

New record of the unstalked crinoid *Tropiometra macrodiscus* (Crinoidea: Comatulida: Tropiometridae) from Korea

Philjae Kim¹, Taekjun Lee^{2,3} and Sook Shin^{2,3,*}

¹Division of Ecological Conservation, Bureau of Ecological Research, National Institute of Ecology, Seocheon-gun, Chungnam 33657, Republic of Korea

²Department of Animal Biotechnology & Resource, Sahmyook University, Seoul 01795, Republic of Korea

³Institute of Marine Life Resources, Sahmyook University, Seoul 01795, Republic of Korea

*Correspondent: shins@syu.ac.kr

Two crinoid specimens of genus *Tropiometra* were collected from Busan and Tongyeong by SCUBA diving on August 2010 and October 2011, respectively. The specimens were identified as *Tropiometra macrodiscus* (Hara, 1895), which belongs to the family Tropiometridae of superfamily Tropiometroidea. The genus *Tropiometra* AH Clark, 1907 comprises four species worldwide at present, and it has not been reported in Korea. *Tropiometra macrodiscus* was first described by Hara (1895) in Japan. It is difficult to distinguish *T. macrodiscus* from *T. afra* (Hartlaub, 1890), there has been confusion with examination of their phylogenetic positions in crinoid morphological classification. Despite this, *T. macrodiscus* can be distinguished from *T. afra* based on longer arms, stouter whole-body parts, magnificently larger numbers of cirrus, and numerous segments. The morphological characteristics of *T. macrodiscus* collected in Korea have been described, and DNA barcode region representing cytochrome *c* oxidase subunit I was obtained for its molecular phylogenetic analysis.

Keywords: crinoid, DNA barcode, phylogenetic position, *Tropiometra afra*, *Tropiometra macrodiscus*, Tropiometridae

© 2022 National Institute of Biological Resources
DOI:10.12651/JSR.2022.11.1.022

INTRODUCTION

The phylum Echinodermata comprises five major classes, one of which is class Crinoidea. All living crinoid species belong to subclass Articulata (Ausich and Messing, 1998), commonly known as sea lilies and feather stars, and called stalked crinoids and unstalked crinoids, respectively. The crinoids existing in modern marine environments belong to the order Comatulida, which is the dominant group of living unstalked crinoids (Messing, 1997). They have a body structure like a cup form and 5 to 200 arms (Towle, 1989). Many pinnules are located on the arms of the comatulids, and they use the movement of arms and pinnules for suspension-feeding behavior.

The genus *Tropiometra* AH Clark, 1907 is the only one in the family Tropiometridae AH Clark, 1908 of the superfamily Tropiometroidea AH Clark, 1908, and comprises four species worldwide. In previous studies, the genus *Tropiometra* has never been reported in Korea

(Won and Shin, 2002; Shin, 2001; 2002; 2004; 2005; 2013; Kim, 2020). This genus is known to have an irregular distribution and is extremely limited locally. *Tropiometra macrodiscus* (Hara, 1895) is a species belonging to the genus *Tropiometra* and has been confused in species-level identification with *Tropiometra afra* (Hartlaub, 1890) in many morphological characteristics. After it was first recorded in 1895, *T. macrodiscus* was recorded under *T. afra* for consideration as a subspecies or reported as *T. afra*. In 1947, differences in the distribution range and morphological characteristics were used to determine the classification key to *T. macrodiscus* and *T. afra* (see Clark, 1947). Therefore, currently, *T. macrodiscus* and *T. afra* are classified as different species despite their similar morphologies.

In the case of species that are difficult to distinguish, DNA barcoding techniques have been extremely helpful in identifying the species (Hebert *et al.*, 2003). In animals, the 658 bp mitochondrial cytochrome *c* oxidase subunit I (COI) is known as the most powerful DNA

(Thompson *et al.*, 1997). Phylogenetic tree construction was conducted with the dataset produced according to the minimum length among the 15 COI sequences, including two *T. macrodiscus* and 13 crinoids (JX236097, GU327867, JX236095, GU480555, JX236082, JX236089, JX236083, KC626526, KC626581, KC626585, KC626589, KC626529, and KC626556). The COI sequences of 449 bp were analyzed, and the pairwise genetic distances were calculated according to the Kimura 2-parameter (K2P) model (Kimura, 1980; Collins *et al.*, 2012) with 1,000 bootstrapping in MEGA software version 7.0 (Kumar *et al.*, 2016) (Table 1). A neighbor-joining tree was constructed using MEGA7.0 (Kumar *et al.*, 2016), and the stalked crinoid *Holopus alidis* (KC626556) was chosen as the outgroup (Fig. 2).

