Two new species of *Parahesione* (Annelida: Hesionidae) associated with ghost shrimps (Crustacea: Decapoda) and their phylogenetic relationships (#82630)

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Two new species of *Parahesione* (Annelida: Hesionidae) associated with ghost shrimps (Crustacea: Decapoda) and their phylogenetic relationships

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Abstract

Two new species of Hesionidae, *Parahesione pulvinata* sp. nov. and *Parahesione apiculata* sp. nov. are described based on materials collected at tidal flats in Okinawa (Japan) from burrows of the ghost shrimps *Neocallichirus jousseaumei* and *Glypturus armatus*. The two new species *Parahesione pulvinata* sp. nov. and *Parahesione apiculata* sp. nov. are characterized by: *i*) having eight enlarged cirri; *ii*) having flattened dorsal cirrophores; *iii*) lacking median antenna; and *iv*) having biramous parapodia. *Parahesione apiculata* sp. nov. is distinguished from *P. pulvinata* sp. nov. by having digitate lobes on the posterior margin of the dorsal cirrophores

(absent in *P. pulvinata* sp. nov.) and having large dorsal cirrophores from chaetiger 1 (from chaetiger 6 in *P. pulvinata* sp. nov.). The two new species were never found outside of the ghost shrimp burrows, suggesting that they are obligate symbionts. Phylogenetic analyses based on four concatenated genes suggest that a symbiotic lifestyle in hesionids has evolved several times.

Introduction

Decapod burrows in tidal flats frequently serve as habitats for different symbionts (Campos et al. 2009; Pillay and Branch 2011). These narrow burrows provide safe environments, but must be shared with decapod hosts, often under hypoxic conditions (Atkinson & Taylor 2005). Nevertheless, some symbionts are adapted to this mode of life (Pillay & Branch 2011); including polynoid and hesionid polychaetes living in burrows of callianassid ghost shrimps and upogebiid mud shrimps (Martin and Britayev 1998).

Hesionidae includes more than 180 species (Rouse et al. 2018, 2022), with about 30 being considered obligate or facultative invertebrate symbionts (mainly echinoderms). Several species inhabit burrows of sipunculids, hemichordates and burrowing polychaetes or are ~~otherwise~~ symbiotic with other animals (Martin and Britayev 1998, 2018; Martin et al. 2017; Rouse et al. 2018), but only two species, *Parahesione luteola* (Webster, 1879) and *Parahesione* sp. were found in mud shrimp burrows (Pettibone 1956; Britayev & Antokhina 2012).

The phylogenetic relationships among hesionids are well known, providing an excellent base to assess the evolution of morphological characters (Ruta et al. 2007; Martin et al. 2015; Bonifacio et al. 2018; Rouse et al. 2018). However, additional studies are required to understand (1) the adaptations to a symbiotic mode of life and (2) the evolutionary consequences of their symbiotic relationships with decapods and their burrows, which may help elucidate their ability to be adapted to different environments.

In this study, we are describing two new species of Hesionidae based on the specimens collected from burrows of callianassid ghost shrimps. We also analyze the phylogenetic relationships within the family based on four concatenated genes in order to understand the evolution of both the symbiotic species and their adaptations to decapod burrows.

Materials & Methods

Hesionid specimens were collected with a yabby pump from inside of the burrows of *Neocallichirus jousseaumei* (Nobili, 1904) and *Glypturus armatus* (A. Milne-Edwards, 1870), which lives in tidal flats throughout the Ryukyu Islands, Japan (Fig. 1). All specimens were fixed and preserved in 70% ethanol. Additional specimens reported as: (1) *Parahesione* sp. used in Britayev & Antokhina (2012); (2) *Parahesione* sp. used in Ruta et al. (2007); (3) *Parahesione* collected from Papua New Guinea collected by Greg Rouse, likely from the burrow of *Calliaxina bulimba* (Poore & Griffin, 1979); and (4) *Parahesione luteola* used in Pettibone (1956) (No. USNM 430 and 28175) were also studied. On GenBank, the Ruta et al (2007)'s sequence is called *Parahesione luteola*. However, in the paper, they concluded it was undescribed species *Parahesione* sp. (Ruta et al. 2007, the first paragraph of pp. 101). The Genbank name is therefore incorrect and is treated as *Parahesione* sp. in this study.

The Japanese specimens were observed using a Nikon SMZ1500 dissecting microscope and a Nikon ECLIPSE Ni-U compound light microscope. Photographs were taken with an Olympus OM-D5 digital camera. The Papua New Guinea specimen was studied with a Leica MZ9.5 stereomicroscope and photographed with a Canon Rebel T3i camera. The type specimens were deposited in the National Museum of Nature and Science, Tsukuba, Japan (NSMT) and the Scripps Institution of Oceanography Benthic Invertebrate Collection, La Jolla, California, USA (SIO-BIC). In the 'Material examined' section, complete specimens are indicated by 'CS'. The length (L) is measured from the anterior margin of the prostomium to the posterior border of the last segment, while the width (W) is taken at the widest segment, including parapodia but excluding chaetae.

For the Japanese specimens, total DNA was extracted using a DNeasy Tissue Kit (Qiagen) from a parapodium of holotype. The reaction mixture [0.2 µL TaKaRa Ex Taq (Takara, Japan), 2 µl of 10 × Ex Taq Buffer (Takara, Japan), 1.8 µL dNTP mixture (Takara, Japan), 1 µL of each primer pair (10 µM), 1 µL of extracted DNA, and 14 µL of distilled water] was used for amplification. PCR amplification was performed with the primer pairs polyLCO (5'-GAYTATWTTCAACAAATCATAAAGATATTGG-3') and polyHCO (5'-TAMACTTCWGGGTGACCAAARAATCA-3') for part of mitochondrial cytochrome *c* oxidase subunit I gene (*COI*) (Carr et al. 2011), 16SarL (CGCCGTTTATCAAAAACAT) and 16SbrH (CCGGTCTGAACTCAGATCACGT) for the part of mitochondrial 16S rRNA gene (*16S*) (Palumbi et al. 1991), 1F (TACCTGGTTGATCCTGCCAGTAG) and 9R (GATCCTTCCGCAGGTTCACCTAC) (Giribet et al. 1996) for the part of nuclear 18S rRNA gene (*18S*), and LSU5 (TAGGTCGACCCGCTGAAYTTAAGCA) and rd5b (CCACAGCGCCAGTTCTGCTTAC) (Littlewood 1994; Schwendinger & Giribet 2005) for the part of nuclear 28S rRNA gene (*28S*) gene , using an Applied Systems 2720 thermal cycler following the protocol: (a) for *COI* and *16S*, preheating at 94°C for 2 min; 35 cycles at 94°C for 40 s, 50°C for 60 s, and 72°C for 60 s; and a final extension at 72°C for 7 min; (b) for *18S* and *28S* preheating at 94°C for 2 min; for 18S and 28S, 35 cycles at 94°C for 40 s, 52°C for 75 s, and 72°C for 60 s; and a final extension at 72°C for 7 min. Nucleotide sequencing was performed using internal primers in addition to the same primer pairs with an ABI BigDye Terminator ver. 3.1 Cycle Sequencing Kit and an ABI 3100 Avant Genetic Analyser (Applied Biosystems). The internal primers were 3F (GTTCGATTCCGGAGAGGGA) and 5R (CTTGGCAAATGCTTTCGC) (Giribet et al. 1996) and 18Sbi (GAGTCTCGTTCGTTATCGGA) and a2.0 (ATGGTTGCAAAGCTGAAAC) (Whiting et al. 1997) for *18S*, and D2F (CTTGAAGAGAGAGTTC) and D3R (ATAGTTCACATCTTTCGG) (Littlewood 1994) for *28S*. For the *Parahesione* collected from Papua New Guinea extraction protocols were followed from Rouse et al. (2018) and *COI* was generated for the specimen. The newly obtained sequences *COI* (625 bp), *16S* (552 bp), *18S* (1677 bp), *28S* (987 bp) were deposited in GenBank. A total of 200 sequences (59 species) were used for molecular analyses.

A total of 190 sequences were downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank), and the remaining sequences were from this study. All sequences were aligned using MAFFT ver. 7.205 according to the E-INS-i strategy (Katoh & Standley 2013). Alignment-ambiguous positions were removed using trimAL with the gappyout method (Capella-Gutiérrez et al. 2009). The trimmed sequences of the four genes, *COI* (657 bp), *16S* (513 bp), *18S* (1654 bp), and *28S* (947 bp), were concatenated using the program Kakusan (Tanabe 2007), which recommended a GTR+G evolutionary model for each of the genes. A phylogenetic tree was constructed using the maximum likelihood (ML) method in the RAxML-VI-HPC program (Stamatakis 2006). The robustness of the ML tree was evaluated by 1,000 bootstrap pseudo-replicates (F option) and the bootstrap support (BS) is indicated as %. Bayesian Inference (BI) analysis was conducted using MrBayes 3.2.2 (Ronquist et al. 2012), with Markov chains of 10 million generations. The model choice for each partition was also based on the Kakusan results. Run convergence was analyzed using Tracer v1.6 (Rambaut et al. 2018); the first one million generations were discarded as burn-in. *Dysponetus caecus* (Langerhans, 1880) was used as the outgroup following Rouse et al. 2018 and Tillic et al. (2022). Additionally, four species were used for calculating K2P genetic distances using MEGAX (Stecher et al. 2020). The hesionids were classified into three groups: obligate and facultative symbionts, according to Martin et al. (2017), and free-living based on previously published data (Table 2).

