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A newly recorded sea urchin, *Araeosoma owstoni* Mortensen, 1904 (Echinoidea; Echinothurioida; Echinothuriidae), from the Korea Strait

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Araeosoma owstoni Mortensen, 1904, a newly recorded sea urchin, was collected from the Korea Strait by trawling during a research expedition in April 2017. The specimen was damaged and was severely peeled off on the aboral side during trawling. However, a test and the surface of the oral side of the sample were well preserved, allowing us to successfully identify it. The species was distinguished by the large and flexible test, the tiny apical section, and the interambulacra width which is twice of the ambulacra. Pedicellaria tridentate and triphyllous were presents, but tetradactyle pedicellaria was absent due to severe peeling on the aboral side. Moreover, a length of 1,212 bp sequence from mitochondrial *COI* gene was obtained and this sequence covered the general DNA barcoding region. The mean of interspecific divergence within *A. owstoni* from Korea and other eight species of *Araeosoma* from the GenBank was 6.8%. This value indicated that our species was clearly distinguishable from the others. Thus, the first *Araeosoma* species occurring in South Korea is presented in this study.

Keywords: *COI*, Echinodermata, echinoids, echinothuriids, taxonomy

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Introduction

Taxa of the family Echinothuriidae Thomson, 1872 usually lives at depths of 40–700 m. Their distinct morphological characteristic is the large flexible test with poisonous spines (Shigei, 1981). Echinothuriidae consists of three subfamilies (i.e., Echinothuriinae, Hygrosomatinae and Sperosomatinae), and contain 52 species (Kroh and Mooi, 2022). Among them, the subfamily Echinothuriinae Thomson, 1872 is characterised by the arrangement of ambulacral pores into three discrete columns on both the oral and aboral surfaces, an apical system based on a contiguous ring of ocular and genital plates, hoofs on the adoral primary spines, and teeth with a bluntly angled tip (Anderson, 2013). Echinothuriinae included the genera *Araeosoma* Mortensen, 1903, *Asthenosoma* Grube, 1868, *Calveriosoma* Mortensen, 1934, and *Hapalosoma* Mortensen, 1903 in the present (Kroh and Mooi, 2022). Of these, *Asthenosoma* is the only known taxon in the Korean fauna, *A. ijimai* Yoshiwara, 1897 (Shin *et al.*, 2006; Shin, 2011). A single specimen of echinothuriid was collected from the Korea Strait by trawling during a research expedition in April 2017, and it clearly presented echinothuriid morphological characters, but did not match with *A. ijimai*, which has been previously reported from Korea. This new echinoid showed morphological characteristics of the genus *Araeosoma*, which has not been recorded yet in the Korean fauna.

Genus *Araeosoma* has a flexible large test and inhabits the deep waters (usually 70–1,000 m). Therefore, the collection of undamaged specimens of *Araeosoma* is rather difficult and it brings difficulty in the morphological identification of *Araeosoma*. So, many mitochondrial cytochrome *c* subunit I (*COI*) data of genus *Araeosoma* are registered without species identification in the GenBank. Moreover, *A. owstoni* Mortensen, 1904, *A. thetidis* (H.L. Clark, 1909) and *Phormosoma* sp. are provided as a habitat for the deep-sea shrimp, *Echinopericlimenes hertwigi* (Balss, 1913) (Hayashi and Ohtomi, 2001). As a result, a supplement of DNA barcoding is required for the proper species identification of *Araeosoma* in further taxonomical studies. Sequence variation in a 658 bp region of the *COI* gene was discovered by DNA barcoding and used for species identification (Hebert *et al.*, 2003). An integrative approach to taxonomy, using both morphological identification and DNA barcoding, has become necessary for assessing species diversity and species boundaries (Puillandre *et al*., 2012).

In this study, a morphological description of a newly recorded sea urchin is presented, together with high-resolution images and a key to the genus. Moreover, a partial sequence of *COI* gene for DNA barcoding was obtained and registered in the GenBank.

