

# A New Species and a New Genus of the Family Pennellidae (Copepoda: Siphonostomatoida) Parasitic on North Pacific Lightfish *Maurolicus japonicus* (Actinopterygii: Stomiiformes: Sternoptychidae) Collected from Suruga Bay, Japan

Nanami Yumura<sup>1,2,4</sup>, Jun Nishikawa<sup>3</sup>, and Susumu Ohtsuka<sup>2</sup>

<sup>1</sup> Atmosphere and Ocean Research Institute, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8564, Japan  
E-mail: yumura.nanami@s.nenv.k.u-tokyo.ac.jp

<sup>2</sup> Fisheries Laboratory, Blue Carbon Innovation Division, Seto Inland Sea Carbon Neutral Research Center, Hiroshima University, 5-8-1 Minato-machi, Takehara, Hiroshima 725-0024, Japan

<sup>3</sup> Department of Marine Biology, School of Marine Science and Technology, Tokai University, 3-20-1 Orido, Shimizu, Shizuoka 424-8610, Japan

<sup>4</sup> Corresponding author

(Received 7 September 2023; Accepted 27 December 2023)

<https://zoobank.org/C1C34CBC-A3B0-4A3D-B4AA-D872848EFA31>

Four ovigerous females of the parasitic copepod *Pseudosarcotretes omorii* gen. et sp. nov. (Siphonostomatoida: Pennellidae) were found on North Pacific lightfish *Maurolicus japonicus* Ishikawa, 1915, collected from Suruga Bay, eastern Japan. *Pseudosarcotretes* gen. nov. shares some features with pennellids parasitic on mesopelagic fish such as *Cardiodectes* Wilson, 1917, *Protosarcotretes* Ohtsuka, Lindsay, and Izawa, 2018, and *Sarcotretes* Jungersen, 1911. The morphological differences between the new genus and other related genera are having a pair of lateral cephalothoracic holdfast, the presence of distinct caudal rami, and the absence of leg 4. The hatching stage of the new genus is the copepodid. A molecular phylogenetic analysis suggested that the reduction in the number of developmental stages in pennellid life cycles had occurred at least in two cases. According to the molecular data, the new genus consists of a clade accommodating *Cardiodectes*, *Lernaeenicus* Lesueur, 1824 and *Pennella* Oken, 1815. All these genera have a distinct cephalothoracic holdfast embedded into the host's muscle tissues, which seems to be advantageous for expanding the range of the hosts to small mesopelagic fish.

**Key Words:** parasitic copepods, Siphonostomatoida, new genus, Pennellidae, lightfish.

## Introduction

Bodies of adult females of copepods belonging to the family Pennellidae are highly modified which makes the phylogenetic relationships of pennellid genera ambiguous (Boxshall 1986; Boxshall and Halsey 2004; Yumura et al. 2022). The majority of the genera (*Allotrifur* Yamaguti, 1963, *Cardiodectes* Wilson, 1917, *Creopelates* Shiino, 1958, *Haemobaphes* Steenstrup and Lütken, 1861, *Impexus* Kabata, 1972, *Lernaeenicus* Lesueur, 1824, *Lernaeocera* Blainville, 1822, *Lernaeolophus* Heller, 1865, *Nagasawanus* Uyeno, 2015, *Ophiolernaea* Shiino, 1958, *Pennella* Oken, 1815, *Peroderma* Heller, 1865, *Phrioxcephalus* Wilson, 1908, *Protosarcotretes* Ohtsuka, Lindsay, and Izawa, 2018, *Sarcotretes* Jungersen, 1911, *Serpentisaccus* Blasiola, 1979, and *Trifur* Wilson, 1917) are mesoparasitic, with the anterior part of the body (to the dominant number of somites) embedded in the host tissues and the posterior part exposed to the water (Kabata 1976; Piasecki 2022). The concept of mesoparasitism was introduced by Kabata (1976) and recently redefined by Piasecki et al. (2022). Some pennellid genera are reported

exclusively from small mesopelagic and bathypelagic fish (e.g., Yamaguti and Utiumi 1953; Shiino 1958; Boxshall 1998; Ohtsuka et al. 2018a, b). Yumura et al. (2022) revealed the phylogenetic relationships among the seven genera and 12 species of Pennellidae based on molecular analysis and suggested that pennellids have expanded the host range by acquiring of mesoparasitic way of life. It is noteworthy that we can deduce what kinds of morphological traits are advantageous for expanding the host range.

An unidentified pennellid copepod was found on a mesopelagic sternoptychid fish, *Maurolicus japonicus* Ishikawa, 1915, stranded on the beach of Suruga Bay, eastern Japan (Fig. 1). The present study deals with the taxonomy and molecular phylogeny of this unidentified taxon of the family Pennellidae and discusses the shortened life cycle (in terms of the number of stages) and advantageous traits for infection of parasitic copepods on small mesopelagic host fishes.

