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Two new species of symbiotic copepods from sea pens (Anthozoa: Octocorallia: Pennatulacea) collected in the Johor Straits, Singapore

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Abstract. Two new species of symbiotic copepods, *Pennatulicola robustclavus* new species (Cyclopoida: Rhynchomolgidae) and *Enalcyonium kohsiangi*, new species (Cyclopoida: Lamippidae), are described from a sea pen, *Pteroeides* sp. (Octocorallia: Pennatulacea: Pennatulidae), collected from the Johor Strait, Singapore. *Pennatulicola robustclavus* new species is distinguished from its congeners by the following: the third pedigerous somite overlaps the fourth pedigerous somite in dorsal view in female; legs 1 to 3 with middle endopodal segments bearing pronounced outer process; armature formula of terminal exopodal segment of leg 4 bearing II, I, 5; and the maxilliped of male lacks elevated ridges. *Enalcyonium kohsiangi* new species, is different from its congeners by the following characters of female: legs 1 and 2 bearing three spines on exopods and the innermost one of which is smallest; absence of any process at rostral area and between caudal rami; and the caudal rami with four similar setae. The finding of *Pennatulicola robustclavus* new species, from Singaporean waters represents a new record of the genus from the north Pacific Ocean. A key to species of *Pennatulicola* is provided.

Key words. symbiotic copepods, new species, sea pen, Singaporean waters, the Johor Straits

INTRODUCTION

Copepoda (Crustacea: Maxillopoda) is one of the most speciose animal groups in the hydrosphere. It is composed of more than 12,000 species in about 220 families, whose members occur in various environments in the sea and fresh waters (Otsuka & Komai, 2008). Some of them have been reported from specific environments, such as abyssal zone, semi-terrestrial habitats, hot springs, and glacial meltwaters (Boxshall & Halsey, 2004). Many of them are known to be free-living species, but symbiotic copepods attaching on/in other multicellular organisms make up more than 4200 species in 120 families (Ho, 2001; Boxshall & Halsey, 2004). Copepods inhabit many kinds of organisms and environments, but the copepod fauna are poorly known in Southeast Asia, which is a crucible of biodiversity. Since Singapore is located centrally in Southeast Asian waters and a gateway to the Pacific Ocean and the Indian Ocean, the copepod diversity is expected to be extremely rich and unusual. Thus, faunal surveys are strongly required in Singaporean waters.

A field survey, the Johor Strait Workshop was held as a part of the Comprehensive Marine Biodiversity Survey (CMBS) in Singaporean coast of the Johor Strait from 15 October to 2 November 2012. In the workshop, many fishes and invertebrates species were collected from intertidal to subtidal habitats by various methods, such as gill nets, cast nets, hand nets, snorkeling, bottom trawl and hand sampling. More than 40 species in 12 families of symbiotic copepods were found from various hosts, such as, sponges, nudibranchs, sea cucumbers and tunicates. In this paper, two new species of cyclopoid copepods are described from the sea pen, *Pteroeides* sp. (Octocarallia: Pennatulacea: Pennatulidae), collected from intertidal mud flat and subtidal muddy bottom in the Johor Straits.

MATERIAL AND METHODS

Specimens of the sea pen, *Pteroeides* sp., were collected by bottom trawl and hand sampling. Copepods were collected by cutting and shaking sea pen hosts in freshwater and filtering, and copepod specimens were preserved in 80% ethanol. Selected copepod specimens were soaked in lactophenol for 4 h before dissection and observations were made using the method proposed by Humes & Gooding (1964). Drawings were made with the aid of a drawing tube. Measurements given are in micrometers with the range followed by the mean and standard deviation in parentheses. All examined specimens are deposited in the crustacean collection at the Zoological Reference Collection, Lee Kong Chian Natural History Museum, National University of Singapore (LKCNHM).

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Table 1. Leg armature formula.

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

TAXONOMY

Rhynchomolgidae Humes & Stock, 1972

Pennatulicola Humes & Stock, 1972

Pennatulicola robustclavus new species
(Figs. 1–3)

Etymology. The specific name of the new species, *robustclavus*, refers to paired pointed projections on the genital complex.

Material examined. Holotype: adult female (ZRC 2015.0009), ex *Pteroeides* sp. (Pennatulacea: Pennatulidae), off northern side of Pulau Ubin (1°25.570′N, 103°56.524′E to 1°25.492′N, 103°56.765′E)(DW20), the Johor Strait, Singapore, 10.3–10.6 m depth, 17 October 2012.

Allotype: adult male (ZRC 2015.0010), collection data same as those of the holotype.

