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A New Record of Intertidal Sea Anemone, *Anthopleura asiatica* (Anthozoa: Hexacorallia: Actiniaria: Actiniidae), from Korea

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ABSTRACT

The genus *Anthopleura*, one of the most species-rich taxon belonging to family Actiniidae, primarily is abundant in the intertidal zone and shallow subtidal waters. In 2022, a previously unrecorded species of *Anthopleura* was discovered during a survey of the intertidal regions of Ulleungdo. Specimens were later collected from Chujado in 2023 and Udo in 2024 on Jeju Island. This unrecorded species has been identified as *Anthopleura asiatica* through an examination of its external anatomy and cnidae, as well as comparisons of mitochondrial 16S rRNA and COIII sequences with those of related species. This study reports that five species of the genus *Anthopleura* have been documented in Korean waters to date.

Keywords: temperate sea anemone, *Anthopleura asiatica*, taxonomy, 16S rRNA, COIII

INTRODUCTION

The genus *Anthopleura* Duchassaing de Fonbressin and Michelotti, 1860, belonging to the family Actiniidae, was established with the type species *Anthopleura krebsi* Duchassaing and Michelotti, 1860, collected from the Antilles, an archipelago located in the West Indies and surrounded by the North Atlantic Ocean and the Caribbean Sea (Duchassaing and Michelotti, 1860). Actiniidae is the largest family of Actiniaria, containing 57 genera (Rodríguez et al., 2024), with the genus *Anthopleura* being one of the most species-rich. There are 49 species in this genus, but only four have been reported in Korea: *A. fuscoviridis* Carlgren, 1949; *A. japonica* Verrill, 1899; *A. kurogane* Uchida and Muramatsu, 1958; and *A. pacifica* Uchida, 1938 (see Song, 1984; Rodríguez et al., 2024).

Sea anemones in the genus *Anthopleura*, similar to other Actiniidae species, primarily attach to rocks in the intertidal zone and shallow subtidal waters (Song, 1992; Ramírez-Orellana et al., 2024; Vassallo-Avalos et al., 2024). Most *Anthopleura* species are found in temperate seas, although a few have been reported in tropical regions (Daly and Den Hartog, 2004; Fautin, 2005; Spano and Häussermann, 2017). These anemones can be identified by their verrucae and acrorhagi, which are projections located between the oral disc and the column (Daly, 2003).

During the 2022 survey of the intertidal regions of Ulleungdo, researchers identified an unrecorded species of *Anthopleura*. This same species was later discovered in Jeju Island's Chujado and Udo in 2023 and 2024, respectively. Through morphological analysis and comparisons of nucleotide sequences with related species, the unrecorded species was identified and described as *Anthopleura asiatica* Uchida and Muramatsu, 1958.

MATERIALS AND METHODS

Specimens were collected from the intertidal regions of Ulleungdo (in 2022) in Gyeongsangbuk-do, as well as from Chujado (in 2023) and Udo (in 2024) in Jeju Island. Before fixation, live sea anemones found on rocky shores were photographed with a digital camera (Tough TG-5; Olympus Digital Solution Corporation, Tokyo, Japan) to document their morphological and ecological characteristics. After collection, the specimens were placed in a plastic container with seawater and subjected to 4 hours of anesthesia using powdered

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menthol. Anesthetized specimens were preserved in 5% neutral buffered formalin (v/v) for morphological examinations and in 99% alcohol (v/v) for molecular analysis.

For identification, cnidae types from the tissues of each part of the sea anemone were examined using a light microscope (Axio Lab.A1; Carl Zeiss, Jena, Germany). The detailed external morphological features of the sea anemone were observed with a stereomicroscope (SteREO Discovery V8; Carl Zeiss). Digital images were captured during the examination using cameras equipped with CMOS sensors (KCS-2000SS; Korea Lab Tech, Seongnam, Korea). An image analyzer (OptiView; Korea Lab Tech) was employed for all measurements.

Additionally, molecular identification was performed to assess the genetic distances between related *Anthopleura* species, particularly *A. buddemeieri* Fautin, 2005. Total DNA was extracted from ethanol-preserved specimens using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions.