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Results

Systematics

Ophiodrominae Pleijel, 1998

Amphidurini Pleijel et al., 2012

***Parahesione* Pettibone, 1956**

Diagnosis (emended). Body ~~flattened~~, reddish when alive. Prostomium with two lateral antennae, without median antenna, two pairs of eyes. Palps with or without palpophores. Anterior enlarged cirri as six or eight pairs. Dorsal cirrophores cylindrical or f[illegible] Parapodia biramous. Notopodia with numerous capillary chaetae. Neuropodia with numerous compound chaetae.

Parahesione pulvinata sp. nov.
[New Japanese name: ana-yadori-otohime]
(Figs. 2–5)
Parahesione sp.: Britayev and Antokhina 2012: 33, Pl. 9 C, D
Zoobank LSID: urn:lsid:zoobank.org:act:2E42DB94-DF8C-447A-A7F8-8C2FDA9FF4CA

Material examined. Holotype: NMST-Pol H-893 (museum numbers will be provided by NSMT after accepting this manuscrip[illegible] Genbank No.: COI OP404166, 16S OP407585, 18S OP407566, 28S OP407536, CS, L18 mm, W 4 mm for 45 chaetigers, East China Sea, Iriomote Island, Uehara, intertidal area, burrow of *Neocallichirus jousseaumei*, 5 September 2020, collected by HN. Paratypes: NSMT-Pol P-894, CS, L 20 mm, W 5 mm for 45 chaetigers, collection data same as holotype (from another burrow of *N. jousseaumei*). Paratypes: NSMT-Pol P-895, CS, L 17 mm, W 4 mm for 39 chaetigers, East China Sea, Iriomote Island, Uehara, Todomari Beach, 1–2 m in depth, burrow of unknown crustaceans, 24 January 2021, collected by TS. Paratypes: NSMT-Pol P-896, CS, L 18 mm, W 4 mm for 36 chaetigers, East China Sea, Okinawa Island, Sunabe, intertidal, burrow of unknown crustaceans, 20 September 2021, collected by TS. Paratypes: NSMT-Pol P-897, CS, L 12 mm, W 3 mm for 24 chaetigers, East China Sea, Okinawa Island, Kouri, intertidal, burrow of unknown crustaceans, 27 February 2021, collected by HN. SIO-BIC A13742, 1 specimen, Madang Lagoon, Tab Island, Madang Province, Papua New Guinea, 5.17° S; 145.84° E, likely in burrow of *Calliaxina bulimba,* 13 December 2012, collected by Art Anker and Greg Rouse.

Additional material: IPEE RAS – Pol. 2004/01, 1 specimen in four fragments, L 19.5 mm, W 4.4 mm for 48 chaetigers, South China Sea, Nhatrang Bay, River Be estuary, intertidal, sandy silt, burrows of *Upogebia* sp., 18 April 2004, collected by Marin.

Description of holotype. Body ~~flat~~, tapered in posterior region, reddish when alive, pinkish after fixation (Figs. 2, 3). Prostomium rectangular, wider than long (Figs. 3E, 4A). Antennae cylindrical, distally tapering. Palpophores cylindrical, shorter than antennae. Palpostyles ~~cylindrical~~, thinner than palpophores, shorter than antennae (2/3 ~~of~~ antennae length). Two pairs of eyes, brownish after fixation, inconspicuous when alive (Fig. 3E).

Dorsal enlarged cirri on segments 1–5, longest one reached chaetiger 8, ventral enlarged cirri on segments 1–4. Chaetae absent from segments 1–[illegible]. Cirrophores of enlarged cirri segments cylindrical, basally fused.

Parapodia with chaetal lobes cylindrical, truncate, longer than wide. Chaetiger 1 uniramous. Chaetiger 2 and following chaetigers biramous. Dorsal cirrophores of chaetigers 1–5 cylindrical, normal hesionid shape (Fig. 4B). From chaetiger 6, dorsal cirroph[illegible] large, pillow shape, extremely flattened, partially covering subsequent segment (Fig. 3C). Dorsal cirrostyles long, conical, smooth. Ventral cirrophores not developed, fused to parapodia. Ventral cirrostyles short, conical, smooth (Fig. 3D). Noto- and neuro aciculae brownish, tip of aciculae reddish after fixation, unknown in alive.

Notopodia small, conical, with about 40 simple capillary notochaetae. Neuropodia large, truncated, with prechaetal lobes and a postero-dorsal digitiform projection (Fig. 4C) and about 30 compound neurochaetae with unidentate serrated blades; blades of most ventral chaetae shorter than in most dorsal (Fig. 4D, 4E). Pygidium with two long anal cirri, smooth.

Etymology. The specific name "pulvinata", derived from the Latin *pulvinus* (cushion, pillow) and, referring to the shape of dorsal cirrophores. The specific name is an adjective in the nominative case.

Remarks. *Parahesione* sp. from Vietnam and Papua New ~~Genia~~ (Fig. 5) are morphologically identical to the Japanese materials, therefore confirming that they belong to *P. pulvinata* sp. nov. The COI sequences for the Japanese and Papua New Guinea were only slightly divergent and the haplotype network is shown in Figure 9.

Distribution and habitat. Ryukyu Islands (Japan, East China Sea), Nhatrang Bay (Vietnam, South China Sea), and Madang Lagoon, Papua New Guinea (Southwestern Pacific Ocean), in intertidal mud flats, living inside burrows of *N. jousseaumei* (Axiidea: Eucalliacidae) (Japan) and *Upogebia* sp. (Gebiidea: Upogebiidae) (Vietnam), or at 1–5 m inside burrows of *Calliaxina bulimba* (Axiidea: Eucalliacidae).

Parahesione apiculata sp. nov.
[New Japanese name: toge-ana-yadori-otohime]
(Figs. 6–8)
Zoobank LSID: urn:lsid:zoobank.org:act:1AB8DAA4-2268-445D-A3A6-9AE9C085A856

Material examined. Holotype: NSMT-Pol H-898, Genbank No.: COI OP404167, 16S OP407586, 28S OP407537, specimen with posterior part lost, L 12 mm, W 4 mm for 28 chaetigers, Philippine Sea, Okinawa Island, Shikenbaru beach, 1–2 m in depth, burrow of *Glypturus armatus*, 23 December 2019, collected by TS. Paratype: NSMT-Pol P-899, specimen with posterior part lost, L 8 mm, W 4 mm for 20 chaetigers, Philippine Sea, Okinawa Island, Nanjo, Ou beach, intertidal, burrow of *G. armatus*, 20 August 2021, collected by HN. Paratype: NSMT-Pol P-900, specimen with posterior part lost, L 9 mm, W 3 mm for 24 chaetigers, East China Sea, Okinawa Island, Kujyuzaki, intertidal, burrow of *G. armatus*, 22 August 2021, collected by TS.

Description of holotype. Body ~~flat~~, tapering in posterior region, reddish when alive, pale orange after fixation (Figs. 6, 7).

Prostomium rectangular, wider than long (Fig. 8A). Antennae cylindrical, tapering distally. Palpophores cylindrical. Palpostyles cylindrical, thinner than palpophores, shorter than antennae (5/7 ~~of~~ antennae length). Eyes present, two pairs, brownish after fixation, unknown in alive (Fig. 7E).

Dorsal enlarged cirri on segments 1–5, ventral enlarged cirri on segments 1–4, longest one reaching chaetiger 8 in holotype. Chaetae absent on segments 1–4. Cirrophores of enlarged cirri segments cylindrical, basally fused.

Parapodia with chaetal lobes cylindrical, truncate, longer than wide. Chaetiger 1 uniramous. Chaetiger 2 and following chaetigers biramous. From chaetiger 1, dorsal cirrophores of chaetiger 1 and following chaetigers large, extremely flattened, partially covering subsequent segment, with a digitate lobe on posterior side margin (Figs. 7C, 8B). Dorsal cirrostyle long, conical, smooth. Ventral cirrophore fused with parapodia. Ventral cirrostyle short, conical, smooth. Noto- and neuroaciculae brownish, with reddish tips after fixation, unknown in alive. Notopodia small, conical, with about 40 simple capillary notochaetae. Neuropodia large, truncated, with pre- and post-chaetal lobes and a digitiform projection present on postero-dorsal part (Fig. 7C, 8B). About 30 compound neurochaetae with unidentate serrated blades; blades in most ventral chaetae shorter than in most dorsal side chaetae (Fig. 8C, 8D). Pygidium with two long anal cirri, smooth.

Etymology. The specific name "apiculata", derived from the Latin *apiculatus* (short pointed) and referring to the digitate lobe on posterior side margin of dorsal cirrophores, is an adjective in the nominative case.

Remarks. *Parahesione apiculata* sp. nov. is characterized by the following features: i) having digitate lobes on posterior margin of dorsal cirrophores (absent in *P. pulvinata* sp. nov.), ii) having large dorsal cirrophores from the first chaetiger (from chaetiger 6 in *P. pulvinata* sp. nov.), iii) living in the burrows of *G. armatus* (compared to *N. jousseaumei, C. bulima* and *Upogebia* sp. in *P. pulvinata* sp. nov.).

Distribution and habitat. Ryukyu Islands (Japan, Philippine Sea and East China Sea), in intertidal mud flats, living inside burrows of *G. armatus*.

