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

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New psychropotid species (Echinodermata: Holothuroidea: Elasipodida) of the Western Pacific with phylogenetic analysis

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Abstract

Holothurians of the family Psychropotidae are widely distributed, but remain least studied deep-sea holothurians. On an expedition in the Western Pacific, six psychropotid specimens were collected by the Jiaolong human operated vehicle (HOV). Through morphological examination, four of them were identified as a new species, *Benthodytes jiaolongi* **sp. nov.**, which was characterized as having minute papillae, narrow brims, and terminal anus; the ossicles were either rods or primary crosses. The remaining two specimens were identified as *Psychropotes verrucicaudatus* Xiao, Gong, Kou & Li, 2019, which was first recorded at the Kyushu-Palau Ridge. The phylogenetic analysis confirmed the classification status of *B. jiaolongi* and *P. verrucicaudatus*, and indicated a paraphyletic relationship within the genus *Benthodytes*. The new species clustered with *Benthodytes sanguinolenta* and was separated from the clade containing the other *Benthodytes* species.

Key words

Benthodytes, holothurians, new record, paraphyletic group, *Psychropotes*, Western Pacific

Introduction

Holothurians of the family Psychropotidae (Elasipodida) were first identified by Théel (1882) who defined four genera of the deep-sea sea cucumbers discovered on the H.M.S. Challenger Expedition. Subsequently, Hérouard (1909) and Vinogradov (1969) erected *Triconus* Hérouard and *Nectothuria* Belyaev and Vinogradov, which were later regarded as synonyms of *Psychropotes* by Hansen (1975). Psychropotidae comprises three genera and 37 species. Hansen (1975) distinguished the three genera by the presence or absence of an unpaired dorsal appendage, the position of the anus, and the presence or absence of circum-oral (or post-oral) papillae. Although, taxonomists have

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long worked on this family, Psychropotidae are still the least studied deep-sea holothurians. Thus, the phylogenetic relationships within Psychropotidae remain unclear.

An expedition of the Jiaolong HOV concentrated on furthering our understanding of the biodiversity, connectivity, and conservation value of the Western Pacific. During sampling, six specimens of Psychropotidae were collected from seamounts on the Kyushu-Palau Ridge and Weijia Guyot (Fig. 1). Based on an analysis of the external morphological characters and ossicles, we identified four specimens as a new species (*Benthodytes jiaolongi* **sp.nov.**) and the other two as new records *Psychropotes verrucicaudatus* Xiao, Gong, Kou & Li, 2019.

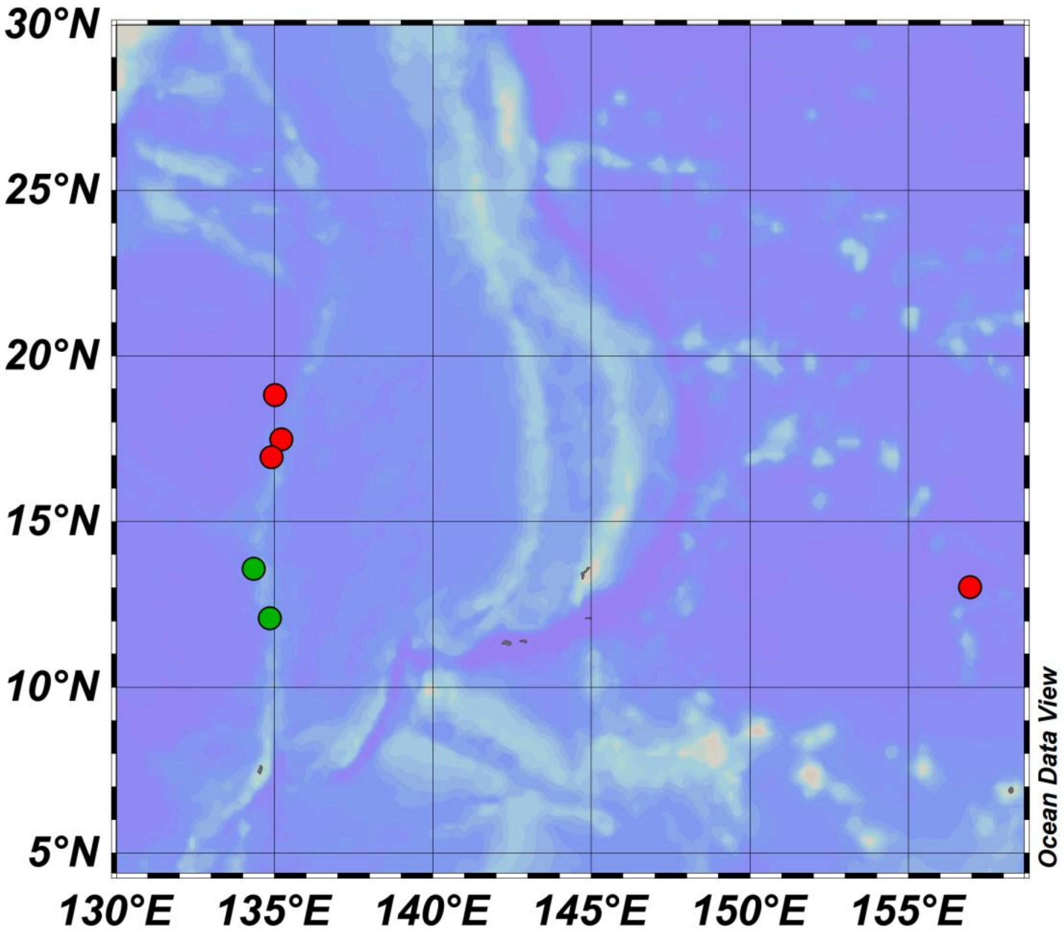

Figure 1. Red dots show the location of *Benthodytes. jiaolongi* **sp.nov.** and green dots indicated *Psychropotes. verrucicaudatus*.

