http://e-ASED.org
eISSN: 2234-8190

Volume 42 Number 1 January 2026
Animal Systematics, Evolution and Diversity

# ASED

## *Corhiza ulleungensis* sp. nov.

**Cover Story**

***Corhiza ulleungensis*** **sp. nov., a new hydrozoan from Ulleungdo waters, Korea, with holotype in situ and paratype specimen illustrated** **(See Page 57, 58, Figs. 1A, 2A).**

The Korean Society of Systematic Zoology

ASED http://e-ASED.org
Animal Systematics, Evolution and Diversity

eISSN 2234-8190

**Volume 42 Number 1 January 2026 (Quarterly)**
**Published on 31 January 2026**
**Published by Korean Society of Systematic Zoology**

## Aims & Scope

***Animal Systematics, Evolution and Diversity*** **(Anim Syst Evol Divers, ASED)** publishes original contributions of systematics, conservation biology, and evolutionary biology with a preference of systematic works such as discovery of new taxa, taxonomic reviews and revisions, phylogenetics and population genetics, biogeography and phylogeography, and biodiversity studies concerning animal taxa of the Korean Peninsula and surrounding areas of East Asia. It is published quarterly (Jan 31st, Apr 30th, Jul 31st, and Oct 31st) by the Korean Society of Systematic Zoology. It has been published since 1985 with a title of the Korean Journal of Systematic Zoology. Its title was changed from the year of 2012 as *Animal Systematics, Evolution and Diversity.*

Publications of this journal are partially supported by funds from the Korean Federation of Science and Technology Societies and the National Research Foundation of Korea (NRF). ASED is indexed by the Korea Citation Index (KCI), CrossRef, KoreaScience, DBpia, BIOSIS Previews, Zoological Record, Biological Abstracts and DOAJ.

**Editors-in-Chief** **Jongwoo Jung** Ewha Womans University, Korea
**Ho Young Suk** Yeungnam University, Korea

**Executive Editors** **Kee-Jeong Ahn** Chungnam National University, Korea
**Cheon Young Chang** Daegu University, Korea
**Rony J. Huys** Natural History Museum, UK
**Mann Kyoon Shin** University of Ulsan, Korea
**Yong-Jin Won** Ewha Womans University, Korea

**Managing Editors** **Jin-Kyung Choi** Daegu National University of Education, Korea
**Sung-Jin Hwang** Woosuk University, Korea
**Jongok Lim** Wonkang University, Korea
**Myung Hwa Shin** National Marine Biodiversity Institute of Korea, Korea

**Editorial Board**

Xiao-Lin Chen (Institute of Zoology, Chinese Academy of Sciences, China)
Soowon Cho (Chungbuk National University, Korea)
Michael Cummings (University of Maryland, USA)
Shan Gao (Ocean University of China, China)
Ho-Yeon Han (Yonsei University, Korea)
Jianping Jiang (Chengdu institute of Biology, CAS, China)
Warren Johnson (National Cancer Institute, USA)
Il-Hoi Kim (Gangneung-Wonju National University, Korea)
Won Kim (Seoul National University, Korea)
Hyun Sook Ko (Silla University, Korea)
Tomoyuki Komai (Natural History Museum and Institute, Japan)
Hang Lee (Seoul National University, Korea)
Liang-Kong Lin (Tunghai University, Taiwan)
Shu-Jin Luo (Peking University, China)
Mi-Sook Min (Seoul National University, Korea)
Masaharu Motokawa (Kyoto University, Japan)
Stephen J. O'Brien (St. Petersburg State University, Russia)
Hidetoshi Ota (University of Hyogo, Japan)
Joong-Ki Park (Ewha Womans University, Korea)
Kwang-Tsao Shao (Biodiversity Research Center, Academia Sinica, Taiwan)
Sung Joon Song (Seoul National University, Korea)
Zhen Zhen Yi (South China Normal University, China)

**Contact *Animal Systematics, Evolution and Diversity***

**Editors-in-Chief** Jongwoo Jung and Ho Young Suk

**Editorial Office**
Room 305-3, The Korea Science and Technology Center,
22, 7 gil, Teheran-ro, Gangnam-gu, Seoul, Korea
**Tel:** +82 2 569 0133 **E-mail:** systzoology@hanmail.net

**E-Printing** Junghaengsa, Inc. **Tel:** +82 2 2232 3281~2
**Manuscript Editing** InfoLumi Co. **Tel:** +82 70 8839 8800
**Design** Seo-Hyeong Publishing Co. **Tel:** +82 2 2209 3671

**Copyright ©2026 Korean Society of Systematic Zoology**

CC It is identical to the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/).

This journal was supported by the Korean Federation of Science and Technology Societies Grant funded by the Korean Government.

ASED http://e-ASED.org
Animal Systematics, Evolution and Diversity

*Anim. Syst. Evol. Divers. Vol. 42, No. 1: 1-11, January 2026*
https://doi.org/10.5635/ASED.2026.42.1.030

# New Record of *Eunice flavopicta* (Annelida: Eunicidae) from Korean Waters, with Notes on Korean *Eunice aphroditois*

Ha-Eun Lee[1], Taeseo Park[2,†,*], Gi-Sik Min[1,†,*]

[1]Department of Biological Sciences and Bioengineering, Inha University, Incheon 22212, Korea
[2]National Institute of Biological Resources, Incheon 22689, Korea

## ABSTRACT

While most Korean giant eunicids have previously been regarded as *Eunice aphroditois*, *E. flavopicta*, originally described from Japan, has been newly reported in South Korea. The Korean specimens corresponded to the diagnostic features of *E. flavopicta* in the articulation of the prostomial appendages and peristomial cirri, distribution and shape of branchiae, shape of dorsal cirri, and types of chaetae. While *E. flavopicta* was previously considered as a junior synonym of *E. aphroditois*, they were distinguished from *E. aphroditois sensu stricto* in the shape of their dorsal cirri and subacicular hooks. This study provides morphological and molecular data for Korean *E. flavopicta* specimens, along with taxonomic notes on *E. aphroditois* from South Korea.

**Keywords:** eunicids, molecular analysis, polychaeta, taxonomy

## INTRODUCTION

*Eunice* Cuvier, 1817 is the type genus of the family Eunicidae Berthold, 1827 and comprises approximately 250 species (WoRMS Editorial Board, 2025). Members of the genus are characterized by five smooth or irregular prostomial appendages, a pair of peristomial cirri, a flat mandible, pectinate branchiae, dark subacicular hooks, and the absence of lateral black dots between the posterior parapodia (Zanol et al., 2014).

*Eunice aphroditois* (Pallas, 1788), the type species of the genus, is a giant eunicid originally described from Sri Lanka. This species has long been recognized for its large body size, branchiae with numerous filaments, and dark aciculae and subacicular hooks (Zanol and Hutchings, 2022). Giant eunicids bearing these features, especially those from the Indian and Pacific Oceans, have frequently been identified or synonymized with *E. aphroditois* (Fauchald, 1992; Salazar-Vallejo et al., 2011; Zanol and Hutchings, 2022). Likewise, East Asian giant eunicid specimens with those features were identified as *E. aphroditois* (Izuka, 1912; Wu et al., 1975; Paik, 1976), and two Japanese species, *Eunice flavopicta* Izuka, 1912 and *Eunice ovalifera* Fauvel, 1936, were reclassified as junior synonyms of *E. aphroditois* by Miura (1986). However, taxonomic revisions by Fauchald (1992) and molecular studies by Zanol et al. (2010, 2014) revealed the underestimation of species diversity within eunicids and showed the need for re-examination to confirm their true identity (Zanol and Hutchings, 2022). For example, giant eunicid specimens from Australia that had almost always been identified as *E. aphroditois* were found to exhibit high intraspecific diversity; consequently, an additional new species, *Eunice dharastii* Zanol and Hutchings, 2022, similar to *E. aphroditois*, has been described from Australian waters (Fauchald, 1992; Salazar-Vallejo et al., 2011; Zanol and Hutchings, 2022).

Korean giant eunicids were identified as *E. aphroditois* by Paik (1976) and subsequent studies and checklists have followed this identification (Paik, 1976, 1982, 1984, 1986, 1987, 1989; Rho and Lee, 1988). Since there has been no re-exam-

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.

†Taeseo Park and Gi-Sik Min contributed equally to this work.
***To whom correspondence should be addressed**
Tel: 82-32-590-7360, Fax: 82-32-590-7250
E-mail: polychaeta@gmail.com (T. Park)
Tel: 82-32-860-7692, Fax: 82-32-874-6737
E-mail: mingisik@inha.ac.kr (G.-S. Min)

**eISSN 2234-8190**

Copyright © The Korean Society of Systematic Zoology

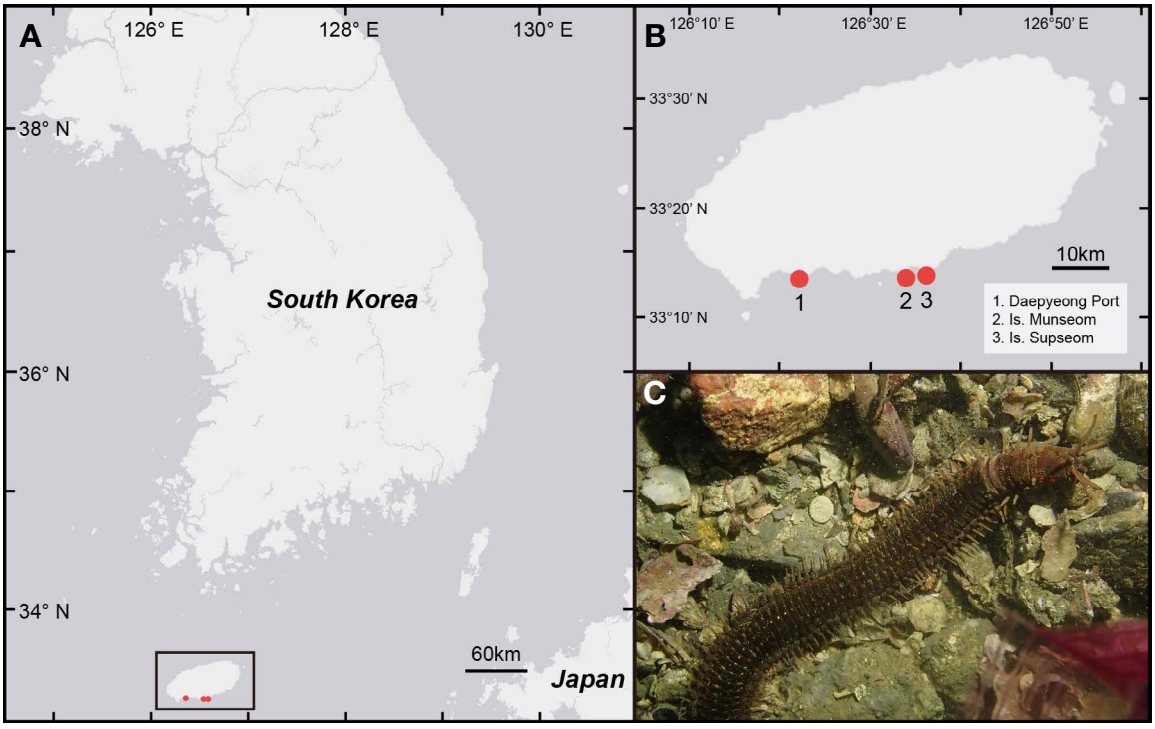


**Fig. 1.** Collection sites of Korean *Eunice flavopicta* (A, B) and alive specimen (C). Live specimen photographed in situ on rocky substrate (25 m depth, Munseom Islet, Jejudo Island). Iridescent reddish brown coloration visible.

ination on the Korean giant eunicids, previously identified as *E. aphroditois* following the revision by Fauchald (1992), it is necessary to reassess their true identity. Thus, we re-examined the morphological features of Korean giant eunicid specimens and obtained sequences of cytochrome c oxidase I (COI), 16S ribosomal DNA (rDNA), and 18S rDNA for molecular analysis.

## MATERIALS AND METHODS

### Sampling and morphological observations

Newly collected giant eunicid worms and several previously deposited specimens labeled as *Eunice aphroditois* at the National Institute of Biological Resources (NIBR), Incheon, South Korea were examined in this study. Samples were collected from the subtidal zone of Jejudo Island, South Korea by SCUBA diving (Fig. 1). Morphological characteristics were observed under a stereoscopic microscope (Leica S8APO; Leica, Wetzlar, Germany), and photographs of the specimens were taken using a digital camera (Dhyana 400DC; Tucsen, Fuzhou, China) and Mosaic capture software (Mosaic version 15; Tucsen). Some specimens were dissected to observe the maxillary apparatus and shape and composition of the parapodia. The dissected parapodia were mounted on glass slides and observed under an optical microscope (DM2500; Leica). Photographs were taken using a digital camera (EOS 6D Mark II; Canon, Tokyo, Japan). Voucher specimens were deposited at NIBR.

### Molecular analysis

DNA was extracted from three ethanol-preserved specimens (NIBRIV0000262417, NIBRIV0000927833, and NIBRIV0000927834) using LaboPass Tissue Mini (Cosmo GENETECH, Seoul, Korea), according to the manufacturer's instructions. Partial sequences of COI, 16S rDNA, and 18S rDNA were amplified using the following primer sets: PolyLCO/PolyHCO for COI; 16Sar-L/16Sbr-H for 16S rDNA; and 1F/BI and 18F509/9R for 18S rDNA (Table 1). The obtained COI, 16S rDNA, and 18S rDNA sequences were edited and aligned using Geneious 8.1.9 (Biomatters, Auckland, New Zealand). Intra- and interspecific genetic distances were calculated as p-distances using Geneious 8.1.9 (Table 2). Newly determined DNA sequences were registered in GenBank (PX435308–10 for COI, PX560176–8 for 16S rDNA, and PX435319–21 for 18S rDNA). Maximum likelihood (ML) and Bayesian inference (BI) analyses were performed on these three genes (Fig. 4, Appendix 1). Sequences of *Diopatra dentata* Kinberg, 1865 were used as the outgroup taxon. ML analyses were conducted in IQ-TREE v1.6.1 using the

**Table 1.** List of primers used for PCR amplification and sequencing in this study

| Primer | Direction | Sequence 5′-3′ | References |
|---|---|---|---|
| COI | | | |
| PolyLCO | Forward | GAYTATWTTCAACAAATCATAAAGATATTGG | Carr et al. (2011) |
| PolyHCO | Reverse | TAMACTTCWGGGTGACCAAARAATCA | Carr et al. (2011) |
| 16S rDNA | | | |
| 16Sar-L | Forward | CGCCTGTTTATCAAAAACAT | Palumbi et al. (1991) |
| 16Sbr-H | Reverse | CCGGTCGAAACTCAGATCACGT | Palumbi et al. (1991) |
| 18S rDNA | | | |
| 1F | Forward | TACCTGGTTGATCCTGCCAGTAG | Giribet et al. (1996) |
| BI | Reverse | GAGTCTCGTTCGTTATCGGA | Whiting et al. (1997) |
| 18F509 | Forward | CCCCGTAATTGGAATGAGTACA | Struck et al. (2002) |
| 9R | Reverse | GATCCTCCGCAGGTTCACCTAC | Giribet et al. (1996) |

PCR, polymerase chain reaction; COI, cytochrome c oxidase I; rDNA, ribosomal DNA.

**Table 2.** Interspecific genetic distances of three genes (COI, 16S rDNA, and 18S rDNA) among available *Eunice* species and an outgroup species, *Leodice harassii*

| Species | Interspecific genetic distance | | | | | | | GenBank accession No. | References |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | |
| COI (621 bp aligned) | | | | | | | | | |
| 1 *E. flavopicta* (Korea) | | | | | | | | PX435308 | Present study |
| 2 *E.* cf. *aphroditois* (Japan) | 0.005 | | | | | | | LC756630 | Shinomiya et al. (2023) |
| 3 *E. aphroditois* (Singapore) | 0.084 | 0.092 | | | | | | MN690243 | Ip et al. (2019) |
| 4 *E.* cf. *violaceomaculata* | 0.187 | 0.190 | 0.192 | | | | | GQ497542 | Zanol et al. (2010) |
| 5 *E. norvegica* | 0.192 | 0.197 | 0.203 | 0.195 | | | | GQ497541 | Zanol et al. (2010) |
| 6 *E. roussaei* | 0.198 | 0.209 | 0.195 | 0.177 | 0.221 | | | GQ497543 | Zanol et al. (2010) |
| 7 *E. filamentosa* | 0.206 | 0.208 | 0.205 | 0.180 | 0.221 | 0.208 | | GQ497545 | Zanol et al. (2010) |
| 8 *Leodice harassii* | 0.206 | 0.203 | 0.22 | 0.194 | 0.215 | 0.222 | 0.221 | GQ497535 | Zanol et al. (2010) |
| 16S rDNA (529 bp aligned) | | | | | | | | | |
| 1 *E. flavopicta* (Korea) | | | | | | | | PX560176 | Present study |
| 2 *E.* cf. *aphroditois* (Japan) | 0.002 | | | | | | | LC756644 | Shinomiya et al. (2023) |
| 3 *E.* cf. *violaceomaculata* | 0.105 | 0.103 | | | | | | GQ478148 | Zanol et al. (2010) |
| 4 *E. roussaei* | 0.122 | 0.120 | 0.116 | | | | | GQ478149 | Zanol et al. (2010) |
| 5 *E. norvegica* | 0.201 | 0.199 | 0.172 | 0.202 | | | | GQ478147 | Zanol et al. (2010) |
| 6 *E. impexa* | 0.207 | 0.205 | 0.188 | 0.212 | 0.183 | | | GQ478156 | Zanol et al. (2010) |
| 7 *E. filamentosa* | 0.208 | 0.206 | 0.217 | 0.234 | 0.201 | 0.192 | | GQ478155 | Zanol et al. (2010) |
| 8 *Leodice harassii* | 0.225 | 0.223 | 0.226 | 0.249 | 0.235 | 0.23 | 0.242 | GQ478140 | Zanol et al. (2010) |
| 18S rDNA (1,689 bp aligned) | | | | | | | | | |
| 1 *E. flavopicta* (Korea) | | | | | | | | PX435319 | Present study |
| 2 *E. roussaei* | 0.003 | | | | | | | GQ497495 | Zanol et al. (2010) |
| 3 *E.* cf. *aphroditois* (Japan) | 0.004 | 0.008 | | | | | | LC756664 | Shinomiya et al. (2023) |
| 4 *E.* cf. *violaceomaculata* | 0.006 | 0.004 | 0.011 | | | | | GQ497494 | Zanol et al. (2010) |
| 5 *E. norvegica* | 0.006 | 0.004 | 0.011 | 0.000 | | | | GQ497493 | Zanol et al. (2010) |
| 6 *E. filamentosa* | 0.007 | 0.005 | 0.011 | 0.001 | 0.001 | | | GQ497500 | Zanol et al. (2010) |
| 7 *E. impexa* | 0.007 | 0.005 | 0.011 | 0.004 | 0.004 | 0.005 | | GQ497501 | Zanol et al. (2010) |
| 8 *Leodice harassii* | 0.014 | 0.012 | 0.019 | 0.008 | 0.008 | 0.008 | 0.012 | GQ497486 | Zanol et al. (2010) |

COI, cytochrome c oxidase I; rDNA, ribosomal DNA.

TIM2e + I + G4 model for concatenated genes with 1,000 replicates (Kalyaanamoorthy et al., 2017). BI analyses were performed using MrBayes v3.2.6 (Ronquist and Huelsenbeck, 2003) with the GTR + I + G model selected by the Akaike information criterion implemented in jModelTest v2.1.10 (Darriba et al., 2012).

## SYSTEMATIC ACCOUNTS

Phylum Annelida Lamarck, 1809
Class Polychaeta Grube, 1850
Order Eunicida Berthold, 1827
Family Eunicidae Berthold, 1827
Genus *Eunice* Cuvier, 1817

### [1]**Eunice flavopicta* Izuka, 1912

*Eunice flavopicta* Izuka, 1912: 121, Pl. 14, figs. 1–5; Imajima and Hartman, 1964: 251; Fauchald, 1992: 146, tables 27, 29, 30.

*Eunice* cf. *aphroditois*: Shinomiya et al., 2023: 318, figs. 1–12, table 2.

**Material examined.** South Korea, Jeju-do: 1 spec., Seogwipo-si, Supseom Islet, NIBRIV0000137768, *Eunice aphroditois*, det. T Park, 27 Feb 2009, 10.3 m depth, coll. KJ Lee, HJ Kil and T Park; 4 spec., same locality, NIBRIV0000137612, 139999–140001, *Eunice aphroditois*, det. T Park, 30 Apr 2009, 10 m depth, coll. T Park and HJ Kil; 5 spec., Seogwipo-si, Bomok-dong, NIBRIV0000262417–262421, *Eunice aphroditois*, 18 Jun 2010, coll. and det. SH Kim; 1 spec., Seogwipo-si, Daepyeong Port, NIBRIV0000927832, 16 Jul 2021, coll. T Park; 2 spec., Seogwipo-si, Munseom Islet, NIBRIV0000927833, NIBRIV0000927834, 11 Jun 2024, 25 m depth, coll. T Park.

**Diagnosis.** Five prostomial appendages and a pair of peristomial cirri thick, slightly wrinkled with blunt tip. Branchiae present from chaetiger 5 or 6 to near posterior end; pectinate with branchial filaments up to about 20–25 at the best development. Dorsal cirri digitiform, with slightly enlarged base and blunt distal tip. Aciculae paired, dark, slightly bent at distal end. Subacicular hooks dark, bidentate, with two laterally directed teeth. Pygidium with a pair of anal cirri.

**Description.** Longest specimen (NIBRIV0000139999) complete, with about 480 chaetigers, 121.5 cm long, 1.5 cm wide at chaetiger 10 (Fig. 2A). Other specimens are 47.7 cm long with 335 chaetigers (NIBRIV0000137768, complete with regenerating posterior region), 43.9 cm long with 315 chaetigers (NIBRIV0000927833, complete), 37.7 cm long with 215 chaetigers (NIBRIV0000262417, incomplete), and 21.5 cm long with 129 chaetigers (NIBRIV0000140001, incomplete). Live specimens iridescent reddish brown or purple with two or three whitish transverse bands on around chaetiger 4. After ethanol fixation, specimens light brown or purple (Figs. 1C, 2A, B). Prostomial appendages, peristomial cirri, and dorsal cirri light brown with paler distal ends in live condition; beige after alcohol fixation.

Prostomium shorter than peristomium, with two dorsal and two ventral buccal lips (Fig. 2C, E). Prostomium with five appendages; three antennae and one pair of palps. Prostomial appendages thick, slightly wrinkled, with blunt tip (Fig. 2C). Median antenna reaching back to chaetiger 1–2 and lateral antennae and palp reaching back to chaetiger 1 and second peristomial ring, respectively. One pair of black eyes present behind base of palps, often covered by peristomial fold (Fig. 2C). Peristomium cylindrical; separation between first and second rings visible only on dorsal and ventral sides (Fig. 2D, E). The first peristomial ring 4–5 times longer than the second one, with muscular ventrolateral lips. Second peristomial ring with one pair of peristomial cirri; cirri digitiform with blunt tip, slightly wrinkled, not outreaching beyond anterior margin of peristomium (Fig. 2C, D).

Maxillary formula 1 + 1, 5 + 5(6), 6(7) + 0, 3 + 8, 1 + 1, and 1 + 1 (Fig. 3A). Maxillae (Mx) III located only on left side, positioned behind MxII and beneath MxIV. MxVI small, slender plate existed below MxV on both side of maxillae. Mandibles flat, black, with calcareous cutting plates (Fig. 3B).

Branchiae first appear from chaetiger 5 or 6 to end of body (Fig. 2F). Branchiae pectinate, with up to about 20–25 filaments around chaetiger 30–50, then reduced to single filament in posteriormost chaetigers (Figs. 2G, 3C). Branchial stem thick, erect, slightly tapering, longer than dorsal cirri (Figs. 2G, 3C). Filaments digitiform, shorter and slenderer than dorsal cirri and branchial stems (Figs. 2G, 3C).

Parapodium equipped with a dorsal cirrus, a ventral cirrus and a branchia (Fig. 3C). Chaetal lobes rounded and bearing a bundle of chaetae. Pre- and postchaetal lobes lower than chaetal lobes (Fig. 3C–E). Dorsal cirri erected, digitiform, with blunt distal tip and slightly enlarged bases ventrally (Figs. 2F, G, 3C). Dorsal cirri with notoaciculae (Fig. 3F, I). Ventral cirri short conical in a few anteriormost and posteriormost chaetigers, becoming inflated to oval bases with digitiform tips; division between base and tip more distinct in anterior region (Fig. 3C–F).

Parapodia bearing bundle of five chaetal types; capillary chaetae, compound falcigerous chaetae, pectinate chaetae, aciculae, and subacicular hooks (Fig. 3F–L). Capillary chaetae and pectinate chaetae positioned at dorsal side of aciculae. Compound chaetae positioned between aciculae and subacicular hooks. Pectinate chaetae with 6–12 teeth; all teeth similar length (Fig. 3H). Compound falcigerous chaetae bidentate with guards (Fig. 3I). Aciculae generally paired, dark, tapering, slightly bent distally (Fig. 3J). Subacicular hooks mainly single, dark, bidentate with guards; two teeth slightly bent laterally (Fig. 3K). Subacicular hooks

Korean name: [1]*막대두수염왕털갯지렁이 (신칭)

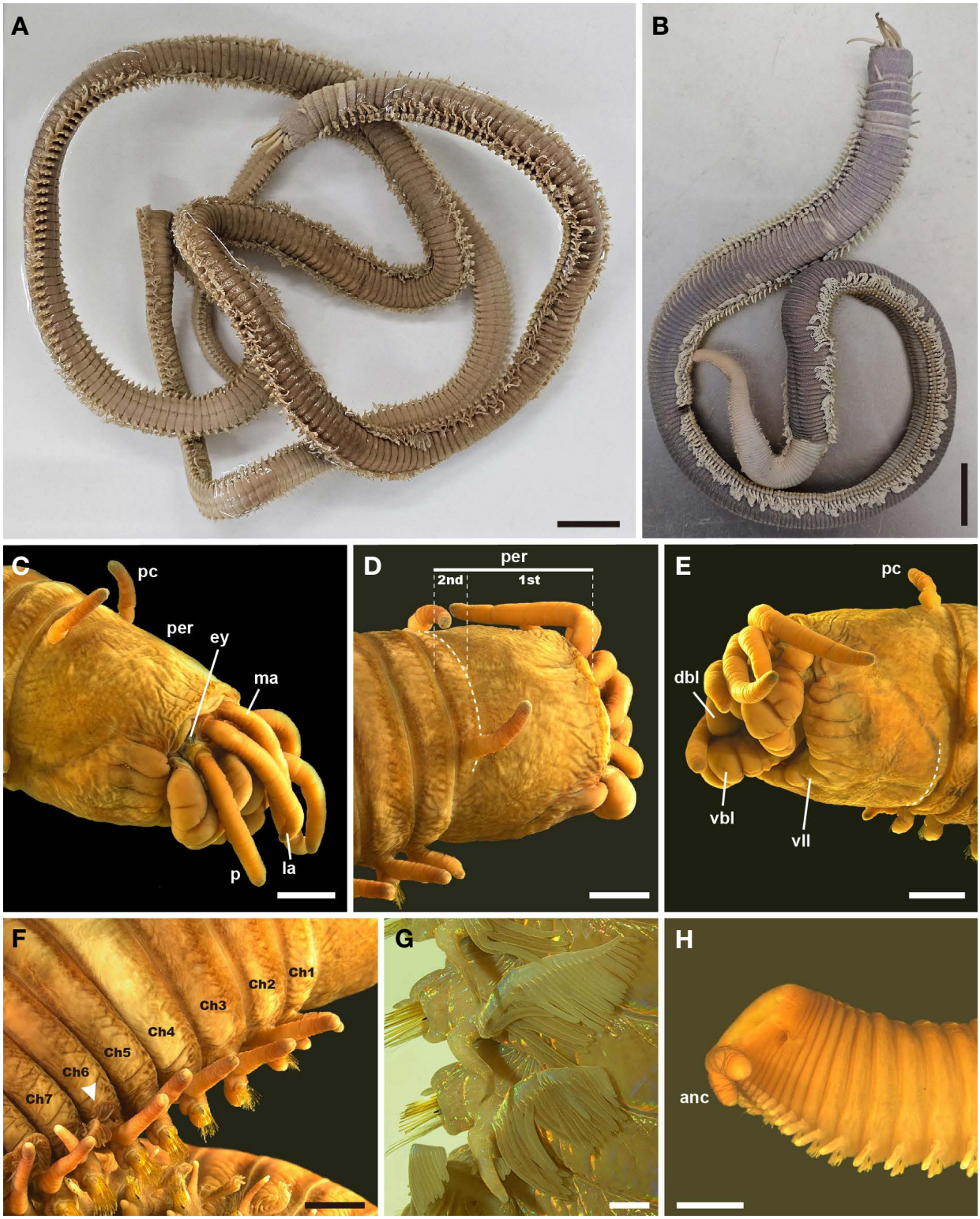


**Fig. 2.** *Eunice flavopicta* from Korea. A, G, NIBRIV0000139999; B, NIBRIV0000137768; C–F, H, NIBRIV0000140001. A, Whole body; B, Whole body with regenerated posterior segments; C, D, Anterior region, dorsolateral view; E, Anterior region, ventrolateral view; F, Chaetiger 1–7, dorsal view; G, Midbody parapodia, dorsal view; H, Pygidium. anc, anal cirri; ch, chaetiger; dbl, dorsal buccal lip; ey, eye; la, lateral antenna; ma, medial antenna; p, palp; pc, peristomial cirri; per, peristomium; vbl, ventral buccal lip; vll, ventrolateral lip. Scale bars: A–F = 2 mm, G, H = 1 mm.

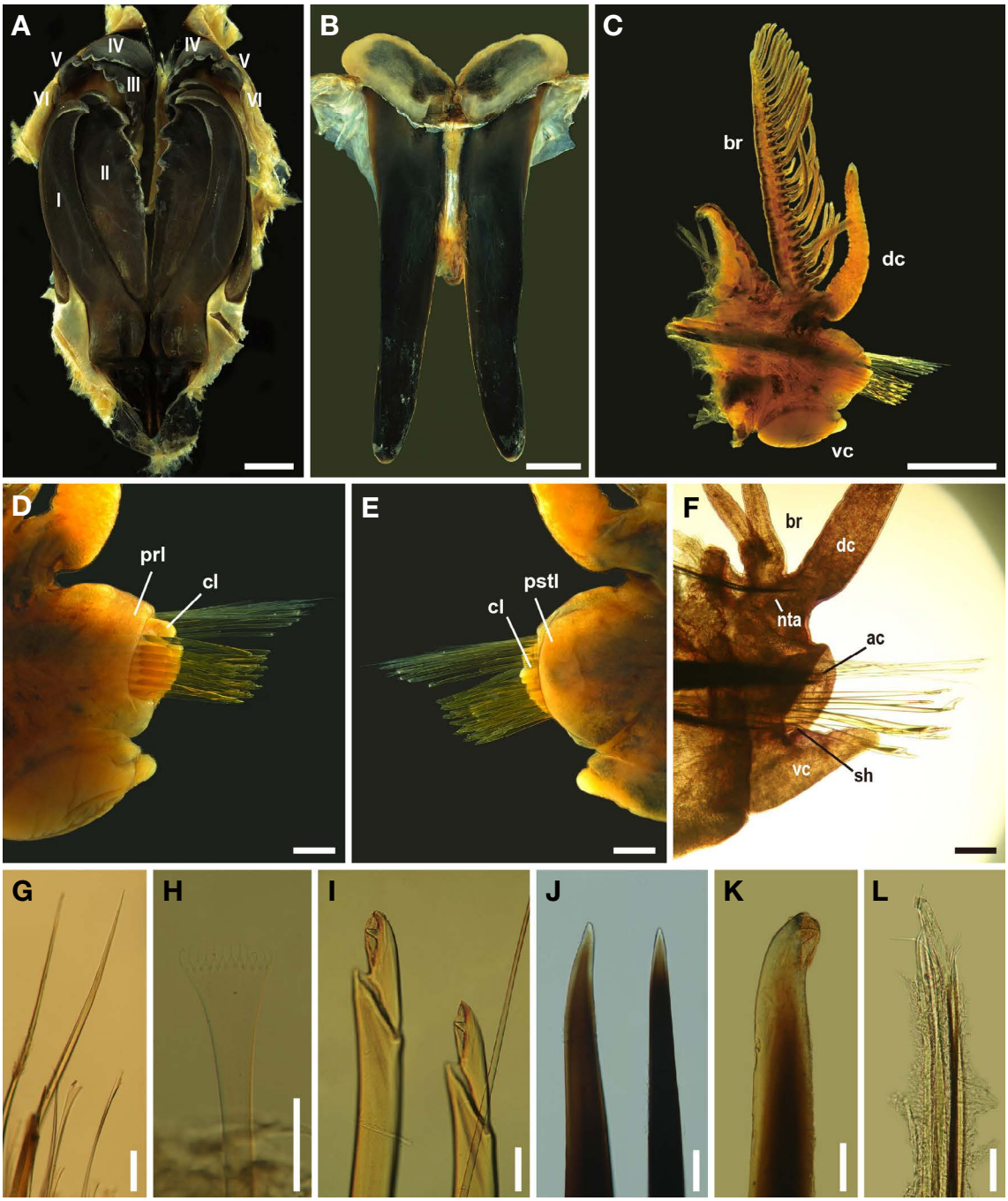


**Fig. 3.** *Eunice flavopicta* from Korea. A, NIBRIV0000137768; B, NIBRIV0000262417; C–F, J, L, NIBRIV0000927833; G–I, K, NIBRIV0000927832. A, Maxillae; B, Mandible; C, D, Dissected anterior parapodium, anterior view; E, Dissected anterior parapodium, posterior view; F, Dissected posterior parapodium, anterior view; G, Capillary chaetae; H, Pectinate chaeta; I, Compound falcigerous chaetae with guards; J, Aciculae; K, Subacicular hook with guards; L, Notoaciculae. ac, acicula; br, branchia; cl, chaetal lobe; dc, dorsal cirrus; nta, notoaciculae; prl, prechaetal lobe; pstl, postchaetal lobe; sh, subacicular hook; vc, ventral cirrus. Scale bars: A–C = 2 mm, D, E = 0.5 mm, F = 0.2 mm, G = 100 μm, H–L = 50 μm.

appear at first from about chaetiger 50 to 200 depending on body size.

Pygidium with two short and stout anal cirri (Fig. 2H).

**Habitat and distribution.** This species is distributed in Japan and South Korea. It was previously collected from rocky shores in the intertidal zones of Japan. In this study, the spe-

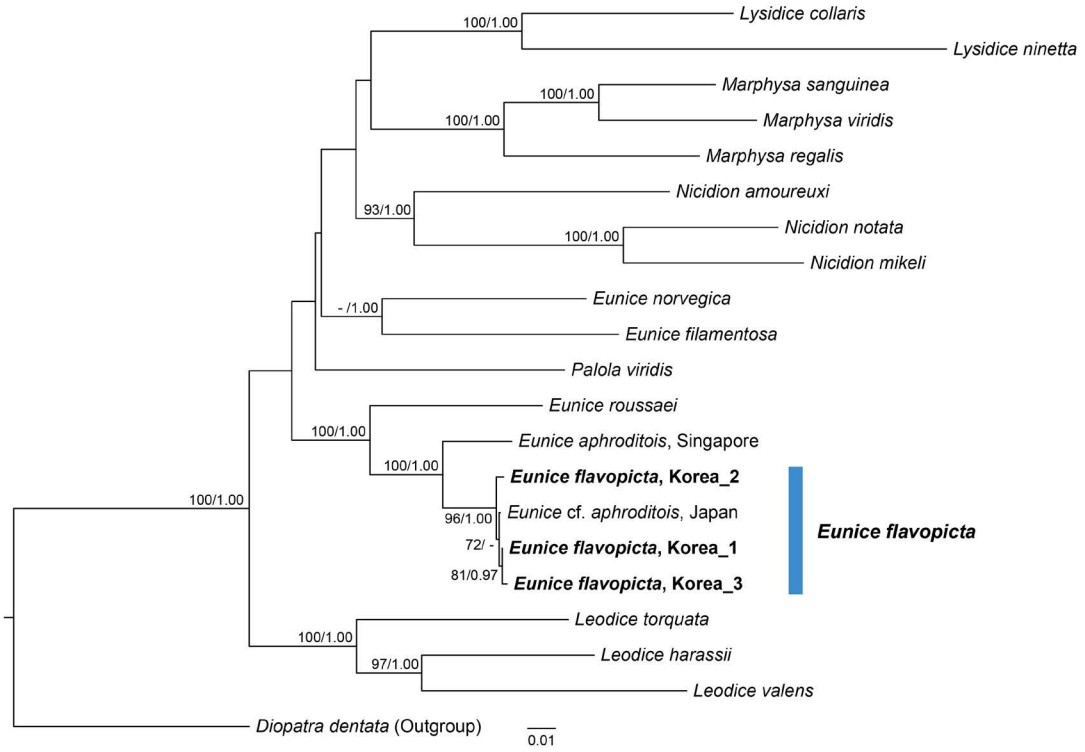


**Fig. 4.** Tree based on maximum likelihood (ML) and Bayesian inference (BI) analyses using COI+16S+18S rDNA sequences. The numbers at nodes represent the ML bootstrap values of ≥70% and the BI posterior probabilities of ≥0.95. DNA sequences of Korean specimens obtained in this study are in bold. *Diopatra dentata* was used as an outgroup taxon.

cies was also found under rocks on the rocky subtidal shores at Jejudo Island, South Korea (Fig. 1).

**Genetics.** Sequences of COI, 16S rDNA, and 18S rDNA were obtained from three Korean specimens of *E. flavopicta*. Maximum intraspecific divergences were 1.2% for COI (658 bp), 0.6% for 16S rDNA (509 bp), and 0.1% for 18S rDNA (1,689 bp). Genetic divergences between Korean specimens and Japanese *Eunice* cf. *aphroditois sensu* Shinomiya et al. (2023) were 0.6–1.3% for COI, 0.2–0.4% for 16S rDNA and 0.4–0.5% for 18S rDNA (due to 9 ambiguous bases 'n' in the Japanese 18S rDNA sequence). Korean specimens differ from *E. aphroditois* from Singapore in that the genetic divergence was 8.5–9.2% in COI (623 bp) (accession No. MN690243) (Ip et al., 2019). The interspecific divergence between the Korean specimens and other *Eunice* species ranged from 8.4–20.6% for COI (621 bp), 10.5–20.8% for 16S rDNA (529 bp), and 0.3–0.7% for 18S rDNA (1,689 bp) (Table 2).

**Remarks.** The morphological characteristics of the Korean giant *Eunice* specimens corresponded well with the original description of *E. flavopicta* by Izuka (1912) and another description by Imajima and Hartman (1964) including the following features: (1) prostomial appendages and peristomial cirri faintly articulated, (2) branchiae present from chaetiger 5 or 6, (3) branchial filaments numbering around 20–25 at maximum development, (4) dorsal cirri erect, digitiform, with blunt tip and slightly enlarged bases (see Pl. XIV, fig. 1 in Izuka, 1912), (5) pectinate chaetae with about 10 teeth of similar length, and (6) subacicular hooks bidentate with guards, with two teeth direct laterally. In addition, the Korean specimens matched well with the descriptions of *E. flavopicta* by Imajima and Hartman (1964) and *Eunice* cf. *aphroditois* by Shinomiya et al. (2023), based on the characteristics described above and the following additional characteristics: (7) compound falcigers bidentate, (8) aciculae and subacicular hooks dark to black. Although compound falcigers were initially described by Izuka (1912) as having two large teeth with six smaller teeth between them, they are now regarded as bidentate, with the damaged margin of the hood possibly appearing as subdentations (see Pl. XIV, fig. 4 in Izuka (1912) and remarks in Miura, 1986). The maxillary formula shows slight differences between the Korean specimens and *E. flavopicta* in Izuka (1912) and Imajima and Hartman (1964) with MxII having 5+5(6) in the former and 4+5 in the latter. However, Shinomiya et al. (2023) described *Eunice* cf. *aphroditois*—which is genetically close

to the Korean specimens and likely represents the same species—as having 4+5 teeth in the juvenile form and 5+5 in the adult form. Thus, the maxillary formula may exhibit slight variations depending on growth.

The Korean giant eunicid specimens observed in this study differ from *E. aphroditois* as redescribed by Fauchald (1992), in the shape of the subacicular hooks (slightly bent and teeth directed laterally vs. both teeth directed distally) and the articulation of the prostomial appendages, peristomial cirri, and dorsal cirri (slightly annulated vs. not articulated). In addition, we consider that the shape of the dorsal cirri of the Korean specimens differs from that of *E. aphroditois*, although both have been described as having thick dorsal cirri with slightly inflated bases. In illustrations (see tab. V, figs. 6, 7 in Pallas, 1788 and fig. 13c, d in Fauchald, 1992) the dorsal cirri of *E. aphroditois* appear to have more inflated bases and pointed tips. In contrast, the Korean and Japanese specimens possess erected, digitiform dorsal cirri with blunt tips and less inflated bases (see Pl. XIV, fig. 1 in Izuka, 1912, fig. 1 in Miura, 1986, and fig. 2f, g in this study).

## DISCUSSION

The Korean giant *Eunice* species collected from Jejudo Island were identified as *E. flavopicta*, originally described from Japan, because the Korean specimens well matched to the original description of *E. flavopicta* by Izuka (1912) and another description by Imajima and Hartman (1964) in the following characteristics: slightly annulated prostomial and peristomial appendages and dorsal cirri, the branchiae occurring from chaetiger 5 or 6, branchial filaments up to around 20−25, and the chaetal types. While the Korean specimens also similar to *E. aphroditois* by having numerous branchial filaments, and dark aciculae and subacicular hooks, the Korean specimens differ from the original description of *E. aphroditois* by Pallas (1788) and detailed description by Fauchald (1992) in the articulation of the prostomial appendages, peristomial cirri, and dorsal cirri, the branchiae starting position, the shape of dorsal cirri, and the shape of the subacicular hooks (Pallas, 1788; Fauchald, 1992).

*Eunice flavopicta* was previously considered as a junior synonym of *E. aphroditois* by Miura (1986), along with *Eunice ovalifera* Fauvel, 1936, because of similarities in morphology and coloration, and his consideration that the observed differences among these species might represent size-dependent variations. However, Fauchald (1992) reinstated *E. flavopicta* as a distinct species considering the complex synonymy of *E. aphroditois* at that time. In a comparison between the description of *E. flavopicta* by Izuka (1912) and *E. aphroditois* by Pallas (1788) and Fauchald (1992), *E. flavopicta* differs from *E. aphroditois sensu stricto* in the shape of subacicular hooks, the articulation of the prostomial and peristomial appendages and dorsal cirri, and the shape of the dorsal cirri.

The Korean *Eunice flavopicta* specimens were also corresponded well to the Japanese *Eunice* cf. *aphroditois* specimens by Shinomiya et al. (2023), and the genetic variations between them were low enough to indicate that they belonged to the same species in the present study (Fig. 4, Table 2). Shinomiya et al. (2023) insisted that the Japanese giant eunicid specimens differ from *E. aphroditois*, originally described by Pallas (1788), in terms of collection locality and branchial morphology such as branchiae starting position and branchial distribution. In addition, some *E. aphroditois* specimens from the Philippines and Indonesia have been reported to inhabit sandy substrates and are known as ‘sand strikers’ or ‘bobbit worms,’ which are sit-and-wait type predators (Lachat and Haag-Wackernagel, 2016; Shinomiya et al., 2023). However, both Korean and Japanese specimens have been found under rocks on rocky shores (Shinomiya et al., 2023, present study). Despite the morphological and ecological differences, Shinomiya et al. (2023) remained their name as *Eunice* cf. *aphroditois* by considering the absence of type material and other available specimens from the type localities of both *E. aphroditois* and *E. flavopicta* for comparison. However, their differences from the original description of *E. aphroditois* in branchiae starting position (chaetiger 5 or 6 vs. 7) and the shape of dorsal cirri (slightly inflated base and blunt tip vs. strongly inflated base and tapering tips), it can be concluded that the Korean specimens and Japanese *E.* cf. *aphroditois* should be identified as *E. flavopicta* Izuka, 1912.

To date, Korean giant eunicids with numerous branchial filaments, and dark aciculae and subacicular hooks have consistently been considered as *E. aphroditois*. However, we have identified that the Korean giant eunicid specimens, either newly collected from Jejudo Island or deposited at the NIBR, correspond to *Eunice flavopicta* Izuka, 1912. Therefore, we also reviewed previous records of *E. aphroditois* from South Korea. The first record of Korean *E. aphroditois* was provided by Paik (1976) based on a single specimen collected from Seongsanpo, Jejudo Island. Additional morphological information was provided in descriptions by Paik (1989) and in simplified identification keys by Paik (1982, 1984) based on specimens collected from Jejudo Island and Ulleungdo Island. However, these descriptions are insufficient, and no voucher specimens collected by Paik or from the same localities are currently available. Because we could not examine specimens from Seongsanpo and Ulleungdo Islands, it was difficult to determine whether all Korean giant

eunicids are *E. flavopicta*. However, based on the description and illustrations provided by Paik (1976, 1982, 1989), the Korean *E. aphroditois* corresponds well with the present Korean giant eunicid specimens in having digitiform dorsal cirri with slightly inflated bases and blunt distal tips. Thus, we consider that the previously reported Korean *E. aphroditois* may not be *E. aphroditois sensu stricto* but rather *E. flavopicta*. However, further studies with newly collected specimens from Seongsanpo, Jejudo Island, and Ulleungdo Island are required to confirm the identity of Korean *E. aphroditois*.

## ORCID

Ha-Eun Lee: https://orcid.org/0000-0002-4694-4889
Taeseo Park: https://orcid.org/0000-0003-2057-9411
Gi-Sik Min: https://orcid.org/0000-0003-2739-3978

## CONFLICTS OF INTEREST

The authors declare no conflict of interest. Although the authors, Taeseo Park and Gi-Sik Min, serve as editors for the Animal Systematics, Evolution and Diversity, they had no involvement in the editorial or peer reviews of this manuscript.

## ACKNOWLEDGMENTS

This study was supported by the research funds from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBRE202402 and NIBR202402103). This study was also supported by the Inha University.

## REFERENCES

Carr CM, Hardy SM, Brown TM, Macdonald TA, Hebert PDN, 2011. A tri-oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. PLoS ONE, 6:e22232. https://doi.org/10.1371/journal.pone.0022232

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

Fauchald K, 1992. A review of the genus *Eunice* (Polychaeta: Eunicidae) based upon type material. Smithsonian Contributions to Zoology, 523:1-422. https://doi.org/10.5479/si.00810282.523

Giribet G, Carranza S, Baguñà J, Riutort M, Ribera C, 1996. First molecular evidence for the existence of a Tardigrada+Arthropoda clade. Molecular Biology and Evolution, 13:76-84. https://doi.org/10.1093/oxfordjournals.molbev.a025573

Imajima M, Hartman O, 1964. The polychaetous annelids of Japan. Occasional Papers of the Allan Hancock Foundation, 26:1-452.

Ip YCA, Tay YC, Gan SX, Ang HP, Tun K, Chou LM, Huang D, Meier R, 2019. From marine park to future genomic observatory? Enhancing marine biodiversity assessments using a biocode approach. Biodiversity Data Journal, 7:e46833. https://doi.org/10.3897/BDJ.7.e46833

Izuka A, 1912. The errantiate polychaeta of Japan. Journal of the College of Science, Imperial University of Tokyo, 30:1-262.

Kalyaanamoorthy S, Minh BQ, Wong TKF, Von Haeseler A, Jermiin LS, 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods, 14:587-589. https://doi.org/10.1038/nmeth.4285

Lachat J, Haag-Wackernagel D, 2016. Novel mobbing strategies of a fish population against a sessile annelid predator. Scientific Reports, 6:33187. https://doi.org/10.1038/srep33187

Miura T, 1986. Japanese polychaetes of the genera *Eunice* and *Euniphysa*: taxonomy and branchial distribution patterns. Publications of the Seto Marine Biological Laboratory, 31:269-325.

Paik EI, 1976. The polychaetous annelids in Korea (IV). Bulletin of Father Jeon's 60th Anniversary, pp. 231-242.

Paik EI, 1982. Taxonomic studies on polychaetous annelids in Korea. Research Bulletin of the Hyosung Women's University, 24:745-913 (in Korean).

Paik EI, 1984. Polychaetous annelid worms from Ulreung Island and its adjacent waters. Research Bulletin of the Hyosung Women's University, 29:127-173 (in Korean).

Paik EI, 1986. Distribution of polychaetous annelid worms from Ulnung-do coast. Bulletin of the Korean Fisheries Society, 19:363-367 (in Korean).

Paik EI, 1987. Checklist of Korean polychaetous annelids. Journal of the Basic Science Research Institute - Catholic University of Taegu-Hyosung, 1:77-92 (in Korean).

Paik EI, 1989. Illustrated encyclopedia of fauna & flora of Korea Vol. 31. Polychaeta. Ministry of Education, Seoul, pp. 1-764 (in Korean).

Pallas PS, 1788. Marina varia nova et rariora. Nova Acta Academiae Scientiarum Imperialis Petropolitanea, 2:229-249.

Palumbi S, Martin A, Romano S, MacMillan WO, Stice L, Grabowski G, 1991. The simple fool's guide to PCR. Version 2.0. University of Hawaii, Honolulu, HI, pp. 1-45.

Rho BJ, Lee JW, 1988. A systematic study on the errantiate Polychaeta in Cheju Island. Korean Journal of Systematic Zoology, 4:121-136.

Ronquist F, Huelsenbeck JP, 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics, 19:1572-1574. https://doi.org/10.1093/bioinformatics/btg180

Salazar-Vallejo SI, Carrera-Parra LF, de León-González JA, 2011. Giant Eunicid Polychaetes (Annelida) in shallow tropical and temperate seas. Revista de Biología Tropical, 59:1463-1474.

Shinomiya C, Kobayashi G, Seike K, Yamashita M, Yamamori L, Sugiyama T, Kawamura M, Nishikawa K, Goto R, 2023. Molecular and morphological assessment of juvenile and adult forms in the giant worm *Eunice* cf. *aphroditois* (Annelida: Eunicidae) and its phylogenetic position in the family. Zoological Science, 40:314-325. https://doi.org/10.2108/zs220075

Struck T, Hessling R, Purschke G, 2002. The phylogenetic position of the Aeolosomatidae and Parergodrilidae, two enigmatic oligochaete-like taxa of the 'Polychaeta', based on molecular data from 18S rDNA sequences. Journal of Zoological Systematics and Evolutionary Research, 40:155-163. https://doi.org/10.1046/j.1439-0469.2002.00200.x

Whiting MF, Carpenter JC, Wheeler QD, Wheeler WC, 1997. The Strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. Systematic Biology, 46:1-68. https://doi.org/10.1093/sysbio/46.1.1

WoRMS Editorial Board, 2025. World Register of Marine Species [Internet]. World Register of Marine Species, Ostend, Accessed 11 Sep 2025, <http://www.marinespecies.org>.

Wu BL, Shen SP, Chen M, 1975. Preliminary report of polychaetous annelids from Xisha Islands, Guangdong Province, China. Studia Marina Sinica, 10:65-104 (in Chinese).