Paratypes: 59 adult females and 25 adult males (ZRC 2015.0011), collection data same as those of holotype; 24 adult females and 9 adult males (ZRC 2015.0012), ex *Pteroeides* sp. (Pennatulacea: Pennatulidae), off northern side of Pulau Ubin (1°25.794′N, 103°55.897′E to 1°25.647′N, 103°55.592′E)(DW18), the Johor Strait, Singapore, 6.2–12.9 m depth, 17 October 2012; 59 adult females and 42 adult males (ZRC 2015.0013), ex *Pteroeides* sp. (Pennatulacea: Pennatulidae), off west side of Pulau Ubin (1°24.983′N, 103°56.021′E)(SW48), East Johor Strait, Singapore, 0 m depth, 20 October 2012.

Description. Holotype adult female. Body (Fig. 1A) 1226 long, typical cyclopiform, dorsoventrally depressed. Cephalothorax ovoid (Fig. 1A), length almost same as width, 795 × 843, with paired pointed posterolateral corners, and composed by fusion of cephalosome and first pedigerous somite. Second to fourth pedigerous somites and urosomites free, progressively narrower posteriorly; third pedigerous somite with paired depressions on posterior margin, broadened posteriorly and in dorsal view overlapping anterior part of fifth pedigerous somite. Fourth pedigerous somite not visible in dorsal view of body. Genital double-somite (Fig. 1B) wider than long, 155 × 227, and widest at posterior third, bearing paired pointed conical processes (Fig. 2G). Abdomen 99 long, composed of three free somites. Caudal ramus (Fig. 1B, C), length almost same as width, 34 × 35, with six naked setae. Egg sacs (Fig. 1A) multiseriate.

Antennule (Fig. 1D) seven-segmented; armature formula 4, 13, 6, 3, 5, 2 + 1 asethetasc, 7 + 1 aesthetasc; all setae naked, except 1 posterodistal seta of sixth segment and 3 setae on posterior margin of distal segment plumose. Antenna (Fig. 1E) four-segmented, composed of coxobasis and 3-segmented endopod; coxobasis bearing one distal naked seta; first endopodal segment rod-like, longer than coxobasis, and armed with one medial naked seta; second endopodal segment with three naked setae on anterior margin; terminal endopodal segment armed with one seta on sub-terminal margin and two claw-like spines and four naked setae on distal tip. Labrum (Fig. 1F) broad, bearing concave middle region of posterior margin with paired lateral protrusions. Mandible (Fig. 1G) one-segmented, with deep proximal notch and elongated distal lash; inner margin with row of long spinules; outer margin bearing one elongate lappet armed with rows of spinules. Paragnath (Fig. 1H) quadrate, unarmed. Maxillule (Fig. 1I) represented by simple lobe armed with two small and one long, naked setae. Maxilla (Fig. 1J) indistinctly two-segmented, composed of syncoxa and basis; syncoxa unarmed; basis bearing one seta on posterior surface, one long spinose seta, and terminal lash armed with row of long spinules along one side. Maxilliped (Fig. 2A) three-segmented, composed of unarmed syncoxa, and armed basis and endopod; middle segment (basis) bearing two unequal, naked setae; terminal segment (endopod) terminating in pointed apical process, bearing one small seta and one spiniform seta.

Legs 1 to 4 (Fig. 2B–E) biramous; legs 1-3 with three-segmented rami; leg 4 with three-segmented exopod and two-segmented endopod. Leg armature formula as shown in Table 1.

Intercoxal sclerite of legs 1 to 4 unarmed. Coxa of legs 1 to 3 bearing one row of spinules on outer distal tip. Basis of legs 1 to 3 bearing single row of hairs along inner margin; outer seta on basis of leg 3 strongly reduced, setule-like (Fig. 2D). Exopod of legs 1 to 4 bearing plumose setae and stout, serrated spines with single subterminal flagellum; inner margin of each segment with row of hairs; proximal and middle segment with serrated outer margin; proximal and middle segment of legs 1 to 4 bearing 2 projections on outer margin, except proximal segment of leg 1 without projection; terminal segment bearing 3 outer projections on legs 1 and 4 and 4 processes on legs 2 to 3. Endopod of legs 1 to 4 bearing plumose setae and serrated spines; middle and terminal segments of legs 1 to 4 bearing row of hair along inner margin; proximal and second segment of legs 1 to 4 with 1 outer projection; outer margins of terminal segments

