0.19 times body length; pleonites 1–4 with several lateral plumose setae. Telson (Fig. II-VI-4H) oval, margins smooth, with simple seta laterally and 16 distal simple setae.

Antennula (Fig. II-VI-2F) with three peduncular articles and four flagellar articles. Peduncular article 1 longest, with outer simple seta; article 2 with two outer distal plumose sensory setae, and one outer distal, one mid-dorsal, and one inner distal simple setae; article 3 with five distal simple setae. Flagellar article 1 with distal plumose sensory seta; article 2 length 2.50 times width, naked; article 3 naked; article 4 with two outer

distal simple setae and prominent aesthetasc.

Antenna (Fig. II-VI-2G) with five peduncular articles and eight flagellar articles. Peduncular article 1 naked; article 2 with two distal and one inner middle simple setae; article 3 with two inner distal simple setae; article 4 with one outer and one distal plumose sensory setae and two distal simple setae; article 5 with one outer and one distal plumose sensory setae, and one inner middle and three distal simple setae. Flagellar articles 1–8 with zero, three, four, four, three, four, three, and five distal simple setae, respectively.

Mandible (Fig. II-VI-2H) with triarticulate palp. Palp article 1 with distal simple seta; article 2 longest, with distal simple seta; article 3 with three distal spiniform setae. Incisor with two cusps; lamina dentata with two teeth, molar rounded.

Maxilla (Fig. II-VI-2I) with one strong and five smaller distal teeth.

Maxilliped (Fig. II-VI-2J) with 5-articulate palp. Palp article 1 naked; article 2 with inner distal simple seta; article 3 with three distal simple setae; article 4 with two distal simple setae; article 5 with four distal simple setae. Endite overreaching distal margin of palp article 2, with distal simple seta and distal seta (tip broken).

Pereopod 1 (Fig. II-VI-3A) subchelate, robust. Basis with ventrodistal simple seta. Ischium with one dorsal and one ventrodistal simple setae. Merus with two dorsodistal and three ventrodistal simple setae. Carpus triangular, not produced ventrodistally, with one ventroproximal and one ventrodistal transparent membrane-like processes, and two ventral spiniform setae and two subdistal simple setae. Propodus broad, with inner spiniform seta, and one outer proximal, three dorsodistal, and one ventrodistal simple setae. Palm with one ventroproximal and one mid-ventral transparent membrane-like processes, two ventral spiniform setae, and two ventro-subproximal and three ventro-subdistal simple setae. Dactylus with ventrodistal short spiniform seta and one mid-ventral, five subdistal, and three distal simple setae. Unguis naked, length nearly two-

thirds dactylus length.

Pereopod 2 (Fig. II-VI-3B) subchelate, as robust as pereopod 1. Basis with dorsal plumose sensory seta, and two dorsal and two ventral simple setae. Ischium with ventrodistal simple seta. Merus with dorsodistal spiniform seta, and one dorsodistal and three ventrodistal simple setae. Carpus triangular, with ventrodistal prolongation, one ventroproximal and one ventrodistal transparent membrane-like processes, one mid-ventral and one ventrodistal spiniform setae, and one outer and three ventral simple setae. Propodus with dorsal plumose sensory seta and two subproximal, two dorsal, and three dorsodistal simple setae. Palm with one ventroproximal, one mid-ventral, and one ventro-subdistal transparent membrane-like processes, mid-ventral bifurcate spiniform seta, one ventro-subproximal and one ventro-subdistal spiniform setae, and three ventro-subdistal simple setae. Dactylus with two middle and six distal simple setae. Unguis naked, length nearly half dactylus length.

Pereopod 3 (Fig. II-VI-3C) subchelate, similar to pereopod 2 except in number of simple setae.

Pereopod 4 (Fig. II-VI-3D) with basis, ischium, merus similar to those of pereopod 3 but narrower and bearing different numbers of plumose sensory setae and simple setae. Carpus trapezoidal, with four small ventral processes, ventrodistal spiniform seta, dorsodistal plumose sensory seta, and one dorsodistal and four ventral simple setae. Propodus with dorsodistal plumose sensory seta and two dorsodistal simple setae. Palm with several small ventral processes, ventrodistal spiniform seta, and one inner and four ventrodistal simple setae. Dactylus narrow, with one ventro-subproximal, one dorso-subdistal, and nine distal simple setae. Unguis naked, length nearly one-quarter dactylus length.

Pereopods 5–7 (Fig. II-VI-3E–G) similar to pereopod 4, except for number of

plumose sensory setae and simple setae.

Pleopod 1 (Fig. II-VI-4A) protopod with two inner hooks and outer simple seta. Exopod not operculiform, length 1.82 times width, with 22 plumose setae (one broken). Endopod length 1.80 times width, 0.96 times exopod length, with nine plumose setae. Protopod, exopod, and endopod partly covered with fine setae.

Pleopods 2–5 (Fig. II-VI-4B–E) similar to pleopod 1, except for number of plumose setae on endopod (nine, eight, nine, eight plumose setae on endopods 2–5, respectively).

Uropodal endopod (Fig. II-VI-4F) length 1.64 times width, with three distal plumose sensory setae and 34 simple setae. Exopod (Fig. II-VI-4G) oval, widest at proximal third, length 1.47 times width, with 15 simple setae (two with tip broken); inner margin smooth, without serration or crenulation; outer margin not concave.

Remarks. The uropodal exopod in female *K. tamago* **sp. nov.** is widest at the proximal third; the inner margin is smooth, without serration or crenulation; the outer margin is not concave. Females of *K. tamago* **sp. nov.** share these features with females of *K. gidgee* and *K. indonesica*, but differ from them as follows (character states in parentheses, *K. gidgee*; *K. indonesica*): uropodal exopod broader, with the length/width ratio 1.47 (2.1; 2.4); uropodal endopod broader, with the length/width ratio 1.64 (2.1; not mentioned); telson margins smooth (dentate distally; paired prominent dentations on distolateral margins) (Poore and Lew Ton 1988; Annisaqois and Wägele 2021).

The description of the tailfan of female *K. serritelson* is insufficient, but *K. tamago* **sp. nov.** differs from this species in having a more-elongate antennular flagellum article 2 (length 2.50 times the width in *K. tamago* **sp. nov.**; 1.44 times in *K. serritelson*), a more-elongate antennal peduncle article 5 (length 2.14 times the width in *K. tamago* **sp. nov.**;

1.56 times in *K. serritelson*), and the carpus of pereopod 1 lacking the ventrodistal prolongation present in pereopods 2 and 3 (ventrodistal prolongation present on the carpus of pereopod 1 in *K. serritelson*) (Wägele 1981b).

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Chapter II-VII. *Cruranthura viridis* sp. nov. (Paranthuridae) from the Miyako Islands

INTRODUCTION

The paranthurid genus *Cruranthura* Thomson, 1946 differs from the other six confamilial genera by the combination of following characters: (1) pereopod 7 absent in non-manca individuals, (2) pleonite 6 delineated dorsally from an oval telson, (3) the head longer than wide, and (4) pleonites 1 free and 2–5 fused dorsally (Poore 1984, 2001; Shiraki et al. 2022). It currently contains four valid species: *Cruranthura caeca* (Mezhov, 1976), known from the Kurile Islands, Russia (Mezhov 1976); *Cruranthura furneauxi* (Poore, 1981), occurring in Tasmania and New South Wales, Australia (Poore 1981, 1984); *Cruranthura peroni* (Poore, 1981) occurring in Brisbane, Queensland, and New South Wales, Australia (Poore 1981, 1984); and the type species *Cruranthura simplicia* Thomson, 1946, occurring in Western Australia (Thomson 1946). The first two species occupy intertidal or subtidal marine habitats, whereas the latter two are estuarine.

Here I describe a new *Cruranthura* species collected subtidally around Irabu Island, Miyako Islands, Okinawa, Japan and sequenced part of its cytochrome *c* oxidase subunit I (COI) gene.

MATERIALS AND METHODS

Four individuals were collected from coral rubble at 10–20 m depth on the north reef of Irabu Island (24°51'57.7"N 125°09'41.9"E), Miyako Islands, Okinawa, southwestern Japan, on 25 April 2022. They were photographed and fixed in 70% ethanol on 25 April

2022, and transferred to 99% ethanol on 12 May 2022. The methods used for dissection, preparation of slides, light microscopy, and drawing were as described in Shiraki et al. (2021). One ovigerous female was bisected into anterior and posterior parts in ethanol, treated with hexamethyldisilazane (HMDS; Nation 1983), and observed at 15 kV accelerating voltage with an S–3000N scanning electron microscope (SEM; Hitachi, Tokyo, Japan). Body length was measured from the tip of the anterolateral lobe of the head to the tip of the telson, and body width at the widest portion of pereonite 6. Measurements were made axially from digital images by using Adobe Illustrator CC and ImageJ (Schneider et al. 2012). The specimens examined have been deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Japan.

DNA was extracted from right pereopod 1 of the holotype specimen by using a NucleoSpin Tissue XS Kit (Macherey-Nagel, Germany). The COI sequence was amplified by PCR with primers LCO-1490 and HCO-2198 (Folmer et al. 1994) under the following conditions with KOD One (Toyobo, Japan): 45 cycles of 98°C for 10 seconds, 56°C for 5 seconds, and 68°C for 1 second. The nucleotide sequence was determined by direct sequencing with a BigDye Terminator Cycle Sequencing Kit ver. 3.1 and a 3730 Genetic Analyzer (Life Technologies, USA). The COI sequence was deposited in the International Nucleotide Sequence Database (INSD) through the DNA Data Bank of Japan (DDBJ).

TAXONOMY

Family Paranthuridae Menzies & Glynn, 1968

Genus *Cruranthura* Thomson, 1946

***Cruranthura viridis* sp. nov.**

[New Japanese name: Kappa-ashitarazu-uminanafushi]

(Figs II-VII-1–II-VII-5)

Diagnosis. Body small (length 2.53–3.38 mm) and moss green in color. Eyes present. Pleonite 1 longer than pleonite 2. Telson length 2.06–2.33 times telson width. Propodus of pereopod 1 with 6–10 spiniform setae. Endopods of pleopods 2–5 uniarticulate, with plumose setae.

Etymology. The specific name is from the Latin adjective *viridis* (green), referring to the moss green body color.

Material examined. Holotype: ovigerous female (ICHUM8565, 8 slides and 1 vial), body length 3.08 mm, coral rubble, 10–20 m depth, Irabu Island (24°51'57.7"N 125°09'41.9"E), Miyako Islands, Okinawa, southwestern Japan, collected by Shoki Shiraki, 25 April 2022. Paratypes: two ovigerous females, ICHUM8566 (body length 3.38 mm; 12 slides and 1 vial) and ICHUM8567 (body length 2.80 mm; 1 slide and 1 vial), and one female lacking oostegites, ICHUM8568 (body length 2.53 mm; 2 slides and 1 vial), collection data for paratypes as for holotype.

Sequence. Partial COI sequence (658 bp) from holotype specimen (accession number LC775393).

Description of female based primarily on holotype. Body (Figs II-VII-1A–D, II-VII-2A, B) color moss green, length 11.54 times body width, slender. Head (Fig. II-

VII-2C) length 1.35 times head width; rostrum not extending past anterolateral lobes; eyes dorsolateral, small, black; eye length 0.13 times head length. Pereonites 1–7 (Fig. II-VII-2A) with length ratios 1.18:1.07:1.19:1.25:1.22:0.78:0.15 relative to head length; pereonite 7 (Figs II-VII-2A, B, II-VII-5C, D) reduced, not hidden laterally but rarely visible, lacking pereopod 7 in adults. Pleonite 1 free; pleonites 2–5 articulated laterally but fused dorsally and indicated by folds (Figs II-VII-2A, B, II-VII-5C, D); pleonite 1 1.72 times as long as pleonite 2. Pleonite 6 delineated dorsally, 0.53 times as long as combined length of pleonites 1–5, delineated dorsally from telson. Telson (Fig. II-VII-4H) oval, length from basal narrowest point to the tip 2.12 times width, with six dorsal simple setae and twelve distal simple setae.

Antennula (Fig. II-VII-2D) with three peduncular articles and one flagellar article. Peduncular article 1 longest, with two outer plumose sensory setae; article 2 with two distal plumose sensory setae and distal simple seta; article 3 with distal plumose sensory seta and three distal simple setae. Flagellar article with four distal aesthetascs and three distal simple setae.

Antenna (Fig. II-VII-2E) with five peduncular articles and one flagellar article. Peduncular article 1 naked; article 2 longest, with two distal simple setae and distal seta (tip broken); article 3 with two distal simple setae; article 4 with two distal plumose sensory setae and two simple setae; article 5 with outer plumose sensory seta, seven simple setae, and distal seta (tip broken). Flagellar article with numerous distal simple setae.

Mandible (Fig. II-VII-5A, B) not articulated with head, without palp.

Maxilla (Fig. II-VII-2F) slender, with 14 teeth and narrow lamella.

Maxilliped (from paratype ICHUM8566; Figs II-VII-2G, II-VII-5A, B) biarticulate; article 1 (presumably corresponding to basis and palp, except for terminal

palp article) with seven simple setae; article 2 minute, with four simple setae. Epipod (Fig. II-V-5A, B) oval.

Pereopod 1 (Fig. II-VII-3A) subchelate. Basis with dorsal plumose sensory seta and one dorsal and one ventrodistal simple setae. Ischium with ventrodistal simple seta. Merus with two dorsal and two ventrodistal simple setae. Carpus triangular, with five ventral simple setae. Propodus broad, with 10 inner spiniform setae and one outer and two dorsodistal simple setae. Palm with proximal trapezoidal projection and 10 simple setae. Dactylus with three ventral and four inner simple setae. Unguis naked, length about 0.4 times dactylus length.

Pereopod 2 (Fig. II-VII-3B) subchelate, narrower than pereopod 1. Basis with two dorsal plumose sensory setae, four simple setae, and dorsal seta (tip broken). Ischium with two ventral simple setae. Merus with two dorsal and three ventral simple setae. Carpus triangular, with three ventrodistal simple setae. Propodus oval, with dorsodistal plumose sensory seta and two dorsodistal simple setae. Palm with five spiniform setae with sensory bristle sensu Negoescu (1994) and three simple setae. Dactylus with two ventral, three ventrodistal, and four inner simple setae. Unguis naked, length about one-third dactylus length.

Pereopod 3 (Fig. II-VII-3C) similar to pereopod 2 except in number of simple setae.

Pereopod 4 (Fig. II-VII-3D) narrow. Basis with three dorsal plumose sensory setae and three simple setae. Ischium with two ventral simple setae. Merus with two dorsal and two ventrodistal simple setae. Carpus trapezoidal, with two ventral spiniform setae with sensory bristle, dorsal plumose sensory seta, and one dorsal and two ventrodistal simple setae. Propodus with two ventral spiniform setae with sensory bristle, dorsodistal plumose sensory seta, and two dorsodistal and three ventral simple setae.

Dactylus with one ventral, three ventrodistal, and three outer simple setae. Unguis naked, length about one-third dactylus length.

Pereopod 5 (Fig. II-VII-3E) similar to pereopod 4 except in number of plumose sensory setae and simple setae on basis.

Pereopod 6 (Fig. II-VII-3F) similar to pereopod 4 except in number of plumose sensory setae on basis and simple setae on merus and propodus. Propodus with two dorsodistal trifurcate spiniform setae.

Pleopod 1 (Fig. II-VII-4A) protopod with three inner hooks. Exopod operculiform, with sinuate outer distal margin and 13 plumose setae (possibly 14; one pore observed); three simple setae on surface. Endopod with six plumose setae.

Pleopod 2 (Fig. II-VII-4B) protopod with two inner hooks. Exopod with five plumose setae and outer simple seta. Endopod uniarticulate, with three plumose setae.

Pleopods 3–5 (Fig. II-VII-4C–E) similar. Protopods with one or two simple setae. Exopods with four plumose setae and outer simple seta. Endopods uniarticulate, with three plumose setae.

Uropod (Fig. II-VII-4F, G) with triangular-prism-shaped protopod bearing dorsodistal simple seta, and three inner ventral and four outer ventral plumose setae. Exopod (Fig. II-VII-4F) broad, oval, with 24 plumose setae (possibly 25; one pore observed), seven simple setae, and two setae (tip broken). Endopod (Fig. II-VII-4G) with eight plumose sensory setae, three plumose setae, 11 simple setae, and five setae (tip broken).

Variation. Table II-VII-1 indicates differences in length measurements and ratios among the four specimens examined. All specimens shared the following characters: (1) the body color was moss green; (2) eyes were present; and (3) pleonite 1

was longer than pleonite 2. The ratio of telson length to telson width ranged from 2.06 to 2.33 among three specimens (it could not be measured for ICHUM8567, which was used for SEM observation). The number of spiniform setae on the propodus of pereopod 1 ranged from 6 to 10 among seven pereopods of four specimens (it was not counted for right pereopod 1 of the holotype, which was used for DNA extraction).

Genetic information. A partial COI sequence (658 bp, encoding 219 amino acids; LC775393) was determined from the holotype specimen. In BLAST searches (Altschul et al. 1990), the COI sequence most similar to mine was from the insect "Cecidomyiidae sp." (accession number OM561009.1; identity score 80.66%, query cover 99%; Bukowski et al. 2022) rather than from a paranthurid, though there are several paranthurid COI sequences in the database. In a BLAST search with the "Organism" option set to "Anthuridea", the sequence most similar to mine was from *Colanthura pigmentata* Kensley, 1980 (accession number KR095339.1; identity score 81.03%, query cover 88%; Song and Min 2015).

Remarks. *Cruranthura viridis* **sp. nov.** differs from congeners in the suite of characters listed in Table II-VII-2. This is the first report of *Cruranthura* from Japan.

All paranthurid isopods have piercing-type mouthparts specialized for sucking (Poore 2001), but the degree of specialization differs among genera. I can roughly divide them into two groups: a moderately specialized group (*Deltanthura* **gen. nov.**, *Paranthura* Bate & Westwood, 1866, and *Pseudanthura* Richardson, 1911); and a highly specialized group (*Califanthura* Schultz, 1977, *Colanthura* Richardson, 1902, *Cruranthura*, and *Cruregens* Chilton, 1882). The mouthparts in the former group retain the mandibular palp, the articulation between the mandible and head, and the articulation

between the maxillipedal basis and palp (cf. Shiraki and Kakui 2022: fig. 1B). The mouthparts in the latter group lack these three structures (cf. Fig. II-VII-5A, B; Annisaqois and Wägele 2021: fig. 31). A piercing apparatus that is fixed on the head and lacks joints and unnecessary structures (such as the mandibular palp) will have higher stability and strength, which may enable species in the latter group to pierce more strongly and may have increased the range of possible prey in the group. Poore (2001) suggested that genera in the moderately specialized group (information on the phylogenetic position of *Deltanthura* **gen. nov.** in Paranthuridae is unavailable) are more basal within Paranthuridae than genera in the highly specialized group. If this is true, the specialization of piercing mouthparts may have steadily increased in the evolution of Paranthuridae. Molecular phylogeny can test this hypothesis.

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Chapter II-VIII. *Deltanthura palpus* gen. et sp. nov. (Paranthuridae) from the Kumanonada Sea

INTRODUCTION

Species in the anthuroid isopod family Paranthuridae Menzies & Glynn, 1968, have the mouthparts acutely produced; the palm of pereopod 1 lacks a proximal tooth; and statocysts are absent (Poore 2001). Paranthuridae originally comprised six genera: *Paranthura* Bate & Westwood, 1866; *Pseudanthura* Richardson, 1911; *Califanthura* Schultz, 1977; *Colanthura* Richardson, 1902; *Cruranthura* Thomson, 1946; and *Cruregens* Chilton, 1882. The species of the former two genera possess pereopod 7 in adults (non-neotenous) and an acute mandible with tri-articulate palp. On the other hand, the latter four are neotenous genera in which non-manca individuals lack pereopod 7, as is the case for manca-stage individuals, and share the character combination of having a blunt mandible lacking a palp and an extremely reduced pereonite 7.

Here I describe a new paranthurid species collected from off the southern coast of Mie Prefecture, and establish a new genus for the species.

MATERIALS AND METHODS

A total of five specimens were collected from off the southern coast of Mie Prefecture, Japan. Among the five, one was collected with a 3-m beam trawl at a depth between 805 and 852 m, during cruise KT 08-3 of RV *Tansei-maru* in 2008. The specimen was fixed in 5–10% borate-buffered formalin in seawater and preserved in 70% ethanol. The remaining four were collected by means of biological dredge during research cruises of

the TRV *Seisui-maru* (Mie University, Japan) in 2023, three of which during Cruise 2312 on 27 June and the remaining one during Cruise 2325 on 26 October. The four specimens were photographed and then fixed and preserved in 99% ethanol. Methods for dissection, observation, and drawing were as described in Shiraki et al. (2021). Body length was measured from the tip of the anterolateral lobe of the head to the tip of the telson, and body width at the widest portion of the tergites (pereonite 4 or 5). Head length was measured from the tip of the rostrum to the posterior edge of the head. The length of pereopods excluded the basis length and was measured as the sum of articular measurements from the center of the proximal edge to the center of the distal edge for each article and the dactylus-unguis (Fig. II-VIII-1). Definitions of the manca and male stages followed Shiraki and Kakui (2024). A specimen that was neither manca nor male was treated as a female lacking oostegites (see the Remarks section following the description). In the text, length to width ratios are abbreviated as "L/W". The study specimens have been deposited in the Seto Marine Biological Laboratory (SMBL-V0645) or the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Japan (ICHUM8848–8851).

TAXONOMY

Family Paranthuridae Menzies & Glynn, 1968

Deltanthura gen. nov.

[New Japanese name: Sankaku-ashitarazu-uminanafushi-zoku]

Diagnosis. Eyes present. Body relatively slender (body L/W = 7.43–9.43). Pereonite 7 reduced but not hidden laterally. Pleonites 1–5 fused but with sutures. Pleonite

6 entirely fused dorsally to telson; pleotelson triangular, without statocyst. Mandible acute, with 3-articulate palp. Maxilliped with 4-articulate palp; endite present. Carpus of pereopod 1 strongly protruding ventrodistally. Pereopod 7 present in non-manca individuals, shorter than pereopods 4–6. Uropodal exopod elongate-triangular, tapering.

Type species. *Deltanthura palpus*, by original designation.

Etymology. The generic name is a combination of the Greek letter *delta* (δέλτα), which is triangular in shape, referring to the shape of pleotelson, prefixed to *anthura*, from Greek *anthos* (a flower) and *oura* (a tail). The gender is feminine.

Remarks. Among paranthurid genera, *Deltanthura* **gen. nov.** is most similar to *Pseudanthura* in sharing (1) the pleonite 6 dorsally fused to the triangular telson, (2) maxillipedal endite, and (3) tapering uropodal exopod. However, the new genus is different from *Pseudanthura* in having (1) eyes (absent in *Pseudanthura*; Poore 2001), (2) the carpus of pereopod 1 strongly protruding (not protruding in *Pseudanthura*, except for *Pseudanthura recifensis* Kensley, 1982 with a weakly protruding carpus; Kensley 1982), and (3) a proportionally shorter body (body L/W = 7.43–9.43 in *Deltanthura* **gen. nov.**; 10.9–25.4 in *Pseudanthura*).

***Deltanthura palpus* sp. nov.**

[New Japanese name: Sankaku-ashitarazu-uminanafushi]

(Figs II-VIII-2–II-VIII-7)

Etymology. The specific name, *palpus* (Latin: palp), is a singular noun in the

nominative case, referring to the presence of a mandibular palp, a unique trait in paranthurid neotenous genera.

Material examined. Holotype: manca (SMBL-V0645; 12 slides and 1 vial), body length 7.03 mm, body width 0.91 mm, Mie prefecture, off the southern coast, Shima Spur, Stn. SM-01-(1), 34°00.83'N 136°53.79'E to 34°01.42'N 136°51.80'E, depth 805–852 m, 3 April 2008, Tadashi Akiyama leg. Non-types: female lacking oostegites (ICHUM8848; 1 slide and 1 vial), body length 13.67 mm, 802–812 m depth, the Kumanonada Sea (33°59.73'N, 136°56.92'E to 33°59.89'N, 136°57.22'E), Stn 4D, off Mie Prefecture, Japan, 27 June 2023; adult male (ICHUM8849; 2 slides and 1 vial), body length 16.72 mm, 747–779 m depth, the Kumanonada Sea (34°02.62'N, 136°53.40'E to 34°02.79'N, 136°53.73'E), Stn 5D, off Mie Prefecture, Japan, 27 June 2023; manca (ICHUM8850; 1 vial), body length 7.49 mm, collection data as for ICHUM8849; subadult male (ICHUM8851; 2 slides and 1 vial), body length 12.87 mm, 739–802 m depth, the Kumanonada Sea (34°02.62'N, 136°53.29'E to 34°02.13'N, 136°52.01'E), Stn 6', off Mie Prefecture, Japan, 26 October 2023.

Description of holotype manca. Body relatively slender (Figs II-VIII-2, II-VIII-3A–C), L/W = 7.69. Head (Fig. II-VIII-3A) length 1.02 times head width, roughened, irregular dorsally; rostrum protruding as much as anterolateral lobes; eyes dorsolateral, with scattered ommatidia. Pereonites 1–7 (Fig. II-VIII-3A, B) roughened, irregular dorsally, with length ratio 1.00:1.02:1.26:1.19:1.08:0.78:0.18; pereonite 7 (Fig. II-VIII-3B, C) reduced, hidden laterally, lacking pereopod 7. Pleonites 1–5 (Fig. II-VIII-3B, C) fused but with sutures, length 0.06 times body length. Pleonite 6 entirely fused dorsally to telson (Figs II-VIII-3B, C, II-VIII-5H); pleotelson triangular, length 1.04 times width,

with four dorsal and six apical simple setae.

Antennula (Fig. II-VIII-3D) with three peduncular and six flagellar articles. Peduncular article 1 with one inner and two outer plumose sensory setae; article 2 with inner simple seta; article 3 with three inner and one outer simple setae. Flagellar articles 1 and 2 naked; article 3 with distal aesthetasc and three distal simple setae; article 4 with distal aesthetasc; article 5 with distal aesthetasc and simple seta; article 6 with four distal simple setae.

Antenna (Fig. II-VIII-3E) with five peduncular and nine flagellar articles. Peduncular article 1 with outer simple seta; article 2 with two distal simple setae; article 3 with two inner simple setae; article 4 with two distal plumose sensory setae and three simple setae; article 5 with four distal plumose sensory setae and four inner simple setae. Flagellar articles 1–9 with four, four, seven, four, three, three, two, four and zero distal simple setae, respectively.

Mandible (Fig. II-VIII-3F) with 3-articulate palp. Palp article 1 naked; article 2 with distal simple seta; article 3 with eleven simple setae. Molar absent. Incisor acute.

Maxilla (Fig. II-VIII-3G) slender, with twelve teeth and narrow lamella.

Maxilliped (Fig. II-VIII-3H) with 4-articulate palp. Palp articles 1 and 2 long, 3 and 4 short, with one, four, one, and three simple setae, respectively. Endite present, reaching middle of article 2, with distal simple seta. Epipod oval.

Pereopod 1 (Fig. II-VIII-4A) subchelate, robust. Basis with three dorsal plumose sensory setae and ventrodistal seta (tip broken). Ischium with one outer and one ventrodistal simple setae. Merus with two dorsal, one outer, and one ventral simple setae. Carpus strongly protruding ventrodistally, with three inner mid-ventral spiniform setae and four simple setae. Propodus broad, with two inner proximal spiniform setae, and one outer, four dorsal, and one distal simple setae. Palm with five outer spiniform setae and

seven simple setae. Dactylus and unguis fused, with two ventral and five middle simple setae.

Pereopod 2 (Fig. II-VIII-4B) narrow. Basis with two dorsal plumose sensory setae, two simple setae, and dorsal seta (tip broken). Ischium with three simple setae. Merus with two dorsal and two ventrodistal simple setae. Carpus triangular, longer than wide, with three ventrodistal simple setae and ventrodistal seta (tip broken). Propodus with dorsal plumose sensory seta, three ventral spiniform setae, and two dorsal and three ventral simple setae. Dactylus with ventrodistal thick seta and three ventral, three ventrodistal, and four inner distal simple setae. Unguis naked.

Pereopod 3 (Fig. II-VIII-4C) similar to pereopod 2 except in number of setae.

Pereopods 4–6 (Fig. II-VIII-4D–F) narrower than pereopod 2, but similar to it except in number of setae and shape of carpus. Carpus rectangular, with ventrodistal spiniform seta.

Pleopod 1 (Fig. II-VIII-5A) protopod with inner simple seta and outer seta (tip broken). Exopod operculiform, distal margin serrate, with 15 marginal plumose setae and four simple setae on surface. Endopod 0.53 times longer than exopod, with three distal simple setae.

Pleopods 2–5 (Fig. II-VIII-5B–E) similar to one another. Protopod with one or two simple setae. Exopod with five to seven distal plumose setae and outer simple (pleopods 2–4) or plumose (pleopod 5) seta. Endopod with three distal plumose setae.

Uropod (Fig. II-VIII-5F, G) with protopod bearing two outer and one inner plumose setae and outer simple seta. Exopod elongate triangular, tapering, with five (right) and 10 (left) simple setae. Endopod with two distal projections, five outer and three distal plumose sensory setae, and 12 distal simple setae.

Partial description of manca (ICHUM8850). Body relatively slender, body L/W = 7.91; pereonites 3–7 reddish internally when fresh (Fig. II-VIII-6A). Pereonite 7 reduced but not hidden laterally. Eye length 0.33 times head length. Pereopod 7 lacking.

Partial description of female lacking oostegites (ICHUM8848). Body relatively slender, body L/W = 7.78 (Fig. II-VIII-6B). Pereonite 7 reduced but not hidden laterally. Eye length 0.35 times head length.

Pereopod 7 (Fig. II-VIII-7C) present, with fewer setae relative to pereopods 4–6; combined length of ischium–unguis 0.47 times those in pereopods 4–6. Basis with four dorsal plumose sensory setae and ventral simple seta. Ischium and merus naked. Carpus with ventrodistal simple seta. Propodus naked. Dactylus with ventrodistal bifurcate spiniform seta and ventrodistal spiniform seta. Unguis tiny, round-tipped, naked.

Partial description of subadult male (ICHUM8851). Body relatively slender, body L/W = 7.43; pereonites 3–7 reddish internally when fresh (Fig. II-VIII-6C). Pereonite 7 reduced but not hidden laterally (Fig. II-VIII-6E). Eye length 0.35 times head length.

Antennula (Fig. II-VIII-7A) with three peduncular and eight flagellar articles. Peduncular article 1 with one inner and two outer plumose sensory setae and four dorsal simple setae; article 2 with five distal plumose sensory setae and dorsal simple seta; article 3 with six simple setae. Flagellar article 1 with distal plumose sensory seta; article 2 longest and swollen, naked; article 3 with three aesthetascs and four distal simple setae; article 4 with two aesthetascs and four simple setae; article 5 with aesthetasc and three simple setae; articles 6–8 tiny, with aesthetasc (article 6), aesthetasc and three simple setae (article 7), and two simple setae (article 8).

Pereopod 7 (Figs II-VIII-6E, II-VIII-7D) present, with fewer setae relative to pereopods 4–6; combined length of ischium–unguis 0.45 times those of pereopods 4–6. Basis, ischium, and merus naked. Carpus with ventrodistal simple seta. Propodus with ventrodistal spiniform seta with sensory bristle sensu Negoescu (1994) and ventrodistal simple seta. Dactylus with ventrodistal spiniform seta and three dorsodistal and two ventrodistal simple setae. Unguis tiny, acute-tipped, naked.

Pleopod 2 (Fig. II-VIII-7F) protopod with two inner and one outer simple setae. Exopod with eleven plumose setae and outer simple seta. Endopod with three plumose setae and appendix masculina. Appendix masculina 1.30 times length of endopod, curved outward.

Partial description of adult male (ICHUM8849). Body relatively slender, body L/W = 9.43; pereonites 3–6 reddish internally when fresh (Fig. II-VIII-6D). Pereonite 7 reduced but not hidden laterally (Fig. II-VIII-6F). Eye length 0.34 times head length.

Antennula (Fig. II-VIII-7B) with three peduncular and ten flagellar articles. Peduncular article 1 with plumose sensory seta and two simple setae; article 2 with three plumose sensory setae, two simple setae, and seta (tip broken); article 3 with two distal simple setae. Flagellar article 1 with plumose sensory seta; articles 2–5 with numerous aesthetascs on distal edge, with 0, 0, 1, and 1 distal simple setae, respectively; articles 6 and 7 with several distal aesthetascs and simple seta; article 8 with three simple setae; article 9 tiny, with aesthetasc; article 10 tiny, with aesthetasc and five simple setae.

Pereopod 7 (Figs II-VIII-6F, II-VIII-7E) present, similar to pereopods 4–6 but narrower and shorter; combined length of ischium–unguis 0.65 times those of pereopods 4–6. Basis with five dorsal plumose sensory setae and one dorsal and three ventral simple

setae. Ischium with two ventral simple setae. Merus with two dorsodistal and one ventrodistal simple setae. Carpus with dorsal plumose sensory seta, ventrodistal spiniform seta with sensory bristle, and one dorsodistal and three ventrodistal simple setae. Propodus with dorsodistal plumose sensory seta, four ventral spiniform setae with sensory bristle, and four dorsal and four ventral simple setae. Dactylus with three ventral, four inner dorsal, and four ventrodistal simple setae. Unguis length about two-fifths dactylus length, curved ventrally, acute-tipped, naked.

Pleopod 2 (Fig. II-VIII-7G, H) protopod with two inner and one outer simple setae. Exopod with seven distal and one outer plumose setae. Endopod with three plumose setae and appendix masculina. Appendix masculina 1.63 times length of endopod, curved outward, tip (Fig. II-VIII-7H) weakly bifid, with barb.

Ontogenetic variation. The relative eye size (0.33–0.35 times head length) varied little across the ontogenetic stages examined. The proximal half of the antennular flagellum was swollen in males but not in female or mancae. The antennular flagellum bore numerous aesthetascs and had more articles in the adult male; it lacked tufts of aesthetascs and had fewer articles in the subadult male. The appendix masculina in the adult male was proportionally longer than that in the subadult male and had a barb, which was not observed in the subadult male.

Remarks. I considered specimen ICHUM8848, which had pereopods 7 but lacked male sexual characters, to be a female lacking oostegites rather than a post-manca (cf. ontogenetic stages in Shiraki and Kakui 2024). This was because the body length of this specimen (13.67 mm) was much greater than that of mancae (7.03–7.49 mm; Shiraki et al. 2022), suggesting there are several instars between them.

This study revealed that later ontogenetic stages in *D. palpus* **sp. nov.** (females lacking oostegites or later, and possibly all stages except mancae) bear pereopod 7, which was shorter than pereopod 6. The degree of development of pereopod 7 differed among ontogenetic stages: those in the female lacking oostegites and subadult male had only a few setae and a tiny unguis (here termed "incomplete pereopod 7"); that in the adult male was proportionally longer, similar to a typical pereopod 7, but shorter and narrower, with many setae and a normal, claw-like unguis (here termed "miniature pereopod 7"). The incomplete pereopod 7 closely resembles that generally observed only in post-mancae of anthuroid species with seven paired pereopods (e.g. Wägele 1985: fig. 7). *Deltanthura palpus* **sp. nov.**, however, retains the incomplete pereopod 7 from the stage of females lacking oostegites (possibly from post-mancae) through subadult males. A reduced pereopod 7 is quite uncommon in Anthuroidea. In anthuroid species with seven pairs of pereopods, individuals at the stage of females lacking oostegites or later have pereopod 7 subequal in length to pereopod 6 (e.g. Frutos et al. 2011, fig. 4; Jarquín-Martínez and García-Madrigal 2021, figs 9, 12). A miniature pereopod 7 has previously been reported in male *Pseudanthura albatrossae* Kensley, 1978 (Kensley 1978, fig. 3), suggesting a close phylogenetic relationship between *Deltanthura* and *Pseudanthura*. A complete lack of pereopod 7 in adults is a neotenous condition known in anthuroids, and my study suggests that having an incomplete or miniature pereopod 7 in adults is another form of neoteny.

Habitat. Muddy bottom with rocks. The known depth range is 739–852 m.

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Chapter II-IX. First report of *Paranthura japonica* Richrdson, 1909 (Paranthuridae) from Okinawa, with the observation on the predation

INTRODUCTION

Paranthura japonica Richardson, 1909 was described based on material collected from Muroran, Hokkaido (Richardson 1909), and later reported from various regions around Japan, from Hokkaido to Kumamoto (Table II-IX-1). Since Cohen and Carlton (1995) reported it from San Francisco, USA (as *Paranthura* sp.; Lee II and Reusser 2012), this species has often been reported as an invasive alien species on the West Coast of the United States (Cohen et al. 2005) and in Europe and northern Africa (Taybi et al. 2025; fig. 1), and currently regarded as one of the most widespread non-indigenous species in the Mediterranean (Ulman et al. 2017). It is suggested that it was transported from Japan to non-native regions via oyster seed exports, ship fouling, or ballast water (Cohen et al. 2005; Lavesque et al. 2013; Ulman et al. 2019).

Paranthura japonica has piercing-type mouthparts. Although observations of predation by live anthuroids have been quite limited (Poore and Bruce 2012), there are three previous studies on species with piercing-type mouthparts. Wägele (1981) found that the paranthurid *Paranthura costana* Bate & Westwood, 1868 and *Paranthura nigropunctata* (Lucas, 1849) hunted small arthropods, including tanaidaceans (*Leptochelia* sp. etc.), amphipods, pycnogonids, and chironomid insect larvae, but did not prey on sponges, polychaetes, turbellarians, nemerteans, or non-conspecific isopods. These two *Paranthura* species also appear to engage in cannibalism; when several individuals were put into a single aquarium, only the largest individual remained after a few days (Wägele 1981). Wägele (1985) found that the leptanthurid *Accalathura*

gigantissima Kussakin, 1967 preys on other crustaceans such as amphipods. Matsushima et al. (2025) observed *Accalathura* sp. preys on a tanaidacean but not on a polychaete. These species pierce the prey, open a small hole by maxilla, and suck the internal contents by a pressure produced by oesophagus and stomach (Wägele 1985). Considering the wide range of prey animals for the four anthuroids with piercing-type mouthparts mentioned above, *P. japonica* may have an impact on native ecosystems as a predator on native crustaceans. However, its ecology, including predation, has not been studied yet (Marchini et al. 2014).

In this chapter, I firstly report *P. japonica* from Okinawa and investigate its predation behavior through behavioral observations by using the individuals collected from Oshoro, Hokkaido.

MATERIALS AND METHODS

Sampling. Two *P. japonica* individuals were collected from coral rubble covered with short algae at a depth of 0.5 m in Kaichu-Doro, Uruma, Okinawa (26°19’57.1”N 127°55’22.6”E) on 21 April 2023. The coral rubble collected was rinsed in freshwater to remove the epibionts; the effluent was sieved through a 0.45 mm-mesh plankton net and *Paranthura* individuals were picked from residue. The individuals were photographed live, and fixed and preserved in 99% ethanol.

For behavioral observation, *P. japonica* and six candidate prey species were collected from algae in the intertidal zone along Oshoro Bay, Hokkaido, Japan (43°12’33.5”N 140°51’34.3”E) on 7 July and 20 October 2020, and additional *P. japonica* individual from Muroran, Hokkaido (42°18’50.4”N 140°58’05.3”E) on 5 September 2021. *Apseudes ranma* Matsushima & Kakui, 2024, a candidate prey species

not occurring in Oshoro Bay, was collected from a tank in the Port of Nagoya Public Aquarium, Japan in 2009–2010 and maintained in an aquarium at 25°C in the laboratory in Sapporo (for details, see Kakui and Hiruta 2013). *Apseudes ranma* individuals used in my study were hatched and reared in the laboratory. Details of prey candidates are summarized in Table II-IX-2.

Morphological observation. The methods used for dissection, preparation of slides, light microscopy, and drawing were as described in Shiraki et al. (2021). Ontogenetic staging follows Shiraki and Kakui (2024). Body length was measured from the tip of the anterolateral lobe of the head to the tip of the telson. Measurements were made axially from digital images by using ImageJ (Schneider et al. 2012). The material examined has been deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Hokkaido, Japan (ICHUM8986, 8987).

Molecular analysis. Partial cytochrome *c* oxidase subunit I (COI) sequences (658 bp) were amplified with primers LCO1490 and HCO2198 (Folmer et al. 1994) and sequenced for one individual each from Oshoro and Muroran following the methods described in Munakata et al. (2021) and for two individuals from Okinawa following the methods described in Kakui et al. (2019). These sequences were deposited in the international Nucleotide Sequence Database (INSD) through the DNA Data Bank of Japan (DDBJ), under accession numbers LC661619 (Oshoro), LC661620 (Muroran), and LC876914 and LC876915 (Okinawa). Kimura (1980) 2-parameter (K2P) distances were calculated with MEGA7 (Kumar et al. 2016)

Observation of predation. Predation was examined by placing one or several *P.*

japonica individuals and one prey individual in seawater in a petri dish and observing them under a Nikon SMZ 1500 stereomicroscope. Video recordings were made with a Nikon D5600 digital camera attached to the stereomicroscope. Still images were extracted from the recorded videos with Microsoft Photo and trimmed with Adobe Photoshop CC.

RESULTS

Family Paranthuridae Menzies & Glynn, 1968

Genus *Paranthura* Bate & Westwood, 1866

Paranthura japonica Richardson, 1909

[Japanese name: Yamato-uminanafushi]

(Figs II-IX-1–II-IX-3)

Material examined. Non-type: ovigerous female after spawning mancae (ICHUM8986; 8.5 mm, 3 slides and 1 vial); adult male (ICHUM8987; 8.2 mm, 1 slide and 1 vial); both from coral rubble covered with algae, 0.5 m depth, Kaichu Doro (26°19'57.1"N 127°55'22.6"E), Uruma, Okinawa, Japan, 21 April 2023. Specimens from Muroran and Oshoro were not deposited in Museum.

Remarks. The two specimens collected from Okinawa were identified as the genus *Paranthura* Bate & Westwood, 1866 in having (1) piercing mouthparts, (2) pereopod 7, (3) pleonite 6 marked off dorsally from pleotelson, and (4) pleotelson without statocyst (Poore 2001; Shiraki et al. 2022). Among the known *Paranthura* species, they share the morphological characters with *P. japonica* such as (1) the anterolateral lobe

protruding rostrum, (2) each ommatidium not scattered but placed closely together, (3) the pereonite 6 shorter than the pereonite 5, (4) the pleonites 1–5 fused dorsally, (5) the oval pleotelson with the short apical simple setae, (6) the uropodal exopod with sinuate along dorsodistal margin, and (7) the uropodal endopod with the length similar to the width (Figs II-IX-1, II-IX-2F–H; Richardson 1909; Nunomura 1975; Nunomura and Shimomura 2012; Shiraki et al. 2024). The K2P distance between the COI sequences derived from the two Okinawa specimens was 1.70% and those between them and the topotypic COI sequence (LC661620) were 0.31–1.70%. These values were encompassed in the intraspecific range of isopods (0.00–4.79%; Raupach et al. 2015). Based on the above, the two specimens from Okinawa were identified as *P. japonica*. This is the first record of the species from Okinawa.

Observation of predation. *Paranthura japonica* preyed on all individuals tested, except for a pycnogonid, Ammotheidae sp. (Table II-IX-2; Fig. II-IX-4) When prey touched the antenna of *P. japonica*, the latter quickly grasped the former with pereopod 1 (or sometimes with pereopod 2), pierced its integumental membrane with the maxilla, and began sucking out its internal contents. The maxilla is a dark-brown, needle-shaped appendage bearing sharp marginal barbules (Fig. II-IX-3B–D), making it more difficult for the prey to escape. As it sucked the internal contents from a prey individual, *P. japonica* temporarily kept them in its stomach until a certain amount had accumulated and then passed them into the hindgut.

In preying on the tanaidacean *Zeuxo ezoensis* Okamoto, Oya & Kakui, 2020 (Fig. II-IX-4A–C), a smaller species, *P. japonica* removed all of the internal contents of the prey within 5−6 minutes, leaving behind the translucent exoskeleton (Fig. II-IX-4C). The

prey tanaidaceans became immobile soon after being pierced.

In preying on an isopod, *Cleantiella strasseni* (Thielemann, 1910) (Fig. II-IX-4D), a much larger species, *P. japonica* pierced the integumental membrane on an antenna or pereopod. After predation by one *P. japonica* individual, *C. strasseni* remained alive and did not seem to be weakened.

In preying on an amphipod, Ampithoidae sp. (Fig. II-IX-4E), a species similar in body length, *P. japonica* sucked out about half of the prey's internal contents, leaving those in the posterior half. The amphipod stopped moving about 6 minutes after being pierced. When its pereopod or antenna was pierced, Ampithoidae sp. autotomized these appendages and escaped from predation.

I also observed that *P. japonica* pierced and sucked contents from a Janiridae sp. (Isopoda) individual that had recently died and a living Caprellidae sp. (Amphipoda) individual, but I lost these prey during observations and could not observe additional details.

In a petri dish containing several *P. japonica*, I observed individuals sucking the contents from other, immobile individuals. The latter were dead (Fig. II-IX-4F), but it was unclear whether they had died before or after being pierced.

Paranthura japonica also preyed on the tanaidacean *Apseudes ranma*, a smaller species not known to co-occur with *P. japonica* in the wild (Fig. II-IX-4G). *Paranthura japonica* completely removed the contents of the prey by sucking, leaving the translucent exoskeleton. The tanaidaceans stopped moving about 3 minutes after being pierced.

DISCUSSION

My observations of predation by *P. japonica* were similar to those made for two

congeneric species, *P. costana* and *P. nigropunctata* (Wägele 1981), although there were some minor differences. All three *Paranthura* species consumed various crustaceans such as tanaidaceans, amphipods, and isopods; predation on pycnogonids was observed in *P. costana* and *P. nigropunctata* but not in *P. japonica*. I observed that some *P. japonica* individuals had preyed on immobile conspecific individuals, indicating that cannibalism can occur when they encounter a conspecific individual in the wild. Cannibalism has also been suggested for *P. costana* and *P. nigropunctata*, but no study, including mine, determined whether "prey" individuals were alive before being pierced, leaving open the possibility that these cases involved scavenging rather than predation.

All pierced individuals except *C. strasseni* and Caprellidae sp. (details lacking for the latter) became immobile after being pierced. Prey contents were rapidly sucked out via a small pierced hole; it took ca. 5–6 minutes to suck all the internal content from *Z. ezoensis*. These observations suggest that *P. japonica* may inject a digestive (and possibly toxic) fluid for extra-oral digestion and immobilization.

I have shown that *P. japonica* is an aggressive predator that preys even on much larger animals than itself (e.g. *C. strasseni*). It also preyed on a crustacean species (*Apseudes ranma*) that it does not encounter in the wild. These results suggest that *P. japonica* will have a high impact on ecosystems it invades, as a predator on native crustaceans.

This study provides the first molecular-based evidence that *P. japonica* has a broad distribution from Hokkaido to Okinawa, spanning more than 2,000 km from north to south. However, this does not rule out the possibility that individuals identified as this species that previously reported from various regions of Japan may include cryptic or morphologically similar species. Poore (1984) noted the presence of cryptic species in

Paranthura. Moreover, it also remains possible that the occurrence of this species in Okinawa represents a human-mediated introduction, rather than a natural distribution.

If *P. japonica* is truly an invasive alien species in the United States, Europe, and northern Africa, although the possibility of species misidentification still remains, a factor which enables the species to invade there would be its high tolerance to environmental changes. This study revealed that the species occurs from subarctic Hokkaido to subtropical Okinawa, indicating that it can survive across a broad range of water temperatures. In addition, individuals used in this study remained active and showed no signs of debilitation even after being exposed to freshwater for approximately 5–10 minutes during the washing procedure, whereas other small crustaceans (e.g. some amphipods and other isopods) either died or became severely weakened. This suggests that the species has relatively high tolerance to substantial changes in salinity. Furthermore, this study revealed that it preys on a variety of crustaceans including species that do not occur sympatrically. These characteristics may facilitate the survival and establishment of this species in areas it has newly invaded.

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Chapter II-X. *Paranthura oriens* sp. nov. (Paranthuridae) from Tateyama, Chiba

INTRODUCTION

The paranthurid isopod genus *Paranthura* Bate & Westwood, 1866 comprises relatively large-sized species with around 10 mm in length. All species are marine, but one species, *Paranthura punctata* (Stimpson, 1855), was also reported from a freshwater lake in New Zealand (Chilton 1906; Barnard 1925). *Paranthura* anthuroids occur worldwide, ranging in depth from the intertidal zone to 2707 m (Poore 2001), but most are shallow species (Frutos et al. 2011), found on muddy/sandy bottoms, under rocks, and among algae (Nunomura and Shimomura 2012). They seem to be predators, feeding mainly on crustaceans (Wägele 1981; Shiraki and Kakui 2022).

Paranthura differs from other, confamilial genera in having an acute mandible with a 3-articulate palp, pereopod 7 present in non-manca individuals, pleonite 6 not fused dorsally to the telson, and a leaf-shaped uropodal exopod (Shiraki et al. 2022). It is the most speciose genus in the superfamily Anthuroidea Leach, 1814, with 67 valid species listed in the World Register of Marine Species (WoRMS; Boyko et al. 2024). Among these, *Paranthura australis* Haswell, 1881 has been regarded as a *nomen dubium* (Poore 1978), and *Paranthura verrillii* Richardson, 1902 has been synonymized with *Paranthura infundibulata* Richardson, 1902 (Barnard 1925); *Paranthura* thus contains 65 valid species. *Paranthura* species resemble one another morphologically (Kensley and Schotte 2000) but differ subtly in the shapes of articles of pereopods, uropods, and the telson, and in the number of spiniform setae on article 3 of the mandibular palp (Poore 1984; Negoescu 1997). Poore (1984) suggested that there were still many undiscovered,

endemic, and cryptic species in *Paranthura*. This must be the case for *Paranthura* in Japan. While this is the most-studied anthuroid genus in Japan, with nine species reported (Table II-1), most of their reports were from shallow waters in restricted areas, suggesting that the diversity of *Paranthura* in Japan is underestimated.

Here I describe a new *Paranthura* species collected subtidally at Tateyama, Chiba, Japan. I also present a partial sequence of the cytochrome *c* oxidase subunit I (COI) gene for future DNA barcoding.

MATERIALS AND METHODS

Specimens were collected from sandy mud bottoms covered with algae (Fig. II-X-1) by means of a boat and biological dredge provided by the Institute for Marine and Coastal Research, Ochanomizu University, from off Kouyatsu, Tateyama, Chiba, Japan on 25 February and 1 June 2022; they are fixed and preserved in 70–99% ethanol. The methods used for dissection, preparation of slides, light microscopy, and drawing were as described in Shiraki et al. (2021). Body length was measured from the tip of the anterolateral lobe of the head to the tip of the telson, and body width at the widest point of pereonite 5. Measurements were made axially from digital images by using Adobe Illustrator CC and ImageJ (Schneider et al. 2012). The material examined has been deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Hokkaido, Japan (ICHUM8805–ICHUM8808).

DNA was extracted from pereopod 3 of paratype ICHUM8807 with a NucleoSpin Tissue XS Kit (Macherey–Nagel, Germany). Primers LCO-1490 and HCO-2198 (Folmer et al. 1994) were used for PCR amplification and cycle sequencing of COI.

PCR conditions with KOD One (Toyobo, Japan) were 45 cycles of 98℃ for 10 seconds, 56℃ for 5 seconds, and 68℃ for 1 second. The COI sequence was determined by direct sequencing with a BigDye Terminator Cycle Sequencing Kit ver. 3.1 and a 3730 Genetic Analyzer (Life Technologies, USA), and was deposited in the International Nucleotide Sequence Database (INSD) through the DNA Data Bank of Japan (DDBJ) under accession number LC778095.

TAXONOMY

Family Paranthuridae Menzies & Glynn, 1968

Genus *Paranthura* Bate & Westwood, 1866

Calamura Boone, 1920: 25–26.

Edanthura Boone, 1923: 148.

Diagnosis. See Frutos et al. (2011).

Species composition. Sixty-six valid species (including my new species) in *Paranthura* can be classified into five groups based on the condition of the pleonites (cf. Negoescu 1997; Fig. II-X-2), with two species of uncertain status. The phylogenetic validity of these groups has not been tested.

Group A contains species having free pleonites 1–5 (37 species): *P. acasia* Poore, 1984; *P. alba* Nunomura, 1977; *P. algophila* Kensley & Schotte, 2000; *P. antarctica* Kussakin, 1967; *P. argentinae* Kussakin, 1967; *P. barnardi* Paul & Menzies, 1971; *P.*

bellicauda Miller & Menzies, 1952; *P. boronia* Poore, 1984; *P. brucei* Negoescu, 1999; *P. caesia* Poore, 1984; *P. caribbiensis* Kensley, 1982; *P. ciliata* Whitelegge, 1901; *P. costana* Bate & Westwood, 1866; *P. dryandra* Poore, 1984; *P. elegans* Menzies, 1951; *P. epacris* Poore, 1984; *P. gracilipes* Nordenstam, 1930; *P. infundibulata*; *P. involuta* Whitelegge, 1901; *P. kunzea* Poore, 1984; *P. linearis* (Boone, 1923); *P. lineata* Nunomura, 1977; *P. lobelia* Poore, 1984; *P. longitelson* Wägele, 1984; *P. maculosa* Nunomura, 1974; *P. microtis* Poore, 1984; *P. nigrocaudata* (Nunomura, 1975); *P. nigropunctata* (Lucas, 1846); *P. nordenstami* Kensley, 2003; *P. ostergaardi* Miller & Menzies, 1952; *P. plumosa* Pillai, 1966; *P. porteri* (Boone, 1920); *P. possessia* Kensley, 1980; *P. santiparrai* Frutos, Sorbe & Junoy, 2011; *P. senecio* Poore, 1984; *P. skottsbergi* Nordenstam, 1930; *P. societensis* Müller, 1993.

Group B contains species having free pleonites 1–3 and fused pleonites 4 and 5 (one species): *P. urodentata* Kensley & Schotte, 2000.

Group C contains species having free pleonite 1 and dorsally fused pleonites 2–5 (seven species): *P. amorensis* Jarquín-Martínez & García-Madrigal, 2021; *P. floridensis* Menzies & Kruczynski, 1983; *P. grevillea* Poore, 1984; *P. lifuensis* Stebbing, 1900; *P. setigera* Negoescu, 1997; *P. tientai* Jarquín-Martínez & García-Madrigal, 2021; *P. urochroma* Pires, 1981.

Group D contains species having dorsally fused pleonites 1–3 and free pleonites 4 and 5 (one species): *P. longa* Wägele, 1985).

Group E contains species having pleonites 1–5 dorsally fused (18 species): *P. algicola* Nunomura, 1978; *P. astrolabium* Kensley, 1979; *P. bunakenensis* Negoescu, 1997; *P. californiae* Nunomura, 1978; *P. deodata* Müller, 1990; *P. flagellata* (Chilton, 1882); *P. hasticauda* Nunomura, 1974; *P. japonica* Richardson, 1909; *P. kagawaensis* Nunomura, 1993; *P. kobensis* Nunomura, 1975; *P. laticauda* Nunomura, 1975; *P. latipes*

Barnard, 1955; *P. nana* Nordenstam, 1930; *P. oriens* **sp. nov.**; *P. polynesica* Kensley, 1979; *P. punctata*; *P. seychellensis* Kensley & Schotte, 2000; *P. telopea* Poore, 1984.

Two species have not been assigned to a group, as information is lacking on the segmentation of their pleons: *P. antillensis* Barnard, 1925; *P. neglecta* Beddard, 1886.

Remarks. The conditions of the pleonites for *P. nigropunctata*, *P. punctata*, and *P. seychellensis* were based on Wägele (1982), Barnard (1914), and Chew and Abdul Rahim (2020), respectively, as the original descriptions lacked this information. I treated *P. involuta* as a member of Group A based on a redescription of the holotype specimen (Poore 1984), even though the original description mentioned that the pleonites were fused laterally (Whitelegge 1901). I include *P. flagellata* from New Zealand in Group E, based on the original description (Chilton 1882). Poore (1981) examined other specimens from New Zealand and concluded they were conspecific with *P. flagellata,* though the character states of his material were those of Group C. It appears to me that *P. flagellata* sensu Poore (1981) is not conspecific with *P. flagellata* Chilton and should be regarded as *Paranthura* sp. Drawings of *P. hasticauda* and *P. lineata* accompanying the original descriptions (Nunomura 1974, 1977) indicated that pleonites 1–5 are fused dorsally, but the original descriptions mentioned a ‘‘Demarcation of pleonal somites visible in dorsal view’’ for both species. Nunomura and Shimomura (2012) treated *P. hasticauda* as Group E and *P. lineata* as Group A, and I have followed this treatment. For other species, I used information from the original descriptions.

The condition of pleonites is stable through ontogenetic stages and is useful as a taxonomic character. For example, in Group A, *P. brucei*, *P. costana*, and *P. santiparrai* retain free pleonites 1–5 in females, males, and mancae (Wägele 1982; Negoescu 1999; Frutos et al. 2011). In Group E, *P. bunakenensis* and *P. oriens* **sp. nov.** retain fused

pleonites 1–5 between sexes (Negoescu 1997; this study). To test the monophyly of each Group or even the validity of elevation of each Group to subgenus or genus, comprehensive phylogenetic analysis can solve.

Paranthura oriens sp. nov.

[New Japanese name: Hinode-uminanafushi]

(Figs II-X-3–II-X-7)

Diagnosis. Body with dark brown spots on each segment and orange pigmentation on anterior part of head, bases of antennulae and antennae, and uropod. Pleonites 1–5 subequal in length, segmented laterally but fused dorsally (Group E). Telson with a rounded rather than sharp apical edge. Uropodal endopod triangular, tapering. Uropodal exopod narrow, 2.72–2.84 times longer than wide.

Etymology. The specific name is the nominative singular Latin noun *oriens* (sunrise), referring to the orange coloration as noted above.

Material examined. Holotype: ovigerous female with 47 embryos (ICHUM8805, 13 slides and 1 vial), body length 10.74 mm, 5 m depth, off Kouyatsu (34°58.931′N, 139°49.133′E), Tateyama, Chiba, Japan, collected by Ryuta Yoshida and Mamoru Yamaguchi, 1 June 2022. Allotype: male (ICHUM8806, 7 slides and 1 vial), body length 7.93 mm, collection data as for holotype. Paratypes: female lacking oostegites (ICHUM8807, 10 slides and 1 vial), body length 9.15 mm, 12.0–19.8 m depth, Stn. 1, off Kouyatsu (34°59.313′N, 139°48.595′E to 34°59.230′N, 139°48.433′E), Tateyama, Chiba, Japan, collected by Ryuta Yoshida and Mamoru Yamaguchi, 25

February 2022; ovigerous female (ICHUM8808; 5 slides and 1 vial), body length 10.85 mm, collection data as for holotype.

Habitat. Sandy mud bottom covered with algae (as periphyton?) (Fig. II-X-1).

Sequence. Partial COI (658 bp; LC778095) sequence from paratype ICHUM8807.

Description of female holotype. Body (Figs II-X-3A, B, II-X-4A, B) slender, 11.39 times longer than wide, with dark brown spots on each segment and orange pigmentation on anterior part of head, base of antennulae and antennae, and uropod when alive (Fig. II-X-3A). Head (Fig. II-X-4C) length 1.10 times head width; rostrum not reaching past anterolateral lobes; eyes dorsolateral, relatively large, length 0.22 times head length, with scattered ommatidia. Pereonites 1–7 (Fig. II-X-4A, B) with length ratio 1.21:1.33:1.59:1.70:1.80:1.30:0.82 relative to head length. Pleonites 1–5 (Fig. II-X-4A, B) subequal in length, segmented laterally but fused dorsally. Pleonite 6 distinct from telson dorsally. Telson (Fig. II-X-6H) long-pyriform, tapering, apical edge rounded, with length (tip of the telson to narrowest point) 2.31 times width, with 41 simple setae and three setae (tips broken); base narrow, concave, 0.62 times width.

Antennula (Fig. II-X-4D, E) with three peduncular and six flagellar articles. Peduncular article 1 longest, with one distal plumose sensory seta, five outer and one distal simple setae, and one distal seta (tip broken); article 2 with two outer plumose sensory setae and one outer and one distal simple setae; article 3 with seven distal simple setae. Flagellar article 1 with one distal plumose sensory seta and one inner distal simple seta; article 2 with two aesthetascs and three distal simple setae; article 3 with two

aesthetascs and three distal simple setae; article 4 with five aesthetascs and three distal simple setae; article 5 tiny, naked; article 6 tiny, with five simple setae.

Antenna (Fig. II-X-4F, f1) with five peduncular articles and five flagellar articles. Peduncular article 1 triangular; article 2 with two distal simple setae; article 3 with one inner and two distal simple setae; article 4 with one distal plumose sensory seta and 10 simple setae; article 5 with four distal plumose sensory setae and seven middle and 13 distal simple setae. Flagellar article 1 with numerous inner simple setae; articles 2–5 tiny, with numerous simple setae.

Mandible (Fig. II-X-4G) with 3-articulate palp. Palp article 1 with one distal simple seta; article 2 longest, with one distal simple seta; article 3 with 11 spiniform setae. Molar absent. Incisor acute.

Maxilla (Fig. II-X-4H) slender, with 11 teeth and narrow lamella.

Maxilliped (Fig. II-X-4I) with uniarticulate palp. Palp article 1 long, with 11 simple setae and one proximal seta (tip broken). Endite absent.

Pereopod 1 (Fig. II-X-5A) subchelate. Basis with three dorsal plumose sensory setae and nine dorsal and five ventral simple setae. Ischium with 10 simple setae and two setae (tips broken). Merus with two dorsal and three ventral simple setae. Carpus triangular, with 12 ventral simple setae. Propodus broader proximally and narrower distally, with 11 outer short simple setae, one dorsodistal simple seta, and one dorsodistal seta (tip broken). Palm with proximal trapezoidal projection and 29 simple setae. Dactylus with 10 ventral, one ventrodistal, and three inner simple setae. Unguis nearly one-quarter dactylus length, naked.

Pereopod 2 (Fig. II-X-5B) subchelate, slightly narrower than pereopod 1. Basis with two dorsal plumose sensory setae and 21 short simple setae. Ischium with six dorsal and 13 ventral simple setae. Merus with four dorsal and nine ventral simple setae. Carpus

triangular, with nine simple setae. Propodus oval, with one dorsodistal plumose sensory seta and three dorsal, three dorsodistal, and six outer simple setae. Palm with eight spiniform setae with sensory bristle sensu Negoescu (1994) and 16 simple setae. Dactylus with nine ventral, three ventrodistal, and five inner simple setae. Unguis about one-sixth dactylus length, naked.

Pereopod 3 (Fig. II-X-5C) subchelate, similar to pereopod 2 except in numbers of spiniform setae with sensory bristle and simple setae. Palm with seven spiniform setae with sensory bristle.

Pereopod 4 (Fig. II-X-5D, d1) narrower than pereopods 2 and 3. Basis with four dorsal plumose sensory setae and 10 simple setae. Ischium with two dorsal and 15 ventral simple setae, and ventral seta (tip broken). Merus with two dorsal and 10 ventral simple setae. Carpus rectangular, with three ventral spiniform setae with sensory bristle, three outer dorsodistal tiny spiniform setae, one dorsal plumose sensory seta, and two dorsodistal and eight ventral simple setae. Propodus narrowly rectangular, with three ventral spiniform setae with sensory bristle, one dorsodistal plumose sensory seta, seven dorsal and one ventrodistal simple setae, and 12 ventral small triangular processes. Dactylus with four ventral, four ventrodistal, and four outer simple setae. Unguis about one-sixth dactylus length, naked.

Pereopods 5–7 (Fig. II-X-5E–G, g1) similar to pereopod 4 except in number of spiniform setae with sensory bristle, plumose sensory setae, and simple setae. Carpi with four (pereopod 5) or five (pereopods 6 and 7) ventral spiniform setae with sensory bristle.

Pleopod 1 (Fig. II-X-6A) protopod with six inner hooks. Exopod with 45 plumose setae and 27 simple setae on surface. Endopod with 24 plumose setae.

Pleopods 2–5 (Fig. II-X-6B–E) similar. Protopods with two or three inner hooks and zero or one outer simple seta. Exopods 2–5 with slit in middle and zero or one outer

proximal simple seta; 19, 17, 15, and 14 distal plumose setae, respectively; and zero, one, one, and five short simple setae, respectively. Endopods 2–5 articulate, with nine, seven, six, and seven distal plumose setae, respectively.

Uropod (Fig. II-X-6F, G) protopod triangular-prism shaped, with three outer distal plumose setae, two inner and 20 outer simple setae, and one inner and one outer setae (tips broken). Endopod (Fig. II-X-6F) triangular, tapering, 2.04 times longer than wide, with four distal plumose sensory setae, 46 simple setae, and six setae (tips broken). Exopod (Fig. II-X-6G) narrow, tapering, 2.72 times longer than wide, with 20 plumose setae, 45 simple setae, and 24 setae (tips broken).

Description of male allotype. Body (Fig. II-X-3C) similar to female, with same pigmentation pattern. Eye length 0.20 times head length, with scattered ommatidia. Telson length 2.47 times width; base 0.73 times width.

Antennula (Fig. II-X-7A) with three peduncular and six flagellar articles. Peduncular article 1 longest, with one distal plumose sensory seta and three simple setae; article 2 with two outer plumose sensory setae and three distal simple setae; article 3 with six distal simple setae. Flagellar article 1 with one plumose sensory seta; article 2 naked; article 3 with two aesthetascs and five distal simple setae; article 4 with two aesthetascs and four distal simple setae; article 5 tiny, with one aesthetasc; article 6 tiny, with one aesthetasc and four simple setae.

Antenna with five peduncular articles and several tiny flagellar articles; similar to that in female, except in number of simple setae.

Mandible with 3-articulate palp (Fig. II-X-7B). Palp article 1 naked; article 2 longest, with one seta (tip broken); article 3 with eight spiniform setae.

Maxilla lost during dissection.

Maxilliped similar to that in female.

Pereopod 1 broken.

Pereopods 2 and 3 similar to those in female, dactylus more slender; differing in number of simple setae.

Pereopods 4–7 similar to those in female but more slender; carpus with three (pereopods 4, 5, and 7) or four (pereopod 6) spiniform setae and propodus with three spiniform setae; differing from those in female in number of plumose sensory setae and simple setae.

Pleopod 1 (Fig. II-X-7C) protopod with four inner hooks. Exopod with 28 plumose setae (possibly 30; two pores observed) and 20 simple setae on surface. Endopod with 28 plumose setae.

Pleopod 2 (Fig. II-X-7D) protopod with two inner hooks and one inner simple seta. Exopod with a short outer slit in the middle, with 11 distal plumose setae and three outer simple setae. Endopod not articulate, with distal eight plumose setae and appendix masculina reaching past distal margin of endopod and curving to outside. Pleopods 3–5 broken.

Uropod similar to that in female. Exopod 2.81 times longer than wide.

Variation. The range in the ratio of eye length to head length was 0.20–0.22. The spiniform setae on article 3 of the mandibular palp ranged in number from eight for the allotype and paratype ICHUM8807 to 11 for the holotype and paratype ICHUM8808. The numbers were the same for the left and right palps within specimens. The range in the length to width ratio of the uropodal exopod was 2.72–2.84 among six uropodal exopods for three females and one male; the remaining two uropodal exopods were lost (ICHUM8808) or broken (allotype).

Genetic information. A partial COI sequence (658 bp, encoding 219 amino acids; LC778095) was determined from paratype ICHUM8807. In BLAST searches (Altschul et al. 1990), the COI sequence most similar to that from my species was from the congener *P. japonica* (accession number LC661620.1; identity score 77.51%, query cover 100%; Shiraki and Kakui 2022). No other congeneric COI sequences have yet been deposited in public databases.

Remarks. As *P. oriens* **sp. nov.** has fused pleonites 1–5, this species belongs in Group E. *Paranthura oriens* **sp. nov.** differs from its 17 congeners in Group E in the following combination of characters: (1) the apical edge of the telson is rounded, not sharp, (2) the uropodal endopod is triangular and tapering, and (3) the uropodal exopod is narrow, 2.72–2.84 times longer than wide. While the orange pigmentation on the anterior part of head, bases of the antennulae and antennae, and uropod is also characteristic of the new species, I observed it to be somewhat faded 21 months after preservation, and it may be completely lost after long preservation in ethanol. In contrast, the dark brown pigmentation had not faded after 21 months in ethanol.

The above three characters differentiate *P. oriens* **sp. nov.** from the Group A species *P. lineata*, for which information on the condition of pleonites 1–5 is ambiguous (see the Remarks section for *Paranthura* above). *Paranthura oriens* **sp. nov.** also differs from the two species for which the condition of pleonites 1–5 is unknown: from *P. antillensis* in the shape of the uropodal endopod (triangular, longer than wide in *P. oriens* **sp. nov.** vs. subcircular, as long as wide in *P. antillensis*; Barnard 1925), and from *P. neglecta* in the ratio of the length of pleonite 1 to the combined lengths of pleonites 2–4 (pleonite 1 much shorter than pleonites 2–4 in *P. oriens* **sp. nov.** vs. somewhat longer in

P. neglecta; Beddard 1886).

The Kimura (1980) 2-parameter (K2P) distance for COI between *Paranthura oriens* **sp. nov.** paratype ICHUM8807 (LC778095) and a *P. japonica* topotype specimen (LC661620; Shiraki and Kakui 2022) was 27.2%. This value is greater than the maximum 4.79% intraspecific variation observed among several isopod species (Raupach et al. 2015), supporting my conclusion from morphology that *P. oriens* **sp. nov.** and *P. japonica* in Group E are different species.

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CHAPTER III: TAXONOMIC STUDY OF ANTHUROIDEA IN THE PHILLIPINES

INTRODUCTION

The Philippines, an archipelagic country in the tropics, has extensive coastlines and is known as the center of marine biodiversity (Carpenter and Springer 2005). The high diversity of anthuroids is expected in the country as it is known to be high in shallow waters of tropical regions (Poore and Bruce 2012), however, there are only two previous papers dealing with them, which reported two species so far (Table III-1): *Colanthura kensleyi* Poore, 1984 known from Aliguay Island (erroneously referred to as Alibay Island in the original description; Poore 1984) and Little Santa Cruz Island near Mindanao; and *Stygocyathura filipinica* (Botosaneanu & Sket, 1999) known from a freshwater cave in Bohol Island in Visayas (Fig. III-II-1A; Poore 1984; Botosaneanu and Sket 1999).

In Chapter III, I describe two new species, namely *Amakusanthura camiguinensis* **sp. nov.** from Camiguin Island and *Sauranthura liangaensis* **sp. nov.** from Surigao del Sur. This study updates the anthuroid fauna in the Philippines to four species in four genera and two families.

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Chapter III-I. *Amakusanthura camiguinensis* sp. nov. (Anthuridae) from Camiguin Island

INTRODUCTION

The anthuroid isopod genus *Amakusanthura* Nunomura, 1977 is one of 26 anthurid genera, which is characterized by having (1) free pleonites 1–3 and dorsally fused pleonites 4 and 5, (2) 3-articulate maxillipedal palp with the article 1 wider than long and the oblique article 3, (3) pereopod 1 with stepped palm, (4) pereopods 4–7 with triangular carpus bearing one spiniform seta, and (5) leaf-like uropod (Poore 2001; Chew et al. 2014). With 47 species (Boyko et al. 2025), the genus has been reported worldwide (Poore 2001), with the highest diversity in tropical Australia (Poore and Lew Ton 1988). Most of the species have been collected from shallow waters but some species are known from deep sea up to 620 m depth (Poore 2001).

The Philippines is well known as the center of marine biodiversity (Carpenter and Springer 2005), but there are still many understudied invertebrate groups in the country. Recent expeditions in the southern Philippines led by Jamael Abato, aiming to reveal the marine invertebrate fauna, have been unveiling the high diversity of the overlooked groups (Abato et al. 2023, 2025). In this study, as a part of the project, I describe a new *Amakusanthura* species collected from the shallow water of Camiguin Island, Mindanao, Philippines. I also determined partial sequences of the 18S rRNA (18S) and 28S rRNA (28S) genes from the new species.

MATERIALS AND METHODS

Five individuals were collected from coral rubble covered with algae in the subtidal zone (1–2 m) or on the rock intertidally at Brgy. Liong, Guinsiliban, Camiguin Island, Mindanao, Philippines, on 10 March 2024 (Fig. III-I-1). Collected individuals were photographed and then fixed and preserved in 80–99% ethanol. The methods used for dissection, preparation of slides, light microscopy, and drawing were as described in Shiraki et al. (2021). Ontogenetic staging follows Shiraki and Kakui (2024). Body length was measured from the tip of the anterolateral lobe of the head to the tip of the telson, and body width at the widest portion of pereonite 4. Measurements were made axially from digital images by using ImageJ (Schneider et al. 2012). The specimens examined have been deposited in the Zoology Division, National Museum of the Philippines, Manila, Philippines (NMCR 99221, NMCR 99222, NMCR 99224) or the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Japan (ICHUM9013 and ICHUM9014).

Total DNA was extracted from right pereopod 5 of the holotype specimen using a NucleoSpin Tissue XS Kit (Macherey–Nagel, Düren, Germany). Primers for the 18S and 28S used for PCR amplification and cycle sequencing were listed in Table III-I-1. PCR conditions for 18S and 28S with KOD FX Neo (Toyobo Life Science, Osaka, Japan) were 94℃ for 2 min; 45 cycles of 98℃ for 10 s, 52℃ for 30 s and 68℃ for 75 s (18S) or 90 s (28S); and 68℃ for 3 min. All nucleotide sequences were determined by direct sequencing with a BigDye Terminator Cycle Sequencing Kit ver. 3.1 and a 3730 Genetic Analyzer (Life Technologies, Carlsbad, USA). Determined sequences were deposited in the International Nucleotide Sequence Database (INSD) through the DNA Data Bank of Japan (DDBJ) under accession numbers LC864040 (18S) and LC864041 (28S).