Zanol J, Halanych KM, Fauchald K, 2014. Reconciling taxonomy and phylogeny in the bristleworm family Eunicidae (polychaete, Annelida). Zoologica Scripta, 43:79-100. https://doi.org/10.1111/zsc.12034

Zanol J, Halanych KM, Struck TH, Fauchald K, 2010. Phylogeny of the bristle worm family Eunicidae (Eunicida, Annelida) and the phylogenetic utility of noncongruent 16S, COI and 18S in combined analyses. Molecular Phylogenetics and Evolution, 55:660-676. https://doi.org/10.1016/j.ympev.2009.12.024

Zanol J, Hutchings P, 2022. A new species of giant *Eunice* (Eunicidae, Polychaeta, Annelida) from the east coast of Australia. ZooKeys, 1118:97-109. https://doi.org/10.3897/zookeys.1118.86448

Received September 30, 2025
First Revised October 20, 2025
Second Revised October 27, 2025
Accepted October 28, 2025

**Appendix 1.** List of species used for molecular analysis in this study with GenBank accession numbers of COI, 16S rDNA and 18S rDNA

| Family | Genus | Species | COI | 16S | 18S |
|---|---|---|---|---|---|
| Eunicidae | *Eunice* | *Eunice flavopicta* (Korea) | PX435308 | PX560176 | PX435319 |
| | | *Eunice flavopicta* (Korea) | PX435309 | PX560177 | PX435320 |
| | | *Eunice flavopicta* (Korea) | PX435310 | PX560178 | PX435321 |
| | | *Eunice* cf. *aphroditois* (Japan) | LC756630 | LC756644 | LC756664 |
| | | *Eunice aphroditois* (Singapore) | MN690243 | – | – |
| | | *Eunice filamentosa* | GQ497559 | GQ478155 | GQ497500 |
| | | *Eunice norvegica* | GQ497541 | GQ478147 | GQ497493 |
| | | *Eunice roussaei* | GQ497543 | GQ478149 | GQ497495 |
| | *Leodice* | *Leodice harassii* | GQ497535 | GQ478140 | GQ497486 |
| | | *Leodice torquata* | GQ497539 | GQ478145 | GQ497491 |
| | | *Leodice valens* | GQ497534 | GQ478139 | GQ497485 |
| | *Nicidion* | *Nicidion amoureuxi* | GQ497538 | GQ478144 | GQ497490 |
| | | *Nicidion mikeli* | GQ497558 | GQ478153 | GQ497498 |
| | | *Nicidion notata* | GQ497544 | GQ478152 | GQ497497 |
| | *Marphysa* | *Marphysa regalis* | GQ497562 | GQ497565 | GQ497510 |
| | | *Marphysa sanguinea* | GQ497547 | GQ478157 | GQ497502 |
| | | *Marphysa viridis* | GQ497553 | GQ478163 | GQ497508 |
| | *Palola* | *Palola viridis* | GQ497556 | GQ478167 | GQ497513 |
| | *Lysidice* | *Lysidice collaris* | GQ497557 | GQ478170 | GQ497516 |
| | | *Lysidice ninetta* | GQ497564 | GQ478169 | GQ497515 |
| Onuphidae | *Diopatra* | *Diopatra dentata* | GQ497522 | GQ478129 | GQ497475 |

COI, cytochrome c oxidase I; rDNA, ribosomal DNA.

ASED http://e-ASED.org
Animal Systematics, Evolution and Diversity

*Anim. Syst. Evol. Divers. Vol. 42, No. 1: 12-20, January 2026*
https://doi.org/10.5635/ASED.2026.42.1.002

# Morphology and Phylogeny of Two Tintinnids (Ciliophora, Spirotrichea) from Korea

Ji Hye Moon, Jae-Ho Jung*

Department of Biology, Gangneung-Wonju National University, Gangneung 25457, Korea

## ABSTRACT

This study reports two newly recorded tintinnid species collected from the coastal area of South Korea: *Leprotintinnus pellucidus* (Cleve, 1899) Jörgensen, 1901 and *Stenosemella ventricosa* (Claparède and Lachmann, 1858) Jörgensen, 1924. Morphological characteristics were examined using light and scanning electron microscopy, and phylogenetic relationships were analyzed based on small subunit ribosomal DNA sequences. Morphological observations confirmed that the collected specimens correspond to *Leprotintinnus pellucidus* and *Stenosemella ventricosa*. In addition, phylogenetic analyses reveal the non-monophyly of both genera. The new sequences, accompanied by morphological descriptions, contribute to a better understanding of tintinnid diversity in Korean waters. By integrating morphological observations with molecular data, this study enhances the accuracy of species identification and clarifies their phylogenetic placement within Tintinnida.

**Keywords:** marine ciliates, marine habitat, planktonic ciliates, taxonomy, 18S rDNA sequences

## INTRODUCTION

Tintinnid ciliates are an ecologically important group of planktonic protists commonly found in marine environments, where they play a crucial role in microbial food webs and carbon cycling (Sherr and Sherr, 1988; Pierce and Turner, 1992; Pomeroy et al., 2007). Among them, species belonging to the genera *Leprotintinnus* Jörgensen, 1900 and *Stenosemella* Jörgensen, 1924 are often reported from coastal and oceanic waters worldwide (Brandt, 1907; Kofoid and Campbell, 1929; Hada, 1937; Zhang et al., 2012).

The genus *Leprotintinnus* is characterized by a lorica with an open posterior end and the presence of agglutinated particles (Kofoid and Campbell, 1929). Although early observations of some species lacking these particles led to taxonomic confusion, this issue has been clarified by the discovery of multiple populations (Cleve, 1899; Brandt, 1907; Jörgensen, 1912, 1927; Hada, 1932, 1937). In South Korea, species of *Leprotintinnus* have traditionally been classified under the *Tintinnopsis* complex following the proposal of Alder (1999) (i.e., *T. neriticus* (Campbell, 1926) Alder, 1999, *T. nordqvisti* Brandt, 1906; National Marine Biodiversity Institute of Korea, 2024). However, considering their distinct lorica morphology and their non-monophyly in the small subunit ribosomal DNA (SSU rDNA) sequences phylogeny, further discussion on their taxonomic status is required (Kazama et al., 2012; Gruber et al., 2018).

The genus *Stenosemella* is characterized by an agglutinated bowl and a short, hyaline collar (Jörgensen, 1924). In the genus *Codonellopsis* Jörgensen, 1924, the presence of an agglutinated bowl and a hyaline collar is similar to that of *Stenosemella*, but the collar of *Stenosemella* is significantly shorter, making the two genera clearly distinguishable (Kofoid and Campbell, 1929). From South Korea, three species of *Stenosemella* (i.e., *S. nivalis*, *S. pacifica*, *S. parvicollis*) have been reported (National Institute of Biological Resources, 2024; National Marine Biodiversity Institute of Korea, 2024).

During our survey of tintinnid ciliates from the coastal waters of Korea, we collected two unrecorded species: *Leprotintinnus pellucidus* and *Stenosemella ventricosa*. In this study, we provide detailed morphological descriptions that include scanning electron micrographs and present phylogenetic analyses based on SSU rDNA sequences.

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: 82-33-640-2312, Fax: 82-33-640-2867
E-mail: jhjung@gwnu.ac.kr

**eISSN 2234-8190**

Copyright © The Korean Society of Systematic Zoology

## MATERIALS AND METHODS

### Sample collection, identification, and molecular analyses

The two tintinnid species were collected using a 20 μm plankton net from the coastal area of Gangneung Port, South Korea (37°46′20.124″N, 128°57′4.268″E) on 19 Feb 2024. The water temperature was 5.5–6.0°C and salinity was 31.0–33.4‰. This net-tow sample was fixed using two fixatives: Bouin's fluid (final concentration, 10%) and ethanol (final concentration, 70%) (Coats and Heinbokel, 1982).

The cells were isolated under a stereo microscope (SZ61; Olympus, Tokyo, Japan) from samples fixed in Bouin's fluid and ethanol. The lorica features were investigated at high magnifications using inverted microscopes (12.5–400×, IX73; Olympus) and an upright microscope (1,000×, BX53; Olympus). Most of these observations were conducted using bright-field and differential interference contrast optics. Slide preparation followed the method described in Moon and Jung (2025). Terminology and systematics follow Kofoid and Campbell (1929), Alder (1999), Agatha and Riedel-Lorjé (2006), and Santoferrara and McManus (2020). The methods for DNA extraction, PCR amplification, sequencing, and phylogenetic analyses followed those described in Moon and Jung (2024).

### Scanning electron microscopy

For scanning electron microscope observation, cells were isolated from the Bouin's fluid-fixed samples and then washed in distilled water. Subsequently, cells of the two species were each mounted onto a separate poly-L-lysine-coated coverslip. The cells were dehydrated through a graded ethanol series (30%, 50%, 70%, 90%, and two changes of 100%). The coverslip with *L. pellucidus* was dried using a critical point dryer (EMCPD300; Leica, Wetzlar, Germany), while the coverslip with *S. ventricosa* was dried using an evaporator (CVE-2200; Eyela, Tokyo, Japan) as an air-drying method to prevent specimen loss that often occurs with critical point drying. These coverslips were coated with platinum. The samples were observed in JSM-IT500 (JEOL, Tokyo, Japan) in the Center for Research Facilities at Gangneung-Wonju National University.

## RESULTS AND DISCUSSION

Phylum Ciliophora Doflein, 1901
Subphylum Intramacronucleata Lynn, 1996
Class Spirotrichea Bütschli, 1889
Order Tintinnida Kofoid and Campbell, 1929

[1]*Genus *Leprotintinnus* Jörgensen, 1900 incertae sedis in order Tintinnida

[2]****Leprotintinnus pellucidus*** **(Cleve, 1899) Jörgensen, 1901 (Figs. 1, 2)**

**Description (n = 5).** Lorica 119–327 × 43–53 μm (on average 265.2 × 47.5 μm) in size, agglutinated, cylindrical, with a slightly swollen central portion (often observed), narrowing toward both ends but pronouncedly toward the posterior, then widening at the posterior end, open at both ends (Figs. 1A–C, 2A). Anterior portion 40–46 μm (on average 43.2 μm) in opening diameter; 13–15 right-handed spiral turns, total length 96.1–105.4 μm; each spiral turn 5.0–13.9 μm long (on average 7.72 μm) (Figs. 1D, 2B, D). Posterior portion 29–40 μm (on average 35.5 μm) in opening diameter, 22–27 μm (on average 24.9 μm) at narrowest diameter (i.e., above posterior opening); widening portion 13.2–26.9° (on average 20.9°) at angle to body axis (Figs. 1E, 2C, E).

**Distribution.** Arctic and Greenland Seas (Cleve, 1899; Brandt, 1907), Korsør in Denmark (Jörgensen, 1912), Norwegian coast (Jörgensen, 1927), Okhotsk Sea and Pacific Ocean (Hada, 1932), Akkeshi Bay in Japan (Hada, 1937), Bering Sea (Wang et al., 2022), and South Korea (present study).

**Phylogenetic analyses.** The SSU rDNA sequence of *L. pellucidus* is 1,744 base pairs long with a GC content of 46.6% (GenBank accession no. PX583870). The results showed the non-monophyly of the genus *Leprotintinnus*. Furthermore, the sequence from the Korean population did not cluster with the same species collected from Japan (AB640641–AB640643) (Kazama et al., 2012), which showed a genetic distance of 0.072–0.087 (95–116 nucleotide differences) (Fig. 5).

**Remarks.** The Korean population of *L. pellucidus* closely matches the lorica shape and dimensions of the type population (119–327 × 43–53 μm vs. 240 × 40 μm) (Cleve, 1899). The lorica of the type population was described as lacking foreign particles, but later studies reported that loricae without agglutinated material may also occur depending on environmental conditions (Cleve, 1899; Brandt, 1907). Although the lorica size varies among populations, *L. pellucidus* generally maintains a shape that narrows toward the posterior and then widens at the posterior end (Cleve, 1899; Brandt, 1906, 1907; Jörgensen, 1927; Hada, 1932, 1937; Wang et al., 2022). The Akkeshi Bay population in Japan lacks a swollen central region, but its overall lorica shape and posterior opening diameter partially overlaps with those of the Korean population (Hada, 1937). SSU rDNA sequence comparisons revealed 95–116 base pair differences between the Korean population and a Japanese population studied by Kazama et al. (2012) (AB640

Korean name: [1]*열린소모충속 (신칭), [2]*곤봉열린소모충 (신칭)

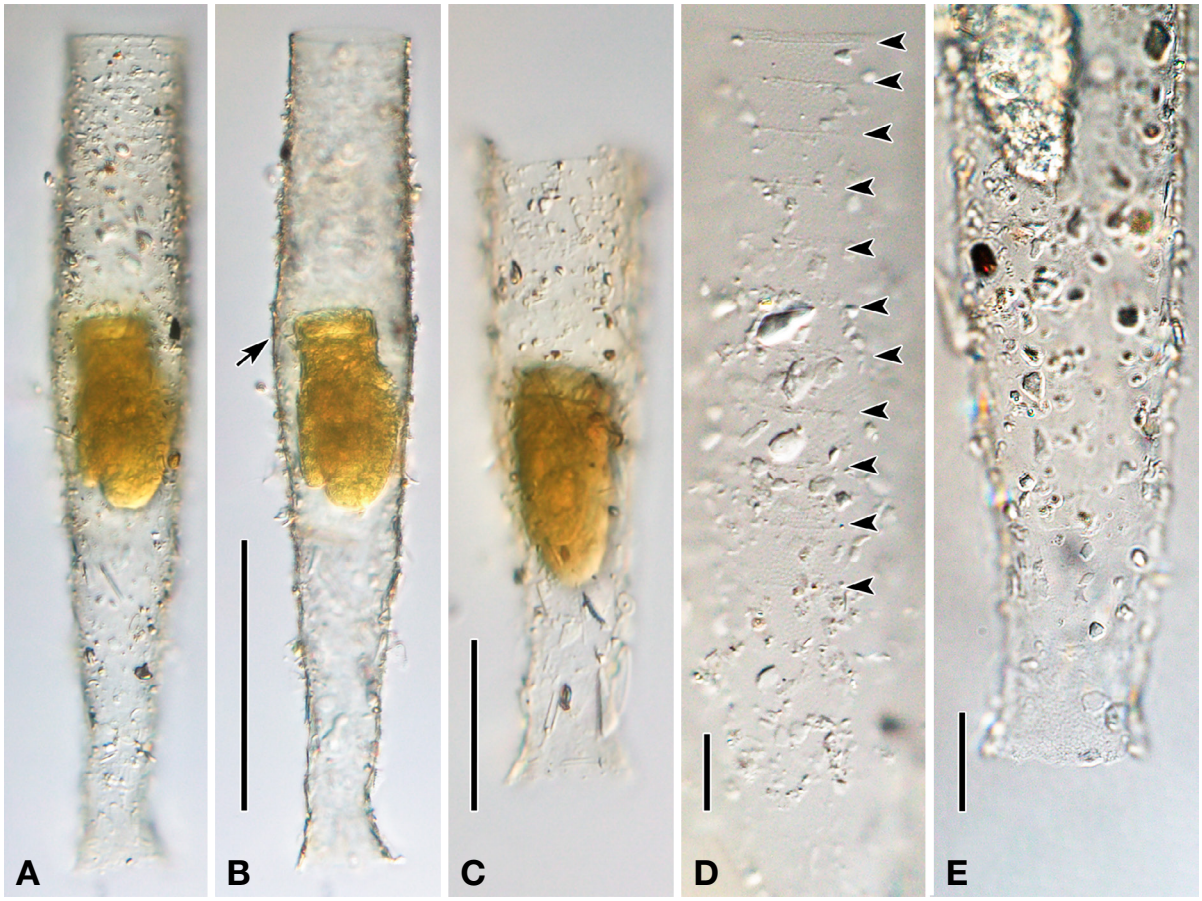


**Fig. 1.** *Leprotintinnus pellucidus* from Bouin's fluid-fixed (A–C) and ethanol-fixed (D, E) samples. A, C, Lateral views of loricae; B, Optical section through the middle of a lorica, arrow showing slightly swollen central portion; D, E, Lateral views of a lorica anterior (D) and posterior (E) portions, arrowheads showing spiral turns. The same scale is applied in A and B for comparison of size variation. Scale bars: B=100 μm, C=50 μm, D=10 μm, E=20 μm.

641–AB640643), suggesting that they are distinct species. Furthermore, the latter Japanese population does not clearly exhibit the characteristic narrowing toward the posterior and subsequent widening at the posterior end seen in the type population, indicating that it is likely a different species (Kazama et al., 2012). As the species of *Leprotintinnus* appear to be non-monophyletic, further studies are needed to clarify their relationships with the genera *Eutintinnus* and *Tintinnopsis*. In addition, it is necessary to investigate their relationships with morphologically similar species that possess an open posterior end.

In comparison with a congener, *L. simplex* Schmidt, 1901 is similar in lorica size to the Korean population of *L. pellucidus* (lorica length: 204 μm vs. 119–327 μm; anterior opening diameter: 41 μm vs. 40–46 μm); however, it can be clearly distinguished by its lorica shape, which is cylindrical with no or only very slight narrowing toward the posterior end, and without a widened posterior opening (Schmidt, 1901). Another congener, *L. nordqvisti* (Brandt, 1906) Kofoid and Campbell, 1929, differs from the Korean population of *L. pellucidus* in having a wider and fragile posterior end, with a notably larger posterior opening diameter (50–60 μm vs. 22–27 μm) (Brandt, 1906, 1907).

**Voucher slides.** Two slides have been deposited at the National Marine Biodiversity Institute of Korea (MABIK) (MABIK PR00045145, MABIK PR00045146).

Family Stenosemellidae Campbell, 1954
Genus *Stenosemella* Jörgensen, 1924

### [1]**Stenosemella ventricosa* (Claparède and Lachmann, 1858) Jörgensen, 1924 (Figs. 3, 4)

**Description (n=9).** Lorica 74–111×66–73 μm (on average

Korean name: [1]*큰짧은목소모충 (신칭)

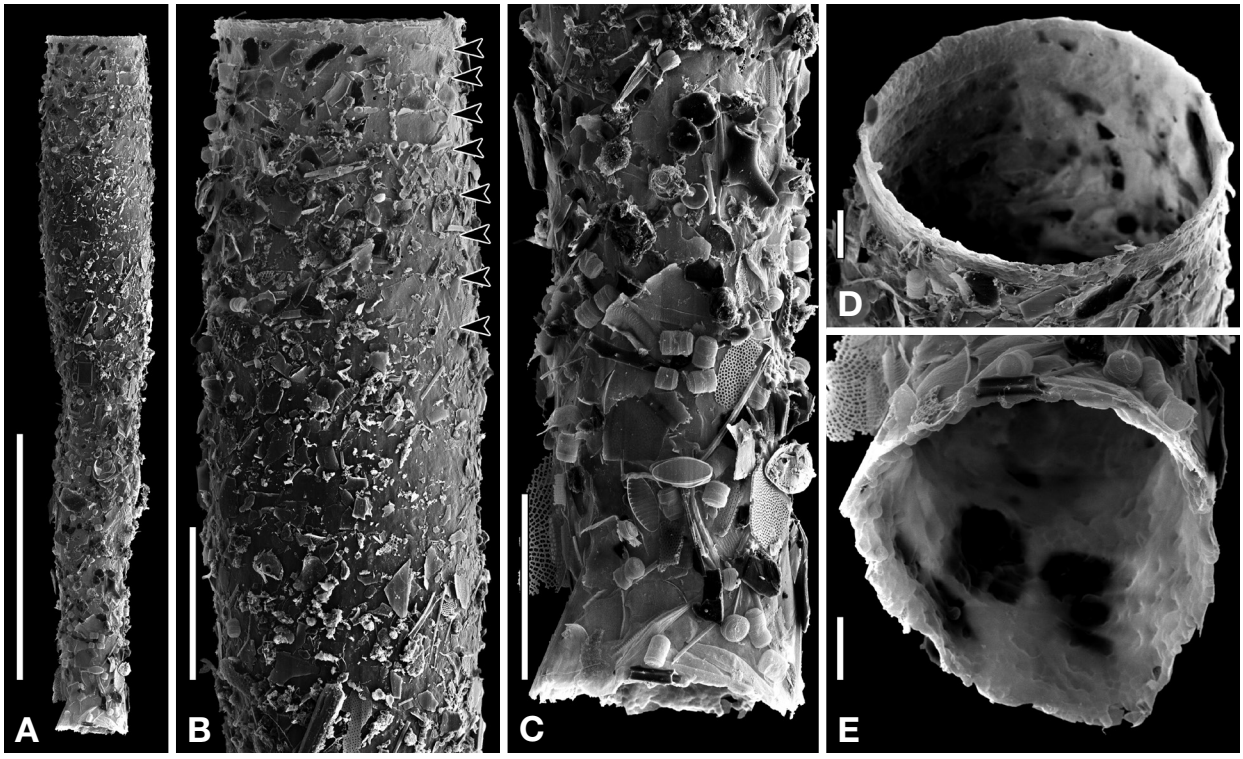


**Fig. 2.** *Leprotintinnus pellucidus* observed from a Bouin's fluid-fixed sample under a scanning electron microscope. A, Lateral view of a lorica; B, C, Lateral views of anterior (B) and posterior (C) portions of the lorica, arrowheads showing spiral turns; D, E, Apical views of anterior (D) and posterior (E) portions of the lorica showing alveoli structures in the lorica wall. Scale bars: A=100 μm, B=50 μm, C=20 μm, D, E=5 μm.

85.4×70.3 μm) in size, usually composed of agglutinated bowl, hyaline collar, and agglutinated secondary collar, rarely with only hyaline collar (Figs. 3A–C, 4A–D). Hyaline collar 31–36 μm wide (on average 34.0 μm) and 2.1–4.5 μm long (on average 3.6 μm), broadly cylindrical, slightly flared; windows 10–12 in number under SEM, often not visible or partially obscured (Figs. 3F, G, 4A, E–G). Agglutinated second collar 29–61 μm wide, and up to 36 μm long (presumed to function as a closing apparatus) (Figs. 3B–D, 4B–D, F–J). Bowl 69–74 μm long (on average 70.4 μm), broadly obovate, rounded to bluntly pointed posterior end; posterior portion 94–115° angle (on average 100.0°); lorica wall usually inconspicuous, occasionally with alveolar structures (Fig. 3D, E).

**Distribution.** Cosmopolitan (Brandt, 1907; Zhang et al., 2012; present study).

**Phylogenetic analyses.** The SSU rDNA sequence of *Stenosemella ventricosa* is 1,745 base pairs long with a GC content of 47.1% (GenBank accession no. PX583871). Phylogenetic analyses revealed that the genus *Stenosemella* and even the *S. ventricosa* sequences themselves were non-monophyletic, forming three distinct clades. The Korean population shows a genetic distance of 0.0006–0.0308 (one to forty-two nucleotide differences) to populations from the United States (EU399538, EU399539), France (JQ408170, JQ408174), and China (KU715764) (Fig. 5).

**Remarks.** The Korean population of *S. ventricosa* matches the lorica morphology described in the type population, although a detailed comparison is not possible by the lack of available dimensional data (Claparède and Lachmann, 1858). Jörgensen (1924) provided detailed measurements when establishing the genus, and the Korean specimens closely correspond to these values (maximum diameter: 65–74 μm vs. 66–73 μm; collar diameter: 31–32 μm vs. 31–36 μm; collar height: 2–3 μm vs. 2.1–4.5 μm). A secondary collar-like structure in *S. ventricosa* was not first observed in this study. A similar structure, referred to as the "Kragen", was described by Hofker (1931), who noted that it separates during cell division. However, since the division process was not observed in the present study, the identity of these structures cannot be confirmed. Nevertheless, we confirmed that the secondary collar in our specimens is capable of opening and closing, whereas Hofker's (1931) figures include not only a structure that appears to close, but also one that seems incapable of closing. This difference suggests that the "Kragen" may represent a dis-

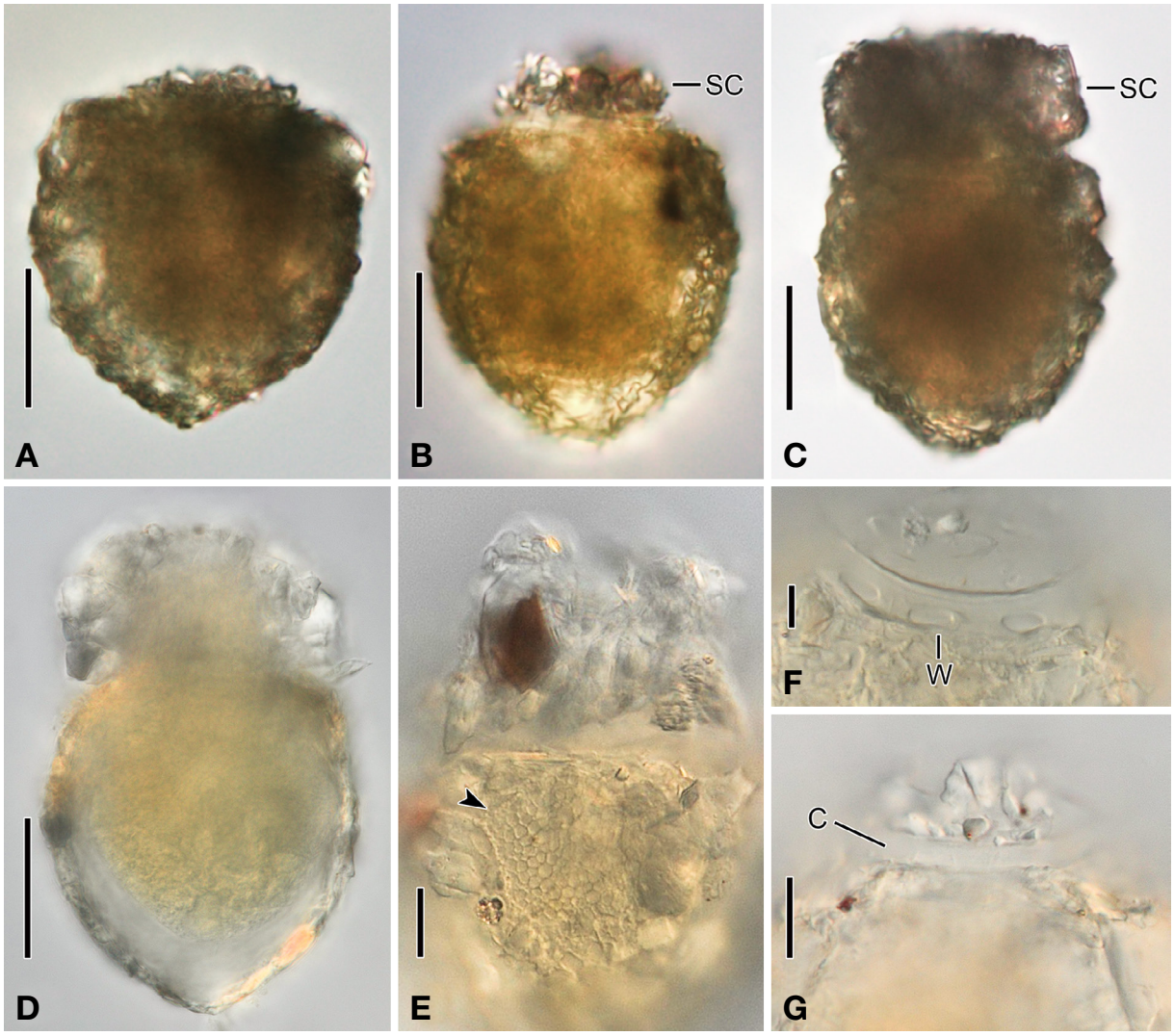


**Fig. 3.** *Stenosemella ventricosa* from a Bouin's fluid-fixed sample. A–C, Lateral views of loricae showing variability in the secondary collar; D, Optical section through the middle of a lorica showing the inner collar diameter; E, Optical section of surface of the lorica wall showing alveoli structures (arrowhead); F, G, Lateral views of a lorica anterior portion showing the presence or absence of windows. C, collar; SC, secondary collar; W, window. Scale bars: A–D = 30 μm, E, G = 10 μm, F = 5 μm.

tinct structure with a different function. Genetic distance analysis revealed relatively high values when the Korean population of *S. ventricosa* was compared with the French (0.026–0.03) and Chinese (0.023) populations. Morphologically, the French population has a taller collar (10 μm vs. 2.1–4.5 μm), and the Chinese population shows a wider collar opening diameter (43 μm vs. 31–36 μm), suggesting that further study may require taxonomic reidentification (Bachy et al., 2012; Zhang et al., 2017).

Compared with its congeners, *S. steini* (Jörgensen, 1912) Jörgensen, 1924 differs morphologically from the Korean population of *S. ventricosa* by having a more developed anterior portion of the bowl and is clearly distinguishable by its collar diameter (43–45 μm vs. 31–36 μm) (Jörgensen, 1912). Another congener, the neotype of *S. pacifica* Kofoid and Campbell, 1929, exhibits a secondary collar and an overall lorica shape similar to that of the Korean population of *S. ventricosa*, but differs markedly in lorica size (45–60 × 35–45 μm vs. 74–111 × 66–73 μm) (Agatha and Tsai, 2008). Phylogenetic analyses indicate that *Stenosemella* is non-monophyletic, it is necessary to further examine its relationships with the other genera that are clustered together. Because the collar of *Stenosemella* is sometimes indistinct under light microscopy, careful species identification is required to establish accurate phylogenetic relationships. For the US population of *S. ventricosa*, dimensional data of the collar are unavailable, and its

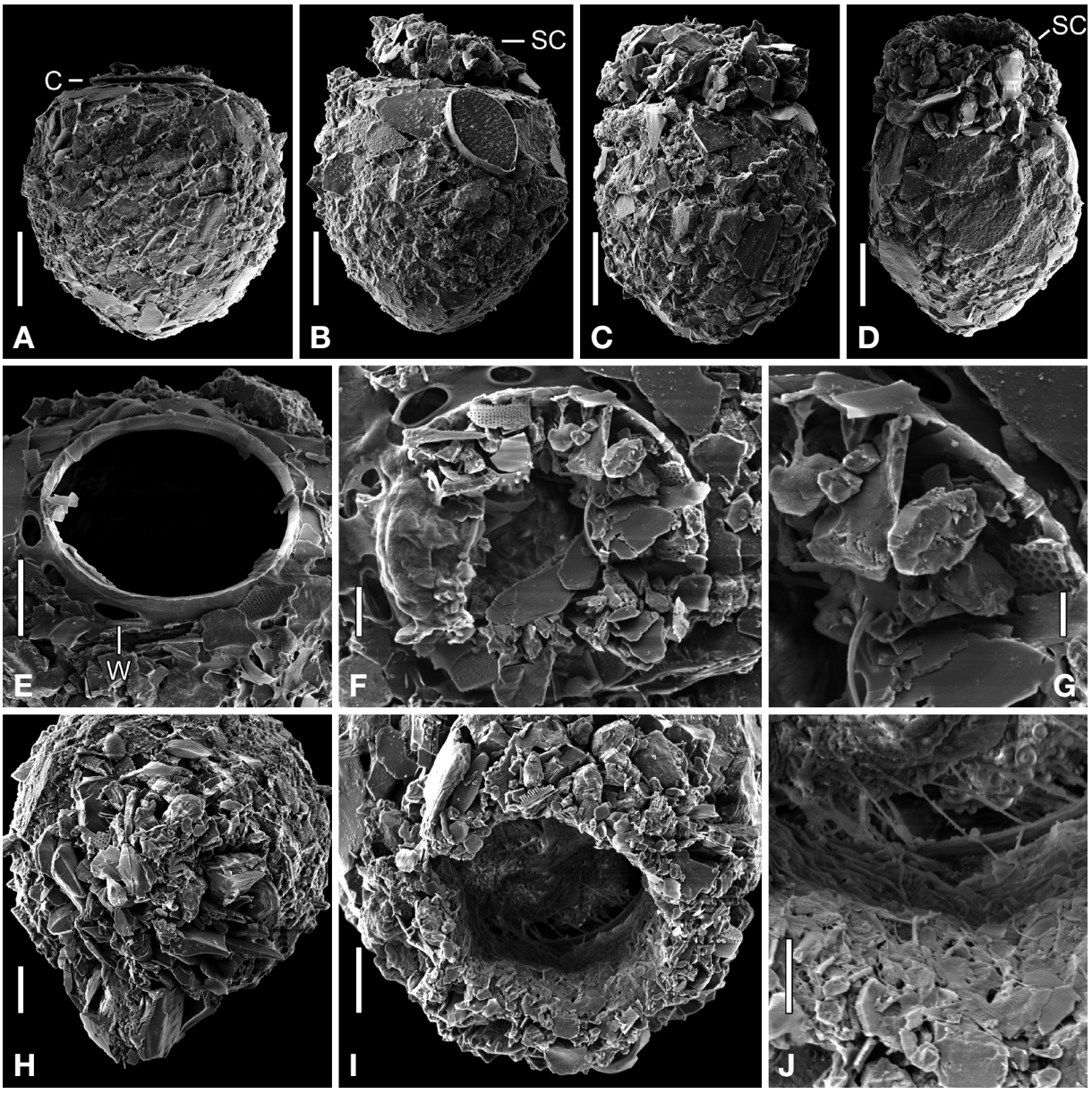


**Fig. 4.** *Stenosemella ventricosa* observed from a Bouin's fluid-fixed sample under a scanning electron microscope. A–D, Lateral views of loricae showing variability in the secondary collar. Fine particles observed on the surface of the bowl are presumed to have formed during the experimental procedure; E–G, Apical views of loricae showing windows (E, F) and a shorter secondary collar (F, G); H–J, Apical views of loricae with a taller secondary collar, showing closure (H) and opening (I, J). C, collar; SC, secondary collar; W, window. Scale bars: A–D = 20 μm, E, H, I = 10 μm, F, J = 5 μm, G = 2 μm.

SSU rDNA sequence differs by only 1–3 base pairs from the Korean population (Strüder-Kypke and Lynn, 2008). Because the available data are insufficient to assume this small difference is simply intraspecific variation, the possibility of a cryptic species cannot be excluded, and further studies including detailed morphological comparisons and analyses of other genetic markers are required.

**Voucher slides.** A total of thirty-eight slides have been deposited: three (MABIK PR00045142–MABIK PR00045144) at the National Marine Biodiversity Institute of Korea (MABIK), and thirty-five (GUC11444–GUC11449, GUC11451, GUC11452, GUC11454–GUC11456, GUC11458–GUC11479, GUC11481, GUC11482) in the Jung Lab (J.-H. Jung) at Gangneung-Wonju National University.

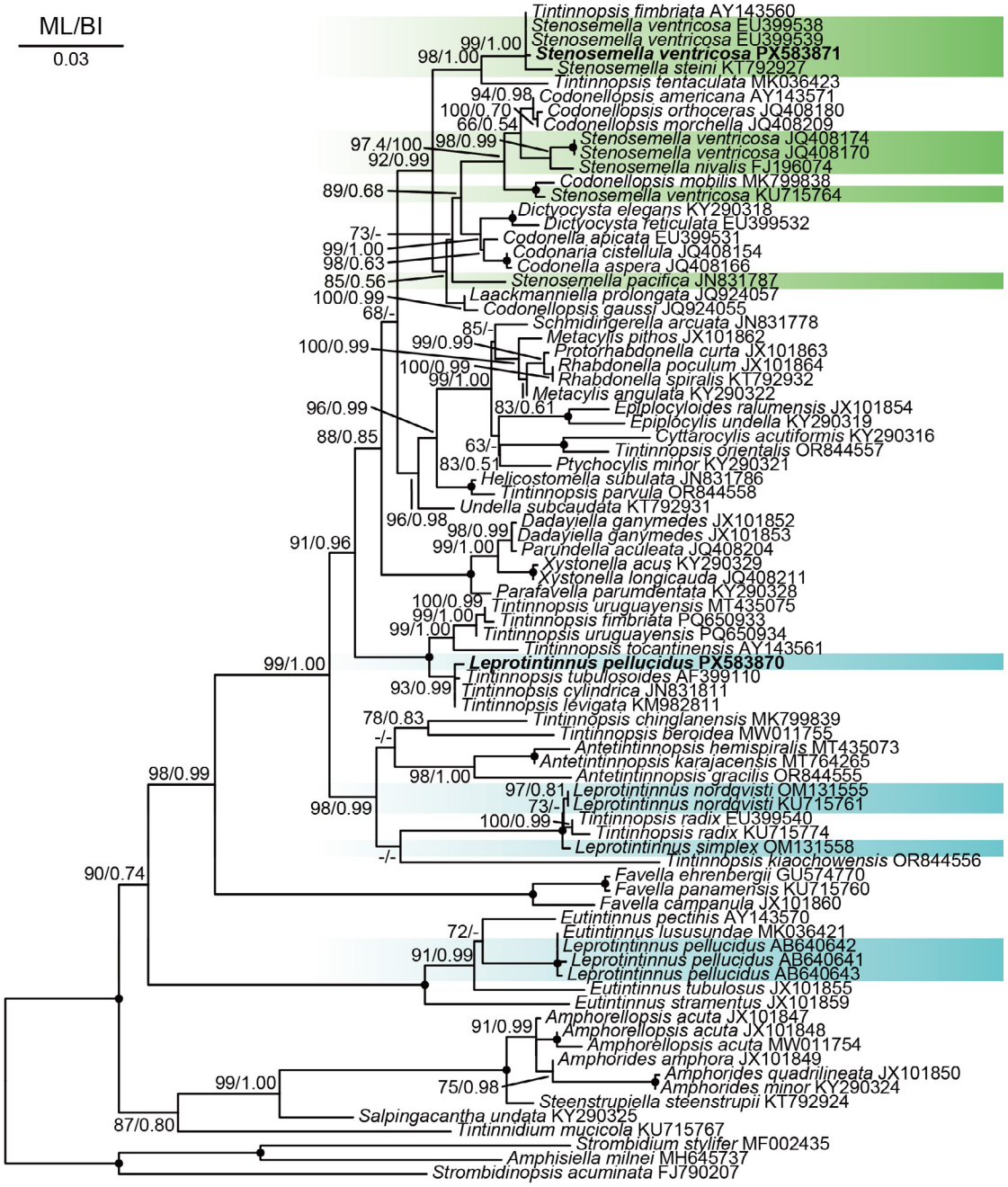


**Fig. 5.** Maximum likelihood (ML) tree based on SSU rDNA sequences, showing the phylogenetic position of *Leprotintinnus pellucidus* and *Stenosemella ventricosa*. Newly obtained sequences are in bold. GenBank accession numbers follow species names. Numbers at the nodes represent the ML bootstrap values and the Bayesian inference (BI) posterior probabilities (solid circles = full supporting values). Dashes indicate posterior probabilities < 0.5, bootstrap values < 50%, or different topologies in ML and BI phylogenies. The scale bar represents 0.03 substitutions per site.

## ORCID

Ji Hye Moon: https://orcid.org/0000-0001-9889-0096
Jae-Ho Jung: https://orcid.org/0000-0001-5497-8678

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

## ACKNOWLEDGMENTS

This work was supported by the management of Marine and Fishery Bio-resources Center (2025) funded by the National Marine Biodiversity Institute of Korea (MABIK).

## REFERENCES

Agatha S, Riedel-Lorjé JC, 2006. Redescription of *Tintinnopsis cylindrica* Daday, 1887 (Ciliophora: Spirotricha) and unification of tintinnid terminology. Acta Protozoologica, 45:137-151.

Agatha S, Tsai SF, 2008. Redescription of the tintinnid *Stenosemella pacifica* Kofoid and Campbell, 1929 (Ciliophora, Spirotricha) based on live observation, protargol impregnation, and scanning electron microscopy. Journal of Eukaryotic Microbiology, 55:75-85. https://doi.org/10.1111/j.1550-7408.2008.00309.x

Alder VA, 1999. Tintinnoinea. In: South Atlantic zooplankton. Vol. 1 (Ed., Boltovskoy D). Backhuys Publishers, Leiden, pp. 321-384.

Bachy C, Gómez F, López-García P, Dolan JR, Moreira D, 2012. Molecular phylogeny of tintinnid ciliates (Tintinnida, Ciliophora). Protist, 163:873-887. https://doi.org/10.1016/j.protis.2012.01.001

Brandt K, 1906. Die Tintinnodeen der Plankton-Expedition. Tafelerklärungen nebst kurzer Diagnose der neuen Arten. Ergebnisse der Plankton-Expedition der Humboldt-Stiftung, 3:1-33.

Brandt K, 1907. Die Tintinnodeen der Plankton-Expedition. Systematischer Teil. Ergebnisse der Plankton-Expedition der Humboldt-Stiftung, 3:1-488.

Claparède É, Lachmann J, 1858. Études sur les infusoires et les rhizopodes. Mémoires de l'Institut National Genevois, 5:1-260.

Cleve PT, 1899. Plankton collected by the Swedish expedition to Spitzbergen in 1898. Svenska Akademiens Handlingar, 32:1-52.

Coats DW, Heinbokel JF, 1982. A study of reproduction and other life cycle phenomena in planktonic protists using an acridine orange fluorescence technique. Marine Biology, 67:71-79. https://doi.org/10.1007/BF00397096

Gruber MS, Strüder-Kypke M, Agatha S, 2018. Redescription of *Tintinnopsis everta* Kofoid and Campbell 1929 (Alveolata, Ciliophora, Tintinnina) based on taxonomic and genetic analyses: discovery of a new complex ciliary pattern. Journal of Eukaryotic Microbiology, 65:484-504. https://doi.org/10.1111/jeu.12496

Hada Y, 1932. The Tintinnoinea from the Sea of Okhotsk and its neighborhood. Journal of the Faculty of Science Hokkaido Imperial University Series 4 Zoology, 2:37-59.

Hada Y, 1937. The fauna of Akkeshi bay: IV. The pelagic ciliata. Journal of the Faculty of Science Hokkaido Imperial University Series 4 Zoology, 5:143-216.

Hofker J, 1931. Studien über Tintinnoidea. Archiv Für Protistenkunde, 75:315-402.

Jörgensen E, 1912. Bericht über die von der schwedischen Hydrographisch-Biologischen Kommission in den schwedischen Gewässern in den Jahren 1909-1910 eingesammelten Planktonproben. Svenska Bydrografisk Biologiska Kommissionens Skrifter, 4:1-20.

Jörgensen E, 1924. Mediterranean Tintinnidae. Report on the Danish Oceanographical Expeditions, 1908-1910, Mediterranean and Adjacent Seas, Vol. II (Biol.), J3:1-110.

Jörgensen E, 1927. Ciliata. Tintinnidae. In: Die Tierwelt der Nord- und Ostsee, Lieferung 8 (Teil II. $c_1$) (Eds., Grimpe G, Wagler E). Akademische Verlagsgesellschaft m.b.H., Leipzig, pp. 1-26.

Kazama T, Ishida S, Shimano S, Urabe J, 2012. Discrepancy between conventional morphological systematics and nuclear phylogeny of tintinnids (Ciliophora: Choreotrichia). Plankton and Benthos Research, 7:111-125.

Kofoid CA, Campbell AS, 1929. A conspectus of the marine and freshwater Ciliata belonging to the suborder Tintinnoinea, with descriptions of new species principally from the AGASSIZ Expedition to the Eastern Tropical Pacific, 1904-1905. University of California Publications in Zoology, 34:1-403.

Moon JH, Jung JH, 2024. New records of three *Tintinnopsis* species and redescription of one *Antetintinnopsis* species (Protozoa: Ciliophora) from coastal waters of Korea. Animal Systematics, Evolution and Diversity, 40:1-15. https://doi.org/10.5635/ASED.2024.40.1.036

Moon JH, Jung JH, 2025. Morphology and molecular phylogeny of controversial tintinnids *Tintinnopsis fimbriata* and *T. uruguayensis* (Protozoa: Ciliophora) collected from coastal waters of South Korea. Journal of Species Research, 14:86-95. https://doi.org/10.12651/JSR.2025.14.1.086

National Institute of Biological Resources (NIBR), 2024. National list of Korea [Internet]. National Institute of Biological Resources, Incheon, Accessed 6 Oct 2024, <http://kbr.go.kr/>.

National Marine Biodiversity Institute of Korea (MABIK), 2024. National list of marine species [Internet]. National Marine Biodiversity Institute of Korea, Seocheon, Accessed 31 Jul 2025, <https://www.mbris.kr/pub/marine/natilist/nationalist.do>.

Pierce R, Turner J, 1992. Ecology of plankton ciliates in marine food webs. Reviews in Aquatic Sciences, 6:139-181.

Pomeroy LR, leB Williams PJ, Azam F, Hobbie JE, 2007. The microbial loop. Oceanography, 20:28-33.

Santoferrara LF, McManus GB, 2020. Diversity and biogeography as revealed by morphologies and DNA sequences. In: Zooplankton ecology (Eds., Teodosio MA, Barbosa AMB). CRC Press, Boca Raton, FL, pp. 85-118.

Schmidt J, 1901. Some Tintinnodea from the Gulf of Siam.Videnskabellige Meddeleljer fra den Natur Historiske Forening i Kjobenhaun, 1901, Kopenhagen, pp. 183-190.

Sherr E, Sherr B, 1988. Role of microbes in pelagic food webs: a revised concept. Limnology and Oceanography, 33:1225-1227. https://doi.org/10.4319/lo.1988.33.5.1225

Strüder-Kypke MC, Lynn DH, 2008. Morphological versus molecular data: phylogeny of tintinnid ciliates (Ciliophora, Choreotrichia) inferred from small subunit rRNA gene sequences. Denisia, 23:417-424.

Wang C, Xu Z, He Y, Yuan C, Li H, Zhao Y, Denis M, Zhang W, Xiao T, 2022. Neritic tintinnid community structure and mixing with oceanic tintinnids in shelf waters of the Pacific Arctic Region during summer. Continental Shelf Research, 239: 104720. https://doi.org/10.1016/j.csr.2022.104720

Zhang Q, Agatha S, Zhang W, Dong J, Yu Y, Jiao N, Gong J, 2017. Three rDNA loci-based phylogenies of tintinnid ciliates (Ciliophora, Spirotrichea, Choreotrichida). Journal of Eukaryotic Microbiology, 64:226-241.

Zhang W, Feng M, Yu Y, Zhang C, Xiao T, 2012. An illustrated guide to contemporary tintinnids in the world. Science Press, Beijing, pp. 1-499.

Received September 1, 2025
Revised October 20, 2025
Accepted November 12, 2025

ASED http://e-ASED.org
Animal Systematics, Evolution and Diversity

*Anim. Syst. Evol. Divers. Vol. 42, No. 1: 21-33, January 2026*
https://doi.org/10.5635/ASED.2026.42.1.003

# A Contribution to Two Newly Recorded *Metopus* Species (Ciliophora: Metopida) in Korea

Quoc Dung Nguyen, Sahr Uzma, Nanda Dwi Kristanti, Mann Kyoon Shin*

Department of Biological Sciences, University of Ulsan, Ulsan 44610, Korea

## ABSTRACT

In this study, we report two species of the genus *Metopus* Claparède and Lachmann, 1858, collected from the marine sediments in Busan and Jindo Island, South Korea. The species identification was carried out using both morphological characteristics and molecular analyses. The Korean population of *Metopus paravestitus* Li et al., 2021 is characterized by body size (80−105 × 30−45 μm) and body shape (elongated ellipsoidal) *in vivo*. The body surface is covered entirely by the ectosymbiotic bacteria, and needle-shaped intracytoplasmic structures are concentrated in the dome region and scattered in the cytoplasm. The adoral zone is comprised 30−38 membranelles, somatic ciliature consisted of 26−32 meridional rows. The Korean population of *Metopus pellitus* Kahl, 1932 is characterized by the body size (60−75 × 25−35 μm) and body shape (ovoid to slightly ellipsoidal) *in vivo*. The body appears hyaline but darker at the anterior end due to the presence of aggregated granules. Caudal cilia are prominently long (40−55 μm), somatic ciliature composed of 21−27 rows; adoral zone comprised 20−25 membranelles. Ectosymbiotic bacteria covered most of the cell surface, except for the dome region. The small subunit ribosomal DNA-phylogenies reconstructed by maximum likelihood and Bayesian inference provide further evidence to support the identification of the two Korean isolates of the *Metopus* species.

**Keywords:** anaerobic ciliates, cytoplasmic structure, ectosymbionts, marine, SSU rDNA

## INTRODUCTION

Free-living ciliates are among the most abundant groups of eukaryotes inhabiting the aquatic environment (Bradley et al., 2010; Gao et al., 2016; Chi et al., 2021; Gong et al., 2021). These organisms play important roles in various ecological functions, including serving as bioindicators, facilitating linkages within food webs, and acting as indicators of water clarity (Abraham et al., 2019; Luo et al., 2019; Song et al., 2019; Liu et al., 2020). Ciliates have been observed to inhabit a wide range of habitats, with the capacity to thrive in conditions characterized by low oxygen levels (Fenchel et al., 1977, 1990, 1995; Finlay, 1982; Finlay et al., 1991; Finlay and Fenchel, 1991; Esteban et al., 1993; Beaudoin et al., 2012; Orsi et al., 2012a, 2012b; Hu, 2014). Recently, research into the adaptation to depleted oxygen conditions, modified mitochondria and the symbionts of anaerobic ciliates has become an interesting subject for researchers (Rotterová et al., 2020; Graf et al., 2021; Méndez-Sánchez et al., 2024; Hao et al., 2025). Anaerobic ciliates have been intensively studied within this decade (Bourland et al., 2014, 2017a, 2017b, 2018a, 2018b, 2020, 2022, 2024; Omar et al., 2017; Li et al., 2021, 2025; Zhuang et al., 2021, 2022, 2024, 2025; Méndez-Sánchez et al., 2022; Feng et al., 2024, 2025; Pomahač et al., 2024). However, our understanding of their lifestyle remains limited compared to that of their aerobic counterparts.

The class Armophorea Lynn, 2004 is recognized as a riboclass, meaning it lacks obvious synapomorphies, and its establishment was based on the small subunit ribosomal DNA (SSU rDNA) sequence data (Lynn, 2008). Phylogenetically, the class Armophorea is non-monophyletic, so the position of some genera is unstable (Bourland et al., 2018a, 2020; Rotterová et al., 2018; Li et al., 2021; Méndez-Sánchez et al., 2022; Pomahač et al., 2024; Kristanti et al., 2025b; Zhuang et al., 2025). The corroboration of morphological and molecular traits step by step will elucidate the validity of the members of this class. While research on anaerobic ciliates has increased globally, studies in Korea remain scarce, with fewer than 10 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: 82-52-259-2396, Fax: 82-52-259-1694
E-mail: mkshin@ulsan.ac.kr

**eISSN 2234-8190**

Copyright © The Korean Society of Systematic Zoology

conducted to date (He and Choi, 2015; Omar and Jung, 2021, 2022; Nguyen et al., 2024, 2026; Nguyen and Shin, 2024; Cahyani et al., 2025; Kristanti et al., 2025a, 2025b). In an effort to expand the documentation of anaerobic ciliate diversity in Korea, this study presents the first records of two marine *Metopus* species—*M. paravestitus* and *M. pellitus*, which were identified through morphological and phylogenetic analyses.

## MATERIALS AND METHODS

### Collecting samples

*Metopus paravestitus* was isolated from the downstream area of the Jangan-cheon stream near Wollae port in Gijang-gun, Busan, South Korea (35°19′49.6″N, 129°16′43.5″E), at a salinity of 25‰, in August 2024. *Metopus pellitus* was collected from the intertidal zone in Namdong-ri, Jindo-gun, Jeollanam-do, South Korea (34°21′51″N, 126°09′48″E), at a salinity of 31‰, in October 2021. Samples were collected with sediments, organic matter, mud and ambient seawater in one litter plastic jars. After collection, the jars were stored at room temperature with lid tightly sealed to maintain the anaerobic condition. Some rice or wheat grains were added to promote bacterial growth and serve as a food source. Observations were conducted after two to four weeks after sampling.

### Morphological observations

Live cells were examined under an Axio Imager A1 microscope (Carl Zeiss, Oberkochen, Germany) using bright field (40−200×) and differential interference contrast (400−1,000×) illuminations. To reveal the ciliary pattern and nuclear apparatus, the cells were impregnated with protargol following the method of Wilbert (1975). All photomicrographs were captured using a CCD camera (AxioCam MRc, Carl Zeiss). Measurements and counts of the protargol-impregnated cells were taken from digital images captured at 1,000× magnification using the calibrated software Carl Zeiss Axiovision 4.8 (Carl Zeiss Microscopy, LLC). Line drawings were generated using Adobe Illustrator 2022. The terminology follows that of Foissner and Agatha (1999), Lynn (2008) and Bourland et al. (2014). Classification follows Jankowski (1964).

### Molecular analysis

Five individuals of each species were isolated using a glass micropipette and rinsed several times with distilled water. Each sample was then transferred to a 1.5-mL EP tube. Genomic DNA was extracted using the RED Extract-N-Amp Tissue PCR Kit (Sigma, St. Louis, MO, USA), following the manufacturer's instructions. Amplification of the SSU rDNA was then performed using TaKaRa ExTaq polymerase (TaKaRa Biomedicals, Japan), with the universal eukaryotic primers, EukA (5′-AAC CTG GTT GAT CCT GCC AGT-3′) and EukB (5′-TGA TCC ATC TGC AGG TTC ACC T-3′) (Medlin et al., 1988). The PCR amplification protocol was performed according to Méndez-Sánchez et al. (2022), involving an initial denaturation step at 94°C for 1 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 60 s, and a final extension cycle at 72°C for 10 min. The PCR products were detected using 1.2% agarose gel electrophoresis and sequenced with the following primers EukA, EukB, 900F (5′-CGA TCA GAT ACC GTC CTA GT-3′), 900R (5′-ACT AGG ACG GTA TCT GAT CG-3′) (Lu et al., 2025), and 528F (5′-CCG CGG TAA TTC CAG CTC-3′) (Alves-de-Souza et al., 2011), on an AB1 automated sequencer (Cosmo Genetech, Seoul, Korea). Geneious Prime ver. 2019.0.4 was used for sequence assembly and generating contigs.

