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Occurrence of Caligid Copepods (Crustacea) in Plankton Samples Collected from Japan and Thailand, with the Description of a New Species

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Adults of four species of the genus *Caligus* (Copepoda: Caligidae) were found in plankton samples collected from the coasts of Japan and Thailand: *Caligus chistos* Lin and Ho, 2003 from the Gulf of Thailand; *C. latigenitalis* Shiino, 1954 from the Suo-nada basin of the Seto Inland Sea, Japan; *C. undulatus* Shen and Li, 1959 off the island of Mukaishima, Hiroshima, Japan; and *C. longiramus* sp. nov. from Amami Island, Kagoshima, Japan. The first two species respectively represent the first reported occurrences in plankton samples, although the third species has been encountered frequently in the pelagic fauna in Japanese waters. *Caligus chistos* also represents the first report of pelagic caligids from Thailand. The present study provides support to the hypothesis that some species of *Caligus* occurring in the plankton alternate between two life modes: on their host fish and free in the water column. *Caligus longiramus* sp. nov. can be distinguished from its congeners by the following combination of features: (1) antennule long with its distal segment twice as long as its proximal one; (2) denticiform process of proximal segment of antenna small and blunt; (3) sternal furca long with sharp tines; (4) exopod of leg 4 armed with I-0; I, III elements; and (5) proximal outer spine on exopod of leg 2 longer than next more distal spine and crossing over it posteriorly.

Key Words: *Caligus*, copepod, fish parasite, new species, plankton, sea lice.

Introduction

The genus *Caligus* Müller, 1785 (Caligidae), well known as sea lice and belonging to the order Siphonostomatoida includes more than 250 species parasitizing marine fishes (Ho and Lin 2004a). Sea lice have a serious impact on cultured fishes around the world (Ho and Lin 2004a; Johnson *et al.* 2004; Rosenberg 2008), but their presence in plankton samples has only recently been highlighted by us (Venmathi Maran and Ohtsuka 2008; Venmathi Maran *et al.* in preparation).

There have been several schools of thought concerning the occurrence of caligid copepods in plankton. Such incidences have been regarded as either accidental, as escapes from the host or as host-switching (Venmathi Maran and Ohtsuka 2008). In particular, the discovery of adults of *Caligus chistos* Lin and Ho, 2003 occurred on *Thunnus maccoyii* (Castelnau, 1872) (Hayward *et al.* 2008) and *Caligus sclerotinosus* Roubal, Armitage and Rhode, 1983 on *Pagrus major* (Temminck and Schlegel, 1843) (Madinabeitia *et al.* in preparation), with no earlier developmental stages found on these hosts, suggested host-switching from different intermediate host fish.

Adults of Caligidae and related families have been reported repeatedly from marine plankton samples around the world (Gnanamuthu 1948; Shen and Li 1959; Heegaard

1972; Pillai 1985; Todd *et al.* 1996; Suárez Morales *et al.* 1998, 2003; Ho and Lin 2004b; Venmathi Maran and Ohtsuka 2008; Venmathi Maran *et al.* in preparation). Recently, three different genera, *Caligus*, *Lepeophtheirus* von Nordmann, 1832, and *Metacaligus* Thomson, 1949, were found in plankton samples off Japan (Venmathi Maran *et al.* in preparation). In addition, *Pandarus* Leach, 1819 (of the caligiform family Pandaridae) has also been recorded as a free-swimming form in Japanese oceanic waters (Venmathi Maran *et al.* in preparation).

In this paper we report on four species of planktonic *Caligus*, including a new species collected from off Amami Island, northern Ryukyu Islands, Japan, and we consider the ecological and adaptive significance of the free-swimming behaviour.

Materials and Methods

Plankton samples were collected using plankton nets of various design at three stations in coastal and oceanic waters of Japan and one station in the Gulf of Thailand (Fig. 1). The collection from off Amami Island, Kagoshima, Japan, was made using a plankton net attached to a beam trawl (BT) (diameter 45 cm; mesh size 0.3 mm) towed along the bottom for 20 min. Samples from all other localities in Japan were collected with a sledge-net (mesh size 0.3 mm)

