

First record of the sea cucumber *Holothuria (Selenkothuria) erinaceus* Semper, 1868 (Holothuroidea: Holothuriida: Holothuriidae) from a Japanese mangrove forest

Shun Kawaida^{1,*}, Yusuke Yamana², Iwao Tanita³, Kusuto Nanjo⁴

Abstract: The tropical epibenthic holothurian *Holothuria (Selenkothuria) erinaceus* Semper, 1868 is reported for the first time; a single specimen has been collected from a subtropical mangrove forest on Ishigaki Island, Yaeyama Islands, southern Japan. The external/internal morphological features, including a calcareous ring and ossicles, are described and compared with literature descriptions of congeners. *Holothuria (Selenkothuria) erinaceus* Semper, 1868 has previously been recognized as a tropical species, distributed widely in the tropical Indian and West Pacific Oceans, including the Philippines and Fiji. Not only is the Ishigaki Island record the northernmost for the species, but also the collection site is the first recorded instance of an epibenthic sea cucumber inhabiting in a mangrove forest habitat.

Key words: Holothurians, Mangrove estuary, Morphology, Ossicles, Subtropical region

Introduction

Mangroves are salt-tolerant woody plants, widely distributed in brackish intertidal zones in tropical and subtropical regions (Kon et al., 2020). Mangrove forests and adjacent tidal flats, defined as mangrove estuaries, provide habitats for various macrobenthic invertebrates

(Lee, 2008). Kintz et al. (2013), for example, reported 156 species of macrobenthic invertebrates from mangrove forests in Malaga Bay (136 km²), Columbia, including crabs, shrimps, bivalves, gastropods and polychaetes. However, there appear to have been no published records of epifaunal sea cucumbers (Echinodermata: Holothuroidea) occurring in a mangrove forest habitat,

¹ Estuary Research Center, Shimane University, 1060 Nishikawatsu, Matsue, Shimane 690-8504, Japan.

² Wakayama Prefectural Museum of Natural History, 370-1 Funo, Kainan, Wakayama 642-0001, Japan.

³ Yaeyama Field Station, Fisheries Technology Institute, Japan Fisheries Research and Education Agency, Ishigaki, Okinawa 907-0451, Japan.

⁴ Department of Applied Aquabiology, National Fisheries University, Japan Fisheries Research and Education Agency, Nagata-Honmachi, Shimonoseki, Yamaguchi 759-6595, Japan.

* Corresponding Author

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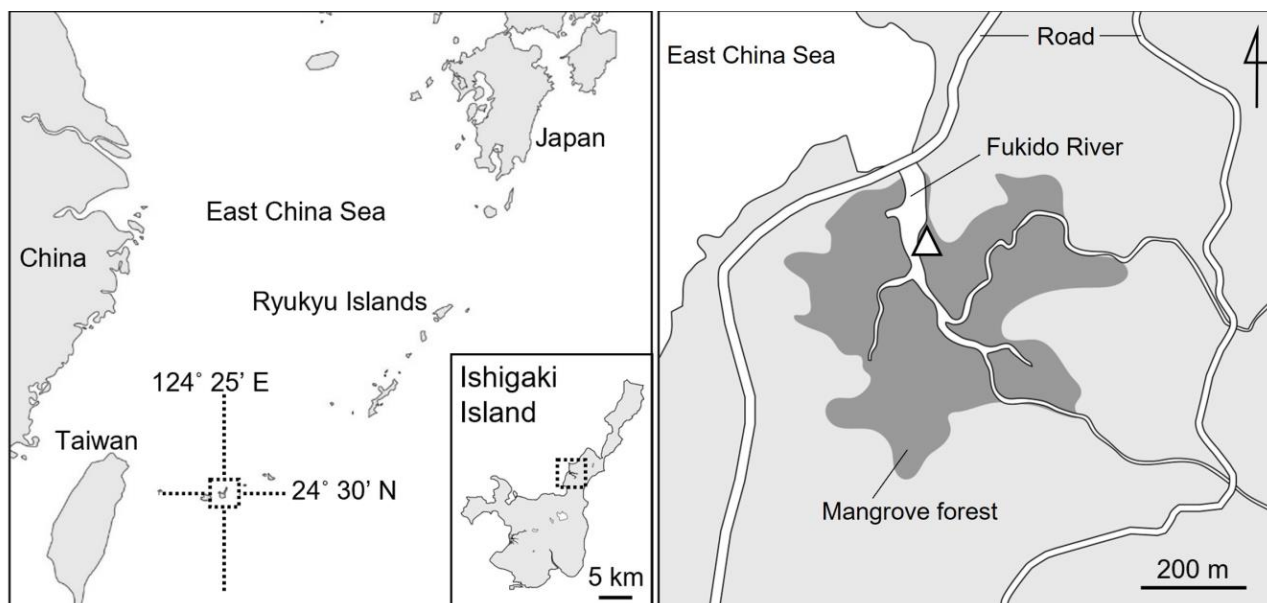