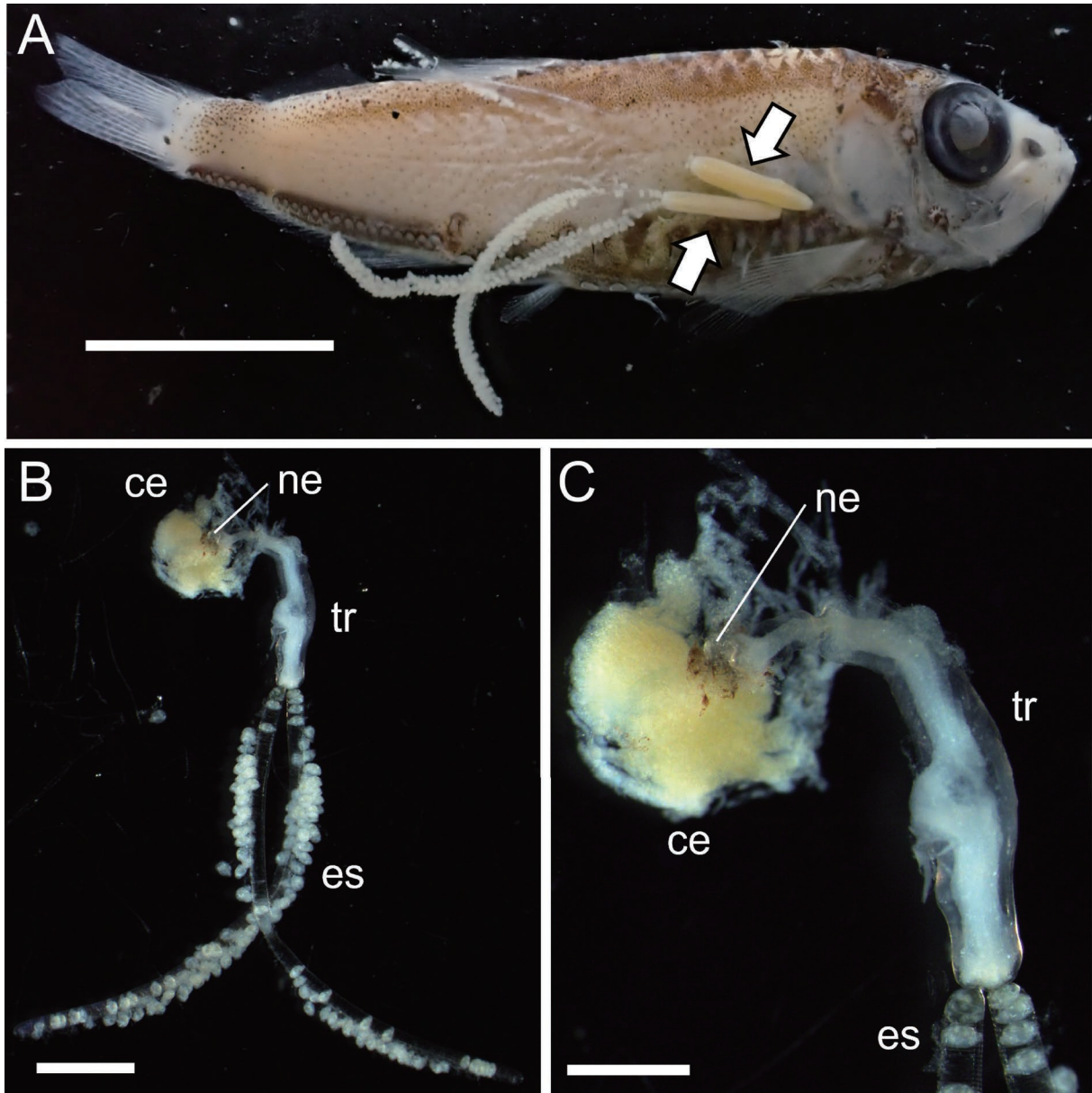


Fig. 1. *Pseudosarcotretes omorii* gen. et sp. nov., adult females. A, Whole specimen (arrowed), in-situ on host, after fixation [above, paratype 1 (NSMT-Cr 31577); below, holotype]; B, whole specimen, dissected out of host, dorsal view [paratype 2 (NSMT-Cr 31578)]; C, body region of specimen (paratype 2). Abbreviations: ce, cephalothorax; es, egg strings; ne, neck; tr, trunk. Scale bars: A, 1 cm; B, 2 mm; C, 1 mm.

## Materials and Methods

**Sample collection and morphological observation.** Three specimens of *M. japonicus* parasitized by mesoparasitic pennellid copepods, stranded at the eastern beach of Miho Peninsula located in Suruga Bay, eastern Japan, were collected on 3–11 February 2019. One specimen of the host with a parasite was preserved in 99.5% ethanol, while the other two were preserved in 10% neutralized formalin/seawater. Since the anterior parts of the parasitic copepods were embedded in the host tissues at the base of the pectoral fin, these were carefully removed from the attachment sites. Two specimens had developed eggs, and juveniles were carefully dissected from the egg strings. The host fish was identified based on morphological features in ac-

cordance with Nakabo (2013). The copepod specimens were observed in lactophenol using Humes and Gooding's (1964) slides and illustrated with the aid of a drawing tube attached to a microscope (BX53, Olympus Co., Ltd., Tokyo, Japan). Morphological terminology of copepods follows Huys and Boxshall (1991) and in some aspects also Kabata (1979).

Type specimens are deposited at the National Museum of Nature and Science, Tsukuba (NSMT) with the collection numbers, NSMT-Cr 31576–31581.

**DNA extraction, PCR, and sequencing.** Total DNA was extracted from parts of the egg strings of paratype 5 (NSMT-Cr 31581) using DNeasy Blood & Tissue Kits (Qiagen, Venlo, Netherlands). The 18S rDNA were amplified using T100 Thermal Cycler (Bio-Rad Laboratories, Inc., CA, USA) with 17.3  $\mu$ L DDW, 2.5  $\mu$ L Ex Taq buffer (10 $\times$ ), 2.0  $\mu$ L dNTP mixture (2.5  $\mu$ M each), 0.3  $\mu$ L of each forward

and reverse primer (20  $\mu$ M), 0.13  $\mu$ L Ex Taq (TaKaRa, Shiga, Japan) and 2.5  $\mu$ L genomic DNA. Used primer sets were 18Sf (5'-TAC CTG GTT GAT CCT GCC AG-3') and 18Sr (5'-TAA TGA TCC TTC CGC AGG TTC AC-3') (Huys et al. 2006). Thermocycling conditions were 94°C for 2 min; 35 cycles at 94°C for 30 s, 56°C for 1 min, and 72°C for 2 min; and a final extension at 72°C for 7 min. Amplicons were purified by ExoSAP-IT express (Thermo Fisher Scientific, MA, USA) following the described protocol. Sequencing reactions were prepared using a BigDye Terminator Cycle Sequence Kit v. 3.1 (Applied Biosystems, MA, USA). The reaction mixtures were purified with a Big Dye XTerminator Purification Kit (Applied Biosystems) and then analyzed on ABI 3500xL Genetic Analyzer (Thermo Fisher Scientific) with following these internal primers; 554f (5'-AAG TCT GGT GCC AGC AGC CGC-3'), 1150f (5'-ATT GAC GGA AGG GCA CCA CCA G-3'), 1282r (5'-TCA CTC CAC CAA CTA AGA ACG GC-3') and 614r (5'-TCC AAC TAC GAG CTT TTT AAC C-3') (Huys et al. 2006). Sequences obtained in this study were deposited in the International Nucleotide Sequence Databases (INSD) through the DNA Data Bank of Japan (INSD accession numbers, LC777452–LC777457).

The details for DNA sequences used in the present analyses were summarized in Table 1. Sequence alignment was performed using MAFFT (Katoh and Standley 2013), and the finally aligned 18S (1673 bp) sequence were obtained. Phylogenetic relationships among the partial 18S sequences were inferred using maximum likelihood (ML) and Bayesian inference (BI) analyses. ML analysis was computed with RAxML v. 8 (Stamatakis 2006; Stamatakis et al. 2008) using RAxML GUI v. 2.0 beta (Edler et al. 2021) under the HKY+G+I model, and the data set was run with 1000 bootstrap (BP) replicates. BI analysis and posterior probabilities (PP) were computed using MrBayes v. 3.2.7 (Ronquist et al. 2012) under the SYM+G+I model for each data subset. Both models were 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 (MC<sup>3</sup>) were conducted for 1000000 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 and trace plots in Tracer v. 1.7.1 (Rambaut et al. 2018). The first 2500 trees (25% of all trees) were discarded as burn-in, and the consensus was formed by 7500 trees. The phylogenetic trees of ML and BI analyses were visualized by FigTree v. 1.4.4 (Rambaut 2012).

