New Psychropotid species (Echinodermata Holothuroidea Elasipodida) of the Western Pacific with phylogenetic analyses

Chuan Yu, Chunsheng Wang, Dongsheng Zhang, Ruiyan Zhang

1   **New Psychropotid species (Echinodermata: Holothuroidea:**

2   **Elasipodida) of the Western Pacific with phylogenetic analyses**

3   Chuan Yu[1, 2], Dongsheng Zhang[1, 2, 3], Ruiyan Zhang[1], Chunsheng Wang[1, 2, 3, 4*]

4   [1] *School of Oceanography, Shanghai Jiao Tong University, Shanghai, China*

5   [2] *Key Laboratory of Marine Ecosystem Dynamics, Second Institute of Oceanography,*

6   *Ministry of Natural Resources, Hangzhou, China*

7   [3] *Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai), Zhuhai,*

8   *Guangdong, China*

9   [4] *State Key Laboratory of Satellite Ocean Environment Dynamics, Ministry of Natural*

10   *Resources, Hangzhou, China*

11   **Abstract**

12   Holothurians of the family Psychropotidae are widely distributed, but are the least

13   studied deep-sea holothurians. On the expedition of the Western Pacific, four

14   holothurians specimens of the family Psychropotidae were collected by the Jiaolong

15   human operated vehicle (HOV). Through morphological examination, two of them

16   were identified as a new species, *Benthodytes jiaolongi* **sp. nov.**, which was

17   characterized as having minute papillae, narrow brims, and terminal anus; the ossicles

18   were either rods or absent. The remaining two specimens were identified as

19   *Psychropotes verrucicaudatus* Xiao, Gong, Kou & Li, 2019, which was firstly recorded

20   at Kyushu-Palau Ridge (KPR). The phylogenetic analysis confirmed the classification

21   status of *B. jiaolongi* and *P. verrucicaudatus*, and indicated a paraphyletic relationship

22   within the genus *Benthodytes*. The new species clustered with *Benthodytes*

23   *sanguinolenta* and was separated from the clade of the other *Benthodytes* species.

24   Furthermore, *B. sanguinolenta* and *B. jiaolongi* **sp. nov.** was found to be different from

25   the other *Benthodytes* species based on their morphology. Therefore, a revision of the

26   genus *Benthodytes* is needed to solve its phylogenetic relationship.

27

28   **Key words**

29   *Benthodytes*, Elpidiidae, new record, paraphyletic group, *Psychropotes*, Western Pacific

30

31   **Introduction**

32

33   Holothurians of the family Psychropotidae (Elasipodida) were first identified by

34   Théel (1882) who defined four genera of the deep-sea sea cucumbers discovered on the

35   H.M.S. Challenger Expedition. Subsequently, Hérouard (1909) and Vinogradov (1969)

36   erected *Triconus* Hérouard and *Nectothuria* Belyaev and Vinogradov, which were later

37   regarded as synonyms of *Psychropotes* by Hansen (1975). Psychropotidae comprises

38   three genera and 37 species. Hansen (1975) distinguished the three genera by the

39   presence or absence of an unpaired dorsal appendage, the position of the anus, and the

40 presence or absence of an unpaired circum-oral papillae. Although, taxonomists have
41 long worked on this family, Psychropotidae are still the least studied deep-sea
42 holothurians. Thus, the phylogenetic relationships within Psychropotidae remain
43 unclear.
44   An expedition of the Jiaolong HOV concentrated on furthering our understanding
45 of the biodiversity, connectivity, and conservation value of the Western Pacific. During
46 sampling, four specimens of Psychropotidae were collected from seamounts on the
47 Kyushu-Palau Ridge and Weijia Guyot. Based on an analysis of the external
48 morphological characters and ossicles, we identified two specimens as a new species
49 (*Benthodytes jiaolongi* **sp.nov.**) and the other two as new records *Psychropotes*
50 *verrucicaudatus* Xiao, Gong, Kou & Li, 2019.

51

Figure 1. Red dots presented the location of *B. jiaolongi* sp.nov. and green dots
indicated *P. verrucicaudatus*.

54

Materials and Methods

Sampling and morphological observations

57   The samples described in the present study were collected by the Jiaolong HOV at
58 a depth of 2408-2602 m, from the Kyushu-Palau Ridge and Weijia Guyot. Before
59 preservation, a Canon EOS 5DII camera (Canon Inc., Tokyo, Japan) was used to take
60 photographs of the specimens on board the ship. Then, a piece of tissue was cut from

61 all specimens and frozen at -20 °C for DNA extraction. Finally, the specimens were
62 fixed in 10% seawater formalin and deposited at the Repository of Second Institute of
63 Oceanography (RSIO). Sodium hypochlorite was used to dissolve some body tissues
64 (tentacles, dorsum, ventrum, brim, dorsal warts and gonads), and ossicles were rinsed
65 five times with purified water. The ossicles were observed using a scanning electron
66 microscope (TM 1000; Hitachi, Ltd., Tokyo, Japan).
67
68 ***PCR amplification and phylogenetic analysis***
69 Total genomic DNA was extracted from 100 mg of muscle tissue using a DNeasy
70 Blood & Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's
71 instructions. Two partial mitochondrial genes, 16S rRNA and COI, were amplified
72 suing primers 16S-arL/brH and COI-ef/er (Miller et al. 2017). The PCR reactions were
73 performed using a 50-μL reagent mix, containing 25-μL 2× Phanta Max Master Mix
74 (Vazyme, Biotech Co., Ltd., Nanjing, China), 20-μL DNase free ddH$_2$O, 2-μL of each
75 primer, 1-μL template DNA, as suggested by the manufacturer. The PCR amplification
76 procedure is shown in Table 1. PCR products were confirmed by 1.5% agarose gel
77 electrophoresis, and purified using an OMEGA PCR kit (Omega, Biotek, Norcross).
78 The purified PCR products were sequenced on an ABI 3730XL sequences (Sangon,
79 Biotec Co., Ltd., Shanghai). Sequence data were edited with Geneious R6.1.6 (Kearse
80 et al. 2012) and deposited in GenBank (Table 2).
81
82 **Table 1.** The PCR amplification procedures.

