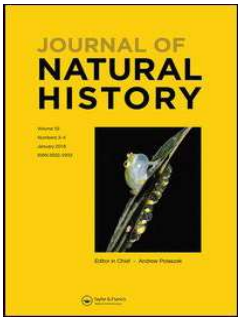

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Two new species of Siphonostomatoida (Copepoda) found on cnidarians in Tokara Islands, Southern Japan

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ABSTRACT

Two new species of siphonostomatoid copepods are described based on specimens collected off Tokara Islands located in the northern part of the Ryukyu Islands, Southern Japan. *Cholomyzon multisetum* sp. nov. (Coralliomyzontidae) found from the orange cup coral *Tubastraea coccinea* Lesson, 1829 (Hexacorallia: Scleractinia: Dendrophylliidae) has the following characters: armature formula of the antennule of the female; the outer lobe of the maxillule; and numbers of elements on the legs 1 to 3. Another species, *Entomopsyllus takara* sp. nov. (Entomolepididae), was found from both the Indo-Pacific blue coral *Heliopora coerulea* (Pallas, 1766) (Octocorallia: Helioporacea: Helioporidae) and the race coral *Distichopora violacea* (Pallas, 1766) (Hydrozoa: Anthoathecata: Stylasteridae). This new species of the male specimen is characterized by the antennule with shortest sixth segment, the endopod of leg 2 bearing two inner setae on the second segment and five setae on the third segment, the third exopodal and endopodal segments of leg 3 bearing three spines and three setae, respectively, and the third exopodal segment of leg 4 bearing four setae. These two species represented the first records of the families Coralliomyzontidae and Entomolepididae from Japanese waters.

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KEYWORDS
Coralliomyzontidae; Entomolepididae; scleractinian coral; hydrozoan coral; octocoral

Introduction

Symbiotic copepods use many diverse animals as their hosts. According to Humes (1985), among invertebrates, Cnidaria is one of the most frequently recorded hosts for copepods, being reported on 160 genera and 350 species. Of the total amount of 416 copepod species associated with cnidarians, 31 species belong to Siphonostomatoida (Humes 1985). Further, more recently, Cheng et al. (2016) recognized 68 species of siphonostomatoids associated on scleractinian corals. During the last three decades, several new siphonostomatoid species were described exploring this living environment. The siphonostomatoid family, Coralliomyzontidae Humes and Stock, 1991, was originally established to accommodate *Coralliomyzon* Humes and Stock, 1991 (Humes and Stock 1991). Despite its similarities with the Asterocheridae Giesbrecht, 1899, the family shows many differences in the maxilliped with

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2-segmented endopod, absence of the exopod of the antenna, legs 4 and 5 reduced into a protuberance bearing one or two setae, and absence of the inner seta on the first and second exopodal segments of leg 1. Such characteristics are also occurred in *Cholomyzon* Stock and Humes, 1969, the genus originally placed in Asterocheridae and then moved to Coralliomyzontidae (see Stock and Humes 1969; Humes and Stock 1991). Later on, Humes (1997a) described two genera, *Temanus* Humes, 1997 and *Tondua* Humes, 1997, both assigned to Coralliomyzontidae. However, Boxshall and Halsey (2004) relegated the family to a junior synonym of Asterocheridae since its characters were considered to be highly apomorphic. Therefore, Coralliomyzontidae represents a derived terminal branch of the Asterocheridae. Consequently, it would be pending a complete phylogenetic revision in order to restore the concept of Coralliomyzontidae or to bring a new proposal. In the most recent taxonomic study regarding to Coralliomyzontidae by Cheng et al. (2011), including the description of *Cholomyzon tubastraeae* Cheng, Dai and Chang, 2011, the authors seem to hesitate to adopt the position of the family. Mahatma et al. (2008) refereed that the revision of morphological study and the phylogenetic analysis are required to resolve the phylogenetic relationship among Coralliomyzontidae, Asterocheidae, and Brychiopontiidae Humes, 1974. Hence, we keep Coralliomyzontidae as a valid family in the present study.

Another siphonostomatoid family Entomolepididae Brady, 1899 was established (as Entomolopidae) based only on *Entomolepis* Brady, 1899, and it is currently composed of seven genera and 15 species. Among them, eight species were known from sponges and few species, e.g. *Entomopsyllus stocki* Kim, 2004 and *Spongiopsyllus redactus* Canário, Neves and Johnsson, 2012, were found from cnidarians (Boxshall and Halsey 2004; Kim 2004; Canário et al. 2012; Lee and Kim 2017). The family also share many plesiomorphic characteristics with Asterocheridae, and thus the phylogenetical revision of Asterocheridae is urgently needed.

In this study, two new species, *Cholomyzon multisetum* sp. nov. (Coralliomyzontidae) and *Entomopsyllus takara* sp. nov. (Entomolepididae), are described based on specimens collected from cnidarians off Tokara Islands, Southern Japan. A key to species of *Cholomyzon* and *Entomopsyllus* McKinnon, 1988 are provided.

