New sea spider species (Pycnogonida: Austrodecidae) from a submarine cave in Japan

Running head: New submarine-cave sea spider

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http://zoobank.org/urn:lsid:zoobank.org:pub:39DB10BF-17ED-4EFC-BDC3-5CC6EF805C96

Abstract

We describe a new sea spider species, *Pantopipetta hosodai* sp. nov., based on one juvenile female collected from a submarine cave ("Akumanoyakata" Cave) at Shimojijima Island, Miyako Island Group, Ryukyu Islands, southwestern Japan. It was collected from the second slope zone of the cave, 80–100 m from the entrance, no light, low salinity, and with rocky substrate. This is the first record globally of a *Pantopipetta* species from a submarine cave and anchialine environment. *Pantopipetta hosodai* sp. nov. resembles *Pantopipetta auxiliata*, *Pantopipetta lenis*, and *Pantopipetta oculata* in having auxiliary claws but differs from them in having a palp with three short distal articles, lateral processes without dorsodistal tubercles, coxae 1 and 3 of legs 1–3 each with one long dorsal tubercle, and one dorsodistal tubercle bearing a seta on each femur. Features of the palp appear to delineate two species groups in *Pantopipetta*, i.e., (1) those having four small distal articles, and a small, basal palp article between the lateral cephalon process and longest palp article (8-articulate palp) and (2) those having three small distal articles, and lacking the small basal article (6-articulate palp), but further detail examination of described species is needed. We discuss the diagnostic characters separating *Pantopipetta* and *Austrodecus* and the generic affiliation of *Austrodecus aconae*. Few pycnogonids from marine or anchialine caves have been identified to species, and it is generally unknown whether cave-dwelling pycnogonids tend to be troglobites.

Key words: Akuma-no-Yakata; Chelicerata; palp; *Pantopipetta*; Pantopoda; Stiripasterida; anchialine

Introduction

Pycnogonida, or sea spiders, are almost exclusively free-living marine invertebrates. With more than 1300 named species (Appeltans et al., 2012; Bamber et al., 2023), pycnogonids occur in all oceans and range in depth from 0–7370 m (Arnaud & Bamber, 1987). Although most studies deal with individuals collected from open marine environments, a few have reported sea spiders from caves (e.g., Akoumianaki & Hughes, 2004; Bamber, 2008; Onorato & Belmonte, 2017; Alvarez & Ojeda, 2018; Gerovasileiou & Bianchi, 2021). Cave-dwelling sea spiders are generally not identified but just listed as Pycnogonida; exceptions are *Pycnogonum coninsulum* Bamber, 2008 from a submarine cave in Hong Kong and *Anoplodactylus batangensis* (Helfer, 1938) from an anchialine cave in Mexico (Bamber, 2008; Alvarez & Ojeda, 2018). Having not yet been collected from open marine environments, *Py. coninsulum* appears to be an endemic cave species (Bamber *et al*., 2008). *Anoplodactylus batangensis* is a cosmopolitan species also found outside the cave, suggesting that the individual in the cave may have been transported through passageways in the anchialine system (Alvarez & Ojeda, 2018).

There are many submarine caves around the Ryukyu Islands, southwestern Japan, but knowledge of their invertebrate faunas is limited. Recent surveys of the invertebrate fauna of "Akumanoyakata" submarine cave at Shimojijima Island have detected new or rare species among poriferans (e.g., Ise, 2019), crustaceans (e.g., Saito & Fujita, 2022), polychaetous annelids (e.g., Worsaae *et al*., 2021), brittle stars (e.g., Okanishi & Fujita, 2019), and bivalves (e.g., Mizuyama *et al*., 2022), but there have been no records of sea spiders to date.