Fig. 1 Map of the Fukido River mangrove estuary, Ishigaki Island, Ryukyu Islands, southern Japan. The specimen of *Holothuria (Selenkothuria) erinaceus* was collected in the downstream section of the river (indicated by white triangle).

despite the former being a common benthic faunal group, and especially diverse in tropical and subtropical coastal environments, including coral reefs, adjacent sandy bottoms and seagrass meadows (e.g., Pawson, 2007; Tanita and Yamada, 2019; Floren et al., 2021).

Tropical macrobenthic invertebrates are often found in the subtropical region of southern Japan, due mainly to larval transport by the Kuroshio Current (Komai et al., 2004; Naruse, 2016). The present report describes a specimen of epibenthic sea cucumber of *Holothuria (Selenkothuria) erinaceus*, a tropical species occurring in the Indian and West Pacific Oceans, including the Philippines and Fiji (Semper, 1868), collected in a mangrove forest in southern Japan. Although several other tropical and subtropical localities have been reported for the species, with the Philippines being the previous northernmost record (Semper, 1868; Rowe and Gates, 1995; Thandar and Samyn, 2004; Honey-Escandón et al., 2012), none have been reported from Japanese waters to date. Following a careful survey of the morphological characteristics of the specimen, we report the first record for *Holothuria (Selenkothuria) erinaceus* from Ishigaki Island in Japan, the present study contributing to a better understanding of macrobenthic invertebrate species diversity in mangrove estuaries.

Materials and Methods

Locality and sampling method

One specimen of the sea cucumber was collected by hand from the muddy sediment surface shaded by an aerial prop root of the red mangrove *Rhizophora stylosa*, a component of the subtropical mangrove forest (24°29'12.3" N, 124°13'49.9" E) along the Fukido River (flowing into the East China Sea), located on northern Ishigaki Island, Yaeyama Islands, southern Japan, between daytime (13:00–17:00) on 29 June 2022 (Fig. 1). The dense and undisturbed mangrove forest, extending along both sides of the river, was dominated by the black mangrove *Bruguiera gymnorhiza*, with patches of *R. stylosa*, with the water depth of the flat being approximately 1.5 m below mean high water spring tide (MHWS) [prop roots inundated at high tide, and exposed together with tidal flats at low tide (Fig. 2a, b)]. Sampling of the sea cucumber was carried out with the permission from the Fishery Division of the Okinawa Prefecture Government.

Morphological observations

The specimen was fixed in 80% ethanol following anaesthetization, using magnesium sulfate (MgSO_4) dissolved in sea water [concentration ca. 3.0% (w/w)].

