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The First Record of *Ophioteichus multispinum* (Echinodermata: Ophiuroidea) from Japan, with Notes on Its Ossicle Description and Ecology

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Three specimens of *Ophioteichus multispinum* H. L. Clark, 1938 are redescribed from approximately 2.3–12.6 m water depth at Sabiura, Kushimoto, Wakayama Prefecture in central Japan. The species occurs completely buried on sandy bottoms. Ossicle morphology is described. Based strictly on images, we present in situ occurrence of the genus *Ophioteichus* H. L. Clark, 1938 from the intertidal regions of Susami, Wakayama Prefecture, and Tosashimizu, Kochi Prefecture.

Key Words: brittle star, scuba diving, ossicle morphology.

Introduction

Ophiuroids (Echinodermata) constitute the largest number of species within the phylum Echinodermata, and they inhabit nearly all marine habitats (Stöhr et al. 2012; Okanishi 2016). Recent investigations by SCUBA diving have revealed many taxonomic discoveries of benthic invertebrates from the subtidal zone, usually at depths greater than 10 m in Japan (e.g., Kise et al. 2017; Naruse and Yoshida 2018; Okanishi and Fujii 2020; Yamana et al. 2022). Kushimoto is located at the southern tip of Honshu (the largest island in Japan) and is a warm region due to the Kuroshio Current flowing in its vicinity (Fig. 1). Kushimoto is the northern limit of tropical coral habitats in Japanese waters and is considered to have high biodiversity (Okanishi and Fujii 2020). The ophiuroid fauna at deeper than 10 m in Kushimoto is unexplored and potentially rich according to previous discoveries of benthic invertebrates in this area.

The genus *Ophioteichus* H. L. Clark, 1938 (Ophintegrida: Amphilepidida: Ophiolepididae) currently comprises 3 valid species, *O. multispinum* H. L. Clark, 1938, *O. parvispinum* H. L. Clark, 1938 and *O. utinomii* (Irimura, 1968) (Pineda-Enriquez et al. 2014). Of these, only *O. utinomii* has been reported from Japanese waters (Irimura 1968; Okanishi 2016). Pineda-Enriquez et al. (2014) redescribed the holotype of *O. utinomii* with a further, detailed examination with close-up photographs of its body parts but without scanning electron microscopy (SEM) images of each isolated ossicle.

Given the recent importance the ossicle morphology has played in understanding the phylogeny and taxonomy of ophiuroids (e.g., Martynov 2010; Thuy and Stöhr 2016; Okanishi and Fujita 2018a; O'Hara et al. 2018), we herein present detailed photographs of all relevant parts of the body, including SEM of the ossicles for the collected specimens of *O. multispuinum*, which has not been recorded from Japanese waters.

Additional occurrences of *Ophioteichus* sp. in Japanese waters were confirmed by in situ observations during this study.

Materials and Methods

Three specimens of *Ophioteichus multispinum* were collected and observed in situ by the last author (IH) during SCUBA diving at Sabiura, Kushimoto, Wakayama Prefecture, central Japan, at 12.6 m depth (20 August 2018), 2.3 m (3 October 2018), and 10.2 m (9 November 2018) (Fig. 1).

Specimens were anesthetized in a 10% aqueous solution of magnesium chloride, then fixed in 99% ethanol. An arm of one specimen (NSMT E-14161) was dissected to remove internal ossicles 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 specimens were sputter-coated with gold-palladium, examined, and photographed with a JEOL JSM 5200LV SEM of the National Museum of Nature and Science, Tsukuba (NSMT). The specimens have been deposited at NSMT.

Additionally, *Ophioteichus* sp. was observed and photographed in situ by the second author (ID) during hand-picking sampling at Susami, Wakayama Prefecture, central Japan, and Tosashimizu, Kochi Prefecture, southwestern Japan, both at intertidal depths, on 6 November 2021, 7 November 2021, 21 November 2021, January 2022 (exact date

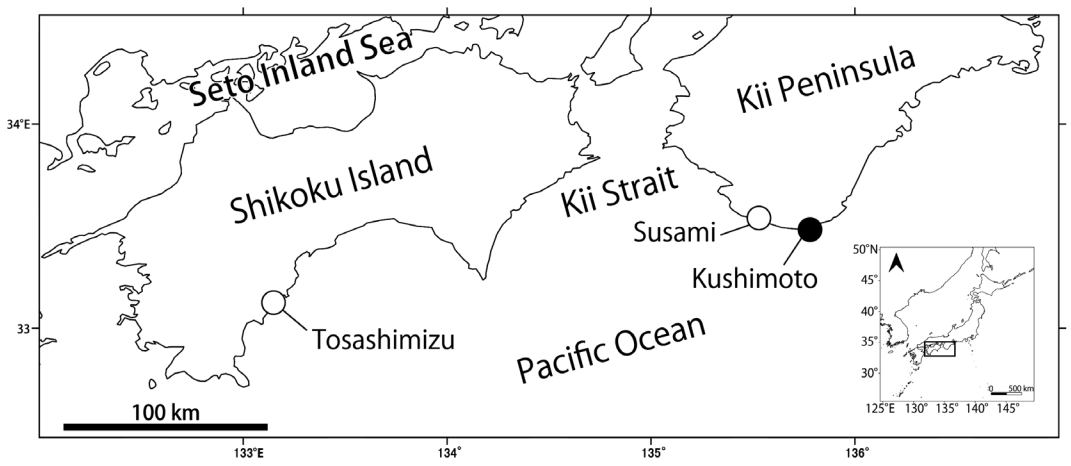

Fig. 1. Sampling locations of *Ophioteichus multispinum* (a solid circle, NSMT E-14161E-14163) and *Ophioteichus* spp. (two blank circles).

unknown), and February 2022 (exact date unknown) at Susami, and on 29 December 2021 at Tosashimizu (Fig. 1). Detailed examination was limited due to non-preservation of the specimens, but they were identified as *Ophioteichus* by observation of their photographs (see Results). The exact number of individuals was not recorded.