Primer	Sequence 5′→ 3′	PCR procedure
COI-ef	ATAATGATAGGAGGRTTTGG	Pre denaturation:95 °C for 3 min
COI-er	GCTCGTGTRTCTACRTCCAT	40 cycles:
		Denaturation:95 °C for 40 s
		Annealing: 45 °C for 40 s
		Extension: 72 °C for 50 s
16S-arL	CGCCGTTTATCAAAAACAT	Pre denaturation:95 °C for 3 min
16S-brH	CCGGTCTGAACTCAGATCACG	35 cycles:
		Denaturation:95 °C for 40 s
		Annealing: 50 °C for 40 s
		Extension: 68 °C for 50 s

83
84 For a more comprehensive phylogenetic analysis, we not only used the sequences
85 of Psychropotidae but also used the mitochondrial sequences of Elpidiidae and two
86 species of Molpadiidae as the outgroup (Table 2). Twenty-five COI and 18 16S
87 sequences were aligned using MAFFT 7 (Katoh and Standley 2013) using the E-INS-I
88 strategy. The 16S and COI alignments were concatenated into the dataset (COI/16S =
89 687/578 bp), while the alignment gaps and missing data were represented as '-' and '?'.
90 Maximum likelihood (ML) and Bayesian inference (BI) analyses were performed using
91 the concatenated dataset. JModelTest 2.1.10 (Darriba et al. 2012) was used to find the
92 best-fit model from 88 competing models using Akaike information criterion (AIC)

93 calculations. In each case, GTR+I+G was the best-fit model for BI analyses. MrBayes
94 3.2 (Huelsenbeck and Ronquist 2001) was used to conduct BI analyses. Markov Chain
95 Monte Carlo (MCMC) iterations were run for 1, 000, 000 generations with sampling
96 every 100 generations. The first 25% of trees were discarded as burn-in, and the
97 consensus trees were summarized in 75% majority-rule trees. RAxML GUI 1.5
98 (Silvestro and Michalak 2012; Stamatakis 2014) was used to perform ML analysis with
99 the GTR+GAMMA+I substitution model for 1000 bootstraps, as recommended by
100 Miller et al. (2017).

101

102 **Table 2.** The gene sequences of the specimens used in this study.

Family	species	GenBank accession number	
		16S	COI
Psychropotidae	*Benthodytes manusensis*	MH627223.1	MH627222.1
	Benthodytes sanguinolenta		HM196507.1
	Benthodytes marianensis	MH049433.1	MH049435.1
	Benthodytes jiaolongi **sp. nov.**	MW992746	MW990356
	Benthodytes jiaolongi **sp. nov.**	MW992747	MW990357
	Psycheotrephes exigual		KX874392.1
	Psychropotes longicauda	DQ777099.1	KU987469.1
	Psychropotes moskalevi	MN310400.1	MN313655.1
	Psychropotes raripes	MN310403.1	MN313656.1
	Psychropotes verrucicaudatus	MH077589.1	MH077588.1
	Psychropotes verrucicaudatus	MW992748	MW980088
Elpidiidae	*Peniagone diaphana*	KX856725.1	KX874384.1
	Peniagone incerta		HM196402.1
	Peniagone sp. AKM-2016	KX856726.1	KX874385.1
	Peniagone vignoni		HM196381.1
	Elpidia glacialis		HM196413.1
	Amperima robusta	KX856728.1	KX874381.1
	Protelpidia murrayi	KX856727.1	KX874382.1
	Scotoplanes sp.TT_2017		LC230158.1
Laetmogone	*Laetmogone wyvillethomsoni*		HM196504.1
	Pannychia moseleyi	KX856731.1	KX874380.1
	Benthogone abstrusa	KX856733.1	KX874374.1
	Enypniastes eximia	KX856730.1	KX874383.1
Molpadiidae	*Molpadia arenicola*	KX856741.1	KX874344.1
	Molpadia musculus AKM_2016	KX856739.1	KX874386.1