TAXONOMY

Family Anthuridae Leach, 1814

Genus *Amakusanthura* Nunomura, 1977

Amakusanthura Nunomura, 1977, p. 79.

Apanthuretta Wägele, 1981a, p. 112 (*nomen nudum*).

Apanthuretta Wägele, 1981b, p. 134.

Type species. *Amakusanthura longiantennata* Nunomura, 1977.

Amakusanthura camiguinensis sp. nov.

(Figs III-I-2–III-I-7)

Diagnosis. Body with brown dotted pigmentation dorsally. Telson with several (four to eleven) long simple setae on surface. Article 2 of mandibular palp with one to three simple setae and article 3 of that with two to three spiniform setae. Maxillipedal endite slightly or moderately exceeding distal margin of maxillipedal palp article 1. Pereopod-1 palm weakly stepped. Pereopod-1 carpus with straight ventral margin. Uropodal exopod dorsodistally deeply concaved (angle 90°).

Etymology. The specific name is an adjective referring to the type locality of the species.

Material examined. Holotype: female lacking oostegites (NMCR 99224; 12 slides and 1 vial), body length 7.66 mm, coral rubble covered with algae, 1–2 m depth,

Brgy. Liong (9°04'43.7"N 124°45'58.7"E), Guinsiliban, Camiguin Island, Mindanao, Philippines, 10 March 2024. Paratypes: female lacking oostegites (ICHUM9013; 6 slides and 1 vial), body length 7.84 mm, collection data same as for holotype; female lacking oostegites (NMCR 99221; 4 slides and 1 vial), body length 5.14 mm, collection data same as for holotype; female lacking oostegites (ICHUM9014; 1 vial), body length 8.91 mm, on rock, intertidally, same sampling site and date as holotype; manca (NMCR 99222; 3 slides and 1 vial), body length 3.04 mm, collection data same as for holotype.

Description of female based on holotype. Body (Figs III-I-2, III-I-3A–E) slender, 11.48 times longer than wide, whitish and with several dorsal brown dots from head through pleonites. Head (Fig. III-I-3A) length 1.22 times head width; rostrum not overreaching anterolateral lobes; eyes dorsolateral, brown, length 0.16 times head length. Pereonites 1–7 (Fig. III-I-3A–C, E) with length ratio 1.39 : 1.43 : 1.19 : 1.50 : 1.46 : 1.28 : 0.90 relative to head length. Pleonites 1–5 (Fig. III-I-3D) together slightly longer than wide, length 1.18 times width, pleonites 1–3 free and pleonites 4 and 5 fused dorsally. Pleonite 6 distinct from telson dorsally, concave on mid-posterior edge. Telson (Fig. III-I-5H) linguiform, with length (from basal narrowest point to apical tip) 1.80 times width, concave at apical edge, with two plumose sensory setae on apical tip, three, two, and two pairs of long simple setae on surface, on posterolateral margin, and on apical tip, respectively, and 45 short simple setae; proximal part with two statocysts.

Antennula (Fig. III-I-3F) with three peduncular and three flagellar articles. Peduncular article 1 longest, with five plumose sensory setae and three outer simple setae; article 2 with three distal plumose sensory setae and five outer long simple setae; article 3 with three distal long simple setae and four distal short simple setae. Flagellar article 1 with simple seta; article 2 with distal simple seta; article 3 with three aesthetascs and four

distal simple setae.

Antenna (Fig. III-I-3G) with five peduncular articles and four flagellar articles. Peduncular article 1 triangular, with simple seta; article 2 with seven simple setae; article 3 with three inner simple setae; article 4 with outer plumose sensory seta and four simple setae; article 5 with three distal plumose sensory setae and six simple setae. Flagellar articles 1–4 with numerous simple setae.

Mandible (Fig. III-I-3H) with 3-articulate palp. Palp article 1 with simple seta; article 2 longest, with two simple setae; article 3 with four membrane-like processes and two distal spiniform setae. Incisor with four cusps. Lamina dentata with four denticulations. Molar round.

Maxilla (Fig. III-I-3I) slender, with seven teeth.

Maxilliped (Fig. III-I-3J) with 3-articulate palp. Palp article 1 trapezoidal, with inner simple seta; article 2 longest, with six inner and one outer simple setae; article 3 oblique, with five inner simple setae. Endite tapering, slightly exceeding distal margin of palp article 1. Epipod lost during dissection.

Pereopod 1 (Figs III-I-4A, III-I-7A) subchelate. Basis with three dorsal plumose sensory setae and nine simple setae. Ischium with nine simple setae. Merus with four simple setae. Carpus trapezoidal, straight on ventral margin, with nine simple setae. Propodus with two dorsodistal, 13 outer short, and seven inner ventral simple setae. Palm weakly stepped, with eleven simple setae. Dactylus with ventrodistal spiniform seta and one ventral, three ventrodistal, and four inner distal simple setae. Unguis nearly as long as dactylus, naked.

Pereopod 2 (Fig. III-I-4B) narrower than pereopod 1. Basis with three dorsal plumose sensory setae and six simple setae. Ischium with two dorsal and two ventrodistal long simple setae and twelve short simple setae. Merus with four dorsal long, one outer

short, and twelve ventral simple setae. Carpus triangular, with ventral short spiniform seta and four ventral simple setae. Propodus with ventrodistal membrane-like process, ventrodistal spiniform seta with sensory bristle sensu Negoescu (1994), ventral short spiniform seta, dorsodistal plumose sensory seta, and 15 dorsal, one mid-outer distal, and 17 ventral simple setae. Dactylus with one ventral, two ventrodistal, and five inner distal simple setae. Unguis about two-fifths dactylus length, naked.

Pereopod 3 (Fig. III-I-4C) similar to pereopod 2 except in numbers of membrane-like processes, short spiniform setae, and simple setae. Dactylus with ventrodistal spiniform seta.

Pereopod 4 (Fig. III-I-4D) slightly narrower than pereopods 2 and 3. Basis with two dorsal plumose sensory setae, five simple setae, and seta (tip broken). Ischium with two dorsal and four ventral long simple setae and ten short simple setae. Merus with three dorsal and six ventral long, and one dorsal and nine ventral short simple setae. Carpus trapezoidal, with ventrodistal spiniform seta, dorsal plumose sensory seta, and one dorsal and eleven ventral simple setae. Propodus with ventrodistal spiniform seta, dorsodistal plumose sensory seta, and eight dorsal and 17 ventral simple setae. Dactylus with ventrodistal spiniform seta and one ventral, two ventrodistal, three outer, and six outer distal simple setae. Unguis about one-third dactylus length, naked.

Pereopods 5–7 (Fig. III-I-4E–G) similar to pereopod 4 except in number of spiniform setae, plumose sensory setae, and simple setae. Pereopod 6 (Figure 4F) with two ventral membrane-like processes on propodus. Pereopod 7 (Figure 4G) with 15 ventral and 22 membrane-like processes on propodus and dactylus, respectively, and two ventrodistal spiniform setae additional to ventrodistal spiniform seta with sensory bristle on propodus.

Pleopod 1 (Fig. III-I-5A) with protopod bearing outer plumose seta and inner

hook. Exopod operculiform, with 37 distal plumose setae (possibly 38; one pore observed) and 31 short simple setae on surface. Endopod with seven plumose setae.

Pleopods 2–5 (Fig. III-I-5B–E) similar. Protopods with two inner hooks, zero or one outer plumose seta, and zero or one outer simple seta. Exopod 2 with slit in middle. Exopods 2 and 4 with 15 distal plumose setae and two and one outer simple setae, respectively. Exopods 3 and 5 lost during dissection. Endopods 2–5 with eight, nine, seven, and seven distal plumose setae, respectively.

Uropod (Fig. III-I-5F, G) protopod triangular-prism-shaped, with eleven outer and six inner plumose setae and six simple setae. Exopod (Fig. III-I-5F) sinuate, dorsodistally deeply concaved (angle 90°), 1.89 times longer than wide, with 63 plumose setae and 78 simple setae. Endopod (Fig. III-I-5G) oval, with distal plumose sensory seta and 67 simple setae.

Variation. All specimens possess the dorsally brown-dotted pigmentation pattern but there is a variation in the number of dots especially on the head (Fig. III-I-6); there were no brown dots in the pereonites 3 and 4 for NMCR 99221 (Fig. III-I-6G, H). The numbers of long simple setae on surface of the telson ranged from four (two pairs) to eleven (Figs III-I-5H, III-I-7F, G), and the numbers of pairs of long simple setae on the apical tip varied from two to three. With respect to mandibular palp, the numbers of simple setae on the article 1, those on the article 2, and the numbers of spiniform setae on the article 3 were one, one to three, and two to three, respectively (Figs III-I-3H, III-I-7D, E). The number of simple setae on the article 3 of the maxillipedal palp was stable (five) in four dissected specimens. The maxillipedal endites were slightly or moderately exceeding the distal margin of article 1 of the maxillipedal palp. The angles of the step on the pereopod-1 palmar margin were obtuse but varied and were nearly 90° in some

pereopods 1 (Fig. III-I-7A–C). The angle of dorsodistal concaved portion on uropodal exopod was stable (90°) in four dissected specimens.

GenBank accession numbers. LC864040 for 18S (2501 bp) and LC864041 for 28S (2475 bp), both from the holotype specimen.

Remarks. *Amakusanthura camiguinensis* **sp. nov.** resembles *Amakusanthura dubia* (Barnard, 1914) and *Amakusanthura vermiformis* Müller, 1992 in having the brown-dotted pigmentation pattern on the body (Figs III-I-2, III-I-3A–E, III-I-6; Barnard 1914; Müller 1992), but differs from them in terms of the pereopod-1 palmar shape: weakly stepped in *A. camiguinensis* **sp. nov.**; strongly projected in *A. dubia*; straight, not stepped in *A. vermiformis*.

Among the other 45 congeners not sharing the body pigmentation-pattern with it, *A. camiguinensis* **sp. nov.** is morphologically similar to *Amakusanthura geminsula* (Kensley, 1982), *Amakusanthura hibbertia* Poore & Lew Ton, 1988, *Amakusanthura libyana* (Negoescu, 1980), and *Amakusanthura magnifica* (Menzies & Frankenberg, 1966) in sharing the maxillipedal endite reaching the distal margin of the palp article 1 and the uropodal exopod dorsodistally deeply concaved (the angle 90°). However, my species differs from the four species in having one to three simple setae on the article 2 of mandibular palp (zero in *A. magnifica* and seven in *A. hibbertia*; Menzies and Frankenberg 1966; Poore and Lew Ton 1988), several (four to eleven) long simple setae on surface of the telson (absent in *A. libyana*; Negoescu 1980), and the straight ventral margin on the carpus of pereopod 1 (sinuate in *A. geminsula*; Kensley 1982), in addition to the dorsal pigmentation pattern on the body.

In the Philippines, two anthuroid species have been reported to date (Poore 1984;

Botosaneanu and Sket 1999): *Colanthura kensleyi* Poore, 1984 known from Aliguay Island and Little Santa Cruz Island near Mindanao; and *Stygocyathura filipinica* (Botosaneanu & Sket, 1999) known from a freshwater cave in Bohol Island in Visayas. This study represents the first report of *Amakusanthura* isopods from the Philippines.

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Chapter III-II. *Sauranthura liangaensis* sp. nov. (Anthuridae) from Surigao del Sur

INTRODUCTION

Sauranthura Poore & Kensley, 1981 is one of the 26 anthurid genera characterized in having pleonites 1–5 together shorter than width, tri-articulate mandibular palp, and uniarticulate maxillipedal palp (Poore 2001, 2009; Chew et al. 2014). It currently contains two species. The type species *Sauranthura goldmanorum* Poore & Kensley, 1981 was described from Australia with the establishment of the genus (Poore and Kensley 1981). Poore (2001) observed additional *Sa. goldmanorum* specimens and amended its diagnosis. The second species *Sauranthura rapanui* Kensley, 2003 was described from Easter Island (Kensley 2003).

In this study, I describe a new *Sauranthura* species collected from shallow water in Lianga, Surigao del Sur, Mindanao, Philippines, amend the generic diagnosis, and synonymize *Sa*. *rapanui* with *Stygocyathura rapanuia* (Botosaneanu, 1987). I also determined the partial sequences of 18S rRNA (18S) and 28S rRNA (28S) from the new species.

MATERIALS AND METHODS

Four individuals were collected at the subtidal zones in Lianga, Surigao del Sur, Mindanao, Philippines, on 18 March 2024 (Fig. III-II-1). Coral rubble covered with algae was collected by SCUBA diving, and rinsed in tap water to remove the epibionts; the effluent was sieved through a 0.45 mm-mesh plankton net and anthuroids were picked

from residue. Three were collected at 4–6 m depth in the Marine Protected Area (MPA) of Brgy. Ganayon (8°39'09.9"N 126°07'32.8"E) and one at 3–6 m depth of Brgy. Payasan (8°36'40.4"N 126°05'18.0"E) (Fig. III-II-1B). Collected individuals were photographed and then fixed and preserved in 80–99% ethanol. The methods used for dissection, preparation of slides, light microscopy, and drawing were as described in Shiraki et al. (2021). Ontogenetic staging follows Shiraki and Kakui (2024). Body length was measured from the tip of the anterolateral lobe of the head to the tip of the telson, and body width at the widest portion of pereonite 5. Measurements were made axially from digital images by using ImageJ (Schneider et al. 2012). The specimens examined have been deposited in the National Museum of the Philippines, Manila, Philippines (NMCR 99220 and NMCR 99223) or the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Japan (ICHUM8983 and ICHUM8984).

Total DNA was extracted from a pereopod using a NucleoSpin Tissue XS Kit (Macherey–Nagel, Düren, Germany). Primers for the 18S and 28S used for PCR amplification and cycle sequencing were listed in Table III-I-1. PCR conditions for 18S and 28S with KOD FX Neo (Toyobo Life Science, Osaka, Japan) were 94℃ for 2 min; 45 cycles of 98℃ for 10 s, 52℃ for 30 s and 68℃ for 75 s (18S) or 90 s (28S); and 68℃ for 3 min. All nucleotide sequences were determined by direct sequencing with a BigDye Terminator Cycle Sequencing Kit ver. 3.1 and a 3730 Genetic Analyzer (Life Technologies, Carlsbad, USA). Determined sequences were deposited in the International Nucleotide Sequence Database (INSD) through the DNA Data Bank of Japan (DDBJ).

TAXONOMY

Family Anthuridae Leach, 1814

Genus *Sauranthura* Poore & Kensley, 1981

Type species. *Sauranthura goldmanorum* Poore & Kensley, 1981.

Amended diagnosis. Body small, less than 4 mm. Head with dark-brown band-like pigmentation between eyes (non-males). Eyes present. Pleonites 1–5 fused, together shorter than wide (non-males). Mandible with tri-articulate palp. Maxillipedal palp articles 1–5 fused, uniarticulate.

Remarks. I added "Mandible with tri-articulate palp" to Poore's (2001) amended diagnosis as the state is crucial to differentiate *Sauranthura* from its sister genus *Pendanthura* Menzies & Glynn, 1968 with uniarticulate mandibular palp (Poore 2001; Chew et al. 2016).

Sauranthura isopods have a pleon shorter than wide (Poore and Kensley 1981, fig. 5; Fig. III-II-3A; erroneously written as "longer than wide" in Poore' [2001] and Chew et al.'s [2014] generic keys). In Anthuridae, the short pleon is known in four genera of Clade 35 sensu Poore (2001), namely, *Cyathura* Norman & Stebbing, 1886, *Pendanthura*, *Sauranthura*, and *Stygocyathura* Botosaneanu & Stock, 1982.

I synonymize *Sauranthura rapanui* Kensley, 2003 with *Stygocyathura rapanuia* (Botosaneanu, 1987). *Sauranthura rapanui* is undoubtedly a member of *Stygocyathura*, not *Sauranthura*, as it has the biarticulate maxillipedal palp, the smooth palmar margin of pereopod-1, the reduced pleopod-1 endopod, and no eyes (Poore 2001). This species has the small body size less than 4 mm, the tip of the slender pleotelson being concave, and the short uropodal endopod being as long as its width, all of which were observed in *St. rapanuia* (Botosaneanu 1987; Kensley 2003). The type localities of the two nominal

species are the same (Easter Island; Botosaneanu 1987; Kensley 2003). Given the above, I concluded that *Sa*. *rapanui* is a junior synonym of *St*. *rapanuia*. Kensley's (2003) specimens, dealt as non-ovigerous females in it, were 1.6–1.9 mm in length and lacked pereopod 7. Botosaneanu's (1987) specimens were 2.3–3.3 mm in length and possessed pereopod 7. These indicate that Kensley's (2003) specimens were manca specimens.

Species composition. Two species: *Sa*. *goldmanorum*; *Sa*. *liangaensis* **sp. nov.**

***Sauranthura liangaensis* sp. nov.**

(Figs III-II-2–III-II-8)

Material examined. Holotype: ovigerous female (NMCR 99220; 12 slides and 1 vial), body length 3.71 mm, coral rubble covered with algae, 4–6 m depth, MPA of Brgy. Ganayon (8°39'09.9"N 126°07'32.8"E), Lianga, Surigao del Sur, Mindanao, Philippines, 18 March 2024. Allotype: subadult male (NMCR 99223; 12 slides and 1 vial), body length 2.98 mm, coral rubble covered with algae, 3–6 m depth, Brgy. Payasan (8°36'40.4"N 126°05'18.0"E), Lianga, Surigao del Sur, Mindanao, Philippines, 18 March 2024. Paratypes: ovigerous female (ICHUM8983; 4 slides and 1 vial), body length 3.20 mm; manca (ICHUM8984; 2 slides and 1 vial), body length 1.89 mm; collection data same as for holotype.

Diagnosis (non-males). Maxillipedal endite short, rounded. Carpus of pereopod 1 trapezoidal, protruded ventrodistally. Carpi of pereopods 4–7 with ventrodistal spiniform seta.

Description of female based on holotype. Body (Figs III-II-2A, B, III-II-3A, B) relatively slender, 7.94 times longer than wide, with several dark-brown dots on pereonites 1–6 (Figs III-II-2A, B, III-II-3A, B). Head (Fig. III-II-3A) length 0.95 times head width, with dark-brown band-like pigmentation between eyes; rostrum overreaching anterolateral lobes; eyes dorsolateral, brown, small, length 0.06 times head length. Pereonites 1–7 (Fig. III-II-3A) with length ratio 0.98:1.01:1.17:1.40:1.42:1.06:0.59 relative to head length. Pleonites 1–5 (Fig. III-II-3A) shorter than width, length 0.61 times width, segmented laterally but fused dorsally, with dorsolateral sutures. Pleonite 6 distinct from telson dorsally, concave on mid-posterior edge. Telson (Fig. III-II-5H) linguiform, with two indentations at apical edge, with two distal plumose sensory setae and 16 simple setae; proximal part with two statocysts.

Antennula (Fig. III-II-3C) with three peduncular and three flagellar articles. Peduncular article 1 longest, with distal seta (tip broken); article 2 with four distal plumose sensory setae and distal simple seta; article 3 with four distal simple setae. Flagellar article 1 with plumose sensory seta; article 2 naked; article 3 with three aesthetascs and four distal simple setae.

Antenna (Fig. III-II-3D) with five peduncular articles and two flagellar articles. Peduncular article 1 triangular, naked; article 2 covered with scale-like structures, with three simple setae; article 3 covered with scale-like structures, with two inner distal simple setae; article 4 with two distal plumose sensory setae and three distal simple setae; article 5 with five distal plumose sensory setae and five distal simple setae. Flagellar article 1 with four simple setae; article 2 with numerous simple setae.

Mandible (Fig. III-II-3E) with tri-articulate palp. Palp article 1 naked; article 2 longest, with distal simple seta and marginal membrane-like process; article 3 with membrane-like process and two distal spiniform setae. Incisor with three cusps. Lamina

dentata with eight denticulations. Molar round.

Maxilla (Fig. III-II-3F) slender, with six teeth.

Maxilliped (Fig. III-II-3G) with uniarticulate palp. Palp long and broad, with five distal long and six short simple setae. Endite short, rounded. Epipod round.

Pereopod 1 (Fig. III-II-4A) subchelate. Basis with three dorsal plumose sensory setae. Ischium with one dorsal and one ventral simple setae. Merus with two dorsal and one ventral simple setae and ventral seta (tip broken). Carpus trapezoidal, protruded ventrodistally, with three ventral simple setae. Propodus with two dorsal and one inner simple setae. Palm with projection in proximal half and weakly bilobed projection in distal half, with five spiniform setae and five simple setae. Dactylus with four inner scale-like structures and one ventral, two ventrodistal, and five inner simple setae. Unguis nearly as long as dactylus, naked.

Pereopod 2 (Fig. III-II-4B) narrower than pereopod 1. Basis with three dorsal plumose sensory setae and three simple setae. Ischium with two dorsal and three ventral simple setae. Merus with two dorsal and three ventral simple setae and ventral seta (tip broken). Carpus trapezoidal, with ventrodistal membrane-like process, ventrodistal spiniform seta with sensory bristle sensu Negoescu (1994), and four simple setae. Propodus with dorsodistal plumose sensory seta and three dorsal and six outer simple setae. Palm with membrane-like process, ventrodistal spiniform setae with sensory bristle, and four simple setae. Dactylus with ventral membrane-like process, ventrodistal spiniform seta, and one ventral, three outer, and six distal simple setae. Unguis about three-quarters dactylus length, naked.

Pereopod 3 (Fig. III-II-4C) similar to pereopod 2 except in numbers of plumose sensory setae and simple setae.

Pereopod 4 (Fig. III-II-4D) slightly narrower than pereopods 2 and 3. Basis with

three dorsal plumose sensory setae and two simple setae. Ischium with one dorsal and three ventral simple setae. Merus with two dorsal and four ventral simple setae. Carpus trapezoidal, with three membrane-like processes, ventrodistal spiniform seta with sensory bristle, dorsal plumose sensory seta, and one dorsal and two ventral simple setae. Propodus with ventral membrane-like process on distal half, ventrodistal spiniform seta with sensory bristle, dorsodistal plumose sensory seta, and one dorsal and three ventral simple setae. Dactylus with one ventral, three ventrodistal, and four outer simple setae. Unguis about two-thirds dactylus length, naked.

Pereopod 5 (Fig. III-II-4E) similar to pereopod 4 except in number of membrane-like processes, plumose sensory setae, and simple setae.

Pereopod 6 (Fig. III-II-4F) similar to pereopods 4 and 5 from basis to merus except in number of plumose sensory setae and simple setae. Distal portion from carpus to unguis lost.

Pereopod 7 (Fig. III-II-4G) longer than pereopods 4 and 5. Basis with two dorsal and two ventral simple setae. Ischium with three ventral simple setae. Merus with two dorsal and six ventral simple setae. Carpus trapezoidal, with three membrane-like processes, ventrodistal spiniform seta with sensory bristle, dorsal plumose sensory seta, and one dorsal and two ventral simple setae. Propodus with two ventral membrane-like processes, ventrodistal spiniform seta with sensory bristle, two ventrodistal three-prolonged spiniform setae, dorsodistal plumose sensory seta, and one dorsal and two ventral simple setae. Dactylus with one ventral, four ventrodistal, and four outer simple setae. Unguis about two-fifths dactylus length, naked.

Pleopod 1 (Fig. III-II-5A) operculiform, with protopod bearing outer plumose seta. Exopod with 29 plumose setae and distal simple seta. Endopod 0.97 times exopod length, with eight plumose setae.

Pleopods 2–5 (Fig. III-II-5B–E) similar. Protopods with zero or two inner hooks, zero or one outer plumose seta, and zero or one outer simple seta. Exopods 2–5 with slit in middle, zero or one outer short plumose seta, and eight, ten, ten, and nine distal plumose setae, respectively. Endopods 2–5 with six, seven, six, and seven distal plumose setae, respectively.

Uropod (Fig. III-II-5F, G) protopod triangular-prism-shaped, with five outer and one inner plumose setae and simple seta. Exopod (Fig. III-II-5F) narrow, 2.82 times longer than wide, with 42 plumose setae and 27 simple setae. Endopod (Fig. III-II-5G) oval, with eight plumose sensory setae and 43 simple setae.

Description of male based on allotype. Body (Figs III-II-2E, III-II-6A–E) relatively slender, 7.33 times longer than wide, with some branched amorphous pigmentations on head, pereonites, and pleon (Figs III-II-2E, III-II-6A–E). Head (Fig. III-II-6A) length 0.97 times head width; rostrum overreaching anterolateral lobes; eyes dorsolateral, dark-brown, large, length 0.35 times head length. Pereonites 1–7 (Fig. III-II-6A–D) with length ratio 1.04:1.02:1.01:1.06:0.96:0.85:0.48 relative to head length. Pleonites 1–5 (Fig. III-II-6D) roughly as long as width, length 1.04 times width, segmented laterally but fused dorsally. Pleonite 6 distinct from telson dorsally, concave on mid-posterior edge. Telson (Fig. III-II-8I) linguiform, with two small indentations at apical edge, with two distal plumose sensory setae and 16 simple setae at the edge and 15 dorsal simple setae; proximal part with two statocysts.

Antennula (Fig. III-II-6F) with three peduncular and six flagellar articles. Peduncular article 1 longest, with distal plumose sensory seta; article 2 with two distal plumose sensory setae and one distal simple setae; article 3 with distal simple seta and two distal setae (tip broken). Flagellar articles 1–4 naked; article 5 with aesthetasc; article

6 with two aesthetascs and two simple setae.

Antenna (Fig. III-II-6D) with five peduncular articles and three flagellar articles. Peduncular article 1 triangular, naked, covered with scale-like structures; article 2 covered with scale-like structures, with three simple setae; article 3 with two distal simple setae; article 4 with two distal plumose sensory setae, four distal simple setae, and seta (tip broken); article 5 with five distal plumose sensory setae and four distal simple setae. Flagellar articles 1–3 with several to numerous simple setae.

Mandible (Fig. III-II-6H) with tri-articulate palp. Palp article 1 naked; article 2 longest, with distal simple seta; article 3 with membrane-like process, two distal spiniform setae, and two distal simple setae. Incisor with three cusps. Lamina dentata with ten denticulations. Molar round.

Maxilla lost.

Maxilliped (Fig. III-II-6I) with uniarticulate palp. Palp long and broad, with 14 simple setae and seta (tip broken). Endite broad, length 0.63 times palp; tip not pointed. Epipod round.

Pereopod 1 (Fig. III-II-7A) subchelate. Basis with four dorsal plumose sensory setae and four simple setae. Ischium with one dorsal and one ventral simple setae. Merus with one dorsal and two ventral simple setae. Carpus trapezoidal, protruded ventrodistally, with three membrane-like processes and three ventral simple setae. Propodus with three dorsal, one inner, and two outer simple setae. Palm with projection in proximal half and weakly bilobed projection in distal half, with nine simple setae. Dactylus with one ventral, two ventrodistal, and four inner simple setae. Unguis as about three-fourth dactylus length, naked.

Pereopod 2 (Fig. III-II-7B) narrower than pereopod 1. Basis with two dorsal plumose sensory setae and four simple setae. Ischium with one dorsal and two ventral

simple setae. Merus with two dorsal and seven ventral simple setae. Carpus trapezoidal, with three ventral membrane-like processes, ventrodistal spiniform seta with sensory bristle, and five simple setae. Propodus with dorsodistal plumose sensory seta and five dorsal and three outer simple setae. Palm with several membrane-like processes, ventrodistal spiniform setae with sensory bristle, and six simple setae. Dactylus with ventrodistal spiniform seta and one ventral, one dorsal, three outer, three inner, and three ventrodistal simple setae. Unguis about half dactylus length, naked.

Pereopod 3 (Fig. III-II-7C) similar to pereopod 2 except in numbers of membrane-like processes, plumose sensory setae, and simple setae.

Pereopod 4 (Fig. III-II-7D) slightly narrower than pereopods 2 and 3. Basis with three dorsal plumose sensory setae, two simple setae, and seta (tip broken). Ischium with one dorsal and four ventral simple setae. Merus with two dorsal and six ventral simple setae. Carpus trapezoidal, with two membrane-like processes, ventrodistal spiniform seta with sensory bristle, dorsal plumose sensory seta, and one dorsal and two ventral simple setae. Propodus with two ventral membrane-like processes, ventrodistal spiniform seta with sensory bristle, dorsodistal plumose sensory seta, and two dorsal and four ventral simple setae. Dactylus with one ventral, two ventrodistal, and four outer simple setae. Unguis about two-fifths dactylus length, naked.

Pereopods 5–7 (Fig. III-II-7E–G) similar to but longer than pereopod 4 except in number of membrane-like processes, plumose sensory setae, and simple setae. Propodus of pereopod 7 with two ventrodistal three-prolonged spiniform setae.

Pleopod 1 (Fig. III-II-8A) operculiform, with protopod bearing four inner hooks and outer plumose seta. Exopod with 30 plumose setae and three simple setae on surface. Endopod 0.88 times exopod length, with ten plumose setae.

Pleopod 2 (Fig. III-II-8B) protopod with three inner hooks and outer simple seta.

Exopod with 15 plumose setae. Endopod 1.09 times exopod length, with six plumose setae and appendix masculina (Fig. III-II-8B, C) overreaching distal margin of endopod and swollen distally.

Pleopods 3–5 (Fig. III-II-8D–F) similar. Protopods with two, three, and zero inner hooks, respectively and outer plumose seta. Exopods 3–5 with slit in middle and zero or one outer short simple seta; 13, twelve, and eleven distal plumose setae, respectively. Endopods 3–5 with seven, seven, and eight distal plumose setae, respectively.

Uropod (Fig. III-II-8G, H) protopod triangular-prism-shaped, with four outer and four inner plumose setae. Exopod (Figure 8G) narrow, 2.85 times longer than wide, with 43 plumose setae and 46 simple setae. Endopod (Fig. III-II-8H) oval, with six plumose sensory setae, 58 simple setae, and two setae (tip broken).

Colour. See the fourth paragraph of the Remarks for the species.

Size. 2.98–3.71 mm in length in non-mancae. 1.89 mm in length in manca.

Distribution. The species is known from Lianga, Surigao del Sur, Mindanao, Philippines.

Habitat. Coral rubble covered with algae (Fig. III-II-1C, D).

Etymology. The specific name is an adjective referring to the type locality.

GenBank accession numbers. LC858703 for 18S (2111 bp) from holotype and LC858704–LC858706 for 28S (all 2513 bp) from NMCR 99220, ICHUM8983, and

NMCR 99223, respectively. In BLAST searches (Altschul et al. 1990), the 18S sequence most similar to mine (LC858703) was from the paranthurid *Paranthura nigropunctata* (Lucas, 1846) (AF279598; identity score 95.78%, query cover 75%; Dreyer and Wägele 2002), and 28S (LC858704) was from the anthurid *Cyathura* sp. (KC428835; identity score 77.96%, query cover 72%; Hurt et al. 2013). Three 28S sequences showed 0.04% of genetic distance (p-distance). 28S sequences from two ovigerous females shared the haplotype and there is one nucleotide substitution between the sequences from subadult male and ovigerous females.

Remarks. *Sauranthura liangaensis* **sp. nov.** is distinguishable from the sole congeneric species *Sa*. *goldmanorum* in non-males having short rounded maxillipedal endite (long and narrowly tapering; character state of *Sa*. *goldmanorum* is in parentheses hereafter), trapezoidal and ventrodistally protruded carpus of pereopod 1 (rounded, not protruded), and ventrodistal spiniform seta on carpus of pereopods 4–7 (no spiniform seta) (Poore and Kensley 1981). These diagnostic characters of my new species are shared in one manca and two females I examined.

I concluded that my subadult male is conspecific with ovigerous females as their 28S sequences are highly similar (only one nucleotide substitution and 0.04% genetic distance in p-distance). My subadult male shared the latter two diagnostic characters with non-male individuals: ventrodistally protruded carpus of pereopod 1; and carpi of pereopods 4–7 each having ventrodistal spiniform seta. *Sauranthura liangaensis* **sp. nov.** showed following sexually dimorphic characters on (character state in parentheses, non-males vs. subadult male): the size of eyes (length 0.06–0.13 times head length vs. 0.35 times), the number of flagellar articles of antennule (three vs. six), the shape of maxillipedal endite (shorter than wide vs. longer than wide), the shape of pereopods 4–7

(narrower in subadult male), and the appendix masculina on pleopod 2 (absence vs. present).

The presence of ventrodistal spiniform seta on carpus of pereopods 4–7 in *Sa. liangaensis* **sp. nov.** contradicts one of the synapomorphies of Clade 35 sensu Poore (2001). Species of the four genera in Clade 35 lack the spiniform seta (Poore 2001). This study denies the character as a synapomorphy of Clade 35.

Poore (2001) mentioned that the pigmentation pattern is species-specific in *Sauranthura*. This study revealed that it is not species-specific. As seen in Fig. III-II-2, non-male specimens (two ovigerous females and one manca) shared dark-brown band-like pigmentation between eyes, but there are pigmentation differences on pereonites and pleon. On pereonites, holotype ovigerous female has several dot-like pigmentations, paratype ovigerous female has several relatively bigger amorphous pigmentations, and manca has no pigmentation. On pleon, both ovigerous females have amorphous pigmentation but manca has no pigmentation. The pattern in subadult male greatly differed from that of non-males. The former lacks band-like pigmentation between eyes but has some branched amorphous pigmentations on the head, pereonites, and pleon instead. The band-like pigmentation between eyes was observed in a manca of *Sa. goldmanorum*, too, suggesting non-males of *Sauranthura* may share this pattern.

I observed that live *Sa. liangaensis* **sp. nov.** individuals strongly grasped other anthuroids with the pereopods 1 when I am sorting isopods during the faunal survey. Anthuroids are assumed to be predators generally based on a few feeding observations (Poore and Bruce 2012). Although there is no feeding data on *Sauranthura* and I could not observe any predations by my new species, considering its aggressiveness, I speculate that the species is also predators and attacks prey using the subchelate pereopods 1 like other anthurids (cf. Horikoshi 2012; observed for *Cyathura*).

This chapter and Chapter III-I reports the third and fourth anthuroid species from the Philippines following *Colanthura kensleyi* Poore, 1984 and *Stygocyathura filipinica* (Botosaneanu & Sket, 1999) (Poore 1984; Botosaneanu and Sket 1999). The four species represent four genera and two families (Table III-1). Given the species numbers reported in other tropical areas, e.g. 15 species in the Fiji Islands (including unnamed species; Kensley 1979; Wägele et al. 1987; Negoescu 1999) and 24 species in Malaysia (Chew and Abdul Rahim 2020), it is likely that the above species number is a fraction of the actual anthuroid diversity in the Philippines, with many species remained undiscovered.

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CHAPTER IV: PARASITES OF ANTHUROIDS

INTRODUCTION

The knowledge on the parasites of anthuroids is quite limited. So far, animals in only three taxa, namely, Epicaridea (Crustacea: Isopoda), Rhizocephala (Crustacea: Thecostraca), and Trematoda (Platyhelminthes: Neodermata), are known to parasitize anthuroid isopods. As for epicarideans, Wägele (1979) reported a non-female unnamed epicaridean parasitic on *Eisothistos macrurus* Wägele, 1979. As for rhizocephalans, Smith (1906) described *Duplorbis calathurae* Smith, 1906 parasitic on *Calathura brachiata* (Stimpson, 1853), which is currently the sole rhizocephalan utilizing anthuroids as hosts. As for trematodes, several microphallid species are known to parasitize three anthurid species (*Anthura gracilis* [Montagu, 1808], *Cyathura polita* [Stimpson, 1855], and *Cyathura carinata* [Krøyer, 1847]) so far (Villot 1879; Burbanck 1962; Reimer 1963; Schulenburg and Wägele 1998; Schulenburg et al. 1999; Jensen et al. 2004; Ferreira et al. 2005a, 2005b). Additionally, the harpacticoid copepod *Cithadius cyathurae* Bowman, 1972 is associated with *Cyathura polita* (Bowman 1972), but in this case, the copepod is just using the *Cyathura* species as the habitat and seems not harmful on the host. To better understand the role of anthuroids in the ecosystem, it is also important to investigate which parasites utilize them as hosts and in what numbers.

In Chapter IV, I firstly report cabiropid epicarideans from anthuroids with the establishment of *Anthuroniscus* **gen. nov.** and the descriptions of three new species (*Anthuroniscus shimomurai* **sp. nov.**, *Anthuroniscus dentatus* **sp. nov.**, and *Anthuroniscus latus* **sp. nov.**), describe the second anthuroid-parasitic rhizocephalan *Duplorbis*

changeling **sp. nov.**, and firstly report trematodes parasitic to *Cyathura muromiensis* Nunomura, 1974.

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Chapter IV-I. Integrative taxonomy of Cabiropidae (Isopoda: Epicaridea) with descriptions of *Anthuroniscus* gen. nov. and the three new species

INTRODUCTION

The parasitic isopod group Epicaridea Latreille, 1825 (containing Bopyroidea Rafinesque, 1815 and Cryptoniscoidea Kossmann, 1880) utilizes crustaceans as both intermediate and definitive hosts (Williams and Boyko 2012; An et al. 2023). As far as is currently known, epicarideans use only planktonic copepods as intermediate hosts, but members of various groups as definitive hosts, including decapods, ostracods, peracarids, and cirripedes (Williams and Boyko 2012). The life cycle contains three larval stages (Williams and An 2009): epicaridium (first stage; pelagic), microniscus (second stage; parasitic on intermediate host), and cryptoniscus (third stage; pelagic or parasitic on definitive host) larvae.

Cryptoniscoidea currently contains twelve valid families (Asconiscidae Bonnier, 1900; Cabiropidae Giard & Bonnier, 1887; Capitoniscidae Boyko & Williams, 2023; Crinoniscidae Bonnier, 1900; Cryptoniscidae Kossmann, 1880; Cumoechidae Boyko & Williams, 2023; Cyproniscidae Giard & Bonnier, 1887; Dajidae G. O. Sars, 1883; Entophilidae Richardson, 1903; Hemioniscidae Bonnier, 1900; Podasconidae Giard & Bonnier, 1895; and Stellatoniscidae Oanh & Boyko, 2020; Boyko et al. 2024a). These families are defined mainly by the host taxa rather than by synapomorphies, and the relationships among them remain unresolved (Boyko 2013; Boyko et al. 2013). Cryptoniscoidea also contains ten monotypic genera treated as family *incertae sedis* (Buhl-Mortensen et al. 2020; Boyko et al. 2024b), nine of which were described only

from cryptoniscus larvae; the remaining one, *Apocumoechus* Nierstrasz & Brender à Brandis, 1931, was based only on females. Cryptoniscoid females have a highly reduced, sac-like, unsegmented body, whereas males retain the segmented body. Morphologically, immature females and males are indistinguishable at the segmented cryptoniscus larval stage (Hosie 2008; Boyko 2013).

Cabiropidae, with 14 genera (Boyko 2013; Buhl-Mortensen et al. 2020; Williams et al. 2024), is one of two cryptoniscoid families parasitic on isopods (Oanh and Boyko 2020). Both males and mature females are known for six genera (*Ancyroniscus* Caullery & Mesnil, 1919; *Arcturocheres* Hansen, 1916; *Bourdonia* Rybakov, 1990; *Cabirops* Kossmann, 1884; *Cirolanoniscus* Pillai, 1966; and *Clypeoniscus* Giard & Bonnier, 1895), whereas only mature females are known for four genera (*Aegoniscus* Barnard, 1925; *Gnomoniscus* Giard & Bonnier, 1895; *Munnoniscus* Giard & Bonnier, 1895; and *Perezina* Nierstrasz & Brender à Brandis, 1930). These genera are classified into four groups based on female morphology (Boyko 2013). The other four genera (*Cironiscus* Nielsen, 1967; *Podoniscus* Bourdon, 1981; *Pleuroniscus* Williams & Boyko in Williams et al., 2024; and *Rolandoniscus* Boyko, 2013) lack information on mature females. Various isopod groups serve as hosts for cabiropids, including asellotes, cymothooid cymothoidans, epicarideans, sphaeromatideans, and valviferans (Table IV-I-1). To date, there are no verified records of cabiropids parasitizing anthuroid isopods, although an epicaridean reported by Wägele (1979) on *Eisothistos macrurus* Wägele, 1979 may be such a case.

During a shallow-water faunal survey in Japan, I collected cabiropids parasitic on anthuroids. This represents the first report of cryptoniscoids parasitizing anthuroid and the first report of cabiropids from Japan. Through morphological observations and a phylogenetic analysis, I detected three different, undescribed species and concluded that a new genus should be established for them. Here I erect the new genus, describe the three

new species and analyze their partial 18S rRNA (18S) gene sequences to infer their phylogenetic position in Epicaridea, and present partial 16S rRNA (16S) gene sequences for future DNA barcoding.

MATERIALS AND METHODS

Sampling, observation, and host identification. Three host anthuroid isopods were collected from three localities in Japan (Fig. IV-I-1). The first host individual, identified as *Mesanthura* sp. and probably parasitized by a cabiropid cryptoniscus larva (the parasite was not recognized when collected, but a female cabiropid appeared later through rearing; see Results), was collected from seaweed at 0.5 m depth at Kaichu Doro, Uruma, Okinawa, southwestern Japan (26°19'57.1"N 127°55'22.6"E), on 21 April 2023. The living host with parasite was maintained for 11 days in a small aquarium filled with seawater from the sampling locality, at room temperature and under ambient light. The host and a female cabiropid were fixed and preserved in 99% ethanol on 2 May 2023 when the host died. The second host individual, identified as *Accalathura* sp. and parasitized by a cabiropid cryptoniscus larva, was collected from coral rubble at 10–15 m depth at Irabu Island, Miyako Islands, Okinawa, southwestern Japan (24°51'54.0"N 125°09'22.2"E), on 23 April 2022, and fixed in 70% ethanol and preserved in 99% ethanol. The third host individual, identified as *Colanthura nigra* Nunomura, 1975 and parasitized by a cabiropid cryptoniscus larva, was collected from seaweed at 1 m depth at Jogashima, Kanagawa, central Japan (35°08'11.4"N, 139°36'39.3"E), on 14 July 2022, and fixed and preserved in 70% ethanol.

The habitus of the female parasite was observed and photographed by using a Nikon SMZ1500 stereomicroscope and an Olympus BX51 digital camera. After removal

of the posterior portion for DNA extraction (see below), the remaining part was mounted on glass slides in glycerin. Exoskeletons of cryptoniscus parasites that were recovered after DNA extraction (see below) were mounted on glass slides in glycerin. Slides were observed with an Olympus BX53 microscope and then sealed with Canada balsam. Illustrations were prepared with Adobe Illustrator CC from draft line drawings made from sequential photographs (female) or with a camera lucida (cryptoniscus larvae). In the cryptoniscus parasites, body length was measured from tip of cephalon to tip of telson, and body width at the widest portion of pereonite 5; the body length of the female was measured from the anterior to posterior ends of the body. I treated segmented cabiropid specimens as cryptoniscus larvae; males in previous studies are treated as cryptoniscus larvae in terms of their morphology in this study, since this larval stage is indistinguishable from males and immature females (Hosie 2008; Boyko 2013).

Host isopods were partly dissected with sharpened tungsten needles under a Nikon SMZ1500 microscope, and their appendages were observed with an Olympus BX53 microscope. Identifications of the host anthuroid species were based on Poore (2001) for *Mesanthura* sp., Poore (2001) and Jarquín-Martínez and García-Madrigal (2021) for *Accalathura* sp., and Nunomura (1975) and Poore (1984) for *C. nigra*. I treated host specimens with slender antennulae as females and those with swollen antennulae as males.

The three parasite specimens and their host anthuroids have been deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Japan. Species in the systematics section are treated in alphabetical order after the type species.

Molecular analysis. Total DNA was extracted from the posterior part (female

parasite) or whole body (cryptoniscus parasites) by using a NucleoSpin Tissue XS Kit (Macherey-Nagel, Germany); cryptoniscus exoskeletons were recovered and mounted after extraction. For the 16S gene, primers Copepod16S_F and Copepod16S_R (Kakui et al. 2023) were used for PCR amplification and cycle sequencing; for 18S, primers18S-a1F and 18S-a9R (Kakui et al. 2011) were used for PCR amplification and 18S-b3F, 18S-b4F, 18S-b4R, 18S-b5F, 18S-b6F, 18S-a6R, 18S-b8R, and 18S-b8F (Kakui et al. 2011; Kakui and Shimada 2017; Kakui et al. 2021) for cycle sequencing. Amplification conditions for 16S with KOD One (Toyobo Life Science, Japan) were as described by Kakui et al. (2023); those for 18S with KOD One (a female and a cryptoniscus larvae from Jogashima) were 45 cycles of 98°C for 10 s, 53°C for 5 s, and 68°C for 10 s. Those for 18S with KOD FX Neo (Toyobo Life Science; the cryptoniscus larva from Irabu) were 94°C for 2 min; 45 cycles of 98°C for 10 s, 52°C for 30 s, and 68°C for 75 s; and 68°C for 3 min. All nucleotide sequences were determined by direct sequencing with a BigDye Terminator Cycle Sequencing Kit ver. 3.1 and 3730 DNA Analyzer (Life Technologies, USA). Sequences were deposited in the International Nucleotide Sequence Database (INSD) through the DNA DATA Bank of Japan (DDBJ).

The 18S dataset for phylogenetic analysis included three sequences I determined, and 14 bopyroid, four cryptoniscoid, and one outgroup sequences (1915–2607 bp; Table IV-I-2) taken from DNA databases. The dataset was aligned by using MAFFT ver. 7 (Katoh and Standley 2013) with the “Q-INS-I” strategy (Katoh and Toh 2008), and then trimmed with MEGA7 (Kumar et al. 2016) to match the shortest length among sequences. Alignment-ambiguous sites were then removed with Gblocks ver. 0.91b (Castresana 2000) in NGPhylogeny.fr (Lemoine et al. 2019) under the “relaxed” parameters described in Talavera and Castresana (2007), for a final length of 1578 positions. Methods for selecting the optimal substitution model (GTR+F+R4), the maximum likelihood (ML)

analysis, estimating nodal support (1000 pseudoreplicates for both SH-like approximate likelihood ratio tests [SH-aLRT] and ultrafast bootstraps [UFBoot]), and drawing the tree were as described by Shimada et al. (2023). I considered nodes having SH-aLRT ≥ 80% and UFBoot ≥ 95% to be well supported.

TAXONOMY

Family Cabiropidae Giard & Bonnier, 1887

[New Japanese name: Waraji-yadori-mushi-ka]

Genus *Anthuroniscus* gen. nov.

[New Japanese name: Uminanafushi-yadori-zoku]

Type species. *Anthuroniscus shimomurai* **sp. nov.**, by original designation.

Etymology. The generic name is a combination of *anthura-* (the stem of Anthuroidea, the host group) and *-oniscus* (a common suffix for cryptoniscoid genera). The gender is masculine.

Diagnosis. Female: body dorsally compressed, elongate, tapering posteriorly; segments fused, with six pairs of lateral bulges. Cryptoniscus larva: eyes lacking; antennular article 1 with eight teeth on posterior margin; uropodal exopod and endopod rectangular, not tapering, endopod longer than exopod; pleotelson trapezoidal, width twice the length.

Anthuroniscus shimomurai sp. nov.

[New Japanese name: Soramame-uminanafushi-yadori]

(Figs IV-I-2–IV-I-4)

Diagnosis. Female: same as for the genus. Male/cryptoniscus larva unknown.

Etymology. The specific name is a noun in the genitive case honoring Michitaka Shimomura, who has greatly contributed to the taxonomy of isopods and related groups in Japan.

Material examined. Holotype: female (ICHUM8643; 1 vial), body length 3.10 mm, 26°19'57.1"N 127°55'22.6"E, 0.5 m depth, Kaichu Doro, Uruma, Okinawa, Japan, 21 April 2023, from marsupium of female *Mesanthura* sp. (ICHUM8644; 2 slides and 1 vial; Anthuridae Leach, 1814).

Attachment site. Inside the marsupium of female *Mesanthura* sp.

GenBank accession numbers. Holotype ICHUM8643: LC794718 (18S) and LC794716 (16S).

Description of holotype female. Body elongate, dorsally compressed, tapering posteriorly, length 3.10 mm, segments fused, with six pairs of lateral bulges (Figs IV-I-3*a*, *b*, IV-I-4). Head with one pair of eyes, and one ventral hook posteriorly (Fig. IV-I-3*c*, *e*). Putative pereopod 1 located between head and first pair of bulges (Fig. IV-I-3*c*, *d*).

Remarks. The female *Mesanthura* sp. from which I collected the holotype

individual was ovigerous on 21 April 2023 (Fig. IV-I-2*b*, *d*), and I did not detect the mature female parasite at that time. On 29 April, however, eggs were observed not in the marsupium but on the bottom of the aquarium, and the mature female parasite was in the marsupium instead (Fig. IV-I-2*c*, *e*). No observations were made between 21 and 29 April. This suggests that, during 8-day period with no observations, the larval parasite molted, pushed out the host's eggs, and grew to occupy >50% of the space of the host marsupium. It should be noted that, as I failed to count the number of eggs on the bottom of the aquarium, the parasite may have eaten some eggs as expected in other cryptoniscoids (Williams et al. 2022) No increase in the size of the parasite was observed between 29 April and 1 May (Fig. IV-I-2*e*, *f*). The host died on 2 May 2023.

***Anthuroniscus dentatus* sp. nov.**

[New Japanese name: Gizagiza-uminanafushi-yadori]

(Figs IV-I-5–IV-I-7)

Diagnosis. Cryptoniscus larva: body teardrop shaped, length 2.89 times body width; pereomeres 1–7 with coxal plate tooth formula of 4, 5, 5, 4, 4, 4, 4; pygidium with eight teeth; propodi of pereopods 6 and 7 with two stout setae. Female/male unknown.

Etymology. The specific epithet *dentatus* (Latin: toothed) is a singular adjective in the nominative case, referring to the toothed pygidium of the pleotelson.

Material examined. Holotype: cryptoniscus larva (ICHUM8645; 1 slide), body length 0.45 mm, 24°51'54.0"N 125°09'22.2"E, 10–15 m depth, Irabu Island, Miyako Islands, Okinawa, Japan, 23 April 2022, from right pereopod 3 of male *Accalathura* sp.

(ICHUM8646; 1 slide and 1 vial; Leptanthuridae Poore, 2001)

Attachment site. Right pereopod 3 of male *Accalathura* sp.

GenBank accession number. Holotype ICHUM8645: LC794719 (18S).

Description of holotype cryptoniscus larva. Body (Fig. IV-I-6*a*, *c*) teardrop shaped, length 0.45 mm, 2.89 times body width, widest at pereonite 5 (0.15 mm). Cephalon anterior margin rounded, with several knobs on anterior part. Eyes absent.

Antennule (Figs IV-I-6*a*, *c*, IV-I-7*a*) article 1 without demarcated area and with two distal simple setae and eight teeth on posterior margin; fifth tooth from inner margin with dorsal cylindrical structure bearing simple seta. Article 2 tridentate on distal margin, with two middle and two distal simple setae. Article 3 rounded, covered ventrally by article 2, with mid-ventral setal area and two conical extensions each bearing two and four simple setae. Antenna (Fig. IV-I-6*a*, *c*) with four peduncular articles and five flagellar articles. Peduncular article 1 broadest; articles 2–4 each with distal simple seta. Flagellar articles 1–4 naked; article 5 with several simple setae. Oral cone (Fig. IV-I-6*a*) triangular, directed ventrally.

Pereomeres 1–7 with coxal plate tooth formula of 4, 5, 5, 4, 4, 4, 4 (Fig. IV-I-6*b*). Pereopods 1 (Fig. IV-I-7*b*; P1) and 2 (Fig. IV-I-7*c*; P2) similar, subchelate, short. Dactylus curved, simple, not bifid, with three outer spiniform projections. Propodus oval, with bifurcate stout seta, simple stout seta, and three (P1) or one (P2) simple setae. Carpus triangular, with distal lamellar projection and two (P1) or one (P2) simple setae. Merus rounded, with outer stout seta bearing seta, and distal simple seta. Ischium with outer lamellar projection. Basis cylindrical, naked. Pereopod 3 (Fig. IV-I-7*d*) subchelate,

narrower than pereopods 1 and 2. Dactylus slender, elongate. Propodus with three stout setae. Carpus trapezoidal, with stout seta and simple seta. Merus with outer stout seta and two simple setae. Ischium with outer ventral and outer dorsal bifurcate lamellar projections. Pereopods 4 (Fig. IV-I-7*e*) and 5 similar to pereopod 3 but propodi narrower and carpi triangular. Pereopods 6 (Fig. IV-I-7*f*) and 7 similar, narrower than pereopods 1–5. Dactylus slender, elongate. Propodus elongate, tapering, with two stout setae. Carpus triangular, with stout seta and simple seta. Merus with outer stout seta and two simple setae. Ischium with outer dorsal bifurcate lamellar projection.

Pleon with five pairs of pleopods. All pleopods similar in shape; sympod rounded, endopod rectangular, exopod pyriform and narrow proximally (Fig. IV-I-6*a*). Pleopod 1 (Fig. IV-I-7*g*) sympod with two inner simple setae. Endopod with three distal simple setae and exopod with four distal simple setae. Uropod (Fig. IV-I-7*h*) biramous, sympod with three outer simple setae. Endopod rectangular, not tapering, with three distal simple setae and two distal triangular lamellar projections. Exopod rectangular, not tapering, 0.56 times as long as endopod, with four distal simple setae, distal seta (tip broken), and two distal triangular lamellar projections. Pleotelson (Fig. IV-I-6*c*) trapezoidal; pygidium (Fig. IV-I-6*d*) with eight teeth.

Remarks. The cryptoniscus larva of *Anthuroniscus dentatus* **sp. nov.** was found on a male anthuroid host (Fig. IV-I-5*a*). As with *A. shimomurai* **sp. nov.** and other cabiropids (Boyko 2013), mature females of *A. dentatus* **sp. nov.** likely inhabit the marsupium of host females. Although little information is available on anthuroid reproduction, Horikoshi (2012) reported, for the anthurid *Cyathura muromiensis* Nunomura, 1974, the temporary cohabitation of a male and a female in a single tube, until female oviposition. During this period of cohabitation, a cabiropid on the male host might

move to inside the marsupium of the female.

Anthuroniscus latus sp. nov.

[New Japanese name: Futo-maru-uminanafushi-yadori]

(Figs IV-I-8–IV-I-10)

Diagnosis. Cryptoniscus larva: body teardrop shaped, length 2.58 times body width; pereomeres 1–7 with coxal plate tooth formula of 3, 4, 4, 4, 4, 4, 4; pygidium roundly projecting distally; propodi of pereopods 6 and 7 without stout seta. Female/male unknown.

Etymology. The specific epithet *latus* (Latin: broad) is a singular adjective in the nominative case, referring to the broad body.

Material examined. Holotype: cryptoniscus larva (ICHUM8647; 1 slide), body length 0.478 mm, 35°08'11.4"N, 139°36'39.3"E, 1 m depth, Jogashima, Kanagawa, Japan, 14 July 2022, from pereonite-3 sternite of female *C. nigra* (ICHUM8648; 1 vial; originally in Paranthuridae Menzies & Glynn, 1968).

Attachment site. Pereonite-3 sternite of female *Colanthura nigra*.

GenBank accession numbers. Holotype ICHUM8647: LC794720 (18S) and LC794717 (16S).

Description of holotype cryptoniscus larva. Body (Fig. IV-I-9*a*, *c*) teardrop

shaped, length 0.48 mm, 2.58 times body width, widest at pereonite 5 (0.19 mm). Cephalon anterior margin rounded, with several knobs on anterior part. Eyes lacking.

Antennule (Fig. IV-I-9*a*) article 1 without demarcated area and with two distal simple setae and eight teeth on posterior margin; fifth tooth from inner margin broadest, with dorsal cylindrical structure bearing simple seta. Article 2 tridentate on distal margin, with two distal simple setae. Article 3 rounded, covered by article 2 ventrally, with mid-ventral setal area and two conical extensions each bearing one and two simple setae. Antenna (Fig. IV-I-10*a*) with four peduncular articles and five flagellar articles. Peduncular article 1 longest and broadest; articles 2–4 with one, two, and one distal simple setae, respectively. Flagellar article 1 with distal simple seta; articles 2–4 naked; article 5 with two distal simple setae. Oral cone (Fig. IV-I-9*a*) triangular, ventrally directed.

Pereomeres 1–7 with coxal plate tooth formula of 3, 4, 4, 4, 4, 4, 4 (Fig. IV-I-9*b*). Pereopods 1 and 2 (Fig. IV-I-10*b*) similar, subchelate, short. Dactylus curved, simple, not bifid. Propodus oval, with bifurcate stout seta and simple stout seta. Carpus triangular, with three stout setae and two simple setae. Merus rounded, with distal simple seta. Ischium and basis naked. Pereopod 3 (Fig. IV-I-10*c*) subchelate, narrower than pereopods 1 and 2. Dactylus slender, elongate. Propodus with three stout setae. Carpus trapezoidal, with distal stout seta. Merus with outer stout seta bearing simple seta. Ischium with distal simple seta and outer ventral and dorsal lamellar projections. Pereopods 4 (Fig. IV-I-10*d*) and 5 similar to pereopod 3 but propodi narrower and carpi triangular. Pereopods 6 (Fig. IV-I-10*e*) and 7 similar, narrower than pereopods 1–5. Dactylus slender, elongate. Propodus elongate, naked. Carpus triangular, with distal stout seta. Merus with outer stout seta and simple seta. Ischium with outer ventral lamellar and dorsal bifurcate lamellar projections.

Pleon with five pairs of pleopods. All pleopods similar in shape; sympod rounded, endopod rectangular, exopod pyriform and narrow proximally (Fig. IV-I-9*a*). Pleopod 1 (Fig. IV-I-10*f*) sympod naked. Endopod and exopod each with five distal simple setae. Uropod (Fig. IV-I-10*g*) biramous, sympod with two outer simple setae. Endopod rectangular, not tapering, with two outer simple setae, three distal simple setae, and two distal triangular lamellar projections. Exopod rectangular, not tapering, 0.55 times as long as endopod, with two distal simple setae and two distal triangular lamellar projections. Pleotelson (Fig. IV-I-9*c*) trapezoidal; pygidium (Fig. IV-I-9*c*) smooth and roundly projecting posteriorly.

Molecular phylogeny. The 18S tree shows two clades in Epicaridea (Fig. IV-I-11), one with full nodal support containing all bopyroid taxa, the other with moderate support (SH-aLRT = 71.4% and UFBoot = 66%) containing all cryptoniscoid taxa. The three new *Anthuroniscus* **gen. nov.** species form a highly supported clade (SH-aLRT = 96.6% and UFBoot = 97%) that is the sister group with full support to Cryptoniscoidea sp., parasitic on the ostracod *Metavargula* sp. (Boyko et al. 2013).

The p-distances for 18S sequences among confamilial genera (1440 positions after the elimination of all positions containing gaps and missing data) were 4.0–11.0%. Pairwise 18S p-distances among the three new *Anthuroniscus* **gen. nov.** species were 0.6% (*shimomurai–dentatus*), 0.8% (*shimomurai–latus*), and 1.3% (*dentatus–latus*). The p-distance for 16S between *A. shimomurai* **sp. nov.** and *A. latus* **sp. nov.** (412 positions) was 36.2%.

DISCUSSION

Based on (1) their host group being Anthuroidea Leach, 1814, (2) 18S p-distances among my three species（0.6–1.3%) lower than the minimum value (4.0%) observed among confamilial genera in my analysis, and (3) morphological similarity between cryptoniscus larvae, I deemed my three species to be congeneric. *Anthuroniscus* **gen. nov.** is placed in Cryptoniscoidea because the cryptoniscus larvae have antenna consisting of four peduncular and five flagellar articles, and females have a highly reduced, sac-like body (Boyko 2013; Boyko and Williams 2015). My phylogenetic analysis (Fig. IV-I-11) supports this conclusion from morphology, showing the clade of *Anthuroniscus* **gen. nov.** within the Cryptoniscoidea clade, and comprising the sister group to the ostracod-parasitic Cryptoniscoidea sp.

Only Cabiropidae and Stellatoniscidae are cryptoniscoid families currently known to be parasitic on isopods (Boyko 2013; Oanh and Boyko 2020). In females, these families can be distinguished by body shape, which is sac-like in Cabiropidae but star-like in Stellatoniscidae. Cryptoniscus larvae in Cabiropidae have the antennular plate without a demarcated area and pereopod 1 similar to pereopod 2, while those in Stellatoniscidae have the antennular plate with a demarcated area and pereopod 1 dissimilar to pereopod 2. *Anthuroniscus* **gen. nov.** shows the character states characteristic of Cabiropidae, and I concluded the genus belongs in this family.

Anthuroniscus **gen. nov.** can be distinguished morphologically from the other 14 cabiropid genera and ten genera treated as family *incertae sedis*. Females have an elongate, dorsally compressed body, and thus *Anthuroniscus* **gen. nov.** belongs in Boyko's (2013) "group 2" in Cabiropidae, containing *Aegoniscus*, *Cirolanoniscus*, and *Clypeoniscus*. *Anthuroniscus* **gen. nov.** differs from these three genera and *Apocumoechus* (family *incertae sedis*) in having a more-elongate, posteriorly tapering body (Figs IV-I-3*a*, *b*, IV-I-4). Cryptoniscus larvae of *Anthuroniscus* **gen. nov.** differ from

the ten cabiropid genera in which cryptoniscus larvae are known in lacking eyes, having eight teeth on antennular article 1, and having a rectangular uropodal endopod.

Cryptoniscus larvae of *Anthuroniscus* **gen. nov.** differ from those of the nine cryptoniscoid genera described from cryptoniscus larvae and treated as "family *incertae sedis*" in having eight teeth on antennular article 1 (two in *Elocryptus* Schultz, 1977; eight in *Chimaeroniscus* Williams, Boyko & Moritaki, 2022; nine in *Osicryptus* Schultz, 1977; none in the other six genera) and the trapezoidal pleotelson twice as wide as long (eight of the nine genera have a different pleotelson; information on the pleotelson is lacking for *Trisopodoniscus* Bourdon, 1981) (Schultz 1977; Bourdon 1981, 1983; Williams et al. 2022). There are also differences in body size and recorded depths. Cryptoniscus larvae of *Anthuroniscus* **gen. nov.** are small (length 0.45–0.48 mm) and were collected from depths of 0.5−15 m, whereas cryptoniscus larvaeof the nine genera are larger (length 1.11–3.10 mm) and were collected from depths of 183–5340 m.

Cryptoniscus larva of *Anthuroniscus dentatus* **sp. nov.** differs from that of *A. latus* **sp. nov.** in the following characters (character state of *A. latus* **sp. nov.** in parentheses): body narrower, length 2.89 times body width (2.58 times); pygidium with eight teeth, not roundly projecting distally (without teeth, roundly projecting distally); pereomeres 1–7 with coxal plate tooth formula of 4, 5, 5, 4, 4, 4, 4 (3, 4, 4, 4, 4, 4, 4); and propodi of pereopods 6 and 7 with two stout setae (without stout setae). Their host species also differ: *A. dentatus* **sp. nov.** is parasitic on *Accalathura* sp. (Leptanthuridae), whereas *A. latus* **sp. nov.** is parasitic on *C. nigra* (Paranthuridae).

I could not morphologically compare female *A. shimomurai* **sp. nov.** (AS) with cryptoniscus larvae of *A. dentatus* **sp. nov.** (AD) and *A. latus* **sp. nov.** (AL) due to the large morphological difference, but the host of *A. shimomurai* **sp. nov.** (*Mesanthura* sp.;

Anthuridae) differed from those of latter two species. The 16S sequences for *A. shimomurai* **sp. nov.** and *A. latus* **sp. nov.** showed a p-distance of 36.2%, much larger than expected for intraspecific 16S sequence variation (up to 10%; Riehl et al. 2018). Given that the 18S p-distances for the pairwise comparisons AS–AD and AS–AL were similar (0.6% and 0.8%, respectively), I concluded that *A. shimomurai* **sp. nov.** was likewise not conspecific with *A. dentatus* **sp. nov.** The elongate, dorsally compressed body in *A. shimomurai* **sp. nov.** may be a synapomorphy for cabiropids parasitic on anthuroids; anthuroid females have a narrow marsupium, which might require the parasites to be elongate.

Prior to my study, Wägele (1979) was the only previous work to report an epicaridean parasitizing an anthuroid isopod (an epicaridean cryptoniscus larvae parasitic on *E. macrurus*). Based on its host, the epicaridean was likely a species of *Anthuroniscus* **gen. nov.** Even so, it was likely a different species from the three *Anthuroniscus* species described herein. Its locality (Gulf of Naples, Italy) was far from Japan, and its host anthuroid family (Expanathuridae Poore, 2001) was different from those of my species (Anthuridae, Leptanthuridae, and Paranthuridae); cryptoniscoid species tend to have high host specificity (Nielsen and Strömberg 1973).

This study added Anthuroidea to the list of cabiropid host groups (Table IV-I-1). Cabiropid parasites remain unknown for various isopod groups, such as Limnoriidea Brandt & Poore, 2002 and Microcerberidea Lang, 1961.

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Chapter IV-II. Description of rhizocephalan barnacle *Duplorbis changeling* sp. nov. (Duplorbidae)

INTRODUCTION

Rhizocephala Müller, 1862 is a group of crustacean-parasitic barnacles with the highly modified body in adults (Høeg et al. 2019). The adult body can be divided into two parts; 'externa' which is external reproductive organ and 'interna (root system)' which is a root-like structure situated inside of host body (Lützen et al. 2016). Most rhizocephalans utilize decapods as their hosts, with few exceptional species parasitizing non-decapod crustaceans: two species in Thompsoniidae Høeg & Rybakov, 1992 and an unnamed rhizocephalan species parasitize stomatopods (Manning 1970; Høeg and Lützen 1993; Lützen and Du 1999); species in Chthamalophilidae Bocquet-Védrine, 1961 parasitize balanomorph barnacles (Walker 2001); and species in Duplorbidae Høeg & Rybakov, 1992 parasitize peracarids such as cumaceans and isopods (Walker 2001).

Duplorbidae is a group of rhizocephalans parasitizing the brood camber (marsupium) of cumacean or isopod crustaceans (Kokasko 2025). It currently includes three genera and six species (Høeg et al. 2020; Kokasko et al. 2025): monotypic *Arcturosaccus* Rybakov & Høeg, 1992 (antarcturid-isopod host; Rybakov and Høeg 1992), monotypic *Cryptogaster* Bocquet-Védrine & Bourdon, 1984 (cumacean host; Bocquet-Védrine and Bourdon 1984), and the type genus *Duplorbis* Smith, 1906 (bopyrid- and anthuroid-isopod hosts; Kokasko et al. 2025) including four species. Diversity, morphology, ecology, and biology of duplorbids are quite less studied as they are rarely collected. Høeg et al. (2020) speculated that Duplorbidae is close allies of Chthamalophilidae and Pirusaccidae Høeg & Glenner in Høeg, Noever, Rees, Crandall &

Glenner, 2019. The first molecular phylogenetic analysis including Duplorbidae showed the sister relationship between Duplorbidae and Chthamalophilidae, but the support values are low (Kokasko et al. 2025).

Among the four *Duplorbis* species, the type species *Duplorbis calathurae* Smith, 1906 is parasitic on the anthuroid *Calathura brachiata* (Stimpson, 1853), whereas the remaining three species *Duplorbis smithi* Nierstrasz & Brender-à-Brandis, 1923, *Duplorbis ocarina* Nierstrasz & Brender-à-Brandis, 1930, and *Duplorbis koru* Kokasko, Williams, Wong & Chan, 2025 are hyperparasitic to bopyrids on decapods (Kokasko et al. 2025). Unnamed *Duplorbis* species have also been reported from several bopyrid hosts so far (Bourdon 1976; Mourey 1991).

In this chapter, I describe the new duplorbid *Duplorbis changeling* **sp. nov.** parasitizing the anthurid isopod *Malacanthura seisuiae* **sp. nov.** collected from the deep-sea bottom in the Kumanonada Sea, Japan. This represents the first report of Duplorbidae from Japan. I determined partial sequences of the 18S rRNA (18S) and 28S rRNA (28S) genes from *D. changeling* **sp. nov.**, and reconstructed the molecular phylogenetic trees to infer the phylogenetic position of my new *Duplorbis* species within Rhizocephala.

MATERIALS AND METHODS

Sample collection. An anthurid parasitized by one rhizocephalan barnacle with three externae was collected from the Kumanonada Sea off the southern coast of Mie Prefecture, Japan, by means of biological dredge during research cruise (Cruise 2423) of the TRV *Seisui-maru* (Mie University, Japan) on 1 November 2024. After photographing the host with the parasite, one of the externae (the most posterior one) was removed from the host, photographed, and fixed and preserved in 99% ethanol. Some pereopods of the

host were detached, and fixed and preserved in 99% ethanol. The remaining was fixed in Bouin's solution (picric acid [trinitrophenol] : paraformaldehyde : acetic acid = 15 : 5 : 1) for about one week and then transferred to 70% ethanol. The material examined is deposited in the Shoki Shiraki's Anthuroid Collection (SSAC).

DNA extraction and PCR. Total DNA was extracted from a part of the ethanol-fixed externa using a NucleoSpin Tissue XS Kit (Macherey–Nagel, Düren, Germany). For 18S, primers 18S-a1F and 18S-a9R (Kakui et al. 2011) were used for PCR amplification, and 18S-b3F, 18S-b4F, 18S-b4R, 18S-b5F, 18S-b6F, 18S-a6R, 18S- b8R, and 18S-b8F (Kakui et al. 2011; Kakui and Shimada 2017; Kakui et al. 2021) for cycle sequencing; for the 28S, primers LSUD1F and 28S_a6R (Lenaers et al. 1989; Shiraki et al. 2025) were used for PCR amplification, and 28S-Rd4.2b, D3AR, 300F, 900F, 28S_a3F, and 28S_a4F (Lenaers et al. 1989; Whiting 2002; Shiraki et al. 2025) for cycle sequencing. Amplification conditions for 18S with KOD FX Neo (Toyobo Life Science, Japan) were 94°C for 2 min; 45 cycles of 98°C for 10 s, 52°C for 30 s and 68°C for 75 s; and 68°C for 3 min; those for 28S with KOD One (Toyobo Life Science) were 45 cycles of 98°C for 10 s, 52°C for 5 s and 68°C for 15 s. All nucleotide sequences were determined by direct sequencing with a BigDye Terminator Cycle Sequencing Kit ver. 3.1 and a 3730 Genetic Analyzer (Life Technologies, Carlsbad, USA). The procedure for the determination of the cytochrome *c* oxidase subunit I (COI) sequence from the host was provided in Chapter II-II.

Molecular phylogeny. Molecular phylogenetic analysis was conducted for the 18S+28S dataset. The dataset contains sequences from 17 rhizocephalan and three thoracican (outgroup) species (Table IV-II-1). The 18S and 28S datasets were

independently aligned using MAFFT (ver. 7, see https://mafft.cbrc.jp/alignment/software/; Katoh and Standley 2013) with the 'Q-INS-I' strategy (Katoh and Toh 2008) and trimmed with Gblocks (ver. 0.91b, see http://phylogeny.lirmm.fr/phylo_cgi/one_task.cgi?task_ type=gblocks; Castresana 2000) in NGPhylogeny.fr (see https://ngphylogeny.fr/; Lemoine et al. 2019) under the 'relaxed' parameters described in Talavera and Castresana (2007). After trimming, the final length of 18S+28S dataset was 2504 bp. The optimal phylogenetic tree was determined for the dataset with the maximum likelihood method implemented in IQtree version 3.0.1 (Wong et al. preprint), with nodal support values obtained through 1000 Shimodaira-Hasegawa-like approximate likelihood ratio tests (SH-aLRT; Guindon et al. 2010) and analysis of 1000 ultrafast bootstrap (UFBoot; Hoang et al. 2018) pseudoreplicates. Clades with SH-aLRT ≥80 and UFBoot ≥95 were considered to be well supported. The optimal substitution model determined under the corrected AIC (Akaike information criterion) option in ModelFinder (Kalyaanamoorthy et al. 2017) was GTR+F+I+R3 for both 18S and 28S partitions. The resulting phylogenetic tree was drawn with FigTree software version 1.4.4 (https://github.com/rambaut/figtree/releases/tag/v1.4.4) and edited with Adobe Illustrator CC. P-distances and Kimura (1980) 2-parameter (K2P) distances were calculated with MEGA7 (Kumar et al. 2016).

TAXONOMY

Family Duplorbidae Høeg & Rybakov, 1992

Genus *Duplorbis* Smith, 1906

Duplorbis changeling sp. nov.

[New Japanese name: Fukuro-fukuromushi]

(Figs IV-II-1–IV-II-2)

Diagnosis. Externae tubular and weakly recurved, not T-shaped, whitish and translucent. Parasitic to anthurid isopods.

Etymology. The specific name is an indeclinable noun derived from the creature 'Changeling' transmitted in Europe folklore, a substitute left by a supernatural being when kidnapping a human child. It refers to the externae of the new species look like the fake offspring of the host anthurids.

Material examined. Holotype: female with three externae (SSAC-04; 2 vials), each externa 2.25 mm, 2.10 mm, and 1.97 mm in longest length (from the anterior to the posterior ones), from ovigerous female of the anthurid isopod *Malacanthura seisuiae* **sp. nov.** (SSAC-03; 9.84 mm in length), the Kumanonada Sea (34°02'24.6"N 136°53'24.6"E to 34°02'24.6"N 136°53'25.2"E), off the southern coast of Mie Prefecture, Japan, 758–759 m, 1 November 2024.