## Results

Order **Siphonostomatoidea** Burmeister, 1835  
 Family **Pennellidae** Burmeister, 1835  
 Genus ***Pseudosarcotretes*** gen. nov.

**Type species.** *Pseudosarcotretes* sp. nov., fixed by the present designation.

**Diagnosis.** *Female.* Body elongated and straight, with reduced abdomen without brush-like structure. Cephalothorax with pair of lateral holdfasts with semicircular expansions. Oral cone on anteroventral tip in form of conically elongated proboscis. Neck comprising pedigers 1–3. Anterior part of trunk slightly narrow, posterior part cylindrical; caudal rami present. Egg string straight and uniseriate.

Antennule incompletely two segmented. Antenna well developed and subchelate, 3-segmented; second segment in form of stout process close to edge; third segment curved inward, forming subchela with preceding segment. Mandible short, with three teeth at tip. Maxillule simple, inner lobe with only one terminal seta; outer lobe absent.

Legs 1 and 2 biramous; leg 3 uniramous; rami distinctly 2-segmented; armature elements are shown in Table 2.

*Male.* Unknown.

**Remarks.** The new genus *Pseudosarcotretes* is defined by a combination of the following features: (1) a pair of cephalothoracic processes in the form of lobate lateral expansions, (2) elongated proboscis, (3) abdomen reduced, (4) the presence of distinct caudal rami, (5) legs 1 and 2 biramous and leg 3 uniramous, and (6) leg 4 absent. Maxilla not observed and may have been lost during detaching from the host.

Adult females of *Pseudosarcotretes* gen. nov. shares some features with *Cardiodectes*, *Protosarcotretes* and *Sarcotretes*. *Pseudosarcotretes* differs from *Cardiodectes* in having elongated proboscis (vs. proboscis not formed); differs from *Protosarcotretes* in absence of leg 4 and endopod of leg 3; differs from *Sarcotretes* in having a distinct caudal ramus (vs. absent or as small swellings) and lacking leg 4 completely (vs. vestige). These differences and more detail information are shown in Table 3.

**Etymology.** The new generic name refers to *pseudo-* (Ancient Greek adjective, meaning counterfeit) and its closely related genus *Sarcotretes*. Gender masculine.

### *Pseudosarcotretes omorii* sp. nov.

(Figs 2–4)

**Type-material.** Holotype (NSMT-Cr 31576), one ovigerous female infecting *Maurolicus japonicus* (standard length 38 mm) collected from the eastern beach of Miho Peninsula in Suruga Bay (34.9899°N, 138.5214°E) on 9 February 2019, cephalothorax partly dissected and mounted on a glass slide, body in a vial. Paratype 1 (NSMT-Cr 31577), from the same host fish as in the holotype; paratype 2 (NSMT-Cr 31578) from another individual host (standard length 38 mm) collected from the eastern beach of Miho Peninsula in Suruga Bay (34.9899°N, 138.5214°E) on 3 February 2019; paratypes 3 and 4 (NSMT-Cr 31579, 31580), two copepodids from egg string of paratype 2; paratype 5 (NSMT-Cr 31581), from another individual host (standard length 46 mm) collected from the eastern beach of Miho Peninsula in Suruga Bay (34.9846°N, 138.5165°E) on 11 February 2019.

**Type locality.** The east coast of Miho Peninsula

Table 1. Pennellid species used in the phylogenetic analysis, with notes on host, sampling date and location, INSD accession numbers of the sequences studied, and references.

Species	Host	Order of host	Sampling date	Sampling location	Accession No.	Reference
<i>Penicullisa shiinoi</i> Izawa, 1965	<i>Canthigaster rivulata</i> (Temminck and Schlegel, 1850)	Tetraodontiformes	28 September 2020	Uwajima of the Seto Inland Sea	LC777454	Present study
<i>Peniculus minuticaudae</i> Shiino, 1956	<i>Stephanolepis cirrhifer</i> (Temminck and Schlegel, 1850)	Tetraodontiformes	11 March 2019	Uwajima of the Seto Inland Sea	LC586436	Yumura et al. (2022)
<i>Peniculus ostraciontis</i> Yamaguti, 1939	<i>Thamnaconus modestus</i> (Günther, 1877)	Tetraodontiformes	9 August 2019	Off Wajima, Sea of Japan	LC586437	Yumura et al. (2022)
<i>Peniculus truncatus</i> Shiino, 1956	<i>Sebastes inermis</i> Cuvier, 1829	Scorpaeniformes	3 March 2020	Teshima of the Seto Inland Sea	LC777453	Present study
<i>Haemobaphes pannosus</i> Kabata, 1979	NA	NA	NA	Off the coast of Gyeongsangbuk-do, South Korea	KR048773	Baek and Hwang (unpublished)
<i>Lernaeocera branchialis</i> (Linnaeus, 1767)	<i>Cyclopterus lumpus</i> Linnaeus, 1758	Scorpaeniformes	19 May 2001	Off the coast of Newfoundland, Canada	AY627030	Lovy and Friend (2020)
<i>Phruxocephalus vipereus</i> Shiino, 1956	<i>Branchiostegus japonicus</i> (Houttuyn, 1782)	Perciformes	23 June 2021	off Ishikawa, Sea of Japan	LC650961	Yumura et al. (2022)
<i>Cardiodectes</i> sp.	<i>Parapercis sexfasciata</i> (Temminck and Schlegel, 1843)	Perciformes	28 May 2019	Off the Tanegashima, Pacific Ocean	LC777455	Present study
<i>Pseudosarcotretes omorii</i> gen. et sp. nov.	<i>Maurollicus japonicus</i> Ishikawa, 1915	Stomiiformes	9 February 2019	Suruga Bay	LC777456	Present study
<i>Lernaeenicus radiatus</i> Lesueur, 1824	<i>Centropristis striata</i> (Linnaeus, 1758)	Perciformes	18 July 2019	Off the coast of southern New Jersey, USA	MN523342	Lovy and Friend (2020)
<i>Lernaeenicus hemirhamphi</i> Kirtisinghe, 1932	<i>Hyporhamphus sajori</i> (Temminck and Schlegel, 1846)	Beloniformes	8 September 2019	Okayama of the Seto Inland Sea	LC586441	Yumura et al. (2022)
<i>Pennella</i> sp.	<i>Cololabis saira</i> (Brevoort, 1856)	Beloniformes	5 October 2018	Off the Hokkaido, Pacific Ocean	LC586438	Yumura et al. (2022)
<i>Lernaeenicus ramosus</i> Kirtisinghe, 1956	<i>Epinephelus awoara</i> (Temminck and Schlegel, 1842)	Perciformes	21 May 2019	Off the Miyazaki, Pacific Ocean	LC586439	Yumura et al. (2022)
<i>Lernaeenicus ater</i> Shiino, 1958	<i>Jaydia lineata</i> (Temminck and Schlegel, 1843)	Perciformes	12 May 2019	Fukuyama of the Seto Inland Sea	LC586440	Yumura et al. (2022)

NA, not available.



