

A Newly Recorded Sea Star of the Genus *Luidia* (Asteroidea: Paxillosida: Luidiidae) from the Korea Strait, Korea

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ABSTRACT

Asteroid specimens of the genus *Luidia* were collected at a depth of 95–100 m in the Korea Strait by bottom trawling in April 2016. The specimens were identified as *Luidia avicularia* Fisher, 1913 (Luidiidae: Paxillosida) based on morphological characteristics and molecular phylogenetic analyses, and the species is new to the Korean fauna. A 648-bp partial nucleotide sequence of mitochondrial cytochrome *c* oxidase I (mt-*COI*) gene was obtained from Korea, and then was compared to sequences of related species stored in GenBank using molecular phylogenetic analyses. No sequence differences were detected between the *L. avicularia* mt-*COI* gene sequences from Korea and China, and the species described in this report was clearly distinct from *L. maculata*, which was previously reported in Korean fauna. Three *Luidia* species have been reported in Korea.

Keywords: sea star, *Luidia avicularia*, Korea Strait, morphological characteristics, molecular phylogenetic analyses, mitochondrial *COI* sequence

INTRODUCTION

Luidiidae Sladen, 1889 is a small family in the class Asteroidea that includes a single valid genus, *Luidia* Forbes, 1839, and 49 valid species (Mah and Blake, 2012). *Luidia* species are widely distributed in the shallow waters of subtropical and tropical seas (Clark and McKnight, 2000), and species live in muddy or sandy substrates (Sloan, 1980). Döderlein (1920) identified the following four major groups in *Luidia* based on the form and appearance of ossicle systems and the development of spines, spinelets, and pedicellariae: Alternata-Group, Ciliaris-Group, Clathrata-Group, and Quinaria-Group. The systematic arrangements of five Chinese *Luidia* species indicated that *L. avicularia*, *L. longispina*, and *L. quinaria* belong to the Quinaria-Group, *L. orientalis* belongs to the Ciliaris-Group, and *L. maculata* belongs to the Alternata-Group. Xiao et al. (2013) studied the molecular phylogenetic relationships of the eight Chinese species based on their mitochondrial cytochrome *c* oxidase I (mt-*COI*) sequences and identified three major clades: clade A included *L. quinaria* from the Sea of Japan; clade B included *L. avicularia*, *L. changi*, *L. hardwicki*, *L. longispina*, *L. orientalis*, *L. quinaria* (from the Yellow Sea), and *L. yesoensis*; clade C included *L. maculata*. Regarding the division of the Alternata

or Quinaria groups, the important features were the presence or absence of pedicellariae close to the mouth and of marked dark parts on the abactinal surface. To date, of the 49 *Luidia* species, two species (*L. maculata* Müller & Troschel, 1842 and *L. quinaria* von Martens, 1865) have been reported in the Korean fauna (Shin and Rho, 1996; Shin, 2010).

Sea stars in the genus *Luidia* were collected from the Korea Strait, at a depth of 95–100 m in the offshore zone (33°41'7.00"N, 127°27'1.00"E) by bottom trawling in April 2016. The specimens were stored in 95% ethanol, and were identified and described based on morphological and molecular analyses. Significant morphological characteristics were photographed using a stereo-microscope (SMZ1000; Nikon, Tokyo, Japan), a scanning electron microscope (JSM-6510; JEOL, Tokyo, Japan), and a digital camera (D7000; Nikon). The specimens were deposited in the Marine Echinoderm Resources Bank of Korea (MERBK), Sahmyook University, Seoul, Korea. Specimen identification based on the description reported by Fisher (1913) and Clark and Rowe (1971). Molecular analyses were based on mt-*COI* sequences that were isolated using universal *COI* primers (F-GGTCACA AATCATAAAGATATTGG and R-TAAACTTCAGGGTGA CCAAAAATCA) (Folmer et al., 1994), and the sequences were used for the accurate molecular identification and com-

