**Benthodytes marianensis**, a new species of abyssal elasipodid sea cucumbers (Elasipodida: Psychropotidae) from the Mariana Trench area

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**Abstract**

*Benthodytes marianensis* sp. nov., is described from the Mariana Trench at the depth of 5567 m. This is the thirteenth species in *Benthodytes* Théel, 1882. The new species has a broad brim around the body, dark violet skin, 9 pairs of large dorsal papillae with other papillae of equal or smaller size situated elsewhere, forming two zigzag rows, and some minute papillae scattered around the anterior region. Body wall ossicles are rods, crosses with three or four arms and central apophyses, and crosses of an unusual type. Crosses with four arms and central bipartite, tripartite and tetrapartite apophyses and peculiar cross-shaped ossicles with high apophyses ending in two horizontal arms are present in the dorsum. Large rod-shaped ossicles with bipartite apophyses and crosses with three or four arms and rudimentary apophyses are present on the ventrum. *B. marianensis* is morphologically most similar to *B. incerta*, but it is distinguished from *B. incerta* by the number and arrangement of dorsal large papillae, the broad brim and details of ossicle morphology. The phylogenetic analyses based on (16S, COI) and nuclear genes (H3) all confirm the morphological identification.

**Key words:** deep-sea holothurians, new species, Mariana Trench, abyssal zone, phylogenetic analysis

**Introduction**

The deep-sea Psychropotidae (Elasipodida) are among the least well-understood holothurians in the world’s oceans (Rogacheva et al. 2009). The family comprises three genera, *Benthodytes*, *Psycheotrephes*, and *Psychropotes*, all erected by Théel in 1882. *Benthodytes* is significantly different from others in having a dorsal anus, emerging circum-oral papillae, soft, pliable, and retractile tentacles, and lacking unpaired dorsal appendages. *Benthodytes* is cosmopolitan and occurs at bathyal and abyssal depths. According to WoRMS (WoRMS, 2018), this genus contains 12 valid species: *Benthodytes abyssicola* Théel, 1882; *B. gosarsi* Gebruk, 2008; *B. incerta* Ludwig, 1894; *B. lingua* Perrier R., 1896; *B. plana* Hansen, 1975; *B. sanguinolenta* Théel, 1882; *B. sibogae* Sluiter, 1901; *B. superba* Koehler & Vaney, 1905; *B. typica* Théel, 1882; *B. valdiviae* Hansen, 1975; *B. violeta* Martinez, Solís-Marín & Penchasadeh, 2014 and *B. wolffi* Rogacheva & Cross in Rogacheva et al., 2009.

Studies on *Benthodytes* have been few due to the general paucity of material (Fujikura, et al. 2012; Gebruk, 2008; Gebruk, et al. 1997; Hansen, 1975; Paolo, 1984; Théel, 1882). The status of many species in this genus remains unclear. Despite this, Hansen (1975) has provided an excellent revision of *Benthodytes*. With the development of technology in deep-sea observation, more and more deep-sea holothurians have been well observed in situ and new species have been discovered (Colgan et al. 1998; Jamieson, et al. 2011; Kuhnz, et al. 2014; Martinez, et al. 2014). In this study, we describe a new species *Benthodytes marianensis* sp. nov. from the Mariana Trench, and provide morphological and molecular data (mitochondrial and nuclear sequences). In addition, we analyze the phylogenetic position of this species within Elasipodida.
Material and methods

Sampling and morphological observations. The sample was collected from the Mariana Trench (11°47.975’N, 142°6.906’E) at a depth of 5567 m from a muddy bottom, during the 37th cruise of China Ocean in June 2016. The specimen was immediately fixed in 95% ethyl alcohol after landing. The living condition in situ was recorded using high definition video camera during the Jiao Long submersible dive. Some details were observed under stereomicroscope. Ossicles were prepared for scanning electron microscopy (SEM), small pieces of the body wall were digested in 15% sodium hypochlorite solution, washed with distilled water several times and then in ethanol 95% and air dried. Finally, ossicles were coated with gold using a Hitachi E-1010 ion sputter coater and observed with a Hitachi S-3400N SEM. The Holotype, catalog number IDSSE-2016-0629-HS01, is preserved in 95% ethanol and deposited at the Institute of Deep-sea Science and Engineering, Chinese Academy of Sciences, Sanya (IDSSE).

