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Two newly recorded echinoderms from the mesophotic zone in Korea

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The region of the marine environment between shallow waters and deep-sea (30−150 m in depth) is referred to as the twilight or mesophotic zone. This zone has been scarcely examined, as these depths are too shallow for safe submersible operation. Since 2018, a survey of mesophotic echinoderms in Korea has yielded many specimens of interest. In this study, we present two newly recorded echinoderms, *Henricia irregularis* and *Parastichopus nigripunctatus* based on morphological redescriptions with high-definition photographs and DNA barcoding data for *P. nigripunctatus*.

Keywords: COI, *Henricia irregularis*, *Parastichopus nigripunctatus*, SEM, taxonomy

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Introduction

The mesophotic zone is frequently defined as the marine region between the shallow waters and deep-sea (30−150 m in depth). This region, where light dependent communities survive in extreme light-limited habitats, has unusual biota, where eurybathic organisms mix with species that only live in deep-water environments (Schneider *et al*., 2019). The obvious hazards and economic challenges posed by in situ surveys and sampling underwater have been and will remain barriers to scientific research at depth. In past centuries, the marine biodiversity surveys had focused mainly on infralittoral and shallow depths, but by the 1970s, submersible technology had allowed surveys of the deep-sea (Grassle *et al*., 1975; Heirtzler and Grassle, 1976; Barnes *et al*., 1977; Corliss *et al*., 1979; Tunnicliffe *et al*., 1986), leading to surprising discoveries of marine life and a dramatic re-thinking of marine biodiversity patterns (e.g., Rex (1981) and Grassle (1985)). However, the mesophotic zone has been scarcely examined as these depths are too shallow for safe submersible operation, yet too deep for conventional SCUBA diving (Pyle, 1996; Hinderstein *et al*., 2010; Kahng *et al*., 2010). A mesophotic survey of echinoderms in Korea was conducted from 2018, using trimix SCUBA diving and fishing net. Intensive investigation of the mesophotic zone has resulted in the discovery of numerous newly recorded echinoderms and more detailed information about their habitat within a shorter period of time compared to previous studies, such as *Aquilonastra doranae* O'Loughlin and Rowe, 2006, *Henricia djakonovi* Chichvarkhin, 2017, and *Ophiacantha scissionis* Lee, Stöhr, Bae and Shin, 2019.

DNA barcoding sequence variation in a 658-base pair (bp) region of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene is a powerful tool for the species identification and discovery (Hebert *et al*., 2003; Ratnasingham and Hebert, 2007). This COI region has been validated as an efficient tool for species discrimination of echinoderms (Ward *et al*., 2008; Hoareau and Boissin, 2010; Layton *et al*., 2016). The COI gene is the most commonly used and accepted barcode for echinoderms. The COI gene has been found to lack a sufficiently conserved region for barcoding using a dataset with more than 4,000 COI sequences covering almost 400 echinoderm species across all five extant classes (Licuanan and Matias, 2022).

Here, we present two newly recorded echinoderms from a mesophotic survey conducted in two regions of South Korea based on morphological description with high-definition photographs. Moreover, we provide DNA barcoding analysis data that can be used to distinguish *Parastichopus nigripunctatus* (Augustin, 1908) from *Apostichopus japonicus* (Selenka, 1867), which has a quite similar appearance to *P. nigripunctatus*.

Materials and Methods

Specimens of *Henricia irregularis* Hayashi, 1940 and *P. nigripunctatus* were collected from Jejudo Island and the East Sea in Korea using fishing nets. Collected specimens were immediately preserved in 99% ethanol solution. Morphological characteristics were examined of *H. irregularis*, such as the size of the disk, upper and proximal portions of arms, number of abactinal spines, shapes of abactinal and actinal skeletons, and number of adambulacral spines. Ossicles of *P. nigripunctatus* were extracted from small pieces of body wall, tentacle, and tube feet, using NaClO solution. Morphological features of specimens were photographed using a digital camera (Sony A6100; Sony Co., Tokyo, Japan) for the whole body, a stereomicroscope (Nikon SMZ1000; Nikon Co., Tokyo, Japan) for the detailed parts of body, and a scanning electron microscope (JSM-6510; JEOL Ltd., Tokyo, Japan) for ossicles. Abbreviations used in the description of *H. irregularis* are as follows: R, which represents the length from the center of the disk to the end of the arm; and r, which represents the length from the center of the disk to the end of interamburacral.