Materials and Methods

Sampling and morphological observations

The samples described in the present study were collected by the Jiaolong HOV at a depth of 2408-2602 m, from the Kyushu-Palau Ridge and Weijia Guyot. Before preservation, a Canon EOS 5DII camera (Canon Inc., Tokyo, Japan) was used to take photographs of the specimens on board the ship. Then, a piece of tissue was cut from all specimens and frozen at -20 °C for DNA extraction. Finally, the specimens were fixed in 10% seawater formalin or 99% alcohol and deposited at the Repository of Second Institute of Oceanography (RSIO). Sodium hypochlorite was used to dissolve some body tissues (tentacles, dorsum, ventrum, brim, dorsal warts and gonads), and ossicles were rinsed five times with purified water. The ossicles were observed using a scanning electron microscope (TM 1000; Hitachi, Ltd., Tokyo, Japan).

PCR amplification and phylogenetic analysis

Total genomic DNA was extracted from 100 mg of muscle tissue using a DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Two partial mitochondrial genes, 16S rRNA and COI, were amplified using primers 16S-arL/brH and COI-ef/er (Miller et al. 2017). The PCR reactions were performed using a 50-μL reagent mix, containing 25 μL 2× Phanta Max Master Mix (Vazyme, Biotech Co., Ltd., Nanjing, China), 20-μL DNase free ddH_2O, 2-μL of each primer, and 1-μL template DNA, as suggested by the manufacturer. The PCR amplification procedure is shown in Table 1. PCR products were confirmed by 1.5% agarose gel electrophoresis, and purified using an OMEGA PCR kit (Omega, Biotek, Norcross). The purified PCR products were sequenced on an ABI 3730XL sequencer (Sangon, Biotech Co., Ltd., Shanghai). Sequence data were edited with Geneious R6.1.6 (Kearse et al. 2012) and deposited in GenBank (Table 2).

Table 1. The PCR amplification procedures.

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

For a more comprehensive phylogenetic analysis, we not only used the sequences of Psychropotidae but also used the mitochondrial sequences of Elpidiidae and two species of Molpadiidae as the outgroup (Table 2). Twenty-five COI and 18 16S sequences were aligned using MAFFT 7 (Katoh and Standley 2013) using the E-INS-I strategy. The 16S and COI alignments were concatenated into the dataset (COI/16S = 687/578 bp), while the alignment gaps and missing data were represented as '-' and '?'. Maximum likelihood (ML) and Bayesian inference (BI) analyses were performed using the concatenated dataset. JModelTest 2.1.10 (Darriba et al. 2012) was used to find the best-fit model from 88 competing models using Akaike information criterion (AIC) calculations. In each case, GTR+I+G was the best-fit model for BI analyses. MrBayes 3.2 (Huelsenbeck and Ronquist 2001) was used to conduct BI analyses. Markov Chain Monte Carlo (MCMC) iterations were run for 1, 000, 000 generations with sampling every 100 generations. The first 25% of trees were discarded as burn-in, and the consensus trees were summarized in 75% majority-rule trees. RAxML GUI 1.5 (Silvestro and Michalak 2012; Stamatakis 2014) was used to perform ML analysis with the GTR+GAMMA+I substitution model for 1000 bootstraps, as recommended by Miller et al. (2017).

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	MW992749	MW980089
	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

Results and Discussion

Morphological observations

Order Elasipodida Théel, 1882

Suborder Psychropotina Hansen, 1975
Family Psychropotidae Théel, 1882
Genus *Benthodytes* Théel, 1882

Diagnosis (according to Hansen, 1975)
Auns dorsal. Unpaired dorsal appendages absent. Circum-oral (or post-oral) papillae present. Tentacles soft, pliable, and retractile.

Benthodytes jiaolongi **sp. nov.**
(Figs 2-5)

Material examined. Catalog numbers: RSIO3710601, RSIO590504, RSIO590506 and RSIO6017101, four adult specimens, collection numbers: DY37-JL106-B01 (156.947° E, 13.017° N), DY59-ROV05-B05 (134.916° E, 16.916° N), DY59-ROV05-B05 (134.916° E, 16.933° N) and DY60-JL180-B04 (134.911° E, 16.935° N).

Diagnosis
Body elongated and subcylindrical when fixed. Skin red with violet, thin and soft. No obvious large papillae arranged on the dorsal surface. Some minute papillae, conical with tips, on the anterior dorsum. Brim narrow, thin, flattened. Mouth ventral, anus terminal. Eighteen tentacles, retracted after fixing; circum oral papillae present. Dorsal ossicles include rods and primary crosses with four arms. Rods present in tentacles. Ossicles of ventrum not observed.

Description of holotype **(RSIO6017101)**
The length of the specimen was approximately 25 cm before being preserved in 10% seawater formalin. Color was violet in life (Fig. 2C); the skin was transparent, thin, soft, and gelatinous after fixing. Brim retracted less than 0.7 cm in width. The dorsal papillae did not develop well, but approximately nineteen pairs of minute papillae were placed closely in two bands along the anterior dorsal radii. Another four single minute papillae were located on the posterior dorsal edge. Midventral tube feet with degeneration arranged in two rows, approximately 28 pairs. Mouth ventral, circum oral papillae present, anus terminal. After the specimen arrived at the sea surface, autolysis began; therefore, tentacles could not be identified (Fig. 2D). Few ossicles were observed. Dorsal ossicles contained primary crosses with spiny arms and spinous rods (Fig. 4A–F). The rods were approximately 400 µm long and the arms of the crosses were approximately 200 µm in length. Rods were presented in tentacles and the length was approximately 400–500 µm (Fig. 4G–J). Ossicles were not be found in the other body parts.