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parison to other Korean *Luidia* species. DNA was extracted from the gonad tissue using a DNeasy Blood and Tissue Kits (Qiagen, Hilden, Germany) and PCR amplification were conducted using the described by Lee and Shin (2011) with minor modification. All PCR products were purified using a QIAquick PCR purification Kit (Qiagen) and were then sequenced using an automated ABI 3100 sequencer (Applied Biosystems, Foster City, CA, USA). The sequence data obtained were analyzed using the basic local alignment search tool (BLAST). Phylogenetic trees for a dataset were inferred by maximum likelihood (ML) and by Bayesian analysis. The GTR + gamma + I model of sequence evolution models was selected for a dataset using jModelTest 2.1.4 (Darriba et al., 2012). The ML tree was estimated using PhyML v3.0 (Guindon and Gascuel, 2003) with GTR + gamma + I model setting, and statistical support estimated using bootstrapping with 1,000 replicates. The Bayesian analysis was conducted in MrBayes 3.2 (Ronquist et al., 2012) with four chains running for 2,000,000 generations, sampling trees every 1,000 generations, and average standard deviation of split frequencies for last 75% of generations. The partial mt-*COI* nucleotide sequences of seven *Luidia* species were used to reconstruct phylogenetic trees using maximum parsimony (MP), ML, and Bayesian inference (BI) methods. The result of the analyses indicated that the specimens were *L. avicularia* Fisher, 1913, which was a newly reported species in the Korean fauna.

Molecular phylogeny

A partial mt-*COI* gene sequence, 648 base pairs in length was isolated for the first time from Korean *L. avicularia* (GenBank accession number: KY305010), and the sequence perfectly corresponded to additional *L. avicularia* sequence data obtained from the National Center for Biotechnology Information (NCBI) (JQ740627; collected in China). We compared the *L. avicularia* mt-*COI* DNA sequences from Korea to those of *L. avicularia* (JQ740627), *L. changi* (JQ740621), *L. hardwicki* (JQ740632), *L. orien-*

talis (JQ740626), *L. quinaria* (JQ740617), *L. yesoensis* (JQ740620), and *L. maculata* (JQ740634, JQ740635) obtained from NCBI, and *Apostichopus japonicus* was used as the outgroup. The phylogenetic trees inferred from the sequence alignment using MP, ML, and BI methods were very similar, with high bootstrap support (ML, 99%) and Bayesian posterior probability of 0.99 (Fig. 1). The phylogenies confirmed that the Korean species was apparently different from the six other *Luidia* species stored at NCBI, and Korean *L. avicularia* data was coincident with Chinese *L. avicularia* (Table 1).

SYSTEMATIC ACCOUNTS

Class Asteroidea de Blainville, 1830
 Order Paxilloidea Perrier, 1884
 Family Luidiidae Sladen, 1889
 Genus *Luidia* Forbes, 1839

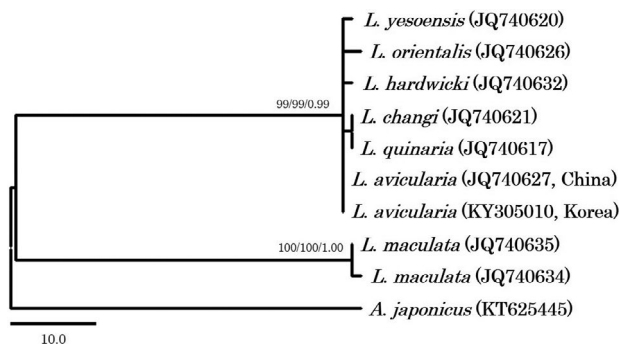


Fig. 1. Maximum parsimony tree inferred from mt-*COI* depicts the phylogenetic relationships among seven *Luidia* species and the *Apostichopus japonicus* outgroup. Bootstrap values obtained from maximum parsimony, maximum likelihood, and Bayesian inference methods are shown on each node, respectively. mt-*COI*, mitochondrial cytochrome c oxidase I.

Table 1. Pairwise genetic distances calculated for mt-*COI* sequences from *Luidia* species

	[1]	[2]	[3]	[4]	[5]	[6]	[7]
mt- <i>COI</i> sequences							
[1] <i>L. avicularia</i> (KY305010)							
[2] <i>L. avicularia</i> (JQ740627)	0.000						
[3] <i>L. quinaria</i> (JQ740617)	0.002	0.002					
[4] <i>L. changi</i> (JQ740621)	0.002	0.002	0.000				
[5] <i>L. hardwicki</i> (JQ740632)	0.002	0.002	0.004	0.004			
[6] <i>L. yesoensis</i> (JQ740620)	0.002	0.002	0.004	0.004	0.004		
[7] <i>L. orientalis</i> (JQ740626)	0.004	0.004	0.007	0.007	0.007	0.007	
[8] <i>L. maculata</i> (JQ740634, JQ740635)	0.271	0.271	0.271	0.271	0.274	0.274	0.274

mt-*COI*, mitochondrial cytochrome c oxidase I.