Materials and methods

Colonies of cnidarians (about 10–15 cm in diameter) were collected by snorkelling and SCUBA diving in coastal waters during cruises using the TR/V *Nansei Maru* of Kagoshima University in August and September 2015. The samples were rinsed with tap water and filtered in a 100-µm mesh. Copepods were carefully sorted under a binocular and then fixed in 80% ethanol. Subsequently, they were soaked in lacto-phenol for about 24 h, dissected by sharpened tungsten needle under a binocular, and examined using a modified version of the wooden slide method by Humes and Gooding (1964) under a compound microscope. Drawings were made with the aid of a drawing tube. Body parts of copepods were measured using an ocular micrometer, and measurements are given in µm. Specimens examined are deposited in the crustacean collection (Cr) of the National Museum of Nature and Science, Tsukuba (NSMT), Japan.

Taxonomy

Family **CORALLIOMYZONTIDAE** Humes and Stock, 1991
Genus ***Cholomyzon*** Stock and Humes, 1969
Cholomyzon multisetum sp. nov.
(Figures 1, 2)

Type material

Holotype adult female (NSMT-Cr 25851), *ex Tubastraea coccinea* Lesson, 1829 (Hexacorallia: Scleractinia: Dendrophylliidae), in Nishinohama Port (29°59′N, 129°54′E), Kuchino-shima Island, Tokara Islands, Japan, 2 m depth, 28 August 2015, leg. D. Uyeno. Paratype: 1 adult female (NSMT-Cr 25852), sampling data same as holotype.

Description of adult female

Body (Figure 1(a)) 896 long. Prosome 637 long, comprising cephalothorax and three free thoracic somites. Cephalothorax (Figure 1(a)) round, wider than long, 518 × 593, flattened dorsoventrally, with pair of protruded posterolateral corner. Second to fourth pedigerous somites (Figure 1(a)) wider than long; fourth pedigerous somite bearing pair of round posterolateral lobes with median notch (Figure 1(c)). Urosome comprising fifth pedigerous somite, genital double somite and abdominal somites. Genital double somite (Figure 1(a)) wider than long, 113 × 176. Abdomen composed of two free post genital somites, 77 × 95 and 70 × 68, respectively. Caudal ramus (Figure 1(b)) 0.80 times long than wide, 18 × 23, with six setae.

Rostral area with round margin without apex. Antennule (Figure 1(d)) 11-segmented, armature formula 1 + 1 aesthetasc, 1 + 1 aesthetasc, 11 + 1 aesthetasc, 3 + 1 aesthetasc, 4 + 1 aesthetasc, 1 + 1 aesthetasc, 2 + 1 aesthetasc, 3, 2, 3, and 7 + 1 aesthetasc; all setae naked. Antenna (Figure 1(e)) composed of coxa, basis, and 2-segmented endopod; coxa unarmed; basis bearing row of hairs; proximal endopodal segment bearing row of spinules and distal segment with terminal slightly curved claw. Mandible (Figure 1(f)) biramous; elongate stylet bearing 13 teeth on distal tip (Figure 1(g)); slender 1-segmented palp bearing long seta with row of fine spinules on inner margin. Oral cone (Figure 1(a)) conical. Maxillule (Figure 1(h)) bilobed with inner lobe (endite) bearing four distal setae and row of spinules and outer lobe (palp) bearing three setae. Maxilla (Figure 2(a)) composed of syncoxa and claw; syncoxa robust, unarmed; claw proximally robust, slender and elongate after patch of hair medially located, row of spinules posteriorly. Maxilliped (Figure 2(b)) subchelate, comprising syncoxa, basis, and unsegmented endopod bearing single median and distal elements and claw.

Legs 1 to 3 (Figure 2(c–e)) biramous; both rami bearing 3-segmented rami. Leg armature formula as follows:

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

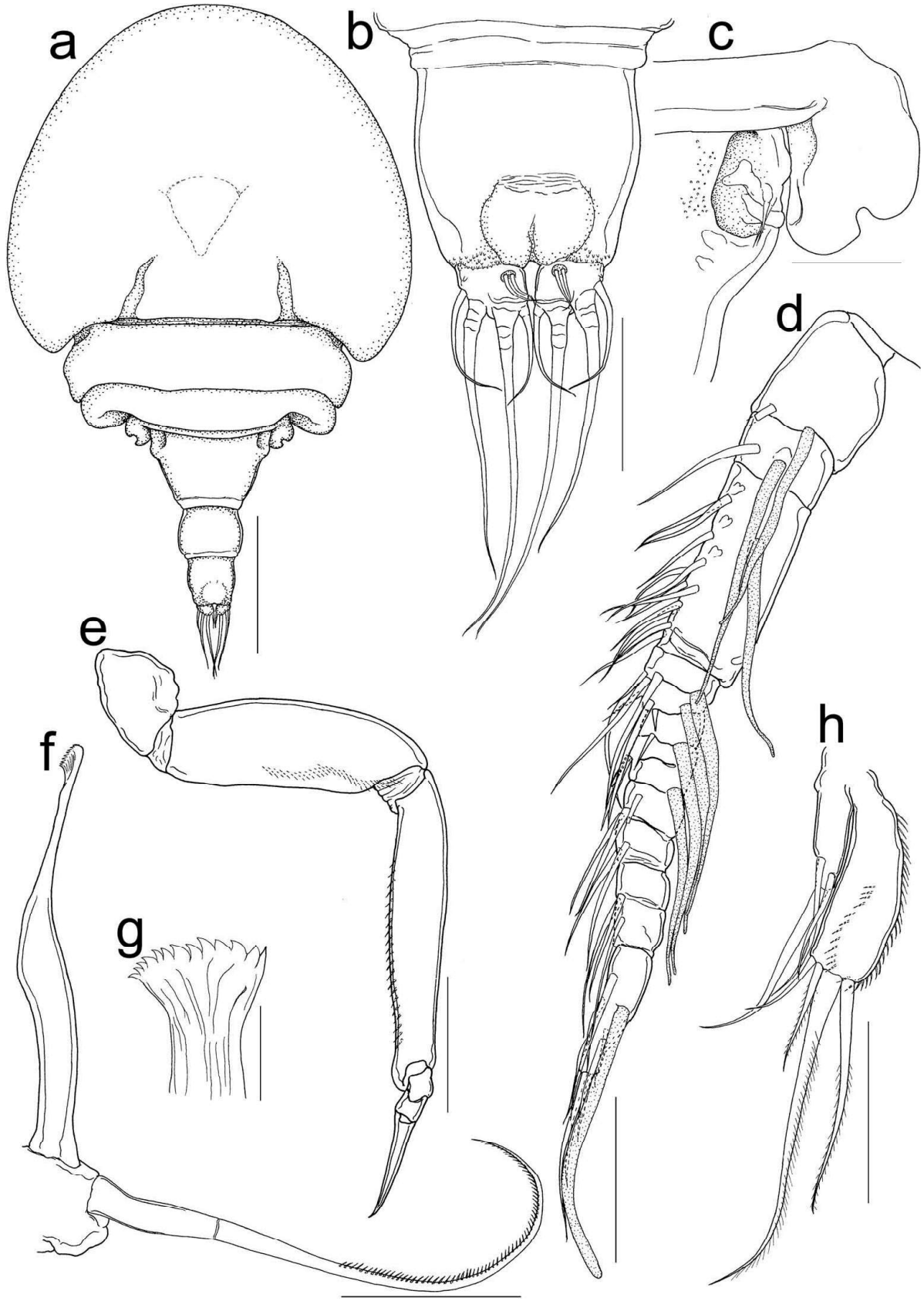

Figure 1. *Cholomyzon multisetum* sp. nov., adult female, NSMT-Cr 25851. (a) habitus, dorsal; (b) abdomen and caudal rami, dorsal; (c) right genital opening, dorsal; (d) right antennule, posterior; (e) left antenna, anterior; (f) right mandible, anterior; (g) distal teeth of left mandible; (h) right maxillule, posterior. Scale bars: (a) 200 µm; (b, d, e, f, h) 50 µm; (c) 30 µm; (g) 10 µm.