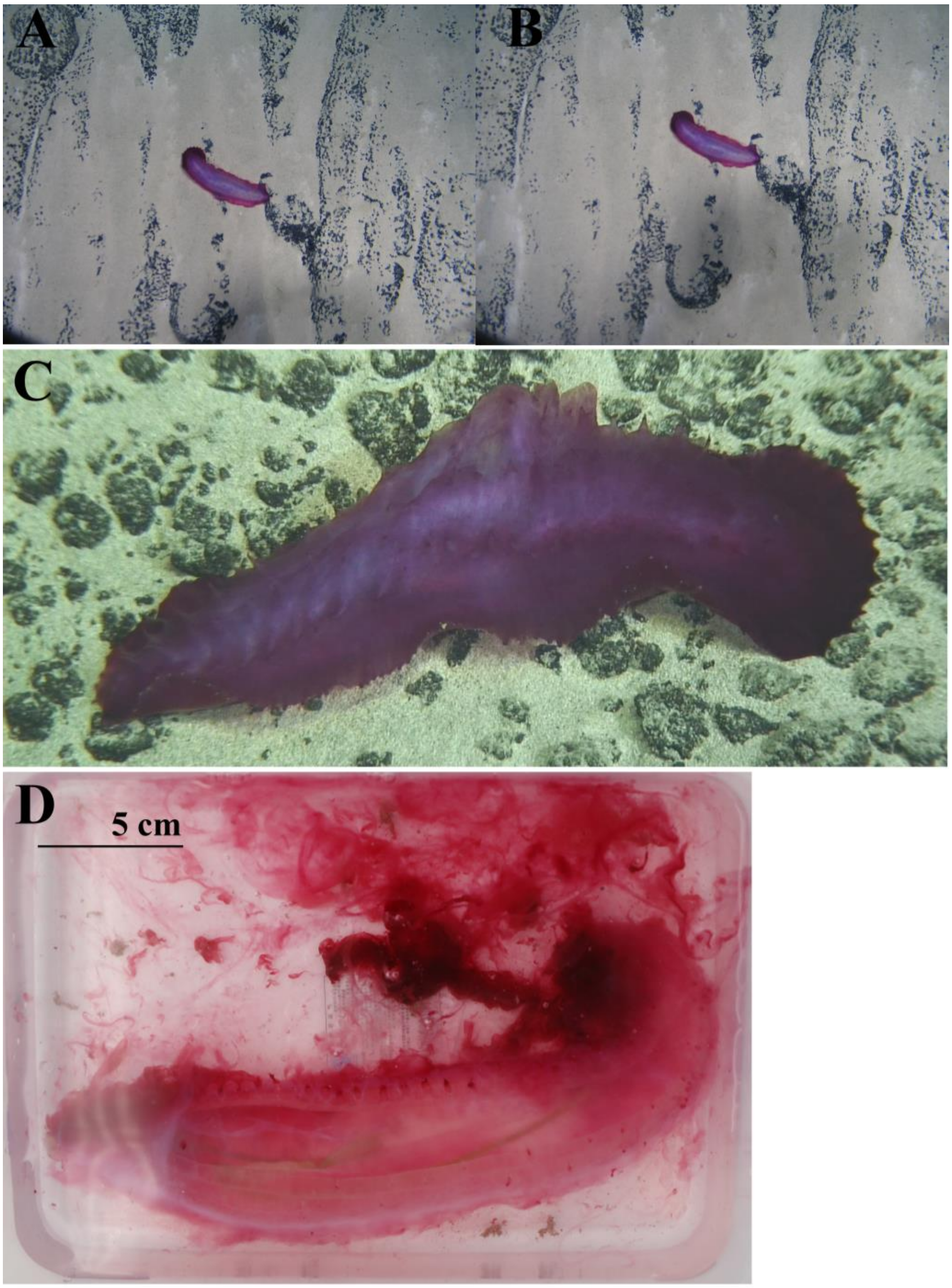

Figure 2. A-B: *Benthodytes jiaolongi* **sp.nov.** in situ on Weijia Guyot (RSIO3710601). C: *Benthodytes jiaolongi* **sp.nov.** in situ on Kyushu-Palau Ridge (RSIO6017101). D: Specimen (RSIO6017101) before preservation in 10% seawater formalin.

