

Morphological description and molecular analysis of newly recorded *Anneissia pinguis* (Crinoidea: Comatulida: Comatulidae) from Korea

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The crinoid specimens of the genus *Anneissia* were collected from Nokdong, Korea Strait, and Moseulpo, Jeju Island. The specimens were identified as *Anneissia pinguis* (A.H. Clark, 1909), which belongs to the family Comatulidae of the order Comatulida. *Anneissia pinguis* was first described by A.H. Clark in 1909 around southern Japan. This species can be distinguished from other *Anneissia* species by a longish and stout cirrus, much fewer arms, and short distal cirrus segments. The morphological features of Korean specimens are as follows: large disk (20–35 mm), 28–36 segments and 32–43 mm length cirrus, division series in all 4 (3 + 4), very stout and strong distal pinnule with 18–19 comb and 40 arms. In Korea fauna, only three species of genus *Anneissia* were recorded: *A. intermedia*, *A. japonica*, and *A. solaster*. In this study, we provide the morphological description and phylogenetic analysis based on cytochrome *c* oxidase subunit I.

Keywords: *Anneissia pinguis*, crinoid, cytochrome *c* oxidase subunit I, morphological feature, phylogeny

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INTRODUCTION

The family Comatulidae Fleming 1828 comprises 105 species, and is abundantly distributed in various habitats (Messing, 2001). This is the most common and diverse taxon of extant crinoids in the adjacent temperate waters (<100 m depth) of the tropical Indo-western Pacific region (Clark and Rowe, 1971; Messing, 1998; 2001; Kogo and Fujita, 2014). The genus *Anneissia* consists of nine species worldwide (Messing, 2020): *A. benhami*, *A. bennetti*, *A. grandicalyx*, *A. intermedius*, *A. japonica*, *A. muelleri*, *A. pinguis*, *A. plectophorum*, and *A. solaster*. Of these, *A. intermedia*, *A. japonica*, and *A. solaster* were reported in Korea (Won and Rho, 2001; Won and Shin, 2002; 2003; Shin, 2001; 2002; 2004; 2005). In Korea, the genus *Anneissia* is usually found from the East Sea to the Korea Strait, including near the waters of Jeju Island (Shin, 2013).

DNA barcoding, a useful molecular identification tool, has been applied to various taxonomical groups. The technique is based on special nucleotides, known as the barcode region (Hebert *et al.*, 2003). The mitochondri-

al cytochrome *c* oxidase subunit I (COI) is usually used in DNA barcoding studies of echinoderms (Ward *et al.*, 2008; Hoareau and Boissin, 2010). In particular, according to previous molecular studies of crinoids, a partial COI sequence was appropriate for the DNA barcode region; the interspecific variation was generally greater than 6% and the intraspecific variation was often less than 3% (Nina Tabitha and Gunalan, 2014; Summers *et al.*, 2017).

At present, only three *Anneissia* species have been recorded in Korea. We report the morphological characteristics and molecular phylogenetic tree of *Anneissia pinguis* (A.H. Clark, 1909) based on 596 bp of the sequence COI from 13 crinoids.

MATERIALS AND METHODS

The seven specimens in this study were collected, immediately fixed in 95% ethanol, and stored in the marine echinoderms resources storage, Sahmyook University, Seoul, Korea. The species identification and morphological description of the specimens followed the description,

illustrations, and taxonomic keys provided by Clark (1909; 1915; 1931), Gislén (1922), and Summer *et al.* (2014). A quarter of the total cirrus was detached for morphological observation of axillaries, and a digital camera (Nikon D7000, Tamron 60 mm macro lens) was used to take photographs.

Partial COI sequences were amplified using the primers LCOcrinoid (5'-TGRTTTTTTCTACDAATCATAAGG-3') and HCOcrinoid (5'-TAAGAATATAAACYT-CAGGATGACC-3'). These primers were modified from the universal COI primer set (LCO1490 and HCO2198) (Folmer *et al.*, 1994). PCR reactions were performed using the AccuPower PCR PreMix kit (Bioneer, Daejeon, Korea), in a total volume of 20 μ L, in accordance with the manufacturer's instructions. First, 1 μ L of template DNA, 0.5 μ L of each primer at 10 pmol, and 18 μ L of distilled water were added to AccuPower PCR PreMix. PCR amplification was conducted with an initial denaturation at 94°C for 3 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 45 s; and a final extension at 72°C for 5 min. The PCR products were analyzed by electrophoresis on 1.5% agarose gel stained with EcoDye™ Nucleic Acid Staining Solution (Biofact, Daejeon, Korea) in 1 \times TBE buffer (Biosesang, Seongnam, Korea); 2 μ L of a 100 bp DNA ladder (Elpis Biotech, Daejeon, Korea) was used for size confirmation. The PCR products were sequenced in both directions (Genotech, Daejeon, Korea) and then aligned using Clustal X (Thompson *et al.*, 1997).