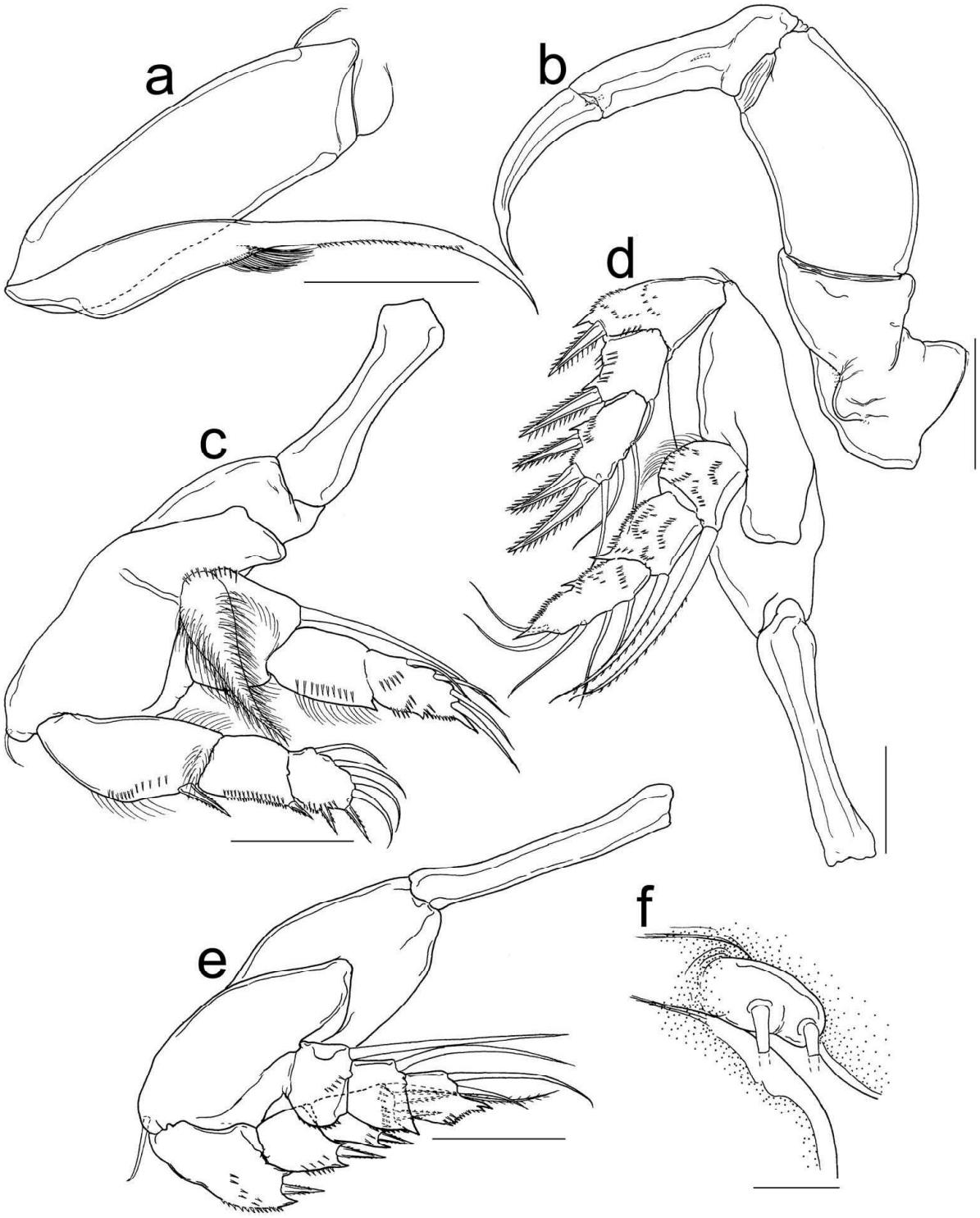

Figure 2. *Cholomyzon multisetum* sp. nov., adult female, NSMT-Cr 25851. (a) right maxilla, posterior; (b) right maxilliped, anterior; (c) right leg 1, anterior; (d) right leg 2, anterior; (e) right leg 3, anterior; (f) left leg 5, ventral. Scale bars: (a, b) 50 µm; (c–e) 30 µm; (f) 10 µm.

Intercoxal sclerites (Figure 2(c–e)) of legs 1 to 3 unarmed. All spines bearing serrated margins. Basis of leg 1 bearing row of spinules on base of thick seta and inner protrusion. Both rami of legs 1 to 3 bearing rows of spinules on outer margins. Leg 1 (Figure 2(c)) bearing distal pointed process on outer margin of

second and third endopodal segments. Legs 2 and 3 (Figure 2(d,e)) bearing pointed process on outer margins of all exopodal and second and third endopodal segments. Third endopodal segments of legs 1 to 3 (Figure 2(c–e)) bearing medial pointed processes on position of outer seta. Leg 4 absent. Leg 5 (Figure 2(f)) represented by constricted protrusion with two setae. Leg 6 (Figure 1(c)) represented by two protrusions with distal seta.

Variability

The morphology of the female paratype as in the holotype. The measurements of the body parts (n = 1) are as follows: body length 847; cephalothorax length 487; cephalothorax width 574; prosome length 620; genital double somite length 112; genital double somite width 150; first urosomite length 77; first urosomite width 103; anal somite length 63; anal somite width 72; caudal ramus length 15; caudal ramus width 23. Caudal ramus 0.66 times longer than wide.

Remarks

Cholomyzon multisetum sp. nov. differs from *C. brevisetigerum* Humes, 1997 and *C. tubastraeae* by the following: third, fourth and fifth segment of the antennule of the female bearing 11, three, and four setae, respectively (vs. seven, two, and two setae); the maxillule bearing the outer lobe with four setae (vs. three setae); distal endopodal segments of legs 2 and 3 bearing five and two elements, respectively (vs. six and three elements, respectively) (see Humes 1997b; Cheng et al. 2011). The new species is also differentiated from *C. palpiferum* Stock and Humes, 1969 by the following characters: absence of leg 4 (vs. represented by thumb-shaped process with two setae); distal endopodal segments of legs 2 and 3 bearing five and two elements, respectively (vs. six and three elements) (see Stock and Humes 1969).

Etymology

The specific name of the new species '*multisetum*' refers to the antennule of the new species bearing more setae than congeners.

Newly established Japanese name

'Iboyagi-gakure-kudakuchi-mijinko-ka' for the family, 'Iboyagi-gakure-kudakuchi-mijinko-zoku' for the genus, and 'Iboyagi-gakure-kudakuchi-mijinko' for the species.

Family **ENTOMOLEPIDIDAE** Brady, 1899
Genus ***Entomopsyllus*** McKinnon, 1988
Entomopsyllus takara sp. nov.
(Figure 3–5)

Type material

Holotype adult male (NSMT-Cr 25853), *ex Heliopora coerulea* (Pallas, 1766) (Octocorallia: Helioporacea: Helioporidae), off Maegomori Port (29°9′N, 129°11′E), Takara Island, Tokara Islands, Japan, 10 m depth, 17 September 2015, leg. D. Uyeno. Paratype: 1 adult male