103

Results and Discussion

104

105 *Morphological observations*
106 Order Elasipodida Théel, 1882

107 Suborder Psychropotina Hansen, 1975
108 Family Psychropotidae Théel, 1882
109 Genus *Benthodytes* Théel, 1882
110
111 **Diagnosis** (according to Hansen, 1975)
112 Auns dorsal. Unpaired dorsal appendages absent. Circumoral (or post-oral) papillae
113 present. Tentacles soft, pliable, and retractile.
114
115 *Benthodytes jiaolongi* **sp. nov.**
116 (Figs 2-3)
117
118 *Material examined.* Catalog numbers: RSIO3710601 and RSIO6017101, two adult
119 specimens, collection numbers: DY37-JL106-B01 (156.947° E, 13.0176° N) and
120 DY60-JL180-B04 (134.911° E, 16.935° N).
121
122 *Diagnosis*
123 Body elongated and subcylindrical when fixed. Skin red with violet, thin and soft.
124 No obvious large papillae arranged on the dorsal surface. Some minute papillae, conical
125 with tips, on the dorsum. Brim narrow, thin, flattened. Mouth ventral, anus terminal.
126 Eighteen tentacles, retracted after fixing; circum oral papillae present. Dorsal ossicles
127 include rods and primary crosses with four arms. Rods present in tentacles. Ossicles of
128 ventrum not observed.
129
130 *Description of holotype* (RSIO6017101)
131 The length of the specimen was approximately 25 cm before being preserved in
132 10% seawater formalin. Color was violet in life (Fig. 2C); the skin was transparent, thin,
133 soft, and gelatinous after fixing. Brim retracted less than 0.7 cm in width. The dorsal
134 papillae did not develop well, but some minute papillae were placed in a single row
135 along the dorsal radii. Midventral tube feet with degeneration arranged in two rows,
136 approximately 18 pairs. Mouth ventral, anus terminal. After the specimen arrived at the
137 sea surface, autolysis began; therefore, tentacles could not be identified (Fig. 2D). Few
138 ossicles were observed. Dorsal ossicles contained primary crosses with spiny arms and
139 spinous rods (Fig. 3A–C). The rods were approximately 400 μm long and the arms of
140 the crosses were approximately 200 μm in length. Rods were presented in tentacles and
141 the length was approximately 400–500 μm (Fig. 3D–G). Ossicles were not be found in
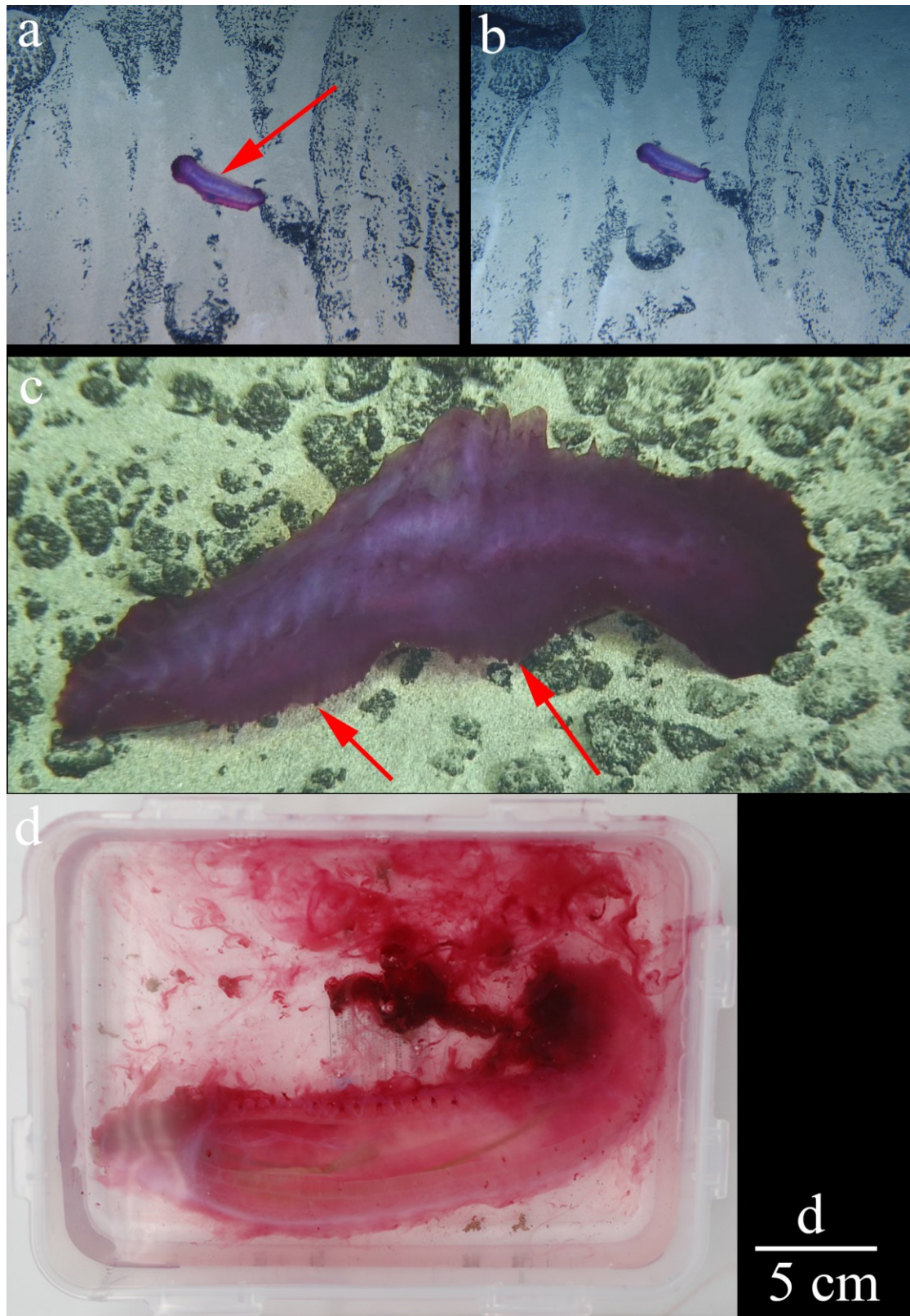142 the other body parts.

Figure 2. a-b: *Benthodytes jiaolongi* sp.nov. (RSIO3710601), red arrows pointed to the minute papillae. c: *Benthodytes jiaolongi* sp.nov. (RSIO6017101), red arrows pointed to the minute papillae. d: Specimen (RSIO6017101) before preservation in 10% seawater formalin.
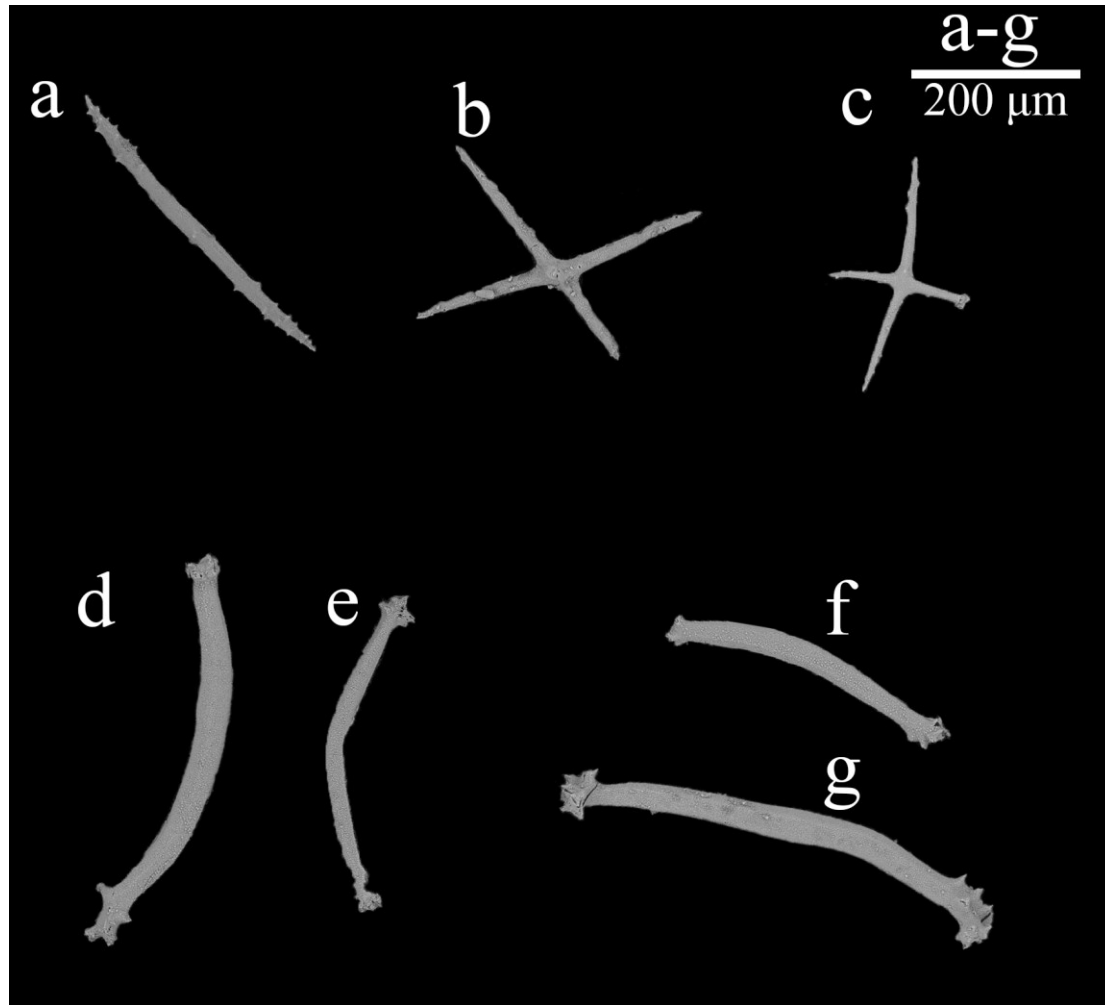
149

