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Developmental stages of an Invasive, Non-indigenous Sea Louce *Caligus sclerotinosus* Roubal, Armitage and Rhode, 1983 (Copepoda: Siphonostomatoida: Caligidae) Parasitic on Red Sea Bream *Pagrus major* (Temminck and Schlegel, 1843) Collected from Japanese Waters

Yusuke Nishida[1], Panakool Thamban Aneesh[1,2,3,*], Yusuke Kondo[2], Ione Madinabeitia[4], Hirofumi Yamashita[5], Sho Shirakashi[6], Kazuo Ogawa[7], Kaori Wakabayashi[1], Kenta Adachi[1,8], and Susumu Ohtsuka[1,2,3,*]

[1]*Graduate School of Integrated Sciences for Life, Hiroshima University, 5–8–1 Minato-machi, Takehara, Hiroshima, 725–0024, Japan. *Correspondence: E-mail: aneesh@hiroshima-u.ac.jp (Aneesh); ohtsuka@hiroshima-u.ac.jp (Ohtsuka)*

E-mail: m213413@hiroshima-u.ac.jp (Nishida); kaoriw@hiroshima-u.ac.jp (Wakabayashi); mf09001x@gmail.com (Adachi)

[2]*Fisheries Laboratory, Blue Innovation Division, Seto Inland Sea Carbon-neutral Research Center, Hiroshima University, 5–8–1 Minato-machi, Takehara, Hiroshima, 725–0024, Japan. E-mail: ykondo@hiroshima-u.ac.jp (Kondo)*

[3]*Universiti Sultan Zainal Abidin, Gong Badak Campus, 21300 Kuala Terengganu, Terengganu, Malaysia*

[4]*Graduate School of Biosphere Science, Hiroshima University, 5–8–1 Minato-machi, Takehara, Hiroshima, 725–0024, Japan (formerly). E-mail: ionemadinabeitia@gmail.com (Madinabeitia)*

[5]*Ehime Prefecture Aquaculture Research Group Promotion Office of Agriculture, Forestry and Fisheries Research Institute, Fisheries Research Center, Uwajima, Ehime 798-0104, Japan. E-mail: yamashita-hirofumi@pref.ehime.lg.jp (Yamashita)*

[6]*Aquaculture Research Institute, Kindai University, 3153 Shirahama, Nishimuro, Wakayama, 649-2211, Japan. E-mail: shirakashi@kindai.ac.jp (Shirakashi)*

[7]*Meguro Parasititological Museum, 4-1-1 Shimomeguro, Meguro-ku, Tokyo 153-0064, Japan. E-mail: ogawak@kiseichu.org (Ogawa)*

[8]*Japan Fisheries Research and Education Agency, Fisheries Technology Institute, Kamiura, Saeki, Oita, 879-2602, Japan*

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The present study describes and illustrates the morphology of naupliar and copepodid stages of *Caligus sclerotinosus* Roubal, Armitage and Rohde, 1983 (Copepoda: Siphonostomatoida: Caligoida), an invasive, non-indigenous parasite collected from commercially important red sea bream *Pagrus major* (Temminck and Schlegel, 1843) (Sparidae) cultured as well as wild populations in western Japan. The life cycle of *C. sclerotinosus* consists of 8 stages: two nauplii, five copepodids (copepodid I to V) and one adult, which exhibited the general pattern of other congeners. Sexual dimorphism was first observed in the urosome and antenna of copepodid IV. The body lengths of the developmental stages of 14 *Caligus* spp. are compared to those of *C. sclerotinosus*. The oral cone of copepodid I is highly unique for its sucker-like shape, widely expanded terminally. All post-naupliar stages were found on the same host fish *P. major*, rejecting the previous hypothesis for the presence of intermediate hosts for the development of *C. sclerotinosus*.

Keywords: Aquaculture, *Caligus sclerotinosus*, Intermediate host, Japan, Life cycle, Non-indigenous, *Pagrus major*

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BACKGROUND

Caligus sclerotinosus was originally described by Roubal (1981) based on a single specimen found on the body surface of a surf bream *Acanthopagrus australis* (Günther, 1859) from northern

New South Wales, Australia. Since the specimen was damaged, Roubal (1981) reported this ectoparasite as an unidentified species of *Caligus*. Two years later, with the additional finding of more specimens from silver seabream *Chrysophrys* (= *Pagrus*) *auratus* (Forster, 1801) caught off Coffs Harbour, New South Wales, Roubal et al. (1983) amended the description and named the parasite '*Caligus sclerotinosus*, sp. nov'. Since the late 1990s, this sea louse species has been observed infecting the body surface of the extensively cultured red seabream *Pagrus major* (Temminck and Schlegel, 1843) in coastal areas of western Japan. Ho et al. (2004) identified and described *C. scleronitosus* based on specimens collected from red seabream cultured in Oita Prefecture. Likewise, the parasite was also reported infecting the external surface of red seabream cultured in Ehime Prefecture (Ohtsuka 2010; Ohtsuka et al. 2018). According to Ho et al. (2004), *C. sclerotinosus* might have been introduced from Australia to Japan together with its hosts due to anthropogenic causes. In 2013, Venmathi Maran et al. recorded the parasite on cultured red seabream from the southern coastal waters of South Korea. The prevalence of *C. sclerotinosus* on farmed sparids has been reported to be high during the warm seasons in both Japan and Korea, causing dermal caligosis to it host (Tanaka et al. 2013; Venmathi Maran et al. 2013; Ohtsuka et al. 2018).

Sea lice infestations in fish farms can be controlled only through the successful tracking and understanding of their life cycle. To date, information on the complete life cycle of the members of the family Caligidae is known only for quiet few species; 21 out of 518 valid species. Those 21 species are divided into four genera, such as *Caligus* (14 species), *Lepeophtheirus* (5 species), *Alebion* Krøyer, 1863 (1 species) and *Paralebion* Wilson C.B., 1911 (1 species) (see Gomzalez and Carvajal 2003; Benz et al. 1992; Venmathi Maran et al. 2013; Khoa et al. 2019; Piasecki et al. 2023). The life cycle of *Caligus rogercresseyi* was successfully tracked by Gomzalez and Carvajal (2003) from the farmed salmon. Recently the influence of temperature and the duration of life cycle of another important pathogen of sea floating cage culture, *Caligus minimus,* infesting the Seabass, *Lates calcarifer* (Bloch, 1790) from, Malaysia was illustrated by Khoa et al. (2019). Despite the frequent occurrence of *C. sclerotinosus* on cultured red seabream, its biology and life cycle are little known. The adult of this caligid species, which is usually found parasitic on the body surface of its host, was also reported free-swimming in marine plankton samples (Gomzalez and Carvajal 2003;

Venmathi Maran and Ohtsuka 2008; Venmathi Maran et al. 2012a b). Previously, only two naupliar, one copepodid and the adult stages of *C. sclerotinosus* have been described, leaving its life cycle incomplete. Therefore, the objective of the present study is to describe the morphology of all developmental stages of *C. sclerotinosus* ectoparasitic on both wild and cultured red seabream from Japanese waters. A morphological comparison of the post-naupliar stages of *C. sclerotinosus* to those of 14 caligid species is included herein. Furthermore, a traditional method of studying the life cycle of parasitic copepods is to rear nauplii hatching from the egg strings of an ovigerous female (see Gomzalez and Carvajal 2003). In the present study, we used the same methods; the nauplii I hatching out from the egg strings of ovigerous female recovered from the wild host, are reared to obtain the naupli II and copepodid I. Subsequent copepodid stages were obtained from the host. We also confirmed the identity by comparing the morphological description, which was supported by the genetic data.

MATERIALS AND METHODS

Post-naupliar stages obtained from farm

Five and ten individuals of *P. major* were obtained from a farm of Kindai University at Shirahama, Wakayama Prefecture, Japan on December 10, 2019 and August 3/4, 2020, respectively. The body surface of the hosts was carefully washed with tap water and gently stroked by hands to remove ectoparasitic copepods. In our preliminary surveys, we have already confirmed the adherence of second to fifth copepodid stages with a frontal filament adhesive to scales of the host body surface. Washing waters were filtered with a small net of 0.2 mm in mesh size to collect copepods and then contaminated blood and mucus of the hosts were removed by relatively strong pouring of tap waters. All copepod specimens were picked up with fine forceps in filtered seawater and finally moved to 99.5% ethanol.

Methods to rear copepodid stages on the host

Egg strings of *Caligus sclerotinosus,* infecting the wild red sea bream collected by fishing from Takehara City, Hiroshima Prefecture during July to October 2021, were incubated at 22°C to obtain nauplii I and II and copepodid I.

Cultures from copepodid stages 1 to 6 were conducted using parasite-free red sea bream (standard length: 61.2–150.4 mm) artificially produced by the Hiroshima Prefectural Farming Fisheries Center. Copepodid I stage hatched and cultured from egg strings of adult females were exposed for 3 hours to red sea bream reared in a tank containing 20 liters of seawater. During the exposure, the seawater was kept at a standstill. After 3 hours, the volume of seawater in the tank was raised to 80 liters and the sea bream were reared under pouring conditions. The growth of *Caligus screlotinosus* was observed daily, with the time when the copepodid I was introduced as 0 hours.

Genetic identification of copepodid stages

Copepodids of *C. sclerotinosus* obtained from Kindai University on December 10, 2019 were genetically identified as well as one adult female collected off Takehara, Hiroshima on June 25, 2020. Specimens of the third to sixth copepodid stages after infection (see Piasecki et al. 2023) were preserved with 99% ethanol, respectively. Total DNA of Copepodid V and adult samples were extracted from whole body using DNeasy Blood & Tissue kits (Qiagen, Venlo, Netherlands). For small sized samples, DNA was extracted from whole body according to the method described by Suyama (2011). DNA was quantified using a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA) and then adjusted to 1 ng μL^{-1} with sterilized water for PCR amplification.

The 28S nuclear ribosomal DNA region (*28S*) for specimens of the above-mentioned development stages after infection were amplified using a Taq PCR master mix kit and primer sets: 28S-F1a (5'-GCG GAG GAA AAG AAA CTA AC-3') and 28S-R1a (5'-GCA TAG TTT CAC CAT CTT TCG GG-3'; Blanco-Bercial et al. 2011). Thermocycling conditions for *28S* were 94°C for 7 min; 35 cycles at 94°C for 45 s, 50°C for 1 min, and 72°C for 1 min; and a final extension at 72°C for 5 min. Amplification results were verified using 2% (w/v) agarose electrophoresis. Excess primers and dNTPs were removed with ExoSAP-IT (Thermo Fisher Scientific, MA, USA), and the sequencing was outsourced commercially (Macrogen Japan, Kyoto, Japan). No difference between sequences (801 bp) were confirmed by the sequences alignment that was performed using CLUSTAL W (Thompson et al. 1994) in MEGA 7 (Kumar et al. 2016). The sequence for *28S* (Accession number; LC596096) was deposited in the DDBJ/EMBL/NCBI GenBank databases

Bayesian inference (BI) analysis was computed using MrBayes v. 3.2.7 (Ronquist et al., 2012) under the GTR+Γ model for each data subset. The model was selected based on Hierarchical likelihood ratio tests (hLRTs) using MrModeltest v. 2.4 (Nylander 2004). Two parallel analyses of Metropolis-Coupled Markov Chain Monte Carlo (MC3) were conducted for 1,000,000 generations, and topologies were sampled every 100 generations. The convergence of MCMC was checked with the value of the average standard deviation of split frequencies (ASDSF) in MrBayes. The phylogenetic tree of BI analysis was visualized by Figtree v. 1.4.4 (Rambaut 2012).

Descriptions and measurement of all developmental stages

The post-naupliar stages of *Caligus sclerotinosus* were collected from the body surface of 15 specimens of cultured red seabream captured on December 10, 2019 and August 3/4, 2020 from a farm of Kindai University, Wakayama Prefecture, Japan. Preliminary surveys during this study have confirmed the adherence of copepodids I–V with a frontal filament to the scales of red seabream. The body surface of the hosts was carefully washed with tap water and gently stroked by hand to remove ectoparasitic copepods. The dislodged debris was filtered with a 0.2 mm mesh size net to recover the detached copepods. All copepod specimens were picked up with a pair of forceps in filtered seawater, preserved in 99.5% ethanol, cleared in lacto-phenol, and observed with a microscope following the methods of Humes and Gooding (1964). Copepods were counted and classified into different stages.

The naupliar (NI–II) and first copepodid stages (CI) of *Caligus sclerotinosus* were obtained via incubation. Ovigerous adult females collected from wild *P. major* caught off Takehara City, Hiroshima Prefecture, Japan, on September 16, 2020 were incubated in conical beaker (water volume: ca. 100 mL) at a temperature of ca. 22.5°C in an incubator (CR-14C, Hitachi, Ltd.). The nauplii and CI first appeared ca. 6 and 48 hours after incubation, respectively. They were preserved in 10% neutralized formalin/seawater for descriptions and measurement.