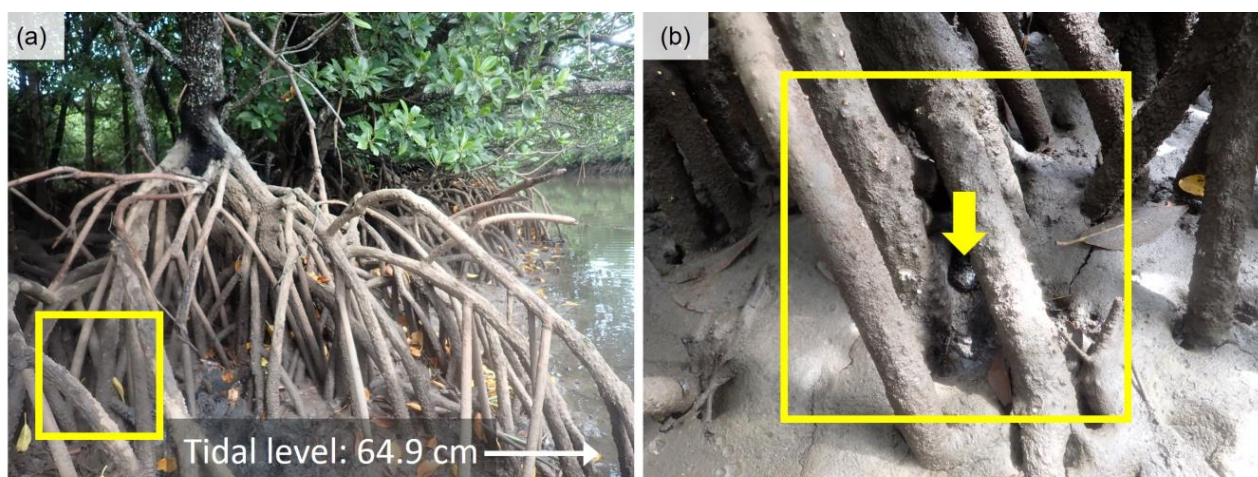


Fig. 2 Habitat of *Holothuria (Selenkothuria) erinaceus*. Prop roots of the red mangrove *Rhizophora stylosa* (a). Tide level based on mean low water spring tide (MLWS) at Ishigaki Island on the measurement date was indicated by white arrow. *Holothuria (Selenkothuria) erinaceus* on exposed muddy substrate between prop roots (indicated by yellow arrow) (b).

The specimen was immersed in the latter for about three hours, after which live color and external characteristics were recorded by digital photographs, prior to fixation.

The specimen was dissected, exposing the tentacles and internal organs, including a calcareous ring, and the eviscerated organs examined under a stereoscopic dissecting microscope (SMZ1270, TREG-DSL32, Nikon Japan Inc., Japan). A small piece of body-wall tissue (ca. 2×2 mm) was sampled from both the mid-dorsal and mid-ventral body wall, and several appendages (five pedicels and five dorsal papillae, plus one dorso-interradial tentacle) extracted for observation of ossicle morphology. Each tissue sample was dissolved using a commercial bleach (Kitchen Bleach, Mitsuei Chemical Co., Ltd., Japan), and most of the extracted ossicles rinsed with distilled water, dried on glass slides, and mounted in polyester resin (RIGOLAC, Showadenko KK., Japan). The slides were examined and ossicles measured (to nearest μm) under a compound microscope (Optiphot XF-NT, Nikon Japan Inc., Japan). Several ossicles from each sample were rinsed with deionized water, dehydrated in 99% ethanol, and mounted on aluminum stubs using conductive tape (NEM Tape, Nisshin EM Co., Ltd.). Samples were then dried at room temperature and observed using a scanning electron microscope (SEM) (JCM-7000, JEOL Ltd., Japan).

The examined ossicles were classified into two types:

‘rods’ (i.e., rod-like ossicles, usually with distal crenation or teeth and few perforations); and ‘plates/endplates’ (multi-perforate flat ossicles). The specimen, glass-slides, and SEM stubs are deposited in the Invertebrate Collection (INV) of the Wakayama Prefectural Museum of Natural History (WMHN), Kainan, Wakayama, Japan.