Molecular data from 38 COI sequences from 12 crinoids under the tribe Comasterini A. H. Clark, 1908 were used (Summer *et al.*, 2014). The stalked crinoid, *Holopus alidis* (Genbank Accession no. KC626556), was used as the outgroup (Hemery *et al.*, 2013). Neighbor-joining (NJ) analysis was conducted using MEGA 7.0 in accordance with the Kimura 2-parameter (K2P) model with 1000 bootstrap replicates (Kimura, 1980; Kumar *et al.*, 2016).

SYSTEMATIC ACCOUNTS

Class Crinoidea Miller, 1821
 Subclass Articulata von Zittel, 1879
 Order Comatulida AH Clark, 1908
 Family Comatulidae Fleming, 1828
 Genus *Anneissia* Summers, Messing & Rouse, 2014

Anneissia pinguis (AH Clark, 1909)

Comanthus pinguis AH Clark, 1909: p. 147; Gislén, 1922: 46; Clark, 1931: 553.

Cenolia (Cenolia) pinguis AH Clark, 1916: 115–195.

Oxycomanthus pinguis Rowe *et al.*, 1986: 248, 270; Kogo, 1998: 42–45.

Anneissia pinguis Summer *et al.*, 2014: 337.

Materials examined. Holotype: Korea, one specimen, Korea, Korea Strait, northeastern Jeju Island, at a depth of 91 m, by trawling, 31 Oct 2017 (GenBank no. MT086598). Paratypes: one specimen, Korea, Jeollanam-do, Goheung-gun, Nokdong Harbor, 11 Sep 2009, by fish trapping; one specimen, Korea, Jeju Island, Moseulpo Harbor, at a depth of 150 m, 2 Oct 2013, by fish trapping; four specimens, Korea, Korea Strait, northeastern Jeju Island, at a depth of 89 m, by trawling, 31 Oct 2017. All of the listed specimens were deposited at Sahmyook University.

Description. Centrodorsal large and thick hemispherical shape. Diameter of centrodorsal 5.0–10.0 mm and diameter of dorsal pole 4.0–7.0 mm (Height: 2.0–4.0 mm). Polar area concave and similar to *A. japonica* and *A. bennetti*. Cirrus socket numerous and arranged in alternating and massed three rows. Cirri long and stout, 28–36 in number, composed of 32–36 segments and 32–43 mm in length. Cirrus segments gradually decrease in length. First cirrus segment shorter than second segment. After the second segment, increase length in gradually until usually the tenth segment. Dorsal surface of segment well developed as carination and opposing spine represented. Terminal claw slightly curved and sharp with weak and small opposing spine, but not always present. Division series flattened and combined with a large centrodorsal. Axillaries, height-low pentagonal shape, almost triangle. Division series broad, smooth, no carination and shown in 4 (3+4) always. IIBr and IIIBr series usually regular presented and always presented in 4 (3+4). IVBr series rarely in body. Arms slightly fragile, 37–40 in number, 100–150 mm in length. Comb structure in pinnules, consisted of well-separated teeth on some terminal segments. P_D (distal pinnule) very stout, strong, 48–57 segments, 18.0–32.0 mm (18–19 comb). P_P (proximal pinnule) less stout basally than P_D, 42–45 segments, 15.0–25.0 mm (16 comb). P₁, 45 segments, 10.0–18.0 mm (15 comb). P₂, short, slender, 4.0–10.0 mm (6–8 comb). P₃, 3.0–8.0 mm (6 comb). P₄, rudimental comb. P₅, no terminal comb. Large Disk, 20.0 to 35.0 mm in diameter, long and centrally placed anal papilla (Height: 6.0–7.0 mm), and marginal mouth.

Size. Centrodorsal discoidal with slightly concave polar area, diameter 5.0–10.0 mm, height 2.0–4.0 mm. Cirrus length 32.0–43.0 mm. Arm length 100–150 mm.