Korean name: *거친검은띠불가사리 (신칭)

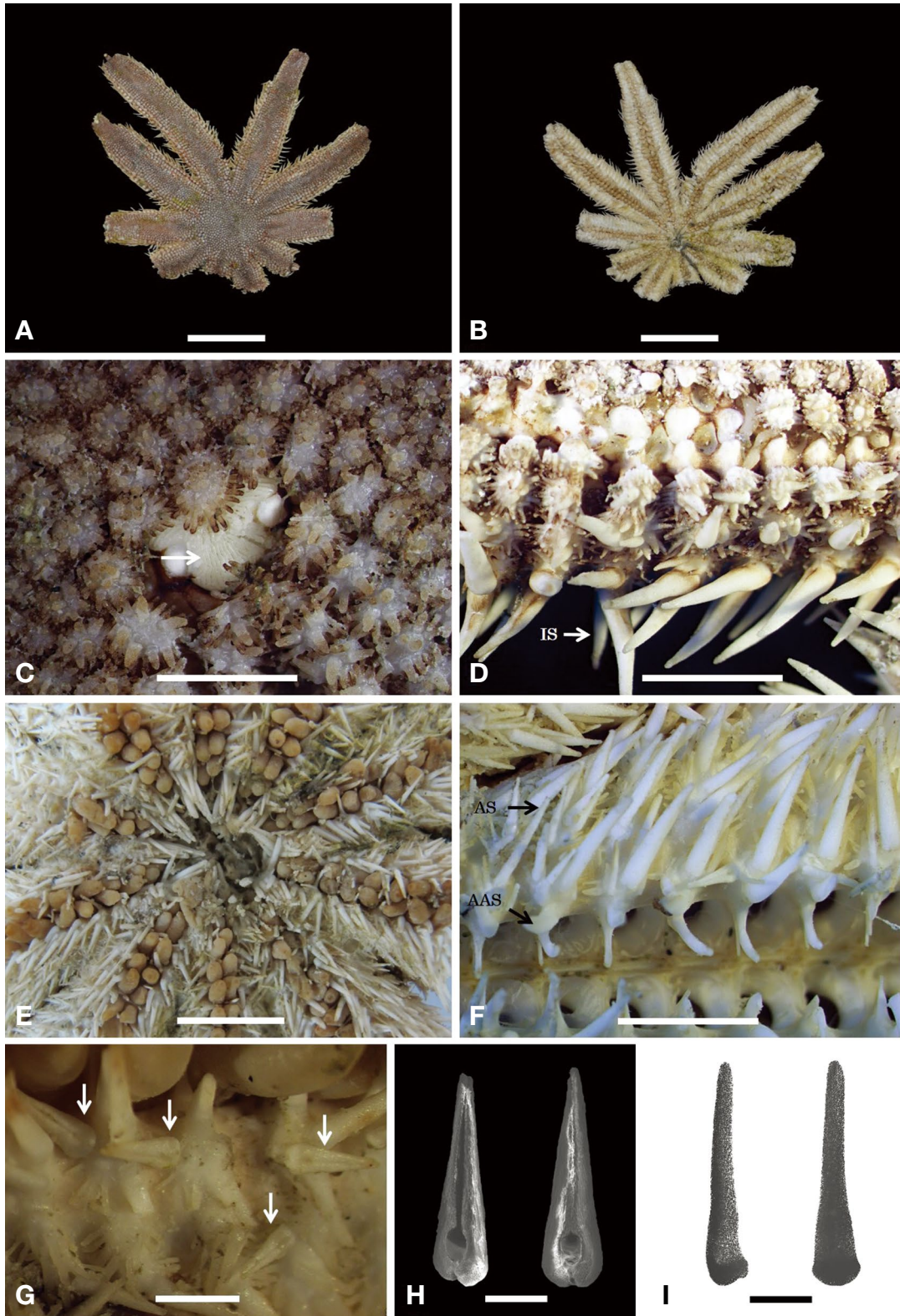


Fig. 2. *Luidia avicularia*. A, Abactinal view; B, Actinal view; C, Madreporite (arrow) and abactinal paxillae; D, Inferomarginal spines (IS) and abactinal paxillae; E, Oral part; F, Adambulacral (AAS) and actinal (AS) spines; G, Bivalve pedicellariae (arrows); H, Bivalve pedicellariae under scanning electron microscope; I, Valves of bivalve pedicellariae under light microscope. Scale bars: A, B=30 mm, C, D, F=200 μ m, E=10 mm, G=1.5 mm, H, I=500 μ m.

¹**Luidia avicularia* Fisher, 1913 (Fig. 2A–H)

Luidia aspera Sladen, 1889: 248.

Luidia moroisoana Goto, 1914: 301.

Luidia avicularia Fisher, 1913: 203; 1919: 172–175, Pl. 43, fig. 1; Clark and Rowe, 1971: 30–44; Hayashi, 1973: 51; Jangoux, 1981: 458; Liao and Clark, 1995: 69, Pl. 3, fig. 3; Rowe and Gates, 1995: 74; Mah, 2008: 213115.

Material examined. Korea: Two specimens, offshore zone (33°41'7.00"N, 127°27'1.00"E), Korea Strait, April 23, 2016, at 95–100 m depth by bottom trawling.

Description. Disc small. Arms long, comparatively narrow, flat, flexible, 10 in number, size R = 8.5–10.0 cm, r = 1.5 cm, R/r = 5.7–6.7.

Abactinal surface completely flat, covered with paxillae (Fig. 2A, B). Paxillae comparatively large, very closely set, arranged in longitudinal rows along length of arm. Madreporite comparatively small, situated close to disk margin between two arms, partially hidden by crowns of surrounding paxillae (Fig. 2C). Superomarginal plates inconspicuous and covered with paxillae, not distinguished from adjoining abactinal plates. Inferomarginal plates large, with 4–5 compressed spines in a transverse series, and numerous, smaller peripheral spines and spinelets (Fig. 2D). Adambulacral plates with three curved and flattened spines. Ventrolateral plates very small, inconspicuous, and each plate bears 12–14 sharp, slender straight spines, 1–2 of which usually larger than others (Fig. 2F). Oral plates narrow, with 14–18 spines projecting upward and toward mouth (Fig. 2E). Tong-shaped, bivalve pedicellariae situated below adambulacral spines (Fig. 2G–I).