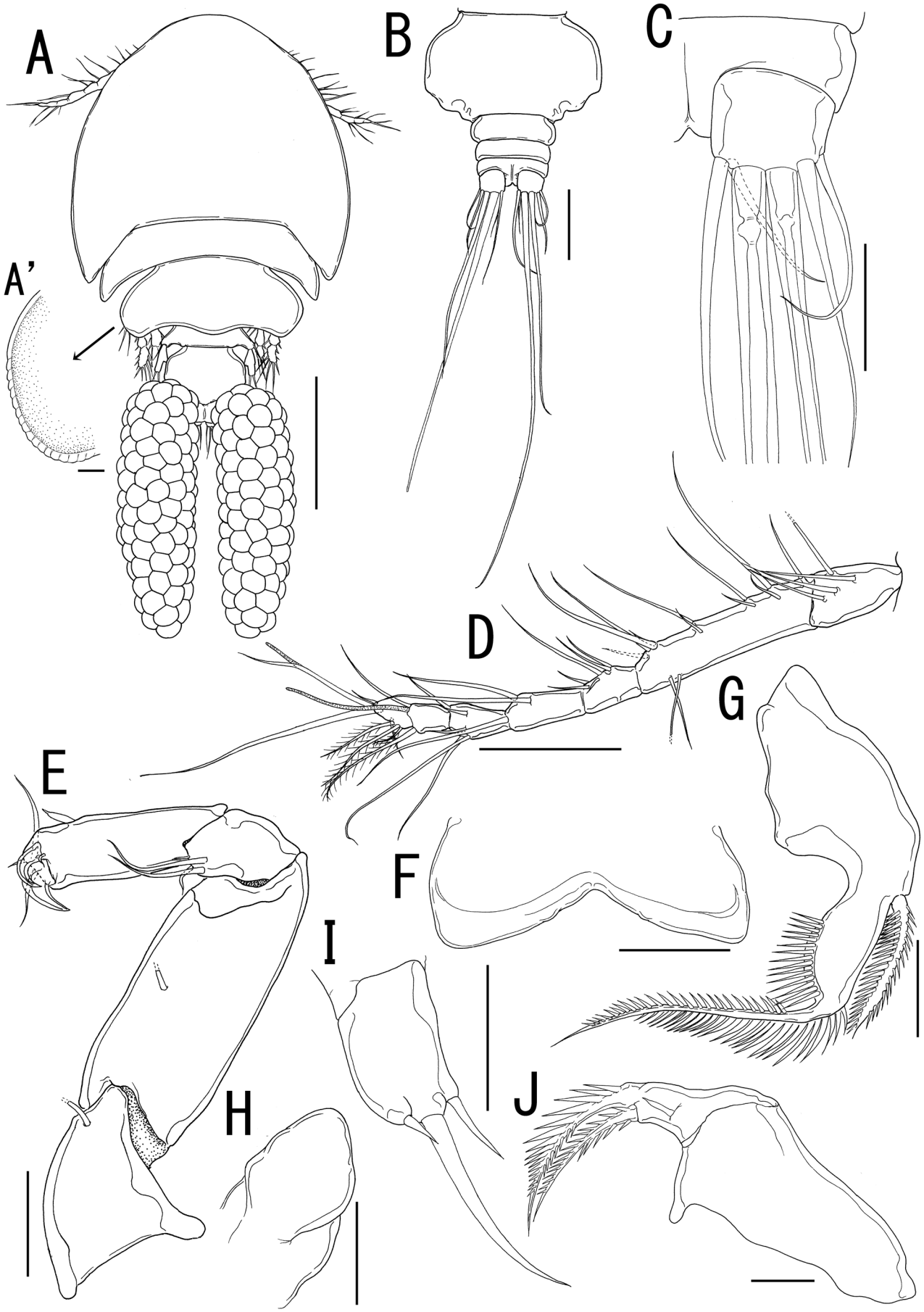

Fig. 1. *Pennatulicola robustclavus* new species, holotype female (ZRC 2015.0009). A, habitus, dorsal; A', magnified view of lateral edge of third pedigerous somite; B, urosome, ventral; C, left caudal ramus, ventral; D, right antennule, posterior; E, left antenna, anterior; F, labrum; G, left mandible; H, left paragnath; I, left maxillule; J, left maxilla. Scale bars = 400 μm [A]; 100 μm [B, D]; 40 μm [C, F]; 50 μm [E]; 20 μm [G–J].