Results

Genus *Holothuria* Linnaeus, 1767

Subgenus *Selenkothuria* Deichmann, 1958

Diagnosis of subgenus (modified after Deichmann, 1958): Soft-skinned forms with numerous cylindrical feet in longitudinal bands forming distinct ventral sole, sometimes arranged in three broad bands; dorsally numerous minute papillae, not conspicuous, rarely forming low warts. Tentacles located at end of body, often bushy. Internal anatomy not remarkable (similar to many other subgenera); respiratory tree branching to both sides of body cavity; some species with numerous stone canals in one or two tufts on both sides of dorsal mesenterium, others with single large stone canal attached to right side; usually one Polian vesicle; gonads apparent as divided threads in tuft behind low calcareous ring; Cuvierian organs present in some forms. Table ossicles of external body wall usually completely lacking, though vestiges of tables sometimes found in young individuals of some

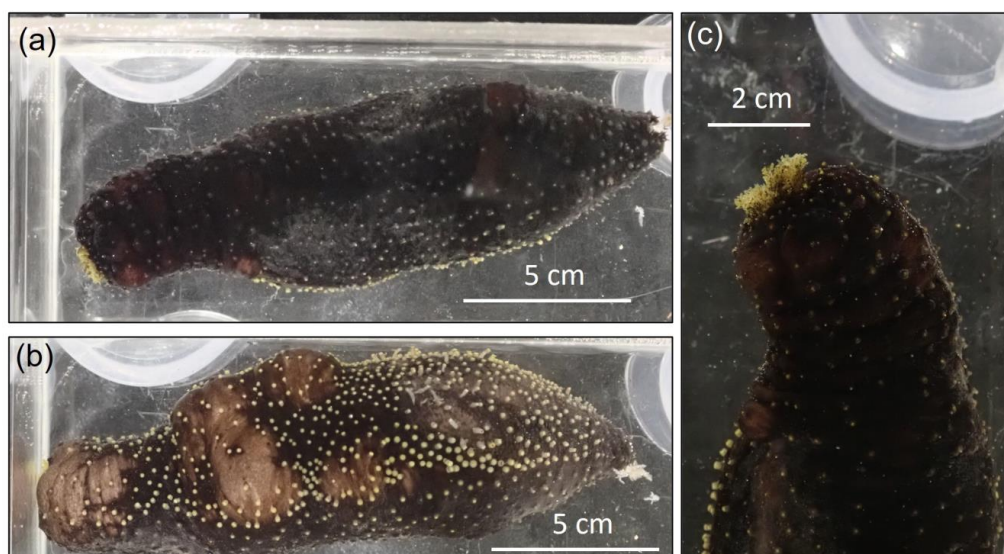


Fig. 3 Live specimen of *Holothuria (Selenkothuria) erinaceus* Semper, 1868 (WMNH-INV-2022-366) collected from the Fukido River mangrove estuary. Dorsal side (a), ventral part (b), around oral disk and tentacles (c).

species. Ossicles of inner body wall consisting of small rods or plates, either smooth or spinous. Ventral feet with single large endplate and walls supported by rods or plates of same size as those in skin, rarely special supporting rods or perforated plates present. Dorsal appendages with or without reduced endplate, often with more curved, shorter rods. Some species with rods present in cloacal retractor muscles and respiratory trees. Color dull gray, dark brown, olive-green or black, with or without two rows of dark spots; anterior and posterior ends darker.

***Holothuria (Selenkothuria) erinaceus* Semper, 1868**
[New Japanese name: Yaeyama-hirugi-namako] (Fig. 3)

Synonymy

- Holothuria erinaceus* Semper, 1868: 91, pi. 30, fig. 24.
H. erinaceus var. *pygmaea* Semper, 1868: 91, pi. 30, fig. 23.
H. marenzelleri Ludwig, 1883: 167.
H. (Halodeima) lubrica var. *marenzelleri* Panning, 1934: 47, fig. 41.
H. (H.) lubrica var. *glaberrima* Panning, 1934: 47, fig. 42 (part).
Selenkothuria erinaceus Deichmann, 1958: 314 (synonymy), 315 (keys).
Holothuria (Selenkothuria) erinaceus Rowe, 1969: 135;

Clark and Rowe, 1971: 178. (distribution), pi. 28, fig. 5.
Holothuria (Sel.) erinacea Thandar, 2007: 29–31, fig. 13.