Habitat. Muddy sand substrates.

Color. Color in life is light reddish brown on abactinal side and light red on actinal side.

Distribution. Korea (Korea Strait), South China, southeastern Japan, southern Taiwan, Philippines, northern Australia, East Indies.

Remarks. *L. avicularia* was distinguished from *L. maculata*, which previously reported in Korea, based on the presence of enlarged central spines or spinelets on the paxillae and by the occurrence of bivalve pedicellariae neighboring the adambulacral spines. The species collected from the Korea Strait was compared to specimens of the same species that were collected from Taiwan. Since Korean *L. avicularia* specimens with severed arms were collected, the R/r ratio (5.7–6.7) was calculated, and the values were lower than those of specimens collected in Taiwan (R/r = 129/16 = 8.1) (Chao, 2000). This species usually inhabits the muddy sand of the deep sea and is distributed in the Northwest Pacific from southeastern Japan to northern Australia. The molecular analyses based on mt-*COI* sequences coupled with our

comprehensive morphological observations and Korean *L. avicularia* data was coincident with *L. avicularia* of NCBI data of family Luidiidae. With the newly recorded *L. avicularia* occurrence, three *Luidia* species have been reported in Korea.

ACKNOWLEDGMENTS

This study was supported by a grant from the National Institute of Biological Resources (NIBR) that was funded by MOE (NIBR201601201), a grant from the Marine Biotechnology Program (MERBK: Marine Echinoderm Resources Bank of Korea), and the program of Management of Marine Organisms Causing Ecological Disturbance and Harmful Effects that was funded by KIMST/MOF, Korea.