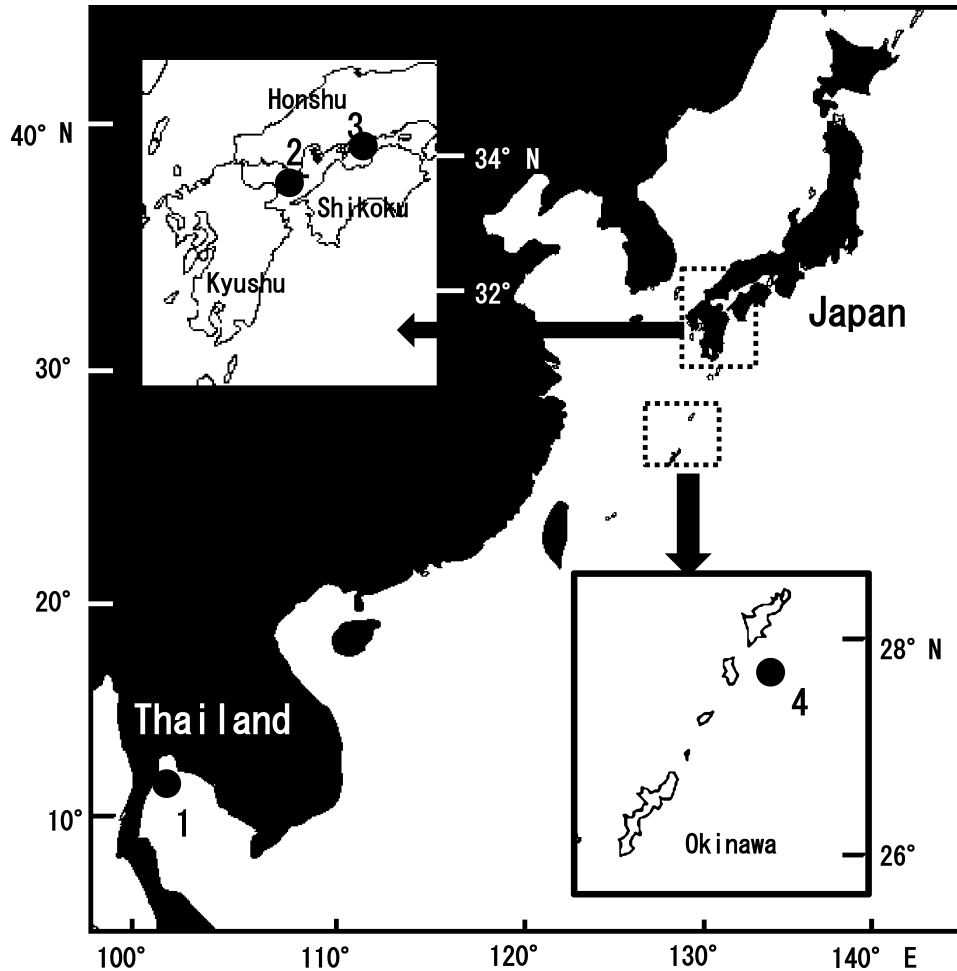


Fig. 1. Collection sites of pelagic caligids including 3 stations in Japanese waters (St. 2–4, 2010) and 1 station in the Gulf of Thailand (St. 1, 2006).

or a small plankton net (0.1 mm), and that from the Gulf of Thailand by using a small plankton net (0.2 mm) towed vertically from the bottom (5 m deep) to the surface. Samples were fixed in 10% neutralized formalin seawater soon after collection. Copepods were sorted out from the samples, and transferred to 70% ethanol. In total, four individuals of the genus *Caligus*, including two males and two females, were found in these samples. Specimens were cleared in lactophenol for 1–2 h and observed using a differential interference contrast microscope (Olympus BX50) at magnifications up to $\times 1000$. All drawings were made with the aid of a drawing tube. The morphological terminology follows Huys and Boxshall (1991) and Ho and Lin (2004a).

The specimens examined are deposited in the Kitakyushu Museum of Natural History and Human History, Kitakyushu, Japan (KMNH IvR 500, 510–513).

Taxonomy

Order **Siphonostomatoida** Thorell, 1859
 Family **Caligidae** Burmeister, 1835
Caligus chiastos Lin and Ho, 2003

Caligus sp.1: Roubal *et al.* 1983: 21.

Caligus chiastos Lin and Ho, 2003: 148; Hayward *et al.* 2008: 57; Venmathi Maran *et al.* 2009: 797.

Material examined. One adult ♂ (KMNH IvR 500, 510), Gulf of Thailand, St.1, 11°46.4'N, 99°48.3'E, day time on 11 November 2006, plankton net.

Description. *Male.* Body 2.55 mm long excluding setae on caudal rami. Cephalothoracic shield longer than wide, 1.48 \times 1.20 mm. Fourth pediger wider than long, 0.24 \times 0.36 mm. Genital complex longer than wide, 0.41 \times 0.42 mm. Abdomen 2-segmented, 0.47 \times 0.19 mm, longer than wide. Caudal ramus longer than wide and armed with 3 long and 3 short setae.

Armature on rami of legs 1–4 summarized in Table 1.