This paper reports one sea spider collected from the completely dark, anchialine zone at 10–20 m depth in the Akumanoyakata Cave. With a slender, pipette-shaped proboscis having distal annulation, it belongs to the family Austrodecidae Stock, 1954. Although the specimen is likely a juvenile female and it is unknown how many articles there are in the adult oviger (one character distinguishing between the austrodecid genera: *Austrodecus* Hodgson, 1907 with six or fewer articles (absent in males of several species) and *Pantopipetta* Stock, 1963 with 10 articles and a terminal claw), we identified the specimen as *Pantopipetta* based on the very slender trunk without dorsomedian tubercles and the palp with three short distal articles (cf. Child, 1994; for detail, see the Discussion section). *Pantopipetta* pycnogonids are relatively rare (Hedgpeth & McCain, 1971) and generally found at considerable depths (Child, 1994), with the shallowest record at 66 m (cf. Hosoda & Kakui, 2020). This is the first record of *Pantopipetta* from a submarine cave or anchialine

environment, and the shallowest depth record at 10–20 m. The specimen can be distinguished from all congeners (16 species; Hosoda & Kakui, 2020), and we describe it here as a new species. Additionally, we discuss the taxonomic significance of the number of palp articles in *Pantopipetta* species and cave pycnogonids in general.

Materials and Methods

A pycnogonid was collected by SCUBA diving on 8 March 2021 in “Akumanoyakata” Cave, located on a reef slope at Shimojijima Island, Miyako Island Group, Ryukyu Island, southwestern Japan (26°51.896’N 128°14.732’E), with the entrance at about 35 m depth; see Osawa & Fujita (2019) for detailed information on the cave. From the second slope zone (Osawa & Fujita, 2019; 80–100 m from the entrance, 10–20 m depth, no light, less than 28‰ salinity, rocky substrate), mud deposited around cnidarians and poriferans on the cave wall was collected with a commercially made aquatic suction sampler (yabby pump). The individual was sorted from the mud sample and preserved in 99% ethanol. The methods used for dissection, preparation of slides, light microscopy, and drawing were as described by Kakui & Angsupanich (2012). Morphological terminology follows Child (1979), except that the term "article" is used instead of "segment" for all appendages (Hosoda & Kakui, 2020). Measurements were made axially (dorsally for the trunk and abdomen; laterally for the palp, proboscis, ocular tubercle, and legs) and are presented in millimeters. Measurements for congeners were obtained from original descriptions or measured from original illustrations. Trunk length was measured from the palp insertion to the base of the abdomen, and trunk width as the width of the segment at the narrowest portion of the trunk. The specimen studied was deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo. To obtain information on male genital openings in *Pantopipetta*, we observed the type series of *Pantopipetta lenis* Hosoda & Kakui, 2020 (ICHUM6038, 6039).

Results

Systematics

Family Austrodecidae Stock, 1954

Genus *Pantopipetta* Stock, 1963

Pantopipetta hosodai sp. nov.

[New Japanese name: Dokutsu-suikuchi-umigumo]

(Figures 1, 2, 3A–D)

Diagnosis (juvenile female)

Trunk segments 2 and 4 short (length/width ratios 1.3 and 2.6); ocular tubercle with swollen tip; lateral processes without dorsodistal tubercle; palp with three short distal articles; coxa 1 of legs 1–3 with long dorsal tubercle (longer than coxa-1 width); coxa 3 of legs 1–3 with long dorsal tubercle (as long as coxa-3 width); all femora with short dorsodistal tubercle (length 0.5 times femur width) bearing seta; auxiliary claws about 0.5 claw length.

Etymology

The specific name is a noun in the genitive case, honoring Yushi Hosoda, who has contributed to the taxonomy of Japanese pycnogonids.

Type material

Holotype. Juvenile female, ICHUM8407; three slides and one vial; second-slope zone in Akumanoyakata Cave (26°51.896'N 128°14.732'E), 10–20 m depth, Shimojijima Island, Miyako Island Group, Ryukyu Islands, Japan, northwestern Pacific Ocean, mud; collected on 8 March 2021 by Yoshihisa Fujita.

Description of holotype (juvenile female)

Trunk (Figures 1A, B, 2A–C) fully segmented, without dorsomedian tubercles; segments 2 and 4 short; segment 3 deformed, flattened by accidentally pinching with forceps (Figure 2A, gray-shaded area with asterisk). Lateral processes long, separated by about their basal diameter (trunk segments 1–3) or about twice their basal diameter (segments 3 and 4), without dorsodistal tubercle. Ocular tubercle (Figure 2B, C) tall, erect, with swollen tip bearing four tiny eyes; tiny distal process present. Proboscis pipette-like, annulated. Abdomen longer than trunk segment 4, with pair of subposterior setae (one broken).