PCR amplification and phylogenetic analysis. Total genomic DNA was extracted from about 30 mg of sea cucumbers muscle tissue using TIANamp Marine Animals DNA kit (TIANGEN, China) and purified using ZYMO Genomic DNA Clean & Concentrator kit (ZYMO, USA) according to the manufacture’s protocols. Two mitochondrial fragments of 16S rRNA and COI gene were amplified using primers: 5’-CCGCGTTTATCAAAAAAT-3’ (16SAR), 5’-CTCCGGTTTGAACCTGATCA-3’ (16SBR) (Kerr et al. 2005) and 5’-ACTGCCACGGCTAGTATGATATTTTTTATGGTNGC-3’ (COIce-F), 5’-TCGTTGCTTACGTTTACGTGRTAATG-3’ (COIceR) (Amin, et al. 2016), respectively. The nuclear gene of H3 was amplified using primers: 5’-ATGGCTCGTACCAAGCAGATG-3’ (H3a) and 5’-ATATCCTRGGCATRATRGTGAC-3’ (H3b) (Colgan et al. 1998). PCR amplification consisted of an initial denaturation step of 95° for 5min, followed by 35 cycles of 94° for 45 s, 50° for 1 min for 16S (50° for COI and 48° for H3) and 72° for 1 min, and a final extension step of 72° for 10min in a 25 µl reaction volume, employing Premix Taq™ version 2.0 DNA polymerase (TaKaRa, Japan) as suggested by the manufacturer. Amplified fragments were sequenced and submitted in GenBank. Our obtained sequences of *Benthodytes marianensis* sp. nov. and the relevant sequences selected from GenBank were aligned using Clustal W. Phylogenetic analysis was constructed using neighbour-joining (NJ) and maximum likelihood (ML) methods by MEGA7 software with Kimura’s two-parameter model and General Time Reversible model, respectively. The bootstrap replicates were set as 1000.

Systematics

Order Elasipodida Théel, 1882

Suborder Psychropotina Hansen, 1975

Family Psychropotidae Théel, 1882

Genus *Benthodytes* Théel, 1882

*Benthodytes marianensis* sp. nov.

(Figures 1–3)

**Diagnosis.** Body long, dorsum inflated, ventrum flattened, anteriorly depressed and tapered slightly posteriorly. Skin dark violet, soft, thick, wrinkled. Dorsal papillae 9 pairs, large, conical, with filiform tips, retracted, in two uneven rows along the dorsal radii. Some minute papillae scattered anteriorly. Some equal or smaller papillae often situated outside the larger papillae. Anus dorsal terminally, mouth ventral but a certain distance from the anterior edge. Brim well developed, with dark violet channels. Tentacles, at least twelve, retracted into stalks. Dorsal ossicles cross-shaped with central bipartite, tripartite, or tetrapartite apophyses. Sparse cross-shaped ossicles with high and spinous apophyses which end in two horizontal branches with downwardly directed spines on the margin, are in the dorsum. Ventral ossicles rod-shaped with central bipartite apophyses and crosses of three or four arms with central rudimentary apophyses. Brim ossicles sturdy and spinous, tripartite with bipartite apophyses and
cross-shaped with tetrapartite or pentapartite apophyses. Apophyses, when divided, present at the basal region. Tentacle ossicles large rods.

**Material examined.** Holotype: 1 adult specimen, catalog number IDSSE-2016-0629-HS01, Genbank accession numbers MH049433, MH049434, MH049435. St. JL-Dive121-S01, Mariana Trench area, 11°47.975’N, 142°6.906’E, 5567 m, muddy sediments, 29 June 2016, coll. Haibin Zhang.