Molecular analyses

Parahesione apiculata sp. nov. and *Parahesione* sp. form a clade with 69% of BS in ML and 0.99 of PP in BI (Fig. 9A). *Parahesione pulvinata* sp. nov. forms a sister clade to the *P. apiculata–Parahesione* sp. clade, with 95% of BS in ML and 1.00 PP. The *Parahesione* clade is sister to the clade *Amphiduros–Amphiduropsis* with 95% of BS in ML and 1.00 PP in BI. K2P genetic distance for the COI sequences between the two new species is 11.0%. Uncorrected genetic distance is 10.1%.

Discussion

Phylogenetic position of two new species and its relationships with *Parahesione* species and other hesionids

The two new species resemble *Parahesione luteola*, which is type species of *Parahesione*, in lacking a median antenna while having a flattened body and living symbiotically with ghost shrimps, while differing by having flattened dorsal cirrophores and eight enlarged anterior cirri, instead of cylindrical dorsal cirrophores and six enlarged anterior cirri. *Parahesione luteola* has been reported from oyster shell and burrows of *Upogebia affinis* (Say, 1818) in the Atlantic coast of the United States of America (Pettibone 1963) and is regarded as a facultative symbiotic species (Martin and Britayev 1998). Unidentified *Parahesione* species have been known from several areas, including: Arctic Sea (Atkinson and Percy 1991), Australia (Gunton et al. 2021), Costa Rica (Maurer, Vargas, and Dean 1988), New Caledonia (Ruta et al. 2007) and Vietnam (Britayev and Antokhina 2012). The specimen of *Parahesione* from Vietnam was reported by Britayev and Antokhina (2012) as having eight pairs of enlarged anterior cirri, unlike *P. luteola* (six pairs of enlarged anterior cirri), which in their opinion should be placed into a new genus. We observed the exact specimen used in Britayev and Antokhina (2012) and confirmed that the specimen should be treated as *Parahesione pulvinata* sp. nov.

According to our data, *Parahesione* sp. and the two new species fall into the same clade. However, the specimen identified by Ruta et al. (2007) as *Parahesione* sp. was collected in New Caledonia (Pacific Ocean), far from the type locality of the single known species of the genus, the eastern Atlantic coast of the United States (Pettibone1956). We reexamined the specimen used in Ruta et al. (2007) and confirmed that the specimen has six enlarged cirri and non-flattened dorsal cirrophores (Fig. 10A, B) and is likely another new species but is not described here. Additionally, we observed the specimens of *P. luteola* in the USNM (Fig. 10C, D). The specimens showed *P. luteola* has six enlarged cirri and non-flattened dorsal cirrophores in according with Pettibone (1956)'s description.

In this situation, we assign these species to the same genus *Parahesione* and modify the diagnosis of the genus. However, the DNA repository data for *Parahesione* sp. used in Ruta et al. (2007) is very limited, with only a single partial sequence of 28S (381 bp). We could not determine the other gene sequences because the specimen was preserved in formalin. Given the situation described above, it is possible that the two morpho-types *Parahesione* (eight enlarged cirri & flattened dorsal cirrophores vs. six enlarged cirri & non-flattened dorsal cirrophores) could be distinguished on a molecular phylogenetic tree if they are re-examined with additional specimens and gene sequences.

Our phylogenetic results (Fig. 9) showed that *Parahesione* as closest to the *Amphiduros-Amphiduropsis* clade consistent with Ruta et al. (2007), and supporting its inclusion in Amphidurini as suggested by Pleijel et al. (2012). *Amphiduros* and *Amphiduropsis* also have enlarged dorsal cirri on segments 1–5, but bear a short median antenna, distinguishing them from *Parahesione*.

Symbiotic relationships and adaptive traits

All specimens of the two new species were always found in the ghost shrimp burrows. No free-living specimens were found on/in the surrounding intertidal bottoms, suggesting that they are obligate symbionts with a high host specificity. *Parahesione apiculata* sp. nov. has always been found in association with *G. armatus*, while *P. pulvinata* was associated with three species *N. jousseaumei* (Axiidea: Eucalliacidae) and *Upogebia* sp. (Gebiidea: Upogebiidae), however, the identification of the latter host was rough and needs to be confirmed (Jimi pers. obs.).

The two new species of *Parahesione*, like many other symbiotic polychaetes (Martin, Britayev 1998, 2018), may have morphological adaptations to living symbiotically. For example, the extreme flatness of their dorsal cirrophores, which is not found in the non-symbiotic species of the *Amphiduropsis*-*Amphiduros* sister clade. We suggest that this flattening facilitates the worm movement between the host body and the walls of the narrow burrows. Flattened bodies have also been reported for symbiotic polynoids living in association with tube dwelling chaetopterids, which also have to move between the host body and the tube wall (Britayev et al. 2017; Britayev and Martin 2021)

The bodies of the two new species also have bright red-colour when alive, that again, is not present in the *Amphiduropsis*-*Amphiduros* species. This suggests that this trait was acquired in *Parahesione*. A bright red colour was also reported for *Hesperonoe* (Polynoidae) species associated with the mud shrimps (Sato et al. 2001; Hong et al. 2017). Some crustacean-associated molluscs have red blood cells, which are considered as an adaptation to live in the burrow hypoxic conditions (Goto et al. 2018). Therefore, we agree with Martin and Britayev (2018) in suggesting that the red colour (likely associated to the presence of dissolved pigment) in the species of *Hesperonoe* may be an adaptation to live in the burrows' hypoxic environment. Further observations based on living specimens are thus required to confirm the presence of these red coelomocytes, and thus, the possible meaning of the red colour as an adaptation to hypoxia, in the species of *Parahesione*.

Conclusions

The genus *Parahesione*, belonging to Hesionidae, is a rare group with poorly understood ecology. We have discovered two new *Parahesione* species associated with ghost shrimps from the northwest Pacific. Through morphological observations and phylogenetic tree construction using four genes, we have revealed how their symbiotic relationship with ghost shrimp has evolved and what morphological characteristics have been acquired as a result. This is the first record of *Parahesione* from Japan.

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Figure 1

Sampling locations for type specimens

Parahesione pulvinata sp. nov. (Red star) and *Parahesione apiculata* sp. nov. (Blue dot)

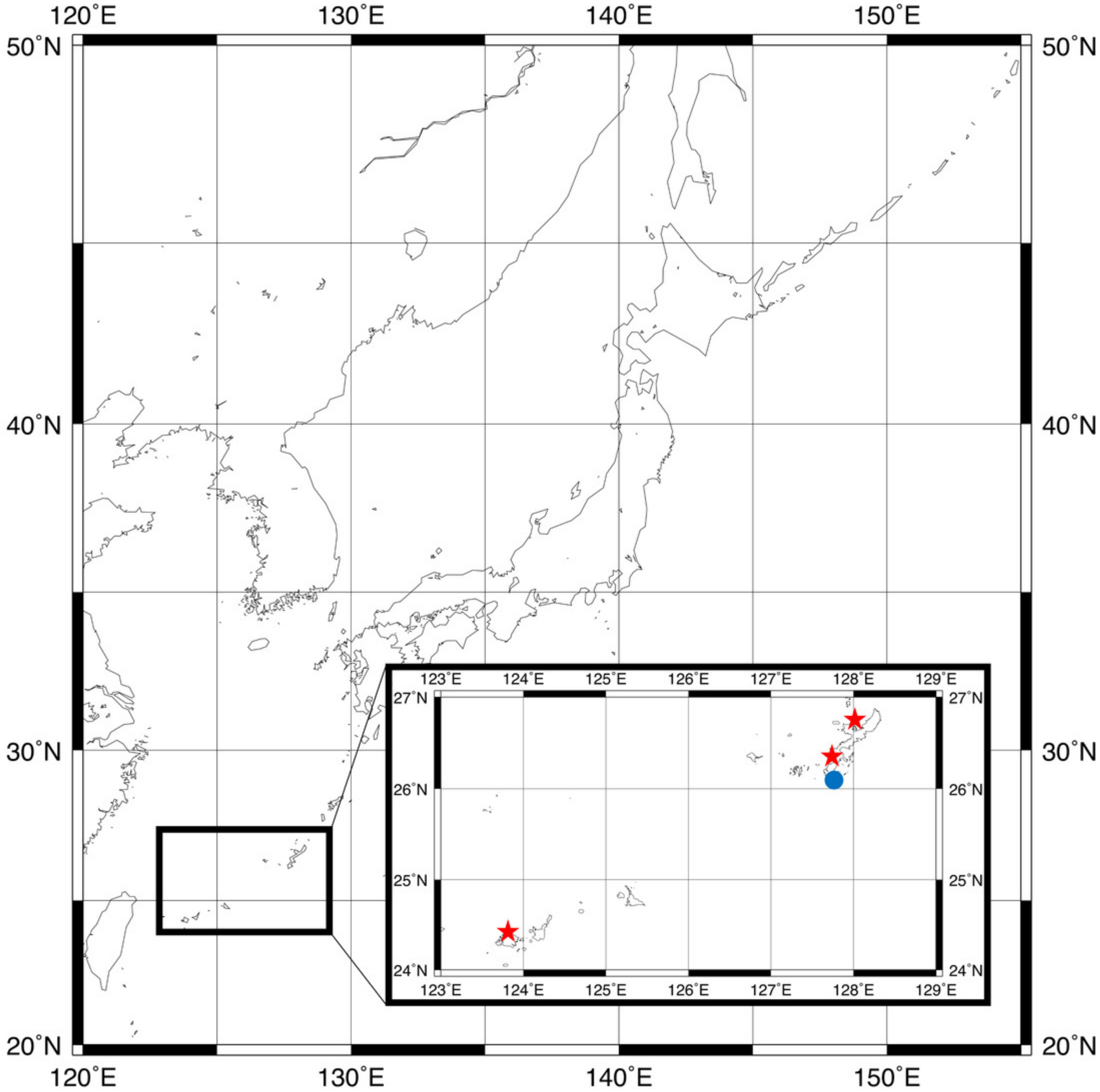

Figure 2

Observations of *Parahesione pulvinata* sp. nov. and its hosts *in situ*

A, sampling at the sandy tidal flat of Uehara; B, host and the new species; C, detail of the host *Neocallichirus jousseaumei*; D, another host with its symbiont (tube); E, dorsal view of the new species of a living specimen (NSMT-Pol H-893); F, dorsal view of a preserved specimen (NSMT-Pol H-893).