Palp (Figures 1C, 2D, E) 6-articulate. Article 1 longest, with two spines. Article 3 with one middle and two strong distal setae; two strong curved spines, one subdistal and one distal. Articles 4–6 (= 3 short distal articles) with three, three, and four distal setae.

Oviger (Figures 1D, 2F) with 1 article, naked.

Legs 1–3 (Figures 2G–I, 3A–C) slender. Coxa 1 with long dorsal tubercle (longer than coxa-1 width). Coxa 2 with tiny dorsal projection and tiny ventro-subdistal genital opening; subdistal seta on legs 1 and 2. Coxa 3 with long dorsal tubercle (longer than coxa-3

width) and ventrodistal seta. Femur with two distal setae and short dorsodistal tubercle (length half femur width) bearing seta. Tibia 1 with two (legs 1 and 2) or one (leg 3) dorsodistal and one ventro-subdistal setae, and dorsodistal robust seta. Tibia 2 with six (leg 1) or four (legs 2 and 3) ventral, two (legs 1 and 3) or one (leg 2) anterior, two (legs 1 and 3) or one (leg 2) posterior setae and mid-dorsal robust seta. Tarsus with two (legs 1 and 2) or one (leg 3) ventral, two (legs 1 and 2) or one (leg 3) anterior, and two (leg 1) or one (legs 2 and 3) posterior setae. Propodus with one dorsodistal and six (legs 1 and 2) or three (leg 3) ventral setae, and two auxiliary claws; with one anterior and one posterior setae on leg 1; auxiliary claws similar in size, about half claw length. Cement gland opening not observed.

Leg 4 (Figures 2J, 3D) slender, much shorter than legs 1–3. Coxa 1 with short dorsal tubercle (shorter than half coxa-1 width). Coxa 2 similar to those in legs 1–3; tiny ventro-subdistal genital opening present. Coxa 3 with short dorsal tubercle (shorter than half coxa-3 width) and ventrodistal seta. Femur with distal seta and short dorsodistal tubercle (length half femur width) bearing seta. Tibia 1 similar to those in legs 1 and 2. Tibia 2 with three ventral, one anterior and one posterior setae, and mid-dorsal robust seta. Tarsus with one ventral and one anterior setae. Propodus with one dorsodistal and two ventral setae, and two auxiliary claws; auxiliary claws similar in size, about half claw length. Cement gland opening not observed.

Measurements. Trunk length 0.46; width across second lateral processes 0.30; proboscis length 0.66; ocular tubercle length 0.33; abdomen length 0.22; length/width of trunk segments 2 and 4, 0.11/0.08, 0.12/0.05; length of palp articles 1–6, 0.30, 0.05, 0.20, 0.02, 0.04, 0.01 (0.62 in total); length of leg-1 articles (from coxa 1; including claw), 0.07, 0.18, 0.08, 0.30, 0.32, 0.33, 0.04, 0.16, 0.08 (1.55 in total); length of leg-2 articles (ditto), 0.08, 0.15, 0.07, 0.28, 0.27, 0.31, 0.03, 0.16, 0.08 (1.41 in total); length of leg-3 articles (ditto), 0.06, 0.14, 0.07, 0.26, 0.24, 0.28, 0.03, 0.17, 0.08 (1.33 in total); length of leg-4 articles (ditto), 0.05, 0.11, 0,05, 0.21, 0.18, 0.24, 0.03, 0.15, 0.07 (1.09 in total).

Pantopipetta lenis Hosoda & Kakui, 2020

(Figure 3E)

Material examined

Holotype, male, ICHUM6038. Paratype, male, ICHUM6039.

Supplementary information on male genital openings

Coxa 2 of legs 1–3 without genital opening. Coxa 2 of leg 4 with ventro-subdistal genital opening (Figure 3E).

Discussion

Staging and sexing

Our specimen has the leg 4 much shorter than the leg 3, with a length of about 0.82 times of that of the leg 3. Although Austrodecidae lacks information on ontogenetic development after the postlarval stage that bears unarticulated legs 4, the above condition was reported in non-adult individuals in several other families (e.g., Okuda, 1940; Brenneis et al., 2011; Miyazaki & Hoshino, 2019). The oviger of our specimen comprises one naked article. Uniarticulate ovigers have been reported in three austrodecid species, namely, *Austrodecus* (*Microdecus*) *fryi* Child, 1994, *Austrodecus palauense* Child, 1983, and *Austrodecus varum* Child, 1994, but the latter two were species described based on juveniles (Child, 1983, 1994). The oviger of *A.* (*M.*) *fryi* bears setae, not naked. Naked uniarticulate ovigers were reported in non-adults of other families (e.g., Okuda, 1940; Brenneis et al., 2011; Miyazaki & Hoshino, 2019).