**Description of the holotype.** As the specimen was seriously damaged during collection, the exact numbers of tentacles, circum-oral papillae and mid-ventral tube feet were difficult to determine. The present description was mainly derived from the living individual recorded by HD video.

The specimen was about 45cm in length and 15cm in width in freshly caught condition and 15cm in length and 7cm in width after preservation in 95% alcohol for several days.

Body about three times as broad, dark violet and semicircular in cross-section, flattened at the anterior end, inflated in following region, and peaked slightly at posterior end (Fig.1). Anus dorsal. Skin soft, thick, gelatinous, covered with mucus. Dorsal papillae broadly conical with filiform tips, retractile, arranged almost symmetrically in two rows along the radii, with some minute papillae confined to the anterior (Fig.1C). In each row are 9 large papillae, often with equal or smaller ones situated elsewhere. Paired and smaller papillae render the dorsal skin wrinkled and uneven-coloured. Brim thin, usually bent upwards, well developed, and strongly pigmented with canals. Brim strongly expanded anteriorly, with retracted water-vascular canals forming serrated margin (Fig.1A). Mouth ventral, a short distance from the anterior edge. Tentacles, at least twelve, retracted into the tentacle stalks and forming a large and flat oral disc.

**FIGURE 1.** A: Individual *in situ*. The black arrow indicates flattened and serrated anterior brim. The white arrow indicates filiform tips of dorsal papillae. The yellow arrow points at anus. B: Specimen before preservation in 95% alcohol. The arrow indicates almost retracted papillae. C: Dorsal view.

After preservation in 95% alcohol for several days, skin became reddish violet but tentacles dark violet. Skin is covered with a very superficial pigmented layer and dorsal papillae almost disappear or retract into the thick skin.
FIGURE 2. SEM images of ossicles. A: the dorsum, arrow indicates the peculiar cross-shaped ossicles. B: the ventrum, arrow represents the large rod-shaped. C: the brim. D: tentacles. Scale 100 µm.

Ossicles in dorsal body wall cross-shaped, of two types. One type is about 190–300 µm in width with central spinous, bipartite, tripartite or tetrapartite apophyses. Each arm is 90–180 µm in length, slightly curved downwards tapered distally. Spines on arms commonly distributed on distal part, or throughout the arms. The other dorsal ossicle type is peculiar, cross-shaped, and uncommon. The arms are about 100µm in length, with multiple spines, and curve downwards. The central apophysis with spines is longer than arms of the cross and ends in two horizontal arms which possess downwardly vertical spines along their edge (Fig.2A, the ossicle with arrow; Fig.3f). Ossicles in ventral body wall include rods, and crosses with three and four arms. The rods are about 500 µm in length with large bipartite apophyses situated slightly off the central. Crosses of three and four arms are
about 230–410 µm and 260–320 µm, respectively, and both possess central rudimentary apophyses (Fig.2B). Ossicles in brim comprise tripartite and cross-shaped types with less curved arms. The tripartite type is about 290–375 µm across with a large and strong central bifurcate apophysis. The cross-shaped type is 240–330 µm, usually with very large, spinous central tetrapartite and sometimes pentapartite apophyses. Compared to ossicles in body wall, brim ossicles usually develop rather robust and larger spines on arms and apophyses (Fig.2C). The division sites of apophyses in *B. marianensis* all occur at the basal region. Ossicles in tentacles are large rods about 830 µm in length with spines only in their distal part (Fig.2D).

**FIGURE 3.** *Benthodytes marianensis* sp. nov. dorsal ossicles. a–c: lateral view. d: ventral view. e: dorsal view. f: lateral view of the peculiar cross-shaped ossicle. Scale 50 µm.
FIGURE 4. Neighbour-joining (NJ) tree based on 16S rRNA sequences. The bootstrap values are NJ/ML at each node and the values below 50% were cut off.