150 **Figure 3.** a-c: ossicles of dorsum. d-g: ossicles of tentacles.

151

152 ***Description of paratype* (RSIO3710601)**

153   The specimen was approximately 22 cm in length and 5 cm wide at the maximum
154 point. Color was red-violet at the seabed (Fig. 2A, B); after arriving at the sea surface,
155 it became pale violet, and the skin appeared transparent, with a white color after
156 preservation in 10% seawater formalin for 5 years. The normal paired dorsal papillae
157 were absent, and approximately 23 pair minute papillae were arranged in a single row
158 from the anterior of the body to the posterior end (Fig. 2A, C). Owing to the long-term
159 preservation, the quantity of the midventral tube feet could not be determined, but the
160 tube feet were arranged in two rows; the brim could not be distinguished. Mouth ventral,
161 anus terminal. Eighteen tentacles, were retracted to the stalk. Ossicles were not
162 observed in the specimen.

163

164 ***Etymology.*** The name is derived from the first Chinese HOV 'Jiaolong'.

165

166 ***Type locality.*** Weijia Guyot, tropical Western Pacific. Depth 2408 m, cobalt-rich crust
167 with sediments, May 1, 2016, Bo Lu. Kyushu-Palau Ridge, tropical Western Pacific.
168 Depth: 2602 m, muddy sediments, January 21, 2021, Ruiyan Zhang, Bo Lu.

169

170   ***Distribution.*** Known from Weijia Guyot and Kyushu-Palau Ridge.

171

172   ***Remarks.*** According to the taxonomy of Hansen (1975), *Benthodytes jiaolongi* **sp. nov.**
173   was close to *Benthodytes sanguinolenta* Théel, 1882 and *Benthodytes typica* Théel,
174   1882 based on the minute dorsal papillae and rod or missing ossicles. However, the
175   morphology of *B. sanguinolenta* and *B. typica* deviated within *Benthodytes* compared
176   with other species, and the taxonomy of *Benthodytes* needs to be revised.

177

178   Based on the description by Hansen (1975), the minute dorsal papillae of *B. typica*
179   were few and placed along the anterior part of the dorsal radii; rods were present in the
180   specimens and scattered in the ventrum, dorsum and tentacles. *B. sanguinolenta* was
181   described by Théel in 1882 based on Challenger Expedition. Hansen (1975) and
182   Rogacheva et al. (2009) re-examined *B. sanguinolenta* and the main characteristics
183   could be described as: minute dorsal papillae arranged in two bands or between the two
184   bands; approximately 1–4 papillae placed in a band, narrowing to 1 or -2 papillae at
185   posterior end; ossicles were not found or a few rods present in the midventral tube feet
186   and tentacle disc. The differences in characteristic between the new species *B. jiaolongi*
187   and the other species *B. typica* and *B. sanguinolenta* can be listed as follows: (1) the
188   ossicles of the new species were only presented in the tentacles and dorsum with rods
189   and primary crosses, but *B. typica* only possessed rods scattered in the ventrum, dorsum
190   and tentacles, and *B. sanguinolenta* did not possess ossicles. (2) The dorsal papillae of
191   *B. typica* present in the anterior body part and *B. sanguinolenta* are arranged into two
192   bands. These of the new species were arranged in a single row from the anterior body
193   to the posterior end.

194

195 Genus *Psychropotes* Théel, 1882

196

197 **Diagnosis** (according to Hansen, 1975)
198 Anus Ventral. Unpaired dorsal appendages present. Circumoral (or post-oral) papillae
199 absent. Tentacle discs of fixed shape, rounded out in line with marginal knobs.

200

201 *Psychropotes verrucicaudatus*
202 (Figs 4-6)
203 *Psychropotes verrucicaudatus* Xiao, Gong, Kou & Li, 2019:421-430.

204

205   ***Material examined.*** Catalog numbers: RSIO6017006 and RSIO6018004, Two adult
206   specimens, collection numbers: DY60-JL170-B05 (134.860° E, 12.079° N) and DY60-
207   JL180-B04 (134.352° E, 13.569° N), Genbank accession numbers of RSIO6017005
208   MW980089, MW992749.

209

210   ***Diagnosis***
211   Body elongate, anteriorly depressed; ventrum flattened with tiny tube feet arranged
212   in the mid-ventrum. Sixteen tentacles. Brim broad. Mouth and anus ventral. Large

