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1	New sea spider species (Pycnogonida: Austrodecidae) from a submarine cave in Japan
2	
3	Running head: New submarine-cave sea spider
4	
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20 Abstract

- 21 We describe a new sea spider species, *Pantopipetta hosodai* sp. nov., based on one juvenile
- 22 female collected from a submarine cave ("Akumanoyakata" Cave) at Shimojijima Island,
- 23 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,
- 27 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
- 29 tubercles, coxae 1 and 3 of legs 1–3 each with one long dorsal tubercle, and one dorsodistal
- 30 tubercle bearing a seta on each femur. Features of the palp appear to delineate two species
- 31 groups in *Pantopipetta*, i.e., (1) those having four small distal articles, and a small, basal palp
- 32 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),
- 34 but further detail examination of described species is needed. We discuss the diagnostic
- 35 characters separating *Pantopipetta* and *Austrodecus* and the generic affiliation of *Austrodecus*
- 36 *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.
- 38
- Key words: Akuma-no-Yakata; Chelicerata; palp; *Pantopipetta*; Pantopoda; Stiripasterida;
 anchialine

41 Introduction

42 Pycnogonida, or sea spiders, are almost exclusively free-living marine invertebrates. With

- 43 more than 1300 named species (Appeltans et al., 2012; Bamber et al., 2023), pycnogonids
- 44 occur in all oceans and range in depth from 0–7370 m (Arnaud & Bamber, 1987). Although

45 most studies deal with individuals collected from open marine environments, a few have

46 reported sea spiders from caves (e.g., Akoumianaki & Hughes, 2004; Bamber, 2008; Onorato

47 & Belmonte, 2017; Alvarez & Ojeda, 2018; Gerovasileiou & Bianchi, 2021). Cave-dwelling

48 sea spiders are generally not identified but just listed as Pycnogonida; exceptions are

49 Pycnogonum coninsulum Bamber, 2008 from a submarine cave in Hong Kong and

50 Anoplodactylus batangensis (Helfer, 1938) from an anchialine cave in Mexico (Bamber,

51 2008; Alvarez & Ojeda, 2018). Having not yet been collected from open marine

52 environments, *Py. coninsulum* appears to be an endemic cave species (Bamber *et al.*, 2008).

53 Anoplodactylus batangensis is a cosmopolitan species also found outside the cave, suggesting

54 that the individual in the cave may have been transported through passageways in the

55 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.

62 This paper reports one sea spider collected from the completely dark, anchialine 63 zone at 10–20 m depth in the Akumanoyakata Cave. With a slender, pipette-shaped proboscis 64 having distal annulation, it belongs to the family Austrodecidae Stock, 1954. Although the 65 specimen is likely a juvenile female and it is unknown how many articles there are in the 66 adult oviger (one character distinguishing between the austrodecid genera: Austrodecus 67 Hodgson, 1907 with six or fewer articles (absent in males of several species) and 68 Pantopipetta Stock, 1963 with 10 articles and a terminal claw), we identified the specimen as 69 Pantopipetta based on the very slender trunk without dorsomedian tubercles and the palp 70 with three short distal articles (cf. Child, 1994; for detail, see the Discussion section). 71 Pantopipetta pycnogonids are relatively rare (Hedgpeth & McCain, 1971) and generally 72 found at considerable depths (Child, 1994), with the shallowest record at 66 m (cf. Hosoda & 73 Kakui, 2020). This is the first record of Pantopipetta from a submarine cave or anchialine

- real 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
- 77 *Pantopipetta* species and cave pycnogonids in general.
- 78