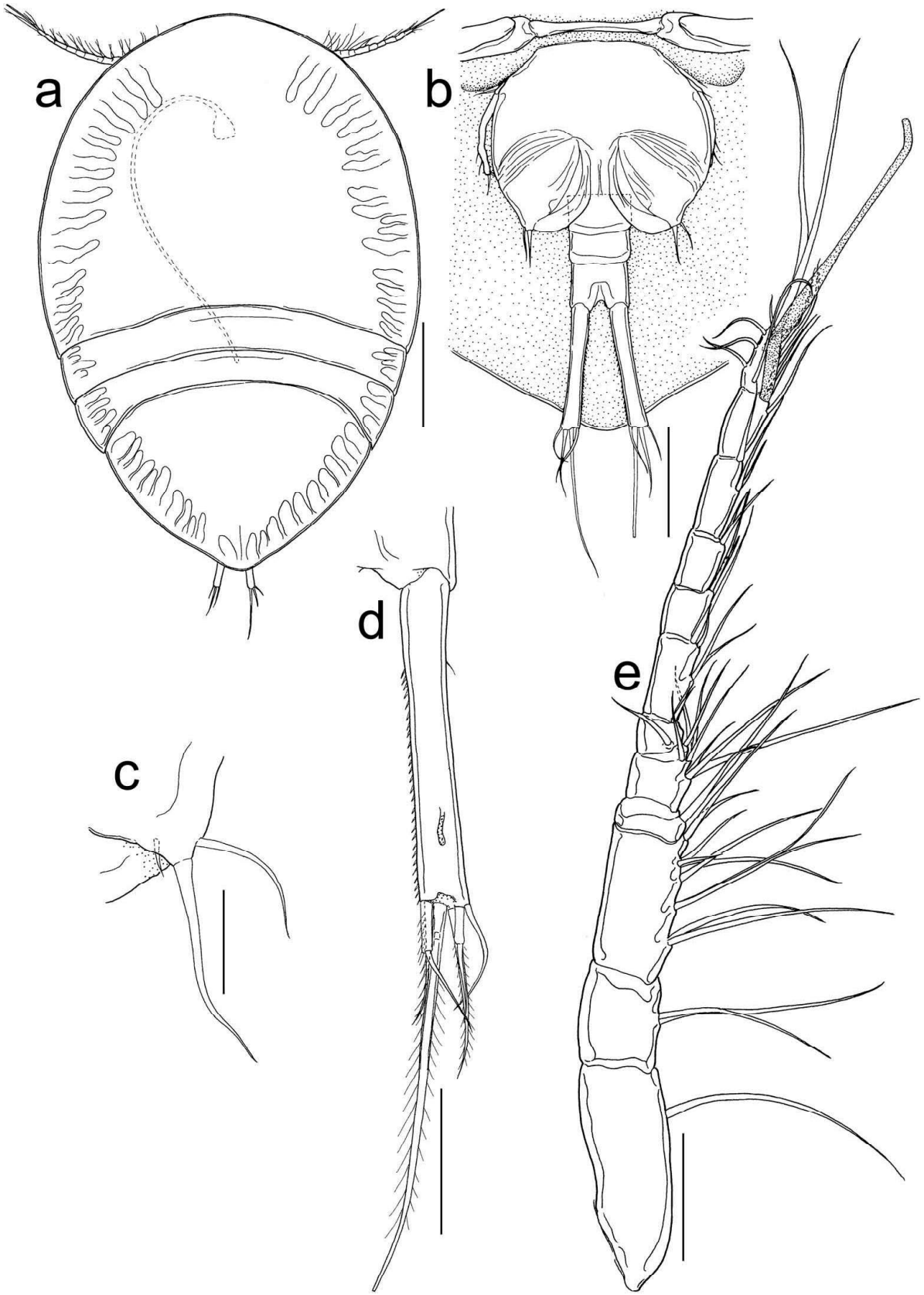

Figure 3. *Entomopsyllus takara* sp. nov., adult male, NSMT-Cr 25853. (a) habitus, dorsal; (b) urosome, ventral; (c) left leg 6; (d) right caudal ramus, dorsal; (e) right antennule, posterior. Scale bars: (a) 200 µm; (b) 80 µm; (c, e) 20 µm; (d) 50 µm.

