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

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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 are characterized by having eight enlarged cirri, dorsal cirrophores with dorsal foliose lobe and biramous parapodia, and by lacking median antenna. *Parahesione apiculata* sp. nov. has digitate lobes on the posterior margin of the dorsal foliose lobe (absent in *P*. *pulvinata* sp. nov.) and dorsal foliose lobe from chaetiger 1 (from 6 in *P*. *pulvinata* sp. nov.). The two new species were never found outside the ghost shrimp burrows, suggesting they are obligate symbionts. Phylogenetic analyses based on four concatenated genes suggest that the symbiotic lifestyle has evolved several times in Hesionidae.

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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 are characterized by having eight enlarged cirri, dorsal cirrophores with dorsal foliose lobe and biramous parapodia, and by lacking median antenna. *Parahesione apiculata* sp. nov. has digitate lobes on the posterior margin of the dorsal foliose lobe (absent in *P. pulvinata* sp. nov.) and dorsal foliose lobe from chaetiger 1 (from 6 in *P. pulvinata* sp. nov.). The two new species were never found outside the ghost shrimp burrows, suggesting they are obligate symbionts. Phylogenetic analyses based on four concatenated genes suggest that the symbiotic lifestyle has evolved several times in Hesionidae.

Introduction

The narrow burrows excavated by decapods in tidal flats are frequently occupied by different symbionts (Campos et al. 2009; Pillay and Branch 2011). However, these safe habitats, but must be shared with decapod hosts, often under hypoxic conditions (Atkinson & Taylor 2005) to which some symbionts become adapted (Pillay & Branch 2011). These include polynoid and hesionid polychaetes living in burrows of callianassid ghost shrimps and upogebiid mud shrimps (Martin and Britayev 1998).

Hesionidae includes more than 199 species (Rouse et al. 2018, 2022; Read & Fauchald 2023), with about 30 being considered obligate or facultative invertebrate symbionts, mainly living in association with echinoderms, but also with burrowing sipunculids, hemichordates and polychaetes, among others (Martin and Britayev 1998, 2018; Martin et al. 2017; Rouse et al. 2018). However, only *Parahesione luteola* (Webster, 1880) and *Parahesione* sp. are known from mud shrimp burrows (Pettibone 1956; Britayev & Antokhina 2012). *Parahesione* was proposed by Pettibone (1956) for *Podarke luteola* Webster, 1879 (type species), whose type material was lost, and *Hesione agilis* Webster & Benedict, 1884. The former was found on an oyster bank in Great Egg Harbor, New Jersey, whereas the latter was found living commensally with *Upogebia affinis* (Say, 1818). These two species were regarded by Pettibone (1963) as synonyms and has been considered as facultative symbionts (Martin & Britayev 1998).

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 nature of their adaptations to a symbiotic mode of life and (2) the evolutionary consequences of their symbiotic relationships with burrowing decapods to try to elucidate their adaptability to differential environmental conditions.

In this paper, we describe two new species of Hesionidae living inside burrows of callianassid ghost shrimps and analyze the phylogenetic relationships within the family, based on four concatenated genes, to assess the evolution of both symbiotic species and their adaptations to living inside host burrows.

Materials & Methods

The specimens were collected with a yabby pump from inside of the burrows of *Neocallichirus jousseaumei* (Nobili, 1904) (Axiidea: Eucalliacidae) and *Glypturus armatus* (Milne-Edwards, 1870) (Gebiidea: Upogebiidae), living in tidal flats throughout the Ryukyu Islands, Japan (Fig. 1). All specimens were fixed and preserved in 70% ethanol. Additional studied specimens were reported as: (1) *Parahesione* sp. (Britayev & Antokhina 2012), (2) *Parahesione* sp. (Ruta et al. 2007, first paragraph, page 101), reported as *P. luteola* in Genbank, (3) *Parahesione* from Papua

New Guinea, collected by GR likely from burrow of *Calliaxina bulimba* (Poore & Griffin, 1979) (Axiidea: Eucalliacidae), and (4) *P. luteola* (Pettibone, 1956) (No. USNM 430 and 28175).

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 observed with a Leica MZ9.5 stereomicroscope and photographed with a Canon Rebel T3i camera.

Type specimens are 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). The following abbreviations are used in the 'Material examined' section: CS (complete specimens), L (length, measured from the anterior margin of the prostomium to the posterior border of the last segment), W (width the widest segment, including parapodia but excluding chaetae).

The total DNA of the Japanese holotype was extracted from a dissected parapodium using a DNeasy Tissue Kit (Qiagen). DNA extraction, sequencing, alignment, and removing ambiguous positions were carried out for the method of Jimi et al. (2021). The *Parahesione* from Papua New Guinea were extracted following from Rouse et al. (2018) and *COI* was generated for the specimen. All newly obtained sequences *COI* (625 bp), *16S* (552 bp), *18S* (1677 bp), *28S* (987 bp) were deposited in GenBank. 198 sequences (59 species) were used for molecular analyses, including 191 downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank) (Table 1). Phylogenetic analyses were also carried out for the method of Jimi et al. (2021). *Dysponetus caecus* (Langerhans, 1880) was used as the outgroup following Rouse et al. (2018) and Tilic et al. (2022). Additionally, four species were used for calculating K2P genetic distances using MEGAX (Stecher et al. 2020). To understand the evolution of symbiosis in the family Hesionidae, we divided them into the following three groups: obligate and facultative symbionts, according to Martin et al. (2017), and free-living based on previously published data (Table 2). In the molecular phylogenetic tree, we mapped species with symbiotic ecology based on this table.

The map of figure 1 was generated by using GMT 6 (Wessel et al. 2019).

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Results

Systematics

Ophiodrominae Pleijel, 1998

Amphidurini Pleijel et al., 2012

***Parahesione* Pettibone, 1956**

Diagnosis (emended). Body depressed, reddish when alive. Prostomium with two lateral antennae, without median antenna, two pairs of eyes. Palps simple or biarticulate. Six or eight pairs of tentacular cirri. Dorsal cirrophores fused with or without dorsal foliose lobe extending to base of parapodia. Parapodia biramous. Notopodia with numerous capillary chaetae. Neuropodia with numerous compound chaetae: homogomph and/or heterogomph falcigers, and heterogomph spinigers (after Pettibone 1956).

Remarks. *Parahesione* resembles *Amphiduros* and *Amphiduropsis* in having enlarged dorsal cirri on segments 1–5, but differs in lacking median antenna (having a short one in *Amphiduros* and *Amphiduropsis*). Two species of the genus, type species *P. luteola* and *Parahesione* sp. from New Caledonia (Ruta et al. 2007) have six enlarged tentacular cirri and cilindrical dorsal cirrophores, while two new species, *P. pulvinata* and *P. apiculata* have eight pairs of tentacular cirri and dorsal cirrophores fused with dorsal foliose lobe extending to base of parapodia. We assign these species to the same genus *Parahesione* and modify the diagnosis of the genus. However, since the DNA repository data for *Parahesione* sp. used in Ruta et al. (2007) is very limited, and for the type species *P. luteola* is unavailable (formaldehyde fixation), it is possible that the two morpho-types *Parahesione* (eight enlarged cirri & dorsal cirrophores with dorsal foliose lobe vs. six enlarged cirri & dorsal cirrophores without dorsal foliose lobe) would be assigned to different genera if molecular sequences for additional specimens of first morpho-type would be obtained.