Color. Various body colors were observed in 95% ethanol, such as bright blue, white, yellow, and dark brown, often with a central line of white on the division series and arms. Body colors were not the same as the pinnules or cirri. Disk color was same as the body color; the centrodorsal and cirrus were light brown and ivory. Most of the specimens have rays on every arm from the centrodorsal to the arm tip, and the rays were colored ivory, white, or the body color. Pinnule color is usually similar to body color, but some specimens had a greenish pinnule



Fig. 1. *Anneissia pinguis* (AH Clark, 1909). (A) oral view; (B) aboral view; (C) centrodorsal; (D) oral surface and distal pinnules; (E) aboral side; (F) cirrus. AP, anal papilla; C, centrodorsal; CS, cirrus socket; CR, cirrus; M, mouth; PC, pinnule comb; Pp, distal pinnule. Scale bars = 10 mm.

tip. When alive, the body colors varied from yellow and orange to dark brown and blue with white and ivory ray on arms (Fig. 1).

Distribution. Korea (Korea Strait), Japan (Goto Islands, Kagoshima Gulf, Sagami Bay, Tokyo Bay), Hong Kong.

Remark. The genus *Oxycomanthus* Hoggett, Birtles &

Vail, 1986 consisted of 13 species. However, among them, four species were accepted as the genus *Clarkcomanthus* Rowe, Hoggett, Birtles & Vail, 1986 and nine species was newly classified as the genus *Anneissia* by Summer *et al.* (2014). These *Anneissia* species are distributed in the Indo-western Pacific Ocean, from Japan, China, Australia,

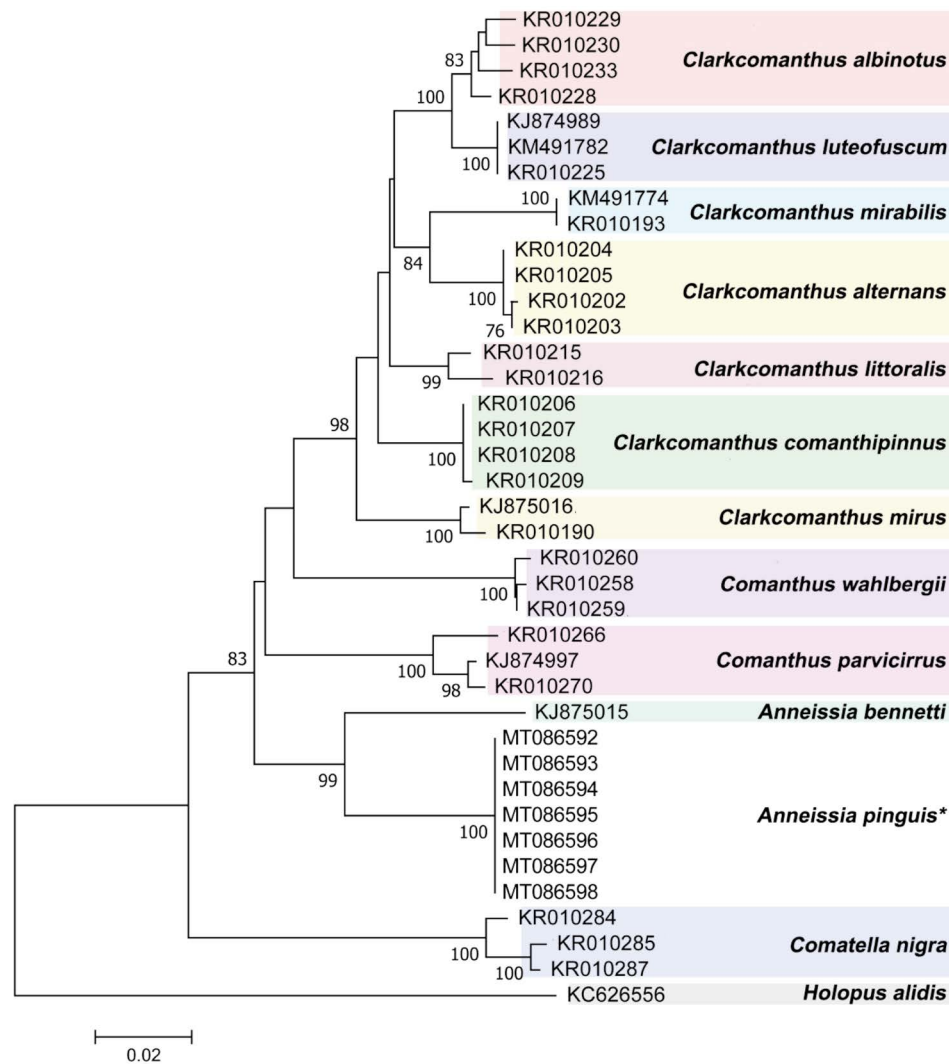


Fig. 2. Neighbor-joining tree of the aligned 596 bp COI sequence for 12 species of tribe Comasterini based on the Kimura 2-parameter model. The GenBank accession numbers used are indicate on each node. Bootstrap values were supported at ≥ 70 .