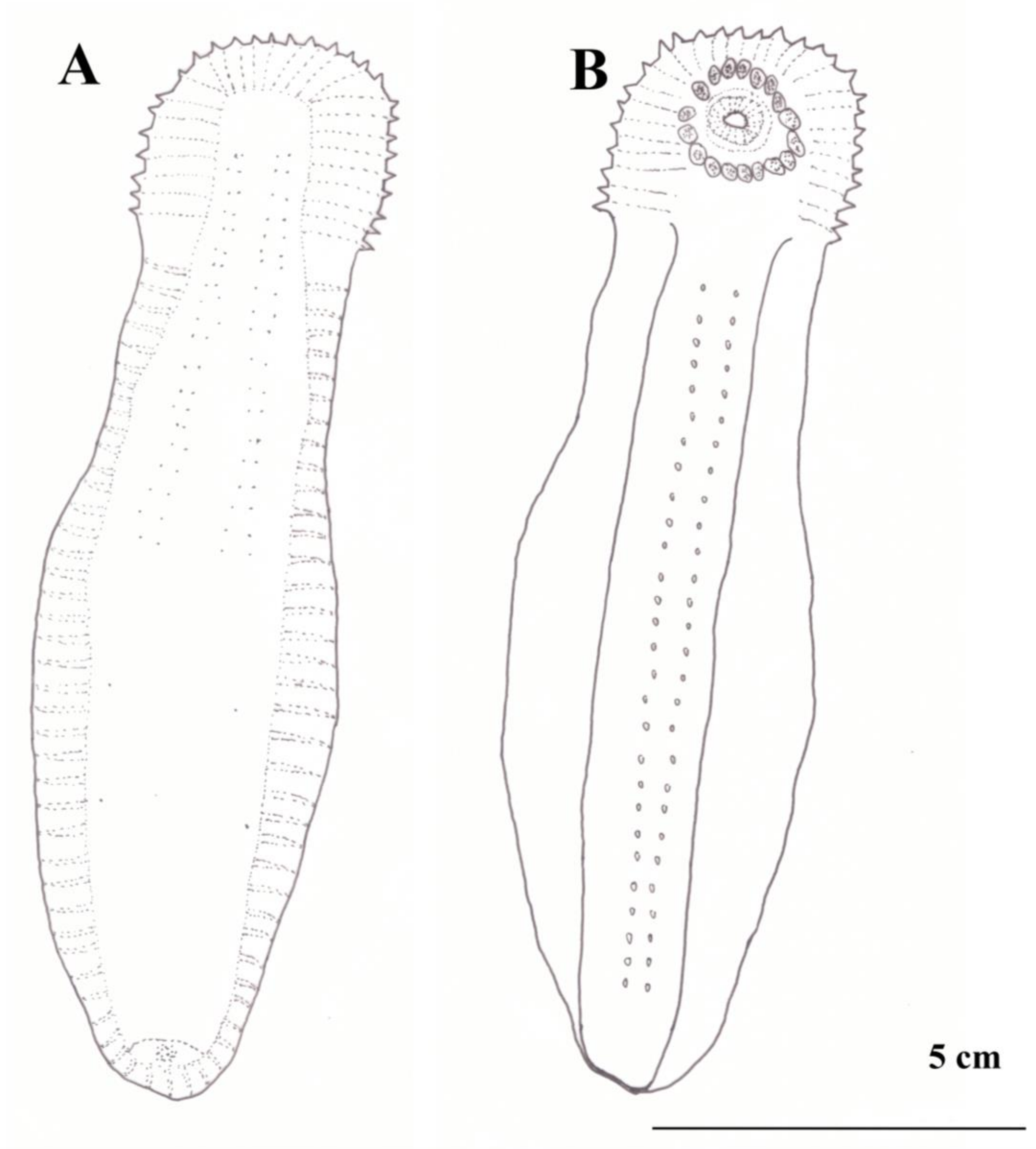

Figure 3. *Benthodytes jiaolongi* **sp.nov.** A. Dorsal view. B: Ventral view.

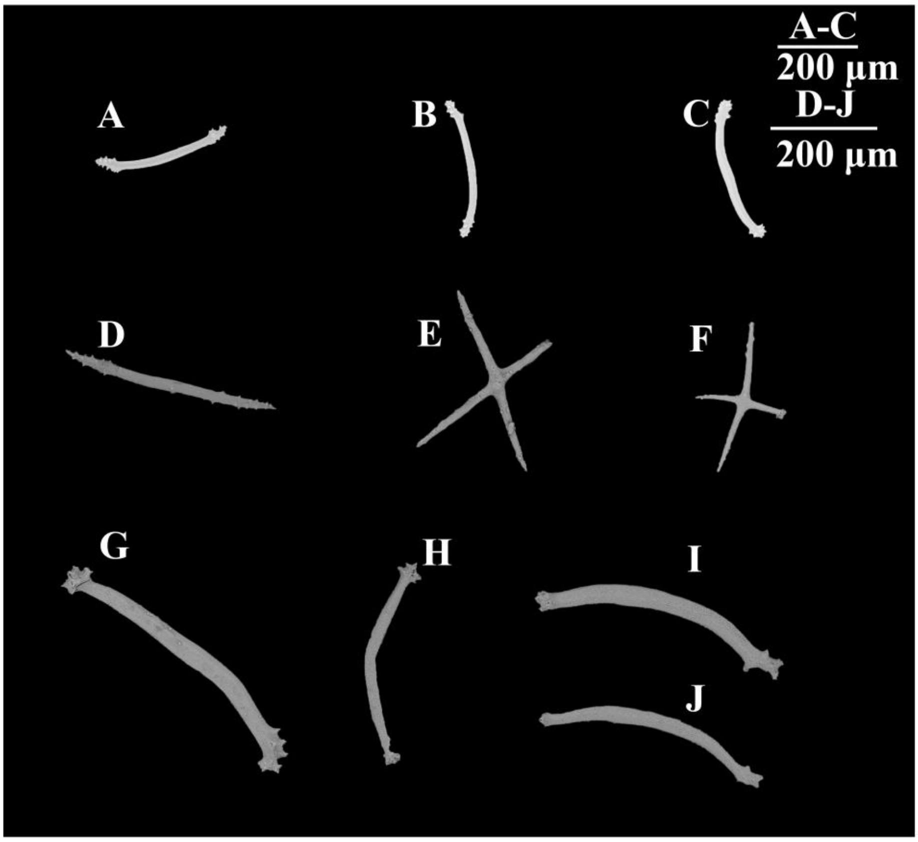

Figure 4. A-C: Scanning electron micrographs of dorsal body wall ossicles from *Benthodytes. jiaolongi*, RSIO590506. D-F: Dorsal body wall ossicles from *Benthodytes.jiaolongi*, RSIO6017101. G-J: Ossicles of tentacles.

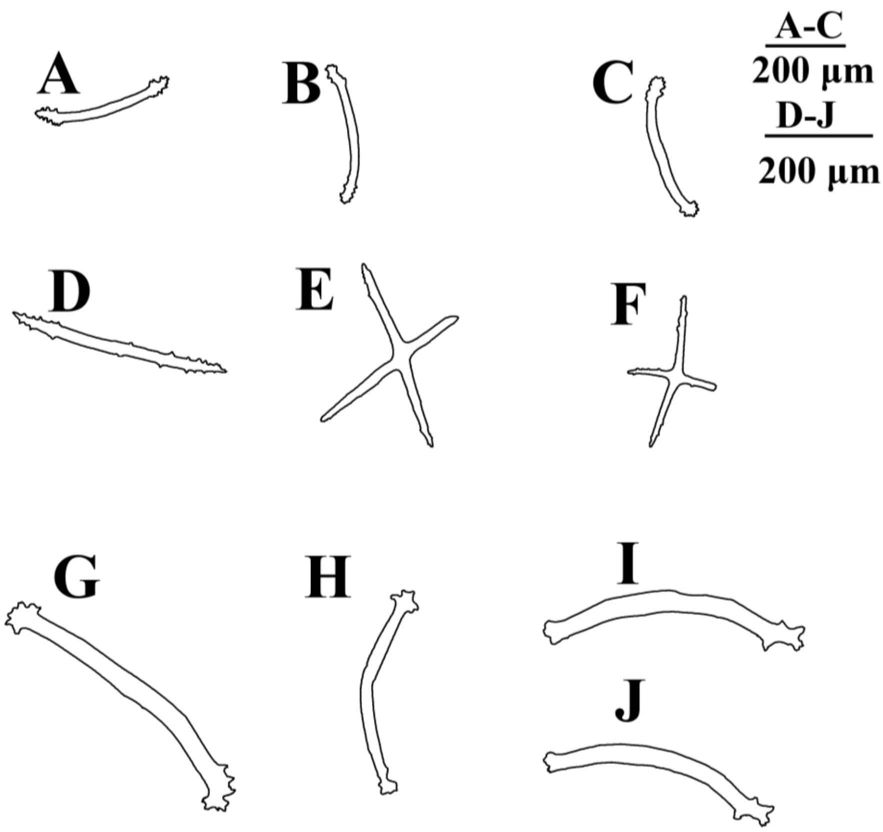

Figure 5. *Benthodytes jiaolongi* **sp.nov.** ossicles from, A-F: dorsal body wall, G-J: tentacles.