Description of externae. Mature externae entirely filled with larvae inside. Three externae arranged in row along host's antero-posterior axis, each arose from pereonites 3–5, respectively, occupying almost entire host marsupium (Fig. IV-II-1b, c). Each externa similar in shape, whitish and translucent, weakly recurved, not T-shaped, outline tubular, with lateral expansions broadly rounded. Peduncle of externa relatively short, positioned in middle of long axis. External opening positioned close to peduncle but rotated 90 degrees relative to antero-posterior axis. Interna (root system) yellow,

extending from pereonite 2 to pereonite 6 of host, mostly in pereonites 3–5 where externae originated.

Distribution. So far, known only from its type locality.

Host and infection site. Marsupium (brood chamber; for externae) and pereon (for interna) of the anthurid isopod *Malacanthura seisuiae* **sp. nov.**

DNA sequences. 2501 bp for 18S and 2475 bp for 28S, both sequences derived from the holotype's posterior externa.

Remarks. Among the four *Duplorbis* species, *D. changeling* **sp. nov.** is most similar to *D. calathurae* in sharing (1) the externae being tubular and nearly straight, not T-shaped and (2) the host taxa being free-living anthuroid isopods, not parasitic bopyrids (Smith 1906; Kokasko et al. 2025). However, *D. changeling* **sp. nov.** shows morphological differences from *D. calathurae* in the externae being whitish and translucent (red in *D. calathurae*) and weakly recurved (completely straight in *D. calathurae*) (Smith 1906). Additionally, my new species is also different from *D. calathurae* in (1) the host family (*Malacanthura seisuiae* **sp. nov.** in Anthuridae for *D. changeling* **sp. nov.** vs. *Calathura brachiata* in Leptanthuridae for *D. calathurae*), (2) the distribution (northwestern Pacific off the coast of Japan for *D. changeling* **sp. nov.** vs. Arctic off the coast of Greenland for *D. calathurae*), and (3) the inhabiting depth (758–759 m for *D. changeling* **sp. nov.** vs. 50 fathoms = 91 m for *D. calathurae*) (Smith 1906).

Molecular phylogeny. My 18S+28S phylogenetic tree (Fig. IV-II-3) showed

that the anthurid-parasitic *D. changeling* **sp. nov.** is sister to the bopyrid-hyperparasitic *D. koru* with full support (SH-aLRT = 100% and UFBoot = 100%), supporting the monophyly of the genus *Duplorbis*. To test the monophyly of the family Duplorbidae, molecular data from the antarcturid-parasitic *Arcturosaccus* and the cumacean-parasitic *Cryptogaster* is required.

Høeg et al. (2020) suggested that Chthamalophilidae, Duplorbidae and Pirusaccidae are closely related based on their synapomorphic unique sexual system with spermatogenic islets. Kokasko et al. (2025), the first study molecularly investigating the phylogenetic position of Duplorbidae, retrieved Duplorbidae as the sister taxon to Chthamalophilidae in their 18S tree, but its support value was low. My analysis also failed to resolve the sister family of Duplorbidae due to the low support values. As with Kokasko et al.'s (2025) tree, Chthamalophilidae, Clistosaccidae, Duplorbidae, Polysaccidae, and Thompsoniidae formed a moderately supported clade (SH-aLRT = 95.4% and UFBoot = 92%), suggesting their phylogenetic closeness.

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Chapter IV-III. First report of trematode flatworm *Microphallus* sp. from *Cyathura muromiensis*

INTRODUCTION

Because the life cycles of parasitic trematode platyhelminths are generally complicated, they have not been fully elucidated for most trematodes. Trematodes typically utilize a definitive host and two intermediate hosts. One group that trematodes utilize as secondary intermediate hosts is the isopod superfamily Anthuroidea Leach, 1814, but among ca. 600 anthuroid species, only *Anthura gracilis* (Montagu, 1808), *Cyathura polita* (Stimpson, 1855), and *Cyathura carinata* (Krøyer, 1847) have been reported in this role. *Levinseniella brachysoma* (Creplin, 1837) utilizes *A. gracilis* (Villot 1879), unidentified *Gynecotyla* (sic; possibly the misspelling of *Gynaecotyla* Yamaguti, 1939) species often occurs in *Cy. polita* (Burbanck 1962), and several microphallid species such as *Maritrema subdolum* Jägerskiöld, 1909 and *Microphallus claviformis* (Brandes, 1889) utilize *Cy. carinata* (Reimer 1963; Schulenburg and Wägele 1998; Schulenburg et al. 1999; Jensen et al. 2004; Ferreira et al. 2005a, 2005b). All the above-mentioned trematodes belong to Microphallidae Ward, 1901 and the definitive hosts of its members are mainly birds (Bray et al. 2008).

Here I firstly report trematode cysts in *Cyathura muromiensis* Nunomura, 1974 from the Muromi River estuary, Fukuoka, Japan. This is the first anthuroid species from Japan found to be parasitized by trematodes, and the fourth species worldwide. I determined a partial sequence of the 28S rRNA (28S) gene and five partial sequences of the "ITS1 cluster," from the 3′ region of the 18S rRNA to the 5′ region of internal transcribed spacer I gene (ITS1), to investigate conspecificity of the cysts, to infer their

phylogenetic position within Trematoda, and for future DNA barcoding. Additionally, based on qualitative samples, I investigated the parasite frequency among hosts, intensity (density) per host individual, cyst locations in the hosts, and relationships between cyst intensity and host sex and size.

MATERIALS AND METHODS

Sampling of hosts and parasites. *Cy. muromiensis* isopods were collected on a brackish tidal flat in the Muromi River estuary (33°34'48.2"N 130°20'13.7"E), Fukuoka, Japan (the species' type locality) (Nunomura 1974), on 7 March (52 specimens) and 9 March (two specimens) 2023. Bottom sediment was filtered with a 1 mm-mesh sieve, and isopods retained in the residue were recovered by hand; this process was carried out repeatedly. Some individuals collected on 7 March 2023 were fixed in 99% ethanol on the same day as collection. All other individuals collected on 7 and 9 March 2023 were brought back to my laboratory alive. Some of these were photographed, dissected to obtain live cysts, and fixed in 99% ethanol; the others were fixed in 99% ethanol.

The head length of each *Cyathura* isopod was measured as a proxy for host size, since head length is linearly related to body length in congeneric *Cy. carinata* (Marques et al. 1994). Fixed isopods were dissected with chemically sharpened tungsten wire needles under a Nikon SMZ1500 stereomicroscope to examine the trematode infection. The number of cysts and the host body segment where they were located (head, pereonites 1–7, pleon, and telson) were recorded. The diameters of the extracted cysts were measured.

An extracted, living cyst was opened with chemically sharpened tungsten wire needles in a 1.0% saline solution, and the metacercaria was removed, observed with an

Olympus BX51 microscope under a cover glass, and subsequently fixed in 70% ethanol. Measurements of host head length, cyst diameter, and the length of excysted metacercaria and its body structures were made from digital images by using ImageJ (Schneider et al. 2012). *Cyathura* individuals were sexed according to the presence (males) or absence (females) of the appendix masculina on the endopod of pleopod 2.

Statistical analyses. Statistical analyses were performed with R software version 4.3.2, except that Excel 2016 was used for linear regression. The number of components in a normal mixture distribution of cyst size was estimated with the mclust package (Scrucca et al. 2023) for two datasets: Dataset A containing measurements of cysts from all infested hosts (378 of 389 extracted cysts; 11 were lost before measurement) and Dataset S containing measurements of cysts from the single host specimen showing the highest intensity (70 of 71 extracted cysts; one was lost before measurement). The value of the Bayesian information criterion (BIC; Schwarz 1978) was used to infer the number of components (Fraley 2003). Differences in the number of cysts by host sex were tested with the non-parametric Mann–Whitney U test; prior to the test, the data were assessed for normality for both female and male hosts with the Shapiro–Wilk test. The relationship between the number of cysts and the size of the host was examined by linear regression, with the regression line and coefficient of determination calculated with Microsoft Excel 2016. Figures were constructed with Excel 2016 (Figs IV-III-2, IV-III-3, and IV-III-5) or R (Fig. IV-III-4), and were edited with Adobe Illustrator CC.

DNA extraction and polymerase chain reaction (PCR). DNA was extracted from the whole body of five cysts (172.6, 205.0, 206.9, 228.8, and 252.1 μm in diameter),

each from a different *Cyathura* individual collected on 7 March 2023, by using a NucleoSpin Tissue XS Kit (Macherey–Nagel, Germany), after the removal of host tissue with needles. 28S sequence was determined for a single cyst out of the five cysts and ITS1 cluster sequences for the five cysts. The primers used for PCR and cycle sequencing are listed in Table IV-III-1. PCR amplification conditions for 28S and the ITS1 cluster with TaKaRa Ex Taq DNA polymerase (Takara Bio, Japan) were 95℃ for 1 min; 30 cycles of 95℃ for 30 sec, 50℃ for 30 sec, and 72℃ for 1 min; and 72℃ for 7 min. All nucleotide sequences were determined by direct sequencing with a BigDye Terminator Cycle Sequencing Kit ver. 3.1. and a 3730 DNA analyzer (Life Technologies, USA). The sequences I obtained were deposited in the International Nucleotide Sequence Database (INSD) through the DNA Data Bank of Japan (DDBJ), under accession numbers LC808145 (28S) and LC808146–LC808150 (ITS1 cluster; cysts with diameters 228.8, 206.9, 252.1, 172.6, and 205.0 μm, respectively).

Molecular phylogenetic analysis. A molecular phylogenetic analysis of 28S sequences from thirty-four microphallids was conducted to identify my parasite; sequences from two lecithodendriid and one macroderoidid species (the sequence of AF433673 is likely *Macroderoides spiniferus* Pearse, 1924, not *Plesiocreadium typicum* Winfield, 1929; Truong et al. 2023) were included as outgroup taxa. The sequences were aligned by using the MAFFT online platform version 7 (Katoh and Standley 2013) with the “L-INS-i” strategy (Katoh et al. 2005) and were trimmed with MEGA7 (Kumar et al. 2016) to match the shortest length among them. Ambiguous sites were removed with Gblocks version 0.91b (Castresana 2000). The optimal phylogenetic tree was determined with the maximum likelihood (ML) method implemented in IQtree version 2.1.2 (Minh et al. 2020), with nodal support values obtained through 1000 Shimodaira-Hasegawa-like

approximate likelihood ratio tests (SH-aLRT; Guindon et al. 2010) and analysis of 1000 ultrafast bootstrap (UFBoot; Hoang et al. 2018) pseudoreplicates. Clades with both SH-aLRT ≥ 80 and UFBoot ≥ 95 were considered to be well supported. The optimal substitution model determined under the corrected AIC (Akaike information criterion) option in ModelFinder (Kalyaanamoorthy et al. 2017) was GTR+F+R3. The resulting phylogenetic tree was viewed with FigTree software version 1.4.4 (https://github.com/rambaut/figtree/releases/tag/v1.4.4) and edited with Adobe Illustrator CC.

RESULTS

Trematode cysts. In all, 389 cysts were obtained from 52 of 54 specimens of *Cy. muromiensis* (Fig. IV-III-1A, B; 50 were collected on 7 March 2023 and two on 9 March 2023); the sample prevalence was 96.3%. Hosts contained one to 71 cysts each (mean intensity = 7.5; median intensity = 4.0). The cysts (Fig. IV-III-1C) were the round, encysted metacercarial stage and were situated inside the host body (Fig. IV-III-1B).

Cysts were found in host pereonites 2–7 and the pleon, with the higher occurrences in pereonites 3–6; no cysts were found in the head, pereonite 1, or the telson (Fig. IV-III-2A). A similar distribution was evident among the 71 cysts extracted from the single host with the highest intensity (Fig. IV-III-2B).

Range in cyst diameter (N = 378) was 172.3–252.1 μm (mean = 211.1 μm; median = 210.6 μm; Fig. IV-III-3A). For both Datasets A and S (Fig. IV-III-3), the model with the optimal BIC value (−2971.462 under both the equal and unequal models for Dataset A; –533.3162 under both models for Dataset S) had one component.

Description of excysted metacercaria. Description and measurements are based on two excysted metacercariae. Excysted metacercaria (Fig. IV-III-1D) linguiform, flattened. Body length 567 μm and body width 276–323 μm under a cover glass. Oval oral sucker (length 40.4–43.8 μm, width 49.1–52.3 μm) larger than round ventral sucker (length 38.1 μm, width 33.0–34.9 μm). Prepharynx present but short, 7.4–36.9 μm. Pharynx oval, near oral sucker, length 22.3–24.1 μm and width 15.8–20.2 μm. Esophagus narrow, length 114–200 μm. Caecum bifurcating in center of body and terminating near lateral side of midbody. Testes oval, locating symmetrically, postovarian. Right testis (ovary side) length 70.9–86.6 μm and width 61.7–70.3 μm. Left testis length 71.8–86.4 μm and width 49.4–61.3 μm. Cirrus sac arched, locating between caeca and ventral sucker. Ovary oval, locating between cirrus sac and right testis, length 56.2–65.9 μm and width 47.8–48.9 μm.

Differences in prevalence depending on host sex. The sex ratio for 47 of 54 isopods collected was 40 females to seven males, or 5.71 F:M. All the females were not ovigerous, 38 of which were parasitized (sample prevalence = 95%), with one to 71 cysts each (mean intensity = 8.1; median intensity = 5.5). All seven males were parasitized, with one to 18 cysts each (mean intensity = 7.0; median intensity = 4.0). The number of cysts was not normally distributed for females, but was for males with low p-value (Shapiro-Wilk test; $p < 0.001$ for females; $p = 0.05254$ for males). The number of cysts was not significantly different between host females and males (Fig. IV-III-4; Mann-Whitney U test, $p = 0.7299$).

Relationship between host size and the number of cysts. There was no correlation between host head length (HL) and the number of cysts (NC) among 47 sexed host individuals (Fig. IV-III-5). The regression line was NC = 7.4256HL + 0.4918 (0.736 ≤ HL ≤ 1.316), but the coefficient of determination was quite low (R^2 = 0.0052). There was also no correlation between HL and NC when this was examined separately by host sex. The coefficients of determination were low for both female (R^2 = 0.0114; NC = 13.74HL – 5.2645) and male hosts (R^2 = 0.001; NC = –1.1886HL + 8.2264).

Genetic information and phylogenetic analysis. ITS1 cluster sequences (865 bp) from five cysts differed by 0–2 nucleotide substitutions, giving p-distances of 0–0.2%. In BLAST searches (Altschul et al. 1990), the ITS1 sequence most similar to that in my ITS1 cluster (LC808146) was from an unidentified digenean parasite in *Cy. carinata* (AJ001831; identity score 76.85%, query cover 100%; Schulenburg et al. 1999).

A 28S sequence (LC808145; 1694 bp) was determined from one cyst (diameter 228.8 μm). In the optimal maximum-likelihood tree (Fig. IV-III-6), my taxon is embedded in a clade containing only sequences from *Microphallus* Ward, 1901 (except for an unidentified microphallid), with high nodal support (SH-aLRT = 98.2%, UFBoot = 99%). While "*Mi. fusiformis* Reimer, 1963" lies outside the *Microphallus* clade, Galaktionov and Blasco-Costa (2018) considered that the specimen from which this sequence (AY220633) derived was likely misidentified. Within the *Microphallus* clade, my taxon was the sister taxon to a microphallid metacercaria from the tanaidacean *Longiflagrum nasutus* (Nunomura, 2005) reported from Japan (Kakui 2014), with moderate support (SH-aLRT = 94.4%, UFBoot = 91%). BLAST searches also suggested that my 28S sequence (LC808145) was most similar to that of microphallid from the tanaidacean species (AB974360; identity score 97.05%, query cover 98%; Kakui 2014).

DISCUSSION

This is the first report of trematode metacercariae from *Cyathura muromiensis*. I concluded that all the metacercariae obtained were conspecific, because the ITS1 cluster sequences from five cysts ranging in size from nearly the lowest diameter to the greatest diameter in my sample varied by zero to two nucleotide substitutions, and the model fitted to the cyst-size distribution contained only one component.

The 28S phylogeny indicated that my cysts belong to a species in *Microphallus*. My 28S sequence differed by 3.1–7.4% from the other sequences in the *Microphallus* clade (Fig. IV-III-6; Table IV-III-2), much greater than the minimum interspecific genetic distance observed in my dataset, 0.2% between *Mi*. *minutus* Johnston, 1948 (KT355822) and *Mi*. *calidris* Belopolskaya & Rizhikov, 1963 (HM584124), and within the range of interspecific differences reported in *Microphallus* (e.g., 0.4–8.6%; Galaktionov and Blasco-Costa 2018). The observed distances suggest that my trematode species is not conspecific with any of the *Microphallus* species included in the analysis. Interestingly, two metacercariae from peracarid hosts (my sequence, LC808145, from a *Cyathura* isopod and Microphallidae sp., AB974360, from a tanaidacean) formed a moderately supported clade, even though microphallids use various crustaceans as second intermediate hosts (Galaktionov and Blasco-Costa 2018), such as decapods for *Mi*. *minutus* (Kudlai et al. 2015) and *Mi*. *basodactylophallus* (Bridgman, 1969) (Bridgman 1969; Gehman et al. 2021). This may imply a single origin of the utilization of peracarids as secondary intermediate hosts in *Microphallus*. That my ITS1 cluster sequence was most similar to the ITS1 sequence from a digenean parasitic in *Cy*. *carinata* may support the idea. However, information on the second intermediate host is lacking for most

species, and more data on *Microphallus* from peracarids are needed to test this hypothesis.

I found cysts in hosts' pereonites 2–7 and the pleon, but not the head, pereonite 1, or telson. The head bears several appendages and has little space for parasites, and the telson is thin, which may explain the lack of cysts in these locations. No infections in pereonite 1 and low infection in pereonite 2 may be related to pereonites 1–2 and 2–3 being connected by a narrow joint in anthuroid isopods (cf. Kakui and Shiraki 2021; Fig. IV-III-2A, E–G), which might prevent or impede parasites from entering pereonites 1 and 2 from adjacent segments. I found most cysts in pereonites 2–7, with a few in the pleon, a distribution similar to that observed in *Levinseniella* sp. and *Ma*. *subdolum*/*Mi*. *claviformis* parasitic in *Cy*. *carinata* under experimental conditions (Ferreira et al. 2005b). Interestingly, in a different experiment (Ferreira et al. 2005a), most cysts of *Ma*. *subdolum* were found in the pleon of *Cy. carinata*. In these two experiments, individuals of *Cy. carinata* came from same locality, perhaps indicating that the preferred location of the parasite can change with factors such as season, water temperature, or substrate.

The definitive host for my trematode species may be birds, since microphallids chiefly use birds as the definitive host (Bray et al. 2008). Although information is lacking regarding predators of *Cy*. *muromiensis*, the small sandpiper *Calidris alpina* (Linnaeus, 1758) preys on the congener *Cy*. *carinata* (Santos et al. 2005). The Muromi River estuary is rich in birds, and *Ca*. *alpina* has been observed there (iNaturalist 2022). A speculative but plausible life cycle for my trematode species might involve a cercaria emerging from the first intermediate host (possibly a gastropod), the cercaria entering a *Cy*. *muromiensis* isopod and giving rise to encysted metacercariae, and (followed by predation of the isopod by the definitive host, possibly a shorebird), the metacercariae becoming sexually mature adults.

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CHAPTER V: MOLECULAR PHYLOGENY OF ANTHUROIDEA

INTRODUCTION

Anthuroidea Leach, 1814 is a benthic isopod group with about 600 species in 63 genera (previously known 62 and *Deltanthura* **gen. nov.**). Its family-level classification system currently follows Poore's (2001) six-family system, consisting of Antheluridae Poore & Lew Ton, 1988, Anthuridae Leach, 1814, Expanathuridae Poore, 2001, Hyssuridae Wägele, 1981, Leptanthuridae Poore, 2001, and Paranthuridae Menzies & Glynn, 1968. Poore's (2001) system was proposed by morphology-based phylogenetic analyses; in his phylogenetic tree, Hyssuridae first branched off, then Anthuridae did; the clade containing the remaining comprised Leptanthuridae + Paranthuridae and Antheluridae + Expanathuridae subclades (Fig. V-1a). This system is not fully supported by the other morphology-based phylogenetic analyses conducted by Wägele (1981a, 1989). In Wägele's (1981a, 1989) analyses, four of six families were not monophyletic: some expanathurids were sister to Hyssuridae; the anthurid *Apanthuroides* Menzies & Glynn, 1968 was not encompassed in Anthuridae but sister to a clade including leptanthurids and paranthurids; and neither leptanthurids nor paranthurids were monophyletic (Fig. V-1b). To date, only the three morphology-based studies referred to above have conducted exhaustive phylogenetic analyses targeting Anthuroidea.

Based on the morphology of the mouthparts, two major groups have traditionally been recognized in Anthuroidea: the group with biting-type mouthparts and the group with piercing-type mouthparts (Norman and Stebbing 1886; Barnard 1925; Wägele 1981b; Cadien and Brusca 1993; Poore 2001). In the currently accepted classification, species in four families (Antheluridae, Anthuridae, Expanathuridae, and Hyssuridae)

have biting-type mouthparts whereas species in the remaining two families (Leptanthuridae and Paranthuridae) have piercing-type mouthparts (Poore 2001). All of three phylogenetic analyses (Wägele 1981a, 1989; Poore 2001) suggested that the piercing-type group was monophyletic and encompassed in the paraphyletic biting-type group, which indicates that biting-type mouthparts is ancestral and piercing-type mouthparts is derived. Piercing-type mouthparts can be further divided into two subgroups based on the mandible's morphology: 'a moderately specialized subgroup' with sharpened mandible bearing palp and 'a highly specialized subgroup' with blunt mandible lacking palp (cf. Annisaqois and Wägele 2021; Shiraki and Kakui 2024). The difference in mouthpart morphology is considered to reflect the difference in feeding habits. Though knowledge of their feeding habits is quite limited (Kensley 1982; Poore and Bruce 2012), it was observed that species with biting-type mouthparts fed on various stuff such as polychaetes, detritus, diatoms, living amphipods, dead conspecific isopods, dead shrimps, or dead fishes (Burbanck 1959; Burbanck and Burbanck 1979; Wägele 1981a; Wägele et al. 1981; Horikoshi 2012) and species with moderately specialized piercing-type mouthparts fed mainly on crustaceans (observed for *Paranthura* Bate & Westwood, 1866 and *Accalathura* Barnard, 1925; Wägele 1981a, 1985; Shiraki and Kakui 2022; Matsushima et al. 2025). The feeding habits of species with highly specialized piercing-type mouthparts remain unknown.

The number of statocysts (gravity-receptive organs) in the pleotelson was used as one of important characters in anthuroid familial identification (Poore and Lew Ton 1988a). Although there are some exceptions, zero statocyst in Expanathuridae, Hyssuridae, and Paranthuridae, one in Antheluridae and Leptanthuridae, and two in Anthuridae (Poore 2001). Two evolutionary scenarios of statocyst number have been proposed: one is that the last common ancestor of Anthuroidea lacked statocysts, the

common ancestor of the non-hyssurid clade containing Anthuridae got two-statocyst condition, then the group branched from Anthuridae become one-statocyst condition, and the secondary loss of statocyst occurred in expanathurid and paranthurid clades independently (Wägele 1981a, 1989; Poore 2001); the other is that the acquisition of two statocysts occurred within Anthuridae independently from the acquisition of one statocyst in the clade with Antheluridae, Expanathuridae, Leptanthuridae, and Paranthuridae (Poore 2001).

Most anthuroid species have seven pairs of pereopods (pereopods 1−7) in adults but those in some genera lack pereopod 7. The lack of pereopod 7 is the condition known in "manca," the individual at the first (and also possibly second) instar after released from mother's brood pouch, thus the species having adults lacking pereopod 7 is considered as neotenous state ('true-neotenous' hereafter). True-neotenous species is known in six genera in the three families, namely, *Leipanthura* Poore, 2009 in Anthuridae (Poore 2009), *Curassanthura* Kensley, 1981 in Leptanthuridae (Alvarez et al. 2019), and *Califanthura* Schultz, 1977, *Colanthura* Richardson, 1902, *Cruranthura* Thomson, 1946, and *Cruregens* Chilton, 1882 in Paranthuridae (Poore 1984, 2001). Another neotenous condition is known in Anthuroidea, namely, adults possess pereopod 7 apparently smaller than the pereopod 6 ('para-neotenous' hereafter). Para-neotenous condition is observed in paranthurid *Deltanthura palpus* **sp. nov.** and *Pseudanthura albatrossae* Kensley, 1978 (Chapter II-VIII; Kensley 1978).

The objectives of this chapter are (1) to test the monophyly of each family and genus, (2) to examine the relationships among families and genera, and (3) to illustrate evolutional history of (3a) mouthpart morphology, (3b) the number of statocysts, and (3c) neotenous conditions. For these objectives, I constructed the first comprehensive

molecular phylogenetic tree of Anthuroidea based on partial sequences of the four gene markers, 18S rRNA (18S), 28S rRNA (28S), 16S rRNA (16S), and cytochrome *c* oxidase subunit I (COI) genes, obtained from 37 Japanese species representing at least 20 genera and all current six families.

MATERIALS AND METHODS

Sampling and morphological observations. Samplings were conducted from 2016 to 2024 in the intertidal zones by hand, in the subtidal zones by free or SCUBA diving, and in the deep sea by dredge around Japan (Fig. V-2; see Table V-1 for detailed sampling localities or coordinates; see Figs V-3, V-4, Table V-2 for collected species). For the samplings in the intertidal zones and the subtidal zones, anthuroid individuals were collected by washing substrates with seawater (for algae and sand) or freshwater (for coral rubble). For the samplings in the deeper sea, individuals were collected with a dredge during Cruises 2312, 2325, 2409, and 2423 of TRV *Seisui-maru* and during 23rd JAMBIO (Japanese Association for Marine Biology) Coastal Organism Joint Survey by the decantation method as described in Akiyama et al. (2008). Collected individuals were fixed and preserved in 70–99% ethanol after photographed as fresh. To use as outgroups for the phylogenetic analysis, three valviferan isopods (Antarcturidae sp., *Symmius caudatus* Richardson, 1904, and *Symmius planus* Nunomura, 1984) were also collected. Antarcturidae sp. was collected on 23 September 2023 at depth of 5795–5798 m of the Japan Trench (st. F14; 39°27'59.0"N 144°48'58.9"E) with a beam-trawl during Cruise KH-23-5 of RV *Hakuho-maru*. *Symmius caudatus* was collected on 9 July 2024 at depth of 243–246 m of the Kumanonada Sea (34°33'43.2"N 137°49'31.8"E to 34°33'36.6"N 137°49'27.6"E), off Mie, Japan during Cruise 2409 of TRV *Seisui-maru*. *Symmius planus*

was collected on 10 June 2022 at depth of 82.4–87.6 m of Misaki (35°08'54.4"N 139°34'40.6"E to 35°09'08.0"N 139°34'32.2"E), Kanagawa, Japan during 23rd JAMBIO Coastal Organism Joint Survey.

For the identification of the collected anthuroids, specimens were partially dissected, mounted on slides, and observed as described in Shiraki et al. (2021). For the genus-level identification of collected anthuroids, I followed Poore (2001), Chew et al. (2014), Jarquín-Martínez and García-Madrigal (2021), and Chapter II-VIII.

DNA extraction, PCR amplification, and sequencing. DNA was extracted from pereopod(s) or whole body of each specimen by using a NucleoSpin Tissue XS Kit (Macherey-Nagel, Germany). For the amplifications of 18S, 28S, 16S, and COI, polymerase chain reaction (PCR) was performed with primer pairs 18S-a1F and 18S-a9R (Kakui et al. 2011), LSUD1F and 28S_a6R (Lenaers et al. 1989; Shiraki et al. 2025), Cope16S_F and Cope16S_R (Kakui et al. 2023), and LCO1490 and HCO2198 (Folmer et al. 1994), respectively (Table V-3). The PCR conditions for 18S and 28S with KOD FX Neo (Toyobo Life Science, Japan) were 94°C for 2 min; 45 cycles of 98°C for 10 s, 45°C (18S) or 52°C (18S or 28S) for 30 s, and 68°C for 75 s (18S) or 90 s (28S); and 68°C for 3 min, and with KOD One (Toyobo Life Science) were 45 cycles of 98°C for 10 s, 53°C (18S) or 52°C (28S) for 5 s, and 68°C for 10 s (18S) or 15 s (28S). Those for 16S and COI with KOD One were 45 cycles of 98°C for 10 s, 50°C (16S) or 56°C (COI) for 5 s, and 68°C for 1 s, and with TaKaRa Ex Taq DNA polymerase (TaKaRa Bio, Japan) were 94°C for 1 min; 35 cycles of 98°C for 10 s, 38–50°C (COI) or 45°C (16S) for 30 s, and 72°C for 50 s; and 72°C for 2 min. The sequencing reaction was prepared mainly with the primers listed in Table V-3; the specific internal primers were newly designed and used for some specific species. All nucleotide sequences were determined by direct

sequencing with a BigDye Terminator Cycle Sequencing Kit (ver. 3.1) and 3730 DNA Analyzer (Life Technologies, Carlsbad, CA, USA). For most species used in this study, the determined sequences were derived from a single specimen. For *Cyathura muromiensis* Nunomura, 1974, *Kupellonura tamago* **sp. nov.**, *Colanthura nigra* Nunomura, 1975, and *Cruranthura viridis* **sp. nov.**, the determined sequences were derived from two specimens which were collected from (almost) the same localities.

Phylogenetic analysis. The partial sequences of the four gene markers (18S, 28S, 16S, and COI) were determined for 37 anthuroids and three outgroup valviferan species (Antarcturidae sp., *Sy*. *caudatus*, and *Sy*. *planus*) (Figs V-3, V-4; Table V-2). The sequences were aligned by using MAFFT ver. 7 (Katoh and Standley 2013) with the "Q-INS-I" strategy (Katoh and Toh 2008) for 18S, 28S, 16S, and COI, respectively. The ambiguous sites were removed with Gblocks ver. 0.91b (Castresana 2000) in NGPhylogeny.fr (Lemoine et al. 2019) under the "relaxed" parameters described in Talavera and Castresana (2007). The concatenated dataset from the four gene markers was 4060 bp long (1686 bp for 18S, 1353 bp for 28S, 363 bp for 16S, and 658 bp for COI). Phylogenetic analysis was performed with the maximum likelihood (ML) method implemented in IQTree version 2.1.2 (Minh et al. 2020) with nodal support values obtained through 1000 Shimodaira-Hasegawa-like approximate likelihood ratio tests (SH-aLRT; Guindon et al. 2010) and analysis of 1000 ultrafast bootstrap (UFBoot; Hoang et al. 2018) pseudoreplicates. Clades with both SH-aLRT ≥ 80 and UFBoot ≥ 95 were considered to be highly supported, and clades supported only by one of two values (SH-aLRT ≥ 80 or UFBoot ≥ 95) were considered to be moderately supported. The optimal substitution model determined under the corrected AIC (Akaike information criterion) option in ModelFinder (Kalyaanamoorthy et al. 2017) was SYM+R3 (for 18S), SYM+R4

(for 28S), TVM+F+I+G4 (for 16S), TPM3+F+G4 (third codon position in COI), TIM2+F+R3 (first codon position in COI), and GTR+F+R2 (second codon position in COI). The resulting phylogenetic tree was viewed with FigTree software version 1.4.4 (https://github.com/rambaut/figtree/releases/tag/v1.4.4) and edited with Adobe Illustrator CC.

RESULTS

Phylogenetic tree of Anthuroidea. My phylogenetic tree recognized five major clades (Figs V-5, V-6). Clade 1 contained *Colanthura* and *Cruranthura* with high support (SH-aLRT = 99.9% and UFBoot = 100%). Clade 2 contained *Accalathura*, *Deltanthura* **gen. nov.**, and *Paranthura* with moderate support (SH-aLRT = 96% and UFBoot = 68%). Clade 3 contained two leptanthurid species with full support (SH-aLRT = 100% and UFBoot = 100%). Clade 4 contained all anthurid species with moderate support (SH-aLRT = 99.2% and UFBoot = 72%). In Clade 4, two subclades each including eight species were recovered, with moderate and less support, respectively (SH-aLRT = 86.1% and UFBoot = 75% and SH-aLRT = 69% and UFBoot = 28%). Clade 5 contained anthelurids, expanathurids, and hyssurids with moderate support (SH-aLRT = 98% and UFBoot = 76%).

Among the current six anthuroid families, the monophyly of Anthuridae and Hyssuridae were moderately or fully supported, respectively, but those of the remaining four families were not supported (Fig. V-6). Species in Leptanthuridae and Paranthuridae were contained in each of three clades (Clades 1–3), and the three clades did not form an exclusive monophyletic clade. Clade 4 composed all species of Anthuridae (Clade 4), formed a low support clade with Clades 1–3. Clade 5 containing Antheluridae,

Expanathuridae, and Hyssuridae. In Clade 5, monophyly of a clade including monophyletic Hyssuridae and non-monophyletic Expanathuridae was with high support (SH-aLRT = 98% and UFBoot = 99%); Antheluridae was paraphyletic; *Eisothistos* Haswell, 1884 was sister to Hyssuridae with moderate support (SH-aLRT = 81.9% and UFBoot = 79%), making Expanathuridae paraphyletic.

Among eight genera containing two or more OTUs, monophyly of *Accalathura*, *Eisothistos*, *Expanathura* Wägele, 1981, and *Paranthura* were fully supported and that of *Cyathura* was highly supported (SH-aLRT = 100% and UFBoot = 99%). *Apanthura* Stebbing, 1900, *Cruranthura*, and *Mesanthura* Barnard, 1914 were not recovered as monophyletic.

Mouthparts. In my phylogenetic analysis (Figs V-5, V-7a, d), both the biting-type and piercing-type were not monophyletic. Of the two subgroups of piercing-type mouthparts, species in the moderately specialized subgroup did not form a monophyletic clade and were contained in Clades 2 and 3, whereas species in the highly specialized subgroup were monophyletic and grouped in Clade 1. Species with biting-type mouthparts were contained in Clades 4 and 5. No clades contained more than two types of mouthparts.

The number of statocysts. Species with two statocysts formed a moderate support clade (Clade 4; Anthuridae; SH-aLRT = 99.2% and UFBoot = 72%). Species with one and zero statocysts were contained in several clades (Figs V-5, V-7b, d): one-statocyst species in Clades 2, 3, and 5; statocyst-lacking species in Clades 1, 2, and 5.

Neoteny. True-neotenous group and non-neotenous group were both non-

monophyletic (Figs V-5, 7c, d). The single para-neotenous species included in my analysis was in Clade 2. Three true-neotenous species formed Clade 1, and the other was placed in Clade 4 along with non-neotenous species.

DISCUSSION

This study presents a comprehensive molecular phylogeny of Anthuroidea for the first time and provides new insights into its systematics based on the resulting tree and morphological characters. The obtained topology is, although the most basal branches are not well supported, different from the morphology-based phylogenetic analyses (Fig. V-1; Wägele 1981a, 1989; Poore 2001). Out of the current six families, monophyly of Anthuridae and Hyssuridae was moderately/fully supported, but that of Antheluridae, Expanathuridae, Leptanthuridae, and Paranthuridae was not supported (Figs V-5, V-6). I provide more discussions for each family, clade, and character below.

Antheluridae in Clade 5. The monophyly of Antheluridae was not supported; *Anthomuda iriomotensis* **sp. nov.** was sister to the remaining groups, and *Ananthura* sp. was sister to the Expanathuridae–Hyssuridae clade. Future taxonomic operation regarding the paraphyly of the family requires molecular information from species in the type genus *Anthelura* Norman & Stebbing, 1886, not included in my dataset.

Species in *Anthomuda* possess a short antennal flagellum and four to five spiniform setae on the third article of mandibular palp and are found in shallow water, whereas those in the other two anthelurid genera possess a long antennal flagellum and six to twelve spiniform setae on the third article of mandibular palp and inhabit deep-sea

bottoms (Negoescu and Svavarsson 1997; Poore 2001). These morphological and ecological differences may support the paraphyletic nature of the family.

One of the diagnostic characters of Antheluridae is to possess one statocyst in the pleotelson (Poore and Lew Ton 1988a; Poore 2001). However, some anthelurid species do not share this state. For example, *Ananthura ocellata* (Nunomura, 1992) lacks statocyst (Nunomura 1992), and *Ananthura rigida* Nunomura, 1976 possesses two statocysts (Nunomura 1976).

Anthuridae in Clade 4. The monophyly of Anthuridae was moderately supported (SH-aLRT = 99.2% and UFBoot = 72%). The synapomorphies of the family are to possess (1) the fused pleonites 1–5, (2) two statocysts on the pleotelson, and (3) only a single posterodistal spiniform seta on the propodi of pereopods 2–7 (Poore 2001).

In this study, three anthurid species (Anthuridae sp. 1, Anthuridae sp. 2, and Anthuridae sp. 3; Fig. V-3p–r) were identified only at the family level, as they could not be assigned morphologically to any known genera. The three species shared the following characters: (1) the maxillipedal palp with three articles of dissimilar length, (2) the carpi of pereopods 4–7 rectangular, similar width to propodus bearing few setae, and (3) the leaf-like uropodal exopod. By having those characters, the three species are similar to the three genera *Indanthura* Pillai & Eapen, 1966, *Malacanthura* Barnard, 1925, and *Pilosanthura* Wägele, 1989 (Poore 2001; Chew et al. 2014). However, my species differ from *Indanthura* in having maxillipedal palp with small and narrow article 3, and from *Malacanthura* and *Pilosanthura* in having weak or no maxillipedal endite. *Malacanthura* is one of the most confusing genera (Poore 2001), and especially related to *Indanthura* or *Haliophasma* Haswell, 1881 (Negoescu and Wägele 1984; Poore and Lew Ton 1988b; Poore 2001). When identifying those genera, careful comparison among them is required.

The three species did not form a monophyletic clade in my phylogenetic tree (Fig. V-5).

My dataset contained only eight among 26 anthurid genera. Future phylogenetic analysis including more genera, especially *Apanthuroides* whose phylogenetic position is controversial in the previous studies (Fig. V-1), is needed.

Expanathuridae in Clade 5. *Eisothistos* formed a moderately supported clade with Hyssuridae (SH-aLRT = 81.9% and UFBoot = 79%), which was sister to the expanathurid type genus *Expanathura*. *Eisothistos* shows exceptional uniqueness in some morphological and biological aspects. *Eisothistos* species have (1) the mouthparts produced anteriorly and covered with upper lip dorsally, (2) the linear mandible without palp, (3) the similar pereopods 1–7 (pereopods 1–3 not subchelate), and (4) the serrated uropodal exopod and endopod (Poore 2001). In terms of ecology, *Eisothistos* females invade the polychaete tube and become worm-like (vermiform), grossly elongated body shaped by stretching the intersegmental integument after spawning in the tube (Wägele 1979; Poore 2001; Knight-Jones and Knight-Jones 2002; Poore and Lew Ton 2002). Unlike typical isopods, females with oostegites have never been observed (Poore 2001). These features suggest *Eisothistos* may be phylogenetically distant from the other six expanathurid genera, as suggested in my tree.

Hyssuridae in Clade 5. The monophyly of Hyssuridae is fully supported in my analysis, although I included only two OTUs. Wägele (1981a, 1989) and Poore (2001) placed the family as the sister group to the remaining taxa within Anthuroidea. The synapomorphies of the family are to possess (1) the elongate pleon, (2) the carpi of pereopods 2 and 3 being strongly produced postero-distally, and (3) the pleopods 1–5 similar, not operculiform (Poore and Lew Ton 1988c; Poore 2001).

Leptanthuridae in Clades 2 and 3. Leptanthurids were in two distinct clades, *Accalathura* clade (in Clade 2) and Clade 3 (*Leptanthura* sp. and Leptanthuridae sp.). Clade 3 is the true leptanthurid clade as the type genus *Leptanthura* G. O. Sars, 1897 is encompassed in it. *Accalathura* was encompassed in Clade 2 together with some paranthurids with moderate support (SH-aLRT = 96% and UFBoot = 68%).

Accalathura and its highly similar genus *Negoescuanthura* Jarquín-Martínez & García-Madrigal, 2021 show several morphological differences from the other leptanthurid genera. Pereopod-1 palm of species in these two genera lack rows of pectinate spiniform setae, one of the diagnostic states for Leptanthuridae (Poore 2001). Members of these two genera have dark body pigmentation, whereas the other leptanthurids lack it (Poore 2001; Jarquín-Martínez and García-Madrigal 2021). They also possess a multi-articulated antennal flagellum, whereas the others do not (Poore and Lew Ton 1990; Poore 2001; Jarquín-Martínez and García-Madrigal 2021). Eyes of isopods in these two genera are black and well-developed, and the color is retained in ethanol (cf. Jarquín-Martínez and García-Madrigal 2021; fig. 14). On the other hand, eyes of species in other leptanthurid genera are usually absent or weakly developed (Poore 2001); for *Leptanthura* species, eye color is red when alive, but the color becomes lost in ethanol (Matsushima et al. 2025). These morphological differences and my molecular phylogeny suggest these two genera may be phylogenetically distant from the other confamilial members.

Paranthuridae in Clades 1 and 2. Paranthurids were contained in two major, highly or moderately supported clades: Clade 1 (*Colanthura* and *Cruranthura*) and Clade 2 (*Deltanthura* **gen. nov.** and *Paranthura* with leptanthurid *Accalathura*). Within Clade

2, *Deltanthura* **gen. nov.** was sister to leptanthurid *Accalathura* with weak support (SH-aLRT = 79.4% and UFBoot = 79%), and the paranthurid type genus *Paranthura* was monophyletic with full support.

Monophyly of the four paranthurid genera *Califanthura*, *Colanthura*, *Cruranthura*, and *Cruregens* has traditionally been supported with the synapomorphies such as the blunt mandible without a palp (highly specialized, piercing-type mouthparts) and the absence of pereopod 7 in adults (true-neotenous) (Poore 1984, 2001; Wägele 1989). These characters can morphologically define Clade 1 and clearly distinguish the four genera not only from the other paranthurids but also from the other anthuroid genera. Given the above, it may be better to remove those four genera from Paranthuridae and establish a new family for them; however, as basal branches in my tree lack high support, I refrain from establishing it. Morphology-based phylogenetic analyses (Poore 1984, 2001; Wägele 1989) recovered the paranthurid type genus *Paranthura* as the sister taxon to the four genera, which was not supported by my molecular phylogenetic analysis.

Mouthparts. Morphology-based phylogenetic analyses (Wägele 1981a, 1989; Poore 2001) regarded biting-type mouthparts as ancestral and piercing-type as derivative. In my tree, no clades contained more than two types of mouthparts, each type was not grouped in a single well-supported clade but in multiple clades, and the relationship among clades was unclear. The evolutionary history of mouthpart morphology remains an open question.

In the piercing type, the highly specialized subgroup was monophyletic (Clade 1) but the moderately specialized one was not (Clades 2 and 3). I initially assumed that each of two subgroups has an independent origin, but my tree cannot rule out that the moderately specialized one evolved multiple times. The morphological differences

between the two subgroups suggest that species in the highly specialized subgroup may use prey items that differ from those of species in the moderately specialized subgroup.

Statocyst number. Clades 1, 3, and 4 each composed of species showing the same statocyst number (zero, one, and two, respectively), but Clades 2 and 5 contained species with zero or one statocyst. It suggests that the number of statocysts would not be so stable and the gain/loss of statocysts may often occur. Due to the lack of basal branch supports, the evolutionary history of statocyst-number change also remains unsolved.

Wägele (1981a) and Nunomura and Shimomura (2012) suggested that the loss of the statocyst is associated with the gain of the ability to climb substrates well and the loss of digging capability. This means that species living in unstable substrates, such as sand and mud, possess statocyst(s) to recognize their spatial position and to maintain balance, whereas species living on stable substrates, such as algae and coral rubble, lack statocyst as they can maintain balance by grabbing the substrates with their pereopods. However, a strong correlation between the number of statocysts and the inhabiting substrates was not detected in my collected species. From coral rubble, species with two (e.g. *Mesanthura miyakoensis* Nunomura, 1979), one (e.g. *Accalathura* sp.), and zero (e.g. *Cruranthura viridis* **sp. nov.**) statocysts were collected. Also, from algae, species with both two (e.g. *Mesanthura nigrodorsalis* Nunomura, 1977) and zero (e.g. *Paranthura japonica* Richardson, 1909) statocysts were collected. As well, from sand, species with both two (e.g. *Cy. muromiensis*) and zero (e.g. *Paranthura alba* Nunomura, 1977) statocysts were collected.

I assume that the swimming ability or the frequency of swimming may also be related to the number or the absence/presence of statocyst(s), i.e., the species that swim well tend to possess statocyst(s) and vice versa. For deep-sea macrostylid isopods which

possess a pair of statocysts in the pleotelson and perform long-distance dispersal by water current, Bober et al. (2018) suggested that statocysts may play a role in perceiving gravity in the water column. In anthuroids, long-distance dispersal is not known and have low swimming ability in general (Wägele 1981a), but they occasionally swim by mainly using their pleopod 1 (S Shiraki personal observation). *Accalathura* species with one statocyst swims a short distance (Wägele 1985). Hyssurids with small pleopod 1 (Fig. II-VI-4), indicating the less swimming ability, do not possess statocyst. From my experience, anthuroids seem to show species-dependent differences in swimming frequency. Also, the size of statocyst(s) may be affected by the degree of necessity of gravity perception. By accumulating the knowledge on swimming frequency, swimming ability, and utilizing microhabitat for each anthuroid species, complex interactions between the number/size of statocysts and swimming ability or preferred substrate types will be revealed, which resolves the evolution of statocysts.

In isopods, statocysts have been reported only from Anthuroidea and the asellote family Macrostylidae (Poore and Lew Ton 1988a; Wägele 1989, 1992; Sekiguchi and Terazawa 1997). Macrostylidae is phylogenetically distant from Anthuroidea (Thomas Thorpe 2024; Barta et al. preprint), and their statocysts are of independent origin.

Neotenous character. My result showed that the true-neotenous trait has evolved at least twice within Anthuroidea; one in Clade 1 and the other in the anthurid *Leipanthura* in Clade 4 (Fig. V-7d). The leptanthurid *Curassanthura*, another true-neotenous genus (Alvarez et al. 2019), was not included in this analysis. In addition to the six true-neotenous genera, *Apanthuroides* contains true-neotenous species, even though it is not the generic diagnostic character (e.g. *Apanthuroides aldabrae* Kensley & Schotte, 2000; Kensley and Schotte 2000; Poore 2001). Considering these points, the

true-neotenous trait must have evolved more frequently within Anthuroidea. Para-neotenous trait was independently acquired in *Deltanthura* **gen. nov.** (Fig. V-7d). The ancestral state of Anthuroidea is likely non-neotenous, as most anthuroid species across various families exhibit non-neotenous trait.

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SUMMARY

As a terminal of my research journey on anthuroids during my doctoral course, I summarize the thesis here. Based on the samples collected, I conducted taxonomy of Anthuroidea in Japan (Chapter II) and in the Philippines (Chapter III), examined the parasitic animals parasitic on/in anthuroids (Chapter IV), and conducted molecular phylogeny of Anthuroidea (Chapter V). In Chapter I, I provided an overview of Anthuroidea such as the morphology, taxonomy, ecology, and systematics.

Chapter II dealt with the taxonomy of Anthuroidea in Japan. Before this study, 39 species in ten genera and five families had been recorded from Japan (Table II-1). Here I established a new genus (*Deltanthura* **gen. nov.**), described eight new species (*Anthomuda iriomotensis* **sp. nov.**, *Malacanthura seisuiae* **sp. nov.**, *Mesanthura sol* **sp. nov.**, *Expanathura monile* **sp. nov.**, *Kupellonura tamago* **sp. nov.**, *Cruranthura viridis* **sp. nov.**, *Deltanthura palpus* **sp. nov.**, and *Paranthura oriens* **sp. nov.**), and extended the distribution records for four species (*Mesanthura cinctula*, *Mesanthura miyakoensis*, *Mesanthura nigrodorsalis*, and *Paranthura japonica*), representing the first reports of Hyssuridae and six genera from Japan. Additionally, I examined the ontogenetic change in pigmentation pattern in *M. miyakoensis* to address the taxonomic utility of pigmentation pattern in *Mesanthura* taxonomy, and investigated the predation behavior in *P. japonica*. This study updates the Japanese anthuroid fauna to 47 species in 16 genera and six families.

Chapter III dealt with the taxonomy of Anthuroidea in the Philippines. Before this study, only two anthuroid species in two genera and two families were reported in the country (Table III-I). I conducted sampling in Minadanao, and described two new species

(*Amakusanthura camiguinensis* **sp. nov.** and *Sauranthura liangaensis* **sp. nov.**). This study updates the fauna to four species in four genera and two families.

Chapter IV dealt with the parasites of anthuroids. Before my study, animals in only three taxa, such as epicaridean isopods, rhizocephalan barnacles, and trematodes, were known to parasitize anthuroid isopods. Here I firstly reported cabiropid epicarideans from anthuroids with the establishment of *Anthuroniscus* **gen. nov.** and the descriptions of three new species (*Anthuroniscus shimomurai* **sp. nov.**, *Anthuroniscus dentatus* **sp. nov.**, and *Anthuroniscus latus* **sp. nov.**), described the second anthuroid-parasitic rhizocephalan *Duplorbis changeling* **sp. nov.**, and firstly reported trematodes parasitic in *Cyathura muromiensis*.

Chapter V dealt with the molecular phylogeny of Anthuroidea based on the four gene markers (18S rRNA gene [18S], 28S rRNA gene [28S], 16S rRNA gene [16S], and cytochrome *c* oxidase subunit I gene [COI]), obtained from 37 Japanese species representing at least 20 genera and all current six families. The resulting tree recovered five major clades: Clade 1 contained paranthurid *Colanthura* and *Cruranthura*; Clade 2 contained the other paranthurids and the leptanthurid *Accalathura*; Clade 3 contained the other leptanthurids; Clade 4 consisted of all anthurids; and Clade 5 contained Antheluridae, Expanathuridae, and Hyssuridae. The result showed the monophyly of Anthuridae and Hyssuridae with moderate and full supports, respectively, but not support that of the remaining four families. Inter-familial relationship was not resolved in the analysis.

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FIGURES AND TABLES

Figure II-I-1. *Anthomuda iriomotensis* **sp. nov.**, holotype, female lacking oostegites, dorsal view of ethanol-fixed specimen. Scale bar: 1 mm.

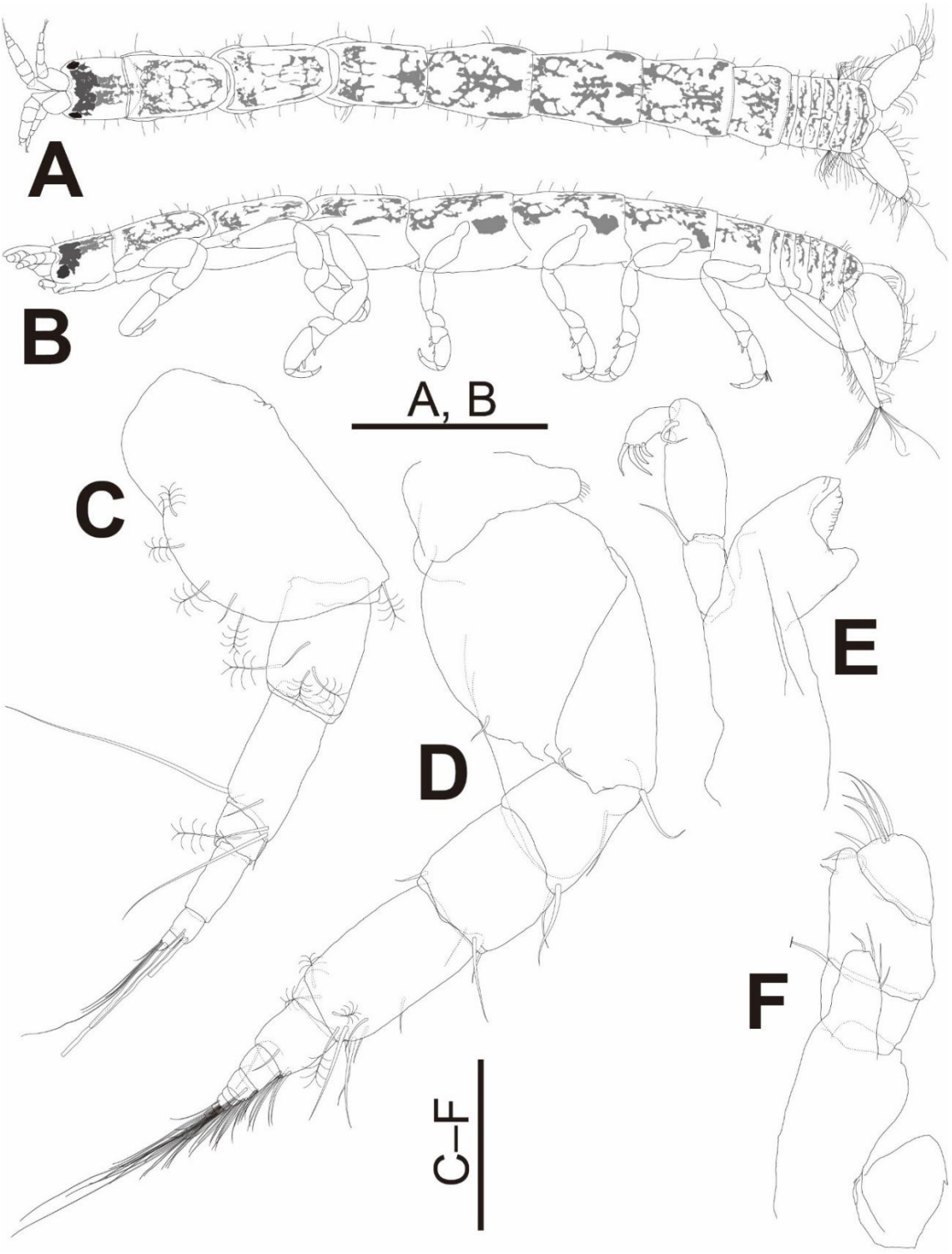

Figure II-I-2. *Anthomuda iriomotensis* **sp. nov.**, holotype, female lacking oostegites. A, dorsal view; B, lateral view; C, right antennula; D, right antenna; E, right mandible; F, right maxilliped. Scale bars: 1 mm (A, B); 100 μm (C–F).

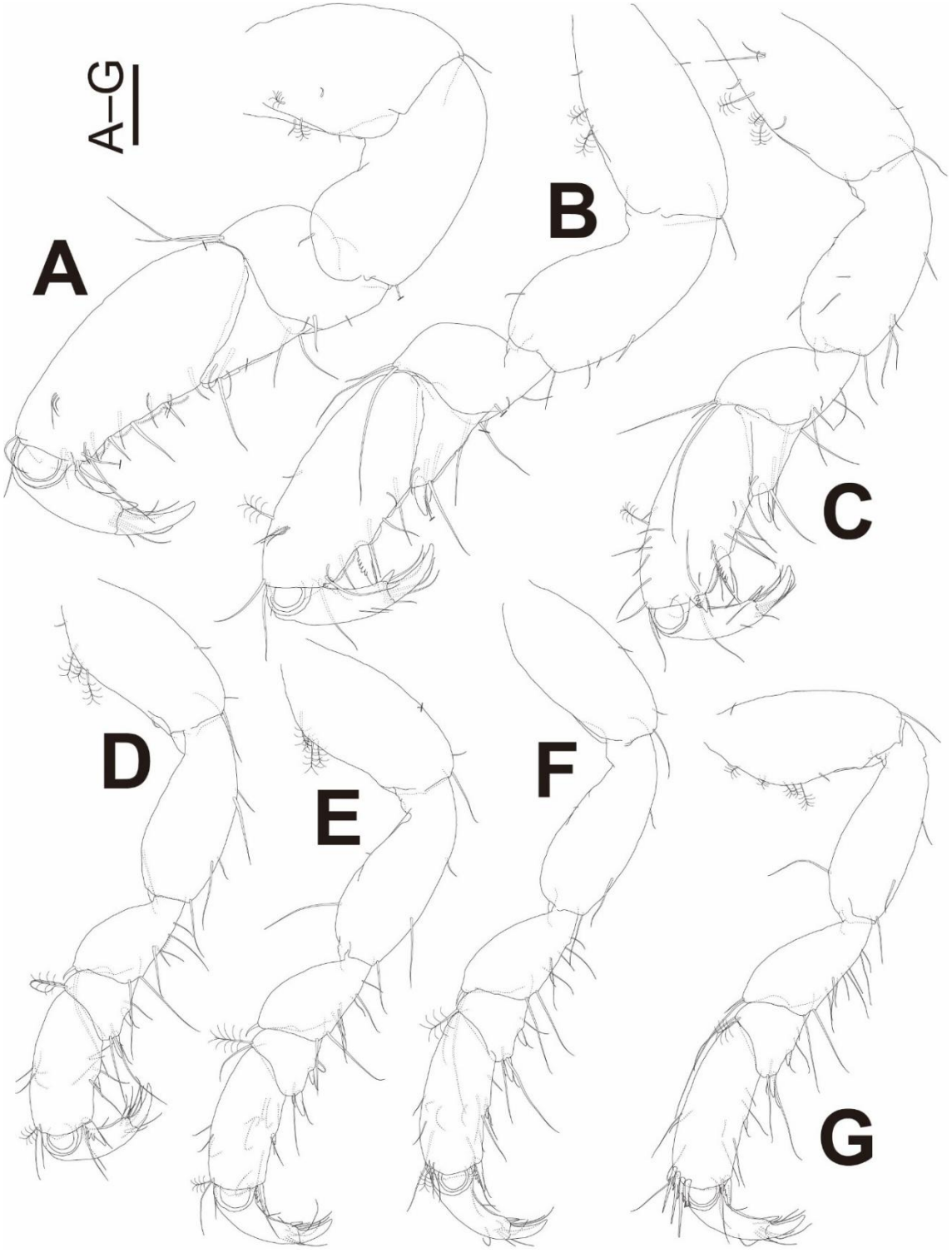

Figure II-I-3. *Anthomuda iriomotensis* **sp. nov.**, holotype, female lacking oostegites. A–G, left pereopods 1–7. Scale bar: 100 μm.

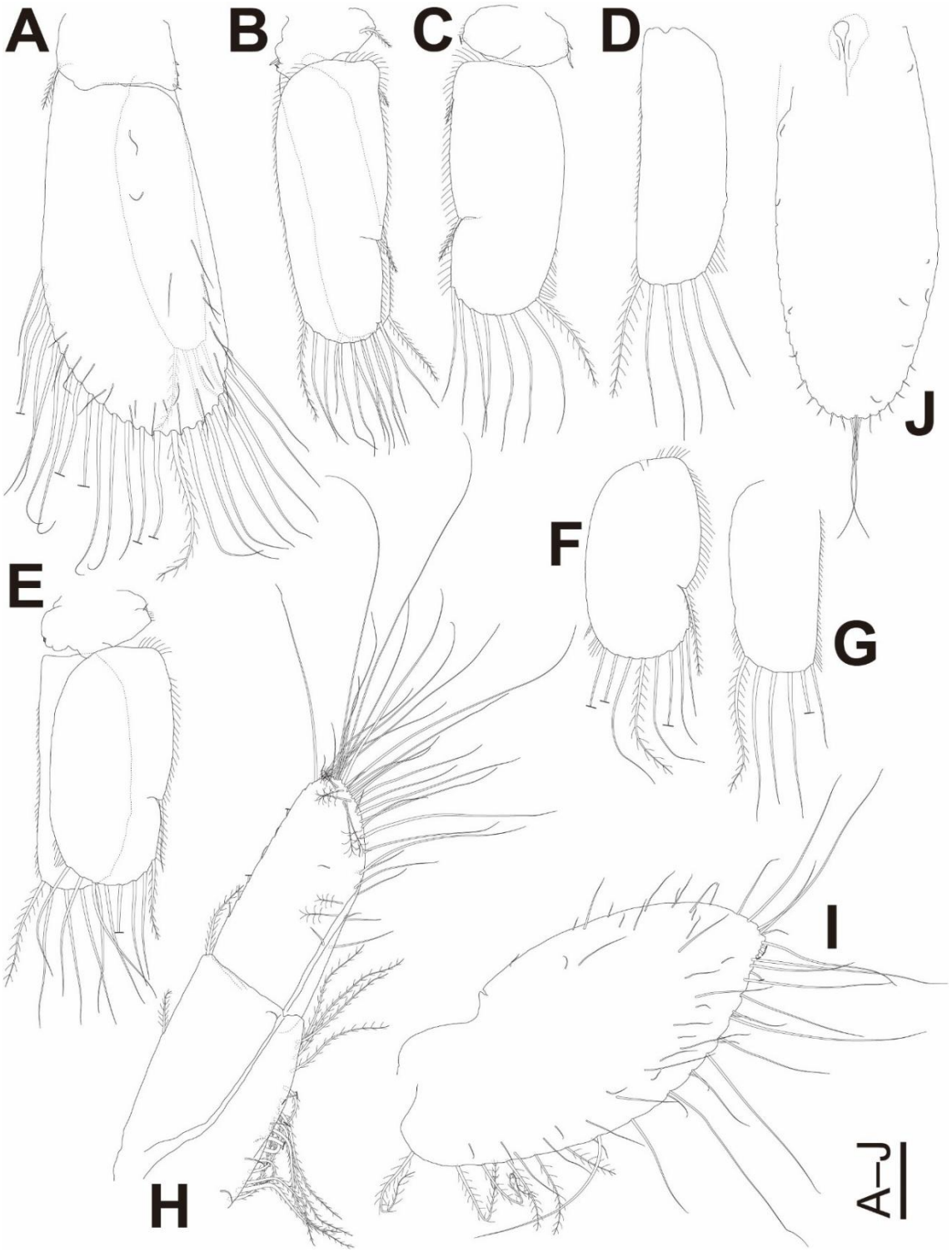

Figure II-I-4. *Anthomuda iriomotensis* **sp. nov.**, holotype, female lacking oostegites. A, right pleopod 1; B, E, left pleopods 2, 4; C, D, left pleopod 3, exopod (C) and endopod (D); F, G, right pleopod 5, exopod (F) and endopod (G); H, left uropodal endopod; I, right uropodal exopod; J, telson. Scale bar: 100 μm.

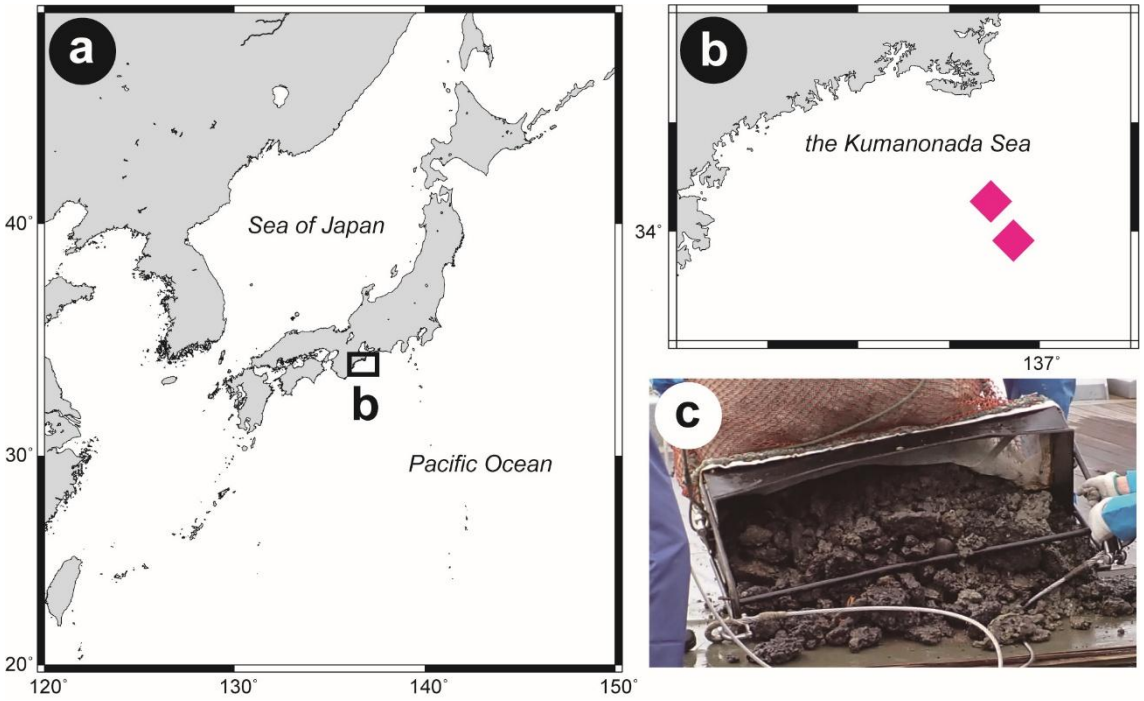

Figure II-II-1. Samling locality in this study and the inhabiting environment of *Malacanthura seisuiae* **sp. nov.** a, map of Japan; b, map of the Kumanonada Sea, pink diamonds indicate the sampling sites; c, inhabiting substrates.

Figure II-II-2. *Malacanthura seisuiae* **sp. nov.**, holotype female lacking oostegites, fresh individuals. a, b, dorsal and lateral views. Scale bar: 1 mm.

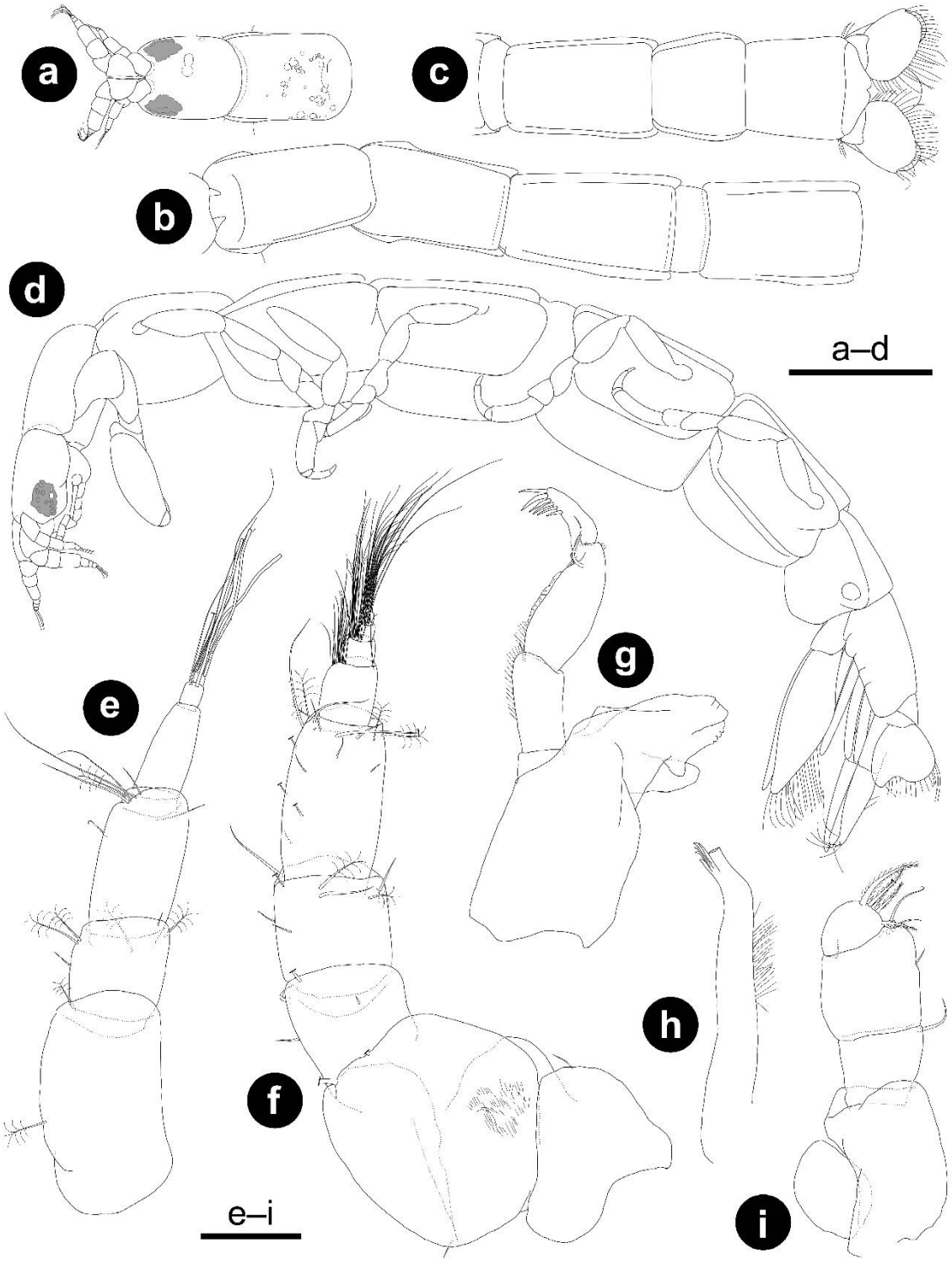

Figure II-II-3. *Malacanthura seisuiae* **sp. nov.**, holotype female lacking oostegites. a, head and pereonite 1, dorsal view; b, pereonites 2–5, dorsal view; c, pereonites 6, 7, and pleon, dorsal view; d, lateral view; e, left antennula; f, left antenna; g, right mandible; h, right maxilla (one big tooth broken); i, left maxilliped. Scale bars: a–d, 1 mm; e–i, 100 μm.

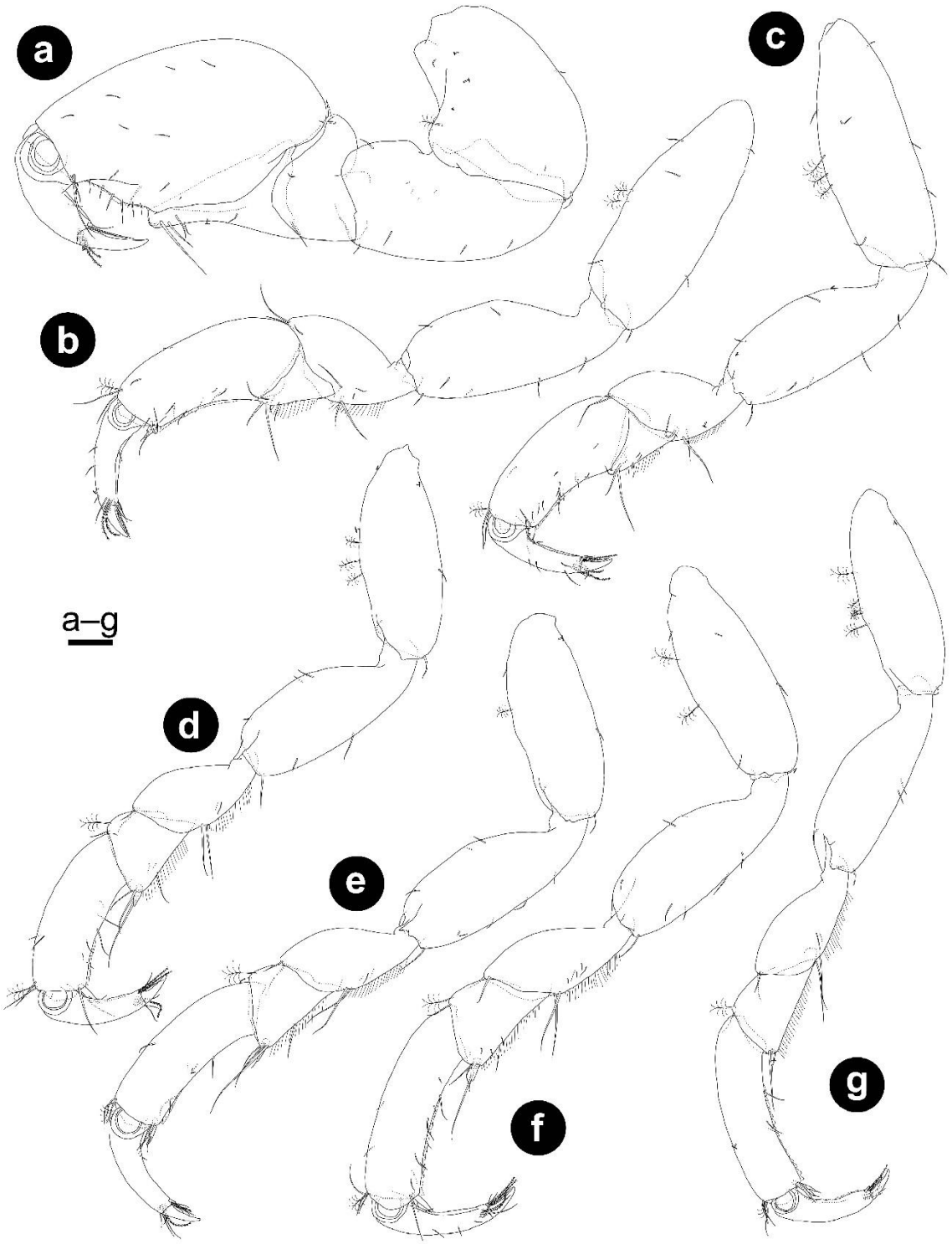

Figure II-II-4. *Malacanthura seisuiae* **sp. nov.**, holotype female lacking oostegites. a–e, g, left pereopods 1–5, 7; f, pereopod 6. Scale bar: 100 μm.