Primer sets (ANEM16SA and ANEM16SB for 16S rRNA; COIIIF and COIIIR for COIII), previously used in molecular phylogenetic analyses of actiniarians, were employed to amplify partial 16S rRNA and COIII sequences (Geller and Walton, 2001; Glon et al., 2021). Amplification was conducted using the TaKaRa Ex Taq (Takara Bio Inc., Kusatsu, Japan) and a MiniAmp thermal cycler (Thermo Fisher Scientific Inc., Waltham, MA, USA) in 50 μL reactions. The PCR conditions were set as follows: 10 min at 94°C, followed by 35 cycles of 94°C for 30 s, annealing at 47°C for 40 s, and extension at 72°C, culminating in a final extension step at 72°C for 10 min.

Genetic distance analysis was conducted using the Kimura 2-parameter model, and neighbor-joining (NJ) trees were constructed with the Molecular Evolutionary Genetics Analysis (MEGA) software version 11 (Tamura et al., 2021). A total of 15 16S rRNA sequences were analyzed from *Anthopleura* (8 species) and *Epiactis prolifera* Verrill, 1869 (outgroup), which included four sequences from the present study. Additionally, 17 COIII sequences from *Anthopleura* (7 species) and *E. prolifera* (outgroup) were analyzed, incorporating six sequences from the present study. The remaining sequences were sourced from GenBank, and the NJ trees included the accession numbers for these sequences.

Six specimens (MABIK CN00081552–CN00081557) were deposited at the National Marine Biodiversity Institute of Korea in Seocheon, while the remaining specimens were stored separately in the Cnidaria Bioresources Bank of Korea and the Marine Biodiversity and Conservation Laboratory at Woosuk University in Jincheon.

SYSTEMATIC ACCOUNTS

Phylum Cnidaria Hatschek, 1888
Class Hexacorallia Haeckel, 1896
Order Actiniaria Hertwig, 1882
Family Actiniidae Rafinesque, 1815
Genus *Anthopleura* Duchassaing de Fonbressin and Michelotti, 1860

Key to species of the genus *Anthopleura* from Korea

1. Solitary ········· 2
– Clonal ········· 4
2. Verrucae compound; no black granules in column and tentacles ········· 3
– Verrucae simple; small black granules in column and tentacles ········· *Anthopleura kurogane*
3. Column with green warts ········· *A. fuscoviridis*
– Column with greyish brown warts ········· *A. japonica*
4. Column and tentacles reddish brown ········· *A. pacifica*
– Column brown to olive green with red spots in longitudinal rows ········· ***A. asiatica***

[1]****Anthopleura asiatica*** **Uchida and Muramatsu, 1958 (Tables 1–3, Figs. 1–4)**

Anthopleura asiatica Uchida and Muramatsu, 1958: 117–119, figs. 4, 5; Fautin, 2005: 379, 387–389.

Material examined. Korea: 2 individuals, Gyeongsangbuk-do: Ulleung-gun, Buk-myeon, Hyeonpo 2-gil (37°31′20″N, 130°48′49″E), 20 Apr 2022, Sim SH, intertidal zone; 3 individuals, Ulleung-gun, Seo-myeon, Seodal-gil (37°30′51″N, 130°47′38″E), 24 May 2023, Hwang SJ, Lee SJ, intertidal zone; 5 individuals, Jeju-do: Jeju-si, Chuja-myeon, Daeseo 5-gil, Hupo beach (33°57′58″N, 126°17′17″E), 30 Nov 2023, Lee CJ, intertidal zone; 6 individuals, Jeju-si, Udo-myeon, Udohaean-gil (33°31′8″N, 126°56′56″E), 28 May 2024, Lee SJ, Yu HJ, Seo SY, intertidal zone.

