

# A Newly Recorded Sea Star of the Genus *Luidia* (Asteroidea, Paxillosoidea, Luidiidae) from Jeju Island, Korea, Based on Morphological and Molecular Analysis

Taekjun Lee<sup>1,2</sup>, Sook Shin<sup>1,3,\*</sup>

<sup>1</sup>Marine Biological Resource Institute, Sahmyook University, Seoul 01795, Korea

<sup>2</sup>Division of Life Sciences, College of Life Sciences and Biotechnology, Korea University, Seoul 02841, Korea

<sup>3</sup>Department of Chemistry Life Science, Sahmyook University, Seoul 01795, Korea

## ABSTRACT

The genus *Luidia* belongs to the family Luidiidae in the order Paxillosoidea. It is a common littoral taxon, and the depth range of the Korean fauna is generally 0–100 m. However, specimens of *Luidia* were collected from Jeju Island in Korea at 150–160 m depth and identified as *Luidia sagamina sagamina* Döderlein, 1920. This species is determined by morphological characteristics and mitochondrial cytochrome c oxidase subunit I sequence analysis. *Luidia sagamina sagamina* is a very rare sea star worldwide and it only collected previously from Sagami Bay, Japan, and is newly reported in Korea. Four species of *Luidia*, including *L. s. sagamina*, are recorded in the Korean fauna.

**Keywords:** taxonomy, morphology, COI, molecular identification

## INTRODUCTION

The genus *Luidia* Forbes, 1839 is the only genus in the family Luidiidae Sladen, 1889 of order Paxillosoidea Perrier, 1884 and consists of 49 species worldwide (Mah, 2018). This genus has worldwide distribution in tropical and temperate waters (D'yakonov, 1968; Clark and McKnight, 2000). It is a common littoral species that usually inhabits sand or muddy-sand substrate, and the depth range of the Korean fauna is 0–100 m (Shin, 2010; Kim et al., 2017). *Luidia* generally feeds on mollusks, various small invertebrates, and fishes (D'yakonov, 1968; Xiao et al., 2013).

Döderlein (1920) identified the following four main groups in *Luidia* based on the form and appearance of ossicle arrangements, and the morphological characteristics of spine, spinelet, and pedicellaria: *Alternata*, *Ciliaris*, *Clathrata*, and *Quinaria*. Clark and Downey (1992) declared that the limits of the four main groups and some of the subgenera within them were ambiguous and did not adopt the taxonomic treatment of Fell (1963). In 1963, Fell lumped with subgenus *Alternaster* and *Armaster*, then perfunctorily raised all the

others to generic rank but it has been failed to gain acceptance (Clark, 1982), notably from Blake (1973), who undertook a thorough study of the ossicles of Luidiidae and related asteroids. In the northwest Pacific, particularly around Far East Asia and Russia, 11 species have been recorded (Fisher, 1911, 1919; Döderlein, 1920; Hayashi, 1940; D'yakonov, 1968; Shin, 2010; Xiao et al., 2013; Kim et al., 2017) (Table 1).

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 identification and discovery of species (Hebert et al., 2003; Ratnasingham and Hebert, 2007), and the region of the COI sequence is validated as an effective tool for species discrimination in echinoderms (Ward et al., 2008; Hoareau and Boissin, 2010; Layton et al., 2016). More than 16,000 COI sequences from echinoderms have been deposited in the GenBank, providing useful data for various research studies and molecular identification of species. Among them, 73 COI sequences of *Luidia* and mitochondrial complete genome of *L. quinaria* von Martens, 1865 are registered and have been used for various studies

