The First Bopyrid Isopod from Hydrothermal Vents: Pleurocryptella shinkai sp. nov. (Isopoda: Epicaridea) Parasitizing Shinkaia crosnieri (Decapoda: Anomura)

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The First Bopyrid Isopod From Hydrothermal Vents: *Pleurocryptella shinkai* sp. nov. (Isopoda: Epicaridea) Parasitizing *Shinkaia crosnieri* (Decapoda: Anomura)

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Deep-sea hydrothermal vents are ‘extreme’ environments harboring diverse animal communities, powered by chemosynthesis. Though vent-endemic macrofauna have been a center of interest since their discovery in 1977, macroparasites have received little attention. Here, we report a bopyrid epicaridean isopod infesting the symbiotic munidopsid squat lobster *Shinkaia crosnieri* Baba and Williams, 1998 from three vent fields of Okinawa Trough, and describe it as *Pleurocryptella shinkai* sp. nov. Although morphologically close to the congeners *Pleurocryptella formosa* Bonnier, 1900, *Pleurocryptella wolffi* Bourdon, 1972, and *Pleurocryptella altalis* Williams, Boyko, and Marin, 2020, both females (via body proportion and characters of barbula, antennae, pleopods, and pleomeres) and males (via features of the head, pleomeres, and uropods) of the new species exhibit distinctive characters. Phylogenetic reconstruction based on the 18S rRNA gene (850 bp) was insufficiently resolved to clarify the relationship of different epicaridean lineages or the exact position of *P. shinkai* sp. nov., but it recovered *P. shinkai* sp. nov. in a distant position from the type genus of Pseudioninae, indicating non-monophyly of this subfamily. Crustaceans are successful in vents but this is only the second vent epicaridean reported, after *Thermaloniscus cotylophorus* Bourdon, 1983 from the East Pacific Rise described from a single cryptoniscus larva. As such, this is the first report of a vent bopyrid isopod and the first vent epicaridean with a known host. Some epicaridean lineages have adapted to tolerating the conditions of hydrothermal vents, and future research will likely uncover more epicarideans and other interesting parasites from these extreme habitats.

Key words: crustacean, Cymothoida, hydrothermal vent, new species, parasites, Okinawa Trough, Pseudioninae

INTRODUCTION

First discovered in 1977 on the Galápagos Rift (Corliss et al., 1979), deep-sea hydrothermal vents are home to chemosynthesis-based ecosystems characterised by not only very high productivity (Van Dover, 2000) but also endemicity, due to the ‘extreme’ environmental conditions requiring animals to be well adapted in order to survive and thrive (Wolff, 2005). Similar chemosynthetic systems were later discovered in hydrocarbon seeps and organic falls, with some species being shared across different types of chemosynthetic ecosystems (Wolff, 2005; Chapman et al., 2019). Crustaceans are often dominant members of vent and seep fauna, especially remarkable are some highly specialised decapods that have established obligatory symbioses with chemoautotrophic bacteria, such as shrimps of the genus *Rimicaris* and squat lobsters of the genus *Kiwa* (Roterman et al., 2018; Methou et al., 2020). The munidopsid squat lobster *Shinkaia crosnieri* Baba and Williams, 1998 is one such species known from vents and seeps in the Indo-West Pacific, and is known for ‘farming’ chemoautotrophic bacterial ectosymbionts on the dense setae growing on the ventral surface of its body (Watsuji et al., 2015). It relies on these bacteria energetically, and is one of the most dominant and emblematic species in chemosynthetic ecosystems of the region (Kojima

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and Watanabe, 2015).

Unlike charismatic, large vent animals such as *S. crosnieri*, parasitic animals that infest them are poorly studied. Although a number of vent-endemic macroparasites such as copepods, acanthocephalans, leeches, polychaetes, and nematodes have been identified, a great deal more remain to be discovered and named (De Buron and Segonzac, 2006). When sorting materials collected from Okinawa Trough vents, we discovered some individuals of *S. crosnieri* exhibiting obvious swellings on either the left or right sides of their branchial chambers. Such condition is commonly induced by the infestation of epicaridean parasitic isopods.

Epicarideans (Isopoda: Epicaridea) are classified into over 800 species (858 species according to the World Register of Marine Species (WoRMS) database, Horton et al., 2022) of unusual parasitic isopods that use crustaceans as hosts throughout their life cycle in two superfamilies: Bopyroidea and Cryptoniscoidea, both dating back to at least the Jurassic (Williams and Boyko, 2012). The parasitic trait is thought to have evolved only once, most probably in species infesting squat lobsters (Boyko et al., 2013). Epicaridea in turn belongs to the suborder Cymothoida, which also includes the fish-parasitic superfamily Cymothooidea (Boyko et al., 2013). Epicaridean isopods exhibit a high degree of sexual dimorphism with larger females and dwarf males. The vast majority of species first use copepods as the intermediate host for their epicaridium larva, which metamorphoses into a microniscus larva that then leaves the copepod host as a cryptoniscus larva and infests larger crustaceans (Williams and Boyko, 2012). Only one species, *Entoniscoides okadai* Miyashita, 1940, is known to not use a copepod host due to its abbreviated development (Miyashita, 1940) but since the life cycle of many species is unclear this could be more widespread. The definitive host crustaceans infested by cryptoniscus larvae usually belong to various groups of decapods, and the modes of infestation, location of attachment, and location of the adult parasites depend on the specific group of epicaridean.

To date, the only epicaridean known from hydrothermal vents is *Thermaloniscus cotylophorus* Bourdon, 1983, a member of Cryptoniscoidea described from the 13°N vent field on the East Pacific Rise based on a single cryptoniscus larva (Bourdon, 1983). Whether this species infests a vent-endemic crustacean and its specific niche in the vent ecosystem, however, remain unclear since there is no information on its host. On the other hand, the dissection of our *S. crosnieri* individuals with dorsal swellings revealed male and female adults of an epicaridean species belonging to Bopyridae in the superfamily Bopyroidea – an undescribed species in the genus *Pleurocryptella*.

Bopyridae is the largest epicaridean family, with over 600 species distributed globally (635 species according to the WoRMS database, Horton et al., 2022), ranging from the intertidal to the deep sea (Williams and Boyko, 2012). Bopyrids are mostly ectoparasitic in the branchial chambers of their definitive decapod hosts and typically infestation triggers the host to produce a characteristic swelling in the carapace that is clearly identifiable from external morphology (Williams and Boyko, 2012). The specificity on definitive hosts is typically quite high, but little is known about specificity for the intermediate copepod hosts.

The bopyrid genus *Pleurocryptella* Bonnier, 1990 in the subfamily Pseudioninae specialises on anomuran squat lobsters, with nine described species and one subspecies (Williams et al., 2020). It is thought to be the sister to the remaining bopyrid epicarideans, and is characterised by features such as both sexes exhibiting biarticulated maxilliped palps and males having well-developed pleopods and articulated uropods (Williams et al., 2020). The type species is *P. formosa* Bonnier, 1900 described from 946 m deep off the Canary Islands (Bonnier, 1900). The genus is now known to be widely distributed across the Atlantic, Pacific, and Indian oceans with the known depth range between approximately 55 m (*Pleurocryptella infecta* Nierstrasz and Brender à Brandis, 1923) to 5130 m (*Pleurocryptella altalis* Williams, Boyko and Marin, 2020), the latter being the deepest record of all described bopyrids. All hosts of species in the genus are squat lobsters in the superfamily Galatheoidea, including the munidid genera *Munida* and *Agononida*, the galatheid genus *Galathea*, and the munidopsid genus *Munidopsis* (Bourdon, 1972, 1976, 1981; Markham, 1974; Page, 1985; Williams et al., 2020). Some species have been shown to have the flexibility of infesting different definitive host species, for example *P. infecta* is known to infest *Munida militaris* Henderson, 1885 in Indonesia (Nierstrasz and Brender à Brandis, 1923), *Munida gracilis* Henderson, 1885 in New Zealand (Page, 1985), and *Munida japonica* Stimpson, 1858 in Japan (Shiino, 1937), although this flexibility appears to be restricted to one host genus.

Here, we examine and characterise the new bopyrid species infesting *S. crosnieri* to formally give it a name, and discuss the significance of finding the first member of Boyproidea in chemosynthetic ecosystems.

MATERIALS AND METHODS

Sample collection

A deep-sea squat lobster *S. crosnieri* infested with bopyrid parasitic isopods was collected using the Human Occupied Vehicle (HOV) *SHINKAI 2000* on-board R/V *NATSUSHIMA* from the Original Site of the Iheya North hydrothermal vent field, Okinawa Trough (Nakamura et al., 2015a) and two further such specimens were collected from the Daiyonyonaguni Knoll hydrothermal vent field, Okinawa Trough (Nakajima et al., 2014). These specimens were initially fixed in 10% formalin and later transferred to 70% ethanol for long-term preservation, and were used for morphological observations. A further individual of *S. crosnieri* infested by the bopyrid was collected using the remotely operated vehicle (ROV) *Hyper-Dolphin* on-board *R/V NATSUSHIMA* in the Hakurei site, Izena Hole vent field, Okinawa Trough (Kawagucci et al., 2010). This specimen was preserved in 99.5% ethanol and the extracted female bopyrid was used for DNA extraction and sequencing. A map of these vent localities is shown in Fig. 1.