Remarks. The body length of 2.55 mm is smaller than in previously recorded males: 2.70 mm off Taiwan (Lin and Ho 2003), 2.82 mm off Malaysia (Venmathi Maran *et al.* 2009). *Caligus chiastos* is characterized by the armature of legs 2 and 4: the proximal outer spine on the exopod of leg 2 crosses over and is longer than the subsequent spine, which is unusual in the genus *Caligus*; and the exopod of leg 4 is armed with elements I-0; I, III.

This is the first report of *C. chiastos* from a plankton sample and also the first record of the occurrence of any pelagic caligid from Thailand. *Caligus chiastos* was first reported on

Table 1. *Caligus chiastos* Lin and Ho, 2003: armature on rami of legs 1–4. Roman and Arabic numerals indicate spines and setae, respectively.

	Exopod	Endopod
Leg 1	I-0; III, I, 3	(vestigial)
Leg 2	I-1, I-1, II, I, 5	0-1; 0-2; 6
Leg 3	I-0; I-1; III, 4	0-1; 6
Leg 4	I-0; I, III	(absent)

the wild reef-associated snapper *Chrysophrys auratus* (Forster, 1801) from Australia (Roubal *et al.* 1983), followed by a report from wild reef-associated crescent sweetlip *Plectrohynchus cinctus* (Temminck and Schlegel, 1843) in Taiwan (Lin and Ho 2003).

In culture farms of southern bluefin tuna *Thunnus maccoyii* in Australia, a large number of adults (maximum intensity: 42 individuals per host; maximum prevalence: up to 20%) were recovered, while chalimus stages occurred not on this host but on different benthic fishes living near the cages; this implies ontogenetic host-switching (Hayward *et al.* 2008). Recently, adults of this species were also found on cultured snapper *Lutjanus johni* (Bloch, 1792) in Malaysia (Venmathi Maran *et al.* 2009).

Caligus latigenitalis Shiino, 1954

(Fig. 2A)

Caligus latigenitalis Shiino, 1954a: 21; Izawa and Choi 2000: 995; Izawa 2004: 329; Kim 1998: 653. Not *Caligus latigenitalis* Shiino 1960: 471.

Caligus dieuzeidei Brain, 1933: Shiino 1954b: 268; Lin *et al.* 1994: 253.

Material examined. One adult ♂ (KMNH IvR 500, 511), the Suo-nada basin of the Seto Inland Sea, Japan, St. 2, 33°51.3'N, 131°22.6'E, 25–26 m deep, 28 October 2010, sledge-net.

Description. *Male.* Body (Fig. 2A) 4.45 mm long excluding setae on caudal rami. Cephalothoracic shield longer than wide, 2.84×2.50 mm. Fourth pediger wider than long, 0.15×0.28 mm. Genital complex wider than long, 0.78×0.93 mm. Abdomen 2-segmented, longer than wide, 0.47×0.19 mm, second segment longer than first segment. Caudal ramus 0.18×0.20 mm, more or less equal in length and width and armed with 3 long terminal setae, 3 short subterminal setae.

Armature on rami of legs 1–4 as in *C. chiastos* (see Table 1).

Remarks. According to Izawa and Choi (2000), males of this species are larger than females (4.1–6.9 mm vs 3.22–4.33 mm). They recognized two size classes based on body length, a large class of 6.10±0.45 mm and a small class of 4.58±0.36 mm. The present adult male is 4.45 mm long and falls within the latter group. Our male specimen is morphologically similar to the descriptions of Izawa and Choi (2000), except for a minor difference in the post-antennal

process. It is weakly curved and pointed in our specimen, but well curved and less pointed in the description of Izawa and Choi (2000). This minor difference might be due to the large size (6.4 mm) of the specimen described by Izawa and Choi (2000).

Males of *C. latigenitalis* are characterized by a maxilliped with the myxal area of its stout corpus comprising three unequal protuberances. This species is closely similar to *Caligus acanthopagri* Lin, Ho and Chen, 1994 in general body structure and the maxilliped; however, it differs from *C. acanthopagri* in the accessory processes at the distal end of the exopod of leg 4, which are simple with a hyaline membrane in *C. acanthopagri* (cf. Lin *et al.* 1994; Ho and Lin 2004a), but strong and sharply indented marginally in *C. latigenitalis* (Izawa and Choi 2000).

Caligus latigenitalis was first described by Shiino (1954a) based on a single female infecting black seabream *Sparus macrocephalus* (Basilewsky, 1855) (= *Acanthopagrus schlegelii schlegelii* Bleeker, 1854) collected at Momotori, Mie Prefecture, Japan. Shiino (1954b) also reported *Caligus dieuzeidei* Brian, 1933 infecting black seabream, but this was subsequently stated to be a misidentified *C. latigenitalis* (cf. Lin *et al.* 1994). Izawa and Choi (2000) suggested that specimens referred to *C. latigenitalis* by Shiino (1960) (hosts were star-spotted smooth-hound sharks *Mustelus manazo* Bleeker, 1854 and brown guitar-fish *Rhinobatos schlegelii* Müller and Henle, 1841, landed at Hazima, Japan) represented a species other than *C. latigenitalis*, although the specific identity remains unclear.