Parahesione pulvinata Jimi, Gonzalez, Rouse and Britayev sp. nov.
[New Japanese name: ana-yadori-otohime]
(Figs. 2–5, supplementary figure 1)
Zoobank LSID: urn:lsid:zoobank.org:act:2E42DB94-DF8C-447A-A7F8-8C2FDA9FF4CA

Parahesione sp.: Britayev and Antokhina (2012): 33, Pl. 9 C, D

Diagnosis. *Parahesione* with dorsal foliose lobe from chaetiger 6 and eight tentacular anterior cirri.

Material examined. Holotype: NMST-Pol H-893, 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, associated to *N. jousseaumei*, 5 September 2020, collected by HN. Paratypes: NSMT-Pol P-894, CS, L 20 mm, W 5 mm for 45 chaetigers, same collection data as holotype, but 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, associated to an unknown crustacean, 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, associated to an unknown crustacean, 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, associated to an unknown crustacean, 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 associated to *C. bulimba,* 13 December 2012, collected by Art Anker and GR. 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, associated to *Upogebia* sp., 18 April 2004, collected by Ivan Marin.

Description of holotype. Body depressed, tapered in posterior region, reddish when alive (Fig. 2), pinkish after fixation (Fig. 3). Prostomium rectangular, wider than long (Figs. 3E, 5A). Lateral antennae slightly shorter than head length, cylindrical, with distally tapering style and short cylindrical ceratophores. Palps 2/3 antennae length, with cylindrical distally tapering palpostyles and short cylindrical palpophores. Two pairs of eyes, dark reddish in alive, brownish after fixation.

Elongated dorsal cirri on segments 1–5; tentacular cirri eight pairs, on segments 1–4, cirrophores of tentacular cirri cylindrical, basally fused; longest dorsal cirri reaching chaetiger 8, longest ventral cirri reaching chaetiger 5. Chaetae absent from segments 1–4.

Dorsal cirrophores from chaetiger 6 fused with dorsal foliose lobe extending to base of parapodia, partially covering subsequent segment (Fig. 3C); all dorsal cirrostyles long, twice as long or longer than neuropodial lobe with chaetae, conical, smooth (Fig. 5C). Ventral cirrophores fused to parapodia; ventral cirrostyles short, slightly extending beyond neuropodial lobe, conical, smooth (Fig. 3D; 5C). Noto- and neuro aciculae brownish, tip of aciculae not seen in vivo, reddish after fixation.

All chaetigers biramous (Fig. 4A) except chaetiger 1 (uniramous notopodia small, conical, pointed, with about 40 simple capillary very fine notochaetae (Fig. 4B); neuropodia large, truncated, longer than wide, with prechaetal lobes and a postero-dorsal digitiform projections (Fig. 5C) and about 30 compound heterogomph chaetae, supraacicular spinigers (Fig. 4C) and subacicular falcigers (Fig. 4D) with unidentate blades faintly serrated in spinigers and superior falcigers (Fig. 5H, 5I); smooth in most inferior falcigers; length of blades in bundle decreases from superior to inferior neurochaetae (Fig. 5D, 5E). Pygidium with two smooth anal cirri twice as long as dorsal cirri.

Variation. Body length 12.0–19.5 mm; number of chaetigers 24–48. Morphology of paratypes corresponds to description of holotype; anterior pair of dark red eyes was visible in specimen from Nhatrang when alive.

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

Remarks. *Parahesione pulvinata* sp. nov. resembles *P. luteola*, the type species of the genus and the single previously known, in lacking the median antenna while having a flattened body and living symbiotically with ghost shrimps. However, it differs in having flattened dorsal parapodial extension and eight tentacular anterior cirri (without extension and and six tentacular cirri in *P. luteola*). *Parahesione* sp. from Vietnam (Fig. 5F–5I) and Papua New Guinea (Supplementary figure 1) 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.

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* (Japan) and *Upogebia* sp. (Vietnam), or at 1–5 m inside burrows of *C. bulimba*.

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

Diagnosis. *Parahesione* with dorsal foliose lobe from chaetiger 1 and eight tentacular anterior cirri.

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 *G. 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 depressed, tapering in posterior region, reddish when alive, pale orange after fixation (Figs. 6, 7).

Prostomium rectangular, wider than long (Fig. 9A). Lateral antennae as long as head length, cylindrical, with distally tapering style and short cylindrical ceratophores. Palps 5/7 antennae length, with cylindrical distally tapering palpostyles and short cylindrical palpophores. Two pairs of eyes (Figs. 7E, 9A), inconspicuous when alive (Fig. 6D), brownish after fixation (Fig. 7E).

Elongated dorsal cirri on segments 1–5; tentacular cirri eight pairs, on segments 1–4, cirrophores of tentacular cirri cylindrical, basally fused; longest dorsal cirri reaching chaetiger 8, longest ventral cirri reaching chaetiger 7. Chaetae absent from segments 1–4.

All dorsal cirrophores cylindrical; from chaetiger 1 fused with dorsal foliose lobe extending to base of parapodia, partially covering subsequent segment (Figs. 7C, 8A, 9B); dorsal foliose lobe with dorso-lateral digitate extension; dorsal cirrostyle long, equal, or slightly longer than neuropodia with chaetae, conical, smooth (Fig. 7C). Ventral cirrophore fused with parapodia; ventral cirrostyle short, slightly extending beyond neuropodial lobe, conical, smooth. Noto- and neuroaciculae not seen in vivo, brownish with reddish tips when preserved.

All chaetigers biramous except chaetiger 1 (uniramous). Notopodia small, conical, with about 40 simple capillary notochaetae (Fig. 8B), faintly serrated. Neuropodia large, truncated, longer than wide, with pre- and post-chaetal lobes and a digitiform projection present on postero-dorsal part (Fig. 7C, 9B). About 30 heterogomph chaetae, supraacicular spinigers (Fig. 8C) and subacicular falcigers (Fig. 8D) with unidentate blades; faintly serrated in spinigers and superior falcigers; smooth in most inferior falcigers; length of blades in bundle decreases from superior to inferior neurochaetae (Fig. 9C, 9D). Pygidium with two long anal cirri, smooth.

Etymology. The specific name "*apiculata*", derives from the Latin *apiculatus* (meaning short pointed) and referring to the digitate extension on dorso-lateral margin of dorsal foliose lobe, is as an adjective in the nominative case.

Remarks. Like *P. pulvinata* sp. nov., *P. apiculata* sp. nov. resemble *P. luteola* in lacking the median antenna, having a flattened body and living symbiotically with ghost shrimps, while differing in having dorsal foliose lobe and eight tentacular anterior cirri (absent and six in *P. luteola*). *Parahesione apiculata* sp. nov. differs from *P. pulvinata* sp. nov. in having digitate extansion on posterior margin of dorsal foliose lobe (absent in *P. pulvinata* sp. nov.) and, having dorsal foliose lobe from chaetiger 1 (from chaetiger 6 in *P. pulvinata* sp. nov.), as well as in living in association with *G. armatus* (*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*.

Key to species of *Parahesione*

1. Parapodia with dorsal foliose lobe ...2
- Parapodia without dorsal foliose lobe... *P. luteola* (Webster, 1879)
2. Dorsal foliose lobe from chaetiger 1, dorsal foliose lobe with digitate extension in opposite side of body... *P. apiculata* sp. nov. This study
3. Dorsal foliose lobe from chaetiger 6, dorsal foliose lobe without digitate extension in opposite side of body... *P. pulvinata* sp. nov. This study

Molecular analyses

Parahesione apiculata sp. nov. and *Parahesione* sp. from Papua New Guinea form a clade, sister to *P. pulvinata* sp. nov. All together, they consitute the *Parahesione* clade which, in turn, is sister to the *Amphiduros–Amphiduropsis* clade (Fig. 10). K2P genetic distance between the two new species is 11.0% (10.1% uncorrected).