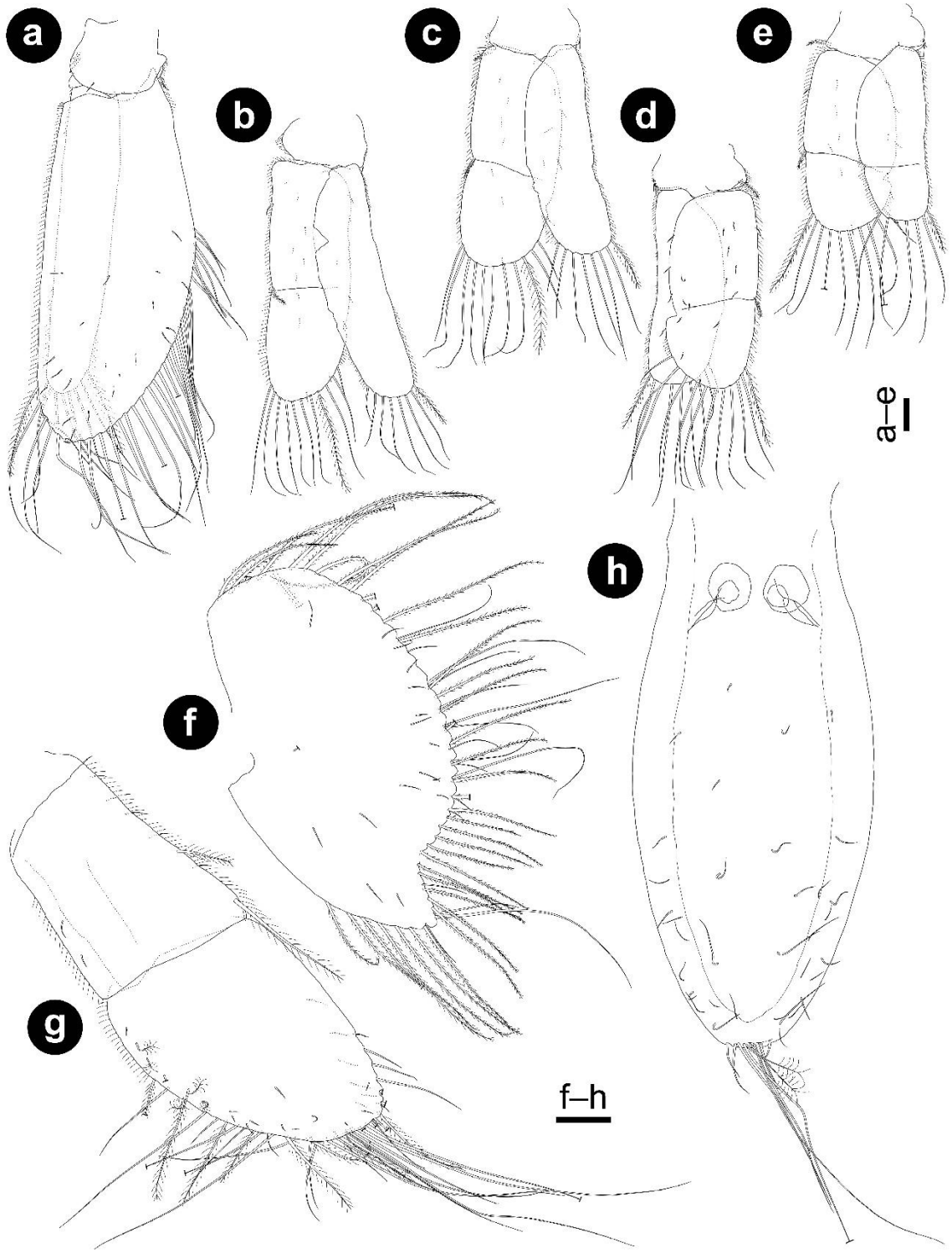

Figure II-II-5. *Malacanthura seisuiae* **sp. nov.**, holotype female lacking oostegites. a–e, left pleopods 1–5; f, left uropodal exopod; g, left uropodal endopod; h, telson. Scale bars: 100 μm.

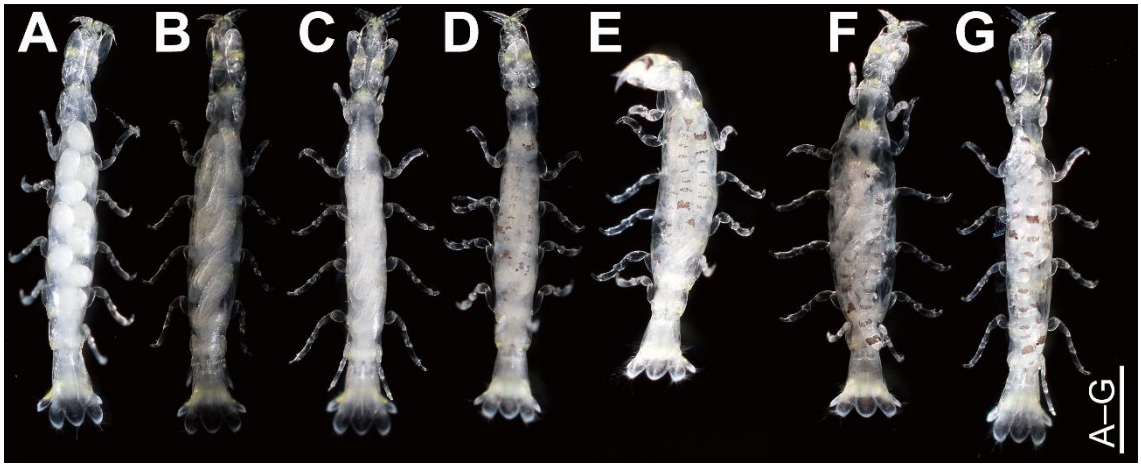

Figure II-III-1. Development, hatching, and brooding of mancae in *Mesanthura miyakoensis*. **(A–G)** Ventral views of ovigerous female on various dates in 2022. **(A)** 10 October, embryos inside marsupium. **(B)** 20 October, embryos. **(C)** 23 October, embryos. **(D)** 28 October, mancae. **(E)** 31 October, mancae. **(F)** 6 November, mancae. **(G)** 9 November, mancae. Scale bar: 1 mm.

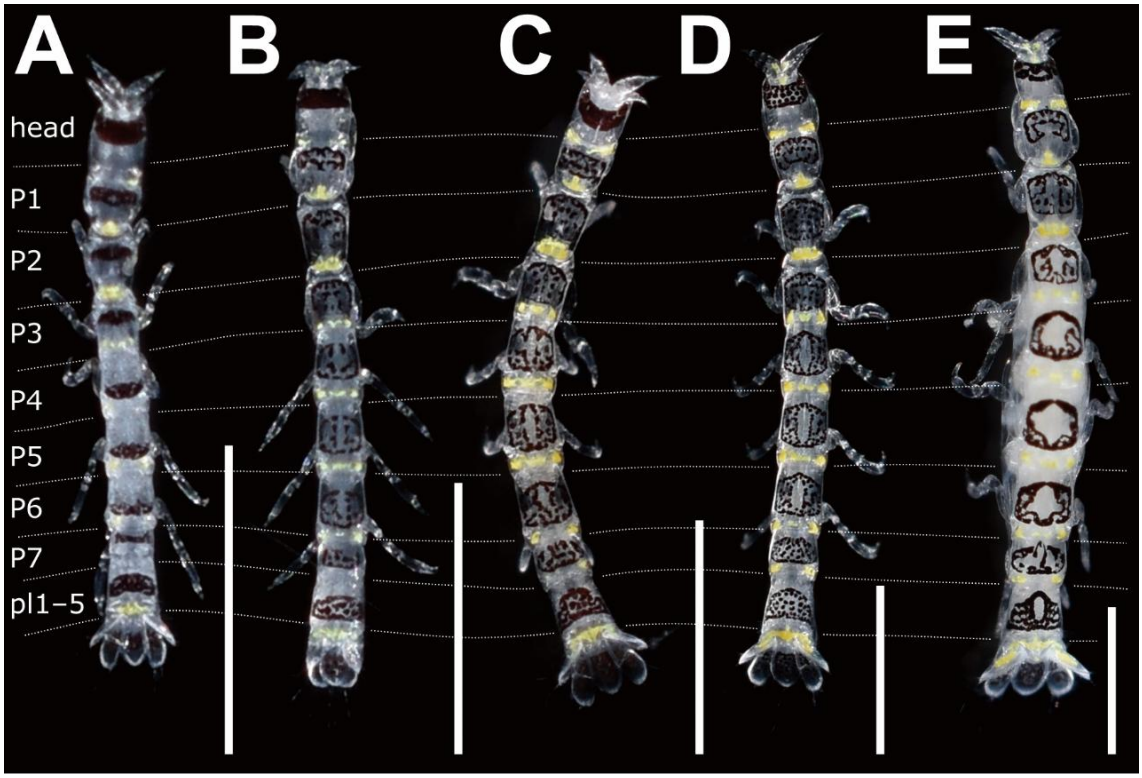

Figure II-III-2. Pigmentation patterns of female *Mesanthura miyakoensis*, living individuals, dorsal view. **(A)** Manca, first instar, Type-A pigmentation pattern. **(B)** Manca, second (?) instar, Type-B pigmentation pattern. **(C)** Post-manca. **(D)** Female lacking oostegites. **(E)** Ovigerous female. P1–7, pereonites 1–7; pl1–5, fused pleonites 1–5. Scale bars: 1 mm (note that the scale bars are progressively smaller; at the same scale, the individuals illustrated would size increase in size from left to right).

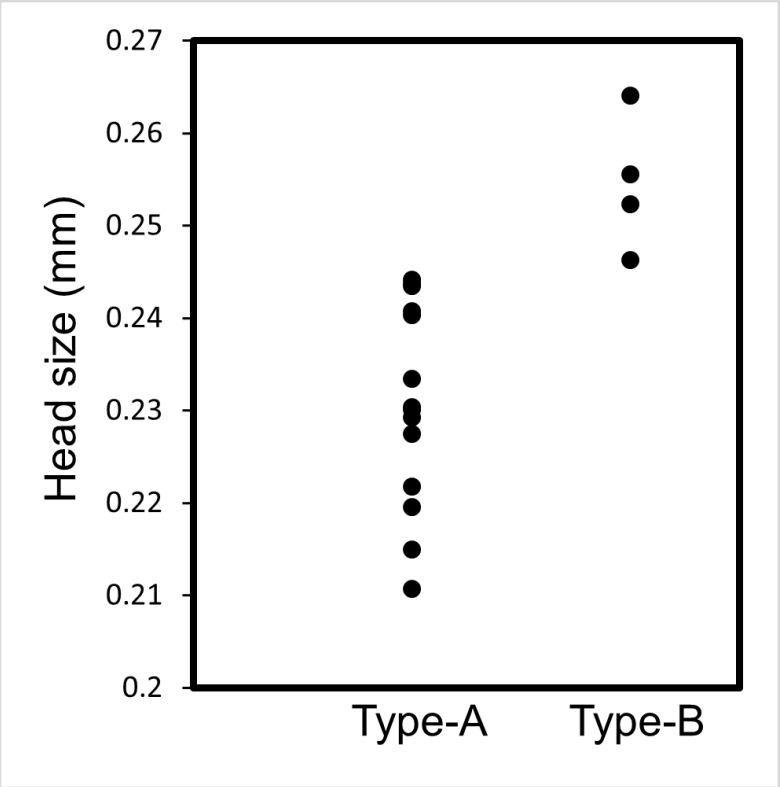

Figure II-III-3. Distribution of head lengths for mancae of Type A (*n* = 14) and Type B (*n* = 4) pigmentation patterns in *Mesanthura miyakoensis*.

Figure II-III-4. Changes in pigmentation pattern in a male *Mesanthura miyakoensis*; living individual, dorsal views. **(A)** Subadult male on 10 October 2022. **(B–E)** Same individual as an adult male, 1 day **(B)**, 5 days **(C)**, 19 days **(D)**, and 27 days **(E)** after molting to adult on 13 October. Scale bar: 1 mm.

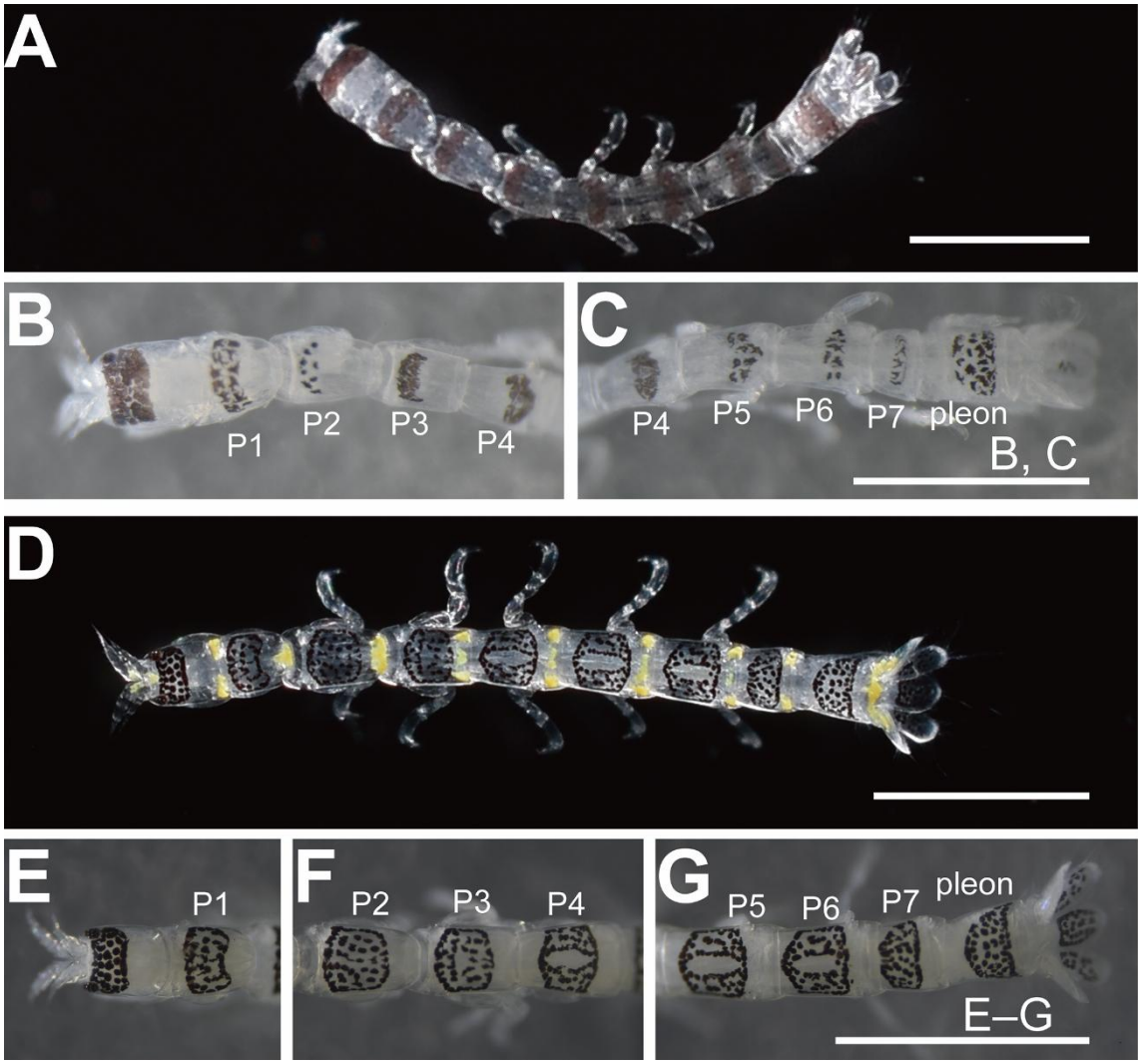

Figure II-III-5. Pigmentation pattern of living and ethanol-fixed specimens of *Mesanthura miyakoensis*. **(A–C)** Manca released on 6 November 2022. **(A)** Living individual. **(B, C)** Anterior and posterior parts of same individual photographed on 21 November 2023, after ca. 360 days in ethanol. **(D–G)** Female lacking oostegites. **(D)** Living individual. **(E–G)** Anterior, middle, and posterior parts of same individual photographed on 23 November 2023, after ca. 410 days in ethanol. P1–7, pereonites 1–7. Scale bars: 0.5 mm **(A–C)**, 1 mm **(D–G)**.

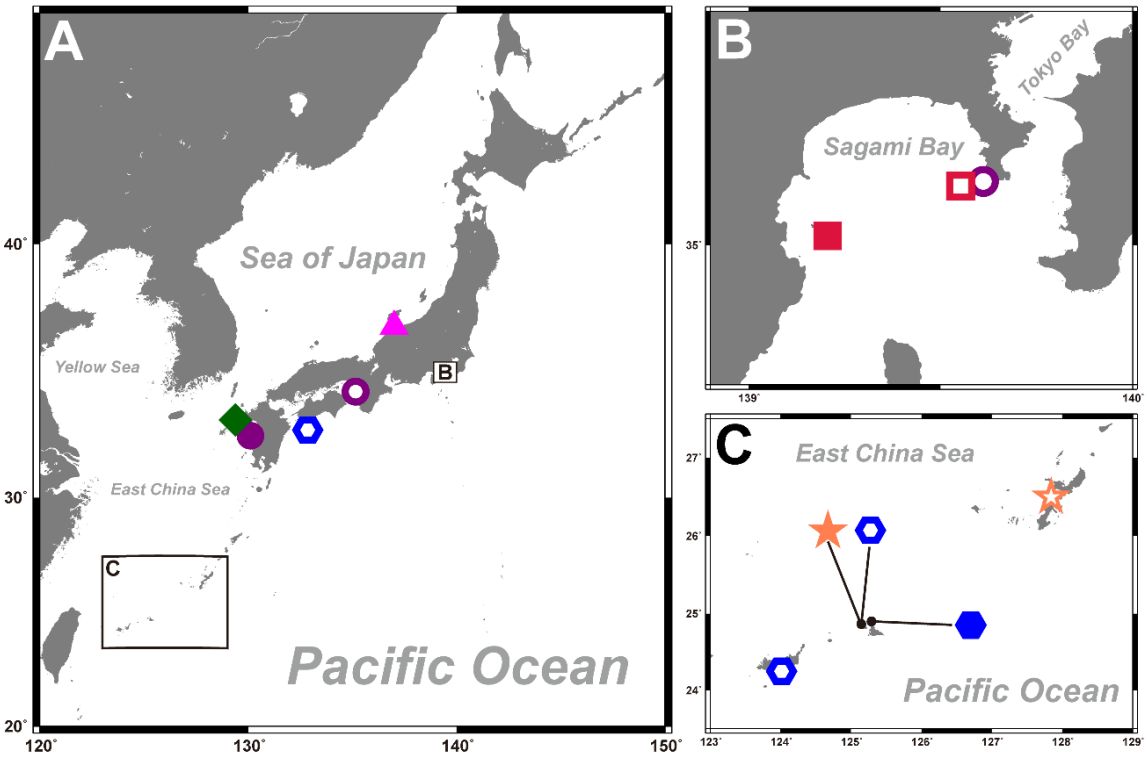

Figure II-IV-1. Distribution of six *Mesanthura* species in Japan: (A) map of Japan; (B) map of Sagami Bay; (C) map of southwestern Japan. Symbols: purple circles, *M. nigrodorsalis*; blue hexagons, *M. miyakoensis*; pink triangle, *M. atrata*; red squares, *M. cinctula*; green diamond, *M. saikaiensis*; orange stars, *M. sol* **sp. nov.**; solid symbols, type locality; open symbols, locality reported after original description.

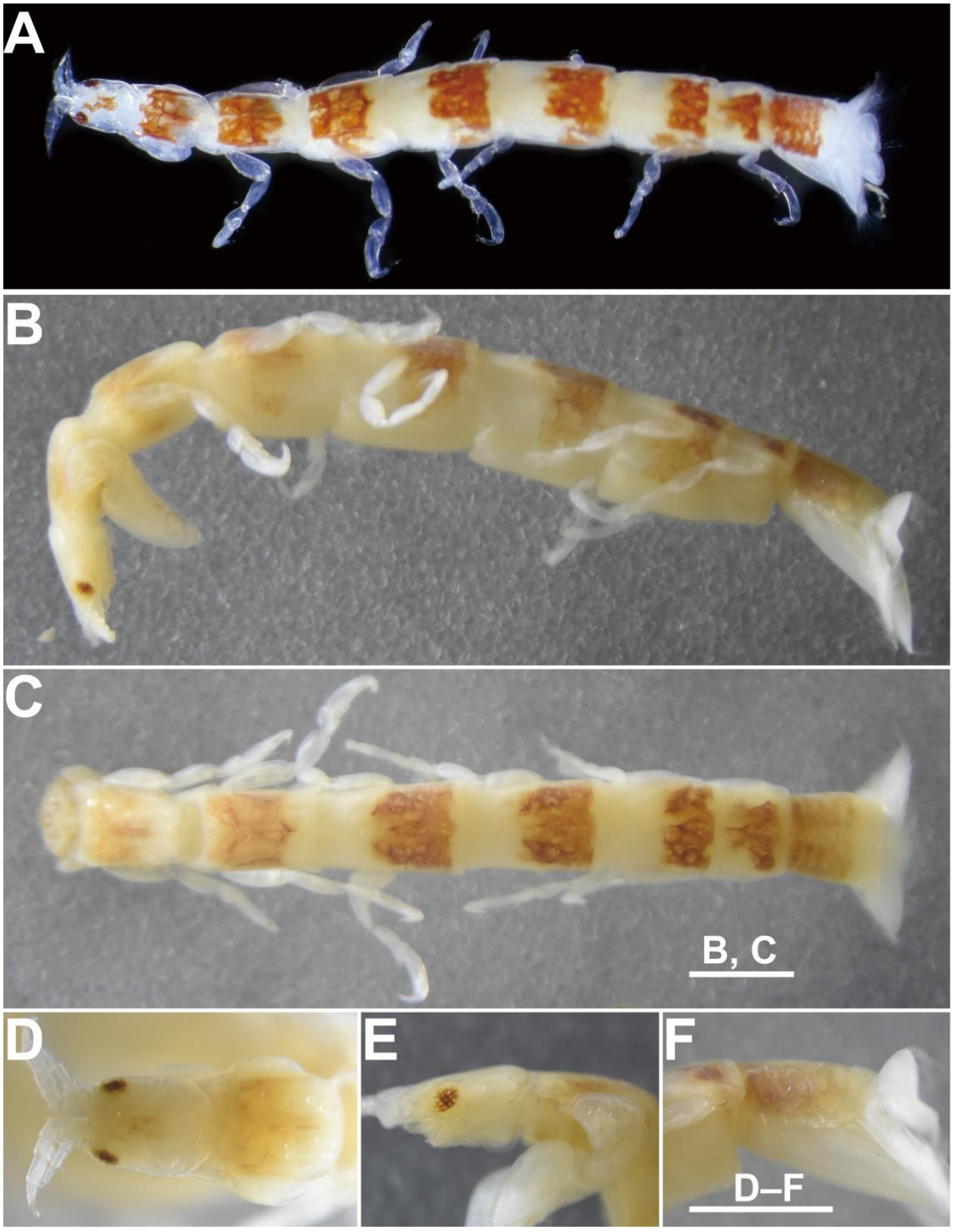

Figure II-IV-2. *Mesanthura cinctula* Nunomura, 2006, female lacking oostegites, living individual (A) and fixed specimen (B–F; ICHUM8940): (A) dorsal view (photographed by Hisanori Kohtsuka; no museum deposition), scale unavailable; (B, C) lateral and dorsal views; (D–F) head in dorsal and lateral views and pleon in lateral view. Scale bars: 1 mm.

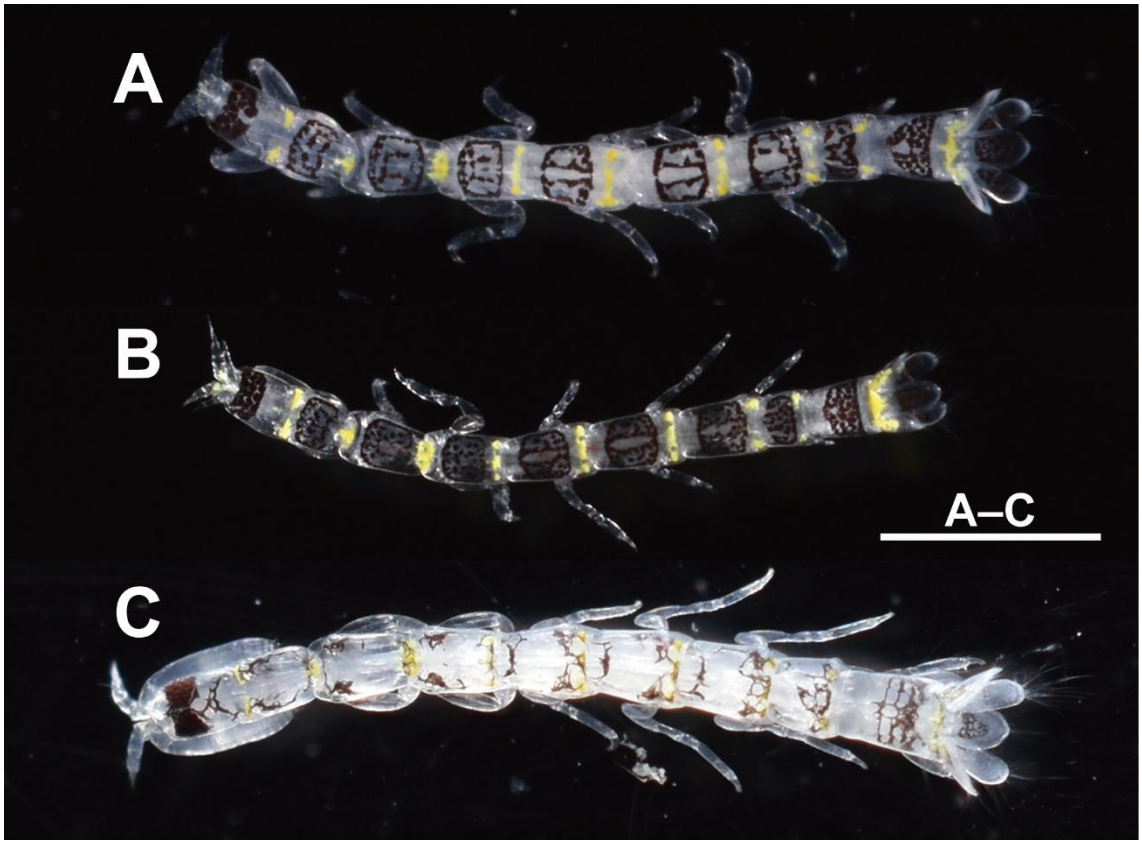

Figure II-IV-3. *Mesanthura miyakoensis* Nunomura, 1979, dorsal views of living individuals: (A) female lacking oostegites (ICHUM8944) from Tatsukushi (photographed by Yuki Oya); (B) female lacking oostegites (ICHUM8943) from Irabu Island; (C) subadult male (ICHUM8945) from Tatsukushi (photographed by Yuki Oya). Scale bar: 1 mm.

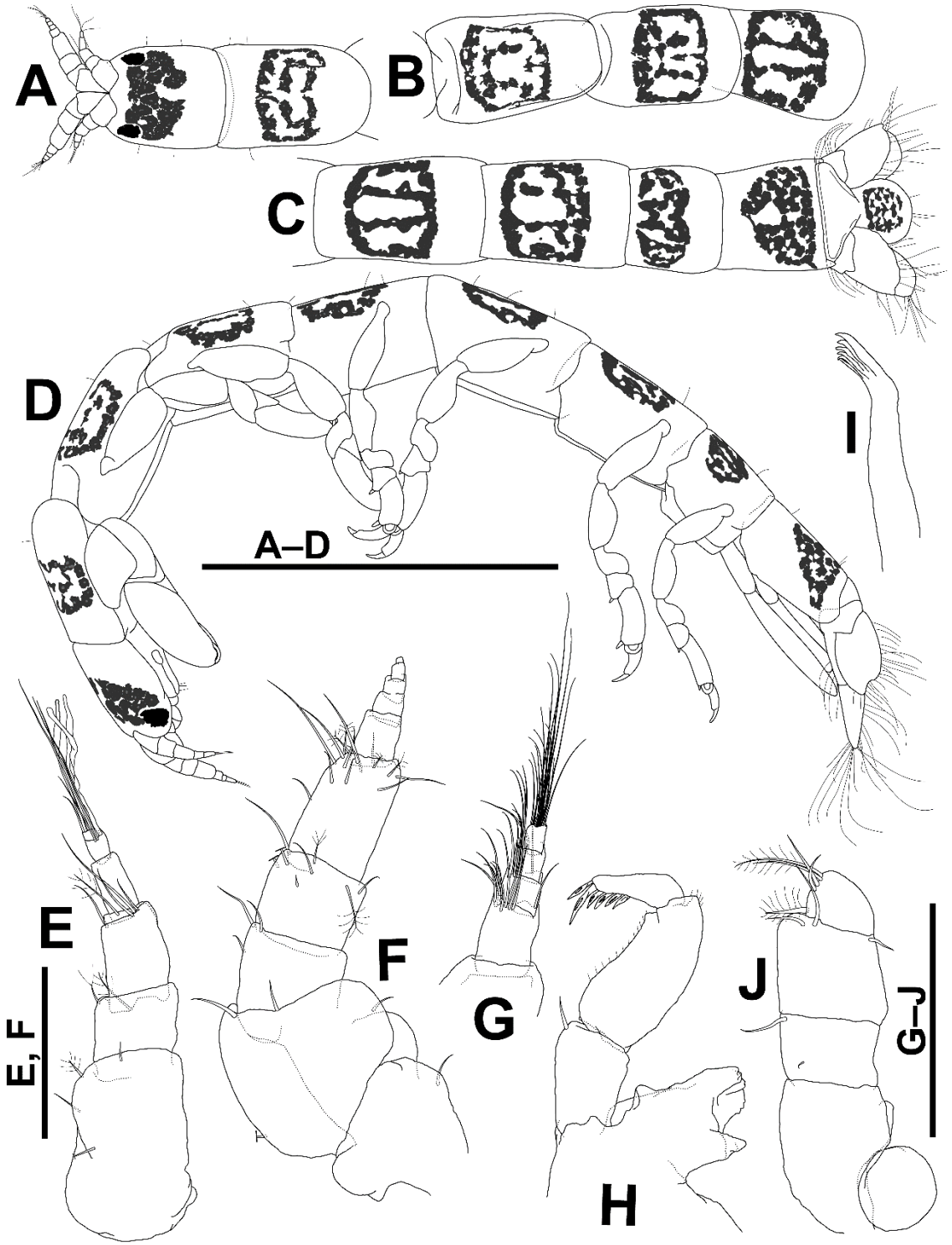

Figure II-IV-4. *Mesanthura miyakoensis* Nunomura, 1979, female lacking oostegites (ICHUM8944): (A) head and pereonite 1, dorsal view; (B) pereonites 2–4, dorsal view; (C) pereonites 5–7 and pleon, dorsal view; (D) body, lateral view; (E) left antennula; (F) right antenna; (G) flagellum of right antenna; (H) left mandible; (I) left maxilla; (J) maxilliped. Scale bars: A–D, 1mm; E–J, 100 μm.

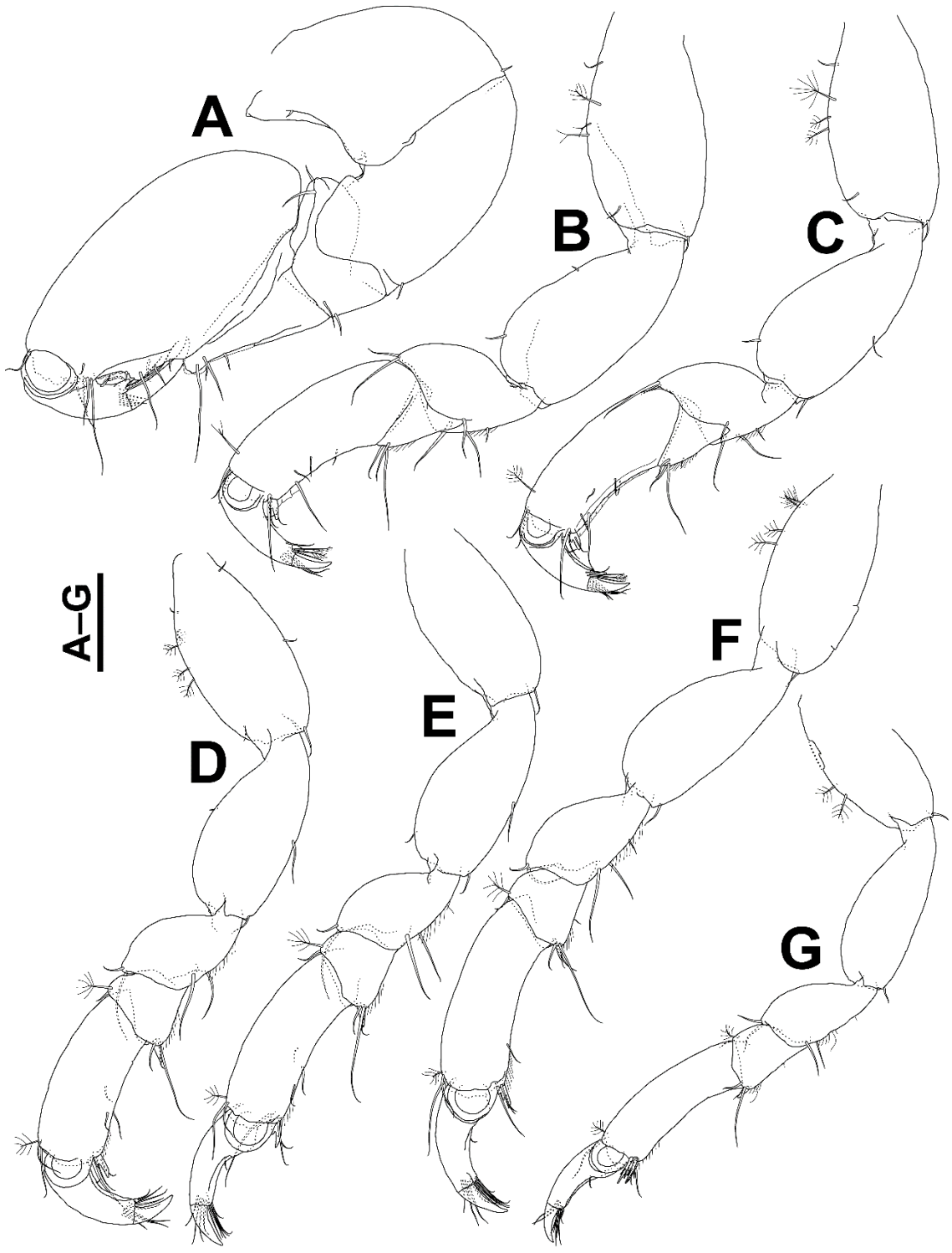

Figure II-IV-5. *Mesanthura miyakoensis* Nunomura, 1979, female lacking oostegites (ICHUM8944): (A–G) left pereopods 1–7. Scale bar: 100 μm.

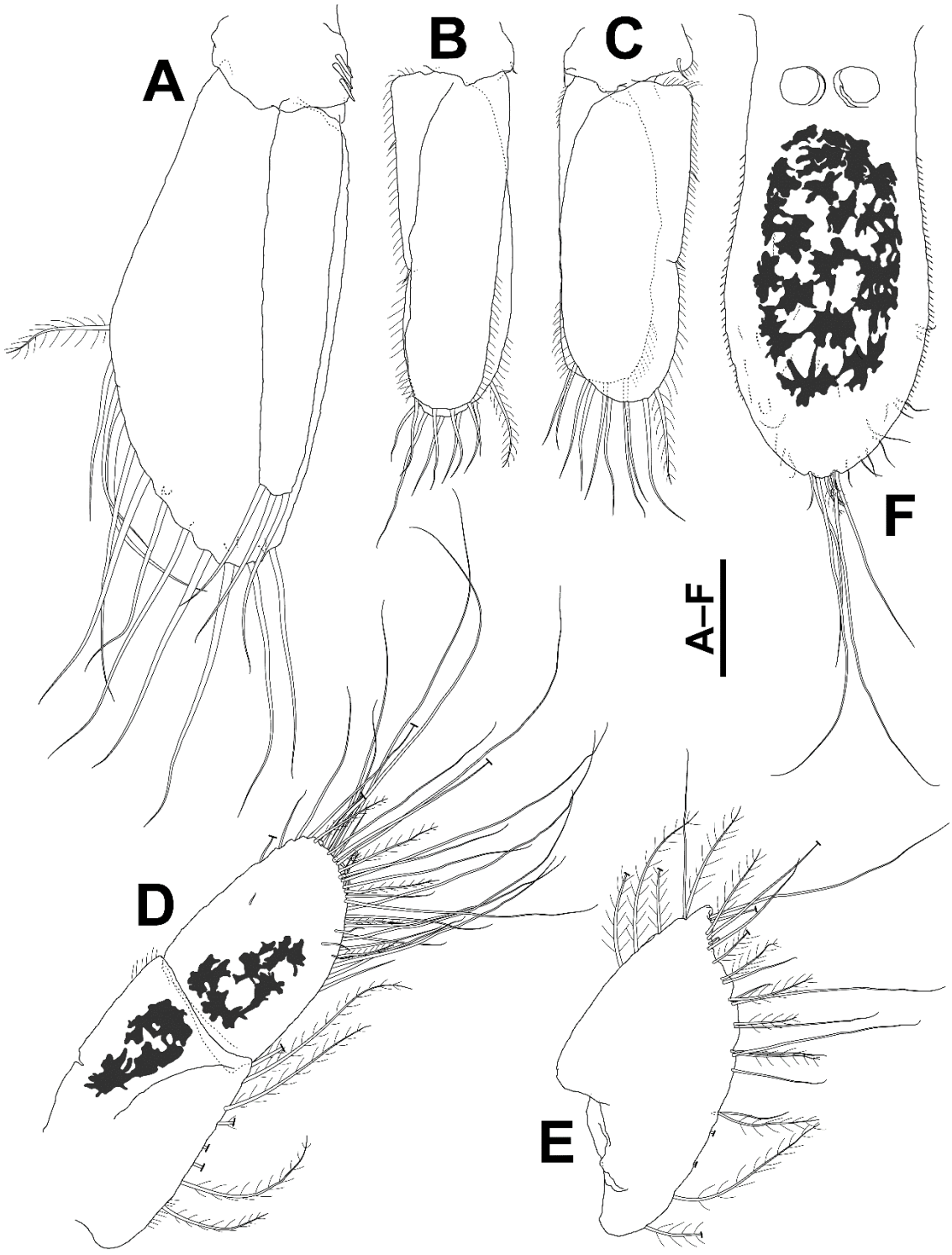

Figure II-IV-6. *Mesanthura miyakoensis* Nunomura, 1979, female lacking oostegites (ICHUM8944): (A–C) left pleopods 1–3; (D) right uropodal endopod; (E) right uropodal exopod; (F) telson. Scale bar: 100 μm.

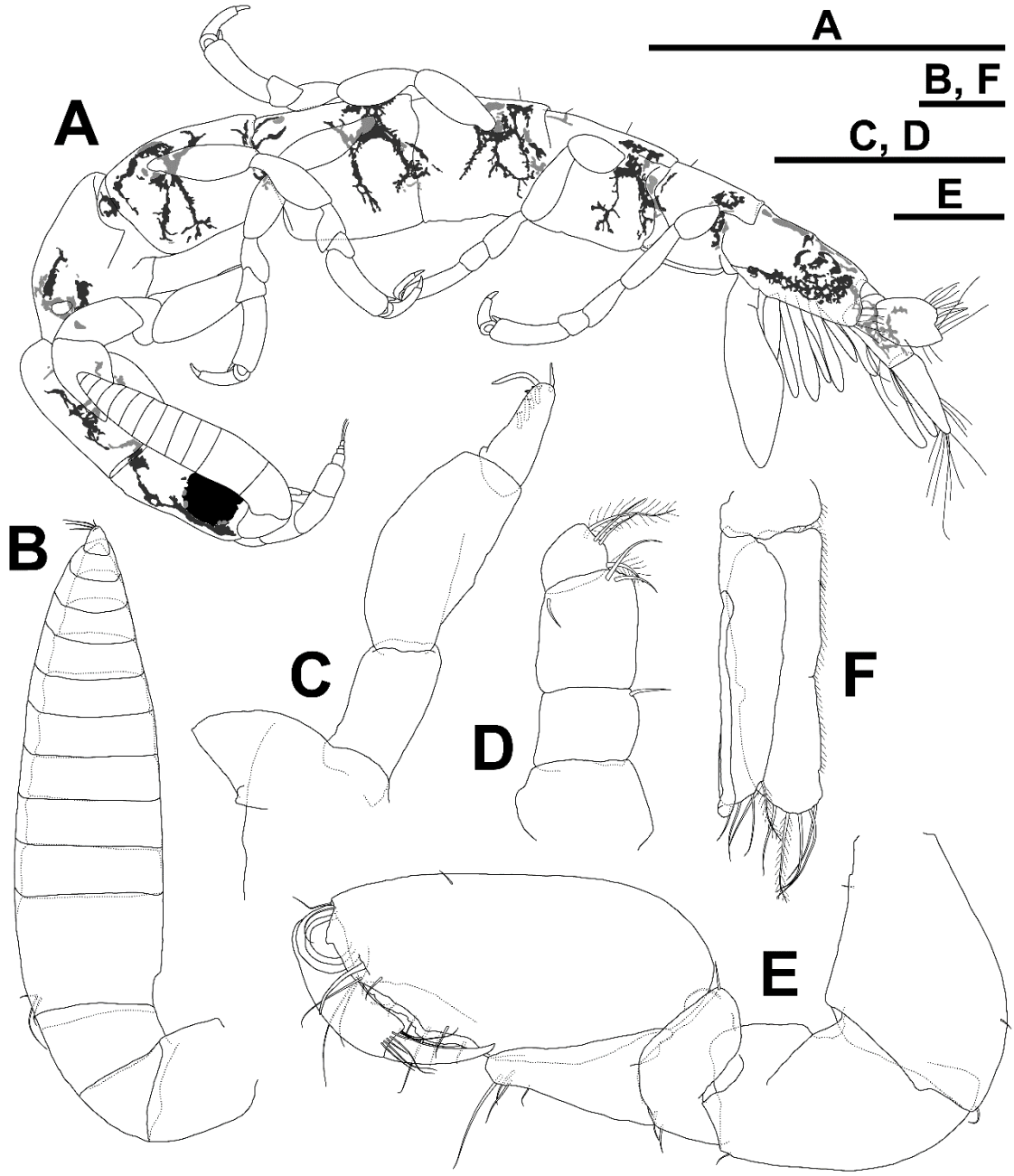

Figure II-IV-7. *Mesanthura miyakoensis* Nunomura, 1979, subadult male (ICHUM8945): (A) lateral view; (B) left antennula; (C) right mandible; (D) right maxilliped; (E) right pereopod 1; (F) right pleopod 2. Scale bars: A, 1 mm; B–F, 100 μm.

Figure II-IV-8. *Mesanthura nigrodorsalis* Nunomura, 1977, females lacking oostegites, living individual (A; ICHUM8949) and fixed specimen (B–E; ICHUM8948): (A) dorsal view of individual from Araihama (photographed by Aoi Tsuyuki); (B) lateral view; (C) head, dorsal view; (D, E) pleon, dorsal and lateral views. Scale bars: A, B, 1 mm; C–E, 500 μm.

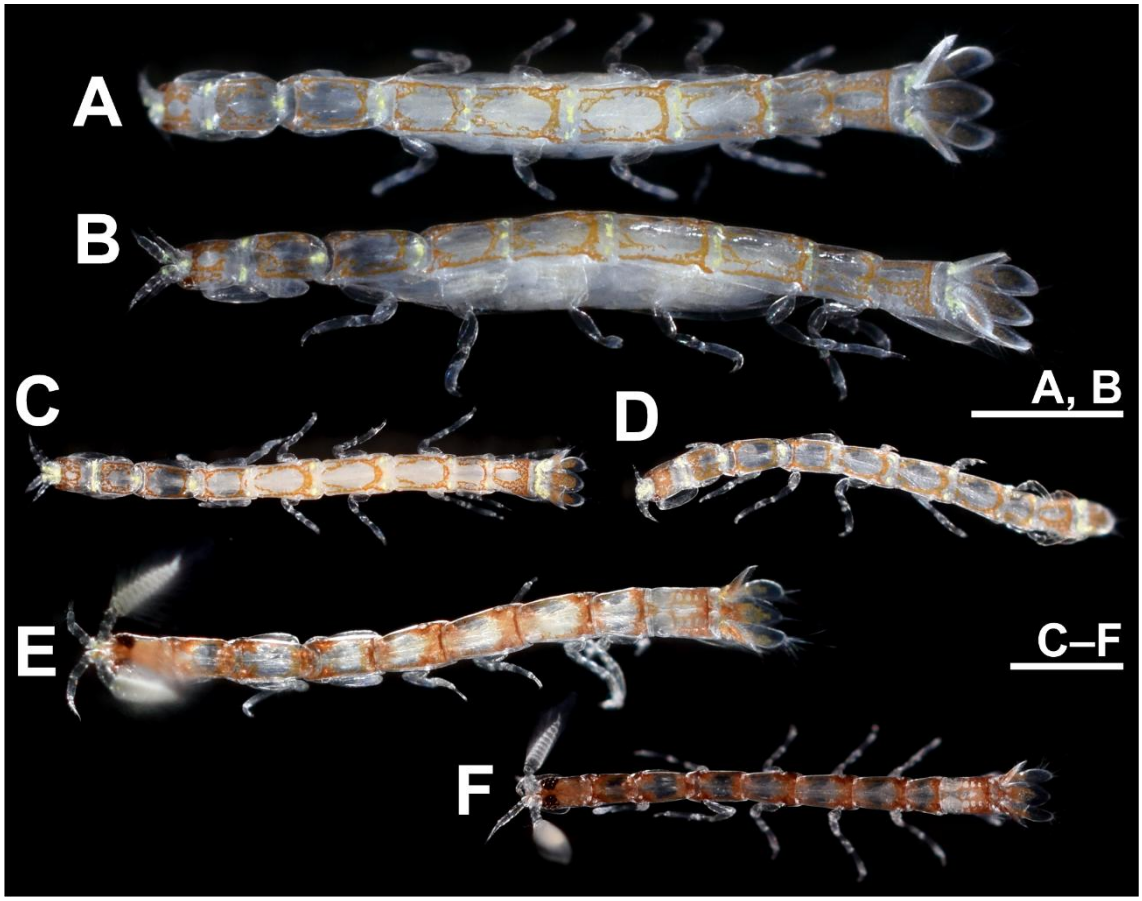

Figure II-IV-9. *Mesanthura sol* **sp. nov.**, living individuals: (A, B) dorsal and lateral views of holotype ovigerous female from Irabu (ICHUM8951); (C, D) dorsal views of paratype females lacking oostegites from Onna Village (ICHUM8952 and ICHUM8953, respectively); (F, F) dorsal views of paratype adult males from Onna Village (ICHUM8954 and ICHUM8955, respectively). Scale bars: 1 mm.

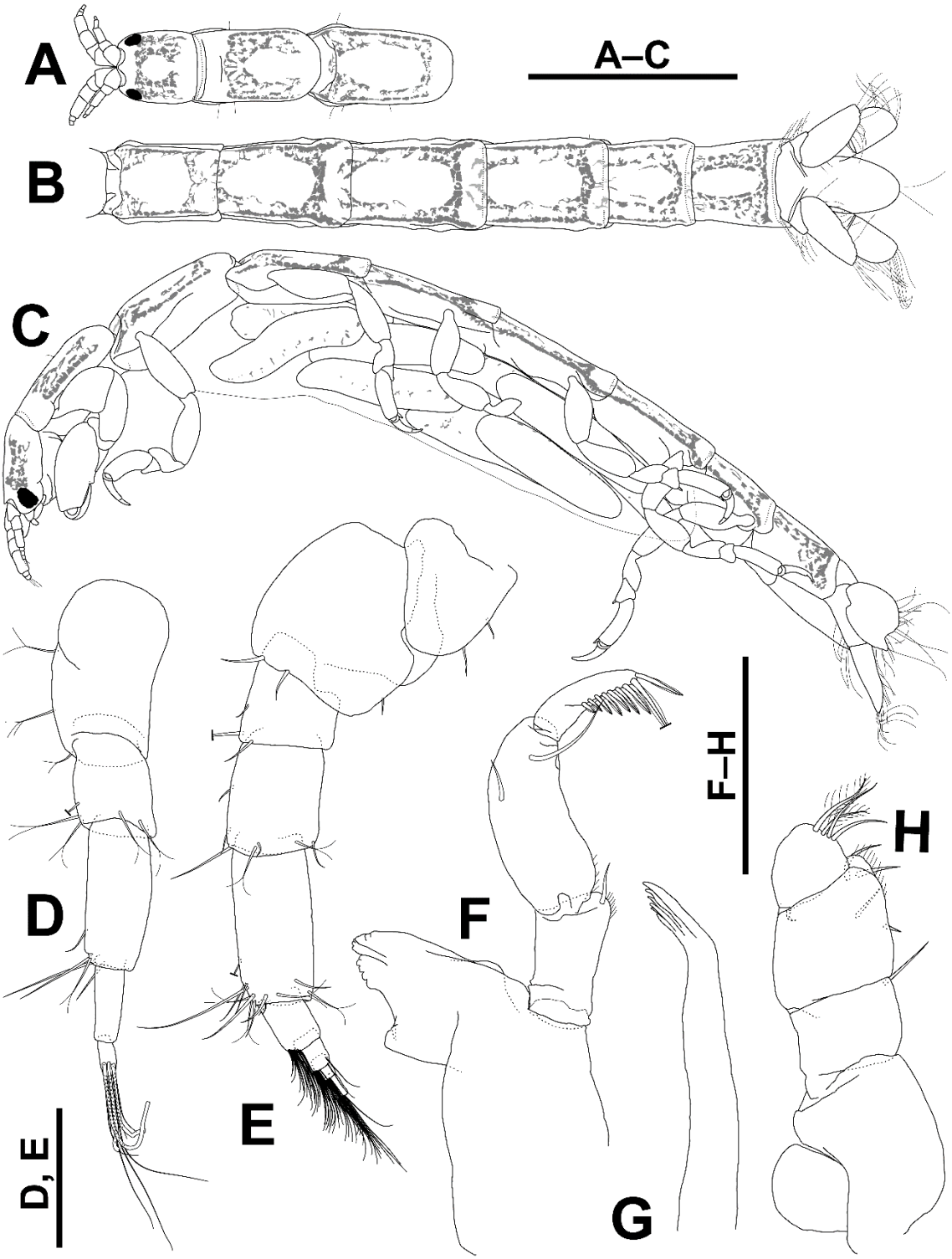

Figure II-IV-10. *Mesanthura sol* **sp. nov.**, holotype ovigerous female from Irabu (ICHUM8951): (A) head and pereonites 1, 2, dorsal view; (B) pereonites 3–7 and pleon, dorsal view; (C) lateral view; (D) left antennula; (E) right antenna; (F) left mandible; (G) maxilla; (H) maxilliped. Scale bars: A–C, 1 mm; D–H, 100 μm.

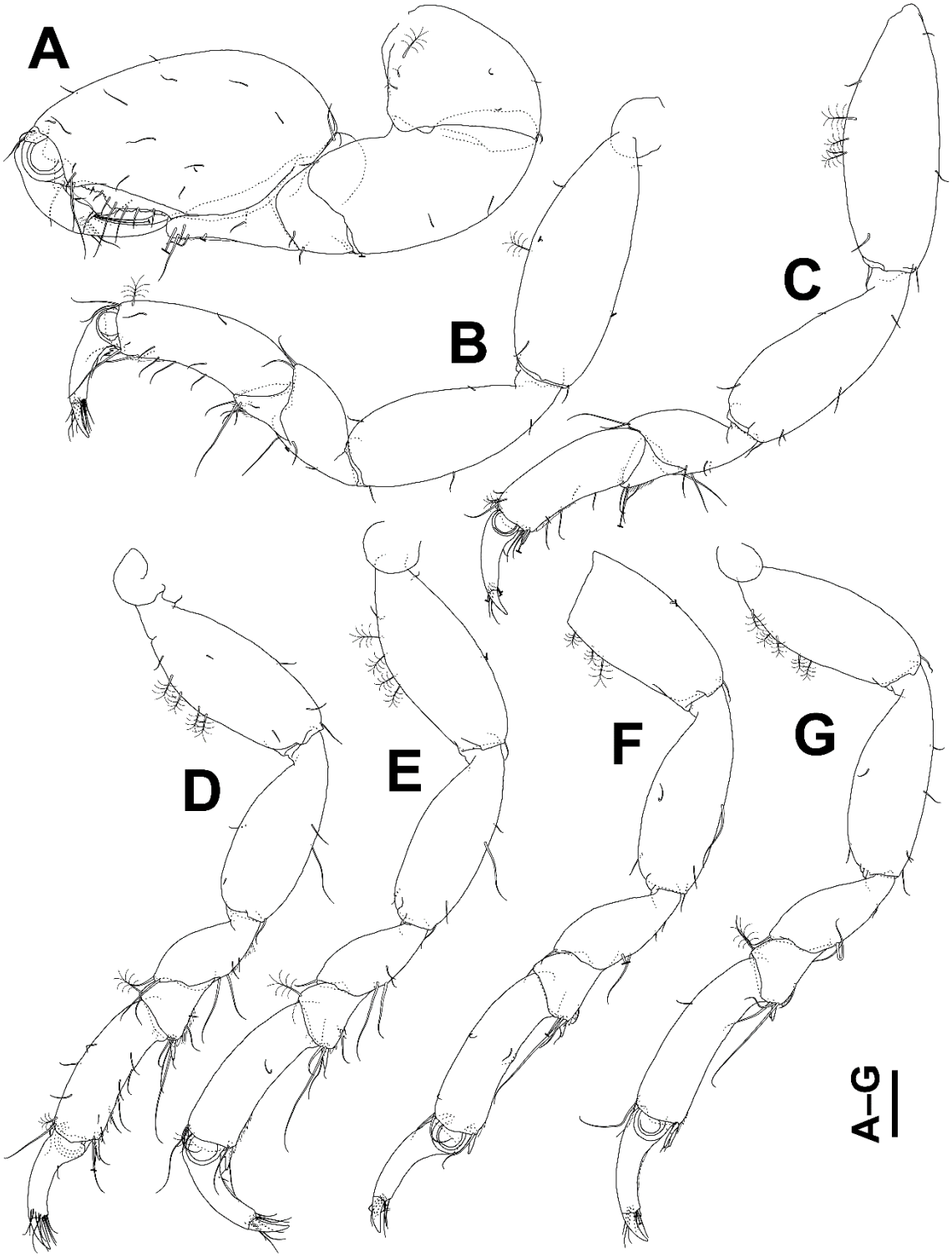

Figure II-IV-11. *Mesanthura sol* **sp. nov.**, holotype ovigerous female from Irabu (ICHUM8951): (A–C, E–G) left pereopods 1–3 and 5–7; (D) right pereopod 4. Scale bar: 100 μm.

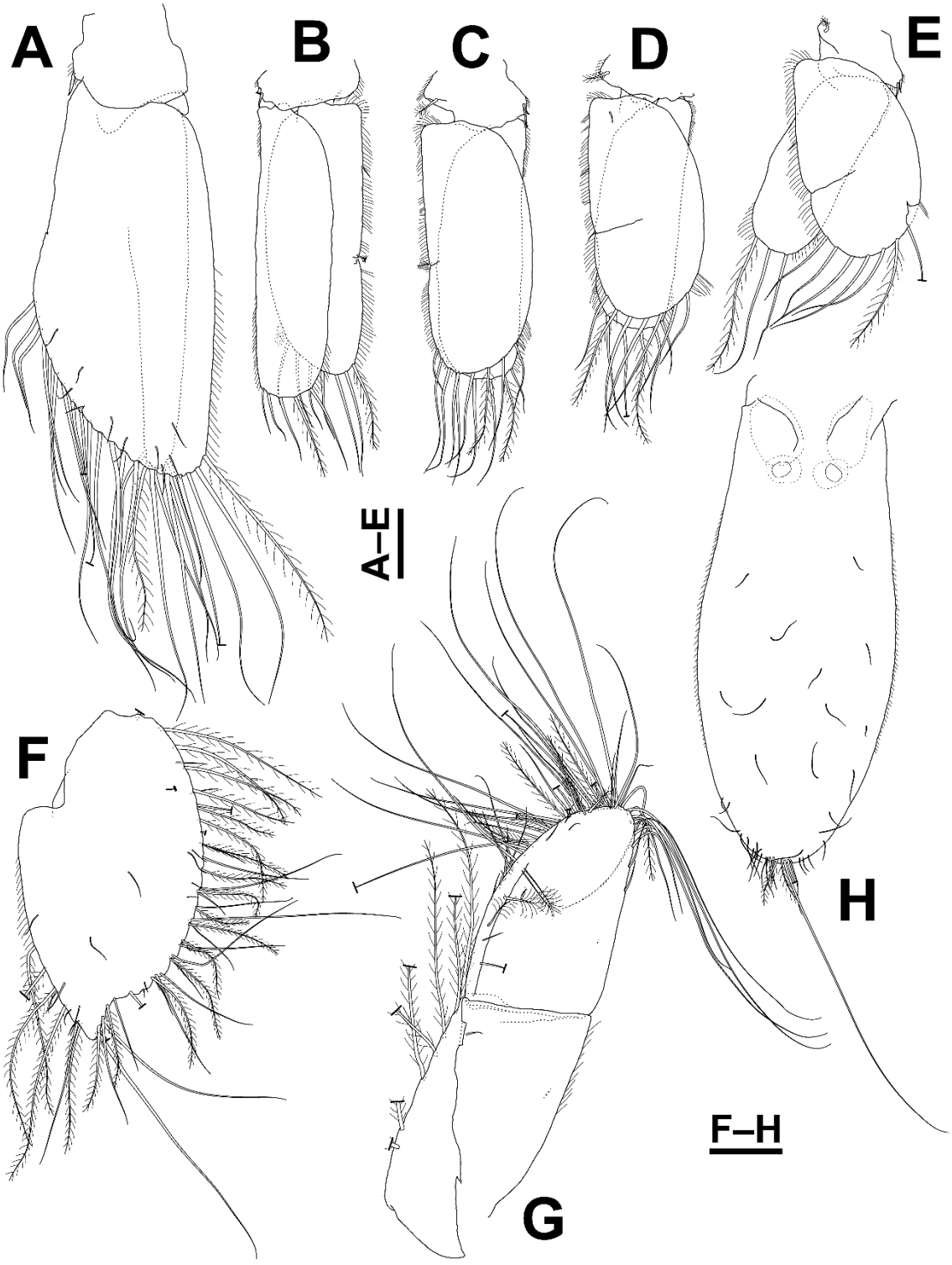

Figure II-IV-12. *Mesanthura sol* **sp. nov.**, holotype ovigerous female from Irabu (ICHUM8951): (A–E) right pleopods 1–5; (F) left uropodal exopod, pigmentation omitted; (G) right uropodal endopod, pigmentation omitted; (H) telson, pigmentation omitted. Scale bars: 100 μm.

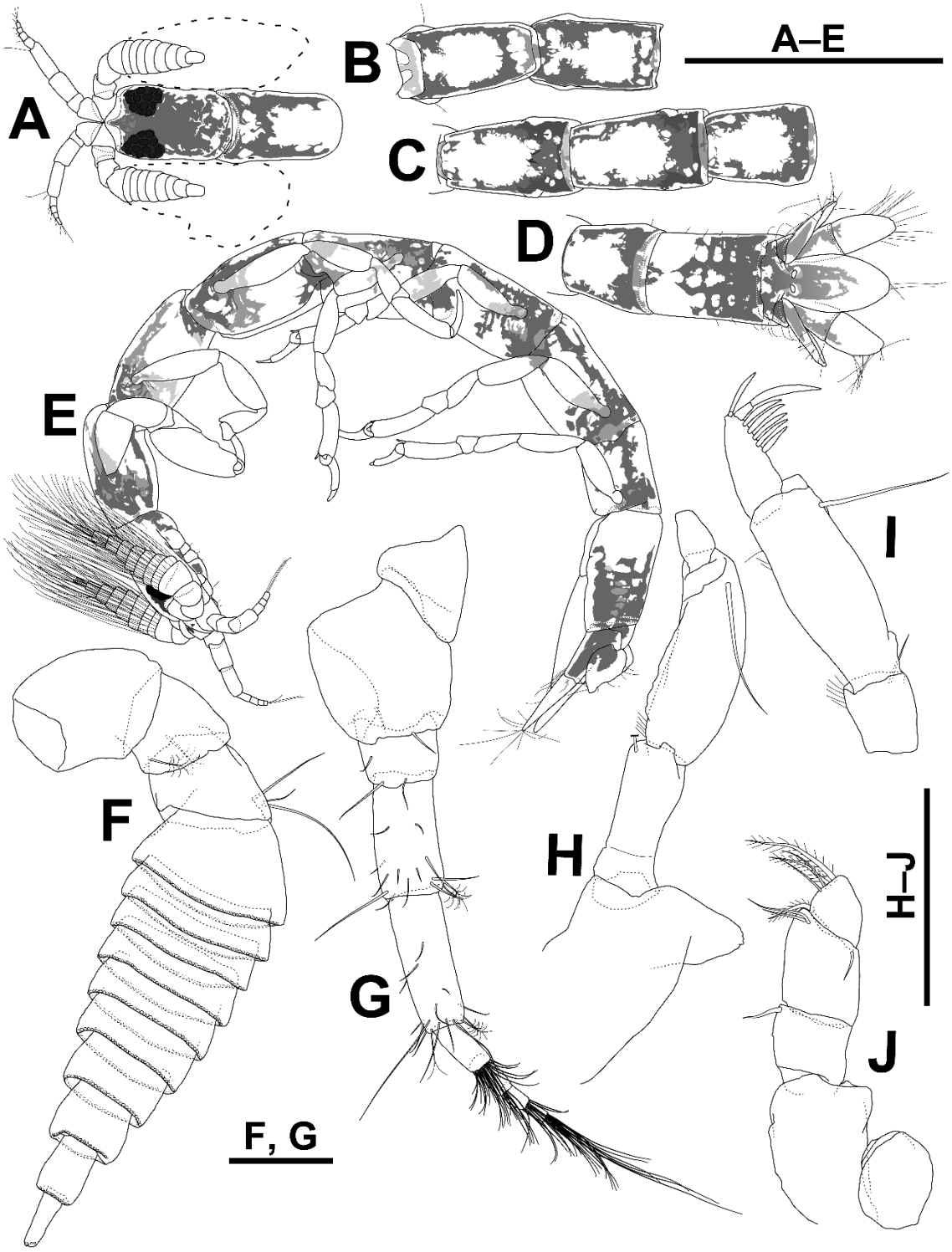

Figure II-IV-13. *Mesanthura sol* **sp. nov.**, paratype adult male (ICHUM8955) from Onna Village: (A) head and pereonite 1, dorsal view; (B) pereonites 2, 3, dorsal view; (C) pereonites 4–6, dorsal view; (D) pereonite 7 and pleon, dorsal view; (E) lateral view, (F) left antennula, (G) right antenna; (H) right mandible; (I) left mandibular palp; (J) maxilliped. Scale bars: A–E, 1 mm; F–J,100 μm.

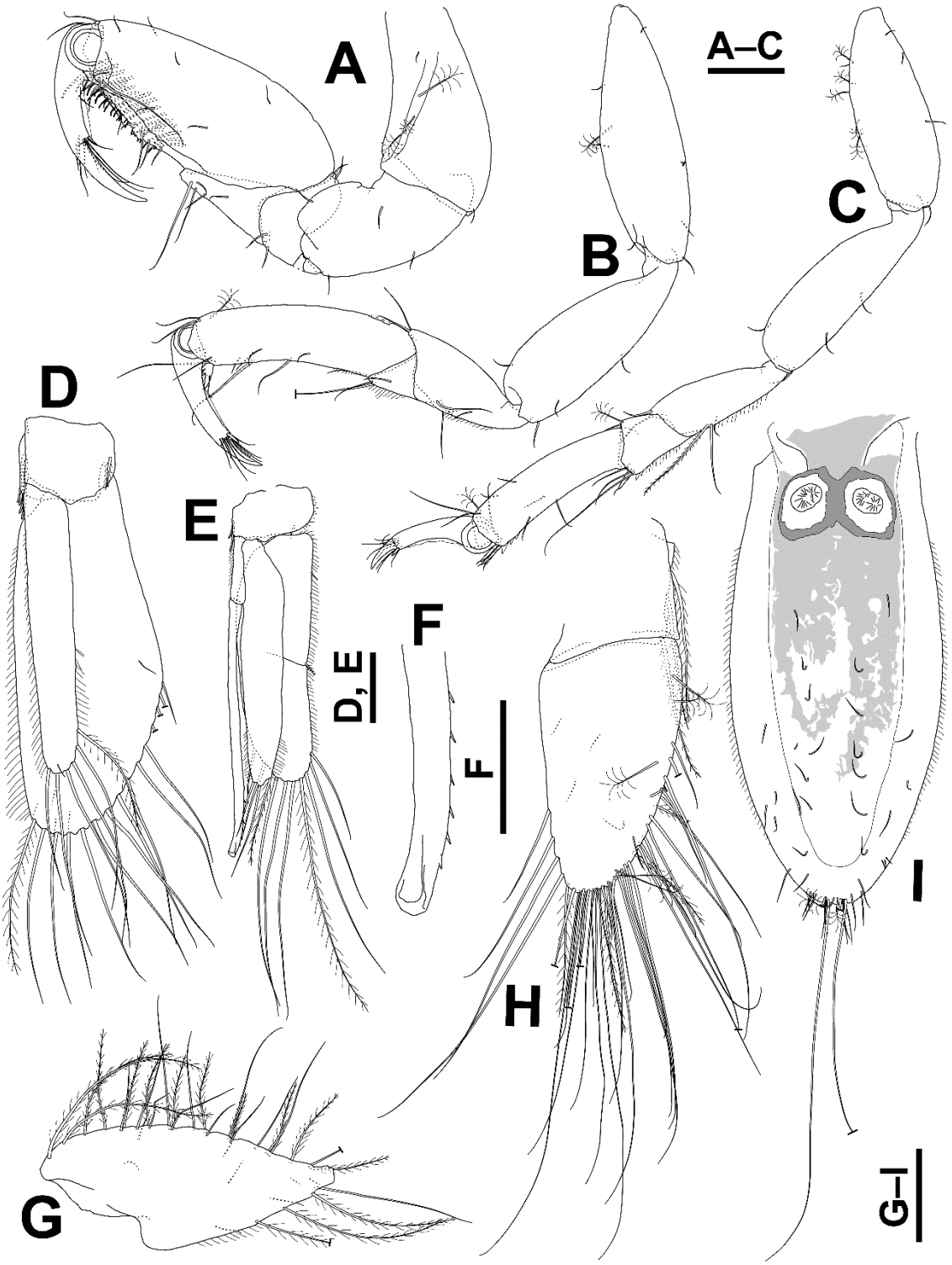

Figure II-IV-14. *Mesanthura sol* **sp. nov.**, paratype adult male (ICHUM8955) from Onna Village: (A) left pereopod 1; (B) right pereopod 2; (C) left pereopod 7; (D, E) right pleopods 1, 2; (F) tip of appendix masculina on right pleopod 2; (G) right uropodal exopod; (H) right uropodal endopod; (I) telson. Scale bars: A–E, G–I = 100 μm; F = 50 μm.

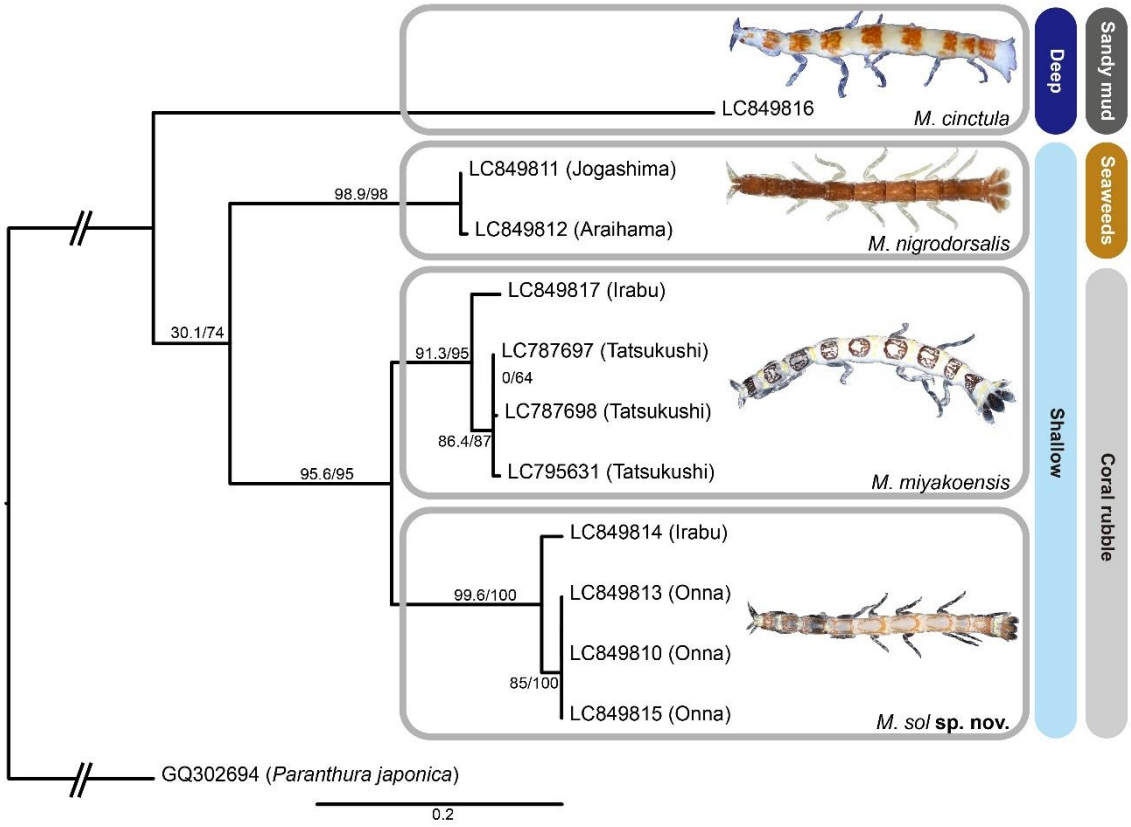

Figure II-IV-15. Maximum likelihood tree for *Mesanthura* species based on 16S rRNA gene sequences (396 bp), with the information on their depth range (deep or shallow) and substrate (sandy mud, seaweeds, or coral rubble). Numbers near nodes are SH-aLRT/UFBoot support values (clades having SH-aLRT ≥80% and UFBoot ≥95% were considered to be well supported).

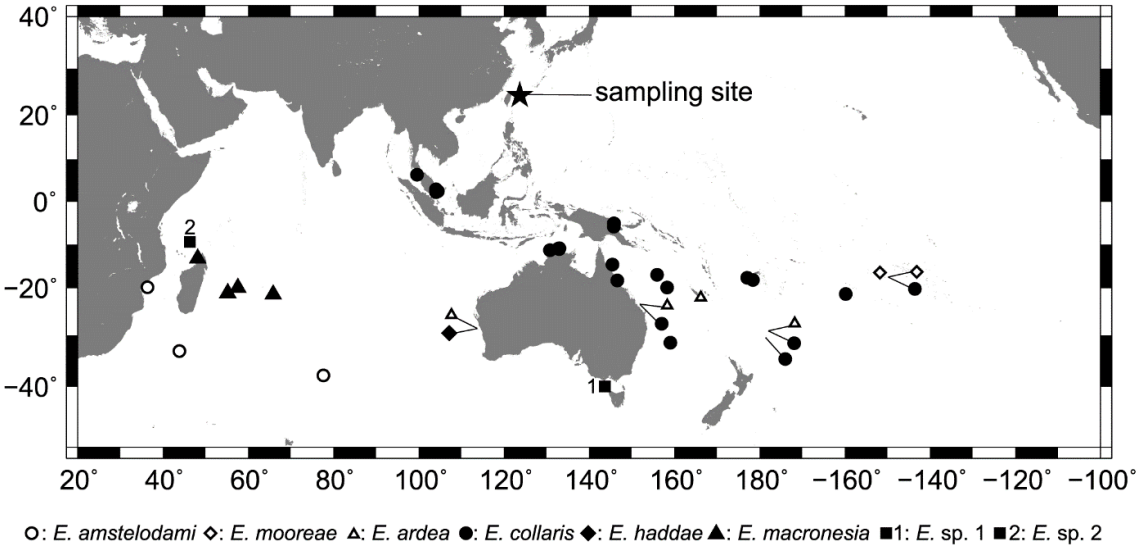

Figure II-V-1. Global distribution of the genus *Expanathura*, including *E.* sp. 1 and *E.* sp. 2 of Poore and Lew Ton (2002). Open symbols, first group; solid symbols, second group.