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

**\*To whom correspondence should be addressed**  
Tel: 82-2-3399-1717, Fax: 82-2-3399-1729  
E-mail: shins@syu.ac.kr

**Table 1.** Distribution and sequence information of species of genus *Luidia* and species used as outgroup

Species	Location	GenBank accession No.	References
<i>L. sagamina sagamina</i>	Jeju Island, Korea	MG940977	This study
<i>L. avicularia</i>	Korea Strait, Korea	KY305010	Kim et al. (2017)
<i>L. avicularia</i>	South China Sea	JQ740627	Xiao et al. (2013)
<i>L. changi</i>	Yellow Sea	JQ740621	Xiao et al. (2013)
<i>L. foliolata</i>	Queen Charlotte Sound, Canada	HM473921	Corstorphine (2010)
<i>L. hardwicki</i>	East China Sea	JQ740633	Xiao et al. (2013)
<i>L. longispina</i>	Tonkin Gulf	JQ740629	Xiao et al. (2013)
<i>L. maculata</i>	East China Sea	JQ740634	Xiao et al. (2013)
<i>L. orientalis</i>	South China Sea	JQ740624	Xiao et al. (2013)
<i>L. quinaria</i>	Yellow Sea	JQ740612	Xiao et al. (2013)
<i>L. quinaria</i>	Toyama Bay, Japan	AB183558	Matsubara et al. (2005)
<i>L. sarsi</i>	North Sea	KX458989	Laakmann et al. (2016)
<i>L. yesoensis</i>	Yellow Sea	JQ740618	Xiao et al. (2013)
<i>Craspidaster hesperus</i>	East China Sea	JQ740636	Xiao et al. (2013)
<i>Aquilonastra batheri</i>	Jeju Island, Korea	MG970141	This study

**Fig. 1.** Collection sites of *Luidia sagamina sagamina* from previous studies (▲) and present study (★).

(Knott and Wray, 2000; Matsubara et al., 2005; Corstorphine, 2010; Xiao et al., 2013; Cortes et al., 2015; Laakmann et al., 2016).

In the present study, we redescribed *Luidia sagamina sagamina* Döderlein, 1920, and provided detailed photographs and COI sequence. Furthermore, we compared this species with the Atlantic *L. s. aciculata* Mortensen, 1933 based on morphological characteristics.

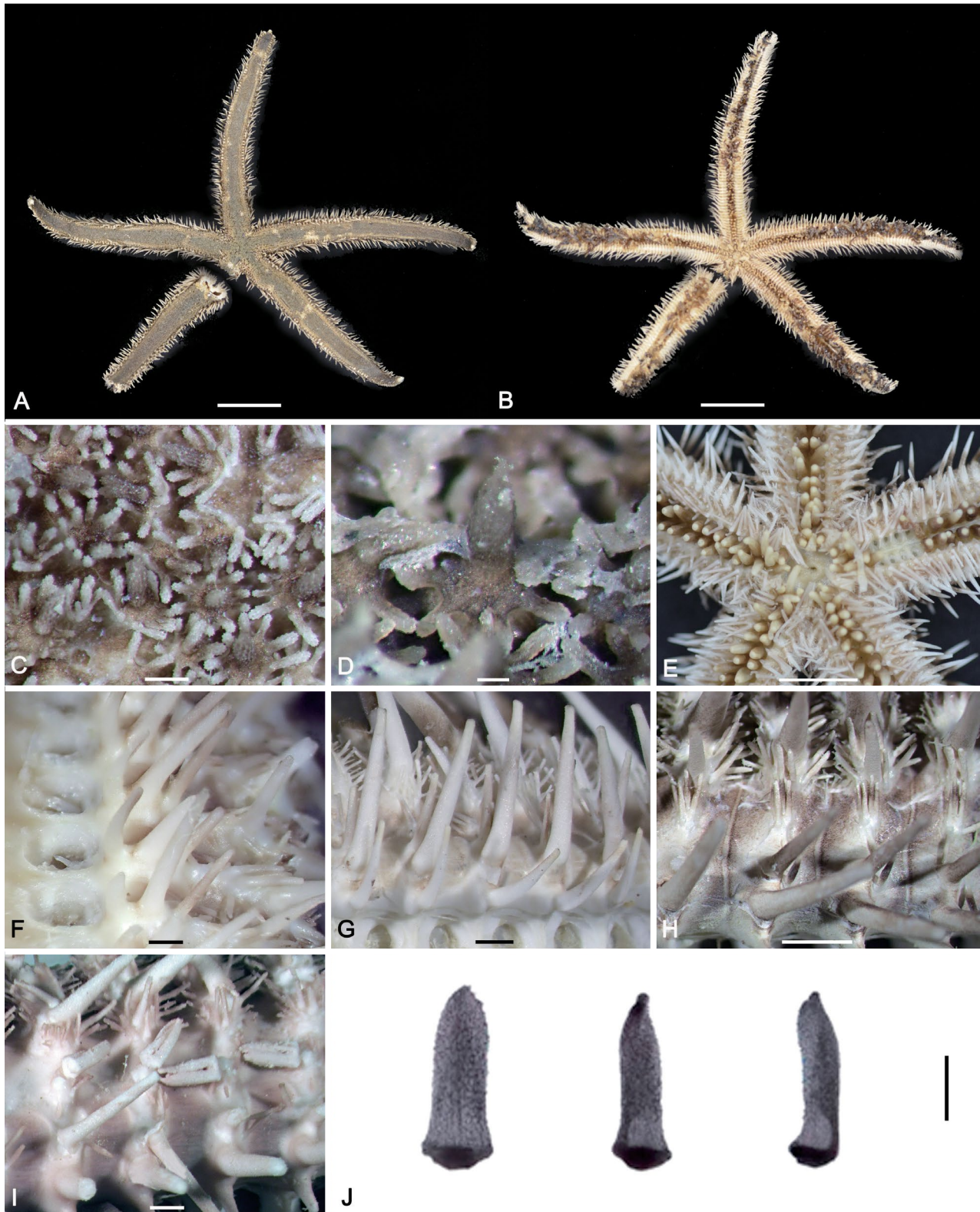
Three specimens were collected in adjacent waters of Moseulpo and Hanrim in Jeju Island, Korea (Fig. 1). They were collected with fishing nets from a depth of 150–160 m. All specimens were preserved in ethyl alcohol solution (>95%) and stored in the Marine Echinoderms Resources Bank of Korea (MERBK) with registered numbers.