Description. Column cylindrical and low in height; smooth; brown to olive green color when alive; red spots in longitudinal rows along column wall; larger individuals up to 14 mm high and 15 mm wide (Fig. 1E). Oral disc usually brown, sometimes with radiating white lines from mouth toward tentacles, centered mouth sometimes slightly elevated, smaller than pedal disc, 4–13 mm wide (Fig. 1C). Tentacles somewhat thick at base and taper to the tip, brown and sometimes with white horizontal pattern; larger individual with up to 104 in number arranged in over five cycles; inner (4–8 mm long) longer than outer (2–7 mm) (Fig. 1C). Acrorhagi at margin between oral disc and column, with color

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Korean name: [1]*빨강세로점꽃해변말미잘 (신칭)

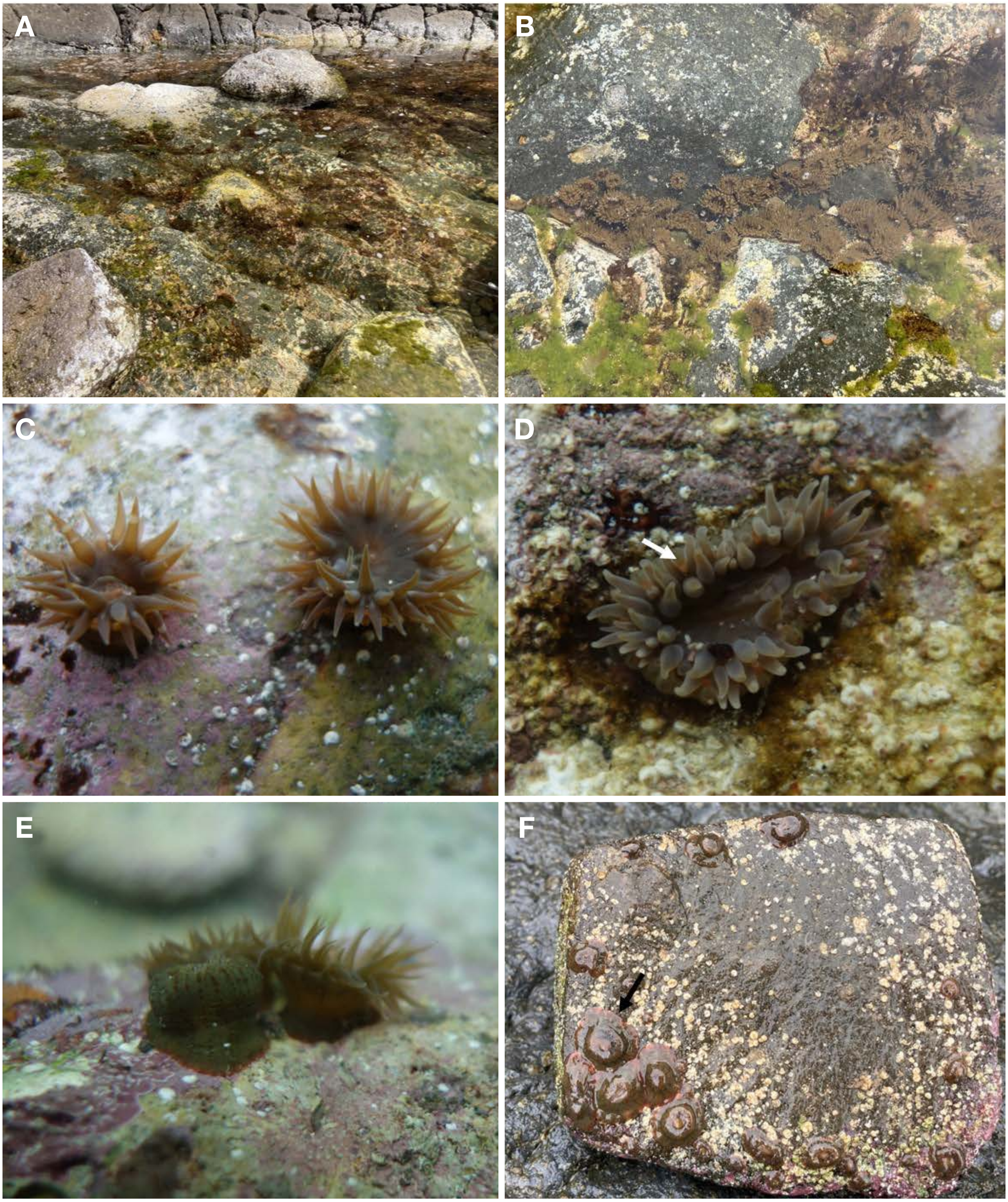

Fig. 1. The habitat and living *Anthopleura asiatica*. A, Rocky Intertidal habitat with waves at Ulleungdo; B, Clonal individuals in group; C, Fully expanded two sea anemones; D, A rather shrunken sea anemone showing light orange acrorhagi (arrow) well; E, Red spots in longitudinal rows along column wall; F, Completely shrunk sea anemones showing pink boundaries of pedal discs (arrow).

variation beige, pink, or slightly light orange (Fig. 1D). Pedal disc, especially contracted, prominent larger than column, 7-15 mm wide; marginal boundary pink (Fig. 1F).

