

A New Species of the Genus *Ophiomonas* Djakonov (Echinodermata: Ophiuroidea: Amphilepididae) from the Deep-Sea of Japan

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A new species, *Ophiomonas shinseimaruae*, is described based on five specimens collected from deep water settings, southeast of Cape Erimo, Hokkaido, Japan. *Ophiomonas shinseimaruae* sp. n. is distinguished from other congeners based on the following characters: elongate semi-circular and separated radial shields; triangular oral shields; flat and broad tentacle scales on the second tentacle pore; octagonal dorsal arm plates, approximately three times wider than long on proximal portion of the arm; and three arm spines present proximally on the arm. This is the first record of the genus *Ophiomonas* from Japanese waters. The COI nucleotide sequence for *Ophiomonas shinseimaruae* sp. n. is provided.

Key words: Brittle star, COI, DNA barcoding, Abyssal zone, Gnathophiurina, Taxonomy, *Ophiomonas shinseimaruae* sp. n.

BACKGROUND

The Ophiuroidea (Echinodermata) consists of approximately 2,100 extant species, which have been recorded from intertidal to abyssal depths (3,000–6,000 m, e.g., Priede, 2017). However, only 116 species have been recorded from depths greater than 3,500 m (Stöhr et al. 2012), due to the bias in sampling from deep-sea versus shallow water settings (Stöhr et al. 2012). In Japanese waters, only 11 of the ~350 known species have been collected from waters deeper than 3,000 m: *Amphilepis tenuis* Lyman, 1879, *Amphiophiura sculptilis* (Lyman, 1878), *Astrodia abyssicola* (Lyman, 1879), *Ophiacantha bathybia* H. L. Clark, 1911, *Ophiomusa granosum* (Lyman, 1878), “*Ophiophthalmus*” *normani* Lyman, 1879, *Ophiosphalma cancellatum* (Lyman, 1878), *Ophiura bathybia* H. L. Clark, 1911, *Ophiura quadrispina* H. L. Clark, 1911, and *Ophiura leptoctenia* H. L. Clark,

1911, *Silax cernuus* (Lyman, 1879) (Lyman 1878 1879; Irimura 1990 1991; Fujita et al. 2009; Okanishi 2016). Therefore, increased taxon sampling at these greater depths (> 3,000 m) will contribute substantially to our understanding the diversity of ophiuroids in Japanese waters.

Ophiomonas (Echinodermata: Ophiuroidea) is a rarely encountered deep-water genus. It contains only three species, each of which has only been collected on a single occasion: *O. bathybia* Djakonov, 1952 from off the Kamchatka Peninsula, at 4,161 m deep; *O. protecta* Koehler, 1904 from the Gulf of Boni, Sulawesi Island, Indonesia, at 1,158 m deep; and *O. remittens* Koehler, 1922 from off Buru Island, Indonesia, at 1,280 m deep (Koehler 1904 1922; Djakonov 1952). The genus has never been recorded within Japanese waters until now.

In this study, five specimens of *Ophiomonas* were collected from abyssal depths (3,143–3,176 m) southeast of Hokkaido, Japan. They were identified as

an undescribed species based on their morphology, and molecular sequences of the *COI* barcoding region were obtained.

MATERIALS AND METHODS

Sample collections and morphological observation

The five specimens described herein were collected by the R/V Shinsei-Maru, operated by the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) with a 3 m beam trawl in the southeast of Cape Erimo, Hokkaido, Japan on 17 August 2020 (Fig. 1). Specimens were deposited at the National Museum of Nature and Science, Tsukuba, Japan (NSMT).

Living specimens were photographed on board with a Nikon D7200 digital camera (Fig. 2A, B), then

fixed in 99% ethanol. Ossicles of the holotype (NSMT E-13546) and a paratype (NSMT E-13550) were isolated using domestic bleach (approximately 5% sodium hypochlorite solution), washed in deionized water, dried in air, and mounted on SEM stubs using double-sided conductive tape. The SEM preparations were examined with a JEOL JSM-5510LV at the Misaki Marine Biological Station (University of Tokyo) and with a JEOL JSM-6380 at NSMT.

The morphological terminology used herein follows Stöhr et al. (2012) and Hendler (2018). In this study, taxonomic position follows that of O'Hara et al. (2018).

DNA barcoding

We obtained a partial sequence of the mitochondrial *COI* gene from the holotype (NSMT E-13546). DNA extraction and PCR parameters

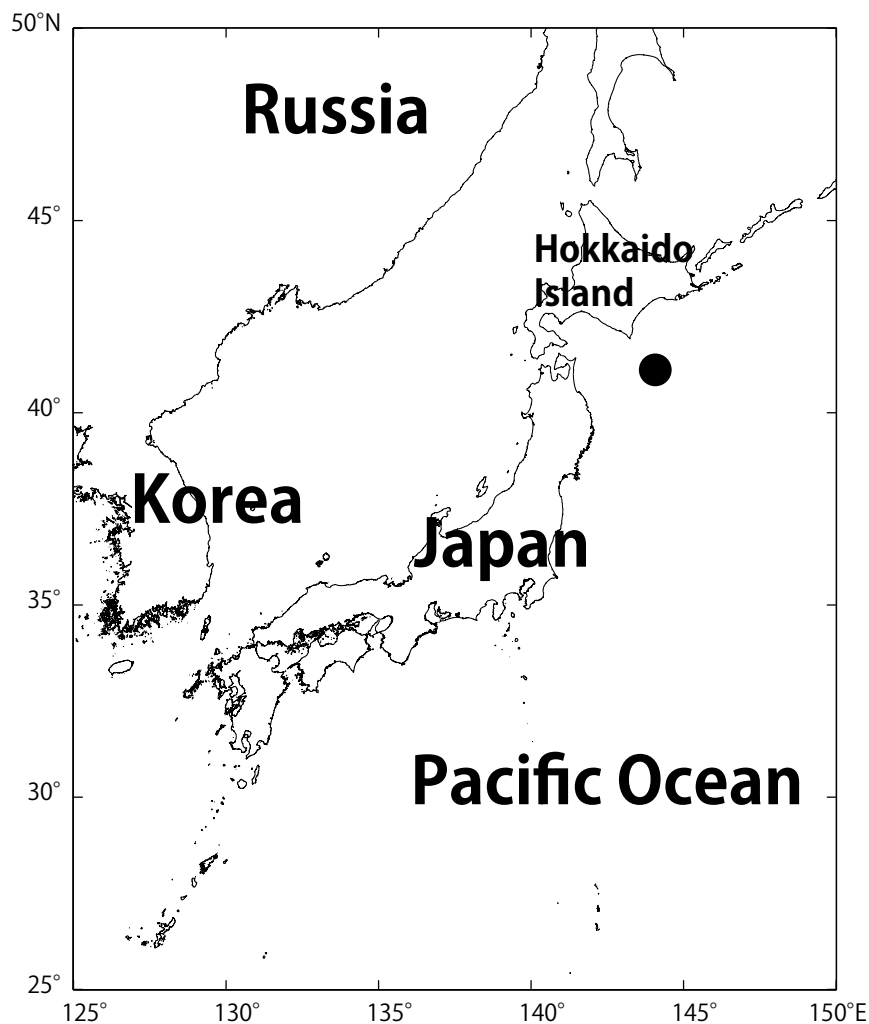


Fig. 1. Sampling locality (solid black circle) of examined specimens.