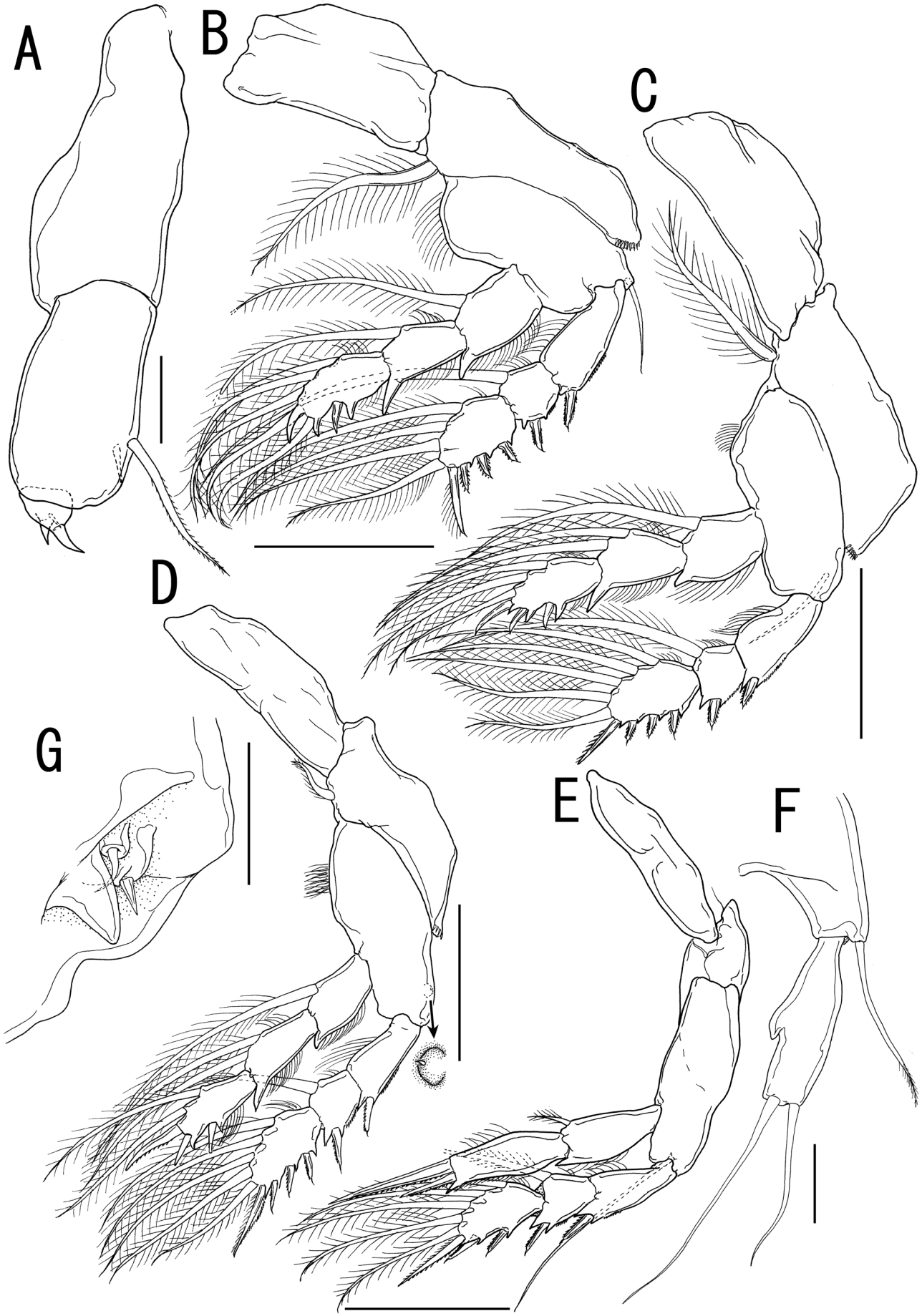

Fig. 2. *Pennatulicola robustclavus* new species, holotype female (ZRC 2015.0009). A, left maxilliped; B, left leg 1, anterior; C, left leg 2, anterior; D, left leg 3, anterior; E, left leg 4, anterior; F, right leg 5, dorsal; G, genital apparature and leg 6. Scale bars = 20 μm [A]; 100 μm [B–E]; 40 μm [F–G].