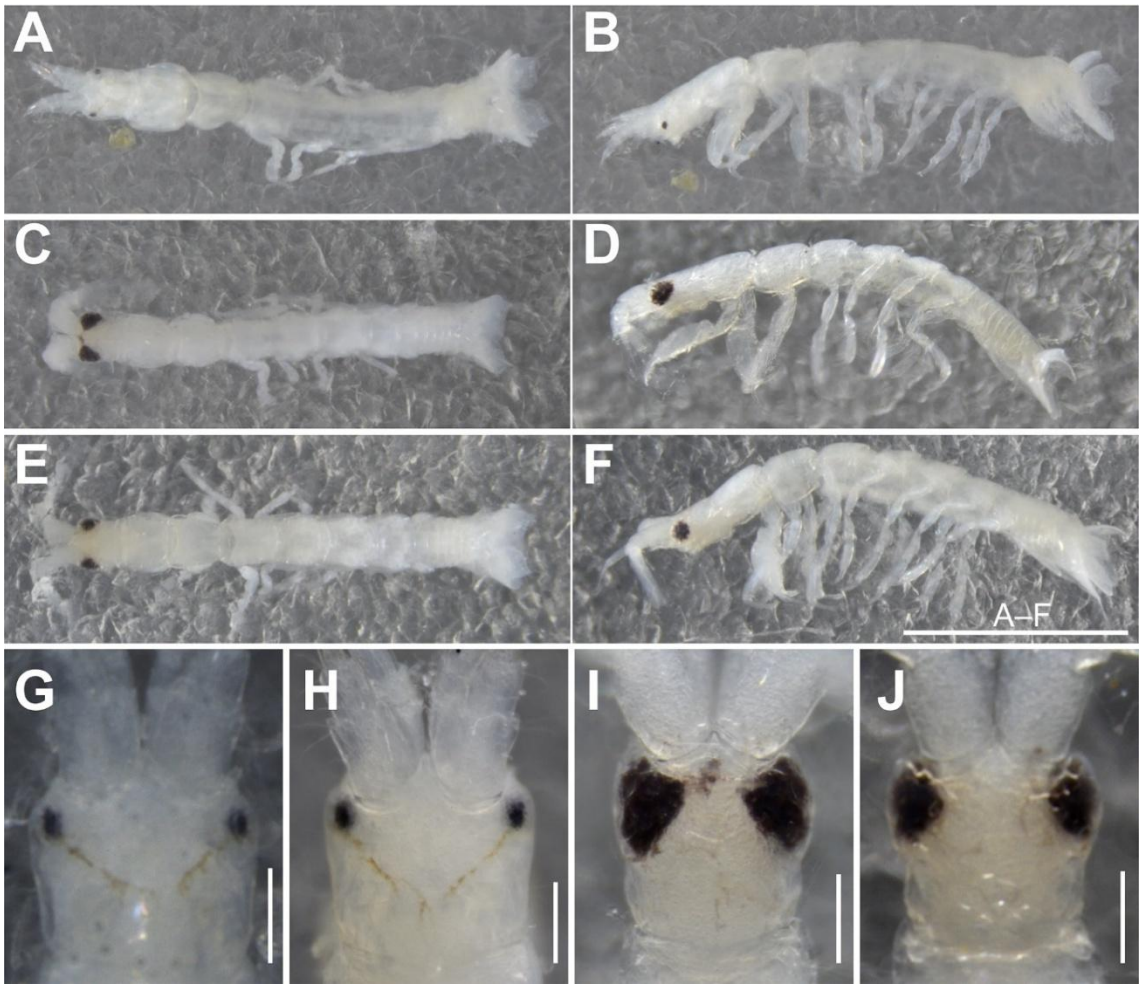

Figure II-V-2. *Expanathura monile* **sp. nov.** Photomicrographs of fixed specimens: A, B, G, paratype (female with fully developed marsupium; SMBL-V0631); C, D, I, allotype (male); E, F, J, paratype (male); H, holotype (female with fully developed marsupium). A, C, E, body, dorsal view; B, D, F, body, lateral view; G–J, head, dorsal view. Scale bars: 1 mm (A–F); 100 μm (G–J).

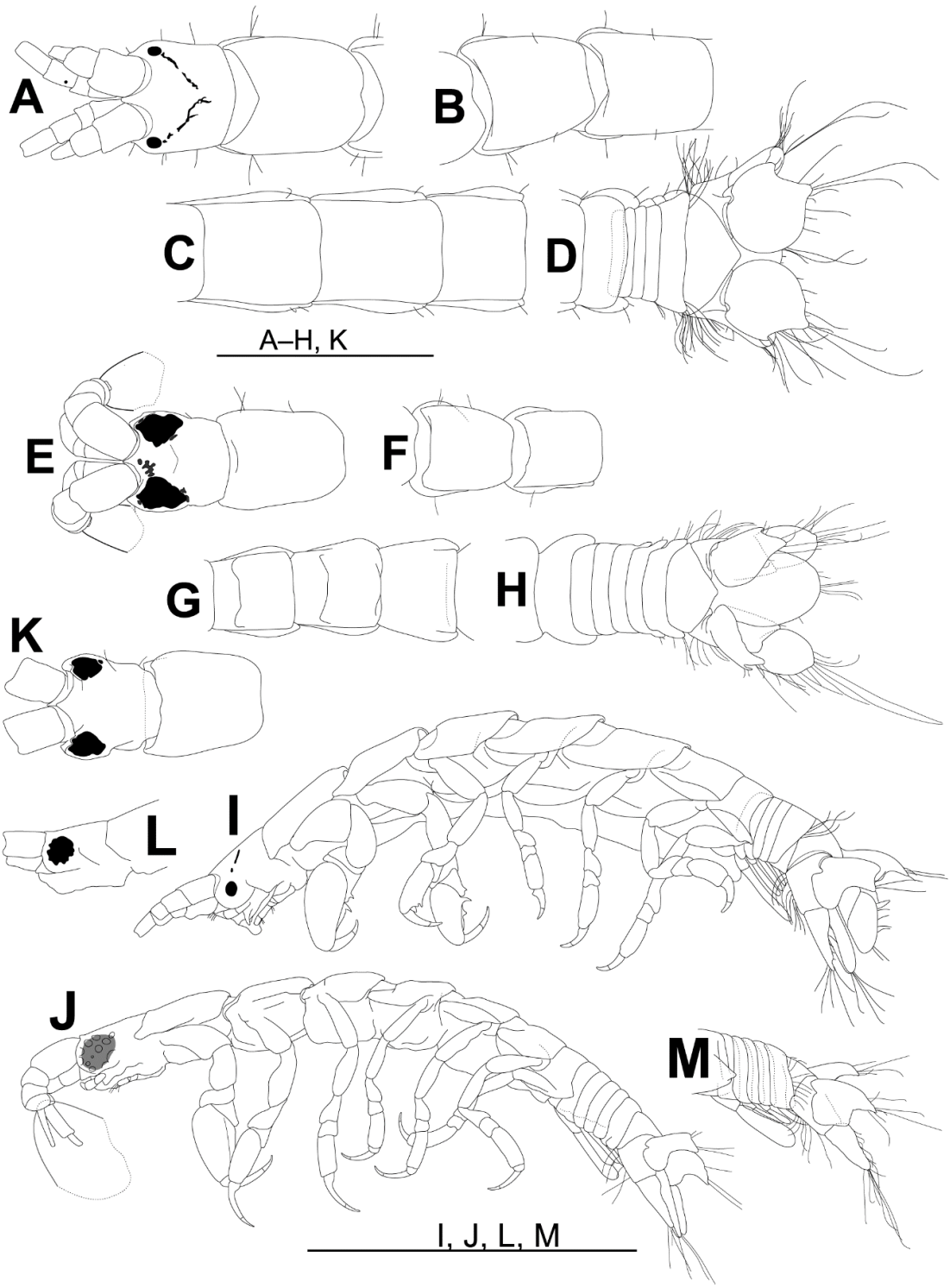

Figure II-V-3. *Expanathura monile* **sp. nov.** A–D, I, holotype (female with fully developed marsupium); E–H, J, allotype (male); K–M, paratype (male). A–H, K, dorsal view; I, J, L, M, lateral view. A, E, K, head and pereonite 1 (most appendages in K omitted); B, F, pereonites 2 and 3; C, G, pereonites 4–6; D, H, pereonite 7 and pleon; I, J,

body; L, head (appendages omitted); M, pleon. Scales: 500 μm (A–H, K); 1 mm (I, J, L, M).

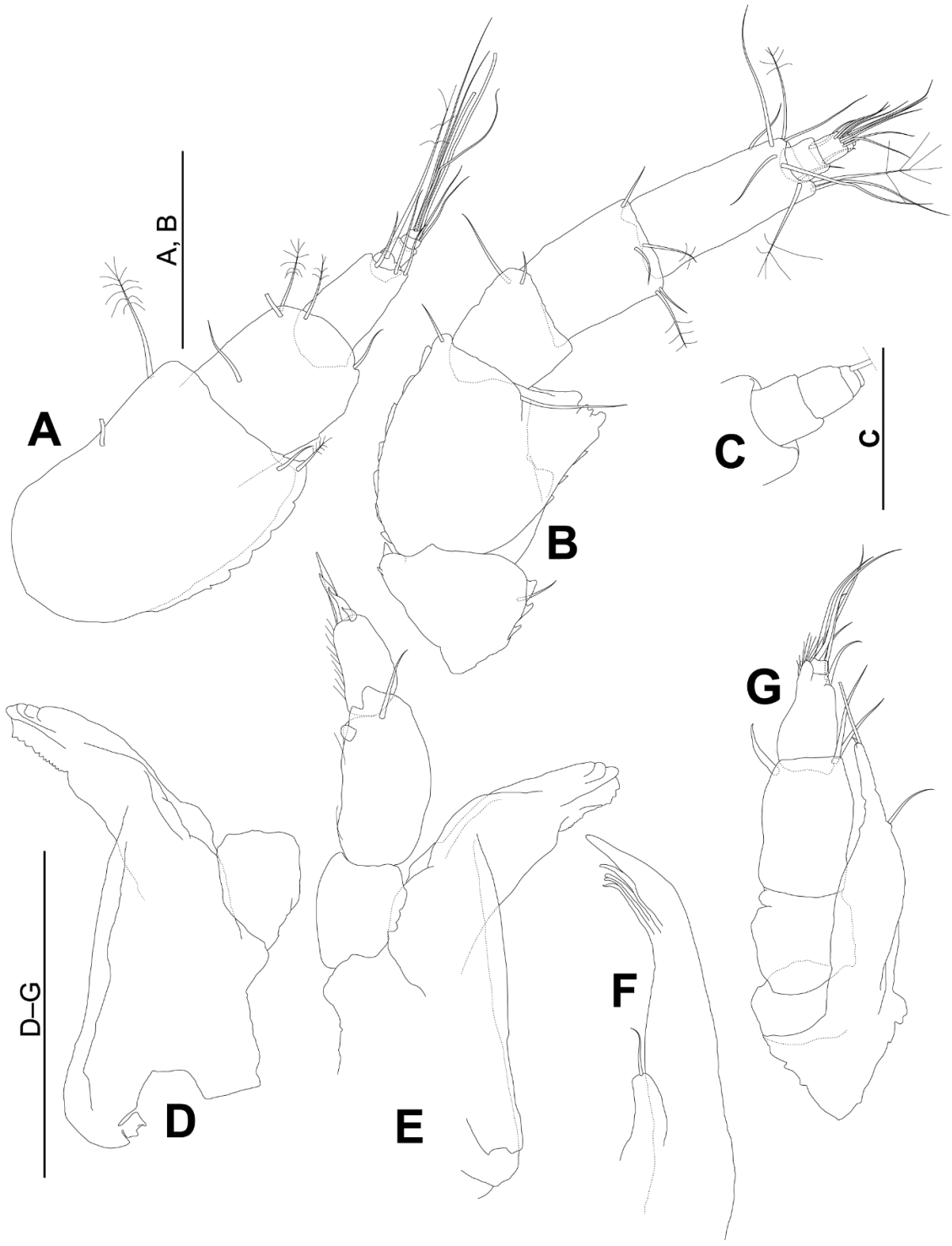

Figure II-V-4. *Expanathura monile* **sp. nov.**, female with fully developed marsupium. A–C, G, holotype; D–F, paratype (SMBL-V0631). A, left antennula; B, left antenna; C, flagellum of left antenna; D, left mandible; E, right mandible; F, left maxilla; G, left maxilliped. Scale bars: 100 μm (A, B, D–G); 50 μm (C).

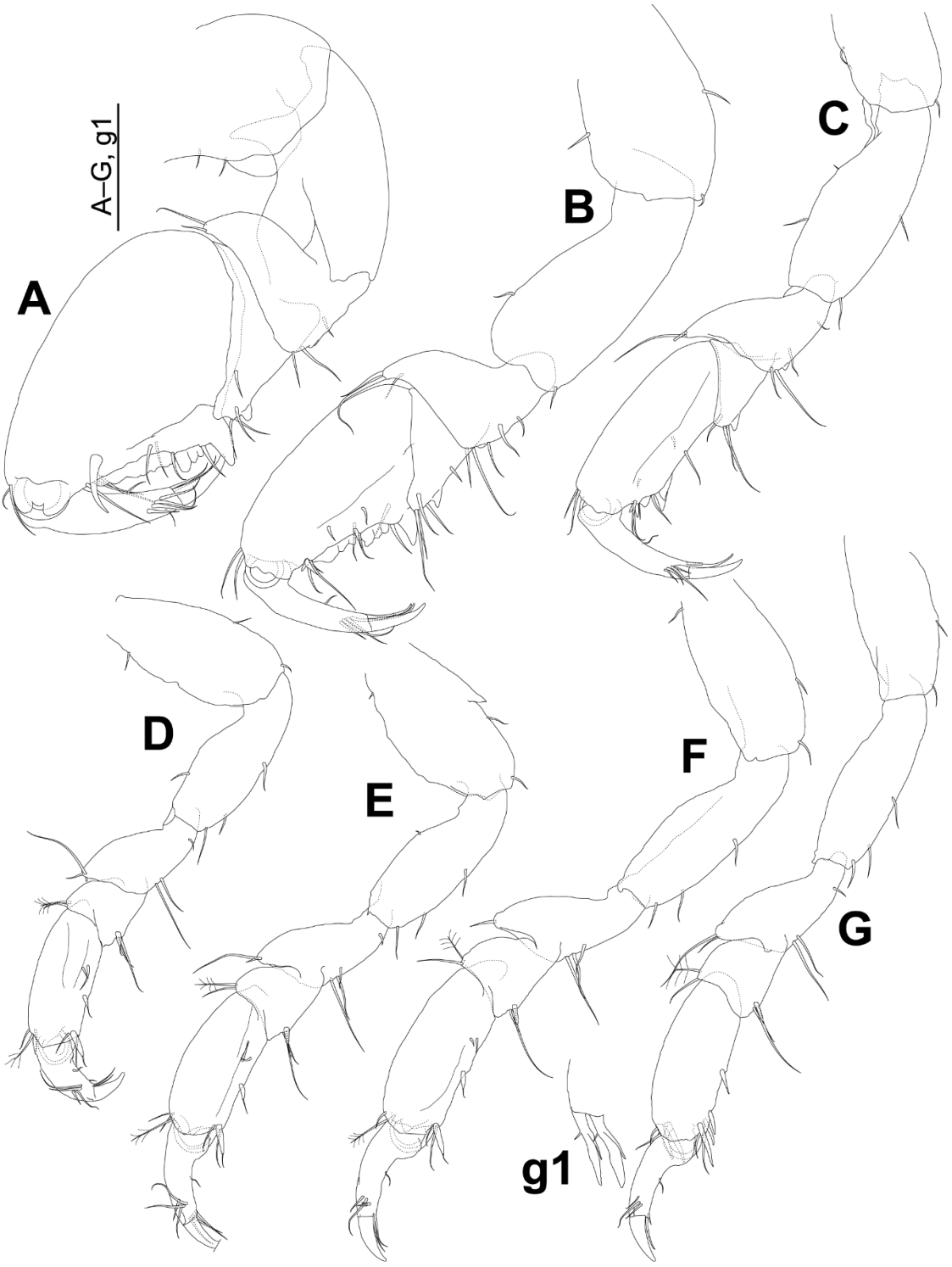

Figure II-V-5. *Expanathura monile* **sp. nov.**, holotype female with fully developed marsupium. A, C–G, right pereopods 1 and 3–7; B, left pereopod 2; g1, three-pronged spiniform setae on pereopod 7 propodus. Scale bar: 100 μm (A–G); 50 μm (g1).

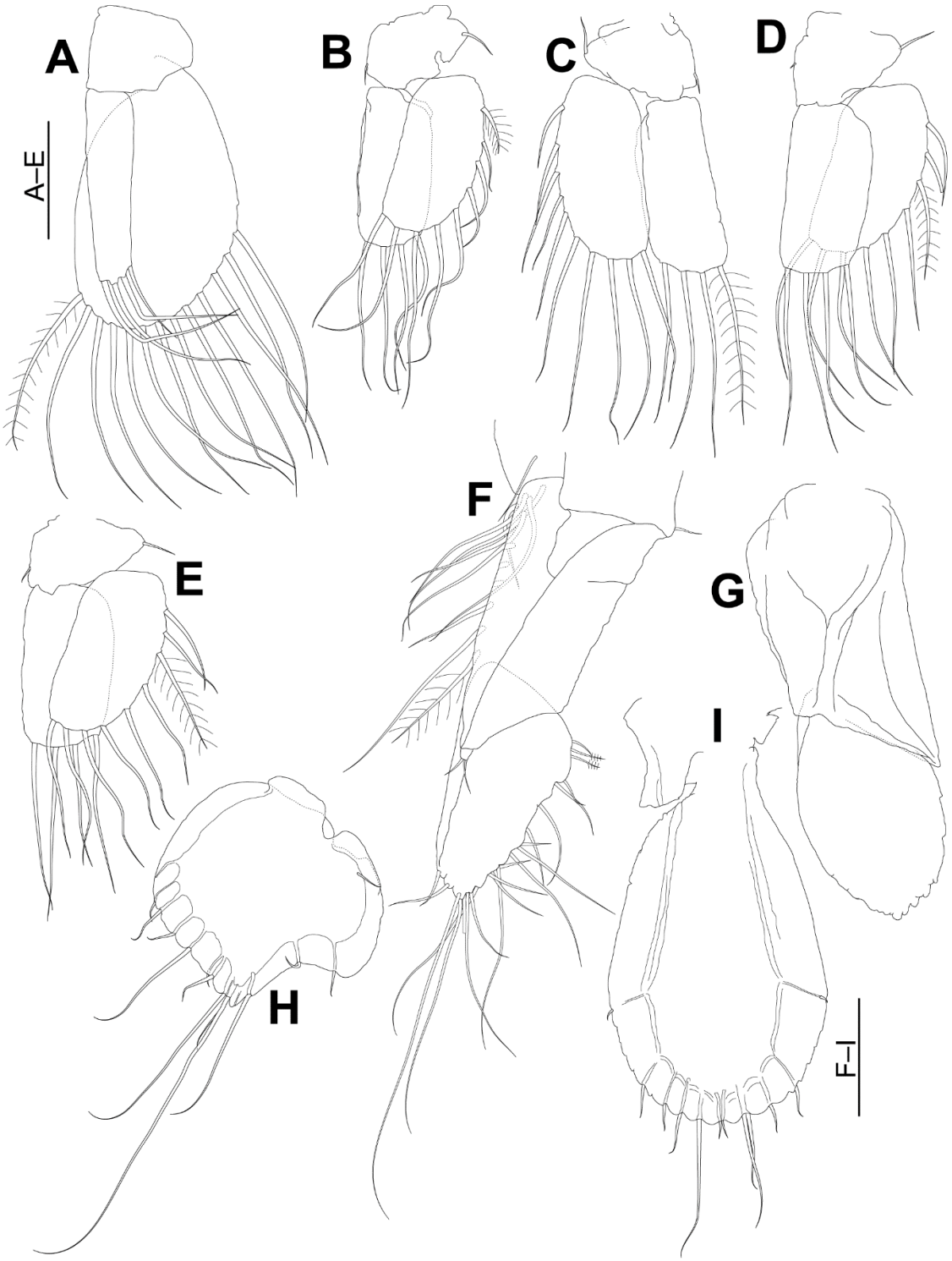

Figure II-V-6. *Expanathura monile* **sp. nov.**, female with fully developed marsupium. A, C–G, holotype; B, H, I, paratype (SMBL-V0631). A, C, D, right pleopods 1, 3 and 4; B, E, left pleopods 2 and 5; F, right uropodal endopod, ventral view; G, left uropodal endopod, lateral view (setae omitted); H, right uropodal exopod; I, telson. Scale bars: 100 μm.

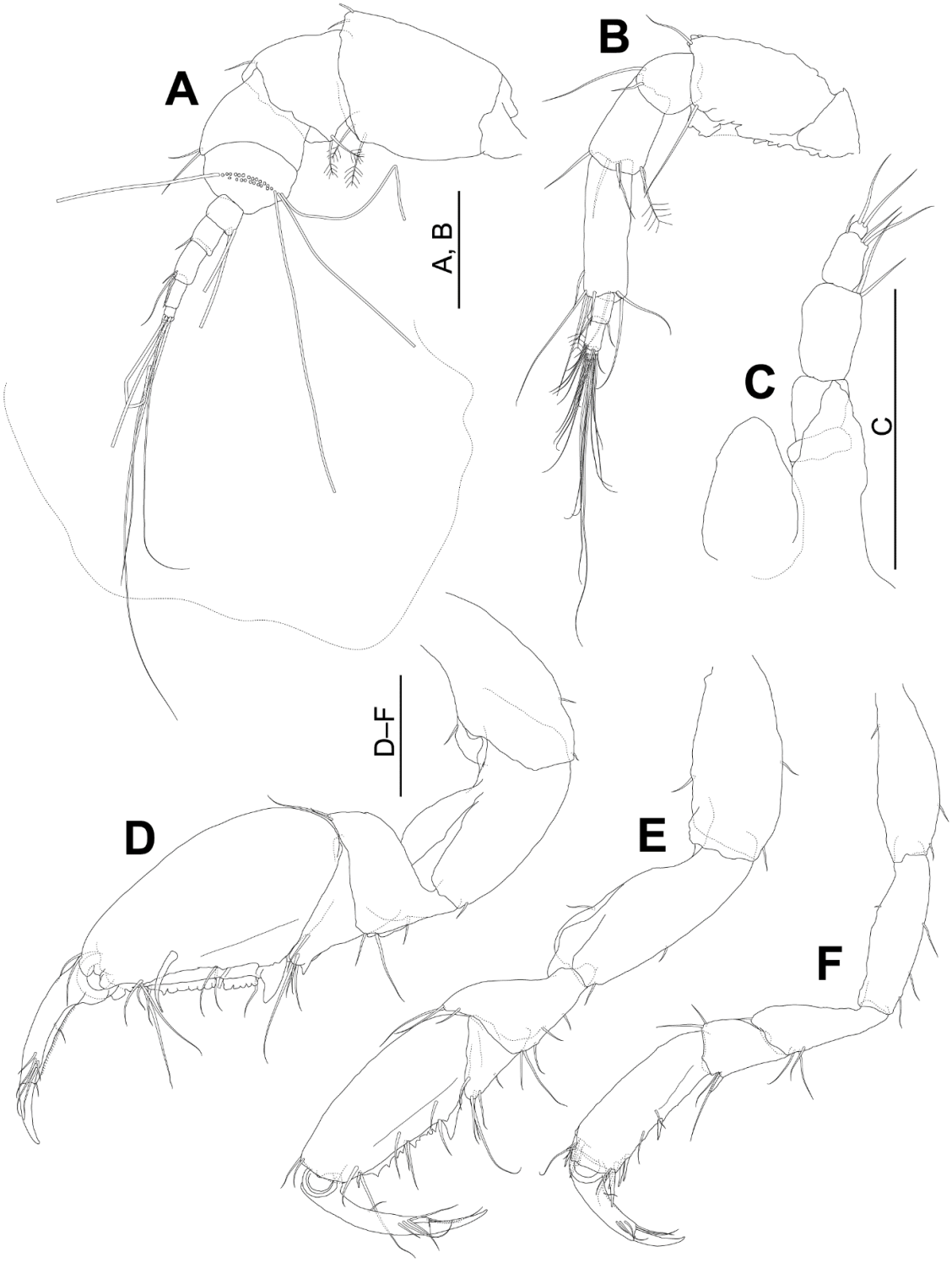

Figure II-V-7. *Expanathura monile* **sp. nov.**, allotype male. A, right antennula (most aesthetascs omitted); B, right antenna; C, left maxilliped; D–F, right pereopods 1, 2 and 7. Scale bars: 100 μm.

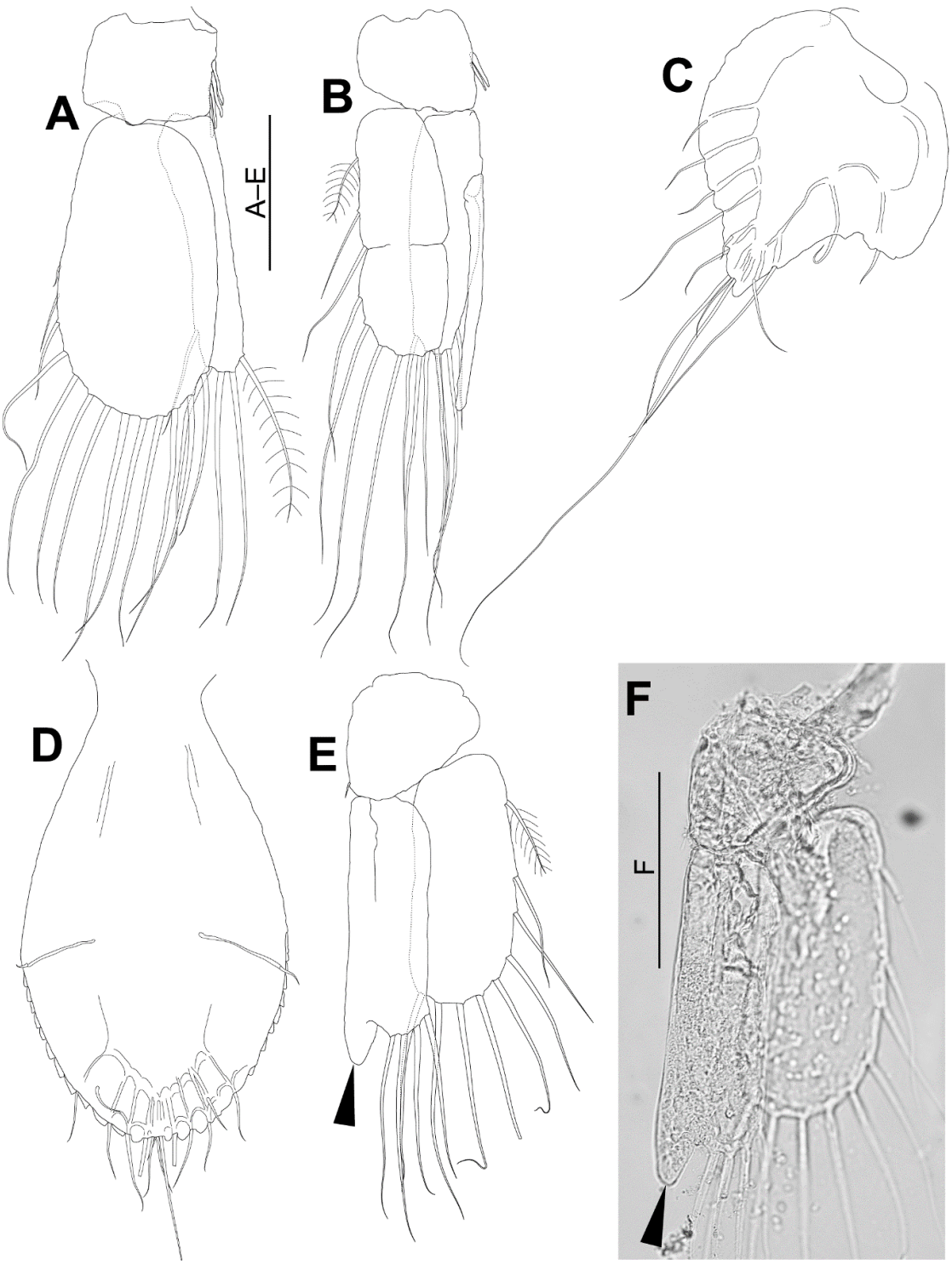

Figure II-V-8. *Expanathura monile* **sp. nov.** A–D, allotype male; E, F, holotype female. A, B, right pleopods 1 and 2; C, left uropodal exopod; D, telson; E–F, drawing and photomicrograph of right pleopod 2. Arrowheads, appendix-masculina-like projection. Scale bars: 100 μm.

Figure II-VI-1. *Kupellonura tamago* **sp. nov.**, holotype, female without oostegites, lateral view of fixed specimen. Scale bar: 1 mm.

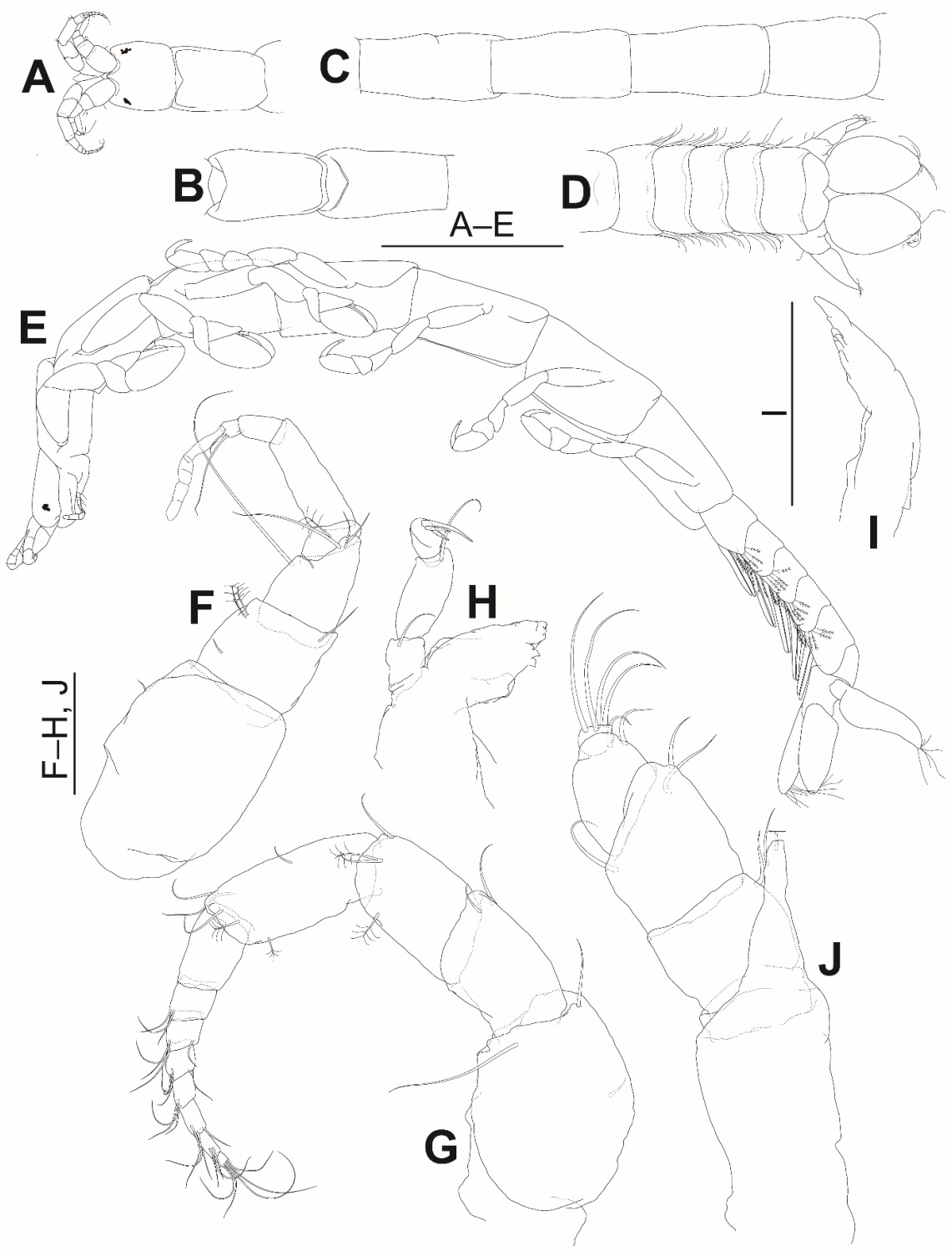

Figure II-VI-2. *Kupellonura tamago* **sp. nov.**, holotype, female without oostegites. A–D, dorsal view; E, lateral view. A, head and pereonite 1; B, pereonites 2 and 3; C, pereonites 4–7; D, pleon; E, body; F, left antennula; G, right antenna; H, right mandible; I, maxilla; J, left maxilliped. Scale bars: 1 mm (A–E); 100 µm (F–J).

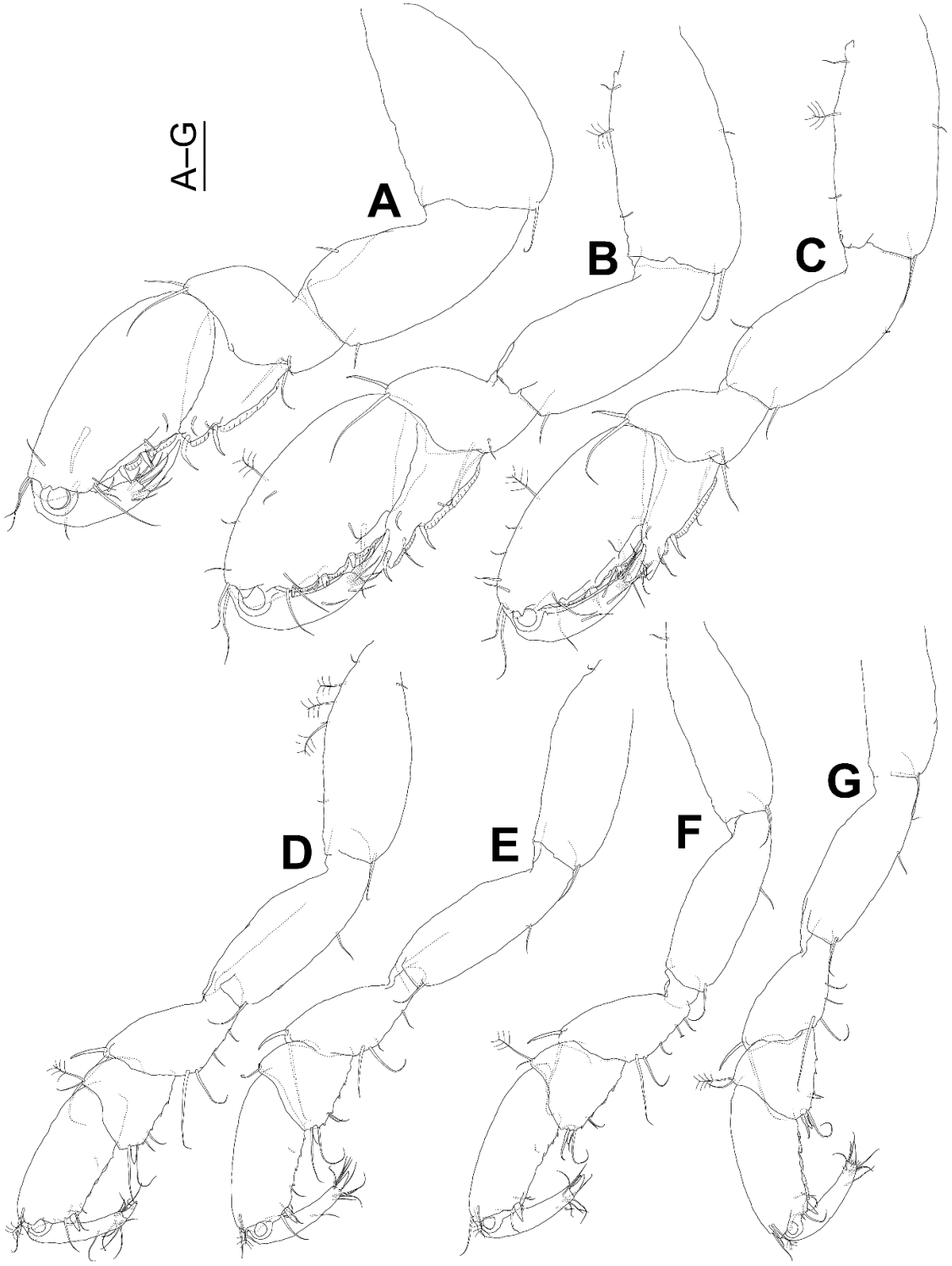

Figure II-VI-3. *Kupellonura tamago* **sp. nov.**, holotype, female without oostegites. A–G, left pereopods 1–7. Scale bar: 100 µm.

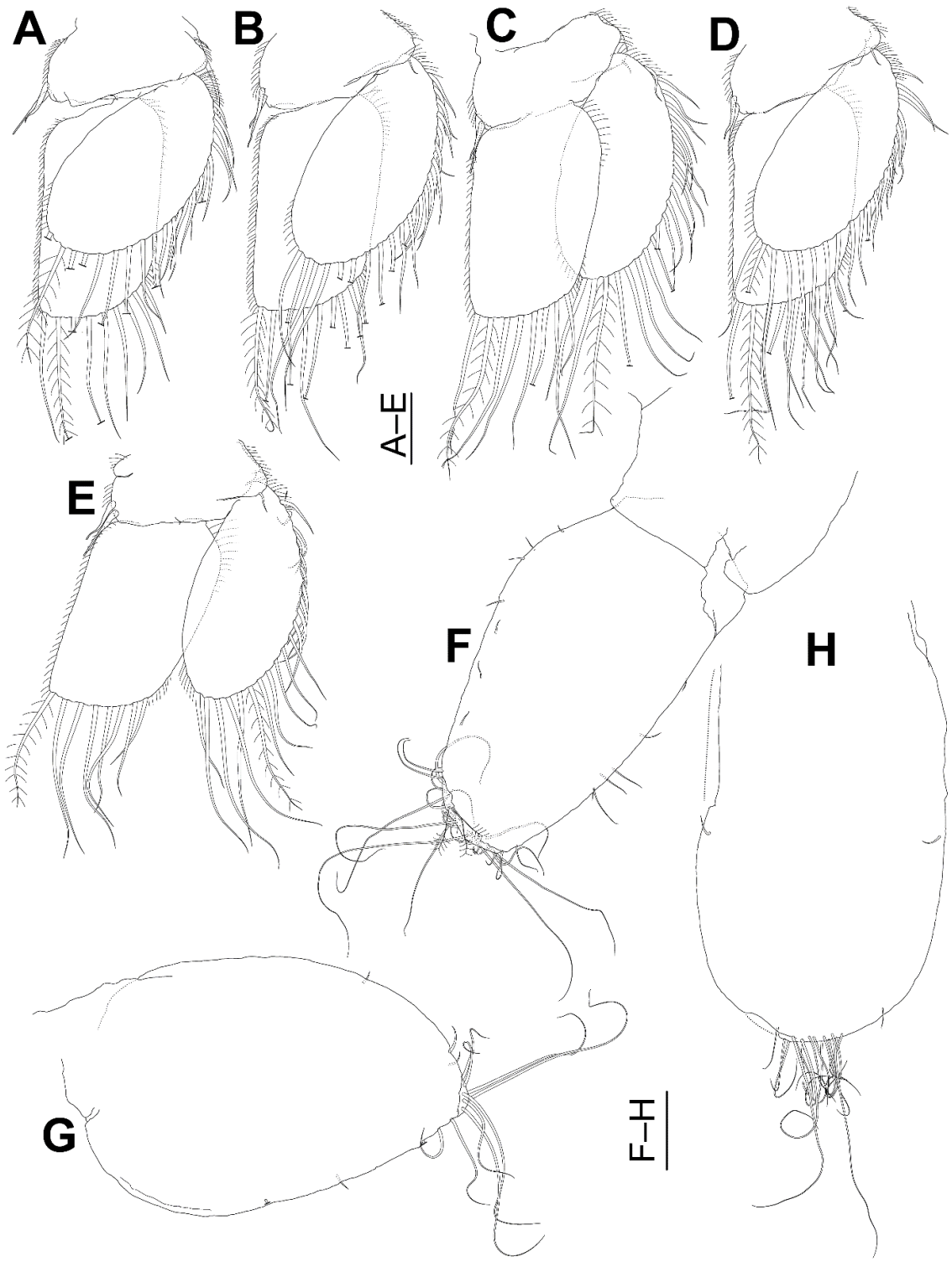

Figure II-VI-4. *Kupellonura tamago* **sp. nov.**, female without oostegites. A, B, D, E, left pleopods 1, 2, 4 and 5; C, right pleopod 3; F, left uropodal endopod; G, left uropodal exopod; H, telson. Scale bars: 100 µm.

Figure II-VII-1. *Cruranthura viridis* **sp. nov.**, holotype, ovigerous female. A, B, Dorsal and right views of living individual; C, D, dorsal and left views of fixed specimen. Scale bars: 1 mm.

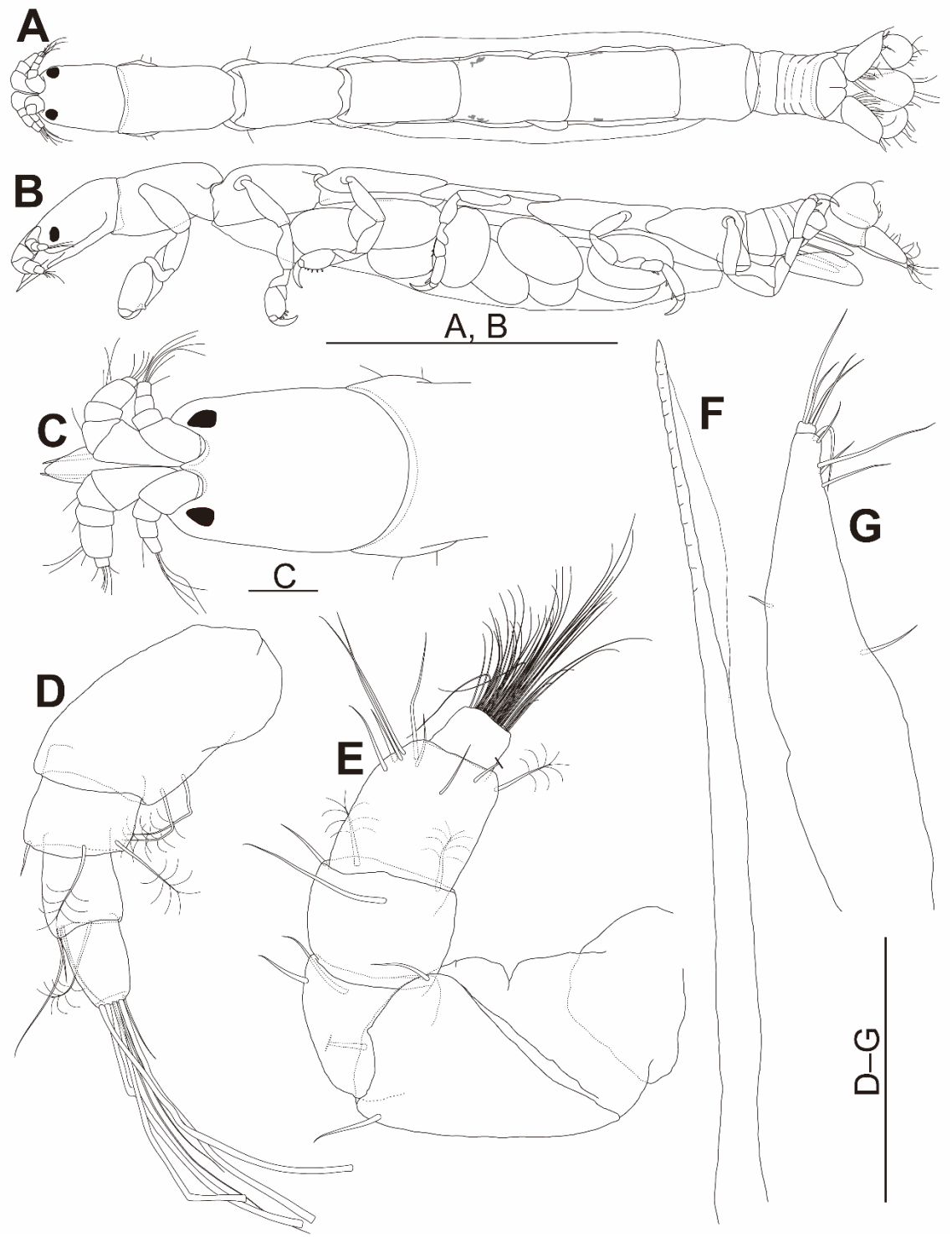

Figure II-VII-2. *Cruranthura viridis* **sp. nov.**, ovigerous females, holotype (A–F), and paratype (ICHUM8566: G). A, B, Body, dorsal and lateral views; C, dorsal view of head; D, right antennula; E, right antenna; F, left maxilla; G, maxilliped. Scale bars: A, B, 1 mm; C–G, 100 μm.

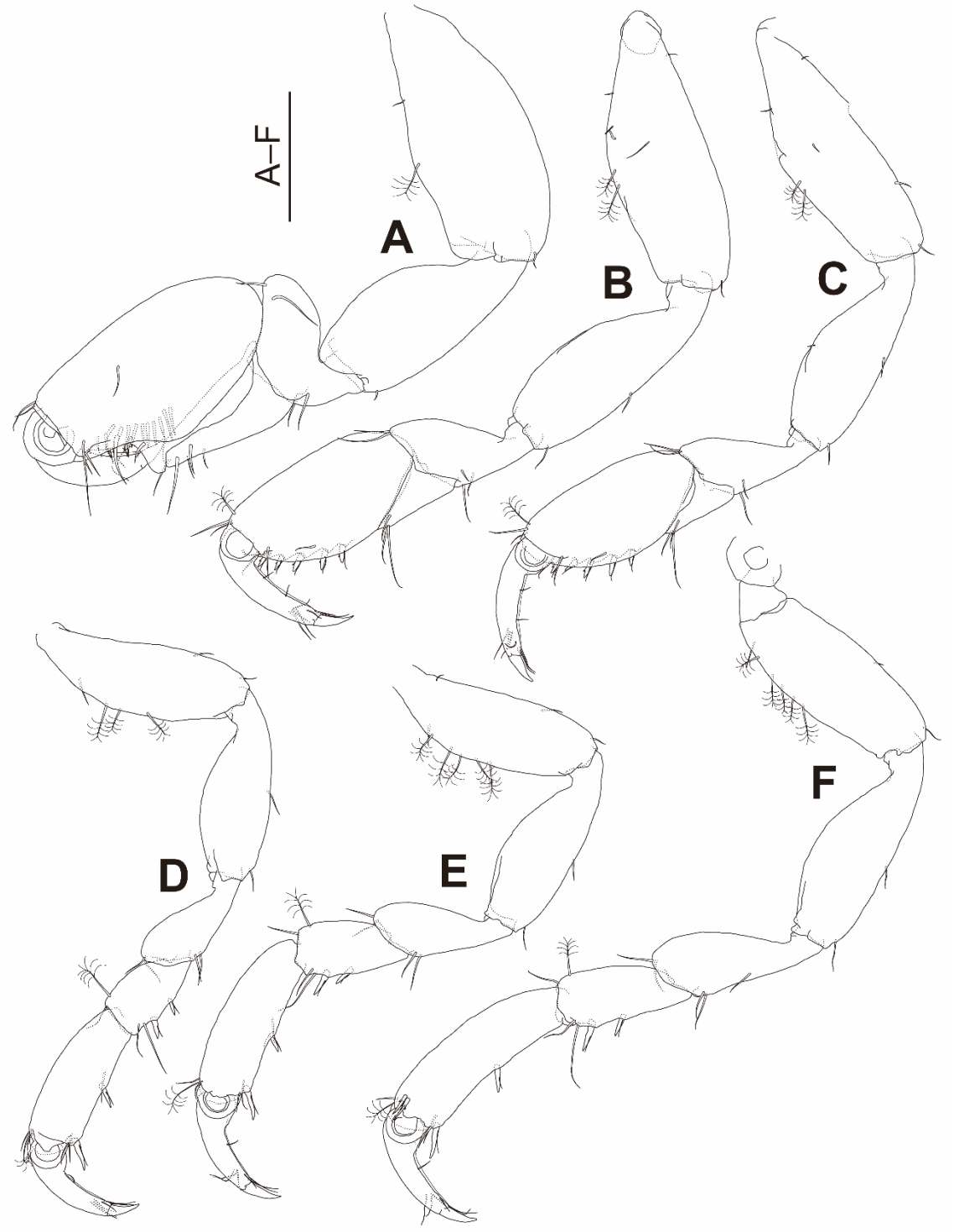

Figure II-VII-3. *Cruranthura viridis* **sp. nov.**, holotype, ovigerous female. A–F, Left pereopods 1–6. Scale bar: 100 µm.

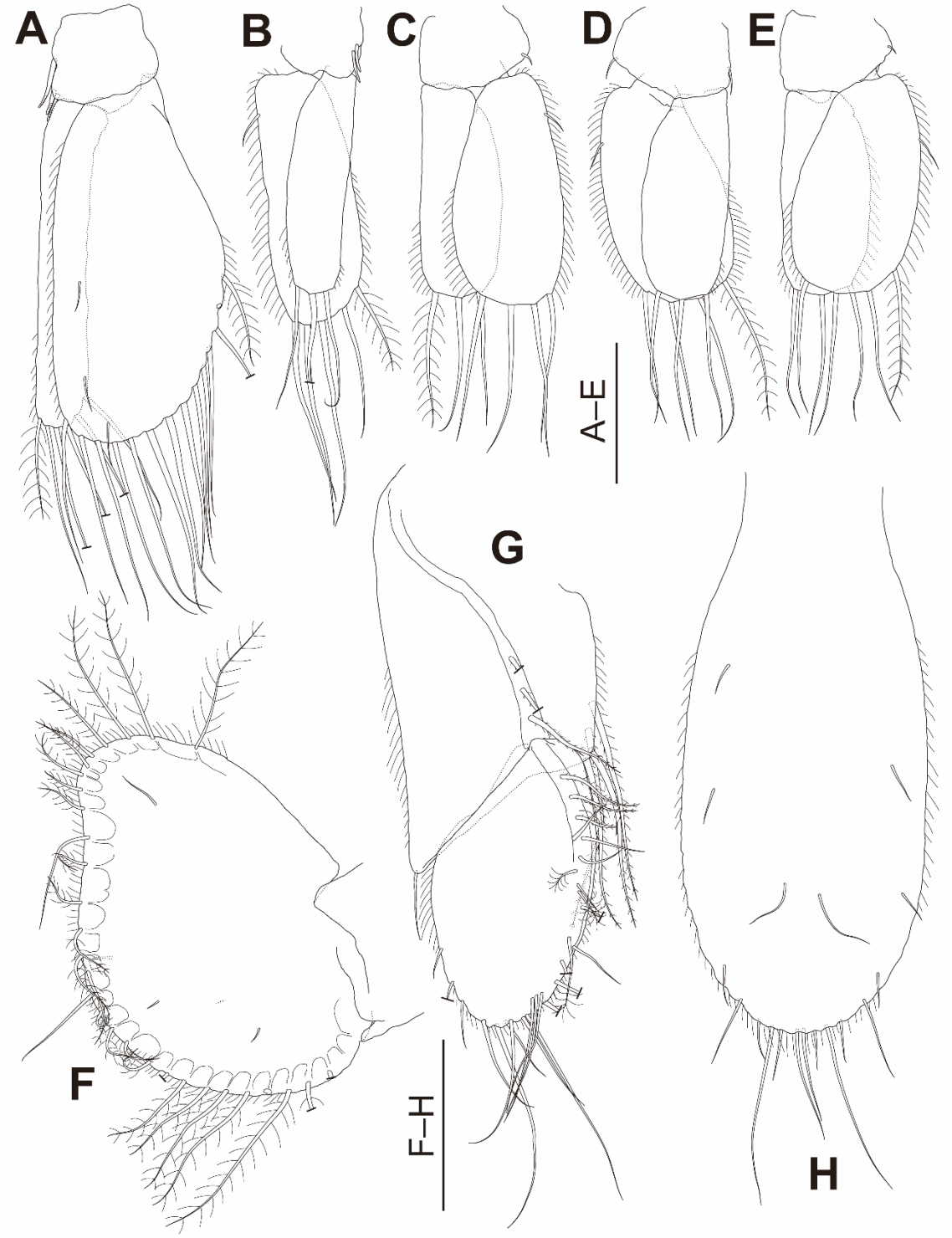

Figure II-VII-4. *Cruranthura viridis* **sp. nov.**, holotype, ovigerous female. A–E, Left pleopods 1–5; F, left uropodal exopod; G, right uropodal endopod; H, telson. Scale bars: 100 μm.

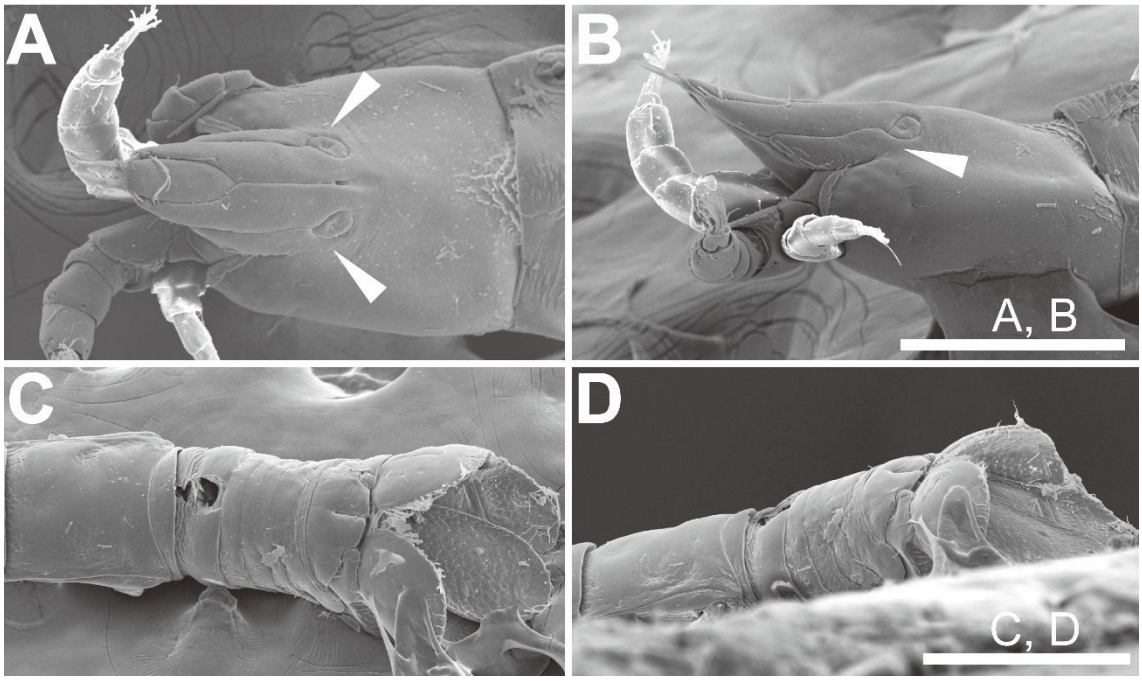

Figure II-VII-5. SEM images of *Cruranthura viridis* **sp. nov.**, paratype (ICHUM8567), ovigerous female, pereopods detached. A, B, Ventral and right views of head; C, D, dorsal and left views of pleon (the hole on pleonite 1 was made by an accidental needle prick during dissection). Arrowheads, the fusion between the mandible and head. Scale bars: A, B, 200 μm; C, D, 300 μm.

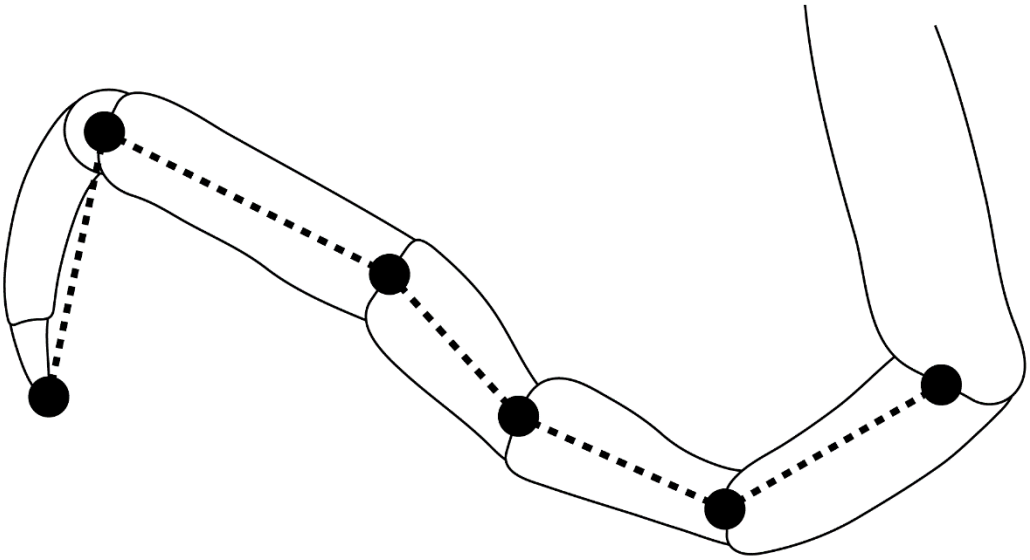

Figure II-VIII-1. Schematic drawing of pereopod 6 showing length measurements made on pereopods. Black circles indicate center of distal edge of each article. Total pereopod length was obtained by summing lengths represented by dotted lines.

Figure II-VIII-2. *Deltanthura palpus* **sp. nov.**, holotype, manca, lateral view of fixed specimen. Scale bar: 1 mm.

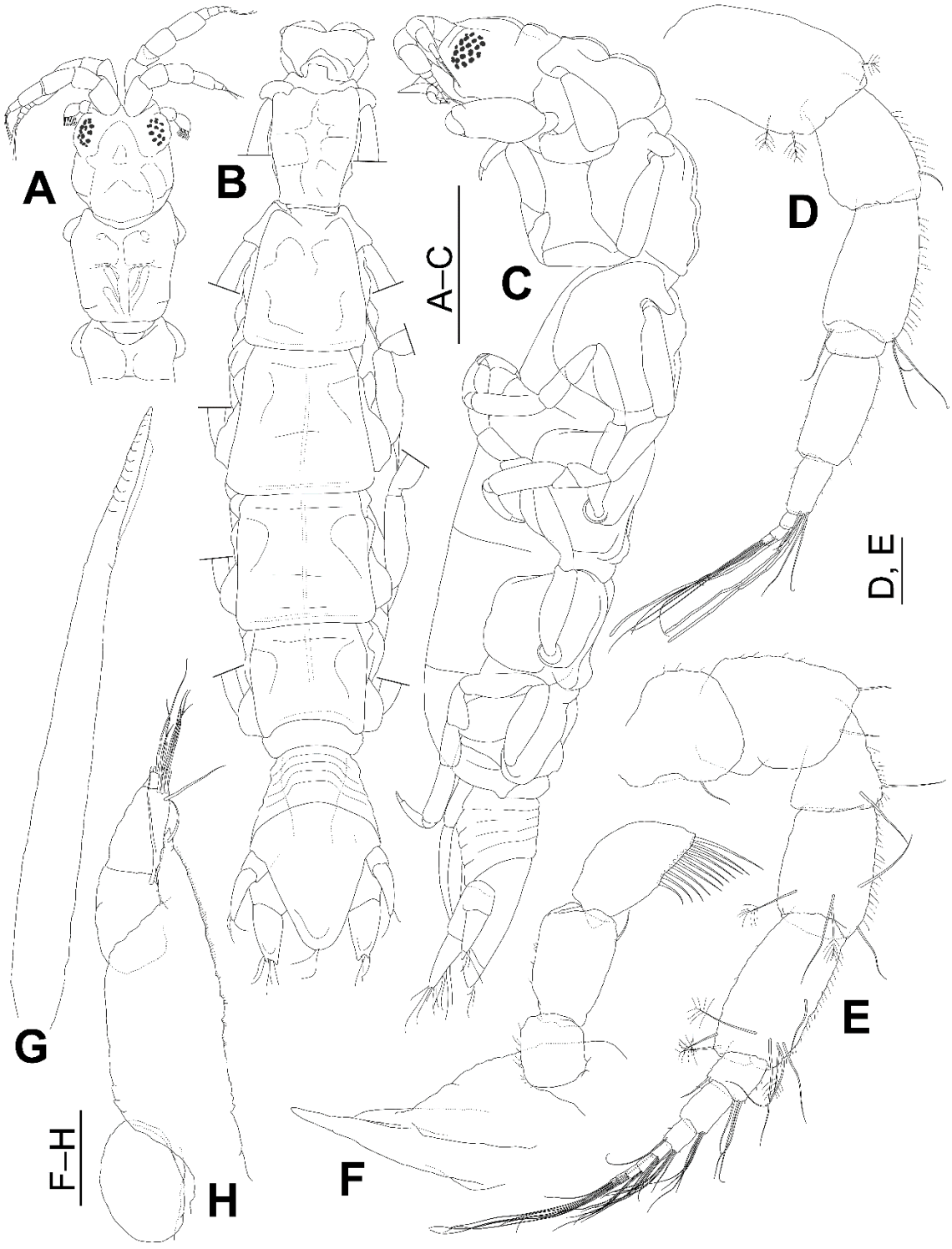

Figure II-VIII-3. *Deltanthura palpus* **sp. nov.**, holotype, manca. A, dorsal view of head and pereonite 1; B, dorsal view from pereonite 2 to telson; C, lateral view; D, left antennula; E, left antenna; F, right mandible; G, right maxilla; H, right maxilliped. Scale bars: 1 mm (A–C); 100 µm (D–H).

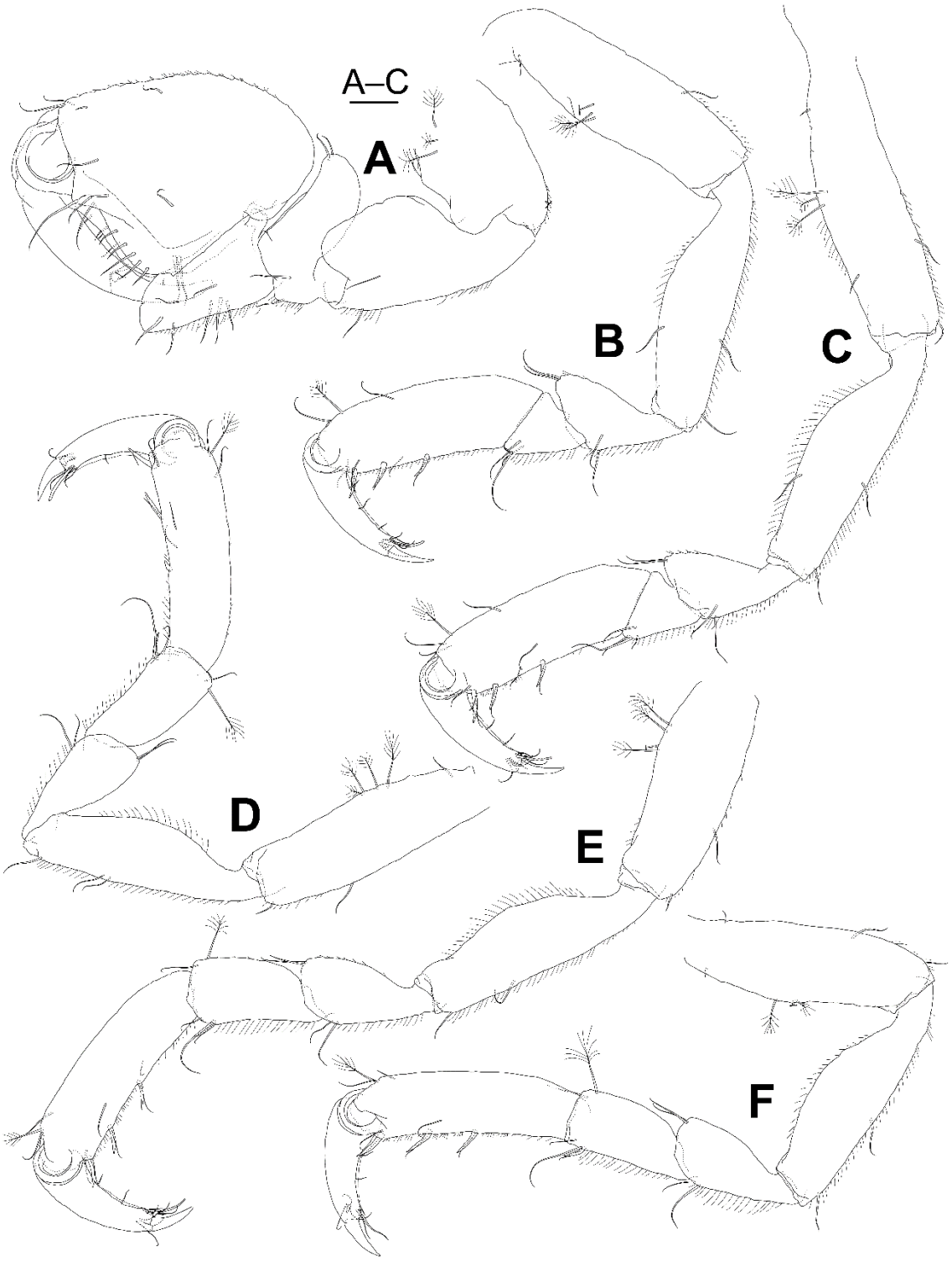

Figure II-VIII-4. *Deltanthura palpus* **sp. nov.**, holotype, manca. A–F, left pereopods 1–6. Scale bar: 100 µm.

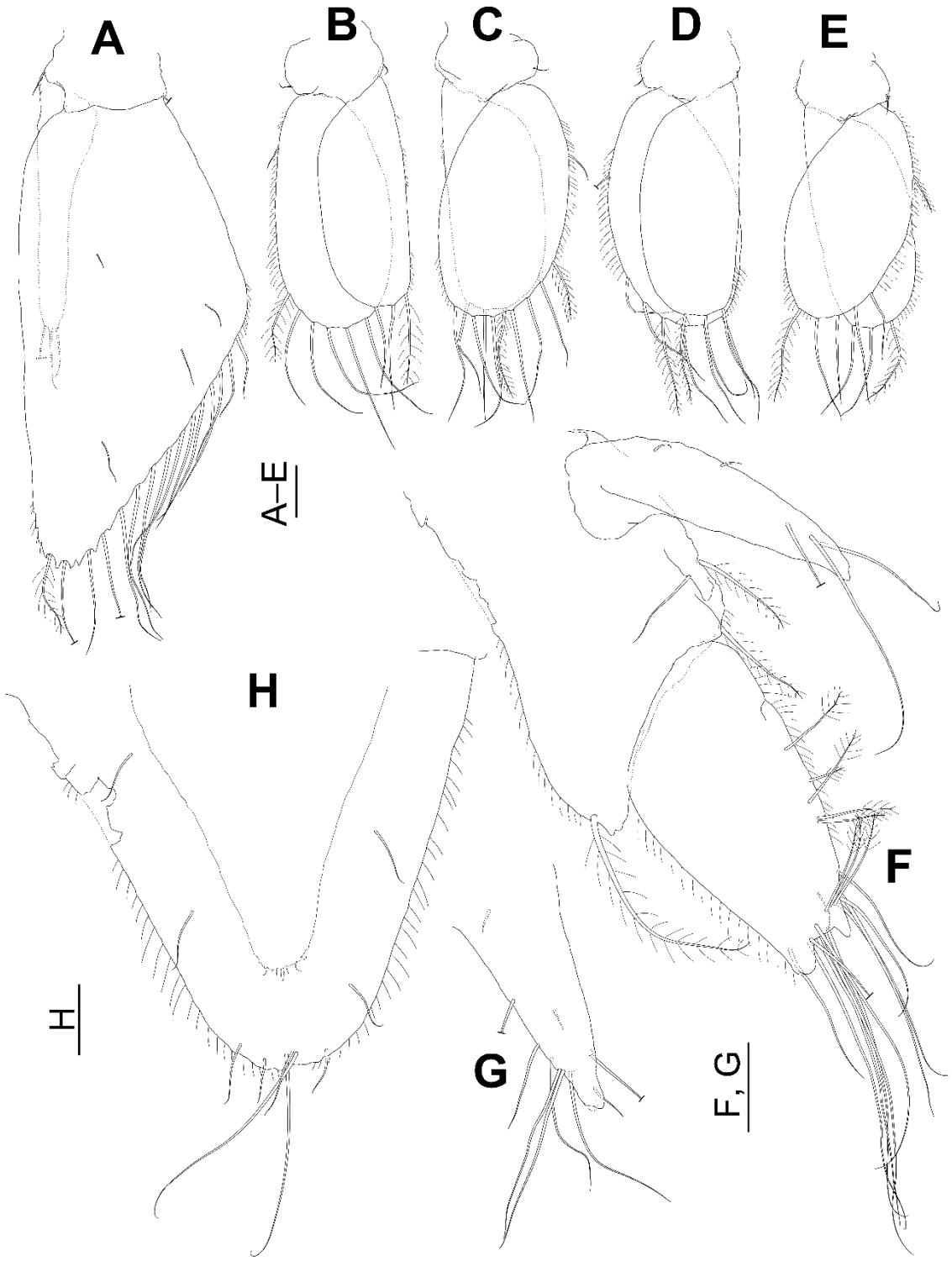

Figure II-VIII-5. *Deltanthura palpus* **sp. nov.**, holotype, manca. A–E, left pleopods 1–5; F, right uropodal endopod and exopod; G, left uropodal exopod; H, telson. Scale bars: 100 μm.

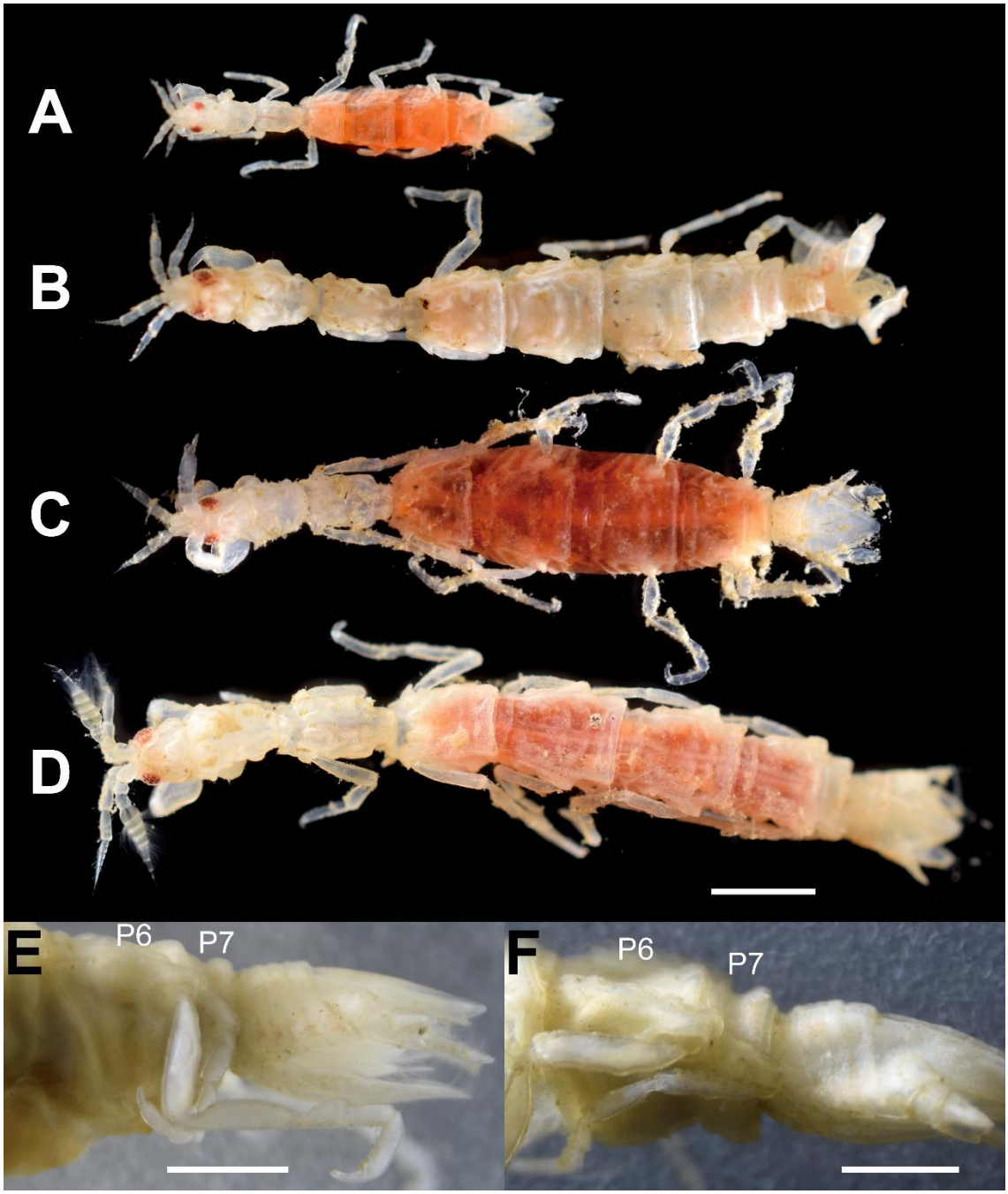

Figure II-VIII-6. *Deltanthura palpus* **sp. nov.** A–D, body in dorsal view, fresh specimens; E, F, posterior part of body in lateral view, ethanol fixed specimens. A, manca (ICHUM8850); B, female lacking oostegites (ICHUM8848); C, E, subadult male (ICHUM8851); D, F, adult male (ICHUM8849). P6, 7, pereonites 6, 7. Scale bars: 2 mm (A–D); 1 mm (E, F).