Original description (Semper, 1868: 91): Twenty short tentacles. Body cylindrical, slightly tapered towards both ends. Numerous small feet on abdominal side. Very dense fine and long papillae on dorsal side, length of body 18–20 cm, maximum thickness 3.75 cm. Color of specimen plain grey-brown, anal field black. Specimen collected from Bohol Island, on shore, Viti Islands. One Polian vesicle; 2 cm long stone canal, somewhat similar to that of *Holothuria scabra* Jäger. Whole reproductive organs of specimen 10 cm long in liquid, 2 cm from calcareous ring, gonadal organs attached with very long 2–4 tubules; body of male specimen 5–6 cm shorter and significantly thinner than that of female described above. Cuvierian tubules exceptionally small, in tuft at bottom of respiratory trees. Calcareous bodies of the specimens from Bohol Island lost completely due to acetic acid. Specimen from Viti Islands no stool like ossicles, numerous knotty rod like ossicles, extremely similar to those of *Holothuria glaberrima* Selenka, but not unlikely both forms of ossicles observed together for specimens from other regions. C-shaped rod-like ossicles and plates observed in pedicels, and rudimentary plates observed in body wall skin. Calcareous ring ribbon-like. Axillary



Fig. 4 Preserved specimen of *Holothuria (Selenkothuria) erinaceus* Semper, 1868 (WMNH-INV-2022-366).

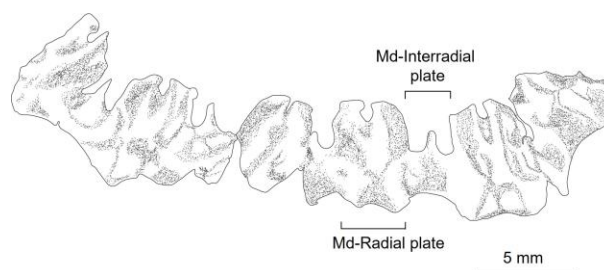


Fig. 5 Elements of calcareous rings of *Holothuria (Selenkothuria) erinaceus* (WMNH-INV-2022-366) viewed from body cavity (anterior uppermost). Md, mid-ventral radial zone.

cord in Cuvierian tubules not cellular, and scattered cells in fibrous connective tissue [originally described by Deutsch in Semper, 1868: 91 p.+ Plate XXX, f 24, mainly translated from original sentences to modern English by a language services provider (Crimson Interactive Japan Co., Ltd.) with native German-speaking professionals].

Type locality: Bohol Island, Philippines; Viti island, Republic of Fiji (Semper, 1868).

Species diagnosis (emended by Tortonese, 1980: pp. 105–106): Body becoming narrower anteriorly. Uniformly brown, but clear ring around small buccal tentacles in 7 mm wide group. Podia crowded on abdominal surface, much smaller and scattered on back, where skin much folded. Ossicles include rods only (generally more elongated than figured in original description). Median axis apparent, somewhat recurved. Shape very variable, surface smooth, edge more or less spiny, holes restricted at extremities or arranged in series along one or both sides of axis. Larger rods forming perforated plates, smaller rods button-like; no true buttons or tables.

Material examined: One specimen, 176 mm long, 51 mm wide; body and eviscerated internal organs fixed and preserved in 80% ethanol (Registered number: WMNH-INV-2022-366).

External morphology: Oral disk slightly inclined to ventral side. Tentacles 20 short (ca. 7.6 mm in preserved state), forming single circle surrounding oral opening,

protected by narrow rhombus-shaped skin. Color of living specimen: tentacles semi-transparent yellow, ventral skin black-brown with three bands of scattered broad pale yellowish pedicels (greatest density < 10 pedicels /cm²), dorsal skin black-purple with dorsal yellowish papillae in about seven indistinct latitudinal lines (Fig. 3). Overall external morphology similar to that of *Holothuria (Semperothuria) roseomaculata* (reported by Kerr, 2013). Color well maintained in preserved specimen after six months (Fig. 4).