The first copepodid stage (CI) was observed with a scanning electron microscope (JSM-6510LV, Jeol Co., Ltd.). Before electron microscopy, specimens were fixed in 10% neutralized formalin/seawater for 10 days and soaked in distilled water for ca. 3 hrs, dehydrated through a graded ethanol series from 70% to 100% (twice), critical-pointed fried, and coated with gold. Intact specimens of second to fifth copepodid stages (CII–CV) were illustrated with the aid of a drawing tube attached to a differential interference microscope (BX50, Olympus Co., Ltd.). Body lengths of nauplii and copepodids were measured from the anterior to the posterior tips of bodies, and from the anterior margin of the cephalothorax to the terminal ends of caudal rami excluding caudal setae, respectively.

This study follows the terminology of the developmental stages of caligids recently been proposed by Piaseck et al. (2023). According to Piasecki et al. (2023), since chalimi and pre-adults of caligids were not strictly defined, these should be replaced by standardized terms used in free-living copepods: copepodids. Instead, the post-naupliar stages of caligids should be simply called first to fifth copepodid stages (CI–V) and adults (CVI).

RESULTS

Genetic identification

All copepodids (CIII 3 indiv.; CIV 6 indiv.; CV 6 indiv.) showed the exactly same nucleoride sequence of *28S* in 801 bp as that of the adult female, which leads to a conclusion that these are developmental stages of *C. sclerotinosus* (see Fig. 12). Although the first and second copepodid stages (CI, CII) were not analyzed by the molecular technique, they were morphologically identified. The morphology of CI was directly confirmed through nauplii via incubation of live ovigerous adult females distinctly identified as *C. sclerotinosus*. CII was morphologically determined in comparison between CI and CIII.

Description of developmental stages

Nauplius I (Figs. 1 and 10)

Shape of newly hatched larva with very short cylinder, resembles shape of egg. Larva eventually attaining definite elongated, oval body (Fig. 1A), average length 0.35 ± 0.02 mm (range: 0.32–0.37 mm, N = 4) (Fig. 10). Anterior end of body wider with three pairs of appendages antero-ventrally. Posterior end, narrower and blunt, equipped with three pairs of appendages antero-ventrally; paired balancers on posterolateral margin curved outwards; and visible nauplius eye dorsally.

Antennule (Fig. 1B) uniramous, two-segmented: proximal segment short with 2 unequal naked setae on outer surface; distal segment 1.5 times longer than proximal segment, distinctly separated from proximal by ridge articulation; distal segment armed apically with 2 large serrated setae, 1 small aesthetasc arising between setae and 3 subapical spines on sides. Antenna (Fig. 1C) biramous, with protopod indistinctly divided into coxa and basis; basis not separated from proximal segment of rami; endopod two-segmented, distal segment with one subterminal seta and two long terminal setae ornamented with serrated and plumose margins, third element short and blunt; exopod 4-segmented, segments 2–4 carrying a total of 4 long setae, ornamented with serrated, outer and plumose inner margins. Mandible (Fig.1D) biramous, with un-segmented protopod, not separated from proximal-most exopodal segment; endopod with two long terminal setae and one subterminal short spiniform seta; exopod four-segmented, each segment with one long seta setae, ornamented with serrated outer and plumose inner margins.

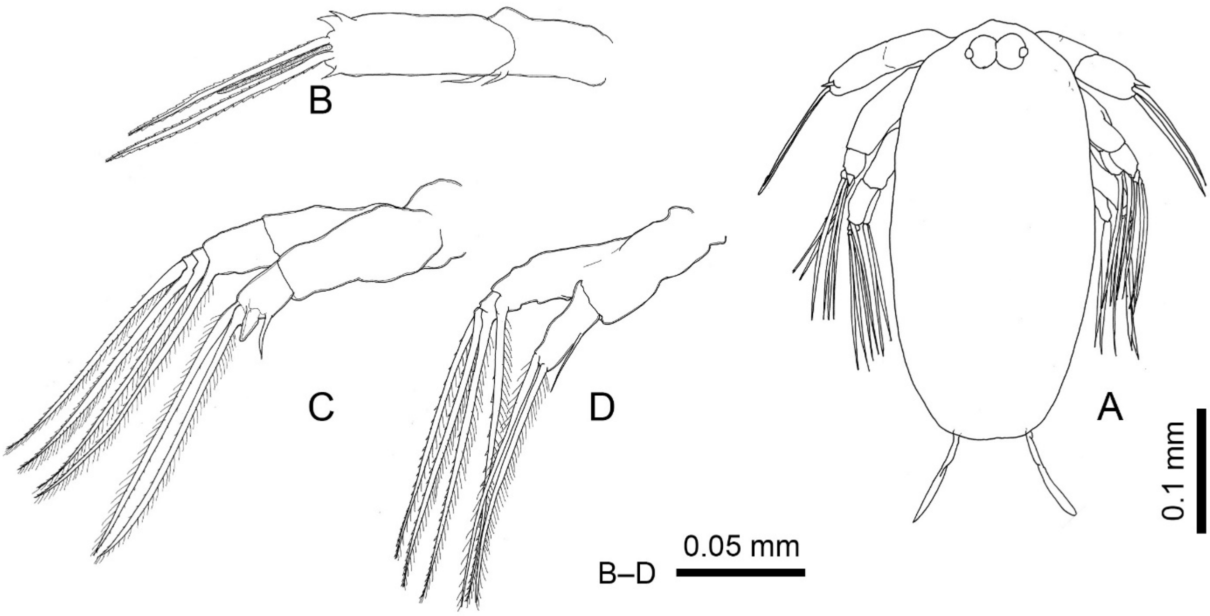

Fig. 1. *Caligus sclerotinosus*, first nauplius. A, Habitus, dorsal view; B, Antennule; C, Antenna; D, Mandible.

Nauplius II (Figs. 2 and 10)

Resembling previous stage, with body (Fig. 2A) longer than first nauplius. Average size 0.40 ± 0.02 mm (range: 0.42–0.37 mm, N = 5) (Fig. 10), with three appendages antero-ventrally as seen in nauplius I; paired balancers on posterolateral margin curved outwards; nauplius eye present. In older specimens, next stage (copepodid) visible inside.

Antennule (Fig. 2B) as in preceding stage; uniramous, two-segmented, segments of equal length, with additional small element terminally and additional minute spinule on distal margin; surface of both segments with 2-3 patches of minute spinules. Antenna (Fig. 2C) as in preceding stage with patches of minute spinules on surface of sympod and endopod. Mandible (Fig. 2D) as in preceding stage. Anlage of paired maxillipeds [sensu Piasecki (1996)] (Fig. 2E) first appearing in this stage, consisting of pair of slender, posteriorly directed processes.

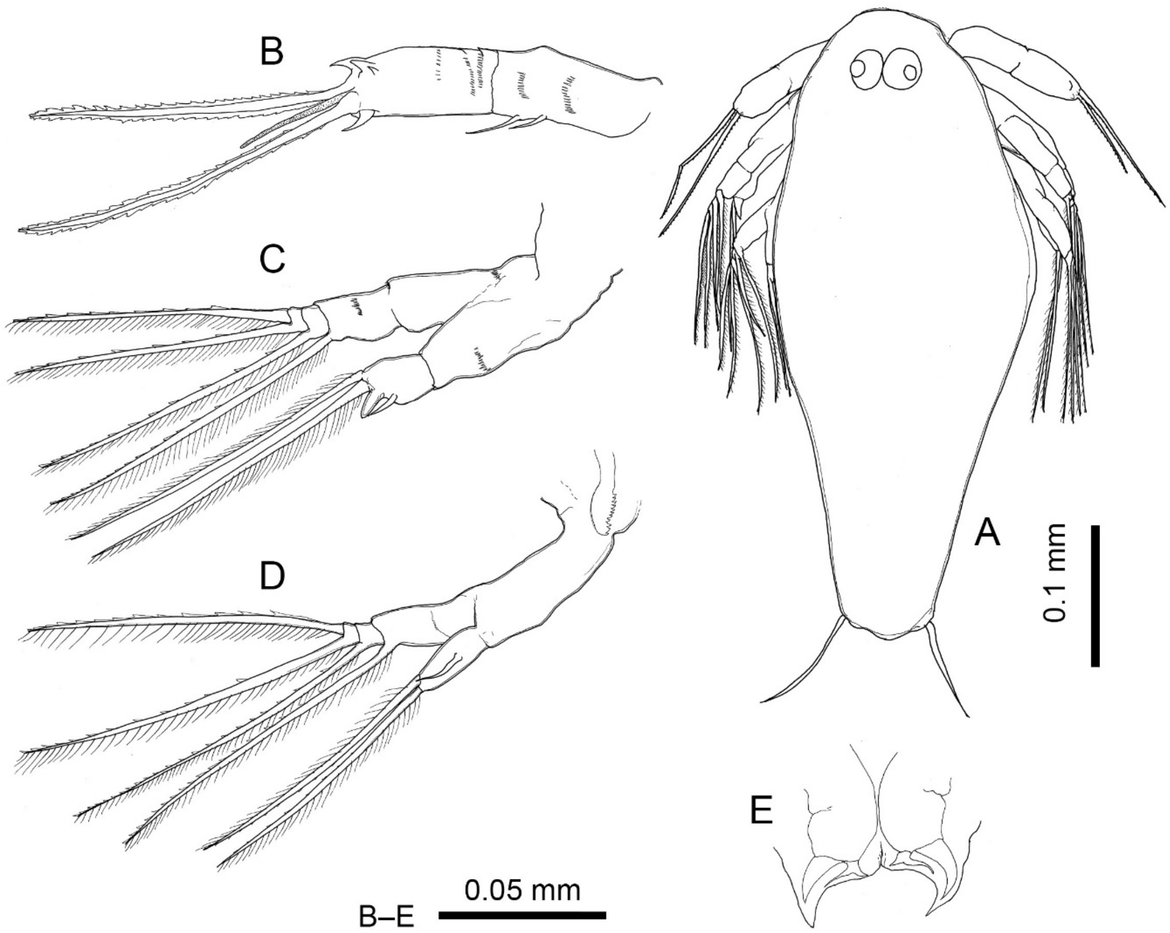

Fig. 2. *Caligus sclerotinosus*, second nauplius. A, Habitus, dorsal view; B, Antennule; C, Antenna; D, Mandible; E, Buds of maxilliped.

Copepodid I (Figs. 3 and 10)

Body (Fig. 3A, B) elongated with average length of 0.55 ± 0.03 mm (range: 0.47–0.60 mm, N = 22) (Fig. 10). Body with indistinct segmentation, consisting of cephalothorax, free thoracic leg bearing somites, genital complex and caudal rami. Cephalothorax incorporating first pedigerous somite, longer than wide. Second and third pedigerous somites much narrower than cephalothorax. Genital complex indistinctly separated from fourth somite and abdomen. Anal region bearing short caudal rami, each armed with 5 unequal plumose setae and one short aesthetasc (Fig. 3N). Mouth cone large, forming trumpet-shape (sucker-like structure), comprising flat labrum and posterior expanded labium (Fig. 3C). In younger specimens coiled frontal filament (Fig. 3A) seen inside of anterior part of cephalothorax. After attaching to their host, older copepodids show frontal filament extruded from anterior margin, with rounded frontal plate at anterior corners (Fig. 3D).

Antennule (Fig. 3E) two-segmented; proximal segment armed with 3 setae; distal segment bearing 2 aesthetascs and 11 setae, of which 4 setae are terminally bifurcated. Antenna (Fig. 3F) three-segmented; basal segment smallest, unarmed; second segment largest, unarmed; terminal segment recurved claw armed with minute spiniform element at inner base; post-antennal process (Fig. 3G-a) represented by a simple, blunt knob. Mandible (Fig. 3H) rod-shaped gnathobase, four-sectioned; proximal section with vestige of naupliar palp; third section longest; terminal section serrated on medial margin of flatten blade, equipped with 11 teeth. Oral cone (Figs 3C, 4) widely expanded terminally, forming sucker-like structure. Maxillule (Fig. 3G-b) with dentiform process and papilla armed with 3 setae. Maxilla (Fig. 3J) two-segmented; proximal segment (lacertus) robust, unarmed; distal segment (brachium) slender with terminal claw-like calamus and sub-terminal canna possessing serrated membrane. Maxilliped (Fig. 3I) sub-chelate, two-segmented; proximal protopodal segment (corpus) with a pointy process; shaft long and slender, with sub-terminal bifid element and terminal claw. Post-oral process (Fig. 3G-c) represented by simple, pointed knob.