We observed genital openings on the coxa 2 of legs 1–4 of our specimen, but they appeared to be smaller than those reported in confamilial adults (Loman, 1908; Miyazaki, 2004), suggesting that they may not be fully formed. In Austrodecidae, genital openings were found on legs 1–4 in females (e.g., Loman, 1908; Turpaeva, 1955; Miyazaki, 2004) and only on leg 4 in *Austrodecus* males (Miyazaki, 2004). Male genital openings had not been described in *Pantopipetta* until now. Here we showed that males of *Pantopipetta lenis* bear genital openings only on the coxa 2 of leg 4 as do *Austrodecus* males. Cement gland openings were not observed in our specimen.

Given the above, we concluded that our specimen is a juvenile female having immature leg 4 and oviger.

Generic affiliation

We identified our specimen as a member of *Pantopipetta* mainly based on that its palp has three short distal articles. All known *Pantopipetta* species have three or four short distal articles on the palp whereas all *Austrodecus* species have one or two, except *Austrodecus*

aconae (Hedgpeth & McCain, 1971) having three short distal articles.

Austrodecus aconae was originally described as a member of *Pantopipetta*. Hedgpeth & McCain (1971) speculated their specimens that have four- or five-articulate oviger (but see below) may be immature and described them as a member of *Pantopipetta*. Hedgpeth & McCain (1971: p. 218) stated that "In all species of *Austrodecus* so far described the terminal joint [= terminal short distal article] of the palp is set at an angle on the penultimate joint [= penultimate short distal article]; this feature is not found in the species of *Pantopipetta*," which appears to be the major reason why they put their species in *Pantopipetta*. It should be noted that, in the palp of *Pantopipetta*, the second short distal article is set at an angle on the first short distal article but not so between the terminal and penultimate short distal articles (e.g., Figure 2D, E; Hedgpeth & McCain, 1971, fig. 6f; Child, 1994, fig. 15E).

Stock (1991: p. 270) wrote "*A.* [=*Austrodecus*] *aconae* (Hedgpeth & McCain, 1971), originally described as a species of *Pantopipetta*" and transferred the species into *Austrodecus* without providing any reason. Child (1994) followed this view and put the species in *Austrodecus*. The author observed its holotype and paratypes (two females and five males) and found that females bear four-articulate ovigers but males lack any trace of ovigers; a five-articulate oviger was not observed (note: Hedgpeth & McCain (1971) observed four females). Male austrodecids lacking ovigers have been reported only in two *Austrodecus* species, *A.* (*Tubidecus*) *excelsum* Stock, 1991 and *A.* (*T.*) *latum* Stock, 1991, but they have palps with two short distal articles (Stock, 1991).

The generic affiliation of *Austrodecus aconae* can vary depending on whether researchers emphasize the number of short distal articles on the palp or that of the oviger. In this study, although we refrain from returning *A. aconae* into *Pantopipetta*, we deemed the number of short distal articles on the palp to be one of the diagnostic characters to distinguish *Austrodecus* (one or two) and *Pantopipetta* (three or four) and identified our specimen as a member of *Pantopipetta*.

Morphological comparisons

Because we concluded that our specimen was a juvenile female with immature oviger and leg 4, we did not use the character states for these two appendages to distinguish our species from congeners. In having auxiliary claws, *Pantopipetta hosodai* sp. nov. resembles *Pantopipetta auxiliata* Stock, 1968 from off the eastern coast of South Africa (68–69 m