Morphology

Male and female bopyrids were removed from the branchial cavity of the host and their gross morphological features were drawn using a camera lucida attached to a stereomicroscope (SZX7, Olympus, Japan). For observation and drawing of details the specimens were dissected, mounted in a solution of glycerine and ethanol, and drawn with a camera lucida on a compound light microscope (CKX41, Olympus, Japan). For scanning electron microscopy (SEM), dissected parts were washed with distilled water and freeze-dried (Aqua FD-6500, Kyowa Vacuum Engineering, Japan) prior to observation with a TM-3000 (Hitachi, Japan)

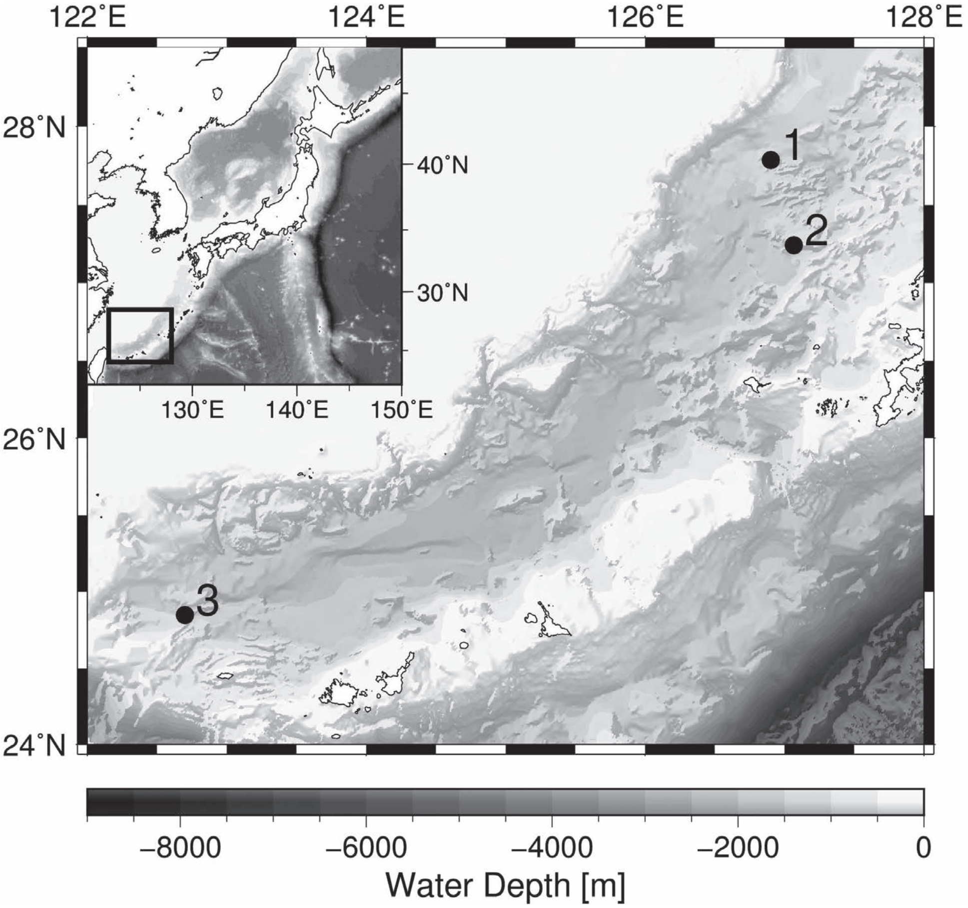

Fig. 1. Map showing hydrothermal vents in the Okinawa Trough where *Pleurocryptella shinkai* sp. nov. was collected: **1**, Original site, Iheya North vent field; **2**, Hakurei site, Izena Hole; **3**, Daiyonyonaguni Knoll vent field.

scanning electron microscope. Terminology used herein follows Williams et al. (2020). Sizes of the bopyrids are indicated by the total length from the anteromedial margin of the head to the posterior margin of the pleon (Cash and Bauer, 1993; Romero-Rodríguez and Román-Contreras, 2008) and the total width, while for the size of the host squat lobster we followed Komai et al. (2017) and measured the carapace length including rostrum from the anterior margin of the head to the posterior margin of the sternum (CL) and carapace width across the widest point of the host specimens (CW). Measurements were made to the nearest 0.1 mm using a calliper for the hosts and an objective micrometer for the parasites.

A whole host squat lobster with parasitic isopods was subjected to X-ray microcomputed tomography (CT) using a ScanXmate-D160TSS105 (Comscantecno, Japan) scanner in Japan Agency for Marine-Earth Science and Technology (JAMSTEC). Amira ver. 2019.3 (Thermo Fisher Scientific, USA) was used to process the scan, following published methods (Sasaki et al., 2018).

Type specimens and their hosts are deposited in the National Museum of Nature and Science, Tsukuba (NSMT), Japan and Seto Marine Biological Laboratory, Kyoto University, Japan (SMBL).

Genetics

In order to understand the phylogenetic position of the bopyrid infesting *S. crosnieri*, we determined partial sequences of its 18S rRNA gene (Machida and Knowlton, 2012), following Boyko et al. (2013). Muscle tissue of a female bopyrid was cut from the ethanol-preserved specimen. Total DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The 18S rRNA gene was amplified by polymerase chain reaction (PCR) using the primer pair #1 and #5_RC designed by Machida and Knowlton (2012). PCR was performed according to the manufacturer's instructions in a mixture containing (TaKaRa *Premix Ex Taq* Hot Start Version 10 µL; template < 500 ng; primer 0.5 µM; sterile purified water up to 20 µL) under the following conditions: 2 min at 95°C, followed by 35 cycles of (45 s at 94°C, 45 s at 60°C, and 2.5 min at 72°C), with a final extension at 72°C for 7 min. The PCR products were purified using ExoSAP-it Express (Thermo Fisher Scientific, USA) and provided to FASMAC Corporation for Sanger sequencing using the primers #1, #3_RC, #4, and #5_RC (Machida and Knowlton, 2012). The obtained data were visualized by the software Geneious Prime 2021.2.2 (https://www.geneious.com/) to manually correct the sequence. The newly obtained 18S rRNA gene sequence was deposited to GenBank under the accession number OL829930.

Phylogenetic reconstruction of the suborder Cymothoida, including the superfamilies Bopyroidea, Cryptoniscoidea, and Cymothooidea, was done using the 18S rRNA gene by adding the sequence of the *Shinkaia*-infested bopyrid to the sequences included in a previously published Cymothoida phylogeny (Boyko et al., 2013) downloaded from GenBank. The sequences were aligned using MAFFT ver. 7.450 (multiple alignment using fast Fourier transform; Katoh and Standley, 2013), resulting in a 1236 bp alignment, which was then imported to MEGA ver. 7.0.26 (Kumar et al., 2016) and gaps and ambiguous regions were removed resulting in an 850 bp-length final alignment. The model selection was done using MEGA ver. 7.0.26 (Kumar et al., 2016) for the maximum likelihood (ML) algorithm, which selected the Kimura 2 Parameter (K2P) + Gamma distribution model, and phylogenetic reconstruction for ML was also done in MEGA with 2000 bootstrap replicates. For phylogenetic reconstruction using Bayesian inference, MrBayes ver. 3.2.6 (Ronquist et al., 2012) was used and since K2P is not implemented in MrBayes we used HKY85 + Gamma distribution as the closest possible model. Metropolis-coupled Monte Carlo Markov chains were run for one million generations, with the topologies sampled every 200 generations and burn-in value at 500. The software Tracer ver. 1.6 (Rambaut et al., 2013) was used to check that the burn-in value was adequately selected. Following Boyko et al. (2013), we used the tanaidacean *Tanais dulongii* to root our tree.

RESULTS

Position of the parasitic isopod

Dissection of each *S. crosnieri* exhibiting bopyridiform swelling revealed a pair of parasitic isopods in either the left or right side of the hosts' gill chambers (Fig. 2A). Males of *Pleurocryptella shinkai* sp. nov. were found to hook onto the dorsal side of the females using their dactyli (Fig. 2B). A 3D reconstruction of the positions of the isopods inside the squat lobster host showed the anterior ends of the parasites directed posteriorly on the host (Fig. 3). The female isopod's ventral side (marsupium) is in contact with the host's branchial cuticle and the head faces the direction of the host's pleon (Fig. 3).