Recently, this species was redescribed by Izawa and Choi (2000) and Izawa (2004) on the basis of adult females and males, including some chalimus stages. It has so far been reported from Japan and Korea (cf. Nagasawa *et al.* 2010), and commonly infects black seabream (Shiino 1954a, b; Kim 1998; Izawa and Choi 2000; Nagasawa *et al.* 2010). This is the first record of the occurrence of *C. latigenitalis* in a plankton sample.

Caligus longiramus sp. nov.

(Figs 2B–E, 3, 4)

Material examined. Holotype: One adult ♀ (KMNH IvR 500, 512), Amami Island, Kagoshima Prefecture, Japan, St. 4, 27°58.3'N, 129°24.1'E, 399–403 m deep, 24 May 2010, beam trawl.

Description. *Female.* Body (Fig. 2B) 5.12 mm long excluding caudal setae. Cephalothorax triangular, 2.65×2.10 mm, longer than wide. Fourth pediger 0.43×0.54 mm, wider than long. Genital complex 1.25×0.81 mm, vase-shaped, longer than wide. Abdomen 0.65×0.34 mm, longer than wide. Caudal ramus (Fig. 2C) 0.23×0.07 mm, longer than wide, with 1 long, 1 short, and 1 thick setae terminally (Fig. 2C) (on right side, long seta broken off).

Antennule (Fig. 2D) 2-segmented; proximal segment armed with 27 setae; distal segment twice as long as proximal one, armed with subterminal seta on posterior margin and 11 setae plus 2 aesthetascs around apex. Antenna (Fig.