depth), *P. lenis* from Japan (140.7–151.5 m depth), and *Pantopipetta oculata* Stock, 1968 from the Andaman Islands (66 m depth). It differs from the latter three species (character state in parentheses) in having the palp with three short distal articles (four) and in lacking a short palp article articulated with the cephalon (article present) (see the following section). In addition, *P. hosodai* sp. nov. differs from *P. auxiliata* in having lateral processes without dorsodistal tubercles (with one tall, knobby spur in *P. auxiliata*), coxa 1 of legs 1–3 with one dorsal tubercle (two in *P. auxiliata*), and the auxiliary claw on the legs about 1/2 claw length (about 1/3 in *P. auxiliata*); from *P. lenis* in having coxa 1 of legs 1–3 with one long dorsal tubercle (no tubercles in *P. lenis*), a long dorsal tubercle on coxa 3 of legs 1–3, as long as coxa-3 width (short, half coxa-3 width in *P. lenis*), and the femur of the legs with one short dorsodistal tubercle bearing a seta (no tubercle in *P. lenis*); and from *P. oculata* in having coxa 1 of legs 1–3 with one dorsal tubercle (four in *P. oculata*), the dorsodistal tubercle on the femur of the legs short, half femur width (long, longer than femur width in *P. oculata*), and the auxiliary claw on the legs about 1/2 claw length (about 1/3 in *P. oculata*).

Pantopipetta hosodai sp. nov. differs from *A. aconae* in having lateral processes without dorsodistal tubercles (with one short tubercle in *A. aconae*), coxa 1 of legs 1–3 with one dorsal tubercle (two in *A. aconae*), and auxiliary claws (no auxiliary claws in *A. aconae*). It also differs from three *Austrodecus* species having uniarticulate ovigers (*A.* (*M.*) *fryi*, *A. palauense*, and *A. varum*) by the number of short distal articles on the palp.

Palp base and number of palp articles

Hosoda & Kakui (2020) found that the palp base (the short article-like structure proximal to the longest palp article) is actually the first palp article in *P. lenis*. In *P. hosodai* sp. nov., however, the palp base is not articulated with the cephalon, but instead is a lateral process of the cephalon (Figure 1C); the long article (the first palp article in *P. hosodai* sp. nov.) that appears homologous to the second palp article in *P. lenis* articulates with the lateral process. The condition in *P. hosodai* sp. nov. is equivalent to Child's (1994: p. 82) description, "no suture or segmentation lines at all around their [= palp bases'] root".

The connection between the lateral process of the cephalon and the palp has not generally been described in detail, but a short palp article distal to the process has been illustrated in the original descriptions or re-descriptions for eight species: *P. auxiliata* (Stock, 1968, fig. 8b); "*Pantopipetta brevicauda* Stock, 1963" in Turpaeva (1990, fig. 6-3; Child [1982] synonymized this species with *Pantopipetta longituberculata* Turpaeva, 1955);

Pantopipetta brevipilata Turpaeva, 1990 (Turpaeva, 1990, fig. 8-2); *Pantopipetta capensis* (Barnard, 1946) in Stock (1963, fig. 8a); *Pantopipetta gracilis* Turpaeva, 1993 (Turpaeva, 1993, fig. 4-1); *P. oculata* (Stock, 1968, fig. 7b); *Pantopipetta weberi* (Loman, 1904) in Loman (1908, fig. 14-194 and 14-197); and *P. lenis* (Hosoda & Kakui, 2020, fig. 3B, C). All of these have a palp bearing four short distal articles. A short basal article has so far not been illustrated in the descriptions of *Pantopipetta* species that have a palp with three short distal articles (*Pantopipetta armata* Arnaud & Child, 1988; *Pantopipetta armoricana* Stock, 1978; *Pantopipetta bilobata* Arnaud & Child, 1988; *Pantopipetta clavata* Stock, 1994; and *P. hosodai* sp. nov.). This suggests that *Pantopipetta* species comprises two species groups: (i) species with an eight-articulate palp having a short article distal to the lateral process of the cephalon and four short distal articles and (ii) species with a six-articulate palp lacking a short article distal to the process and having three short distal articles. To confirm this hypothesis, the connection between the lateral process of the cephalon and the palp needs to be reexamined in known species.