Cnidom: spirocysts, basitrichs, holotrichs, microbasic b-mastigophores, microbasic p-mastigophores (Fig. 2). Distribution and detailed measurements of cnidae in Table 1.

Table 1. Comparison of size of cnidae of *Anthopleura asiatica* and *A. buddemeieri*, including original descriptions and this study, size in μm

Tissue and cnidae	*Anthopleura asiatica* Uchida and Muramatsu, 1958 (original description)	*Anthopleura buddemeieri* Fautin, 2005 (original description)	This study (mean, number of cnidae)
Tentacles			
Spirocysts	14.4–30.0×1.7–3.3	15.9–30.1×1.6–3.2	15.6–33.1×2.1–3.7 (26.1×3.0, 60)
Basitrichs	17.8–25.6×2.2–3.3	17.4–25.2×1.8–3.5	22.0–26.7×2.2–3.5 (23.8×2.7, 52)
Acrorhagi			
Spirocysts	17.8–31.1×1.7–3.3	17.2–33.5×1.9–3.5	27.4–37.6×2.6–3.3 (31.3×3.0, 16)
Basitrichs	13.3–15.6×1.7–2.2	11.1–19.5×1.8–3.3	16.3–20.5×2.1–3.2 (17.8×2.5, 33)
Holotrichs	43.3–54.4×3.3–5.6	33.5–58.5×2.3–5.7	39.4–56.5×3.5–5.6 (47.0×4.6, 32)
Holotrichs	45.6–50.0×4.4–6.7		
Column			
Basitrichs	8.9–12.2×2.2–2.8	10.2–17.2×1.1–3.2	10.7–10.9×1.6–2.0 (10.8×1.8, 2) 15.3–21.5×2.0–2.9 (18.2×2.5, 55)
Basitrichs	13.3–17.8×1.1–2.2		
Basitrichs	20.0–24.4×1.7–2.2		
Holotrichs	8.2–12.0×1.7–2.3	Not observed	Not observed
Actinopharynx			
Spirocysts	Not observed	Not observed	18.0–31.4×1.9–3.4 (26.9×2.9, 8)
Basitrichs	24.4–28.9×1.7–2.3	21.9–32.0×1.9–3.9	21.5–32.4×2.2–3.9 (27.4×3.2, 17)
Microbasic p-mastigophores	21.1–24.4×4.4–5.6	20.5–28.2×3.2–4.3	21.0–27.1×4.8–6.2 (25.0×5.2, 12)
Mesenterial filaments			
Spirocysts	Not observed	Not observed	21.5–32.8×2.6–3.6 (27.2×3.0, 15)
Basitrichs	10.0–13.3×1.7–2.2	10.4–16.1×1.3–3.0	10.4–14.4×2.0–2.8 (12.9×2.4, 37)
Basitrichs	15.6–18.8×1.7–2.2		14.1–18.7×2.8–3.1 (16.5×2.9, 4)
Basitrichs	15.6–23.2×3.5–5.7		21.6–24.5×2.0–2.9 (22.7×2.5, 16)
Microbasic b-mastigophores	Not observed	24.6–34.4×3.2–5.5	25.2–28.5×3.7–3.8 (26.8×3.8, 2)
Microbasic p-mastigophores	15.3–20.2×1.8–2.8	13.3–25.9×2.3–5.7	19.6–29.1×4.1–5.7 (24.8×4.8, 43)
Microbasic p-mastigophores	17.8–25.0×3.3–5.6		

Ecology and habitat. This species, which attaches to rocks via pedal disc muscles, inhabits in groups in the intertidal zone with coastal waves (Fig. 1A, B). It has zooxanthellae, endosymbionts, in tentacles, actinopharynx and mesenterial filaments, 6.4–10.4 μm (mean 8.3 μm).