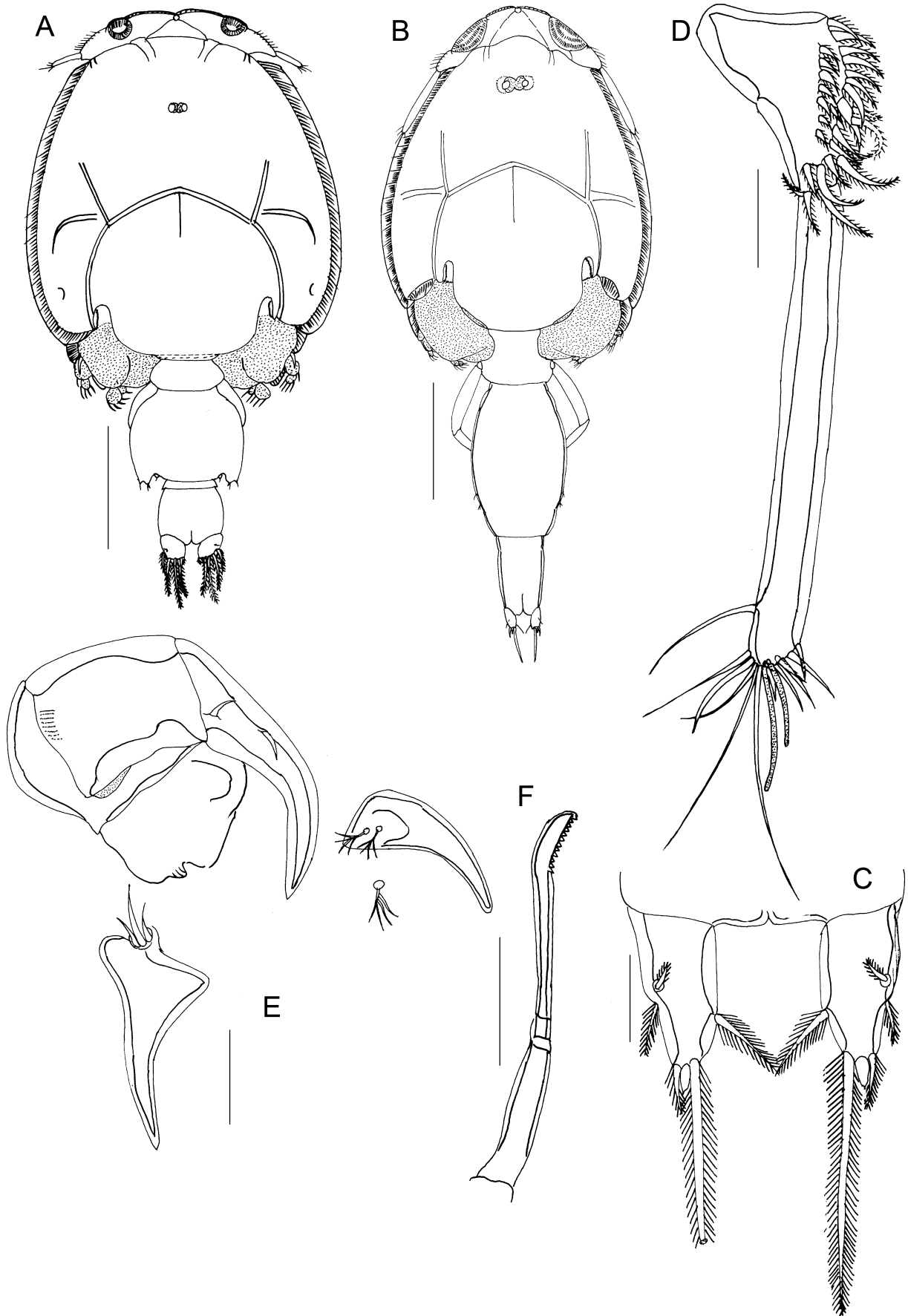


Fig. 2. A, *Caligus latigenitalis* Shiino, 1954, male (KMNH IvR 500, 510), habitus, dorsal view; B–F, *Caligus longiramus* sp. nov., holotype, female (KMNH IvR 500, 511): B, habitus, dorsal view; C, caudal rami, dorsal view; D, antennule, ventral view; E, antenna, postantennal process, and maxillule, ventral view; F, mandible. Scale bars: 1 mm (A, B); 0.1 mm (C–F).