213 spinous unpaired dorsal appendage approximately 1/5 of the body length and conical
214 located at 1/4 body length from anterior end. Warts enshrouded dorsal appendage and
215 skin. Dorsal ossicles included primary crosses with spiny arms and short central
216 apophysis; ossicles with spinous crosses scattered on brim and ventrum. Rods present
217 in tentacles, but with differences in morphology. Warts possessed huge crosses with
218 strongly curved, spinous arms and high central apophysis.
219
220 ***Description of holotype*** (**RSIO6018004**)
221 The external morphology of the specimen resembled a barbell after collection and
222 was approximately 20 cm in length before being preserved in 10% seawater formalin
223 (Fig. 4C, D). Before preservation, the height of the appendage was approximately 50
224 mm, and the width was approximately 30 mm at the base (Fig. 4C, D). The dorsal skin
225 was transparent with a brownish red color on the seabed and dark brown on the deck.
226 Warts covered the dorsal skin and appendage, and the giant ossicles can be seen in the
227 warts (Fig. 4E-F). Approximately 30 pairs of tube feet were arranged in two rows along
228 the middle of the ventrum were degenerated. Sixteen tentacles were formed in a circle.
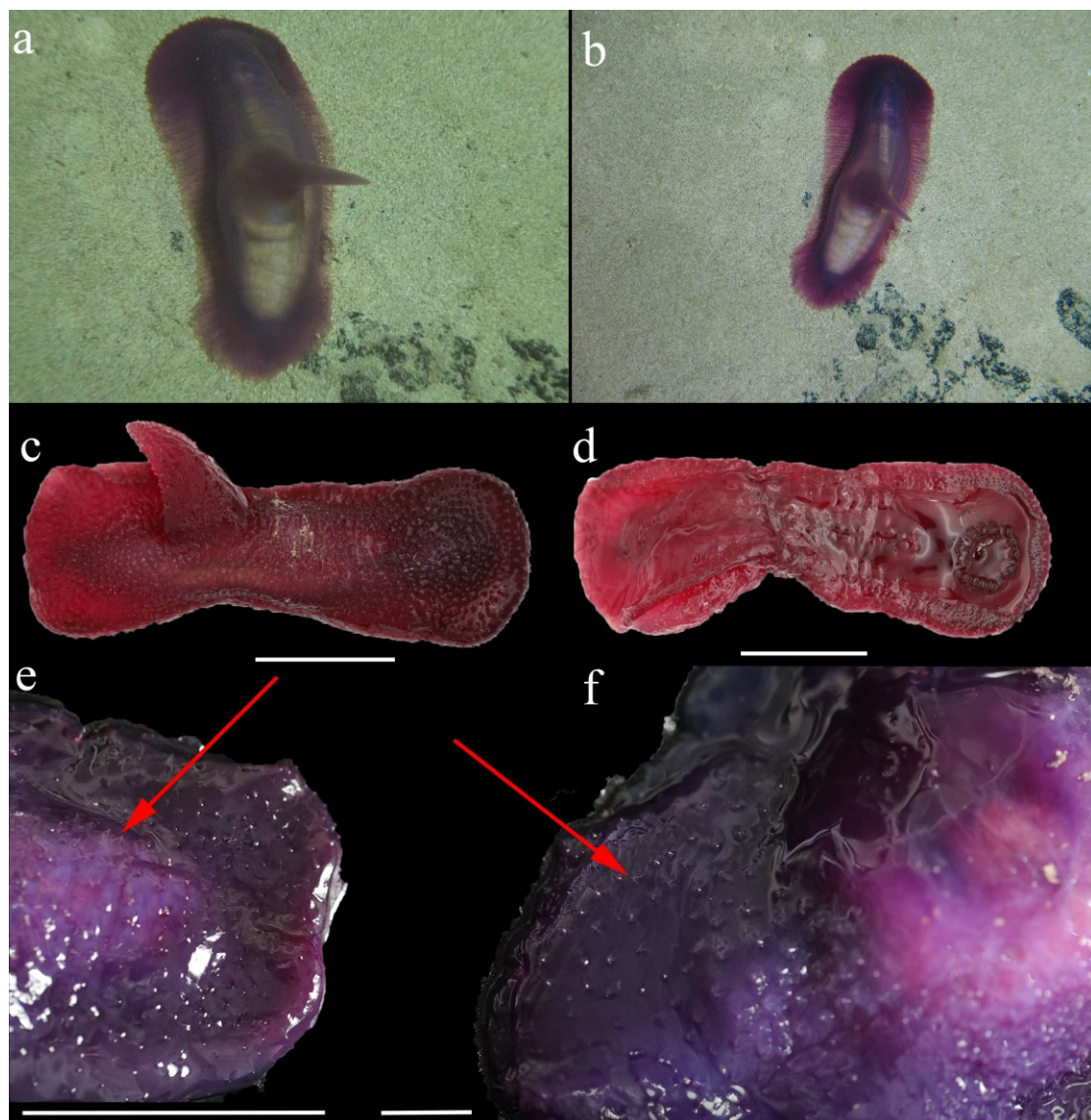229 The brim was broad and covered with warts on the dorsum.

230

231 **Figure 4.** a-b: species in situ. c-d: Specimen before preservation. Scale bar: 5 cm. e-f:
232 Red arrow points to the giant ossicles, specimen after preservation in 10% seawater
233 formalin. Scale bar: 5 cm and Scale bar: 1 cm.
234