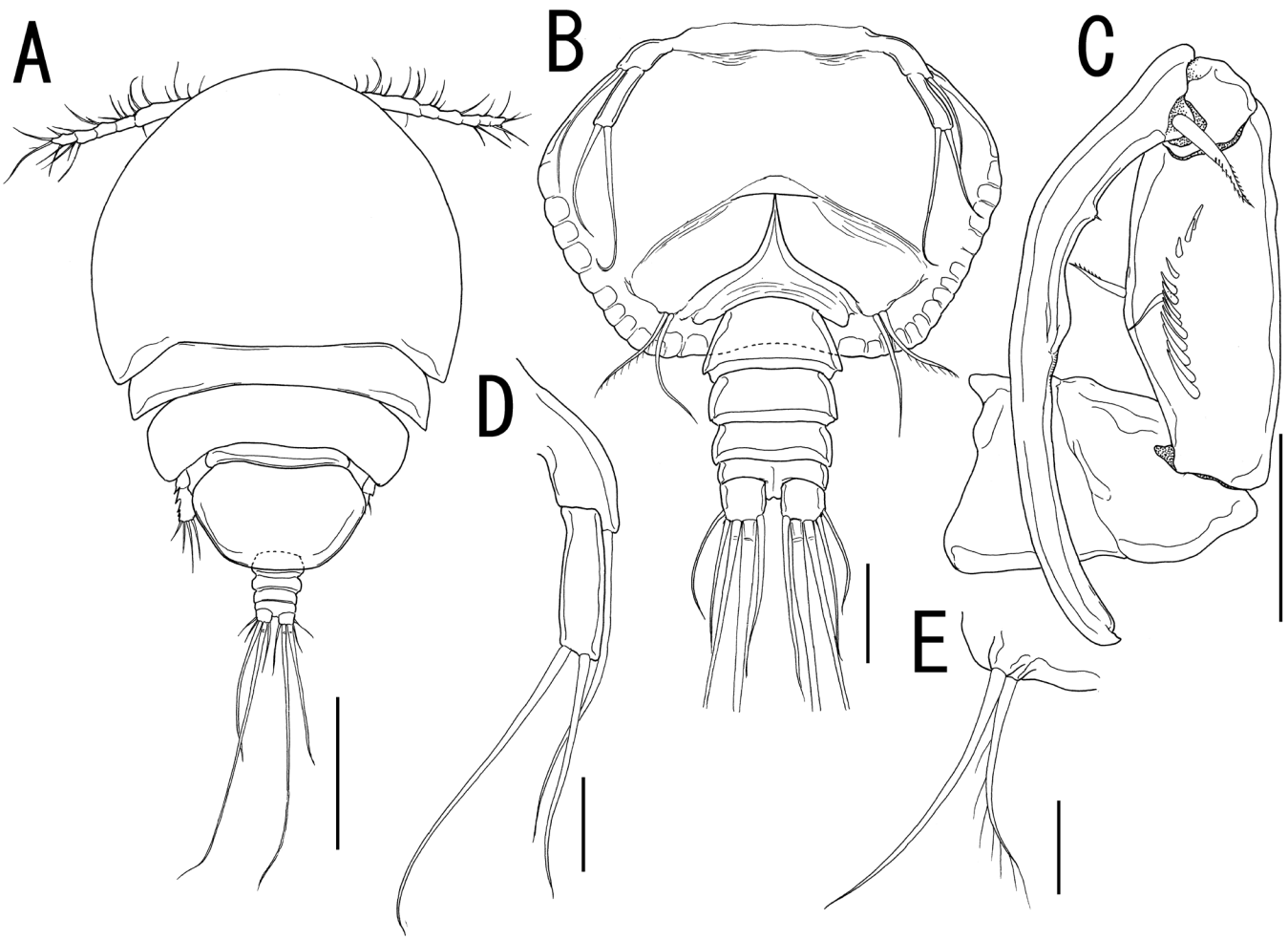

Fig. 3. *Pennatulicola robustclavus* new species, allotype male (ZRC 2015.0010). A, habitus, dorsal; B, urosome, ventral; C, left maxilliped; D, left leg 5, ventral; E, left leg 6. Scale bars = 200 μm [A]; 50 μm [B]; 40 μm [C]; 20 μm [D–E].

bearing 3 projections on legs 1 to 2 and 2 projections on leg 3. Leg 5 (Fig. 2F) composed of outer plumose seta on somite and free segment (exopod) bearing one median blunt process on inner margin and two long naked distal setae. Leg 6 (Fig. 2G) located on posterodorsal surface of genital double somite near one conical pointed projection, composed of two small lobes bearing distal seta and spine, respectively.

Allotype adult male: Body (Fig. 3A) 719 long. Cephalothorax (Fig. 3A) ovoid, wider than long, 409 × 424, with paired pointed posterolateral corners, and composed of cephalosome and first pedigerous somite. Second to fourth pedigerous somites and urosomites free, narrowing posteriorly. Genital somite (Fig. 3B) wider than long, 140 × 229, hexagonal, and with paired genital opercula located posteroventrally. Abdomen 82 long, composed of four free somites. Caudal ramus length almost same as width, 18 × 18, armed as in female.

Antennule, antenna, mandible, paragnath, maxillule and maxilla as in female. Maxilliped (Fig. 3C) four-segmented, developed as grasping organ; proximal segment (syncoxa) large, unarmed; second segment (basis) bearing two median setae and one row of long spinules; third (first endopodal) segment small-and unarmed; terminal (distal endopodal) segment curved, claw-like, bearing one seta proximally and one small knob on inner margin.

Legs 1 to 4 as in female. Leg 5 (Fig. 3D) consisting of naked outer basal seta and free segment (exopod) bearing two long distal naked setae. Leg 6 (Fig. 3E) represented by 1 long naked and 1 long pinnate setae on genital operculum.