### Phylogenetic analysis

A data set comprising two newly determined SSU rDNA sequences and 79 sequences retrieved from the NCBI GenBank was generated for phylogenetic analysis. Species belonging to the classes Heterotrichea and Karyorelictea were used as outgroup. The sequences were aligned using the MAFFT web server with the G-INS-1 method and the default parameters (Katoh et al., 2019). The alignments were then edited and trimmed manually using MEGA 7, and the final data set contains 1,754 characters. Phylogenetic trees were reconstructed using the maximum likelihood (ML) and Bayesian inference (BI) methods. The ML analysis was performed using IQ-TREE2 v.2.3.3 (Minh et al., 2020). The best evolutionary model (SYM+R5) was selected using ModelFinder implemented by IQ-TREE2 under Akaike information criterion correction (AICc) (Kalyaanamoorthy et al., 2017). Node support was assessed by ML analysis of 1,000 standard bootstrap replicates. BI analysis was performed using MrBayes 3.2.6 (Ronquist et al., 2012). The GTR+I+G model was selected as the best evolutionary model using AICc in jModeltest ver.2.1.7 (Posada and Buckley, 2004; Posada, 2008; Darriba et al., 2012). Markov Chain Monte Carlo simulations were run for 10,000,000 generations, with a sampling frequency of 100 generations until the mean standard deviation of the split frequencies was less than 0.01. The first 25% of trees were removed as burn-in. Phylogenetic tree topologies were visualized and edited using FigTree v.1.4.4 (Rambaut, 2018).

## RESULTS

### Classification

Class Armophorea Lynn, 2004

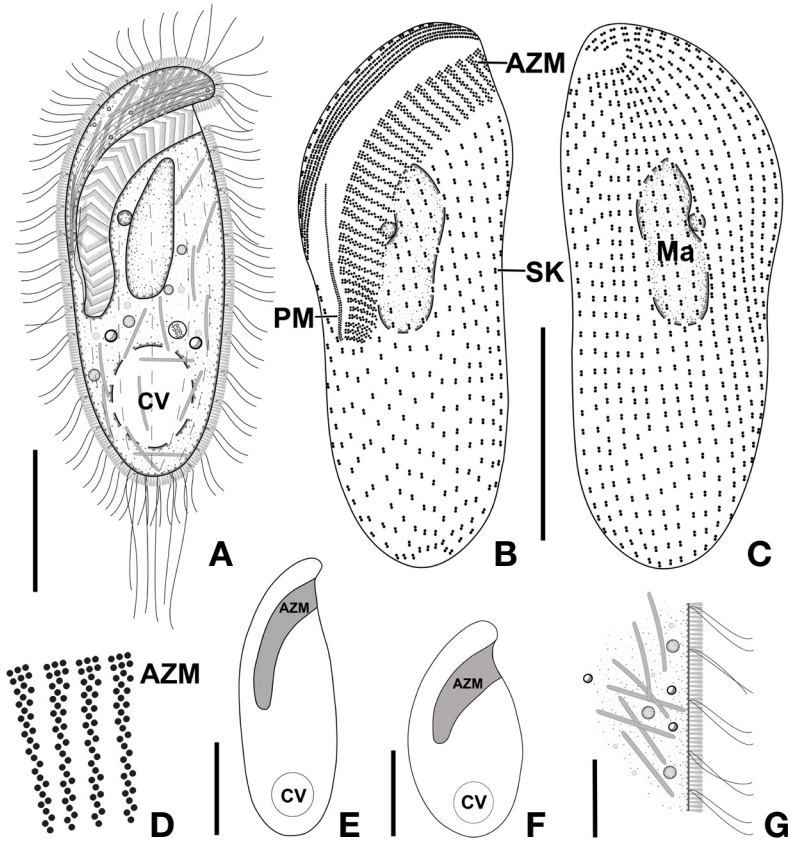


**Fig. 1.** *Metopus paravestitus* from life (A, E–G) and after protargol impregnation (B–D). A, Ventral view of a representative specimen, showing the general body shape and elongated caudal cilia at the posterior end of the cell; B, C, Ventral and dorsal views of the same specimen showing the ciliatures and nuclear apparatus; D, Detail of adoral membranelles; E, F, Different cell shapes; G, Detail showing the ectosymbiotic bacteria covering the cell surface and scattered intracytoplasmically. AZM, adoral zone of membranelles; CV, contractile vacuole; Ma, macronucleus; PM, paroral membrane; SK, somatic kineties. Scale bars: A–C, E, F = 30 μm, G = 10 μm.

Order Metopida Jankowski, 1980
Family Metopidae Kahl, 1927
Genus *Metopus* Claparède and Lachmann, 1858

[1]***1. Metopus paravesitus* Li et al., 2021 (Table 1, Figs. 1, 2)**
*Metopus paravestitus* Li et al., 2021: 2, figs. 1, 2, table 1.

**Material examined.** Sediments were collected from the downstream of the Jangan-cheon stream near Wollae port in Gijang-gun, Busan, South Korea (35°19′49.6″N, 129°16′43.5″ E) in August 2024, salinity (25‰).
**Voucher specimens.** One slide of protargol-impregnated voucher specimens (registration number: UBL20251001003) is deposited at the Biodiversity lab, University of Ulsan, Ulsan, Korea.
**Description.** Body size 80–105 × 30–45 μm *in vivo*, with a body length to width ratio approximately 2.6:1, after protargol impregnation size 70–100 × 23–36 μm. Body shape generally elongated ellipsoidal, twisted anteriorly, posterior end rounded (Figs. 1A–C, E, F, 2A, B). Preoral dome flattened, distinctly twisted leftward. Macronucleus located at anterior half, broadly ellipsoidal shape, sometimes appeared tear-dropped shape, size of fixed specimens on average 31 × 12 μm (Figs. 1B, C, 2I–K). Single micronucleus attached to the macronucleus, usually circular shape, size 4 μm across (Fig. 2I, J). Entire cell surface covered by a layer of rod-shaped ectosymbiotic bacteria, perpendicularly oriented, each rod-shaped length about 2 μm (Figs. 1A, G, 2E, F). Cortex flexible; cytoplasm colorless, containing needle-shaped intracytoplasmic structures scattered throughout the cytoplasm, and densely concentrated at the anterior pole, size 11 × 1 μm (Figs. 1A, G, 2C, D). Contractile vacuole located terminally. Swimming moderate.

Ordinary cilia length about 11 μm; caudal cilia length distributed at cell end, 25–40 μm long. Somatic ciliature com-

Korean name: [1]*유사덮힌고개돌린섬모충

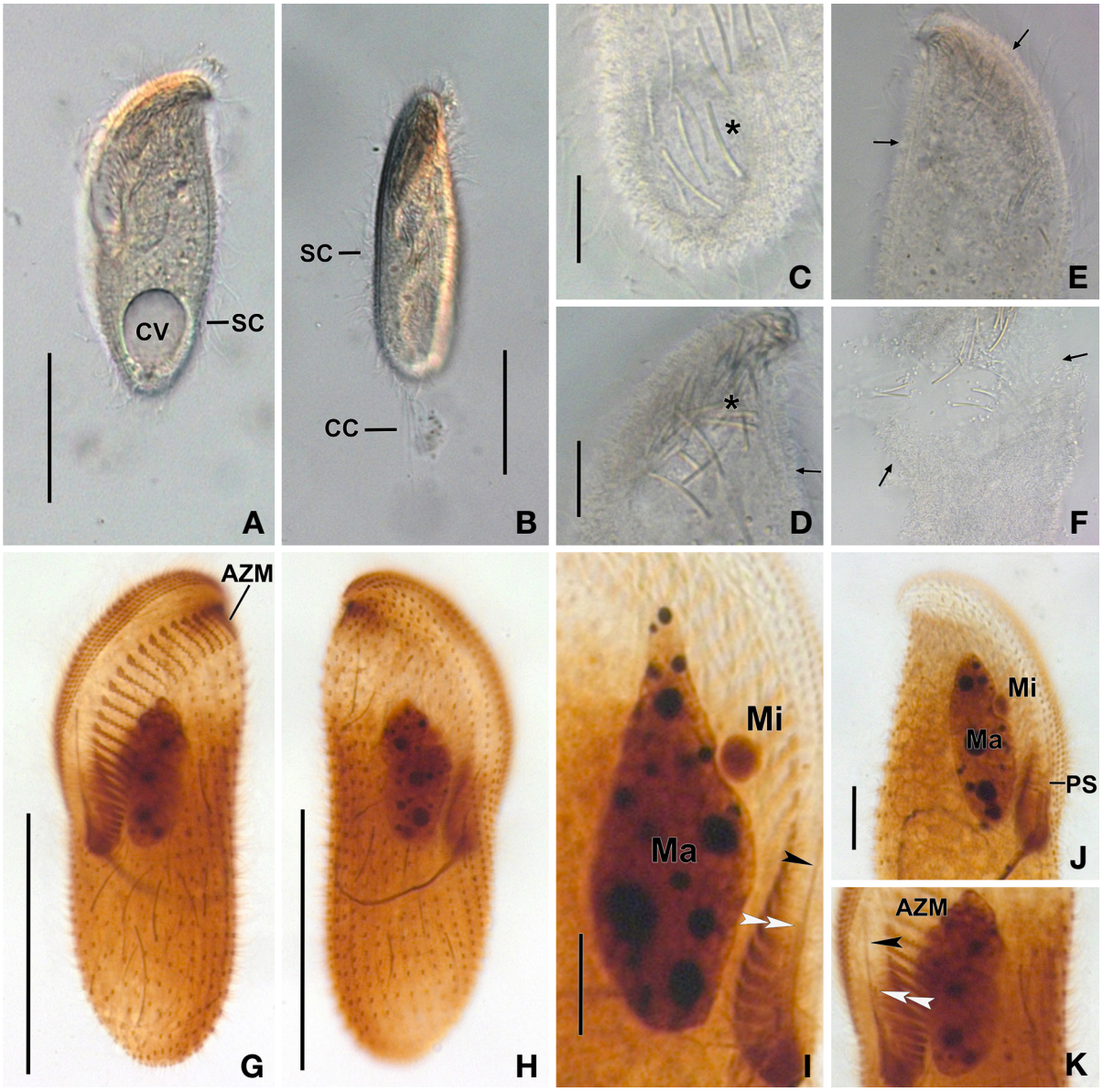


**Fig. 2.** Photomicrographs of *Metopus paravestitus* from life (A–F) and after protargol impregnation (G–K). A, Ventral view of a representative specimen; B, Right lateral view of a cell with elongated caudal cilia at the posterior end; C, D, Asterisks showing intracytoplasmic structures scattered throughout the cytoplasm (C) and concentrated in the dome region (D), black arrow indicating ectosymbiotic bacteria; E, Black arrows indicating the ectosymbiotic bacteria covering the entire cell surface; F, Arrows indicating the ectosymbiotic bacteria detached after cell burst; G, H, Ventral and dorsal views of the same specimen showing the ciliatures and nuclear apparatus; I, K, Showing the macronucleus and micronucleus, black arrowhead shows paroral membrane 1 and the white double arrowhead shows paroral membrane 2; J, Showing the end of the perizonal stripes. AZM, adoral zone of membranelles; CC, caudal cilia; CV, contractile vacuole; Ma, macronucleus; Mi, micronucleus; PS, perizonal stripes; SC, somatic cilia. Scale bars: A, B, G, H = 40 μm, C, D, I, J = 10 μm.

posed of dikinetids arranged in 26–32 meridional rows including dome kineties (Figs. 1B, C, 2G, H). Perizonal stripes invariably five rows, not forming "false kineties", perizonal stripes cilia length 14 μm (Fig. 2G, J).

Adoral zone spiraling, occupying about 55% of body length, composed of 30–38 membranelles (Figs. 1B, D, 2G). Paroral membrane composed of two files of basal bodies (PM1 and PM2), PM1 length 24 μm, and PM2 length 13 μm (Fig. 2I, K).

**Distribution.** China (Shenzhen), Korea (present study).

**Table 1.** Morphometric data of *Metopus paravestitus* (upper lines) and *M. pellitus* (lower lines)

| Characters | Mean | M | SD | CV | Min | Max | n |
|---|---|---|---|---|---|---|---|
| Body, length | 84.7 | 84 | 9.9 | 11.6 | 70 | 100 | 17 |
| | 62.8 | 63 | 7.5 | 11.9 | 46 | 81 | 22 |
| Body, width | 30.7 | 31 | 3.7 | 12.1 | 23 | 36 | 17 |
| | 24.3 | 25 | 3.3 | 13.4 | 16 | 34 | 22 |
| Body length to width, ratio | 2.8 | 2.7 | 0.3 | 10.8 | 2.1 | 3.2 | 17 |
| | 2.6 | 2.6 | 0.3 | 10.7 | 2.1 | 3.1 | 22 |
| Anterior body end to proximal end of adoral zone, distance | 48.5 | 50 | 4.7 | 9.8 | 40 | 56 | 17 |
| | 36.5 | 36 | 2.6 | 7.2 | 31 | 41 | 22 |
| Distance anterior body end to proximal end of adoral zone, % of body length | 57.4 | 57.1 | 2.3 | 4.0 | 53 | 62 | 17 |
| | 58.6 | 58.5 | 4.6 | 7.8 | 47.9 | 67 | 22 |
| Anterior body end to distal end of adoral zone, distance | 7.8 | 8 | 1.5 | 18.8 | 5 | 10 | 16 |
| | 7.7 | 8 | 1.3 | 16.8 | 5 | 10 | 21 |
| Distance anterior body end to distal end of adoral zone, % of body length | 9.3 | 9.5 | 2.2 | 24.0 | 5 | 12.7 | 16 |
| | 12.5 | 12.3 | 1.8 | 14.0 | 9.6 | 15.6 | 21 |
| Anterior body end to posterior end of macronucleus, distance | 45.8 | 46 | 4.7 | 10.3 | 37 | 54 | 17 |
| | 28.2 | 29.0 | 3.5 | 12.3 | 22.0 | 33.0 | 20 |
| Distance anterior body end to posterior end of macronucleus, % of body length | 54.3 | 53.5 | 3.7 | 6.9 | 47.4 | 60 | 17 |
| | 45.0 | 44.5 | 3.8 | 8.5 | 38.7 | 54.5 | 20 |
| Macronucleus, length | 31.7 | 30 | 4.6 | 14.7 | 23 | 42 | 16 |
| | 17.7 | 17 | 2.2 | 12.6 | 14 | 22 | 20 |
| Macronucleus, width | 12.1 | 11 | 4.5 | 36.9 | 8 | 26 | 16 |
| | 11.2 | 11 | 1.8 | 16.3 | 7 | 14 | 20 |
| Micronucleus, diameter | 3.8 | 4 | 0.8 | 20.0 | 3 | 5 | 13 |
| | 3.4 | 3.2 | 0.5 | 14.6 | 2.7 | 4.3 | 11 |
| Paroral membrane 1, length | 24.0 | 23 | 2.2 | 9.0 | 22 | 30 | 15 |
| | 14.3 | 13 | 2.9 | 20.3 | 12 | 20 | 10 |
| Paroral membrane 2, length | 13.4 | 14 | 1.7 | 12.6 | 10 | 15 | 8 |
| | – | – | – | – | – | – | – |
| Adoral membranelles, number | 33.2 | 33 | 2.4 | 7.2 | 30 | 38 | 15 |
| | 22.4 | 22.5 | 1.5 | 6.7 | 20 | 25 | 20 |
| Somatic kineties, number | 28.9 | 29 | 1.5 | 5.1 | 26 | 32 | 16 |
| | 24.1 | 24 | 1.6 | 6.6 | 21 | 27 | 19 |
| Perizonal stripes, number | 5 | 5 | 0 | 0 | 5 | 5 | 17 |
| | 5 | 5 | 0 | 0 | 5 | 5 | 22 |

All measurements in μm. All data are based on protargol-impregnated specimens.
M, arithmetic mean; SD, standard deviation; CV, coefficient of variation (%); Min, minimum value; Max, maximum value; n, number of individuals investigated.

### [1]*2. *Metopus pellitus* Kahl, 1932 (Table 1, Figs. 3, 4)

*Metopus contortus* var. *pellitus* - Kahl, 1932: 417, fig. $70_{39}$.
*Metopus pellitus* Kahl, 1932 - Carey, 1992: 156, fig. 590.

**Material examined.** Sediments were collected from the intertidal zone in Namdong-ri, Jindo-gun, Jeollanam-do, South Korea (34°21′51″N, 126°09′48″E) in October 2021, salinity (31‰).

**Voucher specimens.** One slide of protargol-impregnated voucher specimens (registration number: UBL20251001004) is deposited at the Biodiversity lab, University of Ulsan, Ulsan, Korea.

**Improved diagnosis.** Including data from the Korean population, Kahl (1932), and Carey (1992). Size about 60–100 × 25–50 μm *in vivo*. Body shape ovoid to slightly ellipsoidal with rounded posterior region. Ectosymbiotic bacteria about 3 μm in length; covering on the cell surface, except for the dome region. Caudal cilia prominent and straight, up to 70% of body length. Adoral zone about 22 membranelles; large; broadening from the proximal to the distal region. Somatic kineties approximately 24 rows.

**Description.** Body size 60–75 × 25–35 μm *in vivo*, with a body length to width ratio approximately 2.2:1, and after protargol impregnation size 46–81 × 16–34 μm. Cells fairly hya-

Korean name: [1]*두툼고개돌린섬모충

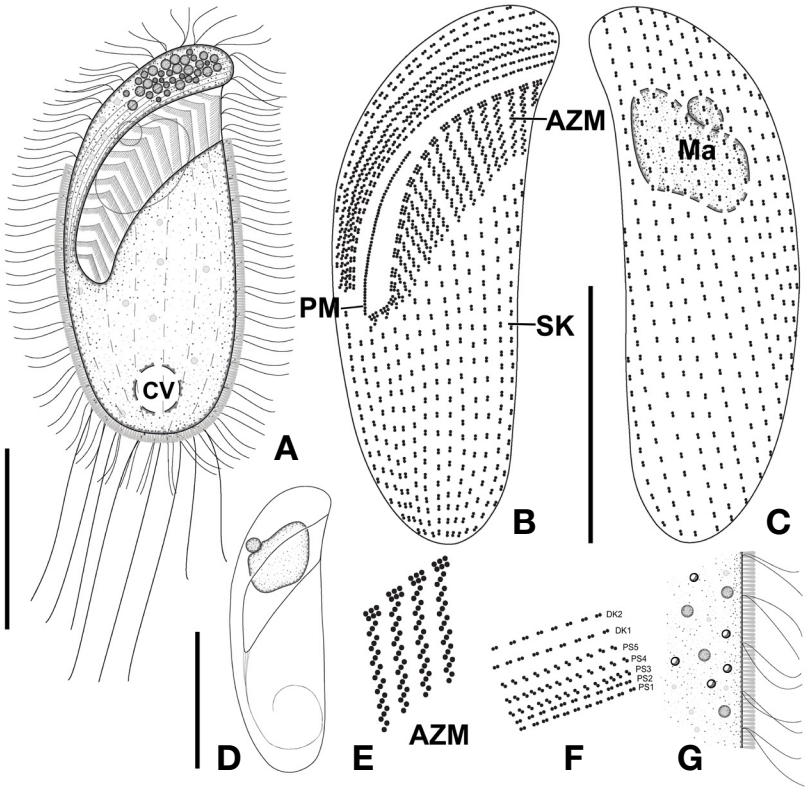


**Fig. 3.** *Metopus pellitus* from life (A, G) and after protargol impregnation (B–F). A, Ventral view of a representative specimen showing the general body shape and prominent caudal cilia at the posterior end of the cell; B, C, Ventral and dorsal views of the same specimen showing the ciliatures and nuclear apparatus; D, Showing the nuclear apparatus and coiled pharyngeal fibers; E, Detail of the adoral membranelles; F, Arrangement of the perizonal stripes and dome kineties; G, Showing the ectosymbiotic bacteria covering the cell surface. AZM, adoral zone of membranelles; CV, contractile vacuole; DK, dome kineties; Ma, macronucleus; PM, paroral membrane; PS, perizonal stripes; SK, somatic kineties. Scale bars: A–D = 30 μm.

line but appearing darker at the anterior end due to the aggregated granules in the preoral dome region. Body shape ovoid to slightly ellipsoidal, wider posteriorly than anteriorly; both margins parallel, at cell equator right margin leaned leftwards, resembling a right-trapezoidal (Figs. 3A–C, 4A, B, E, F). Preoral dome flat, roughly the same width as the body at the cytostome, twisted to left, occupying about 60% of the body length. Macronucleus located in the upper mid-body, sometimes extending to the dome, typically broadly ellipsoidal shape, somewhat bean-shaped, size 17 × 11 μm on average. Micronucleus located in the anterior half to one-third of the left side of macronucleus, spherical shape, size about 3.5 μm across (Figs. 3C, D, 4G). Contractile vacuole terminal. Cortex flexible, cell surface, except for the dome region, covered perpendicularly by ectosymbiotic bacteria, each bar-shaped 3 μm long (Figs. 3A, G, 4B, C). Cytoplasm colorless, filled with some spherical granules size about 1–2 μm. The anterior end comprised cytoplasmic aggregated granules packed densely with sizes 1–2 μm (Figs. 3A, 4A, B, D). Swimming pace moderate, rotating along the body axis.

Ordinary somatic cilia about 10 μm, while perizonal stripe cilia longer about 15 μm in length; caudal cilia very conspicuous and straight, distributed posteriorly, some strands curved rightwards making a bundle-like at the end, length *in vivo* 40–55 μm (Figs. 3A, 4A, D), about 70% of the body length. Somatic ciliature composed of dikinetids, arranged in 21–27 longitudinally rows (Figs. 3B, C, 4E, F). Perizonal stripes composed of dikinetids arranged in five rows, stripe rows 1, 2 and 3 closed together, densely ciliated, while rows 3–5 separated from each other (Figs. 3F, 4H).

Adoral zone occupied about 47% of the body length, commencing from the distal end of perizonal stripes across the ventral side to the right margin where it curved rightwards to enter the buccal cavity (Figs. 3B, E, 4H). Adoral zone comprised 20–25 membranelles; with the widest membranelles at the middle area of adoral zone about 9 μm in protargol-impregnated specimens (Figs. 3E, 4H). Paroral membrane length on average 13 μm (Fig. 4H). Cytopharyngeal fibers long and coiled in the protargol impregnation (Fig. 3D).

**Distribution.** Germany (Kiel) and Korea (this study).

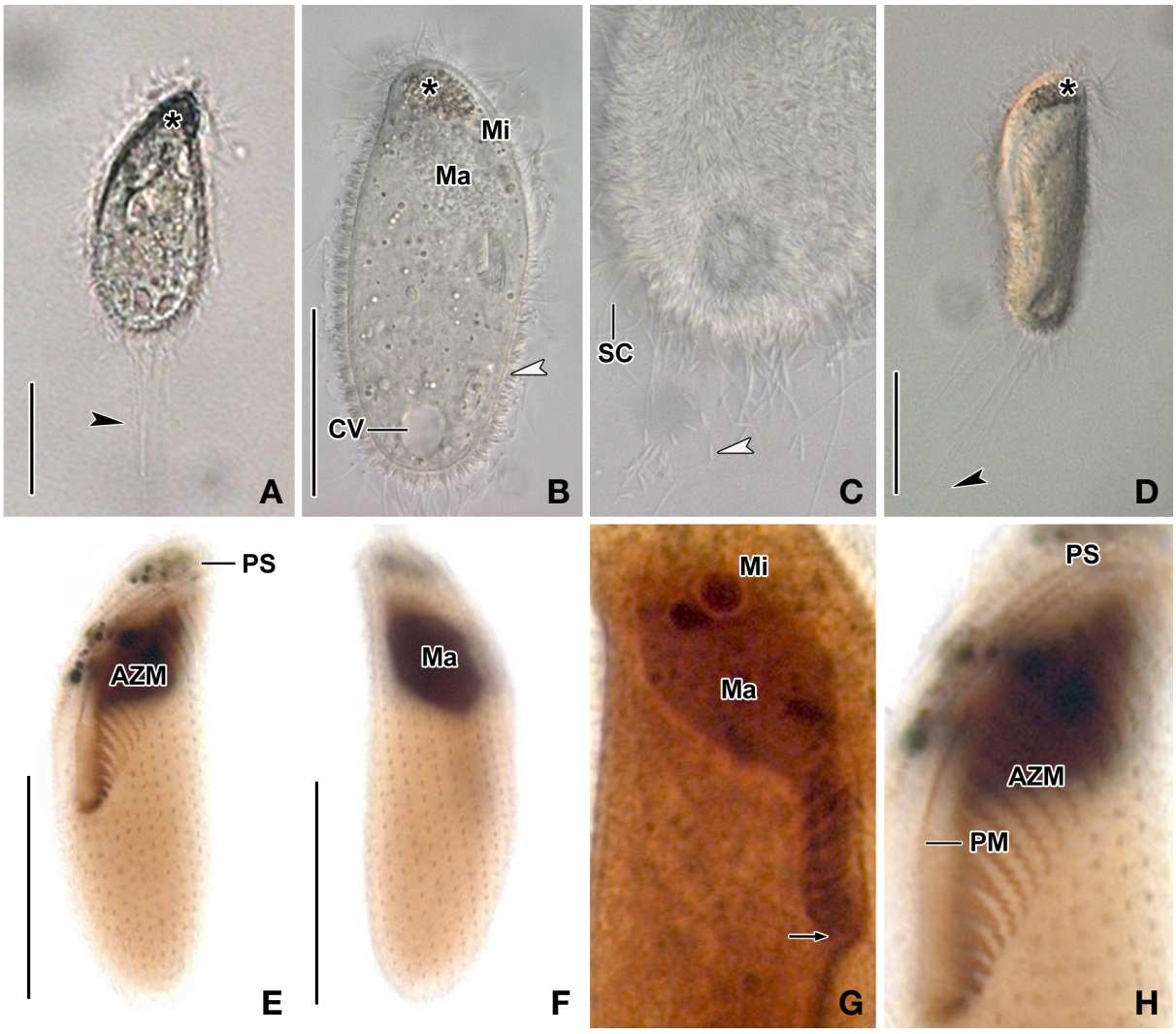


**Fig. 4.** Photomicrographs of *Metopus pellitus* from life (A–D) and after protargol impregnation (E–G). A, Ventral view showing the general shape, the dome packed with aggregated granules (asterisk), and prominent caudal cilia (black arrowhead); B, Showing the nuclear apparatus, the dome with aggregated granules (asterisk) and the ectosymbiotic bacteria (white arrowhead); C, Cell surface covered by rod-shaped ectosymbiotic bacteria, with some spreading under pressure (white arrowhead); D, Ventral-lateral view showing the dome of aggregated granules (asterisk) and the distribution of caudal cilia (black arrowhead); E, F, Ventral and dorsal views of a representative specimen showing the ciliatures and nuclear apparatus; G, Detail of the nuclear apparatus and the proximal end of adoral zone of membranelles (black arrow); H, Ventral view showing the adoral zone of membranelles, perizonal stripes, and paroral membrane. AZM, adoral zone of membranelles; CV, contractile vacuole; Ma, macronucleus; Mi, micronucleus; PM, paroral membrane; PS, perizonal stripes; SC, somatic cilia. Scale bars: A, B, D–F = 30 μm.

## Molecular analysis

The partial SSU rDNA sequences of the two species have been obtained. The Korean isolate of *Metopus paravestitus* (accession number: PX593253) has a length of 1,720 bp and a GC content of 44.5%. While the Korean isolate of *M. pellitus* (accession number: PX593254) has a length of 1,716 bp and a GC content of 44.4%.

The phylogenetic trees reconstructed by ML and BI analyses are mostly congruent; therefore, only the ML tree is shown here, with the supporting values derived from both analyses indicated at the corresponding nodes (Fig. 5). The tree topologies show that family Metopidae Kahl, 1927 is non-monophyletic. All marine *Metopus* species are nested together, forming a clade that is distinct from their freshwater congeners, with maximum support (100% ML/1.00 BI). Within this clade, *M. paraes* Zhuang et al., 2022 is in a basal position (100% ML/1.00 BI) between two sister clades. The first of these includes *M. contortus* (Quennerstedt, 1867) Kahl, 1932,

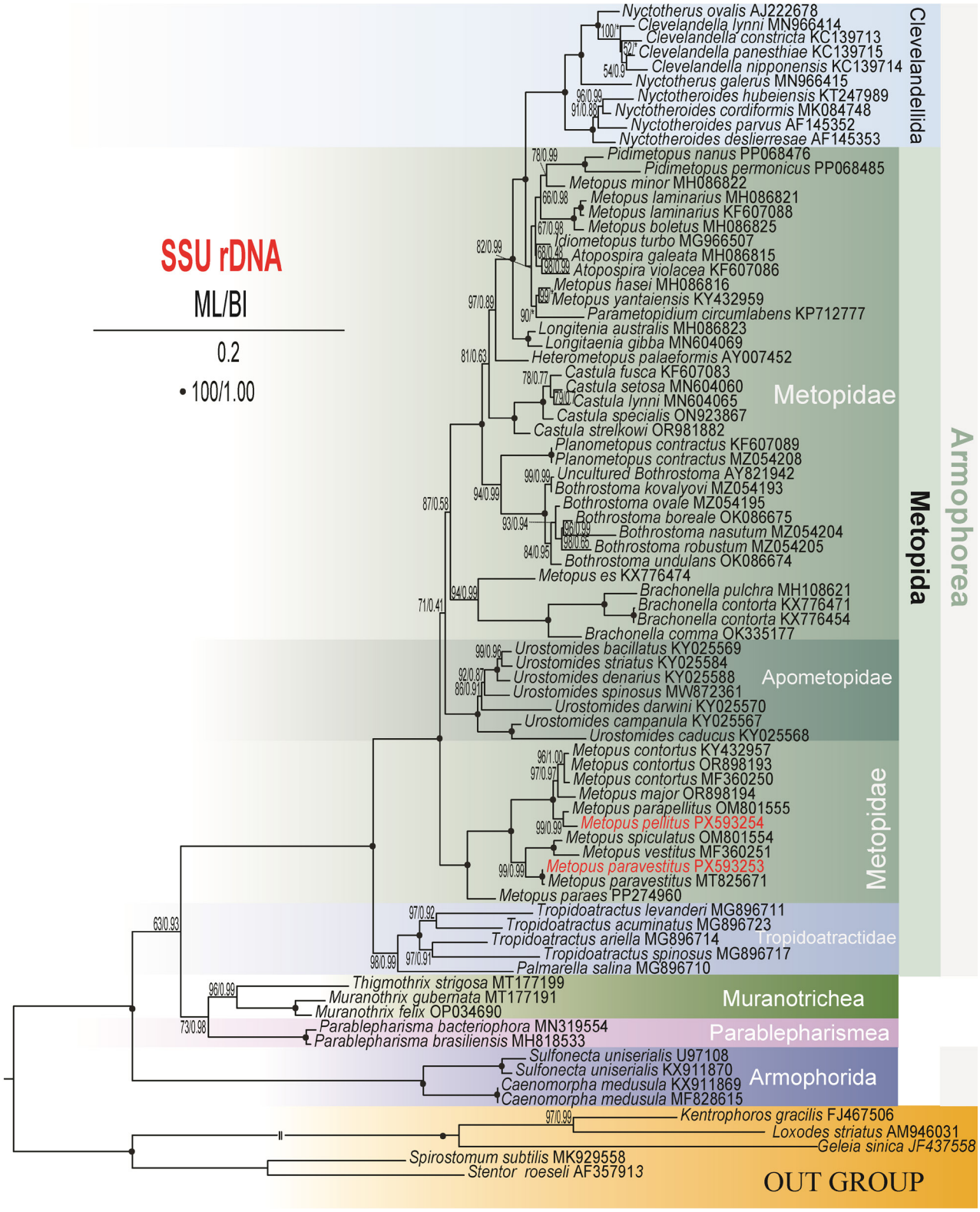


**Fig. 5.** Phylogenetic tree based on small subunit ribosomal DNA (SSU rDNA) sequences, showing the phylogenetic position of the Korean population of *Metopus paravestitus* and *M. pellitus*. The values at the nodes represent the maximum likelihood bootstrap values and the Bayesian inference posterior probabilities. Asterisks (*) indicate disagreements in topology between the two methods. The two newly obtained sequences are marked in red. The scale bar represents 20 changes per 100 nucleotide positions.

*M. major* Kahl, 1932, *M. pellitus*, and *M. parapellitus* Zhuang et al., 2022, with full support (100% ML/1.00 BI). The second includes *M. spiculatus* Zhuang et al., 2022, *M. vestitus* Kahl, 1932, and *M. paravestitus* with strong support (99% ML/0.99 BI). The Korean population of *M. paravestitus* clusters with the Chinese population (MT825671) with maximum support (100% ML/1.00 BI). The two populations share 99.9% of similarity and one nucleotide difference. The Korean population of *M. pellitus* clusters with *M. parapellitus* (99% ML/0.99 BI); the two species share 98.7% similarity and 20 nucleotide differences.

## DISCUSSION

### Morphological comparison of two species from Korea with their marine *Metopus* congeners

Phylogenetic analyses typically show that *Metopus* species cluster into two major clades: a marine clade and a freshwater clade. All *Metopus* species found in saline environments belong to the same clade, with the exception of *Metopus es* (Müller, 1776) Lauterborn, 1916. Therefore, only species inhabiting saline environments will be compared with our species. The Korean population of *Metopus paravestitus* matches its original description by Li et al. (2021) in terms of body shape, the number of adoral membranelles (30−38 vs. 27−37), presence of intracytoplasmic structures and ectosymbionts covering the entire the cell surface. However, our study found that the paroral membrane is composed of two files of basal bodies, which were not mentioned by Li et al. (2021). Furthermore, the Korean population of *M. paravestitus* exhibits fewer somatic kineties than the Chinese population (26−32 vs. 30−42), which is possibly due to their difference in body width (30−45 vs. 25−60 μm). While some features are variable, other aspects strongly support the identification of the Korean population as *M. paravestitus*.

*Metopus paravestitus* can be distinguished from *M. contortus*, *M. major*, and *M. paraes* by the presence of ectosymbiotic bacteria covering the cell surface, and the cytoplasmic structures throughout the cytoplasm, which are absent in the latter three species. The other two species, *M. spiculatus* and *M. vestitus* harbor ectosymbionts and cytoplasmic structures reassembling those of *M. paravestitus*. Nevertheless, *M. spiculatus* can be readily distinguished from *M. paravestitus* by its distinctive preoral dome, which forms a beak-like structure, and the fact that its posterior end tapers into a tail. *Metopus paravestitus* can be differentiated from *M. vestitus* primarily by the body shape (elongated ellipsoidal vs. cone-like), and the tail-like structure at the posterior end (absent vs. present) (Nguyen et al., 2024). *Metopus paravestitus* can be distinguished from *M. parapellitus* by its dark, aggregated granules in the dome (absent vs. present), its intracytoplasmic structure (present vs. absent), and the number of adoral membranelles (30−38 vs. 19−23) (Zhuang et al., 2022). It can also be distinguished from *M. pellitus* by its larger body size *in vivo* (80−105 × 30−45 vs. 60−75 × 25−35 μm), the number of somatic kineties (26−32 vs. 21−27), and the number of adoral membranelles (30−38 vs. 20−25).

The Korean population of *Metopus pellitus* mostly resembles the descriptions of Kahl (1932) and Carey (1992) in terms of body size and shape; ectosymbiotic bacteria covering the cell surface; and long caudal cilia extending up to two-thirds of the body length; marine habitat. The closest species, *Metopus parapellitus*, was described by Zhuang et al. (2022) and is characterized by a marine habitat, rod-shaped ectosymbiotics, and caudal cilia located at the rear and to the right posteriorly. However, the two species can still be differentiated by the number of somatic kineties (21−27 vs. 28−35), the length of the caudal cilia (40−55 vs. 30 μm), and the body size of the Korean population of *M. pellitus*, which is slightly smaller than that of *M. parapellitus* (60−75 × 25−35 vs. 50−100 × 25−40 μm) (Zhuang et al., 2022). *Metopus pellitus* can be distinguished from *M. contortus* and *M. major* by the presence of ectosymbionts, smaller body size, the number of adoral membranelles (20−25 vs. 33−45 vs. 89−112), and number of somatic kineties (21−27 vs. 37−47 vs. 54−74), respectively (Li et al., 2025). *Metopus pellitus* is different from *M. paraes* by the ectosymbionts (present vs. absent), and macronucleus shape (ellipsoidal vs. C-shape to sigmoidal) (Zhuang et al., 2022). The presence of a tail-like structure distinguishes *M. spiculatus* and *M. vestitus* from *M. pellitus*.

### Phylogenetic position

Our analysis shows that the family Metopidae is non-monophyletic, which is consistent with the findings of previous studies (Bourland et al., 2018a, 2020; Rotterová et al., 2018; Li et al., 2021; Méndez-Sánchez et al., 2022; Pomahač et al., 2024). According to the phylogenetic tree, the marine clade of *Metopus* is distinct from members inhabiting freshwater habitats, in accordance with many previous studies (Bourland et al., 2014, 2020; Omar et al., 2017; Li et al., 2021, 2025; Zhuang et al., 2022). A recent study by Li et al. (2025) suggested that the marine *Metopus* species may represent a new genus and that ecological niche differentiation could have driven divergence within the genus *Metopus*.

Within this marine clade, the four species—*Metopus contortus*, *M. major*, *M. parapellitus*, and *M. pellitus*—are grouped together, likely reflecting their morphological similarities, such as their elongated shape, long caudal cilia at the posterior end, and aggregated granules in the dome. While the below clade comprises *M. spiculatus*, *M. vestitus*, and *M. paravestitus* which share the generic features such as the ectosymbiotic

bacteria densely covering the cell surface, and intracytoplasmic structures in the cytoplasm. This suggests that their phylogenetic proximity may be linked to these distinctive traits.

## ORCID

Quoc Dung Nguyen: https://orcid.org/0009-0008-3104-0407
Sahr Uzma: https://orcid.org/0009-0004-1979-3984
Nanda Dwi Kristanti: https://orcid.org/0009-0008-3822-2617
Mann Kyoon Shin: https://orcid.org/0000-0001-6592-1204

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

## ACKNOWLEDGMENTS

This research was supported by the National Research Foundation of Korea, funded by the Ministry of Education of the Republic of Korea (Grant No. NRF-2021R1I1A2048744).

## REFERENCES

Abraham JS, Sripoorna S, Maurya S, Makhija S, Gupta R, Toteja R, 2019. Techniques and tools for species identification in ciliates: a review. International Journal of Systematic and Evolutionary Microbiology, 69:877-894. https://doi.org/10.1099/ijsem.0.003176

Alves-de-Souza C, Cornet C, Nowaczyk A, Gasparini S, Skovgaard A, Guillou L, 2011. *Blastodinium* spp. infect copepods in the ultra-oligotrophic marine waters of the Mediterranean Sea. Biogeosciences, 8:2136-2136. https://doi.org/10.5194/bg-8-2125-2011

Beaudoin DJ, Bernhard JM, Edgcomb VP, 2012. A novel ciliate (Ciliophora: Hypotrichida) isolated from bathyal anoxic sediments. In: Anoxia: evidence for eukaryote survival and paleontological strategies, cellular origin, life in extreme habitats and astrobiology, Vol. 21 (Eds., Altenbach AV, Bernhard JM, Seckbach J). Springer, Dordrecht, pp. 145-154.

Bourland WA, Pomahač O, Čepička I, 2022. Morphology and phylogeny of two anaerobic freshwater ciliates: *Brachonella comma* nov. sp. and widely distributed but little known caenomorphid, *Ludio parvulus* Penard, 1922. Journal of Eukaryotic Microbiology, 69:e12892. https://doi.org/10.1111/jeu.12892

Bourland WA, Pomahač O, Čepička I, 2024. Redescription and molecular phylogeny of the freshwater metopid, *Castula strelkowi* (Jankowski, 1964) from the Czech Republic and synonymization of *Pileometopus* with *Castula*. Protist, 175: 126034. https://doi.org/10.1016/j.protis.2024.126034

Bourland W, Rotterová J, Čepička I, 2017a. Morphologic and molecular characterization of seven species of the remarkably diverse and widely distributed metopid genus *Urostomides* Jankowski, 1964 (Armophorea, Ciliophora). European Journal of Protistology, 61:194-232. https://doi.org/10.1016/j.ejop.2017.07.003

Bourland W, Rotterová J, Čepička I, 2017b. Redescription and molecular phylogeny of the type species for two main metopid genera, *Metopus es* (Müller, 1776) Lauterborn, 1916 and *Brachonella contorta* (Levander, 1894) Jankowski, 1964 (Metopida, Ciliophora), based on broad geographic sampling. European Journal of Protistology, 59:133-154. https://doi.org/10.1016/j.ejop.2016.11.002

Bourland W, Rotterová J, Čepička I, 2018a. Morphologic and molecular characterization of *Brachonella pulchra* (Kahl, 1927) comb. nov. (Armophorea, Ciliophora) with comments on cyst structure and formation. International Journal of Systematic and Evolutionary Microbiology, 68:3052-3065. https://doi.org/10.1099/ijsem.0.002888

Bourland W, Rotterová J, Čepička I, 2020. Description of three new genera of Metopidae (Metopida, Ciliophora): *Pileometopus* gen. nov., *Castula* gen. nov., and *Longitaenia* gen. nov., with notes on the phylogeny and cryptic diversity of Metopid ciliates. Protist, 171:125740. https://doi.org/10.1016/j.protis.2020.125740

Bourland W, Rotterová J, Luo X, Čepička I, 2018b. The little-known freshwater metopid ciliate, *Idiometopus turbo* (Dragesco and Dragesco-Kernéis, 1986) nov. gen., nov. comb., originally discovered in Africa, found on the Micronesian island of Guam. Protist, 169:494-506. https://doi.org/10.1016/j.protis.2018.05.004

Bourland WA, Wendell L, Hampikian G, 2014. Morphologic and molecular description of *Metopus fuscus* Kahl from North America and new rDNA sequences from seven metopids (Armophorea, Metopidae). European Journal of Protistology, 50:213-230. https://doi.org/10.1016/j.ejop.2014.01.002

Bradley MW, Esteban GF, Finlay BJ, 2010. Ciliates in chalkstream habitats congregate in biodiversity hot spots. Research in Microbiology, 161:619-625. https://doi.org/10.1016/j.resmic.2010.05.016

Cahyani N, Sahr U, Shin MK, 2025. Morphology and molecular phylogeny of two newly recorded Plagiopylean ciliate species from South Korea. Animal Systematics, Evolution and Diversity, 41:109-120. https://doi.org/10.5635/ASED.2025.41.2.023

Carey PG, 1992. Marine interstitial ciliates: an illustrated key. Chapman & Hall identification guide 2. Chapman & Hall, London, p. 156-157.

Chi Y, Chen X, Li Y, Wang C, Zhang T, Ayoub A, Warren A, Song W, Wang Y, 2021. New contribution to the phylogeny of the ciliates class Heterotrichea (Protista, Ciliophora): analyses at family-genus level and new evolutionary hypotheses. Science China Life Science, 64:606-620. https://doi.org/10.1007/s11427-020-1817-5

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

Esteban G, Finlay BJ, Embley TM, 1993. New species double the diversity of anaerobic ciliates in a Spanish lake. FEMS Microbiology Letters, 109:93-99. https://doi.org/10.1111/j.1574-6968.1993.tb06149.x

Fenchel T, Bernard C, Esteban GF, Finlay BJ, Hansen PJ, Iversen N, 1995. Microbial diversity and activity in a Danish Fjord with anoxic deep water. Ophelia, 43:45-100. https://doi.org/10.1080/00785326.1995.10430576

Fenchel T, Kristensen LD, Rasmussen L, 1990. Water column anoxia: vertical zonation of planktonic protozoa. Marine Ecology Progress Series, 62:1-10. https://doi.org/10.3354/meps062001

Fenchel T, Perry T, Thane A, 1977. Anaerobiosis and symbiosis with bacteria in free-living ciliates. Journal of Protozoology, 24:154-163. https://doi.org/10.1111/j.1550-7408.1977.tb05294.x

Feng X, Méndez-Sánchez D, Zhuang W, Li R, Pomahač O, Čepička I, Rotterová J, Hu X, 2024. Morphology, morphogenesis, and molecular characterization of *Castula specialis* sp. nov. (Ciliophora, Armophorea, Metopida). Journal of Eukaryotic Microbiology, 71:e13014. https://doi.org/10.1111/jeu.13014

Feng X, Zhuang W, Li R, Hu X, 2025. Ontogeny and phylogeny of the anaerobic genus *Urostomides* with supplementing morphological characterization of the type and one little-known species (Alveolata: Ciliophora: Armophorea). Zoological Journal of the Linnean Society, 203:zlae055. https://doi.org/10.1093/zoolinnean/zlae055

Finlay BJ, 1982. Effects of seasonal anoxia on the community of benthic ciliated protozoa in a productive lake. Archiv für Protistenkunde, 125:215-222. https://doi.org/10.1016/S0003-9365(82)80018-3

Finlay BJ, Clarke KJ, Vicente E, Miracle MR, 1991. Anaerobic ciliates from a sulphide-rich solution lake in Spain. European Journal of Protistology, 27:148-159. https://doi.org/10.1016/S0932-4739(11)80337-8

Finlay BJ, Fenchel T, 1991. An anaerobic protozoon, with symbiotic methanogens, living in municipal landfill material. FEMS Microbiology Letters, 85:169-179. https://doi.org/10.1111/j.1574-6968.1991.tb04709.x-i1

Foissner W, Agatha S, 1999. Morphology and morphogenesis of *Metopus hasei* Sondheim, 1929 and *M. inversus* (Jankowski, 1964) nov. comb. (Ciliophora, Metopida). Journal of Eukaryotic Microbiology, 46:174-193. https://doi.org/10.1111/j.1550-7408.1999.tb04602.x

Gao F, Warren A, Zhang Q, Gong J, Miao M, Sun P, Xu D, Huang J, Yi Z, Song W, 2016. The all-data-based evolutionary hypothesis of ciliated protists with a revised classification of the phylum Ciliophora (Eukaryota, Alveolata). Scientific Report, 6:24874. https://doi.org/10.1038/srep24874

Gong R, Jiang Y, Vallesi A, Gao Y, Gao F, 2021. Conjugation in *Euplotes raikovi* (Protista, Ciliophora): new insights into nuclear events and macronuclear development from micronucleate and amicronucleate cells. Microorganism, 8:162. https://doi.org/10.3390/microorganisms8020162

Graf JS, Schorn S, Kitzinger K, Ahmerkamp S, Woehle C, Huettel B, Schuber CJ, Kuypers MMM, Milucka J, 2021. Anaerobic endosymbiont generates energy for ciliates host by denitrification. Nature, 591:445-450. https://doi.org/10.1038/s41586-021-03297-6

Hao T, Su H, Quan Z, Zhang R, Yu M, Xu J, Li S, Warren A, Al-Farraj SA, Yi Z, 2025. Distinct evolutionary origins and mixed-mode transmissions of methanogenic endosymbionts are revealed in anaerobic ciliated protists. Marine Life Science and Technology, 7:700-716. https://doi.org/10.1007/s42995-025-00295-9

He Y, Choi JK, 2015. First record of four soil ciliates from Korea. Korean Journal of Environmental Biology, 33:215-222. https://doi.org/10.11626/KJEB.2015.33.2.215

Hu X, 2014. Ciliates in extreme environments. The Journal of Eukaryotic Microbiology, 61:410-418. https://doi.org/10.1111/jeu.12120

Jankowski AW, 1964. Morphology and evolution of Ciliophora. III. Diagnoses and phylogenesis of 53 sapropelebionts, mainly of the order Heterotrichida. Archiv für Protistenkunde, 107: 185-294.

Kahl A, 1932. Urtiere oder Protozoa, 1: Wimpertiere oder Ciliata (Infusoria), 3. Spirotricha. Die Tierwelt Deutschlands und der angrenzenden Meeresteile. Gustav Fischer Verlag, Jena, 25: 399-650.

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS, 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Method, 14:587-589. https://doi.org/10.1038/nmeth.4285

Katoh K, Rozewicki J, Yamada KD, 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics, 20:1160-1166. https://doi.org/10.1093/bib/bbx108

Kristanti ND, Cahyani N, Nguyen QD, Shin MK, 2025a. Expanding the family Tropidoatractidae: characterization of two new *Tropidoatractus* species (Armophorea: Metopida) from South Korea based on morphology and molecular phylogeny. Journal of Eukaryotic Microbiology, 72:e70048. https://doi.org/10.1111/jeu.70048

Kristanti ND, Nguyen QD, Shin MK, 2025b. Exploring the diversity of *Brachonella*: morphological and molecular analysis with the discovery of a new species, *Brachonella tenuicauda* nov. spec. (Ciliophora, Armophorea, Metopida). European Journal of Protistology, 99:126142. https://doi.org/10.1016/j.ejop.2025.126142

Li S, Zhuang W, Feng X, Warren A, Gong J, 2025. Morphology and molecular phylogeny of four anaerobic ciliates (Protista, Ciliophora, Armophorea), with report of a new species and a unique arrangement pattern of dikinetids in family Metopidae. Microorganisms, 13:240. https://doi.org/10.3390/microorganisms13020240

Li S, Zhuang W, Pérez-Uz B, Zhang Q, Hu X, 2021. Two anaerobic ciliates (Ciliophora, Armophorea) from China: morphol-

ogy and SSU rDNA sequence, with report of a new species, *Metopus paravestitus* nov. spec. Journal of Eukaryotic Microbiology, 68:e12822. https://doi.org/10.1111/jeu.12822

Liu W, Shin MK, Yi Z, Tan Y, 2020. Progress in studies on the diversity and distribution of planktonic ciliates (Protista, Ciliophora) in the South China Sea. Marine Life Science and Technology, 3:28-43. https://doi.org/10.1007/s42995-020-00070-y

Lu L, Wang T, Ye L, Tong Z, Lin Z, Luo X, Paiva TS, Fan X, 2025. Speciation caught in the act: derived ciliary patterns budded off from the *Oxytricha granulifera*-like lineages (Alveolata, Ciliophora). Zoologica Scripta, 54:730−751. https://doi.org/10.1111/zsc.12733

Luo X, Huang JA, Li L, Song W, Bourland WA, 2019. Phylogeny of the ciliate family Psilotrichidae (Protista, Ciliophora), a curious and poorly-known taxon, with notes on two algae-bearing psilotrichids from Guam, USA. BMC Evolutionary Biology, 19:125. https://doi.org/10.1186/s12862-019-1450-z

Lynn DH, 2008. The ciliated protozoa: characterization, classification, and guide to the literature. Springer, New York, pp. 1-605.

Medlin L, Elwood HJ, Stickel S, Sogin ML, 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. Gene, 71:491-499. https://doi.org/10.1016/0378-1119(88)90066-2

Méndez-Sánchez D, Pomahač O, Rotterová J, Bourland WA, Čepička I, 2022. Diversity and phylogenetic position of *Bothrostoma* Stokes, 1887 (Ciliophora: Metopida), with description of four new species. Protist, 173:125887. https://doi.org/10.1016/j.protis.2022.125887

Méndez-Sánchez D, Schrecengost A, Rotterová J, Koštířová K, Beinart RA, Čepička I, 2024. Methanogenic symbionts of anaerobic ciliates are host and habitat specific. ISME Journal, 18:wrae164. https://doi.org/10.1093/ismejo/wrae164

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R, 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Molecular Biology and Evolution, 37:1530-1534. https://doi.org/10.1093/molbev/msaa015

Nguyen QD, Cahyani N, Shin MK, 2024. Morphological description of three anaerobic ciliates unrecorded in Korea. Animal Systematics, Evolution and Diversity, 40:211-220. https://doi.org/10.5635/ASED.2024.40.3.004

Nguyen QD, Kristanti ND, Cahyani N, Shin MK, 2026. Step-by-step exploration the diversity of the genus *Bothrostoma* Stokes, 1887 (Ciliophora: Armophorea) in Korea using morphological and molecular analyses. European Journal of Protistology, 102:126174. https://doi.org/10.1016/j.ejop.2025.126174

Nguyen QD, Shin MK, 2024. First records of two *Bothrostoma* species (Ciliophora: Armophorea: Metopidae) from Korea. Animal Systematics, Evolution and Diversity, 40:323-331. https://doi.org/10.5635/ASED.2024.40.4.017

Omar A, Jung JH, 2021. New record of 21 ciliate species (Protozoa, Ciliophora) from South Korea. Journal of Species Research, 10:301-320. https://doi.org/10.12651/JSR.2021.10.3.301

Omar A, Jung JH, 2022. New record of five anaerobic ciliates species from South Korea. Journal of Species Research, 11:108-116. https://doi.org/10.12651/JSR.2022.11.2.108

Omar A, Zhang Q, Zou S, Gong J, 2017. Morphology and phylogeny of the soil ciliate *Metopus yantaiensis* n. sp. (Ciliophora, Metopida), with identification of the intracellular bacteria. Journal of Eukaryotic Microbiology, 64:792-805. https://doi.org/10.1111/jeu.12411

Orsi W, Charvet S, Vd'ačný P, Bernhard JM, Edgcomb VP, 2012a. Prevalence of partnerships between bacteria and ciliates in oxygen-depleted marine water columns. Frontier Microbiology, 3:341. https://doi.org/10.3389/fmicb.2012.00341

Orsi W, Edgcomb V, Faria J, Foissner W, Fowle WH, Hohmann T, Suarez P, Taylor C, Taylor GT, Vd'ačný P, Epstein SS, 2012b. Class Cariacotrichea, a novel ciliate taxon from the anoxic Cariaco Basin, Venezuela. International Journal of Systematic and Evolutionary Microbiology, 62:1425-1433. https://doi.org/10.1099/ijs.0.034710-0

Pomahač O, Méndez-Sánchez D, Čepička I, 2024. Bit to bit toward the diversity of metopids: description of the genus *Pidimetopus* n. gen. (Ciliophora: Armophorea). Journal of Eukaryotic Microbiology, 71:e13034. https://doi.org/10.1111/jeu.13034

Posada D, 2008. JModelTest: phylogenetic model averaging. Molecular Biology and Evolution, 25:1253-1256. https://doi.org/10.1093/molbev/msn083

Posada D, Buckley TR, 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Systematic Biology, 53:793-808. https://doi.org/10.1080/10635150490522304

Rambaut A, 2018. FigTree v1.4.4 [Internet]. Institute of Evolutionary Biology University of Edinbrugh, Edinbrugh, Accessed Jul 2025, <https://github.com/rambaut/figtree/releases/tag/v1.4.4>.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP, 2012. MrBayer 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

Rotterová J, Bourland W, Čepička I, 2018. Tropidoatractidae fam. nov., a deep branching lineage of Metopida (Armophorea, Ciliophora) found in diverse habitats and possessing prokaryotic symbionts. Protist, 169:362-405. https://doi.org/10.1016/j.protis.2018.04.003

Rotterová J, Salomaki E, Pánek T, Bourland W, Žihala D, Táborský P, Edgcomb VP, Beinart RA, Kolísko M, Čepička I, 2020. Genomics of new ciliate lineages provides insight into the evolution of obligate anaerobiosis. Current Biology, 30:2037-2050. https://doi.org/10.1016/j.cub.2020.03.064

Song W, Xu D, Zhang Q, Liu W, Warren A, Song W, 2019. Taxonomy and phylogeny of two poorly studied genera of marine oligotrich ciliates including descriptions of two new species: *Cyrtostrombidium paraboreale* sp. n. and *Apostrombidium orientale* sp. n. (Ciliophora: Spirotrichea). European Journal

of Protistology, 70:1-16. https://doi.org/10.1016/j.ejop.2019.05.001

Wilbert N, 1975. Eine verbesserte Technik der Protargolimprägnation für Ciliaten. Mikrokosmos, 64:171-179.