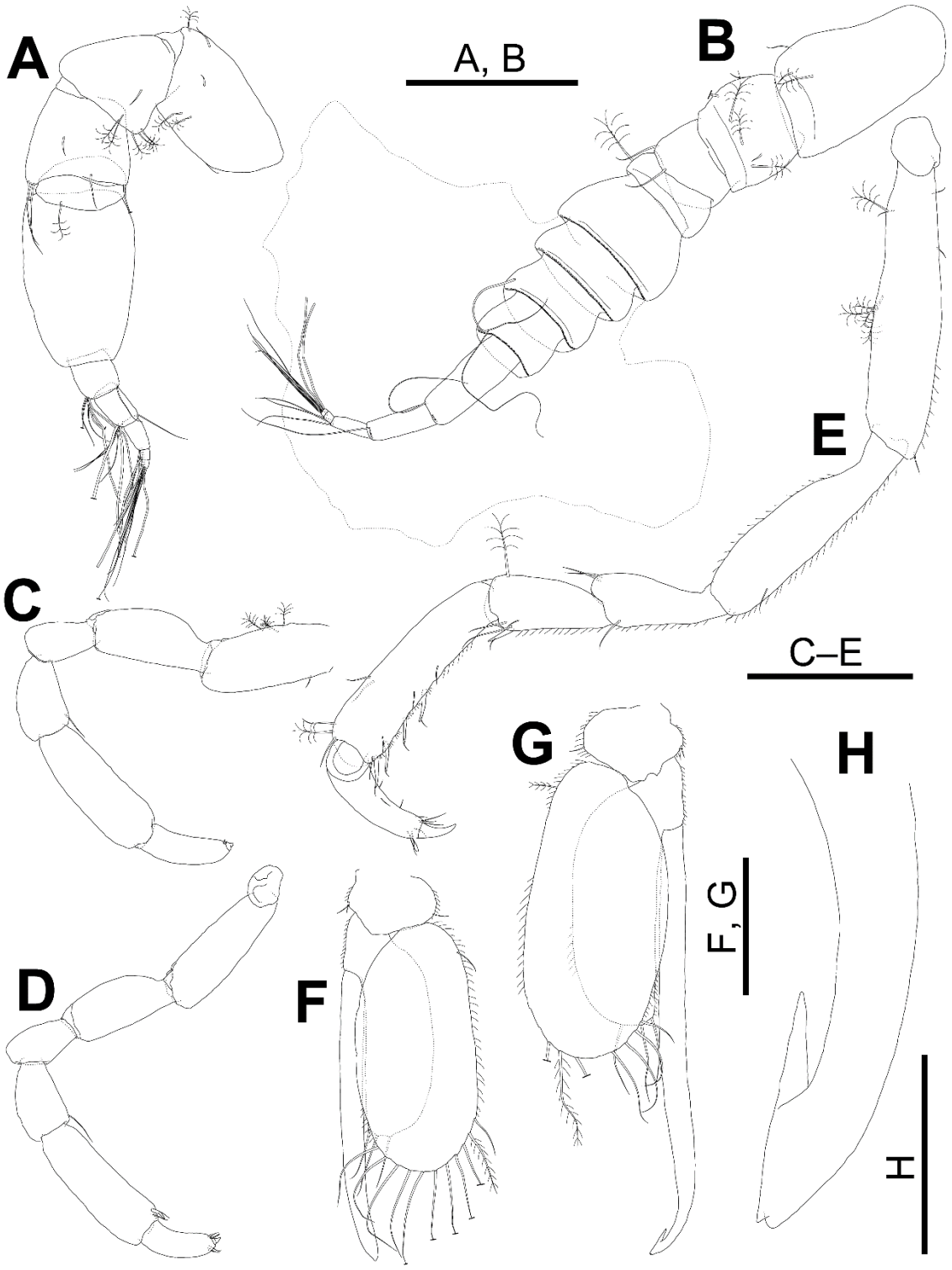

Figure II-VIII-7. *Deltanthura palpus* **sp. nov.** A, D, F, subadult male (ICHUM8851); B, E, G, H, adult male (ICHUM8849); C, female lacking oostegites (ICHUM8848). A, B, left antennule (most aesthetascs omitted from B); C–E, left pereopods 7; F, G, left and right pleopods 2; H, tip of appendix masculina. Scale bars: 500 µm (A–G); 100 µm (H).

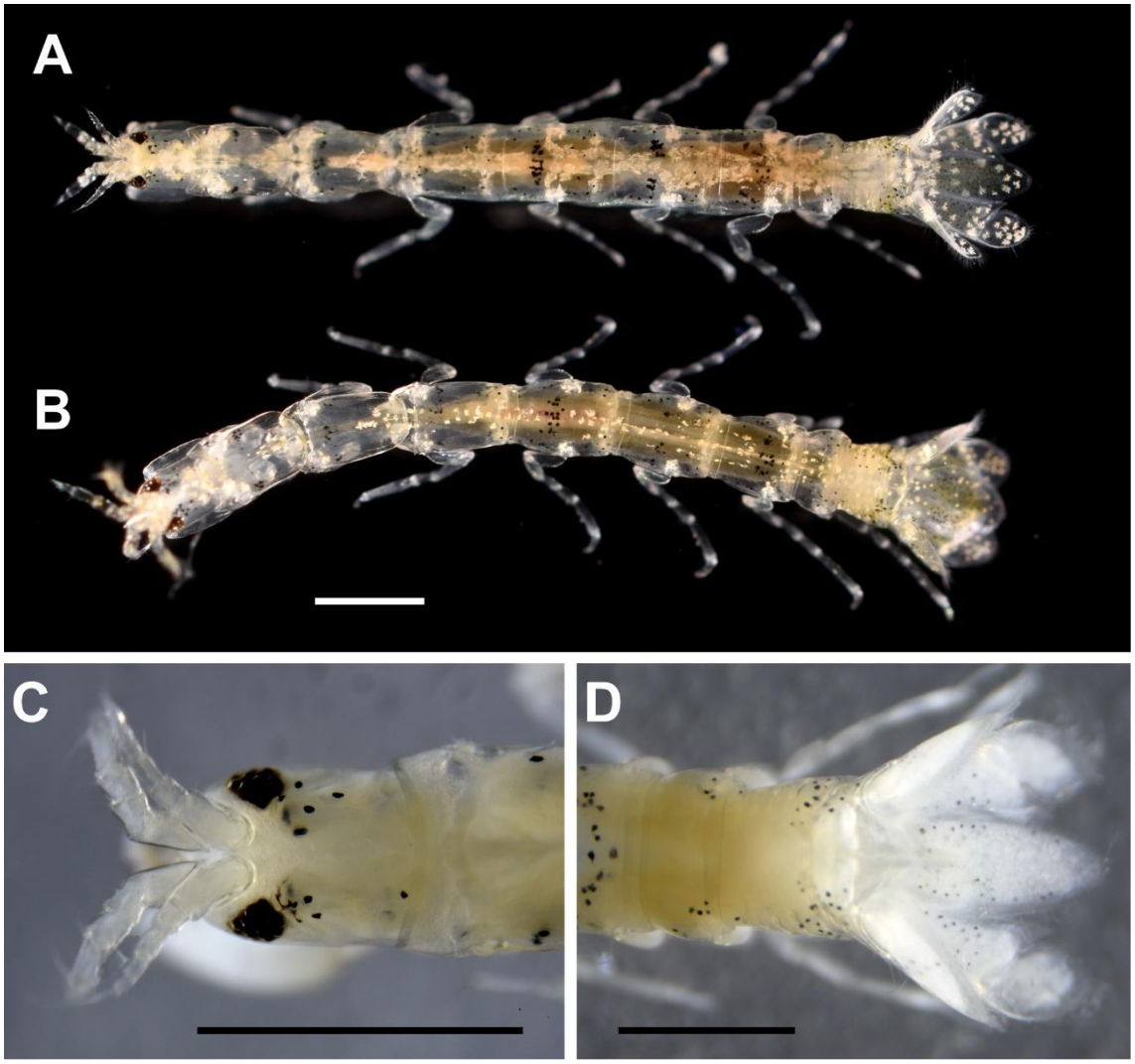

Figure II-IX-1. *Paranthura japonica*. A, C, D, ovigerous female after spawning, ICHUM8986; B, adult male, ICHUM8987. A, B, living individuals in dorsal view; C, head in dorsal view; D, pereonite 7 and pleon in dorsal view. Scale bars = 1 mm.

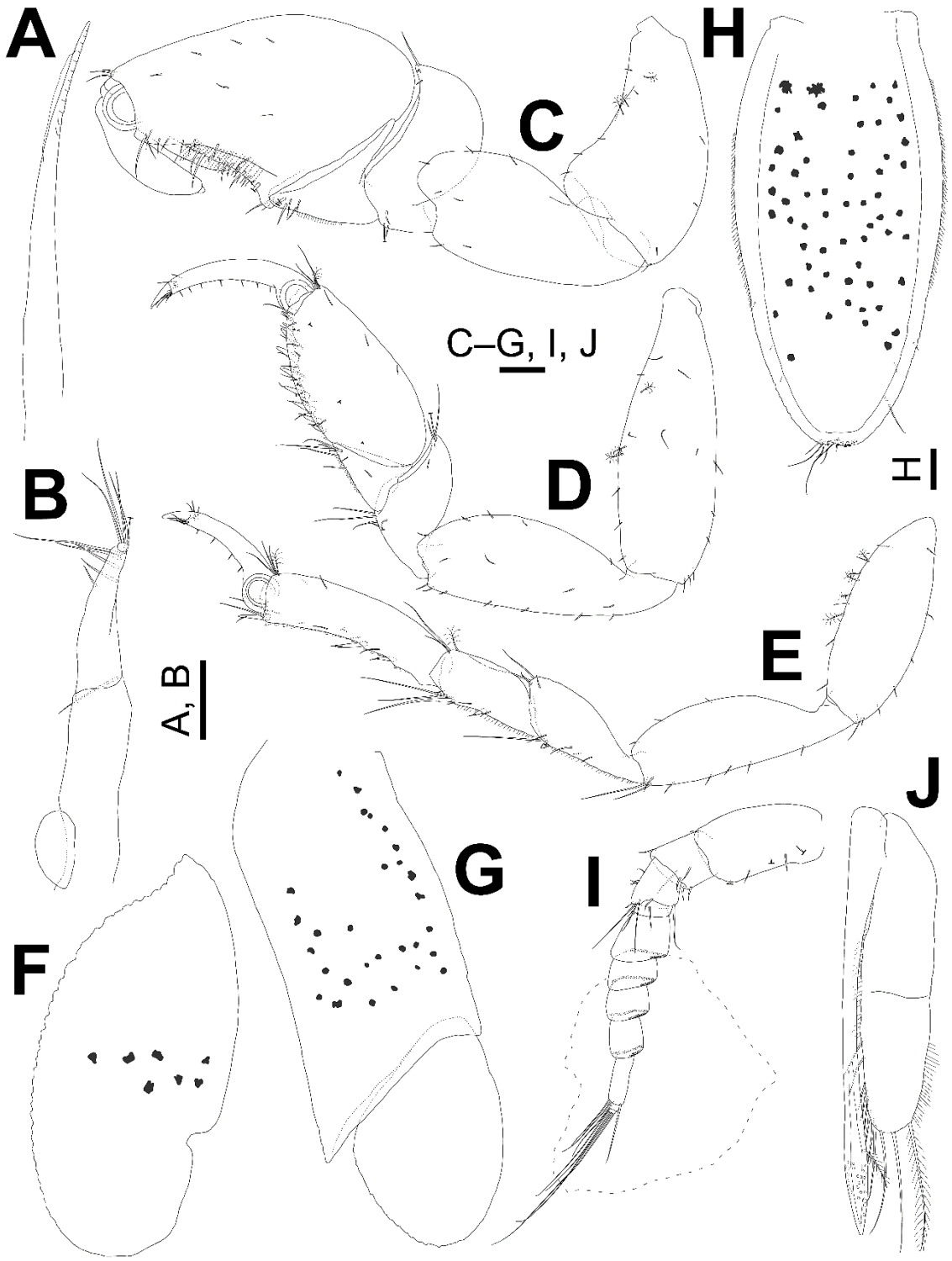

Figure II-IX-2. *Paranthura japonica*. A–H, ovigerous female after spawning, ICHUM8986; I, J, adult male, ICHUM8987. A, left maxilla; B, left maxilliped; C–E, left pereopods 1, 2, 7 (dactylus–unguis of pereopod 1 partly broken); F, G, left uropodal exopod and endopod (setae omitted); H, telson; I, left antennula (aesthetascs omitted); J, right pleopod 2 (exopod lost). Scale bars = 100 μm.

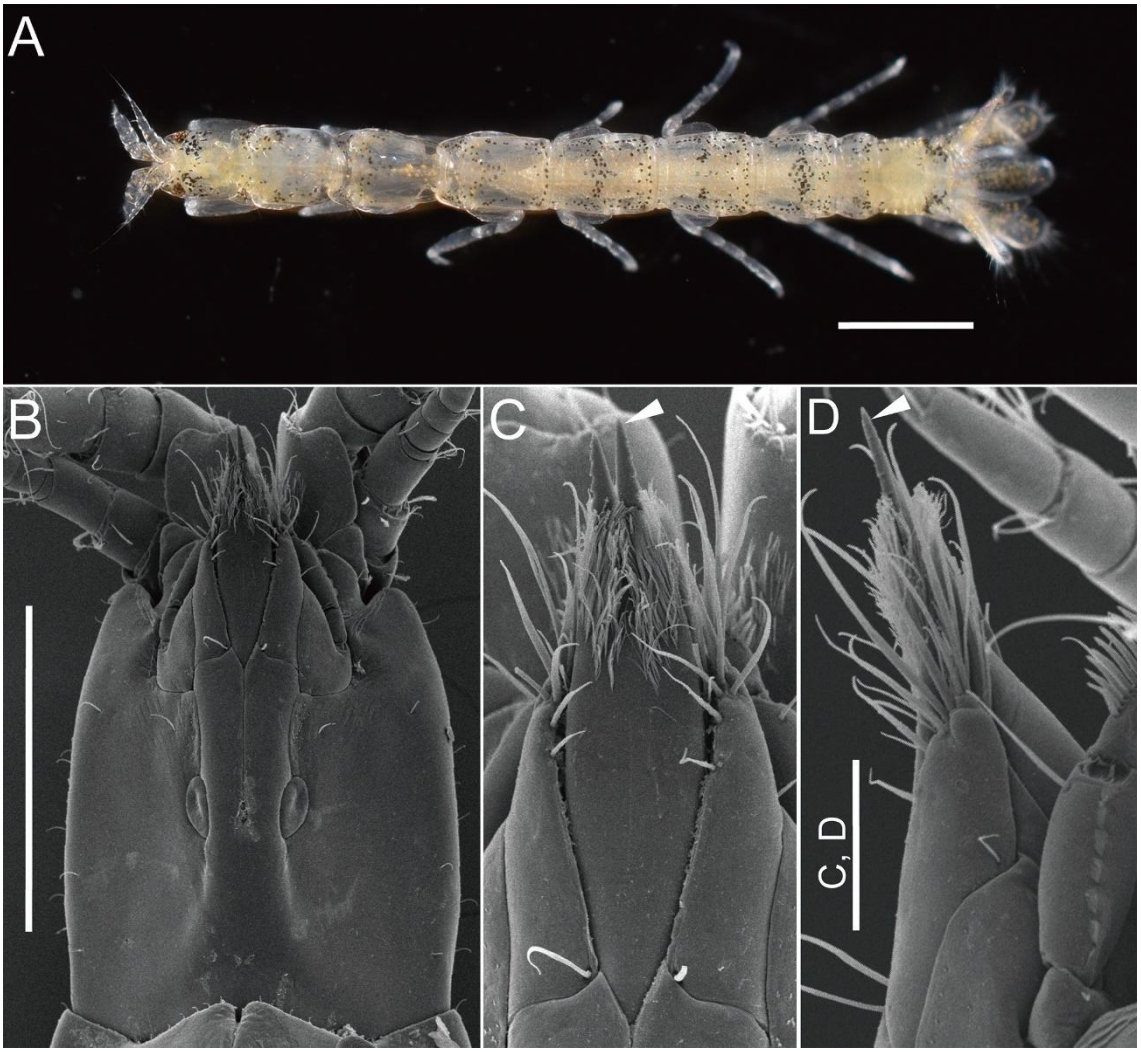

Figure II-IX-3. *Paranthura japonica* and its mouthparts. **(A)** Living specimen collected from Muroran and used for molecular work (accession number LC661620). **(B–D)** SEM images of mouthparts from an individual collected from Oshoro on 7 July 2020; **(B)** ventral view, **(C)** enlargement from (B); **(D)** lateral view. Arrowheads, maxilla. Scale bars: 1 mm **(A)**, 0.5 mm **(B)**, 0.1 mm **(C, D)**.

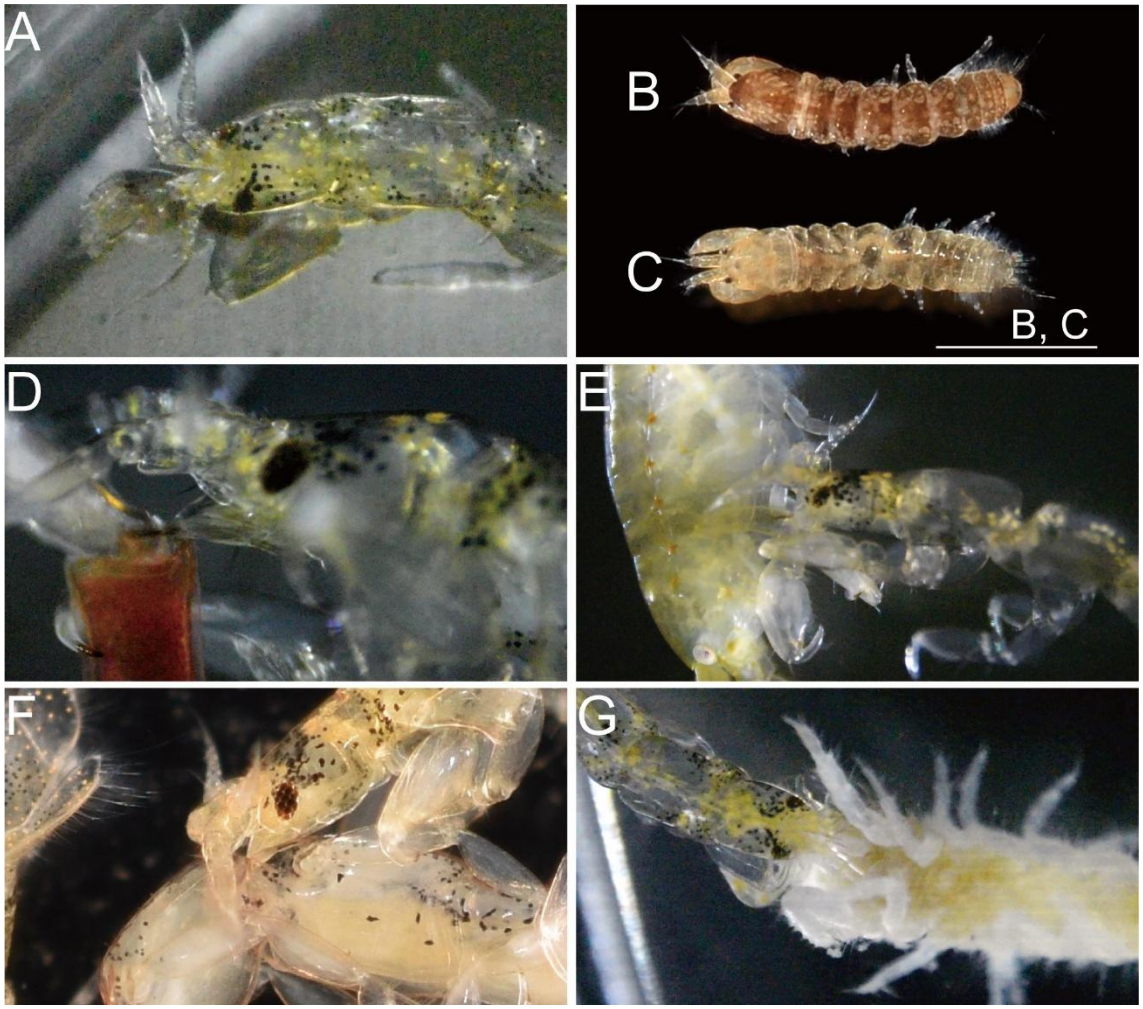

Figure II-IX-4. Predation on various species by *Paranthura japonica*. **(A)** *Paranthura japonica* feeding on *Zeuxo ezoensis*. **(B, C)** *Zeuxo ezoensis* individual before **(B)** and after **(C)** removal of internal contents by sucking. **(D)** *Paranthura japonica* feeding on the antenna of *Cleantiella strasseni*. **(E)** *Paranthura japonica* feeding on Ampithoidae sp. **(F)** *Paranthura japonica* feeding on *P. japonica*. **(G)** *Paranthura japonica* feeding on *Apseudes ranma*. Scale bar: 1 mm **(B, C)**; scale not available for the other panels.

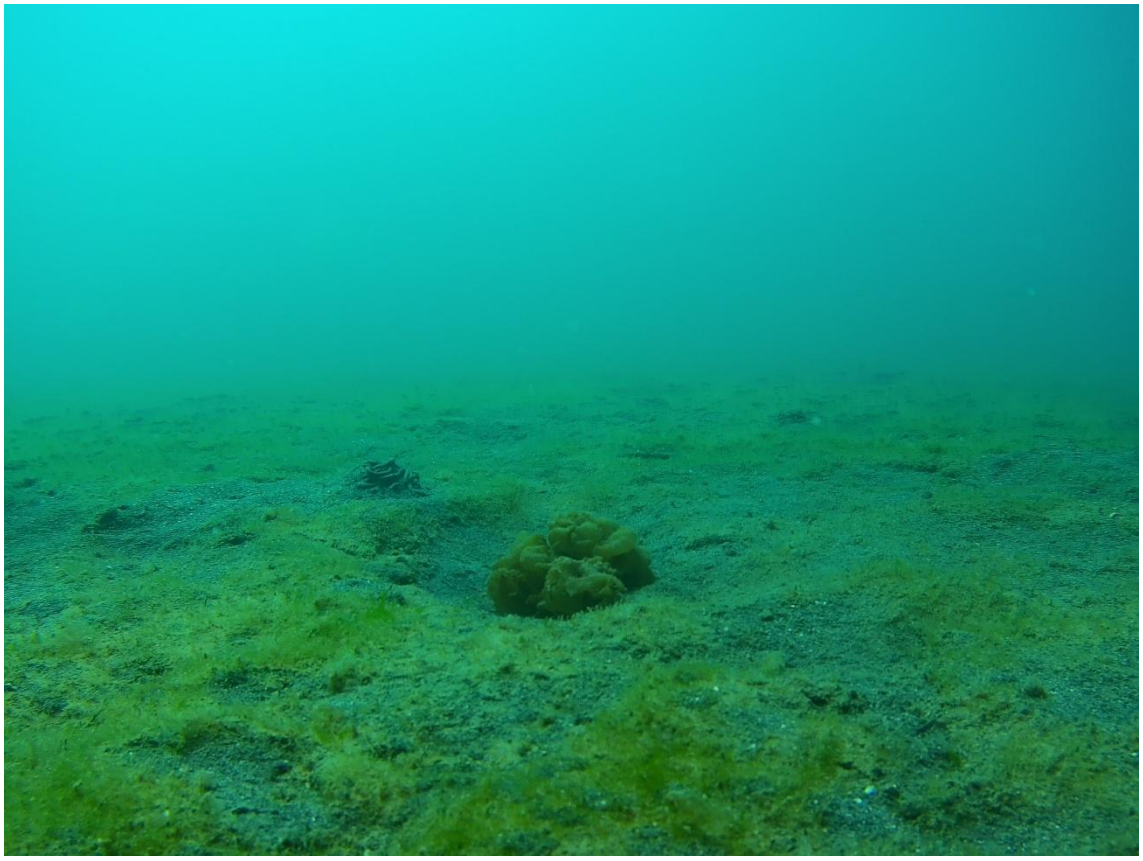

Figure II-X-1. Habitat of *Paranthura oriens* **sp. nov.** at the type locality, photographed by an underwater drone at 6.5 m depth off Kouyatsu (34°58.933′N, 139°49.136′E), Tateyama, Chiba, Japan, on 20 April 2021.

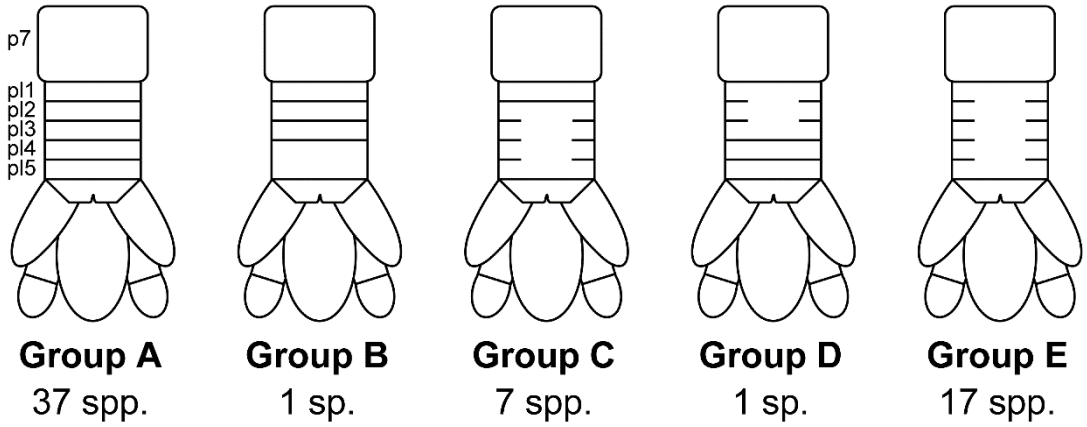

Figure II-X-2. Five species groups in *Paranthura* based on the condition of the pleonites. Group A (37 species), free pleonites 1–5; Group B (one species), free pleonites 1–3 and fused pleonites 4 and 5; Group C (seven species), free pleonite 1 and dorsally fused pleonites 2–5; Group D (one species), dorsally fused pleonites 1–3 and free pleonites 4 and 5; Group E (17 species), pleonites 1–5 dorsally fused. Two species in *Paranthura* remain unplaced due to a lack of relevant information. Abbreviations: p7, pereonite 7; pl1–5, pleonites 1–5.

Figure II-X-3. *Paranthura oriens* **sp. nov.** A, holotype, ovigerous female, dorsal view, fixed specimen. B, paratype ICHUM8807, female lacking oostegites, dorsal view, living animal. C, allotype male lateral view, fixed specimen. Scale bars: 1 mm.

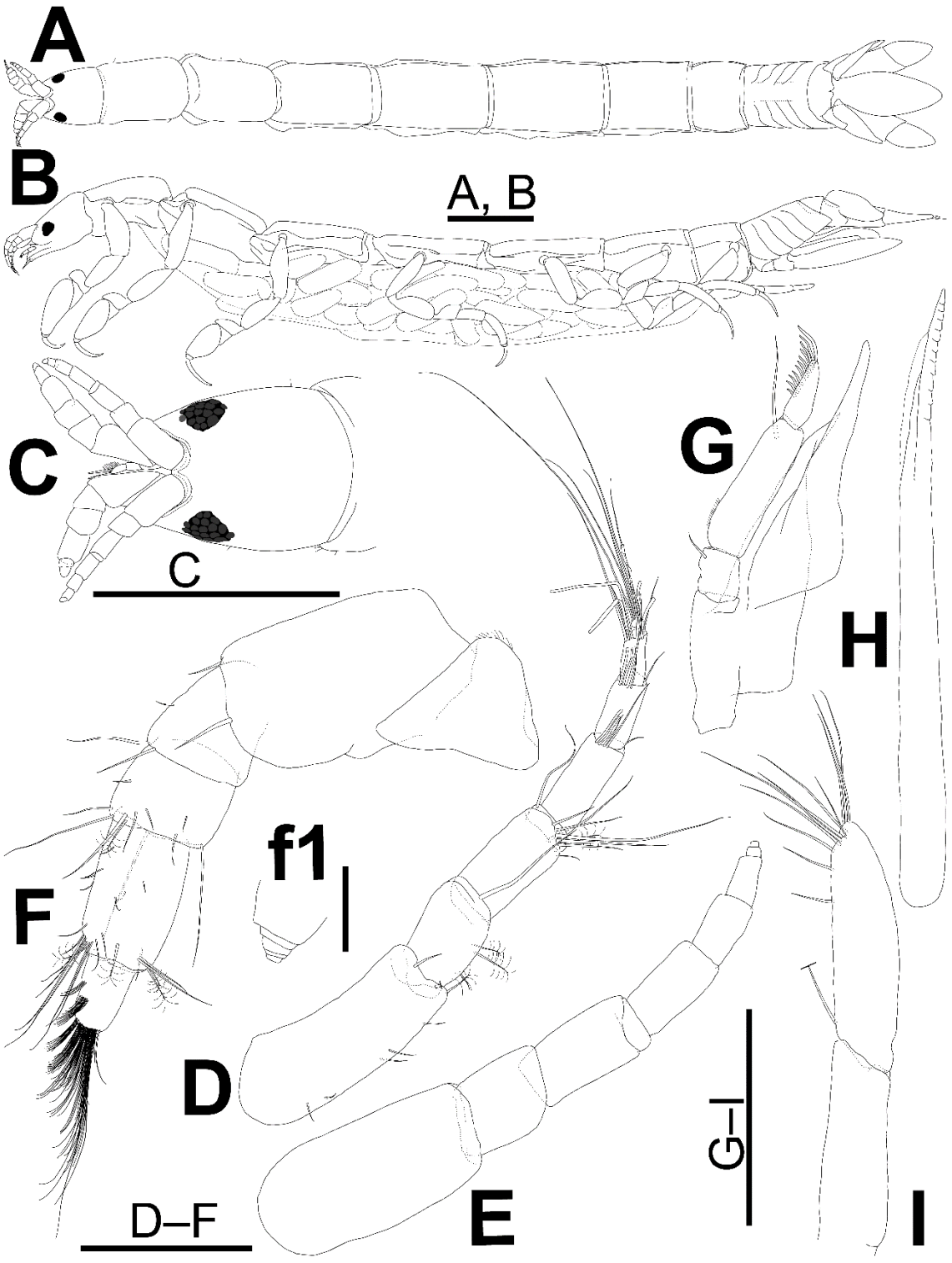

Figure II-X-4. *Paranthura oriens* **sp. nov.**, holotype, ovigerous female. A, B, body, dorsal and lateral views. C, head, dorsal view. D, right antennula. E, right antennula from another angle (setae omitted). F, right antenna; f1, tip of flagellar article 1 of right antenna, with tiny articles 2–5 (setae omitted). G, left mandible. H, right maxilla. I, maxilliped. Scale bars: 1 mm (A–C); 200 µm (D–I); 50 µm (f1).

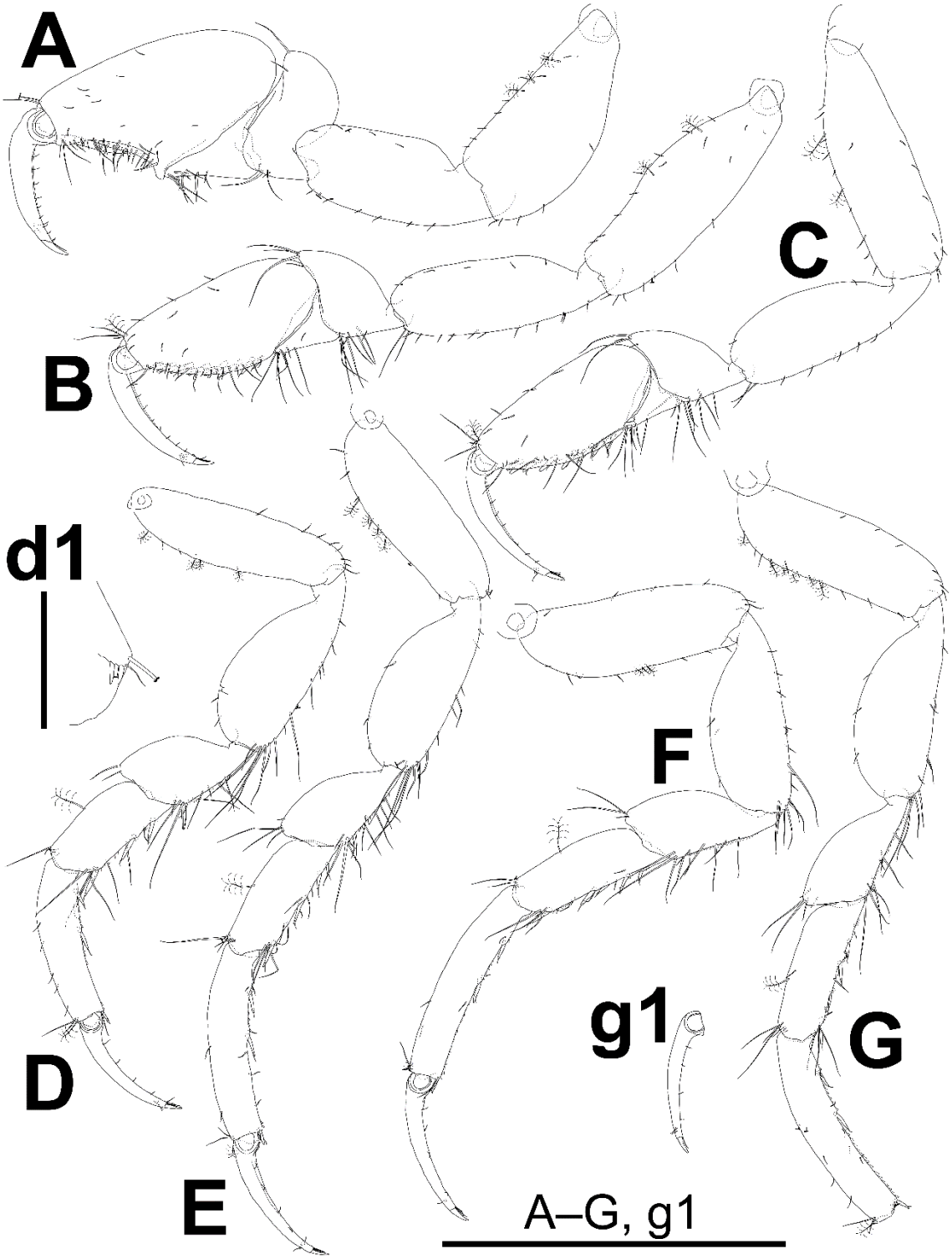

Figure II-X-5. *Paranthura oriens* **sp. nov.**, holotype, ovigerous female. A–F, left pereopods 1–6; d1, three outer dorsodistal tiny spiniform setae on carpus of left pereopod 4 (outer view). G, right pereopod 7; g1, dactylus and unguis of right pereopod 7. Scale bars: 1 mm (A–G, g1); 100 μm (d1).

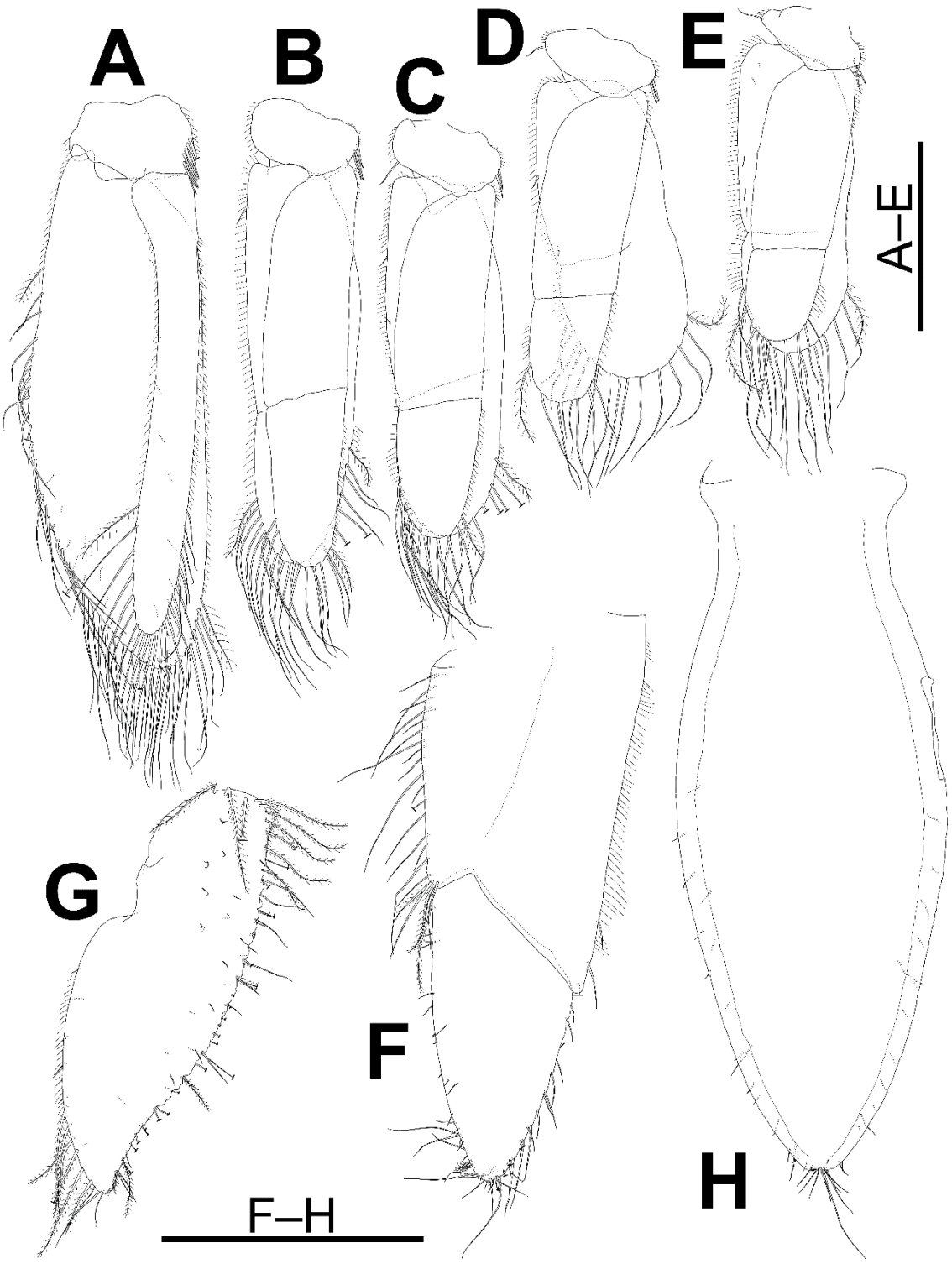

Figure II-X-6. *Paranthura oriens* **sp. nov.**, holotype, ovigerous female. A–E, left pleopods 1–5. F, right uropodal endopod. G, right uropodal exopod. H, telson, ventral view. Scale bars: 500 μm.

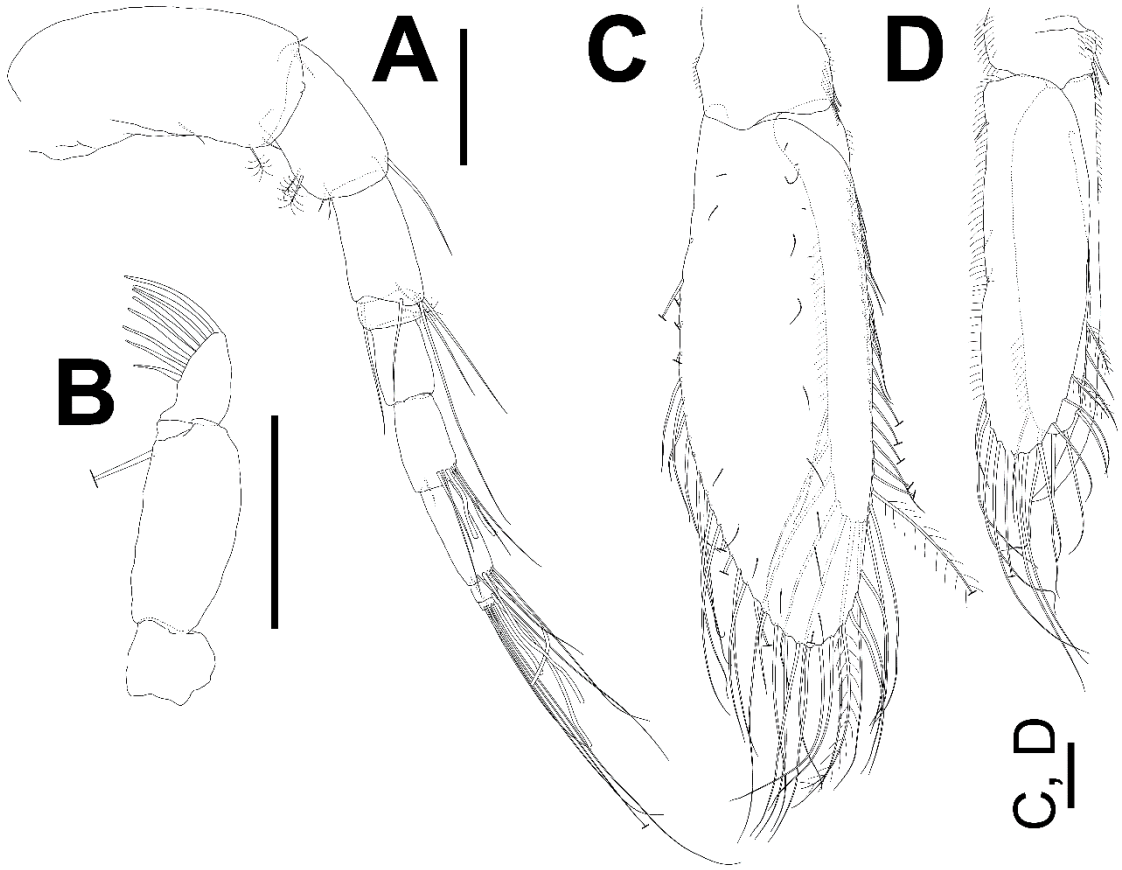

Figure II-X-7. *Paranthura oriens* **sp. nov.**, allotype, male. A, left antennula. B, left mandibular palp. C, right pleopod 1. D, left pleopod 2. Scale bars: 100 μm.

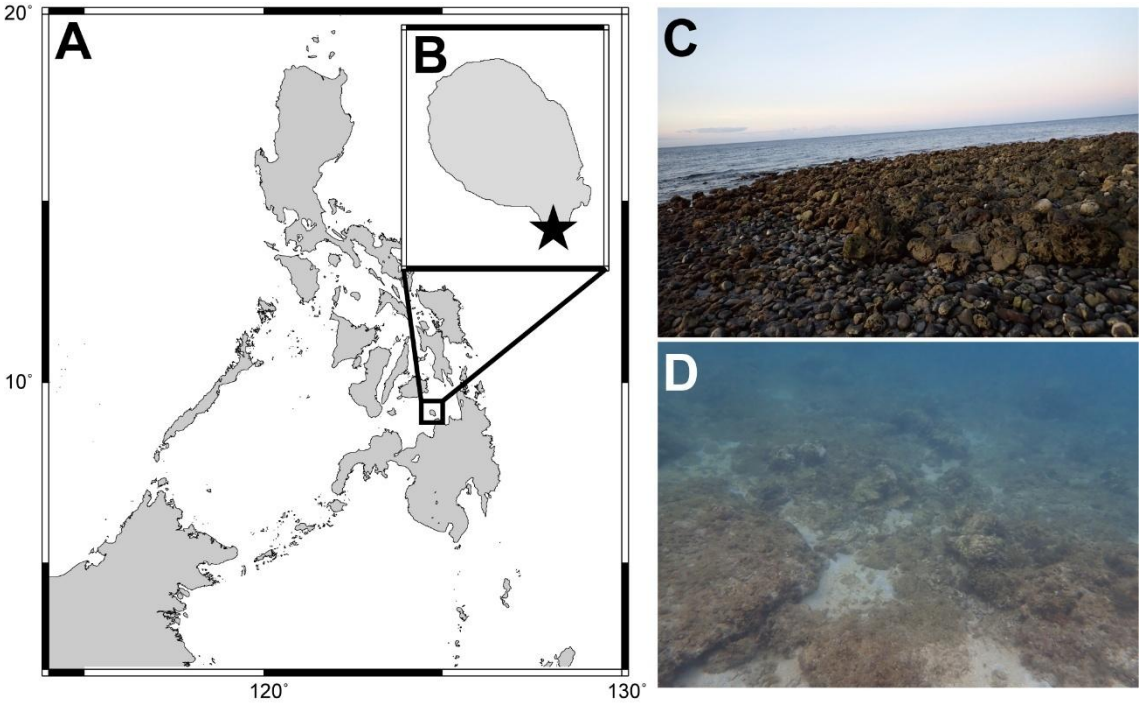

Figure III-I-1. Sampling site in this study. (A) map of the Philippines; (B) map of Camiguin Island, star indicates the sampling site at Brgy. Liong, Guinsiliban; (C, D) the environmental habitats of *Amakusanthura camiguinensis* **sp. nov.** at the intertidal (C) and subtidal (D) zones.

Figure III-I-2. *Amakusanthura camiguinensis* **sp. nov.**, fresh holotype individual, female lacking oostegites. (A, B) dorsal and lateral views. Scale bar = 1 mm.

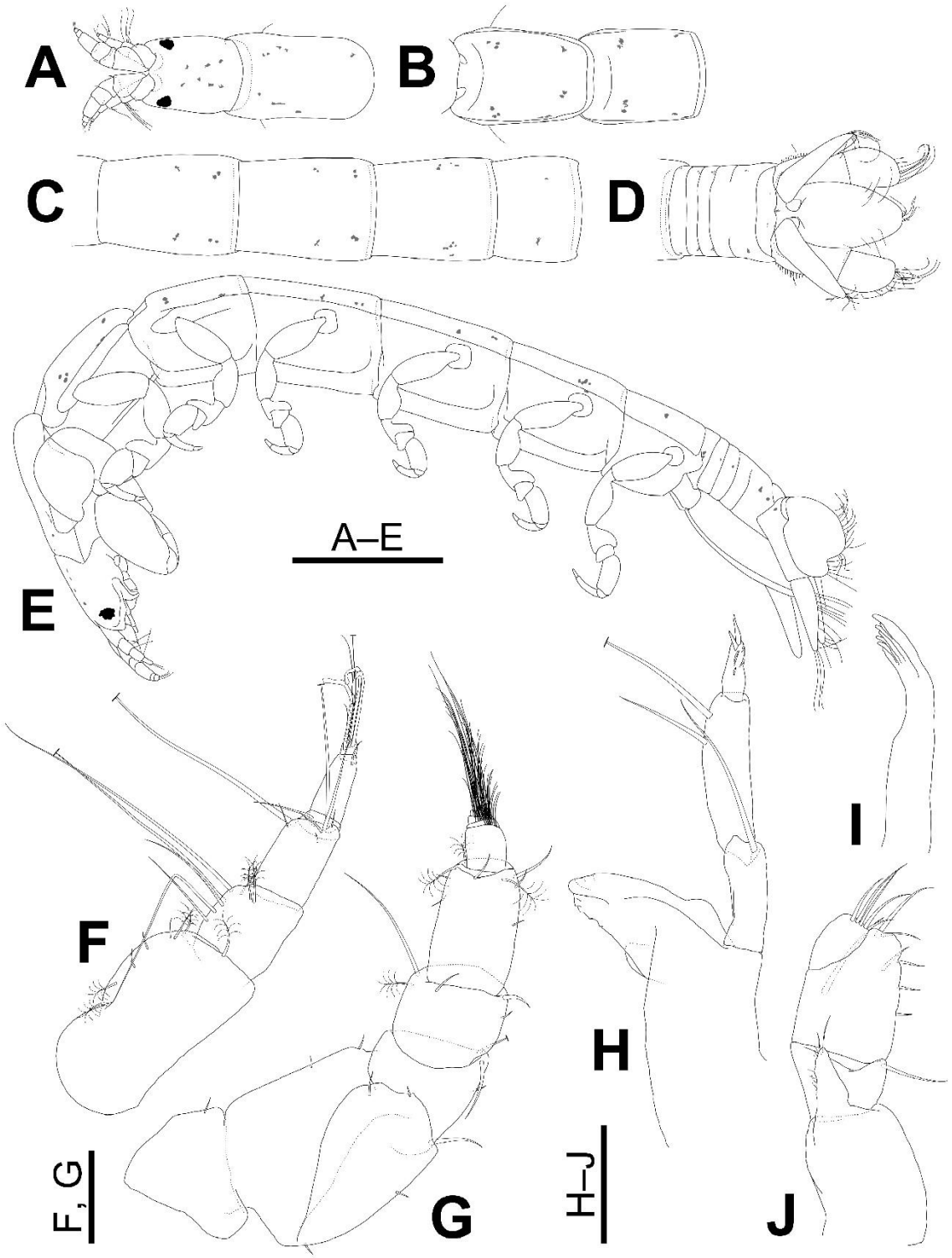

Figure III-I-3. *Amakusanthura camiguinensis* **sp. nov.**, holotype, female lacking oostegites. (A) head and pereonite 1 in dorsal view; (B) pereonites 2 and 3 in dorsal view; (C) pereonites 4–7 in dorsal view; (D) pleon in dorsal view; (E) lateral view; (F) left antennula; (G) left antenna; (H) left mandible; (I) right maxilla; (J) left maxilliped. Scale bars = 1 mm (A–E); 100 μm (F–J).

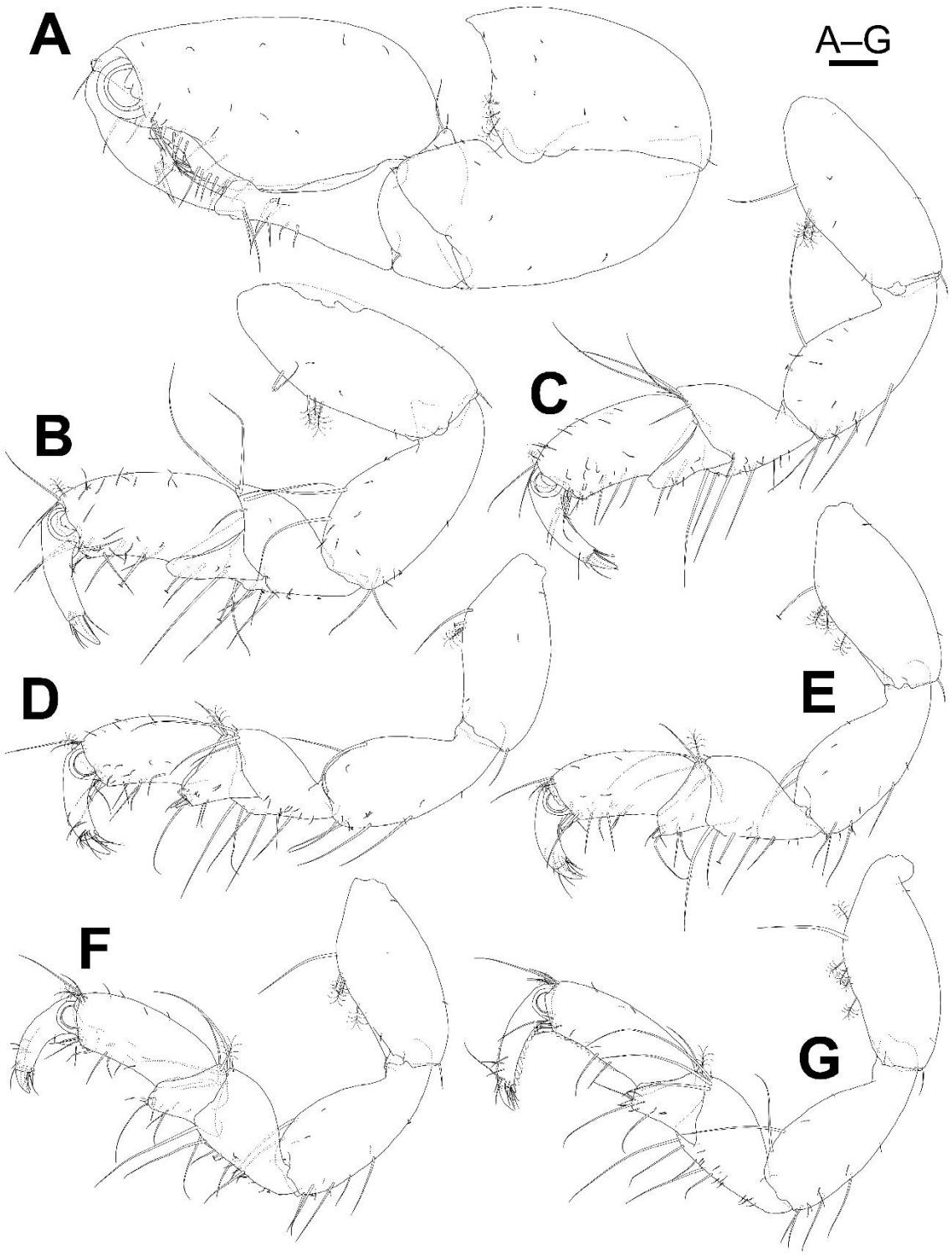

Figure III-I-4. *Amakusanthura camiguinensis* **sp. nov.**, holotype, female lacking oostegites. (A–C, E, F) left pereopods 1–3, 5, 6; (D, G) right pereopods 4 and 7. Scale bar = 100 μm.

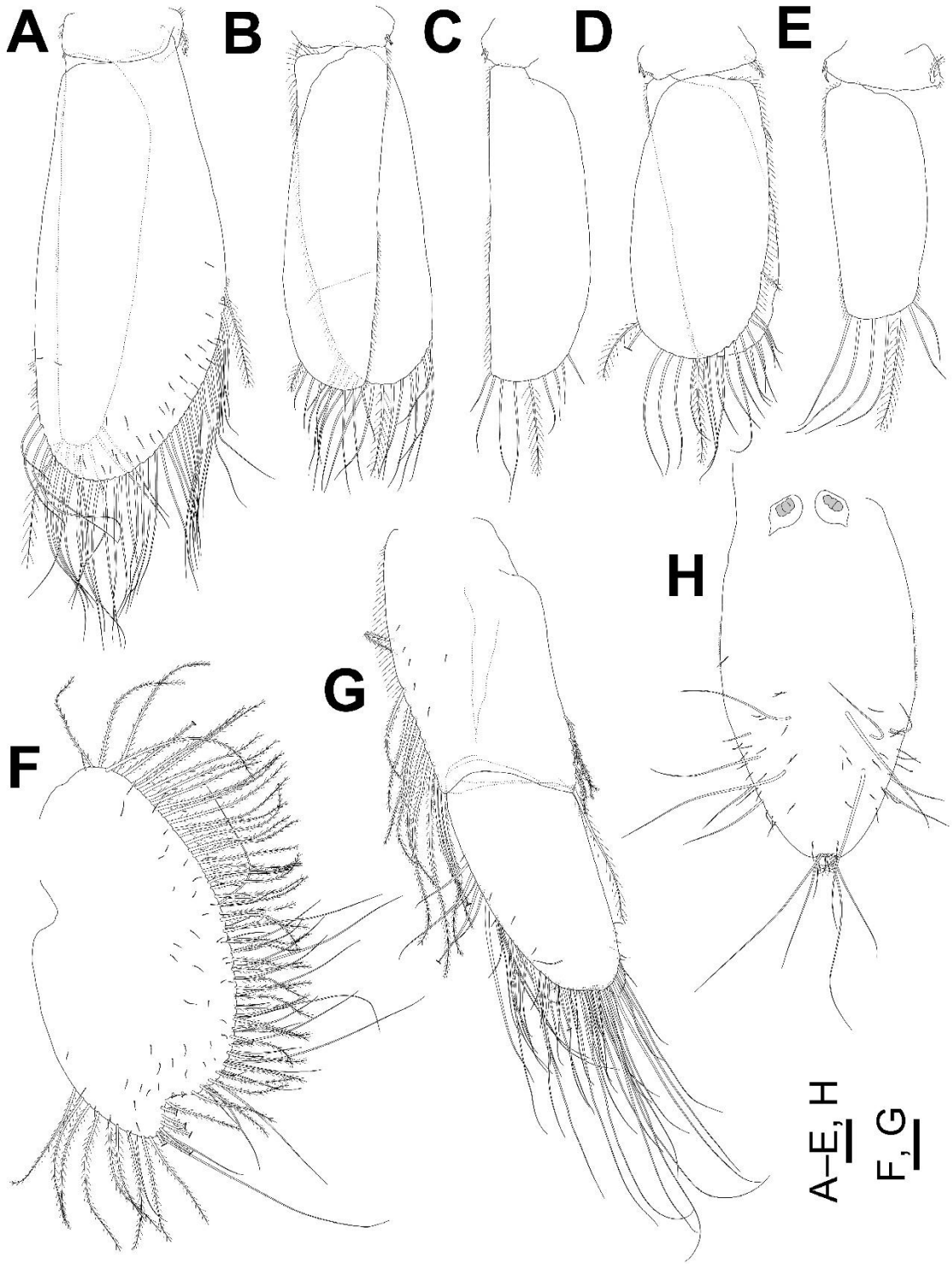

Figure III-I-5. *Amakusanthura camiguinensis* **sp. nov.**, holotype, female lacking oostegites. (A–E) left pleopods 1–5 (exopod lost for pleopods 3 and 5); (F) left uropodal exopod; (G) right uropodal endopod; (H) telson. Scale bars = 100 μm.

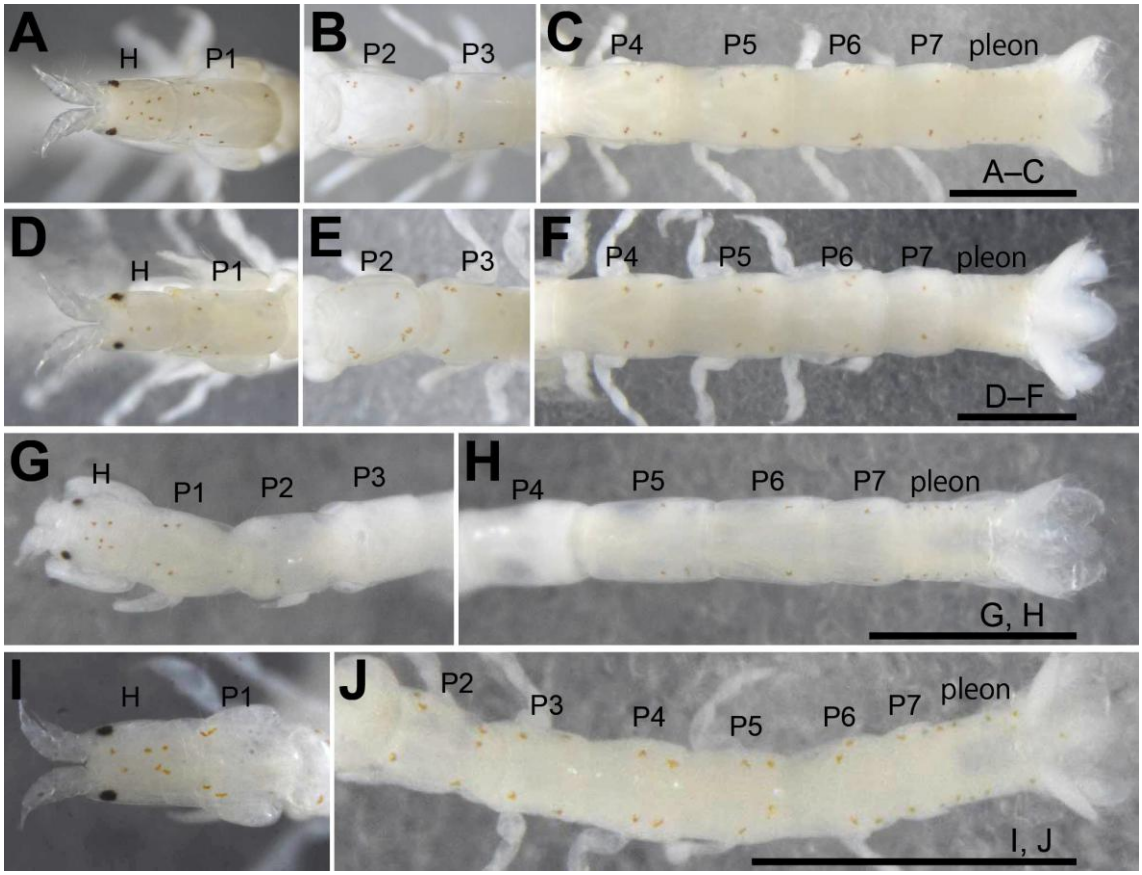

Figure III-I-6. The variation in dorsal pigmentation patterns in *Amakusanthura camiguinensis* **sp. nov.** among four fixed specimens in ethanol. (A–C) female lacking oostegites (holotype); (D–F) female lacking oostegites (paratype ICHUM9013); (G, H) female lacking oostegites (paratype NMCR 99221); (I, J) manca (paratype NMCR 99222). Abbreviations: H, head; P1–7, pereonites 1–7. Scale bars = 1 mm.

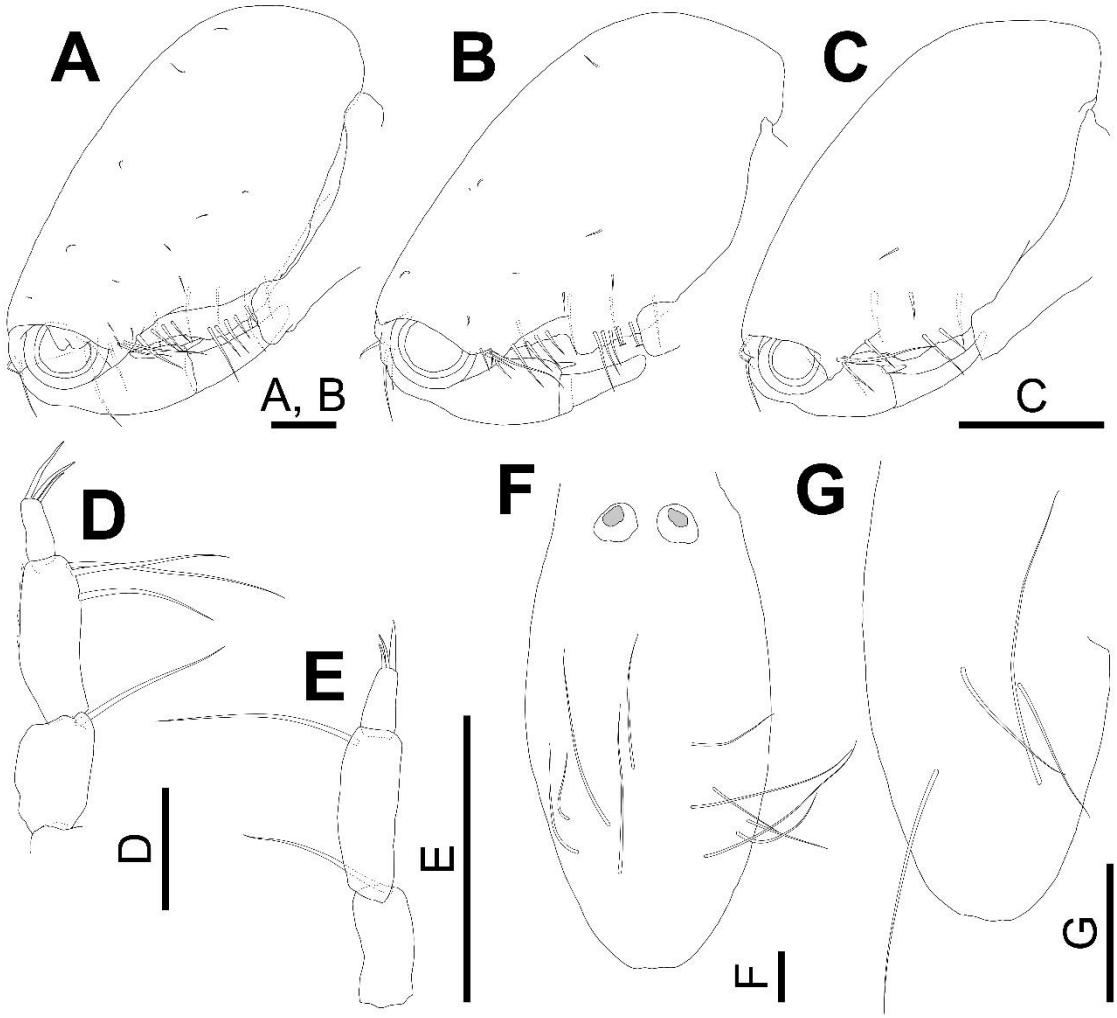

Figure III-I-7. The variation in some morphological characters in *Amakusanthura camiguinensis* **sp. nov.** (A–C) left pereopods 1 (from the carpus to the unguis) from holotype female lacking oostegites, paratype female lacking oostegites (ICHUM9013), and manca (paratype NMCR 99222), respectively (simple setae on dactylus and carpus omitted); (D) left mandibular palp from paratype female lacking oostegites (ICHUM9014); (E) right mandibular palp from manca (paratype NMCR 99222); (F, G) telsons from paratype female lacking oostegites (ICHUM9014) and manca (paratype NMCR 99222), respectively, only long simple setae on the surface are shown. Scale bars = 100 μm.

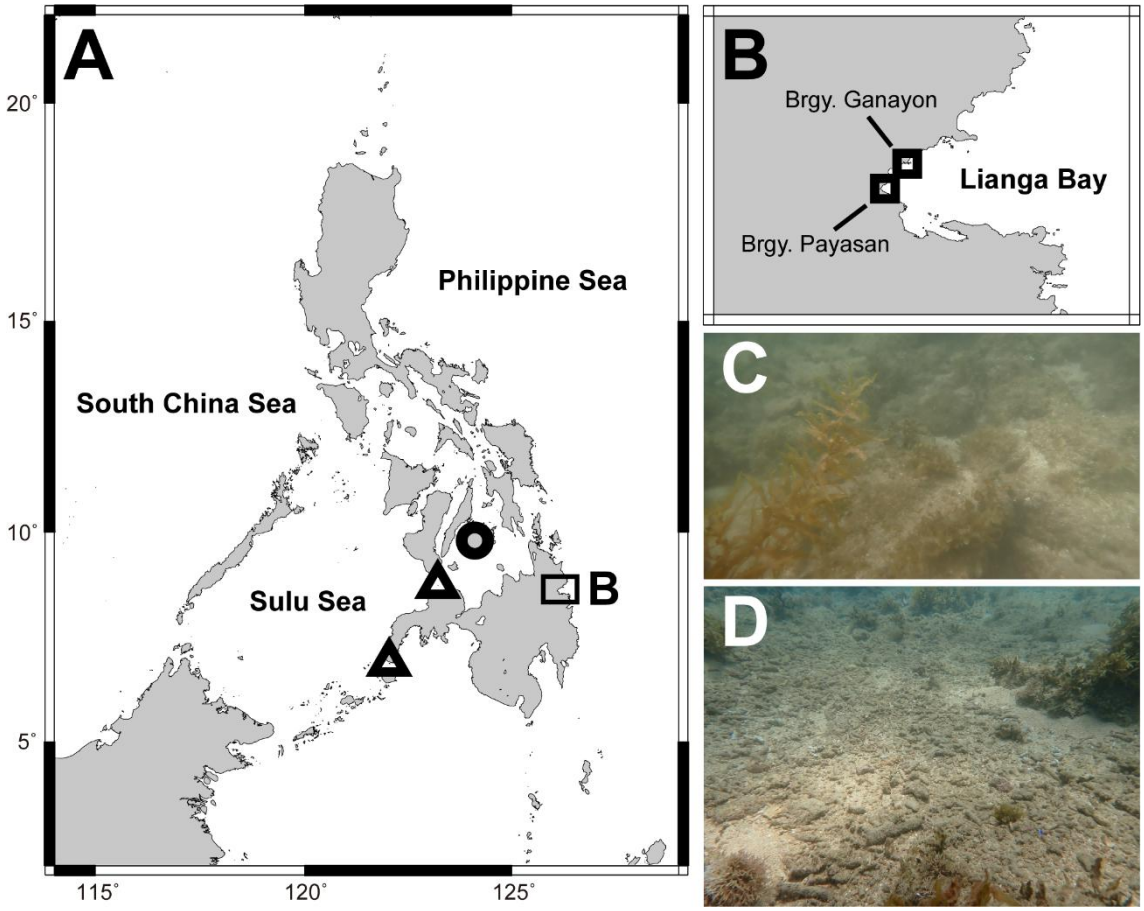

Figure III-II-1. Distribution of anthuroid species in the Philippines and sampling localities in this study. A, map of the Philippines with the distributional records of anthuroid species, circle represents *Stygocyathura filipinica* and triangles represent *Colanthura kensleyi*; B, sampling localities (MPA of Brgy. Ganayon and Brgy. Payasan) in Lianga, Mindanao; C, D, the inhabiting environments of *Sauranthura liangaensis* **sp. nov.** in Brgy. Ganayon and Brgy. Payasan, respectively.

Figure III-II-2. *Sauranthura liangaensis* **sp. nov.**, fresh individuals. A, B, dorsal and lateral views of holotype ovigerous female (NMCR 99220); C, dorsal view of ovigerous female (paratype ICHUM8983); D, dorsal view of manca (ICHUM8984); E, dorsal view of allotype subadult male (NMCR 99223). Scale bar = 1 mm.

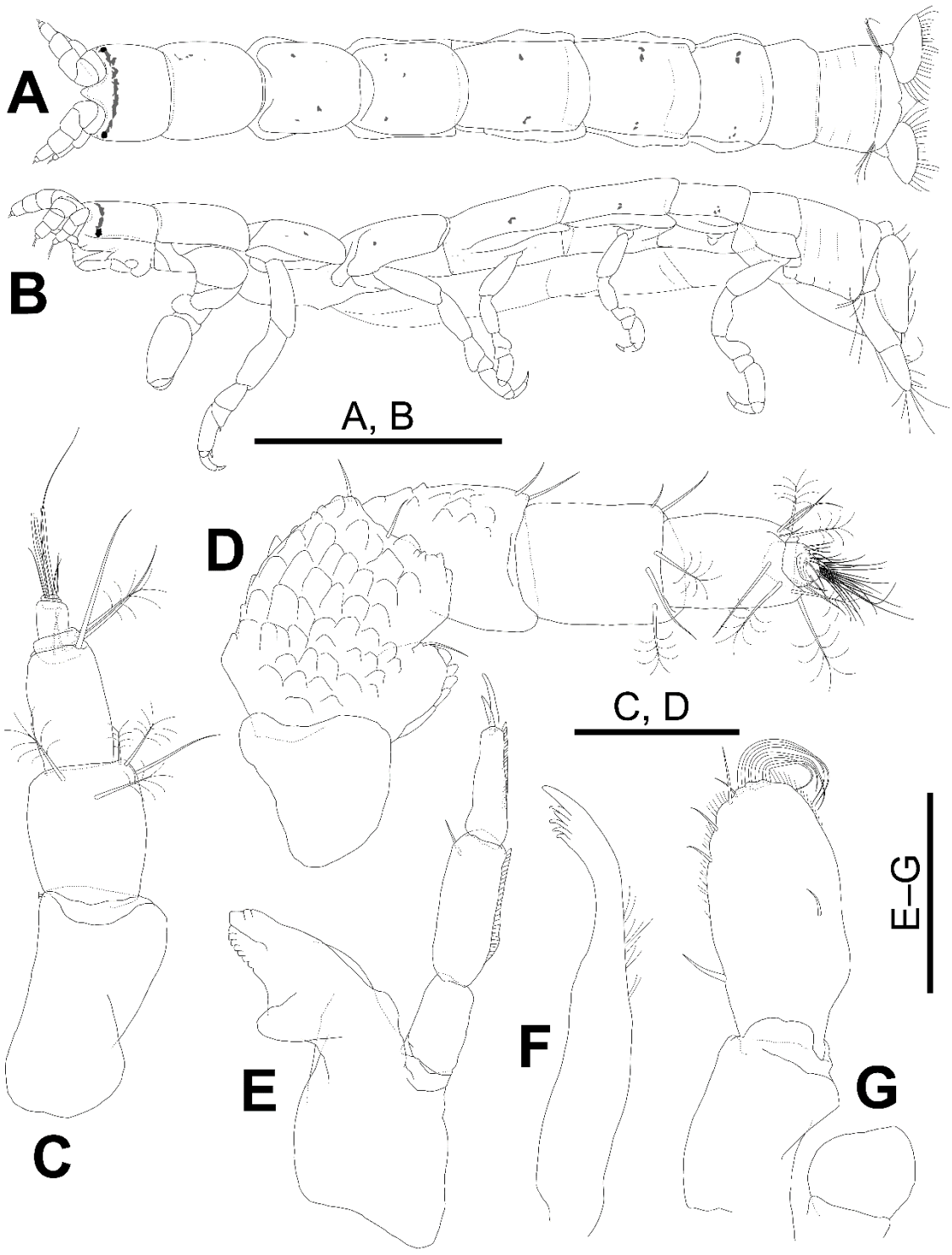

Figure III-II-3. *Sauranthura liangaensis* **sp. nov.**, holotype, ovigerous female, NMCR 99220. A, B, body, dorsal and lateral views; C, left antennula; D, left antenna; E, right mandible; F, left maxilla; G, left maxilliped. Scale bars = 1 mm (A, B); 100 μm (C–G).

