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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
24	slope zone of the cave, 80-100 m from the entrance, no light, low salinity, and with rocky
25	substrate. This is the first record globally of a Pantopipetta species from a submarine cave
26	and anchialine environment. Pantopipetta hosodai sp. nov. resembles Pantopipetta auxiliata,
27	Pantopipetta lenis, and Pantopipetta oculata in having auxiliary claws but differs from them
28	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 <i>Pantopipetta</i> , 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)
33	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,
37	and it is generally unknown whether cave-dwelling pycnogonids tend to be troglobites.
38	
39	Key words: Akuma-no-Yakata; Chelicerata; palp; Pantopipetta; Pantopoda; Stiripasterida;
40	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

74 environment, and the shallowest depth record at 10-20 m. The specimen can be distinguished 75 from all congeners (16 species; Hosoda & Kakui, 2020), and we describe it here as a new 76 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, 82 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 85 was collected with a commercially made aquatic suction sampler (yabby pump). The 86 individual was sorted from the mud sample and preserved in 99% ethanol. The methods used 87 for dissection, preparation of slides, light microscopy, and drawing were as described by 88 89 Kakui & Angsupanich (2012). Morphological terminology follows Child (1979), except that the term "article" is used instead of "segment" for all appendages (Hosoda & Kakui, 2020). 90 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]

(Figures 1, 2, 3A–D)

106

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
127	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).
133	Palp (Figures 1C, 2D, E) 6-articulate. Article 1 longest, with two spines. Article 3
134	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.
137	Legs 1-3 (Figures 2G-I, 3A-C) slender. Coxa 1 with long dorsal tubercle (longer
138	than coxa-1 width). Coxa 2 with tiny dorsal projection and tiny ventro-subdistal genital
139	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 143 144 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) 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

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

172

173	
174	Supplementary information on male genital openings
175	Coxa 2 of legs 1-3 without genital opening. Coxa 2 of leg 4 with ventro-subdistal genital
176	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
182	that of the leg 3. Although Austrodecidae lacks information on ontogenetic development after
183	the postlarval stage that bears unarticulated legs 4, the above condition was reported in non-
184	adult individuals in several other families (e.g., Okuda, 1940; Brenneis et al., 2011; Miyazaki
185	& Hoshino, 2019). The oviger of our specimen comprises one naked article. Uniarticulate
186	ovigers have been reported in three austrodecid species, namely, Austrodecus (Microdecus)
187	fryi Child, 1994, Austrodecus palauense Child, 1983, and Austrodecus varum Child, 1994,
188	but the latter two were species described based on juveniles (Child, 1983, 1994). The oviger
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 having
200	immature leg 4 and oviger.
201	
202	Generic affiliation
203	We identified our specimen as a member of <i>Pantopipetta</i> mainly based on that its palp has
204	three short distal articles. All known Pantopipetta species have three or four short distal
205	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). 227 The generic affiliation of Austrodecus aconae can vary depending on whether 228 researchers emphasize the number of short distal articles on the palp or that of the oviger. In 229 this study, although we refrain from returning A. aconae into Pantopipetta, we deemed the 230 number of short distal articles on the palp to be one of the diagnostic characters to distinguish 231 Austrodecus (one or two) and Pantopipetta (three or four) and identified our specimen as a 232 member of Pantopipetta. 233 234 Morphological comparisons 235 Because we concluded that our specimen was a juvenile female with immature oviger and leg 236 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 237 Pantopipetta auxiliata Stock, 1968 from off the eastern coast of South Africa (68–69 m 238

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 272 273 (Barnard, 1946) in Stock (1963, fig. 8a); Pantopipetta gracilis Turpaeva, 1993 (Turpaeva, 274 1993, fig. 4-1); P. oculata (Stock, 1968, fig. 7b); Pantopipetta weberi (Loman, 1904) in 275 Loman (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 283 284 hypothesis, the connection between the lateral process of the cephalon and the palp needs to 285 be reexamined in known species. 286 Cave-dwelling pycnogonids 287 288 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). 296 297 It is unknown whether pycnogonids inhabiting caves are troglobites (obligate cave-298 dwelling species). Two species, Py. coninsulum and P. hosodai sp. nov., were reported based 299 on a single individual each from two different caves, and it is not known whether they also 300 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
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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
430	
431	Fig. 1. Pantopipetta hosodai sp. nov., holotype, ICHUM8407, juvenile, ethanol-fixed
432	specimen. A, habitus, dorsal view; B, habitus, left view; C, cephalon, right view; D, right
433	oviger. Abbreviations: lpc, lateral process of cephalon; ov, oviger; p1, palp article 1.
434	
435	Fig. 2. Pantopipetta hosodai sp. nov., holotype, ICHUM8407, juvenile. A, habitus, dorsal
436	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 <i>Pantopipetta</i> . A–D, <i>P. hosodai</i> sp. nov., holotype, juvenile female;
442	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.