Morphological terminology used in this study follows Stöhr et al. (2012), Okanishi and Fujita (2018b), and Hendler (2018). The systematics used in this study follow O'Hara et al. (2018).

Taxonomy

Family **Ophiolepididae** Ljungman, 1867
Genus ***Ophioteichus*** H. L. Clark, 1938
[New Japanese name: Joheki-kumohitode-zoku]
Ophioteichus multispinum H. L. Clark, 1938
[New Japanese name: Tashin-joheki-kumohitode]
(Figs 2–6)

Ophioteichus multispinum H. L. Clark, 1938: 360–362, pl. 25, fig. 5; A. M. Clark and Rowe 1971: 129; Pineda-Enriquez 2014: 242, fig. 2.

Non *Ophioteichus multispinum*: Starmer 2003: 557 (= *Ophiomora elegans* Koehler, 1907).

Material examined. NSMT E-14161: one specimen, Sabiura, Arita, Kushimoto, Wakayama Prefecture, central Japan, depth 12.6 m, SCUBA diving, collected by IH, 20 August 2018. NSMT E-14162: one specimen, same locality as NSMT E-14161, 2.3 m, 3 October 2018. NSMT E-14163: one specimen, same locality as NSMT E-14161, 10.2 m, 9 November 2018.

Diagnosis (from Pineda-Enriquez 2014). Disc nearly circular in outline, higher than the arms, and covered with a coat of numerous swollen, smooth larger plates; each plate surrounded by a belt of polygonal smaller flat scales. Disc scales arranged in an identical pattern on dorsal and ventral surfaces. Radial shields smooth and flat, each pair separated by 2 conspicuous tubercles. In each interradius, the disc margin is encircled by 3 or 4 large tubercles. Accessory dorsal and ventral arm plates fragmented. Minute pores present on the lateral margins of the dorsal arm plates. Infradental papilla, secondary infradental papilla, buccal scale, adoral shield spine, secondary adoral shield spines, Lyman's ossicle on each side. Arm spines up to 11, acute, slender, and closely spaced. Each tentacle pore with 2 or 3 tentacle scales of which abradial two scales are flat and of the same size, while the third adradial one is sometimes reduced.

Description of external morphology (NSMT E-14161). *Disc.* Circular, 9.7 mm in diameter (Fig. 2), covered by scales of two sizes. On dorsal surface, polygonal or circular larger scales, approximately 300–800 μm in length, surrounded by smaller polygonal scales, approximately 50–80 μm in length (Fig. 3A–C). Radial shields hexagonal, slightly longer than wide, approximately 1000 μm in length and 900 μm in width, separated by smaller scales (Fig. 3C). A pair of tubercles between a pair of radial shields (Figs 2C, 3C). A trio of larger hemispherical tubercles, approximately 1500 μm in length and 1100 μm in width, on marginal edges of each interradius (Figs 2C, 3A, D). On ventral surface, adoral shields slightly curved parallelogram, wider than long, approximately 1000 μm in length, 250 μm in width inner side, 350 μm in width outer side (Fig. 3E–G). Oral plates triangular, approximately 450 μm in length, 200 μm in width inner side, 50 μm in width outer side, in contact with each other (Fig. 3F). Oral shields pear-shaped, slightly rounded, and convex on distal side, approximately 900 μm in length, 700 μm in width, and pointed inward (Fig. 3F, G). One oral shield slightly larger in size, centrally concaving, serving as madreporite (Fig. 3E). Adoral shield oblong, wider than long, contacting each other adradially (Fig. 3F). Interradial ventral disc also covered by larger scales, approximately 250–700 μm in length, surrounded by and smaller scales, approximately 40–120 μm in length, like dorsal disc (Fig. 3F, G). Genital slits long, almost extending to the disc edge, approximately 0.01 mm in width (Fig. 3H). Approximately 6 small plates arranged parallel on their adradial edge. One trapezoidal infradental papilla, 1 polygonal secondary infradental papilla, 1 trapezoidal buccal scale, 1 circular secondary adoral shield spine, 1 trapezoidal adoral shield spine and