The present study describes a new record of *T. macrodiscus* (Hara, 1895) belonging to the family Tropiometridae, the superfamily Tropiometridae, in South Korea. In the Korean fauna, the family Tropiometridae is reported for the first time, and the morphological characteristics of this species are described.

SYSTEMATIC ACCOUNTS

Class Crinoidea Miller, 1821
 Subclass Articulata Zittel, 1879
 Order Comatulida AH Clark, 1908
 Superfamily Tropiometroidea AH Clark, 1908
 Family Tropiometridae AH Clark, 1908
 Genus *Tropiometra* AH Clark, 1907

Tropiometra macrodiscus (Hara, 1895)

Antedon macrodiscus Hara, 1895: 115; Clark, 1908: 481;
 Hara and Okada, 1921: 33.

Tropiometra macrodiscus Clark, 1907: 349; Clark, 1908:
 316.

Tropiometra afra Clark, 1908: 315; 1912: 176; Gislén,
 1934: 6.

Tropiometra afra var. *macrodiscus* Gislén, 1922: 90; 1922:
 44, 51, 53, 78, 90.

Tropiometra afra macrodiscus Clark, 1947: 272.

Material examined. Korea: One specimen, waters adjacent to Busan (34°53'25.0"N, 128°57'22.4"E), Aug 3, 2010, with SCUBA; One specimen, waters adjacent to Tongyeong (34°39'12.9"N, 128°34'42.5"E), Oct 20, 2011, with SCUBA.

Description. Specimen from Busan; a total of 10 arms, 310 mm long. Centrodorsal width 12 mm, height 4 mm. Dorsal pole 6 mm in diameter. Centrodorsal, very thick disk with slightly sloping side, middle of dorsal pole clearly concave form. Cirrus sockets arranged in three irregular rows. Cirrus XLV, 32–42 segments, 35–50 mm

long, stout and smoothly curved, non-spine development. Terminal claw blunt and short. P₁ 31 mm length, 39 segments, slender, non-genital pinnule. P₂ slightly stouter than P₁, 34 mm in length, 40 segments. P₃, similar to P₂, 36 mm in length 44 segments. The entire body parts solid and non-flexible, even the pinnules or cirrus. The axillaries nearest to arms very stout, solid, and strong; the terminal parts of arms becoming slender. IB_{R1} longish rectangular shape. IB_{R2} (axillaries) broadly pentagonal, low height, and almost triangular. Syzygies occurring between the brachial 3 + 4, 8 + 9, and 13 + 14. The specimen was collected in Tongyeong; 10 arms, robust, and longish. Large stout body. All arms were broken and some regenerated, with a length of 210 mm. Centrodorsal width 13 mm height 6 mm. Dorsal pole 5 mm in diameter. Cirrus sockets arranged in three irregular rows. Cirrus XLIII, 33–41 segments, 35–53 mm long and stout, smoothly curved. In cirrus segments, no opposing spines or dorsal spines. Terminal claws short and curved, and blunt never sharp. P₁ 33 mm in length, 39 segments, slender, and non-genital part. P₂, 36 mm in length, 35 segments, slightly stouter than P₁. P₃, 38 mm in length, 37 segments, similar to P₂. IB_{R1} longish oblong shape. IB_{R2} (axillaries), similar with triangle-like pentagons with edges against adjacent axillaries, very short. Syzygies shown in 3 + 4 and usually 8 + 9.

Size. Arms = 310 mm (Busan) and 210 mm (Tongyeong). Centrodorsal = 12 mm (Busan) and 13 mm (Tongyeong). Cirrus = 50 mm (Busan) and 53 mm (Tongyeong).

Color. Body yellowish dark brown in 95% ethanol, regenerated parts bright yellow.

Distribution. Korea (Korea Strait), Japan (Sagami Bay, Ogasawara Is), China, Hong Kong.