Total genomic DNA of *P. nigripunctatus* was isolated from ethanol-preserved gonad tissues using a DNeasy blood and tissue DNA isolation kit (Qiagen, Hilden, Germany) according to the manufacturer instructions. Genomic DNA quality and concentration were determined using a Nanodrop One-C spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). All genomic DNA samples were stored at −20°C until further use. Partial sequence of mitochondrial COI gene (648 bp) was amplified using a pair of primers conserved in echinoderms, COIeF and COIeR (Arndt *et al*., 1996). PCR was performed using an AccuPower PCR PreMix kit (Bioneer, Daejeon, Korea) in a total volume of 20 μL in accordance with the manufacturer's instructions. After 1.5 μL of template DNA, 1 μL of each primer at 10 pmol, and 16.5 μL of distilled water were added to AccuPower PCR PreMix, polymerase chain reaction (PCR) was performed with the following amplification parameters: an initial denaturation at 94°C for 3 min, 35 cycles of denaturation at 94°C for 30 sec, annealing at 48°C for 45 sec, and extension at 72°C for 1 min, followed by a final extension step at 72°C for 7 min. PCR product quality was determined by 1.0% agarose gel electrophoresis followed by staining with an EcoDyeTM Nucleic Acid Staining Solution (Biofact, Daejeon, Korea). PCR products were directly sequenced in both directions using an ABI Big Dye Terminator kit and an ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

For the molecular analysis, we used mitochondrial COI sequences (599 bp) from five species of stichopodids, including *P. nigripunctatus*, and two species of genus *Cucumaria* de Blainville, 1830 were used as an outgroup. We assembled and aligned COI sequences for this study with Geneious Prime v.2022.2.2 (Biomatters Limited, Auckland, New Zealand). Pairwise genetic distances were calculated using MEGA 11 (Tamura *et al*., 2021) with the Kimura-2 parameter model (Kimura, 1980). Gaps and missing data were completely deleted.

Taxonomic Accounts

Phylum Echinodermata Klein, 1778
Class Asteroidea de Blainville, 1830
Order Spinulosida Perrier, 1884
Family Echinasteridae Verrill, 1867
Genus *Henricia* Gray, 1840

***Henricia irregularis* Hayashi, 1940 (Figs. 1–3)**
불규칙애기불가사리 (신칭)
Henricia irregularis Hayashi, 1940: 164.

Material examined. One specimen, adjacent water of Jumunjin: Gangneung-si: Gangwon-do, Korea (37°52′13″N, 128°58′11″E), February 27, 2018, Lee T., a depth of 85 m, collected with a fishing net, deposited in the National Marine Biodiversity Institute of Korea (MABIK; MABIK_IV00172917) (Fig. 1).

Description. Arms five, long, flexible, gradually tapering to tips (R = 202 mm). Disk small (R = 5.8r) (Fig. 2). Abactinal skeleton weakly reticulated structure with narrow meshes (Fig. 3A, 3B). Abactinal plate small, low, rod-like shape with one or two well discernible tubercles, with ambiguous series. Abactinal paxillae small, composed eight to ten spines. Abactinal papular areas narrow, with one or two papulae in an area, irregular in shape, and subdivided by small ossicles. Madreporite circular, slightly convex, near center of disk, containing scattered spines like those found on adjacent spines. Superomarginal plates elongated cross shape, with rows extending near middle of arm and gradually bending upward toward base. Intermarginal plates rod- or lobe-shape, not in regular series, with a narrow papular area and a single papula in each area; intermarginal areas spacious. Inferomarginal plates crescent-shaped, not forming a regular series. Ventrolateral plates rounded and some slightly elongated shape, larger than adjacent plates, not in regular series. Adambulacral plates elongated cross shape, covered with 13–20 slender spines with blunt tips and arranged transversely in three uneven rows (Fig. 3D). Furrow spines double (Fig. 3C, 3D). Two oral spines with spatulate tip, three marginal spines, four or five sub-oral spines in oral part (Fig. 3C).

Habitat. This species was collected at a depth of 85 meters. It is likely to inhabit the mixed substrate (coarse sand