Legs 1 (Fig. 3K) and 2 (Fig. 3L) biramous, with indistinctly two-segmented sympod. Distal segment with medium-sized pinnate seta on lateral margin. Both rami unsegmented, equal in size. Exopod flat and oval; endopod sub-rectangular having unarmed lateral margin. Leg 3 (Fig. 3M) rudimentary, with two unequal setae on postero-lateral corner of second free somite. Armature formula of legs 1 and 2 as follows (Roman numerals indicating spines and Arabic numerals, setae):

	Sympod	Exopod	Endopod
Leg 1	0-0; 1-0	I, III, 4	6
Leg 2	0-0; 1-0	I, II, 4	6

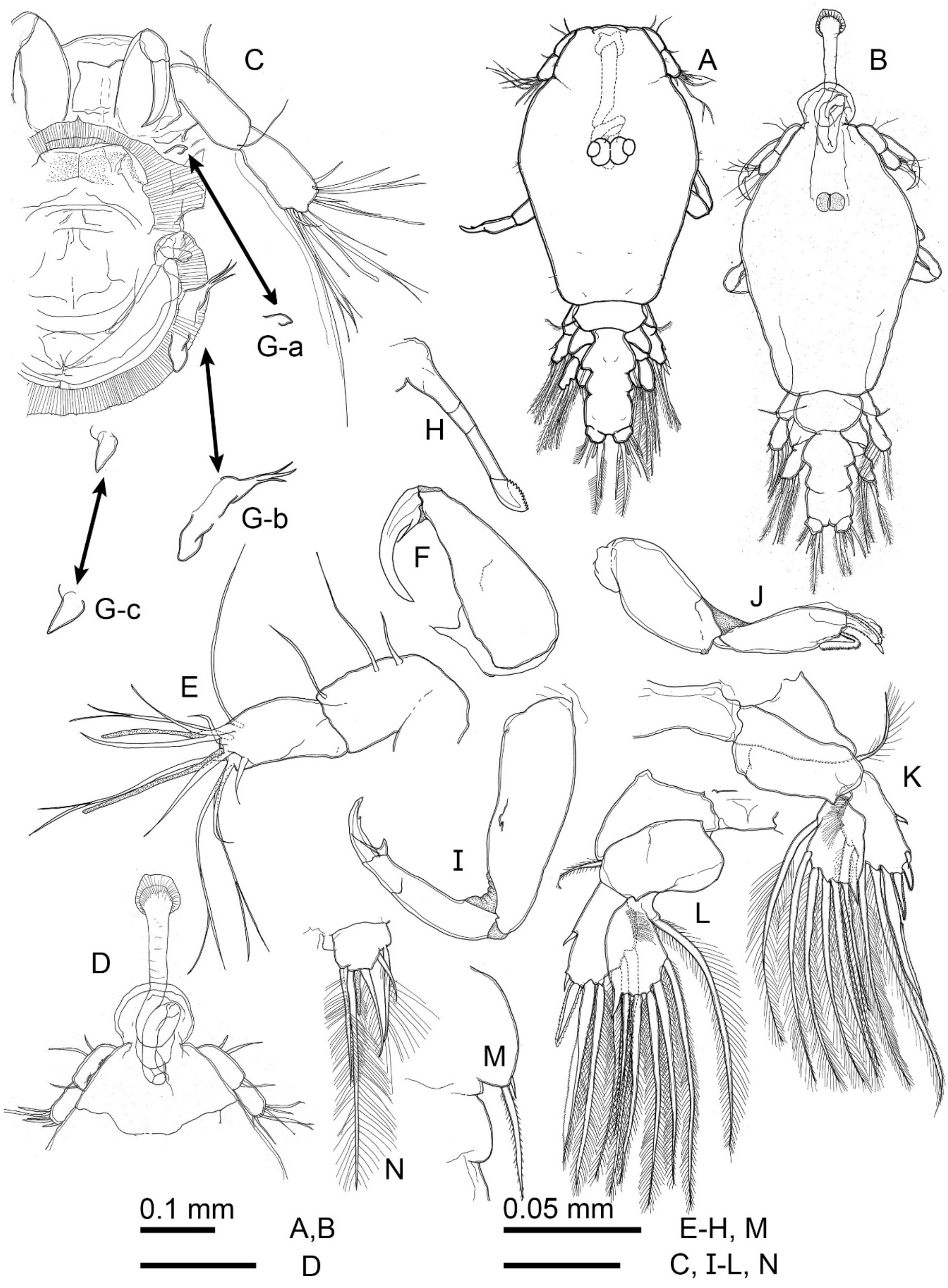

Fig. 3. *Caligus sclerotinosus*, first copepodid. A, Habitus, before ejection of frontal filament, dorsal view; B, Habitus, after ejection of frontal filament, dorsal view; C, Antennule; C, Oral cone and mouthpart appendages; D, Ejected frontal filament; E, Antennule; F, Antenna; G, Postantennal process (a), Maxillule (b), postoral process (c): H, Mandible; I, Maxilla; J, Maxilliped; K, Leg 1; L, Leg 2; M, Leg 3; N, Caudal ramus.

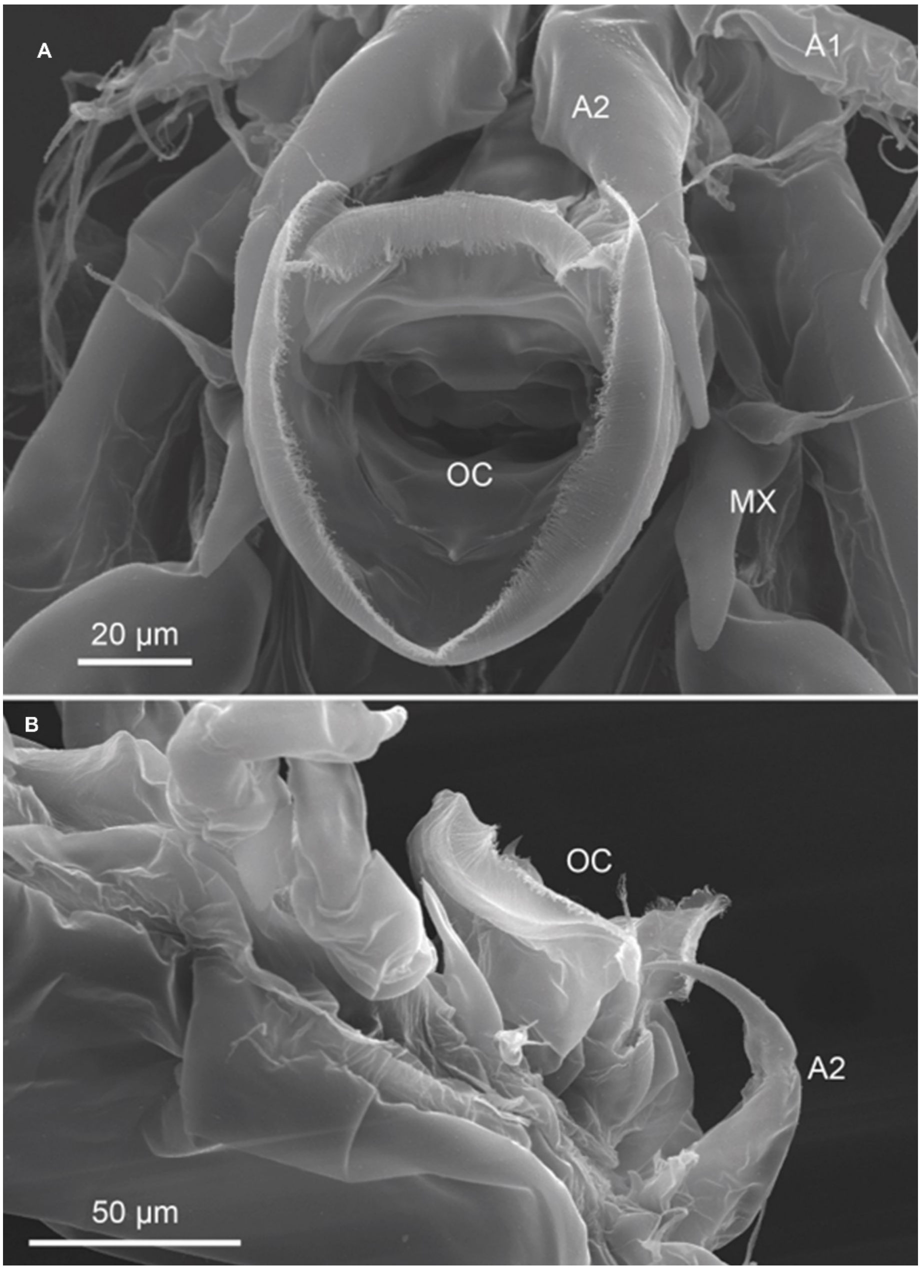

Fig. 4. SEM micrographs of oral cone of first copepodid of *Caligus sclerotinosus*. A, Ventral view; B, Lateral view. Abbreviations: A1. Antennule; A2. Antenna; MX. Maxillule; OC. Oral cone.

Copepodid II (Figs. 5 and 10)

Body (Fig. 5A) with average length of 0.67 ± 0.05 mm (range: 0.58–0.80 mm, N = 21) (Fig. 10). Frontal filament present for attachment to the host. Base of frontal filament (Fig. 5B) with signs of transverse subdivisions. Cephalothorax protruded anteriorly and emarginated posteriorly; about 1.5 times longer than posterior tagma. Second and third pedigerous somites much narrower than cephalothorax; fourth pedigerous somite unarmed. Genital complex distinctly separated from fouth pedigerous somite. Anal somite bearing small caudal rami (Fig. 5L) each armed with 6 unequal naked setae.

Antennule (Fig. 5C) two-segmented; proximal segment with 3 naked setae; distal segment with 11 simple naked setae and 2 aesthetascs. Antenna (Fig. 5D) non-prehensile, with robust base and small distal segment weakly sclerotized and carrying 4 marginal processes. Mandible (Fig. 5E) four-segmented, bearing 12 teeth on distal blade. Maxillule (Fig. 5F) comprising blunt dentiform process and basal papilla bearing 3 unequal setae. Maxilla (Fig. 5G) two-segmented, terminal segment lacking flabellum, canna and calamus bearing serrated membrane. Maxilliped (Fig. 5H) indistinctly three-segmented; first segment robust; distal endopodal segment as subchela in form of large, curved claw and short inner seta. Sternal furca absent.

Legs 1 (Fig. 5I), 2 (Fig. 5J) and 3 (Fig. 5K) biramous, with segmented rami legs 1 and 2 bearing bulbous processes at inner proximal corner of coxa. Leg 3 (Fig. 5K) unsegmented and rudimentary, but with anlagen of rami distinct. Setae on legs 1–3 simple. Armature formula of legs 1–3 as follows:

	Sympod	Exopod	Endopod
Leg 1	1-0	1, 7	1
Leg 2	1-0	1, 6	4, 1
Leg 3	0-0	4	rudiment

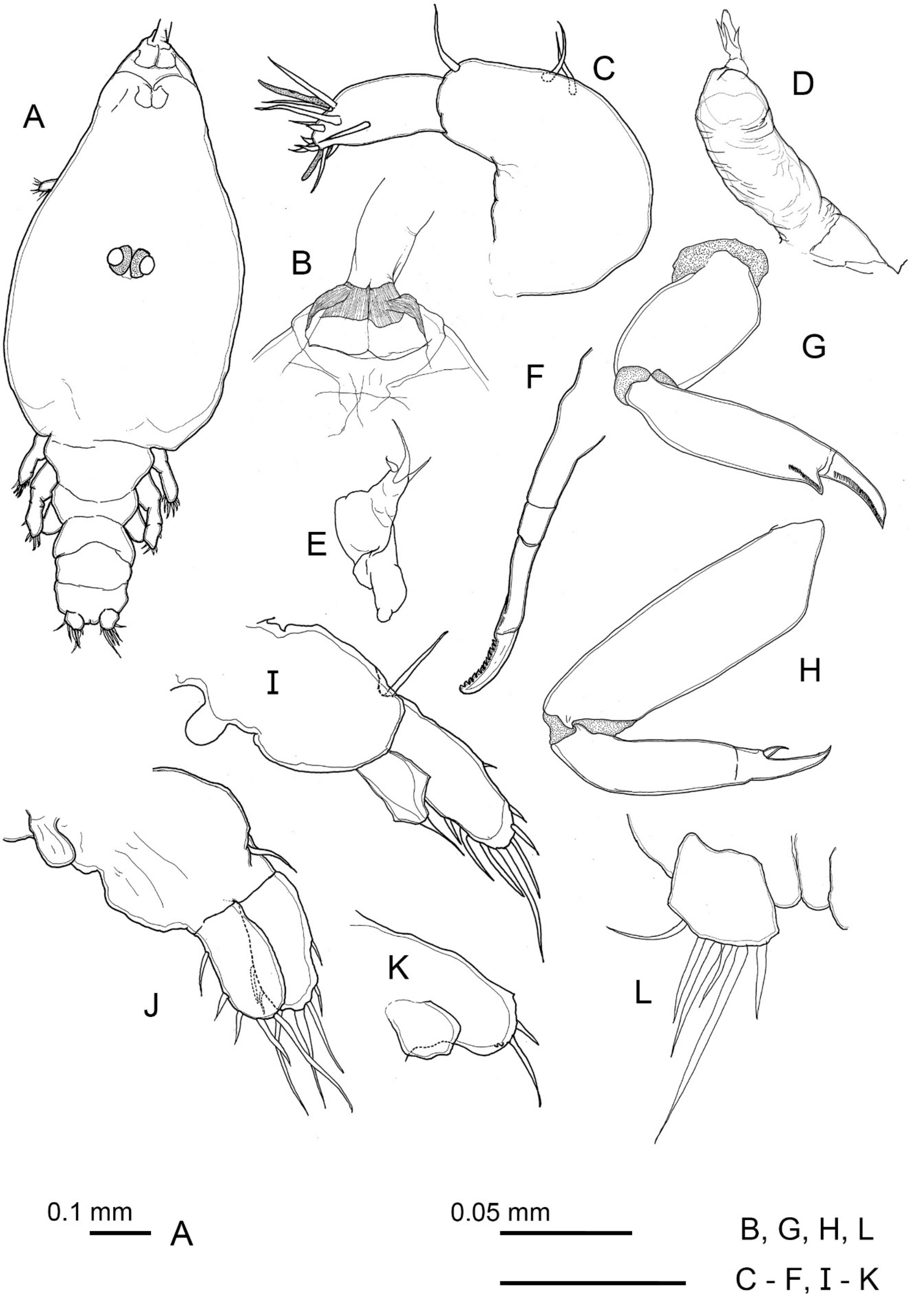

Fig. 5. *Caligus sclerotinosus*, second copepodid. A, Habitus, dorsal view; B, Extension lobes at base of frontal filament; C, Antennule; D, Antenna; E, Mandible; F, Maxillule; G, Maxilla; H, Maxilliped; I, Leg 1; J, Leg 2; K, Leg 3; L, Caudal ramus.

Copepodid III (Figs. 6, 7 and 10)

Body (Figs. 6, 7A) average length of 0.92 ± 0.06 mm (range: 0.88–1.03 mm, N = 6) (Fig. 10). Body with cephalothorax laterally expanded incorporating both first and second pedigerous somites without visible suture dorsally; anterior margin with frontal filament (Fig. 7B) bearing 2 basal lobes; cephalothorax about 3 times longer than indistinctly three-segmented posterior tagma; attenuated distally, bearing anlagen of leg 4 (Fig. 7L) ventro-laterally; caudal rami broader than previous stage, with 3 plumose setae and 3 naked setae.

Antennule (Fig. 7C) two-segmented; proximal segment bearing 7 simple setae; distal segment armed with 11 setae and 2 terminal aesthetascs. Antenna (Fig. 7D) indistinctly two-segmented; proximal segment small; distal segment with indistinct segmentation; distal tip slender, with 2 small spiniform elements and 2 bifurcate processes. Anlagen of post-antennary process (Fig. 7D) simple knob bearing basal papilla with 2 sensilla. Mandible (Fig. 7E), maxillule (Fig. 7F) and maxilla (Fig. 7G) as in preceding stage. Maxilliped (Fig.7H) slightly larger than in CII stage.

Legs 1 (Fig. 7I), 2 (Fig. 7J) and 3 (Fig. 7K) biramous; leg 1 with two-segmented exopod; leg 2 biramous, indistinctly two-segmented; leg 3 biramous, both rami unsegmented; leg 4 (Fig. 7L) uniramous, bearing 2 terminal setae. Armature formula of legs 1–4 as follows:

	Sympod	Exopod	Endopod
Leg 1	1-1	1-0; 7	1
Leg 2	1-0	1-0; 7	0-1; 7
Leg 3	0-0	8	4
Leg 4	0-0	2	(absent)

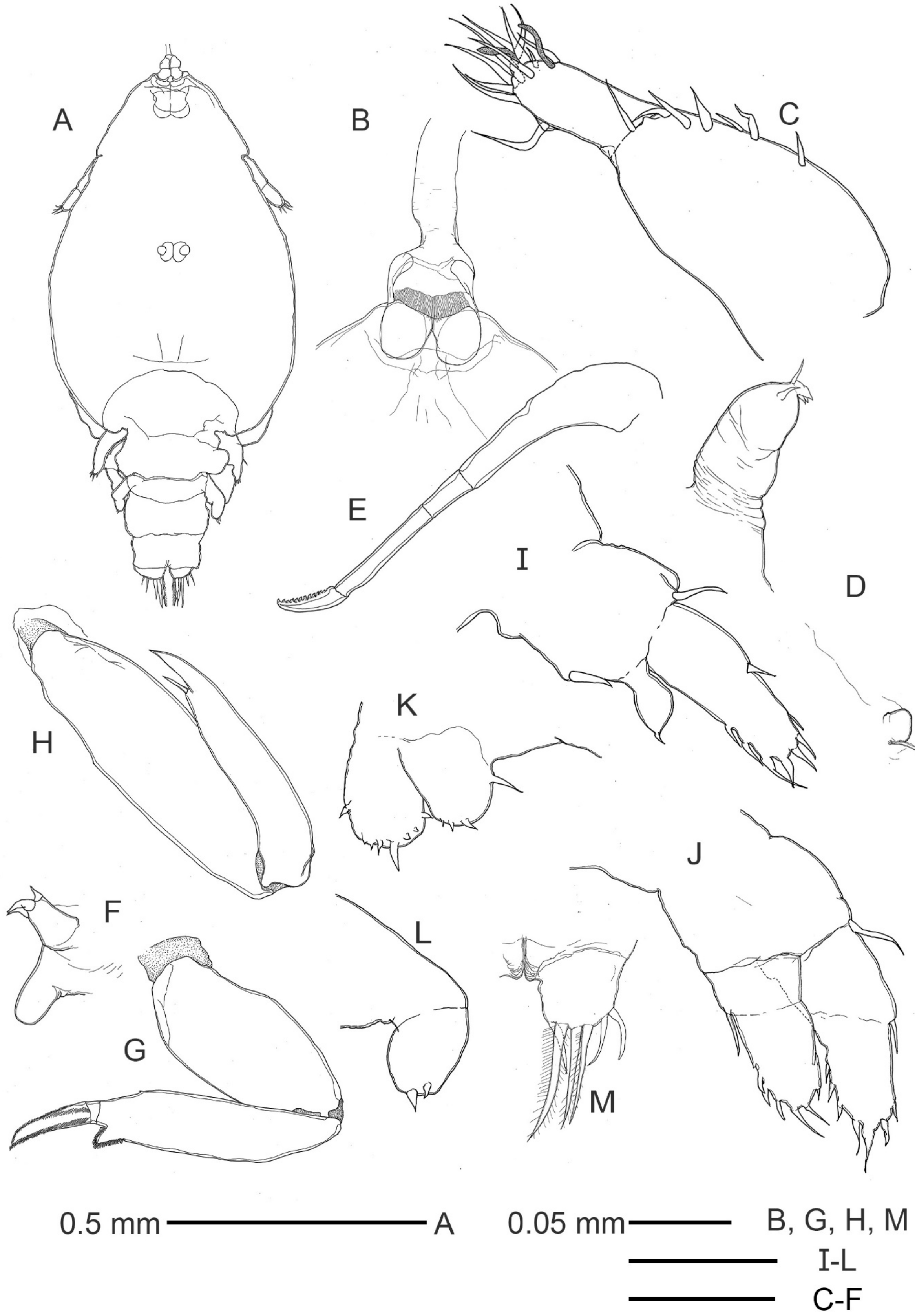

Fig. 6. *Caligus sclerotinosus*, third copepodid, dorsal view. Frontal filament adhering to host scale. Abbreviations: FF. Frontal filament; SC: Scale of host fish.

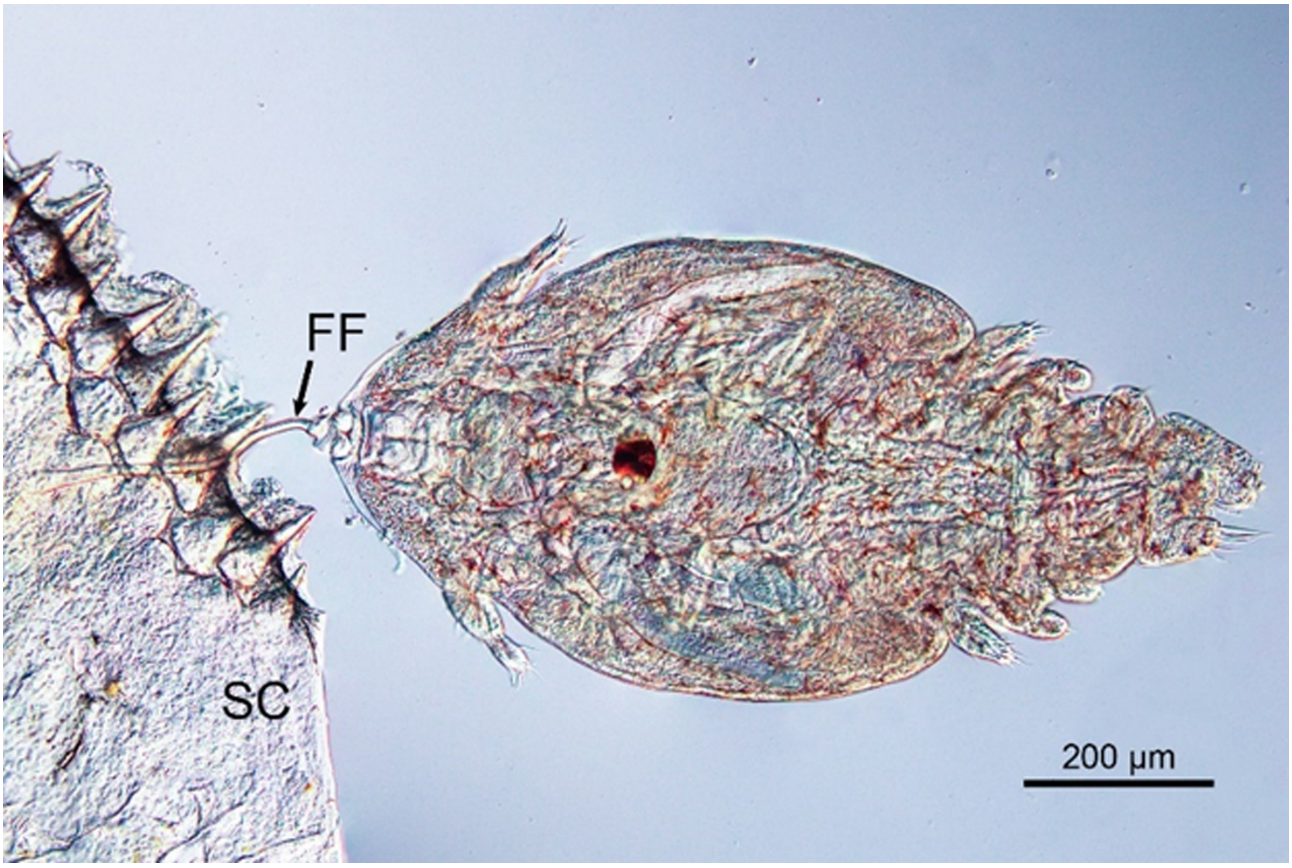

Fig. 7. *Caligus sclerotinosus*, third copepodid. A, Habitus, dorsal view; B, Extension lobes at base of frontal filament; C, Antennule; D, Antenna and postantennal process; E, Mandible; F, Maxillule; G, Maxilla; H, Maxilliped; I, Leg 1; J, Leg 2; K, Leg 3; L, Leg 4; M, Caudal ramus.

Copepodid IV (Figs. 8 and 10)

Female: Body with average length of 1.38 ± 0.09 mm (range: 1.28–1.49 mm, N= 4) (Fig. 10). Body (Fig. 8A) showing full development of caligid cephalothorax with incorporation of third pedigerous somite and development of frontal plates. Cephalothorax widest at mid-level, about 2.5 times longer than posterior tagma; frontal filament (Fig. 8C) having 3 basal bulbs. Genital complex (Fig. 8A, O) widest mid-way, with protruded postero-lateral region. Caudal rami (Fig. 8A, Q) armed with 4 plumose and 2 naked short setae.