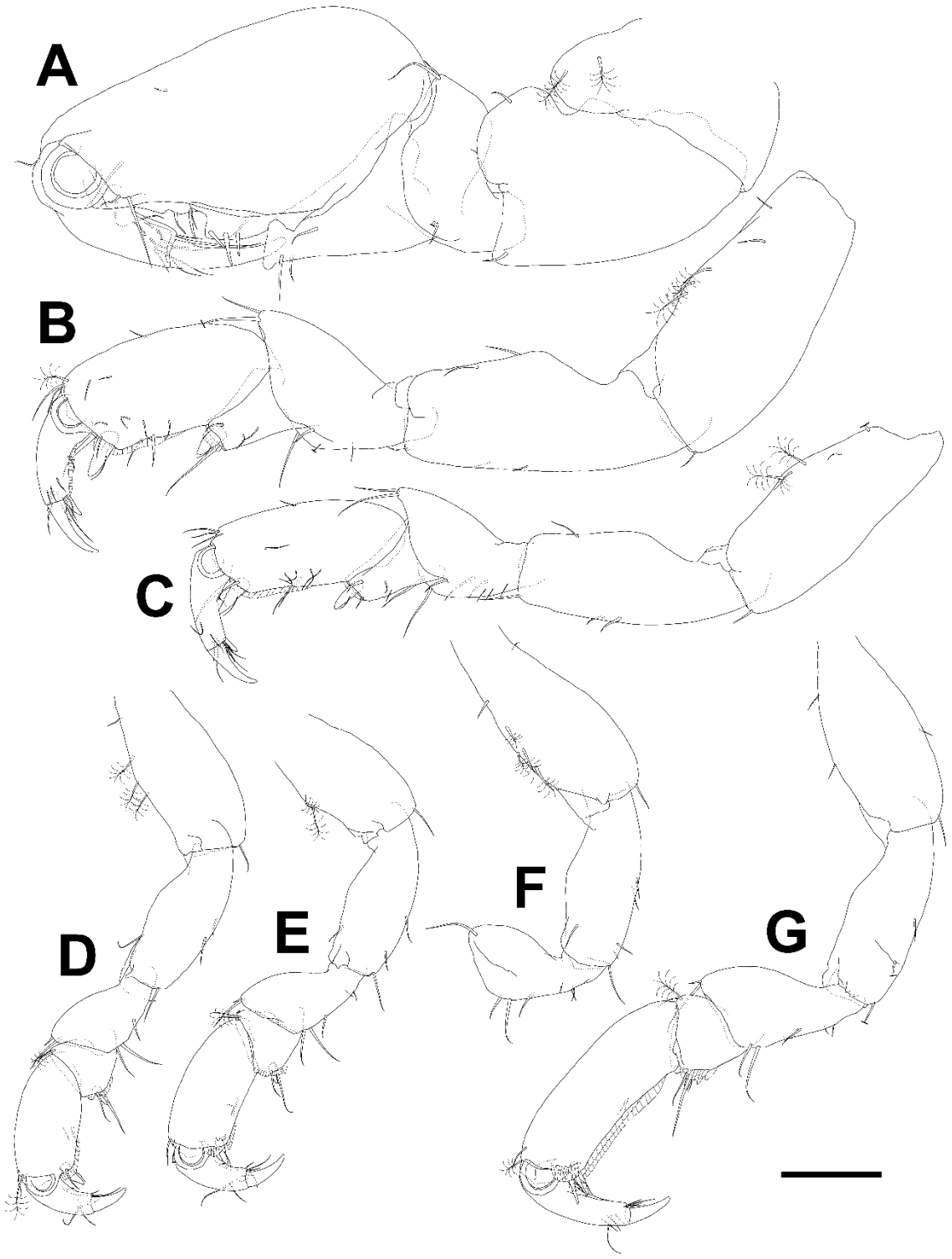

Figure III-II-4. *Sauranthura liangaensis* **sp. nov.**, holotype, ovigerous female, NMCR 99220. A–E, G, left pereopods 1–5, 7; F, right pereopod 6 (from carpus to unguis lost). Scale bar = 100 μm.

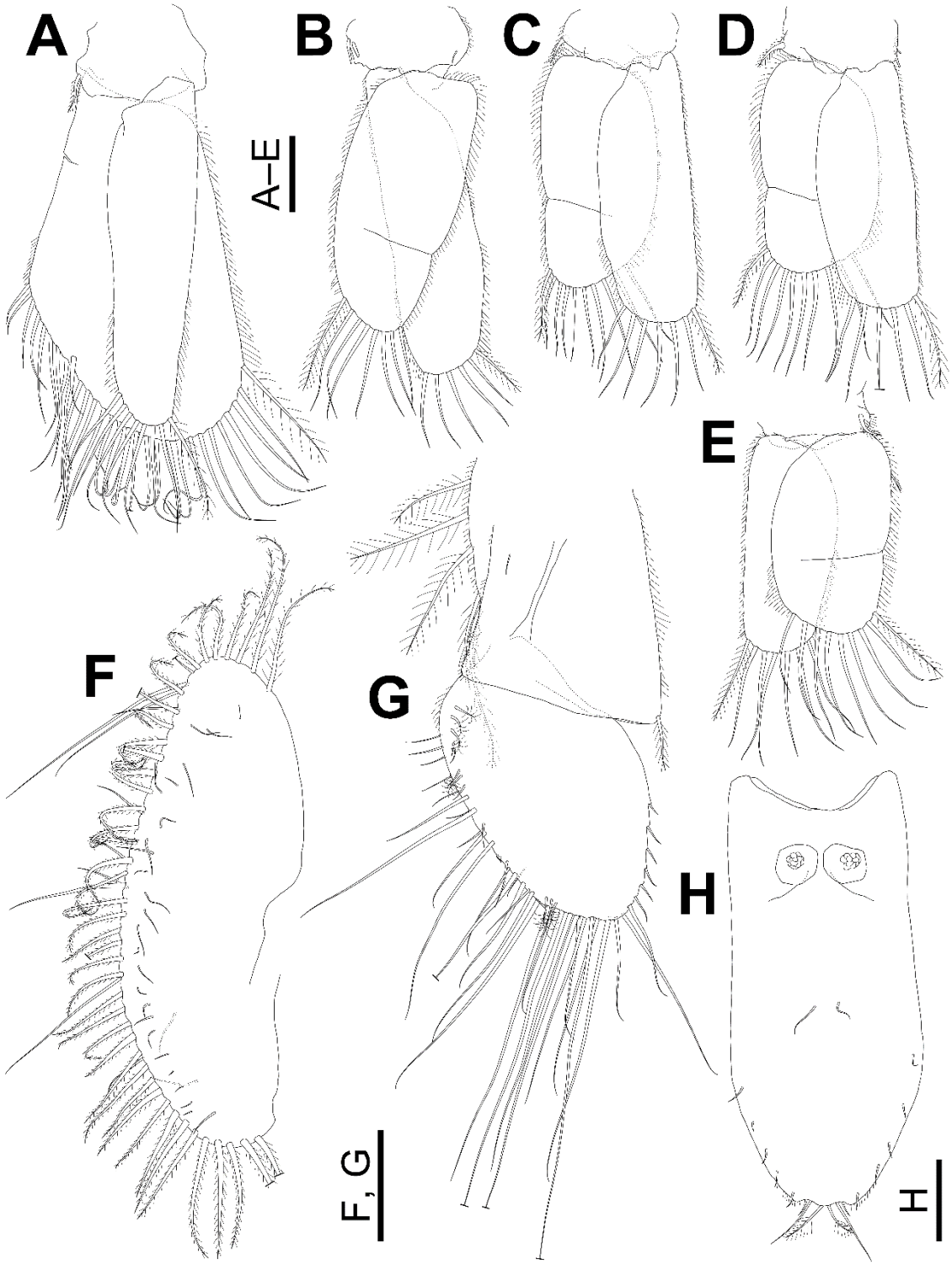

Figure III-II-5. *Sauranthura liangaensis* **sp. nov.**, holotype, ovigerous female, NMCR 99220. A–E, left pleopods 1–5; F, right uropodal exopod; G, right uropodal endopod; H, telson. Scale bars = 100 μm.

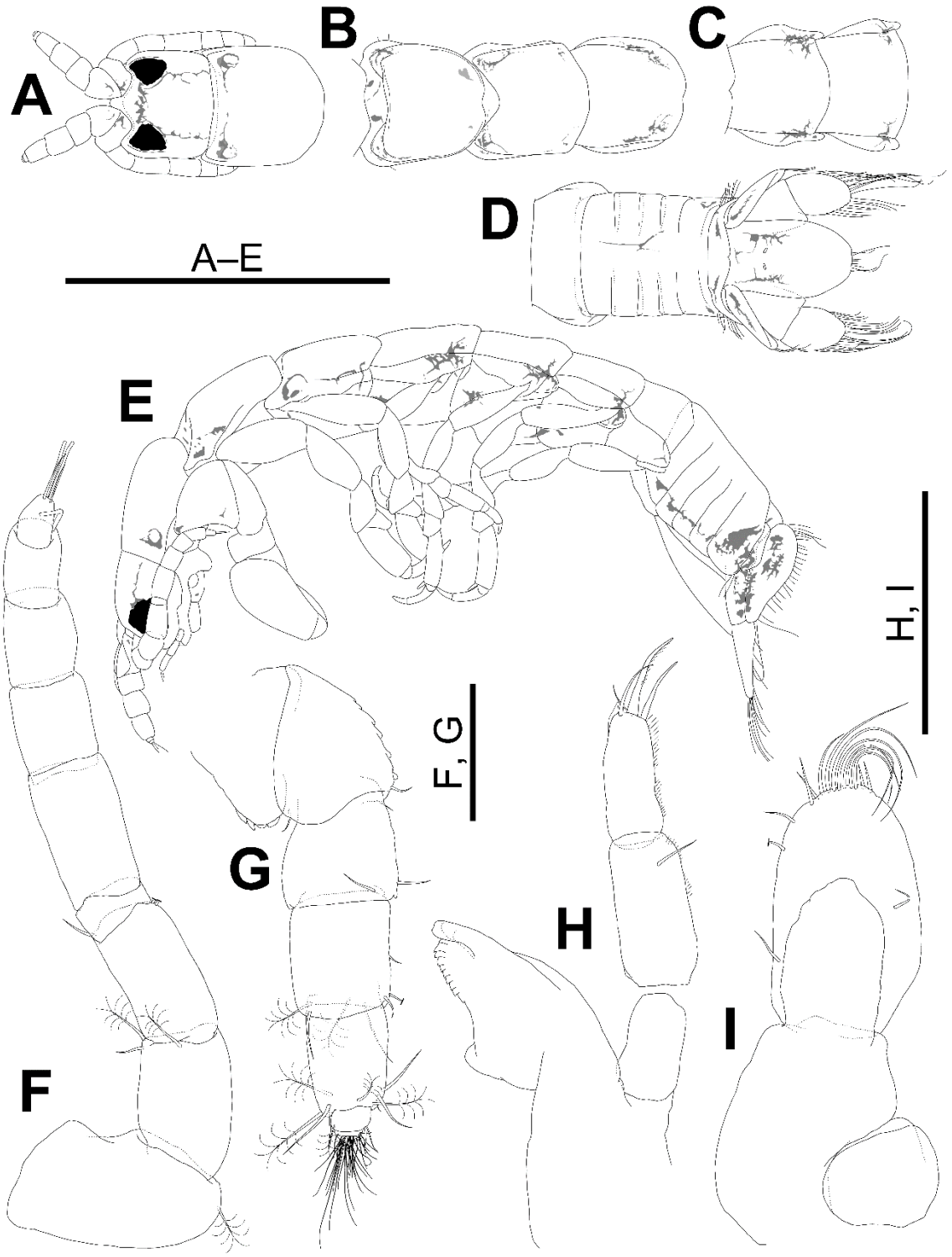

Figure III-II-6. *Sauranthura liangaensis* **sp. nov.**, allotype, subadult male, NMCR 99223. A, head and pereonite 1 in dorsal view; B, pereonites 2–4 in dorsal view; C, pereonites 5 and 6 in dorsal view; D, pereonite 7 and pleon in dorsal view; E, body, lateral view; F, right antennula; G, right antenna; H, left mandible; I, left maxilliped. Scale bars = 1 mm (A–E); 100 μm (F–I).

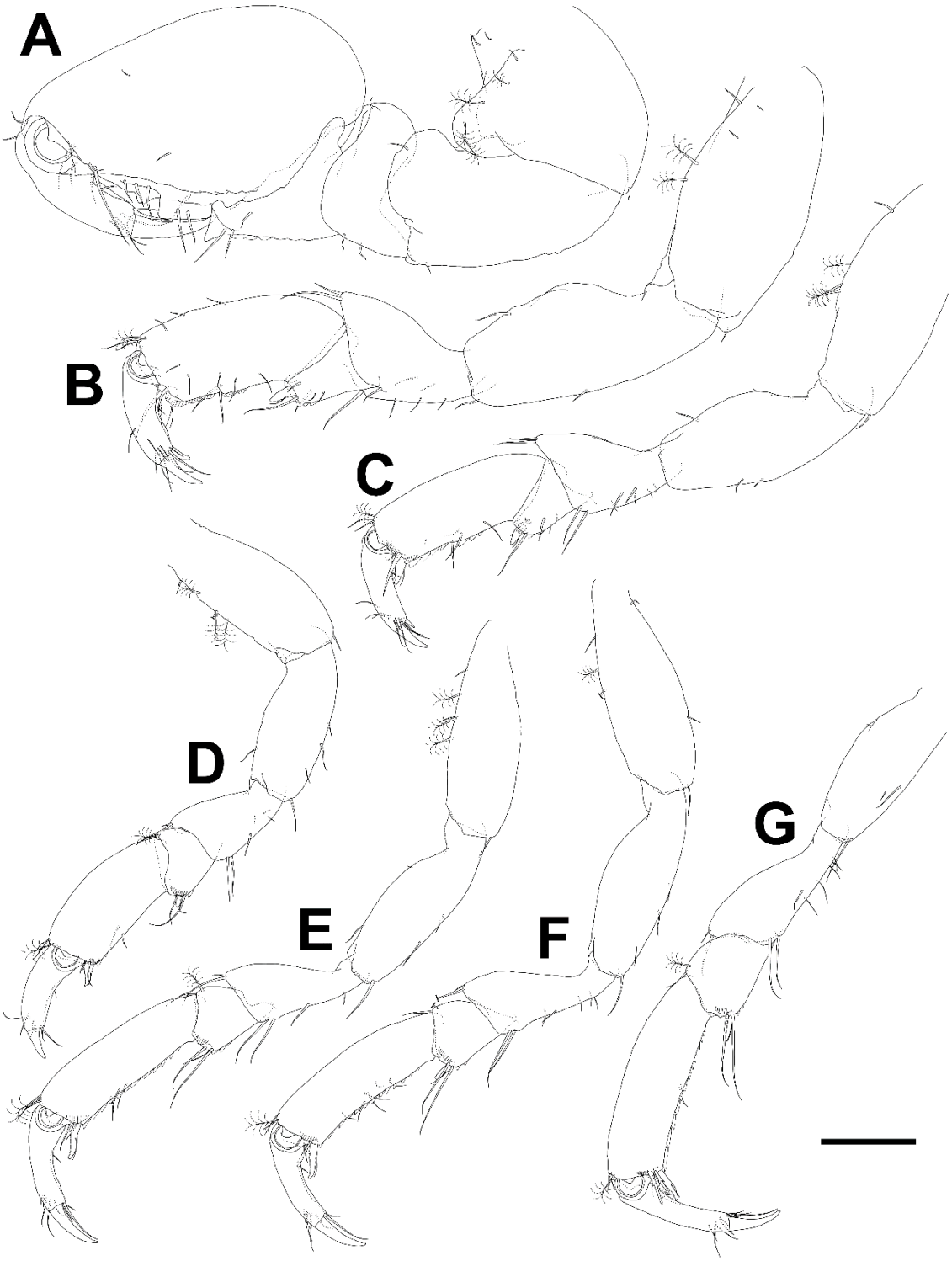

Figure III-II-7. *Sauranthura liangaensis* **sp. nov.**, allotype, subadult male, NMCR 99223. A–G, left pereopods 1–7. Scale bar = 100 μm.

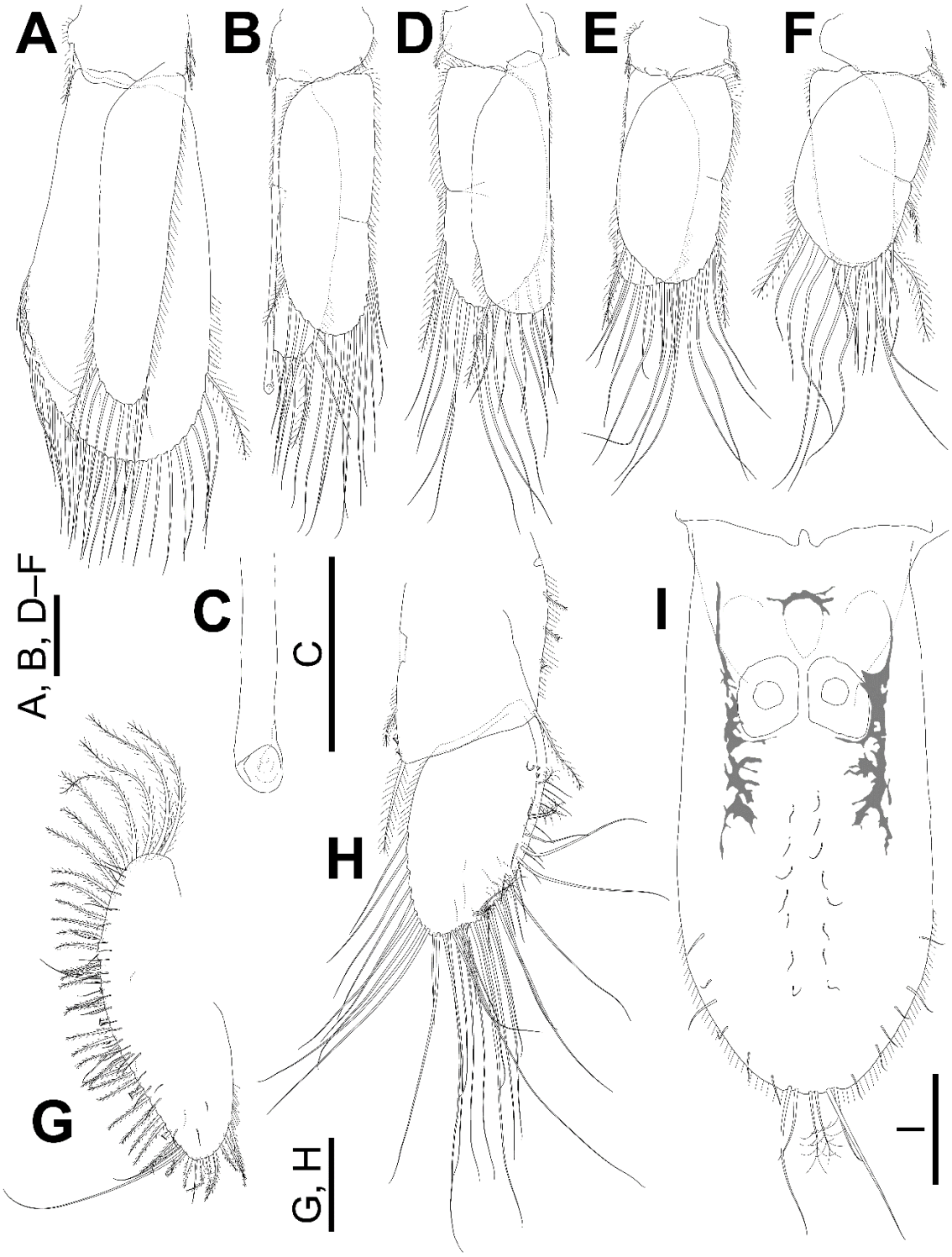

Figure III-II-8. *Sauranthura liangaensis* **sp. nov.**, allotype, subadult male, NMCR 99223. A, B, D–F, left pleopods 1–5; C, appendix masculina on left pleopod 2; G, right uropodal exopod; H, right uropodal endopod; I, telson. Scale bars = 100 μm (A, B, D–I); 50 μm (C).

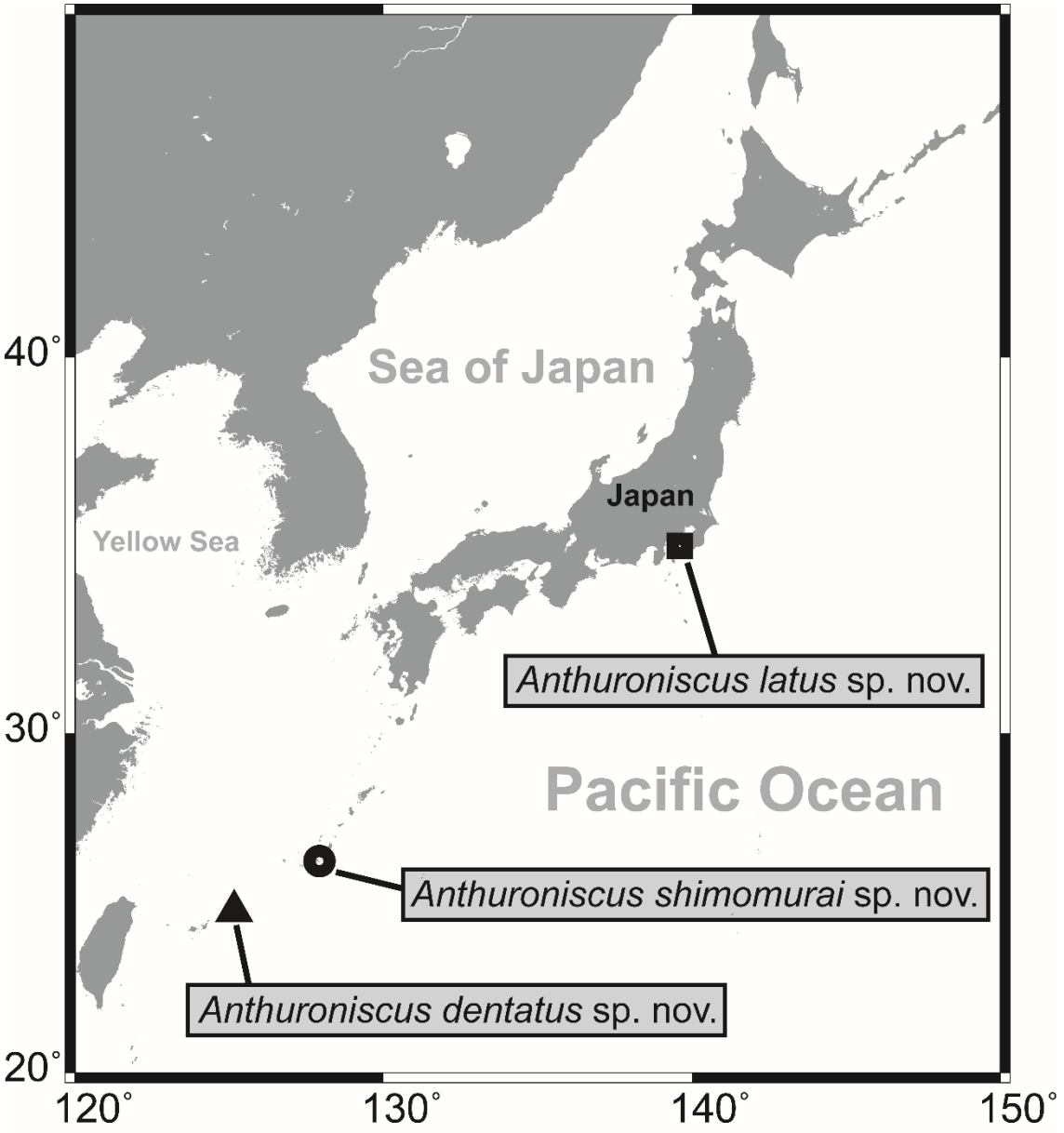

Figure IV-I-1. Map showing sampling sites in Japan, indicating the *Anthuroniscus* **gen. nov.** species detected at each site. Circle: Kaichu Doro, Uruma, Okinawa, southwestern Japan. Triangle: Irabu Island, Miyako Islands, Okinawa, southwestern Japan. Square: Jogashima, Kanagawa, central Japan.

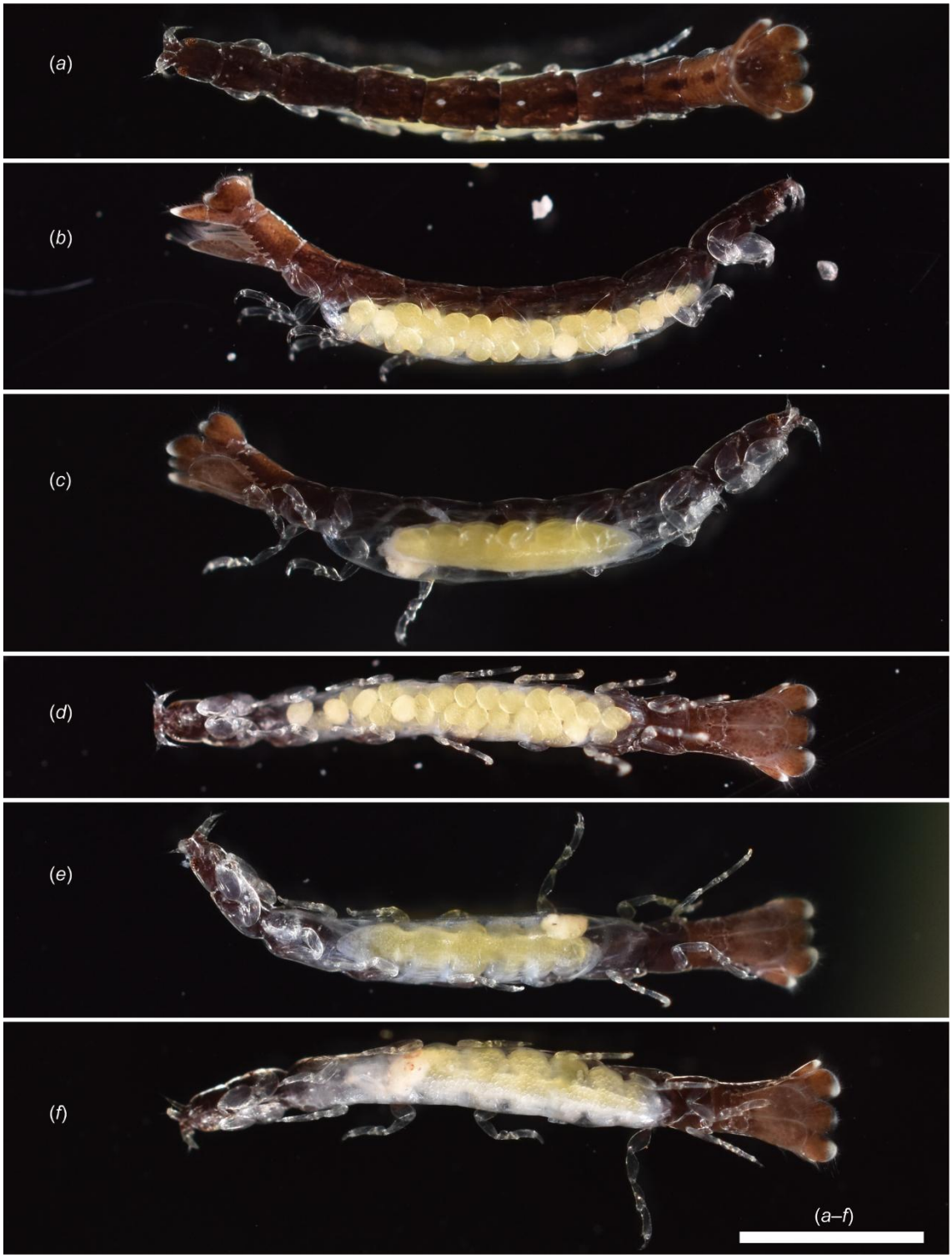

Figure IV-I-2. *Mesanthura* sp. (ICHUM8644) parasitized by female *Anthuroniscus shimomurai* **sp. nov.** (ICHUM8643) during rearing; living specimen. (*a*) Dorsal view. (*b*, *c*) Lateral views. (*d–f*) Ventral views. (*a*, *c*, *e*, *f*) Holotype female of *A. shimomurai* **sp. nov.** in the marsupium on 29 April 2023 (*a*, *c*, *e*) and 1 May 2023 (*f*); (*b*, *d*) marsupium filled with eggs on 21 April 2023. Scale bar, 2 mm.

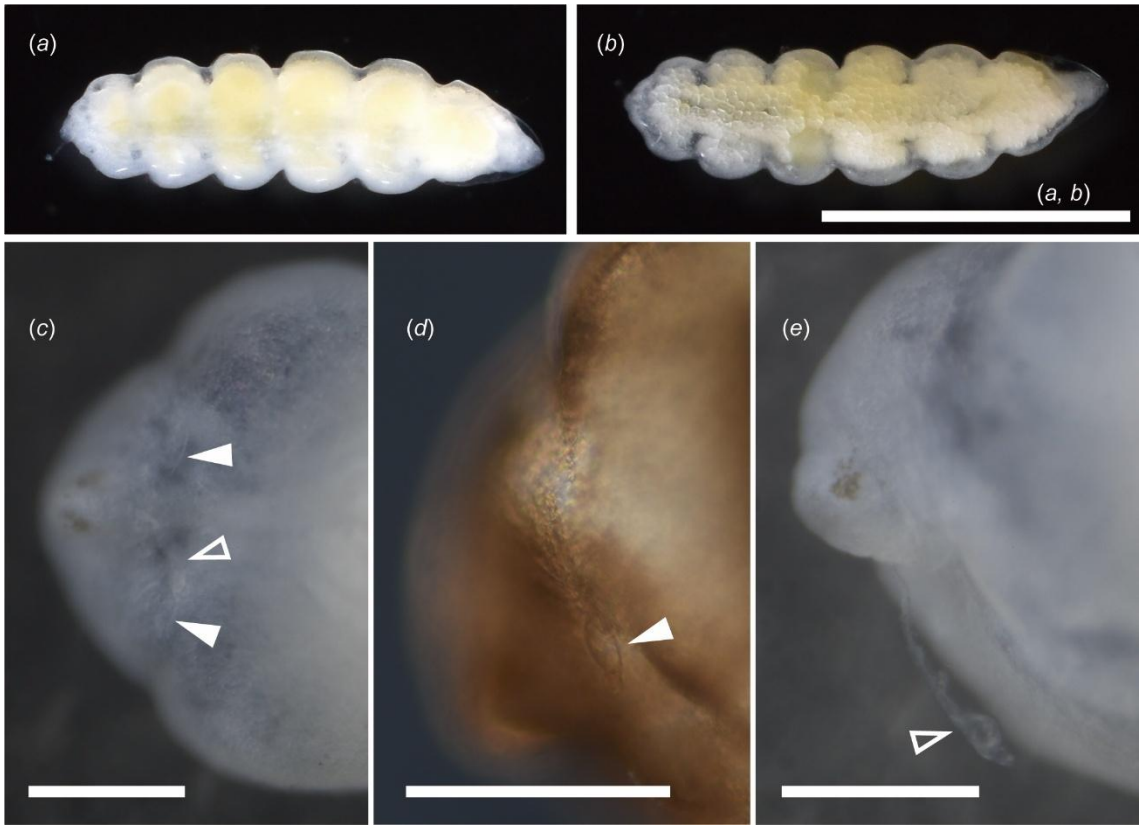

Figure IV-I-3. *Anthuroniscus shimomurai* **sp. nov.** (ICHUM8643), holotype female. (*a*) Ventral view. (*b*) Dorsal view. (*c*) Anterior end, ventral view. (*d*, *e*) Anterior end, left lateral view. White arrowheads, putative pereopod 1; open arrowheads, ventral hook. Scale bars, 2 mm (*a*, *b*), 200 μm (*c*–*e*).

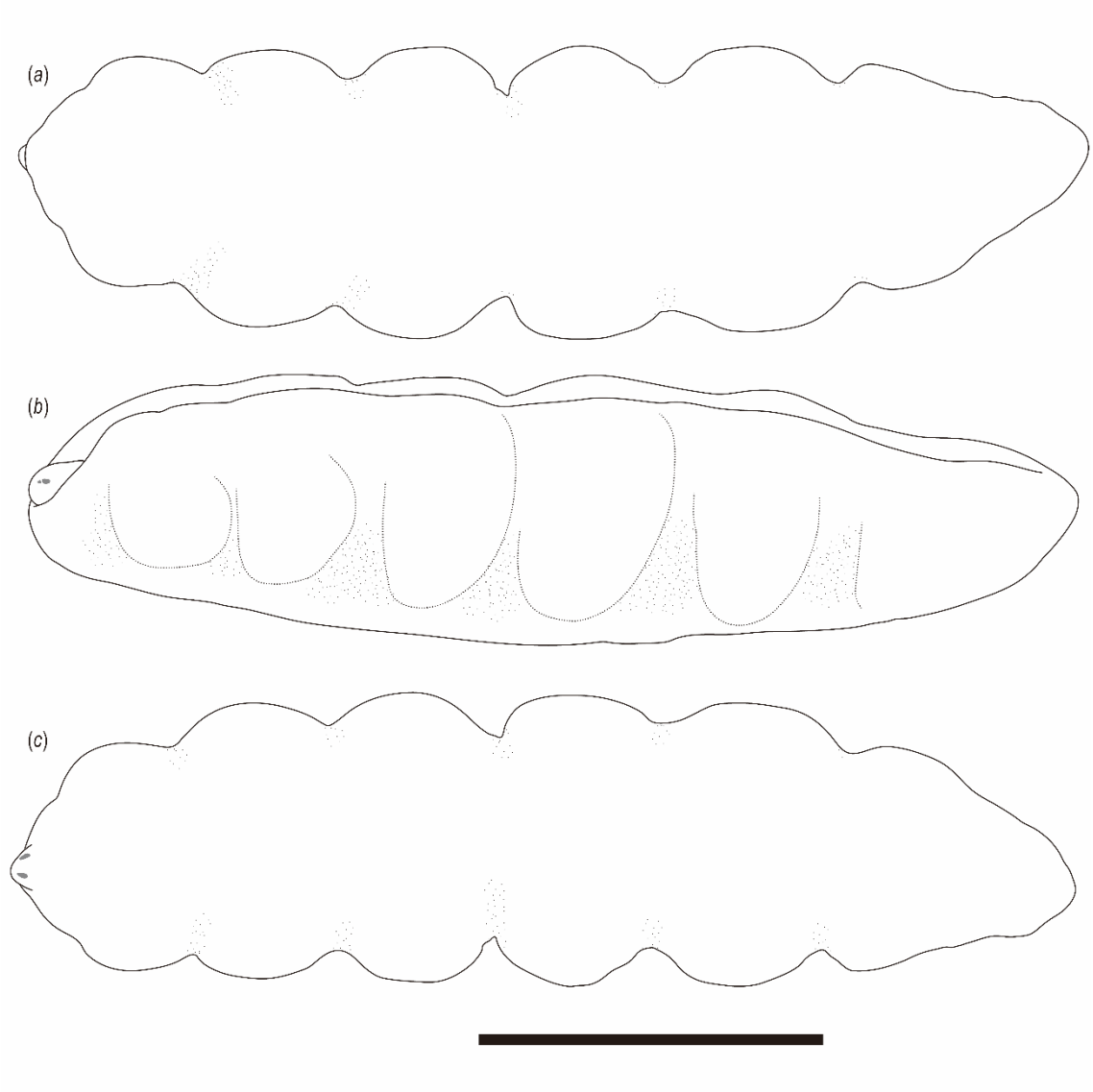

Figure IV-I-4. *Anthuroniscus shimomurai* **sp. nov.** (ICHUM8643), holotype female. (*a*) Dorsal view. (*b*) Right lateral view. (*c*) Ventral view. Scale bar, 1 mm.

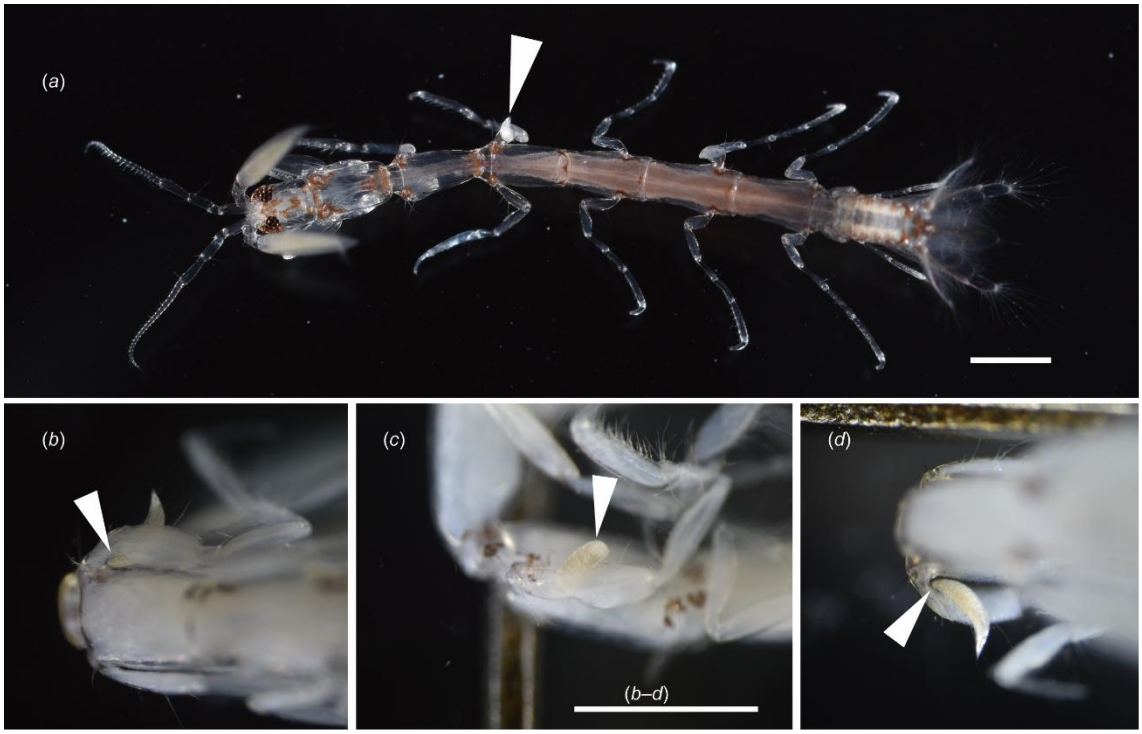

Figure IV-I-5. *Anthuroniscus dentatus* **sp. nov.** (ICHUM8645), holotype cryptoniscus larva, attached to *Accalathura* sp. (ICHUM8646). (*a*) Dorsal view of host; living specimen. (*b–d*) Dorsal, right lateral, and ventral views of *Anthuroniscus dentatus* **sp. nov.**; photomicrographs of fixed specimens. Arrowheads, parasite attached to right pereopod 3. Scale bars, 1 mm.

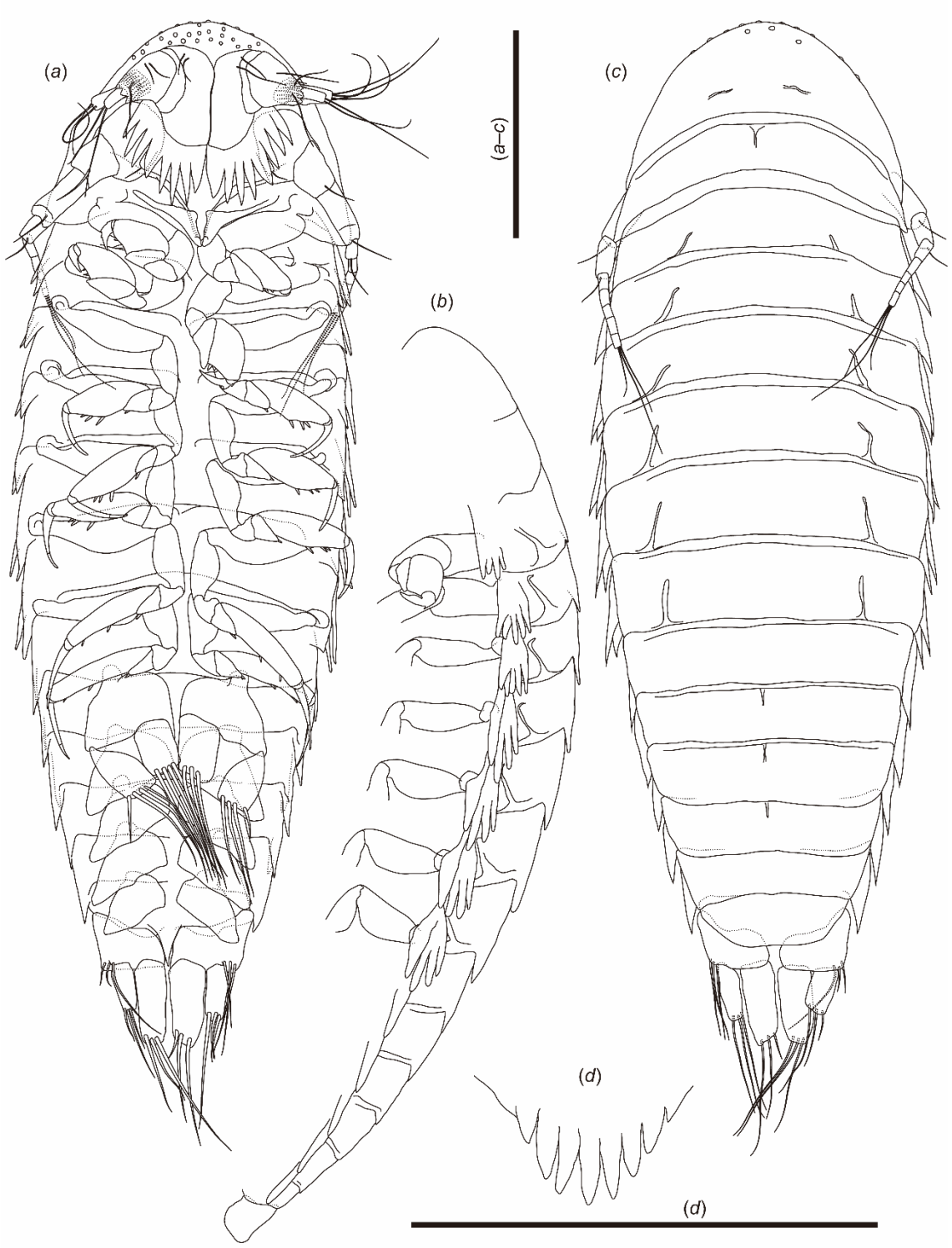

Figure IV-I-6. *Anthuroniscus dentatus* **sp. nov.** (ICHUM8645), holotype cryptoniscus larva. (*a*) Ventral view. (*b*) Left lateral view (cephalon and most appendages omitted). (*c*) Dorsal view; pygidium not illustrated. (*d*) Pygidium. Scale bars, 100 μm.

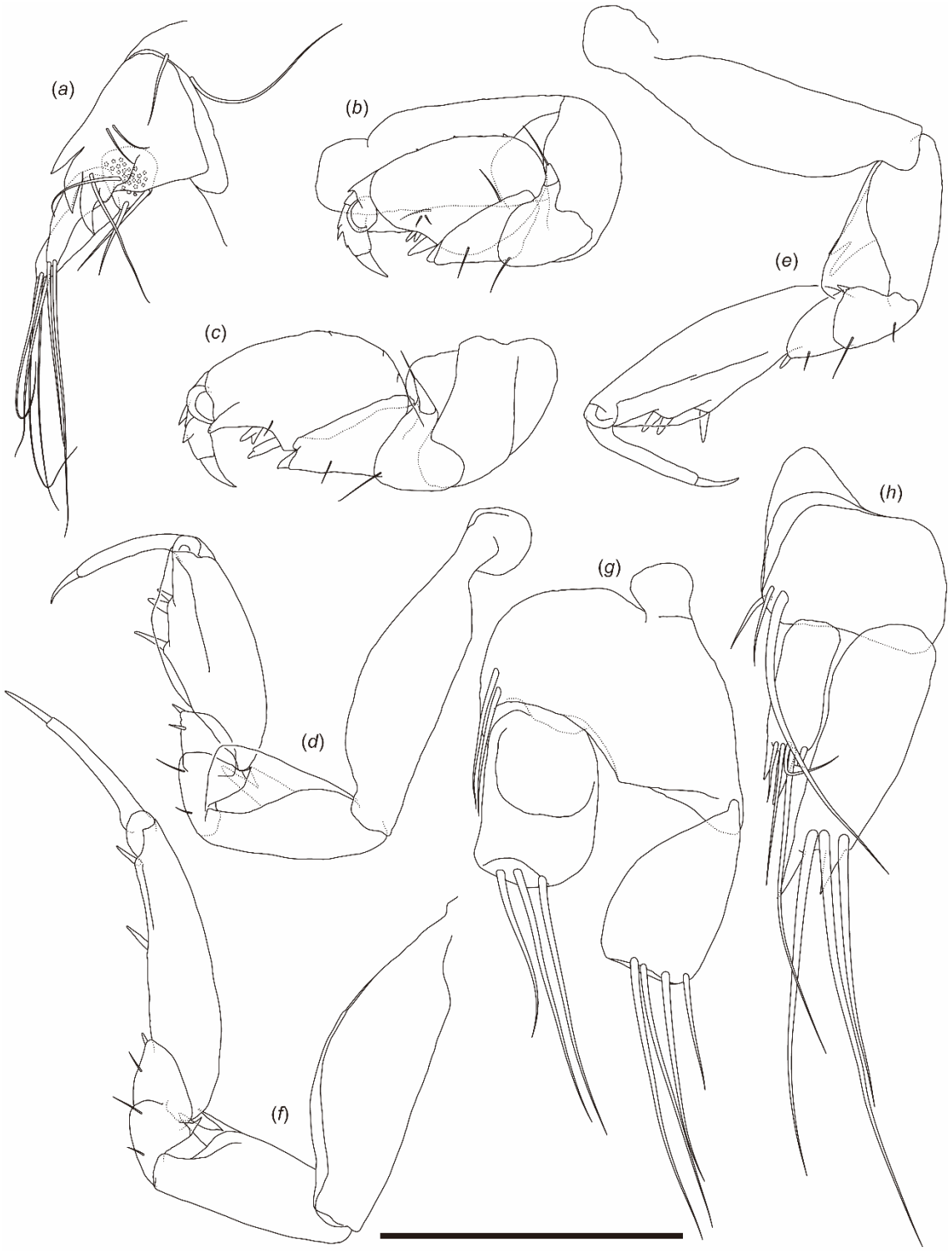

Figure IV-I-7. *Anthuroniscus dentatus* **sp. nov.** (ICHUM8645), holotype cryptoniscus larva. (*a*) Ventral view of right antennule. (*b–f*) Ventral views of right pereopods 1, 2, 3, 4, 6. (*g*) Ventral view of left pleopod 1. (*h*) Ventral view of right uropod. Scale bar, 50 μm.

Figure IV-I-8. *Anthuroniscus latus* **sp. nov.** (ICHUM8647), holotype cryptoniscus larva, attached to *Colanthura nigra* (ICHUM8648); living specimen. (*a*) Lateral view of host. (*b*) Ventral view of host. Arrowheads, parasite attached to pereonite-3 sternite. Scale bar, 1 mm.

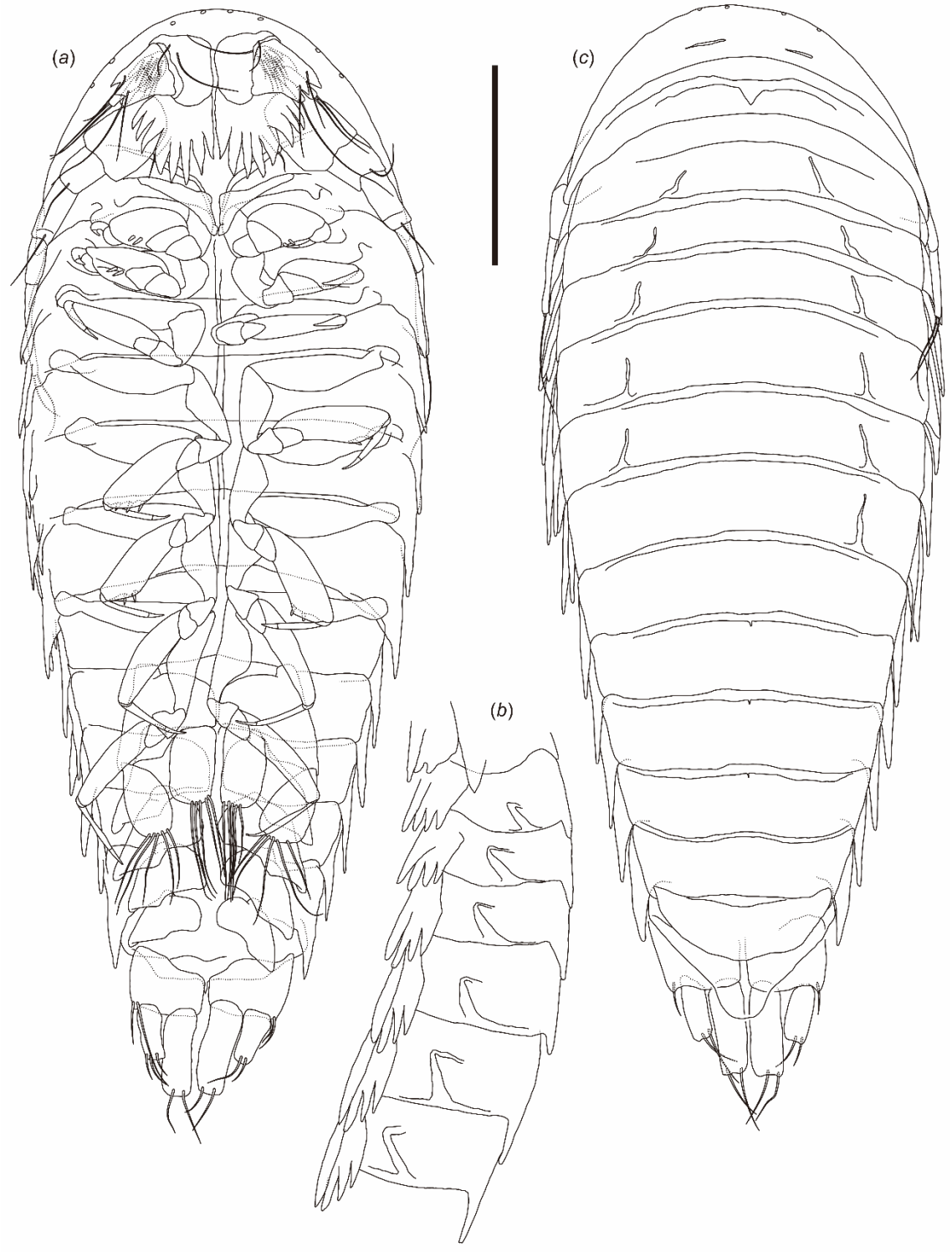

Figure IV-I-9. *Anthuroniscus latus* **sp. nov.** (ICHUM8647), holotype cryptoniscus larva. (*a*) Ventral view. (*b*) Lateral view of left side (cephalon, appendages, and pleon omitted). (*c*) Dorsal view. Scale bar, 100 μm.

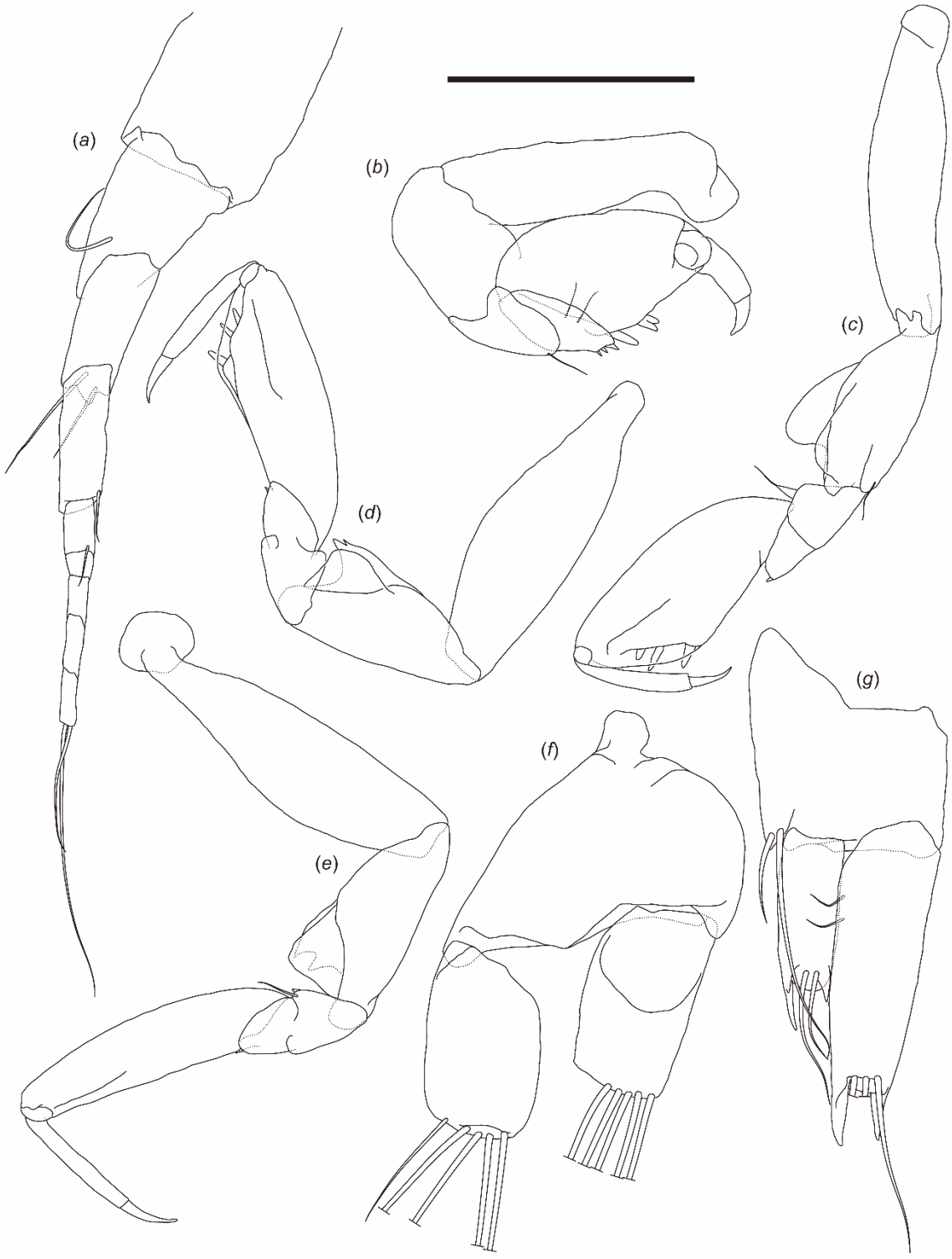

Figure IV-I-10. *Anthuroniscus latus* **sp. nov.** (ICHUM8647), holotype cryptoniscus larva. (*a*) Dorsal view of left antenna. (*b–e*) Ventral view of right pereopods 2, 3, 4, 6. (*f*) Ventral view of right pleopod 1 (setae omitted). (*g*) Ventral view of right uropod. Scale bar, 50 μm.

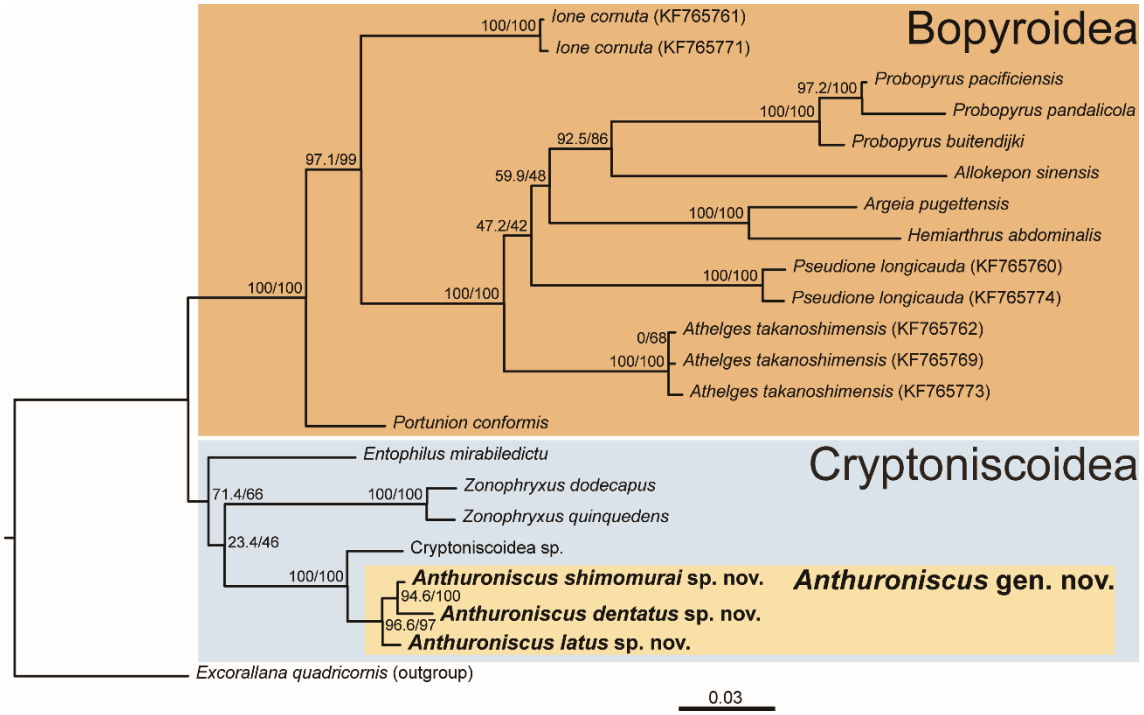

Figure IV-I-11. Maximum likelihood (ML) tree based on 1578 positions of the 18S rRNA gene. Values near nodes are SH-aLRT/UFBoot (clades having SH-aLRT ≥ 80% and UFBoot ≥ 95% were considered to be well supported). Dark orange shading, Bopyroidea; blue shading, Cryptoniscoidea; light orange shading, *Anthuroniscus* **gen. nov.** Taxa for which sequences were obtained in this study are in bold font. The scale bar indicates branch length in number of substitutions per site.

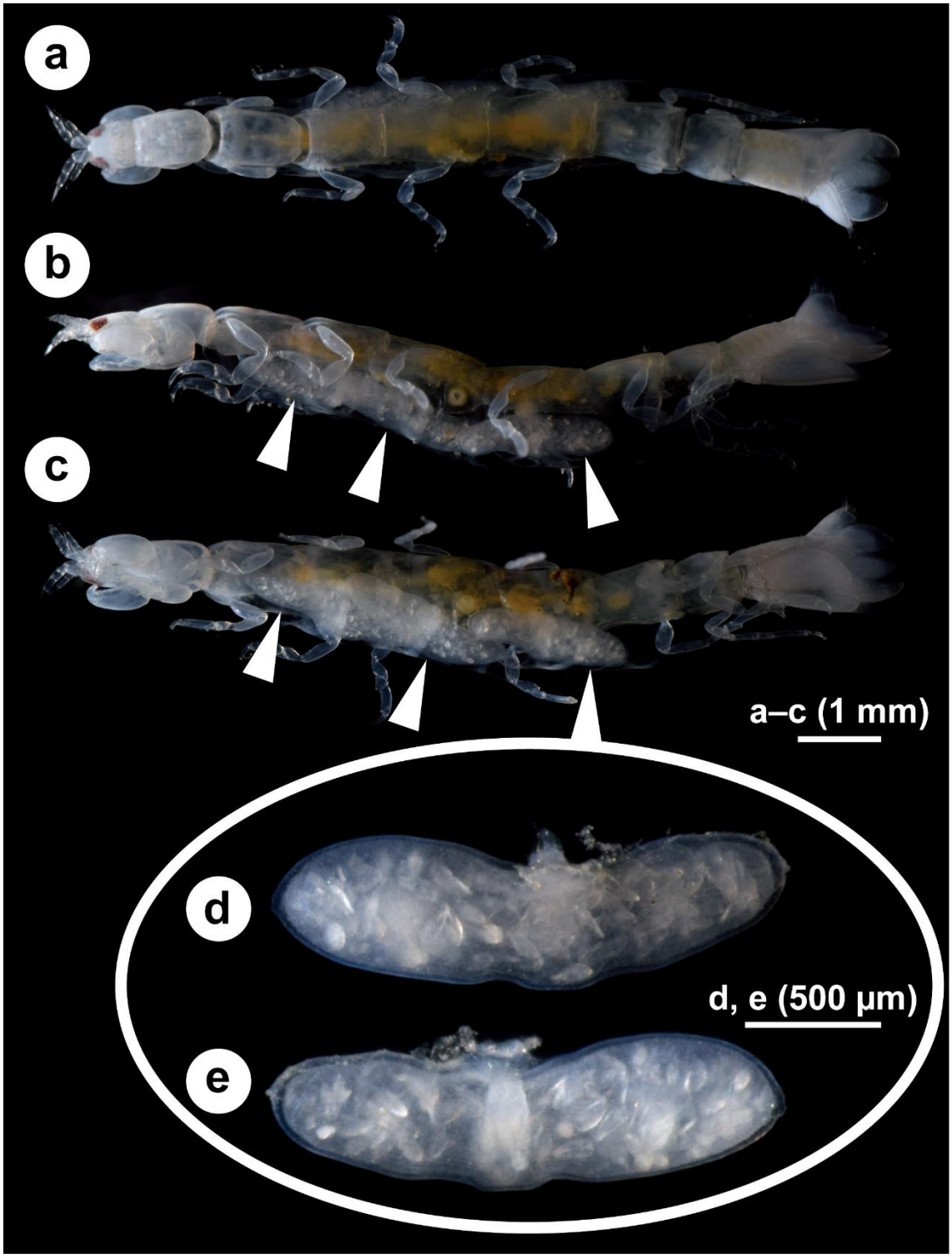

Figure IV-II-1. Rhizocephalan *Duplorbis changeling* **sp. nov.** parasitizing anthurid host *Malacanthura seisuiae* **sp. nov.**, fresh individuals. a–c, dorsal, lateral, and ventral views of infected host, arrow heads showing each externa of *Duplorbis changeling* **sp. nov.**; d, e, detached posterior externa.

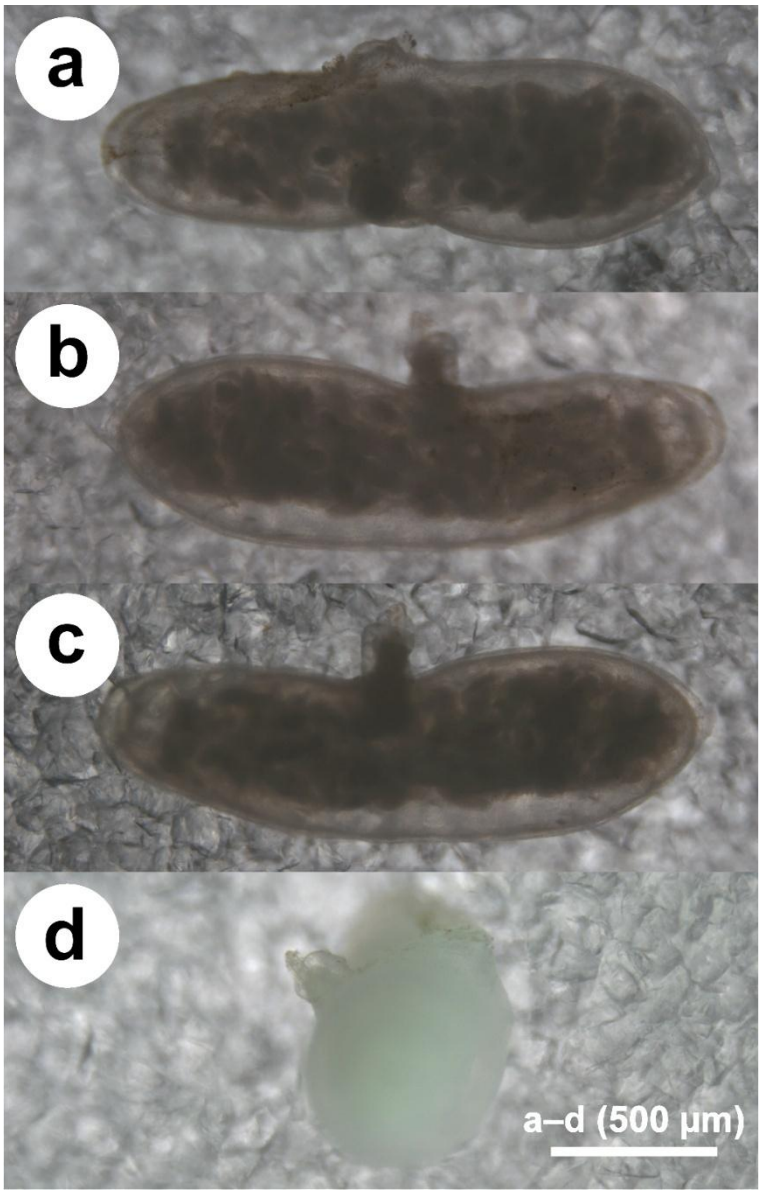

Figure IV-II-2. Posterior externa of *Duplorbis changeling* **sp. nov.**, fixed in ethanol. a–d, dorsal, right lateral, left lateral, and posterior views, the direction relative to the host.

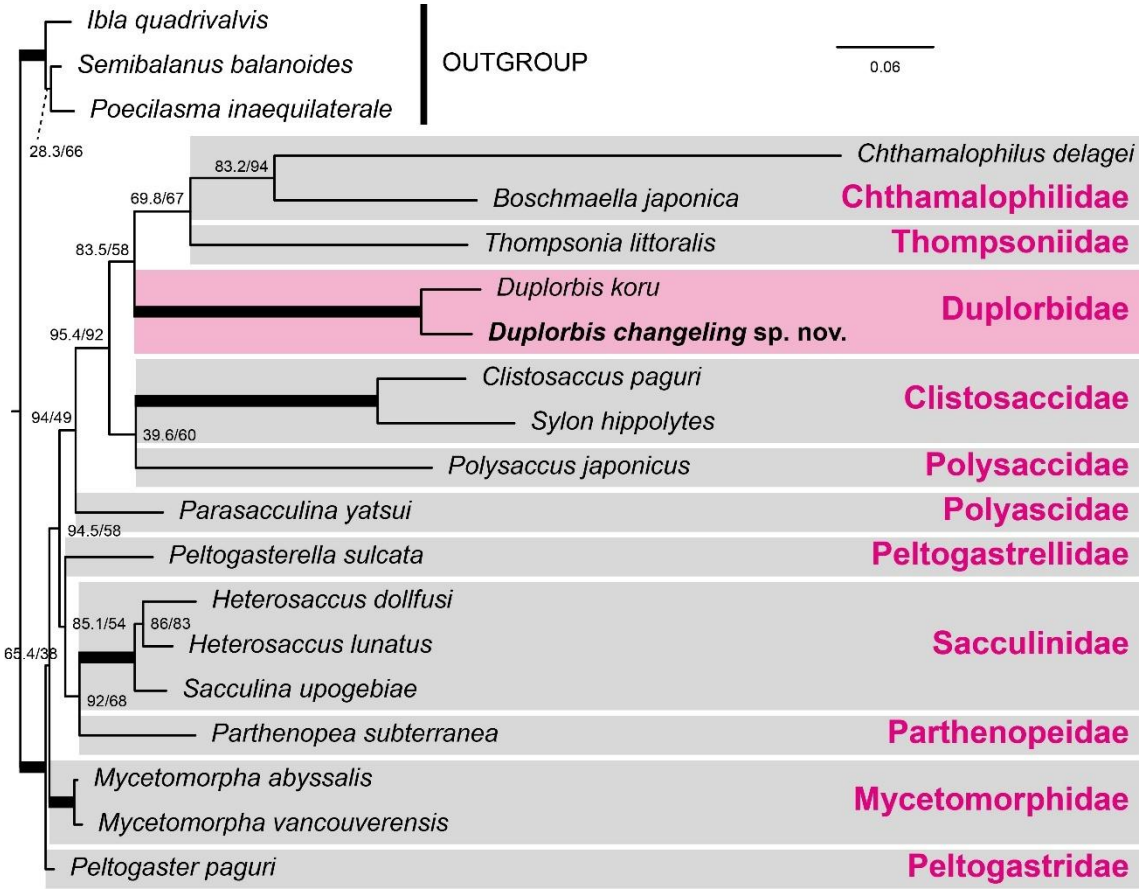

Figure IV-II-3. Maximum likelihood (ML) tree based on the 18S-28S concatenated dataset. Values near nodes are SH-aLRT/UFBoot (clades having SH-aLRT ≥80% and UFBoot ≥95% were considered to be well supported). Thickened branch indicates full support (both SH-aLRT and UFBoot are 100%). Our new species is in bold font and the family Duplorbidae is in pink square. The scale bar indicates branch length in number of substitutions per site.

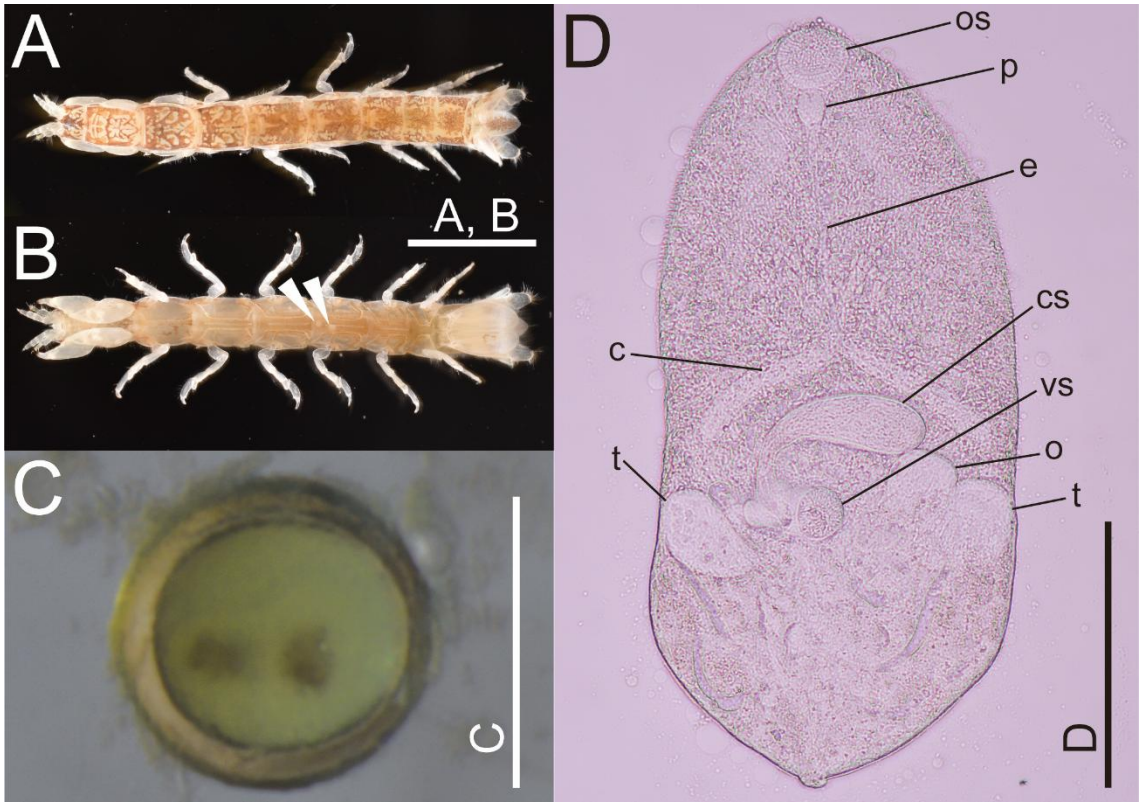

Figure IV-III-1. The isopod *Cyathura muromiensis* and parasitic trematode metacercariae. (A, B) Dorsal (A) and ventral (B) views of *Cy*. *muromiensis*, living individual (arrowheads indicate cysts). (C) Extracted encysted metacercaria, fixed specimen. (D) Excysted metacercaria, living individual. Scale bars: 5 mm (A, B), 200 µm (C, D). Abbreviations: c, caecum; cs, cirrus sac; e, esophagus; o, ovary; os, oral sucker; p, pharynx; t, testis; vs, ventral sucker.

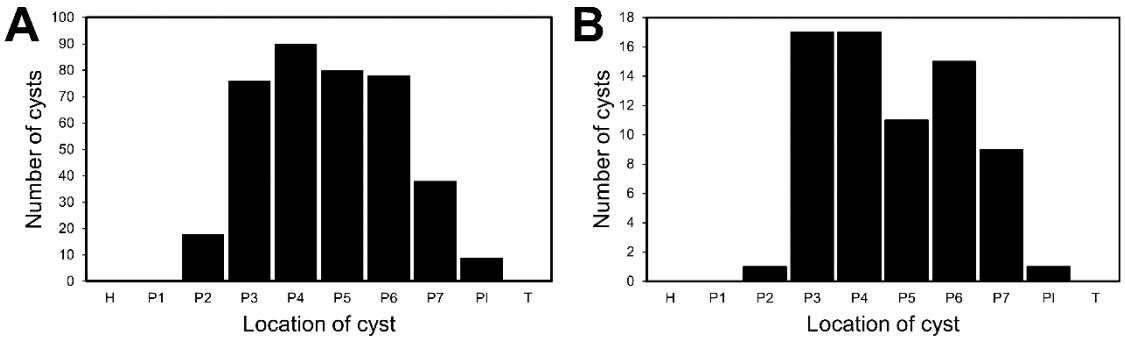

Figure IV-III-2. Frequency distributions of metacercarial cysts among segments of the host *Cyathura muromiensis*. (A) Distribution for 389 cysts from 52 host individuals. (B) Distribution of 71 cysts from the single host with the highest intensity. Abbreviations: H, head; P1–7, pereonites 1–7; Pl, pleon; T, telson.

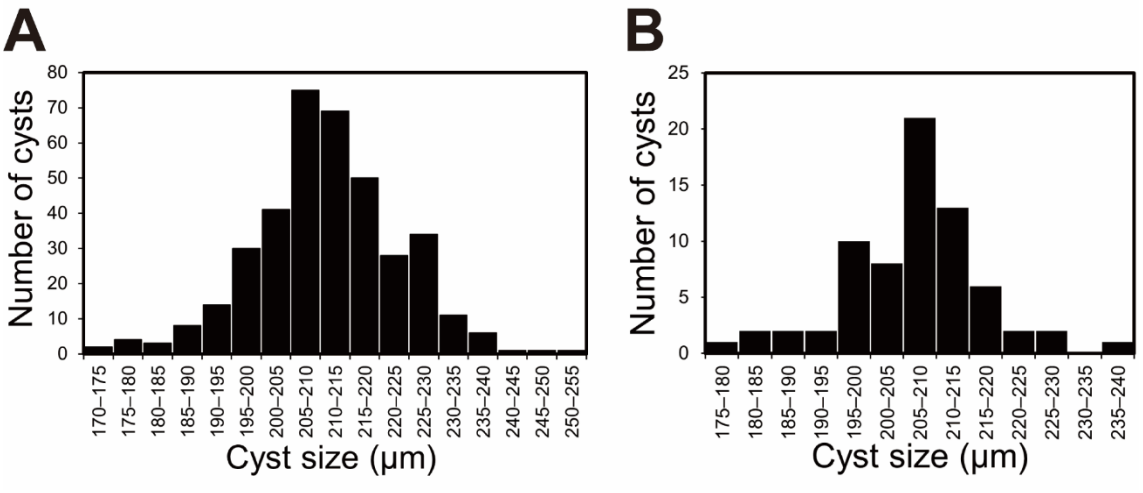

Figure IV-III-3. Size frequency distributions of metacercarial cysts from the host *Cyathura muromiensis.* (A) Distribution for 378 cysts extracted from 52 host individuals. (B) Distribution for 70 cysts extracted from the single host individual with the highest intensity.