followed the methodology outlined by Okanishi and Fujita (2013). Primer sets of *COI005* (5'-TTAGGTAA AHWAAACCAVYTKCCTTCAAAG-3') and *COI008* (5'-CCDTANGMDATCATDGCRTACATCATTCC-3') (Okanishi and Fujita 2013) were used for PCR. The PCR products were separated from excess primers and oligonucleotides using Exo-SAP-IT (Applied Biosystems) following the manufacturer's protocol. The sample was sequenced bidirectionally by 3730xI DNA Analyzer (Thermo Fisher Scientific). It was accessioned in the DNA Data Bank of Japan (DDBJ) under LC586826.

The sequences were aligned using the Clustal W algorithm in MEGA X (Thompson et al. 1994; Kumar et al. 2018). The closest sequences to *Ophiomonas shinseimaruae* sp. n. in GenBank were selected by a BLAST search from the DDBJ website (<http://blast.ddbj.nig.ac.jp/blastn?lang=ja>). Genetic distance of the *COI* between *Ophiomonas shinseimaruae* sp. n. and its closest sequences in GenBank was computed using MEGA X according to the Kimura 2-parameter model (Kimura 1980).

RESULTS

TAXONOMY

Family Amphilepididae Matsumoto, 1915 Genus *Ophiomonas* Djakonov, 1952

Ophiomonas shinseimaruae sp. n.

(Figs. 2–8)

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Material examined: Holotype. NSMT E-13546, southeast of Cape Erimo, Hokkaido, Japan (41°17.69'N, 144°08.01'E–41°15.66'N, 144°07.92'E), 3,143–3,176 m depth (Fig. 1), 19 August 2020, R/V Shinsei-Maru of JAMSTEC, 3 m beam trawl.

Paratypes: NSMT E-13547, NSMT E-13548, NSMT E-13549, and NSMT E-13550 (each one specimen), the same locality as the holotype.

Etymology: The specific name was derived from R/V Shinsei-Maru of JAMSTEC.

Diagnosis: Radial shields elongate semi-circular and separated; oral shields triangular; tentacle scales at the second tentacle pore flat and broad, two in number; dorsal arm plates octagonal, three times wider than long on proximal portion of arm; arm spines three in number on proximal portion of arm; arm spines on the middle to distal portion of arm acute, spinose.

Description of external morphology (holotype,

NSMT E-13546): Disc circular, 9.0 mm in diameter (Fig. 2A, B). Dorsal disc completely covered by a circular central primary plate of 1.80 mm in length, other primary plates approximately 1.25–1.80 mm in length, and semi-circular, smooth with imbricating disc scales, approximately 0.60–1.25 mm in length (Fig. 2C, D). Radial shields elongate semi-circular in shape, entirely separated from each other, approximately three times longer than wide; the length approximately half disc radius (Fig. 2C, E). Interradial ventral disc covered by imbricating scales, similar to those on dorsal surface in shape but smaller, approximately 0.20–0.28 mm in length (Fig. 2F, G). Genital slits elongate, from edge of oral shield to lateral edge of disc, approximately 0.15 mm in width and 3.20 mm in length (Fig. 2G). Oral shields triangular, slightly rounded on distal-lateral side, approximately 1 mm in length and width (Fig. 2F). One smaller, hexagonal oral shield with small pores, suggesting it serves as a madreporite (Fig. 2F). Adoral shields triangular, approximately 3 times wider than long (Fig. 2F). Oral plates polygonal, approximately as wide as long, in contact to each other (Fig. 2F). Teeth four and wide, forming a vertical row on dental plate; the ventral top tooth triangular, secondary one trapezoid, and more dorsal ones flat and square (Fig. 2F). A pair of infradental papillae on the ventralmost position of the dental plate (Fig. 2F). One buccal scale situated at ventral edge of each oral plate approximately 2.5 times wider than long (Fig. 2F). Second oral tentacle pores opening on ventral edge of adoral shield with accompanying two flat and broad adoral spines (Fig. 2F).

Arms five, up to 22 mm in length (Fig. 2A, B). Proximally 1.5 mm in width and 1.2 mm in height, rectangular in cross section. Arms tapering gradually toward the tip (Fig. 2A, B). Dorsal arm plates slightly separated from one another along the full distance of the arm. On proximal portion of arm, dorsal arm plates quadrangular, three times wider than long (Fig. 2H). Dorsal arm plates change midway to a more octagonal shape, twice as wide as long (Fig. 2I). Distally, dorsal plates become fan-shaped, as wide as long and gradually decreasing in size towards arm tip (Fig. 3A). Proximally, ventral arm plates are pentagonal, 1.5 times wider than long and in direct contact (Fig. 3B). Midway along the arm length, the ventral plates remain in direct contact but are slightly longer than wide (Fig. 3C). Distally, these plates become approximately twice as long as wide but separate from each other (Fig. 3D).

Arm spines cylindrical, decreasing overall from three proximally to two distally. On the proximal arm region the ventralmost spine longest, with the remaining two spines in series slightly shorter in length than the ventralmost spine (Fig. 3E). Midway along the