79 Materials and Methods

- 80 A pycnogonid was collected by SCUBA diving on 8 March 2021 in "Akumanoyakata" Cave,
- 81 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
- 83 Osawa & Fujita (2019) for detailed information on the cave. From the second slope zone
- 84 (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
- 86 was collected with a commercially made aquatic suction sampler (yabby pump). The
- 87 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
- 89 Kakui & Angsupanich (2012). Morphological terminology follows Child (1979), except that
- 90 the term "article" is used instead of "segment" for all appendages (Hosoda & Kakui, 2020).
- 91 Measurements were made axially (dorsally for the trunk and abdomen; laterally for the palp,
- 92 proboscis, ocular tubercle, and legs) and are presented in millimeters. Measurements for
- 93 congeners were obtained from original descriptions or measured from original illustrations.
- 94 Trunk length was measured from the palp insertion to the base of the abdomen, and trunk
- 95 width as the width of the segment at the narrowest portion of the trunk. The specimen studied
- 96 was deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM),
- 97 Sapporo. To obtain information on male genital openings in *Pantopipetta*, we observed the
- 98 type series of *Pantopipetta lenis* Hosoda & Kakui, 2020 (ICHUM6038, 6039).
- 99

100 **Results**

- 101 Systematics
- 102 Family Austrodecidae Stock, 1954
- 103 Genus Pantopipetta Stock, 1963
- 104 Pantopipetta hosodai sp. nov.
- 105 [New Japanese name: Dokutsu-suikuchi-umigumo]
- 106 (Figures 1, 2, 3A–D)

107

- 108 Diagnosis (juvenile female)
- 109 Trunk segments 2 and 4 short (length/width ratios 1.3 and 2.6); ocular tubercle with swollen
- 110 tip; lateral processes without dorsodistal tubercle; palp with three short distal articles; coxa 1
- 111 of legs 1–3 with long dorsal tubercle (longer than coxa-1 width); coxa 3 of legs 1–3 with long
- 112 dorsal tubercle (as long as coxa-3 width); all femora with short dorsodistal tubercle (length
- 113 0.5 times femur width) bearing seta; auxiliary claws about 0.5 claw length.
- 114
- 115 *Etymology*
- 116 The specific name is a noun in the genitive case, honoring Yushi Hosoda, who has
- 117 contributed to the taxonomy of Japanese pycnogonids.
- 118
- 119 *Type material*
- 120 Holotype. Juvenile female, ICHUM8407; three slides and one vial; second-slope zone in
- 121 Akumanoyakata Cave (26°51.896'N 128°14.732'E), 10–20 m depth, Shimojijima Island,
- 122 Miyako Island Group, Ryukyu Islands, Japan, northwestern Pacific Ocean, mud; collected on
- 123 8 March 2021 by Yoshihisa Fujita.
- 124

125 Description of holotype (juvenile female)

126 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,
- 128 gray-shaded area with asterisk). Lateral processes long, separated by about their basal
- 129 diameter (trunk segments 1–3) or about twice their basal diameter (segments 3 and 4),
- 130 without dorsodistal tubercle. Ocular tubercle (Figure 2B, C) tall, erect, with swollen tip
- 131 bearing four tiny eyes; tiny distal process present. Proboscis pipette-like, annulated. Abdomen
- 132 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
- 135 distal. Articles 4–6 (= 3 short distal articles) with three, three, and four distal setae.
- 136 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

140 width) and ventrodistal seta. Femur with two distal setae and short dorsodistal tubercle 141 (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) 142 or four (legs 2 and 3) ventral, two (legs 1 and 3) or one (leg 2) anterior, two (legs 1 and 3) or 143 144one (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) 145 posterior setae. Propodus with one dorsodistal and six (legs 1 and 2) or three (leg 3) ventral 146 147 setae, and two auxiliary claws; with one anterior and one posterior setae on leg 1; auxiliary 148 claws similar in size, about half claw length. Cement gland opening not observed.

149 Leg 4 (Figures 2J, 3D) slender, much shorter than legs 1–3. Coxa 1 with short dorsal 150 tubercle (shorter than half coxa-1 width). Coxa 2 similar to those in legs 1-3; tiny ventrosubdistal genital opening present. Coxa 3 with short dorsal tubercle (shorter than half coxa-3 151 152 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, 153 154 one anterior and one posterior setae, and mid-dorsal robust seta. Tarsus with one ventral and 155 one anterior setae. Propodus with one dorsodistal and two ventral setae, and two auxiliary 156 claws; auxiliary claws similar in size, about half claw length. Cement gland opening not observed. 157

158 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 159 160 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, 161 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), 162 163 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 164 (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-4 165 articles (ditto), 0.05, 0.11, 0,05, 0.21, 0.18, 0.24, 0.03, 0.15, 0.07 (1.09 in total).