Remarks. The large crinoids, *T. macrodiscus* and *T. afra*, are confused in species-level classification because of their similar morphological characteristics. When *Antedon macrodiscus* was first recorded (Hara, 1895), it was regarded as a unique species separated from *A. afra* at the species level. Hara (1895) only confused the specimens with *Amphimetra miberti* accepted as *Amphimetra tessellate* because of their morphological similarities, such as pinnules, brachials, and intersyzygial interval. The specimens are distinguished by the number of cirri and the presence/absence of spines on cirrus segments. After the establishment of *Tropiometra*, *A. macrodiscus* was synonymized into *T. macrodiscus*. *Tropiometra macrodiscus* specimens from the Sagami Bay in Japan were reported in April 1908, and these were identified as *T. afra* depending on the ambiguous morphological differences with the specimens collected in Australia (Clark, 1908). Clark placed *T. macrodiscus* under the synonymy of *T. afra*, and reported the specimen collected in the Korea Strait as *T. afra* (Clark, 1947). Gislén (1922) recognized the difference between the two species, but suggested that the several

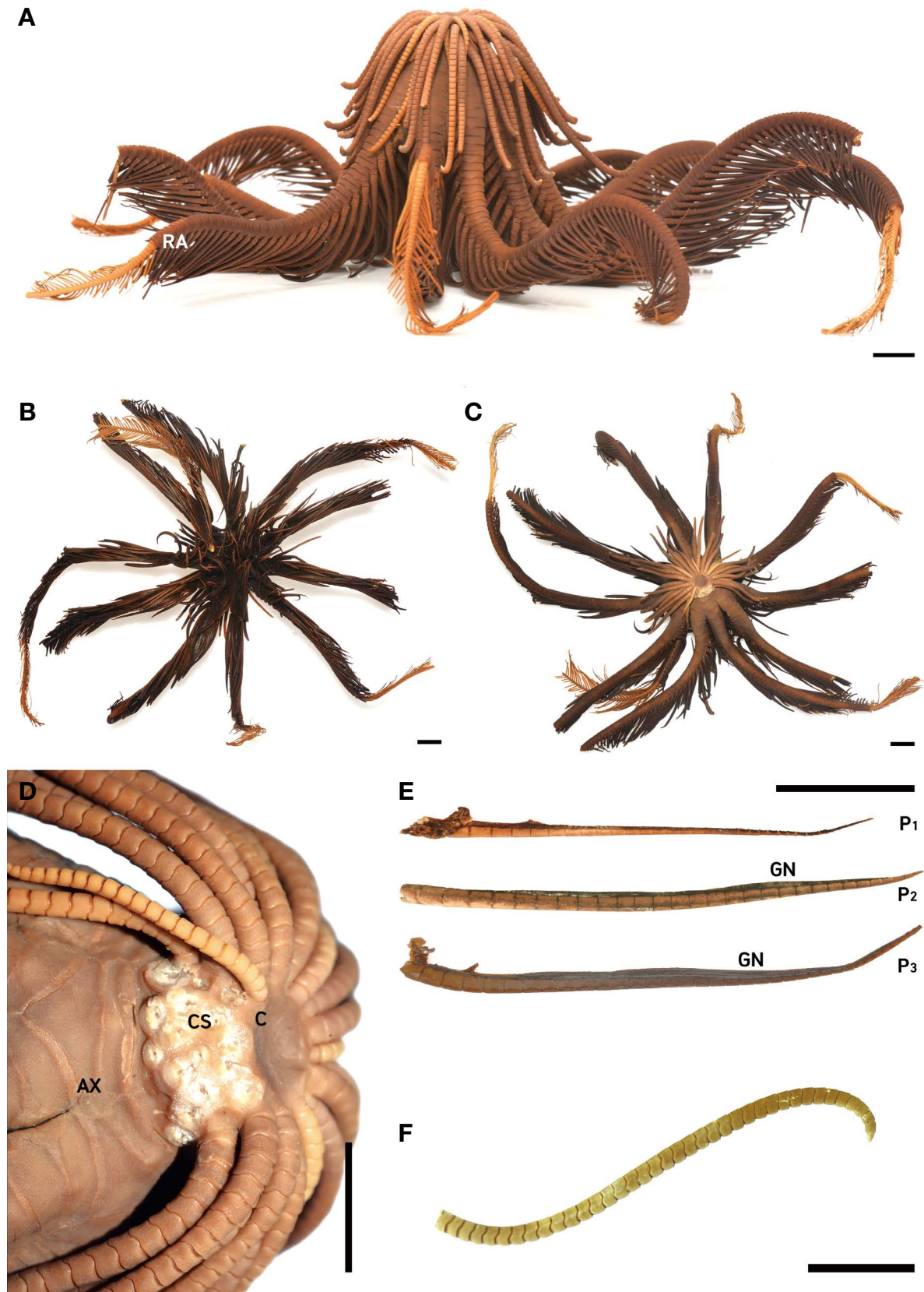


Fig. 1. *Tropiometra macrodiscus* (Hara, 1895). (A) Lateral view; (B) Oral view; (C) Aboral view; (D) centrodorsal, axillaries; (E) pinnules; (F) cirrus. AX, axillaries; C, centrodorsal; CS, cirrus socket; RA, regenerated arm; GN, gonad. Scale bars = 10 mm.

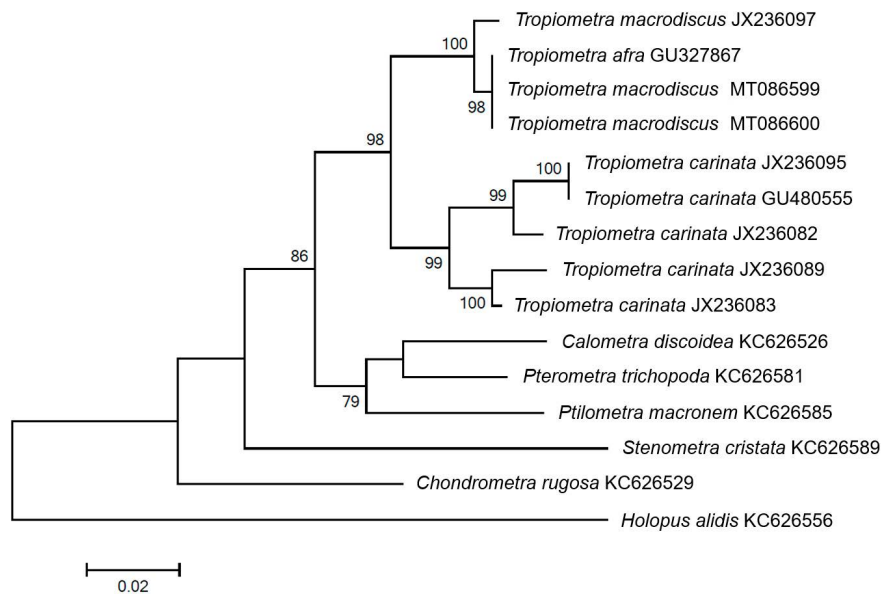


Fig. 2. Neighbor joining tree of the aligned 449 bp partial COI sequences for two *Tropiometra macrodiscus* and 13 crinoids obtained from GenBank. GenBank accession numbers of the species are provided. Bootstrap resampling values were supported at ≥ 70 . Scale bar represents the genetic distance.