FIGURE 5. Neighbour-joining (NJ) tree based on COI sequences. The bootstrap values are NJ/ML at each node and the values below 50% were cut off.

**Relationship.** *Benthodytes marianensis sp. nov.* is unique in the number and arrangement of dorsal papillae: 9 pairs of large broadly conical papillae with equal or smaller papillae situated outside in two zigzag rows. Dorsal deposits most closely resemble those of *B. incerta* Ludwig, 1894.

**Etymology.** This species is named after the location (Mariana Trench) where the species was discovered.

**Habitat.** Muddy sediments.

**Distribution.** Known only from the type locality of the Mariana Trench, western Pacific Ocean, Depth 5567 m.

**Remarks.** Morphologically, *B. marianensis* is very close to *B. incerta* Ludwig, 1894. They share two rows of large dorsal papillae, and peculiar ossicles (Fig. 2A, the ossicle with arrow). *B. incerta* was described by Ludwig in
1894 based on two specimens from the eastern Pacific (one large and one small). The original description lacked illustrations of dorsal ossicles. Also, it was not clear if the type specimens had the peculiar type of ossicles. The papillae ossicles of the type specimens are cross-shaped with high apophyses and the apophyses are bipartite at the top. In addition, there are long side branches on the arms of ventral ossicles and in the high apophyses of papillae ossicles. In contrast, in *B. marianensis*, the apophyses are bipartite, tripartite and tetraptartite at the base part. The long side branches are absent in the ossicles of the Mariana specimen.

Hansen (1975) re-examined type specimens and assigned his *Galathea* specimens to *B. incerta* based on external morphology. The ossicles in the *Galathea* specimens are larger and more regular than those in the type specimens. The Mariana specimen and Hansen's *Galathea* specimens differ in the following features: (1) In *Galathea* specimens, the crosses are much larger than those of Mariana specimen. (2) In *Galathea* specimens, the apophyses of crosses are bipartite at least half the length; in Mariana specimen, the apophyses are bipartite, tripartite or tetraptartite at the base part. (3) In *Galathea* specimens, the apophyses of the peculiar ossicles are smooth; in Mariana specimen, the apophyses of the peculiar ossicles are spinous.

*Benthodytes gotoi* Ohshima, 1915 is known from four specimens, taken in the Okhotsk Sea. Hansen (1975) considered this species to be a synonym of *B. incerta*. The crosses of Ohshima's specimens differ from those of Mariana specimen in their large size. The arms of the crosses are usually 300–800 µm, and the apophyses of crosses are usually bipartite for one-half of its length. In the Mariana specimen, the arms of the crosses are usually 90–180 µm, and the apophyses of crosses are bipartite, tripartite or tetraptartite at the base part.

In addition, the Mariana specimen differs from the descriptions of *B. incerta* and its synonym *B. gotoi* in number and arrangement of dorsal large papillae: In *B. incerta*, there are 6–9 (5–8) large papillae in a single row along each dorsal ambulacrum, while in Mariana specimen there are 9 papillae with equal or smaller ones situated elsewhere, forming a zigzag row. Therefore, based on the external morphology and internal ossicles, the Mariana specimen can be assigned to a new species.

16 16S rRNA gene sequences, 14 COI and 14 H3 gene sequences were selected from GenBank, including almost all species in the Elasipodida especially those in the Psychropotidae. The topological structures of NJ and ML tree were all highly similar to each other based on these genes separately (Fig.4–6). The phylogenetic analysis based on mitochondrial (16S and COI) and nuclear genes (H3) were all consistent with the result of morphological identification, embedding the new species within the Psychropotidae clade. However, *Benthodytes* was paraphyletic according to mitochondrial genes (Fig.4, 5), which is inconsistent with the traditional classification system. The results showed poor support, likely due to the limited sequences available for the Psychropotidae. Sequences obtained in the present study will enrich the database and provide more data for future research.

**FIGURE 6.** Neighbour-joining (NJ) tree based on H3 sequences. The bootstrap values are NJ/ML at each node and the values below 50% were cut off.
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