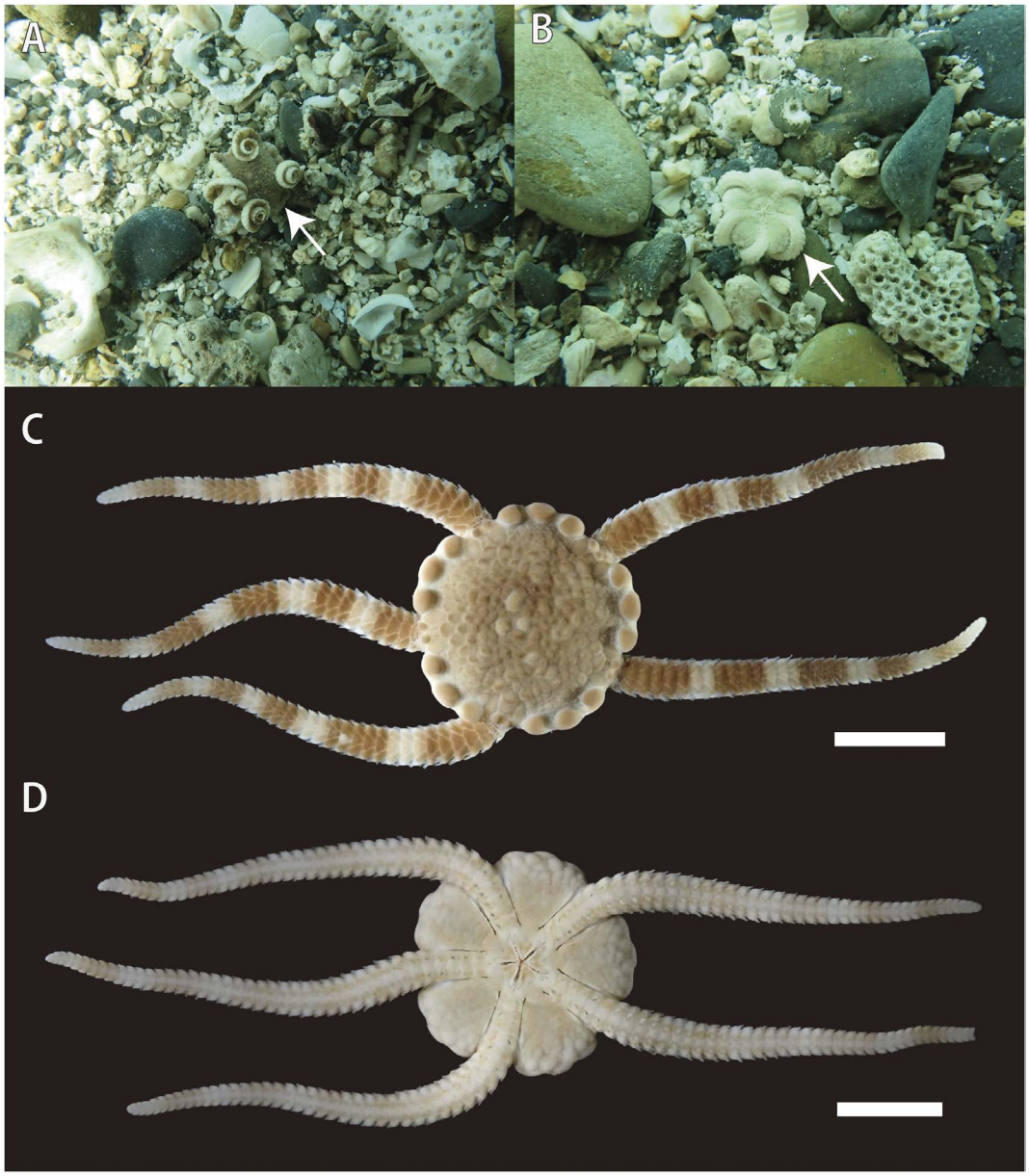

Fig. 2. *Ophioteichus multispinum* (NSMT E-14161), living, in situ, exposed from sand ground (A, B) and anesthetized (C, D) states. A, C, Dorsal view; B, D, ventral view. Scale bars: 0.5 cm (C, D).

1 triangular Lyman's ossicle on each side of jaw (Fig. 3F). Of these 6 ossicles, inner one smaller and becoming larger toward the outer side (Fig. 3F). Teeth cylindrical, pointed, forming vertical row on dental plate (Fig. 3F). Second tentacle pore completely inside the mouth slit.

Arms. Five in number, 34–37 mm in length, 4.2 mm wide and 1.1 mm high in proximal portion, oblong in section. Arms tapering gradually toward the arm tip (Fig. 2).

Each arm segment with a dorsal arm plate, 2 lateral arm plates and a ventral arm plate (Fig. 4). On proximal portion of the arm, dorsal arm plates fan shaped with straight proximal edge, in contact with each other (Fig. 4A). On proximal portion of arm, each dorsal arm plate carries 1 or 2 small accessary plates which decrease in number to 1 on middle portion of arm and disappear on distal portion (Fig. 4A–C). No tubercles on dorsal arm plates. On middle portion of the arm, dorsal arm plates sharpen, triangular, separated and gradually decreasing in size toward the arm tip (Fig. 4A, B). Dorsal arm plates two times wider than long on proximal portion of arm and as wide as long on middle to distal portion (Fig. 4A–C). Lateral arm plates thick, protruding from arm, widely separated by dorsal and ventral arm plates on proximal portion of arm (Fig. 4A, D), in contact with each other on middle to distal portion of arm at proximal edge on both dorsal and ventral sides (Fig. 4B, C, E). On middle to distal portion of arm, dorsal side of lateral arm plates carries smaller accessory pores, 8–10 in number on middle portion, and the number decreasing toward distal portion of arm (Fig. 4B, C). Ventral arm plates squarish fan-shaped with slightly concave lateral edges, almost as wide as long, contiguous on proximal portion of arms and separated on subsequent segments (Fig. 4D, E). On proximal portion of