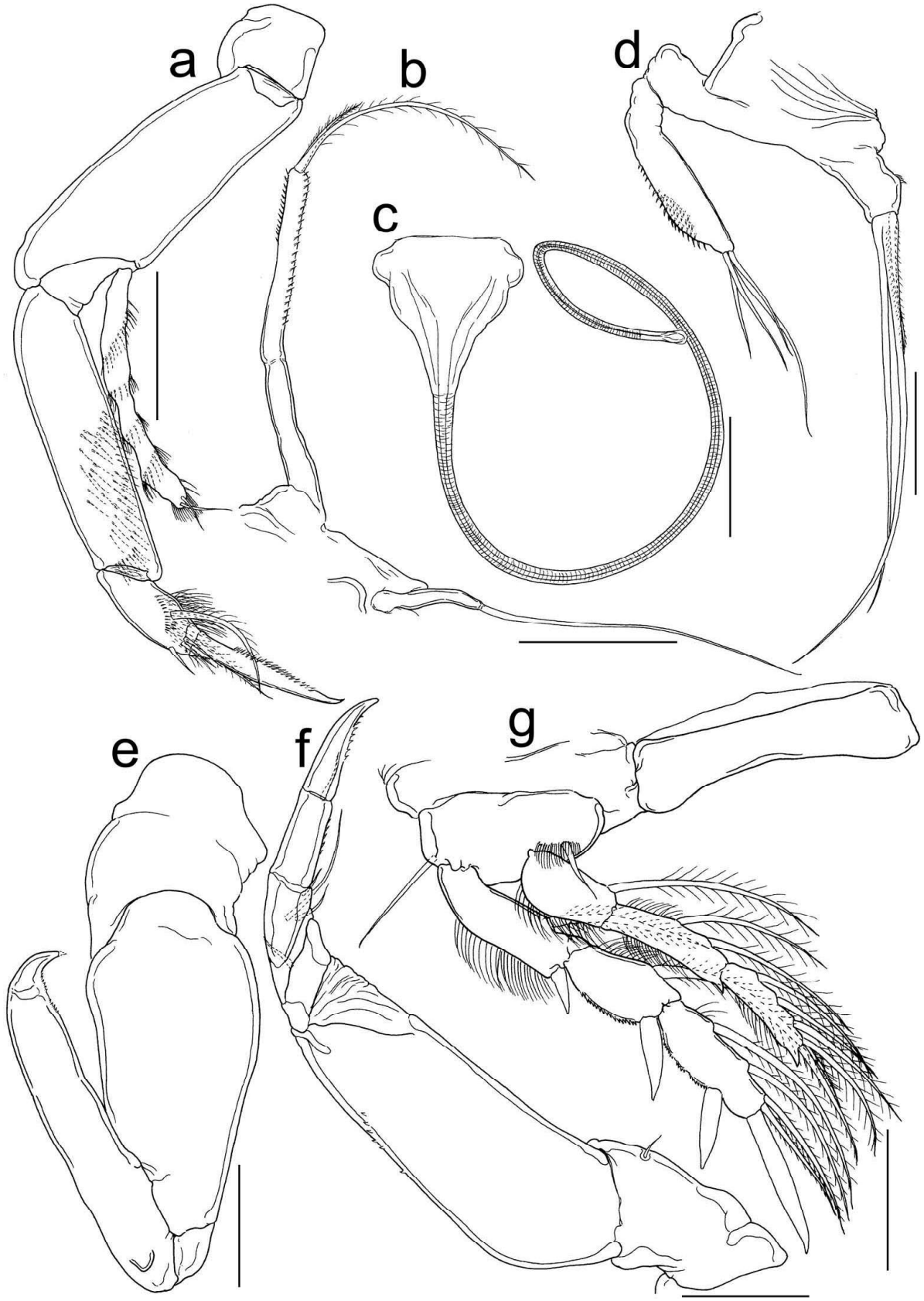

Figure 4. *Entomopsyllus takara* sp. nov., adult male, NSMT-Cr 25853. (a) right antenna, anterior; (b) right mandible, posterior; (c) oral cone; (d) left maxillule, posterior; (e) right maxilla, posterior; (f) left maxilliped, anterior; (g) right leg 1, anterior. Scale bars: (c) 80 µm; (b) 50 µm; (a, d) 40 µm; (e–g) 50 µm.