Cave-dwelling pycnogonids

At least 15 pycnogonid species have been recorded from marine or anchialine caves in the Mediterranean (e.g., Gerovasileiou & Bianchi, 2021), Caribbean (Alvarez & Ojeda, 2018), or northwestern Pacific (Bamber, 2008; this study). Among these species, only three were identified to the species level, each collected from a different environment. *Pycnogonum coninsulum* inhabited a submarine cave at 33‰ salinity, with no information on light provided (Bamber, 2008; Morton et al., 2008). *Anoplodactylus batangensis* came from among vegetation in the illuminated anchialine pool of a cenote (1.63 salinity; Alvarez & Ojeda, 2018). *Pantopipetta hosodai* sp. nov. came from a completely dark, anchialine environment inside a submarine cave (less than 28‰ salinity; cf. Osawa & Fujita, 2019).

It is unknown whether pycnogonids inhabiting caves are troglobites (obligate cave-dwelling species). Two species, *Py. coninsulum* and *P. hosodai* sp. nov., were reported based on a single individual each from two different caves, and it is not known whether they also occur outside the caves. Faunal surveys have been conducted intermittently in Akumanoyakata Cave across a span of 10 years but our *P. hosodai* sp. nov. specimen is the first pycnogonid found. This suggests a very low abundance of pycnogonids in caves, or at least in Akumanoyakata Cave. More comprehensive sampling in both inside and outside caves is needed to ascertain the degree of their dependence on caves.

Acknowledgments

We thank Masaru Mizuyama (National Institution of Advanced Industrial Science and Technology), Kyo Yunokawa (Ie-jima Diving Service), Atsuo Shioiri (Diving Service "COLORCODE"), Hiroki Ichi (Irabu-jima Fishermen's Association), Hirotaka Ichi, and Go Tomitani (Diving Service "Marines Pro Miyako"), Naoki Shirakawa (Diving Service "DOLPHIN KICK") for assistance in scuba sampling in the submarine cave; and Matthew H. Dick (Hokkaido University) for reviewing the manuscript and editing our English. This study was supported by the Japan Society for the Promotion of Science (JSPS) under KAKENHI grant JP20H03313.

Author contributions

KK conceived and designed the study, and made morphological observations; YF collected the pycnogonid; KK and YF wrote the manuscript, and read and approved the final draft.

Financial support

This study was supported in part by a KAKENHI grant (grant number JP20H03313) from the Japan Society for the Promotion of Science (JSPS).

Conflict of interest

The authors declare no conflict of interests.

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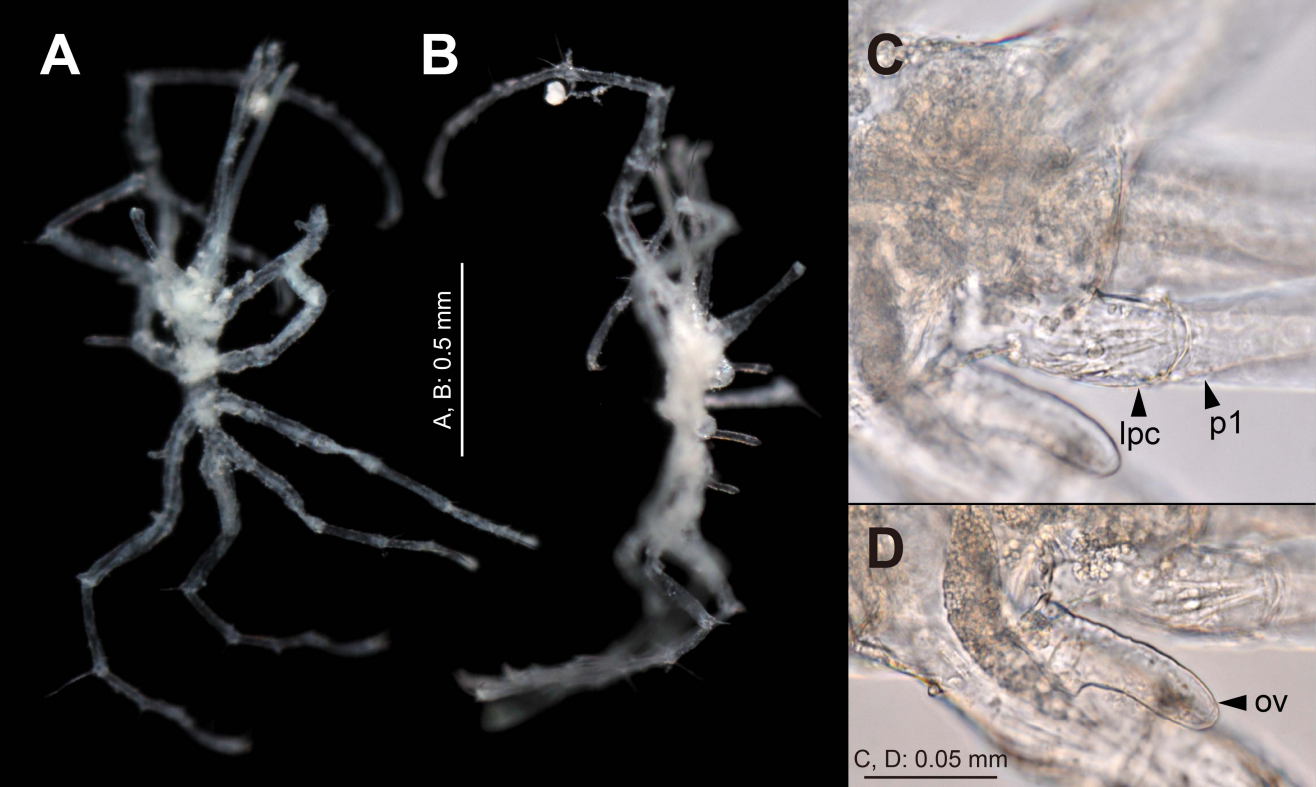
Figure legends