Table 2. Segmentation and setation of legs of *Pseudosarcotretes omorii* gen. et sp. nov.

Life stage	Leg	Protopod	Exopod	Endopod
Adult female	1	1-0	0-1, 5	0-1, 7
	2	0-0	0-1, 6	0-0, 7
	3	0-0	0-0, 4	absent
Copepodid	1	0-0	I, 4	I, 4
	2	0-0	II, 3	I, 5

Table 3. The differentiation of four pennellid genera.

Genus	Cephalothoracic holdfast	Proboscis	Caudal rami	Endopod of leg 3	Leg 4
<i>Pseudosarcotretes</i> gen. nov.	A pair of lateral expansions	Elongated	Bilobate	Absent	Absent
<i>Cardiodectes</i> Wilson, 1917	Lateral lobes and mass of small processes	Not forming	Absent	Absent	Absent*
<i>Protosarcotretes</i> Ohtsuka, Lindsay, and Izawa, 2018	A pair of lateral expansions	Not forming	Bilobate	Present	Present
<i>Sarcotretes</i> Jungersen, 1911	A paired of bulbous swellings	Elongated	Absent or swelling	Absent	Vestige

\* Except *C. rubosus* Leigh-Sharpe, 1934.

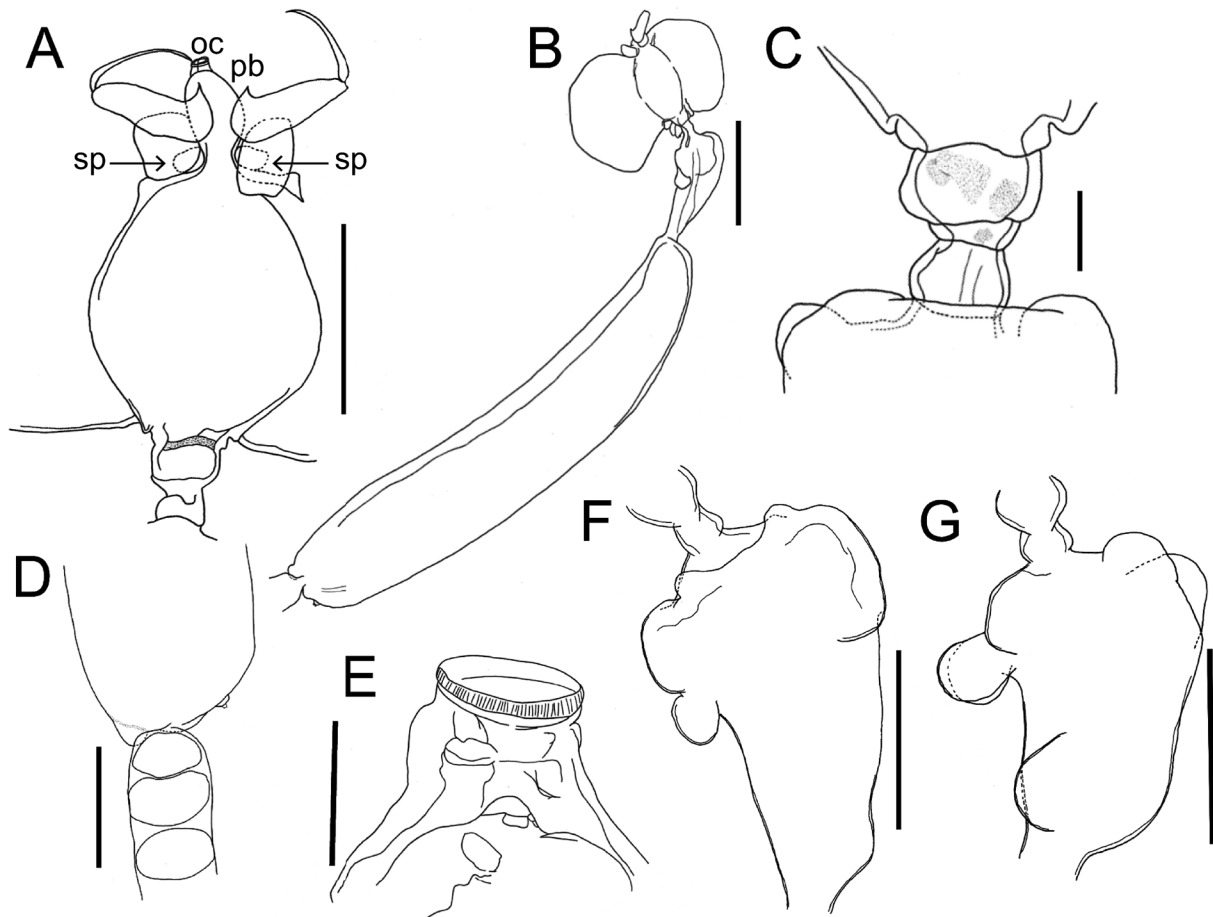


Fig. 2. *Pseudosarcotretes omorii* gen. et sp. nov., ovigerous adult females, holotype (A–F), and paratype (1, NSMT-Cr 31577: G). A, Anterior part of cephalothorax, dorsal view; B, habitus, dorsal view (trunk twisted); C, pedigers 1–3, dorsal view; D, posterior part of trunk, lateral view; E, oral cone, lateral view; F, anterior part of trunk, dorsal view; G, anterior part of trunk, dorsal view. Abbreviations: oc, oral cone; pb, proboscis; sp, semicircular processes. Scale bars: A, D, F, G, 0.5 mm; B, 1 mm; C, 0.1 mm; E, 0.05 mm.

(34.9847°N, 138.5164°E), Japan.

**Host.** *Maurolicus japonicus* Ishikawa, 1915.

**Attachment site.** The cephalothorax, neck, and anterior part of the trunk of the parasite were embedded in the host's

musculature from posterior to right pectoral fin.

**Description.** *Ovigerous female.* Body (Fig. 2B) comprising laterally expanded cephalothorax, relatively slim neck, and trunk with anterior part constricted and posterior cy-

lindrical. Total length ca. 5.5–7.1 mm from anterior tip of cephalothorax to posterior end of caudal ramus. Anterior part of trunk embedded into host tissue. Cephalothorax produced lateral paired semicircle holdfasts (Fig. 2B). First pedigerous somite incorporated into cephalosome (Fig. 2C). Oral cone (Fig. 2E) in the form of elongated proboscis; pair of semicircular processes on constricted area between proboscis and cephalothorax (Fig. 2A). Neck (Fig. 2C) consisting of pedigers 2 and 3, and anterior part of trunk,

0.2–0.3 mm in length. Trunk (Fig. 2B) 3.4–4.4 mm in length, about 1.8 times as long as the cephalothorax and neck; abdomen moderately reduced (Fig. 2D); caudal ramus (Fig. 3F) bilobate; setae missing, probably due to stranding damages. Egg string (Fig. 2D) straight and uniseriate. Number of eggs per string ranging from 298 to 324.