Zhuang W, Feng X, Li R, Al-Farraj SA, Hu X, 2024. Morphogenesis of an anaerobic ciliate *Heterometopus palaeformis* (Kahl, 1927) Foissner, 2016 (Ciliophora, Armophorea) with notes on its morphological and molecular characterization. Protist, 174:126007. https://doi.org/10.1016/j.protis.2023.126007

Zhuang W, Feng X, Li R, Hu X, 2025. Molecular phylogeny and taxonomy of three anaerobic ciliates including *Bothrostoma aporobustum* nov. spec. (Ciliophora, Metopida). European Journal of Protistology, 100:126155. https://doi.org/10.1016/j.ejop.2025.126155

Zhuang W, Li R, Feng X, Al-Farraj SA, Hu X, 2022. New contribution to the diversity of the anaerobic genus *Metopus* (Ciliophora, Armophorea), with descriptions of three new marine species. Frontier in Marine Science, 9:884834. https://doi.org/10.3389/fmars.2022.884834

Zhuang W, Li S, Bai Y, Zhang T, Al-Rasheid SA, Hu X, 2021. Morphology and molecular phylogeny of the anaerobic freshwater ciliate *Urostomides spinosus* nov. spec. (Ciliophora, Armophorea, Metopida) from China. European Journal of Protistology, 81:125823. https://doi.org/10.1016/j.ejop.2021.125823

Received September 2, 2025
First revised October 27, 2025
Second revised December 9, 2025
Accepted December 12, 2025

ASED http://e-ASED.org
Animal Systematics, Evolution and Diversity

*Anim. Syst. Evol. Divers. Vol. 42, No. 1: 34-42, January 2026*
https://doi.org/10.5635/ASED.2026.42.1.006

# Two Newly Recorded Species of the Genus *Holothuria* (Holothuroidea: Holothuriida: Holothuriidae) from Jejudo Island, Korea

Jinho Lee[1], Taekjun Lee[1,2,*]

[1]Marine Animal Biodiversity Center, Sahmyook University, Seoul 01795, Korea
[2]Department of Animal Resources Science, Sahmyook University, Seoul 01795, Korea

## ABSTRACT

In 2024, intertidal surveys on the southern coast of Jejudo Island yielded 15 sea cucumbers that are morphologically distinct from the three *Holothuria* species previously documented in Korea. Through examination of ossicles using light microscopy and scanning electron microscopy analysis of ossicles, we identified two species newly reported for the national fauna: *Holothuria* (*Mertensiothuria*) *leucospilota* and *H.* (*Selenkothuria*) *moebii*. *Holothuria* (*Mertensiothuria*) *leucospilota* is distinguished by the absence of tentacle ossicles, the presence of tables with flat smooth discs, and complete buttons; the ossicle assemblages of the body wall, tube feet, and longitudinal muscles are consistent with established diagnoses. *Holothuria* (*Selenkothuria*) *moebii* is characterized by a lack of tables, the presence of H-shaped plates, and coarsely granulated body-wall rods with rough or bifurcated tips; additionally, tentacle rods with terminal spines are observed, and longitudinal muscles are devoid of ossicles. The collected specimens consist of eight *H.* (*M.*) *leucospilota* and seven *H.* (*S.*) *moebii*. These findings increase the total number of *Holothuria* species recorded in Korea from three to five and reveal the presence of Indo-Pacific tropical elements on Jejudo Island.

**Keywords:** sea cucumber, morphology, scanning electron microscopy (SEM)

## INTRODUCTION

The genus *Holothuria* Linnaeus, 1767 is classified within the family Holothuriidae Burmeister, 1837, order Holothuriida Miller et al., 2017, and class Holothuroidea de Blainville, 1834 (WoRMS Editorial Board, 2025). As the most species-rich genus of sea cucumbers, it currently comprises 18 subgenera and over 160 valid species, which are broadly distributed across tropical and subtropical marine ecosystems worldwide (WoRMS Editorial Board, 2025). Diagnostic features of this genus include a single tuft of gonadal tubules positioned to the left of the mediodorsal mesentery and a diversity of ossicle forms in the body wall, such as tables, buttons, rods, and rosettes (Deichmann, 1958; Shin, 2012; Smirnov, 2012).

In South Korea, only three species of *Holothuria* have been formally documented: *H.* (*Mertensiothuria*) *hilla* Lesson, 1830, *H.* (*Lessonothuria*) *pardalis* Selenka, 1867, and *H.* (*Stauropora*) *pervicax* Selenka, 1867 (see Rho and Shin, 1986; Shin and Suh, 2006; Marine Biodiversity Institute of Korea, 2025). Nevertheless, the diversity of this genus in Korea is not well characterized, especially in southern insular areas such as Jejudo Island, which is located at the northern edge of the subtropical Indo-Pacific biogeographic zone.

During a recent survey of the intertidal zone along the southern coast of Jejudo Island, a total of 15 specimens belonging to the genus *Holothuria* were collected. These specimens displayed morphological traits that distinguish them from the three known Korean species, suggesting the presence of previously unreported taxa in this locality.

This study aims to document and describe two newly reported species of the genus *Holothuria* from Jejudo Island, utilizing comprehensive morphological analysis via scanning electron microscopy. This work helps to address existing gaps in baseline data regarding holothuroid diversity within Korean waters.

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: 82-2-3399-1751, Fax: 82-2-3399-1762
E-mail: leetj@syu.ac.kr

eISSN 2234-8190

Copyright © The Korean Society of Systematic Zoology

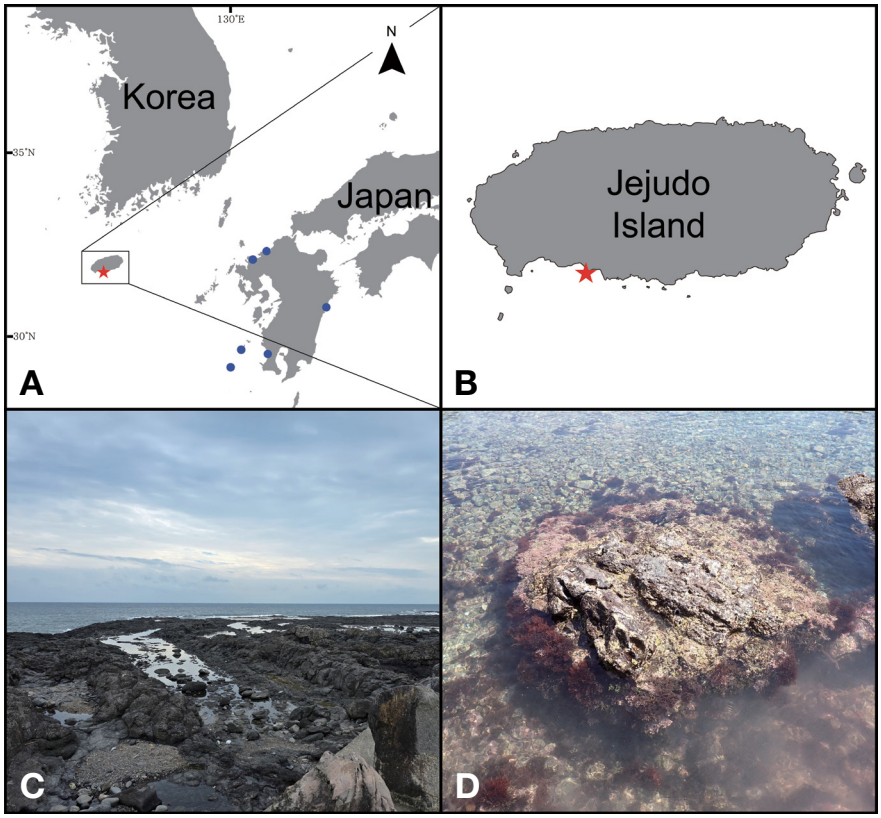


**Fig. 1.** Collection localities for two *Holothuria* species examined in this study. A, B, Sites sampled in this study are denoted by red asterisks, and blue circles indicate previously reported distribution localities; C, D, Sampling locality in this study (Jungmun, Jejudo Island).

## MATERIALS AND METHODS

Fifteen specimens of the genus *Holothuria* were collected from the intertidal zone on the southern coast of Jejudo Island during low tide (33°13′48.9″N, 126°22′30.5″E) on 17–19 October 2024 (Fig. 1). The specimens were immediately preserved in 95% ethanol. Major external morphological features were documented with a digital camera (Sony A6400; Sony Co., Tokyo, Japan). Ossicles were isolated from tissue samples obtained from principal anatomical regions, including the tentacles, dorsal and ventral body walls, dorsal papillae, and ventral tube feet, using a 5% sodium hypochlorite (NaClO) solution. The ossicles were examined using a scanning electron microscope (JSM-6510; JEOL Ltd., Tokyo, Japan).

## RESULTS

Phylum Echinodermata Klein, 1778
Subphylum Echinozoa Haeckel in Zittel, 1895
Class Holothuroidea de Blainville, 1834
Subclass Actinopoda Ludwig, 1891
Order Holothuriida Miller, Kerr, Paulay, Reich, Wilson, Carvajal & Rouse, 2017
Family Holothuriidae Burmeister, 1837
Genus *Holothuria* Linnaeus, 1767

### [1]**Holothuria (Mertensiothuria) leucospilota* (Brandt, 1835)

*Stichopus* (*Gymnochirota*) *leucospilota* Brandt, 1835: 51.
*Holothuria leucospilota*: Lampert, 1885: 71; H.L. Clark, 1920: 149; 1923: 423; Endean, 1956: 131; Tortonese, 1980: 108; James, 1983: 93; WoRMS Editorial Board, 2025: 1672774.
*Holothuria vagabunda*: Selenka, 1867: 334; Théel, 1886: 180, 281; Pearson, 1910: 181.
*Holothuria lamperti*: Ludwig, 1886: 6; Panning, 1934b: 72.
*Holothuria oxurropa*: Sluiter, 1887: 190.
*Holothuria infesta*: Sluiter, 1901: 20.
*Holothuria homoea*: H.L. Clark, 1938: 533.
*Holothuria gelatinosa*: Heding, 1939: 213–216.
*Mertensiothuria leucospilota*: Deichmann, 1958: 297.

Korean name: [1]*말랑해삼 (신칭)

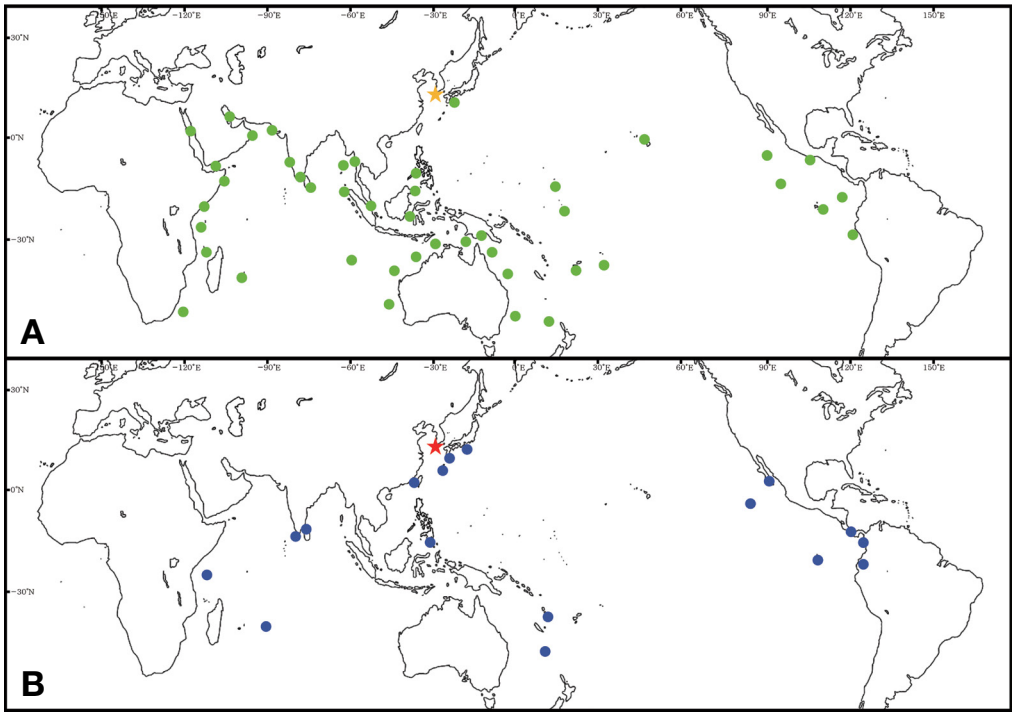


**Fig. 2.** Distribution of two *Holothuria* species in the present study (asterisk) and previous reports (circle). A, Distribution of *H.* (*Mertensiothuria*) *leucospilota* referred from Liao (1980), Clark and Rowe (1971), Cherbonnier (1988), and Alvarado et al. (2009); B, Distribution of *H.* (*Selenkothuria*) *moebii* referred from Clark and Rowe (1971) and Sloan et al. (1979).

*Holothuria* (*Mertensiothuria*) *leucospilota*: Rowe, 1969: 148; Rowe and Doty, 1977: 233; Levin, 1979: 21; Sloan et al., 1979: 122; Tortonese, 1980: 108; Liao, 1980: 115; Rowe, 1983: 156; Chao and Chang, 1989: 119; Marsh et al., 1993: 64; Kerr, 1994: 168; Rowe and Gates, 1995: 293; James, 1995: 190; Massin, 1999: 27; Samyn, 2003: 46; Samyn and Massin, 2003: 2502.

**Material examined.** Eight specimens (MERBK-H-0063–0070); the intertidal zone of Jungmun, Haye-dong, Seogwipo-si, Jejudo Island, Korea (33°14′18.9″N, 126°23′43.5″E) (Fig. 1), 17–19 Oct 2024. Collected by Jinho Lee.

**Description.** Body cylindrical. Coloration in life dark brown to black (Fig. 3A). Body wall thin, less than 1 mm in ethanol. Mouth ventrally directed, surrounded by 20 large black tentacles (Fig. 3C). Anus terminal, discharging thin elongate Cuvierian tubules. Papillae on dorsal surface. Tube feet more numerous ventrally, scattered across surface. Calcareous ring with radial plates two to three times longer than interradial plates. Polian vesicles three to four, length up to 50–60 mm. Longitudinal muscles bifid, well developed. Cuvierian tubules numerous, easily discharged, about 1 mm wide.

Dorsal and ventral body wall containing similar ossicles. Tables and buttons present (Fig. 4A, B). Tables with smooth or spinose disc edges, outline square or circular, four to six pairs of perforations. Four short pillars forming crown-shaped spire with single large central hole. Buttons with smooth rims, three to four pairs of perforations, sometimes with additional small hole near margin.

Ventral tube feet containing tables, buttons, and plates, similar to body wall ossicles (Fig. 4C). Dorsal papillae and ventral tube feet with same ossicle types (Fig. 4C, D). Longitudinal muscles with various ossicles. Tentacles lacking ossicles.

**Size.** Body: length 52–78 mm, width 18–23 mm.

Ossicles: Body wall: Table width 31–41 μm; Button length 48–55 μm, width 18–24 μm.

Tube feet: Table width 33–41 μm; Button length 42–55 μm, width 22–27 μm; Plate length 54–79 μm, width 30–33 μm.

Papillae: Table width 26–42 μm; Button length 39–45 μm, width 25–27 μm; Plate length 37–56 μm, width 38–41 μm.

**Distribution.** Korea (Jejudo Island), and the tropical to subtropical Indo-Pacific, ranging from the Red Sea and Arabian/Persian Gulf across East Africa and the Indian Ocean, through East and Southeast Asia, Australia, Melanesia, Micronesia, Polynesia, and into the eastern Pacific (Clipperton, Revillagigedo, Galápagos, and Peru) (Fig. 2A).

**Remarks.** Within Korea, three congeners of *Holothuria* have been documented: *H.* (*Mertensiothuria*) *hila*, *H.* (*Lessonothuria*) *pardalis*, and *H.* (*Stauropora*) *pervicax* (Rho and Shin,

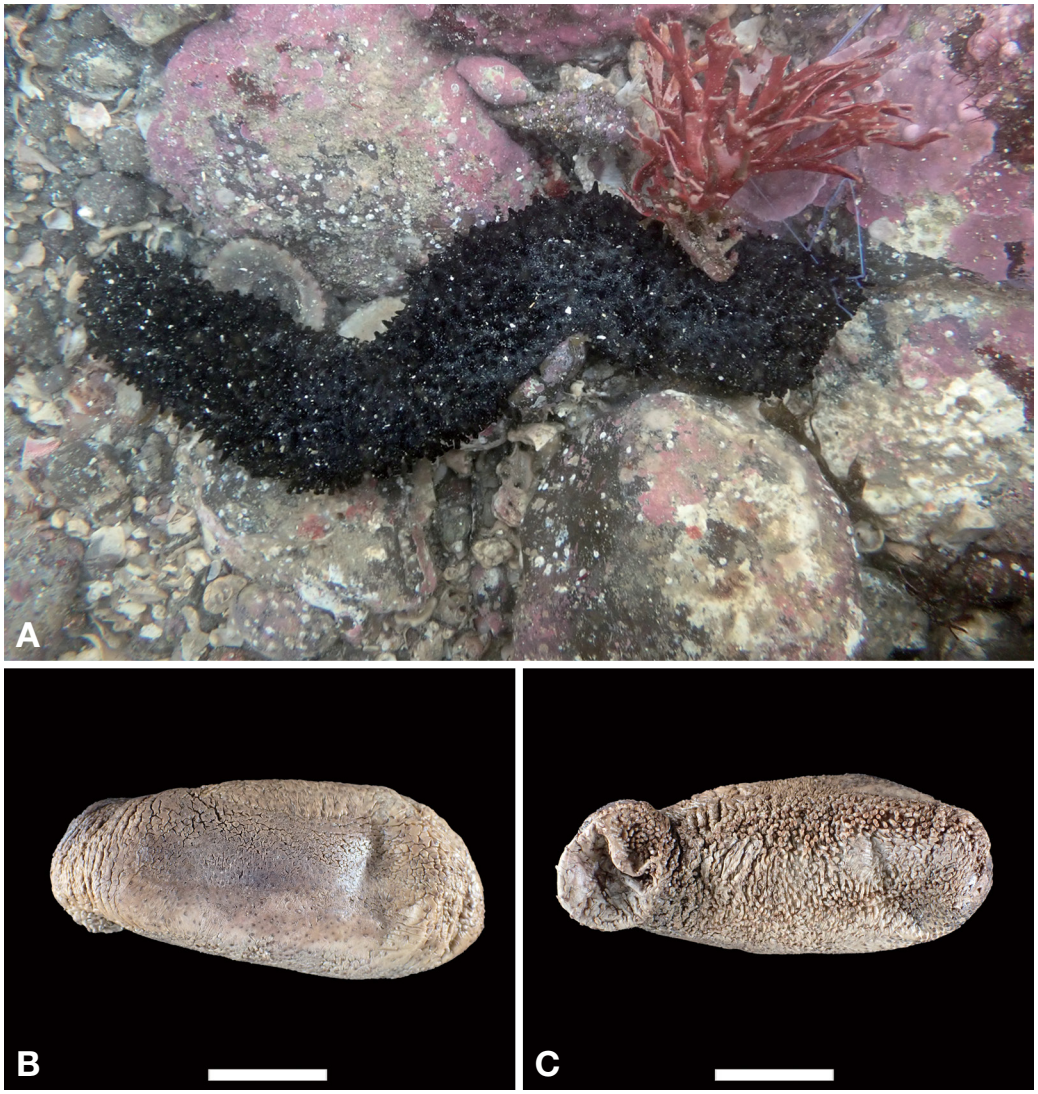


**Fig. 3.** *Holothuria* (*Mertensiothuria*) *leucospilota* (Brandt, 1835) observed in this study. A, Living specimen; B, Dorsal side of ethanol-preserved specimen; C, Ventral side of ethanol-preserved specimen. Scale bars: B, C = 2 cm.

1986; Shin and Suh, 2006; Marine Biodiversity Institute of Korea, 2025). The Jejudo specimens differ from these species based on a consistent set of morphological characteristics (Table 1): tentacle ossicles are absent (present in *H.* (*M.*) *hilla*), tables display flat and smooth discs (sculptured in *H.* (*L.*) *pardalis*), and buttons are complete (incomplete or irregular in *H.* (*S.*) *pervicax*).

Within *Holothuria* (*Mertensiothuria*) Deichmann, 1958, diagnostic traits include the presence of tables with low spires and a distinct combination of ossicles within the longitudinal muscles (Deichmann, 1958; Samyn and Massin, 2003). The Jejudo specimens exhibit these features; the ossicle profiles in their body wall, tube feet, and longitudinal muscles are consistent with those of *H.* (*M.*) *leucospilota* (Table 1). Accordingly, these comparative features support the identification of the Jejudo material as *H.* (*M.*) *leucospilota*.

[1]***Holothuria* (*Selenkothuria*) *moebii* Ludwig, 1883**

*Holothuria moebii* Ludwig, 1883: 171; Théel, 1886: 206.
*Holothuria* (*Selenkothuria*) *moebii*: Deichmann, 1958: 314, 315; Rowe, 1969: 135; 1983: 158; Clark and Rowe, 1971: 179; Sloan et al., 1979: 122; Chao, 1998: 143.
*Holothuria lubrica* var. *moebii*: Mitsukuri, 1912: 89–98.
*Holothuria* (*Holothuria*) *lubrica* var. *moebii*: Panning, 1934a: 46.

Korean name: [1]*뫼비우스해삼 (신칭)

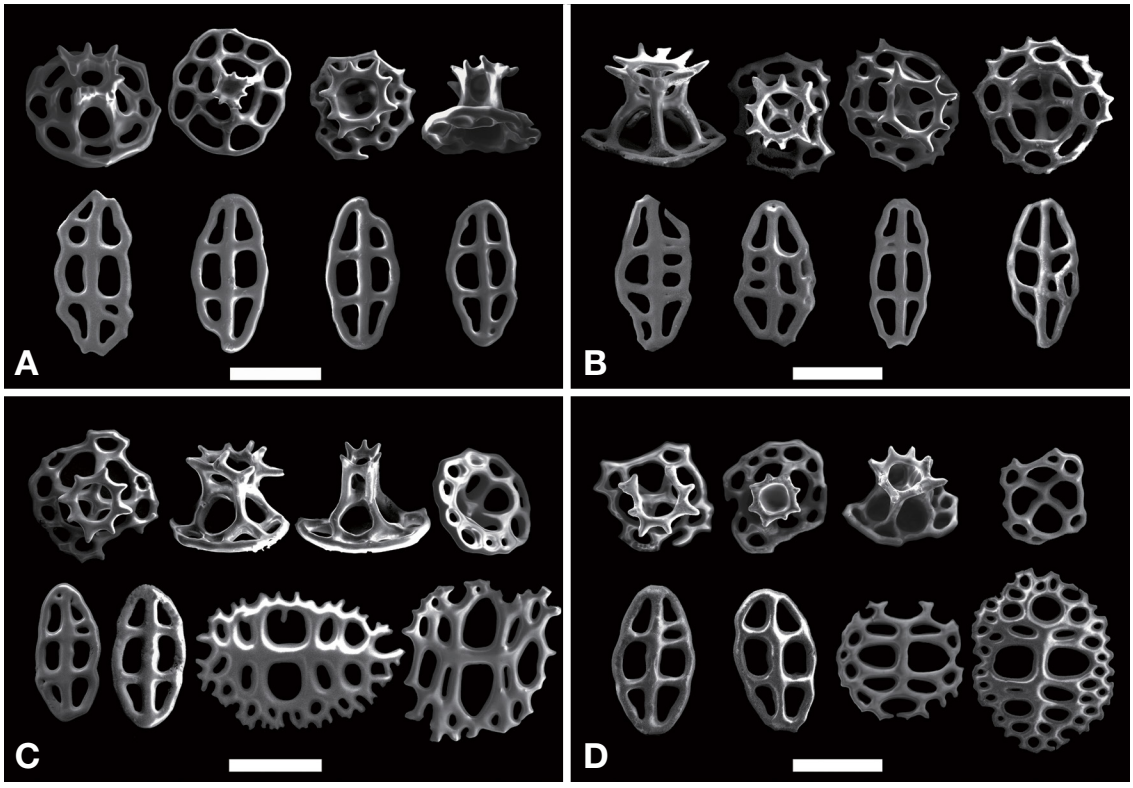


**Fig. 4.** Ossicles of *Holothuria* (*Mertensiothuria*) *leucospilota* (Brandt, 1835) analyzed in this study. A, Ossicles in dorsal body-wall; B, Ossicles in ventral body-wall; C, Ossicles in ventral tube feet; D, Ossicles in dorsal papillae. Scale bars: A–D = 30 μm.

**Table 1.** Morphological comparison between *Holothuria* (*Mertensiothuria*) *leucospilota* examined in this study and additional species *Holothuria* (*Mertensiothuria*) from previously published studies

| Species | Ossicles within tentacles | Rod morphology in dorsal papillae | Ossicles within tube feet | Ossicles within longitudinal muscle | Reference |
|---|---|---|---|---|---|
| *H*. (*M*.) *leucospilota* | Absent | Smooth | Plates, buttons, tables | Rods, C-shaped, rings, buttons | This study |
| *H*. (*M*.) *albofusca* | Present | N/A | Rods, irregular buttons, plates, tables | Rods, rings | Samyn and Massin (2003) |
| *H*. (*M*.) *artensis* | Present | Spine | Rosettes, X-shaped rods | N/A | Cherbonnier and Féral (1984) |
| *H*. (*M*.) *aphanes* | Present | Spine | Rods, buttons, plates, tables | Rings, C-shaped | Samyn and Massin (2003) |
| *H*. (*M*.) *fuscorubra* | Absent | Spine | Plates, buttons, tables | Rods, rings | Samyn and Massin (2003) |
| *H*. (*M*.) *hilla* | Present | Smooth | Plates, buttons, tables | Rings, C-shaped, irregular buttons | Samyn and Massin (2003) |
| *H*. (*M*.) *isuga* | Present | N/A | Tables, buttons | Absent | Mitsukuri (1912) |
| *H*. (*M*.) *viridiaurantia* | Present | Spine | Tables, buttons, plates | Absent | Borrero-Pérez and Vanegas-González (2019) |

N/A, no available.

**Material examined.** Seven specimens (MERBK-H-0071–0077); the intertidal zone of Jungmun, Haye-dong, Seogwipo-si, Jejudo Island, Korea (33°14′18.9″N, 126°23′43.5″E), 17–19 Oct 2024. Collected by Jinho Lee.

**Description.** Body cylindrical. Dorsal surface slightly flattened. Body color dark brown in life (Fig. 5A), brownish

**Fig. 5.** *Holothuria* (*Selenkothuria*) *moebii* Ludwig, 1833 observed in this study. A, Living specimen; B, Dorsal side of ethanol-preserved specimen; C, Ventral side of ethanol-preserved specimen. Scale bars: B, C = 1 cm.

gray when preserved in ethanol (Fig. 5B, C). Dorsal papillae arranged in four longitudinal rows. Ventral tube feet arranged in three longitudinal rows. Mouth ventral. Tentacles 20, shield-shaped. Tentacular ampullae short, 6–8 mm. Longitudinal muscles well developed, not bifid. Polian vesicles five, 4–5 mm. Cuvierian tubules thin, numerous.

Body wall ossicles consist of branched rods with broad bifurcated ends and one to three terminal spines. H-shaped plates also present. Dorsal and ventral body wall ossicles similar (Fig. 6A, B). Tube feet ossicles include branched rods, H-shaped plates, and end plates (Fig. 6C). Rods and plates similar to body wall. End plates coarsely knobbed and similar in size to rods. Papillae with branched rods and H-shaped plates (Fig. 6D). Tentacle ossicles consist of branched rods with bifurcated ends and terminal spines (Fig. 6E). No ossicles observed in longitudinal muscles.

**Size.** Body: length 38–42 mm, width 10–18 mm.

Ossicles: Body wall: Rod length 72–136 μm, width 21–46 μm; Plate length 75–101 μm, width 51–149 μm.

Tube feet: Rod length 113–143 μm, width 15–37 μm; Plate length 123–143 μm, width 93–113 μm.

Papillae: Rod length 66–113 μm, width 15–22 μm; Plate length 134–189 μm, width 93-113 μm.

Tentacle: Rod length 107–162 μm, width 19–59 μm.

**Distribution.** Distributed in Korea (Jejudo Island), as well as throughout the tropical to subtropical Indo-Pacific, from the western Indian Ocean across South and East Asia to Australia and the southwest Pacific, and further into the eastern Pacific

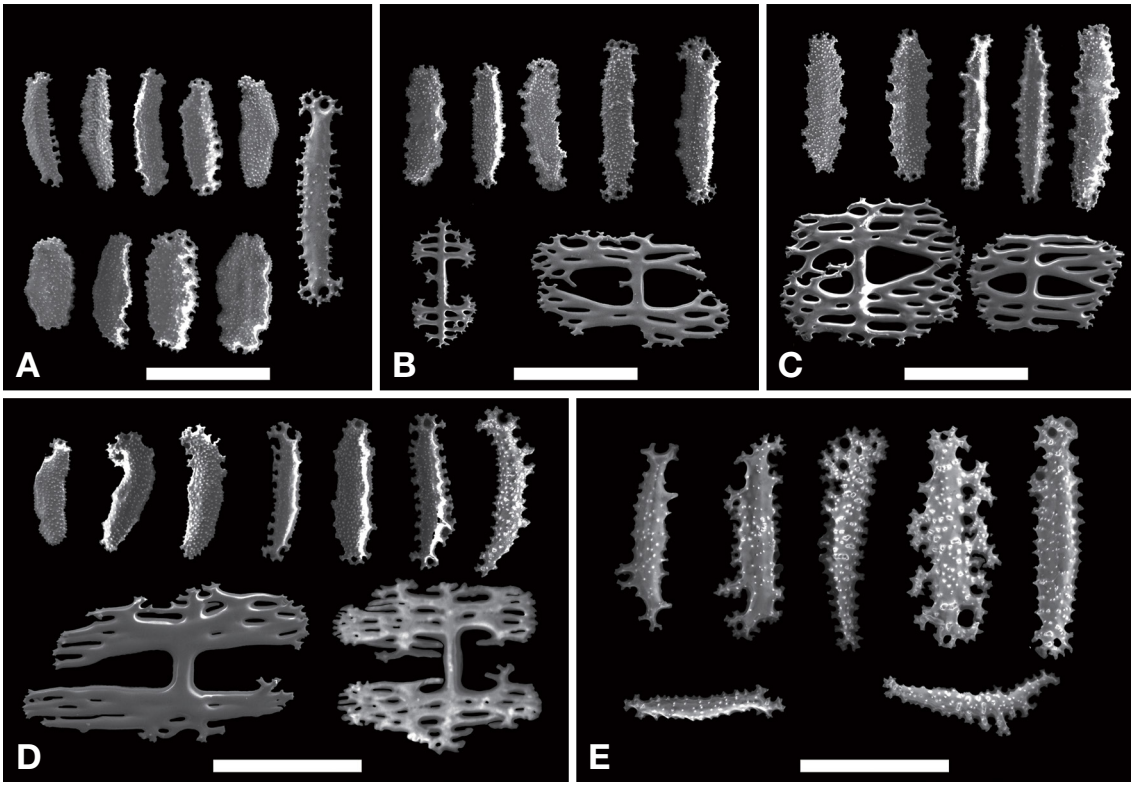


**Fig. 6.** Ossicles of *Holothuria* (*Selenkothuria*) *moebii* Ludwig, 1833 analyzed in this study. A, Ossicles in dorsal body-wall; B, Ossicles in ventral body-wall; C, Ossicles in ventral tube feet; D, Ossicles in dorsal papillae; E, Ossicles in tentacles. Scale bars: A–E = 100 μm.

**Table 2.** Morphological comparison between *Holothuria* (*Selenkothuria*) *moebii* analyzed in this study and other *Holothuria* (*Selenkothuria*) specimens described in previous reports

| Species | Ossicles within the body wall | Morphology of rod ossicles in the body wall | Reference |
|---|---|---|---|
| *H.* (*S.*) *moebii* | Rods | Rods displaying irregular spines with roughened tips | This study |
| *H.* (*S.*) *bacilla* | Rods | Granular with coarse texture | Cherbonnier (1988) |
| *H.* (*S.*) *carere* | Absent | Smooth surface | Honey-Escandón et al. (2011) |
| *H.* (*S.*) *erinaceus* | Rods | Smooth with slight undulation | Kawaida et al. (2023) |
| *H.* (*S.*) *glaberrima* | Rods | Densely distributed micro-spines | Massin (2013) |
| *H.* (*S.*) *lubrica* | Rods | Surface ranges from smooth to spinous | Caso (1954) |
| *H.* (*S.*) *mactanensis* | Rods | Prominent coarse, S-shaped spines | Massin (1996) |
| *H.* (*S.*) *parva* | Rods | Distinctively large and blunt spines | Massin (2013) |
| *H.* (*S.*) *parvispinea* | Rods | Extremely smooth, nearly lacking spines | Massin (2013) |
| *H.* (*S.*) *portovallartensis* | Rods | Smooth surface with abundant perforations | Caso (1954) |
| *H.* (*S.*) *theeli* | Rods | Curved and smooth | Deichmann (1938) |
| *H.* (*S.*) *sinica* | Rods | Smooth and exhibiting lateral perforations | Massin (1996) |
| *H.* (*S.*) *vittalonga* | Rods and plates | Smooth rods with large, spinoses plates | Cherbonnier (1988) |

region (Mexico to Ecuador) (Fig. 2B).

**Remarks.** The specimens examined in this study exhibit the diagnostic morphological combination characteristic of *H.* (*Selenkothuria*) *moebii*: absence of tables in the body wall, presence of H-shaped plates, coarsely granulated body-wall rods with rough or bifurcated tips; tentacle rods equipped with terminal spines, and lack of ossicles in the longitudinal muscles. This set of features is consistent with the original description and subsequent records of the species.

In *Holothuria* (*Selenkothuria*) Deichmann, 1958, the lack of

tables is characteristic of the subgenus, while species-level distinctions primarily rely on the morphology and ornamentation of body-wall rods and the presence or type of plates. As shown in Table 2, none of the other *H.* (*Selenkothuria*) species examined share the same features observed in the present specimens (for example, knobby thick rods in *H.* (*S.*) *bacilla*; curved rods with marginal spines in *H.* (*S.*) *mactanensis*; flat rods with perforated ridges in *H.* (*S.*) *portovallartensis*; and very smooth, spineless rods in *H.* (*S.*) *parvispinea*). Therefore, the seven specimens investigated here are assigned to *H.* (*S.*) *moebii*, constituting the first record of this species from Korea.

## ORCID

Jinho Lee: https://orcid.org/0000-0003-1257-4178
Taekjun Lee: https://orcid.org/0000-0003-4407-7862

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

## ACKNOWLEDGMENTS

This research was supported by the management of the Marine and Fishery Bio-resources Center (2025) through funding from the National Marine Biodiversity Institute of Korea (MABIK), as well as the Basic Science Research Program of the National Research Foundation of Korea (NRF), funded by the Ministry of Education, Republic of Korea, under grant number [2021R1I1A2058017].

## REFERENCES

Alvarado JJ, Solís-Marín FA, Ahearn CG, 2009. Echinoderm (Echinodermata) diversity in the Pacific coast of Central America. Marine Biodiversity, 40:45-56.

Borrero-Pérez GH, Vanegas-González MJ, 2019. *Holothuria* (*Mertensiothuria*) *viridiaurantia* sp. nov. (Holothuriida, Holothuriidae), a new sea cucumber from the Eastern Pacific Ocean revealed by morphology and DNA barcoding. ZooKeys, 893: 1-19. https://doi.org/10.3897/zookeys.893.36013

Brandt JF, 1835. Echinodermata ordo Holothurina. In: Prodromus Descriptionis Animalium ab H. Mertensio in Orbis Terrarum Circumnavigatione Observatorum. Fasc. I: 75 pps. Petropoli, pp. 42-62.

Caso ME, 1954. Contribución al conocimiento de los Holoturoideos de México: algunas especies de holoturoideos litorales y descripción de una nueva especie, *Holothuria portovallartensis*. Anales del Instituto de Biología, Universidad Nacional Autónoma de México, 25:417-442.

Chao SM, 1998. Two sea cucumbers (Echinodermata: Holothuroidea) newly recorded from Taiwan, with a key for *Holothuria*. Bulletin of the National Museum of Natural Science, 11:141-146.

Chao SM, Chang KH, 1989. The shallow-water holothurians (Echinodermata: Holothurioidea) of southern Taiwan. Bulletin of the Institute of Zoology, Academia Sinica, 28:107-137.

Cherbonnier G, 1988. Échinodermes: Holothurides. Faune de Madagascar, 70:1-292.

Cherbonnier G, Féral JP, 1984. Les Holothuries de Nouvelle-Calédonie. Deuxième contribution (Première partie: Synallactidae et Holothuridae). Bulletin du Muséum National d'Histoire Naturelle de Paris, 6:659-700.

Clark AM, Rowe FWE, 1971. Monograph of shallow-water Indo-West Pacific echinoderms. British Museum (Natural History), London, pp. 1-306.

Clark HL, 1920. Tropical Pacific Holothurioidea. Memoirs of the Museum of Comparative Zoology, 39:121-154.

Clark HL, 1923. The Echinoderm fauna of South Africa. Annals of the South African Museum, 13:221-435.

Clark HL, 1938. Echinoderms from Australia. An account of collections made in 1929 and 1932. Memoir of the Museum of Comparative Zoology, Harvard, 55:1-596.

Deichmann E, 1938. Holothurioidea of the British Museum (Natural History) Expedition to the Indian Ocean 1905. British Museum (Natural History), London, pp. 1-53.

Deichmann E, 1958. The Holothuroidea collected by the Velero III and IV during the years 1932 to 1954. Part. II Aspidochirota. The University of Southern California Publications, Allan Hancock Pacific Expeditions, 11:253-348.

Endean R, 1956. Further records of Echinodermata (excluding Crinoidea). Queensland Faunistic Records, Part IV. Department of Zoology, University of Queensland, Brisbane, pp. 124-139.

Heding SG, 1939. The holothurians collected during the cruises of the MS Monsunen in the tropical Pacific in 1934. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening i København, 102:213-222.

Honey-Escandón M, Solís-Marín FA, Laguarda-Figueras A, 2011. *Holothuria* (*Selenkothuria*) *carere*, a new species of sea cucumber (Echinodermata: Holothuroidea) from the Mexican Pacific. Zootaxa, 2922:27-33. https://doi.org/10.11646/zootaxa.2922.1.3

James DB, 1983. Research on Indian echinoderms: a review. Journal of the Marine Biological Association of India, 25:91-108.

James DB, 1995. Taxonomic studies of the species of *Holothuria* Linnaeus, 1767 from the seas around India. Part 2. Journal of the Bombay Natural History Society, 92:190-204.

Kawaida S, Yanagisawa Y, Tanita I, Nanjo K, 2023. First record of the sea cucumber *Holothuria* (*Selenkothuria*) *erinaceus* Semper, 1868 (Holothuroidea: Holothuriidae) from a Japanese mangrove forest. Laguna, 30:61-70.

Kerr AM, 1994. Shallow-water holothuroids (Echinodermata) of Kosrae, eastern Caroline Islands. Pacific Science, 48:161-174.

Lampert K, 1885. Die Seewalzen (Holothuroidea). In: Reisen im Archipel der Philippinen, 4 (2-3) (Ed., Semper C). C. W. Kreidel, Wiesbaden, pp. 1-312.

Levin VS, 1979. Aspidochirote holothurians of the upper sublittoral zone of Indo-West Pacific: species composition and distribution. Biologya Morya, 5:17-23.

Liao Y, 1980. The aspidochirote holothurians of China with erection of a new genus. In: Echinoderms: present and past. Balkema, Rotterdam, pp. 115-120.

Ludwig H, 1883. Verzeichniss der Holothurien des Kieler Museums. Bericht der Oberhessischen Gesellschaft für Natur- und Heilkunde, 22:155-176.

Ludwig H, 1886. Die von G. Chierchia auf der Fahrt der Kgl. Ital. Corvette "Vittor Pisani" gesammelten Holothurien. Zoologische Jahrbücher. Zeitschrift für Systematik, Geographie und Biologie der Thiere, 2:1-36.

Marine Biodiversity Institute of Korea, 2025. National list of marine species in Korea [Internet]. National Marine Biodiversity Institute of Korea, Seocheon, Accessed 1 Sep 2025, <http://www.mbris.kr>.

Marsh LM, Vail LL, Hoggett AK, Rowe FWE, 1993. Echinoderms of Ashmore Reef and Cartier Island. In: Marine faunal surveys of Ashmore Reef and Cartier Island, North-western Australia (Ed., Berry PF). Records of the Western Australian Museum, Supplement, 44:53-65.

Massin C, 1996. Holothurioidea of the Rumphius Biohistorical Expedition, Ambon (1990). Zoologische Verhandelingen, 307:1-298.

Massin C, 1999. Reef-dwelling Holothuroidea (Echinodermata) of the Spermonde Archipelago (south-west Sulawesi, Indonesia). Zoologische Verhandelingen, 329:1-144.

Massin C, 2013. *Holothuria* (*Selenkothuria*) *parvispinea* n. sp. (Echinodermata: Holothuroidea: Holothuriidae) with key to the sub-genus *Selenkothuria*. Zootaxa, 3609:343-348. https://doi.org/10.11646/zootaxa.3609.3.9

Mitsukuri K, 1912. Studies on Actinopodous holothurioidea. Journal of the College of Science, Imperial University of Tokyo, 29:1-284.

Panning A, 1934a. Die Gattung *Holothuria* (2. Teil). Mitteilungen aus dem Zoologischen Staatsinstitut und Zoologischen Museum in Hamburg, 45:24-50.

Panning A, 1934b. Die Gattung *Holothuria* (3. Teil). Mitteilungen aus dem Zoologischen Staatsinstitut und Zoologischen Museum in Hamburg, 45:65-84.

Pearson J, 1910. Littoral marine fauna: Kerimba Archipelago, Portuguese East Africa. Collected by James J. Simpson, M.A., B.Sc., University of Aberdeen, September 1907-May 1908: Holothurioidea. Proceedings of the Zoological Society of London, 80:167-182. https://doi.org/10.1111/j.1096-3642.1910.tb01890.x

Rho BJ, Shin S, 1986. A systematic study on Holothuroidea in Cheju-do. Korean Journal of Zoology, 29:245-256.

Rowe FWE, 1969. A review of the family Holothuriidae (Holothuroidea: Aspidochirotida). Bulletin of the British Museum of Natural History (Zoology), 18:119-170.

Rowe FWE, 1983. A collection of holothurians in the Leiden Museum from the East Indies and New Guinea, with the description of a new species of *Protankyra* (Holothurioidea: Synaptidae) from Java. Zoologische Mededelingen, 57:149-164.

Rowe FWE, Doty JE, 1977. The shallow-water holothurians of Guam. Micronesica, 13:217-250.

Rowe FWE, Gates J, 1995. Echinodermata. In: Zoological catalogue of Australia. Vol. 33 (Ed., Wells A). CSIRO, Melbourne, pp. 263-337.

Samyn Y, 2003. Shallow-water Holothuroidea (Echinodermata) from Kenya and Pemba Island (Tanzania). Studies in Afrotropical Zoology, 292:1-158.

Samyn Y, Massin C, 2003. The holothurian subgenus *Mertensiothuria* (Aspidochirotida: Holothuriidae) revisited. Journal of Natural History, 37:2487-2519. https://doi.org/10.1080/00222930210144334

Selenka E, 1867. Beiträge zur Anatomie und Systematik der Holothurien. Zeitschrift für wissenschaftliche Zoologie, 17:292-374.

Shin S, 2012. Invertebrate fauna of Korea, Vol. 32, No. 4. Sea cucumber: Echinodermata: Echinozoa: Holothuroidea. National Institute of Biological Resources, Incheon, pp. 1-127.

Shin S, Suh SJ, 2006. A new record of sea cucumber (Holothuroidea: Aspirochirotida) from Jejudo Island, Korea. Animal Systematics, Evolution and Diversity, 22:231-233. https://doi.org/10.5635/KJSZ.2006.22.2.231

Sloan NA, Clark AM, Taylor JD, 1979. The echinoderms of Aldabra and their habitats. Bulletin of the British Museum of Natural History (Zoology), 37:81-128.

Sluiter CP, 1887. Die Evertebraten aus der Sammlung des Königlichen Naturwissenschaftlicher Vereins in Niederländisch Indien in Batavia. Die Echinodermen. 1. Holothuroidea. Natuurkundig Tijdschrift voor Nederlandsch Indië, 47(8e Serie 8):181-220.

Sluiter CP, 1901. Die Holothurien der Siboga-Expedition. In: Siboga Expeditie. Monograph XLIV (Ed., Weber M). E. J. Brill, Leiden, pp. 1-142.

Smirnov AV, 2012. System of the class Holothuroidea. Paleontological Journal, 46:793-832. https://doi.org/10.1134/s0031030112080126

Théel H, 1886. Holothuroidea II. Report on the Scientific Results of the Voyage of the HMS Challenger, Zoology, 39:1-290.

Tortonese E, 1980. Researches on the coast of Somalia. Littoral Echinodermata. Monitore Zoologico Italiano, Supplemento, 13:99-139. https://doi.org/10.1080/00269786.1980.11758550

WoRMS Editorial Board, 2025. World Register of Marine Species [Internet]. WoRMS, Flanders Marine Institute (VLIZ), Ostend, Accessed 8 Jul 2025, <https://www.marinespecies.org>.

Received September 24, 2025
First revised October 24, 2025
Second revised November 12, 2025
Accepted January 13, 2026

ASED http://e-ASED.org
Animal Systematics, Evolution and Diversity

*Anim. Syst. Evol. Divers. Vol. 42, No. 1: 43-48, January 2026*
https://doi.org/10.5635/ASED.2026.42.1.014

# New Record of *Euplotes corsica* from a Saltern in South Korea

Sun Young Kim[1], Jeong Hyeon Yeo[2], Nam Seon Kang[1], Ga Young Kim[1], Seo Woo Son[1], Hyung June Kim[1], Jae-Ho Jung[2,*]

[1]National Marine Biodiversity Institute of Korea, Seocheon 33662, Korea
[2]Department of Biology, Gangneung-Wonju National University, Gangneung 25457, Korea

## ABSTRACT

A population of *Euplotes* was collected from a hypersaline saltern in Taean, Korea (72.37 psu). Live observations indicated that it did not correspond to any previously known species from Korea. To determine its identity, the infraciliature, nuclear apparatus, and silverline system were examined using protargol and silver nitrate impregnation methods. The main diagnostic features are as follows: body size 28−37 × 20−25 μm *in vivo*; body rotund, slightly inflated, dorsal and ventral surfaces smooth, without distinct ridges; dorsum arched; macronucleus C-shaped; adoral zone consisting of 21−26 membranelles, occupying 77% of body length on average; ten frontoventral, five transverse, two marginal, and one caudal cirri; six to seven dorsal kineties, the middle kinety composed of seven to eight dikinetids; dorsal argyrome of the double-*eurystomus* type. These morphological and morphometric features correspond well to those of *Euplotes corsica* Berger and Foissner, 1989. In addition, a nearly complete 18S ribosomal RNA gene sequence was obtained using single-cell polymerase chain reaction. Phylogenetic analyses based on this sequence revealed that the Korean population forms a clade with *E. foissneri*, showing 97.1% sequence similarity.

**Keywords:** 18S ribosomal RNA gene, marine ciliate, salt farm, hypersaline, taxonomy

## INTRODUCTION

The genus *Euplotes* Ehrenberg, 1830 comprises approximately 160 nominal species inhabiting diverse environments, including terrestrial, freshwater, and marine ecosystems (Curds, 1975; Jung et al., 2017; Do et al., 2024; Yeo and Jung, 2024). As a "melting pot" of ciliate diversity, new taxa have been continuously discovered within this genus (Do et al., 2024; Alimenti et al., 2025). These benthic ciliates are widespread unicellular organisms, and to date, 17 *Euplotes* species have been reported from saline environments in Korea (Marine Bio-Resource Information System; http://mbris.kr).

During a field survey of marine ciliates, we isolated an unidentified *Euplotes* morphotype from a hypersaline saltern. Since this morphotype has not previously been reported in Korea, we examined the population based on live observations, silver-impregnated preparations, and molecular analyses. Our results confirm that the Korean population is conspecific with *E. corsica* Berger and Foissner, 1989, originally described from the Etang d'Urbino, Corsica, France (Berger and Foissner, 1989). This study provides the first 18S ribosomal RNA (rRNA) gene sequence of *E. corsica*, which represents an essential molecular marker for species identification and phylogenetic reconstruction in ciliates (Abraham et al., 2019). These findings indicate the knowledge of ciliate diversity in Korea remains incomplete and underscore the need for further taxonomic and molecular investigations.

## MATERIALS AND METHODS

A population of *Euplotes* was isolated from a hypersaline saltern. The samples (approximately 40 mL each) were transferred to the laboratory and distributed into 10 cm Petri dishes. Autoclaved rice grains were added to promote bacterial growth as a food source for ciliates. When the cells became sufficiently abundant, a single cell was isolated using a micropipette, washed five times in autoclaved seawater, and transferred to a new culture dish. Cultured cells were subsequently used for morphological and molecular analyses.

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: 82-33-640-2312, Fax: 82-33-640-2867
E-mail: jhjung@gwnu.ac.kr

eISSN 2234-8190

Copyright © The Korean Society of Systematic Zoology

Morphological observations were conducted on living cells using differential interference contrast microscopy (Olympus BX53, Japan) at a magnification of 50–2,000×. The infraciliature, nuclear apparatus, and silverline system were revealed by protargol and silver nitrate impregnations following Foissner (2014) and Corliss (1953). Protargol powder was synthesized according to Pan et al. (2013) and Kim and Jung (2017). The surface ultrastructure was examined with scanning electron microscopy (SEM) following Phanprasert et al. (2025).

For molecular analyses, polymerase chain reaction (PCR) amplification, cloning, and sequencing of 18S, 5.8S, internal transcribed spacer (ITS) 1, ITS2, and partial 28S regions were performed following Kim et al. (2013) and Kim et al. (2020). Sixty-one *Euplotes* 18S with two outgroup sequences were retrieved from the National Center for Biotechnology Information database and included in phylogenetic analyses. Bayesian inference (BI) and maximum-likelihood (ML) trees were reconstructed using PhyML implemented in the Geneious software (v.9.0.5).