Discussion

Parahesione luteola was 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 symbiont (Martin and Britayev 1998). Unidentified species of *Parahesione* were reported from the 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 Vietnamese specimen is here considered as belonging to *P. pulvinata* sp. nov. as suggested by Britayev and Antokhina (2012) and discussed above in the corresponding *Remarks* section. The specimen of *Parahesione* sp. collected by Ruta et al. (2007) 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 dorsal cirri without dorsal foliose lobe (Supplementary figure 2 A, B) and is likely another new species but is not described here. Additionally, we observed the specimens of *P. luteola* in the USNM (Supplementary figure 2 C, D). The specimens showed *P. luteola* has six enlarged cirri and dorsal cirri without dorsal foliose lobe in according with Pettibone (1956)'s description.

In this situation, we assign these two new 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, rectangle prostomium, dorsal cirri with dorsal foliose lobe vs. six enlarged cirri, trapezoidal prostomium, dorsal cirri without dorsal foliose lobe) could be distinguished on a molecular phylogenetic tree if they are re-examined with additional specimens and gene sequences.

Our phylogenetic results (Fig. 10) 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*.

Despite their obvious morphological and molecular differences, *Parahesione apiculata* sp. nov. and *P. pulvinata* have always been found inside ghost shrimp burrows, suggesting they are obligate symbionts. Moreover, they have always been found in association with *G. armatus* and with *N. jousseaumei* and *Upogebia* sp. (the latter still requiring a more precise identification, Jimi pers. obs.), thus suggesting a high degree of host specificity. Moreover, like many other symbiotic polychaetes (Martin and Britayev 1998, 2018), both species show morphological adaptations to symbiosis. These include flat bodies and dorsal foliose lobes, which is not found in the non-symbiotic species of the *Amphiduropsis*-*Amphiduros* sister clade. Flat bodies have been reported for symbiotic polynoids living in association with tube dwelling chaetopterids, which also have to move between the host body and the tube walls (Britayev et al. 2017; Britayev and Martin 2021). Another interesting adaptation is the extreme flatness of body and dorsal foliose lobe,. We suggest these features may facilitate the worm movement between the host body and the walls of the narrow burrowsand to increase the body surface either to be in contact with the host or with the burrow walls. Body expansions in symbiotic polychaetes have been only previously reported for *Gastrolepidia clavigera* Schamarda, 1861, which shows ventral sucker-like lobes increasing the body surface in contact with the slippery holothurian host body and, combined with body arching, probably have a sucker-like function (Gibbs 1971, Britayev and Zamyshliak 1996). The other possible function of the the dorsal foliose lobes may also be related to the efficiency of gas exchange under conditions of hypoxia in host burrows.

The bodies of the two new species also have bright red-colour when alive. Again, this contrasts with the free-living species of *Amphiduropsis*-*Amphiduros* clade, suggesting this trait was newly acquired in *Parahesione*. A bright red color was also reported for *Hesperonoe* (Polynoidae), which also live in association with mud shrimps (Sato et al. 2001; Hong et al. 2017), while some crustacean-associated mollusks have red blood cells that are considered as an adaptation to thrive in the burrow hypoxic conditions (Goto et al. 2018). Therefore, we agree with Martin and Britayev (2018), who suggested that red bodies (likely associated to the presence of dissolved pigment) in *Hesperonoe* may be an adaptation to live in the burrows' hypoxic environment. Thus, further anatomical and histological studies are needed to confirm the gas exchange function of the foliose lobes and the presence of red bodies and thus assess whether they are adaptations of *Parahesione* to life under hypoxic conditions.

Conclusions

The genus *Parahesione*, belonging to Hesionidae, is a rare group of symbiotic polychaetes living in ghost shrimp burrows with two different sets of tentacular cirri; in one, there are only six, whereas in the other there are 8. Interestingly, the prostomium have different shape being trapezoidal in those species with 6 pairs of tentacular cirri, and rectangular for those having 8 pairs of tentacular cirri. Further, the dorsal parapodial modifications involving a foliose dorsal projection has been only reported in those species with 8 pairs of tentacular cirri, whereas it has not been recorded in the only species having 6 pairs of tentacular cirri. We have discovered two new *Parahesione* species associated with ghost shrimps from the northwest Pacific. Both species are characterized by a flattened body, expanded foliose bases of cirrophores, and a bright red color. We consider these features as adaptations to thrive in the burrow hypoxic conditions. Reconstruction of the phylogenetic tree using four genes revealed their close relationship with non-symbiotic species of the sister clade *Amphiduropsis*-*Amphiduros*, suggested the independent establishment of symbiosis in various clades of the family Hesionidae.

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Figure 1

Sampling locations for type specimens

Parahesione pulvinata sp. nov. (Red star) and *Parahesione apiculata* sp. nov. (Blue dot). The map was generated using GMT 6 (Wessel et al. 2019; https://www.generic-mapping-tools.org/).

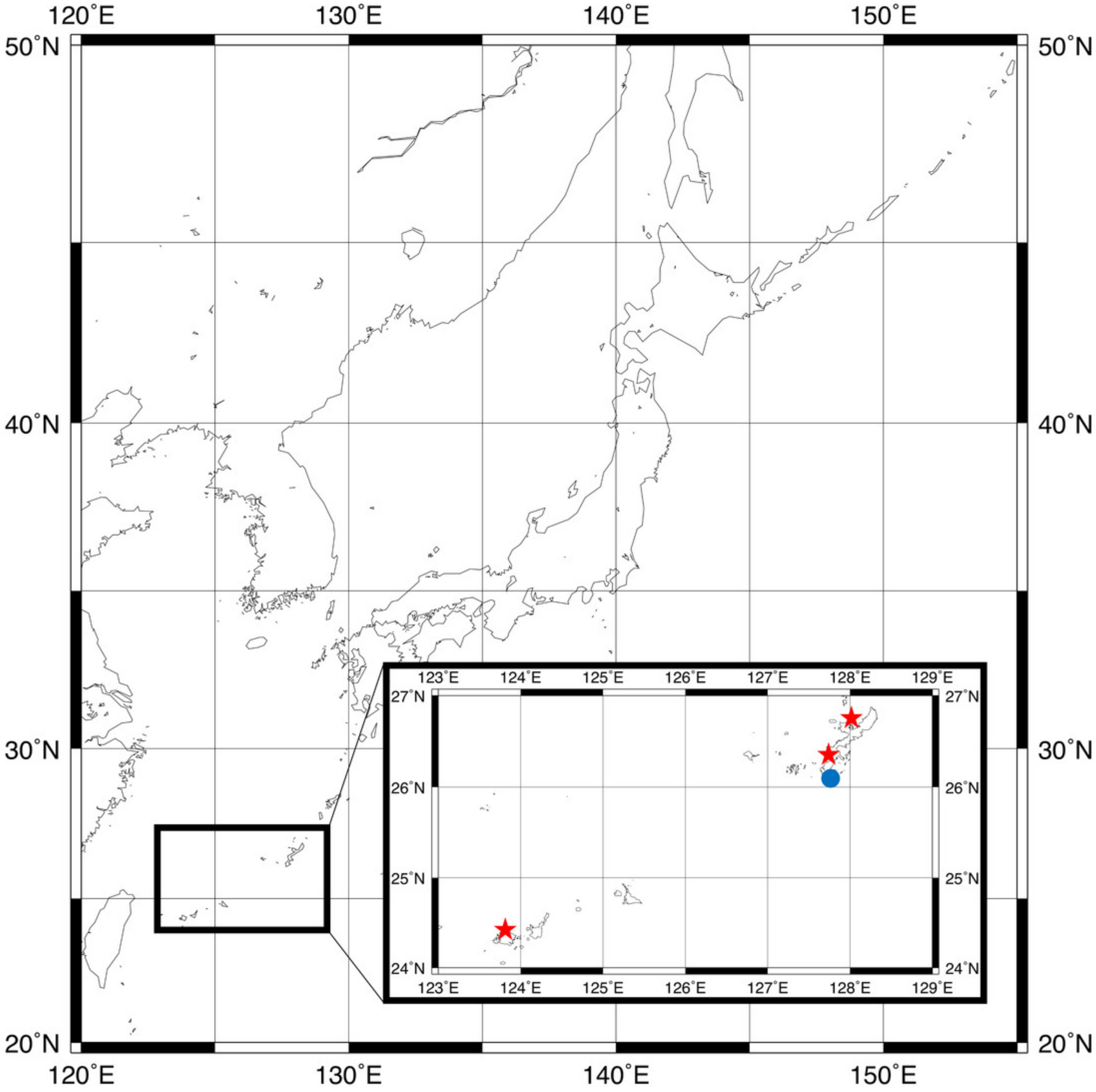
120˚E
130˚E
140˚E
150˚E
50˚N
40˚N
30˚N
20˚N
123˚E
124˚E
125˚E
126˚E
127˚E
128˚E
129˚E
27˚N
26˚N
25˚N
24˚N