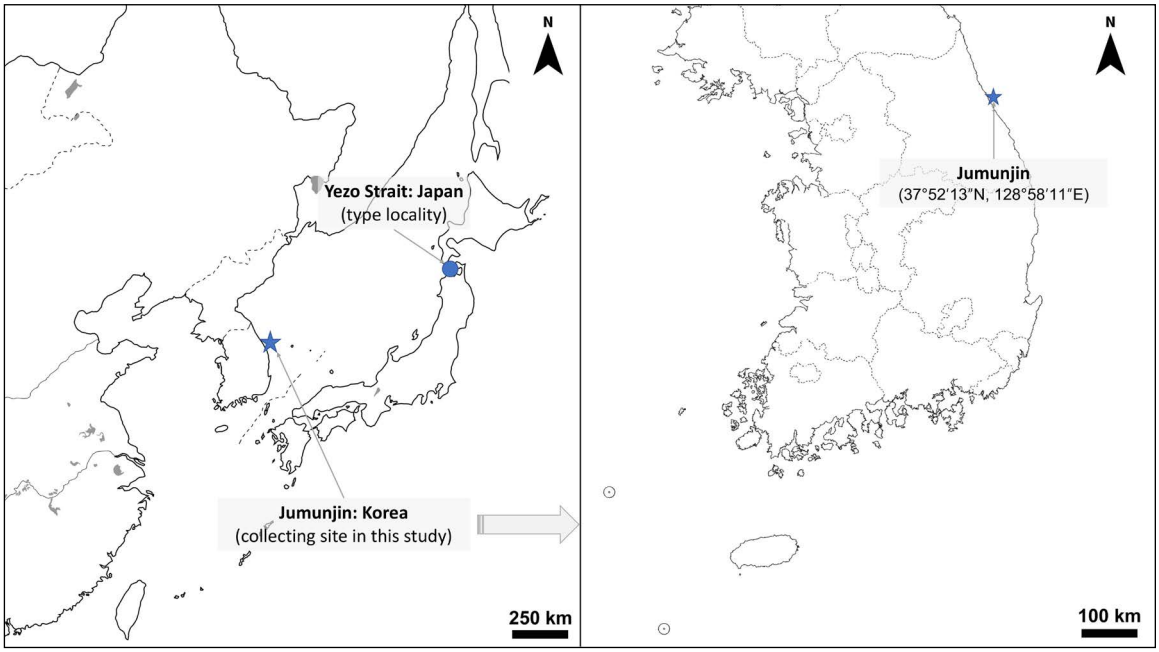

Fig. 1. Distribution of *Henricia irregularis* Hayashi, 1940 based on the present and previous study.

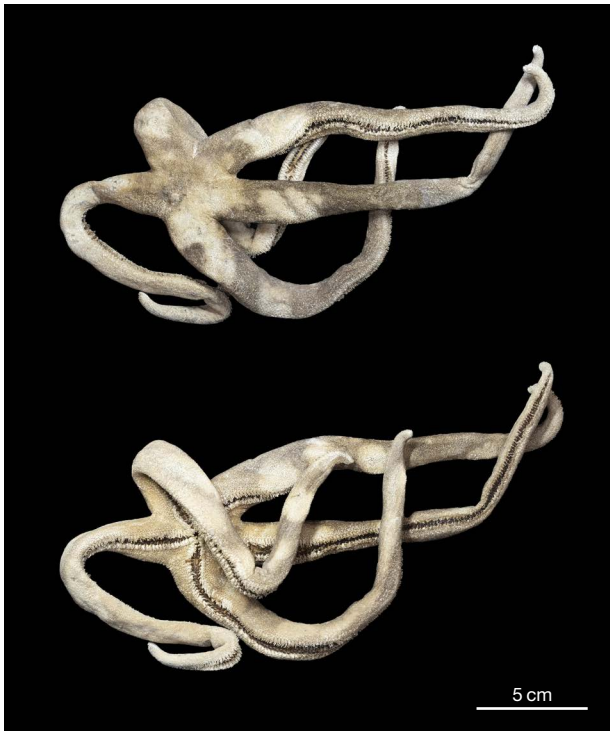

Fig. 2. Abactinal and actinal side of *Henricia irregularis* Hayashi, 1940 in this study.

and rocks), based on other echinoderms collected in the same area.

Distribution. Korea (Jumunjin: the East Sea), Japan (Yezo Strait: Hokkaido).

Remarks. *Henricia irregularis* is similar in appearance to *H. sanguinolenta* Fisher, 1911. Specifically, both species have long arms and a small disk, and their abactinal plates are characterized by narrow meshes. Some morphological differences are the following: 1) abactinal skeleton structure (*H. irregularis*: weak; *H. sanguinolenta*: stout); 2) number of abactinal spines (*H. irregularis*: 8−10; *H. sanguinolenta*: 7−16); 3) number of abactinal papulae (*H. irregularis*: 1 or 2; *H. sanguinolenta*: 1−5); 4) actinal plate series of superomarginal, inferomarginal, ventrolateral (*H. irregularis*: irregular series; *H. sanguinolenta*: regular series).