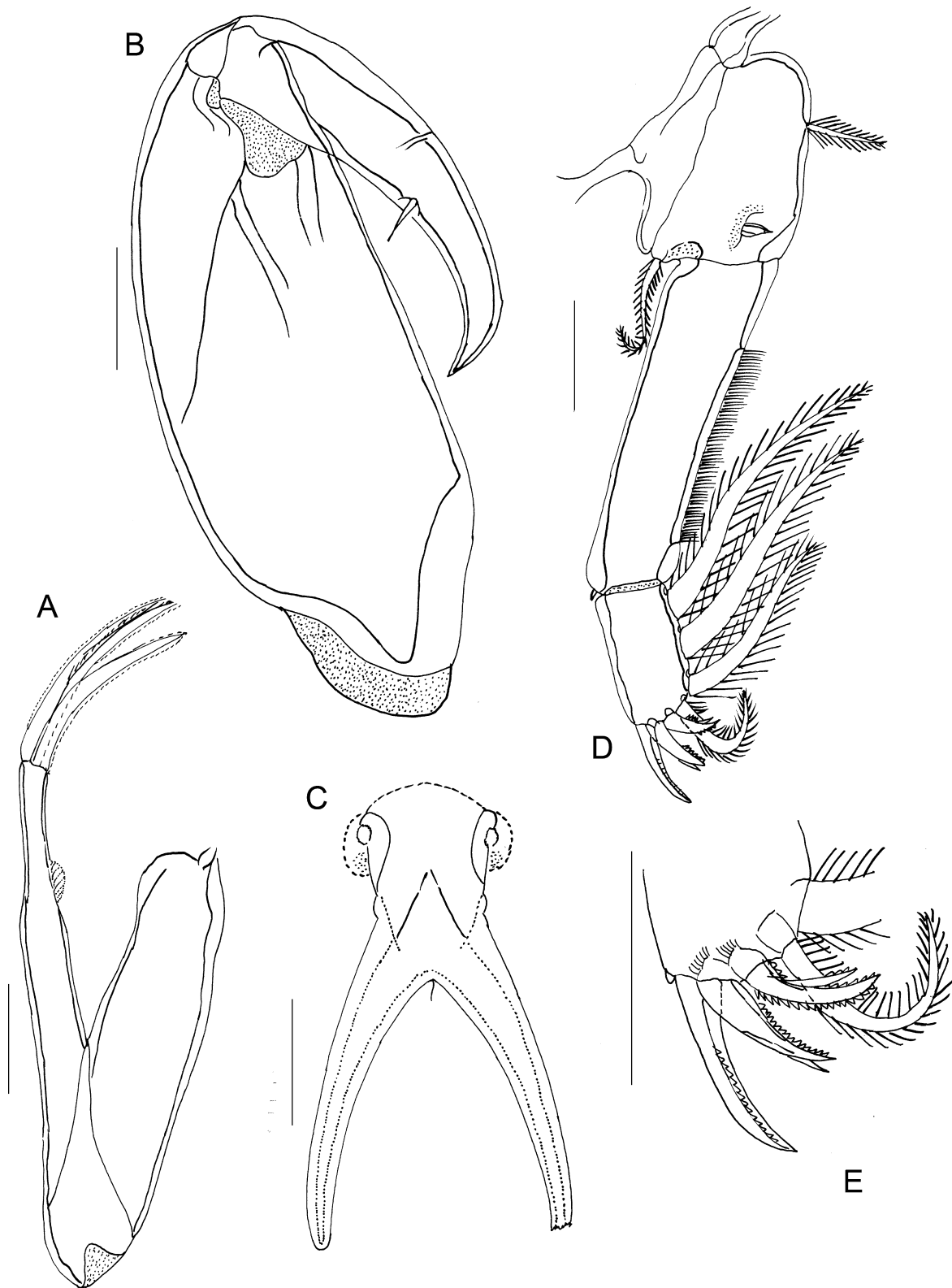


Fig. 3. *Caligus longiramus* sp. nov., holotype, female (KMNH IvR 500,511). A, maxilla, dorsal view; B, maxilliped, dorsal view; C, sternal furca, dorsal view; D, leg 1, ventral view; E, exopod of leg 1 enlarged, ventral view. Scale bars: 0.1 mm.

2E) 3-segmented; proximal segment small, unarmed; middle segment with lateral spinules; distal segment robust, drawn out into curved claw bearing seta laterally and another at midlength. Postantennal process (Fig. 2E) weakly curved, bearing 2 basal papillae, each with 4 setules; another

similar papilla located on nearby sternite. Mandible (Fig. 2F) styloform with 12 teeth subapically. Maxillule (Fig. 2E) robust, curved midlaterally comprising main structure and separate anterior papilla with 3 short setae. Maxilla (Fig. 3A) 2-segmented, slender; proximal segment robust, unarmed;