Antennule (Fig. 8D) two-segmented; proximal segment bearing 20 anterior setae; distal segment with 12 setae and 2 aesthetascs. Antenna (Fig. 8E) indistinctly two-segmented; proximal segment with reduced postero-medial protusion; distal segment protruded into pointed process distally, armed with 2 short sub-terminal elements. Post-antennary process (Fig. 8E) as simple knob with basal papilla bearing 1 sensillum, and similar papilla armed with 2 sensilla nearby in sternum. Mandible (Fig. 8G) as in preceding stage. Maxillule (Fig. 8H) with longer blunt dentiform process than that of preceding stage. Maxilla (Fig. 8I) similar to that of preceding stage. Maxilliped (Fig. 8J) slightly larger than in preceding stage; indistinctly three-segmented.

Leg 1 (Fig. 8K) endopod more atrophied than that of preceding stage; exopod indistinctly two-segmented. Leg 2 (Fig. 8L) biramous; both rami 2-segmented. Leg 3 (Fig. 8M) better developed unsegmented rami than that of preceding stage. Leg 4 (Fig. 8N) uniramous; exopod indistinctly two-segmented, longer than that of preceding stage. Armature formula of legs 1–4 as follows:

	Sympod	Exopod	Endopod
Leg 1	1-1	1-0; 7	(vestigial)
Leg 2	1-1	1-1; 1, 8	0-1; 8
Leg 3	1-1	1-0; 9	0; 1, 5
Leg 4	1-0	4	(absent)

Leg 5 (Fig. 8O) represented by 3 setae on postero-lateral margin of genital complex.

Male: Body (Fig. 8B) average length 1.24 ± 0.16 mm (range: 0.93–1.40 mm, N = 7) (Fig. 10). Sexual dimorphism expressed in a slightly shorter body size, antenna (Fig. 8F), and leg 6 (Fig. 8P). Antenna (Fig. 8F) with distal segment knob-like armed with 3 spiniform elements sub-terminally and blunt apical process. Leg 6 (Fig. 8P) present as small setae on ventro-medial surface of genital complex, posterior to leg 5 (Fig. 8P).

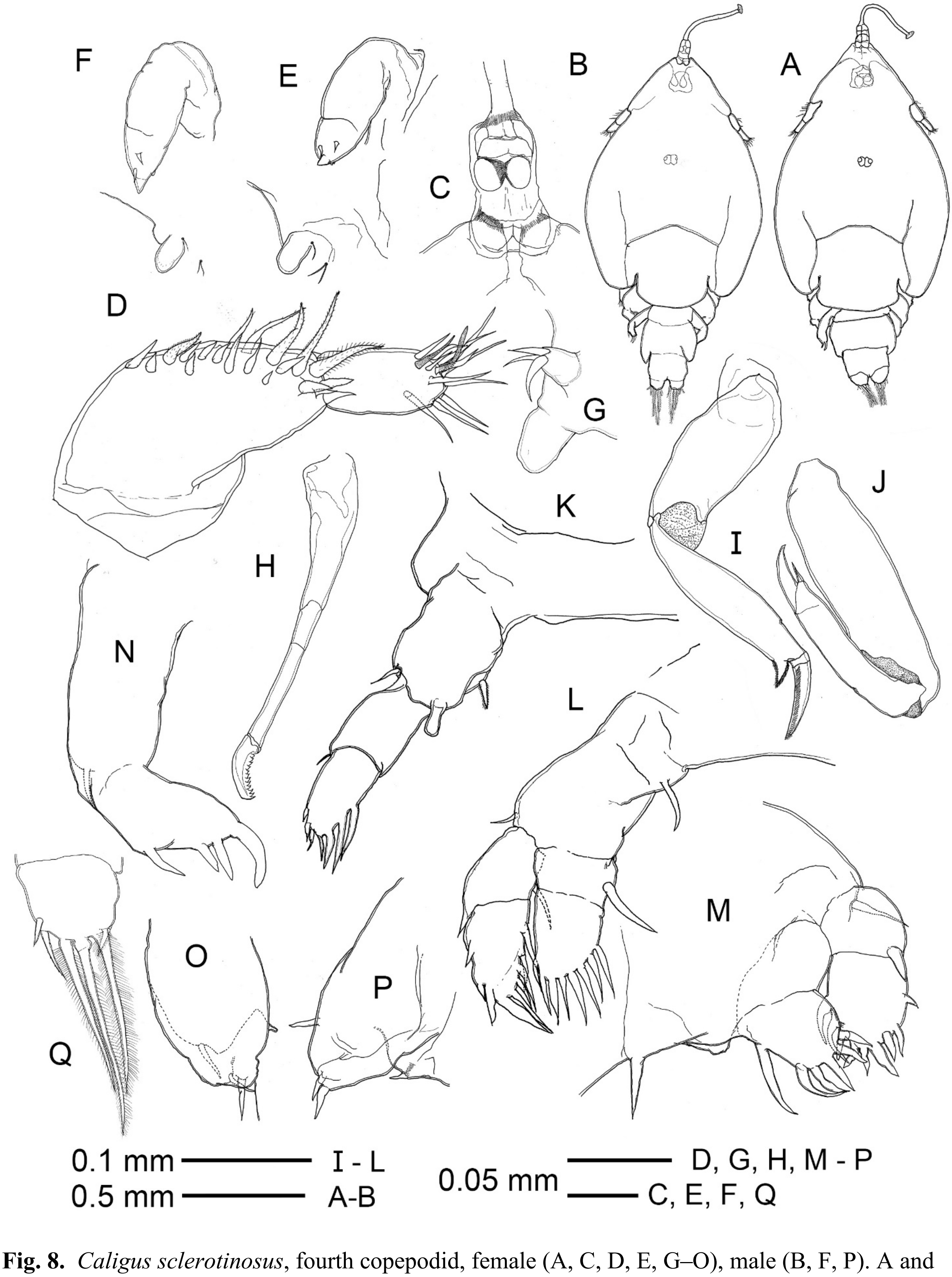

Fig. 8. *Caligus sclerotinosus*, fourth copepodid, female (A, C, D, E, G–O), male (B, F, P). A and B, Habitus, dorsal view; C, Extension lobes at base of frontal filament; D, Antennule; E and F, Antenna and postantennal process; G, Mandible; H, Maxillule; I, Maxilla; J, Maxilliped; K, Leg 1; L, Leg 2; M, Leg 3; N, Leg 4; O and P, Leg 5; Q, Caudal ramus.

Copepodid V (Figs. 9 and 10)

Female: Body length (Fig. 9A) with average length of 1.9 ± 0.2 mm (range: 1.63–2.23 mm, N = 9). General appearance as in preceding stage, but sexual dimorphism more distinctly visible in body size, and shape of genital complex and antenna. Body (Fig. 9A) longer than that of preceding stage; cephalothorax sub-circular, protruded anteriorly, about 3 times longer than following somites and caudal rami combined; distinct frontal plates, H-shaped suture pattern delimiting cephalic, thoracic and lateral zones. Frontal filament (Fig. 9C) with 4 basal bulbs. Rudimentary lunules (Fig. 9A) visible through cuticule of frontal plates. Genital complex expanded laterally; free abdomen 1 segment; caudal ramus (Fig. 9Q) sub-quadrangular armed with 6 plumose setae.

Antennule (Fig. 9D) two-segmented; proximal segment bearing 28 (21 plumose setae + 7 naked setae); distal segment armed terminal 11 naked setae + 2 aesthetascs on distal margin and one sub-terminal seta on posterior margin. Antenna (Fig. 9E) as in preceding stage, but distal endopodal segment with a larger claw. Post-antennary process (Fig. 9E) tapering posteriorly, with 3 basal papillae bearing 3 sensilla. Mandible (Fig. 9G) as in preceding stage. Maxillule (Fig. 9H) with sub-rectangular dentiform process larger than preceding stage; papilla bearing 3 equal naked setae. Maxilla (Fig. 9I) brachiform; proximal segment unarmed; distal segment slender than that of preceding state, carrying 2 unequal elements (short cana and long calamus) terminally. Maxilliped (Fig. 9J) as in preceding stage, three-segmented.

Leg 1 (Fig. 9K) sympod armed with 2 plumose setae; endopod vestigial; exopod distinctly two-segmented, longer than that of preceding stage; proximal segment longer than distal segment, with spiniform element on outer distal edge; distal segment with 3 long naked setae sub-terminally on inner margin; 3 short spiniform elements and short naked seta terminally on outer margin. Leg 2 (Fig. 9L) coxa small with large naked inner seta on posterior edge; basis with small naked seta on outer edge; exopod three-segmented; endopod two-segmented. Leg 3 (Fig. 9M) exopod three-segmented; proximal segment with well-developed spine; endopod indistinctly two-segmented. Leg 4 (Fig. 9N) sympod with short, plumose seta on outer distal corner; exopod indistinctly two-segmented; pecten visible at base of outer-most terminal spine. Armature formula of legs 1–4 as follows:

	Sympod	Exopod	Endopod
Leg 1	1-1	1-0; III, 1, 3	(vestigial)
Leg 2	1-1	0-1; I-1; III, I, 5	0-1; 8
Leg 3	1-1	I-0; I-1; III, 4	0-1; 6
Leg 4	1-0	I-0; IV	(absent)

Leg 5 (Fig. 9O) as in preceding stage, represented by 2 papillae at blunt posterior corner of genital complex, carrying 1 and 2 setae, respectively.

Male: Body (Fig. 9B) average length 1.84 ± 0.18 mm (range: 1.64–2.23 mm, N = 8) (Fig. 10). Sexual dimorphism expressed in body size and shape of genital complex (Fig. 9B), antenna (Fig. 9F), post-antennary process (Fig. 9), abdomen (Fig. 9B) and rudimentary leg 6 (Fig. 9P); other appendages almost same in shape and size as in corresponding-stage female. Antenna (Fig. 9F) with distal segment knob-like armed with 3 spiniform elements sub-terminally, and blunt apical process. Post-antennary process (Fig. 9E) longer than that of corresponding-stage female. Rudimentary leg 6 (Fig. 9P) on ventro-medial surface of genital complex, posterior to leg 5 (Fig. 9P), represented by genital papilla bearing 2 pinnate setules.

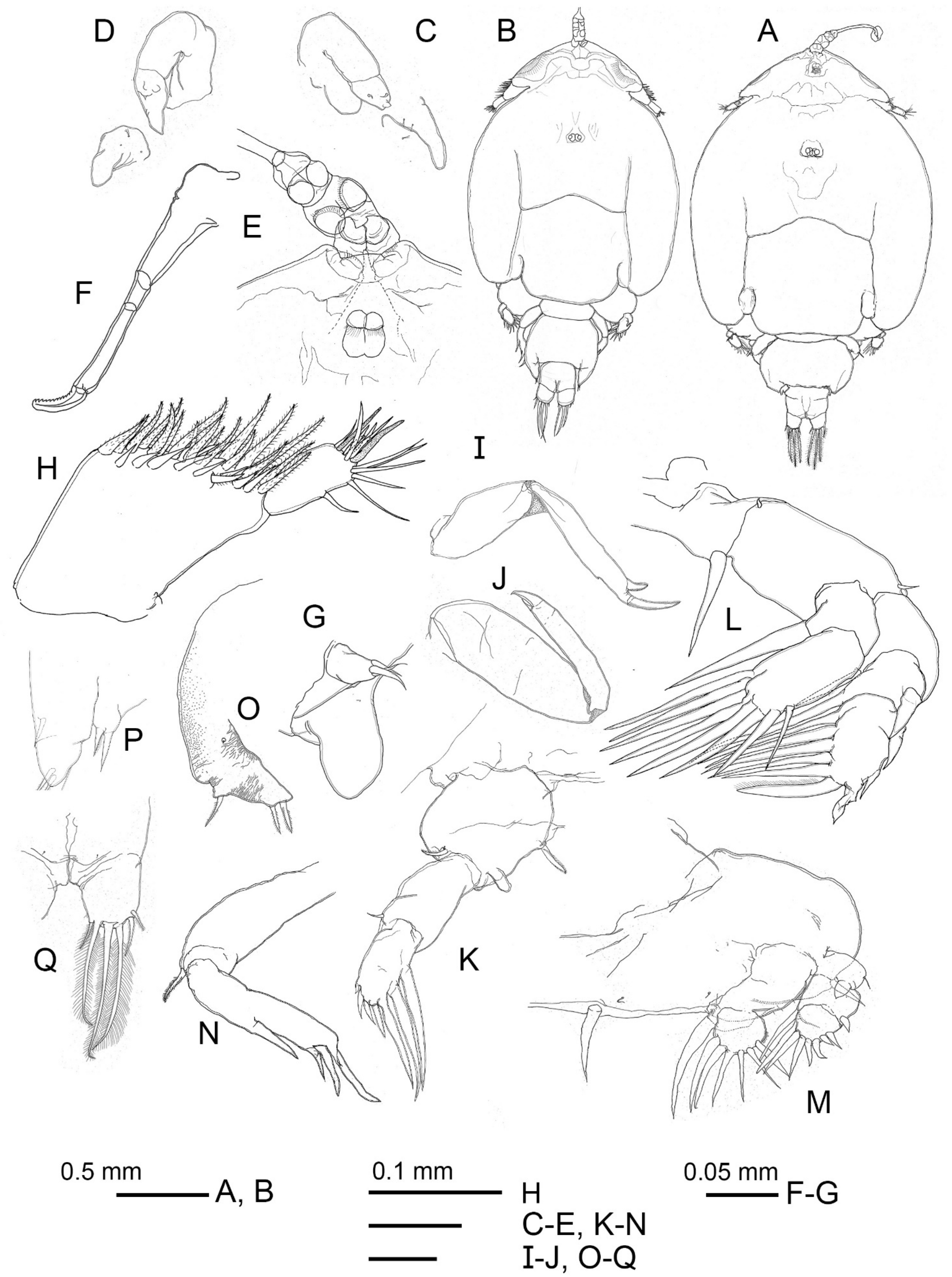

Fig. 9. *Caligus sclerotinosus*, fifth copepodid, female (A, C-E, G–O, Q), male (B, F, P). A and B, Habitus, dorsal view; C, Extension lobes; D, Antennule; E and F, Antenna and postantennal

process; G, Mandible; H, Maxillule; I, Maxilla; J, Maxilliped; K, Leg 1; L, Leg 2; M, Leg 3; N, Leg 4; O and P, Leg 5; Q, Caudal ramus.