Our specimen has slightly different morphological characteristics from the holotype specimen in Hayashi (1940). Hayashi (1940) described the abactinal plate of *H. irregularis* as elongated with a plain appearance, while our specimen shows elongated plates with tubercles. Nevertheless, the major key features in our specimen match a description of Hayashi about the holotype. We conclude that our specimen belongs to the species *H. irregularis*, based on its observed morphological features, which match those of the description of holotype.

Class Holothuroidea de Blainville, 1834
Subclass Actinopoda Ludwig, 1891
Order Synallactida Miller, Kerr, Paulay, Reich, Wilson, Carvajal and Rouse, 2017
Family Stichopodidae Haeckel, 1896
Genus *Parastichopus* H.L. Clark, 1922

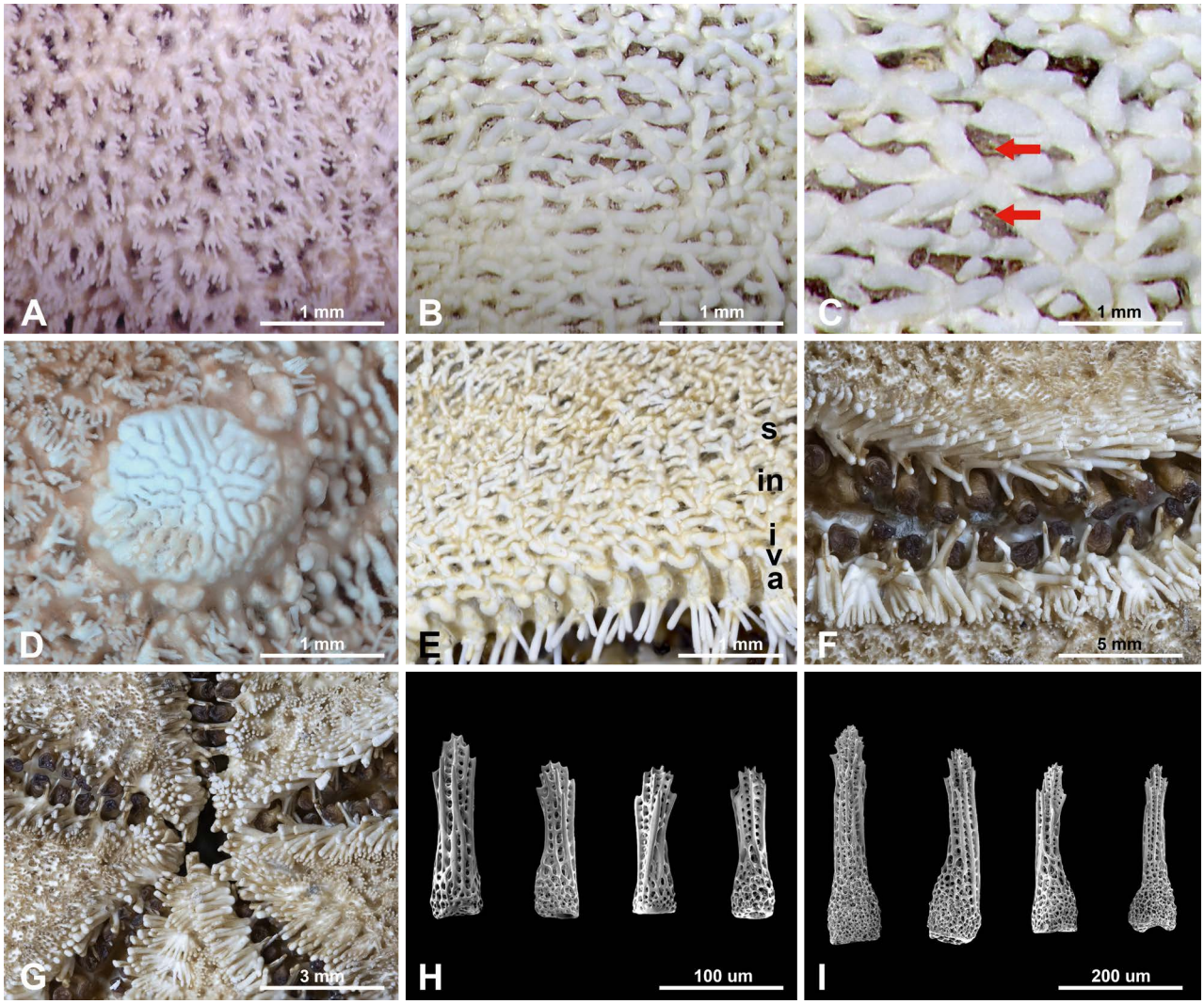

Fig. 3. *Henricia irregularis* Hayashi, 1940 in this study. (A) abactinal paxillae; (B) abactinal skeleton; (C) papulae (arrows); (D) madreporite; (E) actinal skeleton; (F), (I) adambulacral spines; (G) oral part and interradii of actinal side; (H) abactinal spines. Scale bars: A–E = 1 mm, F = 5 mm, G = 3 mm, H = 100 μm, I = 200 μm (H, I, SEM images). Abbreviations: a, adambulacral plates; i, inferomarginal plates; in, intermarginal plates; s, superomarginal plates; v, ventrolateral plates.