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).

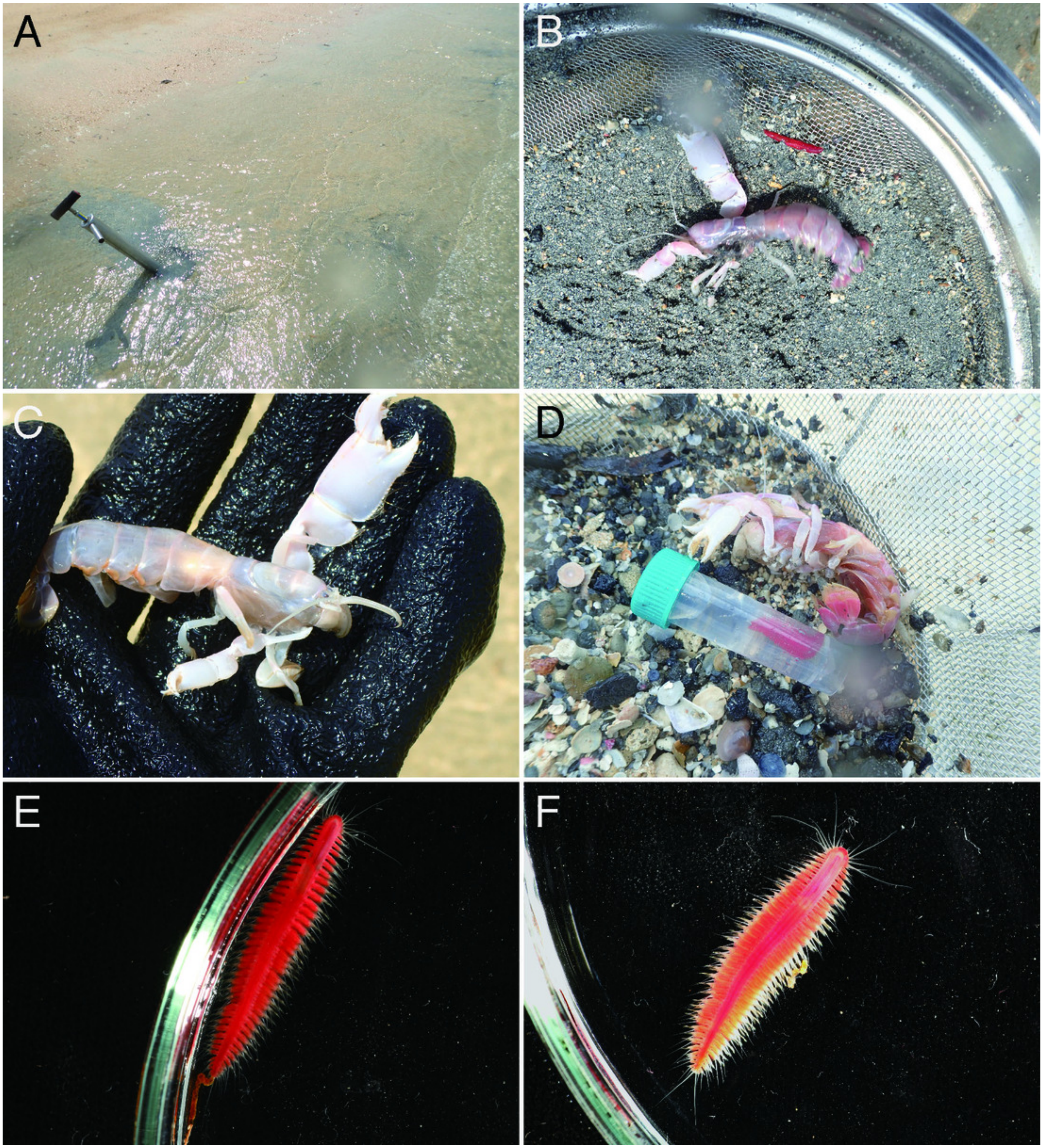
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Figure 3

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.

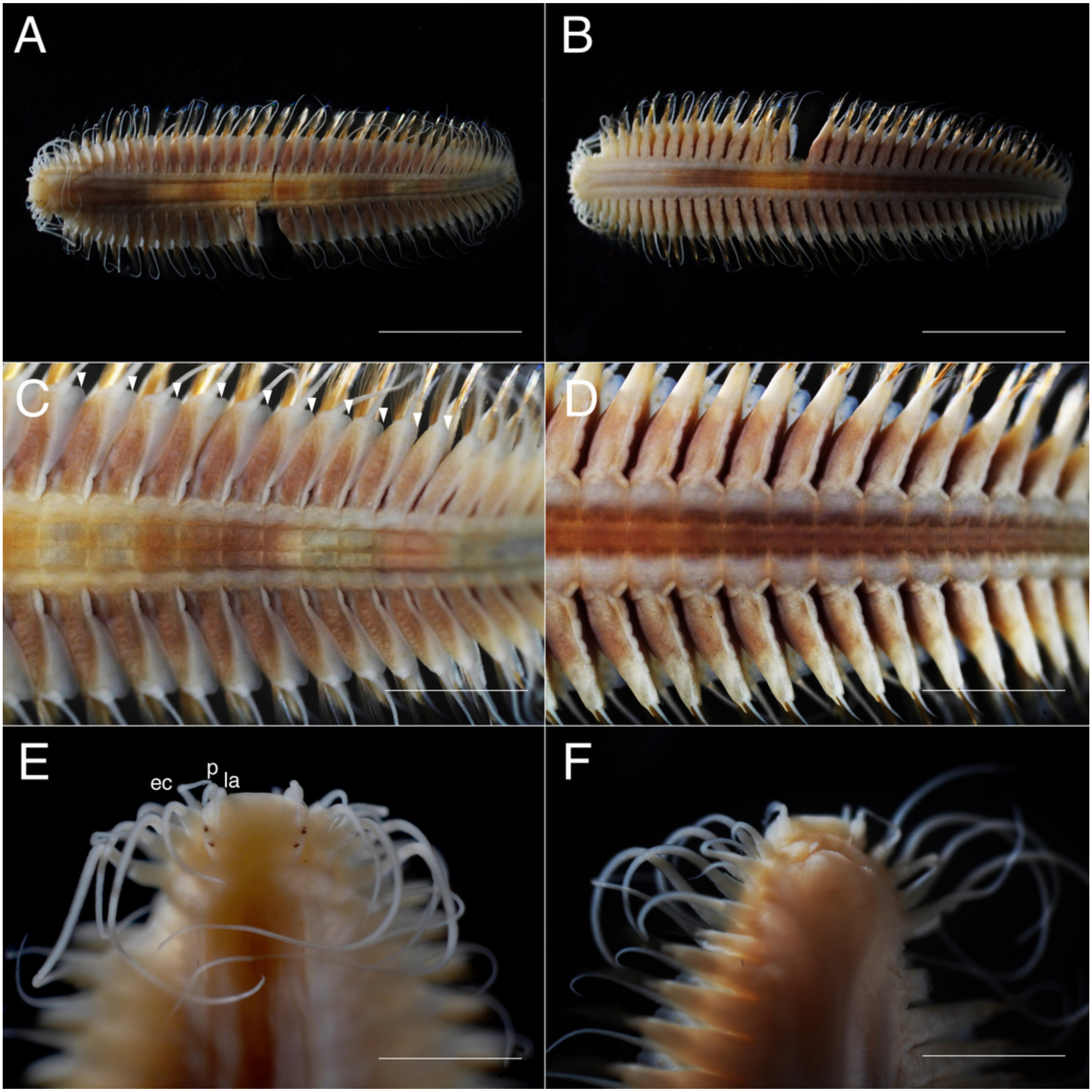
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Figure 4