Important morphological characteristics of specimens were photographed using a digital camera (G12; Canon, Tokyo, Japan). Pedicellariae were extracted from the oral part and

adambulacral plates and treated with a NaClO solution (Shin, 2010). Morphological characteristics were photographed under stereo- and light microscopes (Nikon SMZ 1000, Nikon Eclipse 80i; Tokyo, Japan). The traditional taxonomic characters used for morphological description as described by Döderlein (1920) and Clark and Downey (1992) were applied to confirm the identification of the specimens.

Partial sequences of the mitochondrial COI were amplified using pairs of primers conserved in echinoderms, LCOechFa1 (Layton et al., 2016) and HCO2198 (Folmer et al., 1994). The amplification was conducted in a reaction mixture containing 0.3 µL of exTaq polymerase (Takara, Tokyo, Japan), 2.5 µL of 10× exTaq buffer with MgCl<sub>2</sub>, 1.5 µL of dNTPs (25 nmol), 1.5 µL of each primer (10 pmol), and 1.5 µL of template DNA and DEPC treated-H<sub>2</sub>O to a total volume of 25 µL. Conditions for amplification included an initial denaturation at 95°C for 5 min followed by 35 cycles of 94°C for 30 s, annealing for 45 s at 47°C, extension at 72°C for 1 min, which was followed by a final extension at 72°C for 7 min. Polymerase chain reaction product quality was assessed using a NanoDrop 1000 (Thermo Fisher Scientific, Waltham, MA, USA) and were sequenced using Big Dye Terminator kits (Applied Biosystems, Foster City, CA, USA) on a 3730XL DNA Analyzer (Applied Biosystems).

Mitochondrial COI sequences were assembled with Geneious R11 (Biomatters Limited, Auckland, New Zealand) and alignments were performed using the ClustalX 1.6 software (Thompson et al., 1997) with default parameters. Kimura 2-parameter genetic distances with 1,000 bootstrapping for COI were calculated using MEGA7 (Kumar et al., 2016). The evolutionary models of maximum likelihood (ML), which appropriate model was selected by jModelTest2 (Darriba et al., 2012). As a result, analyses were based on the selected model (GTR + I + G). The ML tree was analyzed with PHYML v.3.0



**Fig. 2.** *Luidia sagamina sagamina*. A, Abactinal side; B, Actinal side; C, D, Abactinal paxillae; E, Oral part; F, G, Adambulacral spines; H, Inferomarginal spines; I, Bivalve pedicellariae on abactinal plates; J, Valves of bivalve pedicellariae under light microscope. Scale bars: A, B=25 mm, C, D, I, J=0.2 mm, E=10 mm, F-H=1 mm.

**Table 2.** Reported species of genus *Luidia* around northwest Pacific (Korea, China, Japan and Russia)

Region	Species										
	<i>avicularia</i>	<i>changi</i>	<i>difficilis</i>	<i>hardwicki</i>	<i>inarmata</i>	<i>longispina</i>	<i>malculata</i>	<i>orientalis</i>	<i>quinaria</i>	<i>sagamina</i>	<i>yesoensis</i>
Korea	●	○	○	○	○	○	●	○	●	★	○
China	●	●	●	●	○	●	●	●	●	○	●
Japan	●	○	○	○	●	○	●	○	●	●	●
Russia	○	○	○	○	○	○	○	○	●	○	○

●, present; ○, absent; ★, newly reported in the present study.