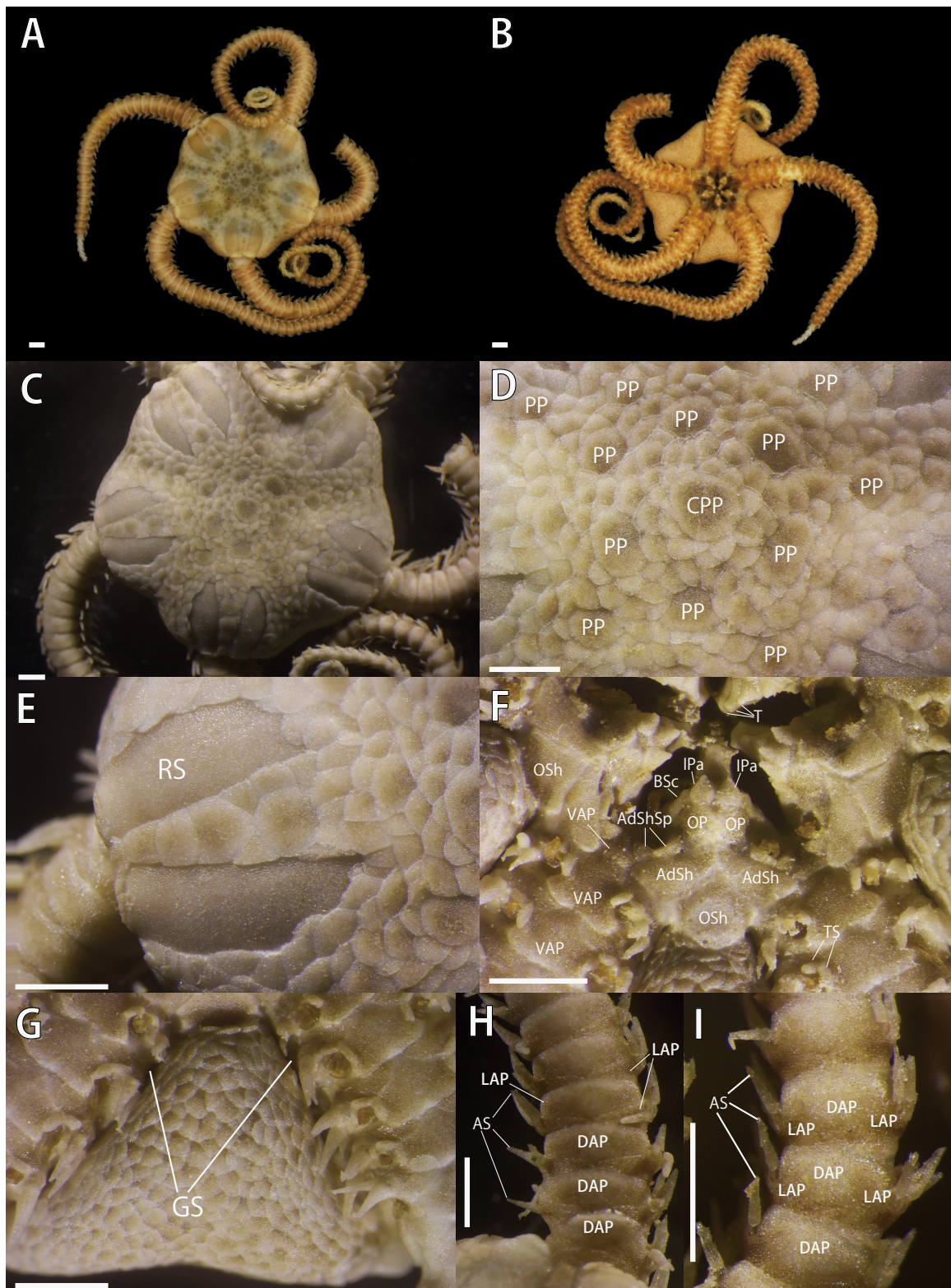


Fig. 2. *Ophiomonas shinseimaruae* sp. n. (Holotype, NSMT E-13546). (A) dorsal whole body, alive; (B) ventral whole body, alive; (C) dorsal disc and proximal portion of arms; (D) central dorsal disc; (E) peripheral dorsal disc; (F) ventral disc; (G) ventral interradial disc; (H) dorsal proximal portion of arm; (I) dorsal middle portion of arm. Abbreviations: AdSh, adoral shield; AdShSp, adoral shield spine; AS, arm spine; BSc, buccal scale; CPP, central primary plate; DAP, dorsal arm plate; GS, genital slit; IPa, infradental papilla; LAP, lateral arm plate; OP, oral plate; OSh, oral shield; PP, primary plate; RS, radial shield; T, tooth; TS, tentacle scale. VAP, ventral arm plate. Scale bars = 1 mm.

arm, the three spines all conical, acutely pointed, and approximately the same length as corresponding arm segment (Figs. 2I, 3C). On the distal portion of the arm, arm spines remain conical and pointed but are half as long as corresponding arm segments as the spine number decreases to two towards arm tip (Fig. 3A, D, F). Tentacle scales two, on proximal and middle portion of the arm, both scales flat and broad at each tentacle pore (Fig. 3B, C). Tentacle scales decreasing in size distally, absent towards arm tip (Fig. 3F).

Description of ossicle morphology (holotype, NSMT E-13546 and a paratype, NSMT E-13550): Vertebrae with zygospondylous articulations (Figs. 4A, B, F, 5A, E, F). On proximal portion of arm, muscle fossae on dorsal sides slightly larger than those on ventral sides on both proximal and distal sides (Fig. 4A, B), becoming clearly wider on dorsal ones than ventral ones on middle to distal portion of arms (Figs. 4F, 5A, E, F). On proximal and middle portion of arms, groove T-shaped on dorsal side (Figs. 4C, 5B) and straight

furrow, in which the radial water vascular canal and radial nerve are place, on ventral side (Figs. 4D, 5C). On distal portion of arms, dorsal groove becoming straight on dorsal side (Fig. 6A) similar to ventral side (Fig. 6B). No distinct channels of lateral openings for canals and nerves recognizable from external view of the ventral furrow (Figs. 4D, 5C, 6B); foot basins visible laterally on ventral side of the vertebra (Figs. 4D, E, 5C, D, 5B, C); protrusions present just externally of the middle of ventral furrow (Figs. 4D, E, 5C, D, 6B, C). A large hole at centre of dorsal groove on distal portion of arm (Fig. 6A).

Lateral arm plates much higher than long on proximal portion of arm (Fig. 6D, E); slightly higher than wide on middle portion of arm (Fig. 6F, G); and approximately twice as wide as high on distal portion of arm (Fig. 6H, I). Distal edge convex with dorsal and ventral edges protruding toward proximal side, and proximal edge concave (Fig. 6D–I). External surfaces smooth; stereom on central portion of proximal edges