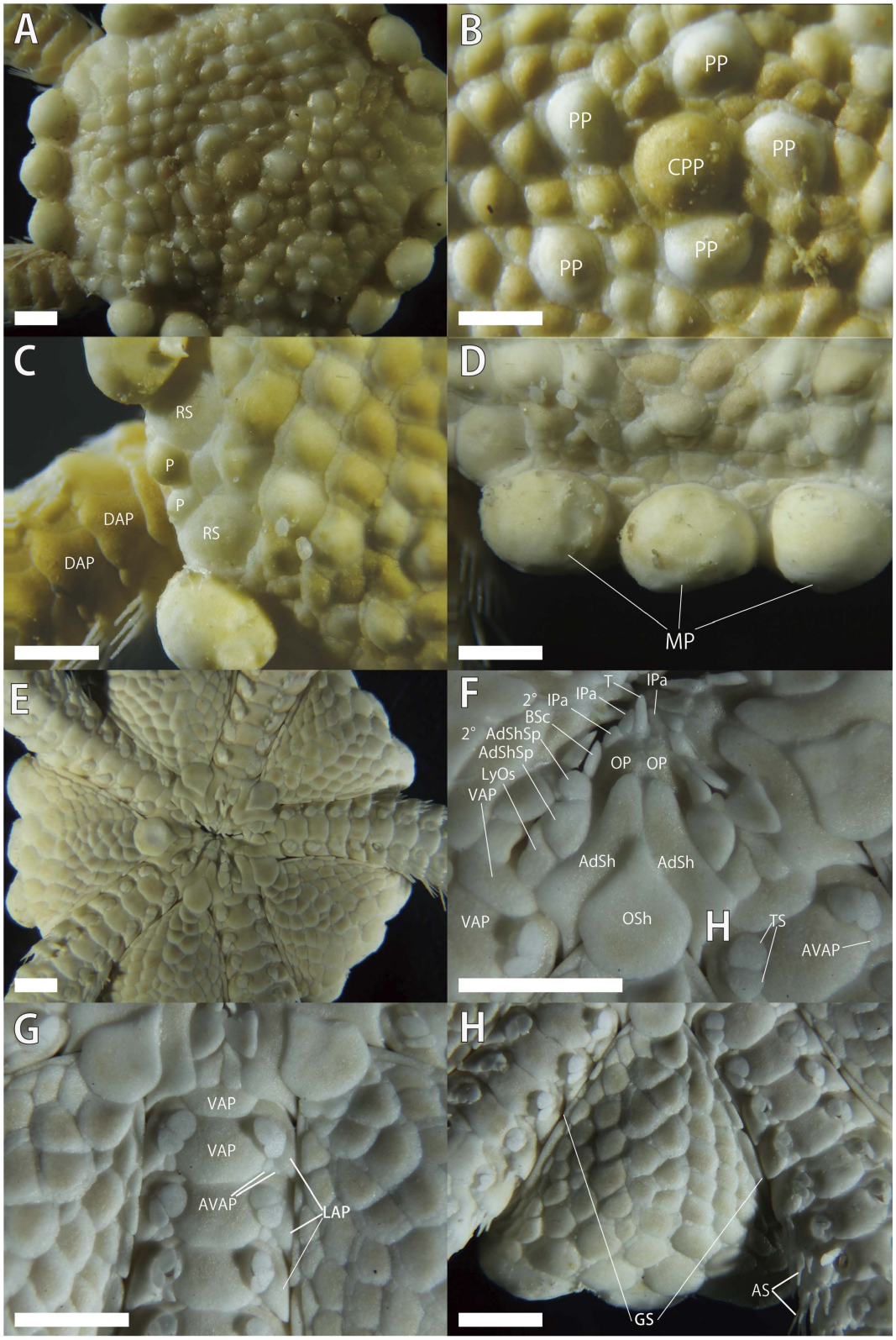

Fig. 3. *Ophioteichus multispinum* (NSMT E-14161). A, Dorsal disc; B, dorsal central part of disc; C, dorsal peripheral part of disc; D, dorsal interradial peripheral part of disc; E, ventral disc; F, jaws; G, ventral disc and proximal portion of arm; H, ventral interradial part of disc. Abbreviations: AdSh, adradial shield; AS, arm spine; AdShSp, adoral shield spine; AVAP, accessary ventral arm plate; BSc, buccal scale; CPP, central primary plate; DAP, dorsal arm plate; GS, genital slit; IPa, infradental papilla; LAP, lateral arm plate; MP, marginal plate; OP, oral plate; OSh, oral shield; P, plate; PP, primary plate; RS, radial shield: TS, tentacle scale; VAP, ventral arm plate; 2°AdShSp, secondary adoral shield spine; 2°IPa, secondary infradental papilla. Scale bars: 1 mm.