Variability. Measurements of paratype adult female (n = 12) are as follows: body length (excluding caudal setae) 1070–1203 (1129 ± 39); cephalothorax length 707–866 (782 ± 47) and width 770–852 (810 ± 24); genital double-somite length 117–171 (144 ± 18) and width 224–260 (244 ± 11); abdomen length 51–81 (70 ± 8); caudal ramus length 31–40 (35 ± 2) and width 31–43 (34 ± 4).

Measurements of paratype adult male (n = 12) are as follows: body length (excluding caudal setae) 685–720 (696 ± 13); cephalothorax length 383–465 (415 ± 20) and width 433–496 (466 ± 17); genital somite length 143–169 (151 ± 10) and width 202–232 (220 ± 8); abdomen length 79–104 (92 ± 8); caudal ramus length 19–27 (23 ± 2) and width 19–26 (21 ± 2).

Remarks. The genus *Pennatulicola* consists of four known species. The new species differs from three congeners, *Pennatulicola pteroidis* (Della Valle, 1880), *P. pterophilus* (Stock, 1962), *P. corallophilus* Nair & Pillai, 1986, in the combination of following features of the female: the third pedigerous somite overlapping in dorsal view the whole fourth pedigerous somite and the anterior part of the fifth pedigerous somite; middle endopodal segments of legs 1 to 3 bearing a pronounced spiniform projection on the outer margin; leg 5 bearing a free segment with a pointed process on the inner margin (see Della Valle, 1880; Stock, 1962; Humes & Stock, 1973; Nair & Pillai, 1986). The new species shares many characters in common with *Pennatulicola serratipes* (Ummerkutty, 1962) but it differs in the antennule of female with four setae on the proximal segment and the maxilliped of male having a second segment without elevated ridges (see Ummerkutty, 1962).

Attachment site. On the entire body surface of sea pens.

Lamippidae Joliet, 1882

***Enalcyonium* Olsson, 1869**

***Enalcyonium kohsiangi* new species**
(Fig. 4)

Etymology. The new species is named after Dr. Tan Koh Siang who is the organiser of the Comprehensive Marine Biodiversity Survey of Singapore.

Material examined. Holotype: adult female (ZRC 2015.0014), ex *Pteroeides* sp. (Pennatulacea: Pennatulidae), off northern side of Pulau Ubin (1°25.570′N, 103°56.524′E to 1°25.492′N, 103°56.765′E) (DW20), the Johor Strait, Singapore, 10.3–10.6 m depth, 17 October 2012.

Allotype: adult male (ZRC 2015.0015), collection data same as those of holotype.

Paratypes: 4 adult females and 5 adult males (ZRC 2015.0016), collection data same as those of holotype.

Description. Holotype adult female. Body (Fig. 4A, B) fusiform, 1394 long, 315 wide, 302 thick, with great width and thickness at middle. Cephalic area (Fig. 4A, C) narrowed anteriorly with conical rostral area and dorsal chitinous frame composed of two angled and one short transverse bars. Genital area (Fig. 4D) projected with round posterior margin. Caudal rami (Fig. 4A, B, E) longer than wide, 66 × 36, tapering posteriorly with one basal and three distal naked setae of similar size and shape.

Antennule (Fig. 4F) unsegmented, bearing seven naked setae on anterior margin, two naked setae on subterminal, and two naked medial and one naked large setae at distal part. Antenna (Fig. 4G) four-segmented, composed of coxobasis and three-segmented endopod; coxobasis and first endopodal segment rod-like, unarmed; second endopodal segment with one naked distal seta; terminal claw (third endopodal segment) incurved. Mandibles, paragnaths, maxillules, maxillae and maxillipeds absent.

Legs 1 to 2 (Fig. 4H, I) biramous, composed of unsegmented exopod with three claw-like spines and lobe-like endopod; outer two exopodal spines larger than innermost one, with distal flagellum. Endopod of leg 2 with one naked seta. Intercoxal sclerite absent in legs 1 and 2.

Allotype adult male: Body (Fig. 4J, K) fusiform, 1418 long, 316 wide, 297 thick, with great width and thickness at 3/5. Cephalic area as in female. Opercula (Fig. 4K) present at genital area. Caudal rami (Fig. 4J, K) longer than wide, 114 × 39, tapering posteriorly, with one basal and three distal naked setae.

Antennule, antenna, and legs 1 to 2 as in female. Mandibles, paragnaths, maxillules, maxillae, and maxillipeds absent.

Variability. Measurements of paratype adult female (n = 4) are as follows: body length (excluding caudal setae) 1185–1526 (1355 ± 143), width 267–397 (321 ± 55) and thickness 267–313 (287 ± 21); caudal ramus length 51–102 (75 ± 21) and width 20–39 (29 ± 9).