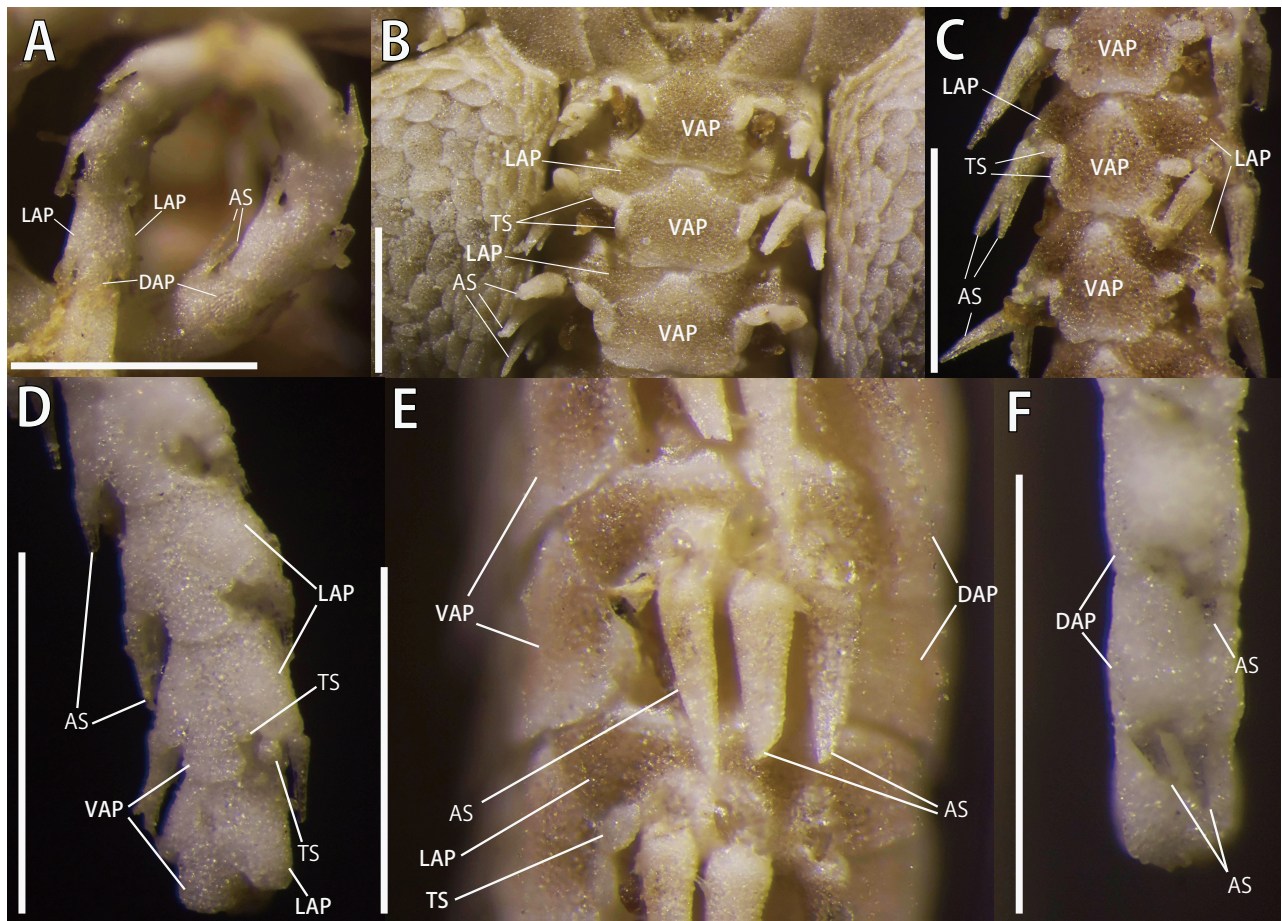


Fig. 3. *Ophiomonas shinseimaruae* sp. n. (Holotype, NSMT E-13546). (A) dorsal distal portion of arm; (B–D), ventral proximal (B), middle (C) and distal (D) portion of arms; (E, F) lateral proximal (E) and distal (F) portion of arms. Abbreviations: AS, arm spine; DAP, dorsal arm plate; LAP, lateral arm plate; TS, tentacle scale; VAP, ventral arm plate. Scale bars = 1 mm.

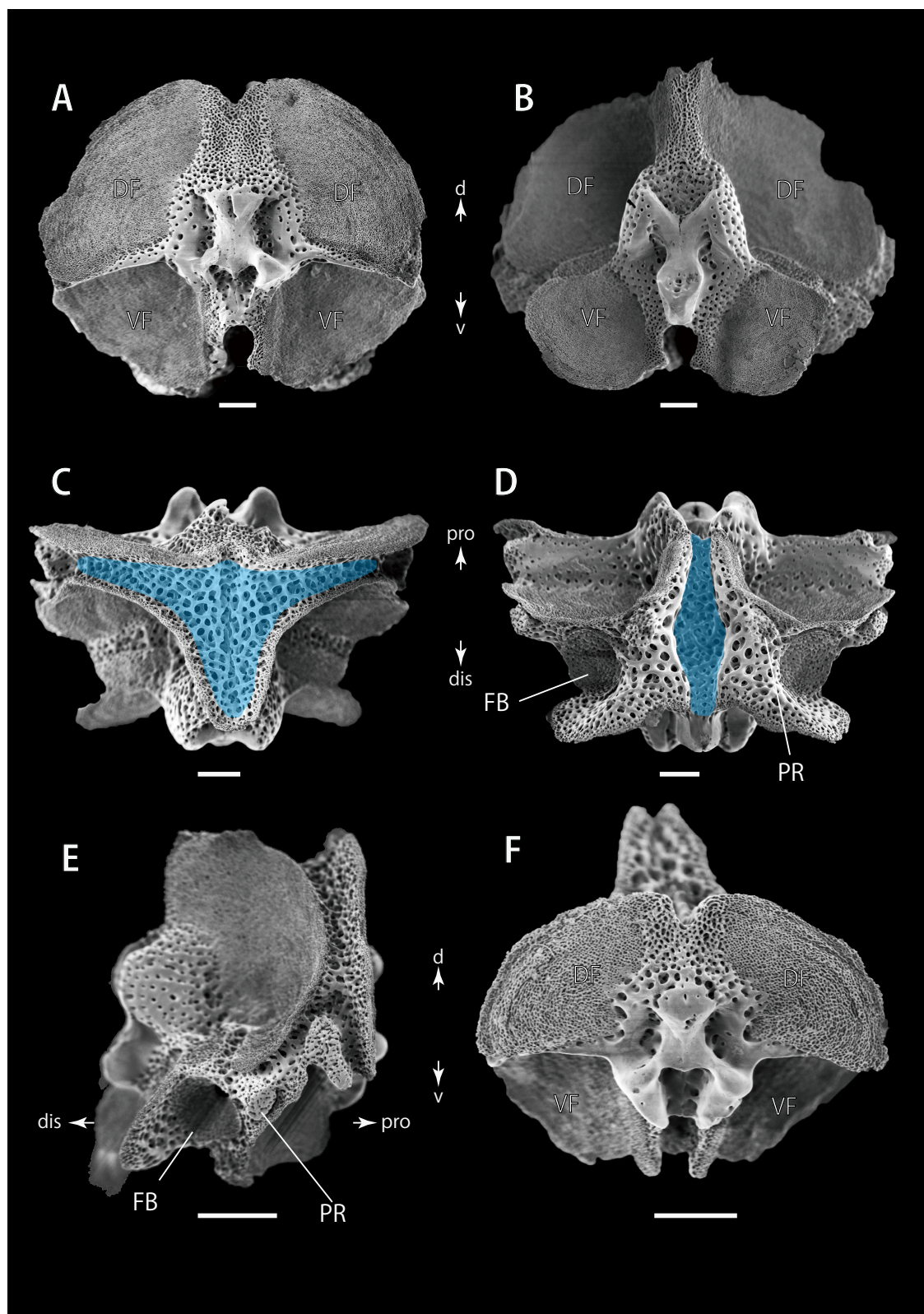


Fig. 4. *Ophiomonas shinseimaruae* sp. n. (Holotype, NSMT E-13546). SEM photographs of vertebrae on proximal (A–E) and middle portion (F) of arms, proximal (A, F), distal (B), dorsal (C), ventral (D) and lateral (E) views. Blue areas indicate grooves. Arrows indicate orientation: d, dorsal; dis, distal; pro, proximal; v, ventral. Abbreviations: DF, dorsal muscle fossae; FB, foot basin; PR, protrusion; VF, ventral muscle fossae. Scale bars = 100 μ m.