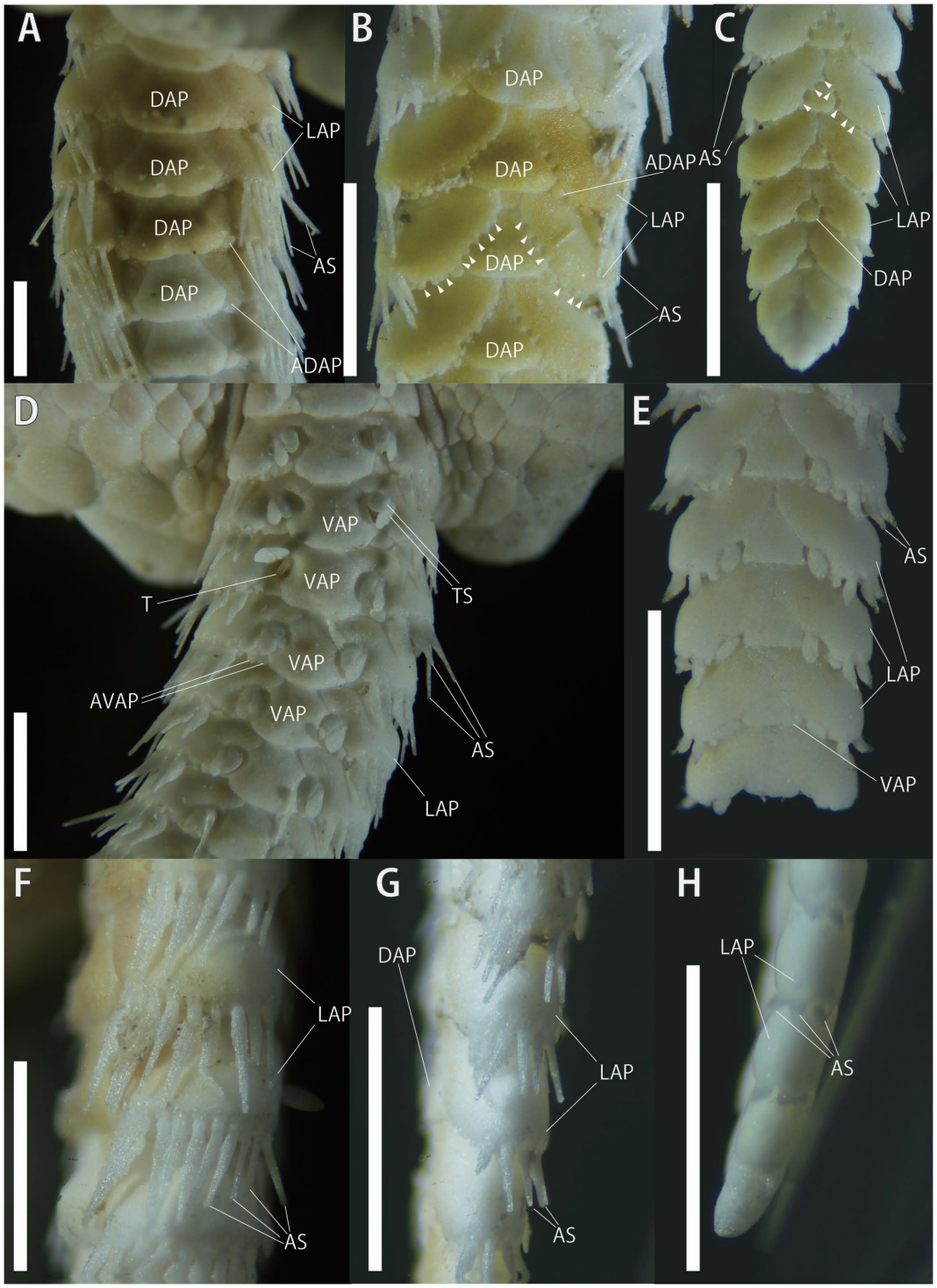

Fig. 4. *Ophioteichus multispinum* (NSMT E-14161). A–C, Dorsal views of arms, proximal (A), middle (B) and distal (C) portions; D, E, ventral views of arms, proximal (D) and distal (E); F–H, lateral views of arms, proximal (F), middle (G) and distal (H) portions. Left and right are oriented as dorsal and ventral, respectively (F–H). Arrowheads indicate pores on dorsal side of lateral arm plates (B, C). Abbreviations: ADAP, accessary dorsal arm plate; AS, arm spine; AVAP, accessory ventral arm spine; DAP, dorsal arm plate; LAP, lateral arm plate; T, tentacle; TS, tentacle scale; VAP, ventral arm plate. Scale bars: 1 mm.

arm, each ventral arm plate carries 1 or 2 small accessary plates which disappear on middle to distal portion (Fig. 4D, E). Ten long, acute arm spines, about the same length as the corresponding arm segment on dorsal-most one and about three-quarters length of the corresponding arm segment on ventral-most one (Fig. 4F); the number decreasing to 4 on the middle portion of arm (Fig. 4G); and decreasing to 3 on distal portion of arm, the length subequal, spear-shaped, about one-sixth length of the corresponding arm segment (Fig. 4H; see detailed descriptions of each ossicle in next section). Two oval tentacle scales at each tentacle pore on proximal portion of arm, subequal in size (Fig. 4D); and the