Antennule (Fig. 3A) incompletely two segmented, bearing middle seta; some elements possibly lost during dissection. Antenna subchelate (Fig. 3B), relatively wide, 3-seg-

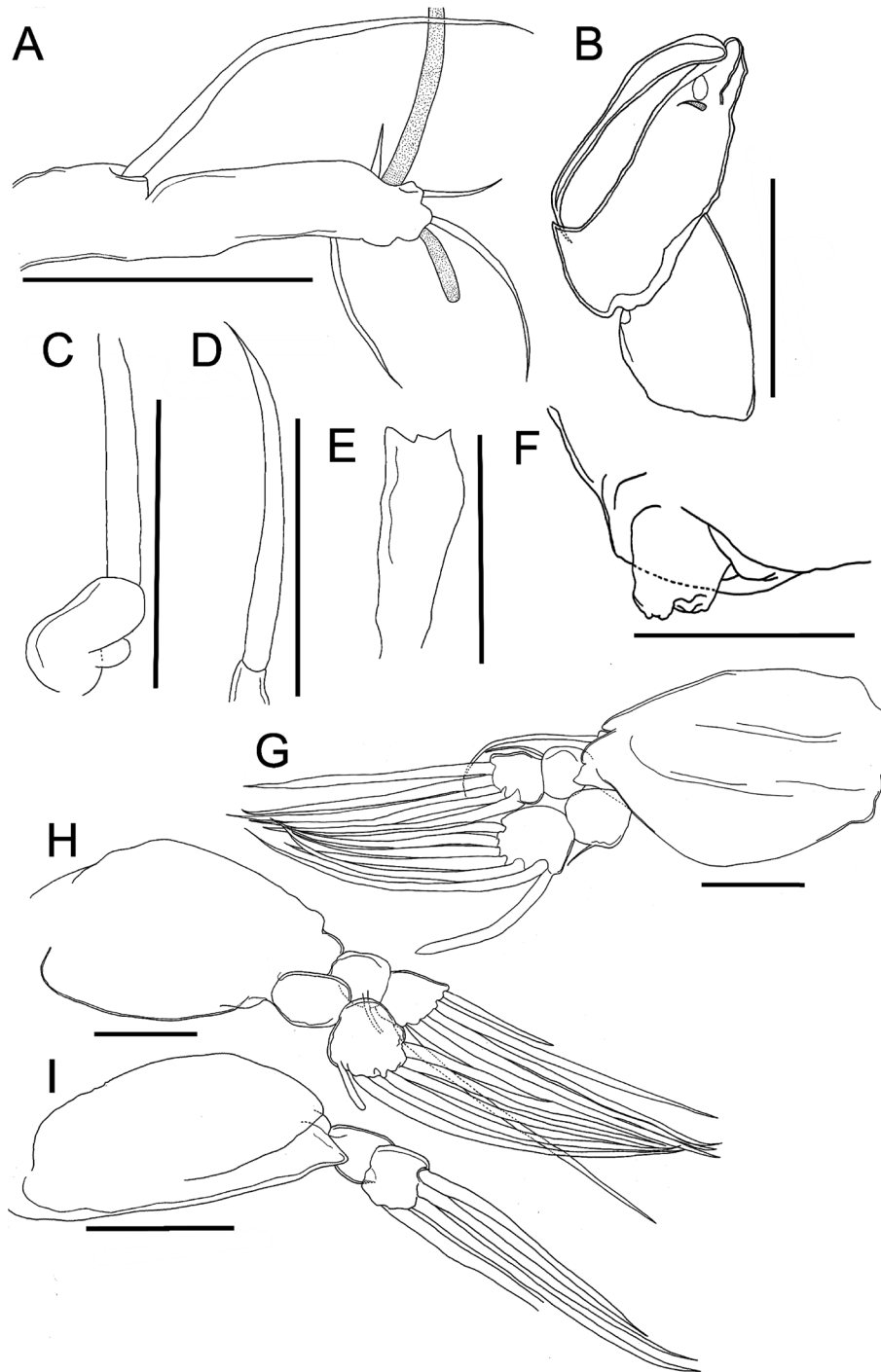


Fig. 3. *Pseudosarcotretes omorii* gen. et sp. nov., ovigerous adult females, holotype (A–F), and paratype (1, NSMT-Cr 31577: G–I). A, Antennule; B, antenna; C, maxillule, basal part; D, maxillule; E, mandible; F, caudal ramus; G, leg 1, anterior surface; H, leg 2, anterior surface; I, leg 3, anterior surface. Scale bars: A, 0.1 mm; B, 0.25 mm; C, D, F, G–I, 0.05 mm; E, 0.01 mm.

mented; second segment produced into robust process near anterior edge; third segment curved inward acting as subchela against preceding segment. Mandible (Fig. 3E) short, spatular, with three teeth. Maxillule (Fig. 3C, D) simple, inner lobe with one terminal seta; outer lobe absent. Maxilla not observed.

Legs 1 and 2 biramous (Fig. 3G, H); rami 2-segmented; leg 3 (Fig. 3I) uniramous; armature elements are shown in Table 2.

*Male.* Unknown.

*Copepodid dissected from pre-hatching egg.* Body comprising cephalosome with incorporated first pedigerous somite, second and third free somites, 2-segmented urosomes, and caudal rami. Cephalothorax (Fig. 4A) ellipsoid, the tip of anterior slightly hollow, oral cone (Fig. 4B) weakly developed. Naupliar eyes conspicuously present. Second pedigerous somite about two-thirds length of third pedigerous somite; urosomite separated vaguely from caudal rami (Fig. 4G).

Antennule (Fig. 4D) 2-segmented. Antenna (Fig. 4C)