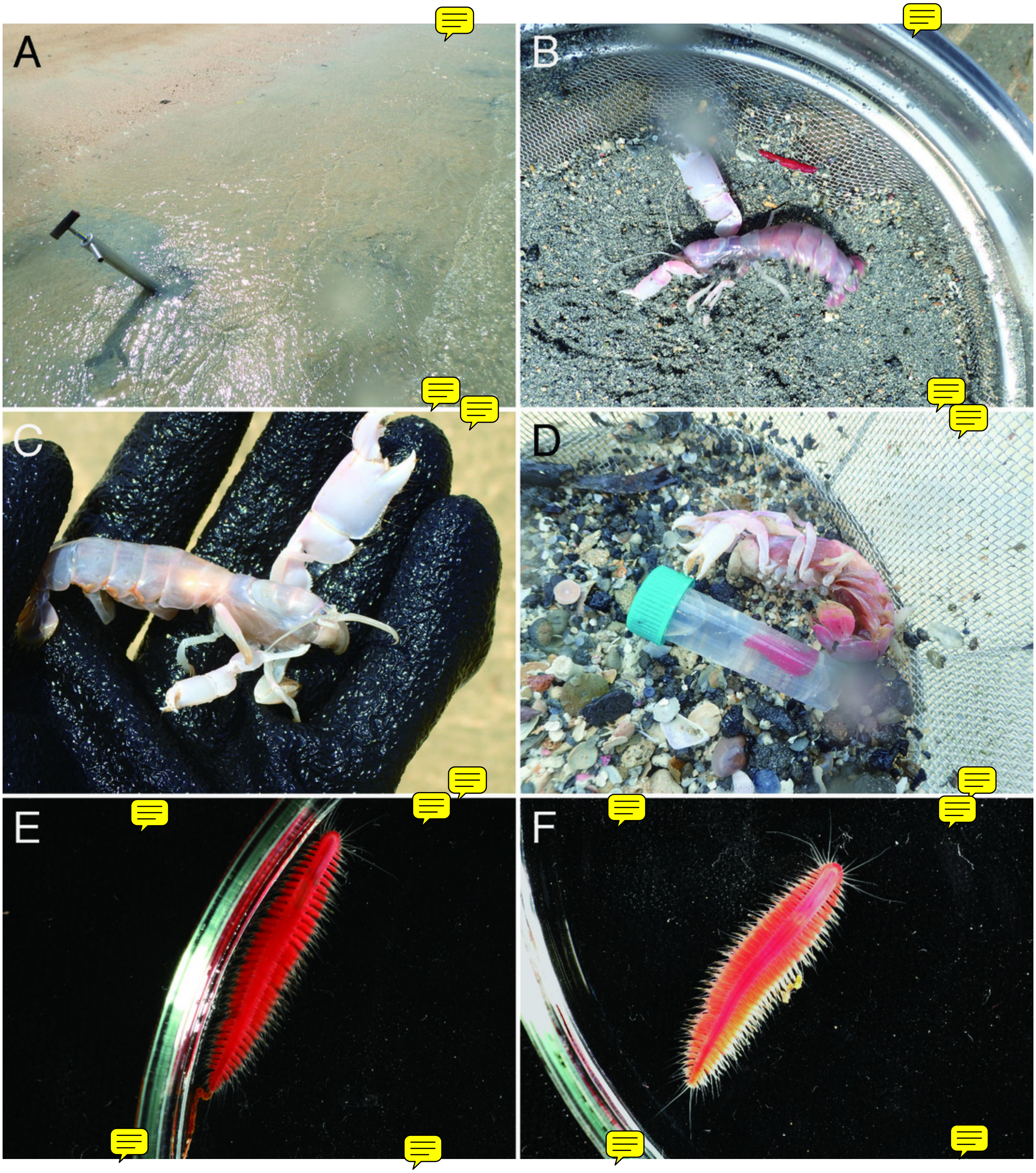
A
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Figure 3

Parahesione pulvinata sp. nov. (NSMT-Pol H-893)

A, whole specimen, dorsal view; B, whole specimen, ventral view; C, middle segments, dorsal view; D, middle segments, ventral view; E, anterior end, dorsal view; F, anterior end, ventral view. White arrows indicate pillow-shaped dorsal cirrophore without digitate lobes. Abbreviation: la, lateral antenna; p, palp; ec, enlarged cirrus. Scale bars: A–B, 5 mm; C–D, 2 mm; E–F, 1 mm.

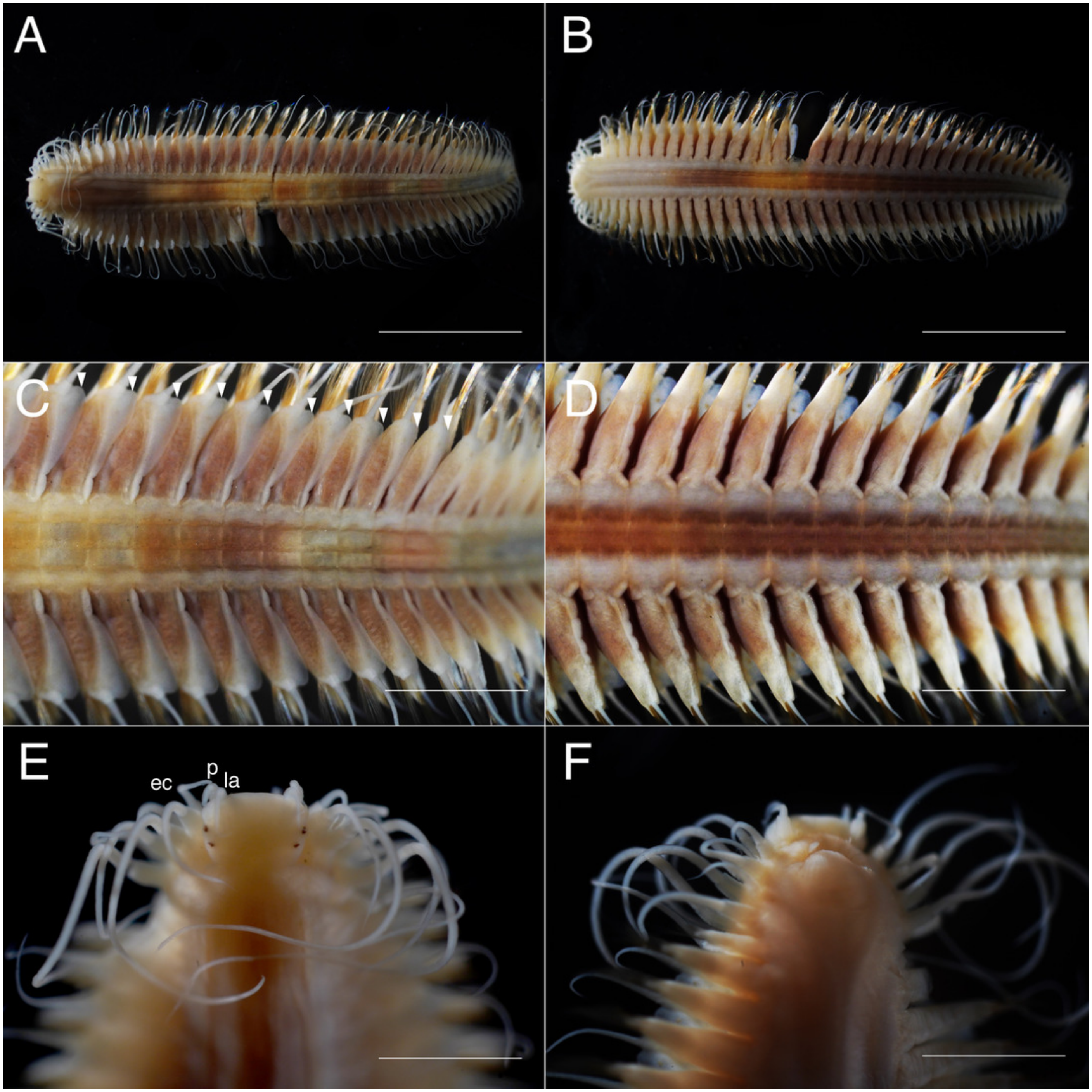
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Figure 4

Parahesione pulvinata sp. nov. (NSMT-Pol H-893)

A, anterior end, dorsal view; B, parapodium of chaetiger 1, rear view; C, parapodium of chaetiger 17, frontal view; D, ~~neurochaet~~a, upper side, chaetiger 17; E, ~~neurochaeta~~, lower side, chaetiger 17. Scale bars: A, 1 mm; B, 100 µm; C, 500 µm; E–F, 100 mm. Abbreviation: la, lateral antenna; p, palp; ec, enlarged cirrus.