New Caledonia, southern New Zealand. The usual habitat depth is very variable, from the shoreline to 330 m. *Anneissia pinguis* was previously reported as a common species in previous localities in the eastern and southern waters of Japan (Clark, 1931). Our specimens were collected in Korea, and our report indicates new information on the distribution of this species. There were no remarkable differences in morphological characteristics compared with the original description (Clark, 1909) and other diagnosis (Clark, 1931), except for coloration. In previous descriptions, the usual color varied from white to dark brown. Most of our specimens were same, however the specimen collected from Jeju Island was blue with white rays, and the specimen that was completely yellow when both alive and preserved in alcohol.

In the phylogenetic analysis, pairwise distances ranged

from 0.0% to 14.7% within the 12 species of Comasterini, and the intraspecific distances was 0.0–1.7%. The average of interspecific distances between *Anneissia* species, *A. bennetti* and *A. pinguis*, was 6.9%, and the intraspecific genetic distances in the COI sequences of *A. pinguis* specimens collected was 0.0% (Table 1). The specimens of *A. pinguis* formed a monophyletic clade at the species level, and were clearly distinguished from their sister groups (Fig. 2). We deposited 658 bp of the COI sequences obtained in this study in GenBank (MT086592–MT086598). These seven sequences of *A. pinguis* were the first to be deposited in GenBank. We have provided morphological descriptions and molecular data, and these results were used to determine the molecular phylogenetic relationship between Korean crinoid fauna.

Table 1. Average of pairwise distances by each species based on 596 bp of mitochondrial COI according to phylogenetic calculations performed using Kimura 2-parameter (K2P) model with 1,000 bootstrap replicates.

Species	GenBank Accession no.	Average of pairwise distance															
		1	2	3	4	5	6	7	8	9	10	11	12	13			
1 <i>Anneissia pinguis</i>	MT086592-MT086598	0															
2 <i>Anneissia bennetti</i>	KJ875015	0.069	-														
3 <i>Clarkcomanthus albinotus</i>	KR010228-KR010230, KR010233	0.105	0.111	0.013													
4 <i>Clarkcomanthus alternans</i>	KR010202-KR010205	0.099	0.103	0.049	0.002												
5 <i>Clarkcomanthus comanthipinnus</i>	KR010206-KR010209	0.093	0.109	0.044	0.049	0.001											
6 <i>Clarkcomanthus littoralis</i>	KR010215, KR010216	0.086	0.096	0.045	0.043	0.039	0.014										
7 <i>Clarkcomanthus luteofuscum</i>	KJ874989, KM49178, KR010225	0.098	0.104	0.021	0.043	0.042	0.040	0									
8 <i>Clarkcomanthus mirabilis</i>	KM491774, KR010193	0.112	0.116	0.056	0.043	0.064	0.054	0.058	0								
9 <i>Clarkcomanthus mirus</i>	KJ875016, KR010190	0.099	0.100	0.054	0.056	0.047	0.049	0.060	0.061	0.007							
10 <i>Comanthus parvicirrus</i>	KR010266, KJ874997, KR010270	0.100	0.098	0.098	0.087	0.090	0.091	0.098	0.090	0.088	0.017						
11 <i>Comanthus walthergii</i>	KR010260, KR010258, KR010259	0.111	0.114	0.089	0.090	0.082	0.095	0.093	0.090	0.085	0.103	0.003					
12 <i>Comatella nigra</i>	KR010284, KR010285, KR010287	0.138	0.140	0.131	0.140	0.132	0.136	0.132	0.124	0.135	0.136	0.147	0.012				
13 <i>Holopus alidis</i>	KC626556	0.210	0.217	0.213	0.216	0.198	0.213	0.217	0.222	0.212	0.211	0.197	0.219	-			