(Guindon and Gascuel, 2003) and 1,000 bootstrap replications. In total, 13 sequences from 11 *Luidia* species were analyzed (Table 1) and the sequence of *L. s. sagamina* (GenBank accession No. MG940977) was included in these analyses. *Aquilonastra batheri* (GenBank accession No. MG970141) and *Craspidaster hesperus* (GenBank accession No. JQ740636) were selected as the outgroup.

## SYSTEMATIC ACCOUNTS

Class Asteroidea de Blainville, 1830

Superorder Valvatacea Blake, 1987

Order Paxillosida Perrier, 1884

Family Luidiidae Sladen, 1889

Genus *Luidia* Forbes, 1839

### <sup>1</sup>\**Luidia sagamina sagamina* Döderlein, 1920 (Fig. 2)

*Luidia sagamina* Döderlein, 1920: 290; Hayashi, 1940: 112; Madsen, 1950: 199, figs. 6, 7.

*Luidia sagamina sagamina* Clark, 1982: 174; Mah, 2018: 224134.

**Material examined.** Korea: One specimen, Jeju Island, adjacent water of Moseulpo, 12 Sep 2012, collected at 150 m with a fishing net, Lee T, Pyo J, Shin S; one specimen, Jeju Island, adjacent water of Hanrim, 24 Apr 2016, collected at 160 m with a fishing net, Kim D, Bae S; one specimen, Jeju Island, adjacent water of Moseulpo, 12 Jul 2017, collected at 150 m with a fishing net, Kim D, Ubagan M.

**Description.** Arms five, long, slender, tapered to tip and slightly narrow at basal part (Fig. 2A, B). Disk small, body flattened and moderately sized (Fig. 2A). Abactinal side covered with paxillae (Fig. 2C). Paxilla with conspicuous conical shaped central spine longer than pillar of paxilla. Central spine almost one and rarely two, number of peripheral spinelets 11–13 in disk and 8–9 in intermediate arm, translucent and digit form (Fig. 2C, D). Pedicellariae very rare or absent and oval shaped with two valves. Madreporite

on margin of interradial disk and concealed with paxillae. Superomarginal paxillae circular, concaved at inferomarginal side, slightly larger than abactinal ones, and three paxillae corresponds with five or six abactinal ones. Its central spines comprised of a conspicuous conical shaped large spine and small spinelets, with various combinations: 1 + 4, 1 + 5, 2 + 3, 2 + 4. Number of peripheral spinelets 16–18 in basal side and 18–20 in intermediate arm. Inferomarginal plates large, crescentic in a cross section; each plate slightly deep ridge separates, filled with many small spinelets. Inferomarginal spines three, considerably long, pointed and alternately sequenced along inferomarginal series, 3 + 2 + 3 + 2 (Fig. 2H): spine of actinal side the shortest, other two almost twice as long and subequal to each other. Adambulacral plate broad with three spines. Median spine straight, longest, tapered to tip, and slightly dull. Innermost spine curved, slightly flattened, and usually a half-length of median one. Outermost spine straight, tapered to tip, slightly dull, and subequal length with innermost one (Fig. 2F, G). Adambulacral pedicellariae with two valves, large and tip slightly dull (Fig. 2I, J). In absence of pedicellaria, five to seven spinelets replaced but emptied in young specimen. Oral plate longer than broad, slightly protuberated. Oral spines long, slender, rod form. Actinal pedicellariae situated under oral plate, large, pyramid-form with three valves and dull tip.

**Size.** R = 150 mm, r = 16 mm, R = 9.3 r; R = 138 mm, r = 15 mm, R = 9.1 r; R = 118 mm, r = 13 mm, R = 9.1 r.

**Body color.** Color is dark brown on abactinal side and pale ivory on actinal side. Inferomarginal spine is dark brown at basal part and tips is white.