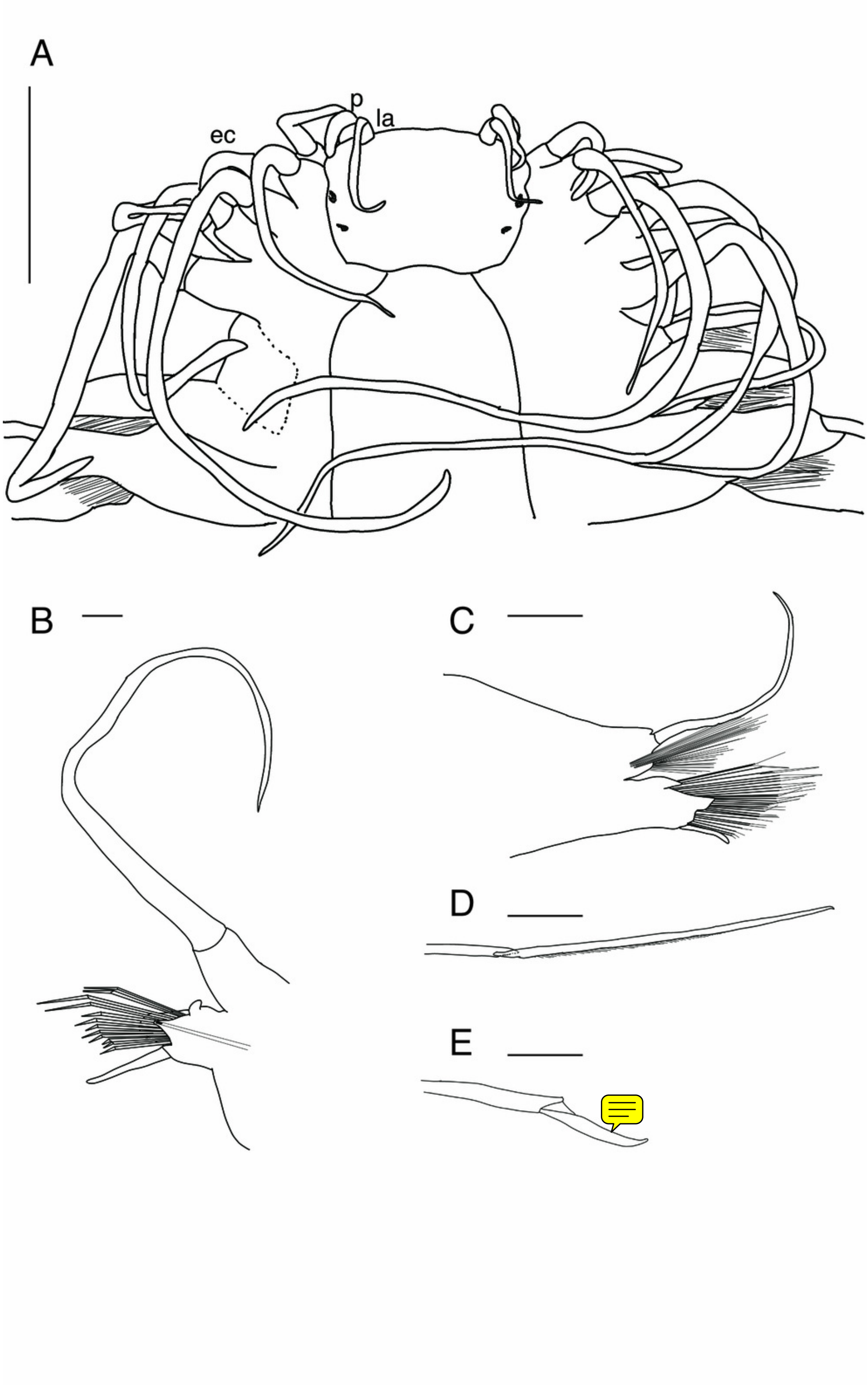
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Figure 5

Parahesione pulvinata sp. nov., live specimen (SIO-BIC A13742, Papua New Guinea)

A, anterior end, dorsal view; B, enlarged view of anterior end, dorsal view; C, middle segments, dorsal view; D, posterior segments, dorsal view. Scale bars: A–B, 5 mm; C–D, 2 mm; E–F, 1 mm.

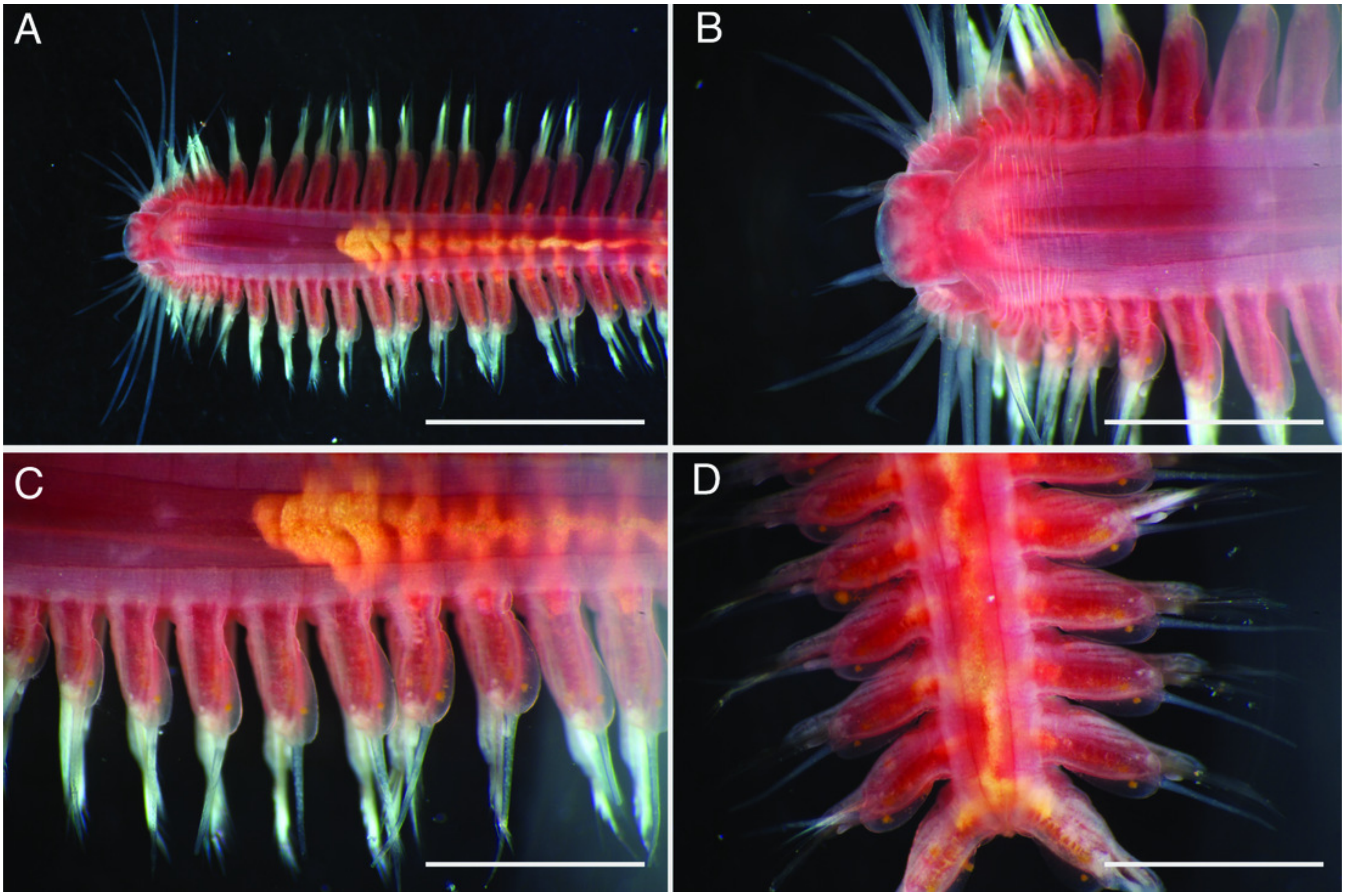

Figure 6

Parahesione apiculata sp. nov. and hosts *in situ*

A, sampling location at the Nanjo sandy tidal flat; B, living specimen of the symbiont; C, living specimen of the *Glypturus armatus* (host); D, dorsal view of a living specimen, lacking posterior most segments (same individual with Fig. 6B, NSMT-Pol P-899).