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

A, parapodium of chaetiger 12, rear view; B, notochaetae, chaetiger 12; C, upper side of neurochaetae, chaetiger 12; D, lower side of neurochaetae. Scale bars: A, 200 μm; B–D, 100 μm.

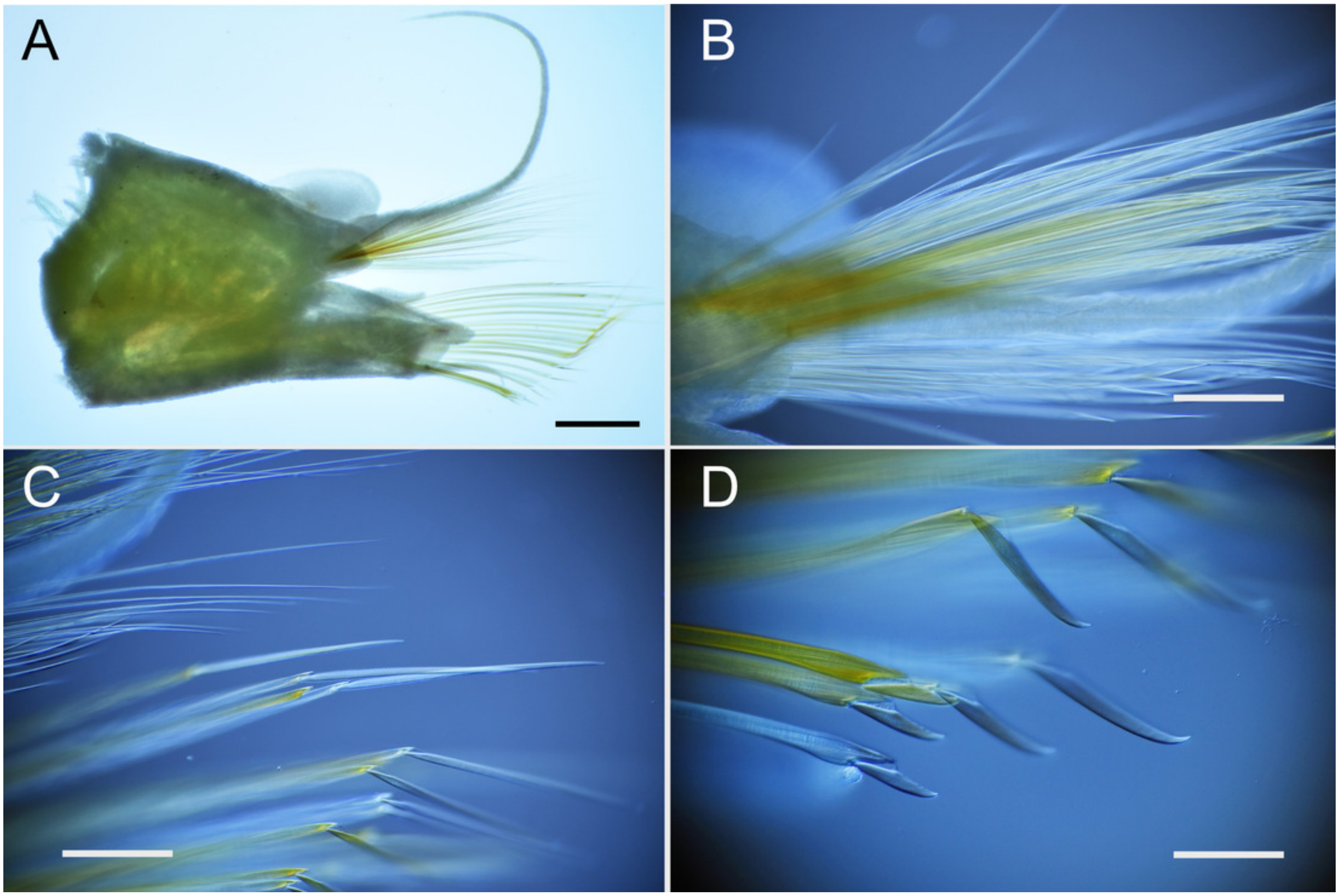

Figure 5

Parahesione pulvinata sp. nov. (NSMT-Pol H-893: A–E, IPEE RAS – Pol. 2004/01: E–I)

A, anterior end, dorsal view; B, parapodium of chaetiger 1, rear view; C, parapodium of chaetiger 17, frontal view; D, neurochaeta, upper side, chaetiger 17; E, neurochaeta, lower side, chaetiger 17; F, parapodium of chaetiger 18, frontal view; G, parapodium of chaetiger 23, rear view; H, supreacicular spiniger; I, uppermost falciger. Scale bars: A, 1 mm; B, 100 µm; C, 500 µm; D–E, 100 mm; F–G, 500 µm; H–I, 100 µm. Abbreviation: la, lateral antenna; p, palp; ec, enlarged cirrus.

