Species Diversity **28**: 189–197
Published online 24 August 2023
DOI: 10.12782/specdiv.28.189

First Specimen Based Record of *Anthessius cucullatus* (Copepoda: Cyclopoida: Anthessiidae) Associated with *Aplysia* spp. (Gastropoda: Aplysiida: Aplysiidae) from Coastal Waters of Kagoshima, Southern Japan

Rina Hashimoto[1,2] and Daisuke Uyeno[1]

[1] Graduate School of Science and Engineering, Kagoshima University, 1-21-35 Korimoto, Kagoshima 890-0065, Japan
E-mail: rina.hashi2021@gmail.com (RH)
[2] Corresponding author

(Received 25 April 2023; Accepted 11 July 2023)

A species of cyclopoid copepod *Anthessius cucullatus* Lee and Kim, 2021 (Anthessiidae) is newly recorded based on both sexes collected from three species of the sea slug genus *Aplysia* Linnaeus, 1767, i.e., *Ap. dactylomela* Rang, 1828, *Ap. juliana* Quoy and Gaimard, 1832, and *Ap. kurodai* Baba, 1937, from coastal waters of Kagoshima Prefecture, southern Japan. The copepod is characterized by having the following characters in the female: 1) the genital double somite covered with a hood-like dorsal lobe; 2) the antenna with four unequal claws and three setae at the distal tip; 3) the maxilla has a spinulose basal lobe; 4) leg 5 with irregular marginal rows of spinules. Furthermore, the male has 5) the third endopodal segment of leg 4 bearing a curved third inner spine. The findings of *An. cucullatus* in this study represent the second record of the copepod from all around the world as well as the first record from Japanese waters with the detailed description of the morphological characters based on the specimens. *Aplysia dactylomela* and *Ap. juliana* are new host records of the copepod.

Key Words: symbiotic copepods, Cyclopoida, Anthessiidae, Aplysiidae, *Aplysia*, Kagoshima, new record of Japan.

Introduction

The genus *Anthessius* Della Valle, 1880, which contains 48 valid species, is known to be the largest genus in the cyclopoid family Anthessiidae (Lee and Kim 2021; Walter and Boxshall 2023). Almost all of the members are associated with marine bivalves and gastropods with several exceptions (Boxshall and Halsey 2004). The copepods of *Anthessius* are actually dominant and commonly occur, and some of the members have been reported from commercially important host species, e.g., *An. isamusi* Uyeno and Nagasawa, 2012 from *Turbo marmoratus* Linnaeus, 1758; *An. pectinis* Tanaka, 1961 from *Mizuhopecten yessoensis* (Jay, 1857) (referred as *Patinopecten yessoensis*); and *An. pinctadae* Humes, 1973 from *Pinctada margaritifera* Linnaeus, 1758 (Humes 1973; Kim 1998; Uyeno and Nagasawa 2012; Uyeno 2020). In this study, *An. cucullatus* Lee and Kim, 2021 is reported based on both sexes from three species of the genus *Aplysia* Linnaeus, 1767 (Gastropoda: Aplysiida: Aplysiidae), i.e., *Ap. dactylomela* Rang, 1828, *Ap. juliana* Quoy and Gaimard, 1832, and *Ap. kurodai* Baba, 1937, collected from coastal waters of Kagoshima Prefecture, southern Japan.

Materials and Methods

The host sea slugs were caught using snorkel and SCUBA and brought to the laboratory, where they were identified, measured for body length (BL), and then rinsed in tap water along with a 100-μm mesh filter. Copepods were sorted from filtered tap water under a dissecting microscope (Olympus SZX16), and then fixed and preserved in 80% ethanol. For the morphological observation, copepods were soaked in lactophenol for 24 hours, dissected using a sharpened insect pin under the dissecting microscope, and observed based on the modified version of the wooden slide method of Humes and Gooding (1964) under a biological microscope (Nikon eclipse E600). Drawings were made with the aid of a drawing tube equipped on the biological microscope. The copepods' bodies were measured using an ocular micrometer, and the measurements were given in milli- and micrometer as the range followed by the mean and standard deviation in parentheses. The materials examined in this study are deposited in the crustacean collection of the National Museum of Nature and Science, Tsukuba (NSMT), Japan (NSMT-Cr 31493–NSMT-Cr 31497). The taxonomy of the gastropods follows Hamatani (2017).

Results

Family **Anthessiidae** Humes, 1986
[Japanese name: Kaiyadori-kenmijinko-ka]
Genus ***Anthessius*** Della Valle, 1880
[Japanese name: Kaiyadori-kenmijinko-zoku]
Anthessius cucullatus Lee and Kim, 2021
[New Japanese name: Amenori-kenmijinko]
(Figs 1–5)

Anthessius cucullatus Lee and Kim, 2021: 198–204, figs 8–11.