Distribution. Pacific Ocean: Korea (Ulleungdo, Chujado, Udo), Japan (middle part of Honshu to Kyushu).

Remarks. *Anthopleura asiatica* was first described by Uchida and Muramatsu in 1958 in Japan. Its distribution ranges from the middle part of Honshu to Kyushu, including areas such as Seto Inland and Sagami Bay (Fujii, 1987; Discover Blue, 2015). In 2005, Fautin reported a new species from New Guinea and Fiji, named *Anthopleura buddemeieri*, which exhibits morphological similarities to *A. asiatica*. Fautin categorized *A. asiatica* as a nomen dubium due to the lack of type specimens, insufficient available information, and the fact that certain materials reported as *A. asiatica* in Japan closely resembled *A. buddemeieri*. Regarding external characteristics, our specimen aligns with the original description of *A. asiatica*. However, there are notable differences in the types of cnidae present in our materials: (1) the absence of holotrichs in the column, (2) the presence of spirocysts in the actinopharynx and mesenterial filaments, and (3) the observation of microbasic b-mastigophores in the mesenterial filaments, although these are quite rare.

Our specimens share similarities in that both *A. buddemeieri* and acrorhagi possess holotrichs of a uniform size, while the columns lack holotrichs. However, our specimen exhibits notable differences: (1) it contains large basitrichs in the mesenterial filaments, (2) microbasic b-mastigophores are quite rare in the mesenterial filaments, and (3) zooxanthellae are present. To accurately identify and classify the Korean specimens and clarify the distinctions between *A. asiatica* and *A. buddemeieri*, we conducted DNA barcoding using 16S RNA and COIII sequences information.

DNA barcoding. Analyses of inter- and intra-species genetic distances were conducted based on the partial sequences of 16S RNA and COIII from *Anthopleura* species and one outgroup (*Epiactis prolifera*). Tables 2 and 3 present the detailed genetic distances within and between the *Anthopleura* species. Intraspecific genetic distances were very low, ranging from 0.0% to 0.3% for both 16S RNA and COIII, excluding the species for which only one sequence was available in GenBank. In contrast, the interspecific genetic