Systematics

Order **Isopoda** Latreille, 1817
Suborder **Cymothoida** Wägele, 1989
Superfamily **Bopyroidea** Rafinesque, 1815
Family **Bopyridae** Rafinesque, 1815
Subfamily **Pseudioninae** Codreanu, 1967
Genus ***Pleurocryptella*** Bonnier, 1900

Type species. *Pleurocryptella formosa* Bonnier, 1900.

Other taxa included (WoRMS database, Horton et al., 2022).

Pleurocryptella altalis Williams, Boyko & Marin, 2020; *Pleurocryptella crassandra* Bourdon, 1976; *Pleurocryptella fimbriata* Markham, 1974, 1974; *Pleurocryptella infecta infecta* Nierstrasz & Brender à Brandis, 1923; *Pleurocryptella infecta tuberculata* Bourdon, 1976; *Pleurocryptella laevis* (Richardson, 1910); *Pleurocryptella latimellaris* (Nierstrasz and Brender à Brandis, 1931); *Pleurocryptella superba* Bourdon, 1981; *Pleurocryptella wolffi* Bourdon, 1972.

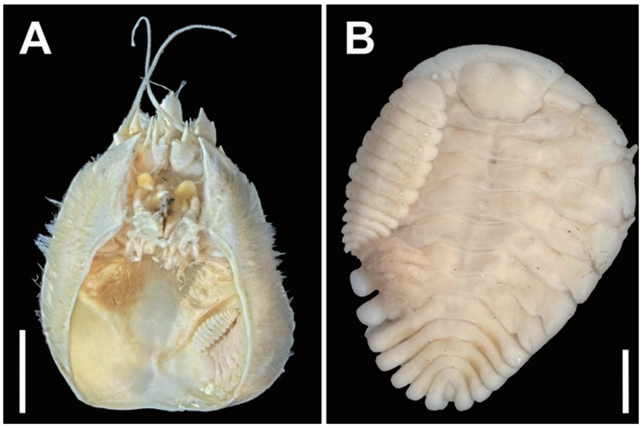

Fig. 2. Photographs of *Pleurocryptella shinkai* sp. nov. **(A)** Holotype female (NSMT-Cr 30591) and allotype male (NSMT-Cr 30592) in vivo in *Shinkaia crosnieri* host (NSMT-Cr 30593); **(B)** female (paratype 5, SMBL-V0642) and male (paratype 6, SMBL-V0644), dorsal view (a piece of host tissue is partially covering the female just below the male). Scale bars: **(A)** 10 mm, **(B)** 2.0 mm.

Pleurocryptella shinkai **sp. nov.**

(Figs. 2–10)

Type material. Holotype (NSMT-Cr 30591; Fig. 2A), mature female (length 11.0 mm, width 8.0 mm), from an ovigerous female *S. crosnieri* (NSMT-Cr 30593; CL 29.4 mm, CW 22.6 mm), Original site, Iheya North vent field, Okinawa Trough (R/V *NATSUSHIMA* cruise NT00-08, HOV *SHINKAI 2000* Dive 2K#1191, 27°47.178′N / 126°54.096′E, 1055 m deep, 17 June 2000). Allotype (NSMT-Cr 30592; Fig. 2A), mature male (length 5.5 mm, width 2.0 mm), from same host as holotype. Paratypes. Paratype 1 (NSMT-Cr 30594), mature female (length 13.0 mm, width 9.0 mm), from ovigerous female *S. crosnieri* (NSMT-Cr 30596; CL 33.2 mm, CW 25.3 mm), Hakurei site, Izena Hole vent field, Okinawa Trough (R/V *NATSUSHIMA* cruise NT11-20, ROV *Hyper-Dolphin* dive 1329, 27°14.5025′N, 127°03.9871′E, 1576 m deep, 5 October 2011), used for genetic sequencing. Paratype 2 (NSMT-Cr 30595), mature male (length 6.5 mm, width 2.2 mm), from same host as paratype 1. Paratype 3 (SMBL-V0639), mature female (length 9.0 mm, width 6.0 mm), from ovigerous female *S. crosnieri* (SMBL-V0640; CL 22.8 mm, CW 17.7 mm) used for micro-CT scan (Fig. 3), Daiyonyonaguni Knoll vent field, Okinawa Trough (R/V *NATSUSHIMA* cruise NT01-05, HOV *SHINKAI 2000* dive 1271, 24°50.934′N, 122°42.012′E, 1332 m deep, 21 May 2001). Paratype 4 (SMBL-V0641), mature male (length 5.0 mm, width 1.2 mm), from same host as paratype 3. Paratype 5 (SMBL-V0642; Fig. 2B), mature female (length 8.5 mm, width 6.5 mm), from female *S. crosnieri* (SMBL-V0643; CL 22.7 mm, CW 17.6 mm), same data as paratype 3. Paratype 6 (SMBL-V0644;

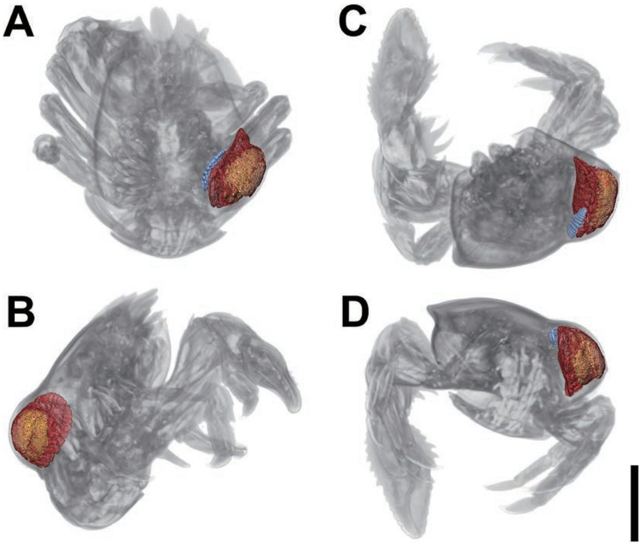

Fig. 3. 3D reconstruction of *Pleurocryptella shinkai* sp. nov. infesting *Shinkaia crosnieri* (NSMT-Cr 30594). Red, female; blue, male; yellow, eggs. **(A)** dorsal view, **(B)** lateral view, **(C)** view from anterior, **(D)** view from posterior. Scale bars: **(A–D)** 10 mm.

Fig. 2B), mature male (length 5.5 mm, width 1.5 mm), from same host as paratype 5.

Description of holotype female. Body (Fig. 4A, B) ovoid, approximately 1.3 times as long as maximum width; pereon slightly deflexed dextrally; left side longer than right side. All body regions and pereomeres distinctly segmented. Head (Fig. 4A) subrectangular, with broad frontal lamina extending beyond pereomere 1; posterior margin convex; anterior margin as well as posterior margin carry indentation; frontal lamina expanded anterolaterally; eyes absent. Barbula (Fig. 4C) with pair of long, tapered outer lobes with round tips; pair of inner lobes narrower than outer lobes, more rounded. Antennule (Fig. 4D) composed of three articles: article 1 without setae; articles 2 and 3 setose. Antenna (Fig. 4E) composed of six articles: articles 4–6 each with short setae distally. Maxilliped (Fig. 4F) with short setae on anterolateral margin: palp segmented, subcylindrical, with short setae distally. Oostegite 1 (Fig. 4G, H) with minute marginal setae: proximal lobe ovate, anteriorly extended; distal lobe approximately 60% narrower than proximal lobe; internal ridge smooth: oostegites 6 and 7 (Fig. 5F, G) extremely reduced, spatula-like, with many fine setae.

Pereon (Fig. 4A) broadest across pereomere 3, gradually tapering anteriorly and posteriorly: pereomere 1 with concave posterior margin; pereomeres 2–4 nearly straight; pereomeres 5–7 sinuous.

Coxal plates and dorsolateral bosses on pereomeres 1–4 (Figs. 4A, 6B). Coxal plates and dorsolateral bosses indistinct on pereomeres 5–7 (Figs. 4A, 6B).

Pereopod 1 (Fig. 5A) shorter than pereopods 2–7; basis moderately narrower than pereopods 2–7; carpus with minute setae distally. Pereopods 2–7 (Fig. 5B–G) subequal in shape and size: bases broad; carpi with minute setae distally. Pleon (Figs. 4A, 6A) with six pleomeres, including pleotelson.