Materials examined. All specimens were collected from coastal waters of Kagoshima Prefecture, Japan: 2 females and 3 males, NSMT-Cr 31493, ex *Ap. dactylomela* (230 mm BL, $n=1$), off Uranomae Port (31°35′36″N, 130°38′28″E), Sakurajima, at a depth of 30 m, 29 May 2021, leg. R. Hashimoto and D. Uyeno; 1 female and 1 male, NSMT-Cr 31494, ex *Ap. kurodai* (182–234 mm BL, $n=4$), off Kamoike (31°33′39″N, 130°33′50″E), at a depth of 1–2 m, 16 April 2021, leg. R. Hashimoto and D. Uyeno; 1 female and 2 males, NSMT-Cr 31495, ex *Ap. dactylomela* (195–225 mm BL, $n=6$), off Marukihama Beach (31°7′07″N, 130°12′42″E), Bonotsu, at a depth of 1–5 m, 16 July 2021, leg. R. Hashimoto, K. Nagayoshi, and D. Uyeno; 3 females and 4 males, NSMT-Cr 31496, ex *Ap. juliana* (108–195 mm BL, $n=10$), off Kamoike (31°33′39″N, 130°33′50″E), at a depth of 1–2 m, 16 February 2022, leg. R. Hashimoto, K. Nagayoshi, Y. Abe, H. Umeki, and D. Uyeno; 24 females and 23 males, NSMT-Cr 31497, ex *Ap. kurodai* (133–211 mm BL, $n=2$), off Bandokoro (31°14′49″N, 130°26′00″E), Wei, at a depth of 1–2 m, 29 April 2022, leg. R. Hashimoto, Y. Abe, H. Umeki, Y. Hatakeyama, N. Higashi, and D. Uyeno.

Adult female. Body (Figs 1A, 5A) cyclopiform, dark brown, 2.33–2.83 (2.65 ± 0.19) mm long ($n=4$). Cephalothorax semilunar, wider than long, 610–920 (790 ± 130) × 1080–1390 (1230 ± 260) μm. Prosome dorsoventrally flattened, 1370–1710 (1550 ± 130) μm long. Urosome (Fig. 1B) 5-segmented, 950–1300 (1150 ± 120) μm long. Fifth pedigerous somite wider than long. Genital double-somite (Fig. 1C) wider than long, 260–310 (280 ± 20) × 390–450 (420 ± 20) μm, composed of wide anterior part and narrow posterior part, with hood-like dorsal lobe. Abdomen 3-segmented; first and second somites wider than long; anal somite almost as long as wide, 170–250 (220 ± 30) × 180–230 (200 ± 20) μm. Caudal ramus (Fig. 1D) 1.78–2.32 (2.07 ± 0.21) times as long as wide, 160–210 (190 ± 20) × 90–100 (90 ± 4) μm, with one median setae on outer margin and five naked and one pinnate distal setae.

Rostral area (Fig. 1E) broad with rounded posterior margin. Antennule (Fig. 1F) 7-segmented, with armature formula: 4, 16, 5, 3, 4 + 1 aesthetasc, 2 + 1 aesthetasc, and 7 + 1 aesthetasc; all setae naked. Antenna (Fig. 1G) 3-segmented: first segment with one small seta distally; second segment with one small seta on inner margin; terminal segment with one short and three longer median setae on inner margin, and one long seta on posterior surface, two setae distantly outside and four unequal claws; innermost claw smallest, second inner claw robust and thicker than others, two outer claws thin, long. Labrum (Fig. 2A) bilobed, with delicate row of serrulation near distal tip. Mandible (Fig. 2B) with five elements on gnathobase: two stout bilobed processes (elements I and II); elongate distal lash (element III) bearing serrated outer margin and spinules on inner margin; small lobe (element IV) with serrated distal margin; elongate seta (element V) with fine spinules along inner margin. Maxillule (Fig. 2C) bilobate: inner lobe bearing two long and three small elements and comb-like process; outer lobe tipped with two small setae. Maxilla (Fig. 2D) 2-segmented; first segment unarmed; second segment bearing spinulose basal lobe, three large and four small spines, and one large spine in middle. Maxilliped (Fig. 2E) indistinctly 2-segmented, digitiform; distal segment tipped with small, pointed process and additional seta.

Legs 1 to 4 (Figs 2F, G, 3A, B) biramous, composed of coxa, basis, and 3-segmented rami. Armature formula shown in Table 1. Inner coxal setae of legs 1 to 4 pinnate, long in legs 1 to 3 and short in leg 4. Outer basal setae of legs 1 to 4 naked. Inner margin of basis of legs 1 to 4 bearing marginal rows of cilia. Intercoxal sclerites of legs 1 to 4 unarmed. Leg 5 (Fig. 3C) 2-segmented: proximal segment bearing small dorsal seta; distal segment free, longer than wide, 180–230 (210 ± 20) × 70–110 (100) μm, length/width ratio of 2.10, bearing three spines and one small seta; inner margin and basis of spines bearing rows of spinules.