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REFERENCES

- Clark, A.H. 1909. On a collection of crinoids from the Zoological Museum of Copenhagen. Vidensk. Medd, Dansk Naturhist Foren 61:115-195.
- Clark, A.M. and F.W.E. Rowe. 1971. Monograph of Shallow-Water Indo-West Pacific Echinoderms. British Museum (Natural History), London. pp. 238.
- Clark, A.H. 1915. A monograph of the existing crinoids, vol. 1. The comatulids, pt. 1: [General]. Bulletin of the United States National Museum. 231pp.
- Clark, A.H. 1931. A monograph of the existing crinoids, Volume 1. The comatulids. Part 3. Superfamily Comasterida. pp. 553-564.
- Clark, A.M. 1982. Echinoderms of Hong Kong. In: B. Morton (ed.), Proceedings of the first international marine biological workshop: The marine flora and fauna of Hong Kong and southern China. Hong Kong University Press, Hong Kong 1:485-501.
- 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. Mol Mar Biol Biotech 3:294-299.
- Gislén, T. 1922. The crinoids from Dr. S. Bock's expedition to Japan 1914. Nova Acta Regiae Soc Sci Upsal Ser 4:1-179.
- Hebert, P.D., A. Cywinska, S.L. Ball and J.R. de Waard. 2003. Biological identifications through DNA barcodes. Proc R Soc London B 270:313-321.
- Hemery, L.G., M. Roux, N. Ameziane and M. Eleaume. 2013. High-resolution crinoid phyyletic inter-relationships derived from molecular data. Cah Biol Mar 54(4):511-523.
- Hoareau, T.B. and E. Boissin. 2010. Design of phylum-specific hybrid primers for DNA barcoding: addressing the need for efficient COI amplification in the Echinodermata. Mol Ecol Resour 10 (6):960e967.
- Hoggett, A.K. and F.W.E. Rowe. 1986. A reappraisal of the family Comasteridae A. H. Clark, 1908 (Echinodermata: Crinoidea), with the description of a new subfamily and a new genus. Zool J Linnean Soc 88:103-142.
- Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111-120.
- Kogo, I. and T. Fujita. 2014. The Feather Stars of Sagami Bay. Tokai University Press, Hadano, 162 pp. [In Japanese with

English abstract].

- Kumar, S., G. Stecher and K. Tamura. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870-1874.
- Messing, C.G. 1998. An initial re-assessment of the distribution and diversity of the East Indian shallow-water crinoid fauna. pp. 187-192. In: R. Mooi and M. Telford (eds.), *Echinoderms: San Francisco*. Balkema, Rotterdam.
- Messing, C.G. 2001. A key to genera of Comasteridae (Echinodermata: Crinoidea) with the description of a new genus. *Bull Biol Soc Washington* 10:277-300.
- Messing, C.G. 2020. World List of Crinoidea. *Anneissia pinguis* (AH Clark, 1909). Accessed through: World Register of Marine Species at: <http://www.marinespecies.org/aphia.php?p=taxdetails&id=829167> on 2020-08-07
- Nina Tabitha, S. and B. Gunalan. 2014. First molecular report and phylogenetic analysis of Crinoidea from Rameswaram Island, south east coast of India. *Ann Biol Res* 5:1-7.
- Shin, S. and J.H. Won. 2002. Two newly recorded species of Colobometridae and Calometridae (Echinodermata, Crinoidea, Comatulida) in Korea. *Anim Syst Evol Diversity* 18:245-252.
- Shin, S. 2001. Four species of the shallow-water Comatulids (Echinodermata, Crinoidea) from Geomundo Island; New records in Korea. *Anim Syst Evol Diversity* 17:251-262.
- Shin, S. 2002. Two new records of Colobometridae (Echinodermata, Crinoidea, Comatulida) in Korea. *Korean J Syst Zool* 18:157-163.
- Shin, S. 2004. A new record of Thalassometridae (Crinoidea, Comatulida, Oligophreata) in Korea. *Korean J Syst Zool* 20:185-189.
- Shin, S. 2005. A new record of antedonid crinoids (Comatulida, Macrophreata, Antedonidae) in Korea. *Anim Syst Evol 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.
- Summers, 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. *Mol Phylogenet Evol* 80:319-339.
- Summers, M.M., C.G. Messing and G.W. Rouse. 2017. The genera and species of Comatulidae (Comatulida: Crinoidea): taxonomic revisions and a molecular and morphological guide. *Zootaxa* 4268:151-190.
- 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 Res* 25:4876-4882.
- Ward, R.D., B.H. Holmes and T.D. O'Hara. 2008. DNA barcoding discriminates echinoderm species. *Mol Ecol Resour* 8:1202-1211.
- Won, J.H. and B.J. Rho. 2001. Taxonomy of the family Antedonidae (Echinodermata, Crinoidea, Comatulida) in Korea. *Korean J Biol Sci* 5:299-302.
- Won, J.H. and S. Shin. 2003. Two new records of Zygometridae and Colobometridae (Echinodermata: Crinoidea: Comatulida) in Korea. *Anim Syst Evol Diversity* 19:125-131.

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