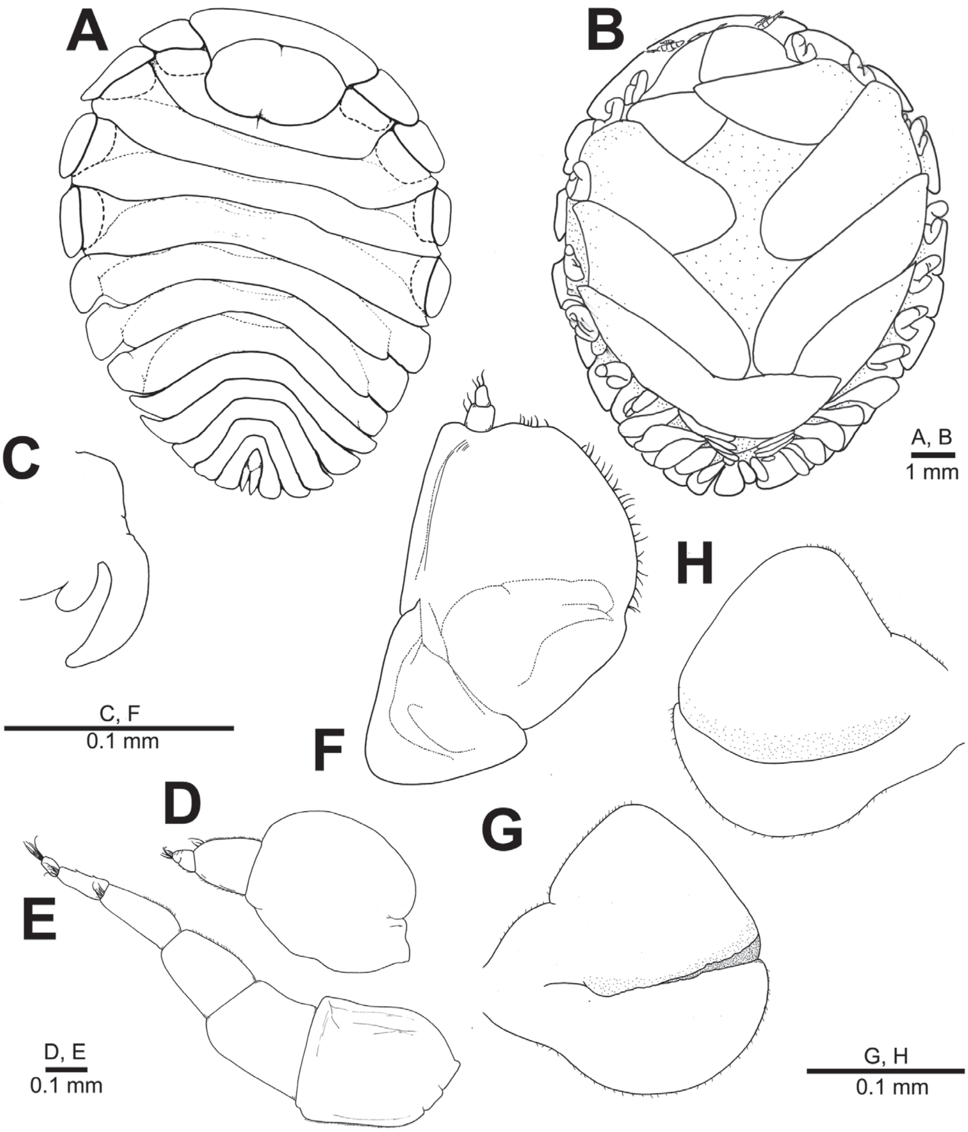

Fig. 4. *Pleurocryptella shinkai* sp. nov., holotype female (NSMT-Cr 30591): **(A)** dorsal view; **(B)** ventral view; **(C)** left barbula, ventral; **(D)** right antennule, external; **(E)** right antenna, external; **(F)** left maxilliped, external; **(G)** left oostegite 1, internal; **(H)** left oostegite 1, external.

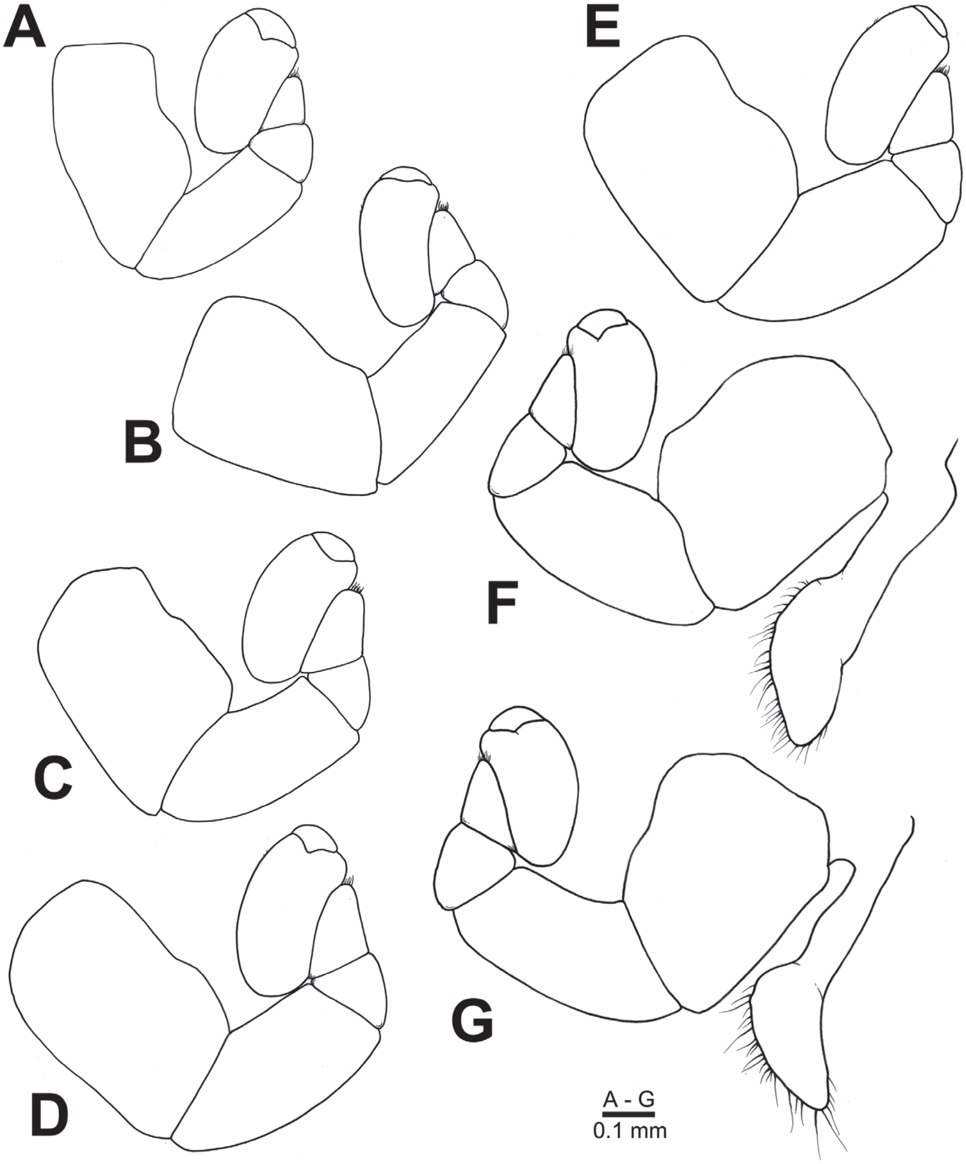

Fig. 5. *Pleurocryptella shinkai* sp. nov., holotype female (NSMT-Cr 30591): **(A)** right pereopod 1, lateral; **(B)** left pereopod 2, medial; **(C)** right pereopod 3, lateral; **(D)** right pereopod 4, lateral; **(E)** left pereopod 5, medial; **(F)** left pereopod 6, with attached oostegite 6, lateral; **(G)** left pereopod 7, with attached oostegite 7, lateral.

Pleomeres curved posteriorly, pleomere 5 surrounds pleotelson slightly. Pleomeres 1–5 (Fig. 6A) with biramous pleopods, progressively smaller posteriorly. Pleopod 5 more than half as large as pleopod 4. Marsupium slightly open with full brood of eggs or epicaridium larvae. Uropod (Figs. 4A, 6A) uniramous, similar to exopodites of pleomeres, scarcely projecting beyond distolateral margins of pleomere 5.

Description of allotype male. Body (Fig. 7A, B) slender, dorsoventrally flattened, approximately 2.7 times as long as maximum width. Head (Fig. 7A) hemispherical, widest near posterior margin, width equal to width of pleomere 1. Antennule (Fig. 7C) of three articles, setae on all articles; setae on terminal article long. Antenna (Fig. 7D) of five articles: articles 1–3 with minute setae; articles 2–5 distally with long setae. Eyes absent.

All pereomeres (Fig. 7A, B) rounded dorsolaterally: pereomeres 1 to 4 (Fig. 7A) increasing in width posteriorly; pereomeres 4–6 subequal in width; pereomeres 6 to pleotelson decreasing in width posteriorly. Pereomeres 1–3 approximately straight; pereomeres 4–7 directed posterolaterally. Pereopods 1 and 2 (Fig. 7E, F) subequal in shape and size, shorter than pereopods 3–7; carpi with short setae distally; propodi with small projection having short teeth distally; dactyli curved inward. Pereopods 3–7 (Fig. 8A–E) subequal in shape and size: carpi with short setae distally; propodi without setae; dactyli small.