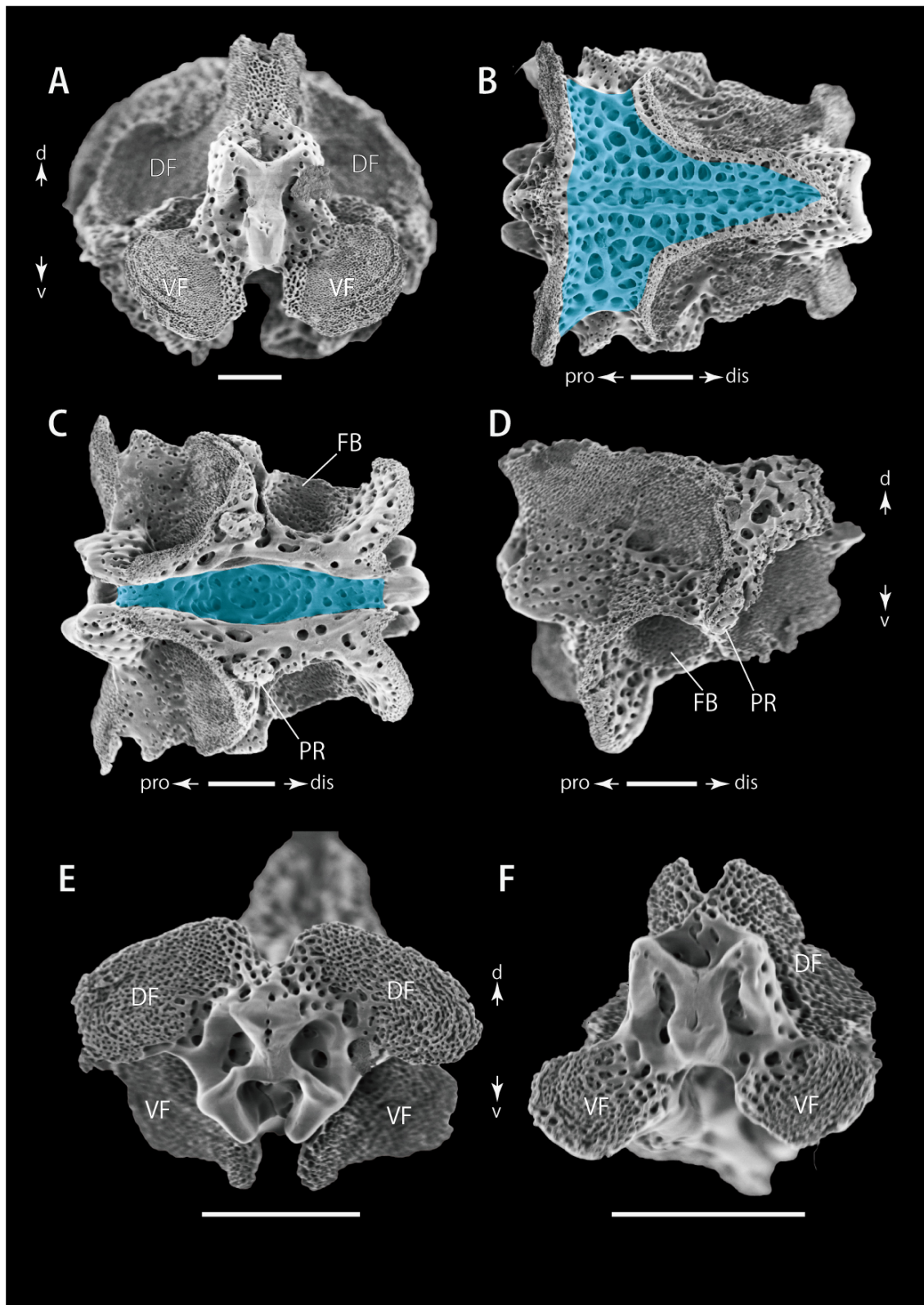


Fig. 5. *Ophiomonas shinseimaruae* sp. n. (Holotype, NSMT E-13546), SEM photographs of vertebrae on middle (A–D) and distal portion (E, F) of arms, proximal (A, F), dorsal (B), ventral (C), lateral (D), distal (E) views. Blue areas indicate grooves. (F) is partly broken. Arrows indicate orientation: d, dorsal; dis, distal; pro, proximal; v, ventral. Abbreviations: FB, foot basin; DF, dorsal muscle fossae; PR, protrusion; VF, ventral muscle fossae. Scale bars = 100 μm.

slightly denser than remaining areas (Fig. 6D, F, H). Internal surfaces with two knobs, composed of more densely meshed stereom than remaining area, situated at centre (Fig. 6E, G, I). On proximal to middle portion of arm, a single perforation next to distal-most knob and a tentacle notch extending to distal-ventral edge (Fig. 6E, G). On distal portion of arm, a tentacle notch extends to ventral-distal edge but no perforation recognizable (Fig. 6I). On proximal to middle portion of arm, three spine articulations on distal edge, with nearly horizontal and parallel dorsal and ventral lobes, merging on proximal edge by depressed bridge, with dorsal lobe wider than ventral lobe (Fig. 6D, F). Distally, the number of articulations decreases to two (Fig. 6H), with dorsal and ventral lobes almost equal in width and merged at proximal edge (Fig. 6H). Surrounding structure of the articulations slightly sunken with denser stereom (Fig. 6D, F, H). Muscle and nerve openings clearly recognized and separated by stereom protuberances on proximal to middle portion of arm (Fig. 6D, F) but absent on distal portion of arm (Fig. 6H).

Dorsal arm plates rectangular with straight distal edges and slightly pointing proximal edge, approximately three times as wide as long on proximal portion of arm (Fig. 7A, B); pentagonal with slightly pointed proximal edge, as wide as long on middle portion of arm (Fig. 7C, D); and oval with slightly pointed proximal edge on distal portion of arm (Fig. 7E, F). Surfaces of the dorsal arm plates smooth (Fig. 7A–F).

Ventral arm plates pentagonal with blunt proximal edge on proximal portion of arm (Fig. 7G, H), and with slightly pointed proximal edge on middle to distal portion of arms (Fig. 7I–L); distal edge slightly convex; slightly wider than long on proximal portion of arm, almost as wide as long on middle portion of arm and twice as long as wide on distal portion of arm (Fig. 7K, L). Surfaces of the ventral arm plates smooth (Fig. 7K, L).