Internal morphology: Calcareous ring thick, with solid radial and interradial plates, former convex, each with one anterior and one posterior depression, latter each with sharp anterior prolongation (Fig. 5). Internally, eight tentacle ampullae, 7–10 mm in length; single Polian vesicle, mid-ventral part close to calcareous ring, cylindrical and expanding posteriorly (36 mm in length); single stone canal, mid-dorsal part close to calcareous ring attached to right side of mesentery, twisted and long (17 mm), with egg-shaped madreporite (1.2 mm in length; 1.8 mm in breadth); Cuvierian tubules present, small, tuft-like (eviscerated and could not be collected because of the tubules sticking to the mangrove roots).

Ossicle morphology: Tentacle ossicles double-bladed saw-shaped rods in external (distal) part, short rods in proximal (basal) part (Fig. 6a). External spinous rods ranging between 147–205 μm in length, 8–11 μm in breadth (at middle); proximal rods spinous, shorter than external rods, ranging between 56–70 μm in length, 9–11 μm in breadth (at middle) (Table 1). Endplate situated

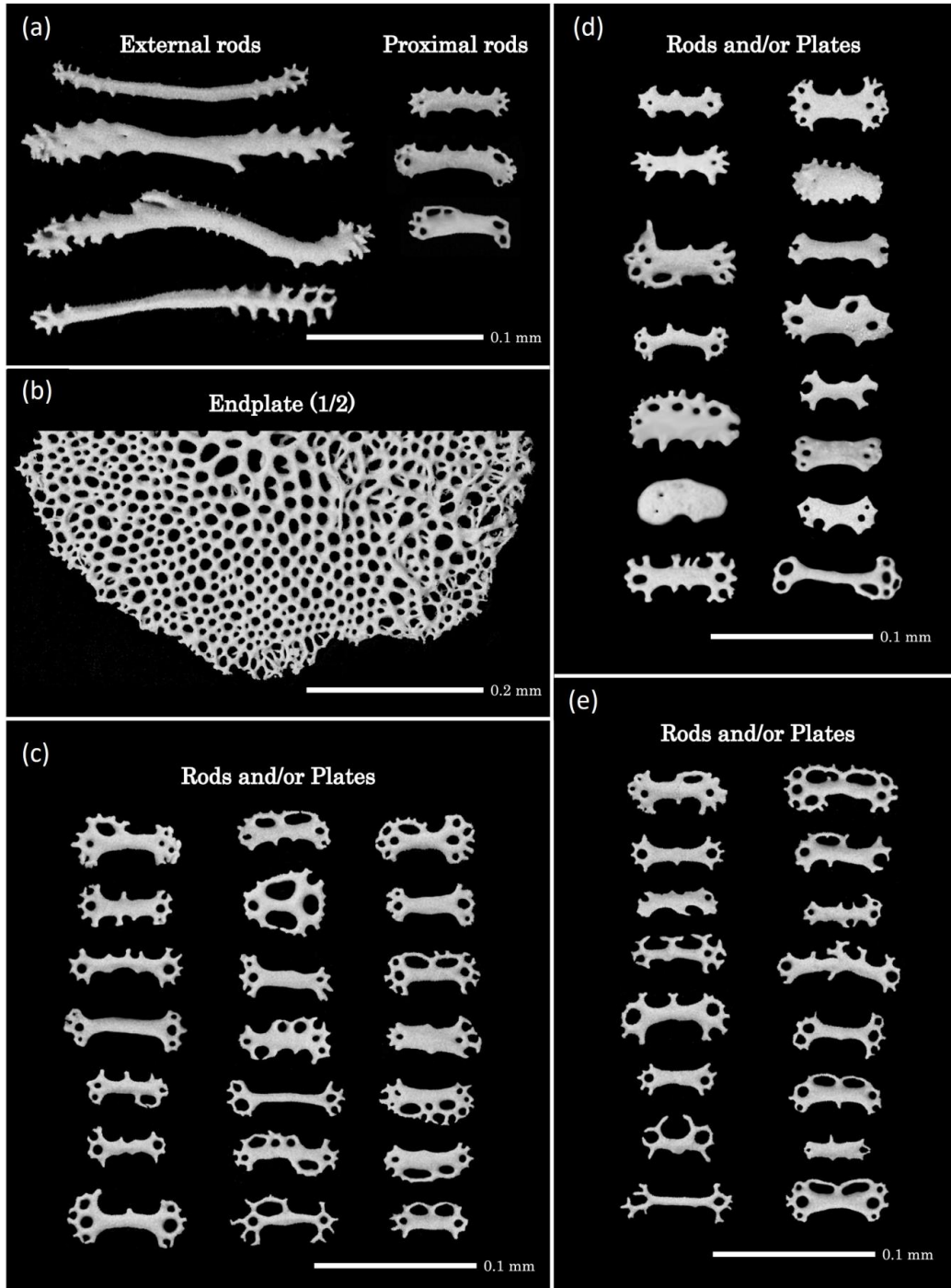


Fig. 6 SEM images of body ossicles of *Holothuria (Selenkothuria) erinaceus* (WMNH-INV-2022-366). External and proximal rods from lateral tentacle (a), endplate from pedicel (b), rods and/or plates from papilla of anterior dorsal body wall (c), ventral body wall (d) and dorsal body wall (e).

Table 1 Counts and measurements of ossicles extracted from a specimen of *Holothuria (Selenkathuria) erinaceus*.

Ossicle type and measurements (μm)	<i>n</i>	Mean \pm sd	Range
Tentacle external rods			
Length	4	177 \pm 25	147–205
Width ^a	4	10 \pm 2	8–11