Copepodid VI (= adults)

Since both females and males of adult *C. sclerotinosus* were well described by Roubal (1981), Roubal et al. (1983), Ho et al. (2004), Boxshall (2018) and Ohtsuka et al. (2018), only the body lengths of both sexes were measured in the present study (Fig. 10).

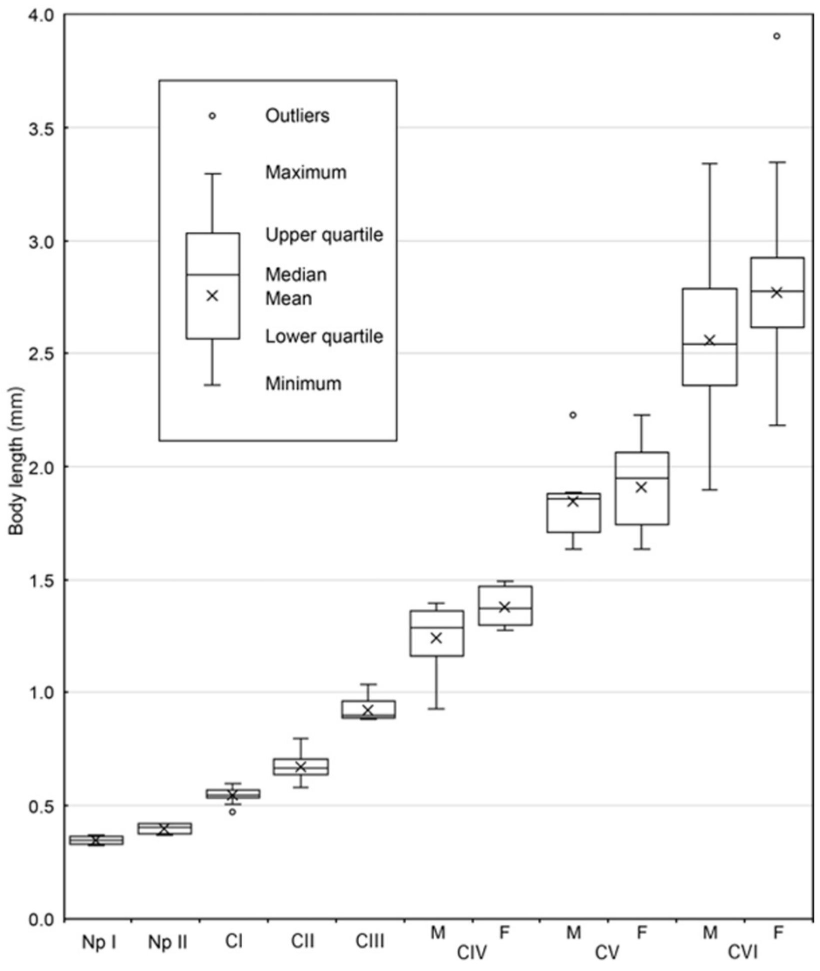

Fig. 10. Body lengths of first naupliar to sixth copepodid stages (adults) of *Caligus sclertotinosus*.

Female: Body average length 2.77 ± 0.21 mm (range: 2.18–3.90 mm, N = 397). Adult females were identified based on the extremely broad shape of the genital complex with its conspicuous leg 5 located at the posterolateral corner.

Male: Body average length 2.56 ± 0.30 mm (range: 1.90–3.34 mm, N = 261). Adult males share the same extremely flattened body form as the female and the leg 5 is conspicuously produced at the posterolateral corner of the genital complex.

DISCUSSION

The developmental stages of *Caligus sclerotinosus* are described for the first time in this study. Both female and male adults have been described previously by Roubal (1981), Roubal et al. (1983), Ho et al. (2004), Boxshall (2018), and Ohtsuka et al. (2018). To date, the life cycle of only 20 species of caligids, out of 518 known species, has been documented and described (Morales-Serna et al. 2015; Walter and Boxshall 2023; present study). In particular, species of the genus *Caligus* (15 species including *C. sclerotinosus*) follow a common pattern in the developmental stages, which are composed of two nauplii, five copepodids and one adult (Kabata 1972; Piasecki 1996; Lin et al. 1996; Ohtsuka et al. 2009; Piasecki et al. 2023). The present work revealed that *C. sclerotinosus* has eight developmental stages separated by a moult: two nauplii (NI–II), five copepodids (CI–V) and one adult (CVI) (see Piasecki et al. 2023). Thus, the life cycle of *C. sclerotinosus* follows the same pattern as that of its congeners.

In this study, the first three stages are free-swimming (NI–II, CI), with CI being infective concomitantly, while the subsequent stages (CII–VI) are ecto-parasitic on the fish host. Furthermore, all post-naupliar stages of *C. sclerotinosus* were found on the same red seabream host, *Pagrus major*. Previous data suggested that there might be one or more intermediate hosts other than *P. major* based on highly skewed composition to adults on farmed host fish. However, the present study clearly showed that the post-naupliar stages of *C. sclerotinosus* essentially infest a single host throughout its life cycle as other congeners do. This fact was confirmed by both field and laboratory data on the rapid growth rates of copepodids and the limited reproductive seasons (Nishida et al. in preparation). However, since an adult female of *C. sclerotinosus* was collected from plankton samples near a red seabream farm in Japanese waters (Venmathi Maran and Ohtsuka, 2008), host-switching may occur in this caligid species. It has been hypothesized that the host-switching behaviour in some adult caligids can be the result of opportunistic behaviour; an escape from irritation or diseases in the host; a change in their life-mode; or accidental detachment from their fish host (Venmathi Maran and Ohtsuka 2008).

Sexual dimorphism in *C. sclerotinosus* is firstly observed at the fourth copepodid stage, as it does in *C. elongates* von Nordmann, 1832, *C. fugu* Yamaguti, 1936, *C. latigenitalis* Shiino, 1954, *C. orientalis* Gusev, 1951, *C. pageti* Russel, 1925, and *C. undulatus* Shen & Li, 1959 (Hwa 1965; Piasecki 1996; Ohtsuka et al. 2009; Madinabeitia and Nagasawa 2011; Aneesh et al. in preparation). Sexual dimorphic differences observed at the fourth copepodid stage of *C. sclerotinosus* include the distal segment of the antenna, the segmentation of the abdomen, the shape of the genital complex, and the presence of leg 6 in males. In the case of adults, the average body length for the females described by Ho et al (2004), 2.98 mm (2.82–3.12 mm, N = 9), is similar to the length observed in the present work, 2.77 mm (2.18–3.90 mm, N = 397) (Table 1). Adult males are shorter than adult females, 2.56 mm (1.90–3.34 mm, N = 261). This study shows females of CIV–VI being relatively larger than the corresponding-stage males, but no significant difference could be found between the average body lengths of both sexes (Mann-Whitney U-test, $p > 0.05$). Such a sexual dimorphic feature, females being significantly larger than males, is found in four caligid species, such as *C. epidemics* Hewitt, 1971, *C. fugu*, *C. punctatus* Shiino, 1955 and *C. rotundigenitalis* Yü, 1933 (Lin et al. 1996; Kim 1993; Ohtsuka et al. 2009; Ho and Lin 2004). On the contrary, significantly larger males than females have been reported for four species, such as *C. centrodonti* Baird, 1850, *C. clemensi* Parker & Margolis, 1964, *C. latigenitalis* and *C. orientalis* (Gurney 1934; Hwa 1965; Kabata 1972; Madinabeitia and Nagasawa 2011). The body lengths of the adult stages of *Caligus* spp. widely range, 2–10 mm (Ho and Lin 2004). In contrast, the body lengths of the nauplii and CI limitedly fall within 0.3–0.7 mm and 0.4–0.8 mm range, respectively (Table 1). This narrow range seems to be related to lecithotrophy during the naupliar and the first copepodid stages (Kearn 2004), which feed solely on the yolk originally contained within the egg. The body lengths of the second to sixth copepodid stages (CII–VI) gradually increased molt by molt at specific and sexual rates. A comparison of the body lengths of two consecutive stages, CIV–VI, revealed that the body becomes approximately 1.3–1.8 times and 1.1–2.0 times larger at each molt in females and males, respectively.

Table 1. Comparison in body lengths of developmental stages among Caligus spp. in the previous and present studies. Generally sexual dimorphism appears in the fourth copepodid stage but in some papers, sexes were not distinguished. -: not available

Species \ Stages	Np I	Np II	C I	C II	C III	C IV		C V		C VI		Reference
						F	M	F	M	F	M	
Caligus sclerotinosus	**0.37 ± 0.02**	**0.40 ± 0.02**	**0.55 ± 0.03**	**0.67 ± 0.05**	**0.92 ± 0.06**	**1.38 ± 0.09**	**1.24 ± 0.16**	**1.90 ± 0.2**	**1.84 ± 0.18**	**2.77 ± 0.21**	**2.56 ± 0.30**	**present study**
C. centrodonti	0.57	-	0.63–0.67	0.7–0.8	1.1–1.2	1.7–1.9		2.77	3.2	-	-	Gurney (1934)
C. clemensi	0.46	0.53	0.66	0.91	1.31	1.31		2.94	3.15	-	-	Kabata (1972)
C. curtus	0.5	0.55	0.7	1.5	-	2.5		-	-	-	-	Heegaard (1947)
C. elongatus	0.448 ± 0.005	0.487 ± 0.02	0.661 ± 0.03	0.82 ± 0.1	1.34 ± 0.13	2.34 ± 0.22		3.79 ± 0.29	3.36 ± 0.28	5.38 ± 0.21	4.33 ± 0.25	Piasecki (1996)
C. epidemicus	0.284	0.295	0.428			1.158	1.052	1.462	1.26	1.877	1.453	Lin et al. (1996)
C. fugu	0.34 ± 0.02	0.37 ± 0.02	0.49 ± 0.03	0.61 ± 0.05	0.95 ± 0.11	1.44 ± 0.13	1.41 ± 0.06	2.20 ± 0.11	2.13 ± 0.16	3.65 ± 0.30	3.05 ± 0.20	Ohtsuka et al. (2009)
C. latigenitalis			0.55	0.77 ± 0.05	1.06 ± 0.06	1.77 ± 0.05	2.15 ± 0.06	2.48 ± 0.07	3.26 ± 0.25	3.36 ± 0.14	3.91 ± 0.12	Madinabeitia and Nagasawa (2011)
C. minimus	0.44	0.74	0.77	1.64	1.29	1.83	-	2.24	-	-	-	Caillet (1979); Madinabeitia and Nagasawa (2011)
C. orientalis	0.270–0.405	0.342–0.475	0.57–0.72	0.81–0.93	1.18–1.41	1.37–2.25		2.35–2.51	2.51–2.75	2.22–3.39	3.72–4.56	Hwa(1965)
C. pageti	0.38 ± 0.014	0.43 ± 0.016	0.67 ± 0.008	0.74 ± 0.08	1.23 ± 0.30	2.17 ± 0.04	-	3.82 ± 0.04	-	-	-	Madinabeitia and Nagasawa (2011); Ben Hassine (1983)
C. punctatus	0.385	0.416	0.565	0.734	1.05	1.64	1.62	2.51	2.38	2.96	2.81	Kim (1993)
C. rotundigenitalis	0.33	0.37	0.5	0.71	0.99	1.42	1.2	1.85	1.5	2.49	1.88	Ho and Lin (2004)
C. spinosus	0.38	0.49	0.7	0.73	0.94	1.16	-	1.63	-	-	-	Izawa (1969)
C. undulatus	0.31 ± 0.01	0.42 ± 0.02	0.64 ± 0.01	0.77 ± 0.09	1.30 ± 0.22	1.94 ± 0.21	1.88 ± 0.25	2.68 ± 0.28	2.61 ± 0.32	-	-	Nawatwa et al. in preparation