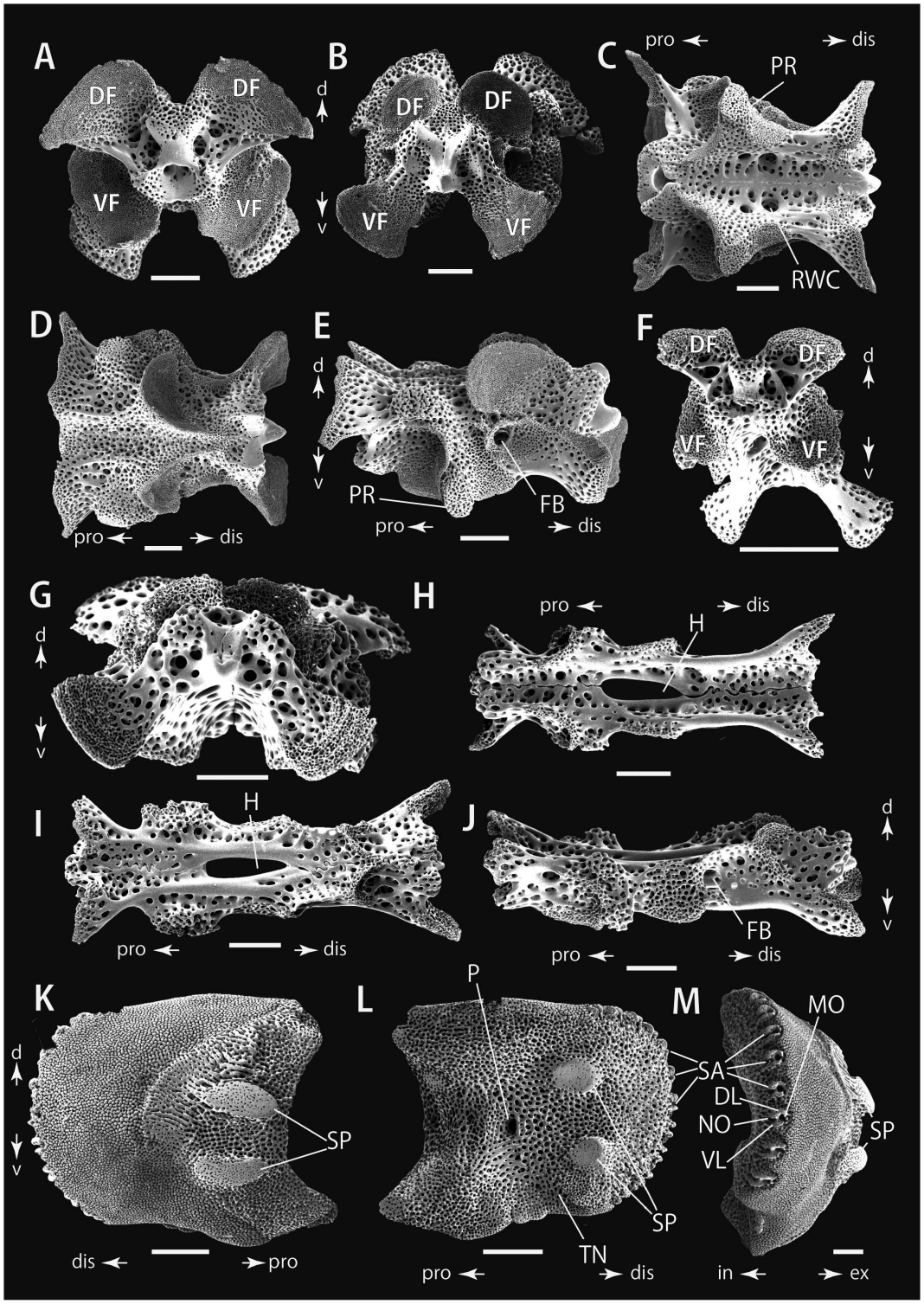

Fig. 5. *Ophioteichus multispinum* (NSMT E-14161), SEM photographs. A–J, Vertebrae from proximal (A–E) and distal (F–J) portions of an arm, distal (A, F), proximal (B, G), ventral (C, H), dorsal (D, I) and lateral (E, J) views; K–M, lateral arm plates from proximal portion of an arm, external (K), internal (L) and distal (M) views. Arrowheads indicate orientation: d, dorsal side; dis, distal side; pro, proximal side; v, ventral side. Abbreviations: DF, dorsal muscle flange; DL, dorsal lobe; FB, depression for tentacle; H, hole; MO, muscle opening; NO, nerve opening; P, perforation; PR, protrusion; RWF, radial water canal; SA, spine articulation; SP, spur; VF, ventral muscle flange; VL, ventral lobe. Scale bars: 100 µm (A–F, M); 50 µm G–J; 200 µm (K, L).

number decreasing to 1 on middle to distal portion of arm (Fig. 4E).

Description of ossicle morphology (NSMT E-14161). Vertebrae with zygospondylous articulation, elongate, large wing-like muscle flanges on ventral-distal side and dorsal-proximal side (Fig. 5A, B, F, G). Longitudinal groove on both dorsal and ventral side (Fig. 5C, D, H, I). A pair of radial water canals opening inside ventral groove (Fig. 5C).

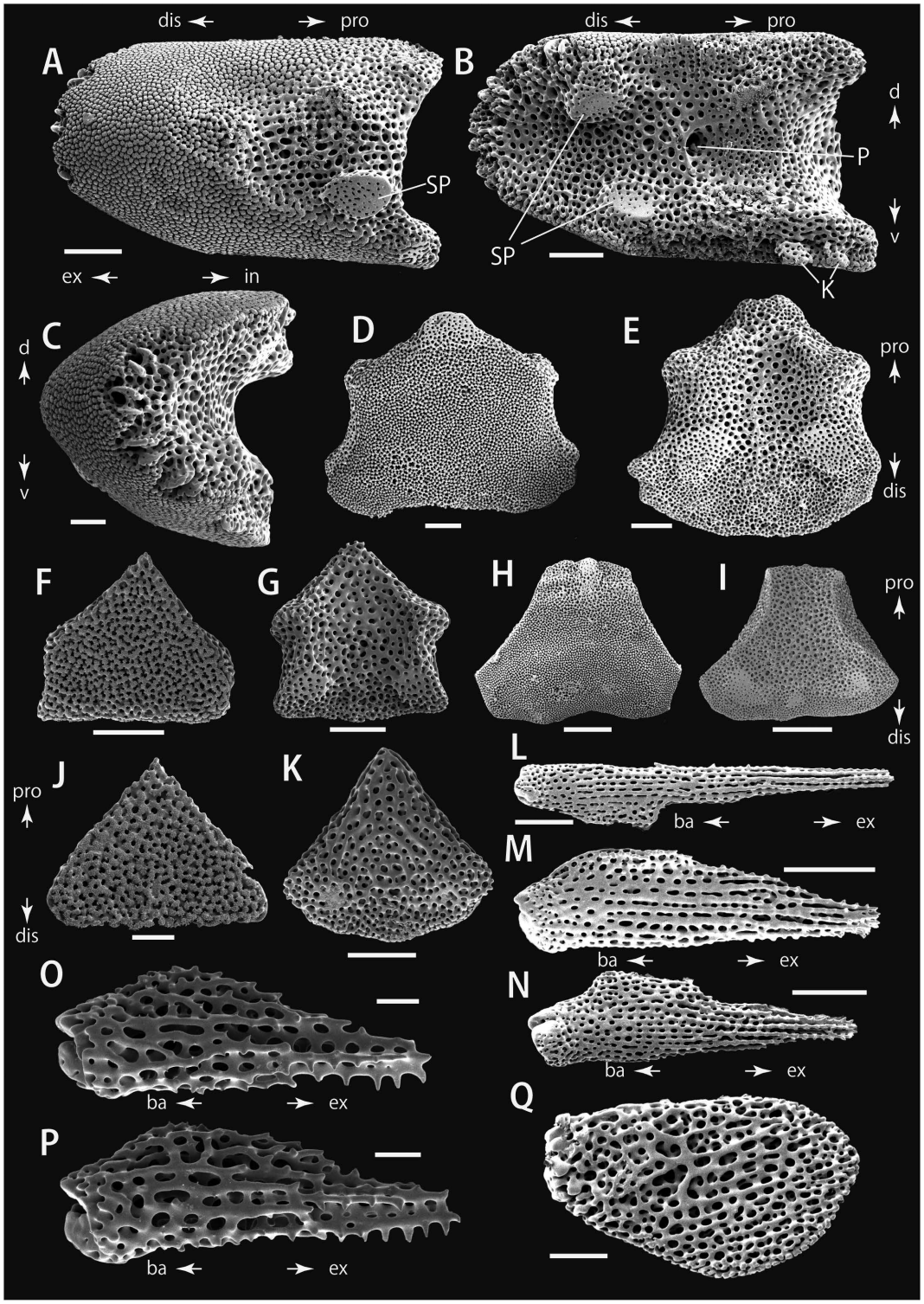

Fig. 6. *Ophioteichus multispinum* (NSMT E-14161), SEM photographs of lateral arm plates (A–C), ventral arm plates (D–G), dorsal arm plates (H–K), arm spines (L–P) and tentacle scale (Q), from proximal (D, E, H, I, L–N, Q) and distal (A–C, F, G, J, K, O, P) portions of arms. A, D, F, H, J, External views; B, E, G, I, K, Q, internal views; C, distal view; L–P, lateral views. Arrowheads indicate orientation: ba, basal side; d, dorsal side; dis, distal side; ex, external side; pro, proximal side; v, ventral side. Abbreviations: DL, dorsal lobe; K, knob; MO, muscle opening; NO, nerve opening; P, perforation; SP, spur; TN, tentacle notch; VL, ventral lobe. Scale bars: 100 μm (A–E, G, I, K–N); 200 μm (H); 50 μm (F, J, Q); 20 μm (O, P).