REFERENCES

- Chao SM, 2000. New records of sea stars (Asteroidea: Echinodermata) from the continental shelf of Taiwan. *Zoological Studies*, 39:275–284.
- Clark AM, Rowe FWE, 1971. Monograph of shallow-water Indo-West Pacific echinoderms. Trustees of the British Museum (Natural History), 690:1–238.
- Clark HES, McKnight DG, 2000. The marine fauna of New Zealand: Echinodermata: Asteroidea (sea-stars), Order Paxilloidea. National Institute of Water and Atmospheric Research (NIWA) Biodiversity Memoires, 116:14–135.
- Darriba D, Taboada GL, Doallo R, Rosada D, 2012. jModel-Test 2: more models, new heuristics and parallel computing. *Nature Methods*, 9:772. <https://doi.org/10.1038/nmeth.2109>
- Döderlein L, 1920. Die Asteriden der Siboga-Expedition 2: Die Gattung *Luidia* und ihre Stammesgeschichte. Siboga-Expedition Monograph, 46:193–293.
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R, 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3:294–299.
- Fisher WK, 1913. New starfishes from the Philippine Islands, Celebes, and the Moluccas. *Proceedings of the United States National Museum*, 46:201–224.
- Fisher WK, 1919. Starfishes of the Philippine seas and adjacent waters. *Bulletin of the United States National Museum*, 3:1–547.
- Goto S, 1914. A descriptive monograph of Japanese Asteroidea. Part I. *Journal of the College of Science, Imperial University of Tokyo, Japan*, 29:1–808.
- Guindon S, Gascuel O, 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, 52:696–704.

- Hayashi R, 1973. The sea-stars of Sagami Bay, collected by His Majesty the Emperor of Japan. Biological Laboratory Imperial Household, Tokyo, pp. 41-53.
- Jangoux M, 1981. Resultats des campagnes MUSORSTOM 1. Philippines. Échinodermes: asteroïdes. Memoires ORSTOM, 91:457-476.
- Lee T, Shin S, 2011. A new record of sea urchin (Echinoidea: Camarodonta: Strongylocentrotidae) based on morphological and molecular analysis in Korea. Korean Journal of Systematic Zoology, 27:213-219. <https://doi.org/10.5635/KJSZ.2011.27.3.213>
- Liao Y, Clark AM, 1995. The echinoderms of southern China. Science Press, Beijing, pp. 63-74.
- Mah C, 2008. *Luidia avicularia* Fisher, 1913. In: Mah CL, 2015. World Asteroidea database [Internet]. Accessed 12 Jan 2017, <<http://www.marinespecies.org/asteroidea/aphia.php?p=taxdetails&id=213115>> .
- Mah CL, Blake DB, 2012. Global diversity and phylogeny of the Asteroidea (Echinodermata). PLoS ONE, 7:e35644. <https://doi.org/10.1371/journal.pone.0035644>
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP, 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology, 61:539-542. <https://doi.org/10.1093/sysbio/sys029>
- Rowe FWE, Gates J, 1995. Echinodermata. In: Zoological catalogue of Australia. Vol. 33 (Ed., Wells A). CSIRO Australia, Melbourne, pp. 1-510.
- Shin S, 2010. Invertebrate fauna of Korea. Vol. 32. Echinodermata: Asterozoa: Asteroidea sea stars. National Institute of Biological Resources, Incheon, pp. 1-150.
- Shin S, Rho BJ, 1996. Illustrated encyclopedia of fauna and flora of Korea. Vol. 36. Echinodermata. Ministry of Education, Seoul, pp. 1-780.
- Sladen WP, 1889. Report on the Asteroidea. Report on the Scientific Results of the voyage of H.M.S. Challenger during the Years 1873-1876, Zoology, 30:1-893.
- Sloan NA, 1980. The arm curling and terminal tube-foot responses of the asteroid *Crossaster papposus* (L.). Journal of Natural History, 14:469-482. <https://doi.org/10.1080/00222938000770411>
- Xiao N, Liu R, Yuan S, Sha Z, 2013. A preliminary phylogenetic analysis of *Luidia* (Paxillosida: Luidiidae) from Chinese waters with cytochrome oxidase subunit I (COI) sequences. Journal of Ocean University of China, 12:459-468. <https://doi.org/10.1007/s11802-013-2158-0>

Received January 4, 2017
 Revised April 10, 2017
 Accepted April 12, 2017