All pleomeres (Fig. 7A, B) directed posterolaterally: sides of all pereomeres curled ventrally and overlapping. Midventral tubercles of pleomeres 1–4 (Fig. 7B) broad, covering almost all space between pleopods; small, hemispherical on pleomeres 5. Pleopods 1–5 (Fig. 7B) small, rounded, partly hidden by curled lateral margins of pleomeres. Pleotelson (Fig. 7B) uniramous, ovate, extending slightly beyond anal cone. Uropod similar to or slightly smaller than

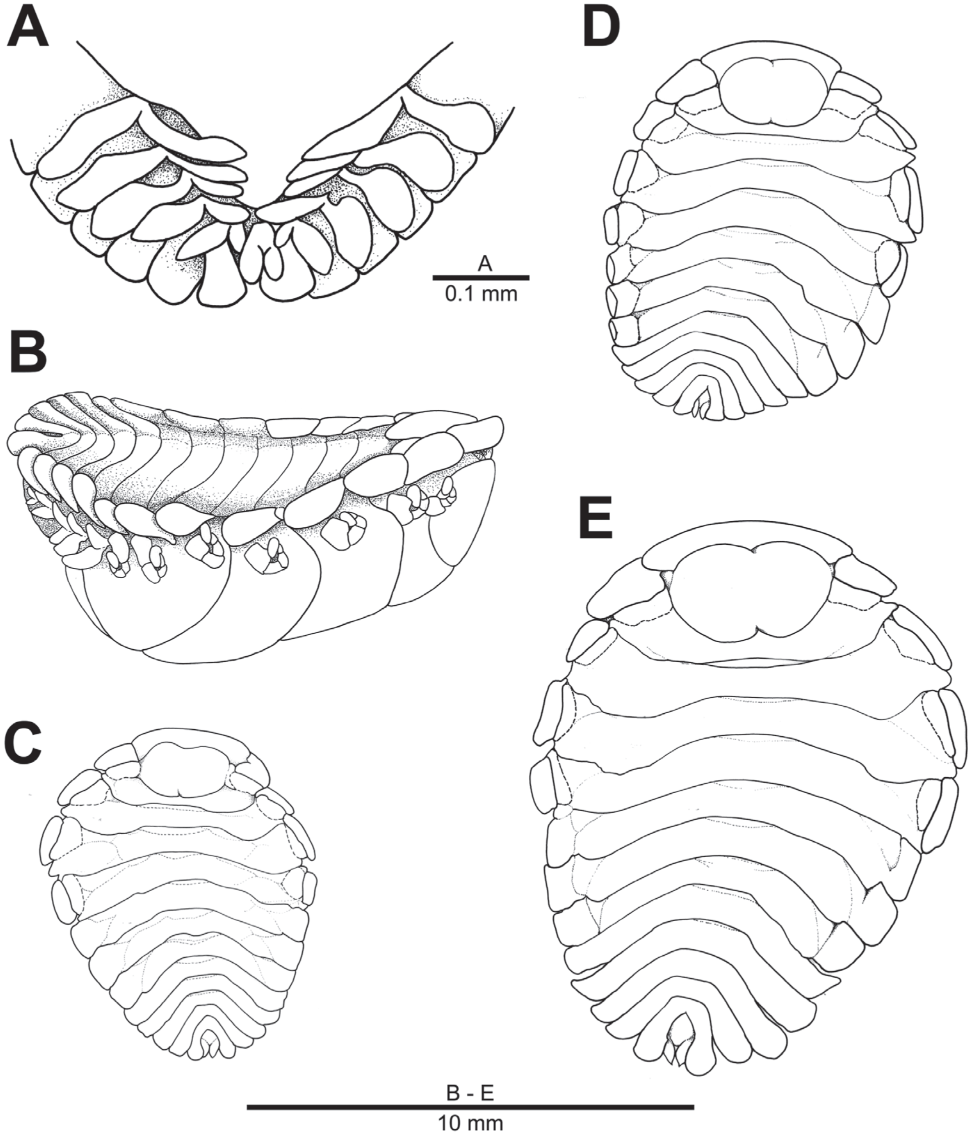

Fig. 6. *Pleurocryptella shinkai* sp. nov.: **(A)** holotype female (NSMT-Cr 30591), abdomen, ventral (1–5 pleopodal exopodites, 1–5 pleopodal endopodites, uropodal exopodites, uropodal endopodites, pleotelson); **(B)** holotype female (NSMT-Cr 30591), lateral view; **(C)** paratype 1 (NSMT-Cr 30594) female, dorsal view; **(D)** paratype 3 (SMBL-V0639) female, dorsal view; **(E)** paratype 5 (SMBL-V0642), dorsal view.

pleopod 5 in size.

Variation. In female paratypes (1, 3, and 5; Fig. 6C–E), there are morphological variations in that the body is approximately 1.3–1.5 times as long as broad, the length of left and right sides vary. The coxal plates and dorsolateral bosses are clearly separated on pereomeres 5–7 only on the left side of one paratype (Fig. 6D), whereas they are fused on all other individuals examined. Among male paratypes, body length varies between 3.0–4.1 times as long as broad.

Etymology. ‘*Shin-kai*’, noun, meaning ‘deep sea’ in Japanese. A name with triple-meaning: 1) in reference to its deep-sea habitat, 2) in honor of the JAMSTEC *Shinkai* submersibles (i.e., HOV *Shinkai 2000* and HOV *Shinkai 6500*) which also collected most of the specimens used herein, 3) also in reference to the genus of the host species *Shinkaia crosnieri*.

Japanese name. ‘*Goemon-no-tankobu*’, with ‘goemon’ being a reference to *S. crosnieri*’s Japanese name ‘goemon-koshioriebi’ (after Goemon Ishikawa, the legendary Japanese chivalrous robber of the Azuchi-Momoyama period who inspired the character Goemon Ishikawa XIII in the manga series *Lupin III*) and ‘tankobu’ literally meaning a lump or bump and figuratively meaning a thorn in one’s side. Named in reference to the bopyridiform swellings formed by this species on the hosts.

Remarks. This new species is morphologically similar to *P. formosa*, *P. wolffi*, and *P. altalis*. The males of these three species are similar to the present new species, with all exhibiting broad midventral tubercles on pleomeres 1–4, covering almost all the space between the pleopods (Fig. 7B) (Williams et al., 2020); males of the other species in the genus have small hemispherical tubercles on the pleomeres, and the space between pleopods is not covered (Bonnier, 1900; Bourdon, 1972; Williams et al., 2020). Males and females of

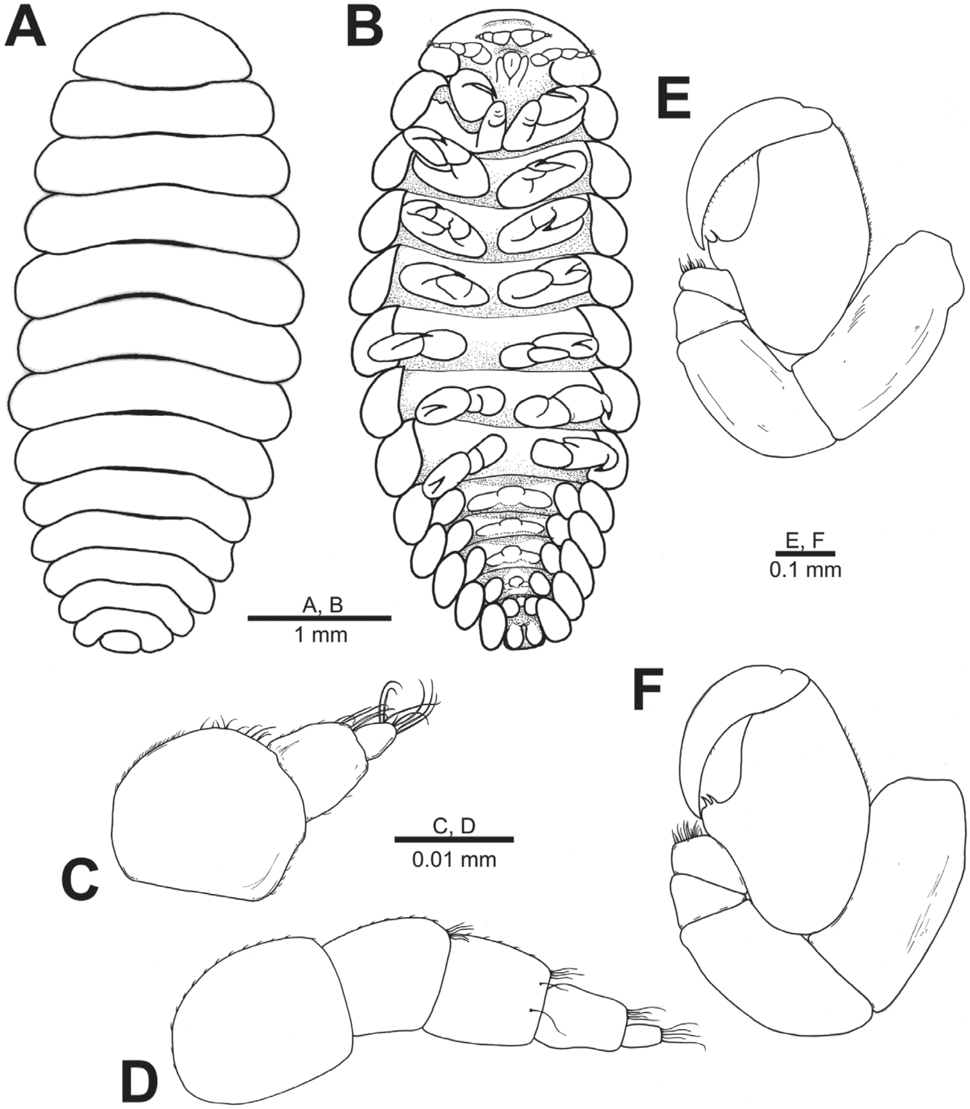

Fig. 7. *Pleurocryptella shinkai* sp. nov., allotype male (NSMT-Cr 30592): **(A)** dorsal view; **(B)** ventral view; **(C)** left antennule, external; **(D)** left antenna, external; **(E)** left pereopod 1, lateral; **(F)** left pereopod 2, lateral.