Adult male. Body (Figs 3D, 5B) cyclopiform, dark brown, 2.33–2.83 (2.65 ± 0.19) mm long ($n=5$). Cephalothorax ovoid, wider than long 610–920 (790 ± 130) × 1080–1390 (1230 ± 260) μm. Prosome elliptical, 1100–1810 (1470 ± 270) μm long. Urosome (Fig. 3E) 6-segmented, 950–1300 (1150 ± 120) μm long. Fifth pedigerous somite composed of narrow anterior and broad posterior parts. Genital somite (Fig. 3D, E) wider than long, 260–310 (280 ± 20) × 390–450 (420 ± 20) μm; genital flap with two apical elements. Anal somite almost as long as wide, 100–240 (180 ± 50) × 140–220 (190 ± 40) μm. Caudal ramus (Fig. 3E) 1.78–2.32 (2.07 ± 0.21) times as longer than wide, 160–210 (190 ± 20) × 90–100 (90 ± 4) μm.

Rostrum as in female. Antennule (Fig. 4A) as in female except second to fourth segments with formula as 15 + 3 aesthetascs, 6, and 3 + 1 aesthetasc. Antenna, labrum, mandible, maxillule, and maxilla as in female. Maxilliped (Fig. 4B) 3-segmented with terminal claw; proximal segment (syncoxa) rod-like bearing conical process on inner margin and patch of spinules distally; middle segment (basis) with two inner setae and patch of numerous fine spinules on inner surface; distal segment (endopod) bearing one long seta and one pointed process; terminal claw bearing one basal seta and marginal row of spinules along inner margin.

Leg 1 as in female, except third endopodal segment bearing armature formula: I, I, 4. Legs 2 and 3 as in female (Fig. 4C). Leg 4 as in female, except third inner spine on third endopodal segment curved (Fig. 4D) (vs. straight in female). Leg 5 (Fig. 4E) basically as in female, slightly slender, 150–