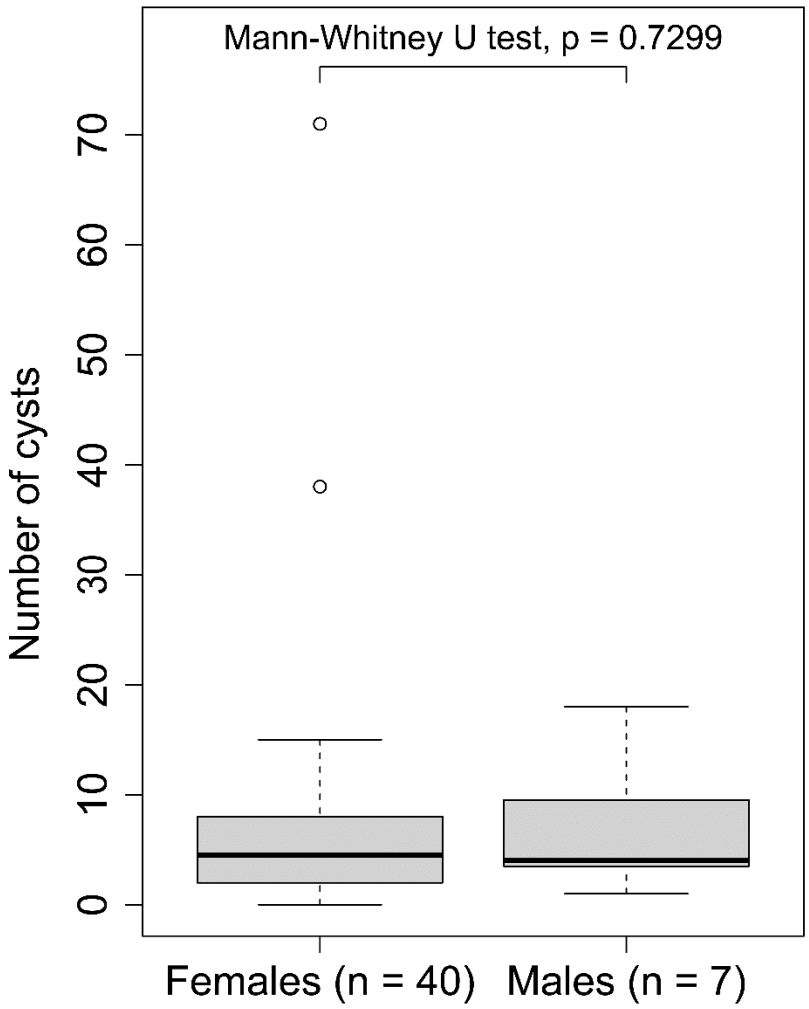

Figure IV-III-4. Numbers of metacercarial cysts for female and male *Cyathura muromiensis* hosts. The boxplots show median values (thick, black bars), first and third quartiles (bottoms and tops of the boxes), outliers (circles), and minimum and maximum values excluding outliers (ends of the whiskers). The Mann–Whitney U test showed no significant difference between the sexes.

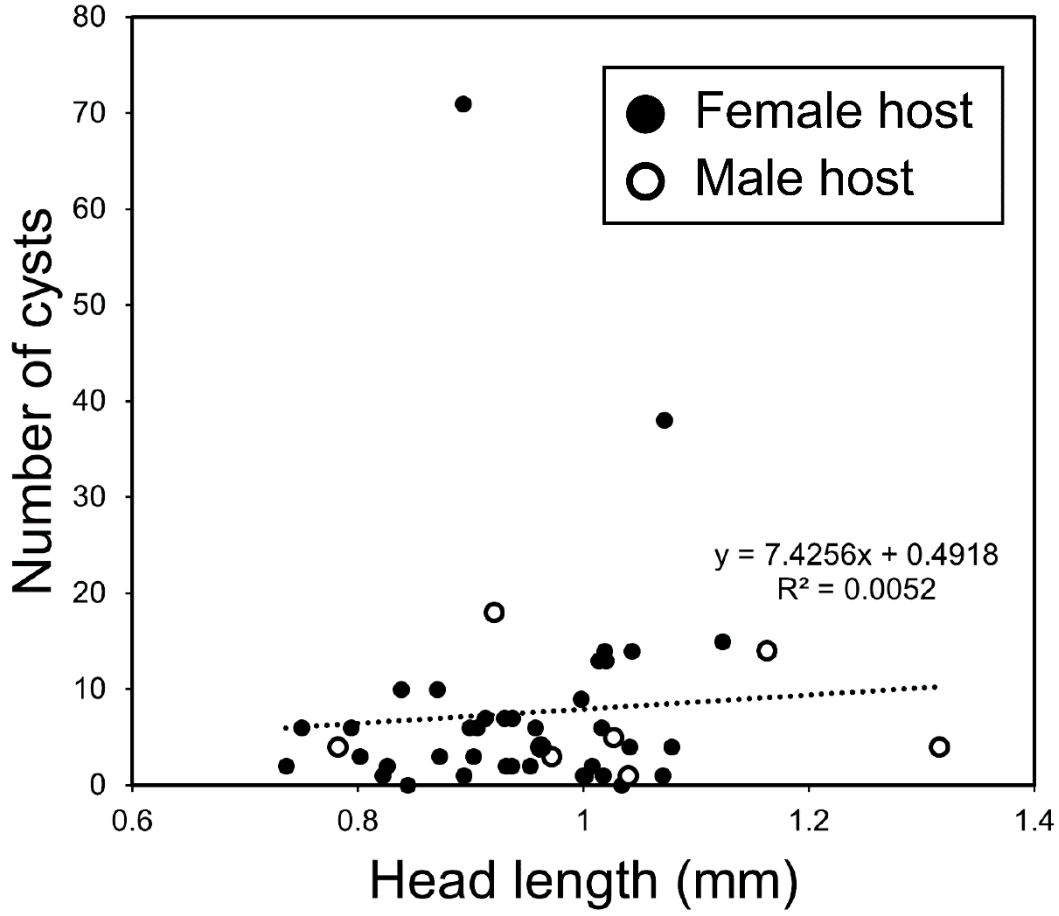

Figure IV-III-5. Relationship between host size and the number of cysts. Solid and open circles denote female and male hosts, respectively. The dotted line is the regression line.

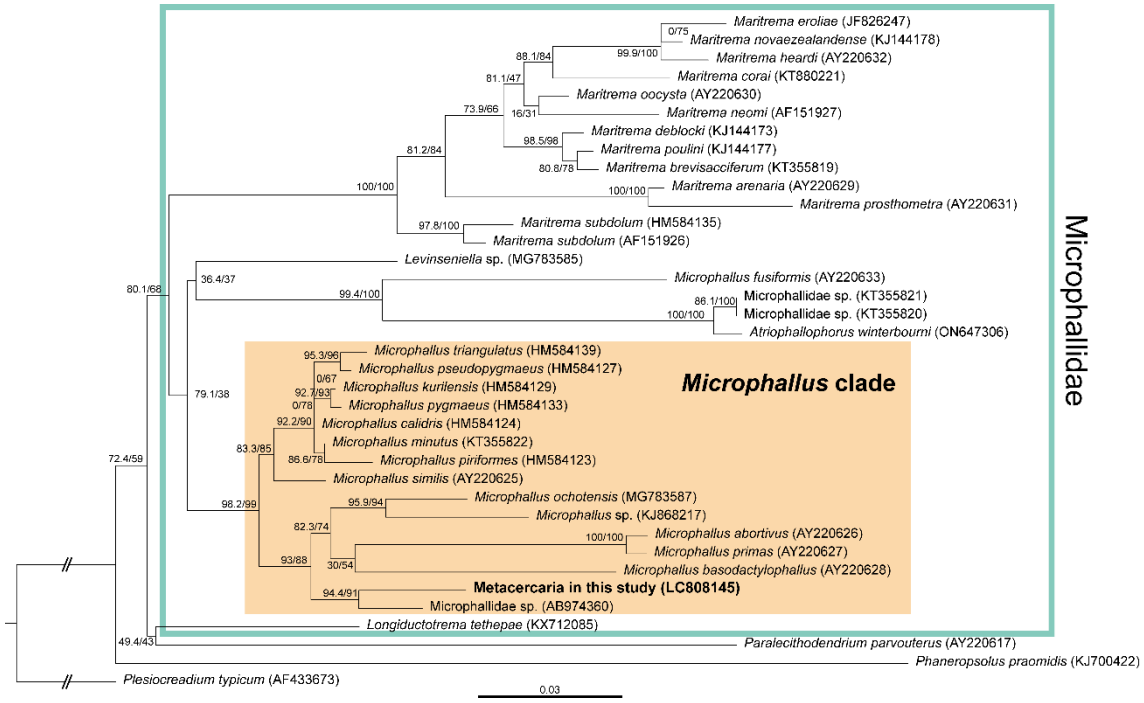

Figure IV-III-6. Maximum likelihood (ML) phylogenetic tree for microphallid trematodes based on 28S rRNA gene sequences (945 bp). Numbers near nodes are SH-aLRT/UFBoot support values. The portion of the tree containing species in Microphallidae is outlined in green; the *Microphallus* clade is shaded orange. Our taxon is in bold font. The scale bar indicates branch length in number of substitutions per site.

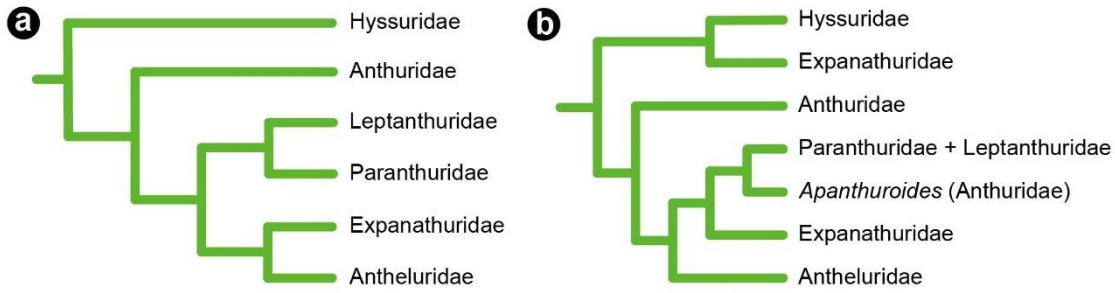

Figure V-1. Phylogenetic relationships within Anthuroidea proposed in previous studies. **(a)** cladogram of the current six families presented by Poore (2001). **(b)** cladogram presented by Wägele (1989), simplified by assigning each genus to the current family. The cladogram presented by Wägele (1981a) is not shown here as the cladogram is almost identical to that of Wägele (1989).

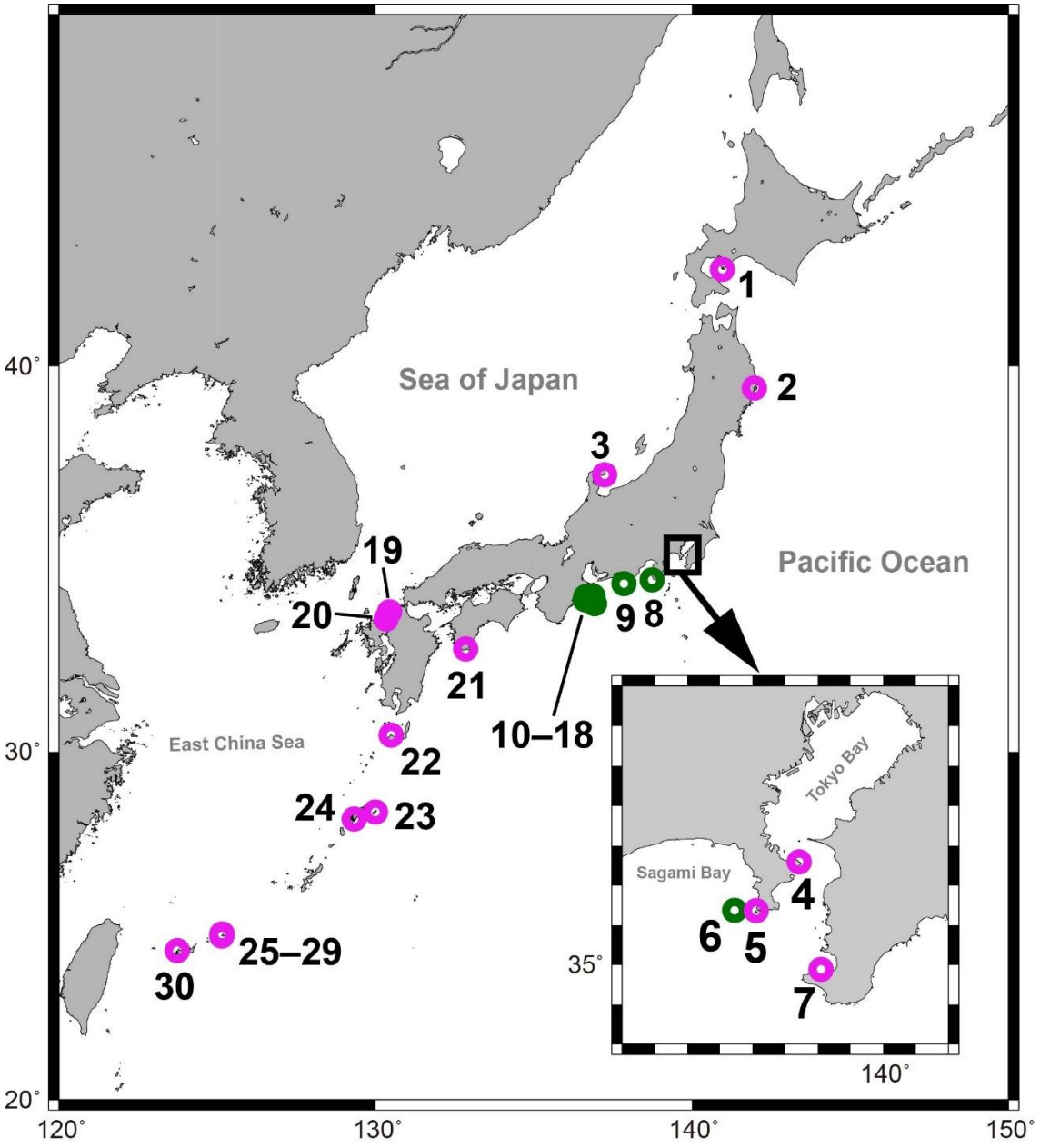

Figure V-2. Map showing the 30 sampling sites in Japan. The numbers of each site correspond to the site numbers in Table 1. Pink circles indicate shallower sampling sites (≤ 21 m depth). Green circles indicate deeper sampling sites (≥ 99 m depth). Part of the area was expanded and shown in lower right.

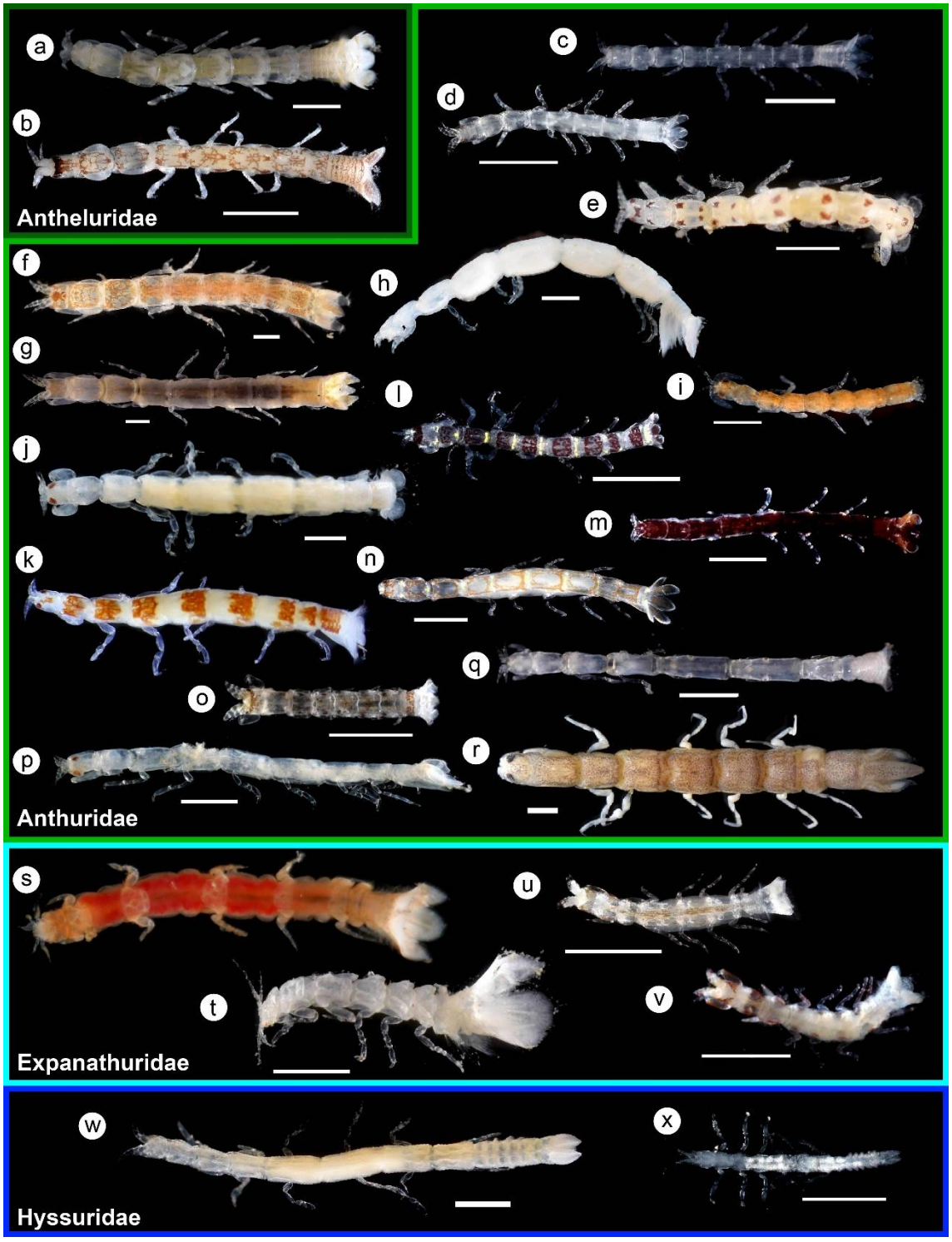

Figure V-3. Anthuroid species used as OTUs in this study. **(a)** *Ananthura* sp. **(b)** *Anthomuda iriomotensis* **sp. nov. (c)** *Amakusanthura* sp. (photograph taken by Yuki Oya). **(d)** *Apanthura* cf. *shikokuensis*. **(e)** *Apanthura* sp. **(f)** *Cyathura muromiensis*. **(g)** *Cyathura sagamiensis*. **(h)** *Haliophasma* sp. **(i)** *Leipanthura* sp. **(j)** *Malacanthura seisuiae* **sp. nov. (k)** *Mesanthura cinctula* (photograph taken by Hisanori Kohtsuka). **(l)**

Mesanthura miyakoensis. **(m)** *Mesanthura nigrodorsalis*. **(n)** *Mesanthura sol* **sp. nov. (o)** *Pendanthura* sp. **(p)** Anthuridae sp. 1. **(q)** Anthuridae sp. 2. **(r)** Anthuridae sp. 3. **(s)** *Eisothistos* sp. 1 (photograph taken by Kotaro Kan). **(t)** *Eisothistos* sp. 2 **(u)** *Expanathura* cf. *monile* **sp. nov. (v)** *Expanathura* sp. **(w)** *Kupellonura tamago* **sp. nov. (x)** Hyssuridae sp. **(a, b)** Antheluridae. **(c–r)** Anthuridae. **(s–v)** Expanathuridae. **(w, x)** Hyssuridae. Scale bars = 1 mm. Scale not available for (k) and (s).

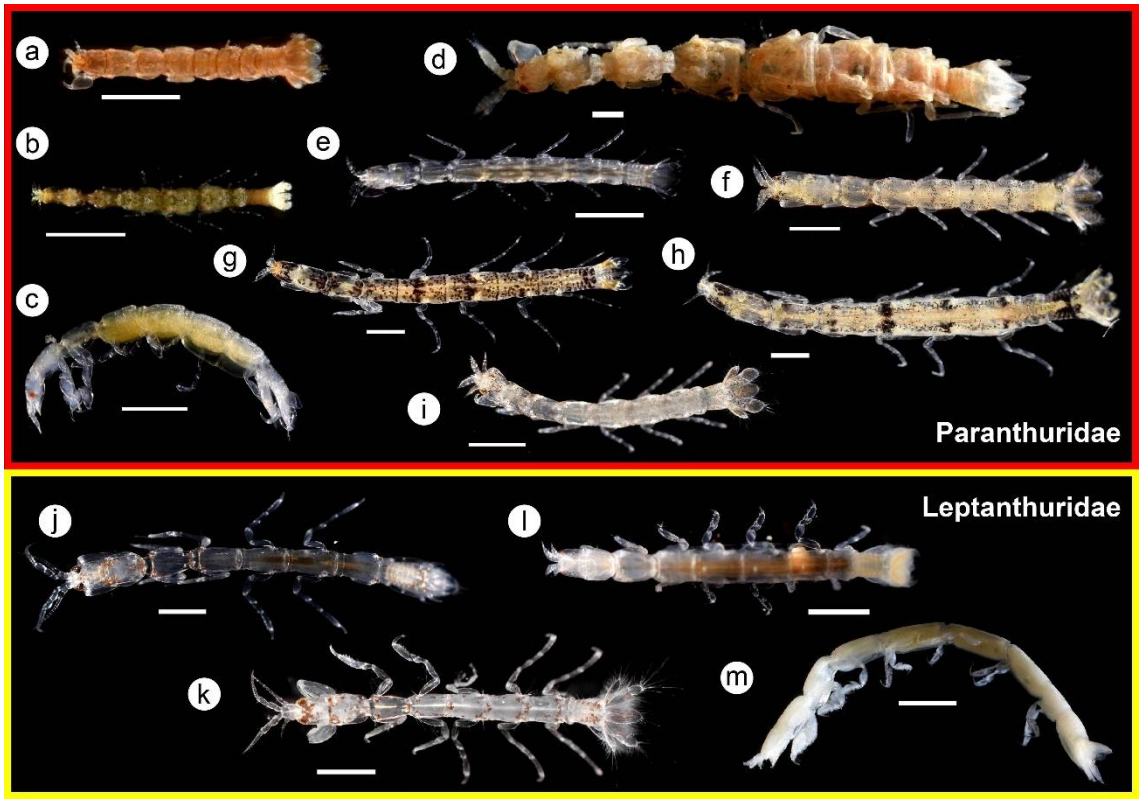

Figure V-4. Anthuroid species used as OTUs in this study. **(a)** *Colanthura nigra*. **(b)** *Cruranthura viridis* **sp. nov. (c)** *Cruranthura* sp. **(d)** *Deltanthura palpus* **sp. nov. (e)** *Paranthura alba* (photograph taken by Yuki Oya). **(f)** *Paranthura japonica*. **(g)** *Paranthura oriens* **sp. nov. (h)** *Paranthura* sp. 1. **(i)** *Paranthura* sp. 2. **(j)** *Accalathura* sp. 1. **(k)** *Accalathura* sp. 2. **(l)** *Leptanthura* sp. **(m)** Leptanthuridae sp. **(a–i)** Paranthuridae. **(j–m)** Leptanthuridae. Scale bars = 1 mm.

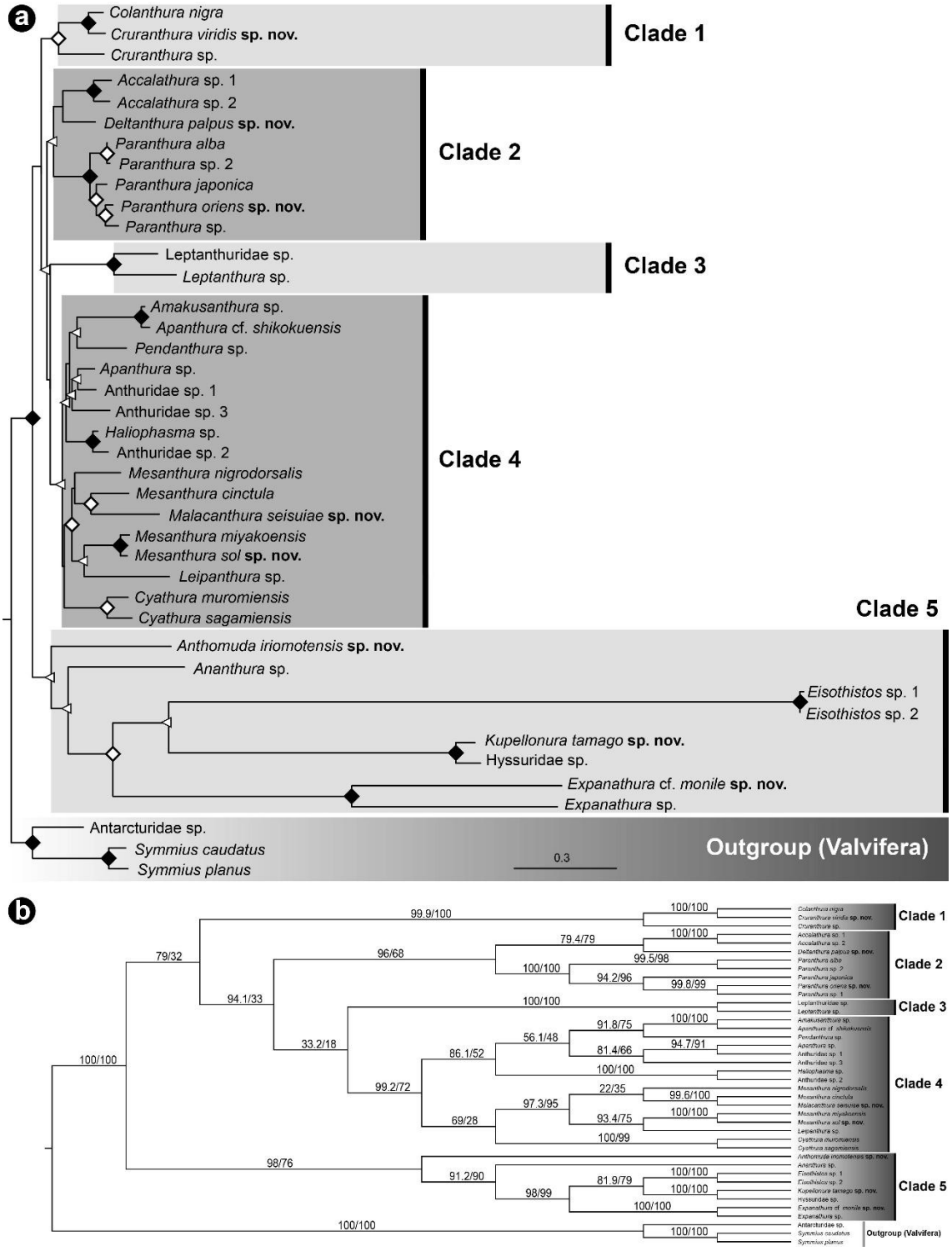

Figure V-5. ML tree based on 4060 positions of concatenated dataset of 18S, 28S, 16S, and COI. **(a)** The resulting tree. Solid black diamond on nodes indicates full support (both SH-aLRT = 100 and UFBoot = 100), open diamond high support (both SH-aLRT ≥ 80 and UFBoot ≥ 95), open triangle moderate support (SH-aLRT ≥ 80 or UFBoot ≥ 95), and no symbol less support (SH-aLRT < 80 and UFBoot < 95). Each clade was squared in

grey color. The scale bar indicates branch length in number of substitutions per site. **(b)** Cladogram of the resulting tree with SH-aLRT/UFBoot values on each branch.

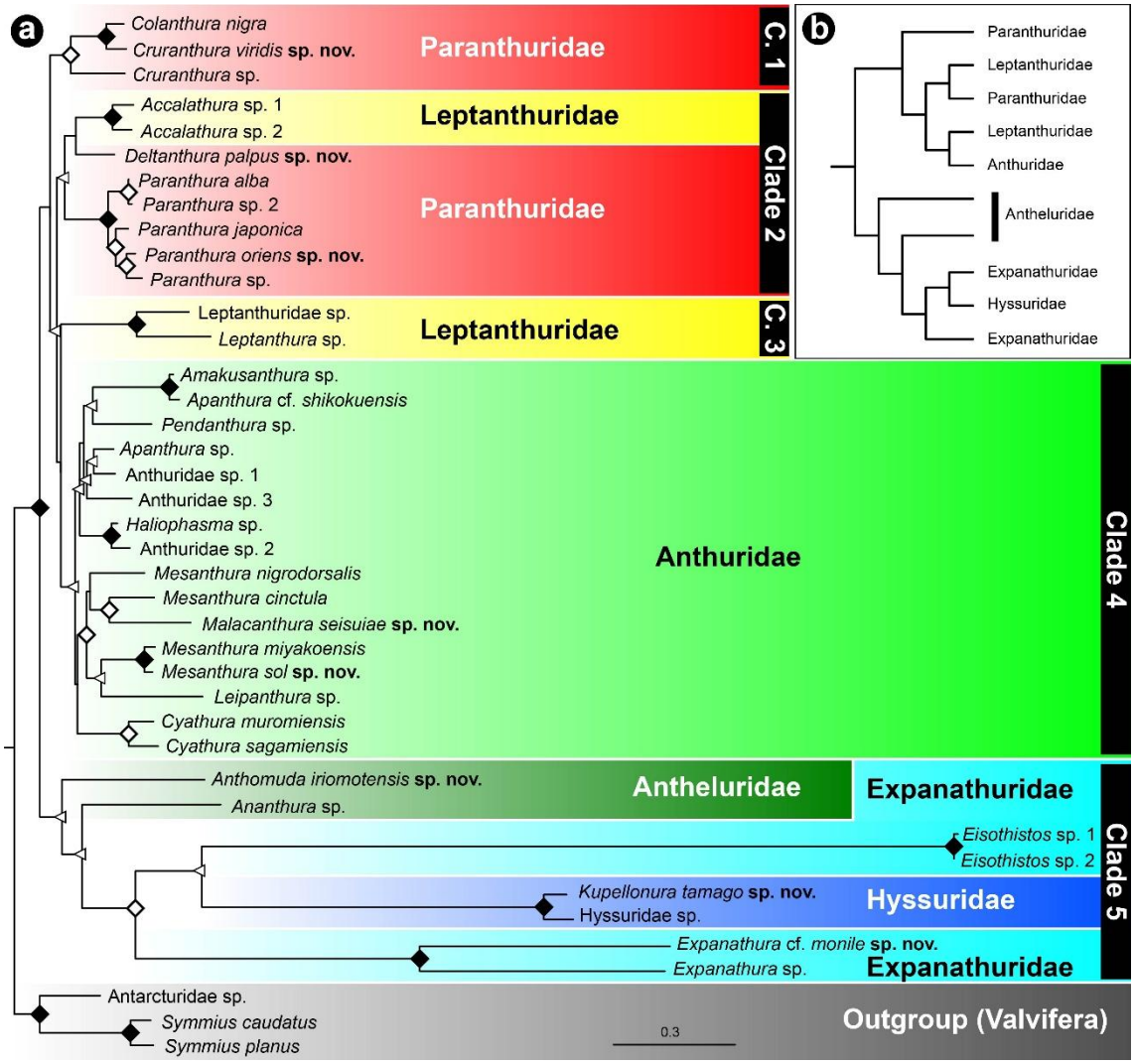

Figure V-6. The resulting tree. **(a)** The tree with the OTUs squared by the family. **(b)** Simplified cladogram.

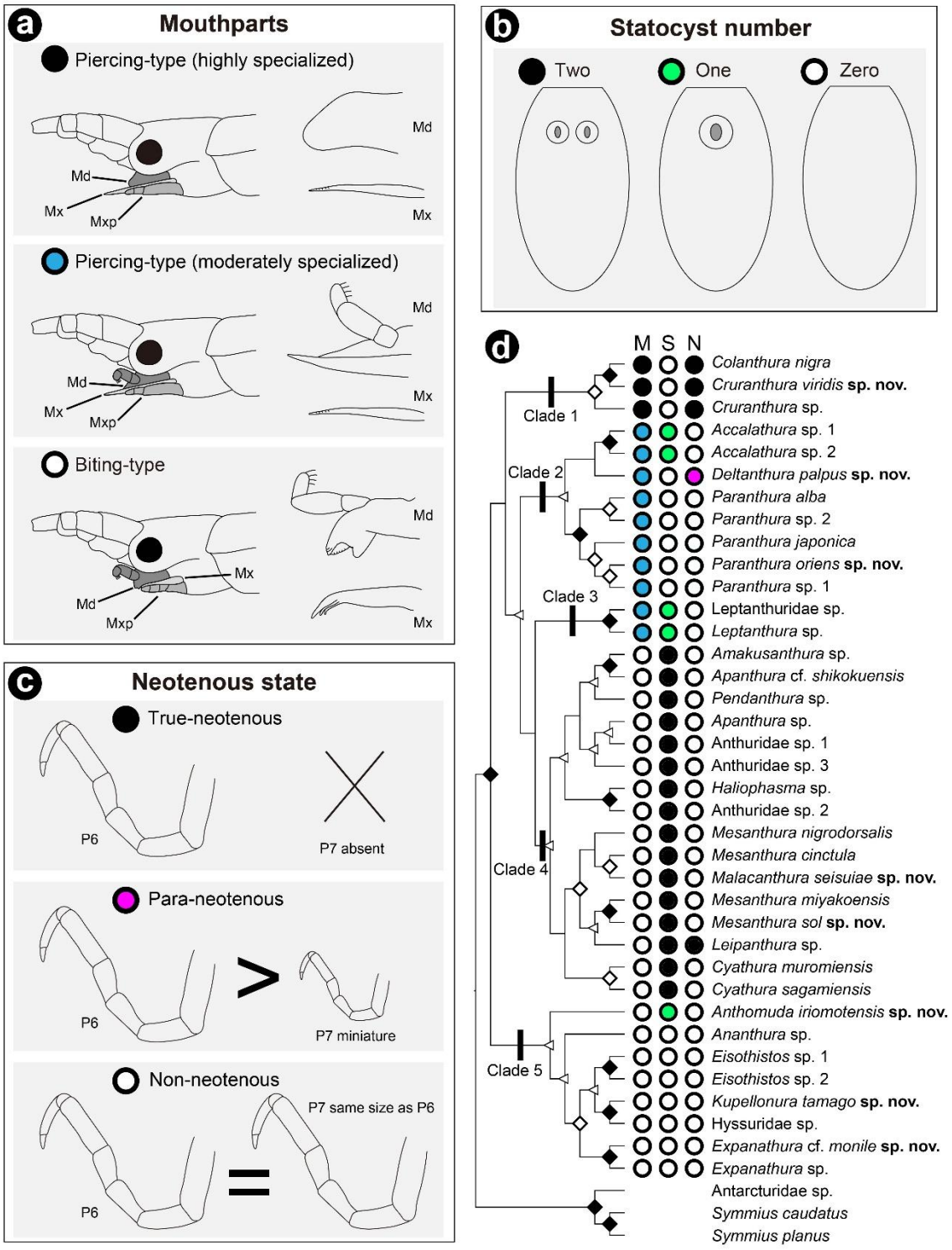

Figure V-7. The traits of three characters focused in this study. (a) Mouthparts. (b) number of statocysts. (c) Neotenous characters (d) The traits of the three characters mapped on the resulting cladogram. Circles in the left line represent the trait of mouthparts (M), those in the middle line that of statocyst number (S), and those in the right line that of neotenous character (N). Black, blue, and white circles on the left line

represent the highly specialized piercing-type mouthparts, moderately specialized piercing-type mouthparts, and biting-type mouthparts, respectively. Black, green, and white circles on the middle line represent two, one, and zero statocysts on the pleotelson, respectively. Black, pink, and white on the right line represent the true-neotenous, para-neotenous, and non-neotenous traits of pereopod 7, respectively.

Table II-1. List of anthuroid species reported from Japan.

Family	Genus	Species	References
Antheluridae	*Ananthura*	*Ananthura ocellata*	Nunomura (1992b)
		Ananthura rigida	Nunomura (1976b), Nunomura (2006)
	Anthomuda	*Anthomuda iriomotensis* **sp. nov.**	**This study**
Anthuridae	*Amakusanthura*	*Amakusanthura aokii*	Nunomura (2004b)
		Amakusanthura azumai	Nunomura (2016a)
		Amakusanthura hiradoensis	Nunomura (2016b)
		Amakusanthura longiantennata	Nunomura (1977)
		Amakusanthura toyamaensis	Nunomura (1992a)
	Apanthura	*Apanthura fusei*	Nunomura (1993)
		Apanthura honshuensis	Wägele (1984)
		Apanthura longiunguis	Nunomura (2014)
		Apanthura shikokuensis	Nunomura (1993)
		Apanthura trioculata	Nunomura (1993)
	Apanthuroides	*Apanthuroides breviantennata*	Nunomura (2013)
	Cyathura	*Cyathura furcata*	Nunomura and Hagino (2000)
		Cyathura higoensis	Nunomura (1977)
		Cyathura kikuchii	Nunomura (1977), Nunomura (1993), Nunomura (2016b)
		Cyathura muromiensis	Nunomura (1974a), Horikoshi et al. (2012), Nunomura (2013), Nunomura (2014), Nunomura (2016b), **This study**
		Cyathura omorii	Nunomura (1992b)
		Cyathura sagamiensis	Nunomura (2006); Kimura et al. (2024)

		Cyathura shinjikoensis	Nunomura (2001)
	Malacanthura	*Malacanthura seisuiae* **sp. nov.**	**This study**
	Mesanthura	*Mesanthura atrata*	Nunomura (1985)
		Mesanthura cinctula	Nunomura (2006), **This study**
		Mesanthura miyakoensis	Nunomura (1979), **This study**
		Mesanthura nigrodorsalis	Nunomura (1977), **This study**
		Mesanthura saikaiensis	Nunomura (2016b)
		Mesanthura sol **sp. nov.**	**This study**
Expanathuridae	*Eisothistos*	*Eisothistos nipponicus*	Nunomura (1984)
	Expanathura	*Expanathura monile* **sp. nov.**	**This study**
Hyssuridae	*Kupellonura*	*Kupellonura tamago* **sp. nov.**	**This study**; Kimura et al. (2024)
Leptanthuridae	*Accalathura*	*Accalathura ochotensis*	Nunomura (1976a)
Paranthuridae	*Colanthura*	*Colanthura angularis*	Nunomura (2016b)
		Colanthura nigra	Nunomura (1975), Nunomura (1977), Nunomura (2004a), **This study**
		Colanthura setouchiensis	Nunomura (1993), Nunomura (2013)
	Deltanthura **gen. nov.**	*Deltanthura palpus* **sp. nov.**	**This study**; Kimura et al. (2024)
	Paranthura	*Paranthura alba*	Nunomura (1977), Nunomura (2016b)
		Paranthura hasticauda	Nunomura (1974b)
		Paranthura japonica	Richardson (1909), Nunomura (1977), Nunomura (1985), Nunomura (1993), Nunomura (2006), Nunomura (2016b), **This study**
		Paranthura kagawaensis	Nunomura (1993), Nunomura (2013)

Paranthura kobensis	Nunomura (1975), Nunomura (1993)
Paranthura laticauda	Nunomura (1975), Nunomura (1993)
Paranthura lineata	Nunomura (1977), Nunomura (2016b)
Paranthura maculosa	Nunomura (1974b)
Paranthura nigrocauda	Nunomura (1974b), Nunomura (1977)
Paranthura oriens **sp. nov.**	**This study**

Table II-IV-1. Specimens used in this study and accession numbers for DNA sequence data; bold font, novel sequences obtained in this study; *, sequences from the Chapter II-III.

Species	Specimen No.	Sampling locality in Japan	Coll. date	Ontogenetic stage	Accession No.
M. cinctula	ICHUM8940	Off Jogashima, Kanagawa	23 May 2018	Female lacking oostegites	-
	ICHUM8941	Off Jogashima, Kanagawa	22 Feb. 2019	Manca	**LC849816**
M. miyakoensis	ICHUM8942	Irabu Island, Okinawa	24 Apr. 2022	Post-manca	**LC849817**
	ICHUM8943	Irabu Island, Okinawa	24 Apr. 2022	Post-manca	-
	ICHUM8944	Tatsukushi, Kochi, Shikoku	26 July 2021	Female lacking oostegites	-
	ICHUM8945	Tatsukushi, Kochi, Shikoku	13 June 2021	Subadult male	LC787697*
	ICHUM8946	Tatsukushi, Kochi, Shikoku	10 Oct. 2022	Female lacking oostegites[1]	LC795631*
	ICHUM8947	Tatsukushi, Kochi, Shikoku	10 Oct. 2022	Post-manca	LC787698*
M. nigrodorsalis	ICHUM8948	Nagamatsu Beach, Osaka	10 Sep. 2022	Female lacking oostegites	-
	ICHUM8949	Araihama, Kanagawa	25 July 2021	Female lacking oostegites	**LC849812**
	ICHUM8950	Jogashima, Kanagawa	12 July 2022	Female lacking oostegites	**LC849811**
M. sol **sp. nov.**	ICHUM8951	Irabu Island, Okinawa	24 Apr. 2022	Ovigerous female	**LC849814**
	ICHUM8952	Onna Village, Okinawa	23 Apr. 2023	Female lacking oostegites	**LC849810**
	ICHUM8953	Onna Village, Okinawa	23 Apr. 2023	Female lacking oostegites	-
	ICHUM8954	Onna Village, Okinawa	23 Apr. 2023	Adult male	**LC849815**
	ICHUM8955	Onna Village, Okinawa	23 Apr. 2023	Adult male	**LC849813**

[1] Specimen ICHUM8946 was an ovigerous female when collected but molted and became a female lacking oostegites through rearing; in this chapter, I treated it as a female lacking oostegites, whereas the Chapter II-III treated it as an ovigerous female.

Table II-IV-2. Genetic distances (as percentages) among partial 16S rRNA sequences from *Mesanthura* specimens; above diagonal, K2P distances; below diagonal, p-distances; intraspecific distances are in bold font.

	1	2	3	4	5	6	7	8	9	10	11
1. *M. cinctula* (LC849816)		41.0	37.5	37.0	37.5	38.5	37.6	40.2	38.7	38.7	38.7
2. *M. miyakoensis* Irabu (LC849817)	30.8		**4.6**	**4.8**	**5.4**	26.3	26.2	20.5	20.8	20.8	20.8
3. *M. miyakoensis* Tatsukushi (LC787697)	28.9	**4.4**		**0.2**	**0.7**	27.5	27.4	19.8	19.5	19.5	19.5
4. *M. miyakoensis* Tatsukushi (LC787698)	28.6	**4.6**	**0.2**		**1.0**	27.9	27.8	20.1	19.8	19.8	19.8
5. *M. miyakoensis* Tatsukushi (LC795631)	28.9	**5.1**	**0.7**	**1.0**		27.9	27.8	19.8	19.5	19.5	19.5
6. *M. nigrodorsalis* Araihama (LC849812)	29.4	21.4	22.1	22.3	22.3		**0.7**	27.7	27.0	27.0	27.0
7. *M. nigrodorsalis* Jogashima (LC849811)	28.9	21.4	22.1	22.3	22.3	**0.7**		27.7	26.9	26.9	26.9
8. *M. sol* **sp. nov.** Irabu (LC849814)	30.6	17.5	17.0	17.2	17.0	22.6	22.6		**3.2**	**3.2**	**3.2**
9. *M. sol* **sp. nov.** Onna (LC849813)	29.9	17.7	16.7	17.0	16.7	22.1	22.1	**3.2**		**0.0**	**0.0**
10. *M. sol* **sp. nov.** Onna (LC849815)	29.9	17.7	16.7	17.0	16.7	22.1	22.1	**3.2**	**0.0**		**0.0**
11. *M. sol* **sp. nov.** Onna (LC849810)	29.9	17.7	16.7	17.0	16.7	22.1	22.1	**3.2**	**0.0**	**0.0**	

Table II-V-1. Comparison of selected characters among species in the second group of *Expanathura*.

Character	*E. collaris*	*E. haddae*	*E. macronesia*	*E. monile* **sp. nov.**
Head pigmentation pattern[a]	U-shaped	No data	Lacking	V-shaped
Telson lateral setae	Several short and 1 long	Subequal, short	Subequal, short	Subequal, short
Telson shape	Oval	Truncate	Intermediate	Oval
Number of articles in antennular flagellum[a]	2–3	3	2	3–4
Outer projection of antennal peduncular article 2	Absent	Absent	Absent	Present
Projection on uropodal exopod[a]	120°	120°	90°	90°
Reference	Kensley (1979), Negoescu (1999)	Kensley and Poore (1982)	Kensley (1980)	This study

[a]State in females.

Table II-VII-1. Length measurements and ratios for the type specimens of *Cruranthura viridis* **sp. nov.** BL, body length; BW, body width; HL, head length; HW, head width; EL, eye length; P1L–P7L, lengths of pereonites 1–7; p1L–p6L, length of pleonites 1–6.

Collection No.	BL (mm)	BW (mm)	BL/BW	HL/HW	EL/HL	HL:P1L:P2L:P3L:P4L:P5L:P6L:P7L:p1L–5[a]:p6L	p1L/p2L
ICHUM8565	3.08	0.27	11.54	1.35	0.13	1.00:1.18:1.07:1.19:1.25:1.22:0.78:0.15:0.62:0.33	1.72
ICHUM8566	3.38	0.27	12.52	1.38	0.16	1.00:1.19:1.08:1.18:1.29:1.30:0.96:0.13:0.66:0.31	1.89
ICHUM8567	2.80	0.24	11.64	1.37	0.14	1.00:1.12:1.02:1.12:1.14:1.11:0.67:0.15:0.65:0.29	1.75
ICHUM8568	2.53	0.22	11.54	1.35	0.14	1.00:1.08:1.02:1.11:1.16:1.05:0.74:0.15:0.65:0.31	2.02

[a] Aggregated length of pleonites 1–5.

Table II-VII-2. Comparison of selected characters among *Cruranthura* species. BL, body length; p1L, p2L, lengths of pleonites 1 and 2; TL, telson length; TW, telson width; —, no data.

Character	*C. caeca*	*C. furneauxi*	*C. peroni*	*C. simplicia*	*C. viridis* **sp. nov.**
BL (mm)	7.5–10.8	5.2–9.1	4.5–9.5	5.25–6.00	2.53–3.38
Color	—	Red brown	Red brown	—[a]	Moss green
Eye	Absent	Present	Present	Present	Present
Relative lengths of p1L and p2L	p1 = p2	p1 < p2	p1 > p2	—	p1 > p2
TL/TW	2.5	2.5	2.2	2.0	2.1–2.3
Number of spiniform setae on propodus of pereopod 1	Ca. 35[b]	Ca. 35	Up to 25	9–12	6–10
Endopods of pleopods 2–5	Uniarticulate	Uniarticulate	Biarticulate	Biarticulate	Uniarticulate
Setae on endopods of pleopods 2–5	Present	Present	Absent	Absent	Present
Habitat	Marine	Marine	Estuarine	Estuarine	Marine
References	Mezhov (1976), Poore (1984)	Poore (1981, 1984)	Poore (1981, 1984)	Thomson (1946), Poore (1984)	This study

[a] Scattered, darkly pigmented patches present on head, but information on body color lacking (Thomson 1946).

[b] Counted from Mezhov (1976: fig. 4).

Table II-IX-1. Records of *Paranthura japonica* from Japan.

Area	Prefecture	Locality	References
Hokkaido	Hokkaido	Rishiri Is.	Nunomura (2004a); Ohta et al. (2020)
		Abashiri	Nunomura (1991)
		Akkeshi Bay	Nunomura (1991)
		Oshoro	Nunomura (1991); Shiraki and Kakui (2022)
		Usu Bay	Nunomura (1991)
		Muroran[1]	Richardson (1909); Shiraki and Kakui (2022)
Tohoku	Aomori	Mutsu Bay	Nunomura (2004b)
	Iwate	Otsuchi Bay	Nunomura (1987)
Chubu	Niigata	Sado Is.	Nunomura (1981)
	Toyama	Toyama Bay	Nunomura (1985a)
	Shizuoka	Shimoda	Nunomura (2004c)[2]; Nunomura (2006)[2]
Kinki	Wakayama	Wakayama	Nunomura (1993)
Shikoku	Kagawa	Sakaide	Nunomura (1985b); Nunomura (1993); Nunomura (2014)
	Kochi	Tosashimizu	Nunomura (2014)
		Otsuki Town	Nunomura (2014)
Kyushu	Nagasaki	Shijiki Bay	Nunomura (2016)
	Kumamoto	Amakusa	Nunomura (1977)
	Okinawa	Uruma	This study

[1]Type locality of the species. [2]The records by Nunomura (2004b) and Nunomura (2006) are likely based on the same specimens, as they share the same collection data.

Table II-IX-2. Candidate prey taxa used in this study. n.o., not observed; +, predation observed; –, predation not observed.

Taxon offered	No. of individuals offered	Predation	Prey condition before/after experiment	Sampling locality
Amphipoda				
Ampithoidae sp.	1	+	alive/dead	Oshoro
Caprellidae sp.	1	+	alive/n.o.	Oshoro
Isopoda				
Cleantiella strasseni	1	+	alive/alive	Oshoro
Janiridae sp.	1	+	dead/dead	Oshoro
Tanaidacea				
Zeuxo ezoensis	4	+	alive/dead	Oshoro
Apseudes ranma	6	+	alive/dead	Aquarium
Pycnogonida				
Ammotheidae sp.	1	–	alive/alive	Oshoro

Table III-1. List of anthuroid species reported from the Philippines.

Family	Genus	Species	References
Anthuridae	*Amakusanthura*	*Amakusanthura camiguinensis* **sp. nov.**	**This study**
	Sauranthura	*Sauranthura liangaensis* **sp. nov.**	**This study**
	Stygocyathura	*Stygocyathura filipinica*	Botosaneanu and Sket (1999)
Paranthuridae	*Colanthura*	*Colanthura kensleyi*	Poore (1984)

Table III-I-1. List of primers used for PCR and cycle sequencing (CS).

Gene	Primer	Sequence (5′→3′)	Reaction	Direction	Reference
18S rRNA	18S-a1F	GGYGAAACCGYGAAWGGYTC	PCR	Forward	Kakui et al. (2011)
	18S-b3F	CCTGAGAAACGGCTACCACAT	CS	Forward	Kakui and Shimada (2017)
	18S-b4F	TGCGGTTAAAAAGCTCGTAGTTG	CS	Forward	Kakui et al. (2011)
	18S-b4R	TCCAACTACGAGCTTTTTAACC	CS	Reverse	Kakui et al. (2011)
	18S-b5F	GATCGAAGGCGATYAGATACC	CS	Forward	Kakui et al. (2021)
	18S-b6F	CCTGCGGCTTAATTTGACTC	CS	Forward	Kakui et al. (2011)
	18S-a6R	AACGGCCATGCACCAC	CS	Reverse	Kakui et al. (2011)
	18S-b8R	TCTAAGGGCATCACAGACCTG	CS	Reverse	Kakui et al. (2011)
	18S-b8F	GGTCTGTGATGCCCTTAGATG	CS	Forward	Kakui et al. (2011)
	18S-a9R	CCTTGTTACGACTTTTAGTTCC	PCR	Reverse	Kakui et al. (2011)
28S rRNA	LSUD1F	ACCCGCTGAATTTAAGCATA	PCR	Forward	Lenaers et al. (1989)
	28S-RD1.3f	GGATTCCCTYAGTAAGKGCG	CS	Forward	Drumm (2010)
	300F	CAAGTACCGTGAGGGAAAGTTG	CS	Forward	Lockyer et al. (2003)
	300R	CAACTTTCCCTCACGGTACTTG	CS	Reverse	Lockyer et al. (2003)
	900F	CCGTCTTGAAACACGGACCAAG	CS	Forward	Lockyer et al. (2003)
	28S_a3F	ACCCGAAAGATGGTGAWCTATG	CS	Forward	This study
	28S_a4F	ACGAGTTTCATCCTGTAAAGMKAATG	CS	Forward	This study
	28S_a6R	AGCCGAGTGCTTGCTACTAC	PCR	Reverse	This study

Table IV-I-1. Host isopods for each genus in Cabiropidae. a, Barnard (1920) reported, but did not name, two larval specimens attached on *Lanocira capensis* Barnard, 1914 (Corallanidae Hansen, 1890; junior synonym of *Lanocira gardineri* Stebbing, 1904; cf. Bruce and Sidabalok 2011), which may belong in *Clypeoniscus*, but Nielsen (1967) was doubtful about their affiliation. nd, no host information.

Cabiropid genus	**Host isopods**			**References**
	Suborder	**Superfamily**	**Family**	
Aegoniscus	Cymothoida	Cymothooidea	Aegidae	Barnard (1925)
Ancyroniscus	Cymothoida	Cymothooidea	Aegidae	Bourdon and Bruce (1980)
	Sphaeromatidea	Sphaeromatoidea	Sphaeromatidae	Caullery and Mesnil (1919)
Arcturocheres	Valvifera	(no superfamily rank)	Arcturidae	Schultz (1980)
			Austrarcturellidae	Schultz (1980)
			Holidoteidae	Schultz (1980)
Bourdonia	Epicaridea	Bopyroidea	Bopyridae	Rybakov (1990)
Cabirops	Epicaridea	Bopyroidea	Bopyridae	Boyko (2013)
Cirolanoniscus	Cymothoida	Cymothooidea	Cirolanidae	Pillai (1966)
Cironiscus	Cymothoida	Cymothooidea	Cirolanidae	Nielsen (1967)
Clypeoniscus	Asellota	Stenetrioidea	Stenetriidae	Barnard (1920)
	Cymothoida[a]	Cymothooidea[a]	Corallanidae[a]	Barnard (1920)
	Valvifera	(no superfamily rank)	Idoteidae	Bourdon (1967)
Gnomoniscus	Epicaridea	Cryptoniscoidea	Podasconidae	Giard and Bonnier (1895)
Munnoniscus	Asellota	Janiroidea	Munnopsidae	Giard and Bonnier (1895)
Perezina	Non-isopod (Rhizocephala)			Nierstrasz and Brender à Brandis (1929)
Pleuroniscus	Epicaridea	Bopyroidea	Bopyridae	Williams et al. (2024)

Podoniscus	nd	nd	nd	Bourdon (1981)
Rolandoniscus	Epicaridea	Bopyroidea	Bopyridae	Boyko (2013)
Anthuroniscus **gen. nov.**	Cymothoida	Anthuroidea	Anthuridae	**This study**
			Leptanthuridae	**This study**
			Paranthuridae	**This study**

Table IV-I-2. Taxa included in the phylogenetic analysis of 18S rRNA gene sequences. Information for species sequenced in this study is in bold font.

Family	Species	Locality	Accession	Reference
Bopyroidea				
Bopyridae	*Allokepon sinensis*	Bohol, Philippines	KF765766	Boyko et al. (2013)
	Argeia pugettensis	Coos Bay, Oregon, USA	KF765770	Boyko et al. (2013)
	Athelges takanoshimensis	Discovery Bay, Hong Kong	KF765762	Boyko et al. (2013)
	Athelges takanoshimensis	Discovery Bay, Hong Kong	KF765769	Boyko et al. (2013)
	Athelges takanoshimensis	Discovery Bay, Hong Kong	KF765773	Boyko et al. (2013)
	Hemiarthrus abdominalis	Baltic Sea	AF255684	Dreyer and Wägele (2001)
	Probopyrus buitendijki	Sungai Timun, Negeri Sembilan, Malaysia	KF765767	Boyko et al. (2013)
	Probopyrus pacificiensis	Guerrero, Mexico	AF255683	Dreyer and Wägele (2001)
	Probopyrus pandalicola	Georgia, USA	EU848422	Cho (2012)
	Pseudione longicauda	32°47'N, 130°36'E, Japan	KF765760	Boyko et al. (2013)
	Pseudione longicauda	32°47'N, 130°36'E, Japan	KF765774	Boyko et al. (2013)
Entoniscidae	*Portunion conformis*	Limfjord Inlet, Denmark	KF765764	Boyko et al. (2013)
Ionidae	*Ione cornuta*	Pigeon Point, Oregon, USA	KF765761	Boyko et al. (2013)
	Ione cornuta	Charleston mud flat, Oregon, USA	KF765771	Boyko et al. (2013)
Cryptoniscoidea				
Cabiropidae	***Anthuroniscus shimomurai* sp. nov.**	**Kaichu Doro, Uruma, Okinawa, Japan**	**LC794718**	**This study**
	***Anthuroniscus dentatus* sp. nov.**	**Irabu Is., Miyako Islands, Okinawa,**	**LC794719**	**This study**

	***Anthuroniscus latus* sp. nov.**	**Jogashima, Kanagawa, Japan**	**LC794720**	**This study**
Dajidae	*Zonophryxus dodecapus*	Candelaria, Tenerife, Canary Islands	KF765768	Boyko et al. (2013)
	Zonophryxus quinquedens	Weddell Sea, Antarctica	DQ008451	Raupach and Thatje (2006)
Entophilidae	*Entophilus mirabiledictu*	Royal Diving Club, Aqaba, Red Sea	KF765763	Boyko et al. (2013)
Unidentified	Cryptoniscoidea sp.	No data	KF765772	Boyko et al. (2013)
Cymothooidea				
Cymothoidae	*Excorallana quadricornis*	Venezuela	AF255688	Dreyer and Wägele (2001)

Table IV-II-1. Taxa included in the phylogenetic analysis of 18S and 28S gene sequences. GB., GenBank; TBA, to be assigned.

Family	Species	18S GB. No.	28S GB. No.	Reference
Clistosaccidae	*Clistosaccus paguri*	GU190697	GU190709	Glenner et al. 2010
	Sylon hippolytes	DQ826564	GU190700	Glenner and Hebsgaard 2006; Glenner et al. 2010
Chthamalophilidae	*Boschmaella japonica*	-	GU190701	Glenner et al. 2010
	Chthamalophilus delagei	GU190696	GU190710	Glenner et al. 2010
Duplorbidae	*Duplorbis changeling* **sp. nov.**	TBA	TBA	**This study**
	Duplorbis koru	PV521855	-	Kokasko et al. 2025
Mycetomorphidae	*Mycetomorpha abyssalis*	LC799152	LC799153	Kakui 2024
	Mycetomorpha vancouverensis	MH974514	MH974515	Høeg et al. 2019
Parthenopeidae	*Parthenopea subterranea*	DQ826566	FJ790317	Glenner and Hebsgaard 2006; Lützen et al. 2009
Peltogastridae	*Peltogaster paguri*	DQ826570	EU082335	Perez-Losada et al. 2008; Glenner et al. 2010
Peltogastrellidae	*Peltogasterella sulcata*	DQ826572	EU082336	Glenner and Hebsgaard 2006; Perez-Losada et al. 2008
Polyascidae	*Parasacculina yatsui*	MG604305	MG604305	Kobayashi et al. 2018
Polysaccidae	*Polysaccus japonicus*	DQ826565	GU190708	Glenner and Hebsgaard 2006; Glenner et al. 2010
Sacculinidae	*Heterosaccus dollfusi*	EU082413	EU082333	Perez-Losada et al. 2008
	Heterosaccus lunatus	EU082414	EU082334	Perez-Losada et al. 2008
	Sacculina upogebiae	KF539758	KF539760	Lützen et al. 2016
Thompsoniidae	*Thompsonia littoralis*	DQ826573	-	Glenner and Hebsgaard 2006
Out group	*Ibla quadrivalvis*	AY520655	AY520621	Perez-Losada et al. 2004
	Poecilasma inaequilaterale	AY520654	AY520620	Perez-Losada et al. 2004
	Semibalanus balanoides	AY520626	EU370440	Perez-Losada et al. 2004; von Reumont et al. 2009

Table IV-III-1. List of primers used in this study.

Gene marker	Primer	Reaction	Position	Sequence	Reference
28S	U178	PCR/CS	F	GCACCCGCTGAAYTTAAG	Lockyer et al. (2003)
	300F	CS	F	CAAGTACCGTGAGGGAAAGTTG	Lockyer et al. (2003)
	300R	CS	R	CAACTTTCCCTCACGGTACTTG	Lockyer et al. (2003)
	U1148	CS	F	GACCCGAAAGATGGTGAA	Lockyer et al. (2003)
	L1642	PCR/CS	R	CCAGCGCCATCCATTTTCA	Lockyer et al. (2003)
ITS1 cluster	M1780F	PCR/CS	F	ACACCGCCCGTCGCTACTA	Galaktionov et al. (2012)
	ITS1_inF	CS	F	AACATTGGCAGTGCTAGGC	This study
	ITS1_inR	CS	R	TGAACTGCACGCTCAACACTA	This study
	M 5·8R	PCR/CS	R	GGCTGCGCTCTTCATCGACA	Galaktionov et al. (2012)

CS, cycle sequencing; F, forward; R, reverse.

Table IV-III-2. P-distances among partial 28S rRNA sequences (945 bp) from species in the *Microphallus* clade in the phylogenetic tree.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. *Microphallus triangulatus* (HM584139)														
2. *Microphallus pseudopygmaeus* (HM584127)	0.007													
3. *Microphallus kurilensis* (HM584129)	0.013	0.012												
4. *Microphallus pygmaeus* (HM584133)	0.014	0.013	0.003											
5. *Microphallus calidris* (HM584124)	0.011	0.007	0.004	0.005										
6. *Microphallus minutus* (KT355822)	0.013	0.010	0.006	0.007	0.002									
7. *Microphallus piriformes* (HM584123)	0.020	0.019	0.014	0.015	0.012	0.010								
8. *Microphallus similis* (AY220625)	0.026	0.025	0.022	0.023	0.018	0.016	0.025							
9. *Microphallus ochotensis* (MG783587)	0.055	0.053	0.051	0.050	0.047	0.049	0.053	0.049						
10. *Microphallus* sp. (KJ868217)	0.063	0.060	0.060	0.059	0.056	0.058	0.063	0.058	0.040					
11. *Microphallus abortivus* (AY220626)	0.078	0.077	0.074	0.073	0.070	0.070	0.073	0.068	0.074	0.074				
12. *Microphallus primas* (AY220627)	0.078	0.077	0.074	0.073	0.070	0.070	0.072	0.068	0.074	0.077	0.008			
13. *Microphallus basodactylophallus* (AY220628)	0.073	0.071	0.067	0.066	0.064	0.065	0.070	0.069	0.066	0.078	0.090	0.091		
14. Microphallidae sp. (AB974360)	0.046	0.042	0.038	0.039	0.035	0.035	0.038	0.040	0.046	0.057	0.068	0.069	0.064	
15. Metacercaria, this study (LC808145)	0.056	0.055	0.052	0.051	0.048	0.050	0.053	0.049	0.048	0.061	0.068	0.069	0.074	0.031

Table V-1. Detailed localities of sampling sites in Japan in this study. For the locality of dredge, we showed the coordinates of starting point of dredging.

Site	Locality	Coordinates	Depth (m)	Date
1	Muroran, Hokkaido	42°18'50.4"N 140°58'05.3"E	0	5 Sep. 2021
2	Yamada Bay, Iwate	39°27'48.8"N 141°58'30.6"E	0	15 July 2021
3	Tsukumo Bay, Ishikawa	37°18'18.2"N 137°14'33.6"E	0.2	17 Sep. 2022
4	Kannonzaki, Kanagawa	35°15'32.2"N 139°44'34.0"E	0.5	16 Mar. 2021
5	Jogashima, Kanagawa	35°08'11.0"N 139°36'40.5"E	1	12 July 2022
6	Misaki, Kanagawa	35°08'15.0"N 139°32'40.0"E	178–235	9 June 2022
7	Tateyama, Chiba	34°59'18.8"N 139°48'35.7"E	12–19.8	25 Feb. 2022
8	Shimoda, Shizuoka	34°36'31.1"N 138°44'46.6"E	255–260	13 Oct. 2020
9	Enshū Sea, off Shizuoka	34°30'31.8"N 137°51'22.2"E	457–468	8 July 2024
10	Kumano Sea, off Mie	34°10'51.0"N 136°52'45.6"E	152–213	24 Nov. 2021
11	Kumano Sea, off Mie	34°10'30.0"N 136°45'18.0"E	105–99	29 June 2023
12	Kumano Sea, off Mie	34°10'14.4"N 136°44'00.6"E	138–137	26 Nov. 2021
13	Kumano Sea, off Mie	34°10'00.0"N 136°38'42.0"E	186–183	28 June 2023
14	Kumano Sea, off Mie	34°05'33.0"N 136°37'22.2"E	457–459	25 Nov. 2021
15	Kumano Sea, off Mie	34°04'54.0"N 136°40'00.0"E	548–506	29 June 2023
16	Kumano Sea, off Mie	34°02'47.4"N 136°53'15.6"E	743	31 Oct. 2024
17	Kumano Sea, off Mie	34°02'36.0"N 136°53'24.0"E	747–779	27 June 2023
18	Kumano Sea, off Mie	33°59'12.6"N 136°55'40.8"E	765–768	26 Oct. 2023
19	Tsuyazaki, Fukuoka	33°46'28.4"N 130°26'58.7"E	18	4 July 2023
20	Muromi river, Fukuoka	33°34'48.2"N 130°20'13.7"E	0	9 Mar. 2023

21	Tatsukushi, Kochi	32°47'09.3"N 132°51'22.2"E	0–1	13 June 2021, 26 July 2021, 10 Oct. 2022
22	Yakushima Is., Kagoshima	30°27'15.7"N 130°29'52.8"E	1	6 May 2023
23	Kikaijima Is., Kagoshima	28°19'56.3"N 130°00'08.7"E	0	18 May 2023
24	Amami Is. Kagoshima	28°07'54.3"N 129°19'53.1"E	2	20 May 2023
25	Irabu Is., Okinawa	24°51'58.3"N 125°09'47.8"E	10–15	24 Apr. 2022
26	Irabu Is., Okinawa	24°51'57.7"N 125°09'41.9"E	10–20	25 Apr. 2022
27	Irabu Is., Okinawa	24°51'55.5"N 125°08'39.6"E	10–15	24 Apr. 2022
28	Irabu Is., Okinawa	24°51'54.0"N 125°09'22.2"E	10–15	23 Apr. 2022
29	Irabu Is., Okinawa	24°48'16.7"N 125°08'30.8"E	10–15	24 Apr. 2022
30	Iriomote Is., Okinawa	24°23'02.6"N 123°44'14.7"E	21	12 Aug. 2016

Table V-2. List of species included in our phylogenetic analysis with their character states on mouthparts, number of statocysts, and neotenous character. Familial affiliation followed Poore (2001). TM, type of mouthparts; B, biting-type; MP, moderately specialized piercing-type; HP, highly specialized piercing-type; NS, number of statocysts; NC, state of neotenous character; NN, non-neotenous; TN, true-neotenous; PN; para-neotenous; TBA, to be assigned. [1], sequences from Katsuragawa et al. (in press); [2], sequences from Shiraki and Kakui (2025); [3], sequence from Shiraki and Kakui (2024); [4], sequence from Shiraki and Kakui (2022); [5], sequence from Shiraki et al. (2024).

Current family	Species	TM	NS	NC	Locality	18S	28S	16S	COI
Antheluridae	*Ananthura* sp.	B	0	NN	16	TBA	TBA	TBA	
	Anthomuda iriomotensis **sp. nov.**	B	1	NN	30	TBA	TBA		
Anthuridae	*Amakusanthura* sp.	B	2	NN	21	TBA		TBA	
	Apanthura cf. *shikokuensis*	B	2	NN	21	TBA	TBA		
	Apanthura sp.	B	2	NN	6	TBA	TBA		
	Cyathura muromiensis	B	2	NN	20	LC866769[1]	LC866780[1]	TBA	TBA
	Cyathura sagamiensis	B	2	NN	10	TBA	TBA	TBA	TBA
	Haliophasma sp.	B	2	NN	15	TBA	TBA		
	Leipanthura sp.	B	2	TN	11	TBA	TBA		
	Malacanthura seisuiae **sp. nov.**	B	2	NN	16	TBA	TBA		TBA
	Mesanthura cinctula	B	2	NN	8	TBA	TBA	LC849816[2]	
	Mesanthura miyakoensis	B	2	NN	27	TBA	TBA	LC849817[2]	
	Mesanthura nigrodorsalis	B	2	NN	5	TBA	TBA	LC849811[2]	TBA

	Mesanthura sol **sp. nov.**	B	2	NN	27	TBA	TBA	LC849814[2]	
	Pendanthura sp.	B	2	NN	25	TBA	TBA		TBA
	Anthuridae sp. 1	B	2	NN	16	TBA	TBA		
	Anthuridae sp. 2	B	2	NN	18	TBA	TBA		
	Anthuridae sp. 3	B	2	NN	19	TBA	TBA	TBA	
Expanathuridae	*Eisithistos* sp. 1	B	0	NN	2	TBA	TBA	TBA	TBA
	Eisithistos sp. 2	B	0	NN	9	TBA	TBA		
	Expanathura cf. *monile* **sp. nov.**	B	0	NN	29	TBA			
	Expanathura sp.	B	0	NN	22	TBA	TBA	TBA	
Hyssuridae	*Kupellonura tamago* **sp. nov.**	B	0	NN	13, 14	TBA	TBA	TBA	
	Hyssuridae sp.	B	0	NN	11	TBA	TBA	TBA	
Leptanthuridae	*Accalathura* sp. 1	MP	1	NN	28	TBA	TBA	TBA	TBA
	Accalathura sp. 2	MP	1	NN	23	TBA	TBA		TBA
	Leptanthura sp. 114	MP	1	NN	24	TBA	TBA	TBA	TBA
	Leptanthuridae sp.	MP	1	NN	12	TBA	TBA	TBA	
Paranthuridae	*Colanthura nigra*	HP	0	TN	4	TBA	TBA	TBA	
	Cruranthura viridis **sp. nov.**	HP	0	TN	26		TBA		LC775393[3]
	Cruranthura sp.	HP	0	TN	18	TBA	TBA	TBA	
	Deltanthura palpus **sp. nov.**	MP	0	PN	17	TBA	TBA		

	Paranthura alba	MP	0	NN	21	TBA	TBA		TBA
	Paranthura japonica	MP	0	NN	1	LC866770[1]	LC866781[1]	TBA	LC661620[4]
	Paranthura oriens **sp. nov.**	MP	0	NN	7	TBA	TBA		LC778095[5]
	Paranthura sp. 1	MP	0	NN	7	TBA	TBA		TBA
	Paranthura sp. 2	MP	0	NN	3	TBA	TBA	TBA	TBA
Outgroup	Antarcturidae sp.	-	-	-	-	TBA	TBA		
	Symmius caudatus	-	-	-	-	TBA	TBA	TBA	
	Symmius planus	-	-	-	-	TBA	TBA	TBA	

Table V-3. List of primary primers used in this study.

Gene	Primer name	Sequence (from 5′ to 3′ direction)	Direction	Reaction	Reference
18S	18S-a1F	GGYGAAACCGYGAAWGGYTC	Forward	PCR	Kakui et al. (2011)
	18S-b3F	CCTGAGAAACGGCTACCACAT	Forward	CS	Kakui and Shimada (2017)
	18S-b4F	TGCGGTTAAAAAGCTCGTAGTTG	Forward	CS	Kakui et al. (2011)
	18S-b4R	TCCAACTACGAGCTTTTTAACC	Reverse	CS	Kakui et al. (2011)
	18S-b5F	GATCGAAGGCGATYAGATACC	Forward	CS	Kakui et al. (2021)
	18S-b6F	CCTGCGGCTTAATTTGACTC	Forward	CS	Kakui et al. (2011)
	18S-a6R	AACGGCCATGCACCAC	Reverse	CS	Kakui et al. (2011)
	18S-b8R	TCTAAGGGCATCACAGACCTG	Reverse	CS	Kakui et al. (2011)
	18S-b8F	GGTCTGTGATGCCCTTAGATG	Forward	CS	Kakui et al. (2011)
	18S-a9R	CCTTGTTACGACTTTTAGTTCC	Reverse	PCR	Kakui et al. (2011)
28S	LSUD1F	ACCCGCTGAATTTAAGCATA	Forward	PCR	Lenaers et al. (1989)
	28S-RD1.3f	GGATTCCCTYAGTAAGKGCG	Forward	CS	Drumm (2010)
	300F	CAAGTACCGTGAGGGAAAGTTG	Forward	CS	Lockyer et al. (2003)
	300R	CAACTTTCCCTCACGGTACTTG	Reverse	CS	Lockyer et al. (2003)
	900F	CCGTCTTGAAACACGGACCAAG	Forward	CS	Lockyer et al. (2003)
	28S_a3F	ACCCGAAAGATGGTGAWCTATG	Forward	CS	Shiraki et al. 2025
	28S_a4F	ACGAGTTTCATCCTGTAAAGMKAATG	Forward	CS	Shiraki et al. 2025
	28S_b6F	AAGCGACTAGCCCTGAAAATG	Forward	CS	This study
	28S_a6R	AGCCGAGTGCTTGCTACTAC	Reverse	PCR	Shiraki et al. 2025
16S	Cope16S_F	CGCCTGTTTATCAAARACWY	Forward	PCR/CS	Kakui et al. (2023)
	Cope16S_R	TCGATTTGAACTCAAATCAWG	Reverse	PCR/CS	Kakui et al. (2023)

COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	Forward	PCR/CS	Folmer et al. (1994)
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Reverse	PCR/CS	Folmer et al. (1994)