P. shinkai sp. nov. can be distinguished from *P. formosa*, *P. wolffi*, and *P. altalis* by the following characters.

Females of *P. shinkai* sp. nov. have a body that is longer than wide (Fig. 4A), whereas in *P. formosa*, the total body length and width are subequal (Bonnier, 1900). In *P. shinkai* sp. nov. the barbula consists of two lobes of different lengths (Fig. 4C), the outer lobe is tapered and rounded at the tip, being wider than the inner lobe, which is shorter and more rounded (Fig. 4C). This differs from *P. altalis*, in which the two lobes are approximately the same length and the barbula is sharp and elongated (Williams et al., 2020). The antennae of the *P. shinkai* sp. nov. female consist of six articles (Fig. 4E), whereas there are five articles in females of *P. formosa*, *P. wolffi*, and *P. altalis* (Bonnier, 1990; Bourdon, 1972; Williams et al., 2020). Pleopod 5 of the *P. shinkai* sp. nov. female is over half the size of pleopod 4 and is biramous (Fig. 6P), while in *P. altalis* it is less than half the size of pleopod 4 and uniramous (Williams et al., 2020). In *P. shinkai* sp. nov. females the pleomeres are curved posteriorly, with pleomere 5 slightly surrounding the pleotelson (Fig. 4A), whereas in *P. altalis* although the pleomere is also curved it does not surround the pleotelson (Williams et al., 2020); in *P. formosa*, the pleomeres are not curved at all (Bonnier, 1900).

In males of *P. shinkai* sp. nov. the lateral margins of the pleomeres are curled laterally in ventral view but do not completely cover the pleopods (Fig. 7B), which differs from *P. altalis* males where the laterally curved pleomeres cover the pleopods completely (Williams et al., 2020). The width of the head is almost equal to the width of pleomere 1 in males of *P. shinkai* sp. nov. (Fig. 7A), while in *P. formosa* the male head is much narrower than pleomere 1 (Bonnier, 1900). The uropods of male *P. shinkai* sp. nov. are about the same size or slightly smaller than pleopod 5 (Fig. 7A), but in *P. wolffi* males they are distinctly smaller than pleopod 5 and in *P. altalis* males they are much larger than pleopod 5 (Bourdon, 1972; Williams et al., 2020).

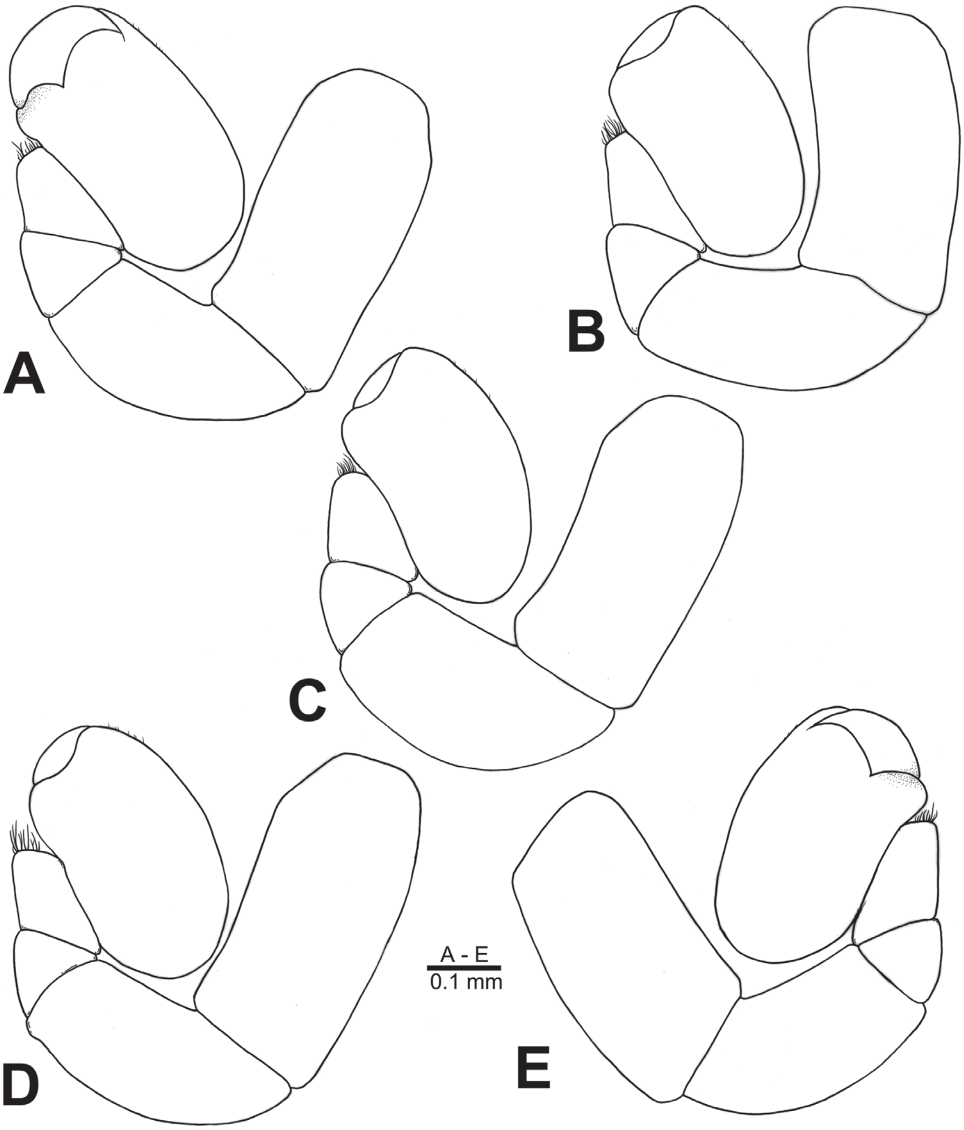

Fig. 8. *Pleurocryptella shinkai* sp. nov., allotype male (NSMT-Cr 30592): **(A)** left pereopod 3, lateral; **(B)** right pereopod 4, medial; **(C)** right pereopod 5, medial; **(D)** right pereopod 6, medial; **(E)** right pereopod 7, lateral.

Scanning electron microscopy

Observation of *P. shinkai* sp. nov. males under SEM showed numerous spinulose scales on the surface of the antennae, and that the surface and setae were covered with filamentous bacteria exhibiting numerous septa separating the individual cells upon close-up (Fig. 9A–D). We found that the number of articles on the antennae was different in a single individual, indicating that the tip of one antenna was damaged and was possibly under regeneration through moulting (Fig. 9C). Spinulose scales were observed on the surfaces of the propodi of *P. shinkai* sp. nov. (Fig. 9E). The surfaces of the propodi were also covered with filamentous bacteria (Fig. 9F).

Dissection of *P. shinkai* sp. nov. females revealed eggs in varying stages of development, depending on the individual. Paratype 3 was carrying unhatched eggs, each approximately 300 µm in diameter. The marsupium of the holotype female was found to contain epicaridium larvae (Fig. 10), each of which had a 7-segmented pereomere and a 6-segmented pleomere (Fig. 10A). The antennules were found to point towards the anterior, while the antennae were directed posteriorly (Fig. 10A, B). The antennae were almost as long as the body, with the tips carrying three potentially sensory setae each (Fig. 10A, B). Epicaridium larvae each had five pairs of pereopods and five pairs of pleopods; the pereopods were sturdy and the pleopods were biramous at the base (Fig. 10B). As with the antennae, the tips of the pleopods carried three-way forked structures. A large spherical mass (about a third of the body length) was attached posterior to the pleon (Fig. 10D). These epicaridium larvae are likely in the process of moulting, since the antennae lack clear segmentation, similar to pre-moulting epicaridium larvae of other species (Williams and Boyko, 2021).