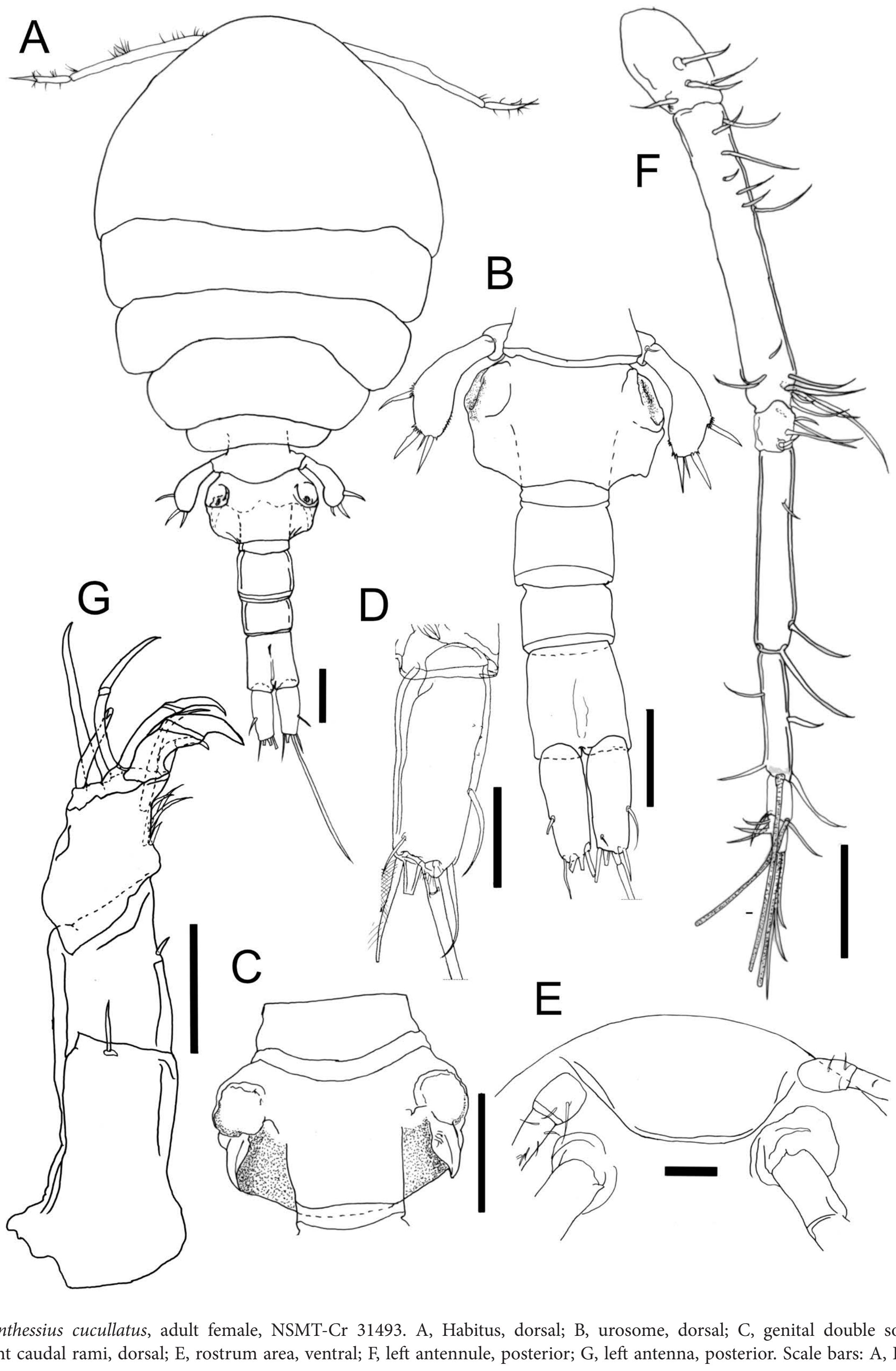

Fig. 1. *Anthessius cucullatus*, adult female, NSMT-Cr 31493. A, Habitus, dorsal; B, urosome, dorsal; C, genital double somite, ventral; D, right caudal rami, dorsal; E, rostrum area, ventral; F, left antennule, posterior; G, left antenna, posterior. Scale bars: A, B = 200 µm; C–G = 100 µm.

190 (160 ± 10) × 60–80 (70 ± 9) µm, length/width ratio of 2.38.

Infection sites. Body surface, especially on the inner side of the mantle and around the gills. Some males were found attached to the urosomites of the females using the maxillipeds (Fig. 5C).

Remarks. *Anthessius cucullatus* is clearly differentiated from other 47 congeners by having the unique hood-like dorsal lobe reaching to the posterior margin of the genital double-somite in the female (Lee and Kim 2021). The species is also differentiated by other characters from the congeners. Twenty species of the genus including *An. cucul-*