## RESULTS AND DISCUSSION

Class Spirotrichea Bütschli, 1889
Subclass Euplotia Jankowski, 1979
Order Euplotida Small and Lynn, 1985
Family Euplotidae Ehrenberg, 1838
Genus *Euplotes* Ehrenberg in Hemprich and Ehrenberg, 1831

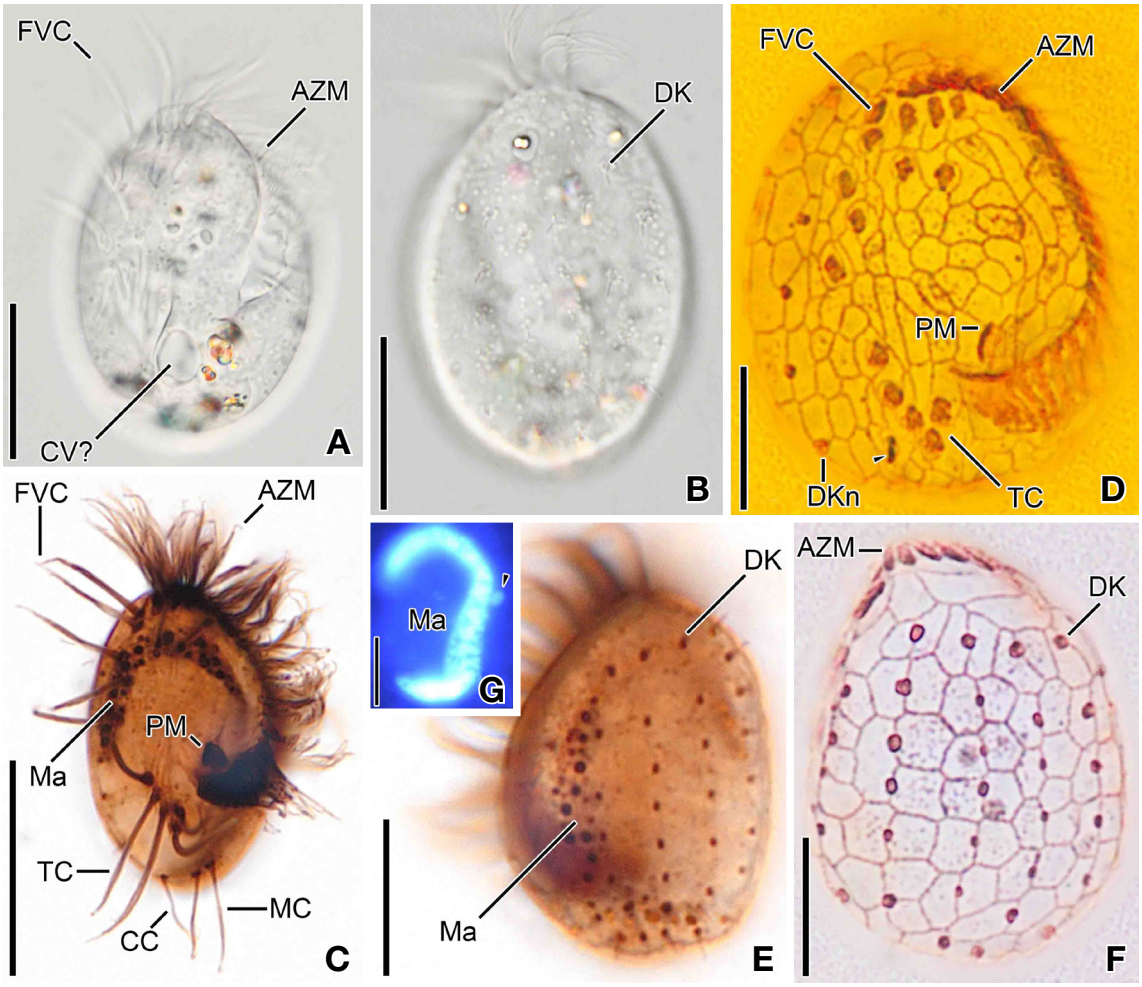


**Fig. 1.** *Euplotes corsica in vivo* (A, B), after protargol (C, E) and Chatton-Lwoff silver nitrate impregnation (D, F), and after DAPI staining (G). A–E, Ventral (A, C, D) and dorsal (B, E) views showing body shape, ventral cirri, oral apparatus, and dorsal kineties; arrowhead indicates the contractile vacuole pore. Cells in A and B slightly compressed; F, Dorsal view showing the arrangement of dorsal kineties and the double-*eurystomus*-type argyrome pattern; G, Nuclear apparatus; arrowhead indicates the micronucleus. AZM, adoral zone of membranelles; CC, caudal cirrus; CV, contractile vacuole; DK, dorsal kineties; DKn, dorsal kinety n (rightmost); FVC, frontoventral cirri; Ma, macronucleus; MC, marginal cirri; PM, paroral membrane; TC, transverse cirri. Scale bars: A–C = 20 μm, D–G = 10 μm.

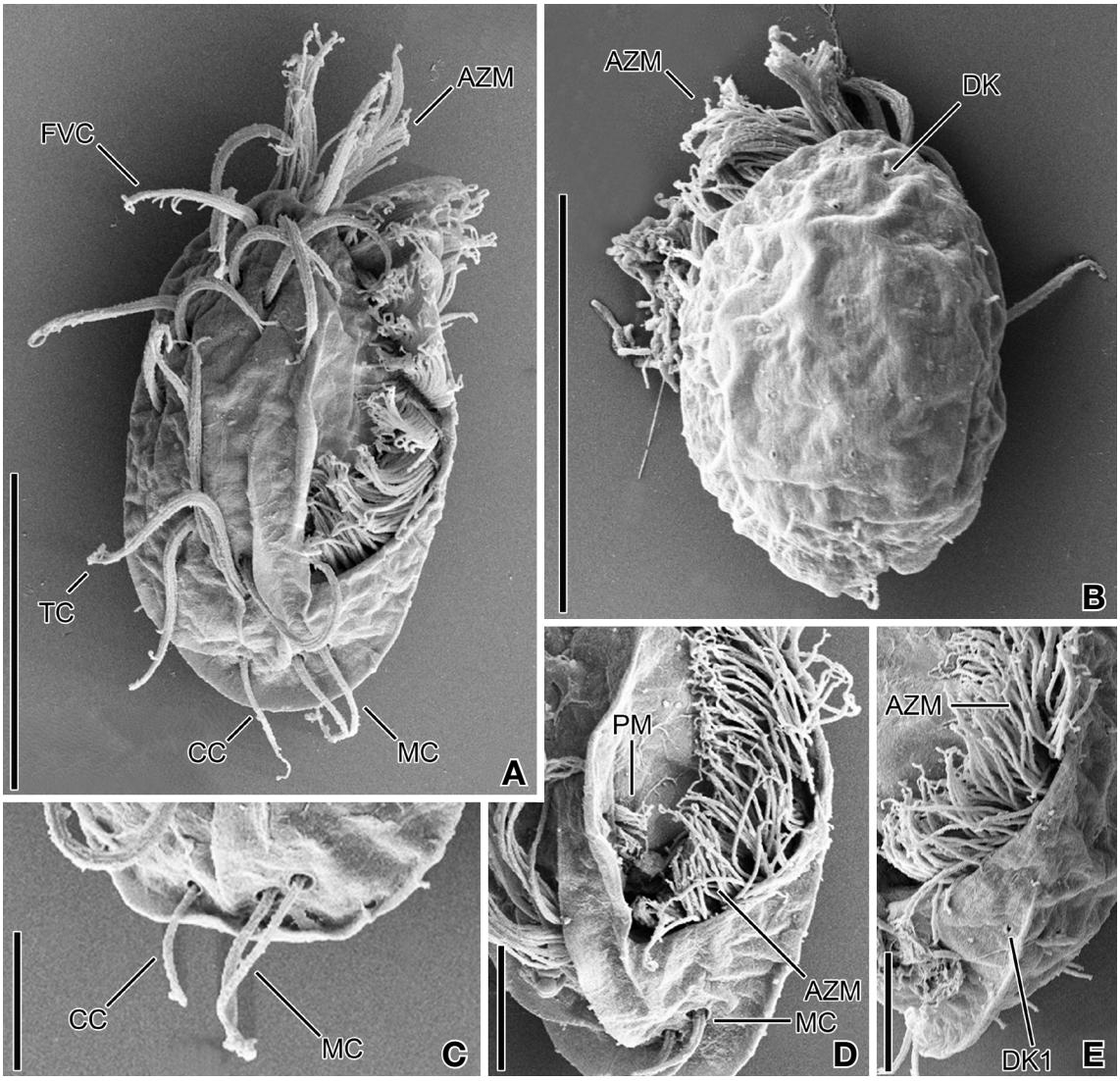


**Fig. 2.** *Euplotes corsica* in the scanning electron microscope. A, C, Ventral views of representative specimens showing body shape, ventral cirri, and posterior end; B, Dorsal view showing the arrangement of dorsal kineties; D, E, Ventrolateral views showing the ventral cirri and dorsolateral kineties. AZM, adoral zone of membranelles; CC, caudal cirrus; DK, dorsal kineties; DK1, dorsal kinety 1; FVC, frontoventral cirri; MC, marginal cirri; PM, paroral membrane; TC, transverse cirri. Scale bars: A, B = 20 μm, C–E = 5 μm.

### [1]**Euplotes corsica* Berger and Foissner, 1989

**Material examined.** Marine water (temperature 15.86°C, salinity 72.37 psu) collected from a saltern, Iwon-myeon, Taean-gun, Chungcheonnam-do, South Korea (36°54′13.17″ N, 126°17′45.4″E) on 27 Mar 2024.

**Description.** Body size 28–37 × 20–25 μm (mean 27 × 22 μm) *in vivo*, 27–34 × 19–27 μm (mean 31 × 23 μm) after protargol impregnation with a length : width ratio of 1.3–1.4 : 1. Body rotund, slightly inflated; dorsal and ventral cortex smooth, without distinct ridges; dorsum arched (Figs. 1, 2A, B). Contractile vacuole not observed, but a pore is present below the transverse cirri (Fig. 1A, D). Cortical granules colorless, 0.6–0.8 μm across *in vivo*; food vacuoles, crystals and lipid droplets measuring 1.7–2.1 μm, 2.2–3.4 μm and 1.7–2.0 μm *in vivo*, respectively (Fig. 1A, B). Macronucleus C-shaped (23–28 × 3–5 μm), with a spherical micronucleus (1.5–3.0 μm across after DAPI staining) attached to macronucleus (Fig. 1C, D, G).

Adoral zone occupying 77% of body length on average after

Korean name: [1]*코르시카자라하모충 (신칭)

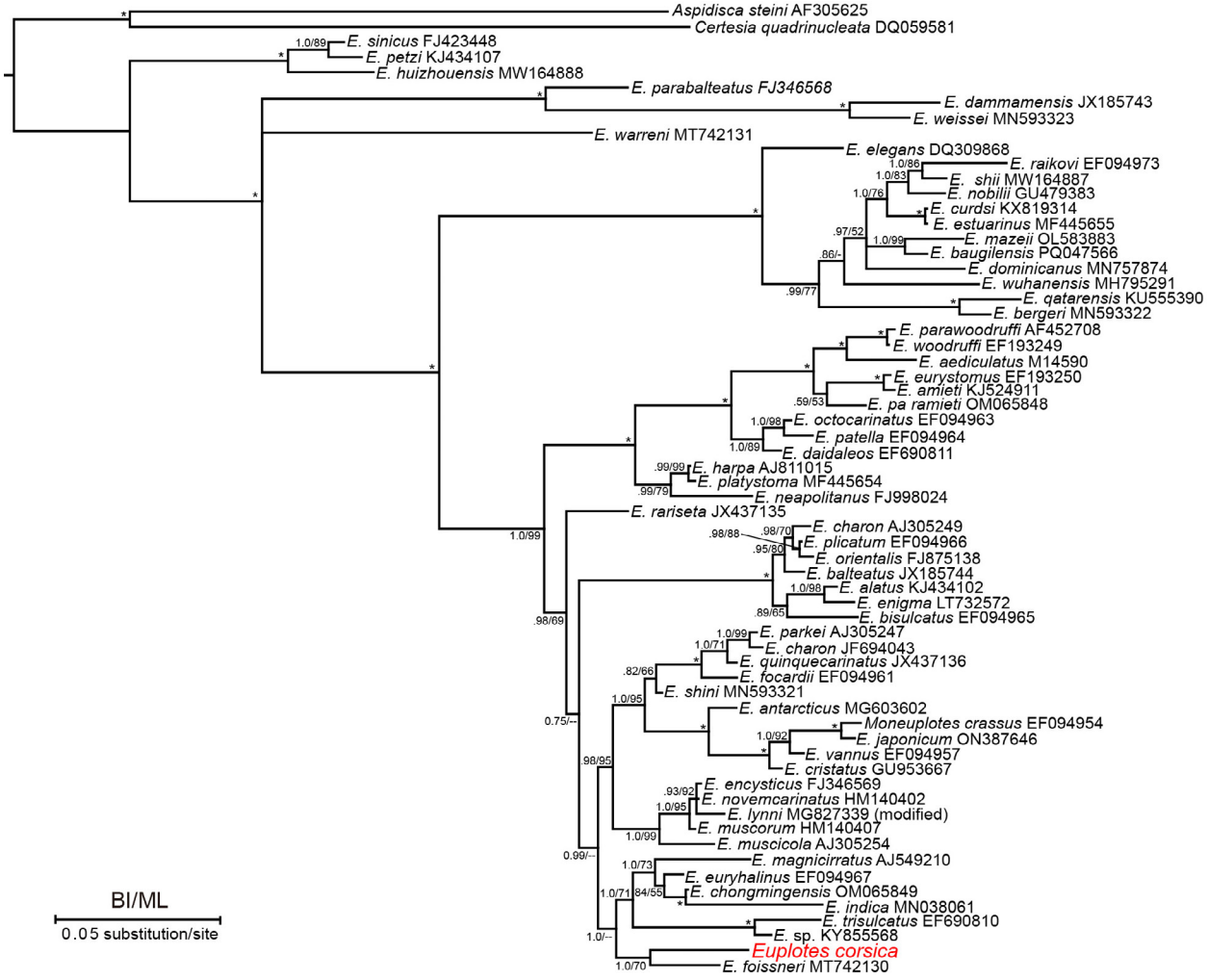


**Fig. 3.** Bayesian inference (BI) tree based on 18S ribosomal RNA sequences showing the relationships among *Euplotes* spp. The new sequence obtained in this study is shown in red. Numbers at the nodes represent support values in the following order: Bayesian posterior probabilities using the MrBayes algorithm (BI) and maximum likelihood (ML) bootstrap percentages from 1,000 replicates. An asterisk (*) denotes nodes with full bootstrap support in both analyses. A hyphen (-) represents support values <50% and incongruence between BI and ML analyses at the corresponding node.

protargol impregnation, composed of 21–26 membranelles (Fig. 1C, D). Paroral membrane located below the buccal lip (Figs. 1C, D, 2D). Ventral cirri composed of ten frontoventral, five transverse, two marginal cirri, and one caudal cirrus (Figs. 1A, C, D, 2A, C–E).

Six to seven dorsal kineties, with the middle kinety consisting of about 7–8 dikinetids; dorsal argyrome of the double-*eurystomus* type (Fig. 1B, E, F).

**Distribution.** France (type locality; Berger and Foissner, 1989) and South Korea (this study).

**Remarks.** The Korean population agrees well with the type population of *E. corsica* (Berger and Foissner, 1989). The principal diagnostic features are as follows: body size after protargol impregnation (27–34 × 19–27 μm vs. 29–42 × 22–32 μm), number of adoral membranelles (21–26 vs. 20–25), number of frontoventral cirri (10 vs. 10), dorsal kineties (6–7 vs. 7–8), and dikinetids (7–8 in the middle kinety vs. 6–9 in kinety 5).

As noted by Berger and Foissner (1989), *Euplotes alatus* Kahl, 1932 resembles *E. corsica*, but can be distinguished by its larger body length (75–90 μm vs. 28–37 μm) (Kahl, 1932). Gelei (1938) redescribed a Hungarian population of *E. alatus* based on silver impregnation, which exhibited a slightly smaller body length than the type population (55–70 μm vs. 75–90 μm) but still differs clearly from *E. corsica* by the number of dorsal kineties (10 vs. 7–8 in the type pop. vs. 6–7 in the Korean pop.). Interestingly, *Euplotes alatus* sensu Borror (1968) corresponds morphologically to *E. corsica* in

body length (36–43 μm vs. 29–42 μm in the type pop.) and in the anterior position of the leftmost frontoventral cirrus. However, Borror's population differs from the Korean population in body length after impregnation (36–43 μm vs. 27–34 μm) and in the number of dikinetids in the middle kinety (10–12 vs. 7–8) (Borror, 1968). Both the type and Korean populations of *E. corsica* possess fewer dorsal dikinetids in the middle kinety than *E. alatus* sensu Borror (1968) (6–9 vs. 7–8 vs. 10–12).

Given that *Euplotes* is species-rich genus and these populations exhibit slight morphological variation, further investigation is necessary. Single-cell PCR of the Korean population yielded a contiguous rRNA gene sequence (GenBank accession No. PX640646) comprising 18S (1,853 bp), ITS1 (122 bp), 5.8S (157 bp), ITS2 (196 bp), and partial 28S (909 bp). In the 18S rRNA-based phylogenetic tree, *E. corsica* forms a well-supported sister relationship with *E. foissneri* Valbonesi et al., 2021 (ML bootstrap 70%, BI posterior probability 1.00) (Fig. 3). Pairwise sequence similarity between the Korean population of *E. corsica* and *E. foissneri* was 97.1%. However, *E. foissneri* can be readily distinguished from *E. corsica* by the number of frontoventral cirri (9 vs. 10) (Valbonesi et al., 2021).

Regarding its habitat, the type population of *E. corsica* was collected from the soil of a saline pool at the Etang d'Urbino, Corsica, France (salinity not reported; Berger and Foissner, 1989). The Korean population was likewise found in a saline environment, though under hypersaline conditions (72.37 psu). As discussed by Syberg-Olsen et al. (2016), subclades within *Euplotes* often correlate with habitat type (freshwater, brackish, marine, hypersaline). Among its congeners, *E. qatarensis* Fotedar et al., 2016 tolerates salinities up to 120 psu; however, *E. qatarensis* and *E. corsica* did not form a clade in our phylogenetic analyses. Although salinity variation can influence the morphology of *Euplotes* species (Jones and Gates, 1994), such effects have not yet been documented in *E. corsica*.

**Voucher slides.** A total of five slides and three SEM stubs were deposited: one protargol preparation (MABIK PR00045140), one Chatton-Lwoff silver nitrate preparation (MABIK PR00045141), and three SEM stubs (MABIK PR00045137–9) at the National Marine Biodiversity Institute of Korea (MABIK); and two protargol preparations (GUC012369, GUC012383) and one Chatton-Lwoff silver nitrate preparation (GUC012373) in the Jung Laboratory (J.-H. Jung) at Gangneung-Wonju National University.

## ORCID

Sun Young Kim: https://orcid.org/0000-0001-5175-648X
Jeong Hyeon Yeo: https://orcid.org/0000-0003-1077-1805
Nam Seon Kang: https://orcid.org/0000-0001-5451-8647
Ga Young Kim: https://orcid.org/0009-0000-8038-6609
Seo Woo Son: https://orcid.org/0009-0007-5531-9448
Hyung June Kim: https://orcid.org/0000-0002-9798-2703
Jae-Ho Jung: https://orcid.org/0000-0001-5497-8678

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

## ACKNOWLEDGMENTS

This study was supported by the National Marine Biodiversity Institute Research Program (2025M00200) and the management of Marine and Fishery Bio-resources Center (2026) funded by the National Marine Biodiversity Institute of Korea (MABIK).

## REFERENCES

Abraham JS, Sripoorna S, Maurya S, Makhija S, Gupta R, Toteja R, 2019. Techniques and tools for species identification in ciliates: a review. International Journal of Systematic and Evolutionary Microbiology, 69:877-894. https://doi.org/10.1099/ijsem.0.003176

Alimenti C, Jiang Y, Di Giuseppe G, Pedrini B, Luporini P, Vallesi A, 2025. Morphology and molecular basis of cell - cell recognition in *Euplotes songi* sp. nov., a phylogenetically early-emerging new species of *Euplotes* (Ciliophora, Spirotrichea). International Journal of Systematic and Evolutionary Microbiology, 75:006907. https://doi.org/10.1099/ijsem.0.006907

Berger H, Foissner W, 1989. Morphology and biometry of some soil hypotrichs (Protozoa, Ciliophora) from Europe and Japan. Bulletin of the British Museum (Natural History), Zoology, 55:19-46.

Borror AC, 1968. Systematics of *Euplotes* (Ciliophora, Hypotrichida): toward union of the old and the new. Journal of Protozoology, 15:802-808. https://doi.org/10.1111/j.1550-7408.1968.tb02218.x

Corliss JO, 1953. Silver impregnation of ciliated protozoa by the Chatton-Lwoff technic. Stain Technology, 28:97-100. https://doi.org/10.3109/10520295309105108

Curds CR, 1975. A guide to the species of the genus *Euplotes* (Hypotrichida, Ciliatea). Bulletin of the British Museum (Natural History) Zoology, 28:1-63.

Do EH, Kwon HI, Yeo JH, Quintela-Alonso P, Jung JH, 2024. Morphology and molecular phylogeny of *Euplotes baugilensis* n. sp. (Ciliophora, Spirotrichea), with an illustrated key to *Euplotes* species with reduced cirri. European Journal of Protistology, 95:126110. https://doi.org/10.1016/j.ejop.2024.126110

Foissner W, 2014. An update of 'basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa'. International Journal of Systematic and Evolutionary Microbiology, 64:271-292. https://doi.org/10.1099/ijs.0.057893-0

Gelei J, 1938. Vollkommene Sinneselemente bei den höheren Ciliaten. I. Studie über die Sinnessorganellen von *Euplates*-Arten. Mathematischer und Naturwissenschaftlicher Anzeiger der Ungarischen Akademie der Wissenschaften, 57:845-887.

Jones TC, Gates MA, 1994. A morphometric study of euryhalinity in marine populations of the ciliate genus *Euplotes*. Journal of Eukaryotic Microbiology, 41:303-316. https://doi.org/10.1111/j.1550-7408.1994.tb06083.x

Jung JH, Park MH, Kim SY, Choi JM, Min GS, Kim YO, 2017. Checklist of Korean ciliates (Protozoa: Ciliophora). Journal of Species Research, 6:241-257. https://doi.org/10.12651/JSR.2017.6.3.241

Kahl A, 1932. Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 3. Spirotricha. Tierwelt Deutschlands, 25:399-650.

Kim JH, Jung JH, 2017. Cytological staining of protozoa: a case study on the impregnation of hypotrichs (Ciliophora: Spirotrichea) using laboratory-synthesized protargol. Animal Cells and Systems, 21:412-418. https://doi.org/10.1080/19768354.2017.1376707

Kim SY, Choi JK, Dolan JR, Shin HC, Lee S, Yang EJ, 2013. Morphological and ribosomal DNA-based characterization of six Antarctic ciliate morphospecies from the Amundsen Sea with phylogenetic analyses. Journal of Eukaryotic Microbiology, 60:497-513. https://doi.org/10.1111/jeu.12057

Kim SY, Xu D, Jung JH, Choi JK, 2020. Phylogeny and genetic/morphological variation of *Strombidinopsis minima*-like species (Ciliophora: Choreotrichia). Journal of Eukaryotic Microbiology, 67:115-124. https://doi.org/10.1111/jeu.12761

Pan X, Bourland WA, Song W, 2013. Protargol synthesis: an in-house protocol. Journal of Eukaryotic Microbiology, 60:609-614. https://doi.org/10.1111/jeu.12067

Phanprasert Y, Kim SY, Kang NS, Jeong M, Kim JI, Shin W, Lee WJ, Kim E, 2025. Morphological and molecular phylogenetic characterization of three new marine goniomonad species. Journal of Eukaryotic Microbiology, 72:e70002. https://doi.org/10.1111/jeu.70002

Syberg-Olsen MJ, Irwin NAT, Vannini C, Erra F, Di Giuseppe G, Boscaro V, Keeling PJ, 2016. Biogeography and character evolution of the ciliate genus *Euplotes* (Spirotrichea, Euplotia), with description of *Euplotes curdsi* sp. nov. PLoS ONE, 11:e0165442. https://doi.org/10.1371/journal.pone.0165442

Valbonesi A, Di Giuseppe G, Vallesi A, Luporini P, 2021. Two new species of *Euplotes* with cirrotype-9, *Euplotes foissneri* sp. nov. and *Euplotes warreni* sp. nov. (Ciliophora, Spirotrichea, Euplotida), from the coasts of Patagonia: implications from their distant, early and late branching in the *Euplotes* phylogenetic tree. International Journal of Systematic and Evolutionary Microbiology, 71:004568. https://doi.org/10.1099/ijsem.0.004568

Yeo JH, Jung JH, 2024. New record of marine scuticociliates and euplotids (Protozoa, Ciliophora) from coastal waters of South Korea. Journal of Species Research, 13:417-422. https://doi.org/10.12651/JSR.2024.13.4.417

Received November 12, 2025
First revised December 9, 2025
Second revised December 22, 2025
Accepted January 8, 2026

ASED http://e-ASED.org
Animal Systematics, Evolution and Diversity

*Anim. Syst. Evol. Divers. Vol. 42, No. 1: 49-53, January 2026*
https://doi.org/10.5635/ASED.2026.42.1.015

# Two New Records of *Pycnoclavella* (Ascidiacea: Clavelinidae) in Korea

Su Yuan Seo*

Natural History Museum, Ewha Womans University, Seoul 03760, Korea

## ABSTRACT

*Pycnoclavella* was classified into the family Pycnoclavellidae (Kott, 1990) based on its distinct morphology. However, molecular data indicated that it forms a single group with *Clavelina*, leading to its reclassification into the Family Clavelinidae. This study enhances our understanding of the biodiversity within the Family Clavelinidae by documenting the first records of two ascidian species, *Pycnoclavella belizeana* and *Pycnoclavella producta*, in the Korean fauna. Both species have three rows of stigmata arranged in a spindle-shaped pattern. *Pycnoclavella belizeana* has zooids measuring about 6 mm with a thorax over two times as long as wide, while *Pycnoclavella producta* has larger zooids (up to 20 mm) with a thorax similar in length and width. Specimens were collected by SCUBA diving in the subtidal zones of Jeju Island and Gyeongsangnam-do, Korea. All specimens were deposited at the National Marine Biodiversity Institute of Korea and the Natural History Museum of Ewha Womans University.

**Keywords:** ascidians, Clavelinidae, *Pycnoclavella belizeana*, *Pycnoclavella producta*, Korean fauna

## INTRODUCTION

The family Clavelinidae Forbes and Hanley, 1848 comprises four recognized genera: *Clavelina* Savigny, 1816; *Euclavella* Kott, 1990; *Nephtheis* Gould, 1856; and *Pycnoclavella* Garstang, 1891 (see Shenkar et al., 2025).

In 1990, Kott proposed that the genus *Pycnoclavella* be elevated to a separate, monotypic family, Pycnoclavellidae. This taxonomic division was based on several unique morphological and reproductive traits observed in *Pycnoclavella*, which Kott argued significantly differentiated it from other genera within Clavelinidae. The new family could be recognized by its small zooids, a replication process involving horizontal division across the abdomen (demonstrated by Trason, 1963), smaller gonads, a limited number of embryos, unique larval tubular adhesive organs, and a reduced otolith (Kott, 1990, 2003, 2005). Despite Kott's clear morphological distinctions, the validity of the genus *Pycnoclavella* and the family Pycnoclavellidae remained a topic of ongoing debate within the ascidian community for many years (Monniot, 1988, 1997; Monniot and Monniot, 1996, 2001; Kott, 2003, 2005).

Pérez-Portela and Turon (2008) conducted a pivotal analysis that illuminated the genetic relationships within the group. Their results indicated that while *Pycnoclavella* and *Clavelina* appeared in genetically distinct clades, they collectively formed a single monophyletic group when assessed alongside representatives of other major Aplousobranchia families, including Didemnidae (Giard, 1872), Holozoidae (Berrill, 1950), Polycitoridae (Michaelsen, 1904), and Polyclinidae (Milne Edwards, 1841). Consequently, the genus *Pycnoclavella* has since been reclassified into the family Clavelinidae (Shenkar et al., 2025), restoring the traditional four-genus composition of the family (Pérez-Portela and Turon, 2008).

The genus *Pycnoclavella* Garstang, 1891, comprises 24 valid species worldwide (Shenkar et al., 2025) and is widely distributed across the Atlantic, Indian, and Pacific Oceans (Garstang, 1891; Julin, 1904; Sluiter, 1904; Berrill and Abbott, 1949; Millar, 1953; Trason, 1963; Monniot, 1988, 1997; Kott, 1990, 2003, 2005; Brunetti, 1991; Goodbody, 1996; Monniot and Monniot, 1996, 2001; Pérez-Portela et al., 2007, 2010; Pérez-Portela and Turon, 2008; Seo, 2023). In the Pacific Ocean, nine species have been identified: *P. atlantica* Pérez-Portela et al., 2007; *P. detorta* (Sluiter, 1904); *P. diminuta* (Kott, 1957); *P. flava* (Monniot, 1988); *P. kottae* Millar, 1960; *P. minuta* Millar, 1953; *P. stanleyi* Berrill and Abbott, 1949; *P. stolonialis* Pérez-Portela et al., 2010; and *P. tabella* Kott, 1990. Most of these species are found in the southern Pacific Ocean (Sluiter, 1904; Berrill and Abbott, 1949; Millar, 1953; Mon-

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: 82-2-3277-3426, Fax: 82-2-3277-2566
E-mail: syseo@ewha.ac.kr

eISSN 2234-8190

Copyright © The Korean Society of Systematic Zoology

niot, 1988; Kott, 1990, 2003; Monniot and Monniot, 2001; Seo, 2023). Notably, *P. atlantica* and *P. stolonialis* have been reported in the marine waters of South Korea (Seo, 2023). This study adds two new species of *Pycnoclavella* to the records from Korean marine waters.

## MATERIALS AND METHODS

In this study, specimens of both *Pycnoclavella belizeana* and *P. producta* were collected by SCUBA diving from the subtidal zones of Seogwipo-si, Jeju Island, and Tongyeong-si, Gyeongsangnam-do, Korea, respectively. After collection, all living specimens were transferred to containers with seawater and anesthetized with menthol powder for 5–6 h. The anesthetized specimens were then fixed in 4% buffered formalin.

For identification, specimens were examined for morphological characteristics under a stereoscopic microscope (SMZ 745T; Nikon, Tokyo, Japan). Images of the preserved specimens were captured using a microscopic camera (UHCCD 05000KPA; Touptek Photonics, Zhejiang, China) and a digital camera (WG-4; Ricoh, Tokyo, Japan). The size of the zooids was measured using an image analyzer (Toupview 3.7; Touptek Photonics) and a ruler.

All specimens examined in this study were deposited at the Natural History Museum of Ewha Womans University (EW NHMAS4323, 4857) and the National Marine Biodiversity Institute of Korea (MABIK IV00175005, MABIK IV00175 006).

## SYSTEMATIC ACCOUNTS

Class Ascidiacea Blainville, 1824
Order Aplousobranchia Lahille, 1886
Family Clavelinidae Forbes and Hanley, 1848

### [1]*Genus *Pycnoclavella* Garstang, 1891

*Archiascidia* Julin, 1904: 489. Type species (TS): *Archiascidia neapolitana* Julin, 1904.
*Pycnoclavella* Garstang, 1891: 265. TS: *Pycnoclavella auriluccens* Garstang, 1891.

### [2]****Pycnoclavella belizeana*** **Goodbody, 1996 (Fig. 1A, B)**

*Pycnoclavella belizeana* Goodbody, 1996: 590–597, figs. 1–4; Pérez-Portela et al., 2010: 57, table 2.

**Material examined.** Korea: 2 individuals (MABIK IV00176 046), Jeju-do: Seogwipo-si, 33°13′40″N, 126°34′05″E, Scuba diving at a depth of 33 m, 9 May 2022, coll. Park SW, Cho SH; 2 individuals (EWNHMA4323), Seogwipo-si, 33°13′38″N, 126°34′15″E, Scuba diving at a depth of 10 m, 10 May 2022, coll. Park SW, Cho SH.

**Description.** Solitary. Zooids found in clusters of porifera, hydrozoa, bryozoa, algae and other detritus. *Pycnoclavella belizeana* mixed with other unreported *Pycnoclavella* species from Korea. Six zooids situated in cluster. Zooids either separate or connected by long stolon at a distance. Posterior abdomen of zooids embedded in cluster. Zooid slightly transparent. Yellow pigment accumulated in endostyle and branchial sac fully visible. Abdomen region semi-translucent and attached fragments of other organisms or debris frequently (Fig. 1A).

Zooids very small. In living, zooid about 5–6 mm long on average, with narrow, long oesophageal neck between thorax and abdomen. Length of thorax about two times as long as widest width of thorax. In preserved specimens, zooids about 3.1 mm long and oesophageal neck almost completely contracted. Thorax about 0.8 mm long and abdomen about 2.3 mm long on average. Zooidal test fragile; stalk test somewhat tough. Muscles on thoracic test of zooid 6–7 in number, extending along length of thorax. Branchial apertures opened at upper terminal of thorax, and atrial aperture opened obliquely at top of thorax (Fig. 1B). Both apertures smooth lipped yellow stains. Tentacles in branchial apertures simple, 14–16. Neural gland opening simple. Dorsal languet fine pointed tongue shape. Three stigmata rows of 12–24 stigmata. In fully expanded branchial sac of living zooid, the three stigmata rows arranged like spindle. Central row oriented along anterior-posterior plane, whereas anterior and posterior rows positioned at angle of 45° or greater (Fig. 1A). Each transverse vessels provided with developed membrane. Stomach situated at posterior end of descending limb of gut loop, box-shaped with four ridges on surface. Thick rectum forming ascending limb of gut loop. Anus opened at level as high as posterior stigmata row. Atrial apertures having smooth border (Fig. 1B). Gonad and larva absent in specimens.

**Distribution.** Korea (present study), Belize (North Channel).

**Remarks.** This species is easily identifiable by its very short zooid length, thorax over two times as long as wide, three rows of stigmata, and the distinctive spindle-like arrangement of the stigmata rows when alive. The small size of the zooids aligns this species with its Pacific congenera, *P. minuta* and *P. stanleyi*. In contrast to the current species, which features either solitary zooids or those connected by a stolon, *P. minuta* forms colonies with zooids that have a basal test mass. Additionally, *P. minuta* is distinguished from the current species by having four rows of stigmata (Millar, 1953; Goodbody, 1996; Pérez-Portela et al., 2010). *Pycnoclavella stanleyi* differs from

Korean name: [1]*꼬마곤봉멍게속, [2]*빗살곤봉멍게 (신칭)

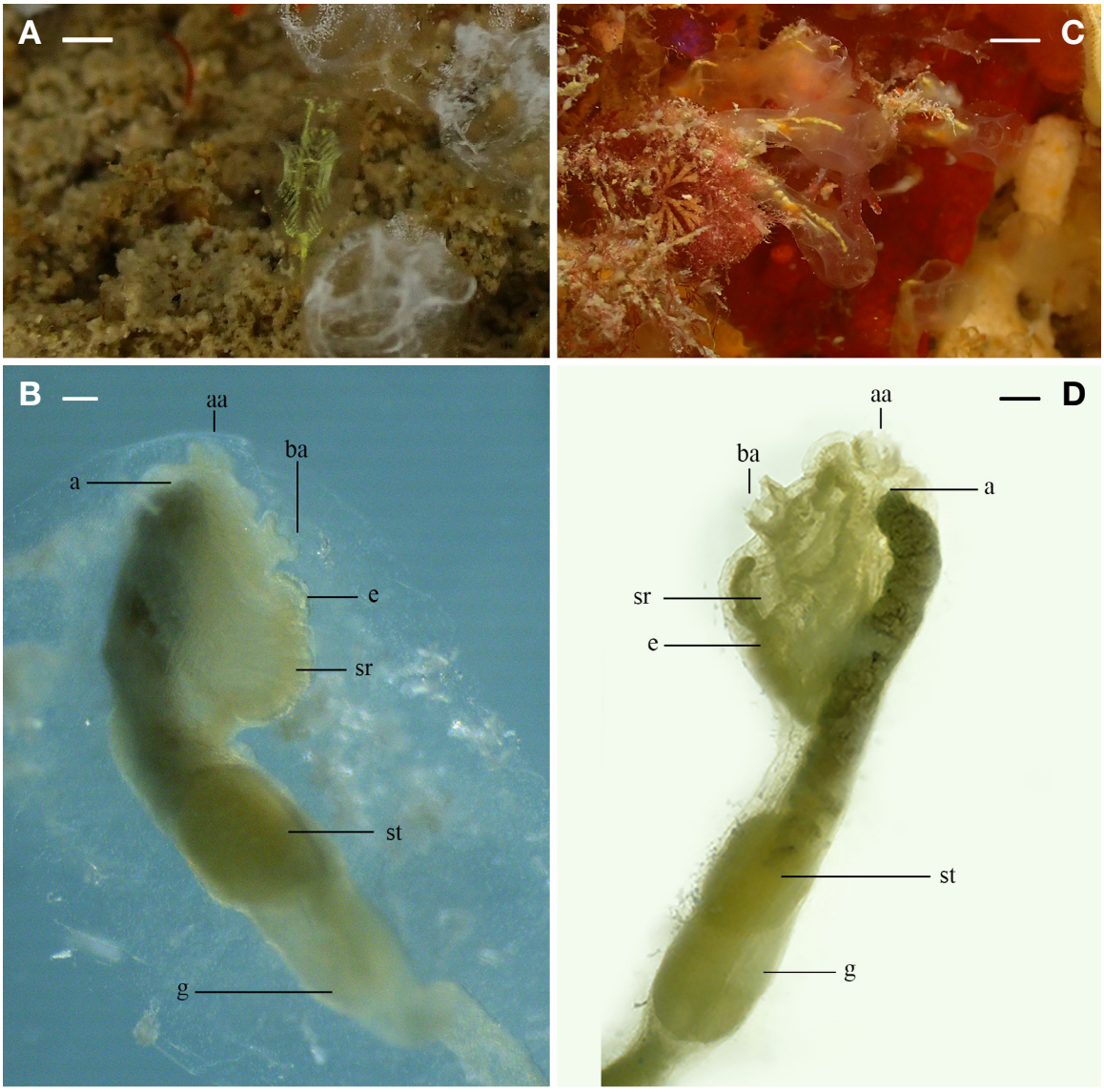


**Fig. 1.** *Pycnoclavella belizeana* (A, B), *Pycnoclavella producta* (C, D). A, Colony in living; B, Zooid in preservative; C, Colony in living; D, Zooid in preservative. a, anus; aa, atrial aperture; ba, branchial aperture; e, endostyle; g, gut; sr, stigmata row; st, stomach. Scale bars: A=1 mm, B=0.2 mm, C=2 mm, D=0.4 mm.

the current species by exhibiting 4–7 rows of stigmata and a consistent orange coloration. The specimens observed here display a yellow coloration, which aligns with *P. belizeana*'s known color variation from white to yellow depending on light intensity (Berrill and Abbott, 1949; Goodbody, 1996).

### [1]*Pycnoclavella producta* (Milne Edwards, 1841) (Fig. 1C, D)

*Pycnoclavella producta* Pérez-Portela and Turon, 2008: 115, fig. 3C, 4; 2010: 57, table 2.
*Archidistoma productum* Monniot, 1970: 145, fig. 1B–D.
*Clavelina producta* Milne Edwards, 1841: 278, Pl. 2, fig. 3.

**Material examined.** Korea: 2 individuals (MABIK IV00176045), Gyeongsangnam-do: Tongyeong-si, 34°32′02″N, 128°43′56″E, Scuba diving at a depth of 25 m, 14 Jul 2024, coll. Park SW, Cho SH; 5 individuals (EWNHMAS4857), Tongyeong-si, 34°32′21″N, 128°43′54″E, Scuba diving at a depth of 25 m, 14 Jul 2024, coll. Park SW, Cho SH.

**Description.** Solitary. Zooids found in tangled clusters of

Korean name: [1]*세실곤봉멍게 (신칭)

hydrozoa, gorgonacea, bryozoa, algae, shell fragments and other detritus. *Pycnoclavella producta* found associated with other previously unrecorded *Pycnoclavella* species and additional *Aplidium* species from Korea. Zooids either separate or connected by long stolon. Posterior abdomen of zooids embedded in cluster. In living zooid, slightly translucent, with diffused white coloration. Esophagus, gut and feces visible inside body (Fig. 1C).

Length of zooids longer compared to other *Pycnoclavella* species. In living, zooid about 15 mm long on average (up to 20 mm), with narrow, long oesophageal neck between thorax and abdomen. Length and width of thorax are similar, resembling an old miniature light bulb. In preserved specimens, zooids about 5.3 mm long and its oesophageal neck contracted. Thorax about 1.8 mm long and abdomen about 3.5 mm long on average. Zooidal test fragile; stalk test somewhat tough. Muscles 6−7 on thoracic test of zooid and extend along length of thorax. Branchial apertures opened at upper terminal of thorax and atrial aperture obliquely opened at top of thorax. Both apertures smooth rimmed (Fig. 1D). Branchial aperture having six lobes with white margins. Tentacles in branchial apertures simple. Neural gland opening simple. Dorsal languet fine pointed tongue shape. Three stigmata rows of 18−28 stigmata. Central row oriented along anterior-posterior plane, whereas anterior and posterior rows inclined at about 45° (Fig. 1C). Spindle-shaped branchial arrangement visible when alive, but not observed in contracted zooid. Transverse vessel each provided with developed membrane. Stomach situated at posterior end of descending limb of gut loop. Stomach having barrel-shaped and smooth surface. Thick rectum forming ascending limb of gut loop. Anus opened at level as high as posterior stigmata row. Atrial apertures having smooth border (Fig. 1D). Gonad and larva absent in specimens.

**Distribution.** Korea (present study), France (English Channel), Spain.

**Remarks.** The present specimens exhibited the diagnostic features of *Pycnoclavella producta*: a stolonial growth habit, thorax with similar length and width, three rows of stigmata, and zooids with a long, thin, cylindrical peduncle (Milne Edwards, 1841). This specimen shares features with the Pacific species *P. kottae* and *P. tabella*, particularly in having three rows of stigmata and slender, elongated zooids. However, this species is structurally distinct: *P. kottae* has zooids embedded in a basal test mass, while *P. tabella* is typically covered with sand grains, except for the exposed thoracic region (Millar, 1960; Kott, 1990).

**Key to the *Pycnoclavella* species from Korean waters**

1. Stigmata in 3 rows ····· 2
− Stigmata in >3 rows····· 3
2. Zooid length 5−6 mm, thorax over two times as long as wide····· *belizeana* Goodbody
− Zooid length up to 20 mm, thorax with similar length and width····· *producta* (Milne Edwards)
3. Number of stigmata rows 5−7, number of muscle 10····· ····· *atlantica* Pérez-Portela, Duran and Turon
− Number of stigmata rows 6−8, number of muscle 6−7····· ·····*stolonialis* Pérez-Portela, Goodwin, Picton and Turon

## ORCID

Su Yuan Seo: https://orcid.org/0000-0003-2597-2351

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

## ACKNOWLEDGMENTS

This work was supported by the management of Marine Fishery Bio-resources Center (2025) funded by the National Marine Biodiversity Institute of Korea (MABIK) and the Natural History Museum of Ewha Womans University in part. I would like to thank scuba instructor Seung-hwan Park for taking the ecological photographs.

## REFERENCES

Berrill NJ, Abbott DP, 1949. The structure of the ascidian, *Pycnoclavella stanleyi* n. sp., and the nature of its tadpole larva. Canadian Journal of Research, 27d:43-49. https://doi.org/10.1139/cjr49d-006

Brunetti R, 1991. *Pycnoclavella taureanensis* n.sp. (Ascidiacea) from the Mediterranean Sea. Vie et Milieu, 41:245-248.

Garstang W, 1891. Note on a new and primitive type of compound ascidian. The Annals and Magazine of Natural History Series 6, 8:265-268. https://doi.org/10.1080/00222939109460434

Goodbody I, 1996. *Pycnoclavella belizeana*, a new species of ascidian from the Caribbean. Bulletin of Marine Science, 58: 590-597.

Julin C, 1904. Recherches sur la phylogénèse des tuniciers. *Archiascidia neapolitana* nov. gen., nov. sp. Mittheilungen aus der Zoologischen Station zu Neapal, 16:489-552.

Kott P, 1990. The Australian Ascidiacea, part 2, Aplousobranhia (1). Memories of the Queensland Museum, 29:1-226.

Kott P, 2003. New syntheses and new species in the Australian

Ascidiacea. Journal of Natural History, 37:1611-1653. https://doi.org/10.1080/00222930110104258

Kott P, 2005. *Pycnoclavella* (Tunicata: Ascidiacea) species from the West Indian Ocean. African Zoology, 40:205-212. https://doi.org/10.1080/15627020.2005.11407319

Millar RH, 1953. On a collection of ascidians from the Gold Coast. Proceedings of the Zoological Society of London, 123:277-325. https://doi.org/10.1111/j.1096-3642.1953.tb00176.x

Millar RH, 1960. Ascidiacea. Discovery reports, Vol. 30. Cambridge University Press, London, pp. 1-160.

Milne Edwards H, 1841. Observations sur les Ascidies composées des côtes de la Manche. Mémoires de l'Académie des Sciences de l'Institut de France, 18:217-326. https://doi.org/10.5962/bhl.title.6289

Monniot C, 1970. Sur quatre Ascidies rares ou mal connues des côtes de la Manche. Cahiers de Biologie Marine, 11:145-152.

Monniot C, 1988. Ascidies de Nouvelle-Caledonie. 5. Polycitoridae du lagon. Bulletin du Muséum National d'Histoire Naturelle, Paris, 10:197-235.

Monniot C, 1997. Les genres *Archidistoma* et *Clavelina* (Ascidiacea, Clavelinidae) dans le canal du Mozambique. Zoosystema, 19:193-209.

Monniot F, Monniot C, 1996. New collections of ascidians from the Western Pacific and Southeastern Asia. Micronesica, 29: 133-279.

Monniot F, Monniot C, 2001. Ascidians from the tropical western Pacific. Zoosystema, 23:201-383.

Pérez-Portela R, Duran S, Palacín C, Turon X, 2007. The genus *Pycnoclavella* (Ascidiacea) in the Atlanto-Mediterranean region: a combined molecular and morphological approach. Invertebrate Systematics, 21:187-205. https://doi.org/10.1071/IS06015

Pérez-Portela R, Goodwin CE, Picton BE, Turon X, 2010. *Pycnoclavella stolonialis* n. sp. (Tunicata: Ascidiacea), with phylogenetic and distributional remarks on the genus in Europe. Zootaxa, 2407:51-66. https://doi.org/10.11646/zootaxa.2407.1.3

Pérez-Portela R, Turon X, 2008. Phylogenetic relationships of the Clavelinidae and Pycnoclavellidae (Ascidiacea) inferred from mtDNA data. Invertebrate Biology, 127:108-120. https://doi.org/10.1111/j.1744-7410.2007.00112.x

Seo SY, 2023. The genus *Pycnoclavella* (Ascidiacea: Aplousobranchia: Clavelinidae) new to Korea with two new records. Journal of Asia-Pacific Biodiversity, 16:1-4. https://doi.org/10.1016/j.japb.2022.12.005

Shenkar N, Gittenberger A, Lambert G, Rius M, Moreira da Rocha R, Swalla BJ, Turon X, 2025. Ascidiacea World Database. *Pycnoclavella* Garstang, 1891 [Internet]. World Register of Marine Species, Flemish Marine Institute (VLIZ), Ostend, Accessed 9 Nov 2025, <https://www.marinespecies.org/aphia.php?p=taxdetails&id=103454>.

Sluiter CP, 1904. Die Tunicaten der Siboga-Expedition. Part 1. Die socialen und holosomen Ascidien. Siboga-Expedition, 56a:1-126.

Trason WB, 1963. The life cycle and affinities of the colonial ascidian *Pycnoclavella stanleyi*. University of California Publications in Zoology, 65:283-326.

Received December 5, 2025
Revised December 12, 2025
Accepted January 8, 2026

ASED http://e-ASED.org
Animal Systematics, Evolution and Diversity

*Anim. Syst. Evol. Divers. Vol. 42, No. 1: 54-62, January 2026*
https://doi.org/10.5635/ASED.2026.42.1.017

# *Corhiza ulleungensis* sp. nov. (Hydrozoa: Leptothecata: Halopteriddidae) from Korea, with the First Record of the Genus *Corhiza*

Hyo-Jin Yu, Sung-Jin Hwang*

Department of Life Science, Woosuk University, Jincheon 27841, Korea

## ABSTRACT

A new species of the genus *Corhiza* (Hydrozoa: Halopteriddidae), *Corhiza ulleungensis* sp. nov., is described from specimens collected in subtidal zones (30−52 m depth) of Ulleungdo and Dokdo, the East Sea of Korea, during 2022. This represents the first record of *Corhiza* from Korean waters. The new species is characterized by polysiphonic stems, irregularly branched hydrocladia, cup-shaped hydrothecae with smooth margins, presence of axillar nematothecae, and 2−4 median nematothecae at intersegments. It is distinguished from similar species *C. suensoni*, *C. complexa*, and *C. patula* by differences in stem structure, hydrocladial arrangement, nematothecal number, and geographical distribution. Molecular analysis of partial 16S rRNA sequences showed minimal intraspecific variation (0.01 ± 0.00) and clear interspecific divergence (0.15) from *C. pauciarmata*—the only other *Corhiza* species among the 11 currently recognized for which 16S sequence data are available. Phylogenetic analysis strongly supports the placement of the new species within *Corhiza*. This discovery extends the distribution of *Corhiza* in the Northwest Pacific northward from Japan's Hirado Strait (Kyushu) to Korean waters, increasing the known halopteridid fauna of Korea to five genera and eight species.

**Keywords:** *Corhiza ulleungensis*, Halopteriddidae, 16S rRNA, Ulleungdo, Dokdo, taxonomy

## INTRODUCTION

The hydrozoan family Halopteriddidae Millard, 1962 currently comprises 16 genera worldwide: *Anarthroclada* Naumov, 1955; *Antennella* Allman, 1877; *Antennellopsis* Jäderholm, 1896; *Astrolabia* Naumov, 1955; *Calvinia* Nutting, 1900; *Cladoplumaria* Ansin Agis, Ramil and Vervoort, 2004; *Corhiza* Millard, 1962; *Diplopteroides* Peña Cantero and Vervoort, 1999; *Gattya* Allman, 1885; *Halopteris* Allman, 1877; *Monostaechas* Allman, 1877; *Monostaechoides* Gil and Ramil, 2021; *Nuditheca* Nutting, 1900; *Pentatheca* Naumov, 1955; *Polyplumaria* Sars, 1874; and *Thamnopteros* Galea, 2020 (see Schuchert et al., 2025). Of these, four genera—*Antennella* Allman, 1877; *Halopteris* Allman, 1877; *Monostaechas* Allman, 1877; *Antennellopsis* Jäderholm, 1896—are currently known from Korean waters, represented by seven species: *Antennella quadriaurita* Ritchie, 1909; *A. secundaria* (Gmelin, 1791); *A. siliquosa* (Hincks, 1877); *A. aurantia* Yu, Lee and Hwang, 2024; *Antennellopsis integerrima* (Jäderholm, 1896); *Halopteris constricta* Totton, 1930; *Monostaechas quadridens* (McCrady, 1859) (see Park, 2010; Yu et al., 2024).

The genus *Corhiza* was established by Millard (1962) based on *Corhiza scotiae* from the South Atlantic Ocean. The genus is characterized by erect, pinnate colonies with alternately arranged hydrocladia arising from a stem that may be either monosiphonic or polysiphonic, depending on the species (Schuchert, 1997). Hydrothecae are present on both stem and hydrocladia and are typically cup-shaped with a smooth or weakly sinuated rim, differentiating *Corhiza* from genera such as *Gattya*, which possess distinctly multicuspid hydrothecal margins (Millard, 1962; Schuchert, 1997). Hydrocladia are generally unbranched and monoserial. A key diagnostic feature of the genus is the absence of phylactocarps, the protective branching structures around gonothecae that characterize genera like *Cladoplumaria* (Ansín Agís et al., 2004). Although *Corhiza* may superficially resemble *Halopteris*, the two genera can be distinguished by a combination of stem structure, hydrothecal morphology, and gonothecal characters

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: 82-43-531-2892, Fax: 82-43-531-2862
E-mail: buteo2@woosuk.ac.kr, buteo2@gmail.com

eISSN 2234-8190

Copyright © The Korean Society of Systematic Zoology

(Schuchert, 1997).