Description of paratype. RSIO3710601.The specimen was approximately 22 cm in length and 5 cm wide at the maximum point. Color was red-violet at the seabed (Fig. 2A, B); after arriving at the sea surface, it became pale violet, and the skin appeared transparent, with a white color after preservation in 10% seawater formalin for 5 years. The normal paired dorsal papillae were absent, and the minute papillae could not be distinguished. Owing to the long-term preservation, the quantity of the midventral tube feet could not be determined, but the tube feet were arranged in two rows; the brim could not be distinguished. Mouth ventral, with circum-oral papillae, anus terminal. Eighteen tentacles, were retracted to the stalk. Ossicles were not observed in the specimen.

RSIO590504.The specimen was approximately 22 cm in length before being preserved in 10% seawater formalin. Color was red-violet on the deck and the skin appeared transparent, with a white color after preservation in 10% seawater formalin. During sampling, the specimen was accidently stained with sponge, meaning that the tentacles could not be determined and the dorsal tips could not be distinguished. Midventral tube feet were identified but the quantity could not be determined. Mouth ventral, anus terminal. Ossicles were not observed in the specimen.

RSIO590506. Specimen was approximately 13 cm in length before being preserved in 99% alcohol and was heavily damaged. Color was red-violet after arriving at the sea surface, and the skin appeared transparent. The specimen was stained with sponge as was RSIO590504 and many external character could not be distinguished. Mouth ventral, anus terminal. Few rods were observed on the dorsal body (Fig. 4A-C). Rods were approximately 400 µm and the spine was terminal. Ossicles were not observed in other the body wall.

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

Type locality. Kyushu-Palau Ridge, tropical Western Pacific. Depth: 2453–2692 m, muddy sediments, July 20, 2020 and January 21, 2021, Ruiyan Zhang, Bo Lu.

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

Remarks. According to the taxonomy of Hansen (1975), *Benthodytes jiaolongi* **sp. nov.** was close to *Benthodytes sanguinolenta* Théel, 1882 and *Benthodytes typica* Théel, 1882.

B. typica was described by Théel in 1882 based on the Challenger Expedition, and was the type species of *Benthodytes*. The original description indicated approximately eight, minute, retractile processes located on each of the dorsal ambulacra and unbranched spinose calcareous spicula scattered on the integument. Hansen (1975) re-examined *B. typica* and reported that the specimens showed considerable variation. The specimens from station 575 showed three or four pairs of minute dorsal papillae placed along the anterior part of the radii, and rods were scattered in the ventrum, dorsum, and tentacles. The specimens from the Bay of Bengal usually possessed three pairs of dorsal papillae on the anterior body, but deposits were not found. Most specimens from the Mozambique Channel did not show the the papillae and six or seven pairs of minute dorsal papillae were presented in the specimens from the northern Indian Ocean.

Benthodytes papillifera Théel, 1882 was identified based on 13 specimens taken from three Pacific Challenger stations. Théel described this species as being similar to *B. sanguinolenta* based on the tentacles and pedicels. Some minute papillae, considered as prominences of the integument, covered the dorsal surface and each papilla contained one or more spicula. Hansen (1975) re-examined specimens from each of the stations and proposed that the variation in *B. papillifera* represented the geographic variation of *B. typica*. In the original description of *Benthodytes glutinosa*, Perrier (1896) indicated that the differences from *B. typica* were the more elongated shape and the complete absence of dorsal papillae. Hansen (1975) considered this species to be a synonym of *B. typica*.

In general, the morphological features of *B. typical* can be summarized as: 3–7 pairs of minute papillae arranged on the dorsal surface, and rods scattered on the body integument and tentacles. *B. jiaolongi* differed from *B. typica* in its arrangement and number of dorsal papillae and by the ossicles. The dorsal minute papillae of *B. jiaolongi* were arranged in two bands along the anterior dorsal ambulacra, and those of *B. typica* were arranged in a row with 3–7 pairs of papillae. The ossicles of *B. jiaolongi* were only presented in the tentacles and dorsum with rods and primary crosses, but *B. typica* only possessed rods scattered on the ventrum, dorsum and tentacles.

The characteristics of *B. sanguinolenta* as described by Théel (1882) were many minute retractile processes scattered on the dorsal surface and dissolved calcareous deposits. According to a re-examination by Hansen (1975), the dorsal minute papillae were arranged in two radial bands, and the rods only presented on the midventral tube feet and tentacle discs within specimens from station 663. Rogacheva et al. (2009) recorded *B. sanguinolenta* and the main characteristics can be described as: minute dorsal papillae arranged in two bands or between the two bands; approximately 1–4 papillae placed in a band, narrowing to one or two papillae at the posterior end; ossicles were not found. The differences in the characteristics between the new species *B. jiaolongi* and *B. sanguinolenta* can be listed as follows: (1) The dorsal papillae of *B. sanguinolenta* are arranged into two bands, whereas those of the new species were arranged in two rows on the anterior dorsal ambulacra. (2) The ossicles of the new species were only presented on the tentacles and dorsum with rods and primary crosses, whereas the ossicles were not found in *B. sanguinolenta*.

Genus *Psychropotes* Théel, 1882

Psychropotes verrucicaudatus
(Figs 6-8)

Psychropotes verrucicaudatus Xiao, Gong, Kou & Li, 2019:421-430.

Material examined. Catalog numbers: RSIO6017006 and RSIO6018004, Two adult specimens, collection numbers: DY60-JL170-B05 (134.860° E, 12.079° N) and DY60-JL180-B04 (134.352° E, 13.569° N).