- 166
- 167
- 168 Pantopipetta lenis Hosoda & Kakui, 2020
- 169 (Figure 3E)
- 170
- 171 Material examined
- 172 Holotype, male, ICHUM6038. Paratype, male, ICHUM6039.

173

174 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).

- 177
- 178

179 **Discussion**

180 Staging and sexing

181 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 182 183 the postlarval stage that bears unarticulated legs 4, the above condition was reported in nonadult individuals in several other families (e.g., Okuda, 1940; Brenneis et al., 2011; Miyazaki 184 185 & Hoshino, 2019). The oviger of our specimen comprises one naked article. Uniarticulate ovigers have been reported in three austrodecid species, namely, Austrodecus (Microdecus) 186 187 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 188 189 of A. (M.) fryi bears setae, not naked. Naked uniarticulate ovigers were reported in non-adults 190 of other families (e.g., Okuda, 1940; Brenneis et al., 2011; Miyazaki & Hoshino, 2019).

191 We observed genital openings on the coxa 2 of legs 1–4 of our specimen, but they 192 appeared to be smaller than those reported in confamilial adults (Loman, 1908; Miyazaki, 193 2004), suggesting that they may not be fully formed. In Austrodecidae, genital openings were 194 found on legs 1-4 in females (e.g., Loman, 1908; Turpaeva, 1955; Miyazaki, 2004) and only 195 on leg 4 in Austrodecus males (Miyazaki, 2004). Male genital openings had not been 196 described in *Pantopipetta* until now. Here we showed that males of *Pantopipetta lenis* bear 197 genital openings only on the coxa 2 of leg 4 as do Austrodecus males. Cement gland openings 198 were not observed in our specimen.

199 Given the above, we concluded that our specimen is a juvenile female having200 immature leg 4 and oviger.

201

202 Generic affiliation

203 We identified our specimen as a member of *Pantopipetta* mainly based on that its palp has

- 204 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

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

207 Austrodecus aconae was originally described as a member of Pantopipetta. 208 Hedgpeth & McCain (1971) speculated their specimens that have four- or five-articulate 209 oviger (but see below) may be immature and described them as a member of Pantopipetta. 210 Hedgpeth & McCain (1971: p. 218) stated that "In all species of Austrodecus so far described 211 the terminal joint [= terminal short distal article] of the palp is set at an angle on the 212 penultimate joint [= penultimate short distal article]; this feature is not found in the species of 213 *Pantopipetta*," which appears to be the major reason why they put their species in 214 Pantopipetta. It should be noted that, in the palp of Pantopipetta, the second short distal 215 article is set at an angle on the first short distal article but not so between the terminal and 216 penultimate short distal articles (e.g., Figure 2D, E; Hedgpeth & McCain, 1971, fig. 6f; Child, 217 1994, fig. 15E).

218 Stock (1991: p. 270) wrote "A. [=Austrodecus] aconae (Hedgpeth & McCain, 1971), 219 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 220 221 species in Austrodecus. The author observed its holotype and paratypes (two females and five 222 males) and found that females bear four-articulate ovigers but males lack any trace of 223 ovigers; a five-articulate oviger was not observed (note: Hedgpeth & McCain (1971) 224 observed four females). Male austrodecids lacking ovigers have been reported only in two 225 Austrodecus species, A. (Tubidecus) excelsum Stock, 1991 and A. (T.) latum Stock, 1991, but 226 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*.