**Distribution.** Korea (Jeju-do), Japan (Sagami Bay).

**Remarks.** Eleven species of genus *Luidia* have been reported in the northwest Pacific, and among them four species distributed in Korea (Table 2). *Luidia sagamina* consists of two subspecies: *L. s. sagamina* and *L. s. aciculata*, of which the latter was first reported as *L. aciculata* by Mortensen, 1933. After that, Madsen (1950) treated *L. s. aciculata* as the Atlantic species and Clark (1982) followed Madsen's

Korean name: <sup>1</sup>\*깊은검은띠불가사리 (신칭)



**Table 4.** Interspecific pairwise distance values for 11 species under genus *Luidia*, calculated using the Kimura-2 parameter distance model, based on partial sequences of the mitochondrial COI gene

	1	2	3	4	5	6	7	8	9	10	11	12	13
1 <i>L. s. sagamina</i>													
2 <i>L. avicularia</i> (Korea)	0.222												
3 <i>L. avicularia</i> (China)	0.222	0.000											
4 <i>L. changi</i>	0.224	0.002	0.002										
5 <i>L. foliolata</i>	0.252	0.227	0.227	0.229									
6 <i>L. hardwicki</i>	0.222	0.000	0.000	0.002	0.227								
7 <i>L. longispina</i>	0.222	0.000	0.000	0.002	0.227	0.000							
8 <i>L. maculata</i>	0.241	0.264	0.264	0.264	0.244	0.264	0.264						
9 <i>L. orientalis</i>	0.224	0.002	0.002	0.003	0.229	0.002	0.002	0.266					
10 <i>L. quinaria</i> (Japan)	0.241	0.131	0.131	0.131	0.226	0.131	0.131	0.258	0.133				
11 <i>L. quinaria</i> (China)	0.224	0.003	0.003	0.002	0.231	0.003	0.003	0.262	0.005	0.133			
12 <i>L. sarsii</i>	0.192	0.218	0.218	0.218	0.224	0.218	0.218	0.239	0.220	0.224	0.218		
13 <i>L. yesoensis</i>	0.228	0.012	0.012	0.011	0.236	0.012	0.012	0.270	0.014	0.133	0.012	0.222	

COI, cytochrome c oxidase subunit I.

possibly only a difference in descriptive expression.

Mitochondrial COI sequence was obtained and registered with the GenBank (accession No. MG940977). The phylogenetic relationships of 13 COI sequences (658-bp) from 11 species under the genus *Luidia* were analyzed (Table 1), corresponding to the general DNA barcoding region of mitochondria. Pairwise genetic distances were calculated by the Kimura-2 parameter (Table 4). Pairwise distances ranged from 0.0% to 26.4% within the 11 *Luidia* species and the average was 16.7%. Pairwise distances between *L. s. sagamina* and other *Luidia* species ranged from 19.2% to 25.2%, and exceeded the average value of *Luidia* species. *Luidia s. sagamina* accomplished a monophyly with *L. sarsi* (Fig. 3) and its sequence is distinguished from other species of *Luidia* (Table 4). Pairwise distances of *L. s. sagamina* ranged from 19.2% to 25.2% within the 11 species of *Luidia* and these values exceeded interspecific divergences (12.0%) reported in a previous study involving DNA barcoding of echinoderms (Layton et al., 2016).

## ACKNOWLEDGMENTS

The present study was supported by the a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) (NIBR 201801201), the Marine Biotechnology Program of the Korea Institute of Marine Science and Technology Promotion (KIMST) funded by the Ministry of Oceans and Fisheries (MOF) (No. 2017 0431), and the 'Development of the methods for controlling and managing marine ecological disturbance-causing and harmful organisms (MEDHO)' program funded by Korea Institute of Marine Science and Technology Promotion (KIMST)/Ministry of Oceans and Fisheries (MOF) of the Republic of Korea.

## REFERENCES

- Clark AM, 1982. Notes on Atlantic Asteroidea. 2. Luidiidae. Bulletin of the British Museum (Natural History), Zoology, 42:157-184.
- Clark AM, Downey ME, 1992. Starfishes of the Atlantic. Chapman and Hall Identification Guides, 3. Chapman and Hall, London, pp. 1-794.
- Clark HES, McKnight DG, 2000. The marine fauna of New Zealand: Echinodermata: Asteroidea (sea-stars). Order Paxillo-sida. Order Notomyotida. NIWA Biodiversity Memoir, 116: 1-196.
- Corstorphine EA, 2010. DNA barcoding of echinoderms: species diversity and patterns of molecular evolution. MSc thesis, University of Guelph, Guelph, Ontario, Canada.
- Cortes MP, Chung HM, Pomory CM, 2015. Sediment preference, salinity tolerance and COX-1 genetic differences in two purportive species of *Luidia* (Echinodermata: Asteroidea). Journal of the Marine Biological Association of the United Kingdom, 95:551-559. <https://doi.org/10.1017/S0025315414001817>
- Darriba D, Taboada GL, Doallo R, Posada D, 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods, 9:772. <https://doi.org/10.1038/nmeth.2109>
- D'yakonov AM, 1968. Sea stars (asteroids) of the USSR seas. [Translation from keys to the fauna of the USSR. No. 34. Zoological Institute of the Academy of Sciences of the USSR, 1950]. Israel Program for Scientific Translations, Jerusalem, pp. 1-152.
- Döderlein L, 1920. die Asteriden der Siboga-Expedition. 2. Die Gattung *Luidia* und ihre Stammesgeschichte. Siboga Expedition, 46:193-291.
- Fell HB, 1963. The phylogeny of sea-stars. Philosophical Transactions of the Royal Society B, 246:381-435. <https://doi.org/10.1098/rstb.1963.0010>
- Fisher WK, 1911. Asteroidea of the North Pacific and adjacent waters. 1. Phanerozoia and Spinulosida. Bulletin of the