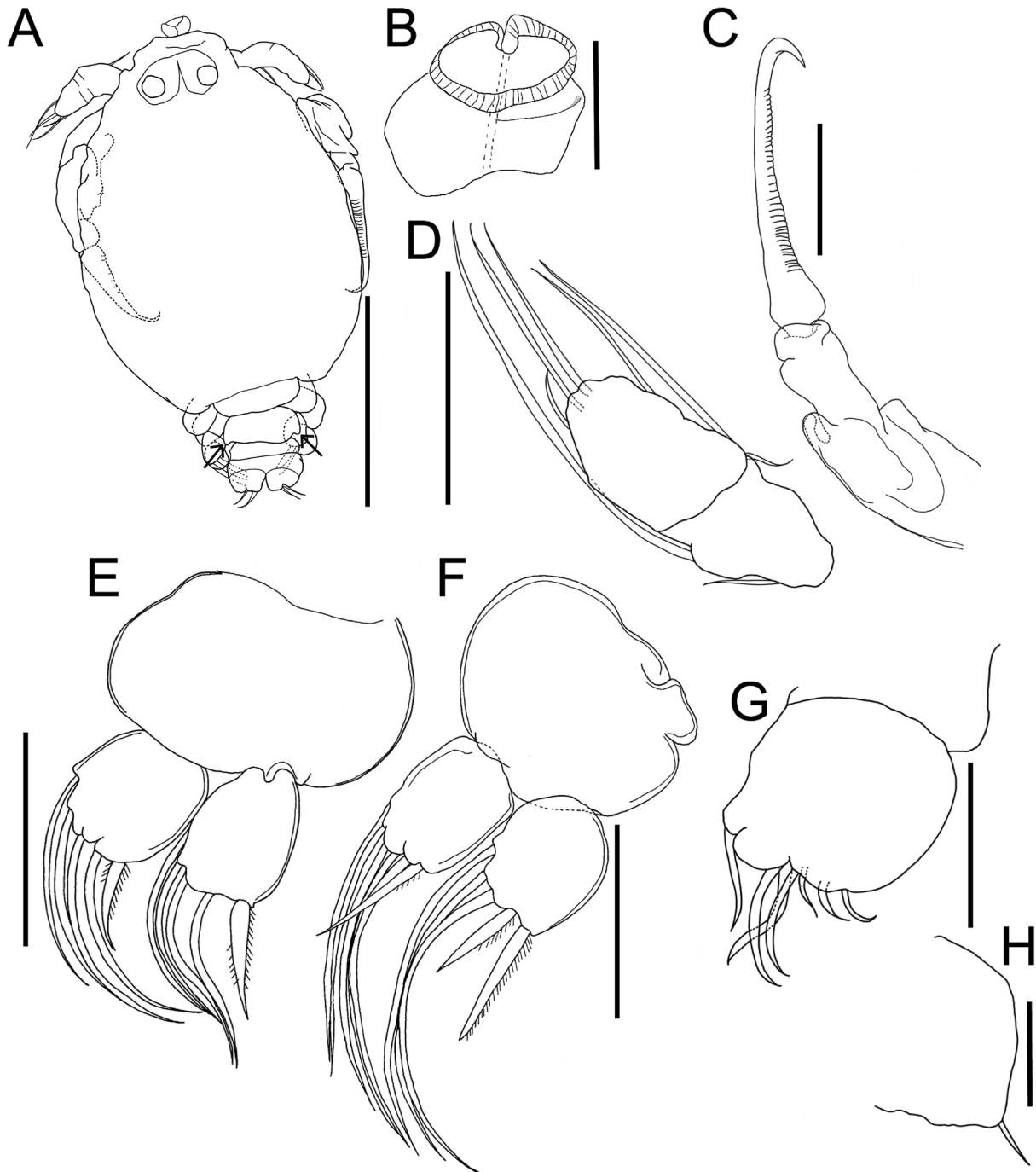


Fig. 4. *Pseudosarcotretes omorii* gen. et sp. nov., copepodids, paratypes (3 and 4, NSMT-Cr 31579, 31580), from the egg string of paratype (2, NSMT-Cr 31578). A, Habitus, dorsal view; B, oral cone; C, antenna; D, antennule; E, leg 1; F, leg 2; G, caudal rami; H, leg 3. Arrows showing bearing parts of leg 3. Scale bars: A, 0.2 mm; B, H, 0.02 mm; C–F, 0.05 mm; G, 0.03 mm.

3-segmented, proximal segment large, having blunt process at mid length, middle segment short, terminal segment curved inward to form subchela with serrate notch. Oral cone (Fig. 4B) short, slightly conical. Oral appendages rudimentary, invisible.

Legs 1 and 2 (Fig. 4E, F) biramous, rami unsegmented; armature elements shown in Table 2; leg 3 (Fig. 4H) represented by single spine on posterior tip of third pedigerous somite.

**Variation.** The number and shape of knobs on the anterior parts of body of adult females vary among specimens. While the holotype has a pair of wing-shaped knobs on the anterior end of trunk, paratype 1 bears two pairs of spherical knobs in addition to one hump near the constricted part (Fig. 2F, G).

**Etymology.** The specific name is named in honor of the late Dr Makoto Omori, who deceased on 4 June 2022, and had been making a great contribution to copepodology of Japan and keen to study the taxonomy and ecology of the pelagic shrimp *Lucensosergia lucens* (Hansen, 1922) in Suruga Bay.

## Discussion

Recently, Yumura et al. (2022) analyzed the phylogenetic relationships among 12 pennellid genera based on nuclear gene sequences, and suggested an evolutionary shift in life mode from ectoparasite to mesoparasite enables the parasites to utilize a wider range of host fish. In the present analysis, molecular data of *Pseudosarcotretes* gen. nov., *Cardiodectes*, and *Peniculisa* Wilson, 1917 were newly added to re-analyze the results of Yumura et al. (2022). The three clades previously proposed by Yumura et al. (2022) were supported, in which *Peniculisa* comprised the same clade

as *Peniculus* von Nordmann, 1832, and then *Pseudosarcotretes* gen. nov. formed a clade together with *Cardiodectes*, *Lernaeenicus*, and *Pennella* with a high bootstrap value and posterior probability (BP = 96, PP = 1) (see Fig. 5). These genera morphologically share cephalothoracic holdfast or anterior parts embedded into the tissues of muscles of their hosts.

Some studies revealed the developmental stages of pennellids (Sproston 1942; Ho 1966; Schram and Anstensrud 1985; Samia 1993; Izawa 1997; Arroyo et al. 2002; Ismail et al. 2013). The hatching stages of *Peniculus*, *Peniculisa*, *Cardiodectes*, *Peroderma*, and *Pseudosarcotretes* gen. nov. are copepodids [*Peniculus minuticaudae* Shiino, 1956 (see Ismail et al. 2013), *Peniculisa shiinoi* Izawa, 1965 (see Izawa 1997), *Cardiodectes* sp. (see Ho 1966), *Peroderma cylindricum* Heller, 1865 (see Samia 1993), *Pseudosarcotretes omorii* gen. et sp. nov. (present study)]. On the other hand, those of *Lernaeocera*, *Lernaeenicus*, and *Pennella* are nauplii [*Lernaeocera branchialis* (Linnaeus, 1767) (see Sproston 1942), *Lernaeenicus sprattae* (Sowerby, 1806) (see Schram and Anstensrud 1985), and *Pennella balaenopterae* Koren and Danielssen, 1877 (see Arroyo et al. 2002)]. Superimposing these developmental features on the phylogenetic tree obtained in the presently reported study indicates that the reduction in the number of stages has occurred at least twice, the *Peniculus-Peniculisa* and the *Cardiodectes-Pseudosarcotretes* clades throughout the pennellid lineage. The copepodids can swim faster than nauplius, and the elimination of the naupliar phase has the advantage of acquiring the host more certainty (Piasecki and Avenant-Oldewage 2008). There may be benefits for the species with the host living in low density because copepodids can be quickly infesting once it finds a host.