***Parastichopus nigripunctatus* (Augustin, 1908)**
(Figs. 4–6) 검은점돌기해삼 (신칭)
Stichopus nigripunctatus Augustin, 1908: 7, 8, pl. 1-fig. 2, text-fig. 5; Ohshima, 1915: 248.
Stichopus depressus Augustin, 1908: 11.
Stichopus owstoni Mitsukuri, 1912: 175.
Apostichopus nigripunctatus: Hayashi, 1940: 164.
Parastichopus nipponensis Imaoka *et al*., 1990: 135.
Parastichopus nigripunctatus Imaoka *et al*., 1991: 186.

Material examined. One specimen, adjacent water of Hanrim: Jeju-si: Jeju-do, Korea (33°26′24″N, 126°8′49″E), 2 October 2013, Lee T., a depth of 80 m, collected with a fishing net, deposited in the National Marine Biodiversity Institute of Korea (MABIK; MABIK_IV00172918); One specimen, adjacent water of Uljin: Uljin-gun: Gyeongsangbuk-do, Korea (36°59′43″N, 129°25′41″E), 25 May 2014, Lee T., a depth of 30 m, collected with a fishing net, deposited in the National Marine Biodiversity Institute of Korea (MABIK; MABIK_IV00172919) (Fig. 4).

Description. Body cylindrical, tapering to end, 36–38 papillae present along body length, and length of 9.7–10.6 cm in ethyl alcohol preserved samples (Fig. 5). Mouth opened nearby end of body and subventral side. Tentacles 20 in number, with tentacular ampullae. Tube feet ventrally developed to form a sole, restricted to three ventral radii when relaxed. Tube feet crowded,

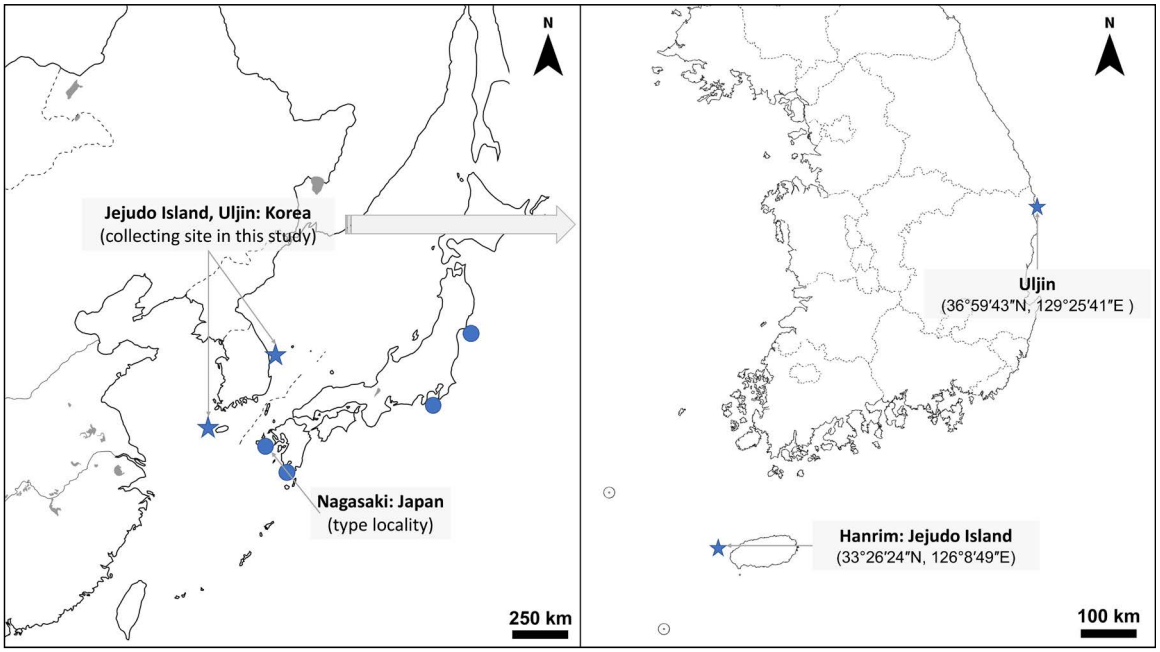

Fig. 4. Distribution of *Parastichopus nigripunctatus* (Augustin, 1908) based on the present and previous study.