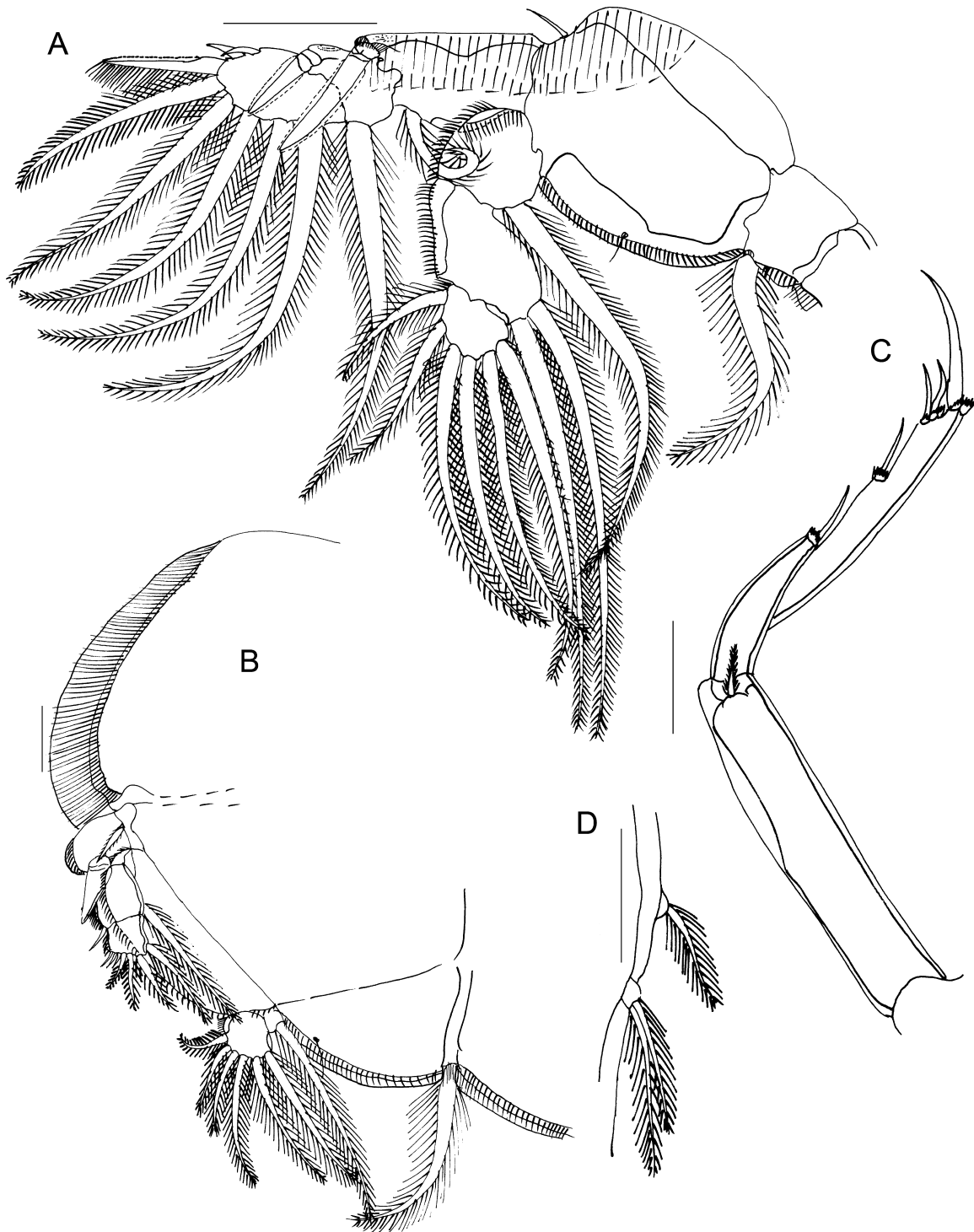


Fig. 4. *Caligus longiramus* sp. nov., holotype, female (KMNH IvR 500,511). A, leg 2, dorsal view; B, leg 3, dorsal view; C, leg 4, dorsal view; D, leg 5, ventral view. Scale bars: 0.1 mm.

distal segment carrying small subterminal hyaline membrane on outer edge and 2 large spinulated elements. Maxilliped (Fig. 3B) indistinctly 3-segmented; proximal segment robust, unarmed; distal subchela robust with claw, armed with small seta at midlength. Sternal furca (Fig. 3C) long with sharp tines (left tine's end slightly broken).

Armature on rami of legs 1–4 as in *C. chiastos* (see Table 1).

Leg 1 (Fig. 3D) with coxa armed with long, plumose outer

(basal) and short, plumose inner (coxal) setae; vestigial endopod tipped with minute seta; middle 2 of 4 terminal elements on distal margin of second exopodal segment each with strong accessory process (Fig. 3E). Leg 2 (Fig. 4A) coxa with large, plumose inner seta; basis with small, naked outer seta and medial papilla bearing long setule; both outer and medial edges of basis fringed with marginal membrane; similar membrane present on outer margin of first segment of exopod; proximal outer spine on distal segment of exo-

pod longer than next more distal spine and crossing over it posteriorly. Leg 3 (Fig. 4B) having protopod with small, plumose outer seta in addition to posterior marginal membranes. Leg 4 (Fig. 4C) with 2-segmented exopod; all outer exopodal spines with pecten at base, outer terminal spine longer than adjacent spines. Leg 5 (Fig. 4D) represented by 2 papillae at posterolateral corners of genital complex tipped, respectively with 1 and 2 plumose setae.

Remarks. The present new species is characterized by the following features: (1) a relatively long antennule with the distal segment twice as long as the proximal segment; (2) a blunt dentiform process of the proximal segment of the antenna; (3) a long sternal furca with a pair of sharp tines; (4) the 2-segmented exopod of leg 4 being armed with I-0; I, III spines; and (5) the proximal outer spine on the exopod of leg 2 being longer than the next more distal spine and crossing over it posteriorly.

Caligus longiramus is closely similar to two other species of *Caligus* in having long antennules: *Caligus aesopus* Wilson, 1921 from Chile, India, Japan, Korea, New Zealand, South Africa, and Taiwan (cf. Choe and Kim 2010) and *Caligus spinosus* Yamaguti, 1939 from Japan (Yamaguti 1939) and Korea (Choe and Kim 2010); however, it differs from these, in characters of the maxillule and leg 4. The tip of the maxillule is bifid in *C. aesopus* and *C. spinosus*, but simply pointed in the new species. In leg 4 the outer spines on the exopod are robust and equal in length in *C. aesopus* and *C. spinosus* (cf. Choe and Kim 2010), while these are slender and the outer terminal spine is longer than the others in *C. longiramus*.

Caligus chiastos is also considered to be one of the closest congeners to *C. longiramus* on the basis of the armature of legs 2 and 4 (Lin *et al.* 1994), but it differs distinctly from the new species in the shorter antennules. Only these two species share a proximal outer spine on the exopod of leg 2 that is longer than the next more distal spine and crosses over it posteriorly. Additional differences between these two species include: (1) the outer spines on the exopod of leg 4 are robust with the outer terminal spine slightly longer than the others in *C. chiastos*, while these are slender with the outer terminal spine distinctly longer than others in *C. longiramus*; (2) the sternal furca has straight tines in *C. chiastos*, but is posteriorly broad in *C. longiramus*; and (3) the dentiform process of the proximal segment of the antenna is small and blunt in *C. longiramus*, but large and sharply pointed in *C. chiastos*.