235   A giant cross with four arms could be seen in each wart. The arms were 800–1000
236 μm in length, and the maximum width between the large arms was approximately 500
237 μm. The arm flexion (presented by the length close to long arms / the length close to
238 short arms at the intersection of the diagonal) was approximately 250 / 400 μm (Fig.
239 5A–D). In addition, the height of the central rudimentary apophyses was approximately
240 200–300 μm. Ventral ossicles can be divided into two types: primary cross with spiny
241 arms and cross with three arms (Fig. 6A–C), and the length of each arm was
242 approximately 200 μm. The primary crosses with spinous arms were present in the
243 dorsum (Fig. 6D–F) and brim (Fig. 6H–J), and the length of the arms was up to 200 μm.
244 Furthermore, dorsal ossicles possessed a spinous rod 170 μm in length (Fig. 6G), and
245 large primary crosses with spiny arms were present in the brim (Fig. 6K). Ossicles of
246 tentacles were rods with an irregular shape (Fig. 6L–R). The large rod with two
247 apophyses at the end was approximately 900–1000 μm in length; the small rod with
248 apophyses in the middle area was approximately 200 μm in length. Other rods with
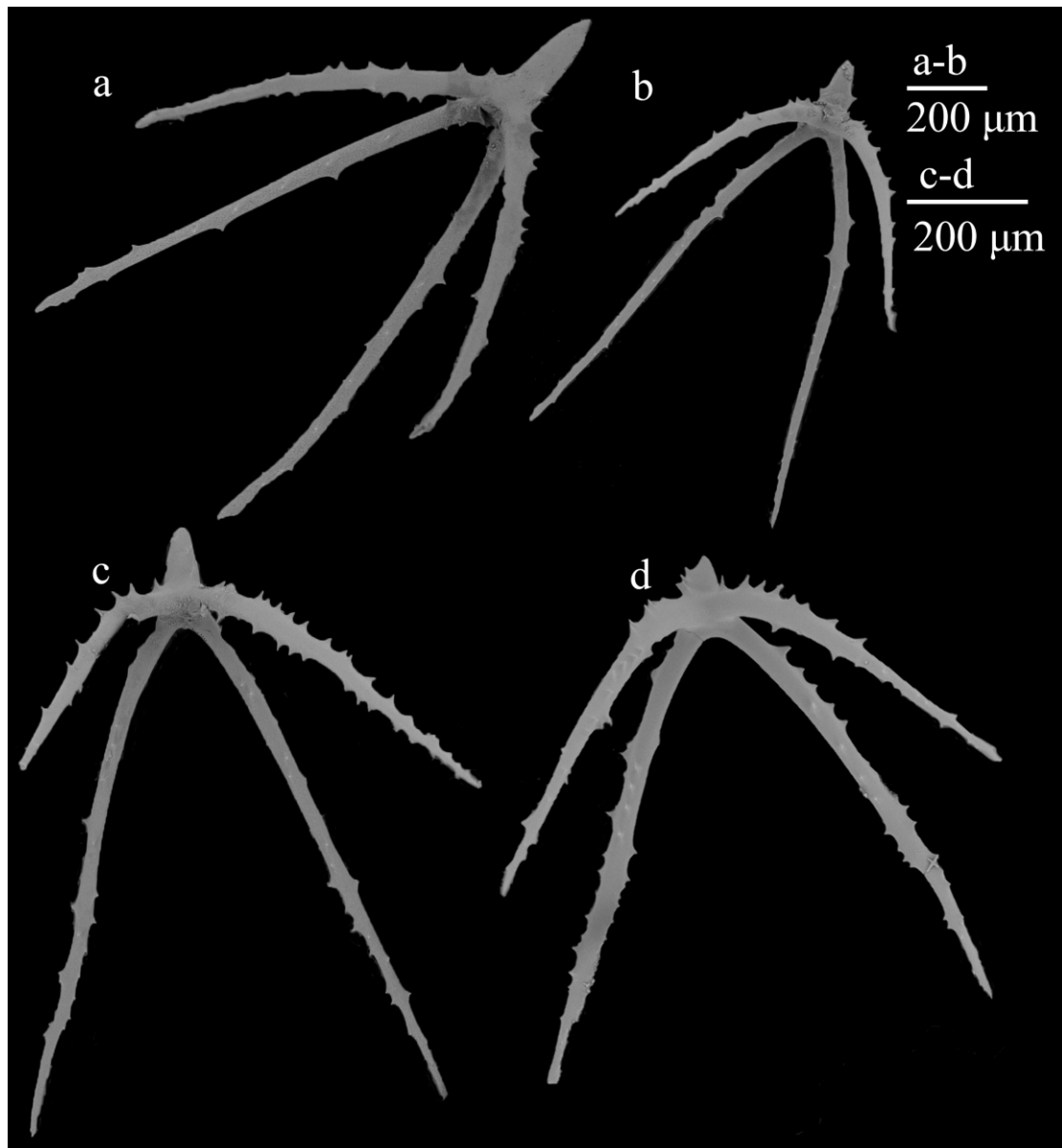249 spiny arms were 500–800 μm in length.
250

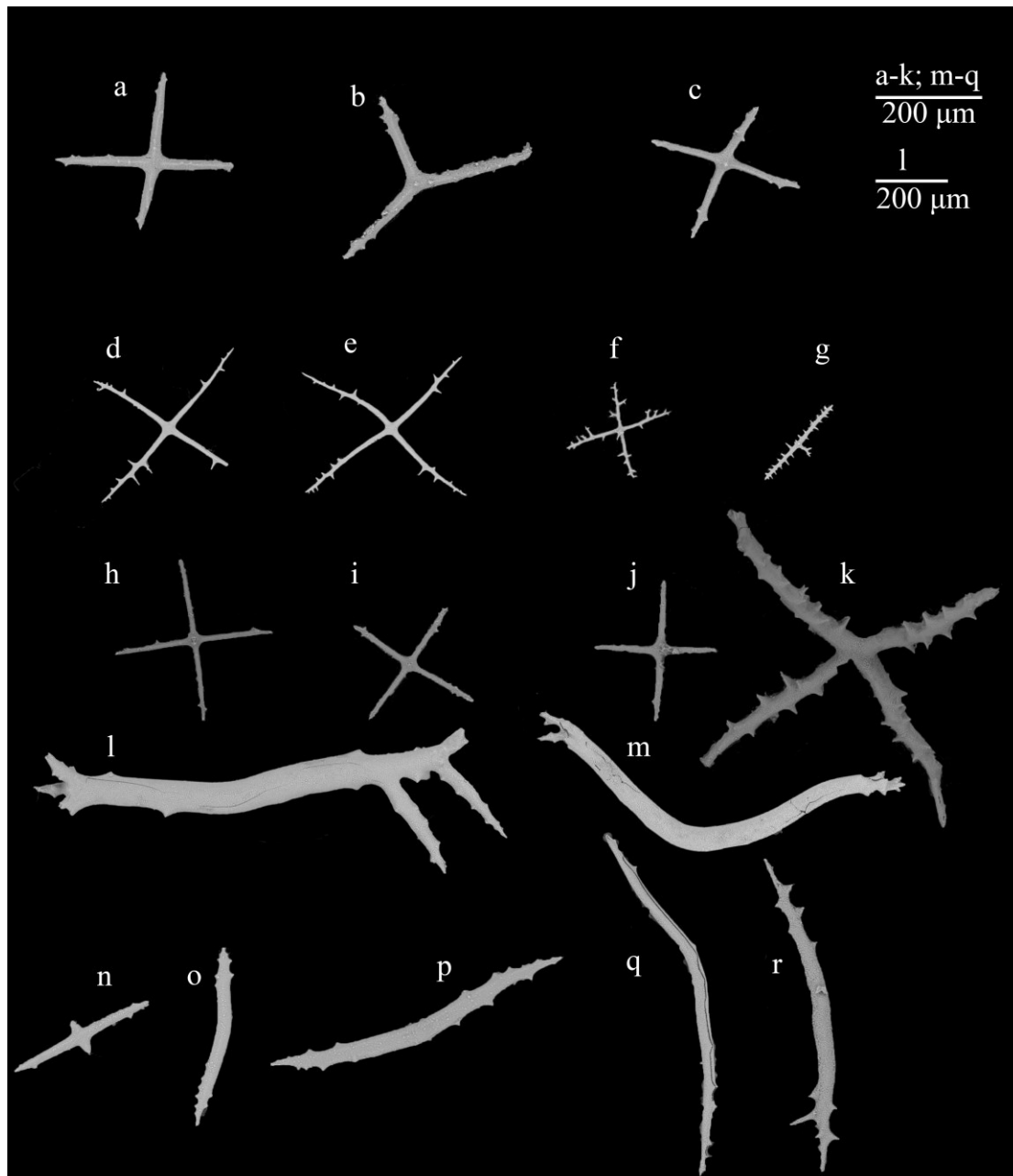**Figure 5.** The giant ossicles in the warts.