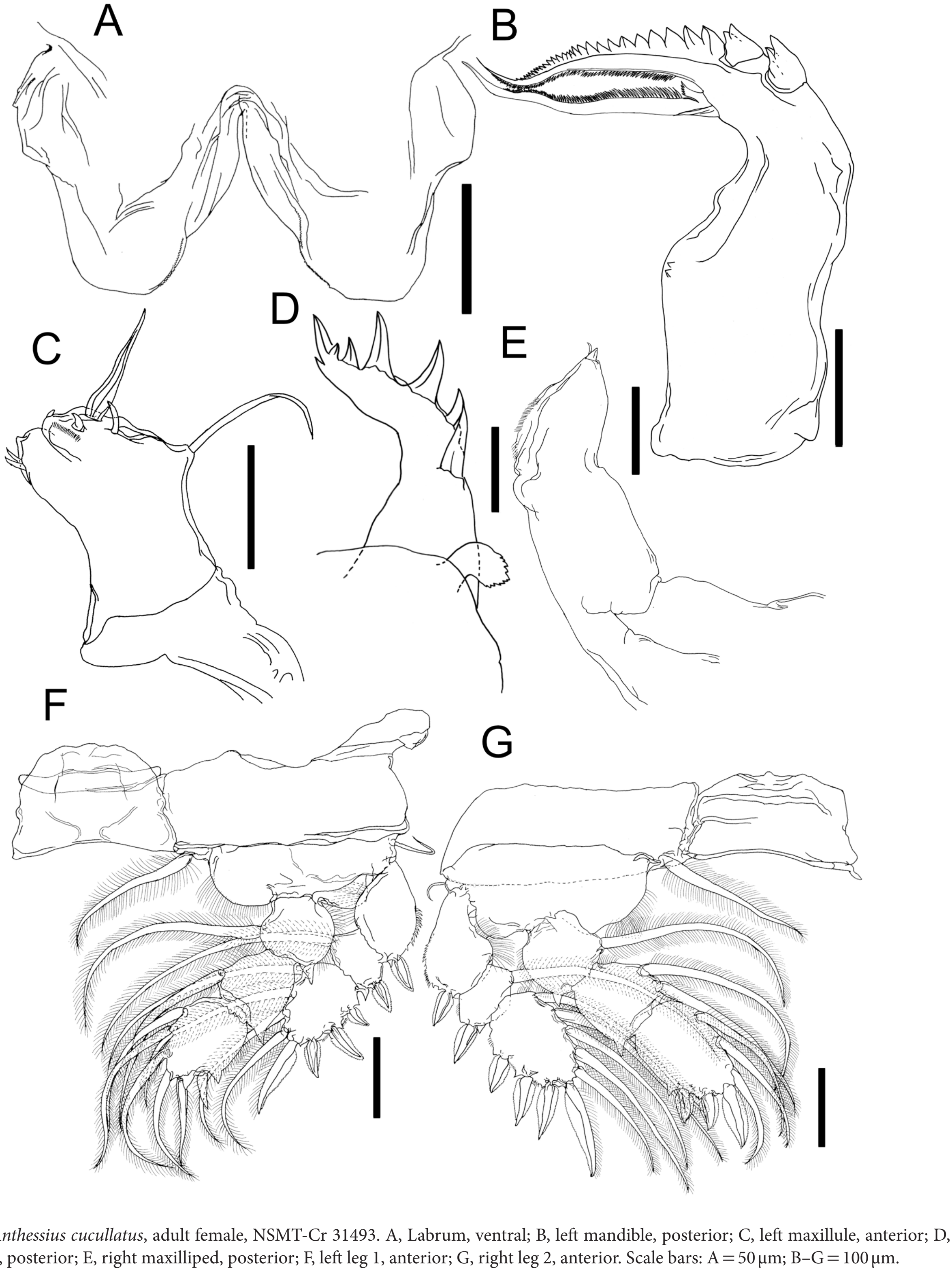

Fig. 2. *Anthessius cucullatus*, adult female, NSMT-Cr 31493. A, Labrum, ventral; B, left mandible, posterior; C, left maxillule, anterior; D, left maxilla, posterior; E, right maxilliped, posterior; F, left leg 1, anterior; G, right leg 2, anterior. Scale bars: A = 50 µm; B–G = 100 µm.

Table 1. Armature of legs 1 to 4 of *Anthessius cucullatus* of female. Number of setae and spines shown in Arabic and Roman numbers, respectively.