The most useful features to differentiate each developmental stage of caligid copepodids were the number of extension lobes at the base of the frontal filament (Piasecki 1996) and the number of setae present on the first segment of the antennule (Kim 1993; Ohtsuka et al. 2009; Piasecki et al. 2023). To date, the number of setae on the first antennulary segment increases following two basic patterns as caligid copepodids develop into adults (CI–VI): 3-3-7-18-26/27/29-27/28/29 and 3-7-13/14-20-27-27 (Ohtsuka et al. 2009). The case of *C. sclerotinosus* was similar to the former pattern but slightly different at CIII: 3-3-7-20-29-29. In the genus *Caligus*, seven species groups are distinguished: *bonito*-, *confusus*-, *diaphanus*-, *macarovi*-, *productus*-, *pseudorhambi*-, and *undulatus*-species groups (Boxshall and El-Rashidy 2009; Boxshall 2018; Ohtsuka and Boxshall 2019; Ohtsuka et al. 2020). The complete life cycle of the *macarovi*-species group is only known for two species, *C. orientalis* (Hwa 1965) and *C. sclerotinosus* (present study). Even though the description of the former species is not accurate enough, the approximate number of setae on the first antennulary segments of CI–VI can be barely counted from the illustrations as follows: 3-3-6-14(?)-24(?)-28(?). This pattern, in particular, that of the early copepodid stages, resembles that of *C. sclerotinosus* rather than the other type. Therefore, it is suggestive that each species group may exhibit its own developmental pattern based in the number of setae on the first segment of antennule. Members belonging to some species groups, such as the *macarovi*-species group, mainly defined on the basis of the segmentation and setation of leg 4 may be convergently assigned. On the other hand, the *bonito*-, *confusus*- and *productus*- species groups seem to be the robust ones with distinct synapomorphies. These species groups should be redefined based not only on genetics but also on developmental biology.

The anlagen of the post-antennary process in *C. sclerotinosus* is visible for the first time at the first copepodid stage (CI) (Figs. 3C, 4). In comparison to other caligid species, the anlagen of the post-antennary process appears for the first time at the third copepodid stage of *C. centrodonti*, *C. elongatus*, *C. fugu*, *C. latigenitalis*, *C. pagetti*, *C. punctatus*, *C. rotundigenitalis*, and *C. spinosus* Yamaguti, 1939. In *C. clemensi*, *C. curtus* Müller, 1785, *C. epidemicus* Hewitt, 1971, the third copepodid stage lacks the post-antennary process, but such structure will appear in later stages. In *C. orientalis*, the process is retained throughout all the copepodid stages (CII–V), except for the first copepodid stage.

The sternal furca located between the right and left maxillipeds is found in most of *Caligus* spp. but absent in *C. absens* and other caligid species (Ho and Lin 2004; Ohtsuka personal observation). The sternal furca is suggested to function as brakes to prevent the caligid copepod from slipping backwards on the host (Kabata and Hewitt 1971; Ho and Lin 2004; Dojiri and Ho 2013). Generally, it first appears at the final moult without the appearance of "bud" in the preceding copepodid stages (Kim 1993; Ho and Lin 2004; Ohtsuka et al. 2009; Madinabeitia and Nagasawa 2011; present study). However, the bud of the sternal furca appears in the fifth copepodid stage in the following species: *C. centrodonti*, *C. clemensi*, *C. elongatus* and *C. orientalis* (Gurney 1934; Hwa 1965; Kabata 1972; Piasecki 1996). Although the adhesive behaviour of the adult caligids to the substrata with the cephalo-thoracic sucker and the lunules has been revealed with a high-speed camera (Ohtsuka et al. 2021), the function of the sternal furca is still unknown. When caligid adults adhere to the substrata, the attachment is accomplished at first with the frontal plate pressing the paired lunules to the substrata, and subsequently, with the cephalo-thoracic sucker (Ohtsuka et al. 2021). Since the tines of the sternal furca are directed backwards, it may be related to the attachment and detachment of the lunules serving as a fulcrum rather than brakes to avoid slipping backwards. The lunules seem to function as supplementary suckers when a small amount of water within a concavity of the lunule enters or leaves via a small anterior pit (Kaji et al. 2012). This seems to be controlled by the muscular movement of the frontal plate (Ohtsuka et al. 2021).

The wide oral cone (Fig. 4) observed on the infective copepodid stage (CI) is found exclusively in *C. sclerotinosus* (Ohtsuka et al. 2018; present study). The oral cone of CI of other congeners has been illustrated in several manuscripts but it has never appeared expanded (*e.g.*, Gurney 1933, 1934; Izawa 1969; Kim 1993; Piasecki 1996). When CI of *Caligus* spp. attaches to the fish host for the first time, it grasps the settlement site with the antennae and maxillipeds at first, which is subsequently replaced by the attachment with the frontal filament as shown in *Lepeophtheirus* spp. (Kearn 2004; Ohtsuka personal observation). Although the function of the expanded oral cone of CI of *C. sclerotinosus* is totally unknown, it is thought to be utilized for supplementary attachment to the host due to its sucker-like shape. This hypothesis may be enhanced by the fact that the second to fifth copepodids (CI–V) of *C. sclerotinosus*, which attach by means of the frontal filament, lack such a specialized oral cone.

Except for the oral cone and the antenna at the first copepodid stage, the oral appendages, including the maxilliped, show few changes in development throughout the copepodid stages (CI–VI) of *C. sclerotinosus*. In contrast, both exo- and endopods of legs 1–4 show an increase in the number of segments and setae ornamentation throughout the developmental stages of CI–VI. Leg 4 firstly appears at the third copepodid stage as in *C. clemensi*, *C. elongatus*, C. *epidemicus*, *C. fugu*, *C. latigenitalis*, *C. punctatus*, and *C. rotundigenitalis*. Meanwhile, in *C. centrodonti*, *C. curtus*, *C. minimus*, and *C. pagetti* leg 4 is visible at the second copepodid stage (see Khoa et al. 2019)). Leg 5 in *C. sclerotinosus* appears at the fourth copepodid stage as it does in *C. elongatus*, *C. fugu*, *C. punctatus*, and *C. rotundigenitalis*. In *C. centrodonti*, *C. curtus*, *C. latigenitalis*, *C. minimus*, and *C. pagetti* leg 5 appears at the third copepodid stage. Leg 5 appears at the fifth copepodid stage in *C. epidemicus*, and at the sixth copepodid stage (= adult) in *C. clemensi*.

In the present study copepodid I of *C. sclerotinosus* took four to five days at 25–26 °C and seven days at 18.6°C to become copepodids VI (adults) under laboratory conditions (see Fig. 11). The influence of temperature on the development of *Caligus minimus* was also previously reported by Khoa et al. (2019). The successful tracking of the duration and the information on the influence of temperature on the development of *C. sclerotinosus*, will be the key to develop further control for this inversive species in aquaculture.

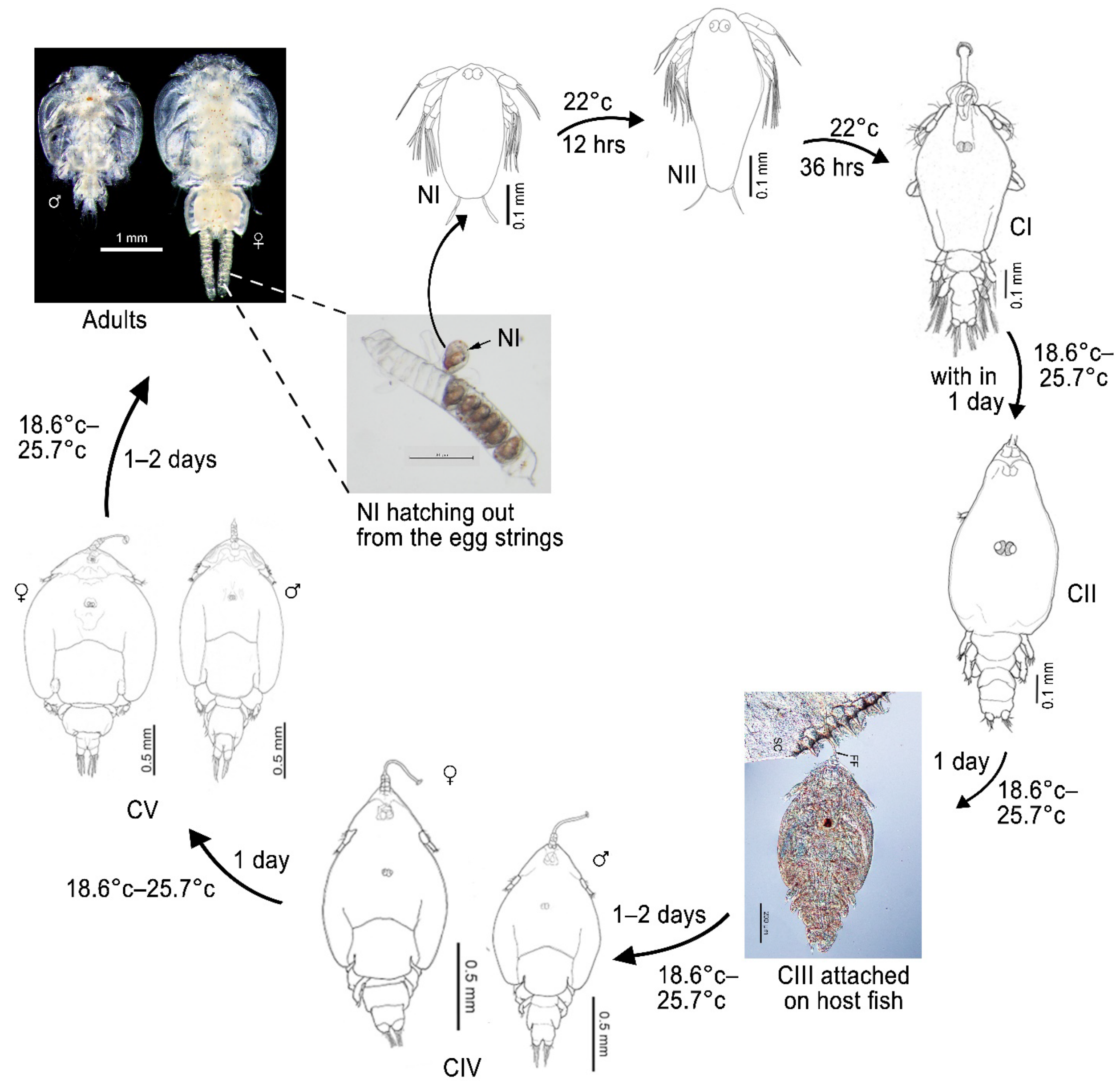

Fig. 11. The life cycle of *Caligus sclertotinosus,* duration and temperature in each stage is also provided. N-1: first nauplius;N-2: second nauplius; C-1: copepodid 1; C-2: copepodid 2; C-3: copepodid 3; C-4: copepodid 4; C-5: copepodid 5.

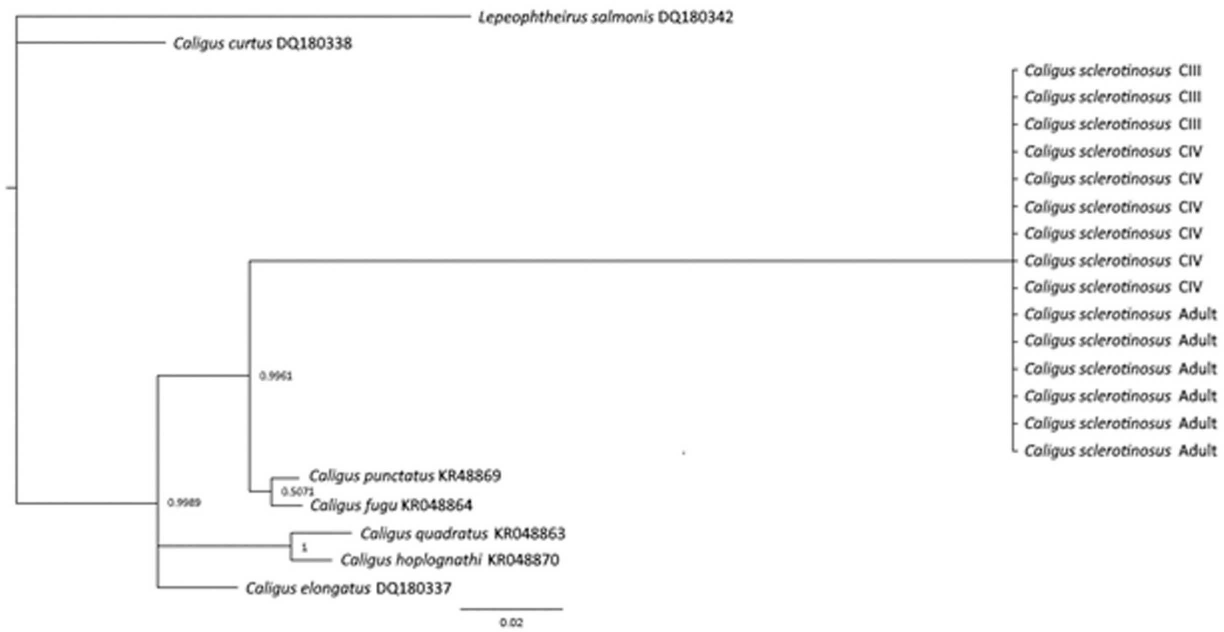

Fig 12. Bayesian inference (BI) tree of the copepodid III, IV and adults of *Caligus sclerotinosus* based on *28S* sequence. Numbers at nodes indicate bootstrap support values. The accession numbers in GenBank are shown after each scientific name.