Description

RSIO6018004. The external morphology of the specimen resembled a barbell after collection and was approximately 20 cm in length before being preserved in 10% seawater formalin (Fig. 6C, D). Before preservation, the height of the appendage was approximately 50 mm, and the width was approximately 30 mm at the base (Fig. 6C, D). The dorsal skin was transparent with a brownish red color on the seabed and dark brown on the deck. Warts covered the dorsal skin and appendage, and the giant ossicles can be seen in the warts (Fig. 6E-F). Approximately 30 pairs of tube feet were arranged in two rows along the middle of the ventrum were degenerated. Sixteen tentacles were formed in a circle. The brim was broad and covered with warts on the dorsum.

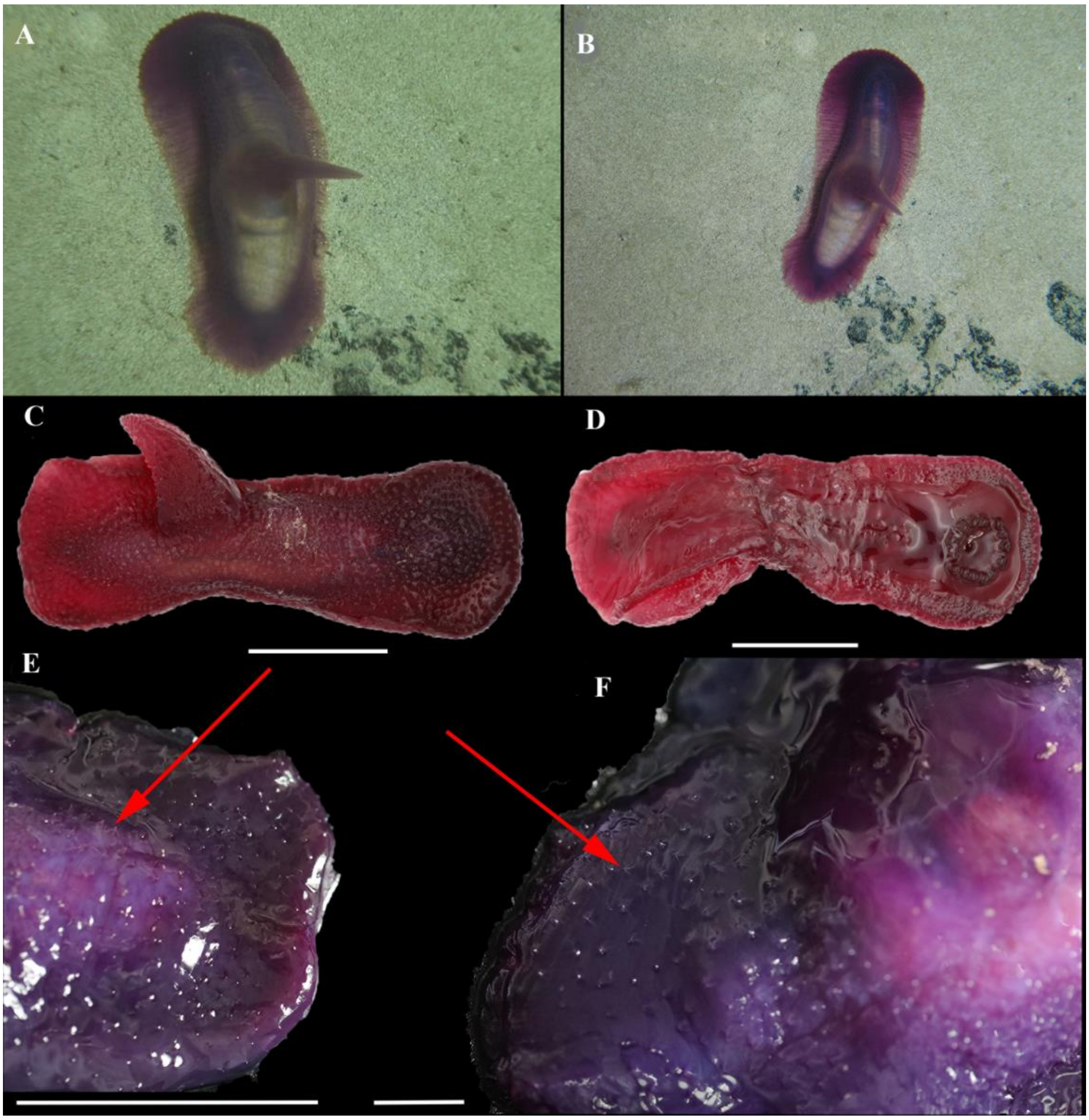

Figure 6. A-B: species in situ. C-D: Specimen before preservation. Scale bar: 5 cm. E-F: Red arrow points to the giant ossicles, specimen after preservation in 10% seawater formalin. Scale bar: 5 cm (C, D, E) and Scale bar: 1 cm (F).

A giant cross with four arms could be seen in each wart. The arms were 800–1000 µm in length, and the maximum width between the large arms was approximately 500 µm. The arm flexion (presented by the length close to long arms / the length close to short arms at the intersection of the diagonal) was approximately 250 / 400 µm (Fig. 7A–D). In addition, the height of the central rudimentary apophyses was approximately 200–300 µm. Ventral ossicles can be divided into two types: primary cross with spiny arms and cross with three arms (Fig. 8A–C), and the length of each arm was approximately 200 µm. The primary crosses with spinous arms were present in the dorsum (Fig. 8D–F) and brim (Fig. 8H–J), and the length of the arms was up to 200 µm. Furthermore, dorsal ossicles possessed a spinous rod 170 µm in length (Fig. 8G), and large primary crosses with spiny arms were present in the brim (Fig. 8K). Ossicles of tentacles were rods with an irregular shape (Fig. 8L–R). The large rod with two apophyses at the end was approximately 900–1000 µm in length; the small rod with apophyses in the middle area was approximately 200 µm in length. Other rods with spiny arms were 500–800 µm in length.