Materials and Methods

The specimen was collected by trawling on a research expedition in April 2017. The collected specimen was preserved in ethyl alcohol solution (>95%) immediately after taking photographs (G7Xmk2, Canon, Tokyo, Japan) and was stored at the Marine Biological Resource Institute of Sahmyook University until it was moved to the National Institute of Biological Resources, Korea (NIBR). Two types of pedicellaria were recognized and collected with fine forceps. They were then bleached with 10% solution of sodium hypochlorite to remove the soft tissue from each pedicellaria. Bleached valves of pedicellaria were directly washed with distilled water and dried completely in a dry oven. High-resolution images of individual pedicellaria and valves were recorded using a scanning electron microscope (SEM) (JSM-microscopes 6510; JEOL, Tokyo, Japan). The detailed structures of the specimen were observed with a stereomicroscope (SZ-61, Olympus, Tokyo, Japan).

Genomic DNA was extracted from gonad tissue that was emerging out of the crack of the specimen, with the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Partial sequences of *COI* were amplified using universal primers of ECOLa and ECOIb (Knott and Wray, 2000). Polymerase chain reaction (PCR) was performed in a reaction volume of 20 μL that contained AccuPower PCR PreMix & Master Mix (Bioneer, Seoul, Korea), 1 μL of each primer (10 mM), and 1.5 μL of DNA template (>50 ng/μL) using the following thermocycling profile: 94°C for 3 min; 35 cycles of 94°C for 30 s, 52°C for 60 s, and 72°C for 60 s; and a final extension of 72°C for 7 min. The assemblies and alignments of sequencing results were performed using Geneious v.11.1.5 (Biomatters, Auckland, New Zealand). Genetic distances of *Araeosoma owstoni*, six other *Araeosoma* species and seven echinothuriids were investigated and all data were obtained from the NCBI, except for *A. owstoni*. The pairwise distance (p-distance) was calculat-

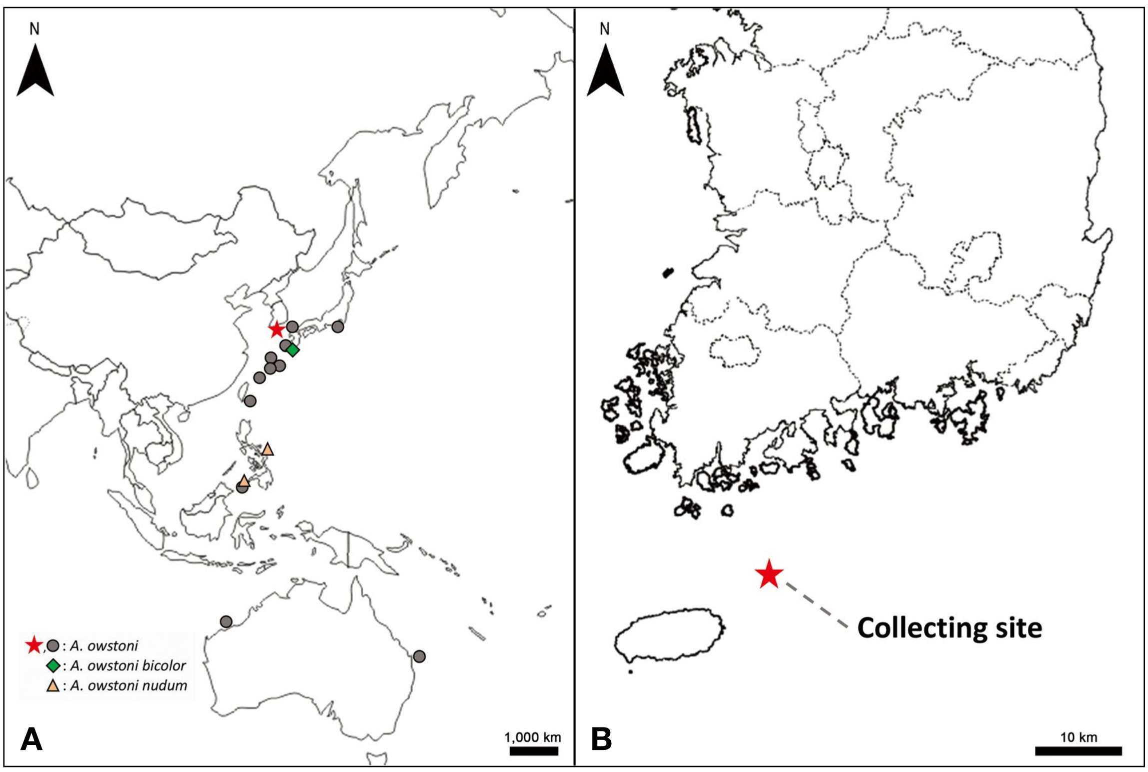

Fig. 1. Distribution of *Araeosoma owstoni* Mortensen, 1904. A, collection localities of *A. owstoni*, *A. owstoni bicolor* (A. Agassiz and H.L. Clark, 1907) and *A. owstoni nudum* Mortensen, 1934 from previous studies; B, collecting site of *A. owstoni* of this study and marked as '★' on the map.