Foot basins located on lateral central part of vertebra (Fig. 5E, J). Protrusions present on lateral middle portion (Fig. 5C, E). A large hole opening on central part of distal vertebrae (Fig. 5H, I).

Lateral arm plates slightly longer than high, dorsal edge straight, and ventral-proximal edge slightly convex (Fig. 5K–M). In external view, proximal edge concave with 2 subequal (proximal portion of arm; Fig. 5K) or 1 (distal portion

of arm; Fig. 6A) well defined, prominent, and horizontally elongated spurs composed of more densely meshed stereom than remaining proximal edge. Bands of conspicuous, fine horizontal striations almost the same length on the distal side of spurs on proximal portion of arm (Fig. 5K). The striations unrecognizable on distal portion of arm (Fig. 6A). Eight to 10 equal-sized spine articulations on distal edge on proximal portion of arm (Fig. 5M) and 5 on distal portion of arm (Fig. 6C), and the articulations composed of parallel, horizontal dorsal and ventral lobes (Figs 5M, 6A). Dorsal lobes larger than ventral lobes and the muscle and nerve openings almost the same size (Figs 5M, 6C).

On inner-distal side, 2 well-defined, round and elevated spurs which are almost the same size (Figs 5L, 6B). On proximal portion of arm, large tentacle notch opening on ventral side and end in center of lateral arm plates, where a single perforation is opening (Fig. 5L). On the other hand, no such distinct tentacle notch recognizable on distal portion of arm (Fig. 6B). Two small knobs on proximal-ventral edge on distal portion of arm (Fig. 6B). Ventral arm plates basically tetragonal, notched on lateral sides (Fig. 6D, E). Proximal edge shaper on distal portion of arm (Fig. 6D, E) than on proximal portion of arm (Fig. 6F, G).

Dorsal arm plates triangular with straight proximal edge on proximal portion of arm and sharpened proximal edge on distal portion of arm (Fig. 6H–K).

Arm spines long, cylindrical with wider basal part (Fig. 6L–P). Longer and more slender at proximal portion of arms than at distal portion of arm (Fig. 6L–P). On proximal arm, dorsal arm spines longer and more slender than ventral ones (Fig. 6L–N), however, they subequal in size in distal portion of arm (Fig. 6O, P). Tentacle scales on proximal portion oval (Fig. 6Q), and decreasing in size toward following segments, and finally disappearing on middle to distal portion of arm.

External surfaces of lateral, ventral and dorsal arm plates with finer porous stereom than inner surfaces (Figs 5K, 6D, F, H, J).

Color in life. On dorsal side, central part of the disc brown and peripheral part creamy white. Marginal tubercles brown (Fig. 2C). Brown transverse bands on 2 to 5 arm segments alternately with creamy white ones on 2 to 3 arm segments (Fig. 2C). Ventral side, uniform creamy white (Fig. 2D).

Variations. Different from the specimen described above [NSMT E-14161: disc diameter (d.d.) = 9.7 mm, up to 10 arm spines], slightly smaller specimens (NSMT E-14162, E-14163: d.d. = 9.3 mm and 9.6 mm, respectively) have up to 11 arm spines on the proximal portion of the arm. The numbers of arm spines and of the ossicles on each side of jaw (infradental papilla, secondary infradental papilla, buccal scale, adoral shield spine, secondary adoral shield spines, Lyman's ossicle, hereafter "oral papillae s.l.") were 10–11 and 6, respectively, in our specimens (d.d. = 9.3–9.7 mm), and both of them were lower than those of the holotype (MCZ 5306: d.d. = 15 mm; Pineda-Enriquez et al. 2014). The difference between our examined specimens and the holotype may be due to size differences between specimens.

Ecological notes. All the collected specimens (NSMT E-14161–E-14163) of *Ophioteichus multispinum* were found under rocks or coral pebbles with burying of their entire body inside sand (Fig. 2A, B), showing sand-dwelling habitat of this species. Our video observations reveal that *O. multispinum* maintained its arms curled up for a while after being dug out of the sand, then slowly extended its arms (Fig. 2; Supplementary Movie 1). This curled-up arm posture does not appear to be the normal condition, but even taking this into account, this species was very sluggish compared to other brittle stars. Other sand-dwelling brittle stars, such as *Ophiopsila xmasilluminans* Okanishi, Oba, and Fujita, 2019 dig back into the sand as soon as they are exposed (Okanishi et al. 2019), but *O. multispinum* remained in place or moved only a short distance (Supplementary Movie 2). Considering this difference, we can expect that the ecology of *O. multispinum* is quite different from that of the other sand-dwelling taxa (e.g., *Ophiopsila* Forbes, 1843 and Amphiuridae), although more periodic field observations and rearing experiments are essential to further elucidation of the ecology and behavior of *O. multispinum*.

Distribution. *Ophioteichus multispinum* is known from: Lindeman Island, Great Barrier Reef, near MacKay, Queensland, Australia (type locality: H. L. Clark 1938, depth unknown; a record of topotype: Pineda-Enriquez et al. 2014, 45.3 m); Padada Beach, Gulf of Davao, Celebes Sea, Phillippines, depth unknown (Pineda-Enriquez et al. 2014); Sabiura, Kushimoto, Wakayama Prefecture, Japan 2.3–12.3 m depth (this study).