different features could be considered as variations that may be due to the intervening territory. At present, *T. macrodiscus* and *T. afra* are understood to be different species with extremely different distribution ranges. However, *T. macrodiscus* specimens from the Sagami Bay reported in Hara (1895) have stouter and larger forms than the specimens recorded as *T. afra*, and have longer and more numerous cirrus segments. These morphological differences according to the distribution range were emphasized as the basis for the species-level classification, and these differences were identified as the main classification keys for the current *Tropiometra* classification.

We compared our specimen with previous descriptions of *T. macrodiscus* and *T. afra*. Our specimens clearly showed differences from *T. afra* described by Clark (1947) in characteristics that are the key to the morphological classification of crinoids: 1) long cirrus and arms, 2) more numerous cirrus segments and cirrus quantity, 3) pentagon-shaped axillaries, and 4) no opposing spines on cirrus. In addition, our specimen differs slightly from *T. macrodiscus* described by Clark (1947), whereas it was in accordance with the description of *T. macrodiscus* Gislén (1922). Furthermore, the collection sites of the specimens we have were included in the Korean Strait known as the distribution range of *T. macrodiscus* (from Hong Kong northward to the Korean Strait and eastward to the Sagami Bay, Japan, and the Bonin Islands), distinct from the distribution range of *T. afra* (Philippines and southward to Bowen, Queensland, and to Fremantle and Geraldton, Western Australia). In the description of *T. macrodiscus*

Clark (1947), the specimens from the Korean Strait showed that the length of the arms was not longer and the number of cirri was less than that of the other specimens. Our specimens were similar to specimens from China and Japan. Therefore, we presumed that our specimens were mature in accordance with obviously long arms and numerous cirri, and we concluded that the *T. macrodiscus* specimens of Clark (1947) are possibly immature specimens, i.e., the length of the arm is shorter than that of our specimens and the slightly fewer number of cirri.