difficult to distinguish, present at mid-ventral radius in six–eight rows and at each lateral radii in three–four rows (Fig. 5). Large and small papillae numerous and scattered on dorsal side. Large papillae on ventrolateral side, 20–21 in number (Fig. 5). Calcareous ring simple. Radial plate adhered to interradial plate, with a notch on anterior central margin. Interradial plate lower than radial one, almost triangular shaped, with acute anterior margin. Both plates show concave posterior margins, with interradial margins more gently curved than radial margins. Ossicles of dorsal body wall with perforated plates and tables (Fig. 6A, 6B). Disk of tables circular (diameter of disk 46–86 μm) with spinose edge and four larger central holes surrounded by numerous smaller peripheral holes, which often decreased in size near table margin (Fig. 6B). Four or five-pillared spire situated on center of table (height of spire 32–48 μm) with each pillar connected by single cross-beam, and crown with robustly pointed spines and single central hole (Fig. 6B). Ossicle of tube feet present tables and irregularly shaped plates with numerous perforations. Ossicles of tentacle contain curved or straight rods of various size (length of 197–473 μm), with numerous tiny thorns at their tips and fewer thorns at center (Fig. 6C).

Habitat. This species was collected at a depth of 30 and 85 meters. It is likely to inhabit the mixed substrate (coarse sand and rocks), based on other echinoderms collected in the same area.

Distribution. Korea (Hanrim: Jejudo Island; Uljin: the East Sea), Japan (Sendai Bay, Sagami Bay, Nagasaki, Kagoshima Bay) (Fig. 4).

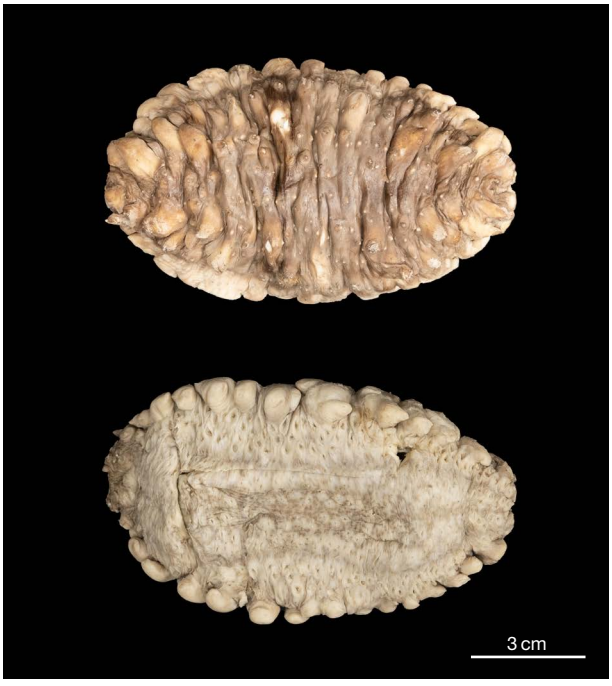

Fig. 5. Dorsal and ventral side of *Parastichopus nigripunctatus* (Augustin, 1908) in this study.

DNA barcoding analysis. We obtained a partial sequence of mitochondrial COI gene and deposited it into GenBank of NCBI (GenBank accession no. OP903144

and OP903339). Pairwise genetic distances (p-distance) were calculated by Kimura-2 parameter (Table 1). The mean interspecific p-distance of genus *Parastichopus* was 16.5% and ranging from 10.5% (*P. nigripunctatus*–*P. californicus*) to 21.4% (*P. californicus*–*P. tremulus*). The p-distance showed that *P. nigripunctatus* from Korea was identical to *P. nigripunctatus* from Japan and quietly close to *A. japonicus*: the mean intraspecific p-distance of *P. nigripunctatus* is 0.3% and the p-distance between *P. nigripunctatus* and *A. japonicus* ranging from 1.5 to 3.1%. DNA barcoding analysis also supported that the newly recorded species of *Parastichopus* from Korea matched with *P. nigripunctatus*.