Japanese Name. The new Japanese name for *Ophioteichus* ("Joheki-kumohitode-zoku") is formed as a compound of "joheki" (meaning "rampart"), referring to the large rampart-like marginal tubercles on their disc (H. L. Clark 1938), and "kumohitode" (meaning "brittle star"), which are translations of Ancient Greek "*teichus*", and "*ophis*", respectively, and "zoku" (meaning "genus").

The new Japanese name for the specific name "*multispinum*" is formed as a compound of "Tashin" (meaning "many spines") which is a translation of the Latin "*multispinum*", referring to the more than 10 arm spines of this species.

Remarks. The observations of the ossicles in this study confirm that the examined specimens are properly placed within the Ophiolepididae, based on the following characteristics (O'Hara et al. 2018): the lateral arm plate has at least 1 spur on the ventral third of the external proximal side (Figs 5K, 6A), arm spine articulation with muscle and nerve openings of similar size (Figs 5M, 6C), and finer porous stereom in external surfaces of lateral, ventral and dorsal arm plates than those of inner surfaces (Figs 5K, 6D, F, H, J).

The 3 specimens fall within the genus *Ophioteichus* by the following characters: a circular disc that is covered with a coat of numerous swollen, smooth larger plates; each plate is surrounded by a belt of polygonal smaller flat scales; the disc scales are arranged in the same pattern dorsally and ventrally; in each interradius, the disc margin is encircled by 3 or 4 large tubercles; accessory dorsal and ventral arms plates; infradental papilla, secondary infradental papilla, buccal scale, adoral shield spine, secondary adoral shield

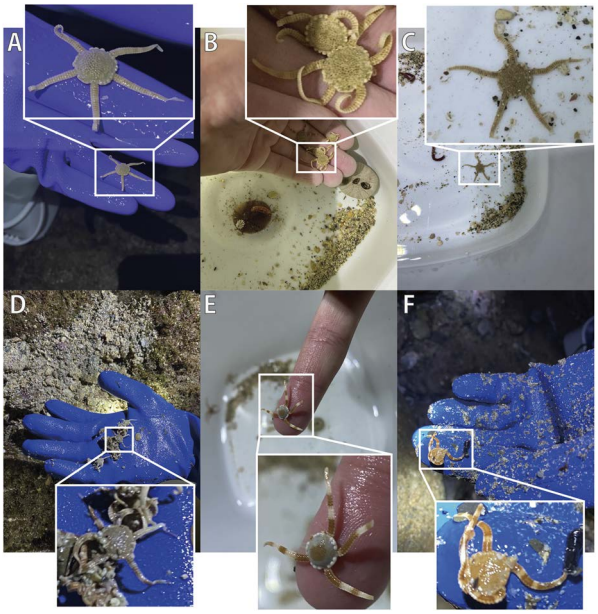

Fig. 7. *Ophioteichus* spp., living state, from intertidal region of Susami, Wakayama Prefecture (A–E) and Tosashimizu, Kochi Prefecture (F). A, B, 6 November 2021; C, 21 November 2021; D, E, 4 December 2021; F, 28 December 2021.

spine, Lyman's ossicle on each side; up to 11 slender, acute, closely spaced arm spines; 2 flat, subequal tentacle scales on each tentacle pore (Pineda-Enriquez et al. 2014).

The long and acute arm spines (Fig. 3F) and minute pores on dorsal margins of the lateral arm plates (Fig. 3B) of the examined material are consistent with diagnostic characters of *O. multispinum* from the holotype from Australia as per H. L. Clark (1938) and Pineda-Enriquez et al. (2014). On the other hand, we observed the following differences between our examined 3 individuals and the holotype: 1) the number of arm spines at the proximal portion of the arm of the holotype is more than 12, but of our examined specimens is 11 in maximum number (see "Variations" above); 2) the holotype has seven oral papillae s.l. [Pineda-Enriquez et al. (2014) did not count Lyman's ossicles as oral papillae s.l.], but the equivalent number is 6 in our examined specimens. We would like to attribute these differences to the immaturity of our examined specimens (d.d. = 9.3–9.7 mm) compared to the holotype (d.d. = 15 mm), and therefore consider these differences of number of arm spines and oral papillae s.l. as an intraspecific variation.

This is the first discovery of this species and the second discovery of a species of *Ophioteichus* from Japanese waters. The distribution of *O. multispinum* now includes Sabiura, at the southern tip of Honshu (Fig. 1). We found *Ophioteichus* in the intertidal sandy zone of Susami, Wakayama Prefecture and Tosashimizu, Kochi Prefecture (Fig. 1), although no specimens were collected. However, their large marginal plates on the lateral side of the disc (Fig. 7) definitely indicate that they were *Ophioteichus*. Although it is necessary to observe the specimens for detailed identification, the accumulation of photographic information may lead to the possibility of further collection of specimens in the future, so we believe that reports of such "sightings" are also valuable.

Supplementary Information

Supplementary Movie 1. *Ophioteichus multispinum* (NSMT E-14161) living, *in situ* states, from Sabiura, Wakayama. Available at https://doi.org/10.6084/m9.figshare.21086377.v1

Supplementary Movie 2. *Ophioteichus multispinum* (NSMT E-14161) living states, from Sabiura, Wakayama. Available at https://doi.org/10.6084/m9.figshare.21086296.v1

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Authors Contributions

Masanori Okanishi: Conceptualization; Resources; Visualization; Supervision; Writing – original draft; Funding acquisition. Ichinosuke Dan: Investigation; Writing – review & editing. Isao Hirabayashi: Investigation; Writing – review & editing.

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Declarations

Competing interests. The authors declare no conflicts of interest.

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