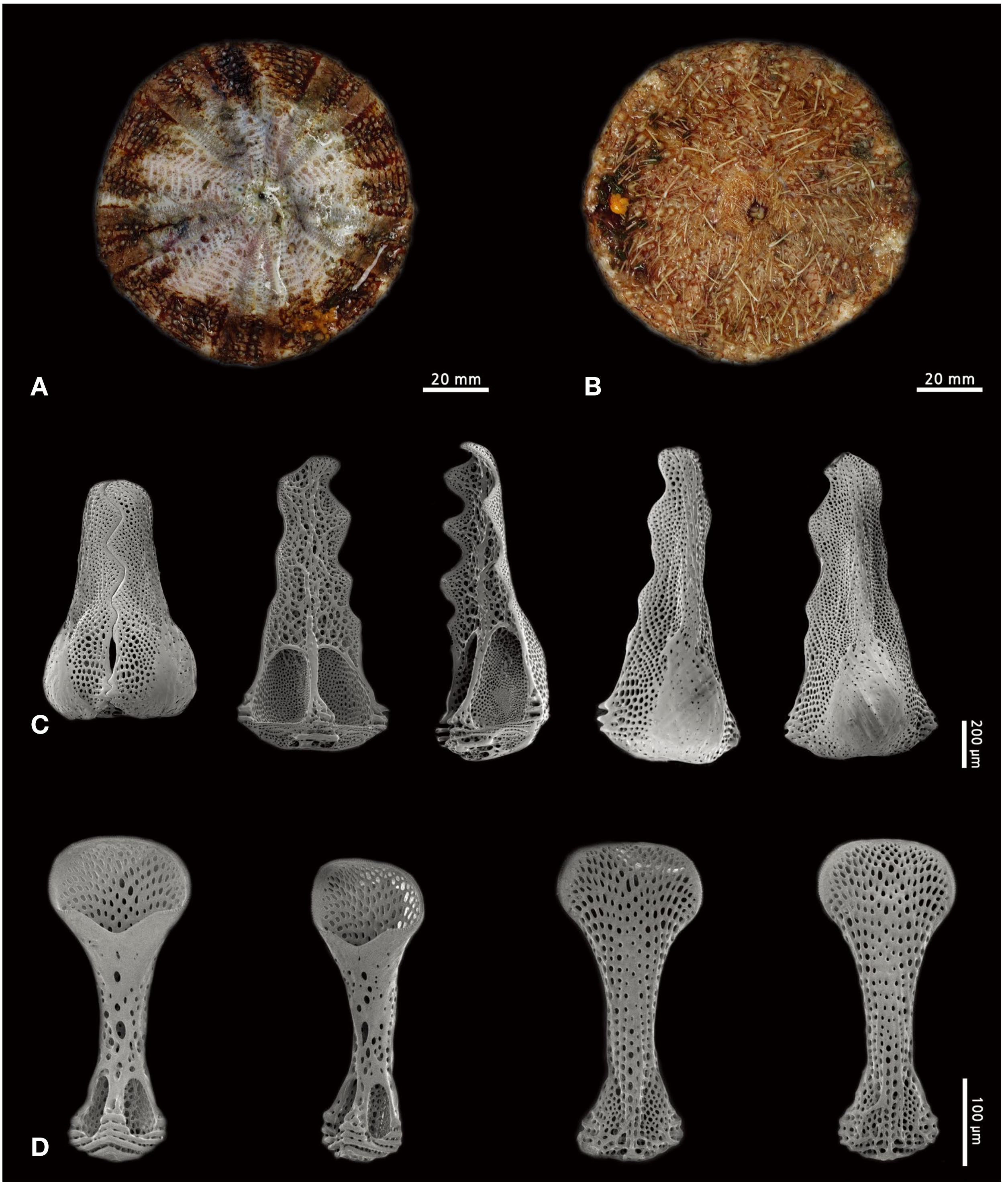

Fig. 2. *Araeosoma owstoni* Mortensen, 1904. A, aboral side; B, oral side. C, tridentate pedicellaria and its valves; D, valves of triphyllous pedicellaria.

ed using the Kimura 2-parameter model (Kimura, 1980) in MEGA X (Kumar *et al*., 2018).