Tentacle proximal rods			
Length	3	62 \pm 7	56–70
Width	3	10 \pm 1	9–11
Pedicel endplate			
Major axis	5	640 \pm 59	581–709
No. holes ^b	5	1024 \pm 125	840–1158
Papilla rods and/or plates			
Length	21	60 \pm 8	48–76
Width	21	12 \pm 4	5–19
Body rods and/or plates (ventral)			
Length	15	60 \pm 9	46–78
Width	15	15 \pm 7	8–31
Body rods and/or plates (dorsal)			
Length	16	57 \pm 10	41–77
Width	16	11 \pm 4	5–18

^a Width of central part of stem.^b Estimated by doubling counts of half plate area.

on tips of pedicels, undulant, with numerous uneven perforations increasing and narrowing marginally, diameter ranging between 587–709 μm , with ca. 840–1158 holes (Fig. 6b, Table 1). Rod and plate ossicles absent from pedicel wall; papillae and ventral/dorsal body wall ossicles comprising derivatives of rod ossicles, including rods, plates and intermediate forms (rod-like plates), primarily exhibiting the shapes namely “Venetian mask-shape rods (Fig. 6c–e)”, ranging between 41–78 μm in length, 5–31 μm in breadth (at middle) (Table 1).

General distribution: Type localities (Semper, 1868); Gulf Albay, Luzon Island, Bohol Island, Philippines, Viti Islands, Fiji, Port Mackay, Australia, and Singapore. Also common from East Africa to Polynesia (Tortonese, 1980), in tropical Indo-West Pacific Ocean (Rowe and Gates, 1995; Thandar and Samyn, 2004), including east, west and southwest Pacific (Domantay, 1957), Ceylon (Sri

Lanka), Bay of Bengal, East Indies, northern Australia, the Philippines and South Pacific Islands (Clark and Rowe, 1971), Australia (Rowe and Gates, 1995), Reunion and Comoros Island, France, Madagascar (Honey-Escandón et al., 2012), and Ryukyu Islands, Japan (present study).