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

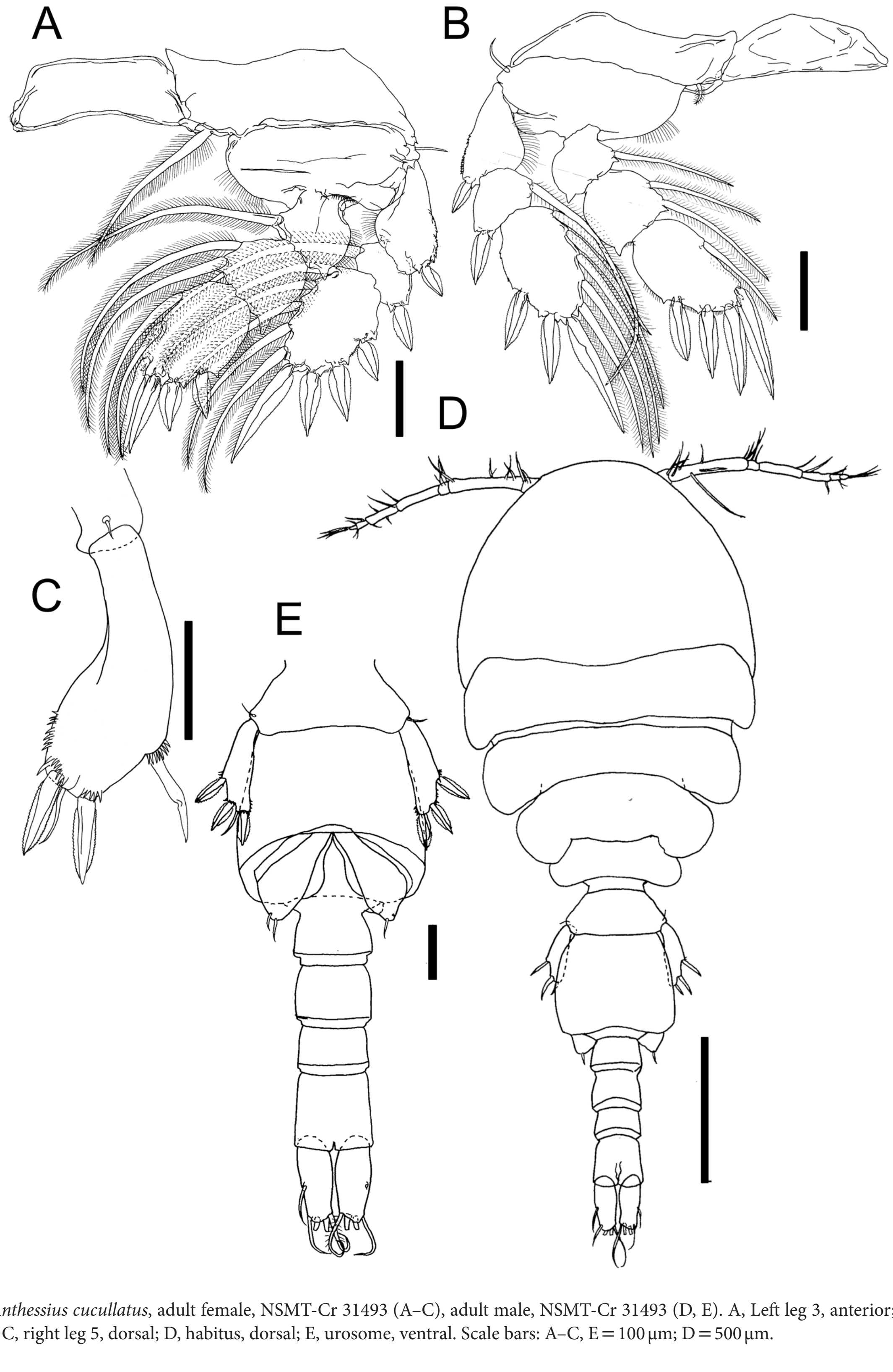

Fig. 3. *Anthessius cucullatus*, adult female, NSMT-Cr 31493 (A–C), adult male, NSMT-Cr 31493 (D, E). A, Left leg 3, anterior; B, right leg 4, anterior; C, right leg 5, dorsal; D, habitus, dorsal; E, urosome, ventral. Scale bars: A–C, E = 100 μm; D = 500 μm.

latus, have three spines on the third exopodal segment of leg 4, whereas the remaining 27 species have four spines (Lee and Kim 2021). Of those 20 congeners, *An. cucullatus* shares short caudal rami (i.e., less than three times long as wide) with the following 11 species: *An. dolabellae* Humes and Ho, 1965, *An. investigatoris* Sewell, 1949, *An. isamusi*, *An. kimjensis* Suh, 1993, *An. longipedis* Ho and Kim, 1992, *An. lophiomi* Avdeev and Kazachenko, 1986, *An. navanacis* (Wilson, 1935), *An. placunae* Devi, 1984, *An. proximus* Stock, Humes, and Gooding, 1963, *An. sensitivus* Stock, Humes, and Gooding, 1963, and *An. varidens* Stock, Humes, and Gooding, 1963 (see Wilson 1935; Sewell 1949; Stock et al. 1963; Humes and Ho 1965; Devi 1984; Avdeev and Kazachenko 1986; Ho and Kim 1992; Suh 1993; Uyeno and