Taxonomic Account

Phylum Echinodermata Bruguière, 1791
[ex Klein, 1734]

Table 1. Pairwise distance (%) within *A. owstoni* from Korea and other 12 echinothuriids from GenBank, based on the Kimura 2-parameter model.

No.	Species	GenBank Accession No.	1	2	3	4	9	10	11	19	25	26	27	28	References
1	*Araeosoma owstoni*	OK094487													This study
2	*Araeosoma thetidis*	GU670318	3.2												iBOL
3	*Araeosoma* sp. 1	GU670303	3.2	0.0											iBOL
4	*Araeosoma* sp. 2	GU670283	7.2	6.8	6.8										iBOL
5	*Araeosoma* sp. 3	GU670320	6.3	6.1	6.1	1.6									iBOL
6	*Araeosoma* sp. 4	HM889125	6.6	6.2	6.2	3.4	3.2								iBOL
7	*Araeosoma* sp. 5	GU670286	6.0	6.0	6.0	3.8	3.8	3.4							iBOL
8	*Araeosoma* sp. 6	GU670296	10.8	9.9	9.9	9.6	8.5	9.7	8.3						iBOL
9	*Araeosoma* sp. 7	GU670298	10.8	9.9	9.9	8.7	8.5	9.0	7.4	1.8					iBOL
10	*Asthenosoma* sp.	JN259399	10.6	12.3	12.3	10.7	10.7	11.4	12.6	16.9	15.9				iBOL
11	*Hygrosoma* sp.	GU670211	13.8	13.5	13.5	14.0	13.8	13.3	13.3	14.7	14.2	16.2			iBOL
12	*Tromikosoma* sp.	GU670304	15.7	15.9	15.9	14.2	14.5	15.4	14.4	16.6	16.1	16.4	12.4		iBOL
13	*Sperosoma biseriatum*	HM542993	16.2	16.2	16.2	14.7	15	15.4	15.2	17.9	17.4	16.9	13.1	1.4	Unpublished

*'iBOL' is the abbreviation for 'International Barcode of Life'.

Class Echinoidea Leske, 1778
Subclass Euechinoidea Bronn, 1860
Order Echinothurioida Claus, 1880
Family Echinothuriidae Thomson, 1872

Key to the genus of Family Echinothuriidae in Korea

1. Ambulacral plates on peristome arranged quadriserially in each zone. Genital pores open in a membranous gap, and not pierce genital plates ··············· ***Araeosoma***
2. Ambulacral plates on peristome arranged biserially in each zone. Gonopores open within genital plates ··· *Asthenosoma*

Genus *Araeosoma* Mortensen, 1903
얇은각성게속 (신칭)

Araeosoma Mortensen, 1903: 63; 1927: 281; Clark, 1925: 59; Rowe and Gates, 1995: 217; Liu, 2008: 864; Anderson, 2013: 523; Kroh and Mooi, 2022: 123396.

Type species. *Calveria fenestrata* Thomson, 1872, by original designation.

Araeosoma owstoni **Mortensen, 1904 (Fig. 2)**
오스톤얇은각성게 (신칭)

Araeosoma owstoni Mortensen, 1904: 82; Clark, 1925: 63; Shigei, 1981: 198; 1986: 35; Rowe and Gates, 1995: 217; Liao and Clark, 1995: 326; Chao and Lee, 2001: 19; Liu, 2008: 864; Kroh and Mooi, 2022: 513119.

Material examined. One specimen, Korea Strait (33°45′36.0″N, 127°10′58.0″E) (Fig. 1B), 24 April 2017, T. Lee and S. Bae, 90 m depth by trawling, deposited voucher of NIBR: NIBRIV0000895385.