CONCLUSIONS

This study is pioneer in adopting the alternative terminology for the developmental stages of caligid copepods proposed by Piasecki et al. (2023). According to Piasecki et al. (2023), since chalimi and pre-adults of caligids were not strictly defined, these should be replaced by standardized terms used in free-living copepods: copepodids. Instead, the post-naupliar stages of caligids should be simply called first to fifth copepodid stages (CI–V) and adults (CVI). Thus, the life cycle of *Caligus sclerotinosus* consists on the following eight stages separated by molts: two naupliar stages, five copepodid stages (CI–V) and one adult (CVI). To avoid further terminological problems, future works on the life cycle of other siphonostomatoid copepods should follow the nomenclature proposed by Piasecki et al. (2023), as done in this study.

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Ethics approval consent to participate: The specimen is not under the listed category of experimental animals, which needs ethics approval.

REFERENCES

Ben Hassine OK. 1983. Les copépodes parasites de poissons Mugilidae en Méditerranée occidentale (côtes Françaises et Tunisiennes). Morphologie, bio-écologie, cycles evolutifs. PhD Dissertation, Université des Sciences et Techniques du Languedoc, France.

Benz GW, Mohan PJ, Crow GL. 1992. Developmental stages of *Paralebion elongatus* from aquarium-held reef whitetip sharks (Triaenodon obesus) captured in Hawaiian waters. J Parasitol **78:**1027–1035. doi:10.2307/3283225.

Blanco-Bercial L, Bradford-Grieve J, Bucklin A. 2011. Molecular phylogeny of the calanoida (Crustacea: Copepoda). Mol Phylogenet Evol **59:**103–113. doi:10.1016/j.ympev.2011.01.008.

Boxshall GA. 2018. The sea lice (Copepoda: Caligidae) of Moreton Bay (Queensland, Australia), with descriptions of thirteen new species. Zootaxa **4398:**1–172. doi:10.11646/zootaxa.4398.1.1.

Boxshall GA, El-Rashidy HH. 2009. A review of the *Caligus productus* species group, with the description of a new species, new synonymies and supplementary descriptions. Zootaxa **2271:**1–26. doi:10.11646/zootaxa.2271.1.1.

Caillet C. 1979. Biologie comparee de *Caligus minimus* Otto 1821 st de *Clavellodes macrotrachelus* Brian, 1906 copepodes parasites de poissons marins. PhD Dissertation, Universite des sciences et Techniques du Languedoc, France.

Dojiri M, Ho JS. 2013. Systematics of the Caligidae, copepods parasitic on marine fishes. Crustac Monogr **18:**1–448.

González L, Carvajal J. 2003. Life cycle of *Caligus rogercresseyi*, (Copepoda: Caligidae) parasite of Chilean reared salmonids. Aquaculture **220:**101–117.

Gurney R. 1933. Notes on some copepoda from Plymouth. J Mar Biol Assoc UK **19:**299–304. doi:10.1017/S0025315400055909.

Gurney R. 1934. The development of certain parasitic copepoda of the families Caligidæ and Clavellidæ. Proc Zool Soc Lond **104:**177–217. doi:10.1111/j.1469-7998.1934.tb07748.x.

Heegaard P. 1947. Contribution to the phylogeny of the arthropods. copepoda. Spolia Zool Musei Haun **8:**1–227.

Ho JS, Gómez S, Ogawa K, Aritaki M. 2004. Two species of parasitic copepods (Caligidae) new to Japan. Syst Parasitol **57:**19–34. doi:10.1023/B:SYPA.0000010681.98484.85.

Ho JS, Lin CL. 2004. Sea lice of Taiwan (Copepoda: Siphonostomatoida: Caligidae). The Sueichan Press, Keelung, Taiwan.

Humes AG, Gooding RU. 1964. A method for studying the external anatomy of copepods. Crustaceana **6:**238–240. doi:10.1163/156854064X00650.

Hwa TK. 1965. Studies on the life history of a fish-louse (Caligus orientalis Gussev). Acta Zool Sin **17:**48–57.

Izawa K. 1969. Life history of *Caligus spinosus* Yamaguchi, 1939 obtained from cultured yellow tail, *Seriola quinqueradiata* T. & S.(Crustacea: Caligoida). Rep Fac Fish Pref U Mie **6:**127–157.

Kabata Z. 1972. Developmental stages of *Caligus clemensi* (Copepoda: Caligidae). J Fish Res Board Can **29:**1571–1593. doi:10.1139/f72-245.

Kabata Z, Hewitt GC. 1971. Locomotory mechanisms in Caligidae (Crustacea: Copepoda). J Fish Board Can **28:**1143–1151. doi:10.1139/f71-169.

Kaji T, Venmathi Maran BA, Kondoh Y, Ohtsuka S, Boxshall GA, Tsukagoshi A. 2012. The lunule of caligid copepods: an evolutionarily novel structure. Evol Dev **14:**465–475. doi:10.1111/ede.12000.

Kearn GC. 2004. Leeches, lice and lampreys. A natural history of skin and gill parasites of fishes. Springer, Dordrecht.

Kim IH. 1993. Developmental stages of *Caligus punctatus* Shiino, 1955 (Copepoda: Caligidae). *In*: Boxshall GA, Defaye D (eds.) Pathogens of wild and farmed fish: sea lice. CRC Press, London, pp. 6–29.

Khoa TND, Mazelan S, Muda S, Shaharom-Harrison F. 2019. The Life Cycle of *Caligus minimus* on Seabass (*Lates calcarifer*) from Floating Cage Culture. Thalassas **35:**77–85. doi:10.1007/s41208-018-0088-8

Kumar S, Stecher G. Tamura, K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol **33:**1870–1874. doi:10.1093/molbev/msw054.

Lin CL, Ho JS, Chen SN. 1996. Developmental stages of *Caligus epidemicus* hewitt, a copepod parasite of tilapia cultured in brackish water. J Nat Hist **30:**661–684. doi:10.1080/00222939600770371.

Madinabeitia I. Nagasawa K. 2011. Chalimus stages of *Caligus latigenitalis* (Copepoda: Caligidae) parasitic on blackhead seabream from Japanese Waters, with discussion of terminology used for developmental stages of caligids. J Parasitol **97:**221–236. doi:10.1645/GE-2615.1.

Morales-Serna FN, Rivas-Salas AI, Gómez S, Fajer-Ávila EJ. 2015. Developmental stages and fecundity of *Lepeophtheirus simplex* (Copepoda: Caligidae) parasitic on bullseye puffer fish (*Sphoeroides annulatus*). Folia Parasitol **62:**1–14. doi:10.14411/fp.2015.004.

Nylander JAA. 2004. MrModeltest. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Ohtsuka S. 2010. Diversity of parasitic copepods with a special reference to sea lice. Jpn J Vet Parasitol **9:**1–8.

Ohtsuka S, Boxshall GA. 2019. Two new species of the genus *Caligus* (Crustacea, Copepoda, Siphonostomatoida) from the Sea of Japan, with a note on the establishment of a new species group. ZooKeys **893:**91–113. doi:10.3897/zookeys.893.46923.

Ohtsuka S, Madinabeitia I, Yamashita H, Venmathi Maran BA, Suárez-Morales E, Ho JS. 2018. Planktonic phases in symbiotic copepods: a review. Bull South Calif Acad Sci **117:**104–119. doi:10.3160/3616.1.

Ohtsuka S, Nawata M, Nishida Y, Nitta M, Hirano K, Adachi K, Kondo Y, Venmathi Maran BA, Suárez-Morales E. 2020. Discovery of the fish host of the 'planktonic' caligid *Caligus undulatus* Shen and Li, 1959 (Crustacea: Copepoda: Siphonostomatoida). Biodivers Data J **8:**e52271. doi:10.3897/BDJ.8.e52271.

Ohtsuka S, Nishida Y, Hirano K, Fuji T, Kaji T, Kondo Y, Komeda S, Tasumi S, Koike K, Boxshall GA. 2021. The cephalothoracic sucker of sea lice (Crustacea: Copepoda: Caligidae): the functional importance of cuticular membrane ultrastructure. Arthropod Struct Dev **62:**101046. doi:10.1016/j.asd.2021.101046.

Ohtsuka S, Takami I, Venmathi Maran BA, Ogawa K, Shimono T, Fujita Y, Asakawa M, Boxshall GA. 2009. Developmental stages and growth of *Pseudocaligus fugu* Yamaguti, 1936 (Copepoda: Siphonostomatoida: Caligidae) host-specific to Puffer. J Nat Hist **43:**1779–1804. doi:10.1080/00222930902993757.

Piasecki W. 1996. The developmental stages of *Caligus elongatus* von Nordmann, 1832 (Copepoda: Caligidae). Can J Zool **74:**1459–1478. doi:10.1139/z96-161.

Piasecki W, Venmathi Maran BA, Ohtsuka S. 2023. Are we ready to get rid of the terms "Chalimus" and "Preadult" in the caligid (Crustacea: Copepoda: Caligidae) life cycle nomenclature?. Pathogens **12:**460. doi:10.3390/pathogens12030460.

Ronquist F, Teslenko M, Mark PVD, Ayres DL, Darling A, Ho¨hna, S, Larget B, Liu L, Suchard MA, Huelsenbeck JP 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol **61:**539–542.

Roubal FR. 1981. The taxonomy and site specificity of the metazoan ectoparasites of the black bream, *Acanthopagrus australias* (Günther), in northern New South Wales. Aust J Zool Suppl Ser **30:**1–100. doi:10.1071/AJZS084.

Roubal FR, Armitage J, Rohde K. 1983. Taxonomy of metazoan ectoparasites of snapper, *Chrysophrys autratus* (family sparidae), from Southern Australia, Eastern Australia and New Zealand. Aust J Zool Suppl Ser **31:**1–68. doi:10.1071/AJZS094.

Suyama Y. 2011. Procedure for single-pollen genotyping. *In*: Isagi Y. Suyama Y (eds.) Single-pollen genotyping. Springer, Tokyo, pp. 7–15.

Tanaka S, Yamamoto S, Ogawa K. 2013. The occurrence of *Caligus sclerotinosus* (Caligidae) infection in cultured red sea bream *Pagrus major* and involvement of phototaxis in fish-to-fish transfer of the adults. Fish Pathol **48:**75–80. doi:10.3147/jsfp.48.75.

Thompson JD, Higgins DG. Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res **22:**4673–4680. doi:10.1093/nar/22.22.4673.

Venmathi Maran BA, Moon SY, Ohtsuka S, Oh SY, Soh HY, Myoung JG, Iglikowska A, Boxshall GA. 2013. The caligid life cycle: new evidence from *Lepeophtheirus elegans* reconciles the cycles of *Caligus* and *Lepeophtheirus* (Copepoda: Caligidae). Parasite **20:**15. doi:10.1051/parasite/2013015.

Venmathi Maran BA, Ohtsuka S. 2008. Descriptions of caligiform copepods in plankton samples collected from East Asia: accidental occurrences or a new mode of life cycle?. Plankton Benthos Res **3:**202–215. doi:10.3800/pbr.3.202.

Venmathi Maran BA, Ohtsuka S, Jitchum P. 2012a. Occurrence of caligid copepods (Crustacea) in plankton samples collected from Japan and Thailand, with the description of a new species. Species Divers **17:** 87–95. doi:10.12782/sd.17.1.087.

Venmathi Maran BA, Ohtsuka S, Shang X. 2012b. Records of adult caligiform copepods (Crustacea: Copepoda: Siphonostomatoida) in marine plankton from East Asia, including descriptions of two new species of *Caligus* (Caligidae). Species Divers **17:**201–219. doi:10.12782/sd.17.2.201.

Walter TC, Boxshall GA. 2023. World of Copepods Database. Caligidae Burmeister, 1835. https://www.marinespecies.org/aphia.php?p=taxdetails&id=135513. Accessed 13 June 2023.