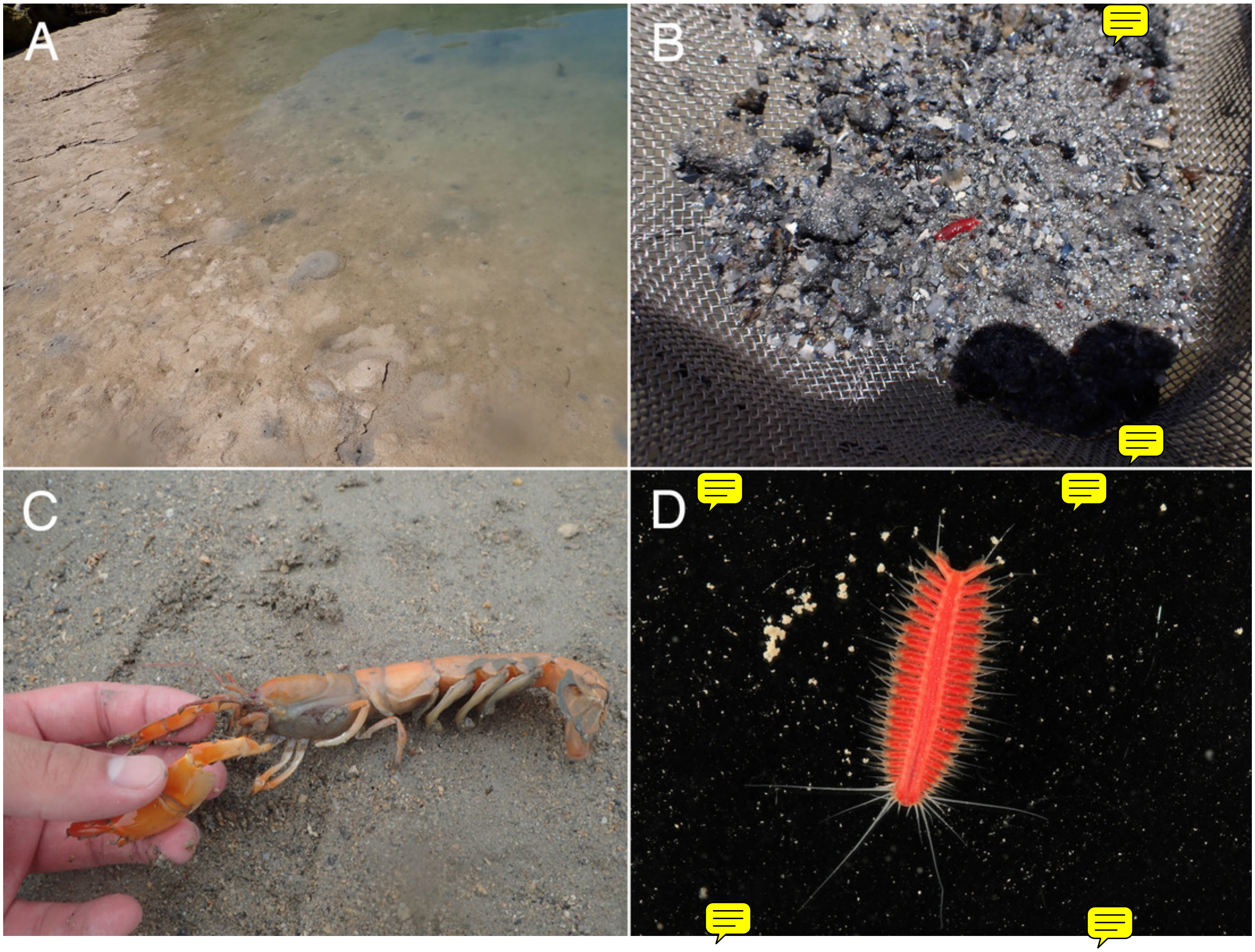

Figure 7

Parahesione apiculata sp. nov. (NSMT-Pol H-898)

A, whole specimen, dorsal view; B, whole specimen, ventral view; C, middle segments, dorsal view; D, middle segments, ventral view; E, anterior end, dorsal view; F, anterior end, ventral view. White arrows indicate digitate lobes. Abbreviation: la, lateral antenna; p, palp; ec, enlarged cirrus. Scale bars: A–B, 3 mm; C–D, 1 mm; E–F, 1 mm.

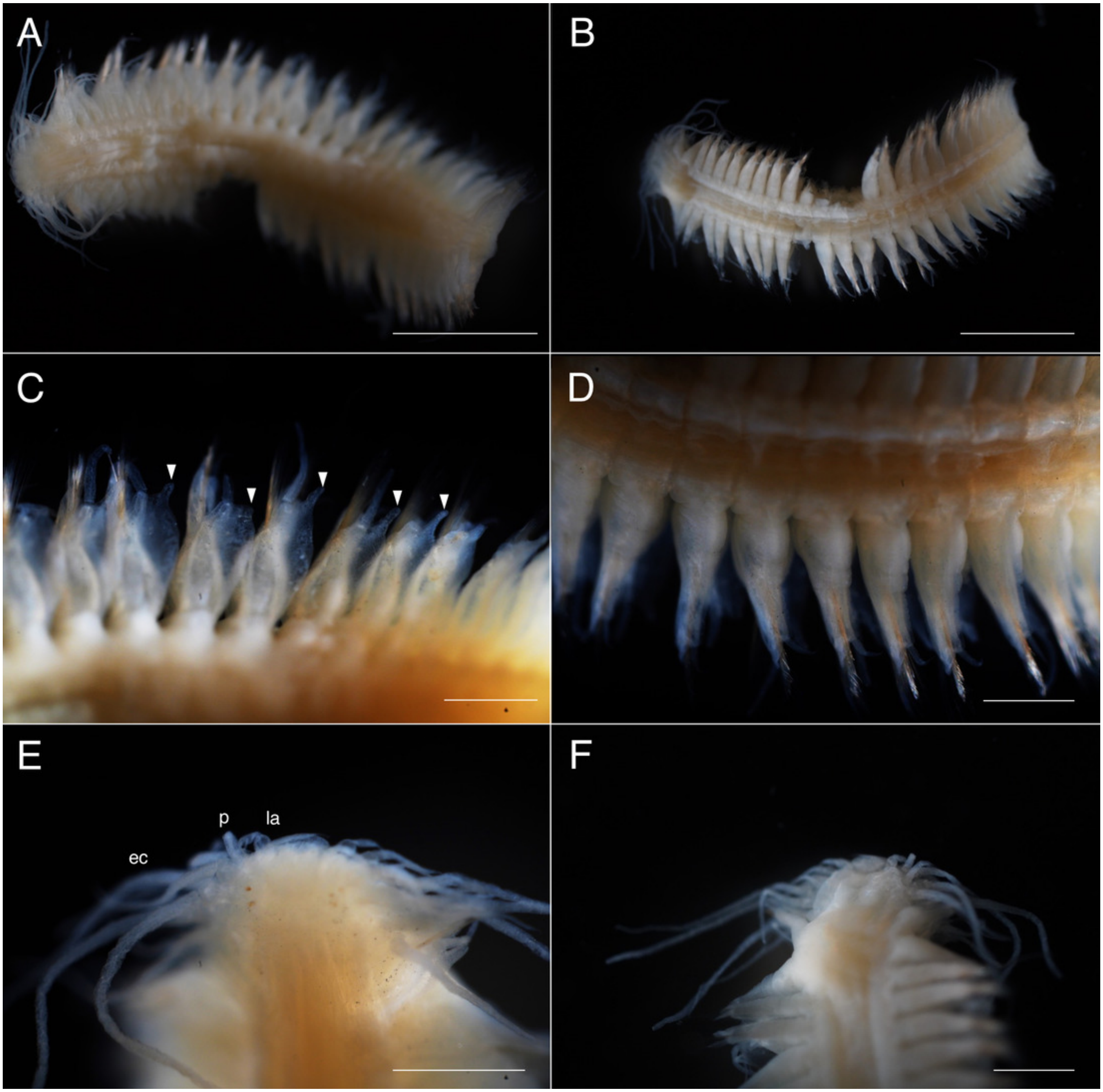
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Figure 8

Parahesione apiculata sp. nov. (NSMT-Pol H-898)

A, anterior end, dorsal view; B, parapodium of chaetiger 17, frontal view; ~~D, neurochaeta~~, upper side, chaetiger 17; D, ~~neurochaeta~~, lower side, chaetiger 17. Black arrow indicates a digitate lobe. Abbreviation: la, lateral antenna; p, palp; ec, enlarged cirrus. Scale bars: A, 1 mm; B, 500 µm; C–D, 100 mm.