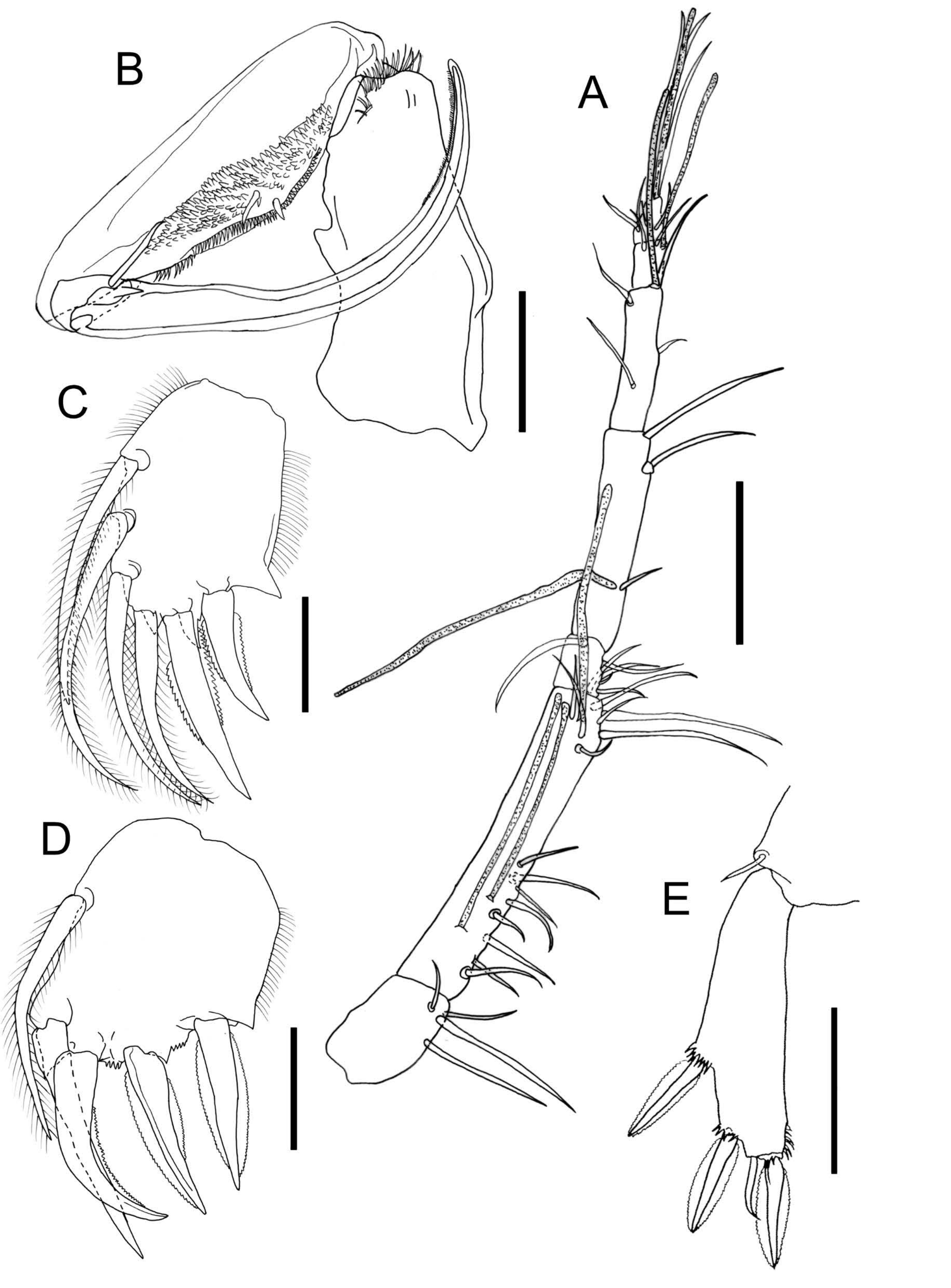

Fig. 4. *Anthessius cucullatus*, adult male, NSMT-Cr 31493. A, Right antennule, posterior; B, left maxilliped, posterior; C, terminal endopodal segment of right leg 1, anterior; D, terminal endopodal segment of right leg 4, anterior; E, left leg 5, dorsal. Scale bars: A–D = 100 μm; E = 200 μm.

Nagasawa 2012; Lee and Kim 2021).

Anthessius cucullatus differs from the following six congeners: *An. kimjensis*, *An. longipedis*, *An. lophiomi*, *An. navanacis*, *An. placunae*, and *An. sensitivus* by having a small basal lobe armed with minute spinules on the maxilla (vs. unarmed) (see Wilson 1935; Illg 1960; Stock et al. 1963; Devi 1984; Avdeev and Kazachenko 1986; Ho and Kim 1992; Suh 1993). *Anthessius cucullatus* differs from *An. isamusi* by having the antenna bearing four unequal claws: the innermost claw is much smaller than others; the secondin-