In the phylogenetic analysis, 15 COI sequences from nine crinoids belonging to the family Tropiometridae were used for species molecular identification. Pairwise distances ranged from 0.0% to 26.1% within the nine Tropiometridae species. The intraspecific variation for *T. macrodiscus* (MT086599, MT086600 from Korea; JX236097 from Japan) and *T. afra* (GU327867 from Japan) ranged from 0.0% to 0.9% (Table 1). According to our molecular data, *T. afra* reported in Japan should be considered as same species, *T. macrodiscus*. The COI sequence of *T. afra* (GU327867 from Japan) was reported by Rouse *et al.* (2013). Rouse *et al.* (2013) only determined the molecular phylogeny status of extant crinoids, and we could not find any information on the morphological description of the source of the sequence. The only additional information available in the paper is the collection site of the specimen. However, the paper was accepted on September 2012, and the corresponding author noticed descriptive notes by personal communication for *T. afra macrodiscus* in World Register of Marine Spe-

cies (Worms, <https://www.marinespecies.org/>) that the two subspecies, *T. afra afra* and *T. afra macrodiscus*, are distinct enough to separate species based on molecular data on 6 June 2013 (Unpublished data, accessed on 4 November 2021). Moreover, Clark (1947) recorded that the significant classification key for *T. afra* distributed in Philippines and Australia, and *T. macrodiscus* distributed in Hong Kong, China, and Japan is the distribution range. Therefore, some questions remain regarding the specimens used in Rouse *et al.* (2013). The specimen collection site location belongs to the distribution range of *T. macrodiscus*, and it makes us to consider that their specimen might be *T. macrodiscus*, not *T. afra*.

The COI sequences of 657 bp obtained in this study were registered in the NCBI GenBank database (<https://www.ncbi.nlm.nih.gov/>) under accession numbers MT086599 (Busan) and MT086600 (Tongyeong). Our results will be useful for examining the molecular phylogenetic relationships of crinoids and establishing genetic differences between *T. macrodiscus* and *T. afra* in the future.

ACKNOWLEDGEMENTS

This study was supported by a grant from the National Institute of Biological Resources (NIBR), which was funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR 202102203), and the project titled “Improvement of management strategies on marine disturbing and harmful organisms (No. 20190518)” funded by the Ministry of Oceans and Fisheries, Korea.