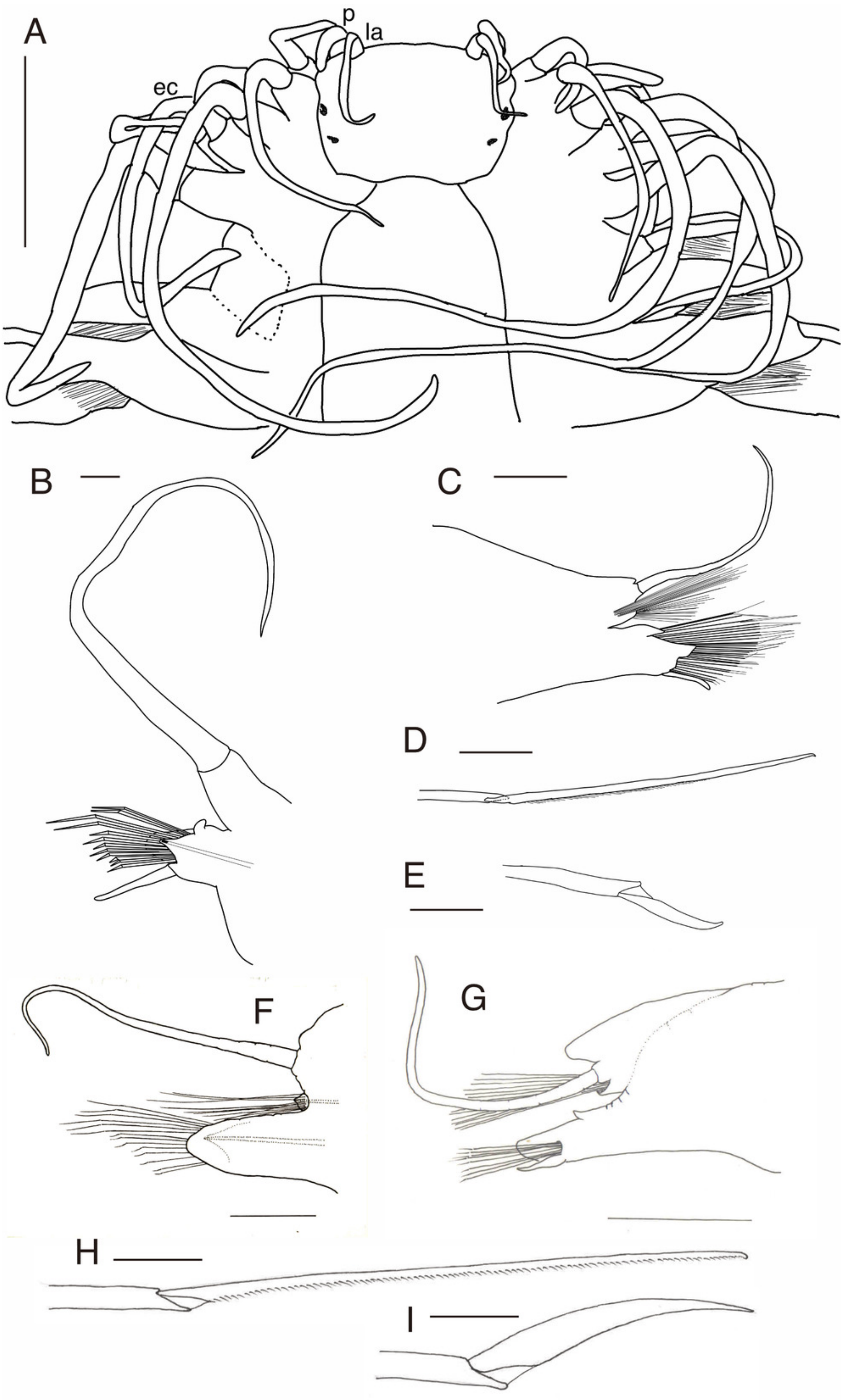
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Figure 6

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).

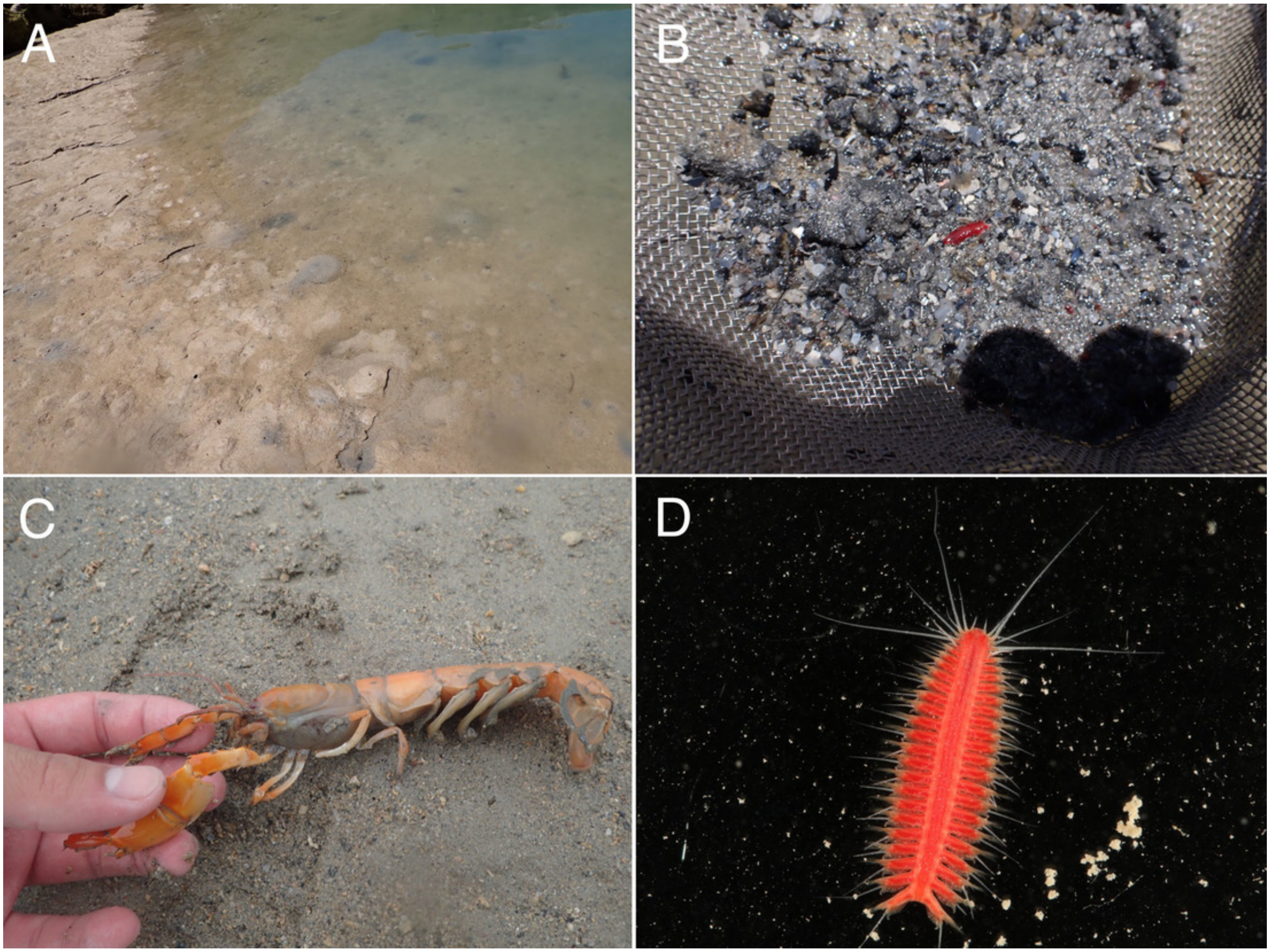

Figure 7

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.