Phylogenetic reconstruction

The reconstructed phylogeny of Cymothoida (including

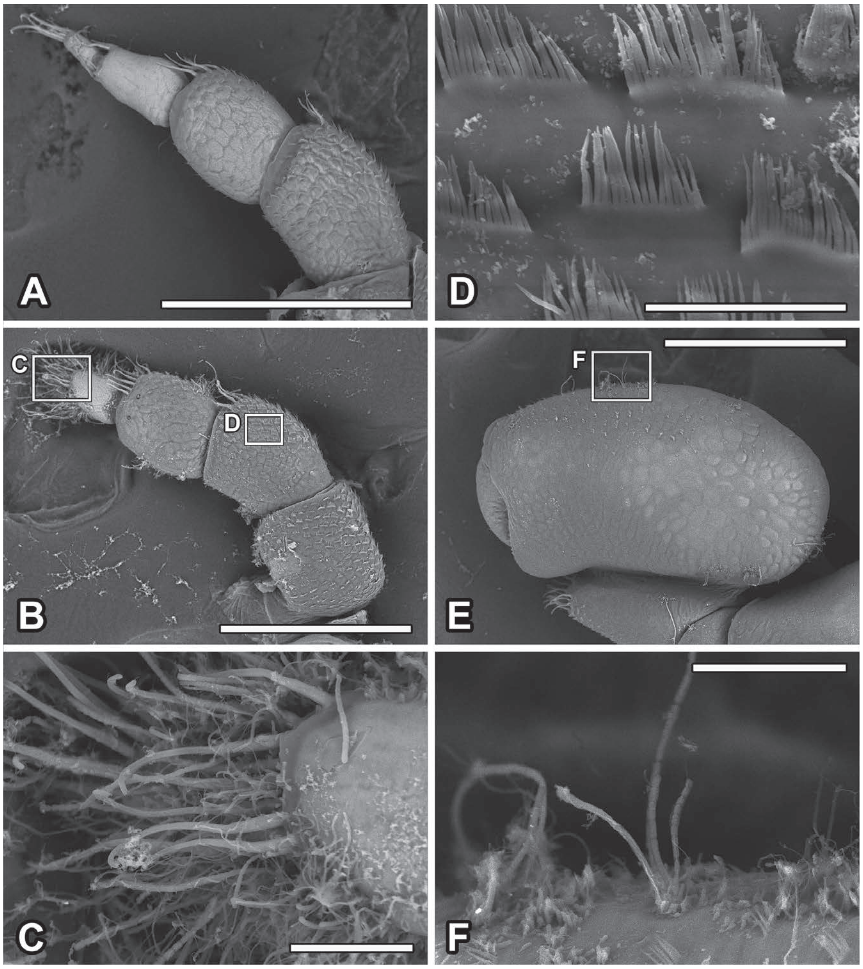

Fig. 9. *Pleurocryptella shinkai* sp. nov., paratype 3 (SMBL-V0639) male: **(A)** left antenna, medial; **(B, C)** right antenna, lateral; **(D)** surface of antenna, lateral; **(E)** right pereopod 7, medial; **(F)** surface of left pereomere 7, medial. Scale bars: **(A, B, E)** 150 μm, **(C, F)** 15 μm, **(D)** 10 μm.

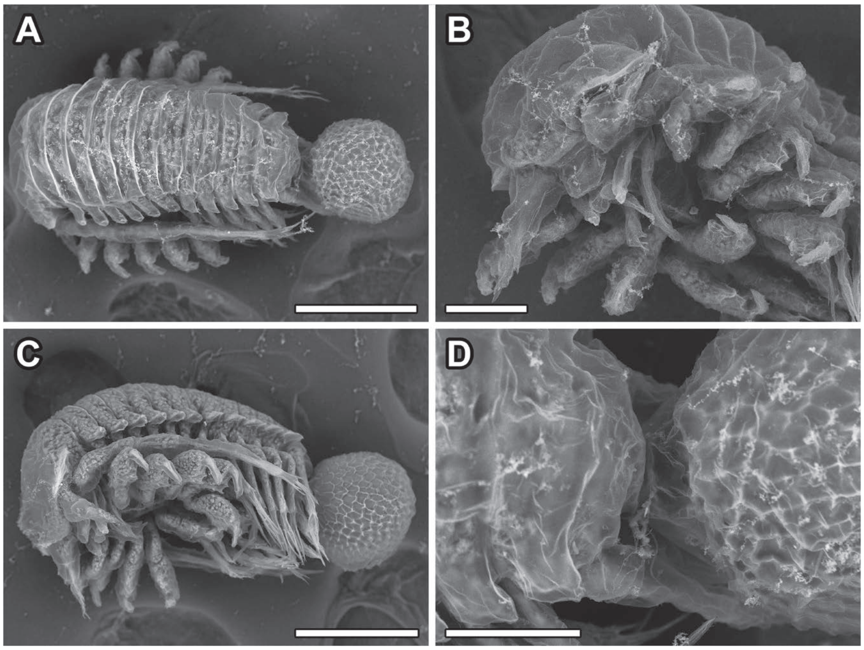

Fig. 10. *Pleurocryptella shinkai* sp. nov., epicaridium larvae from the holotype female (NSMT-Cr 30591): **(A)** habitus, dorsal; **(B)** habitus, ventral; **(C)** cephalon, ventral; **(D)** close-up of the posterior region, dorsal. Scale bars: **(A)** 200 μm, **(B)** 150 μm, **(C)** 50 μm, **(D)** 15 μm.

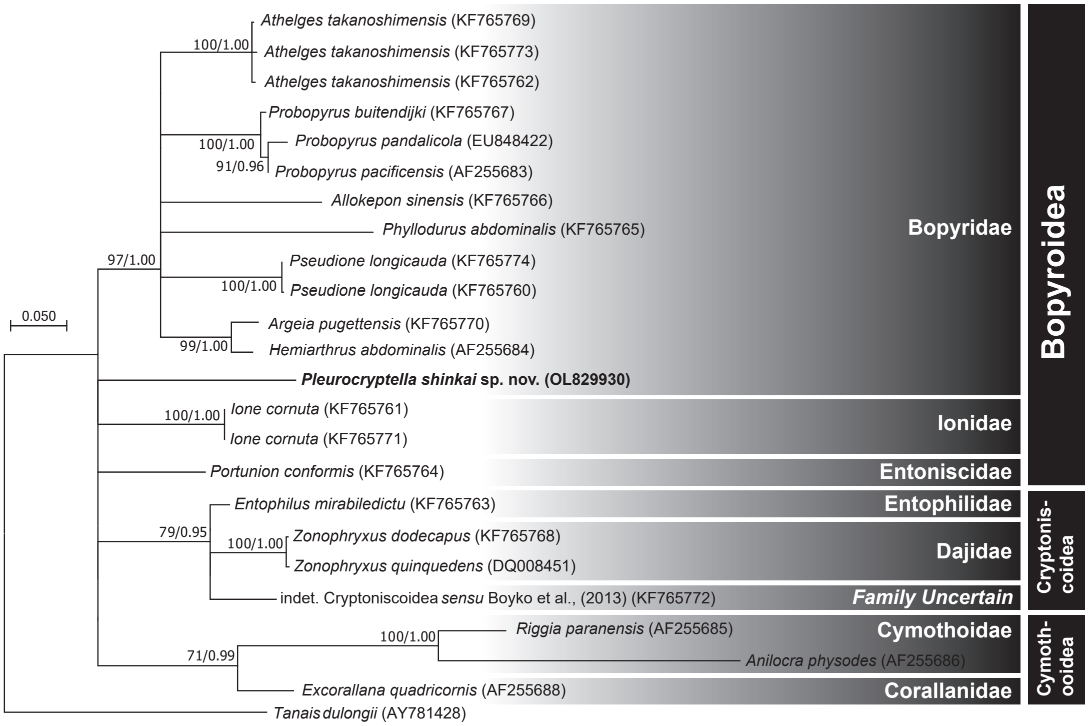

Fig. 11. Phylogenetic reconstruction of Cymothoida (epicarideans + Cymothooidea) using both maximum likelihood (ML) and Bayesian inference based on 850 bp of the 18S rRNA sequence. Node values indicate ML bootstrap values and Bayesian posterior probability, respectively, from left to right. Numbers in brackets indicate GenBank accession numbers.

the epicaridean superfamilies Bopyroidea and Cryptoniscoidea, as well as Cymothooidea) is presented in Fig. 11, where the nodes with support values below 70% bootstrap (BS, for ML) and 0.95 Bayesian Posterior Probability (BPP, for Bayesian) were considered unresolved. Cymothooidea, with three species included, and Cryptoniscoidea, with four species included, were both recovered monophyletic with weak to moderate support (BS = 71, BPP = 0.99 for Cymothooidea; BS = 79, BPP = 0.95 for Cryptoniscoidea). However, we could not recover a monophyletic Bopyroidea, with *P. shinkai* sp. nov. and Ionidae being unresolved. *Pleurocryptella shinkai* sp. nov. fell outside the rest of the included bopyrid species (which formed a well-supported monophyletic clade with BS = 97 and BPP = 1.00) in a polytomy with other non-bopyrid species included. *Pseudione longicauda* Shiino, 1937, the only other representative of Pseudioninae included, was recovered distant from *P. shinkai* sp. nov. As such, the internal structure of Cymothoida remains unresolved in the present phylogeny.