Figure 6. a-c: ventral; d-g: dorsal; h-k: brim; l-r: tentacle.

Description of paratype (RSIO6017005)

The specimen was approximately 18 cm in length, the height of the appendage was approximately 40 mm, and the width was approximately 20 mm at the base. Mouth and anus ventral. The skin was transparent and of a light brown color. Dorsal skin and appendage covered with warts, and the dorsum of the brim also grew warts. Giant ossicles could be seen in the warts. Tentacles could not be clarified due to damage, but there were more than 12. The ossicles were similar to those of the holotype.

Type locality. Kyushu-Palau Ridge, tropical Western Pacific Depth 2360–2468 m, muddy sediments, January 8, 2021 and January, 25 2021, Ruiyan Zhang, Bo Lu.

268   ***Distribution.*** Known from Jiaolong Seamount of South China Sea and Kyushu-Palau
269   Ridge.

270

271   ***Remarks.*** The specimens were clearly a new record of the *P. verrucicaudatus* but with
272   differences in external morphology and ossicles. *P. verrucicaudatus* was described by
273   Xiao et al. (2019). The differences between our specimens and *P. verrucicaudatus*
274   reflected intraspecific difference between growth stages. In addition, owing to the
275   preservation of the samples, Xiao et al. (2019) did not observe the complete ossicles of
276   the warts, but we were able to observe these structures in our specimens.

277

278   The intraspecific differences can be listed as follows: (1) in our specimens, the skin
279   was transparent and the color was darker than that of *P. verrucicaudatus*. (2) The width
280   of the appendage at the base was also larger than that of *P. verrucicaudatus*. (3) The
281   length of the primary crossing arms distributed in the dorsum, ventrum, and brim was
282   longer than that of *P. verrucicaudatus*. Furthermore, the spinous rod of the dorsal
283   ossicles was not present in *P. verrucicaudatus*, and the ventral body wall of the
284   specimens did not possess the tripartite ossicles. (4) Most of the ossicles of the tentacles
285   in our specimens were the same as those of *P. verrucicaudatus*, but longer.

286

287   ***Phylogenetic analyses***
288   Owing to limited genetic sequences, the phylogenetic relationships of Elasipodida
289   are not well studied. Miller et al. (2017) constructed high-level phylogenetic
290   relationships of Holothuroidea and separated Deimatidae from Elasipodida to rebuild
291   new phylogenetic relationships. The remaining families of Elasipodida included
292   Elpidiidae, Laetmogonidae, Pelagothuriidae, and Psychropotidae, but their positions
293   within Elasipodida remained unresolved. Li et al. (2018) used mitochondrial and
294   nuclear genes to perform phylogenetic analyses of Elasipodida, especially the
295   Psychropotidae, and the results showed that *Benthodytes* was a paraphyletic group of
296   Psychropotidae based on the clade of *B. sanguinolenta*.

297

298   To obtain clearer phylogenetic relationships, we concatenated 25 COI and 18 16S
299   sequences into a dataset to build the ML and BI trees. Although the genetic sequences
300   were limited, the topological structures of the ML and BI trees supported the
301   classification status of *B. jiaolongi* **sp. nov.** and *P. verrucicaudatus*. The phylogenetic
302   relationships of Psychropotidae could be divided into four parts and were inconsistent
303   with the traditional classification system based on Hansen (1975). *Benthodytes* were
304   paraphyletic in Psychropotidae and the new species was located in the outmost clade of
305   Psychropotidae. In addition, *Psychropotes* was a sister group to *Psycheotrephes*, and
306   part of *Benthodytes* was placed in the lateral clade of the sister group. *P.*
307   *verrucicaudatus* showed a paraphyletic relationship with *Psychropotes* and was
308   clustered in the clade of *Psycheotrephes*. Elpidiidae can be divided into two clades.
309   *Peniagone* clustered together into a supported monophyletic group, but *Peniagone*
310   *diaphana* was a sister group to other *Peniagone* species. *Peniagone* could be a
311   paraphyletic group. The other four genera of Elpidiidae clustered into a group, and