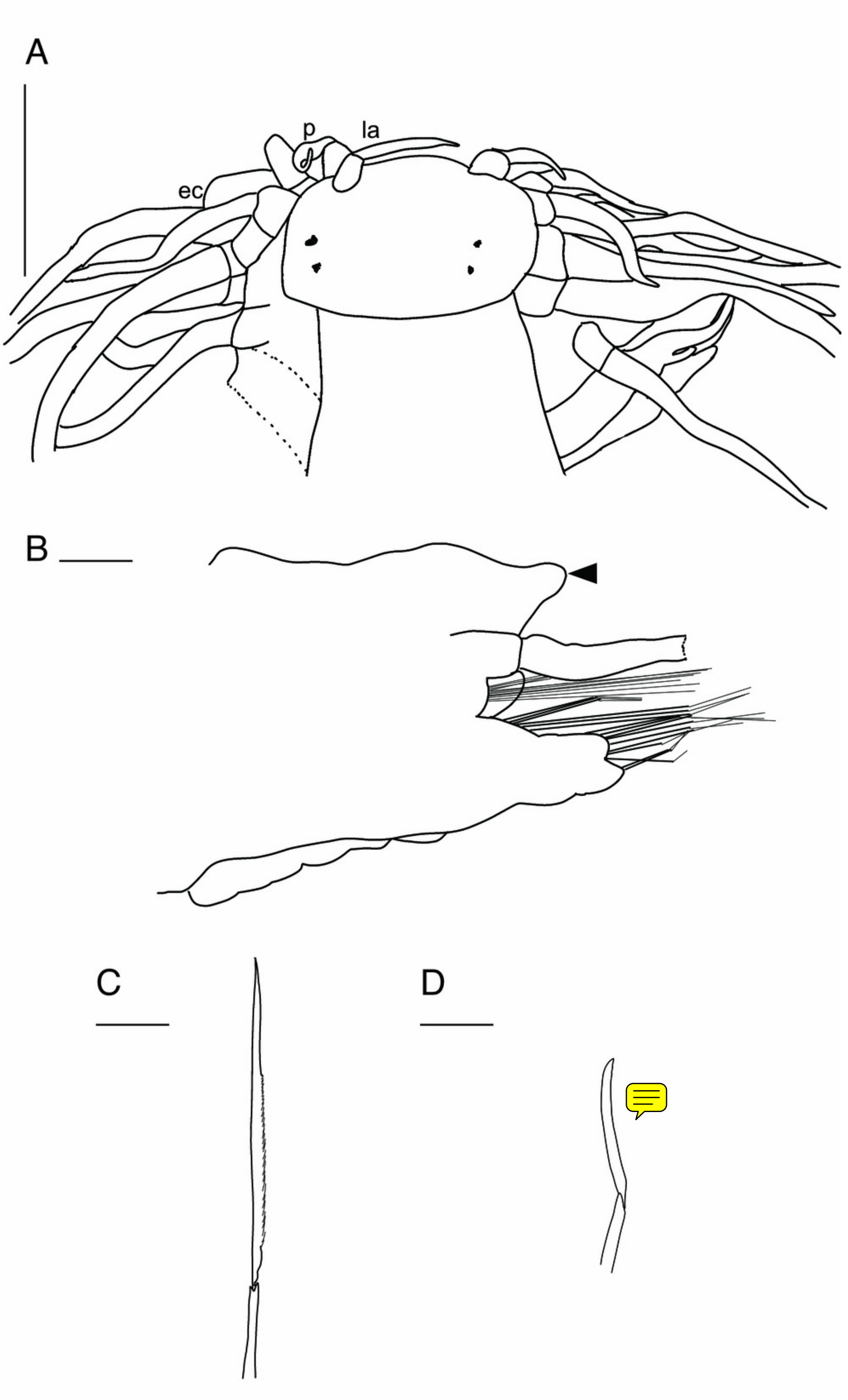
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Figure 9

A. Maximum-likelihood phylogenetic tree of Hesionidae based on *COI*, *16S*, *18S* and *28S* sequences

Nodal bootstrap support (BS) values higher than 50% are indicated for each branch. Posterior probability (PP) of each branch is also shown behind the bootstrap value. * = 100 in BS and 1.00 in PP; - = node absent in the Bayesian tree. Red circles indicate symbiotic species.

Figure 10

Parahesione sp. (A–B) used in Ruta et al. (2007) and *Parahesione luteola* (C–D) used in Pettibone (1956)

A, anterior end, dorsal view; B, anterior end, ventral view; C, anterior end, lateral view; D, anterior end, ventral view.

Table 1(on next page)

List of hesionids included in the phylogenetic analysis and the Genbank accession numbers

Table 1. List of hesionids included in the phylogenetic analysis and the Genbank accession numbers.

Species	18S	16S	28S	COI	References
Dysponetus caecus (Langerhans, 1880)	AY839568	EU555047	EU555028	AF221568	Wiklund et al. (2009); Dahlgren et al. (2000)
Nereis pelagica Linnaeus, 1758	AY340438	AY340470	AY340407	–	Rousset et al. (2007)
Amphiduros fuscescens (Marenzeller, 1875)	DQ442584	DQ442569	DQ442598	DQ442561	Ruta et al. (2007)
Amphiduropsis cf. *axialensis* sensu Rouse, Carvajal & Pleijel (2018)	MG649239	MG523356	MG649243	MG517505	Rouse et al. (2018)
Amphiduros pacificus Hartman, 1961	JN631334	JN631324	JN631345	JN631312	Pleijel et al. (2012)
Elisesione imajimai Jimi, Eibye-Jacobsen & Salazar-Vallejo, 2018	LC361352	–	LC361353	LC361354	Jimi et al. (2018)
Gyptis brunnea (Hartman, 1961)	JN631335	JN631323	JN631346	JN631313	Pleijel et al. (2012)
Gyptis golikovi (Averincev, 1990)	JN631336	JN631321	JN631347	–	Pleijel et al. (2012)
Gyptis hians Fauchald & Hancock, 1981	JN571891	JN571880	JN571900	JX503009	Summers et al. (2015)
Gyptis pacificus (Hessle, 1925)	JN631337	JN631322	JN631348	JN631314	Pleijel et al. (2012)
Gyptis paucilineata Pleijel, Rouse & Nygren, 2009	–	–	–	EU498243	Pleijel et al. (2009)
Gyptis polymorpha Pleijel, Rouse & Nygren, 2009	–	–	–	EU498247	Pleijel et al. (2012)
Gyptis propinqua Marion & Bobretzky, 1875	–	DQ442573	DQ442602	EU498229	Pleijel et al. (2009)
Gyptis robertscrippsi Rouse, Carvajal & Pleijel, 2018	MG649238	MG523360	MG649247	MG517513	Rouse et al. (2018)
Gyptis simpsonorum Pleijel, Rouse &	–	–	–	KU738203	Pleijel et al.

Nygren, 2009					(2009)
Gyptis shannonae Summers, Pleijel & Rouse, 2015	KP745537	KP745534	KP745540	–	Summers et al. (2015)
Hesiolyra bergi Blake, 1985	AM159577	MG523359	–	MG517521	Rouse et al. (2018)
Hesione sp. sensu Ruta et al. (2007)	DQ442617	DQ442615	DQ442619	–	Ruta et al. (2007)
Hesiospina aurantiaca (M. Sars, 1862)	JN852829	JN631319	JN852897	–	Pleijel et al. (2012)
Hesiospina vestimentifera Blake, 1985	JN631330	JN852860	JN631343	JN631310	Pleijel et al. (2012)
Heteropodarke formalis Perkins, 1984	–	KJ855065	KJ855076	–	Martin et al. (2015)
Heteropodarke pleijeli Muona, 2006	–	KY823464	KY823481	–	Martin et al. (2015)
Leocrates chinensis Kinberg, 1866	DQ442589	DQ442575	DQ442605	DQ442565	Ruta et al. (2007)
Leocratides kimuraorum Jimi, Tanaka & Kajihara, 2017	LC480516	LC480518	LC480517	LC258082	Jimi et al. (2017); Goto et al. (2019)
Micropodarke dubia (Hessle, 1925)	JN571888	DQ442576	JN571899	JN571825	Summers et al. (2015)
Neogyptis carriebowcayi Pleijel, Rouse, Sundkvist & Nygren, 2012	JN631338	JN631325	JN631349	JN631315	Pleijel et al. (2012)
Neogyptis fauchaldi Pleijel, Rouse, Sundkvist & Nygren, 2012	JN631339	JN631326	–	JN631316	Pleijel et al. (2012)
Neogyptis hinehina Pleijel, Rouse, Sundkvist & Nygren, 2012	JN631340	JN631328	JN631350	JN631317	Pleijel et al. (2012)
Neogyptis jeffruoccoi Rouse, Carvajal & Pleijel, 2018	JN852831	–	MG649244	MG517514	Rouse et al. (2018)
Neogyptis mediterranea (Pleijel, 1993)	–	DQ442572	DQ442601	DQ442563	Ruta et al. (2007)
Neogyptis rosea (Malm, 1874)	JN571890	DQ442574	DQ442603	JN571826	Ruta et al. (2007); Summers et al. (2015)
Neogyptis julii Summers, Pleijel & Rouse,	KP745538	KP745535	KP745541	KP745532	Summers et al.

2015					(2015)
Neogyptis sp. A sensu Pleijel et al. (2012)	JN631341	JN631327	JN631351	JN631318	Pleijel et al. (2012)
Nereimyra aphroditoides (O. Fabricius, 1780)	–	JF317211	JF317204	JF317198	Pleijel et al. (2012)
Nereimyra punctata (Müller, 1788)	DQ442591	DQ442577	DQ442606	DQ442566	Ruta et al. (2007)
Nereimyra woodsholea (Hartman, 1965)	–	–	JF317207	AY644802	Nygren et al. (2005)
Oxydromus fauveli (Uchida, 2004)	–	–	KJ855078	KJ855071	Martin et al. (2015)
Oxydromus flexuosus (Delle Chiaje, 1827)	DQ442592	DQ442578	DQ442607	DQ442567	Ruta et al. (2007)
Oxydromus okupa (Martin, Meca, Gil, Drake and Nygran, 2017)	KJ855075	KJ855070	KJ855082	–	Martin et al. (2015, 2017, 2019)
Oxydromus microantennatus (Hutchings & Murray, 1984)	–	KJ855067	KJ855079	KJ855072	Martin et al. (2015)
Oxydromus obscurus (Verrill, 1873)	–	KJ855068	KJ855080	KJ855073	Martin et al. (2015)
Oxydromus pallidus Claparède, 1864	DQ442593	DQ442579	DQ442608	–	Ruta et al. (2007)
Oxydromus pugettensis (Johnson, 1901)	DQ790086	KJ855069	KJ855081	KJ855074	Martin et al. (2015)
***Parahesione apiculata* sp. nov.**	–	**OP407586**	**OP407537**	**OP404167**	**This study**
***Parahesione pulvinata* sp. nov.**	**OP407566**	**OP407585**	**OP407536**	**OP404166**	**This study**
Parahesione sp.	–	–	DQ442613	–	Ruta et al. (2007)
Podarkeopsis arenicolus (La Greca, 1946)	JN571889	JN571879	DQ442609	JN571827	Summers et al. (2015)
Podarkeopsis helgolandicus (Hilbig & Dittmer, 1979)	JN631331	–	JN631344	JN631311	Pleijel et al. (2012)
Psamathe fusca Johnston, 1836	DQ442595	DQ442581	DQ442610	DQ513294	Ruta et al. (2007)
Sirsoe dalailamai Rouse, Carvajal & Pleijel, 2018	MG649240	MG523357	MG649245	MG517498	Rouse et al. (2018)
Sirsoe methanicola (Desbruyères &	JN631332	DQ442582	DQ442611	DQ513295	Ruta et al. (2007)