- United States National Museum, 76:1-420.
- Fisher WK, 1919. Starfishes of the Philippine seas and adjacent waters. *Bulletin of the United States National Museum*, 100: 1-547.
- 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.
- 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, 1940. Contributions to the classification of the sea-stars of Japan. I. Spinulosa. *Journal of the Faculty of Science, Hokkaido Imperial University*, 7:107-204.
- Hebert PDN, Cywinska A, Ball SL, deWaard JR, 2003. Biological identifications through DNA barcodes. *Proceeding of the Royal Society B: Biological Sciences*, 270:313-321. <https://doi.org/10.1098/rspb.2002.2218>
- Hoareau TB, Boissin E, 2010. Design of phylum-specific hybrid primers for DNA barcoding: addressing the need for efficient COI amplification in the Echinodermata. *Molecular Ecology Resources*, 10:960-967. <https://doi.org/10.1111/j.1755-0998.2010.02848.x>
- Kim D, Kim M, Shin S, 2017. A newly recorded sea star of the genus *Luidia* (Asterozoa: Paxillosida: Luidiidae) from the Korea Strait, Korea. *Animal Systematics, Evolution and Diversity*, 33:131-135. <https://doi.org/10.5635/ASED.2017.33.2.061>
- Knott KE, Wray GA, 2000. Controversy and consensus in asteroid systematics: new insights to ordinal and familial relationships. *American Zoologist*, 40:382-392. <https://doi.org/10.1093/icb/40.3.382>
- Kumar S, Stecher G, Tamura K, 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33:1870-1874. <https://doi.org/10.1093/molbev/msw054>
- Laakmann S, Boos K, Knebelberger T, Raupach MJ, Neumann H, 2016. Species identification of echinoderms from the North Sea by combining morphology and molecular data. *Helgoland Marine Research*, 70:1-18. <https://doi.org/10.1186/s10152-016-0468-5>
- Layton KKS, Corstorphine EA, Hebert PDN, 2016. Exploring Canadian echinoderm diversity through DNA barcodes. *PLoS ONE*, 11:e0166118. <https://doi.org/10.1371/journal.pone.0166118>
- Madsen FJ, 1950. The echinoderms collected by the Atlantide Expedition 1945-46. 1. Asterozoa. *Atlantide Reports*, 1: 167-222.
- Mah CL, 2018. World Asterozoa database [Internet]. World Register of Marine Species, Accessed 9 Feb 2018, <<http://www.marinespecies.org/aphia.php?p=taxdetails&id=123260>> .
- Matsubara M, Komatsu M, Araki T, Asakawa S, Yokobori S, Watanabe K, Wada H, 2005. The phylogenetic status of Paxillosida (Asterozoa) based on complete mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, 36:598-605. <https://doi.org/10.1016/j.ympev.2005.03.018>
- Ratnasingham S, Hebert PDN, 2007. BOLD: The barcode of life data system (<http://www.barcodinglife.org>). *Molecular Ecology Notes*, 7:355-364. <https://doi.org/10.1111/j.1471-8286.2007.01678.x>
- Shin S, 2010. Invertebrate fauna of Korea. Vol. 32. Echinodermata: Asterozoa: Asterozoa sea stars. National Institute of Biological Resources, Incheon, pp. 1-150.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG, 1997. The CLUSTAL\_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, 25:4876-4882. <https://doi.org/10.1093/nar/25.24.4876>
- Ward RD, Holmes BH, O'Hara TD, 2008. DNA barcoding discriminates echinoderm species. *Molecular Ecology Resources*, 8:1202-1211. <https://doi.org/10.1111/j.1755-0998.2008.02332.x>
- 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 April 2, 2018  
Revised October 10, 2018  
Accepted October 12, 2018