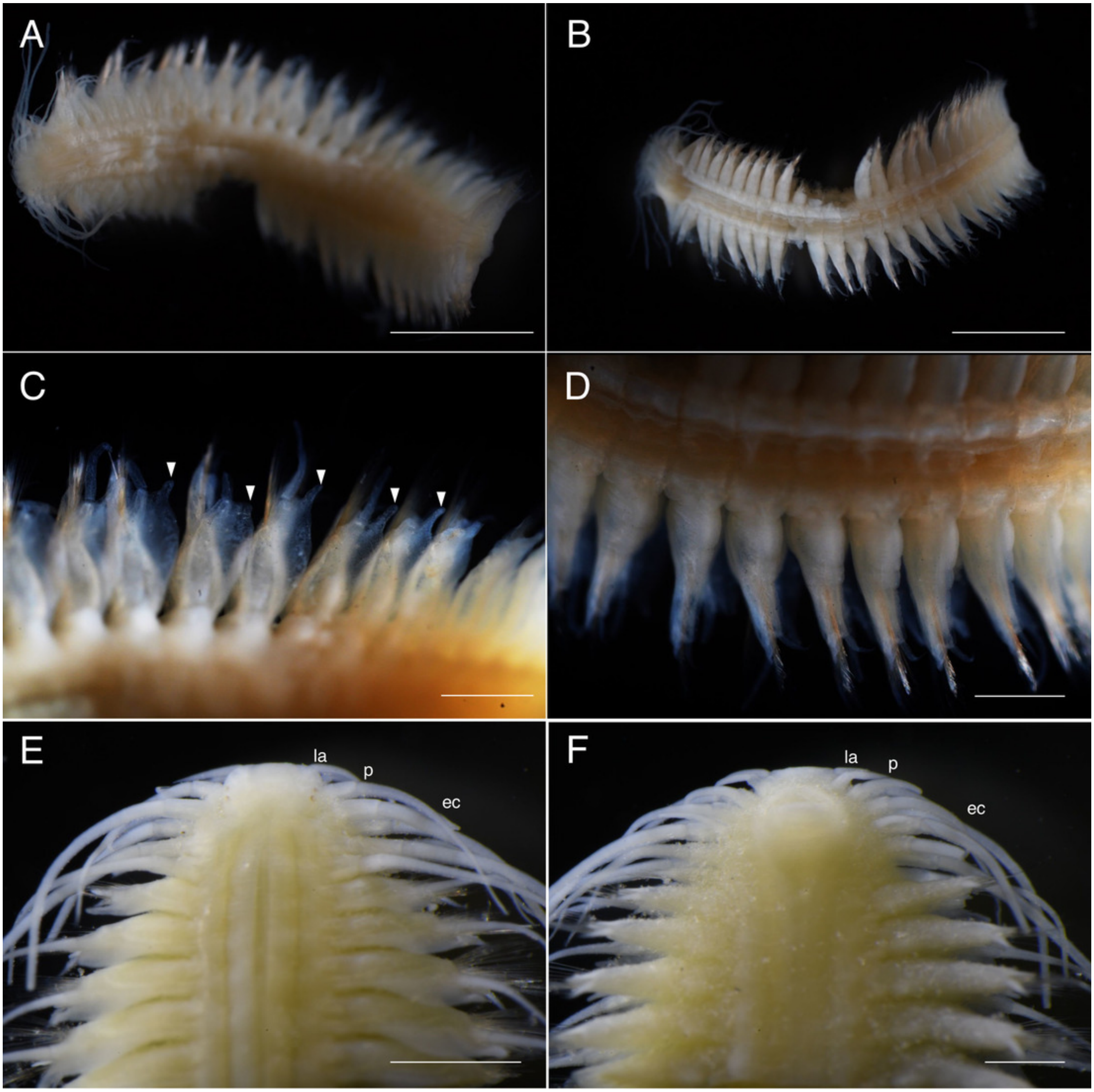
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Figure 8

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

A, parapodium of chaetiger 12, frontal view; B, notochaetae, chaetiger 12; C, upper side of neurochaetae, chaetiger 12; D, lower side of neurochaetae. Scale bars: A, 200 µm; B–D, 100 µm.

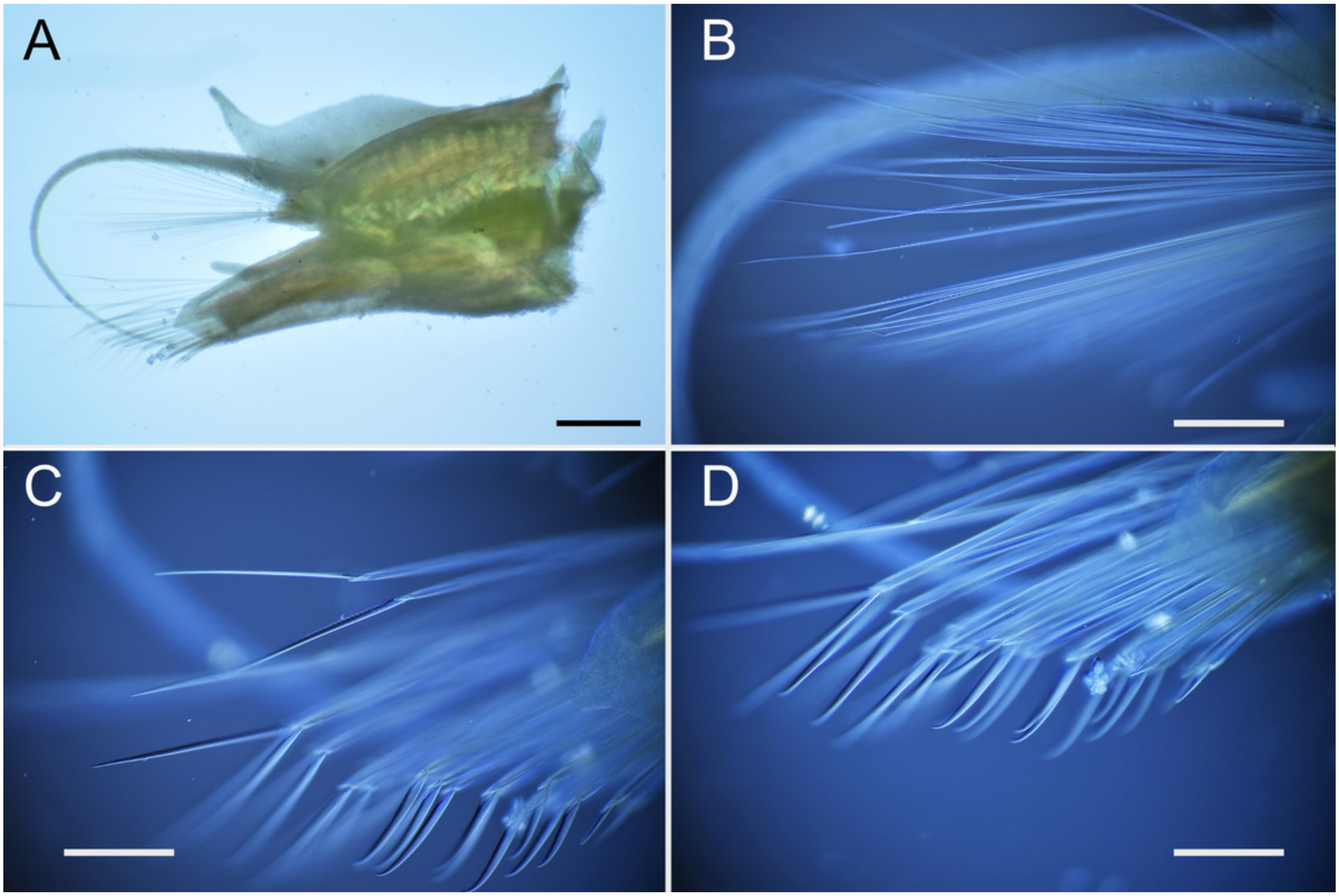

Figure 9

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.