DISCUSSION

The first vent bopyrid epicaridean

Pleurocryptella shinkai sp. nov. is the first hydrothermal vent-dwelling member of Bopyroidea, and the second epicaridean parasite reported from vents after *Thermaloniscus cotylophorus* (in Cryptoniscoidea) from East Pacific Rise. These are also the only epicarideans known from chemosynthetic ecosystems as a whole. As *T. cotylophorus* is only known from a cryptoniscus larva (Bourdon, 1983), *P. shinkai* sp. nov. is also the first vent epicaridean known from adult individuals and with a known host. Previous reports of other vent macroparasites include copepods, leeches, nematodes, and acanthocephalans, although the apparent low parasite diversity in vents has been noted as an artefact of research effort because parasites have received little attention in chemosynthetic ecosystems (De Buron and Morand, 2004; De Buron and Segonzac, 2006). Decapod crustaceans are very successful in vents, with symbiotic taxa such as shrimps (*Rimicaris*) and squat lobsters (*Kiwa*) dominating systems in Atlantic, Indian, and Southern oceans (Rogers et al., 2012) in addition to *S. crosnieri* in the Indo-West Pacific. Our discovery of *P. shinkai* sp. nov. shows that at least one lineage in each of the two epicaridean superfamilies (Cryptoniscoidea and Bopyroidea) have adapted to the 'extreme' vent ecosystem, warranting future research which will likely discover more epicaridean species associated with other vent decapods. Although not dominant, a number of *Munidopsis* species such as *M. ryukyuensis* Cubelio, Tsuchida & Watanabe, 2007 and *M. longispinosa* Cubelio, Tsuchida & Watanabe, 2007 also inhabit Okinawa Trough vents (Nakamura et al., 2015b) and are potential hosts of other *Pleurocryptella* species.

Shinkaia crosnieri is an obligate chemosymbiotic species that live in close vicinity of vent effluents in order to provide energy to the bacteria they 'farm' (Watsuji et al., 2015, 2017, 2018). This means that *P. shinkai* sp. nov. is also subjected to the high-stress environment, and is indicative of its capacity to adapt and thrive in the 'extreme' vent ecosystem. All female *S. crosnieri* parasitized by *P. shinkai* sp. nov. were ovigerous and thus the parasitism does not inhibit host reproduction. We found infested *S. crosnieri* at multiple sites across the Okinawa Trough, showing that this relationship is not a one-off occurrence but a well-established one. *Shinkaia crosnieri* has a very wide distribution in the Indo-West Pacific, being originally described from vents on Edison Seamount in Papua New Guinea (Baba and Williams, 1998) and also known from methane seeps in South China Sea (Feng et al., 2018), off India (Mazumdar et al., 2019), and off Pakistan (Kazmi et al., 2002; Bohrmann et al., 2008; Wang et al., 2017; Wei et al., 2021), which suggests that *P. shinkai* sp. nov. may also have a similarly wide distribution. In the case of *T. cotylophorus*, although it was discovered at a vent (Bourdon, 1983), its link to the vent ecosystem remains unclear due to the host being unknown. The host could be vent-endemic decapods living on the chimneys such as alvinocaridid shrimps, but given the high diversity of hosts in Cryptoniscoidea (Williams and Boyko, 2012) it could also be a non-endemic crustacean only occasionally intruding the vent periphery. As such, identifying the host of *T. cotylophorus* is crucial to understanding its role in the East Pacific Rise vent ecosystems.

Although the intermediate copepod host of *P. shinkai* sp. nov. is unknown, the copepod family Dirivultidae is endemic to vents and the dirivultid copepod *Stygiopontius senckenbergi* Ivanenko and Ferrari, 2013 is known to live in association with *S. crosnieri* as epifauna (Uyeno et al., 2020). Though speculative, it is possible that such dirivultid copepods function as the intermediate host for *P. shinkai* sp. nov., completing its life cycle within the hydrothermal vent ecosystem.

Morphology of *P. shinkai* sp. nov.

The external morphology of *P. shinkai* sp. nov. is not substantially different from that of other members of the genus from non-vent habitats, and we did not find any characters that are directly suggestive of being adapted to this environment. Our SEM observations of a male individual with different number of articles on the antennae of either side suggest that a part of antenna, and possibly of the other appendages as well, of *P. shinkai* sp. nov. is occasionally lost. Though the parasite is protected by the carapace of the host, *S. crosnieri* is known to frequently carry out gill grooming using the chelate P5 as do other squat lobsters (Bauer, 2020), and this behavior may injure the bopyrid parasite. Observation of the epicaridean larva revealed a spherical mass on the pleon, which we speculate is connected to the pleon and has the properties of a nutrient-rich egg yolk (Fig. 10D). We are unsure how close to hatching the larvae observed are, and it is possible that they are still developing and the yolk sac will disappear upon release.

Phylogenetic reconstruction

Our phylogenetic reconstruction (Fig. 11) recovered *P. shinkai* sp. nov. outside the clade including the rest of bopyrid species in the tree, in a polytomy. With this polytomy, the accurate phylogenetic position of *P. shinkai* sp. nov. could not be resolved. Notably, however, *P. shinkai* sp. nov. was clearly distantly related to the only other member of Pseudioninae included, *Pseudione longicauda* Shiino, 1937, with species in other subfamilies found closer to *P. longicauda* than *P. shinkai* sp. nov. with high support (BS = 97, BPP = 1.00). *Pseudione* is the type genus of subfamily Pseudioninae. Although *Pleurocryptella* is currently placed within Pseudioninae, it has a number of unique morphological characteristics (Markham, 1974; Boyko et al., 2013). *Pleurocryptella* has seven oostegites while all other genera except *Pagurocryptella* have only five, and *Pleurocryptella* is also the only genus where males have a segmented maxilliped palp. *Pleurocryptella* has generally been considered the earliest-branching genus of Bopyridae due to having seven oostegites being considered an ancestral trait, but it was unclear if this could instead be a derived feature (Williams et al., 2020). Taken together, these suggest that *Pleurocryptella* likely needs to be moved into a different subfamily or even family (or the establishment of a new one). However, this requires further molecular evidence to be conclusive, especially as its placement in Bopyridae is questioned. If *P. shinkai* n. sp. is indeed is a bopyrid (as suggested by morphology), then its position in our tree would imply that it is sister to all remaining bopyrids and branched off near the root of this family.

Regarding the overall topology of the Cymothoida, our tree agreed with the previous reconstruction by Boyko et al. (2013) in recovering a monophyletic Cryptoniscoidea. However, our phylogeny did not have sufficient resolution to ascertain the monophyly of Epicaridea, which was supported in the Bayesian tree in Boyko et al. (2013) and unresolved in their ML tree. In Boyko et al. (2013), Bopyroidea was recovered as a moderately supported monophyletic clade in the ML tree only but not in the Bayesian tree. We too could not recover a well-supported Bopyroidea, although our topology is not contradictory with its monophyly due to the limited resolution. Nevertheless, these phylogenetic reconstructions are based on a single gene and clearly do not have sufficient signal to resolve all relationships within Cymothoida. As for Cymothooidea, its monophyly was only weakly supported in the previous phylogeny (Boyko et al., 2013), while it was moderately supported in the one presented herein. Distantly related lineages within a clade of parasitic animals often share similar morphological characteristics as a result of convergent evolution, resulting in a discordance between traditional morphological classification and molecular phylogeny (e.g., parasitic copepods, Boxshall et al., 2005; eulimid molluscs, Takano and Kano, 2014). This is likely also the case in epicarideans, and in order to understand the evolutionary relationships among different lineages, molecular phylogenetic analyses with a much higher taxon sampling and coverage are warranted in the future.

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COMPETING INTERESTS

There are no competing interests to be declared.

AUTHOR CONTRIBUTIONS

CC, HKW, and MY conceived and designed the study. NK carried out dissections as well as light and electron microscopy, and made the drawings with the help of HKW and MS. MS and NK carried out identification of the bopyrid species based on morphology. CC, NK, and HKW prepared the specimens for micro-CT and CC carried out the 3D reconstruction. NK and HKW obtained the DNA sequence, which was analysed by HKW. NK, HKW, CC, and MS prepared the figures. NK, CC, and MS drafted the manuscript. All authors contributed to the final manuscript and gave approval for submission and publication.

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