Current taxonomic accounts recognize 11 valid species of *Corhiza* (Schuchert et al., 2025), distributed primarily in temperate and subtropical regions of the Southern Hemisphere. These include three species from the Indian Ocean, two from the South Atlantic, and four from the South Pacific, with depth records ranging from shallow subtidal habitats to several hundred meters (Nutting, 1906; Millard, 1962, 1980; Hirohito, 1995; Schuchert, 1997; Vervoort and Watson, 2003). Two species are known from the North Pacific, but in the northwestern Pacific, only *C. suensoni* has been reported, from the Hirado Strait, Japan (Jäderholm, 1896). To date, no species of *Corhiza* have been documented from Korean waters.

During recent taxonomic surveys of Leptothecate hydroids from Ulleungdo and Dokdo islands in the East Sea of Korea, specimens representing an undescribed species of *Corhiza* were collected. In this study, we provide a detailed morphological description and molecular data of *Corhiza ulleungensis* sp. nov., representing the first record of this genus from the Korean hydroid fauna. With this discovery, the family Halopteriddidae of Korea now comprises five genera and eight species.

## MATERIALS AND METHODS

Specimens were obtained by SCUBA diving from subtidal habitats at depths of 30−52 m around Ulleungdo and Dokdo islands (the East Sea, Korea) during 2022. Prior to sampling, colonies attached to rock surfaces and mussel shells were photographed in situ with a digital camera (Olympus Tough TG-6; Olympus Digital Solution Corporation, Tokyo, Japan) to document natural coloration, colony form, and associated microhabitat. The collected material was relaxed in a menthol-seawater solution for up to two hours and subsequently fixed in 99% ethanol (v/v) for morphological and molecular analyses.

Taxonomic identification followed detailed observations of the colony, stem, hydrocladia, hydrothecae, nematothecae, and gonothecae using a stereomicroscope (SteREO Discovery.V8; Carl Zeiss, Jena, Germany). Measurements were taken from digital images captured with an image analysis system (Opti View; Korea Lab Tech, Seongnam, Korea). Terminology and comparative characters follow standards used in recent halopteridid systematics (Moura et al., 2018; Galea et al., 2020).

Total genomic DNA was isolated from ethanol-preserved material using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following a brief rinse in distilled water. A fragment of the 16S rRNA gene was amplified using primer pair F1Mod (5′-TCG ACT GTT TAC CAA AAA CAT A-3′) and R2 (5′-ACG GAA TGA ACT CAA ATC ATG TAA G-3′) (Cunningham and Buss, 1993). PCRs were performed in a 50 μL reaction volume on a T100 Thermal Cycler (Bio-Rad Laboratories Inc., Hercules, CA, USA) with TaKaRa Ex Taq (Takara Bio Inc., Kusatsu, Japan). After temperature-gradient optimization (40−55°C), the final thermal profile consisted of an initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 30 s, 50°C for 45 s, and 72°C for 1 min; followed by a final extension at 72°C for 10 min.

Sequences were edited and aligned in Geneious Prime version 2025.1.3 (Biomatters, Auckland, New Zealand). The phylogenetic dataset comprised 34 sequences, including 33 halopteridid sequences representing 12 species in four genera, with *Sertularella miurensis* Stechow, 1921, used as the outgroup. Genetic distances were calculated using the Kimura 2-parameter model, and a Neighbor-Joining tree was constructed in MEGA version 11 (Tamura et al., 2021). Three sequences generated in this study—three from *Corhiza ulleungensis* sp. nov. (PX617882, PX617883, PX617884)—were deposited in GenBank; all accession numbers are provided in Fig. 3. English proofreading of the manuscript was assisted by ChatGPT, a generative AI language model.

The holotype (MABIK CN00081705) and two paratypes (MABIK CN00081704, MABIK CN00081706) were deposited in the National Marine Biodiversity Institute of Korea (Seocheon). Additional paratypes (CBB22CnHyE061, MBCL22 CnHyE34) were deposited in the Cnidaria Bioresources Bank of Korea and the Marine Biodiversity and Conservation Laboratory, Woosuk University (Jincheon).

## SYSTEMATIC ACCOUNTS

Phylum Cnidaria Hatschek, 1888
Class Hydrozoa Owen, 1843
Subclass Hydroidolina Collins, 2000
Order Leptothecata Cornelius, 1992
Superfamily Plumularioidea McCrady, 1859
Family Halopteriddidae Millard, 1962

### [1]*Genus *Corhiza* Millard, 1962

**Type species.** *Corhiza scotiae* Millard, 1962, by original designation.

**Diagnosis.** Colonies erect, typically pinnate. Hydrocaulus mono- or polysiphonic, the latter composed of several longitudinal tubes in free communication; stem unbranched or sparsely branched. Hydrocladia inserted alternately along stem; always unbranched and monoserial, arising from apophyses

Korean name: [1]*꼬인뿌리깃히드라속 (신칭)

of stem internodes; in most species irregularly arranged, but in some alternate regularly to left and right. Hydrothecae present on both stem and hydrocladia, cup-shaped to shallowly bell-shaped; rim entire, smooth, or only weakly undulated. Nematothecae occurring in typical halopteridid arrangement, including single median inferior nematotheca and paired laterals; formula varying among species. Gonothecae borne on stem or hydrocladia, generally ovate to elongate, often with one or more nematothecae. Phylactocarps absent, a key feature distinguishing genus from certain related halopteridids.

**Key to the genera of Halopteridide from Korea**

1. Lateral nematothecae laterally fused to hydrothecal wall ····· *Antennellopsis*
− Lateral nematothecae free from hydrotheca ····· 2
2. Colony consisting of solitary, unbranched stems arising directly from creeping hydrorhiza; hydrocladia absent ····· *Antennella*
− Colonies branched, with hydrocladia or secondary stems present ····· 3
3. Colonies with simple, alternately arranged hydrocladia; stem mono- or polysiphonic; hydrocladia never branched ····· *Corhiza*
− Colonies with more complex branching or distinctly pinnate hydrocladia ····· 4
4. Hydrocladia arising irregularly from lower portion of stem; stems typically monosiphonic; each hydrocladium with basal hydrotheca ····· *Monostaechas*
− Hydrocladia arranged in opposite or sub-opposite pairs, forming a regular pinnate colony ····· *Halopteris*

**Key to the species of *Corhiza***

1. Stem monosiphonic ····· *C. suensoni*
− Stem polysiphonic ····· 2
2. Companion tube present ····· *C. sociabilis*
− Companion tube absent ····· 3
3. Stems fascicled, branching sub-dichotomously ····· *C. fascicularis*
− Branching not sub-dichotomous ····· 4
4. Hydrocladia almost absent, arising directly from stem ····· *C. scotiae*
− Hydrocladia well developed, branching in various patterns ····· 5
5. Hydrocladia forming feather-like arrangement ····· *C. splendens*
− Hydrocladia not forming feather-like arrangement ····· 6
6. Hydrocladia short and crowded, colony appearing woolly or shaggy ····· *C. pannosa*
− Colony not woolly or shaggy ····· 7
7. Hydrotheca large, with margin flared outward ··· *C. patula*
− Hydrothecal margin not distinctly flared ····· 8
8. Hydrotheca with adcauline wall completely adnate ····· *C. bellicosa*
− Hydrotheca with adcauline wall not completely adnate ··· 9
9. Adcauline wall adnate for one-third of hydrothecal length ····· *C. megatheca*
− Adcauline wall adnate for one-half of hydrothecal length ····· 10
10. Axillar nematotheca absent ····· *C. pauciarmata*
− Axillar nematotheca present ····· 11
11. Intersegment with 2 nematothecae only ····· *C. complexa*
− Intersegment with 2−4 nematothecae ····· *C. ulleungensis* sp. nov.

### [1]**Corhiza ulleungensis* sp. nov. (Tables 1−4, Figs. 1−3)

**ZooBank LSID.** urn:lsid:zoobank.org:act:652AB195-C1CD-4625-8F34-D86FCD69A6EB.

**Type locality.** Korea: Gyeongsangbuk-do, Ulleung-gun, Buk-myeon, Cheonbu-ri, Ssangjeongcho (37°33′26″N, 130°56′27″E), 51−52 m.

**Material examined.** Korea: Holotype (MABIK CN00081705), 1 colony, Gyeongsangbuk-do: Ulleung-gun, Buk-myeon, Cheonbu-ri, Ssangjeongcho (37°33′26″N, 130°56′27″E), 51−52 m depth, 4 Jul 2022, Park SH, Shim SH; Paratype (MABIK CN00081704), 1 colony, Ulleung-gun, Ulleung-eup, Dodong-ri, Bukjeobawi (37°30′4″N, 130°55′11″E), 20−30 m depth, 30 Jun 2022, Jeong SC, Lee KH, Lee SJ, Yu HJ; Paratype (MABIK CN00081706), 1 colony, Ulleung-gun, Ulleung-eup, Dokdo-ri, Gajebawi (37°14′50″N, 131°51′54″E), 50 m depth, 1 Jul 2022, Park SH, Shim SH. All specimens were collected by SCUBA diving.

**Description.** Colonies erect, stems arising from hydrorhiza, 6−8 cm in height (Figs. 1, 2). Stems strongly fascicled and composed of numerous irregularly twisted vascular tubes originating from hydrorhiza (Figs. 1, 2). Hydrocladia branch irregularly from stem, unbranched, up to 1.7 cm long. Transverse node present at base of hydrocladia, three to seven nematothecae above it (Figs. 1, 2). In hydrocladia, hydrothecae main segments and intersegments arranged alternately (lower part of intersegment divided into transverse node and upper part into oblique node) (Fig. 1). Each hydrothecal segment contains one hydrotheca, one pair of lateral nematothecae, one axillar nematotheca, one median inferior nematotheca, 548−643 μm in length (Fig. 1). Each intersegment with only two or three median nematothecae, rarely four, 481−773 μm in length (Fig. 2). Hydrothecae cup-shaped, with straight abcauline walls and

Korean name: [1]*울릉꼬인뿌리깃히드라 (신칭)

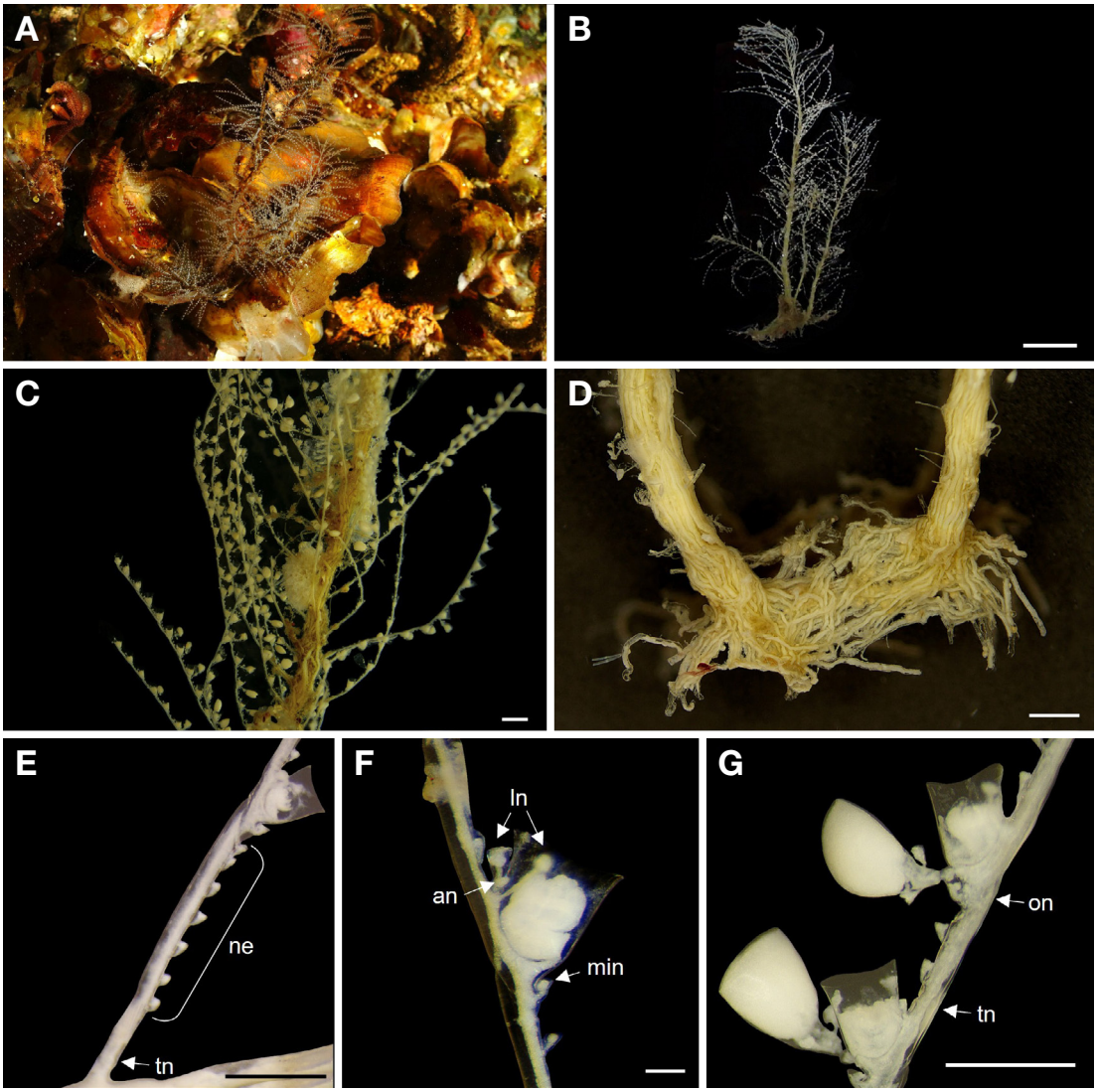


**Fig. 1.** *Corhiza ulleungensis* sp. nov., Holotype; (MABIK CN00081705): A, Living colony in situ; B, Colony after preservation in ethanol; C, Irregularly branched hydrocladia; D, Creeping hydrorhiza and polysiphonic stem; E, Series of seven nematothecae arranged along the basal region of a hydrocladium; F, One axillar nematotheca, a pair of lateral nematothecae, and a median inferior nematotheca; G, Gonothecae arising immediately below the hydrotheca. ne, nematothecae; an, axillar nematotheca; ln, lateral nematothecae; min, median inferior nematotheca; tn, transverse node; on, oblique node. Scale bars: B=1 cm, C, D=1 mm, E, G=0.5 mm, F=0.1 mm.

adcauline walls adnate to hydrocladia approximately halfway along length of hydrothecae, 310–400 μm in height (Fig. 1). Margins of hydrothecae circular, smooth, and slightly turned outward. Median nematothecae not extended beyond base of hydrothecae, and lateral nematothecae not protruded beyond margin of hydrothecae (Fig. 1).

Gonothecae arising from base of hydrothecae, bearing two large nematothecae at base, fig-shaped, with a wide, circular opening at distal end, with pedicels consisted of two segments, about 1.5 times as long as hydrothecae, measuring 579–651 μm (Figs. 1, 2).

Comparison of detailed measurements between type specimens in Table 1.

**Color.** The stems are light brown, whereas the hydrocladia and hydrothecae are white. The coloration becomes somewhat faded after preservation in alcohol (Fig. 1).

**Ecology and habitat.** The colonies attach to barnacle and mussel shells, as well as other hard substrates, by means of creeping hydrorhiza, and occur in subtidal habitats at depths of 30 m or greater. Gonothecae have been observed during the summer months, primarily in June and July.

**Distribution.** Korea (Ulleungdo, Dokdo).

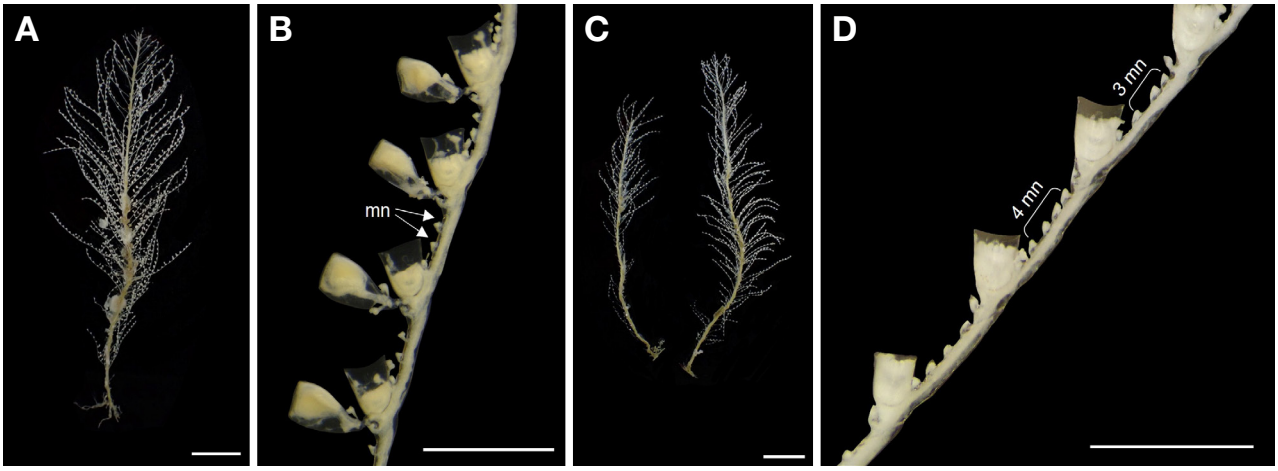


**Fig. 2.** *Corhiza ulleungensis* sp. nov. Paratype; (MABIK CN00081704): A, Photograph of the specimen; B, Gonothecae arising from the base just below hydrothecae, accompanied by two median nematothecae. Paratype; (MABIK CN00081706): C, Photograph of the specimen; D, Intersegmental region showing three to four median nematothecae. mn, median nematothecae. Scale bars: A, C = 1 cm, B, D = 1 mm.

**Table 1.** Comparison of detailed measurements of hydrocladia and stem, hydrothecae, nematothecae and gonothecae in type specimens of *Corhiza ulleungensis* sp. nov.

| Characters | *Corhiza ulleungensis* sp. nov. | | |
|---|---|---|---|
| | Holotype | Paratypes | |
| | MABIK CN00081705 | MABIK CN00081704 | MABIK CN00081706 |
| Stem | | | |
| Height (cm) | 7.5–8 | 6.8–8 | 6.5–8.2 |
| Diameter | 917–957 | 843–854 | 876–1,147 |
| Hydrocladia | | | |
| Length (cm) | 1.4–1.6 | 1.2–1.7 | 0.8–1.2 |
| Segments | | | |
| Intersegment, length | 522–589 | 481–578 | 605–773 |
| Main segment, length | 554–643 | 552–625 | 548–567 |
| Diameter at node | 98–111 | 100–111 | 104–114 |
| Hydrothecae | | | |
| Height | 331–385 | 338–400 | 310–353 |
| Diameter | 342–368 | 321–356 | 311–370 |
| Free adcauline wall length | 201–239 | 223–235 | 176–208 |
| Abcauline wall length | 365–409 | 357–420 | 348–414 |
| Nematotheca | | | |
| Length | 70–79 | 76–78 | 73–78 |
| Diameter | 32–42 | 38–53 | 45–61 |
| Median length | 90–95 | 91–98 | 83–89 |
| Median diameter | 56–70 | 64–66 | 73–87 |
| Laterals length | 110–120 | 115–123 | 121–130 |
| Laterals diameter | 75–95 | 59–85 | 84–94 |
| Gonotheca | | | |
| Length without pedicel | 579–651 | 601–626 | – |
| Width | 262–387 | 233–421 | – |

–, no data; size in μm.

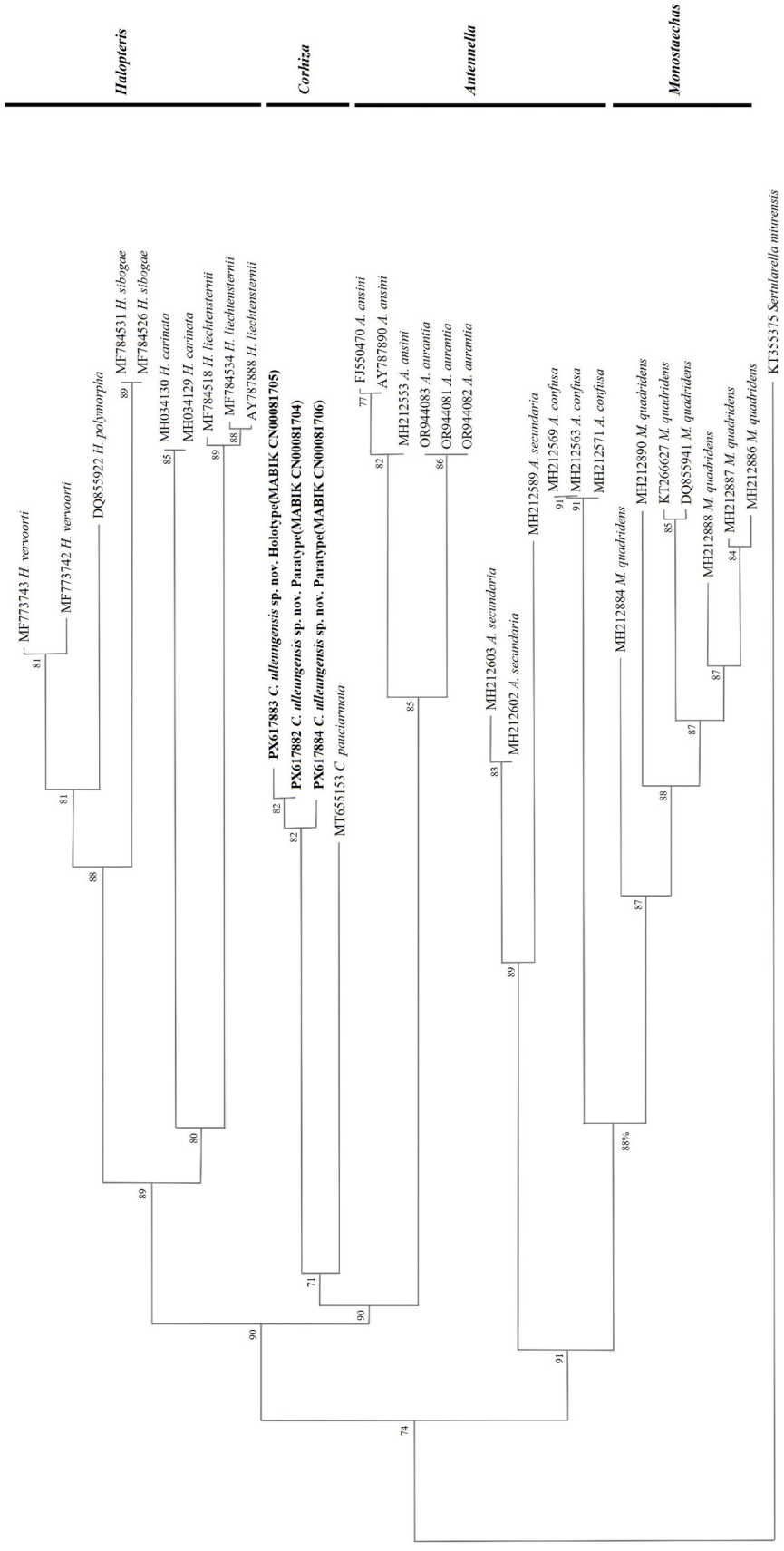


**Fig. 3.** Neighbor-Joining tree based on 16S rRNA sequences from four genera of the family Halopteridae, with *Sertularella miurensis* used as the outgroup. Numbers at the nodes represent bootstrap values (1,000 replicates). Sequences newly generated in this study are shown in bold, and GenBank accession numbers are listed alongside the corresponding species names. Scale bar: 0.050.

**Table 2.** Comparison between of *Corhiza ulleungensis* sp. nov. and similar species

| Characters | *Corhiza ulleungensis* sp. nov. | *C. complexa* | *C. patula* |
|---|---|---|---|
| | This study | (Nutting, 1906) | Galea, 2020 |
| | | Original description | Original description |
| Stem | | | |
| Height (cm) | 6.5–8.2 | 10 | – |
| Diameter | 843–1,147 | – | – |
| Hydrocladia | | | |
| Length (cm) | 0.8–1.7 | – | – |
| Segments | | | |
| Intersegment, length | 481–773 | 400–620 | 550–760 |
| Main segment, length | 548–643 | 350–500 | 580–650 |
| Diameter at node | 98–114 | – | 100–140 |
| Hydrothecae | | | |
| Height | 310–400 | 220 | 440–485 |
| Diameter | 311–370 | 190–250 | 420–475 |
| Free adcauline wall length | 176–239 | 110–150 | 355–385 |
| Abcauline wall length | 348–420 | 170–220 | 695–810 |
| Nematotheca | | | |
| Length | 70–79 | – | 120–190 |
| Diameter | 32–61 | – | 60–105 |
| Median length | 83–98 | – | 120–140 |
| Median diameter | 56–87 | – | 90–115 |
| Laterals length | 110–130 | – | 150–170 |
| Laterals diameter | 59–95 | – | 45 |
| Lateral, apophysis length | – | – | 75–90 |
| Gonotheca | | | |
| Length without pedicel | 579–651 | 330–650 | – |
| Width | 233–421 | – | – |
| Distribution | Northwest Pacific (Korea: Ulleungdo, Dokdo) | Central North Pacific (Hawaiian Islands) | South Pacific (New Caledonia) |

– , no data; size in μm.

**Etymology.** The specific epithet *ulleungensis* is derived from Ulleung, referring to Ulleung-gun, the administrative district encompassing Ulleungdo and Dokdo in the East Sea of Korea, where the species was collected. The name is formed by combining Ulleung with the Latin adjectival suffix *-ensis*, meaning "belonging to" or "originating from," and denotes the species' type locality.

**DNA barcoding.** Genetic distance analyses based on partial 16S rRNA sequences from 12 halopteridid species representing four genera (*Halopteris*, *Corhiza*, *Antennella*, and *Monostaechas*), with *Sertularella miurensis* as the outgroup, revealed clear patterns of intra- and interspecific variation (Table 3). Intraspecific divergence was extremely low (0.000 ± 0.000) for most species with multiple sequences, including *Antennella ansini*, *A. aurantia*, *A. confusa*, *Halopteris carinata*, *H. liechtensternii*, *H. sibogae* and *H. vervoorti*. The new species, *Corhiza ulleungensis* sp. nov., also showed minimal intraspecific variation (0.01 ± 0.00). Slightly higher intraspecific values were observed for *Monostaechas quadridens* (0.08 ± 0.01) and *Antennella secundaria* (0.08 ± 0.01). Species represented by single sequences—*Corhiza pauciarmata*, *Halopteris polymorpha*, and the outgroup—could not be assessed (n/c).

Interspecific genetic distances among halopteridid species ranged widely (0.08–0.37), consistent with previous studies showing the strong discriminatory power of 16S rRNA within the family. Within the genus *Corhiza*, the distance between *C. ulleungensis* sp. nov. and *C. pauciarmata* was 0.15, supporting their recognition as distinct species despite morphological similarities.

The phylogenetic reconstruction (Fig. 3) recovered *Corhiza* as a distinct clade (bootstrap 71%) composed of *C. ulleungensis* sp. nov. (three sequences) and *C. pauciarmata*. Within this clade, the three sequences of the new species formed a well-supported subclade (82%). *Corhiza* was placed sister to *Halop-*

**Table 3.** Genetic distances within and between species of the family Halopteriddidae based on partial 16S rRNA in 602 bp length

| Species (number of sequences) | Within (mean ± SD) | Between *Corhiza ulleungensis* sp. nov. (mean ± SD) |
|---|---|---|
| *Antennella ansini* (3) | 0.00 ± 0.00 | 0.24 ± 0.02 |
| *Antennella aurantia* (3) | 0.00 ± 0.00 | 0.25 ± 0.02 |
| *Antennella confusa* (3) | 0.00 ± 0.00 | 0.27 ± 0.03 |
| *Antennella secundaria* (3) | 0.08 ± 0.01 | 0.24 ± 0.02 |
| *Corhiza pauciarmata* (1) | n/c | 0.15 ± 0.02 |
| ***Corhiza ulleungensis* sp. nov.** (3) | 0.01 ± 0.00 | – |
| *Halopteris carinata* (2) | 0.00 ± 0.00 | 0.22 ± 0.02 |
| *Halopteris liechtensternii* (3) | 0.00 ± 0.00 | 0.25 ± 0.03 |
| *Halopteris polymorpha* (1) | n/c | 0.23 ± 0.02 |
| *Halopteris sibogae* (2) | 0.00 ± 0.00 | 0.21 ± 0.02 |
| *Halopteris vervoorti* (2) | 0.01 ± 0.00 | 0.19 ± 0.02 |
| *Monostaechas quadridens* (7) | 0.08 ± 0.01 | 0.27 ± 0.02 |

SD, standard deviation; n/c, no calculated.

*teris*, which was strongly supported (90%) and comprised two well-defined subgroups: one containing *H. vervoorti*, *H. polymorpha* and *H. sibogae* (bootstrap 81–89%), and another containing *H. carinata* and *H. liechtensternii* (85–89%).

*Antennella* formed a robust monophyletic lineage (bootstrap 90%), divided into two subclades: one including *A. ansini* and *A. aurantia* (77–86%), and another containing *A. secundaria* and *A. confusa* (83–91%).

*Monostaechas quadridens* formed a distinct, well-supported clade (84–88%) separate from both *Antennella* and *Halopteris*, with intergeneric distances ranging from 0.22 to 0.36 (Table 4). Distances between *Antennella* and *Halopteris* were also high (0.23–0.37), reflecting their strong genetic and phylogenetic separation.

The outgroup *Sertularella miurensis* (Sertularellidae) was clearly isolated from Halopteriddidae, with large genetic distances (0.30–0.34) and basal placement in the tree (bootstrap 74%), confirming the monophyly of Halopteriddidae.

Together, the molecular evidence—supported by the clear placement of the new species within *Corhiza* and strong separation from all other halopteridid genera—confirms that the specimens from Ulleungdo and Dokdo represent a new species, *Corhiza ulleungensis* sp. nov. This species constitutes the first confirmed record of *Corhiza* from Korean waters and extends the known distribution of the genus into the northwestern Pacific.

**Remarks.** Species of the genus *Corhiza* are characterized by erect monosiphonic or polysiphonic colonies, unbranched and monoserial hydrocladia inserted alternately, cup-shaped hydrothecae with smooth or only slightly undulated rims, and the absence of phylactocarps. *C. ulleungensis* sp. nov. exhibits all diagnostic features of the genus and is therefore confidently assigned to *Corhiza* (Millard, 1962; Schuchert, 1997).

**Table 4.** Genetic distances within and between genus of the family Halopteriddidae based on partial 16S rRNA in 602 bp length

| Genus (number of sequences) | Within (mean ± SD) | Between genus (mean, min–max) |
|---|---|---|
| *Antennella* (12) | 0.26 ± 0.08 | 0.25–0.30 |
| *Corhiza* (4) | 0.15 ± 0.02 | 0.23–0.27 |
| *Halopteris* (10) | 0.18 ± 0.05 | 0.23–0.34 |
| *Monostaechas* (7) | n/c | 0.27–0.34 |

SD, standard deviation; n/c, no calculated.

Among the 11 valid species currently recorded to *Corhiza*, *C. suensoni* is the species most comparable to *C. ulleungensis* sp. nov. with respect to geographic distribution, as both occur in the northwestern Pacific (Jäderholm, 1896). However, despite this regional overlap, the two species can be clearly differentiated by several diagnostic morphological features. *C. ulleungensis* sp. nov. is readily distinguished from *C. suensoni* by its polysiphonic stem, whereas *C. suensoni* consistently possesses a monosiphonic stem. The branching pattern of the hydrocladia also differs: in the new species, hydrocladia are irregularly branched, while in *C. suensoni*, they arise in a regular, alternate arrangement. Furthermore, *C. ulleungensis* sp. nov. bears axillar nematothecae, a feature absent in *C. suensoni*. These morphological distinctions clearly separate the two species despite their contiguous geographic extents.

*Corhiza ulleungensis* sp. nov. also shows morphological affinities to *C. complexa* (Nutting, 1906) and *C. patula* Galea, 2020, sharing polysiphonic stems, irregularly branched hydrocladia, and the presence of axillar nematothecae (Fig. 1). Nonetheless, clear diagnostic distinctions separate these species.

*Corhiza ulleungensis* sp. nov. possesses 2–4 median nematothecae at the intersegments—significantly more than the condition in *C. complexa* (2) and *C. patula* (1). Furthermore, the geographic distributions of these species differ markedly: *C. complexa* occurs in the Central North Pacific (Hawaiian Islands), *C. patula* in the South Pacific (New Caledonia), whereas *C. ulleungensis* sp. nov. is presently known only from the Northwest Pacific (Korea).

The combination of a polysiphonic stem, irregularly arranged hydrocladia, presence of axillar nematothecae, the occurrence of up to four median nematothecae at intersegments, and the detailed proportions of the hydrothecae and gonothecae—together with its geographic distribution—collectively support the recognition of *C. ulleungensis* sp. nov. as a distinct new species within *Corhiza*. This morphological distinctiveness is consistent with its phylogenetic placement, which recovers *C. ulleungensis* sp. nov. in a clade that includes *C. pauciarmata* within *Corhiza* and clearly separates it from the other congeners included in the 16S rRNA-based reconstruction.

Comparison of detailed measurements and characteristics between *C. ulleungensis* sp. nov. and similar species is shown in Table 2.

## ORCID

Hyo-Jin Yu: https://orcid.org/0000-0002-4143-1101
Sung-Jin Hwang: https://orcid.org/0000-0002-1259-6775

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

## ACKNOWLEDGMENTS

This work was supported by the management of Marine Fishery Bio-resources Center (2025) funded by the National Marine Biodiversity Institute of Korea (MABIK). Additional support was provided by Woosuk University.

## REFERENCES

Ansín Agís JA, Ramil F, Vervoort W, 2004. *Cladoplumaria anomala* gen. nov., sp. nov., a new genus and species of the family Halopteriididae (Cnidaria: Hydrozoa) from the Chesterfield Islands region (Pacific Ocean). Cahiers de Biologie Marine, 45:313-324.

Cunningham CW, Buss LW, 1993. Molecular evidence for multiple episodes of paedomorphosis in the family Hydractiniidae. Biochemical Systematics and Ecology, 21:57-69. https://doi.org/10.1016/0305-1978(93)90009-G

Galea HR, Di Camillo CG, Maggioni D, Montano S, Schuchert P, 2020. A reassessment of *Halopteris polymorpha* (Billard, 1913) (Cnidaria: Hydrozoa), with descriptions of three new species. Revue suisse de Zoologie, 125:21-59. https://doi.org/10.5281/zenodo.1196007

Hirohito, 1995. The hydroids of Sagami Bay II. Thecata. Biological Laboratory Imperial Household, Tokyo, pp. 1-244.

Jäderholm E, 1896. Ueber aussereuropäische Hydroiden des zoologischen Museums der Universität Upsala. Bihang till Kongliga Svenska Vetenskaps-Akademiens Handlingar, Afdelning, 4:1-20.

Millard NAH, 1962. The Hydrozoa of the south and west coasts of South Africa. Part I. The Plumulariidae. Annals of the South African Museum, 46:261-319.

Millard NAH, 1980. Hydroida. The South African Museum's Meiring Naude cruises. Part 11. Annals of the South African Museum, 82:129-153.

Moura CJ, Lessios H, Cortés J, Nizinski MS, Reed J, Santos RS, Collins AG, 2018. Hundreds of genetic barcodes of the species-rich hydroid superfamily Plumularioidea (Cnidaria, Medusozoa) provide a guide toward more reliable taxonomy. Scientific Reports, 8:17986. https://doi.org/10.1038/s41598-018-35528-8

Nutting CC, 1906. Hydroids of the Hawaiian Islands collected by the steamer Albatross in 1902. Bulletin of the United States Fish Commission, 23:931-959.

Park JH, 2010. Invertebrate fauna of Korea, Vol. 4, No. 1. Thecates. National Institute of Biological Resources, Incheon, pp. 1-183.

Schuchert P, 1997. Review of the family Halopteriididae (Hydrozoa, Cnidaria). Zoologische Verhandelingen, 309:1-162.

Schuchert P, Choong H, Galea H, Hoeksema B, Lindsay D, Manko M, Pica D, 2025. World Hydrozoa Database. Halopteriididae Millard, 1962 [Internet]. World Register of Marine Species, Flemish Marine Institute (VLIZ), Ostend, Accessed 11 Nov 2025, <https://www.marinespecies.org/aphia.php?p=taxdetails&id=22795>.

Tamura K, Stecher G, Kumar S, 2021. MEGA11: molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution, 38:3022-3027. https://doi.org/10.1093/molbev/msab120

Vervoort W, Watson JE, 2003. The marine fauna of New Zealand: Leptothecata (Cnidaria: Hydrozoa) (thecate hydroids). NIWA Biodiversity Memoir, 119:1-538.

Yu HJ, Lee SJ, Hwang SJ, 2024. A new species of *Antennella* (Hydrozoa: Leptothecata: Halopteriididae) from Korea. Animal Systematics, Evolution and Diversity, 40:79-87. https://doi.org/10.5635/ASED.2024.40.1.056

Received December 12, 2025
First revised December 19, 2025
Second revised December 26, 2025
Accepted December 26, 2025

ASED http://e-ASED.org
Animal Systematics, Evolution and Diversity

*Anim. Syst. Evol. Divers. Vol. 42, No. 1: 63-81, January 2026*
https://doi.org/10.5635/ASED.2026.42.1.018

# Two New Species of Anenthemonae (Cnidaria: Anthozoa: Actiniaria) and a New Korean Record of *Metridium farcimen* (Enthemonae)

Myeong Kyu Lim[1], Seung-Joon Lee[1,2], Sung-Jin Hwang[1,*]

[1]Department of Life Science, Woosuk University, Jincheon 27841, Korea
[2]Department of Biological Sciences, Dankook University, Cheonan 31116, Korea

## ABSTRACT

During taxonomic surveys of sea anemones conducted in the East Sea of Korea, specimens representing two undescribed species of the suborder Anenthemonae and one previously unrecorded species of the suborder Enthemonae were collected. In this study, we describe two new species—*Tempuractis microarmata* sp. nov. (family Edwardsiidae) and *Isohalcurias aurantimaculatus* sp. nov. (family Halcuriidae)—and report the first Korean record of *Metridium farcimen* (family Metridiidae), based on detailed morphological examinations and DNA barcoding analyses using partial 16S rRNA sequences. *Tempuractis microarmata* sp. nov., collected from Ulleungdo at depths of 12–16 m, represents the first record of the genus *Tempuractis* from Korean waters. This minute edwardsiid exhibits an obligate symbiotic association with homoscleromorph sponges (*Oscarella* sp.) and is distinguished from the type species *T. rinkai* by the presence of microbasic b-mastigophores in both tentacles and column. *Isohalcurias aurantimaculatus* sp. nov., collected from Ulleungdo at depths of 28–30 m, is characterized by a cream-colored column with distinctive orange spots and by the presence of microbasic b-mastigophores in multiple body regions, clearly separating it from all congeners. *Metridium farcimen*, recorded from Goseong, Yeongdeok, and Ulleungdo at depths of 20–36 m, is readily distinguished from *M. senile* by its highly extensible column, with a column height to pedal disc diameter ratio exceeding 2 : 1, and by the presence of microbasic p-mastigophores in the actinopharynx. DNA barcoding analyses revealed variable performance of the 16S rRNA marker among families: it provided effective species-level discrimination in Edwardsiidae and Halcuriidae but showed limited utility for resolving species boundaries within *Metridium*.

**Keywords:** Edwardsiidae, Halcuriidae, Metridiidae, new species, East Sea of Korea, 16S rRNA

## INTRODUCTION

Sea anemones (order Actiniaria Hertwig, 1882), belonging to the phylum Cnidaria, comprise approximately 1,101 valid species assigned to 264 genera and 50 families (Fautin, 2016). Excluding the monotypic suborder Helenmonae Daly and Rodríguez, 2019, which contains only *Relicanthus daphneae* (Daly, 2006), Actiniaria is currently divided into two major suborders: Anenthemonae Rodríguez and Daly, 2014, and Enthemonae Rodríguez and Daly, 2014 (Fautin, 2016; Rodríguez et al., 2025). These two suborders differ fundamentally in their patterns of mesenterial development and associated anatomical features (Rodríguez et al., 2014).

Suborder Anenthemonae is characterized by a distinctive mesenterial arrangement in which mesenteries of the second and subsequent cycles originate in the endocoels of the first cycle, in contrast to the exocoelic development typical of most other actiniarians (Rodríguez et al., 2014). This suborder comprises two superfamilies: Actinernoidea Stephenson, 1922, and Edwardsioidea Andres, 1881.

The superfamily Actinernoidea includes sea anemones with a well-developed pedal disc lacking basilar muscles, one or two siphonoglyphs, and an endocoelic pattern of mesenterial development throughout successive cycles (Carlgren, 1949; Rodríguez et al., 2014; Izumi et al., 2023). Following recent phylogenetic revisions, Actinernoidea is composed of three families: Actinernidae Stephenson, 1922, Halcuriidae Carlgren, 1918, and Isactinernidae Izumi et al., 2023 (see Izumi

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: 82-43-531-2892, Fax: 82-43-531-2862
E-mail: buteo2@woosuk.ac.kr, buteo2@gmail.com

eISSN 2234-8190

Copyright © The Korean Society of Systematic Zoology

et al., 2023). The family Halcuriidae currently contains three genera: *Carlgrenia* Stephenson, 1918; *Halcurias* McMurrich, 1893; and *Isohalcurias* Izumi et al., 2023 (Rodríguez et al., 2025). Species of *Isohalcurias* are distinguished from those of *Halcurias* by the presence of spirocysts in the column and pinnate retractor muscles, whereas *Halcurias* lacks columnar spirocysts and possesses simple retractor muscles. At present, *Isohalcurias* comprises three valid species: *I. carlgreni* (McMurrich, 1901), *I. citreum* Izumi et al., 2023, and *I. malum* Izumi et al., 2023. The species previously reported from Korean waters as *Halcurias carlgreni* (Hwang and Song, 2010) was reassigned to *I. carlgreni* by Izumi et al. (2023), representing the only species of *Isohalcurias* currently known from Korea, while *I. citreum* and *I. malum* are known only from Japan.

The superfamily Edwardsioidea comprises a single family, Edwardsiidae Andres, 1881. Members of this superfamily are burrowing sea anemones inhabiting soft sediments and characterized by an elongate, vermiform body lacking a true pedal disc, typically possessing eight perfect mesenteries in adults (Carlgren, 1949; Daly et al., 2008). The aboral end is modified into a physa or bears a terminal pore, and mesenteries are arranged in four pairs bearing retractor muscles. Edwardsiids occur worldwide from shallow intertidal zones to deep-sea environments (Carlgren, 1892, 1949). The family currently comprises 12 genera, including *Edwardsia* Quatrefages, 1842, and *Tempuractis* Izumi et al., 2018, among others. The genus *Tempuractis* is unique among edwardsiids in its symbiotic lifestyle, inhabiting exclusively within homoscleromorph sponges of the genus *Oscarella* (Izumi et al., 2018). Species of *Tempuractis* are characterized by a very small body size (less than 5 mm in length), a smooth column lacking nemathybomes or tenaculi, sixteen tentacles arranged octamerously in two cycles, and the absence of both siphonoglyph and sphincter muscle (Izumi et al., 2018). At present, the genus is monotypic, containing only *Tempuractis rinkai* Izumi et al., 2018, known from temperate Japanese waters. No species of *Tempuractis* have been previously recorded in Korean waters.

Suborder Enthemonae represents the larger and more diverse lineage within Actiniaria and is characterized by the exocoelic development of mesenteries of the second cycle (Rodríguez et al., 2014). This suborder comprises three superfamilies: Actinioidea Rafinesque, 1815 (20 families); Actinostoloidea Carlgren, 1932 (9 families); and Metridioidea Carlgren, 1893 (29 families), totaling 58 families worldwide (Rodríguez et al., 2025). Members of Enthemonae occupy a wide range of marine habitats, from shallow coastal environments to the deep sea. The family Metridiidae Carlgren, 1893 consists of sea anemones characterized by a smooth, muscular column and a broad oral disc fringed with numerous short tentacles. The column mesoglea is well-developed and muscular, allowing extensive expansion and contraction. Recent molecular phylogenetic studies have demonstrated that Metridiidae is monogeneric, containing only the genus *Metridium* de Blainville, 1824 (Glon et al., 2021, 2023; Daly et al., 2024). Two genera previously assigned to this family, *Isometridium* Carlgren, 1951 and *Paraisometridium* Zamponi, 1978, are now regarded as junior synonyms of *Metridium*, with their respective species synonymized with *M. farcimen* and *M. senile*.

The genus *Metridium* has a complex taxonomic history marked by extensive synonymy, largely due to pronounced phenotypic plasticity, ecophenotypic variation, and broad geographic distributions (Stephenson, 1935; Fautin et al., 1989; Glon et al., 2020). Molecular and biochemical genetic approaches have resolved species boundaries and confirmed the conspecificity of populations previously regarded as distinct species (Bucklin and Hedgecock, 1982; Bucklin, 1985; Glon et al., 2021, 2023). Currently, the genus *Metridium* contains two valid species—*M. senile* (Linnaeus, 1761) and *M. farcimen* (Brandt, 1835), supported by integrated morphological, genetic, and phylogenomic evidence (Bucklin and Hedgecock, 1982; Fautin et al., 1989; Fautin and Hand, 2000; Glon et al., 2021, 2023; Daly et al., 2024). *Metridium senile* exhibits an amphi-boreal distribution in the North Atlantic and North Pacific, with introduced populations in the Southern Hemisphere, whereas *M. farcimen* is restricted to the North Pacific from Kamchatka to the Gulf of California (Fautin and Hand, 2000; Glon et al., 2020, 2023; Daly et al., 2024).

During taxonomic surveys of sea anemones conducted in the East Sea of Korea between 2022 and 2025, specimens representing two undescribed species of the suborder Anenthemonae and one previously unrecorded species of the suborder Enthemonae were collected. In the present study, we provide detailed morphological descriptions and molecular phylogenetic analyses for two new species *Tempuractis microarmata* sp. nov. (Edwardsiidae) and *Isohalcurias aurantimaculatus* sp. nov. (Halcuriidae), and report the first Korean record of *Metridium farcimen* (Metridiidae), supported by both morphological examination and DNA sequence data. These findings expand the known actiniarian fauna in Korean waters and extend the documented distribution of the genus *Tempuractis* into the East Sea of Korea. With these additions, the diversity of Anenthemonae in Korean waters now comprises 3 genera and 4 species, while the overall actiniarian fauna of Korea includes 35 species.

## MATERIALS AND METHODS

Specimens representing three species were collected during SCUBA-based surveys conducted in subtidal zones (12–

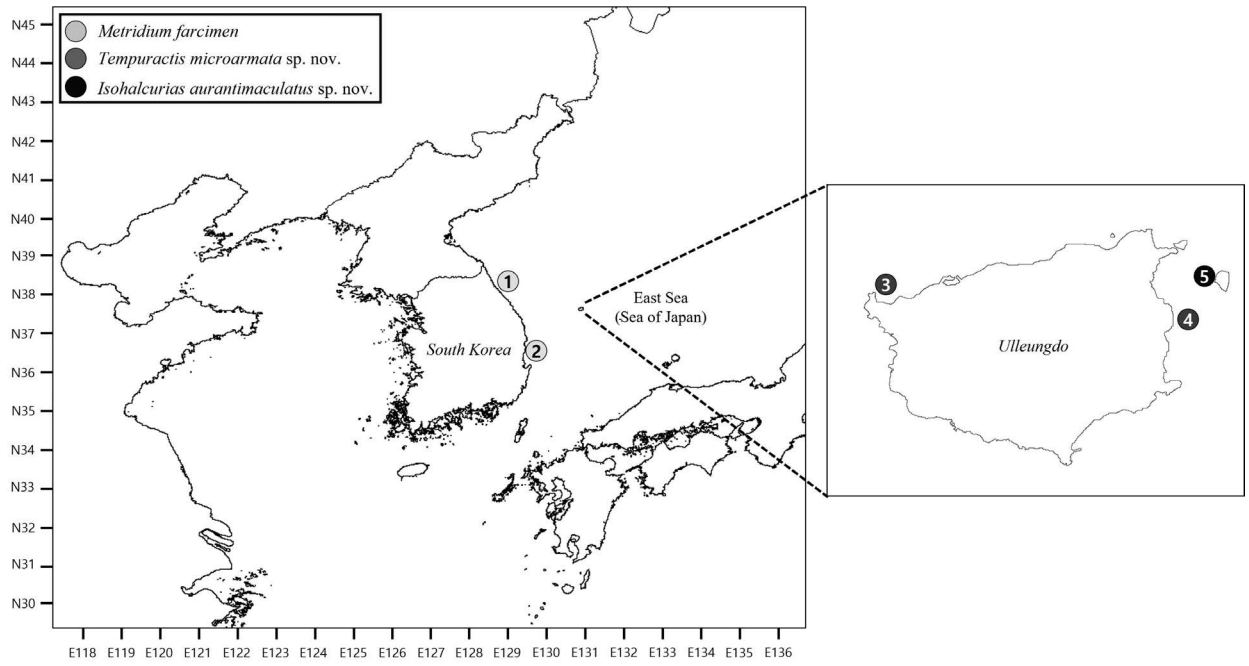


**Fig. 1.** Collection sites of three actiniarian species in the East Sea of Korea. 1, Goseong, Gangwon-do; 2, Yeongdeok, Gyeongsangbuk-do; 3–5, Ulleungdo, Gyeongsangbuk-do (3, Daepunggam; 4, Bukjeobawi; 5, Jukdo).

36 m depth) of Goseong, Yeongdeok, and Ulleungdo Island in the East Sea of Korea between 2022 and 2025 (Fig. 1). Before sampling, sea anemones attached to rocky substrates were photographed in situ using a digital camera (Olympus Tough TG-6; Olympus Digital Solution Corporation, Tokyo, Japan) to record live coloration, external morphology, and associated ectosymbiotic organisms. Collected specimens were anesthetized in a menthol-seawater solution for up to four hours and subsequently preserved in 5% buffered formalin for morphological study and 99% ethanol (v/v) for molecular analyses.

Species identification was based on detailed examination of external morphology, including tentacles, column, and oral and pedal discs, using a stereomicroscope (SteREO Discovery.V8; Carl Zeiss, Jena, Germany). Cnidae in each part were examined using a light microscope (Axio Lab A1; Carl Zeiss). External parts and cnidae were measured from digital images using OptiView software (Korea Lab Tech, Seongnam, Korea).

For molecular analysis, the mitochondrial 16S rRNA gene was selected as the DNA barcoding marker. Although mitochondrial markers such as COX1, COX3, and 16S rRNA have been used in previous DNA barcoding studies of sea anemones (order Actiniaria), the availability of sequence data varies among markers and taxonomic groups (Rodríguez et al., 2014; Glon et al., 2021). For the three genera examined in the present study, available COX1 and COX3 sequence data were insufficient to conduct comprehensive phylogenetic analyses, whereas sufficient 16S rRNA sequences were available in GenBank for all three genera. Therefore, DNA barcoding was performed using the 16S rRNA gene following established protocols for Actiniaria (Izumi et al., 2018, 2023).

Genomic DNA was extracted from ethanol-preserved tissue using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. A partial fragment of the mitochondrial 16S rRNA gene was amplified using the primer pair ANEM16SA (5′-CACTGACCGTGATAATGTAGCGTA-3′) and ANEM16SB (5′-CCCCATGGTAGCTTTTATTCG-3′) (Geller and Walton, 2001). Polymerase chain reactions were carried out in 50 μL volumes using TaKaRa Ex Taq polymerase (Takara Bio Inc., Kusatsu, Japan) on a T100 Thermal Cycler (Bio-Rad Laboratories Inc., Hercules, CA, USA). After optimization of annealing temperatures (45–55°C), amplification conditions consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 55°C for 45 s, and 72°C for 1 min, with a final extension step at 72°C for 10 min.

Sequences were manually edited and aligned using Geneious Prime version 2025.1.3 (Biomatters, Auckland, New Zealand). Pairwise genetic distances were calculated under the Kimura two-parameter (K2P) model, and a Neighbor-Joining (NJ) phylogenetic tree was reconstructed using MEGA version 11 (Tamura et al., 2021). Sequences generated in this study included six from *Tempuractis microarmata*

sp. nov. (PX640996, PX640997, PX699650–PX699653), six from *Metridium farcimen* (PX699632–PX699637), seven from *Metridium senile* (PX699638–PX699644), one from *Isohalcurias carlgreni* (PX700238), two from *Isohalcurias aurantimaculatus* sp. nov. (PX671085, PX647080) and one from *Diadumene lineata* (PX736382); all sequences were deposited in GenBank, and accession numbers are provided in the corresponding NJ tree. English language editing of the manuscript was assisted by ChatGPT, a generative artificial intelligence language model.