Remarks. *Parastichopus nigripunctatus* has been recorded as *Apostichopus nigripunctatus* in WoRMS, which created in 2010 (WoRMS Editorial Board, 2023). However, the nomenclatural act of transferring this species to the genus *Apostichopus* did not fulfill the requirements of Article 8 of the International Code of Zoological Nomenclature (1999), and therefore, to date, this transfer remains unqualified (Woo *et al*., 2017). Thus, we refer to this species as *P. nigripunctatus*. This species is distributed in the co-region with *A. japonicus* in South Korea. Their external morphological characteristics are quite similar to each other, including body color, number of papilla and shaped of tentacle. However, their ossicles of body wall, specifically table shaped ossicles, are clearly different from *A. japonicus*. The genus *Apostichopus* erected by Liao (1980) based on material from the northern East China Sea that was identified as *A. japonicus*. But this genus clearly differs from other genera in the Stichopodidae (including *Parastichopus*) based on reduced table spicules without pillars in the body wall (Liao, 1980). The table shaped ossicle of *P. nigripunctatus* has thorny edge on a plane and spires, whereas *A. japonicus* has smooth edge and less thorny spire in a table. And we also could not observe button-shaped ossicles in body wall from our samples of *P. nigripunctatus*. This is one of unique morphological characteristic compared to other species of *Parastichopus* (Imaoka *et al*., 1991).

DNA barcoding has emerged as a valuable tool for expanding our understanding of species biodiversity (Sonet *et al*., 2022). In our study, we observed a close genetic distance between *P. nigripunctatus* and *A. japonicus*, with p-distance values ranging from 1.5% to 3.1% (with a mean of 1.9%) (Table 1), which raises the possibility of synonymy between these two species. This result contrasts with the typical interspecific DNA barcoding gap observed in echinoderms as reported by Layton *et al*. (2016), who identified 145 barcode index numbers from 999 samples and found a mean intraspecific distance of 0.48% (range: 0.0–7.6%) and a mean interspecific distance of 12.0% (range: 2.0–26.2%).

A similar case to our study is found in the genus *Luidia*

Table 1. Pairwise genetic distances (%) within 14 holothuroids comprising five species of Stichopodidae, including *P. nigripunctatus*, and two species of *Cucumaria* from South Korea and GenBank based on the Kimura 2-parameter model.

No.	Species	GenBank accession no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	*Parastichopus nigripunctatus* (Uljin, Korea)	OP903339														
2	*Parastichopus nigripunctatus* (Jejudo, Korea)	OP903114	0.0													
3	*Parastichopus nigripunctatus* (Japan)	NC_013432	0.5	0.5												
4	*Parastichopus californicus*	NC_026727	10.5	10.5	10.9											
5	*Parastichopus tremulus*	KT206232	20.8	20.8	20.5	21.4										
6	*Apostichopus japonicus*	FJ971399	1.5	1.5	2.0	10.3	20.5									
7	*Apostichopus japonicus*	GH550584	1.5	1.5	2.0	10.7	20.8	0.3								
8	*Apostichopus japonicus*	JN836336	1.7	1.7	1.9	10.7	20.5	0.5	0.2							
9	*Apostichopus japonicus*	KT625443	1.5	1.5	2.0	10.7	20.5	0.3	0.3	0.5						
10	*Apostichopus japonicus*	KT625444	1.5	1.5	2.0	10.7	20.8	0.3	0.3	0.5	0.3					
11	*Apostichopus japonicus*	KT625445	2.6	2.6	3.1	10.5	20.5	1.0	1.4	1.5	1.4	1.4				
12	*Stichopus parvimensis*	NC_029699	10.9	10.9	11.3	5.8	20.8	10.7	11.1	11.1	11.1	11.1	10.7			
13	*Cucumaria frondosa*	NC_065088	22.3	22.3	22.6	21.6	21.6	22.3	22.3	22.1	22.6	22.8	22.3	21.4		
14	*Cucumaria miniata*	AY182376	25.1	25.1	24.8	24.4	22.8	24.8	24.4	24.1	24.6	24.8	25.3	24.7	8.4	