REFERENCES

- Ausich, W.I. and C.G. Messing. 1998. Crinoidea. Sea Lilies and Feather Stars, Version 21, The Tree of Life Web Project, <http://tolweb.org/>.
- Clark, A.H. 1907. New genera of recent free crinoids. Smithsonian Miscellaneous Collection 50(29):343-364.
- Clark, A.H. 1908. New genera of unstalked crinoids. Proceedings of the Biological Society of Washington 21:125-136.
- Clark, A.H. 1947. A monograph of the existing crinoids, Volume 1. The comatulids. Part 4b. - Superfamily Mariametrida (concluded the family Colobometridae) and Superfamily Tropiometrida (except the families Thalassometridae and Charitometridae). Bulletin of the United States National Museum 82:1-7.
- Collins, R.A., L.M. Boykin, R.H. Cruickshank and K.F. Armstrong. 2012. Barcoding's next top model: an evaluation of nucleotide substitution models for specimen identification. Methods in Ecology and Evolution 3(3):457-465.
- Folmer, O., M. Black, W. Hoeh, R. Lutz and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3:294-299.
- Gislén, T. 1922. The crinoids from Dr. S. Bock's expedition to Japan 1914. Nova acta Regiae Societatis Scientiarum Upsaliensis Ser. 4(6):1-179.
- Gislén, T. 1934. A reconstruction problem. Analysis of fossil crinoids from N. America with a survey of all known types of comatulid arm ramifications. Kungliga Fysiografiska Sällskapet, Handlingar, N.F. 45(11):1-59.
- Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41(41):95-98.
- Hara, J. 1895. Description of a new species of Comatula *Antedon macrodiscus*. Zoological Magazine, Tokyo, 7(81):115-116.
- Hara, J. and Y. Okada. 1921. Two new species of Myzostoma. Annotationes Zoologicae Japonenses 10(4):33-39.
- Hartlaub, C. 1890. Beitrag zur Kenntniß der Comatuliden-Fauna des Indischen Archipels (Vorläufige Mittheilung). Nachrichten von der Königl. Gesellschaft der Wissenschaften und der Georg-Augusts-Universität zu Göttingen 1890:68-188.
- Hebert, P.D., A. Cywinska, S.L. Ball and de J.R. Waard. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: Biological Sciences 270(1512):313-321.
- Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16(2):111-120.
- Kim, P. and S. Shin. 2020. Morphological description and molecular analysis of newly recorded *Anneissia pinguis* (Crinoidea: Comatulida: Comatulidae) from Korea. Journal of Species Research 9(4):467-472.
- Kumar, S., G. Stecher and K. Tamura. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7):1870-1874.
- Messing, C.G. 1997. Living Comatulids. Paleontological Society Papers 3:3-30.
- Nina, T.S. and B. Gunalan. 2014. First molecular report and phylogenetic analysis of Crinoidea from Rameswaram Island, South East Coast of India. Annals of Biological Research 5(8):1-7.
- Ratnasingham, S. and P.D.N. Hebert. 2007. BOLD: The Barcode of Life Data System (<http://www.barcodinglife.org>). Molecular Ecology Resources 7(3):355-364.
- Rouse, G.W., L.S. Jermiin, N.G. Wilson, I. Eeckhaut, D. Lantierbecq, T. Oji, C.M. Young, T. Browning, P. Cisternas, L.E. Helgen, M. Stuckey and C.G. Messing. 2013. Fixed, free, and fixed: the fickle phylogeny of extant Crinoidea (Echinodermata) and their Permian-Triassic origin. Mole-

- cular Phylogenetics and Evolution 66(1):161-181.
- Shin, S. 2001. Four species of the shallow-water Comatulids (Echinodermata, Crinoidea) from Geomundo Island; New records in Korea. *Animal Systematics, Evolution and Diversity* 17:251-262.
- Shin, S. 2002. Two new records of Colobometridae (Echinodermata, Crinoidea, Comatulida) in Korea. *The Korean Journal of Zoology* 18:157-163.
- Shin, S. 2004. A new record of Thalassometridae (Crinoidea, Comatulida, Oligophreata) in Korea. *The Korean Journal of Zoology* 20:185-189.
- Shin, S. 2005. A new record of antedonid crinoids (Comatulida, Macrophreata, Antedonidae) in Korea. *Animal Systematics, Evolution and Diversity* 21:67-72.
- Shin, S. 2013. Invertebrate fauna of Korea. Echinodermata: Crinozoa: Crinoidea: Comatulida, Asterozoa, Ophiuroidea: Euryalida Feather stars, Basket Stars. National Institute of Biological Resources, Ministry of Environment 32(5):1-60.
- Shin, S. and J.H. Won. 2002. Two newly recorded species of Colobometridae and Calometridae (Echinodermata, Crinoidea, Comatulida) in Korea. *Animal Systematics, Evolution and Diversity* 18:245-252.
- Summer, M.M., C.G. Messing and G.W. Rouse. 2014. Phylogeny of Comatulidae (Echinodermata: Crinoidea: Comatulida): A new classification and an assessment of morphological characters for crinoid taxonomy. *Molecular Phylogenetics and Evolution* 80:319-339.
- Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin and D.G. Higgins. 1997. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25(24): 4876-4882.
- Towle, A. 1989. *Modern Biology*. Austin, TX: Holt, Rinehart and Winston. ISBN 0-03-013919-8.
- Ward, R.D., B.H. Holmes and T.D. O'Hara. 2008. DNA barcoding discriminates echinoderm species. *Molecular Ecology Resources* 8(6): 1202-121.

Submitted: October 20, 2021

Revised: December 15, 2021

Accepted: December 15, 2021