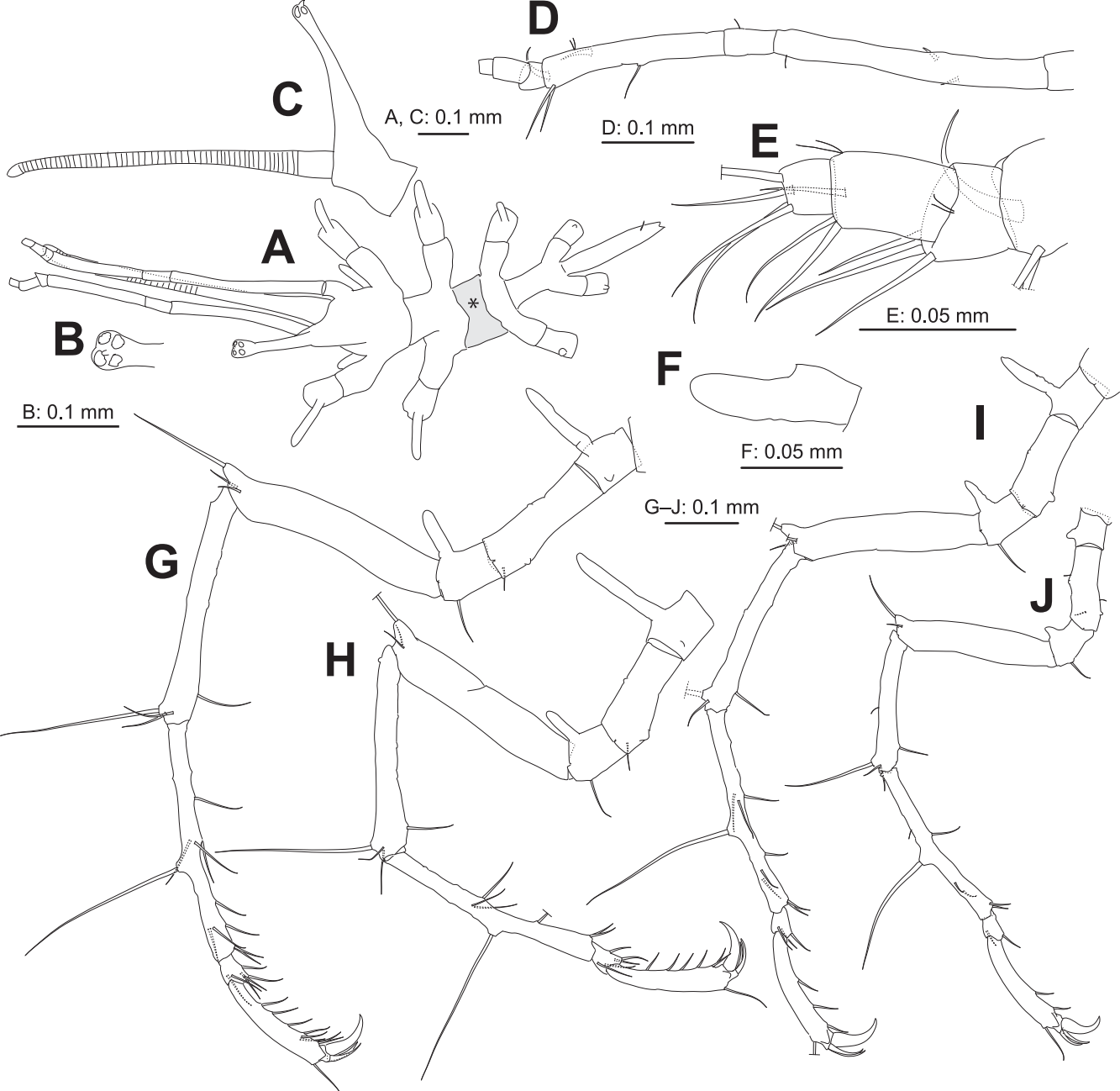
Fig. 1. *Pantopipetta hosodai* sp. nov., holotype, ICHUM8407, juvenile, ethanol-fixed specimen. A, habitus, dorsal view; B, habitus, left view; C, cephalon, right view; D, right oviger. Abbreviations: lpc, lateral process of cephalon; ov, oviger; p1, palp article 1.