Some species of *Cardiodectes* and *Lernaeenicus*, and *Pseudosarcotretes omorii* gen. et sp. nov., which belong to the same clade (see Fig. 5), are known from small, deep-sea

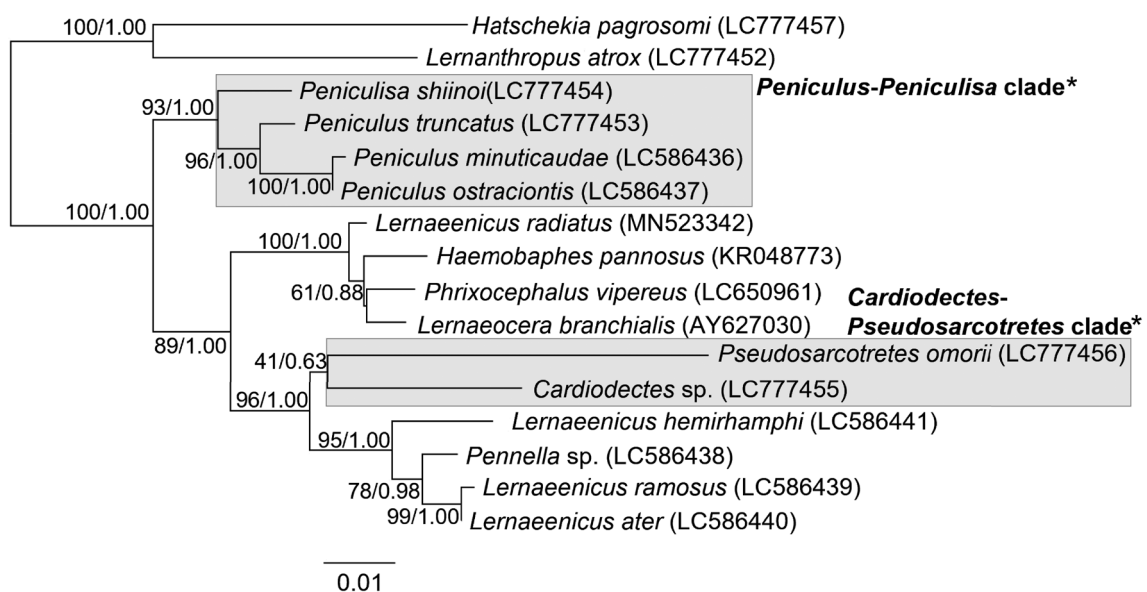


Fig. 5. Phylogenetic tree based on 18S rDNA sequences. INSD accession numbers are shown in parentheses. Arabic numbers of nodes indicate bootstrap values for analyses of maximum likelihood (left) and posterior probabilities (right) for Bayesian inference. The asterisks show that the hatching stages are copepodids.



fishes (< 100 mm) such as cardinal fish and lanternfish (e.g., Yamaguti and Utiumi 1953; Shiino 1958; Boxshall 2000). *Pseudosarcotretes omorii* gen. et sp. nov. shares the infection sites (the tissues of muscles) and the character of having a cephalothoracic holdfast with other genera of the same clade. Several parasitic crustaceans can infect small fishes by adhering to the body surface or the tissues of muscles, but not gills, mouths or fins (e.g., Williams and Bunkley-Williams 1986; Ho and Kim 1989). The relative sizes of the parasites to their small fish hosts seem to be too large to occupy their limited space such as the gills and mouths. Yumura et al. (2022) suggested that pennellids could have expanded the range of host fish with strong swimming ability by acquiring the mesoparasitic way of the infection. Moreover, mesoparasitic ways of the infection, such as embedding into the tissues of muscles and having cephalothoracic holdfast as an anchor, are possibly advantageous to expand the host range to small host fishes.

### Acknowledgments

We would like to thank Dr Naohide Nakayama for the collection of the specimen, and Dr Aki Kato for providing parts of research facilities. We would like to thank Dr Shigeaki Kojima, and Dr Takuya Yahagi for their encouragement during this study, and to Dr Hironori Komatsu for supervising the deposition of the biological specimens in the National Museum of Nature and Science, Tsukuba, Japan.

### Authors Contributions

Nanami Yumura: Investigation; Resources; Data curation; Funding acquisition; Writing – original draft. Jun Nishikawa: Resources; Writing – review & editing. Susumu Ohtsuka: Conceptualization; Resources; Funding acquisition; Supervision; Writing – review & editing.

### Funding

This work was partially supported by grants-in-aid (KAKENHI) from the Japan Society of the Promotion of Science, awarded to SO (Grant Numbers 19H03032), by JST SPRING, awarded to NY (Grant Numbers JPMJSP2108), and a grant-in-aid from School of Marine Science and Technology, Tokai University awarded to JN.