Description. Test large, flexible, low form, rounded edge and oral side flat (Fig. 2A). Apical area rather small (Fig. 2A). Genital and ocular plates widely separated. Genital pores large, and closely situated middle of genital plate. Madreporite plate distinctly larger than other genital plates. Interambulacral areas twice as broad as ambulacral (Fig. 2A). Interambulacra 38 and ambulacra 60 in number. Ambulacral plate with small and these tubercles not forming regular series. Interambulacral plate with two primary tubercles and numerous military tubercles. Length of the primary spines on the aboral side significantly varying. Secondary spines have poison gland near tip of spine. Only one kind of tridentate pedicellariae present and variable in size (Fig. 2C). Triphyllous pedicellariae elongate, narrow form (Fig. 2D). Spicules of tube feet rather large, irregular, thorny, fenestrated plates.

Size. test diameter = 102.3 mm; test height = 28.4 mm; apical system = 17.9 mm; peristome = 25.8 mm; length of longest primary spine = 15.3 mm.

Color. Aboral side was dark brown with reddish brown color on the margin of ambulacral area. Oral side was brown when alive (Fig. 2A, B).

Distribution. Korea (Korea Strait: off eastern Jeju), Japan (Okinoshima, Sagami Bay to Kagoshima Bay, and Okinawa), East China Sea, Taiwan, Philippines, Australia (Cape Lambert, and Brisbane) (Fig. 1A).

Remarks. Despite being peeled off on the aboral side and lacking tetradactyle pedicellarie, the collected specimen provided enough evidence for morphological identification. This specimen conserves its original test shape and has two types of pedicellaria: tridentate and triphyllous. The morphological characteristics of the specimen were consistent with the following major morphological characteristics of *Araeosoma owstoni* in previous studies (Mortensen, 1904; Shigei, 1986): 1) including patterns and numbers of amburacra and interambucra, 2) ratio of diameter with apical systems and test, and 3) pedicellariae forms. *Araeosoma owstoni* has three subspecies so far (Kroh and Mooi, 2022). Among them, morphological characteristics of *A. owstoni bicolor* (A. Agassiz and H. L. Clark, 1907) and *A. owstoni nudum* Mortensen, 1934 were slightly different from *A. owstoni*. For instance, *A. owstoni bicolor* had higher numbers of interambulacral and ambulacral plates than *A. owstoni* and *A. owstoni nudum* had more naked aboral side (Mortensen, 1934; 1948). *Araeosoma owstoni bicolor* distributed from Kagoshima, Japan (Agassiz and Clark, 1907; 1909), and *Araeosoma owstoni nudum* from Philippine to Hong Kong (Mortensen, 1934; 1948), however, their distributions are relatively similar to *A. owstoni* (Fig. 1A). Mortensen (1934) was the first to suggest *Araeosoma owstoni nudum* as a variant of *A. owstoni*. Furthermore, Shigei (1981) synonymised *Asthenosoma bicolor* as *A. owstoni* and did not mention about the presence of all subspecies when he redescribed *A. owstoni* (Shigei, 1986). Accordingly, we present our newly collected echinoid as *A. owstoni*.

DNA barcoding analysis. In terms of morphological identification, the genus Araeosoma is one of the most difficult echinoids. Thus, a partial sequence of mitochondrial *COI* (1,212 bp) was obtained and deposited in the GenBank (accession number: OK094487) for further studies such as DNA barcoding and molecular phylogenetics. The pairwise distances were calculated based on 522 bp sequences of *COI* and this dataset consisted of seven species of *Araeosoma* including *A. owstoni*, and seven other echinothuriids (Table 1). Numerous data of *Araeosoma* in the GenBank were not identified to the species level. Fortunately, *A. thetidis*, a species closely related to *A. owstoni*, was correctly identified and registered. In the result of pairwise distance analysis, divergence between genus *Araeosoma* and other echinothuriids was 14.5% and the mean of interspecific divergence of genus *Araeosoma* is 6.5% (Table 1). The average interspecific divergence between *A. owstoni* and other *Araeosoma* species was 6.8%, ranging from 3.2% with *A. thetidis* to 10.8% with *Araeosoma* sp. 6 and 7 (Table 1). For most cases, the interspecific divergence in echinoderm ranged from 2.5 to 24.2% (Arndt *et al*., 1996; Hart *et al*., 1997; Ward *et al*., 2008) and the mean was around 12.0% (Layton *et al*., 2016). Interestingly, closely related echinoid species have a low interspecific divergence as 2–3% (Palumbi *et al*., 1997). As a result of the DNA barcoding in this study, *A. owstoni* from Korea can be distinguished from other *Araeosoma* species and has a close relationship with *A. thetidis*.

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