Toulmond, 1998)					
Sirsoe munki Rouse, Carvajal & Pleijel, 2018	MG649241	MG523358	MG649246	MG517510	Rouse et al. (2018)
Sirsoe sirikos Summers, Pleijel & Rouse, 2015	JN571893	JN571882	JN571902	JN571829	Summers et al. (2015)
Syllidia armata Quatrefages, 1866	DQ442596	DQ442583	DQ442612	DQ442568	Ruta et al. (2007)
Vrijenhoekia balaenophila Pleijel, Rouse, Ruta, Wiklund & Nygren, 2008	JN631333	DQ513301	DQ513306	DQ513296	Pleijel et al. (2008)
Vrijenhoekia ahabi Summers, Pleijel & Rouse, 2015	JN571898	JN571887	JN571907	JN571876	Summers et al. (2015)
Vrijenhoekia falenothiras Summers, Pleijel & Rouse, 2015	JN571897	JN571886	JN571906	JN571875	Summers et al. (2015)
Vrijenhoekia ketea Summers, Pleijel & Rouse, 2015	JN571896	JN571885	JN571905	JN571838	Summers et al. (2015)
Vrijenhoekia sp. A sensu Summers et al. (2015)	KP745539	KP745536	KP745542	KP745533	Summers et al. (2015)

Table 2(on next page)

Life style of hesionids included in the phylogenetic analysis, indicating the mode of life and the host taxa in case of symbionts

Table 2. Life style of hesionids included in the phylogenetic analysis, indicating the mode of life

Species	mode of life	host	References
Dysponetus caecus (Langerhans, 1880)	free-living	–	Watson et al. (2014)
Nereis pelagica Linnaeus, 1758	free-living	–	McIntosh (1910)
Amphiduros fuscescens (Marenzeller, 1875)	free-living	–	Pleijel (2001)
Amphiduropsis cf. *axialensis* sensu Rouse et al. (2018)	free-living	–	Rouse et al. (2018)
Amphiduros pacificus Hartman, 1961	free-living	–	Pleijel (2001)
Elisesione imajimai Jimi, Eibye-Jacobsen & Salazar-Vallejo, 2018	free-living	–	Jimi et al. (2018)
Gyptis brunnea (Hartman, 1961)	free-living	–	Hartman (1961)
Gyptis golikovi (Averincev, 1990)	free-living	–	Averincev (1990)
Gyptis hians Fauchald & Hancock, 1981	free-living	–	Banse & Hobson (1968)
Gyptis pacificus (Hessle, 1925)	free-living	–	Hessle (1925)
Gyptis paucilineata Pleijel, Rouse & Nygren, 2009	free-living	–	Pleijel et al. (2009)
Gyptis polymorpha Pleijel, Rouse & Nygren, 2009	free-living	–	Pleijel et al. (2009)
Gyptis propinqua Marion & Bobretzky, 1875	free-living	–	Parapar et al. (2005)
Gyptis robertscrippsi Rouse, Carvajal & Pleijel, 2018	free-living	–	Rouse et al. (2018)

and the host taxa in case of symbionts.

Gyptis simpsonorum Pleijel, Rouse & Nygren, 2009	free-living	–	Pleijel et al. (2009)
Gyptis shannonae Summers, Pleijel & Rouse, 2015	free-living	–	Summers et al. (2015)
Hesiolyra bergi Blake, 1985	free-living	–	Blake (1985)
Hesione sp. sensu Ruta et al. (2007)	free-living	–	Ruta et al. (2007)
Hesiospina aurantiaca (M. Sars, 1862)	free-living	–	Pleijel (2004)
Hesiospina vestimentifera Blake, 1985	facultative symbiont	*annelids*	Pleijel (2004)
Heteropodarke formalis Perkins, 1984	free-living	–	Perkins (1984)
Heteropodarke pleijeli Muona, 2006	free-living	–	Pleijel (1999), Muona (2006)
Leocrates chinensis Kinberg, 1866	facultative symbiont	corals	Martin et al. (2017), Wang et al. (2018)
Leocratides kimuraorum Jimi, Tanaka & Kajihara, 2017	obligate-symbiont	sponges	Jimi et al. (2017)
Micropodarke dubia (Hessle, 1925)	free-living	–	Hessle (1925)
Neogyptis carriebowcayi Pleijel, Rouse, Sundkvist & Nygren, 2012	free-living	–	Pleijel et al. (2012)
Neogyptis fauchaldi Pleijel, Rouse, Sundkvist & Nygren, 2012	free-living	–	Pleijel et al. (2012)
Neogyptis hinehina Pleijel, Rouse, Sundkvist & Nygren, 2012	free-living	–	Pleijel et al. (2012)
Neogyptis jeffruoccoi Rouse, Carvajal & Pleijel, 2018	free-living	–	Rouse et al. (2018)

Neogyptis mediterranea (Pleijel, 1993)	free-living	–	Pleijel et al. (2012)
Neogyptis rosea (Malm, 1874)	free-living	–	Pleijel et al. (2012)
Neogyptis julii Summers, Pleijel & Rouse, 2015	free-living	–	Summers et al. (2015)
Neogyptis sp. A sensu Pleijel et al. (2012)	free-living	–	Pleijel et al. (2012)
Nereimyra aphroditoides (O. Fabricius, 1780)	free-living	–	Pleijel et al. (2011)
Nereimyra punctata (Müller, 1788)	free-living	–	Pleijel et al. (2011)
Nereimyra woodsholea (Hartman, 1965)	free-living	–	Pleijel et al. (2011)
Oxydromus fauveli Uchida, 2019	free-living	–	Uchida et al. (2019)
Oxydromus flexuosus (Delle Chiaje, 1827)	Facultative symbiont	starfish, holothuroids, annelids	Martin et al. (2017)
Oxydromus humesi (Pettibone, 1961)	obligate symbiont	bivalves	Pettibone (1961), Martin et al. (2012, 2017)
Oxydromus okupa (Martin, Meca, Gil, Drake and Nygren, 2017)	obligate symbiont	bivalves	Martin et al. (2012, 2017), Meca et al. (2019)
Oxydromus microantennatus (Hutchings & Murray, 1984)	free-living	–	Hutchings & Murray (1984)
Oxydromus obscurus (Verrill, 1873)	facultative symbiont	annelids, holothuroids	Martin & Britayev (1998)
Oxydromus pallidus Claparède, 1864	facultative symbiont	annelids	Martin et al. (2017)
Oxydromus pugettensis (Johnson, 1901)	facultative symbiont	echinoderms, decapods, gastropods, bivalves	Martin & Britayev (1998)

***Parahesione apiculata* sp. nov.**	obligate symbiont	decapods	This study
***Parahesione pulvinata* sp. nov.**	obligate symbiont	decapods	This study
Parahesione sp.	facultative symbiont?	decapods	Ruta et al. (2007)
Podarkeopsis arenicolus (La Greca, 1946)	free-living	–	La Greca (1946)
Podarkeopsis helgolandicus (Hilbig & Dittmer, 1979)	free-living	–	Hilbig & Dittnmer (1979)
Psamathe fusca Johnston, 1836	free-living	–	Parapar et al. (2005)
Sirsoe dalailamai Rouse, Carvajal & Pleijel, 2018	free-living	–	Rouse et al. (2018)
Sirsoe methanicola (Desbruyères & Toulmond, 1998)	free-living	–	Desbruyères & Toulmond (1998)
Sirsoe munki Rouse, Carvajal & Pleijel, 2018	free-living	–	Rouse et al. (2018)
Sirsoe sirikos Summers, Pleijel & Rouse, 2015	free-living	–	Summers et al. (2015)
Syllidia armata Quatrefages, 1866	free-living	–	Ruta & Pleijel (2006)
Vrijenhoekia balaenophila Pleijel, Rouse, Ruta, Wiklund & Nygren, 2008	free-living	–	Pleijel et al. (2008)
Vrijenhoekia ahabi Summers, Pleijel & Rouse, 2015	free-living	–	Summers et al. (2015)
Vrijenhoekia falenothiras Summers, Pleijel & Rouse, 2015	free-living	–	Summers et al. (2015)
Vrijenhoekia ketea Summers, Pleijel & Rouse, 2015	free-living	–	Summers et al. (2015)

Vrijenhoekia sp. A sensu Summers et al. (2015)	free-living	–	Summers et al. (2015)