### Declarations

**Competing interests.** The authors declare no conflicts of interest.

### References

- Arroyo, N. L., Abaunza, P., and Preciado, I. 2002. The first naupliar stage of *Pennella balaenopterae* Koren and Danielssen, 1877 (Copepoda: Siphonostomatoida, Pennellidae). *Sarsia* 87: 333–337. doi:10.1080/0036482021000155785
- Boxshall, G. A. 1986. A new genus and two new species of Pennellidae (Copepoda: Siphonostomatoida) and an analysis of evolution within the family. *Systematic Parasitology* 8: 215–225. doi:10.1007/BF00009890
- Boxshall, G. A. 1998. Host specificity in copepod parasites of deep-sea fishes. *Journal of Marine Systems* 15: 215–223. doi:10.1016/S0924-7963(97)00058-4
- Boxshall, G. A. 2000. Parasitic copepods (Copepoda: Siphonostomatoida) from deep-sea and mid-water fishes. *Systematic Parasitology* 47: 173–181. doi:10.1023/A:1006469117230
- Boxshall, G. A. and Halsey, S. H. 2004. *An Introduction to Copepod Diversity*. The Ray Society, London, xv + vii + 966 pp.
- Burmeister, H. 1835. Beschreibung einiger neuen oder weniger bekannten Shmarotzerkrebse, nebst allgemeinen Betrachtungen über die Gruppe, welche sie angehören. *Nova Acta Physico-Medica Academiae Caesareae Leopoldino-Caroliniae Germanicum Naturae Curiosorum* 17: 269–336.
- Edler, D., Klein, J., Antonelli, A., and Silvestro, D. 2021. raxmlGUI 2.0: A graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods in Ecology and Evolution* 12: 373–377. doi:10.1111/2041-210X.13512
- Ho, J.-S. 1966. Larval stages of *Cardiodectes* sp. (Caligoida; Lernaoceriformes), a copepod parasitic on fishes. *Bulletin of Marine Science* 16: 159–199.
- Ho, J.-S. and Kim, I.-H. 1989. *Lophoura* (Copepoda: Sphyriidae) parasitic on the rattails (Pisces: Macrouridae) in the Pacific, with note on *Sphyrion lumpi* from the Sea of Japan. *Publications of the Seto Marine Biological Laboratory* 34: 37–54. doi:10.5134/176159
- Humes, A. G. and Gooding, R. U. 1964. A method for studying the external anatomy of copepods. *Crustaceana* 6: 238–240. doi:10.1163/156854064X00650
- Huys, R. and Boxshall, G. A. 1991. *Copepod Evolution*. The Ray Society, London, 468 pp.
- Huys, R., Llewellyn-Hughes, J., Olson, P. D., and Nagasawa, K. 2006. Small subunit rDNA and Bayesian inference reveal *Pectenophilus ornatus* (Copepoda *incertae sedis*) as highly transformed Mytilicolidae, and support assignment of Chondracanthidae and Xarifiidae to Lichomolgoidea (Cyclopoida). *Biological Journal of the Linnean Society* 87: 403–425. doi:10.1111/j.1095-8312.2005.00579.x
- Ismail, N., Ohtsuka, S., Maran, B. A., Tasumi, S., Zaleha, K., and Yamashita, H. 2013. Complete life cycle of a pennellid *Peniculus minuticaudae* Shiino, 1956 (Copepoda: Siphonostomatoida) infecting cultured threadsail filefish, *Stephanolepis cirrhifer*. *Parasite* 20: 42. doi:10.1051/parasite/2013041
- Izawa, K. 1997. The copepodid of *Peniculisa shiinoi* Izawa, 1965 (Copepoda, Siphonostomatoida, Pennellidae), a single free-swimming larval stage of the species. *Crustaceana* 70: 911–919. doi:10.1163/156854097X00537
- Kabata, Z. 1976. A rational look at parasitic Copepoda and Branchiura. Pp. 175–181. In: Page, L. A. (Ed.) *Wildlife Diseases (Proceedings of the Third International Wildlife Disease Conference Held at the University of Munich's Institute for Zoology and Hydrobiology in Munich, 1975)*. Plenum Press, New York and London. doi:10.1007/978-1-4757-1656-6\_23
- Kabata, Z. 1979. *Parasitic Copepoda of British Fishes*. The Ray Society, London, xii + 468 pp., 199 pls.
- Katoh, K. and Standley, D. M. 2013. MAFFT multiple sequence

- alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780. doi:10.1093/molbev/mst010
- Lovy, J. and Friend, S. E. 2020. Black sea bass are a host in the developmental cycle of *Lernaenicus radiatus* (Copepoda: Pennellidae): insights into parasite morphology, gill pathology and genetics. *Parasitology* 147: 478–490. doi:10.1017/S0031182019001781
- Nakabo, T. 2013. *Fishes of Japan with Pictorial Keys to the Species. Third Edition*. Tokai University Press, Hadano, xlix + xxxii + xvi + 2428 pp. [In Japanese]
- Nylander, J. A. A. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Ohtsuka, S., Lindsay, D. J., and Izawa, K. 2018a. A new genus and species of the family Pennellidae (Copepoda, Siphonostomatoida) infecting the Pacific viperfish *Chauliodus macouni*. *Parasite* 25: 6. doi:10.1051/parasite/2018003
- Ohtsuka, S., Nishikawa, J., and Boxshall, G. A. 2018b. A new species of *Peniculus* (Copepoda: Siphonostomatoida) parasitizing mesopelagic myctophid fish: first discovery of colonization of the genus in deep water. *Parasite* 25: 58. doi:10.1051/parasite/2018057
- Piasecki, W. and Avenant-Oldewage, A. 2008. Diseases caused by Crustacea. Pp. 1113–1198. In: Eiras, J., Segner, H., Wahli, T., and Kapoor, B. G. (Eds) *Fish Diseases*. Science Publishers, Enfield.
- Piasecki, W., Barcikowska, D., Panicz, R., Eljasik, P., and Kochmański, P. 2022. First step towards understanding the specific identity of fish muscle parasites of the genus *Sarcotaces* (Copepoda: Philichthyidae)—New species and first molecular ID in the genus. *International Journal for Parasitology: Parasites and Wildlife* 18: 33–44. doi:10.1016/j.ijppaw.2022.03.008
- Rambaut, A. 2012. FigTree v1.4. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh. Available at <http://tree.bio.ed.ac.uk/software/figtree/> (15 June 2021).
- Rambaut, A., Drummond, A. J., Xie, D., Baele, G., and Suchard, M. A. 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 67: 901–904. doi:10.1093/sysbio/syy032
- Ronquist, F., Teslenko, M., Mark, P. V. D., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., and Huelsenbeck, J. P. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542. doi:10.1093/sysbio/sys029
- Samia, B. 1993. *Contribution à l'étude de Peroderma cylidricum Heller, 1865, parasite de la sardine, Sardina pilchardus (Walbaum, 1792) des côtes tunisiennes*. Master thesis, Faculty of Science, Université de Tunis, Tunis, 91 pp.
- Schram, T. A. and Anstensrud, M. 1985. *Lernaenicus sprattae* (Sowerby) larvae in the Oslofjord plankton and some laboratory experiments with the nauplius and copepodid (Copepoda, Pennellidae). *Sarsia* 70: 127–134. doi:10.1080/00364827.1985.10420625
- Shiino, S. M. 1958. Copepods parasitic on Japanese fishes. 17. Lernaecidae. Report of the Faculty of Fisheries, Prefectural University of Mie 3: 75–100.
- Sproston, N. G. 1942. The developmental stages of *Lernaecocera branchialis* (Linn.). *Journal of the Marine Biological Association of the United Kingdom* 25: 441–466. doi:10.1017/S0025315400055090
- Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690. doi:10.1093/bioinformatics/btl446
- Stamatakis, A., Hoover, P., and Rougemont, J. 2008. A rapid bootstrap algorithm for the RAxML Web servers. *Systematic Biology* 57: 758–771. doi:10.1080/10635150802429642
- Williams, E. H. and Bunkley-Williams, L. 1986. The first *Anilocra* and *Pleopodias* isopods (Crustacea: Cymothoidae) parasitic on Japanese fishes, with three new species. *Proceedings of the Biological Society of Washington* 99: 647–657.
- Yamaguti, S. and Utiumi, H. 1953. *Lernaenicus quadrilobatus* n. sp. (Copepoda, Lernaecidae) parasitic on the lantern-fish *Diaphus coeruleus*. *Publications of the Seto Marine Biological Laboratory* 3: 51–53. doi:10.5134/174461
- Yumura, N., Adachi, K., Nitta, M., Kondo, Y., Komeda, S., Wakabayashi, K., Fukuchi, J., Boxshall, G. A., and Ohtsuka, S. 2022. Exploring evolutionary trends within the Pennellidae (Copepoda: Siphonostomatoida) using molecular data. *Systematic Parasitology* 99: 477–489. doi:10.1007/s11230-022-10040-w