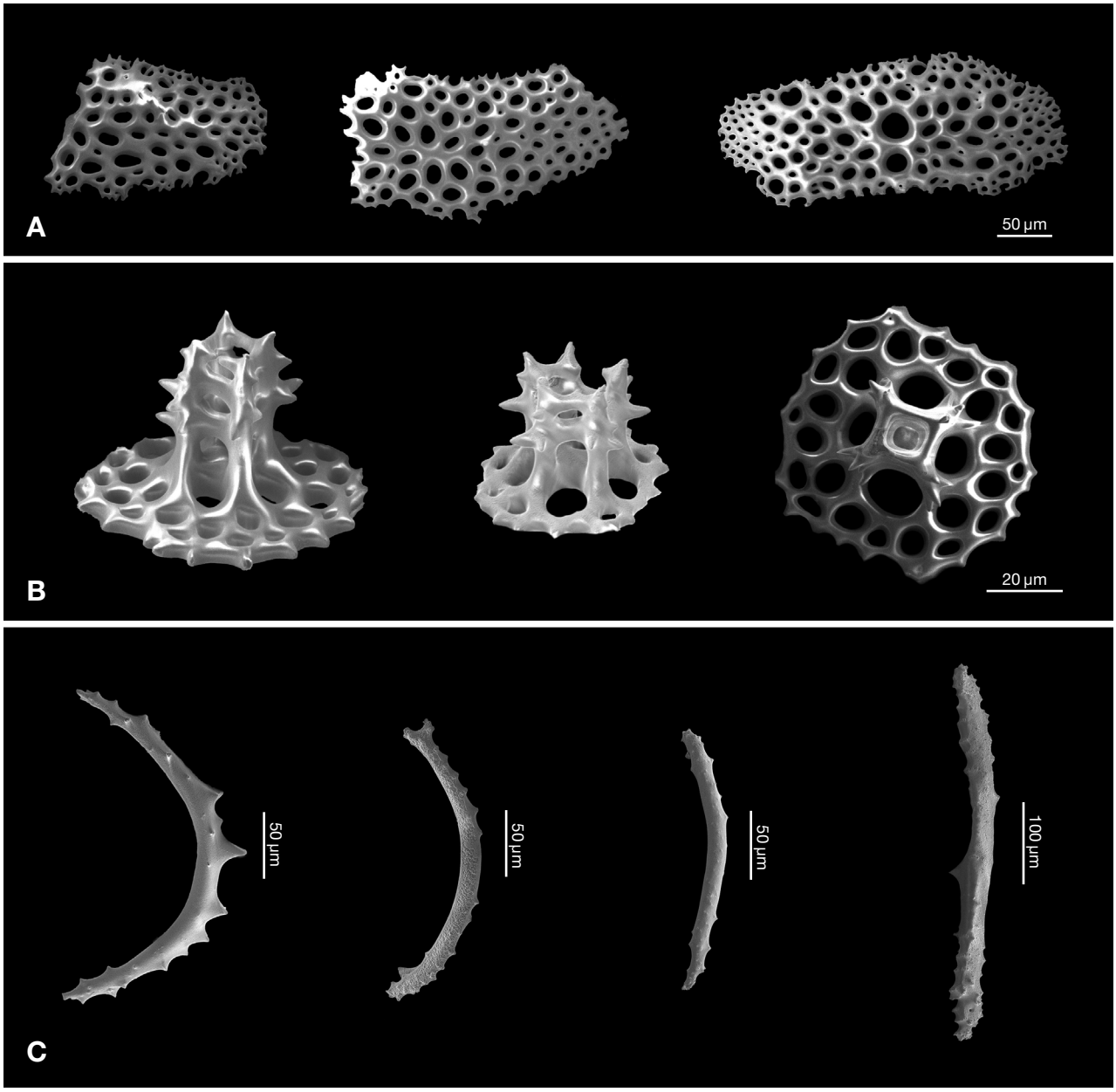

Fig. 6. Ossicles of *Parastichopus nigripunctatus* (Augustin, 1908) in this study. (A) perforated plates in dorsal body wall; (B) tables in dorsal body wall; (C) rods in tentacle.

Forbes, 1839, belonging the class Asteroidea of the phylum Echinodermata, where species delimitation via COI was conducted by Xiao *et al*. (2013). They reported that seven *Luidia* species showed a close genetic pairwise distance ranging from 0.0−1.6%: *L. avicularia* Fisher, 1913; *L. changi* Liu, Liao and Li, 2006; *L. hardwicki* (Gray, 1840); *L. longispina* Sladen, 1889, *L. orientalis* Fisher, 1913, *L. quinarian* von Martens, 1865; and *L. yesoensis* Goto, 1914. Thus, they suggested that these seven species should be re-evaluated for their species status and potential synonymy. However, these seven *Luidia* species have distinct morphological characteristics for taxonomic identification, such as the number of arms, shape of paxillae and pedicellariae, and arrangement of body skeleton. Therefore, these seven species of *Luidia* are still recognized as separate species (WoRMS editorial board, 2023).

In conclusion, we confirm that the *Parastichopus* specimens belong to the species *P. nigripunctatus* based on our study using both morphological and molecular analyses. This finding represents the first record of *P. nigripunctatus* in the Korean fauna.

Acknowledgements

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