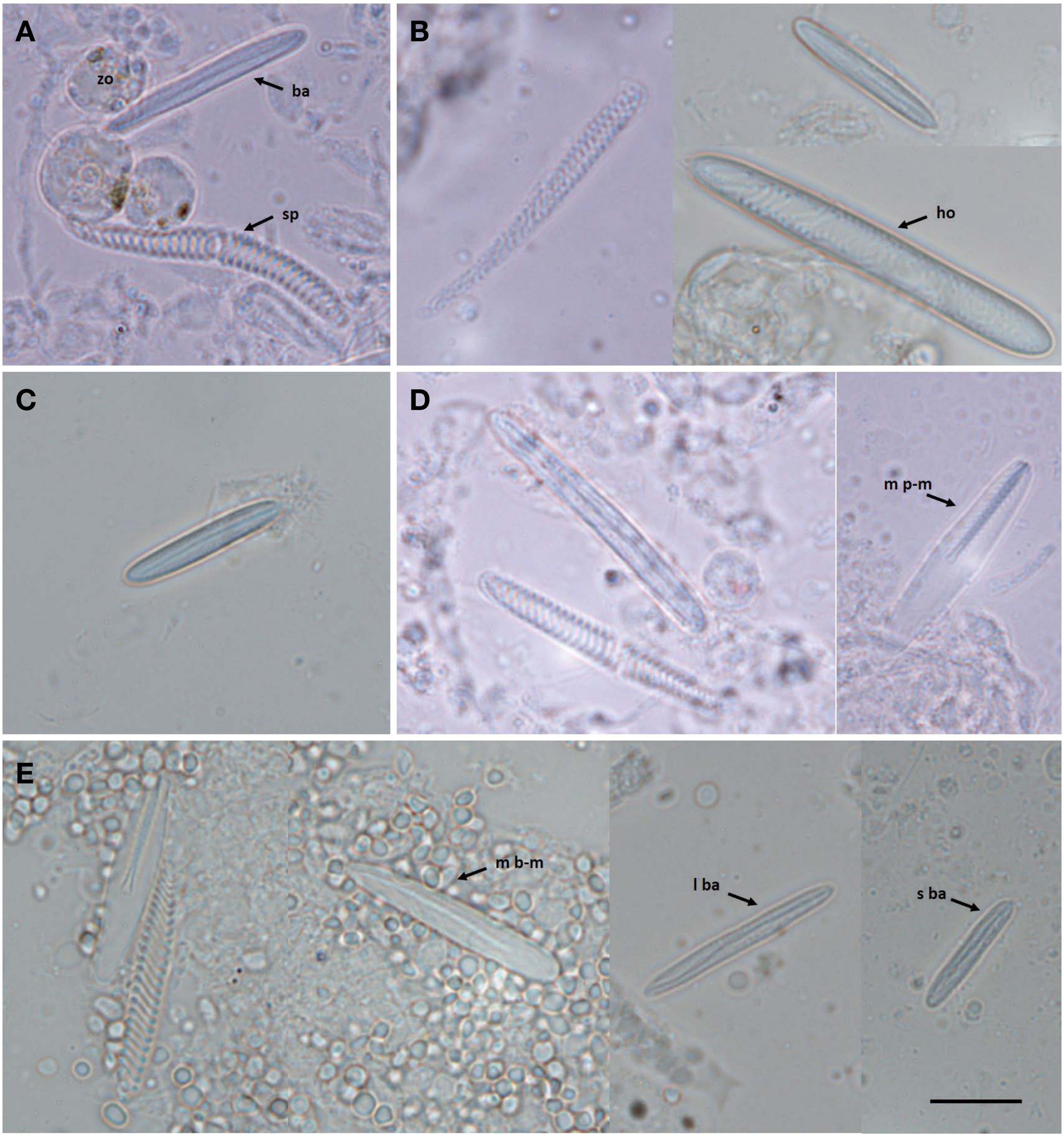

Fig. 2. Cnidae of *Anthopleura asiatica*. A, Tentacles; B, Acrorhagi; C, Column; D, Actinopharynx; E, Mesenterial filaments. ba, basitrich; ho, holotrich; m b-m, microbasic b-mastigophore; l ba, large basitrich; m p-m, microbasic p-mastigophore; s ba, small basitrich; sp, spirpcyst; zo, zooxanthella. Scale bar: A–E=10 μm.

distances among species were relatively high, ranging from 0.3% to 1.8% for 16S RNA and ranging from 0.7% to 4.2% for COIII. Furthermore, the *Anthopleura* species exhibited genetic variations of 3.4–4.0% and 4.5–5.8% in 16S RNA and COIII, respectively, when compared to the outgroup *E. prolifera*. This confirms the previously established understanding in other cnidarians that these two genes show DNA barcoding resolution (Moura et al., 2011; Zheng et al., 2014; Maronna et al., 2016).

The NJ trees show a clear distinction between *A. asiat-*

Table 2. Comparison of interspecific and intraspecific 16S rRNA gene genetic distances of *Anthopleura asiatica* and related species

Species	Accession No. (GenBank)	Within each species (mean±SD)	Between species of *Anthopleura* (Min.–Max.)
Anthopleura asiatica	AF375809.1, **PQ490417,** **PQ490424** **PQ490425,** **PQ519007**	0.003±0.002	0.009–0.017
Anthopleura buddemeieri	OQ357356.1, MW158858.1	0.000±0.000	0.000–0.018
Anthopleura carneola	AF357806.1	n/c	0.010–0.018
Anthopleura dowii	AF375811.1	n/c	0.005–0.013
Anthopleura handi	AF375819.1, OQ357353.1	0.000±0.000	0.000–0.018
Anthopleura anjunae	AF375813.1	n/c	0.003–0.013
Anthopleura sola	AF375818.1	n/c	0.003–0.013
Anthopleura xanthogrammica	AF375820.1	n/c	0.003–0.012
Epiactis prolifera	AF375807.1	n/c	0.034–0.040

Accession numbers of newly sequenced in this study are in bold.
SD, standard deviation; Min., minimum; Max., maximum; n/c, no calculated.