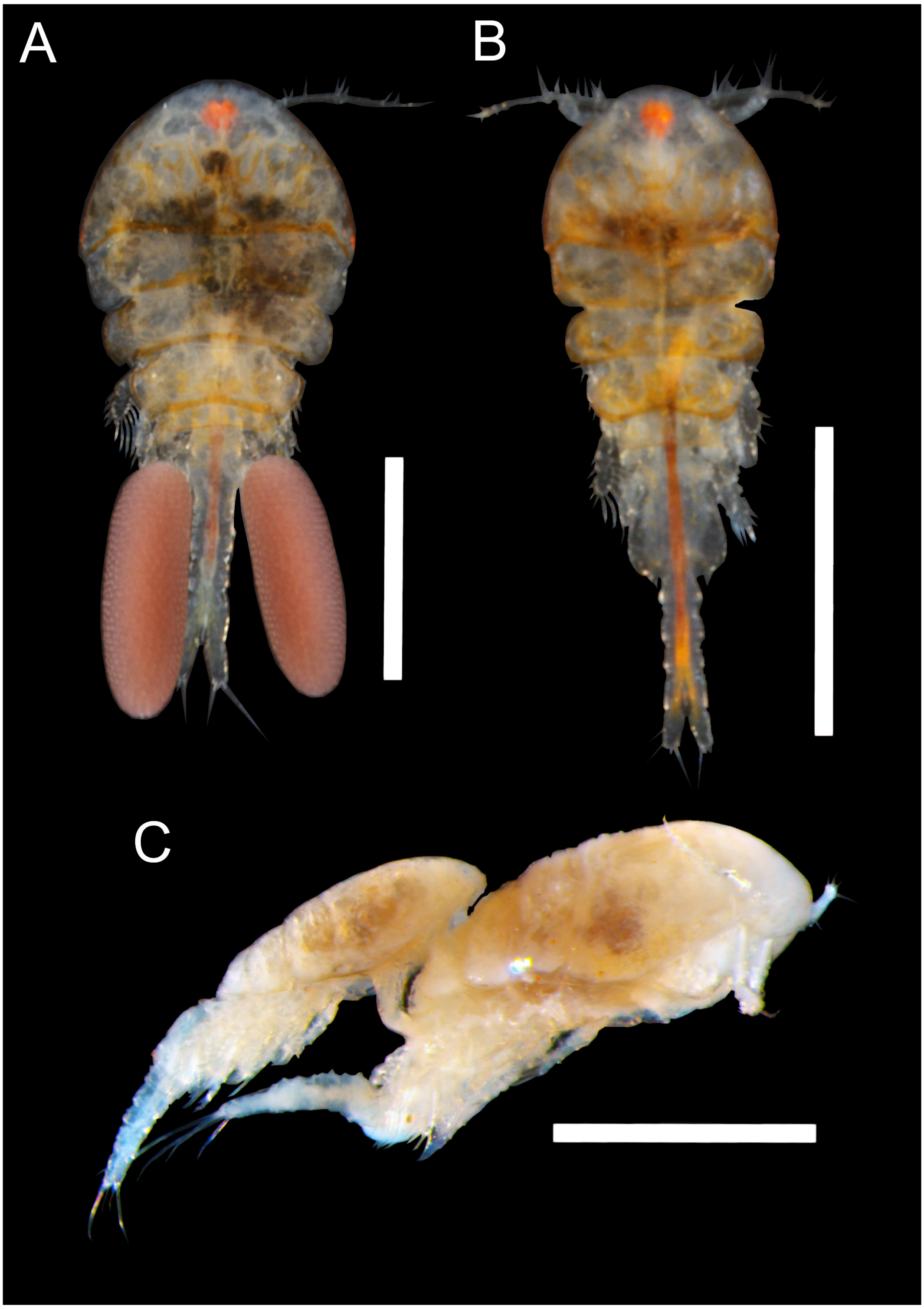

Fig. 5. Coloration of fresh specimens of *Anthessius cucullatus*. A, Adult female, habitus, dorsal; B, adult male, habitus, dorsal; C, mating pair, showing adult male grasping urosome of adult female. Scale bars: 1 mm.

ner claw is thicker and larger than others and one long seta on the posterior surface, and two setae at outer distal corner. The antenna of *An. isamusi* bears two strong hook-like, one long, and one small claws and two long setae (see Uyeno and Nagasawa 2012). *Anthessius cucullatus* is separated from *An. investigatoris*, *An. proximus*, and *An. varidens* by the third endopodal segment of leg 4 in the male bearing a curved inner spine whereas that of the three species are not transformed (see Sewell 1949; Stock et al. 1963; Humes and Ho 1965). *Anthessius cucullatus* shares many characters with *An. dolabellae* but it differs from the latter by having the distal segment of leg 5 lacking fine spinules, because that of *An. dolabellae* has the outer margin ornamented with spinules (see Humes and Ho 1965).

Discussion

Anthessius cucullatus was originally described based on both sexes collected from *Ap. kurodai* in the coastal waters of Korea (Lee and Kim 2021). The findings of *An. cucullatus* from three species of the genus *Aplysia* in this study represent the second record of the copepod with the morphological description based on both sexes. The specimens which Uyeno et al. (2023) used in their molecular analyses were also examined in the present study. Together with the following three congeners, i.e., *An. graciliungus* Do and Kajihara, 1984 from *Mytilus galloprovincialis* Lamarck, 1819 in Hyogo (Do and Kajihara 1984) and in the plankton sample in Nagasaki (Ueda et al. 2006), *An. isamusi* from *T. marmoratus* in Okinawa (Uyeno and Nagasawa 2012), and *An. pectinis* from *Pecten albicans* (Schröter, 1802) in Nagasaki (Tanaka 1961), a total of four species of the genus are now known to be distributed in Japanese waters. *Aplysia dactylomela* and *Ap. juliana* are newly found hosts of *An. cucullatus*. During the field surveys in this study, a single individual of another aplysiid *Bursatella leachii* Blainville, 1817 in Kagoshima Bay was additionally examined, but nonetheless, no sample of *An. cucullatus* was found from the latter aplysiid. Uyeno (2020) mentioned that the number of species of copepods so far reported as associates of mollusks in Japanese waters is overwhelmingly small compared to the high diversity of mollusks. It is expected that the number of species of *Anthessius* could increase in future research in Japan.

Acknowledgments

We thank Kenshiro Nagayoshi, Yuta Abe, Hayata Umeki, Yuki Hatakeyama, Nazuna Higashi, and Midori Matsuoka (Kagoshima University) for their kind help for sampling.

Authors Contributions

Rina Hashimoto: Conceptualization; Writing – original draft; Formal Analysis; Writing – review & editing. Daisuke Uyeno: Supervision; Writing – review & editing.

Funding

Part of this work was supported by "Establishment of Glocal Research and Education Network in the Amami Islands" project of Kagoshima University adopted by the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Declarations

The authors declare no conflicts of interest.

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