The holotypes of *Isohalcurias aurantimaculatus* sp. nov. (MABIK CN00081709), *Tempuractis microarmata* sp. nov. (MABIK CN00081707), paratypes of *I. aurantimaculatus* (MABIK CN00081711) and *T. microarmata* (MABIK CN00081710), together with one specimen of *Metridium farcimen* (MABIK CN00081708), were deposited in the National Marine Biodiversity Institute of Korea (MABIK), Seocheon. Paratypes of the two new species and additional specimens of all three species were deposited in the Cnidaria Bioresources Bank of Korea and the Marine Biodiversity and Conservation Laboratory, Woosuk University, Jincheon.

## SYSTEMATIC ACCOUNTS

Phylum Cnidaria Hatschek, 1888
Subphylum Anthozoa Ehrenberg, 1834
Class Hexacorallia Haeckel, 1896
Order Actiniaria Hertwig, 1882
Suborder Anenthemonae Rodríguez and Daly, 2014
Family Halcuriidae Carlgren, 1918

### [1]*Genus *Isohalcurias* Izumi et al., 2023

**Type species.** *Isohalcurias carlgreni* (McMurrich, 1901).
**Diagnosis.** Mesenteries to 34 pairs, arranged in four cycles (6 primary, 4 secondary, 8 tertiary, 16 quaternary). Ten pairs of macrocnemes, fertile, filamented, with restricted retractor muscles. Microcnemes confined to upper column, some complete. Retractor muscles diffuse, pinnate. Parietal muscles weak to moderate. Tentacles up to 68. Dioecious. Cnidom: spirocysts (particularly present on column), basitrichs (present throughout tissues), and microbasic p-mastigophores (restricted to actinopharynx and filaments).

**Key to species of *Isohalcurias***

1. Column with slightly raised spots ····· 2
– Column smooth ····· 3
2. Microbasic b-mastigophores present in tentacles ····· *I. aurantimaculatus* sp. nov.
– Microbasic b-mastigophores absent in tentacles ····· *I. carlgreni*
3. Microbasic b-mastigophores present in tentacles ··· *I. malum*
– Microbasic b-mastigophores absent in tentacles ··· *I. citreum*

### [2]**Isohalcurias aurantimaculatus* sp. nov.**
**(Tables 1, 2, Figs. 2–4)**

**ZooBank LSID:** urn:lsid:zoobank.org:act:75355CCE-E8D6-4101-8290-E75067E2F4F0.
**Type locality.** Korea: Gyeongsangbuk-do, Ulleung-gun, Ulleung-eup, Jukdo (37°31′54″N, 130°56′10″E), 28–30 m.
**Material examined.** Korea: Holotype (MABIK CN00081709), 1 individual, Gyeongsangbuk-do: Ulleung-gun, Ulleung-eup, Jukdo (37°31′54″N, 130°56′10″E), 28–30 m, 31 Aug 2023, Hwang SJ, Lee SJ, Yu HJ, Bae JK; Paratypes (CBB23CnAnE440, MBCL23CnAnE185), 2 individuals, Ulleung-gun, Ulleung-eup, Jukdo (37°31′54″N, 130°56′10″E), 28–30 m, 31 Aug 2023, Hwang SJ, Lee SJ, Yu HJ, Bae JK; Paratypes (MABIK CN00081711, MBCL25CnAnE077), 2 individuals, Ulleung-gun, Ulleung-eup, Jukdo (37°31′53″N, 130°56′11″E), 30 m, 23 Jun 2025, Lee SJ, Yu HJ, Lim MK. All specimens were collected by SCUBA diving.
**Description.** Column elongate, cylindrical; upper and lower diameters subequal, varying slightly with degree of expansion. Column height reaching up to 15 mm in living specimens; length reduced by ca. 50% after preservation. Column surface nearly smooth, with slightly raised orange spots. Distal column not lobed. Tentacles 28–34 (juveniles with fewer tentacles), arranged alternately in inner and outer cycles (Fig. 2A). Mouth margin folded, slightly protruding beyond oral disc; mouth subcircular. Pedal disc well developed, firmly adherent to hard substrates; disc diameter approximately equal to column diameter.

Size: living specimens—column height 4.5–15.0 mm, column width 2.9–5.2 mm; pedal disc diameter 3.1–5.5 mm; tentacle length 3.4–12.8 mm. Preserved specimens—column height 2.2–7.0 mm, column width 2.3–4.4 mm; pedal disc diameter 2.0–4.3 mm; oral disc diameter 0.8–1.7 mm; tentacle length 1.0–3.6 mm.

Cnidom: spirocysts, basitrichs, microbasic b-mastigophores, and microbasic p-mastigophores (Fig. 3); cnidae distribution and measurements summarized in Table 1.
**Color.** In living specimens, oral disc bright yellow. Tentacle bases semi-transparent, becoming white distally. Column cream-colored with scattered orange spots; spot density variable among individuals (Fig. 2A). When preserved, entire body pale yellow (Fig. 2B).

Korean name: [1]*유사별란말미잘속 (신칭), [2]*주황점유사별란말미잘 (신칭)

**Table 1.** Comparison of size of cnidae among species of the genus *Isohalcurias*, size in μm

| Tissue and cnidae | *Isohalcurias aurantimaculatus* sp. nov. | *Isohalcurias carlgreni* | *Isohalcurias citreum* | *Isohalcurias malum* |
|---|---|---|---|---|
| | This study (mean, number of cnidae) | Hwang and Song, 2010 (number of cnidae) | Izumi et al., 2023 original description (number of cnidae) | Izumi et al., 2023 original description (number of cnidae) |
| Tentacles | Tentacle-tip | Tentacle-tip | Tentacle | Tentacle |
| Spirocysts | 9.3–34.7×2.4–7.0 (21.1×4.3, 150) | 15.0–46.0×2.0–8.0 (162) | 22.5–42.9×2.6–5.9 (43) | 21.8–41.2×4.0–7.3 (25) |
| Basitrichs_S | 13.0–27.5×1.7–3.5 (21.7×2.8, 76) | 20.0–35.0×2.0–4.0 (125) | 27.0–34.8×2.6–4.1 (61) | 15.8–19.9×3.0–3.5 (3) |
| Basitrichs_L | Not observed | Not observed | 47.4×5.2 (1) | 28.9–39.1×2.7–4.4 (37) |
| Microbasic b-mastigophores | 21.0–26.7×3.1–4.6 (24.1×3.7, 31) | Not observed | Not observed | 30.1–36.3×3.4–6.0 (11) |
| | Tentacle-base | Tentacle-base | | |
| Spirocysts | 9.8–32.3×2.5–6.0 (20.8×4.2, 153) | 15.0–43.0×2.5–10.0 (196) | | |
| Basitrichs | 17.4–25.2×1.6–3.0 (21.3×2.6, 34) | 21.0–33.0×2.0–3.0 (59) | | |
| Microbasic b-mastigophores | 21.7–27.2×3.0–3.9 (24.4×3.5, 35) | Not observed | | |
| Column | | | | |
| Spirocysts | 13.8–32.0×2.6–6.8 (21.2×4.9, 105) | 13.0–37.0×2.0–7.0 (72) | 24.7–32.3×4.2–7.0 (69) | 27.8–37.1×4.2–6.1 (27) |
| Basitrichs | 12.6–25.9×1.6–3.0 (21.5×2.3, 104) | 16.0–37.0×1.5–3.0 (163) | 23.6–34.1×2.3–3.5 (24) | 23.8–34.2×2.7–4.1 (37) |
| Actinopharynx | | | | |
| Spirocysts | 15.5–30.7×2.9–7.2 (22.8×4.9, 97) | 20.0–40.0×5.0–8.0 (209) | 22.6–33.9×4.1–7.9 (51) | 31.5–52.0×5.8–9.6 (64) |
| Basitrichs | 15.7–27.7×2.0–3.1 (22.3×2.5, 55) | 20.0–33.0×2.0–4.0 (141) | 22.6–30.8×3.0–4.3 (62) | 25.7–36.2×3.2–4.5 (37) |
| Microbasic b-mastigophores | 20.8–26.2×2.9–4.4 (23.4×3.5, 42) | Not observed | 25.6–28.6×4.3–5.4 (4) | 30.1–37.1×5.6–7.4 (6) |
| Microbasic p-mastigophores | 16.6–25.6×2.4–6.9 (21.0×3.9, 57) | 21.0–30.0×4.0–6.0 (85) | 23.4–29.2×5.2–7.0 (39) | 29.0–35.2×5.3–7.2 (16) |
| Filaments | | | | |
| Spirocysts | 13.1–28.8×2.9–6.6 (22.2×4.6, 41) | 18.0–31.0×3.0–8.0 (46) | 7.5–31.4×1.6–6.9 (71) | 28.3–33.3×5.4–7.1 (4) |
| Basitrichs_S | Not observed | Not observed | 7.0–12.5×1.5–2.8 (47) | 9.9–19.8×1.7–4.4 (48) |
| Basitrichs_L | 15.3–28.3×2.0–4.1 (23.8×2.7, 47) | 20.0–30.0×2.0–3.0 (12) | 25.9–30.1×2.7–4.2 (24) | 26.7–28.0×3.8–5.0 (2) |
| Microbasic b-mastigophores | 22.1–27.6×3.1–4.3 (25.2×3.8, 19) | Not observed | Not observed | Not observed |
| Microbasic p-mastigophores | 11.8–27.1×3.4–6.3 (20.9×5.0, 79) | 18.0–30.0×4.0–6.0 (89) | 23.2–27.7×5.4–7.6 (28) | 25.2–34.8×5.6–9.2 (52) |

**Ecology and habitat.** This species occurs primarily at depths greater than 28−30 m around Jukdo Island, Ulleungdo, in the East Sea of Korea. Individuals attach their pedal discs to small stones and molluscan shells and are commonly observed forming aggregations of multiple individuals.

**Distribution.** Korea (Ulleungdo).

**Etymology.** The specific epithet *aurantimaculatus* is derived from the Latin *aurantium* ("orange-colored") and *maculatus* ("spotted"), referring to the orange spots on the column of the living specimens.

**DNA barcoding.** Genetic distance analyses based on partial 16S rRNA sequences (434 bp) from 10 specimens representing 4 species of the genus *Isohalcurias*, 1 species of *Halcurias* (family Halcuriidae), and the outgroup *Synhalcurias elegans* were conducted to examine patterns of intra- and interspecific variation and to evaluate molecular support for

the new species designation. Analysis of partial 16S rRNA sequences from Halcuriidae indicates that this marker provides moderate utility for species identification within *Isohalcurias*. Although overall genetic divergence values were very low (0.0–0.5%), phylogenetic analyses successfully discriminated most species (Table 2).

The 16S rRNA data strongly support the validity of *Isohalcurias aurantimaculatus* sp. nov., which forms a well-supported monophyletic clade (bootstrap support: 100%) distinct from all congeners and consistently occupies a phylogenetic position sister to the *I. carlgreni*, *I. citreum* clade (Fig. 4). Nevertheless, several limitations of this marker are evident. First, *I. aurantimaculatus* sp. nov. shows zero genetic divergence from *I. malum*, necessitating phylogeny-based rather

**Table 2.** Genetic distances within and between species of the family Halcuriidae based on partial 16S rRNA in 434 bp length

| Species (number of sequences) | Within (mean ± SD) | Between *I. aurantimaculatus* sp. nov. (mean ± SD) |
|---|---|---|
| *Isohalcurias aurantimaculatus* sp. nov. (2) | 0.000 ± 0.000 | – |
| *Isohalcurias citreum* (1) | n/c | 0.005 ± 0.003 |
| *Isohalcurias malum* (2) | 0.000 ± 0.000 | 0.000 ± 0.000 |
| *Isohalcurias carlgreni* (4) | 0.000 ± 0.000 | 0.003 ± 0.002 |
| *Halcurias fragum* (1) | n/c | 0.002 ± 0.002 |

SD, standard deviation; n/c, no calculated.

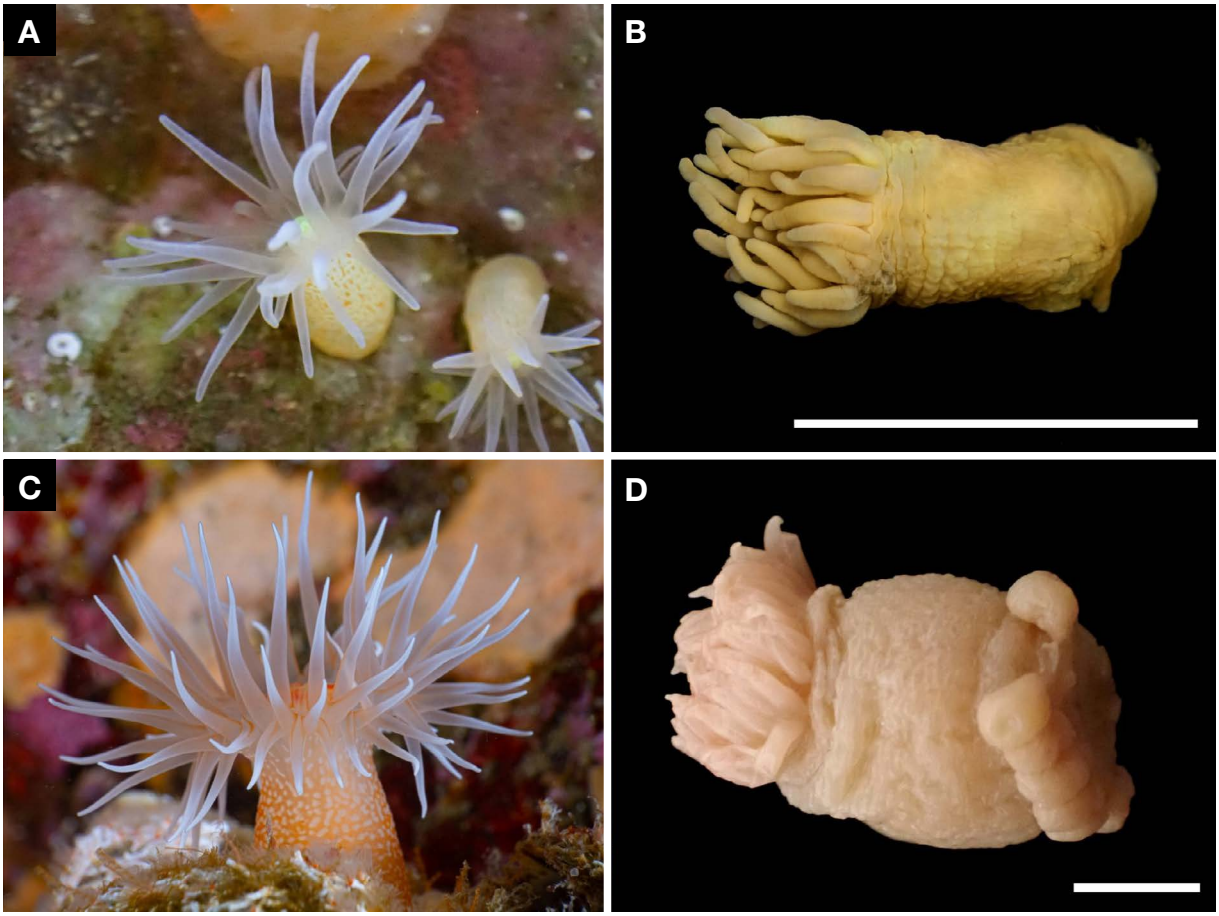


**Fig. 2.** External morphology of *Isohalcurias aurantimaculatus* sp. nov. and comparison with *I. carlgreni*. A, Live colony of *I. aurantimaculatus* sp. nov. from Jukdo, Ulleungdo; B, Holotype (MABIK CN00081709) of *I. aurantimaculatus* sp. nov. preserved in 99% ethanol; C, Live colony of *I. carlgreni*; D, *I. carlgreni* specimen preserved in 5% formalin. Scale bars: B = 5 mm, D = 1 cm.

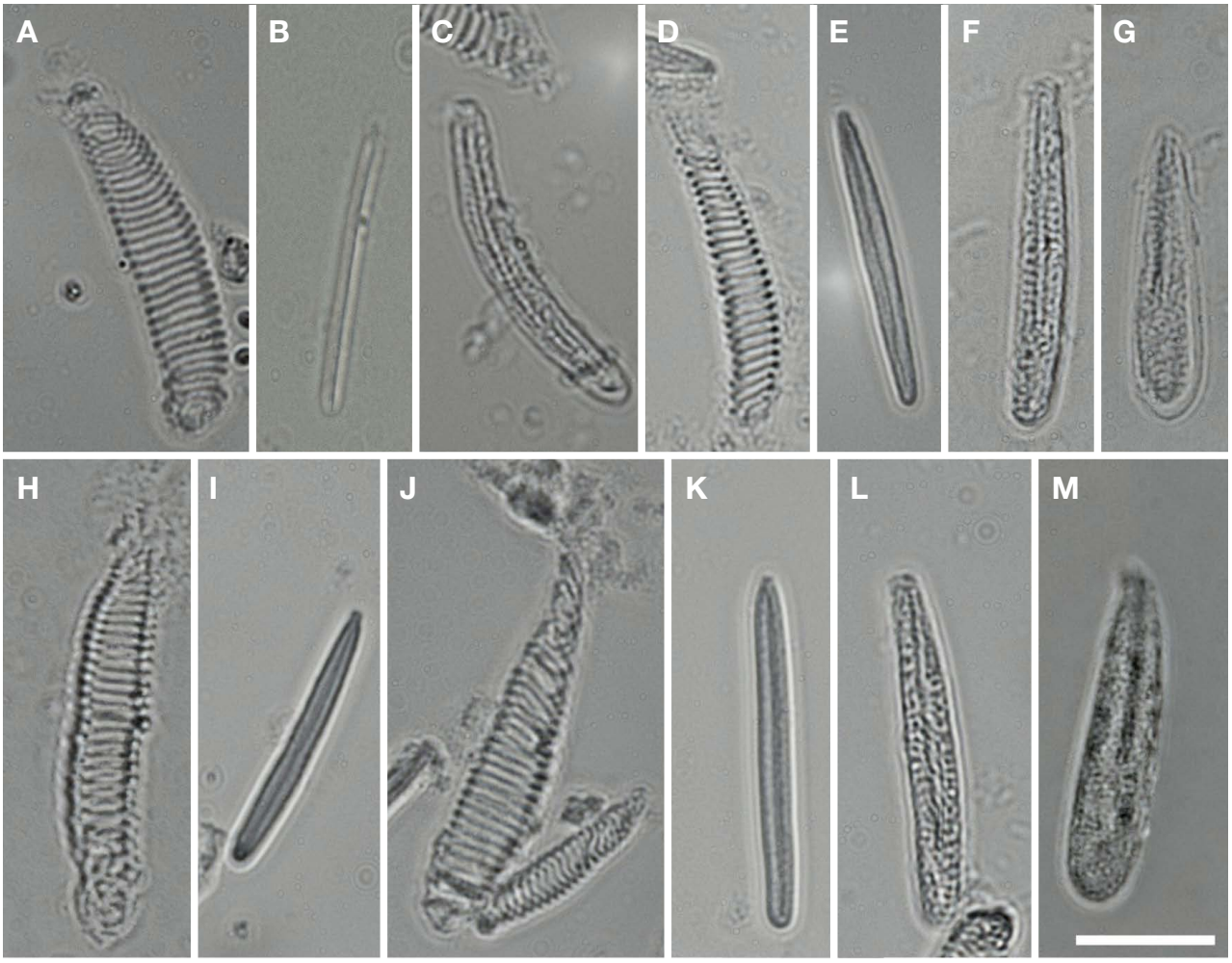


**Fig. 3.** Cnidae of *Isohalcurias aurantimaculatus* sp. nov. A–C, Tentacle; D–G, Actinopharynx; H, I, Column; J–M, Filament. A, Spirocyst; B, Basitrich; C, Microbasic b-mastigophore; D, Spirocyst; E, Basitrich; F, Microbasic b-mastigophore; G, Microbasic p-mastigophore; H, spirocyst; I, Basitrich; J, Spirocyst; K, Basitrich; L, Microbasic b-mastigophore; M, Microbasic p-mastigophore. Scale bar = 10 μm.

than distance-based species identification. Second, interspecific divergence values are well below commonly applied DNA barcoding thresholds (0.3–0.5% versus the ~2% typically recommended) (Hebert et al., 2003; Ward, 2009), a pattern consistent with the slow mitochondrial evolution reported in anthozoans (Shearer et al., 2002). Third, the unexpectedly low intergeneric divergence observed within Halcuriidae (as low as 0.2%) raises questions regarding the current delimitation of generic boundaries within the family.

Despite these limitations, the complete absence of intraspecific variation (0.000 ± 0.000 in all examined species), together with a stable phylogenetic structure and strong bootstrap support, indicates that 16S rRNA remains suitable for routine species identification in Halcuriidae when interpreted using phylogenetic approaches. In this context, the marker effectively corroborates the distinctiveness of *I. aurantimaculatus* sp. nov., providing molecular evidence that complements the morphological and ecological data supporting its description as a new species.

**Remarks.** *Isohalcurias aurantimaculatus* sp. nov. is morphologically and ecologically most similar to the previously recorded species of the genus, particularly *I. carlgreni*, with which it is sympatric at Ulleungdo in the East Sea of Korea (Hwang and Song, 2010). Both species share general features of the genus *Isohalcurias*, but despite this geographic overlap, they can be clearly distinguished based on diagnostic morphological and cnidae composition. The most prominent differences concern the composition of the cnidom: microbasic b-mastigophores are present in the tentacle tip and base, actinopharynx, and filaments of *I. aurantimaculatus* sp. nov.,

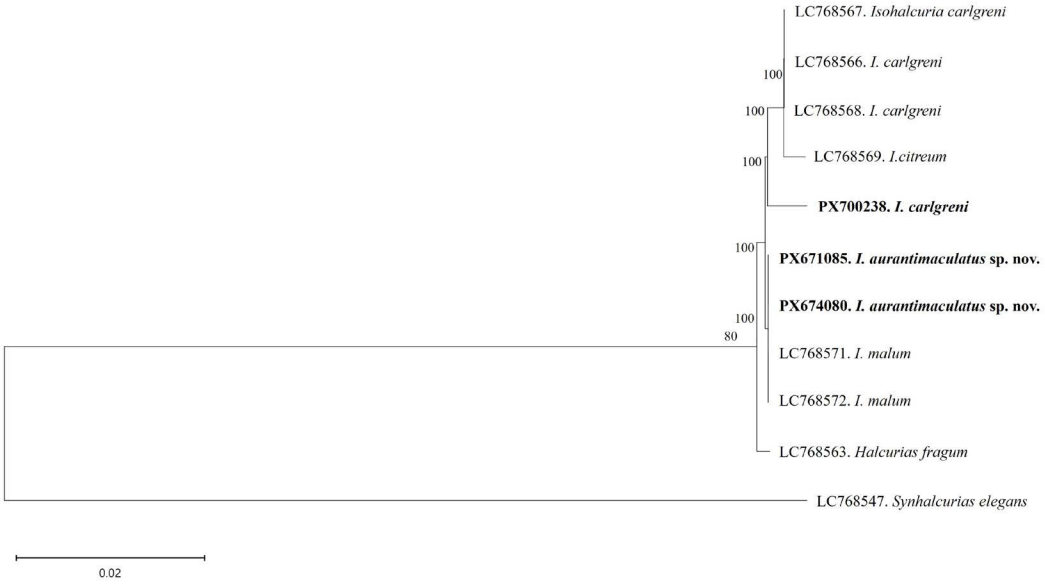


**Fig. 4.** Neighbor-joining phylogenetic tree based on partial 16S ribosomal RNA sequences (434 bp) of *Isohalcurias aurantimaculatus* sp. nov. and related Halcuriidae species. Numbers at nodes indicate bootstrap support values from 1,000 replicates. Sequences newly obtained in this study are shown in bold.

whereas these nematocysts are absent in *I. carlgreni*. Coloration also differs markedly, with *I. aurantimaculatus* sp. nov. exhibiting a cream-colored column with orange spots, white tentacles, and a yellow oral disc, in contrast to the bright orange column with spots and thin orange lines on the tentacles and oral disc of *I. carlgreni* (Hwang and Song, 2010) (Fig. 2C, D).

Based on cnidae composition and 16S rRNA sequence similarity, *I. aurantimaculatus* sp. nov. shows closer affinity to *Isohalcurias malum* Izumi et al., 2023. Nevertheless, clear distinctions are evident: microbasic b-mastigophores are present in the filaments of *I. aurantimaculatus* sp. nov. but absent in *I. malum*, and the latter has a completely smooth column and uniform pale red to pale orange coloration. Ecologically, *I. malum* inhabits deep-sea environments at depths of 200–375 m around Okinawa, Japan, whereas *I. aurantimaculatus* sp. nov. occurs in shallow waters at approximately 30 m depth around Ulleungdo, Korea.

Lastly, *I. aurantimaculatus* sp. nov. can be distinguished from *Isohalcurias citreum* Izumi et al., 2023 by the presence of microbasic b-mastigophores in the tentacles and filaments, which are absent in *I. citreum*. In addition, *I. citreum* has a completely smooth column, a uniformly bright yellow body coloration, and inhabits deeper waters at depths of 60–200 m in southern Japan (Izumi et al., 2023).

Overall, the combination of cnidom composition, column surface texture, coloration, and ecological distribution clearly supports the recognition of *I. aurantimaculatus* sp. nov. as a distinct species within the genus *Isohalcurias*. This morphological distinctiveness is further supported by phylogenetic analyses based on 16S rRNA sequences, which recover *Isohalcurias* species as a monophyletic clade and show *I. aurantimaculatus* sp. nov. forming a distinct lineage clearly separated from all previously recorded species (Fig. 4).

Family Edwardsiidae Andres, 1881

### [1]*Genus *Tempuractis* Izumi et al., 2018

**Type species.** *Tempuractis rinkai* Izumi et al., 2018.
**Diagnosis.** Very small-sized edwardsiids (<5 mm in length). Body vermiform, column smooth, not differentiated into capitulum, scapus, and physa, lacking nemathybomes and tenaculi. Pedal disc absent; aboral end tapered or rounded, not differentiated into a physa. Tentacles 16, arranged octamerously in two cycles. Eight perfect mesenteries present. Siphonoglyph absent. Sphincter muscle absent. Retractor muscles weak. Obligately symbiotic, inhabiting the tissues of homoscleromorph sponges of the genus *Oscarella*. Cnidom: spirocysts, basitrichs, microbasic p-mastigophores (absent or restricted).

Korean name: [1]*해면속살이말미잘속 (신칭)

**Key to species of *Tempuractis***

1. Microbasic b-mastigophores absent in tentacles and column ····· *T. rinkai*
– Microbasic b-mastigophores present in tentacles and column····· *T. microarmata* sp. nov.

[1]***Tempuractis microarmata* sp. nov.**
**(Tables 3, 4, Figs. 5–7)**

**ZooBank LSID:** urn:lsid:zoobank.org:act:29C40A64-3728-4174-BB75-03BC03914D96.

**Type locality.** Korea: Gyeongsangbuk-do, Ulleung-gun, Ulleung-eup, Seo-myeon, Taeha-ri, Daepunggam (37°31′21″N, 130°47′40″E), 12 m.

**Material examined.** Korea: Holotype (MABIK CN0008 1707), 1 individual, Gyeongsangbuk-do: Ulleung-gun, Ulleung-eup, Seo-myeon, Taeha-ri, Daepunggam (37°31′21″N, 130°47′40″E), 16 m, 24 Jun 2025, Lee SJ, Yu HJ, Lim MK; Paratypes (CBB25CnAnF441, MBCL25CnAnF067), 2 individuals, Ulleung-gun, Ulleung-eup, Seo-myeon, Taeha-ri, Daepunggam (37°31′21″N, 130°47′40″E), 16 m, 24 Jun 2025, Lee SJ, Yu HJ, Lim MK; Paratypes (MABIK CN00081710, MBCL25CnAnF074), 2 individuals, Ulleung-gun, Ulleung-eup, Dodong-ri, Bukjeobawi (37°30′04″N, 130°55′14″E), 16 m, 23 Jun 2025, Lee SJ, Yu HJ, Lim MK; 3 individuals, (MBCL22CnAnF097), Ulleung-gun, Ulleung-eup, Dodong-ri, Bukjeobawi (37°30′05″N, 130°55′16″E), 12 m, 4 Jul 2022, Hwang SJ, Jeong SC, Lee KH, Lee SJ, Yu HJ. All specimens were collected by SCUBA diving.

**Description.** Column cylindrical, upper and lower diameters subequal; naked, smooth. When fully extended, column elongate, mesenterial insertions and actinopharynx visible through body wall. Siphonoglyph absent. Pedal disc absent, species inhabiting internal tissue of homoscleromorph sponge *Oscarella* sp. Tentacles 16, arranged in two cycles (8 inner + 8 outer); inner tentacles longer than outer. Mouth subcircular.

Size (preserved specimens): column height 2.0–5.5 mm, column width 0.7–1.3 mm; oral disc diameter 0.5–0.9 mm. Inner tentacles 1.3–2.0 mm long; outer tentacles 1.0–1.8 mm long.

Cnidom: spirocysts, basitrichs, mesobasic b-mastigophores, microbasic b-mastigophores, microbasic p-mastigophores (Fig. 6); cnidae distribution and measurements summarized in Table 3.

**Color.** When alive, oral disc and actinopharynx white. Tentacles semi-transparent orange, becoming more intensely orange distally. Column pale, semi-transparent orange; when tentacles extended beyond sponge tissue, white mesenterial insertions and actinopharynx visible through column. When contracted and withdrawn into sponge, only orange tentacles exposed (Fig. 5A). In preserved, coloration faded; body partially translucent white in places (Fig. 5B, C).

**Ecology and habitat.** This species occurs at depths of 12–16 m around Bukjeobawi and Daepunggam, Ulleungdo Island, in the East Sea of Korea. All observed individuals were embedded within the tissue of a homoscleromorph sponge (*Oscarella* sp.) attached to rocky substrates and were typically found in aggregations of multiple individuals (Fig. 5A). A pedal disc was not observed. Only the oral disc and tentacles are positioned near the sponge osculum, whereas the column remains entirely enclosed within the sponge tissue. In relaxed

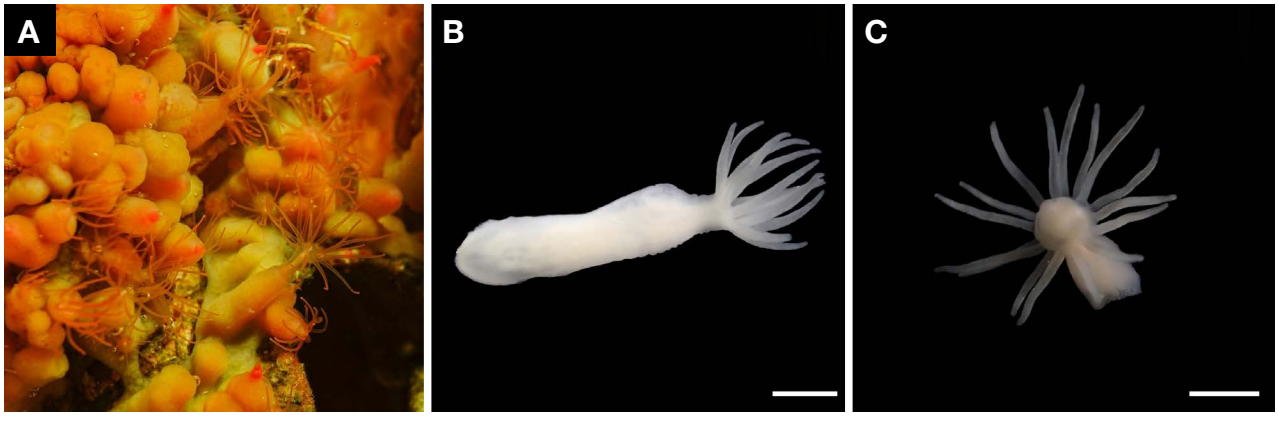


**Fig. 5.** External morphology of *Tempuractis microarmata* sp. nov. A, Live colony of *T. microarmata* sp. nov. from Bukjeobawi, Ulleungdo; B, Holotype (MABIK CN00081707) preserved in 5% formalin; C, Oral disc of Paratype (MABIK CN00081710) showing inner and outer tentacle cycles (8+8). Scale bars: B, C = 1 mm.

Korean name: [1]*해면속살이말미잘 (신칭)

**Table 3.** Comparison of size of cnidae between *Tempuractis microarmata* sp. nov. and *Tempuractis rinkai*, size in μm

| Tissue and cnidae | *Tempuractis microarmata* sp. nov. | *Tempuractis rinkai* |
|---|---|---|
| | This study (mean, number of cnidae) | Izumi et al., 2018 original description (mean, number of cnidae) |
| Tentacles | | |
| Spirocysts | 11.2–24.3×2.7–5.8 (18.2×4.2, 136) | 13.6–24.9×2.3–4.1 (20.1×3.2, 117) |
| Basitrichs | 16.7–22.3×2.9–4.0 (19.4×3.4, 28) | 12.1–18.0×2.1–3.4 (16.1×1.6, 37) |
| Holotrichs | Not observed | 23.6–27.2×3.4–5.0 (25.1×4.3, 19) |
| Microbasic b-mastigophores | 18.4–31.0×4.1–7.2 (25.1×5.0, 78) | Not observed |
| Column | | |
| Spirocysts | Not observed | 17.2–20.7×2.6–3.8 (18.8×3.5, 6) |
| Basitrichs | 9.5–14.8×1.6–3.3 (12.0×2.4, 107) | 8.2–13.6×1.7–2.9 (11.7×2.4, 57) |
| Holotrichs | Not observed | 13.0–30.2×3.1–7.0 (25.0×5.8, 126) |
| Microbasic p-mastigophores | Not observed | 21.0–27.0×4.3–5.7 (23.2×4.8, 8) |
| Mesobasic b-mastigophores | 25.0–32.4×7.0–16.6 (28.7×10.4, 65) | Not observed |
| Microbasic b-mastigophores | 25.2–41.8×5.1–10.4 (31.3×6.8, 93) | Not observed |
| Actinopharynx | | |
| Basitrichs | 9.6–14.4×1.6–5.7 (12.1×3.0, 59) | 6.7–19.6×1.0–4.1 (14.6×4.0, 71) |
| Microbasic p-mastigophores | 18.8–25.6×3.0–6.0 (22.2×4.3, 38) | 21.9–27.7×3.7–5.6 (24.6×4.6, 52) |
| Filaments | | |
| Basitrichs | 9.2–17.0×2.0–4.8 (13.6×3.0, 46) | 10.1–29.8×2.1–7.0 (13.1×2.7, 69) |
| Microbasic p-mastigophores | 17.1–29.7×3.6–5.5 (21.8×4.5, 85) | 21.0–27.0×4.3–5.7 (22.3×4.2, 47) |

**Table 4.** Genetic distances within and between species of the family Edwardsiidae based on partial 16S rRNA in 497 bp length

| Species (number of sequences) | Within (mean±SD) | Between *Tempuractis microarmata* sp. nov. (mean±SD) |
|---|---|---|
| *Tempuractis microarmata* sp. nov. (6) | 0.017±0.004 | – |
| *Tempuractis rinkai* (1) | n/c | 0.010±0.000 |
| *Edwardsianthus amethystus* (1) | n/c | 0.140±0.020 |
| *Edwardsianthus sapphirus* (1) | n/c | 0.110±0.020 |
| *Nematostella vectensis* (1) | n/c | 0.090±0.010 |
| *Scolanthus celticus* (1) | n/c | 0.060±0.010 |

SD, standard deviation; n/c, no calculated.

individuals, the oral disc and tentacles protrude beyond the sponge surface.

**Distribution.** Korea (Ulleungdo).

**Etymology.** The specific epithet *microarmata* is derived from the Latin *micro-* ("small") and *armatus* ("armed"), referring to the presence of tiny and distinctive microbasic b-mastigophores on the tentacles and column.

**DNA barcoding.** Genetic distance analyses based on partial 16S rRNA sequences (497 bp) from six species representing four genera within the family Edwardsiidae (*Tempuractis*, *Edwardsianthus*, *Nematostella*, and *Scolanthus*) revealed clear patterns of intra- and interspecific variation (Table 4). Intraspecific divergence in the newly described species, *Tempuractis microarmata* sp. nov., was low (0.017±0.004), based on six sequences, and was only slightly higher than the range (0.0–0.3%) typically reported for sea anemones (Hwang and Woo, 2025), but well within acceptable limits for DNA barcoding of actiniarians. In contrast, intraspecific variation could not be assessed for species represented by single sequences—*T. rinkai*, *Edwardsianthus amethystus*, *E. sapphirus*, *Nematostella vectensis*, and *Scolanthus celticus*—and was therefore considered not calculable. Interspecific genetic distances within the family Edwardsiidae ranged from 0.01 to 0.14. Within the genus *Tempuractis*, the

**Fig. 6.** Cnidae of *Tempuractis microarmata* sp. nov. A–C, Tentacle; D, E, Actinopharynx; F–H, Column; I, J, Filament. A, Spirocyst; B, Basitrich; C, Microbasic b-mastigophore; D, Basitrich; E, Microbasic p-mastigophore; F, Basitrich; G, Mesobasic b-mastigophore; H, Microbasic b-mastigophore; I, Basitrich; J, Microbasic p-mastigophore. Scale bar = 10 μm.

distance between *T. microarmata* sp. nov. and *T. rinkai* was notably low (0.010 ± 0.000), yet consistently greater than zero, supporting their recognition as distinct species despite close morphological similarity. This small but stable level of divergence, when considered together with diagnostic morphological differences, supports the validity of *T. microarmata* sp. nov. as a separate species.

Genetic distances between *T. microarmata* sp. nov. and representatives of other edwardsiid genera were substantially higher, ranging from 0.060 ± 0.010 (*Scolanthus celticus*) and 0.090 ± 0.010 (*Nematostella vectensis*) to 0.110 ± 0.020 (*Edwardsianthus sapphirus*) and 0.140 ± 0.020 (*E. amethystus*). These values (6–14%) are consistent with intergeneric divergence and demonstrate the strong discriminatory power of the 16S rRNA marker for species-level identification within Edwardsiidae.

**Remarks.** *Tempuractis microarmata* sp. nov. is morphologically and ecologically most similar to the type species of the

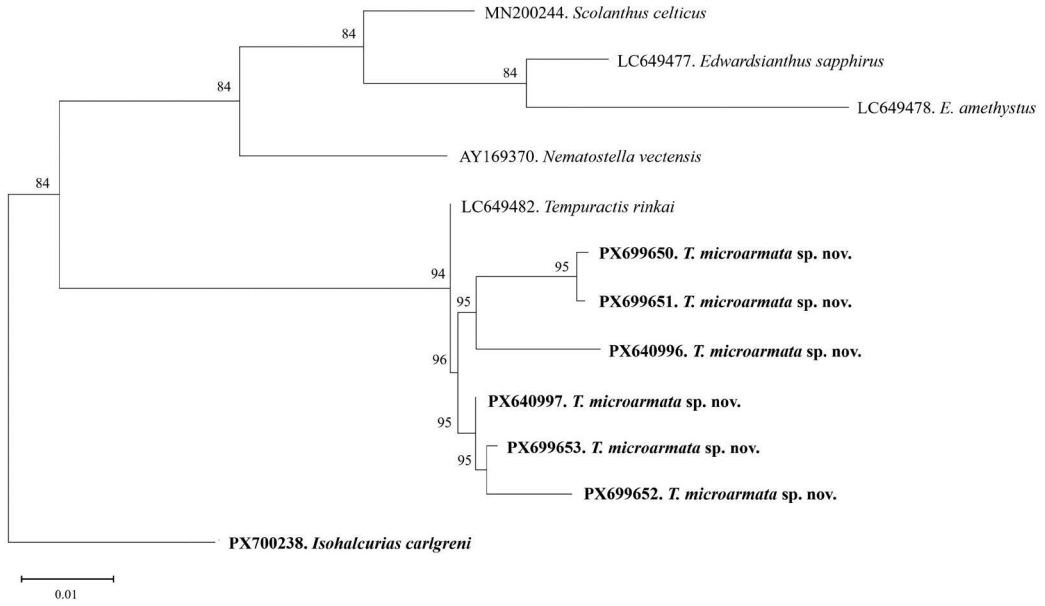


**Fig. 7.** Neighbor-joining phylogenetic tree based on partial 16S ribosomal RNA sequences (497 bp) of *Tempuractis microarmata* sp. nov. and related Edwardsiidae species. Numbers at nodes indicate bootstrap support values from 1,000 replicates. Sequences newly obtained in this study are shown in bold.

genus, *Tempuractis rinkai* Izumi et al., 2018. Both species share the diagnostic characters of *Tempuractis*, including very small body size, a vermiform form, absence of a pedal disc, an octamerous tentacle arrangement (16 tentacles in two cycles), lack of a siphonoglyph and marginal sphincter muscle, and an obligate symbiotic association with homoscleromorph sponges of the genus *Oscarella*. This intimate sponge-associated lifestyle, in which the column is embedded within host tissue and only the oral region and tentacles are exposed, represents a key ecological characteristic defining the genus *Tempuractis*.

Despite these shared generic features, *T. microarmata* sp. nov. can be clearly distinguished from *T. rinkai* by a combination of cnidom composition, external morphology, and geographic distribution. The most prominent difference between the two species concerns the composition of the cnidom. In *T. microarmata* sp. nov., microbasic b-mastigophores are present in both the tentacles and column, whereas these nematocysts have not been observed in *T. rinkai*. Instead, *T. rinkai* possesses holotrichs in the tentacles and column, whereas *T. microarmata* sp. nov. lacks holotrichs. In addition, mesobasic b-mastigophores were observed in *T. microarmata* sp. nov. but have not been reported for *T. rinkai*. Other cnidae types, including spirocysts, basitrichs, and microbasic p-mastigophores, are shared by both species.

The two species also exhibit clear geographic separation. *Tempuractis microarmata* sp. nov. is currently known only from Ulleungdo Island in the East Sea of Korea, where it inhabits subtidal environments at depths of 12–16 m. In contrast, *T. rinkai* has been recorded exclusively from Japanese waters along the Pacific coast of central and northern Honshu, occurring from the intertidal zone to shallow subtidal depths (to approximately 8 m). No overlap in their known distributions has been documented. The discovery of a *Tempuractis* species in Korean waters, together with consistent differences in cnidom composition and allopatric distribution, clearly supports the recognition of *T. microarmata* sp. nov. as a distinct species.

This morphological distinctiveness is further supported by phylogenetic analyses based on 16S rRNA sequences, which recover species of *Tempuractis* as a well-supported monophyletic clade and show *T. microarmata* sp. nov. forming a distinct lineage clearly separated from *T. rinkai*, corroborating its recognition as a separate species (Fig. 7).

Suborder Enthemonae Rodríguez and Daly, 2014
Family Metridiidae Carlgren, 1893

**Genus *Metridium* de Blainville, 1824**

**Key to species of *Metridium***

1. Ratio of column height to pedal disc diameter over 2 : 1 in the extended state, and microbasic p-mastigophores present in the actinopharynx ····· *M. farcimen*

– Ratio of column height to pedal disc diameter less than 2 : 1 in the extended state, and microbasic p-mastigophores absent in the actinopharynx ·····*M. senile*

[1]***Metridium farcimen*** **(Brandt, 1835)**
**(Tables 5, 6, Figs. 8–10)**

*Actinia* (*Polystephanus*) *farcimen* Brandt, 1835: 212.
*Metridium farcimen*: Fautin and Hand, 2000: 1151–1161, figs. 1–4; Eash-Loucks and Fautin, 2012: 41–43, fig. 32; Glon et al., 2021: 1–12, fig. 5; 2023: 9–24; Daly et al., 2024: 1–9.
*Actinia priapus* Tilesius, 1809: 405.
*Heliactis farcimen* Brandt: Andres, 1883: 181.
*Dendractis priapus* Tilesius: Andres, 1883: 364.
*Metridium giganteum* Fautin et al., 1989: 77–85, fig. 1.
*Isometridium rickettsi* Carlgren, 1951: 430.

**Material examined.** Korea: One individual (MABIK CN 00081708), Gangwon-do: Goseong-gun, Munam-ri, Naksandaegi artificial reef (38°17′29.21″N, 128°33′32.88″E), 29 m, 25 Sep 2022, Shim SH; 4 individuals (MBCL23CnAnE279, MBCL23CnAnE280, MBCL23CnAnE286, MBCL23CnAnE296), Goseong-gun, Gajin-ri, Bukhangang (38°22′10.62″N, 128°31′32.40″E), 33 m, 19 Mar 2023, Shim SH; 2 individuals (MBCL23CnAnE295, MBCL23CnAnE283), Goseong-gun, Gajin-ri (38°22′11.94″N, 128°31′35.22″E), 34 m, 19 Mar 2023, Shim SH; 1 individual (MBCL23CnAnF287), Gyeongsangbuk-do: Ulleung-gun, Buk-myeon, Cheonbu-ri, Ssangjeongcho (37°33′23.4″N, 130°56′22.44″E), 20 m, 24 May 2023, Lee SJ, Shim SH; 6 individuals (MBCL23CnAnE272, MBCL23CnAnE278, MBCL23CnAnE284, MBCL23CnAnE291, MBCL23CnAnE292, MBCL23CnAnE294), Yeongdeok-gun, Daejin-ri, artificial reef (36°34′47.77″N, 129°25′50.76″E), 32 m, 22 Apr 2023, Shim SH; 3 indivi-

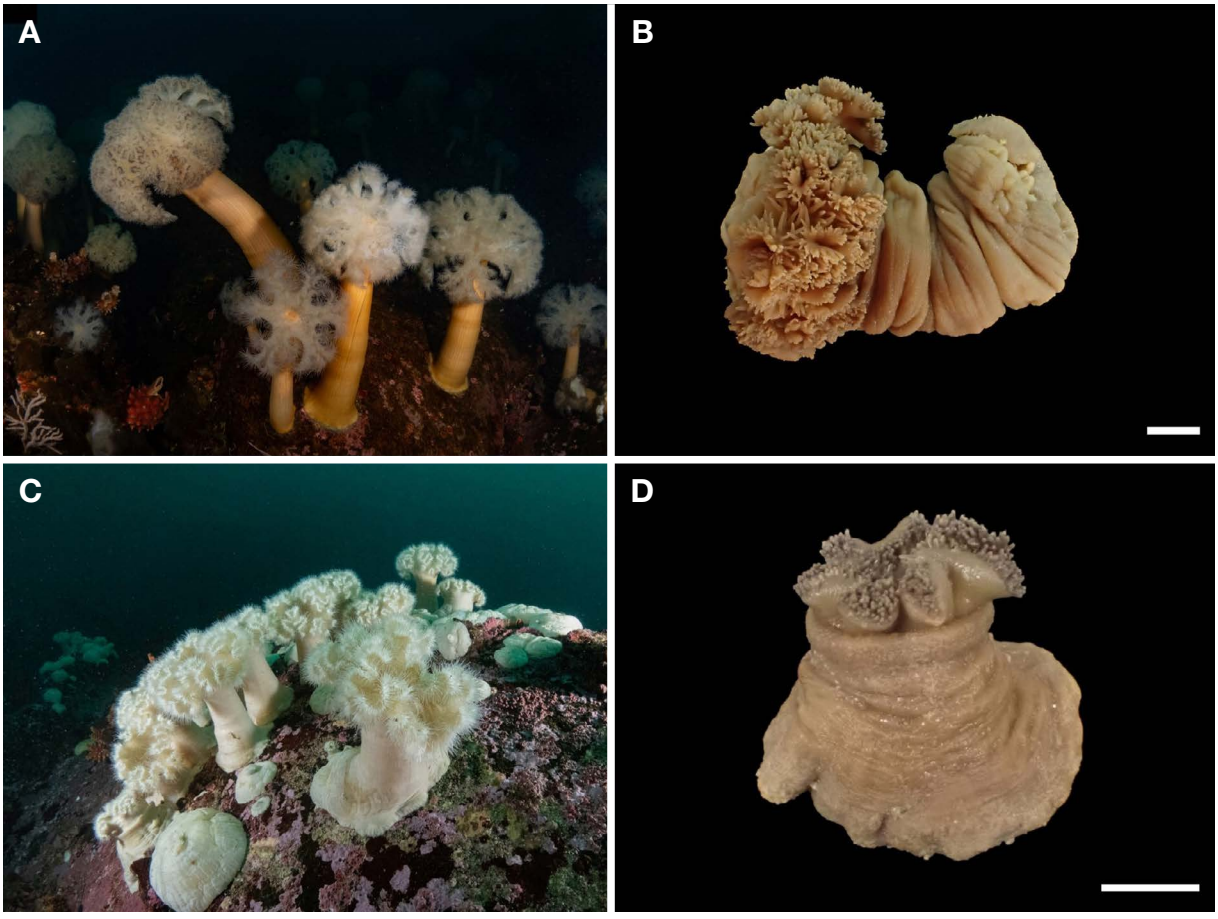


**Fig. 8.** External morphology and comparison of *Metridium* species. A, Live colonies of *M. farcimen* from Goseong, Gangwon-do; B, *M. farcimen* specimen preserved in 5% formalin; C, Live colonies of *M. senile* from Ulleungdo; D, *M. senile* specimen preserved in 5% formalin. Scale bars: B, D = 1 cm.

Korean name: [1]*큰섬유세닐말미잘 (신칭)

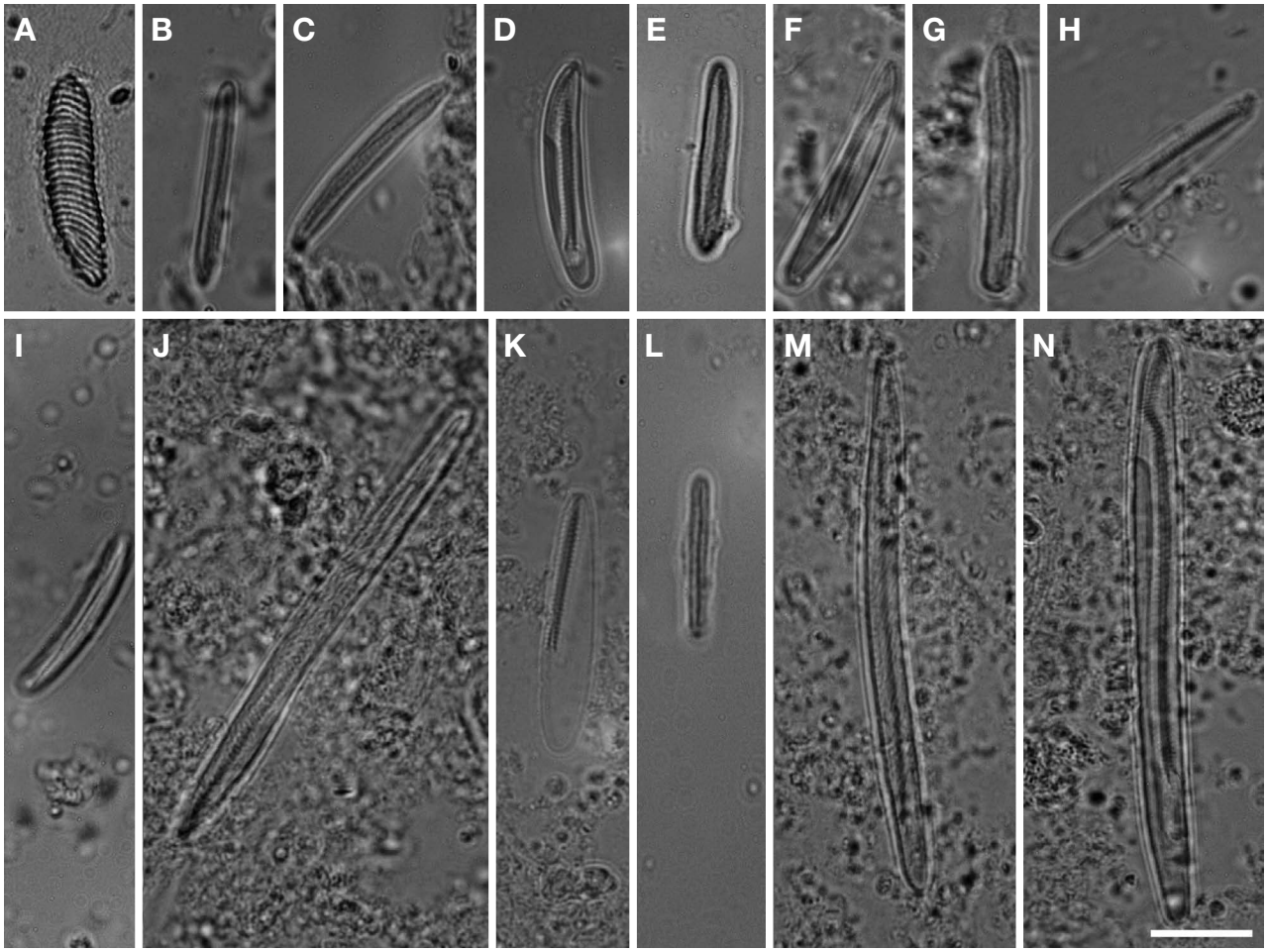


**Fig. 9.** Cnidae of *Metridium farcimen*. A–D, Tentacle; E, F, Column; G, H, Actinopharynx; I–K, Filament; L–N, Acontia. A, Spirocyst; B, Basitrich; C, Microbasic b-mastigophore; D, Microbasic p-mastigophore; E, Basitrich; F, Microbasic p-mastigophore; G, Microbasic b-mastigophore; H, Microbasic p-mastigophore; I, Basitrich; J, Microbasic b-mastigophore; K, Microbasic p-mastigophore; L, Basitrich; M, Microbasic b-mastigophore; N, Microbasic p-mastigophore. Scale bar = 10 μm.

duals (CBB25CnAnE442, MBCL23CnAnF290, MBCL23CnAnF293), Yeongdeok-gun, Daejin-ri (36°34′16.79″N, 129°26′25.17″E), 36 m, 22 Apr 2023, Shim SH. All specimens were collected by SCUBA diving.