Proximally, ventralmost arm spine tapering to acute tip (Fig. 7M) and other two spines tapering to blunt tip (Fig. 7N); distally, arm spine shape similar, tapering gradually to acute tip (Fig. 7O, P).

Dental plates entire without fragmentation, oblong, approximately twice as long as wide, with four sockets for teeth (Fig. 8). Dorsalmost tooth socket large and circular with two perforating oval holes separated by complete septum (Fig. 8). Second dorsalmost tooth socket smaller than the dorsalmost one, with small perforating holes separated by complete septum opening as slit-shaped transverse holes on internal side (Fig. 8). Other two ventral sockets shallow, not penetrating (Fig. 8).

Variation (paratypes, NSMT E-13547, NSMT E-13548, NSMT E-13549 and NSMT E-13550): Some

morphological variation was observed among the four paratypes. Number of tentacle scales on second oral tentacle pores was two for two paratypes (NSMT E-13547, disc diameter [d.d.] = 12.5 mm; NSMT E-13549, d.d. = 11.6 mm), one for one paratype (NSMT E-13548, d.d. = 9.5 mm), and one or two for one paratype (NSMT E-13550, d.d. = 10.9 mm). One paratype (NSMT E-13550) had four arms naturally while other three paratypes have five.

DNA barcoding: We obtained a 774-bp *COI* gene region for the holotype of *Ophiomonas shinseimaru* sp. n. (NSMT E-13546; LC586826). No *COI* sequence of the genus *Ophiomonas* was registered in GenBank, and the closest sequence is from *Amphiura bidentata* H. L. Clark, 1938 (Amphiuridae: KU895046) with 20.0% K2P genetic distance.

Occurrence: Known only from the type locality, southeast of Cape Erimo, Hokkaido, Japan, 3,143–3,176 m deep (Fig. 1).

DISCUSSION

Diagnostic characters of the new species agree with the definition of *Ophiomonas* as follows: having conspicuous radial shields (Fig. 2C, E); a buccal scale (Fig. 2F); and a second oral tentacle pore opening outside of mouth slit (Djakonov 1952; Fell 1960; A. M. Clark 1970). This is the first record of the genus *Ophiomonas* from Japanese waters.

Ophiomonas has historically been placed within the family Amphilepididae based on external morphological features (Fell 1960), although the definition of the Amphilepididae now includes microstructures of dental and lateral arm plates (O'Hara et al. 2018). However, microstructural characters in *Ophiomonas* have not been previously available and the taxonomic status of *Ophiomonas* has not been evaluated with these characters in mind. In this study, we provide for the first time SEM images of dental plates and lateral arm plates for *Ophiomonas*.

The characters suggesting their similarity to the family Amphiuridae include the dental plate with large penetrating oval holes in the dorsalmost tooth socket and smaller oval holes in the second dorsalmost tooth sockets, which have a complete septum (Fig. 8A, B). Holes in the ventral sockets are shallow and not penetrating (Fig. 8A). Lateral arm plates of *Ophiomonas shinseimaru* sp. n. were sickle-shaped with a dorsal arm tip, without surface ornamentation (Fig. 6D–G), and with two knobs on internal side (Fig. 6E, G, I). Arm spine articulations were composed of two smooth, separated, parallel and straight dorsal and ventral lobes (Fig. 6D, F, H). All of these characters