Etymology. The specific name, a noun in apposition, refers to the relatively long antennule of the new species (Latin “*longus*”, meaning long, and “*ramus*”, branch).

Caligus undulatus Shen and Li, 1959

Caligus undulatus Shen and Li, 1959: 12; Pillai 1966: 123; 1985: 360; Montú 1982: 329; Venmathi Maran and Ohtsuka 2008: 202.

Material examined. One adult ♂ (KMNH IvR 500, 513), Mukaishima island, the Seto Inland Sea, Hiroshima

Prefecture, Japan, St. 3, 34°37.1'N, 133°21.2'E, 9 September 2010, plankton net.

Description. *Female.* Body (Fig. 6E) 3.46 mm long, excluding caudal setae. Cephalothoracic shield longer than wide, 1.78×1.11 mm. Fourth pediger 0.17×0.35 mm and slightly constricted anteriorly. Genital complex longer than wide, 0.78×0.53 mm. Abdomen long, 0.62×0.17 mm. Caudal ramus 0.15×0.06 mm, with 3 long terminal and 3 short subterminal setae.

Remarks. The present female is smaller (3.46 mm) than those in previous reports (4.25–4.29 mm) (Venmathi Maran and Ohtsuka 2008; Venmathi Maran *et al.* in preparation). However, the general body structure of this specimen corresponds well to a previous description based on specimens found in plankton samples from off Japan and Korea (Venmathi Maran and Ohtsuka 2008). Later descriptions of *C. undulatus* differ from that of the original material from China (cf. Shen and Li 1959) in the form of the genital complex which lacks prominent ridges, but they are similar to material from India (Pillai 1966), Japan, and Korea (Venmathi Maran and Ohtsuka 2008; Venmathi Maran *et al.* in preparation). *Caligus undulatus* has been found rather frequently in plankton in the world oceans (Ho and Lin 2004b), and it may have unique aspects to its life history, since its host fish has never been reported.

Discussion

Although there have been several findings of pelagic caligiform copepods around the world, starting from Cherniavsky (1868) and continuing up to Venmathi Maran and Ohtsuka (2008), the adaptive significance of the pelagic occurrence of caligids still needs more investigation. Formerly, occurrences of caligids in plankton samples were considered as accidental phenomena (Kabata 1979; Pillai 1985; Suárez Morales *et al.* 1998, 2003), since the mouthparts of caligids are suitable only for feeding on hosts (Kabata 1979). However, this view has been questioned due to a recent study on *C. chiastos* (cf. Hayward *et al.* 2008) in which severe infection of adult *C. chiastos* on farmed southern bluefin tuna was recorded off south Australia with an intensity of up to 42 individuals per host, while no chalimus stages were detected (Hayward *et al.* 2008). Instead, chalimi were found on Degen's leatherjacket *Thamnaconus degeni* (Regan, 1903) and the scad *Trachurus* sp., as well as some elasmobranchs and benthic teleosts living in neighboring waters (Hayward *et al.* 2008). Host-switching is absolutely dependent on active swimming of the adults in the water column. In this study, an adult male of *C. chiastos* was found in a plankton sample collected from the western part of the Gulf of Thailand, possibly caught in the process of host-switching.

Adults of *Caligus sclerotinosus* heavily infect cultured red seabream in western Japan, but no chalimus stage has been found on this host (Madinabeitia *et al.* in preparation). Adults of this species were also found in plankton samples near the cages (Venmathi Maran and Ohtsuka 2008), whereas no intermediate host for the chalimi has yet been found,

despite an intensive search effort (Madinabeitia *et al.* in preparation). It is likely that *C. sclerotinosus* exchanges hosts during its ontogeny as *C. chistos* does. It is noteworthy that ontogenetic host-switching in these two species of *Caligus* has hitherto been observed only in fish farms.

The other present case of pelagic caligidids does not involve ontogenetic host-switching. *Caligus undulatus* has been found frequently in plankton in the world oceans, but it has never been encountered on host fish (Ho and Lin 2004b; Venmathi Maran and Ohtsuka 2008; Venmathi Maran *et al.* in preparation). It has been suggested that adults of this species occupy both in the water column and on host fish, as some species of the family Ergasilidae do (Ohtsuka *et al.* 2004).

The occurrence of planktonic adults is observed not only in the genus *Caligus* but also in other caligiform genera and families such as *Lepeophtheirus*, *Metacaligus*, and *Pandarus* (Venmathi Maran and Ohtsuka 2008; Venmathi Maran *et al.* in preparation). Laboratory experiments are necessary to confirm whether the planktonic occurrence of any particular species of caligidids or other caligiforms represents host switching or a persistent life stage.

Acknowledgments

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