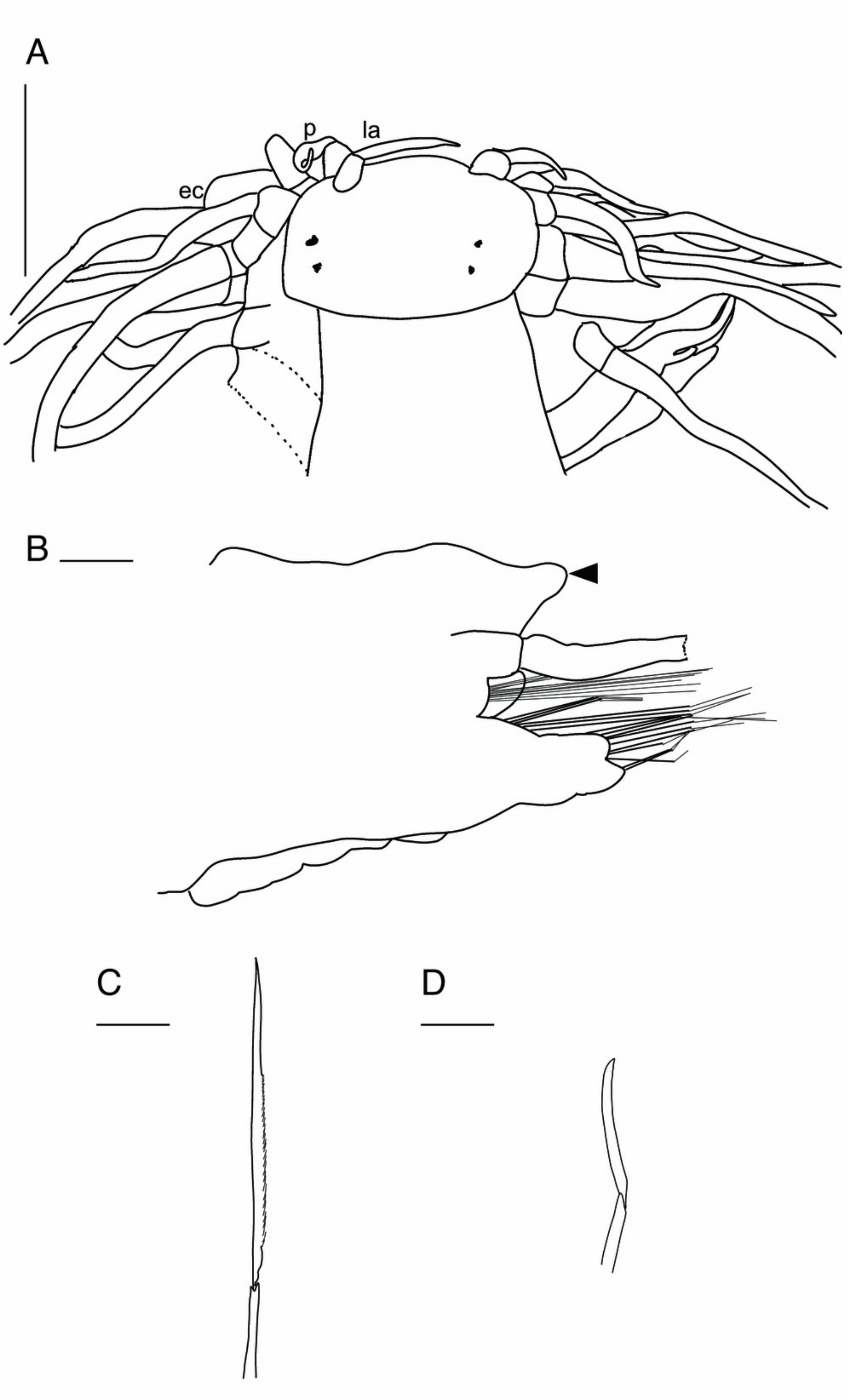
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Figure 10

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.

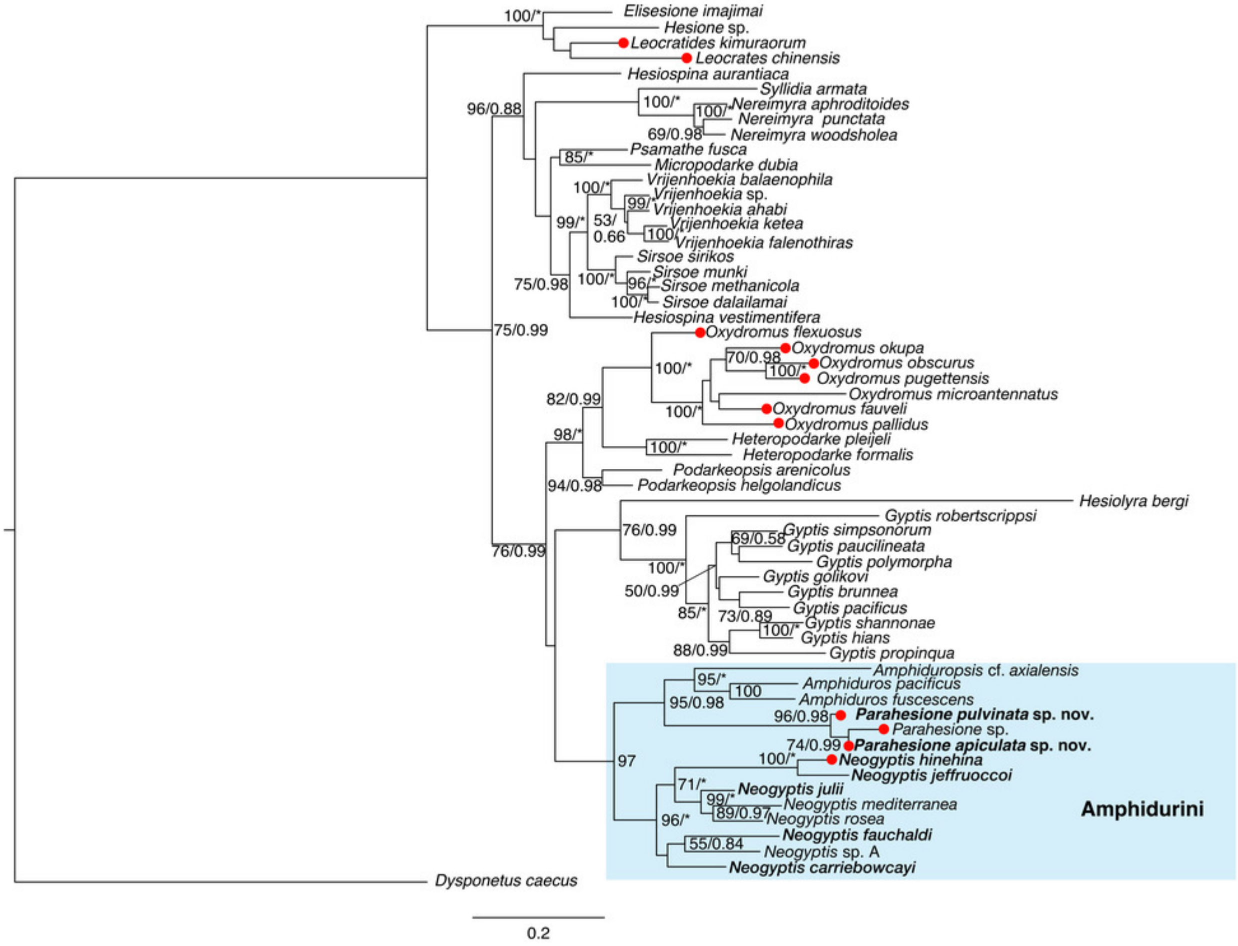

Table 1(on next page)

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 and the host taxa in case of symbionts.

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)

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)