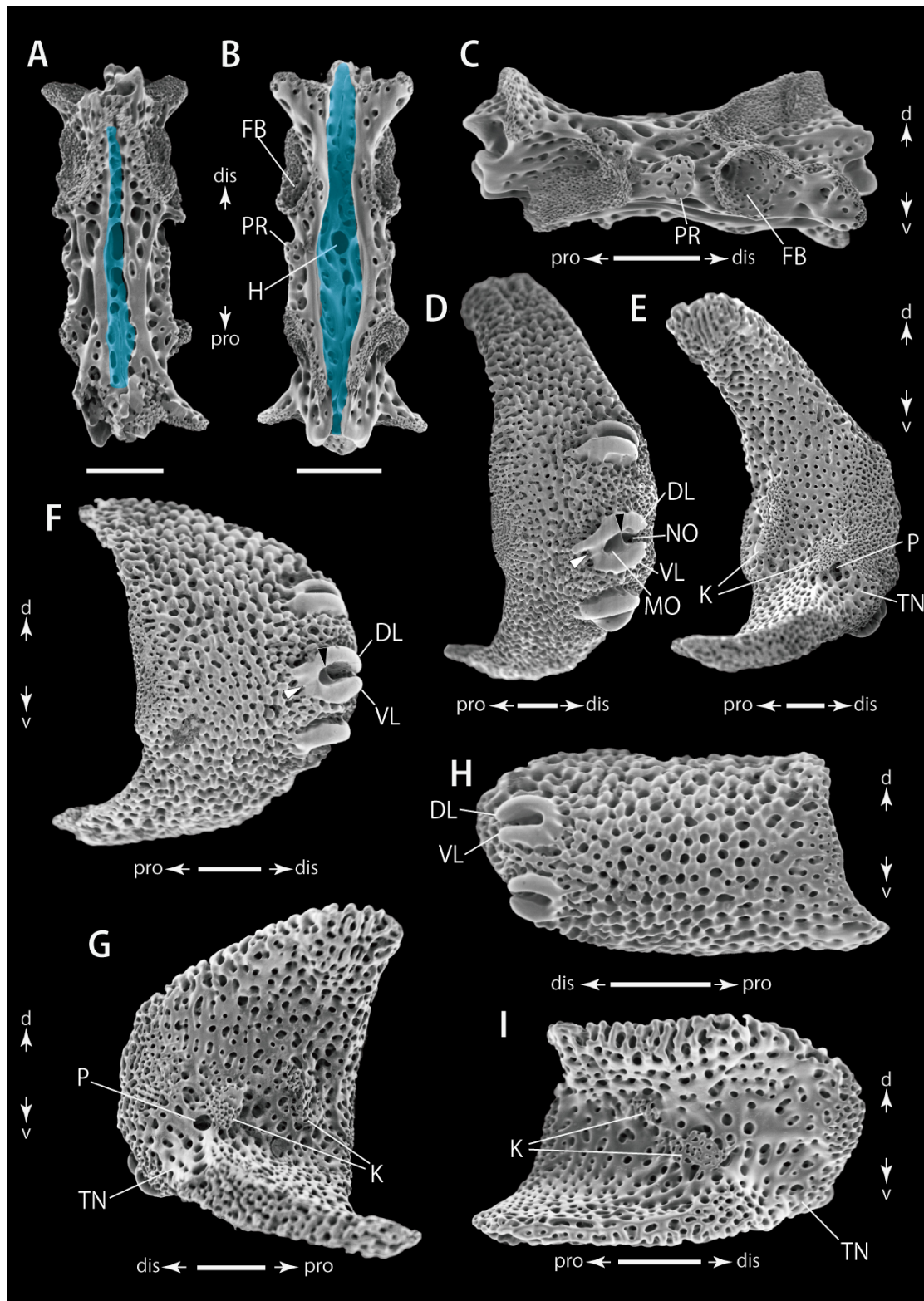


Fig. 6. *Ophiomonas shinseimaruae* sp. n. (Holotype, NSMT E-13546), SEM photographs of vertebrae on distal portion of arm (A–C) and lateral arm plates (D–I) from proximal (D, E), middle (F, G) and distal (H, I) portion of arms, dorsal (A), ventral (B) and lateral (C) views, external (D, F, H) and internal (E, G, I) views. Blue areas indicate grooves (A, B). (A) is partly broken. Arrows indicate orientation: d, dorsal; dis, distal; pro, proximal; v, ventral. Arrowheads indicate depressed bridge (white) and stereom protuberances (black). Abbreviations: K, knob; DL, dorsal lobe; FB, foot basin; H, hole; MO, muscular opening; NO, nerve opening; P, perforation; PR, protrusion; TN, tentacle notch; VL, ventral lobe. Scale bars = 100 μ m.

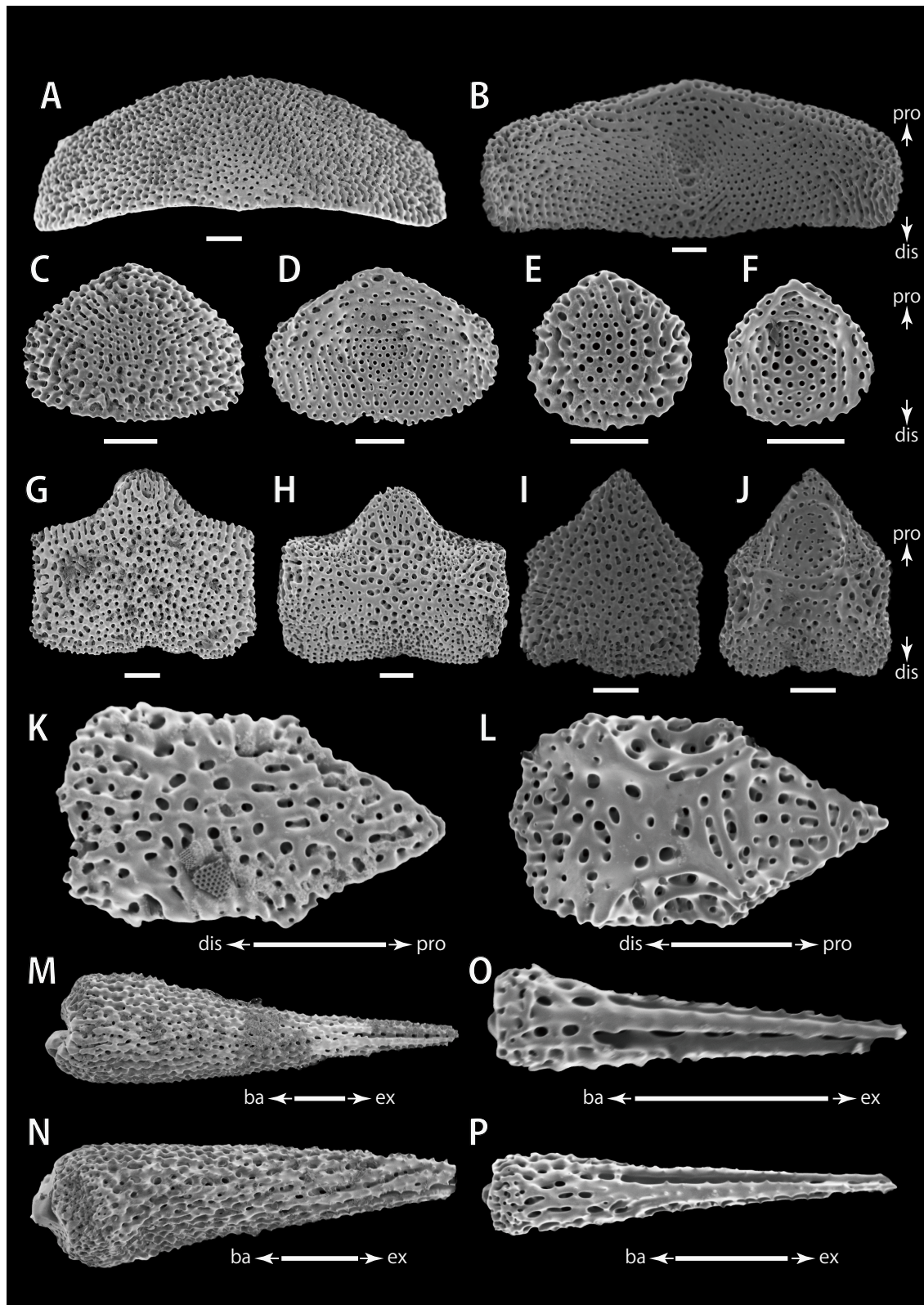


Fig. 7. *Ophiomonas shinseimaruae* sp. n. (Holotype, NSMT E-13546). SEM photographs of ossicles, (A–F), dorsal arm plates from proximal (A, B), middle (C, D) and distal (E, F) portion of arms; (G–L) ventral arm plates from proximal (G, H), middle (I, J) and distal (K, L) portion of arms, external (A, C, E, G, I, K) and internal (B, D, F, H, J, L) views; (M–P) arm spines from proximal (M, N) and distal (O, P) portion of arms, dorsalmost (M, O) and ventralmost (N, P) ones. Arrows indicate orientation: ba, basal; dis, distal; ex, external; pro, proximal. Scale bars = 100 μm.

support membership within the Amphiuridae according to O'Hara et al. (2018).

Furthermore, there is close similarity in *COI* sequences between *O. shinseimaruae* sp. n. and *Amphiura bidentata*, with 20.0% K2P genetic distance among the *COI* sequences of GenBank. Thus, based on these results, *O. shinseimaruae* sp. n. and more broadly the genus *Ophiomonas* may be a member of the family Amphiuridae. However, we here refrain from transferring *Ophiomonas* from Amphilepididae to Amphiuridae due to a lack of molecular data and ossicle morphology of *Ophiomonas bathybia* Djakonov, 1952, the type species of *Ophiomonas*.

Ophiomonas shinseimaruae sp. n. can be distinguished from its three congeners, *O. bathybia* Djakonov, 1952, *O. protecta* Koheler, 1904, and *O.*

remittens Koehler, 1922 by the following five characters:

Radial shields

Long, elongated half-circles and separate in *O. shinseimaruae* sp. n. (Fig. 2C, E) whereas those of *O. bathybia* and *O. remittens* are triangular and in contact at their distal ends (Koehler 1922; Djakonov 1952; A. M. Clark 1970). Those of *O. protecta* are semi-circular and in contact on their distal side (Koehler 1904).