Fig. 2. *Pantopipetta hosodai* sp. nov., holotype, ICHUM8407, juvenile. A, habitus, dorsal view (gray-shaded area marked with asterisk indicates damaged area flattened by accidentally

pinching with forceps); B, distal tip of ocular tubercle, dorsal view; C, cephalon, left view (lateral process of cephalon and leg 1 omitted); D, left palp (ornamentation on short distal articles omitted); E, distal portion of left palp; F, right oviger; G–J, left legs 1–4.

Fig. 3. Genital openings of *Pantopipetta*. A–D, *P. hosodai* sp. nov., holotype, juvenile female; E, *P. lenis* Hosoda & Kakui, 2020, holotype, male. A–E, left legs 1, 2, 3, 4, and 4, respectively. c2, c3, coxae 2 and 3. Arrowheads, genital opening.


A
B
A, B: 0.5 mm
C
lpc
p1
D
ov
C, D: 0.05 mm


A
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I
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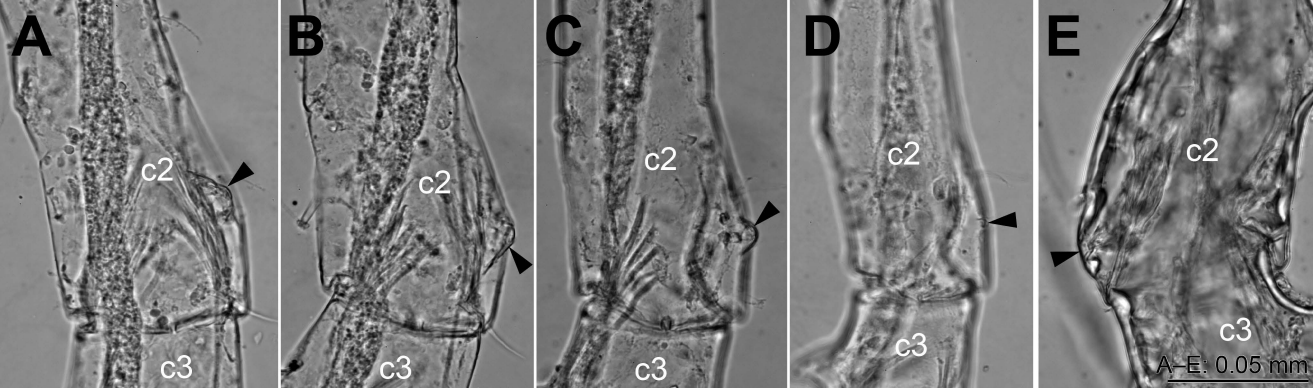
A, C: 0.1 mm
D: 0.1 mm
E: 0.05 mm
B: 0.1 mm
F: 0.05 mm
G–J: 0.1 mm


A
c2
c3
B
c2
c3
C
c2
c3
D
c2
c3
E
c2
c3
A–E: 0.05 mm