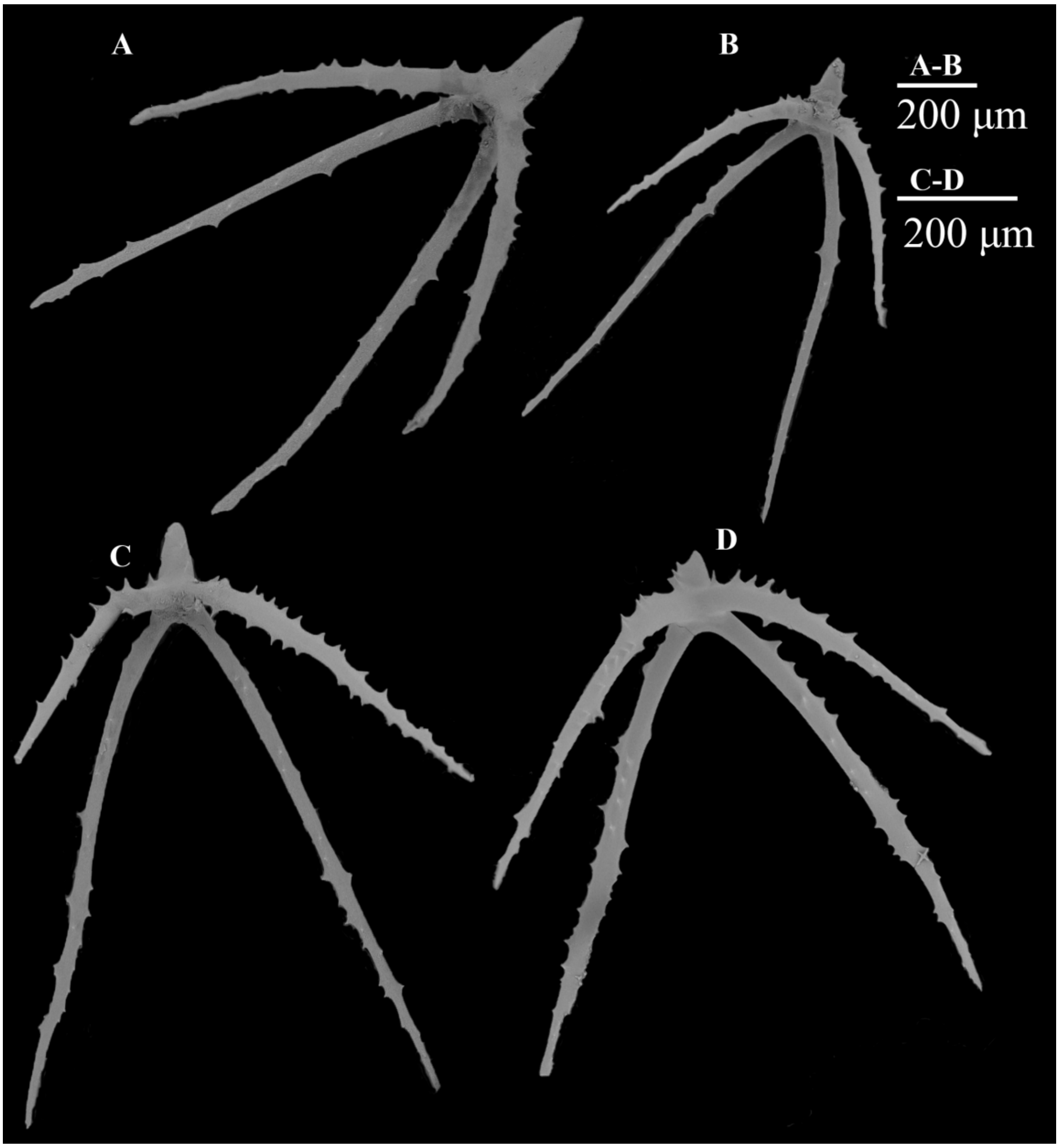

Figure 7. *Psychropotes verrucicaudatus*, A-D: The giant ossicles from the dorsal warts.