Measurements of paratype adult male (n = 5) are as follows: body length (excluding caudal setae) 1348–1576 (1482 ± 83), width 247–316 (276 ± 26) and thickness 247–333 (286 ± 31); caudal ramus length 91–120 (109 ± 12) and width 34–52 (43 ± 8).

Remarks. The new species has legs 1 to 2 each bearing three spines on the exopod. This armatures on the legs are shared with five congeners, *E. auriculatum* Kim, 2004, *E. ciliatum* Stock, 1972, *E. carrikeri* Dudley, 1973, *E. heegaardi* Bouligand, 1960, *E. nudum* Stock, 1973. *Enalcyonium ciliatam* and *E. carrikeri* differs from the new species in the presence a process between bases of slender caudal rami (see Stock, 1972; Dudley, 1973). *E. heegaardi* is differentiable from the new species by having a paired frontal processes (see Heegaard, 1949). Legs 1 and 2 of the new species, *E. auriculatum* and *E. nudum* have three spines but the innermost spine is the smallest in the new species (vs. the outermost one is the smallest in *E. auriculatum* and *E. nudum*) (see Stock, 1973; Kim, 2004). Although the number of elements on legs 1 and 2 has not described in *E. albidum* (de Zulueta, 1908), the caudal ramus in *E. albidum* is armed with two subterminal and two terminal setae (vs. one basal and three terminal setae in the new species, Fig. 4E) (see de Zulueta, 1908).

Attachment site. Gastrovascular cavity of sea pens.

DISCUSSION

So far, all described species of *Pennatulicola* have been found from pennatulid sea pens (Octocorallia: Pennatulacea) and a poritid stony coral (Hexacorallia:Scleractinia) from Indian and Indonesian waters as well as the Mediterranean (Humes & Stock, 1973; Nair & Pillai, 1986). *Pennatulicola*