Table 3. Comparison of interspecific and intraspecific COIII gene genetic distances of *Anthopleura asiatica* and related species

Species	Accession No. (GenBank)	Within each species (mean±SD)	Between species of *Anthopleura* (Min.–Max.)
Anthopleura anjunae	KY789289.1	n/c	0.014–0.036
Anthopleura anneae	KY789293.1	n/c	0.015–0.030
Anthopleura asiatica	AF375794.1, **PQ671483,** **PQ671484,** **PQ671485,** **PQ671486,** **PQ671487,** **PQ671488**	0.000±0.000	0.017–0.034
Anthopleura ballii	KY789281.1	n/c	0.014–0.036
Anthopleura buddemeieri	OQ363522.1, MW158888.1, MW158887.1	0.000±0.000	0.007–0.042
Anthopleura dowii	KY789286.1	n/c	0.028–0.042
Anthopleura handi	OQ363536.1, OQ363547.1	0.000±0.000	0.007–0.042
Epiactis prolifera	KY789287.1	n/c	0.045–0.058

Accession numbers of newly sequenced in this study are in bold.
SD, standard deviation; n/c, no calculated.

ica and *A. buddemeieri* based on genetic differences with 0.9% in 16S RNA and 1.9% in COIII (Figs. 3, 4). This result showed difference between the Korean specimens and *A. buddemeieri* from Singapore, along with a negligible sequence difference from *A. asiatica* collected in Honshu, Japan, across both genetic markers (0.3% in 16S RNA and 0.0% in COIII) (Geller and Walton, 2001; Yap et al., 2020).

Therefore, it was confirmed that externally similar temperate species (*A. asiatica*) and tropical species (*A. buddemeieri*) can be clearly distinguished by nucleotide sequences.

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CONFLICTS OF INTEREST

Sung-Jin Hwang, a contributing editor of the Animal Systematics, Evolution and Diversity, was not involved in the