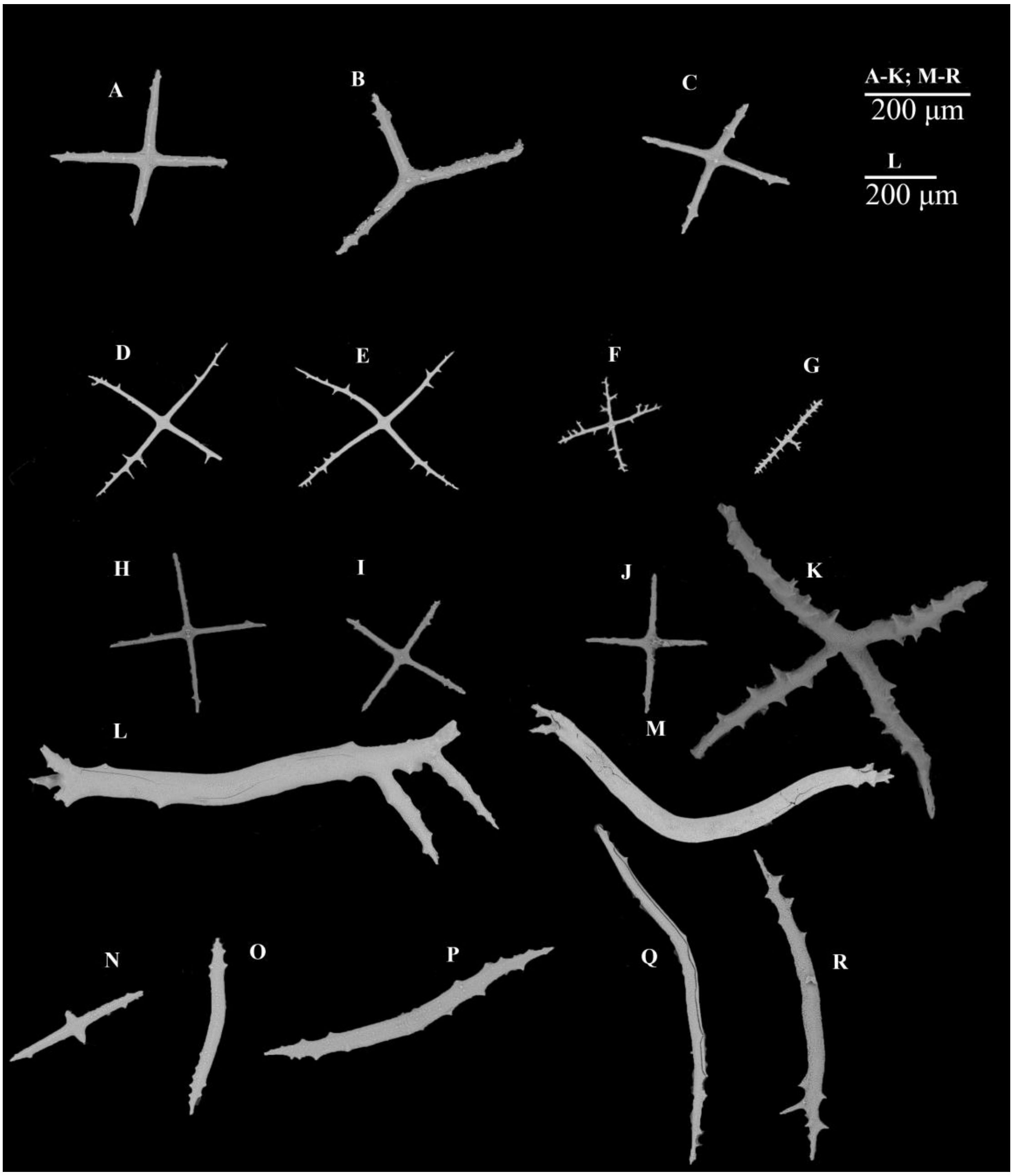

Figure 8. *Psychropotes verrucicaudatus* ossicles from, A-C: ventral body wall; D-G: dorsal body wall; H-K: brim; L-R: tentacle.

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 RSIO6018004.

Type locality. Jiaolong Seamount, South China Sea, western Pacific Ocean, sandy bottom, depth 3615 m.

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

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

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

Phylogenetic analyses

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

To obtain clearer phylogenetic relationships, we concatenated 25 COI and 18 16S sequences into a dataset to build the ML and BI trees. Although the genetic sequences were limited, the topological structures of the ML and BI trees supported the classification status of *B. jiaolongi* **sp. nov.** and *P. verrucicaudatus* (Fig. 9). The phylogenetic relationships of Psychropotidae clustered into four parts and were inconsistent with the traditional classification system based on Hansen (1975). *Benthodytes* were paraphyletic in Psychropotidae and the new species was located in the outmost clade of Psychropotidae. In addition, *Psychropotes* was a sister group to *Psycheotrephes*, and the clade of *B. jiaolongi* **sp. nov**. and *B. sanguinolenta* were placed in the lateral clade of the sister group. *P. verrucicaudatus* showed a paraphyletic relationship with *Psychropotes* and was clustered in the clade of *Psycheotrephes exigua*. Elpidiidae clustered into two clades. *Peniagone* clustered together into a supported group, but *Peniagone diaphana* was a sister group to other *Peniagone* species. The other four genera of Elpidiidae clustered into a group, and *Elpidia glacialis* was distant from the other three genera. *Protelpidia murrayi* and *Scotoplanes* sp. TT 2017 were closer to each other, and *Amperima roubusta* was away from the two genera. Laetmogone was an obvious polyphyletic group, and *Pannychia moseleyi* was placed in the outmost clade of the other three families. *Laetmogone wyvillethomsoni* clustered with Elpidiidae and was situated outside; *Benthogone abstrusa* was clustered with *Enypniastes eximia*, but the possibility of a clade was low.

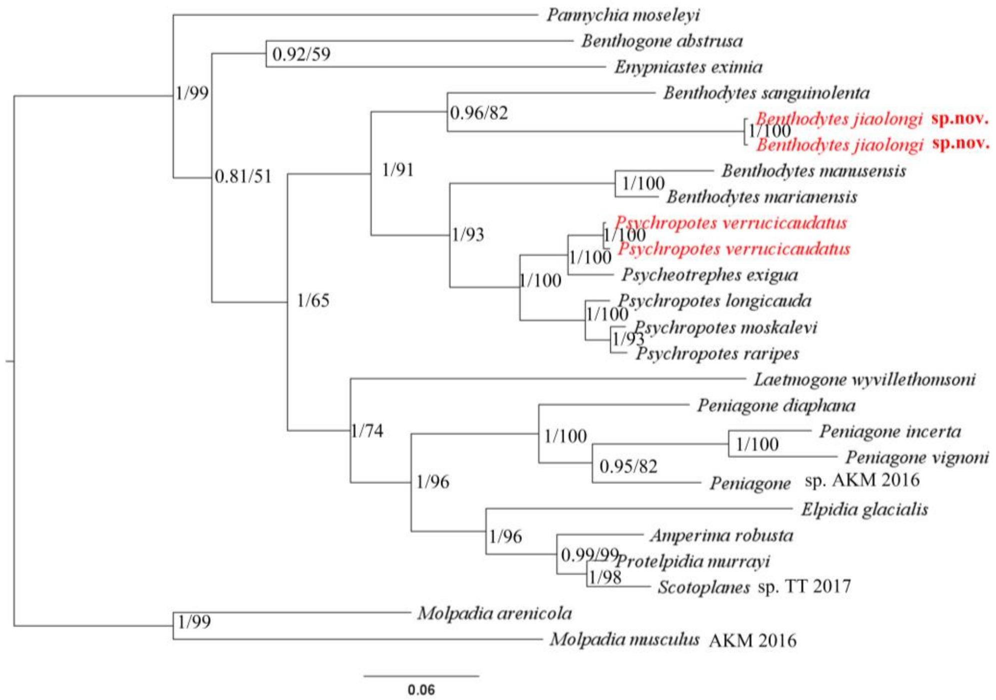

Figure 9. Bayesian inference (BI) and maximum likelihood (ML) trees based on concatenate sequences. The values are BI/ML at each node.

Based on the morphological and phylogenetic analyses, *B. jiaolongi* **sp. nov.** can be identified as a new species closely related to *B. sanguinolenta*. In addition, our specimens provided a new record of *P. verrucicaudatus* in the Western Pacific, broadening its distribution. Our results clarify that *Benthodytes* is paraphyletic and that the clade of *B. sanguinolenta* and *B. jiaolongi* **sp. nov.** is separated from the other species of *Benthodytes*.

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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

Perrier R (1896) Sur les Élasipodes recueillis par le Travailleur et le Talisman. Comptes rendus hebdomadaires des séances de l'Académie des sciences 123(21):900–903.

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 postanalysis 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