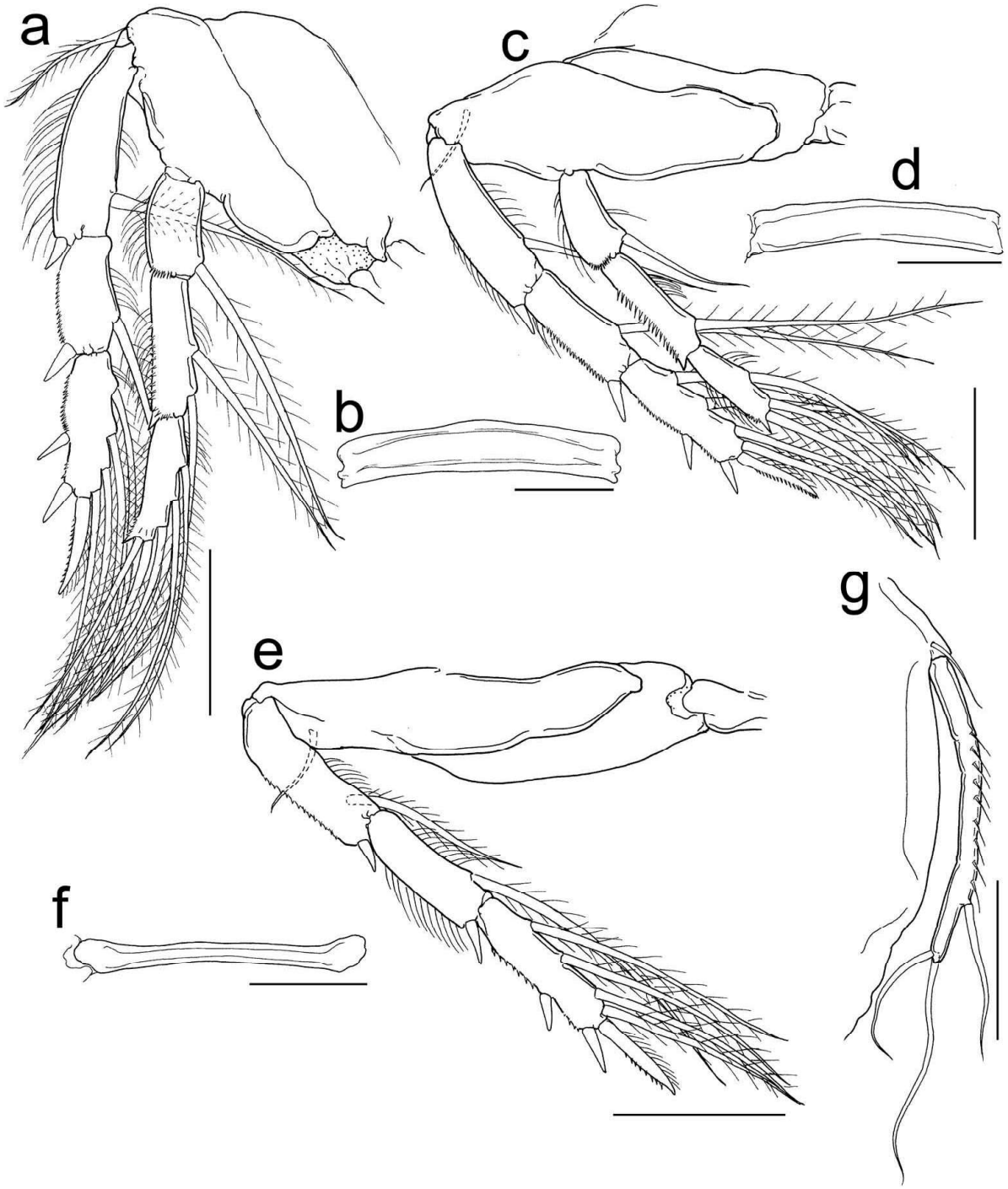

Figure 5. *Entomopsyllus takara* sp. nov., adult male, NSMT-Cr 25853. (a) right leg 2, anterior; (b) intercoxal sclerite of leg 2; (c) right leg 3, anterior; (d) intercoxal sclerite of leg 3; (e) right leg 4, anterior; (f) intercoxal sclerite of leg 4; (g) right leg 5, dorsal. Scale bars: (a–f) 50 μm; (g) 40 mm.

(NSMT-Cr 25854), *ex Distichopora violacea* (Pallas) (Hydrozoa: Anthoathecata: Stylasteridae), locality and date same as that of holotype.

Description of holotype adult male

Body (Figure 3(a)) 1088 long. Prosome 1053 long, comprising cephalothorax and three free thoracic somites. Cephalothorax (Figure 3(a)), wider than long, 647 × 722, flattened dorsoventrally. Second to fourth pedigerous somites (Figure 3(a)) wider

than long; dorsal plate of fourth pedigerous somite covering urosome and caudal rami. Urosome comprising fifth pedigerous somite fused to genital somite and three postgenital somites. Genital somite (Figure 3(b)) wider than long, 174 × 199. Postgenital somites (Figure 3(b)) 3-segmented, 37 × 57, 30 × 56, and 44 × 53, respectively. Caudal rami (Figure 3(b,d)) 6.53 times long than wide, 115 × 18, with six setae and row of spinules on inner margin.

Rostrum absent. Antennule (Figure 3(e)) 13-segmented, armature formula 1, 2, 10, 2, 6, 2, 2, 4, 2, 2, 3, 3 + 1 aesthetasc, and 10; all setae naked. Antenna (Figure 4(a)) composed of coxa, basis, unsegmented exopod, and 2-segmented endopod; coxa and basis unarmed; exopod rod shaped, bearing several rows of hairs and two distal setae; endopod bearing proximal segment with irregular rows of hair and distal segment with terminal claw and five setae. Mandible (Figure 4(b)) biramous, composed of elongate inner stylet (endite) and 2-segmented outer palp bearing two distal setae. Oral cone (Figure 4(c)) elongate and thin. Maxillule (Figure 4(d)) bilobed with inner lobe (endite) bearing three distal setae; outer lobe (palp) indistinctly 2-segmented, bearing 4 setae with patch of spinules on inner margin. Maxilla (Figure 4(e)) 3-segmented, with syncoxa, basis and endopod; syncoxa and basis unarmed; unsegmented endopod forming claw. Maxilliped (Figure 4(f)) 5-segmented, comprising syncoxa, basis, and 3-segmented endopod. Syncoxa bearing minute seta; basis bearing row of spinules; endopod bearing

	Coxa	Basis	Exopod	Endopod
Leg 1	0–0	1–1	I-1; I-1; II, 1, 3	0–1; 0–2; 0, 2, 3
Leg 2	0–0	1–0	I-1; I-1; II, I, 4	0–1; 0–2; 0, 2, 3
Leg 3	0–0	1–0	I-1; I-1; II, I, 4	0–1; 0–1; 0, 2, 1
Leg 4	0–0	1–0	I-1; I-1; II, I, 4	absent

proximal segment with seta, middle segment with three setae, and distal segment with seta and slightly, distally curved claw.

Legs 1 to 3 (Figures 4(g), 5(a–d)) biramous; leg 4 (Figure 5(e,f)) uniramous; both rami bearing 3-segmented rami. Leg armature formula as follows:

Intercoxal sclerites (Figures 4(g), 5(b,d,f)) of legs 1 to 4 unarmed. All setae on both rami plumose. Basis of leg 1 bearing row of hairs close to inner seta and inner protrusion. Rami of legs 1 to 4 bearing rows of spinules on outer margins and hairs on both margins. Endopods of legs 1 to 3 (Figures 4(g), 5(a,c)) bearing pointed posterolateral processes on middle and distal segments. Leg 5 (Figure 5(g)) 2-segmented, protopod fused to pedigerous somite bearing outer seta; exopod rod-like, slightly curved, bearing three setae and row of setules on outer margin. Leg 6 (Figure 3(b,c)) represented by three setae on genital operculum.

Variability

The morphology of the male paratype as in the holotype. The measurements of the body parts (n = 1) are as follows: body length 974; cephalothorax length 593; cephalothorax width 665; prosome length 974; genital somite length 162; genital somite width 187; first urosomite length 25; first urosomite width 51; second

urosomite length 25; second urosomite width 56; anal somite length 42; anal somite width 57; caudal ramus length 102; caudal ramus width 18. Caudal ramus 5.69 times longer than wide.