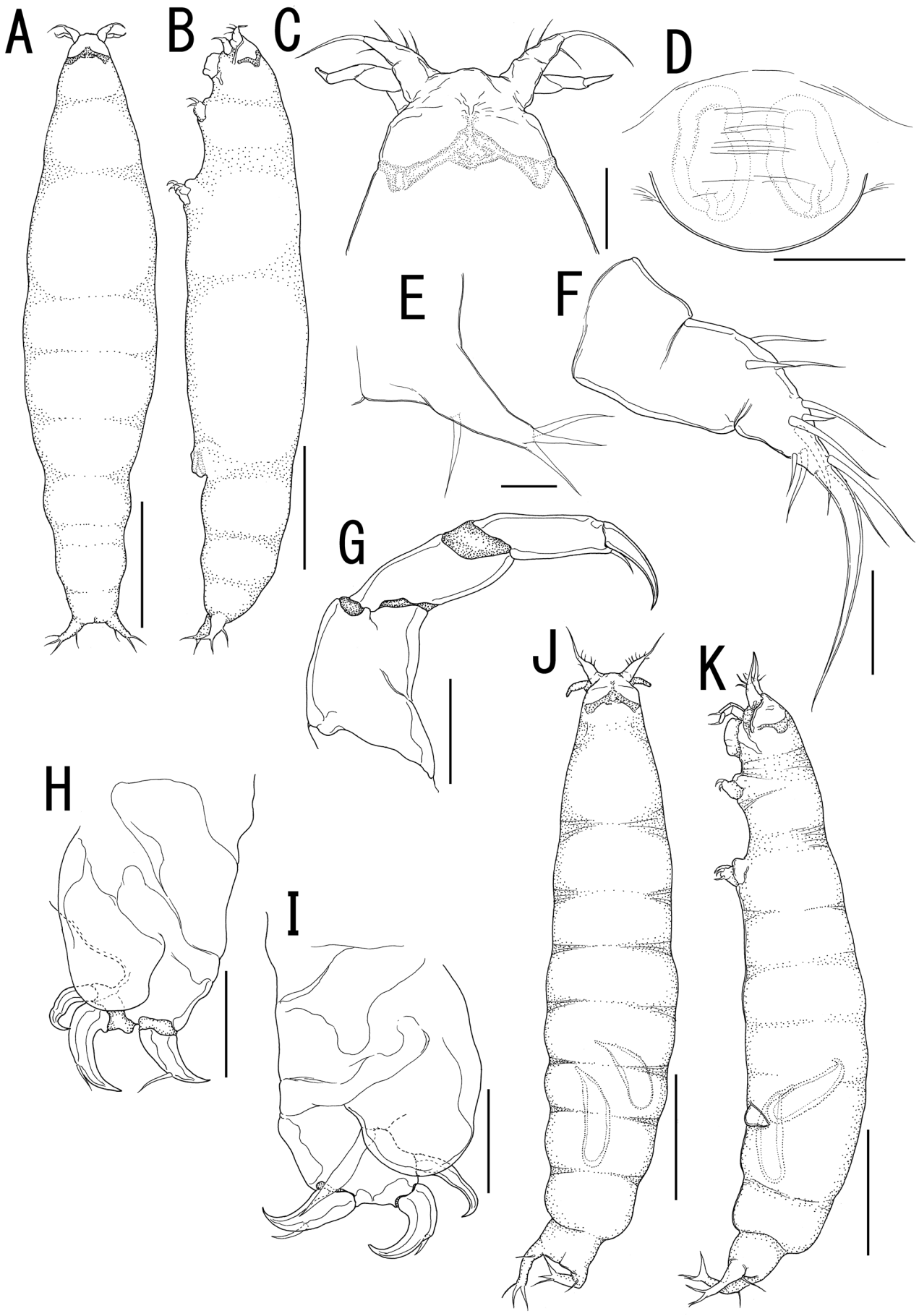

Fig. 4. *Enalcyonium kohsiangi* new species, holotype female (ZRC 2015.0014) (A–I) and allotype male (ZRC 2015.0015) (J–K). A, habitus, dorsal; B, habitus, lateral; C, anterior part of body, dorsal; D, genital area; E, left caudal ramus, ventral; F, left antennule, posterior; G, left antenna; H, right leg 1, posterior; I, left leg 2, posterior; J, habitus, dorsal; K, habitus, lateral. Scale bars = 300 μm [A, B, J, K]; 50 μm [C–D]; 30μm [E]; 20 μm [F–I].

robustclavus new species, was also found from the pennatulid sea pen, *Pteroeides* sp. from Singaporean waters. The discovery of the new species represents a new record of the genus from the North Pacific Ocean. According to the key to genera of Rhynchomolgidae created by Boxshall & Halsey (2004), *Pennatulicola* differs from *Doridicola* Leydig, 1853 by having the mandible with a spinose lappet and the antennule bearing three setae on the first segment. Therefore, the presence of the four setae on the antennules is considered to be one of characteristic features of the new species. Since descriptions of known species include some insufficient and doubtful parts, re-descriptions of congeners are needed.

Key to the species of *Pennatulicola*

1. Fourth pedigerous somite visible in dorsal view of female; armature formula of third exopodal segment of leg 4 III,I, 5; free segment of leg 5 of female without dentation or swelling..... .....*P. corallophilus*
– Third pedigerous somite overlapping in dorsal view fourth pedigerous somite and anterior part of fifth pedigerous somite; armature formula of third exopodal segment of leg 4 II, I, 5; free segment of female leg 5 with process or swelling.....2
2. Third pedigerous somite of female overlapping in dorsal view fifth pedigerous somite and anterior part of genital complex.. 3
– Third pedigerous somite of female not overlapping in dorsal view anterior part of genital complex 4
3. Middle endopodal segments of female legs 1 to 3 with two pointed projections on outer margin; free segment of leg ...5 with obtuse dentation or swelling at posterior two-thirds ....*P. pterophilus*
– Middle endopodal segments of female legs 1 to 3 with one pointed projection on outer margin; free segment of leg 5 with dentation at middle.....*P. pteroidis*
4. Proximal segment of antennule with four setae; second segment of male maxilliped with an elevated ridge subdistally ....*P. serratipes*
– Proximal segment of antennule with three setae; second segment of male maxilliped without such ridge..... .....*P. robustclavus* new species

Species of *Enalcyonium* are endoparasites of sea fans (Octocorallia: Alcyonacea) and sea pens (e.g., de Zulueta, 1908; Humes, 1957; Dudley, 1973; Ho, 1984; Stock, 1972, 1973, 1988; Boxshall & Halsey, 2004; Kim, 2004, 2007, 2009). Most congeners were found from sea fans, and only three species (*E. albidum*, *E. concinnum*, *E. kohsiangi* new species) are known from sea pens (de Zulueta, 1908; Humes, 1957; present study). The genus has a wide geographical distribution throughout the world's oceans from near the Arctic Circle to tropical waters, and range of depth extends from the intertidal to bathypelagic zones (e.g., Heegaard, 1949; Kim, 2004). In addition, from facts that *Enalcyonium* spp. infest many soft coral species and species diversity of soft coral is extremely high in tropical Asian waters surrounding Singapore, the presence of a vast number of potentially undescribed species is expected. Since the genus has highly transformed body and its appendages are reduced or totally absent (Boxshall & Halsey, 2004), species identification is often difficult. Thus, the number and shape of spines on legs 1 to 2 and the shape and position of setae on the caudal rami are very helpful characters for species identification. The presences of processes on the rostral area and between caudal rami are considered to be important characters as well.

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