312 *Elpidia* was distant from the other three genera. *Protelpidia* and *Scotoplanes* were
313 closer to each other, and *Amperima* was away from the two genera. Laetmogone was
314 an obvious polyphyletic group, and *Pannychia moseleyi* was placed in the outmost
315 clade of the other three families. *Laetmogone wyvillethomsoni* clustered with Elpidiidae
316 and was situated outside; *Benthogone abstrusa* was clustered with *Enypniastes eximia*,
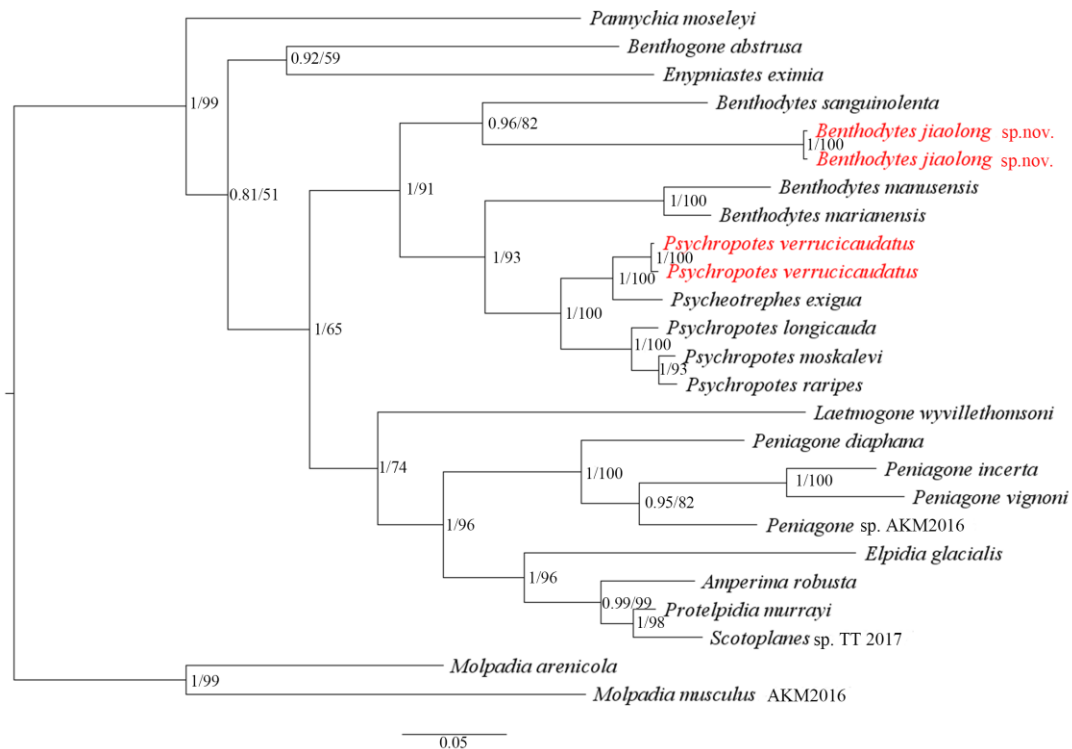317 but the possibility of a clade was low.

318

319 **Figure 7.** Bayesian inference (BI) and maximum likelihood (ML) trees based on
320 concatenate sequences. The values are BI/ML at each node.

321

322 Based on the morphological and phylogenetic analyses, *B. jiaolongi* sp. nov. can
323 be identified as a new species closely related to *B. sanguinolenta*. In addition, our
324 specimens provided a new record of *P. verrucicaudatus* in the Western Pacific,
325 broadening its distribution. Our results clarify that *Benthodytes* is paraphyletic and that
326 the clade of *B. sanguinolenta* and *B. jiaolongi* sp. nov. is separated from the other
327 species of *Benthodytes*. Furthermore, *B. sanguinolenta* and *B. typica* differ from other
328 species of *Benthodytes* based on their morphology, and we suggest that *Benthodytes*
329 could be revised. Théel (1882) established *Benthodytes* according to the type species *B.*
330 *typica*; today, 12 species of *Benthodytes* are recognized by the well-developed dorsal
331 papillae and the cross ossicles of the body wall. A new genus characterized by well-
332 developed dorsal papillae and crossing ossicles of the body wall could be established,
333 and *Benthodytes* could be maintained with *B. typica*, *B. sanguinolenta*, and *B. jiaolongi*.
334 The key to the species of new *Benthodytes* could be the minute dorsal papillae and the
335 rod ossicles or missing ossicles.

336

Acknowledgements

We are grateful to all the scientists and crew on the R/V "Sheng Hai Yi Hao" and "Xiangyanghong9", and the Jiaolong HOV team for help in the collection of the deep-sea specimens. We also thank Dr. Lu Bo for helping process the specimens on board. We would like to thank Dr. Gebruk and Rogacheva for their help and valuable suggestions and comments on this article. This study was supported by the foundation of China Ocean Mineral Resources R & D Association (No. DY135-E2-2-03, No. DY135-E2-2-06), the Project of State Key Laboratory of Satellite Ocean Environment Dynamics, Second Institute of Oceanography (SOEDZZ2002).

References

Belyaev M, Vinigradov E, (1969) A new pelagic holothurian (Elasipoda, Psychropotidae) from abyssal depths in the Kurile-Kamchatka Trench. Zoologicheskii Zhurnal 48(5): 709-716.

Darriba D, Taboada G L, Doallo R (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9(8): 1–2. https://doi.org/10.1038/nmeth.2109

Hansen B (1975). Scientific results of the Danish deep-sea expedition round the world 1950-52. Systematics and biology of the deep-sea holothurians. Part. l. Elasipoda. Vinderup: The Galathea Committee, 1–262.

Hérouard, E. (1909) Triconus, nouveau genre de la famille des Psychropotineae. Bulletin Institut Musée Oceanographique, Monaco 145: 1-5.

Huelsenbeck J P, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17(8): 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Katoh K, Standley D M (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. 30(4): 772–780. https://doi.org/ 10.1093/molbev/mst010

Li Y-N, Xiao N, Zhang L-P (2018) *Benthodytes marianensis*, a new species of abyssal elasipodid sea cucumbers (Elasipodida: Psychropotidae) from the Mariana Trench area. zootaxa 4462(3):443–450. https://doi.org/10.11646/zootaxa.4462.3.10

Matthew L, Richard M, Amy W, Steven S-H (2012) Geneious Basic: An Integrated and Extendable Desktop Software Platform for the Organization and Analysis of Sequence Data. Bioinformatics 28(12): 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Miller A K, Kerr A M, Paulay G (2017) Molecular Phylogeny of Extant Holothuroidea (Echinodermata). Mol Phylogeneti Evol 111(1137): 110–131. https://doi.org/10.1016/j.ympev.2017.02.014

Rogacheva A, Cross I A, Billett D S M (2009) Psychropotid holothurians (Echinodermata: Holothuroidea: Elasipodida) collected at abyssal depths from

around the Crozet Plateau in the Southern Indian Ocean. Zootaxa 2096(1): 460–478. https://doi.org/10.1080/17451000.2018.1546012

Silvestro D, Michalak I (2012) raxmlGUI: a graphical front-end for RAxML. Org Divers Evol 12(4): 335–337. https://doi.org/10.1007/s13127-011-0056-0

Stamatakis A (2014) RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Théel H (1882) Report on Holothurioidea. Pt. I. Report of the Scientific Results of the Voyage of H.M.S. Challenger. Zoology 4(13): 1–176.

Xiao N, Gong L, Kou Q, Li X-Z (2018) *Psychropotes verrucicaudatus*, a new species of deep-sea holothurian (Echinodermata: Holothuroidea: Elasipodida: Psychropotidae) from a seamount in the South China Sea. Bull Mar Sci 95(3):421–430. https://doi.org/10.5343/bms.2018.0041