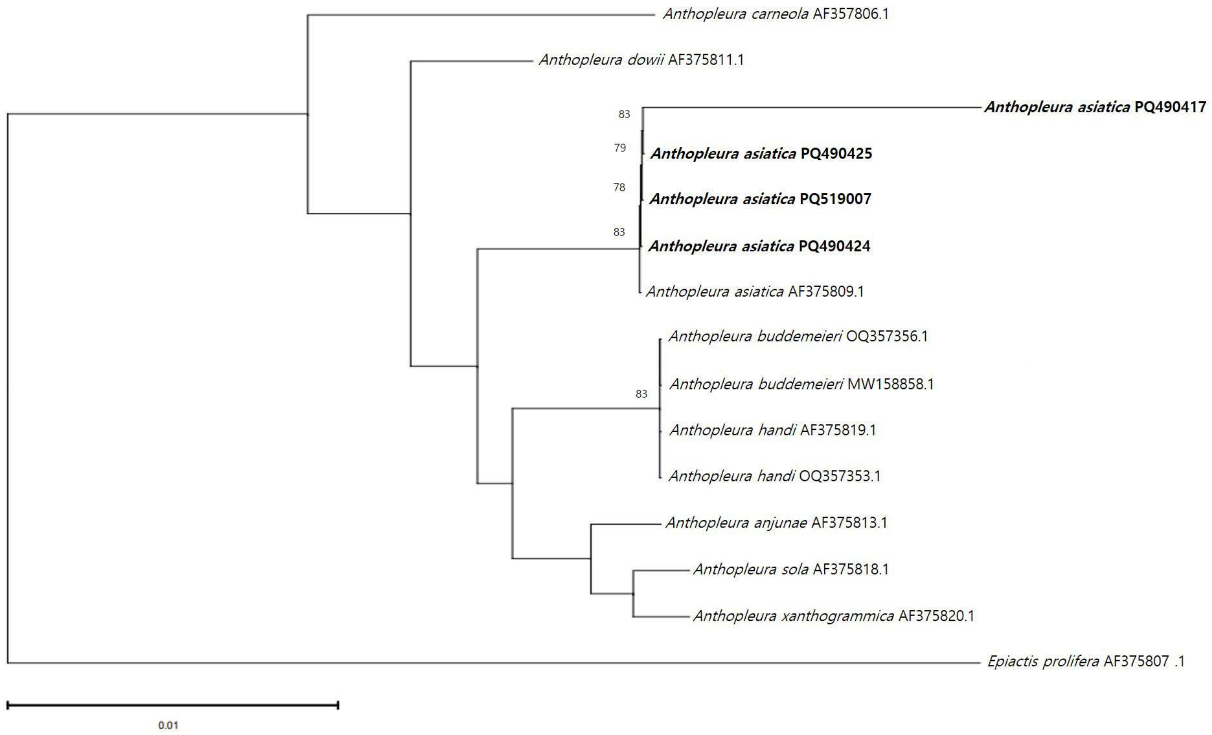

Fig. 3. Neighbor-joining tree constructed from the 16S rRNA sequences of *Anthopleura asiatica* and related species. Numbers at the nodes indicate the percentage occurrences among 1,000 bootstrap values. Newly sequenced in this study are in bold.

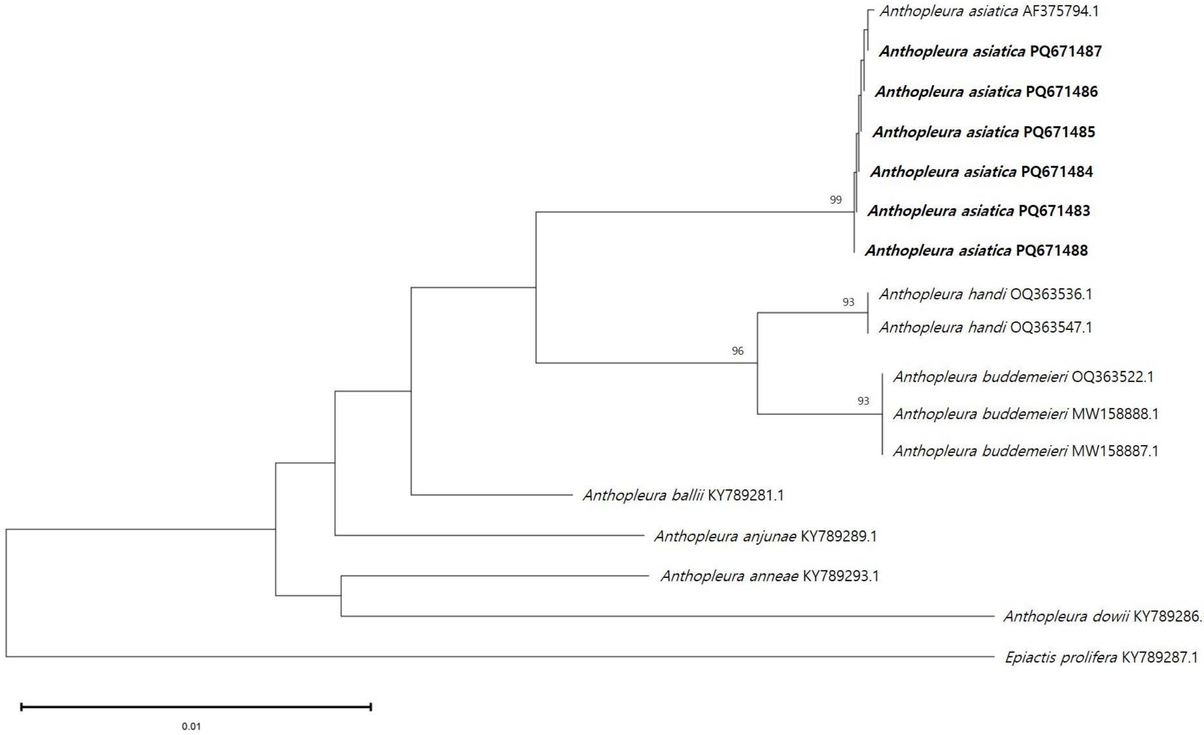

Fig. 4. Neighbor-joining tree constructed from the COIII sequences of *Anthopleura asiatica* and related species. Numbers at the nodes indicate the percentage occurrences among 1,000 bootstrap values. Newly sequenced in this study are in bold.

editorial evaluation or decision to publish this article. The remaining author has declared no conflicts of interest.

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