233

234 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

237 from congeners. In having auxiliary claws, Pantopipetta hosodai sp. nov. resembles

238 Pantopipetta auxiliata Stock, 1968 from off the eastern coast of South Africa (68-69 m

239 depth), P. lenis from Japan (140.7–151.5 m depth), and Pantopipetta oculata Stock, 1968 240 from the Andaman Islands (66 m depth). It differs from the latter three species (character 241 state in parentheses) in having the palp with three short distal articles (four) and in lacking a 242 short palp article articulated with the cephalon (article present) (see the following section). In 243 addition, P. hosodai sp. nov. differs from P. auxiliata in having lateral processes without 244 dorsodistal tubercles (with one tall, knobby spur in *P. auxiliata*), coxa 1 of legs 1–3 with one 245 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 246 247 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 248 249 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 250251 the femur of the legs short, half femur width (long, longer than femur width in *P. oculata*), 252 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.

258

259 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 272 273 (Barnard, 1946) in Stock (1963, fig. 8a); Pantopipetta gracilis Turpaeva, 1993 (Turpaeva, 2741993, fig. 4-1); P. oculata (Stock, 1968, fig. 7b); Pantopipetta weberi (Loman, 1904) in 275Loman (1908, fig. 14-194 and 14-197); and P. lenis (Hosoda & Kakui, 2020, fig. 3B, C). All 276 of these have a palp bearing four short distal articles. A short basal article has so far not been 277 illustrated in the descriptions of *Pantopipetta* species that have a palp with three short distal 278 articles (Pantopipetta armata Arnaud & Child, 1988; Pantopipetta armoricana Stock, 1978; Pantopipetta bilobata Arnaud & Child, 1988; Pantopipetta clavata Stock, 1994; and P. 279 280 hosodai sp. nov.). This suggests that Pantopipetta species comprises two species groups: (i) 281 species with an eight-articulate palp having a short article distal to the lateral process of the 282 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 283 284hypothesis, the connection between the lateral process of the cephalon and the palp needs to

- be reexamined in known species.
- 286

287 Cave-dwelling pycnogonids

288 At least 15 pycnogonid species have been recorded from marine or anchialine caves in the 289 Mediterranean (e.g., Gerovasileiou & Bianchi, 2021), Caribbean (Alvarez & Ojeda, 2018), or 290 northwestern Pacific (Bamber, 2008; this study). Among these species, only three were 291 identified to the species level, each collected from a different environment. Pycnogonum 292 coninsulum inhabited a submarine cave at 33% salinity, with no information on light 293 provided (Bamber, 2008; Morton et al., 2008). Anoplodactylus batangensis came from among 294 vegetation in the illuminated anchialine pool of a cenote (1.63 salinity; Alvarez & Ojeda, 295 2018). Pantopipetta hosodai sp. nov. came from a completely dark, anchialine environment inside a submarine cave (less than 28‰ salinity; cf. Osawa & Fujita, 2019). 296

It is unknown whether pycnogonids inhabiting caves are troglobites (obligate cavedwelling 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

- 301 Akumanoyakata Cave across a span of 10 years but our *P. hosodai* sp. nov. specimen is the
- 302 first pycnogonid found. This suggests a very low abundance of pycnogonids in caves, or at
- 303 least in Akumanoyakata Cave. More comprehensive sampling in both inside and outside
- 304 caves is needed to ascertain the degree of their dependence on caves.

305	
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315	
316	Author contributions
317	KK conceived and designed the study, and made morphological observations; YF collected the
318	pycnogonid; KK and YF wrote the manuscript, and read and approved the final draft.
319	
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324	Conflict of interest
325	The authors declare no conflict of interests.
326	
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429 Figure legends

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- 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

- 437 pinching with forceps); B, distal tip of ocular tubercle, dorsal view; C, cephalon, left view
- 438 (lateral process of cephalon and leg 1 omitted); D, left palp (ornamentation on short distal
- 439 articles omitted); E, distal portion of left palp; F, right oviger; G–J, left legs 1–4.
- 440
- 441 **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,
- 443 respectively. c2, c3, coxae 2 and 3. Arrowheads, genital opening.