Discussion

The specimen collected was identified as *Holothuria (Sel.) erinaceus*; the ossicles were consistent with the original description and other taxonomic studies of the species (Semper, 1868; Clark and Rowe, 1971). It differed from phylogenetically related species (Honey-Escandón et al., 2012): *H. (Sel.) bacilla* (Cherbonnier, 1988), *H. (Sem.) cinerascens* (Ahmed et al., 2016), *H. (Sel.) mactanensis* (Tan Tiu, 1981), and *H. (Sel.) parva* (Thandar, 1977) in the form of the body-wall ossicles, each predominantly

having knobbed rods and thick/stock rods, tables and rods, non-perforated sinuous rods with irregular spinose margin, and straight or irregular non-perforated spinous rods, respectively, whereas *H. (Sel.) erinaceus* has only perforated short flat rods. Furthermore, other external/internal morphological features reported in the present study completely matched the original description (Semper, 1868), confirming the identity of the species.

The present study not only represents the first record for *H. (Sel.) erinaceus* from Japan, but also extends the geographical distribution of the species approximately 700 km north from the former northernmost record (Luzon Island, Philippines) (Semper, 1868). The status of *H. (Sel.) erinaceus* in Japanese subtropical waters, either well-established or a recent arrival, cannot be determined at present, although a common inference can be drawn regarding northward distribution of marine organisms around Japan, due to the impact of the Kuroshio Current. The latter flows northward near the eastern coast of Taiwan, turns eastward to flow near the north side of the Ryukyu Islands, and subsequently flows near the south coast of the Ohsumi Islands, and off Kyushu, Shikoku and Honshu (Asakura, 2021), with the potential to carry eggs and larvae of marine species from tropical regions to southern Japan and Honshu (Sassa et al., 2006; Sassa and Takasuka, 2020). Recent studies have shown that various inland/estuarine brachyuran species with a pelagic larval phase, not previously recorded in Japan, have been transported from tropical areas such as the Philippines and India to the Yaeyama Islands, mainly by the Kuroshio Current (Komai et al., 2004; Naruse, 2016). Furthermore, some studies have indicated likely increased habitat ranges of tropical species northwards, due to recent sea surface temperature rises related to global warming (e.g., Bianchi and Morri, 1994; Hiscock et al., 2004; Crickenberger, 2014). Accordingly, larvae of *H. (Sel.) erinaceus* may be transported northward around Japan from tropical regions, although detailed information on their pelagic duration and genetic structure is necessary to clarify their population dynamics.

The *H. (Sel.) erinaceus* specimen was found on the muddy substrate in a mangrove forest. Although James (1982) reported that *H. (Sel.) erinaceus* inhabits mud flats in the supra-littoral zone, being gregarious in some places on the Andaman coast, India, there have been

no instances of the species inhabiting mangrove forests reported to date. Accordingly, the finding of our study reinforces that mangrove forests contribute to high biodiversity in coastal ecosystems by providing available habitats for various macrobenthic invertebrates, including holothurid sea cucumbers. The physical structure of mangrove vegetation reduces water movement (i.e., wave and tidal current) energy and provides excellent shelter from coastal erosion for various macrobenthic invertebrates (Macintosh and Ashton, 2002). Such water movement would affect the distribution of some species of sea cucumbers such as *Apostichopus japonicus* and *Holothuria scabra*, which are typically found in sheltered areas with less wave and current action (Barkai, 1991; Mercier et al., 2000). Furthermore, several studies have revealed that large amounts of sediment organic matter (comprised mainly mangrove detritus) are an important food source for some deposit feeders (Kawaida et al., 2019; Mackenzie et al., 2020). In general, holothurians are considered to obtain nutrients from organic detritus and sediments from substrate surface (Purcell et al., 2016), intestine of the specimen of *H. (Sel.) erinaceus* being filled with fine mud probably due to feeding on sediment organic matter in the mangrove forest floor. These findings suggest that functions of mangrove forests (i.e., shelter from wave/current impacts and organic-rich environments) are likely beneficial to *H. (Sel.) erinaceus*. Further investigations on the relationships between the spatial distribution of *H. (Sel.) erinaceus* and abiotic environmental properties (e.g., salinity, prop root densities, relative substrate elevation and sediment characteristics) in mangrove forests would contribute to a better understanding of the important role of mangrove estuaries in providing habitats for diverse macrobenthic invertebrates.

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