Remarks

To date, four species of *Entomopsyllus* are known. Two species, *E. nichollsi* McKinnon, 1988 and *E. stocki* Kim, 2004, have a single inner seta on the second endopodal segment of leg 2 (McKinnon 1988; Kim 2004), and another two congeners, *E. adriae* (Eiselt, 1959) and *E. brevicaudatus* Lee and Kim, 2017, share two setae with *E. takara* sp. nov. (see Eiselt 1959; Lee and Kim 2017). The new species differs from *E. adriae* because it shows a shorter sixth segment of the antennule on the male and the third exopodal segment of leg 4 bearing four setae, while *E. adriae* has the longer sixth segment of the antennule and the third exopodal segment bearing three setae (Eiselt 1959). The new species clearly differs from *E. brevicaudatus* by having the following features: leg 2 bearing the third endopodal segment with five setae, and leg 3 bearing the third exopodal segment with three spines and the third endopodal segment with three setae, while *E. brevicaudatus* has leg 2 bearing four setae and leg 3 bearing four spines and two setae (Lee and Kim 2017).

Etymology

The specific name of the new species, '*takara*', refers to the name of an island as type locality in Tokara Islands, Kagoshima, Japan.

Newly established Japanese name

'Kasabuta-kudakuchi-mijinko-ka' for the family, 'Kasabuta-kudakuchi-mijinko-zoku' for the genus, and 'Kasabuta-kudakuchi-mijinko' for the species.

Discussion

In this study, two new species of siphonostomatoids belonging to the families Coralliomyzontidae and Entomolepididae are reported from cnidarians: *Cholomyzon multisetum* sp. nov. found in *Tubastraea coccinea* (Hexacorallia: Scleractinia: Dendrophylliidae) and *Entomopsyllus takara* sp. nov. from *Heliopora coerulea* (Octocorallia: Helioporacea: Helioporidae) and *Distichopora violacea* (Hydrozoa: Anthoathecata: Stylasteridae)].

In Coralliomyzontidae, all eight congeners including the new species have been retrieved from dendrophylliid and fungiid corals (Anthozoa: Scleractinia) (Stock and Humes 1969; Humes and Stock 1991; Humes 1997a, 1997b; Cheng et al. 2011; present study). In Entomolepididae, eight of a total of 16 species were found in poriferans (Boxshall and Halsey 2004; Kim 2004; Canário et al. 2012; Lee and Kim 2017; present study). The hosts of five species are still unconfirmed because several species were found from washing of the biological substrate (e.g. tunicates and pearl oysters) and from planktonic samples (e.g. Brady 1899; Thompson and Scott 1903; McKinnon 1988). Finally, *E. takara* sp. nov., *E. stocki*, and *Spongiopsyllus redactus* were described from cnidarians (Kim 2004; Canário et al. 2012; present

study). Hence, it was suggested that primary hosts of entomolepidids are poriferans and the occurrences on other invertebrates might be accidental (Stock 1992; Boxshall and Halsey 2004). In this study, *E. takara* sp. nov. was collected from washing of two species of cnidarians not closely related. Since sponges, hydrozoans and bryozoans are commonly associated with *H. coerulea* and *D. violacea*, it is possible that the true hosts of the new species are sessile organisms. On the other hand, Boxshall and Halsey (2004) mentioned as an example that the entomolopidid found on a coral in New Caledonia, in addition to *E. stocki*, was found from the stoloniferan coral *Tubipora musica* (Linnaeus, 1758) (Octocorallia: Alcyonacea: Tubiporidae) (Kim 2004). As there are not many examples of findings of the family, further field surveys are required. However, this family might show a host preference for basal sessile metazoans.

Since there are no records of either coralliomyzontids or entomolepidids from Japanese waters, the findings of *C. multisetum* sp. nov. and *E. takara* sp. nov. represent first records of both families. We provide keys to species for both genera.

Key to the species of Cholomyzon

1. Leg 4 present ..... *C. palpiferum*
   Leg 4 absent 2

2. Third segment of female antennule with 11 setae; maxillule bearing inner lobe with four setae; distal endopodal segments of legs 2 and 3 bearing five and three elements, respectively ..... *C. multisetum* sp. nov.
   Third segment of female antennule with 7 setae; maxillule bearing inner lobe with three setae; distal endopodal segments of legs 2 and 3 bearing six and three elements, respectively 3

3. Distal endopodal segment of leg 1 bearing six elements ..... *C. brevisetigerum*
   Distal endopodal segment of leg 1 bearing five elements ..... *C. tubastraeae*

Key to the species of Entomopsyllus

1. Second endopodal segment of leg 2 bearing one seta 2
   Second endopodal segment of leg 2 bearing two setae 3

2. Female antennule 11-segmented; third exopodal segment of leg 4 bearing four inner setae ..... *E. nichollsi*
   Female antennule 15-segmented; third exopodal segment of leg 4 bearing two inner setae ..... *E. stocki*

3. Third exopodal segment of leg 4 bearing three inner setae ..... *E. adriae*
   Third exopodal segment of leg 4 bearing four inner setae 4

4. Leg 2 bearing four setae on third endopodal segment; leg 3 bearing four spines and two setae on third exopodal segment and third endopodal segment, respectively ..... *E. brevicaudatus*
   Leg 2 bearing five setae on third endopodal segment; leg 3 bearing three spines and three setae on third exopodal segment and third endopodal segment, respectively..... ..... *E. takara* sp. nov.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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