Oral shields

Triangular with rounded edges in *O. shinseimaruae* sp. n. (Fig. 2F) and *O. remittens* whereas diamond-shaped in *O. bathybia* and *O. protecta* (Koehler 1904).

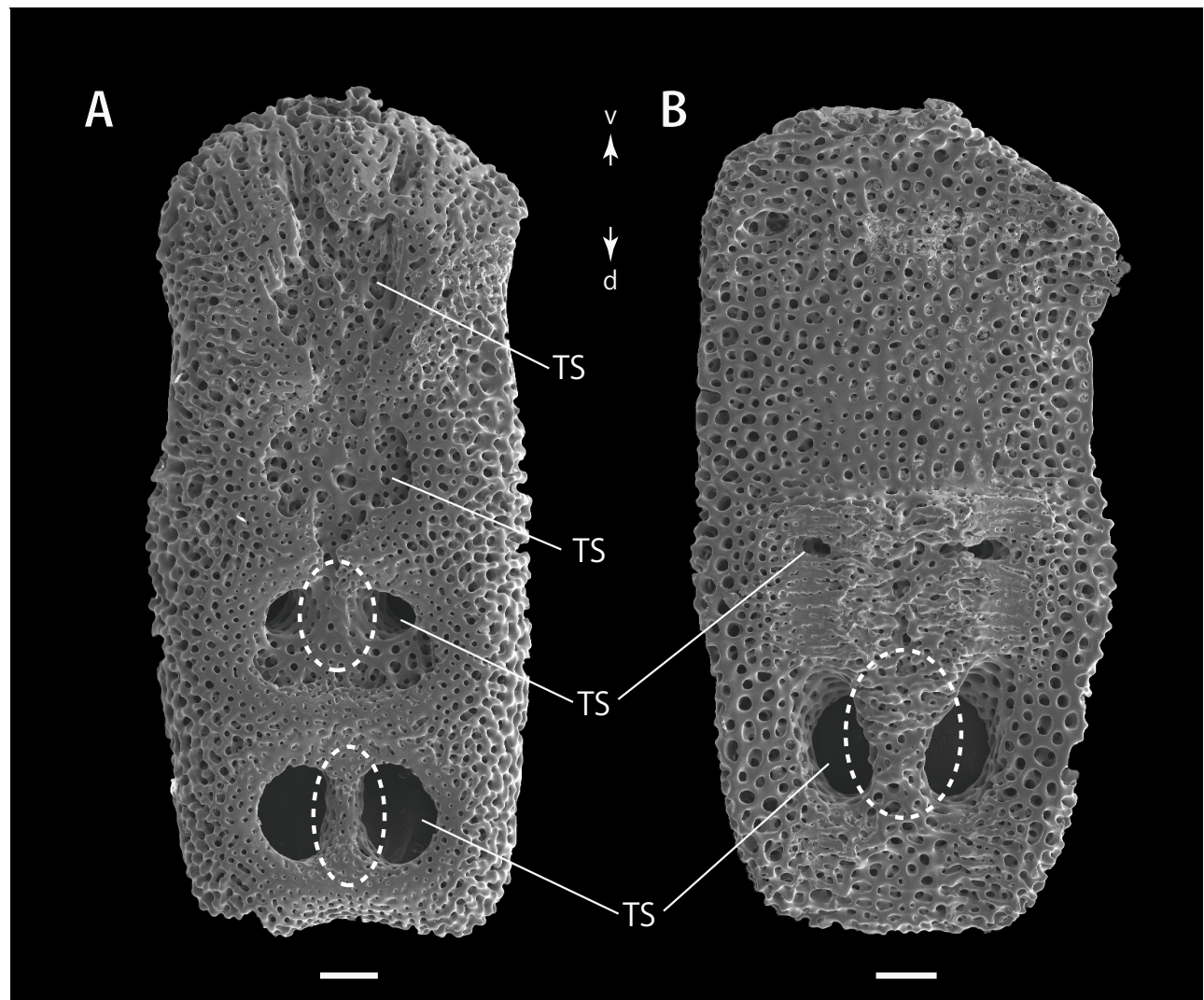


Fig. 8. *Ophiomonas shinseimaruae* sp. n. (A paratype, NSMT E-13550). (A, B) SEM photographs of dental plate, external (A) and internal (B) views. Arrows indicate orientation: d, dorsal; v, ventral. Dashed circles indicate septum. Abbreviations: TS, tooth socket. Scale bars = 100 μ m.

1922; Djakonov 1952; A. M. Clark 1970).

Infradental papillae

Triangular in *O. shinseimaruae* sp. n. (Fig. 2F), while those of *O. bathybia* and *O. protecta* are flat and square, and those of *O. remittens* are cylindrical (Koehler 1904 1922; Djakonov 1952; A. M. Clark 1970).

Tentacle scales

Ophiomonas shinseimaruae sp. n. (Fig. 2F) with broad and flat adoral shield spines (previously known as tentacle scales) on the second tentacle pore, while *O. bathybia*, *O. protecta* and *O. remittens* have small and round adoral shield spines at same position (Koehler 1904 1922; Djakonov 1952; A. M. Clark 1970). Tentacle scales on proximal portion of arm are square in *O. shinseimaruae* sp. n. (Fig. 2G) whereas those of *O. bathybia* and *O. remittens* are oval (Koehler 1922; Djakonov 1952; A. M. Clark 1970).

Dorsal arm plates

Ophiomonas shinseimaruae sp. n. has rectangular dorsal arm plates, approximately three times wider than long, while those of *O. bathybia* and *O. protecta* are diamond-shaped, approximately twice as wide as long (Koehler 1904; Djakonov 1952; A. M. Clark 1970). Those of *O. remittens* are semi-circular, approximately twice as wide as long (Koehler 1922).

CONCLUSIONS

Ophiomonas shinseimaruae sp. n. is described herein and represents the first record of this genus from Japanese waters. It is the 12th known ophiuroid species collected from Japanese abyssal depths (> 3000 m). Along with a morphological description of the new species, molecular information from the DNA barcoding region (*COI*) is provided for future phylogenetic studies.

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Authors' contributions: MO conceived and designed the experiments, sampled examined specimens, performed the experiments, analyzed the data, prepared figures and the original draft, authored and reviewed drafts of the paper, and approved the final draft. TM sampled and photographed living status of the examined specimens, performed dissection of a part of a paratype, authored and reviewed drafts of the paper and approved the final draft. TF authored and reviewed drafts of the paper and approved the final draft.

Competing interests: All of the authors declare they have no conflict of interest.

Availability of data and materials: All examined specimens in this study are deposited in the National Museum of Nature and Science, Tsukuba (NSMT). Sequences of new species were deposited in DNA Data Bank of Japan (DDBJ), accession number: LC586826.

Consent for publication: All of the authors agreed to publish the paper.

Ethics approval consent to participate: Not applicable.

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