**Description.** Column smooth, elongate, cylindrical; upper and lower diameters subequal. Column highly extensible, reaching ca. 1 m in height in fully expanded living individuals; preserved specimens strongly contracted, column height < 100 mm (Fig. 8B). In expanded individuals, ratio of column height to pedal disc diameter exceeding 2 : 1 (Fig. 8A). In contracted condition, column margin may completely conceal oral disc. Oral disc bearing numerous tentacles; disc lobing well developed, forming stiff lobes. Tentacles differentiated into marginal and discal types; discal tentacles thicker and longer than marginal tentacles. Pedal disc well developed, usually firmly attached to hard substrates (Fig. 8A).

Size (preserved specimens): column height 62 mm, column diameter 33 mm; pedal disc diameter 27–28 mm. In incompletely contracted specimens, column height to pedal disc diameter ratio ca. 2 : 1; in fully contracted specimens, approaching 1 : 1. Oral disc diameter 24–25 mm. Marginal tentacles 2–5 mm long, ca. 1 mm wide; discal tentacles 4–8 mm long, 1–2 mm wide, remaining conspicuously thicker and longer than marginal tentacles even after preservation.

Cnidom: spirocysts, basitrichs, microbasic b-mastigophores, and microbasic p-mastigophores (Fig. 9); cnidae distribution and measurements summarized in Table 5.

**Color.** When alive, body yellowish brown to yellowish beige; oral disc concolorous with column or slightly paler; tentacles white (Fig. 8A). In preserved specimens, coloration faded; body ivory to very pale light brown (Fig. 8B).

**Table 5.** Comparison of size of cnidae between *Metridium farcimen* and *Metridium senile*, size in μm

| Tissue and cnidae | *Metridium farcimen* | *Metridium farcimen* (as *Metridium senile fimbriatum*) | *Metridium senile* |
|---|---|---|---|
| | This study (mean, number of cnidae) | Hand, 1955 (number of cnidae) | Cha and Song, 2001 |
| Tentacles | | Ordinary tentacles | |
| Spirocysts | 9.9–32.6×2.5–6.1 (19.6×4.2, 103) | 12.0–30.0×4.0 (50) | 21.0–24.5×2.5–4.0 |
| Basitrichs | 10.5–23.0×1.8–3.3 (20.3×2.6, 39) | 6.0–15.0×1.5 (63) | 21.0–25.0×2.2–3.0 |
| Microbasic b-mastigophores | 17.0–28.2×2.5–4.7 (21.6×3.3, 23) | 12.0–32.0×2.0–3.5 (78) | 23.0–24.0×3.5–4.0 |
| Microbasic p-amastigophores | Not observed | 20.0–37.5×4.0–6.0 (85) | Not observed |
| Microbasic p-mastigophores | 18.4–27.5×3.0–5.0 (24.6×4.0, 48) | Not observed | Not observed |
| Column | | Scapus | |
| Basitrichs | 13.1–26.6×2.2–3.0 (16.9×2.6, 38) | 11.0–23.5×1.5–3.0 (72) | |
| Microbasic p-amastigophores | Not observed | 14.0–30.0×3.0–4.5 (61) | |
| Microbasic p-mastigophores | 23.5–29.7×3.2–5.0 (27.5×3.7, 43) | Not observed | |
| Actinopharynx | | | |
| Spirocysts | Not observed | Not observed | 25.5–28.0×2.5–3.0 |
| Basitrichs | Not observed | Not observed | 32.5–36.0×2.2–3.0 |
| Microbasic b-mastigophores | 25.3–36.0×2.6–3.5 (29.3×3.0, 18) | 26.0–38.5×3.0–4.0 (74) | Not observed |
| Microbasic p-mastigophores | 14.4–32.6×3.2–5.1 (25.4×4.2, 54) | 18.0–27.5×3.0 (51) | Not observed |
| Filaments | | | |
| Basitrichs | 10.8–19.6×1.5–3.1 (15.6×2.2, 30) | 11.0–19.0×1.5 (50) | 31.5–33.5×2.2–2.5 |
| Microbasic b-mastigophores | 44.3–61.9×3.2–4.3 (55.8×3.8, 10) | Not observed | Not observed |
| Microbasic p-amastigophores | Not observed | 17.0–22.0×4.0–5.0 (68) | Not observed |
| Microbasic p-mastigophores | 16.5–31.8×2.5–5.2 (24.4×3.8, 39) | 8.0–19.0×4.0–5.5 (73) | 14.8–42.5×3.5–6.0 |
| Acontia | | | |
| Basitrichs | 11.3–19.3×1.4–2.6 (16.4×1.8, 20) | 8.0–13.5×2.0 (54) | Not observed |
| Microbasic b-mastigophores | 42.4–72.1×3.1–4.7 (54.5×3.7, 62) | 32.0–87.0×4.5–6.0 (132) | 36.8–62.0×3.0–4.0 |
| Microbasic p-amastigophores | Not observed | 48.0–92.0×6.0–9.0 (107) | Not observed |
| Microbasic p-mastigophores | 43.7–71.7×3.3–6.5 (56.9×4.3, 29) | Not observed | 14.0–73.0×3.0–7.0 |

Hand (1955) described microbasic amastigophores; however, following Östman et al. (2010), the term microbasic p-amastigophore is adopted.

**Ecology and habitat.** This species occurs primarily in the subtidal zone of the East Sea of Korea at depths exceeding 30 m. Individuals attach firmly to natural rocky substrates as well as artificial reef structures by means of a well-developed pedal disc and are commonly encountered in aggregations of multiple individuals (Fig. 8A). Seasonal or temperature-related changes in body posture were observed: individuals generally show pronounced expansion, characterized by increased column height and fully extended tentacles, at lower water temperatures (below approximately 10°C), whereas contraction is more frequent under relatively warmer conditions.

**Distribution.** Korea (Goseong-gun, Ulleungdo, Yeongdeok-gun), Russia (Kamchatka Peninsula), USA (Alaska and

California), Mexico (Guerrero and Sonora), and Canada (British Columbia).

**DNA barcoding.** Genetic distance analyses based on partial 16S rRNA sequences (497 bp) from 33 specimens representing two species of the genus *Metridium* (family Metridiidae) were conducted to evaluate the utility of this marker for species identification. Analyses of *Metridium senile* (21 sequences) and *M. farcimen* (12 sequences) revealed extremely low levels of genetic variation both within and between species (Table 6). Intraspecific genetic distances were minimal for both *M. farcimen* (0.002 ± 0.001) and *M. senile* (0.001 ± 0.001), and the mean interspecific distance between the two species was similarly low (0.001 ± 0.001). As a result, no barcoding gap was detected, because interspecific divergence did not exceed intraspecific variation, contrary to a fundamental requirement of DNA barcoding.

The NJ phylogenetic analysis further demonstrated the limited discriminatory power of 16S rRNA in *Metridium* (Fig. 10). Sequences identified as *M. senile* and *M. farcimen* were completely intermixed, with no species-specific clustering or monophyletic grouping observed.

These results indicate that 16S rRNA is unsuitable as a DNA barcode for discriminating species within *Metridium*. The near absence of genetic divergence between species suggests that this mitochondrial region evolves at an extremely slow rate, likely reflecting strong functional constraint and purifying selection that limit accumulation of mutations at the species level, a pattern previously reported to reduce species-level resolution of 16S rRNA in other metazoan taxa (Saccone et al., 1999; Galtier et al., 2009).

**Remarks.** *Metridium farcimen* (Brandt, 1835) and *Metridium senile* (Linnaeus, 1761) are closely related congeners that share many defining features of the genus *Metridium*, including a large, soft-bodied form, numerous tentacles cov-

**Table 6.** Genetic distances within and between species of the family Metridiidae based on partial 16S rRNA in 497 bp length

| Species (number of sequences) | Within (mean ± SD) | Between *Metridium farcimen* (mean ± SD) |
|---|---|---|
| *Metridium senile* (21) | 0.001 ± 0.001 | 0.001 ± 0.001 |
| *Metridium farcimen* (12) | 0.002 ± 0.001 | – |

SD, standard deviation.

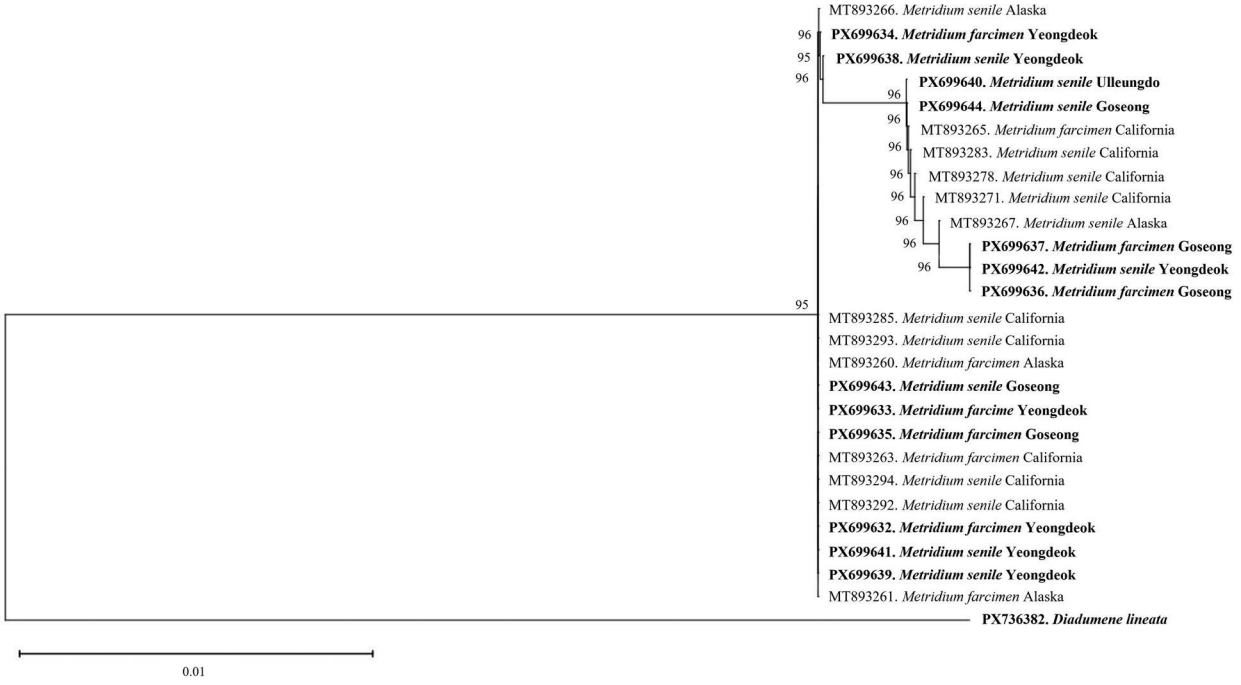


**Fig. 10.** Neighbor-joining phylogenetic tree based on partial 16S ribosomal RNA sequences (497 bp) of *Metridium farcimen* and *M. senile*. Numbers at nodes indicate bootstrap support values from 1,000 replicates. Sequences newly obtained in this study are shown in bold.

ering a lobed oral disc, a well-developed pedal disc, and a similar general body plan (Fig. 8A, C). Despite these similarities, the two species can be reliably distinguished based on a combination of external morphology, cnidom composition, and differences in body size and proportions.

The specimens examined in this study are consistent with previous descriptions of *M. farcimen* in overall external morphology. Although the original description of *M. farcimen* did not include cnidae data, subsequent taxonomic studies clarified that *M. fimbriatum* Verrill, 1865 and *M. senile* var. *fimbriatum* are junior synonyms of *M. farcimen*, and the material examined by Hand (1955) is now recognized as *M. farcimen* (Fautin and Hand, 2000; Östman et al., 2010; Daly et al., 2024). Consequently, the cnidae data reported by Hand (1955) for material previously identified as *M. senile fimbriatum* can be applied to *M. farcimen*. The cnidae composition observed in the present material broadly aligns with that attributed to *M. farcimen*, although minor discrepancies may reflect methodological limitations, particularly the difficulty of distinguishing microbasic p-mastigophores from microbasic p-amastigophores under light microscopy. *Metridium farcimen* and *M. senile* consistently differ in cnidom composition: *M. farcimen* possesses microbasic p-mastigophores in the tentacles and basitrichs in the column and acontia, whereas *M. senile* lacks these nematocysts and instead bears spirocysts and basitrichs in the actinopharynx, providing clear diagnostic separation between the two species.

Field observations further facilitate differentiation. *Metridium farcimen* exhibits an extremely extensible column, with a column height to pedal disc diameter ratio exceeding 2 : 1 in expanded individuals, and can reach a maximum body height of approximately 1 m. In contrast, *M. senile* typically has a ratio near 1 : 1 and a much smaller maximum body height, generally not exceeding 10–30 cm (Fig. 8D). Coloration also differs: *M. farcimen* generally displays a yellowish-brown to yellowish-beige column with white tentacles, while *M. senile* shows substantial color polymorphism, ranging from white and cream to orange, red, brown, and olive green.

DNA barcoding results are consistent with previous studies demonstrating that multiple mitochondrial and nuclear markers—including ITS, 18S rRNA, 28S rRNA, COX3, 12S rRNA, and 16S rRNA—all exhibit low resolution for species discrimination within *Metridium* (Glon et al., 2021). That study showed that sequence-capture approaches were more effective than PCR-based Sanger sequencing or double-digest RADseq methods for distinguishing *Metridium* species. Future studies aimed at resolving species boundaries within Korean *Metridium* populations would therefore benefit from sequence-capture-based genomic approaches.

In summary, the *Metridium* specimens collected from the East Sea of Korea exhibit the characteristic features of *M. farcimen*, allowing clear differentiation from *M. senile* in both field and preserved material. Key distinguishing traits include cnidom composition, body proportions, maximum body size, and coloration, providing a robust basis for accurate identification of *M. farcimen*.

## ORCID

Myeong Kyu Lim: https://orcid.org/0009-0004-5017-3417
Seung-Joon Lee: https://orcid.org/0000-0003-4880-1547
Sung-Jin Hwang: https://orcid.org/0000-0002-1259-6775

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

## ACKNOWLEDGMENTS

This work was supported by the management of Marine Fishery Bio-resources Center (2025) funded by the National Marine Biodiversity Institute of Korea (MABIK). Additional funding was provided by the Korea Institute of Ocean Science & Technology (KIOST) under the project "Strengthening of capacity to analyze and assess marine environment / ecosystem variability in the waters surrounding the Korean Peninsula" (EA0301). Further support was provided by Woosuk University.

## REFERENCES

Andres A, 1881. Prodromus neapolitanae actiniarum faunae addito generalis actiniarum bibliographiae catalogo. Mitteilungen aus der Zoologischen Station zu Neapel, 2:305-371.

Andres A, 1883. Le attinie, Vol. I. Salviucci, Rome, pp. 1-460.

Brandt JF, 1835. Prodromus descriptionis animalium ab H. Mertensio in orbis terrarum circumnavigatione observatorum. Fascic. I. Polypos, Acalephas Discophoras et Siphonophoras, nec non Echinodermata continens. Sumptibus Academiae, Petropoli, pp. 1-75.

Bucklin A, 1985. Biochemical genetic variation, growth and regeneration of the sea anemone, *Metridium*, of British shores. Journal of the Marine Biological Association of the United Kingdom, 65:141-157. https://doi.org/10.1017/S0025315400060860

Bucklin A, Hedgecock D, 1982. Biochemical genetic evidence

for a third species of *Metridium* (Coelenterata: Actiniaria). Marine Biology, 66:1-7. https://doi.org/10.1007/BF00397248

Carlgren O, 1892. Beiträge zur Kenntnis der Edwardsien. Öfversigt af Kongliga Vetenskapsakademiens Förhandlingar, 1892: 451-461.

Carlgren O, 1893. Studien über nordische Actinien. Kungliga Svenska Vetenskapsakademiens Handlingar, 25:1-148.

Carlgren O, 1918. Die Mesenterienanordnung der Halcuriiden. Kungliga Fysiografiska Sällskapet Lundlingar, 29:1-37.

Carlgren O, 1949. A survey of the Ptychodactiaria, Corallimorpharia and Actiniaria. Kungliga Svenska Vetenskapsakademiens Handlingar, 1:1-121.

Carlgren O, 1951. The actinian fauna of the Gulf of California. Proceedings of the United States National Museum, 101:415-449. https://doi.org/10.5479/si.00963801.101-3282.415

Daly M, 2006. The anatomy, terminology, and homology of acrorhagi and pseudoacrorhagi in sea anemones. Zoologische Verhandelingen, 345:89-101.

Daly M, Brugler MR, Cartwright P, Collins AG, Dawson MN, Fautin DG, France SC, McFadden CS, Opresko DM, Rodriguez E, Romano SL, Stake JL, 2008. The phylum Cnidaria: a review of phylogenetic patterns and diversity 300 years after Linnaeus. Zootaxa, 1668:127-182. https://doi.org/10.11646/zootaxa.1668.1.11

Daly M, Glon H, Li Y, 2024. Taxonomy and nomenclature in the widespread and well-known sea anemone *Metridium* de Blainville, 1824. Marine Biodiversity, 54:33. https://doi.org/10.1007/s12526-024-01425-9

Eash-Loucks WE, Fautin DG, 2012. Taxonomy and distribution of sea anemones (Cnidaria: Actiniaria and Corallimorpharia) from deep water of the northeastern Pacific. Zootaxa, 3375:1-80. https://doi.org/10.11646/zootaxa.3375.1.1

Fautin DG, 2016. Catalog to families, genera, and species of orders Actiniaria and Corallimorpharia (Cnidaria: Anthozoa). Zootaxa, 4145:1-449. https://doi.org/10.11646/zootaxa.4145.1.1

Fautin DG, Bucklin A, Hand C, 1989. Systematics of sea anemones belonging to the genus *Metridium* (Coelenterata: Actiniaria), with a description of *M. giganteum* new species. The Wasmann Journal of Biology, 47:77-85.

Fautin DG, Hand C, 2000. *Metridium farcimen*, the valid name of a common North Pacific Sea anemone (Cnidaria: Actiniaria: Acontiaria). Proceedings of the Biological Society of Washington, 113:1151-1161.

Galtier N, Nabholz B, Glémin S, Hurst GDD, 2009. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. Molecular Ecology, 18:4541-4550. https://doi.org/10.1111/j.1365-294X.2009.04380.x

Geller JB, Walton ED, 2001. Breaking up and getting together: evolution of symbiosis and cloning by fission in sea anemones (genus *Anthopleura*). Evolution, 55:1781-1794. https://doi.org/10.1111/j.0014-3820.2001.tb00827.x

Glon H, Daly M, Carlton JT, Flenniken MM, Currimjee Z, 2020. Mediators of invasions in the sea: life history strategies and dispersal vectors facilitating global sea anemone introductions. Biological Invasions 22:3195-3222. https://doi.org/10.1007/s10530-020-02321-6

Glon H, Häussermann V, Brewin PE, Brickle P, Kong S, Smith ML, Daly M, 2023. There and back again: the unexpected journeys of *Metridium* de Blainville, 1824 between the old oceans and throughout the modern world. Biological Bulletin, 244:9-24. https://doi.org/10.1086/723800

Glon H, Quattrini A, Rodríguez E, Titus BM, Daly M, 2021. Comparison of sequence-capture and ddRAD approaches in resolving species and populations in hexacorallian anthozoans. Molecular Phylogenetics and Evolution, 163:107233. https://doi.org/10.1016/j.ympev.2021.107233

Hand C, 1955. The sea anemones of central California: Part 3. The acontiarian anemones. The Wasmann Journal of Biology, 13:189-251.

Hebert PDN, Cywinska A, Ball SL, DeWaard JR, 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: Biological Sciences, 270:313-321. https://doi.org/10.1098/rspb.2002.2218

Hertwig R, 1882. Die Actinien der Challenger Expedition. Verlag von Gustav Fischer, Jena, pp. 1-119.

Hwang SJ, Song JI, 2010. New records of Endocoelantheainea and Nynantheae (Anthozoa: Zoantharia: Actiniaria) from Korea. Korean Journal of Systematic Zoology, 26:223-232. https://doi.org/10.5635/KJSZ.2010.26.3.223

Hwang SJ, Woo S, 2025. A new record of intertidal sea anemone, *Anthopleura asiatica* (Anthozoa: Hexacorallia: Actiniaria: Actiniidae), from Korea. Animal Systematics, Evolution and Diversity, 41:16-23. https://doi.org/10.5635/ased.2025.41.1.024

Izumi T, Fujii T, Yanagi K, Fujita T, 2023. Fluorescent anemones in Japan: comprehensive revision of Japanese Actinernoidea (Cnidaria: Anthozoa: Actiniaria: Anenthemonae) with rearrangements of the classification. Diversity, 15:773. https://doi.org/10.3390/d15060773

Izumi T, Ise Y, Yanagi K, Shibata D, Ueshima R, 2018. First detailed record of symbiosis between a sea anemone and homoscleromorph sponge, with a description of *Tempuractis rinkai* gen. et sp. nov. (Cnidaria: Anthozoa: Actiniaria: Edwardsiidae). Zoological Science, 35:188-198. https://doi.org/10.2108/zs170042

McMurrich JP, 1893. Report on the Actiniæ collected by the United States Fish Commission steamer Albatross during the winter of 1887-1888. Proceedings of the United States National Museum, 16:119-216. https://doi.org/10.5479/si.00963801.16-930.119

McMurrich JP, 1901. Contributions on the morphology of the Actinozoa VI. Halcurias pilatus and Endocoelactis. Biological Bulletin, 2:155-163. https://doi.org/10.2307/1535781

Östman C, Kultima JR, Roat C, 2010. Tentacle cnidae of the sea anemone *Metridium senile* (Linnaeus, 1761) (Cnidaria: Anthozoa). Scientia Marina, 74:511-521. https://doi.org/10.3989/scimar.2010.74n3511

Rodríguez E, Barbeitos MS, Brugler MR, Crowley LM, Grajales A, Gusmão L, Häussermann V, Reft A, Daly M, 2014. Hidden

among sea anemones: the first comprehensive phylogenetic reconstruction of the order Actiniaria (Cnidaria, Anthozoa, Hexacorallia) reveals a novel group of hexacorals. PLoS ONE, 9:e96998. https://doi.org/10.1371/journal.pone.0096998

Rodríguez E, Daly M, 2014. Anenthemonae, a new suborder of Actiniaria (Cnidaria, Anthozoa) including the family Edwardsiidae. Systematics and Biodiversity, 12:323-335.

Rodríguez E, Fautin D, Daly M, 2025. World List of Actiniaria. Actiniaria [Internet]. World Register of Marine Species, Accessed 16 Dec 2025, <https://www.marinespecies.org/aphia.php?p=taxdetails&id=1360>.

Saccone C, De Giorgi C, Gissi C, Pesole G, Reyes A, 1999. Evolutionary genomics in Metazoa: the mitochondrial DNA as a model system. Gene, 238:195-209. https://doi.org/10.1016/S0378-1119(99)00270-X

Shearer TL, Van Oppen MJH, Romano SL, Wörheide G, 2002. Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). Molecular Ecology, 11:2475-2487.

Stephenson TA, 1922. On the classification of Actiniaria. Part III.—Definitions connected with the forms dealt with in Part II. Quarterly Journal of Microscopical Science, 66:247-319.

Stephenson TA, 1935. The British sea anemones, Vol. II. The Ray Society, London, pp. 1-426.

Tamura K, Stecher G, Kumar S, 2021. MEGA11: molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution, 38:3022-3027. https://doi.org/10.1093/molbev/msab120

Tilesius GT, 1809. De nova actiniarum specie gigantea kamtschatica, quam in Portu Petro-Pauli ad vivum pinxit ac. Mémoires de l'Académie Impériale des Sciences de St. Pétersbourg, 1:388-422.

Ward RD, 2009. DNA barcode divergence among species and genera of birds and fishes. Molecular Ecology Resources, 9:1077-1085. https://doi.org/10.1111/j.1755-0998.2009.02541.x

Received December 23, 2025
Revised December 27, 2025
Accepted December 27, 2025

ASED http://e-ASED.org
Animal Systematics, Evolution and Diversity

Anim. Syst. Evol. Divers. Vol. 42, No. 1: 82-87, January 2026
https://doi.org/10.5635/ASED.2026.42.1.009

Short communication

# New Record of a Diogenid Species, *Diogenes pallescens* (Crustacea: Decapoda: Anomura: Diogenidae), from Korea

Jibom Jung[1], Jin-Ho Park[2], Hee-seung Hwang[3,*]

[1]Institute of Data Innovation, Seoul National University, Seoul 08826, Korea
[2]Division of Science Education, Biology Education Major and Institute of Fusion Science, Jeonbuk National University, Jeonju 54896, Korea
[3]School of Biological Sciences and Institute of Biodiversity, Seoul National University, Seoul 08826, Korea

## ABSTRACT

A diogenid hermit crab, *Diogenes pallescens*, collected from Jejudo Island, is reported for the first time in Korea. This species is characterized by an enlarged left cheliped, a broad rostrum, an unarmed propodus of the third pereopods, and the left cheliped bearing numerous setae. In this paper, a detailed description with illustrations, and a key to the Korean *Diogenes* species are provided. As a result of this study, the geographical distribution of the present species has been extended, and five species of diogenid hermit crab including the present one, are now recorded in the Korean fauna. The specimen examined herein was deposited in the National Marine Biodiversity Institute of Korea (MABIK).

**Keywords:** Korean fauna, hermit crab, Crustacea, Decapoda, Diogenidae, *Diogenes*

## INTRODUCTION

Hermit crabs of the superfamily Paguroidea Latreille, 1802 represent key constituents of intertidal and subtidal ecosystems, fulfilling important ecological roles (Williams and McDermott, 2004). Within this superfamily, the genus *Diogenes* Dana, 1851 currently comprises 86 species, predominantly distributed across the Indo-Pacific region (Rahayu, 2015; WoRMS, 2025). Up to date, four *Diogenes* species has been found in Korea: *D. deflectomanus* Wang & Tung, 1980, *D. edwardsii* (De Haan, 1849), *D. nitidimanus* Terao, 1913, and *D. penicillatus* Stimpson, 1858 (see Jung et al., 2018). Diagnostic morphological characteristics of this genus include an enlarged left cheliped, a broad rostrum, and antennules with tapered filaments (McLaughlin, 2003). Among them, *D. pallescens* Whitelegge, 1897, originally described from Funafuti in the South Pacific, exhibits a broad geographical distribution ranging from South Africa and the Red Sea to Japan and French Polynesia (Rahayu, 2022). Historically, morphologically similar species, such as *D. gardineri* Alcock, 1905, and *D. serenei* Forest, 1956, were considered distinct; however, these were synonymized with *D. pallescens* by McLaughlin (2002), who regarded them as morphological variants. In the present study, three specimens of *D. pallescens* were collected from gravelly sand estuarine habitat in northwestern Jejudo Island and preserved in 95% ethyl alcohol. Observations and identifications were conducted using a stereomicroscope (MZ8; Leica, Wetzlar, Germany). Images were captured with a digital camera (Model D7000 & D800E; Nikon, Tokyo, Japan). Shield length (SL) was measured from the tip of the rostrum to the midpoint of the posterior carapace margin using a digital caliper (CD6CSX; Mitutoyo, Kawasaki, Japan) with a resolution of 0.1 mm. The specimens examined were deposited in the National Marine Biodiversity Institute of Korea (MABIK).

## SYSTEMATIC ACCOUNTS

Phylum Arthropoda von Siebold, 1848
Class Malacostraca Latreille, 1802
Order Decapoda Latreille, 1803

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: 82-2-880-6687, Fax: 82-2-872-1993
E-mail: heeseung0212@gmail.com

eISSN 2234-8190

Copyright © The Korean Society of Systematic Zoology

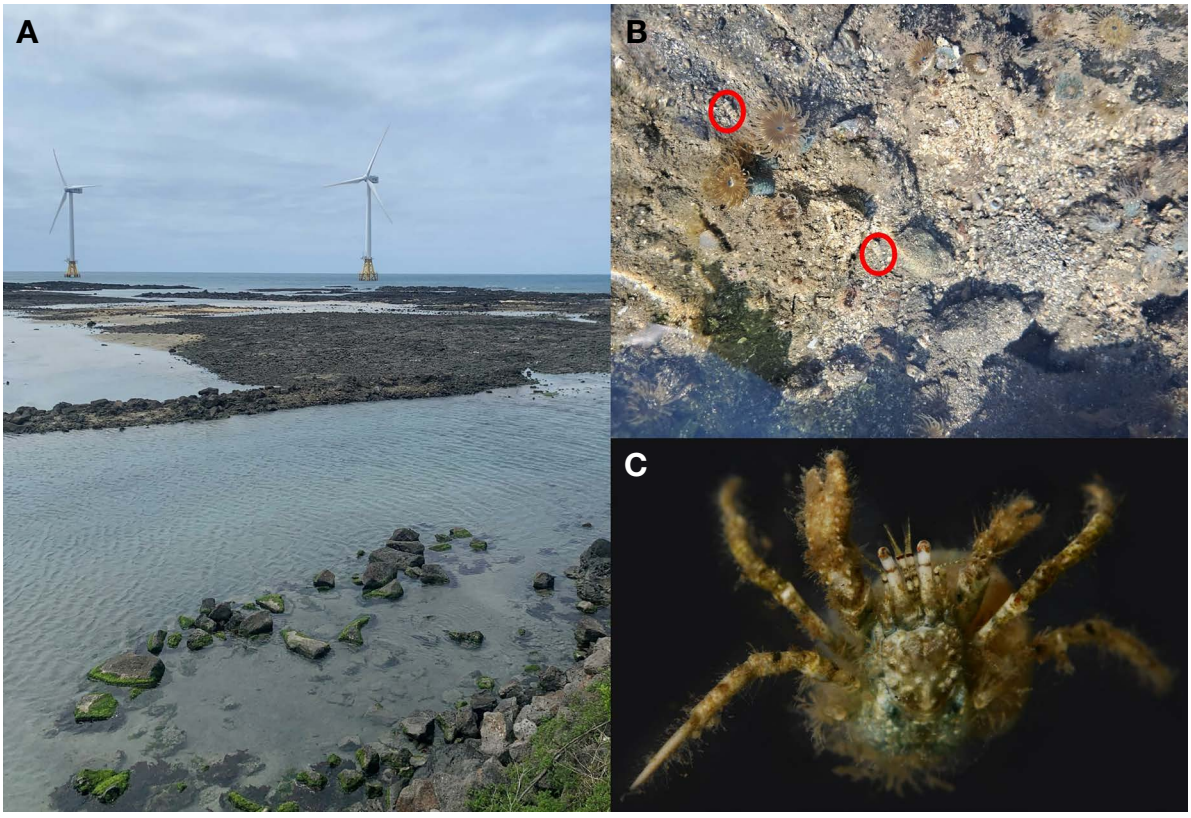


**Fig. 1.** A, Collection site of *Diogenes pallescens* Korea in Jejudo Island; B, Associated anemone of *D. pallescens* Korea; red circles indicate individuals of *D. pallescens* Korea; C, Dorsal view of *D. pallescens* Korea (male, SL 3.1 mm, MABIK CR00258586).

Superfamily Paguroidea Latreille, 1802
Family Diogenidae Ortmann, 1892
Genus *Diogenes* Dana, 1851

[1]***Diogenes pallescens* Whitelegge, 1897 (Figs. 1, 2)**

*Diogenes pallescens* Whitelegge, 1897: 141, 142, Pl. VI, figs. 2a–c; McLaughlin, 2002: 91, 92, figs. 1–4; Abd El-Wakeil et al., 2009: 60 (list), figs. 2q, 2s; Rahayu and Osawa, 2012: 183–185, fig. 2; Arima, 2014: 85, unnumbered figs.; Rahayu, 2022: 349, fig. 9C; Nugroho et al., 2024: 17, fig. 8C.

*Diogenes gardineri* Alcock, 1905: 830, Pl. 68, fig. 1; Nobili, 1907: 366–367.

*Diogenes serenei* Forest, 1956: 530–532, figs. 12–15; Gherardi and McLaughlin, 1994: 643.

**Material examined.** Korea: 1♂ (SL 3.1 mm), Jeju-do: Jeju-si, Hangyeong-myeon, Geumdeung-ri, 33°21′35.0″N, 126°11′14.3″E, depth 0.3 m, 31 Jul 2024, hand-collected by Jibom Jung, MABIK CR00258586; 1♂ (SL 2.8 mm), same data as above, MABIK CR00258587; 1♂ (SL 2.6 mm), same data as above, MABIK CR00258588.

**Description.** Shield (Figs. 1C, 2A) 1.1× longer than wide; lateral margin with 4–7 spines; anterolateral margin with 1–2 small spines near antennal peduncles. Ocular peduncles sub-cylindrical, 0.9× SL, 5× longer than wide. Rostrum broadly rounded, slightly shorter than subtriangular lateral projections with distal spine. Ocular scales subrectangular, widely spaced, distal margin with 3–5 strong spines. Intercalary rostral process acutely triangular, as long as ocular scales, with prominent ventral spine. Antennular peduncles reach base of cornea when fully extended. Antennal peduncles 0.5× SL; antennal scales with 3–5 teeth, slightly longer than last antennal peduncular segment. Maxilliped 3 basally approximate. Thirteen pairs of gills; pleurobranch present above pereopod 2.

Pereopods with abundant long setae. Left cheliped (Figs. 1C, 2B) larger and longer than right. Left chela mostly with numerous small tubercles or spines, 1.7× longer than wider; inner dorsolateral margin and median line of dorsal surface with irregular row of strong spines.

Korean name: [1]*털손넓적왼손집게 (신칭)

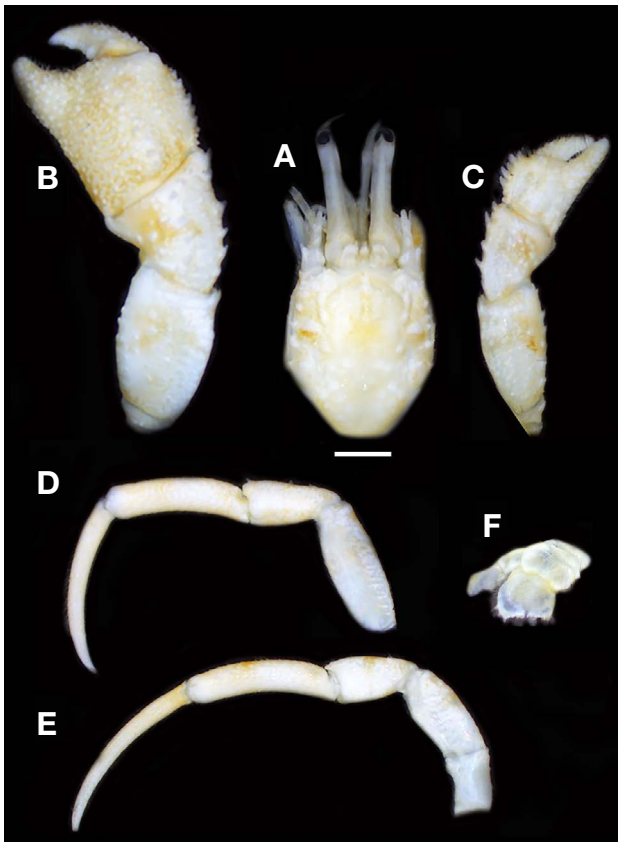


**Fig. 2.** *Diogenes pallescens* Korea (male, SL 3.1 mm, MABIK CR00258586). A, Shield and cephalic appendages, dorsal view; B, Left cheliped, dorsal view; C, Right cheliped, dorsal view; D, Left second pereopod, lateral view; E, Right third pereopod, mesial view; F, Telson, dorsal view. Scale bar: A–F = 1 mm.

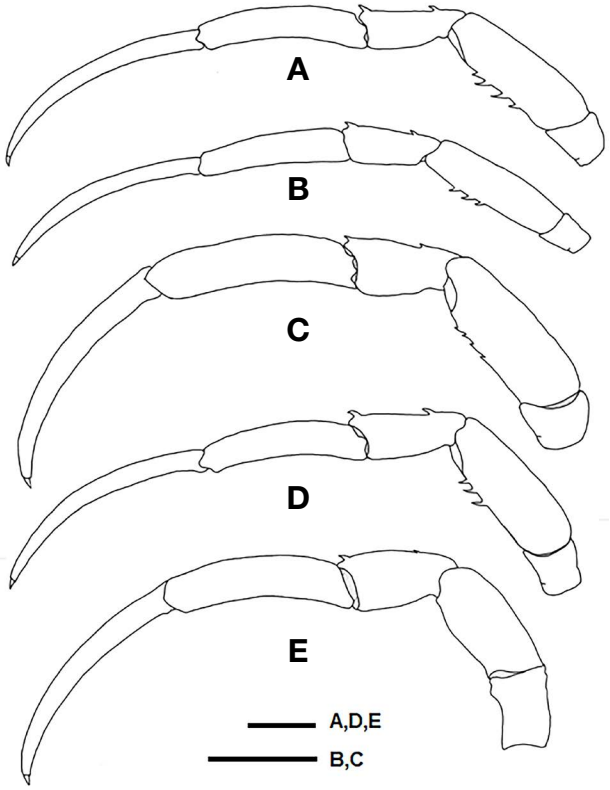


**Fig. 3.** Comparison of left second pereopods of *Diogenes* species (lateral view, setae omitted). A–D, Redrawn from Fig. 3 of McLaughlin (2002): A, A syntype of *D. pallescens* (AMG1402); B, A lectotype of *D. gardineri* Alcock, 1905 (shield length [SL] 1.8 mm, MZCU); C, *D. serenei* Forest, 1956 (SL 2.3 mm, MNHN Pg 1502); D, *D. pallescens* (SL 2.9 mm, USNM 1000219); E, *D. pallescens* Korea (male, SL 3.1 mm, MABIK CR00258586). Scale bars: A–E = 1 mm.

Carpus with row of 5–7 strong spines on inner dorsolateral margin, 2–3 spines on dorsodistal margin, row of 4–5 strong spines on median line of dorsal surface, smaller scattered tubercles on dorsal-outer surface. Merus with 3–4 spines on inner ventrolateral margin, 3 spines on inner dorsolateral margin; 3 spines on inner dorsodistal margin. 3 tubercles on outer ventrolateral margin.

Right cheliped (Figs. 1C, 2C) 0.7× left cheliped length. Right chela mostly covered with numerous small tubercles or spines, 1.4× longer than wide; row of 5–6 strong spines on inner dorsolateral margin, shallow groove on median line of dorsal surface, dactyl with sparse small tubercles. Carpus with row of 5–7 strong spines on inner dorsolateral margin, 3 strong spines on median line of dorsal surface. Merus with row of 6–7 spines on outer lateral margin, strong spine on dorsodistal margin.

Second and third pereopods (Fig. 2D, E) slender, unarmed except for dorsodistal and dorsal spines on carpi; dorsal spine on carpus small in third pereopod compared to that of the first. Abdomen membranous, coiled, without paired pleopods. Uropod asymmetric. Telson (Fig. 2F) asymmetric, with median cleft distinct; a row of spines on distal and lateral margins, lateral one larger.

**Distribution.** Funafuti (type locality), Indo-Pacific region, from East Africa and the Red Sea to Southeast Asia and French Polynesia, including Guam, China, Japan, and Korea (Jejudo Island).

**Coloration.** Body generally mottled cream with scattered brown spots. Posterolateral surface of shield with two distinct dark spots. Ocular peduncles with brown ring near the distal end. Antennular peduncles with dark brown rings on the distal end and proximal end, and two mottled brown rings on the middle part. Antennal peduncles with brown ring near the distal end. Second and third pereopods with dark brown spots on mid-dorsal margin of meri, carpi, and propodi (Fig. 1C).

**Remarks.** The Korean specimens of *D. pallescens* gener-

**Table 1.** Summary of the eight surveyed sites, including environmental characteristics and presence (O) or absence (X) of *Diogenes pallescens* Korea

| Site | GPS | Presence/absence of *Diogenes pallescens* | Presence/absence of unreported anemone |
|---|---|---|---|
| 1 | 33°21′35.0″N 126°11′14.3″E | O | O |
| 2 | 33°21′34.9″N 126°11′13.0″E | X | X |
| 3 | 33°21′35.5″N 126°11′11.5″E | X | X |
| 4 | 33°21′36.1″N 126°11′11.3″E | X | X |
| 5 | 33°21′36.7″N 126°11′13.4″E | X | X |
| 6 | 33°21′37.8″N 126°11′12.1″E | X | X |
| 7 | 33°21′39.2″N 126°11′11.5″E | X | X |
| 8 | 33°21′41.2″N 126°11′10.5″E | X | X |

ally conform to the morphological description provided by McLaughlin (2002), particularly with respect to key diagnostic features such as the shape of the shield, rostrum, and chelipeds. However, a notable difference was observed in the second pereopods: the merus is unarmed in the Korean specimens (Fig. 3), whereas McLaughlin (2002: fig. 3) reported the presence of two to five spines along the ventral margin, a disparity that is considered intraspecific variation. Recent taxonomic studies (Rahayu, 2015, 2022; Asakura, 2020; Nugroho et al., 2024) have revealed substantial morphological diversity among species within the *D. pallescens* group, allowing for clearer delimitation of *D. pallescens* sensu stricto. The present Jejudo Island specimens conform to the diagnosis of *D. pallescens*, particularly in possessing a subrectangular shield, a dorsodistal spine on the merus of the third maxilliped, a continuous row of ventromesial spines on the cheliped ischium, and symmetrical, relatively unarmed pereopods. In contrast, *Diogenes magnus* Asakura, 2020 differs markedly in having an oval shield, the absence of a dorsodistal spine on the third maxilliped, a strongly spinulose left cheliped with double rows of spines on the dactyl and palm, and distinctly asymmetrical setation on the third pereopods—with dense setae restricted to the left side. Moreover, *D. magnus* lacks the ventromesial spine row on the ischium, a key feature in *D. pallescens* Additional comparisons from the Indo-West Pacific region further support the distinction of *D. pallescens*. *Diogenes singaporensis* Rahayu, 2015 exhibits paired spine rows along the carpus of the chelipeds and prominent tufts of long setae on the ambulatory legs—traits that contrast with the modest spinulation and simpler setation in *D. pallescens*. Likewise, *Diogenes moosai* Rahayu & Forest, 1995 is distinguished by its highly setose pereopods, strongly arched dactyli, and more elaborate cheliped ornamentation. Rahayu and Osawa (2012) reaffirmed that *D. gardineri* and *D. serenei*, historically confused with *D. pallescens*, lack the telson morphology and pereopod armature diagnostic of the latter. More recently, Nugroho et al. (2024) recorded *D. pallescens* from Halmahera Island with identical characters—namely, the dorsodistal meral spine on the pereopods, ischial spine rows, and an entire telson lacking a median cleft—thus corroborating the morphological consistency of the species across its broad distribution.

Additionally, our study reveals two ecological features of *D. pallescens* in Korea that have not been previously documented. First, specimens were predominantly collected from estuarine environments characterized by sandy and gravelly substrates at depths of less than 1 m, located within 100 m of the coastline. This contrasts with earlier reports that described *D. pallescens* as inhabiting intertidal zones down to depths of 40 m (Table 1, Fig. 1A). Second, we observed free-living sea anemones occurring exclusively in association with these hermit crabs (Table 1, Fig. 1B). This novel co-occurrence is noteworthy given that previous records have primarily associated *D. serenei* with dead coral substrates and coral reef habitats (Forest, 1956; Rahayu and Komai, 2000; Rahayu, 2022). Additionally, symbiotic relationships have been reported between *D. edwardsii* and *Verrillactis paguri* (Toyota and Tsunoda, 2024), and between *D. pallescens* and sea cucumbers (McLaughlin, 2002).

**Key to the species of the genus *Diogenes* from Korea**

1. Propodus of third pereopods with rows of robust and well-calcified spines visibly projecting from the surface··· 2
– Propodus of third pereopods unarmed or with a row of minute or vestigial spines····· 3
2. Left cheliped with numerous setae ·····*D. penicillatus*
– Left cheliped without setae, usually with anemone ····· ·····*D. edwardsii*
3. Left cheliped with numerous setae ····· ***D. pallescens***
– Left cheliped without setae ····· 4
4. Cutting edge of dactylus of right chela with calcareous teeth····· *D. nitidimanus*
– Cutting edge of dactylus of right chela almost unarmed ··· ····· *D. deflectomanus*

## ORCID

Jibom Jung: https://orcid.org/0000-0002-5074-0002
Jin-Ho Park: https://orcid.org/0000-0001-6522-744X
Hee-seung Hwang: https://orcid.org/0000-0003-0616-1975

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

## ACKNOWLEDGMENTS

This work was supported by a grant from the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (No. RS-2025-02653064). This research was supported by Learning & Academic research institution for Master's PhD students, and Postdocs (LAMP) Program of the National Research Foundation of Korea (NRF) grant funded by the Ministry of Education (No. RS2023-00301976). This work was supported by the management of Marine and Fishery Bio-resources Center (2025) funded by the National Marine Biodiversity Institute of Korea (MABIK).

## REFERENCES

Abd El Wakeil KF, Ahmed ES, Obuid-Allah AH, El Shimy NA, 2009. Hermit crabs (Crustacea: Decapoda: Anomura) inhabiting the intertidal and shallow subtidal region of the Red Sea coast of Egypt. Zootaxa, 2213:57-63. https://doi.org/10.11646/zootaxa.2213.1.3

Alcock A, 1905. Marine Crustaceans XIV. Paguridae. In: The fauna and geography of the Maldive and Laccadive Archipelagoes, being an account of the work carried on and the collections made by an expedition during the years 1899-1901 (Ed., Gardiner JS). Cambridge University Press, Cambridge, pp. 827-835.

Arima H, 2014. Hermit crab illustrated. Seibundo-Shinkosha, Tokyo, pp. 1-223.

Asakura A, 2020. Hermit crabs of the genus *Diogenes* Dana, 1851 (Crustacea: Decapoda: Diogenidae) collected during the Albatross Philippine Expedition, 1907-1910, including descriptions of three new species. Publications of the Seto Marine Biological Laboratory. 45:1-46.

Forest J, 1956. Les pagures du Vietnam I. Le genre Diogenes Dana. Bulletin du Muséum National d'Histoire Naturelle, 28:524-532.

Gherardi F, McLaughlin PA, 1994. Shallow water hermit crabs (Crustacea: Decapoda: Anomura: Paguridea) from Mauritius and Rodrigues Islands, with the description of a new species of *Calcinus*. Raffles Bulletin of Zoology, 42:613-656.

Jung J, Park JH, Kim W, 2018. A systematic study on the paguroidea (Crustacea: Decapoda) in South Korean water: checklist and geographical distribution. Ocean and Polar Research, 40:145-160. https://doi.org/10.4217/OPR.2018.40.3.145

McLaughlin PA, 2002. *Diogenes pallescens* Whitelegge, *D. gardineri* Alcock and *D. serenei* Forest (Decapoda: Anomura: Paguroidea: Diogenidae): distinct species or morphological variants. Raffles Bulletin of Zoology, 50:81-94.

McLaughlin PA, 2003. Illustrated keys to families and genera of the superfamily Paguroidea (Crustacea: Decapoda: Anomura), with diagnoses of genera of Paguridae. Memoirs of Museum Victoria, 60:111-144. https://doi.org/10.24199/j.mmv.2003.60.16

Nobili G, 1907. Ricerche sui Crostacei della Polinesa. Decapodi, Stomatopodi, Anisopodi e Isopodi. Memorie della Reale Accademia delle Scienze di Torino, 57:351-429.

Nugroho DA, Rahayu DL, Pratiwi R, Hernawati RT, Nurhaman U, Ririhena JE, 2024. Hermit crabs (Decapoda: Anomura: Coenobitidae, Calcinidae, Diogenidae, Paguridae) from Halmahera Island, North Maluku, Indonesia. Nauplius, 32:e20240507. https://doi.org/10.1590/2358-2936e20240507

Ortmann AE, 1892. Die Decapoden-Krebse des Strassburger Museum, mit besonderer Berücksichtigung der von Herrn Dr. Doederlein bei Japan und bei den Liu-Kiu-Inseln gesammelten und zur Zeit im Strassburger Museum aufbewahrten Formen. IV. Die Abtheilungen Galatheidea und Paguridea. Zoologischer Jahrbücher, Abteilung für Systematik, Geographie und Biologie der Tiere, 6:241-326. https://doi.org/10.5962/bhl.part.26455

Rahayu DL, 2015. New record and new species of the hermit crab genus *Diogenes* Dana, 1851 (Decapoda: Anomura: Diogenidae) from Singapore. Raffles Bulletin of Zoology, Supplement, 31:182-192.

Rahayu DL, 2022. Hermit crabs of Singapore (Crustacea: Decapoda: Anomura: Diogenidae, Paguridae), with description of two new species. Raffles Bulletin of Zoology, 70:329-363. https://doi.org/10.26107/RBZ-2022-0017

Rahayu DL, Forest J, 1995. Le genre *Diogenes* (Decapoda, Anomura, Diogenidae) en Indonésie, avec la description de six espèces nouvelles. Bulletin du Muséum National d'Histoire Naturelle, Paris, Séries 4, Section A, 16:383-415.

Rahayu DL, Komai T, 2000. Shallow-water hermit crabs (Crustacea: Decapoda: Anomura: Diogenidae and Paguridae) of Phuket, Thailand. Phuket Marine Biological Center Research Bulletin, 63:21-44.

Rahayu DL, Osawa M, 2012. Hermit crabs (Crustacea: Decapoda: Diogenidae) from the KUMEJIMA 2009 Expedition, Japan. Zootaxa, 3367:176-190. https://doi.org/10.11646/zootaxa.3367.1.17

Toyota K, Tsunoda K, 2024. Research of the symbiotic ecology between sea anemones and hermit crabs (*Diogenes edwardsii*). Annals of the Research Institute of Japan Sea Region, 55:25-31.

Whitelegge T, 1897. The atoll of Funafuti, Ellice Group: its zoology, botany, ethnology and general structure VI. The Crustacea. Memoirs of the Australian Museum, 3:127-151.

Williams JD, McDermott JJ, 2004. Hermit crab biocoenoses: a worldwide review of the diversity and natural history of hermit crab associates. Journal of Experimental Marine Biology and Ecology, 305:1-128. https://doi.org/10.1016/j.jembe.2003.12.033

World Register of Marine Species (WoRMS), 2025. Taxonomic details for genus *Diogenes* [Internet]. World Register of Marine Species, Flemish Marine Institute (VLIZ), Ostend, Accessed 14 Apr 2025, <https://www.marinespecies.org/aphia.php?p=taxdetails&id=106843>.

Received October 24, 2025
First Revised December 9, 2025
Second Revised December 19, 2025
Accepted January 8, 2026

ASED http://e-ASED.org
Animal Systematics, Evolution and Diversity

Anim. Syst. Evol. Divers. Vol. 42, No. 1: 88-92, January 2026
https://doi.org/10.5635/ASED.2026.42.1.013

Short communication

# New Record of *Thoracophelia japonica* (Annelida, Opheliidae) from South Korea

Ha-Eun Lee[1], Gi-Sik Min[1,*], Geon Hyeok Lee[1,2,*]

[1]Department of Biological Sciences and Bioengineering, Inha University, Incheon 22212, Korea
[2]Marine Environment Research Division, National Institute of Fisheries Science, Busan 46083, Korea

## ABSTRACT

An opheliid polychaete *Thoracophelia japonica* (Misaka and Sato, 2003), originally described from Japan, is newly recorded from South Korea. The Korean specimens corresponded well to the original description of *T. japonica*, originally described as *Euzonus japonicus*, in having 15 pairs of palmatifid branchiae occurring on setigers 12−26, a pair of lateral transverse rows bearing 8−10 dorsal and 2−3 ventral conical cirri present on setiger 10, ventral conical cirri starting from chaetiger 9, pygidium with lateral lobes fringed with 6−8 anal cirri on each side and a minute midventral anal cirrus, and the absence of a pygidial midventral plate.

**Keywords:** East Sea, Opheliid, Polychaeta, taxonomy

## INTRODUCTION

*Thoracophelia* Ehlers, 1897 is a genus belonging to the family Opheliidae and is characterized by three distinct body regions: (1) the cephalic region consisting of the prostomium and the first two chaetigers, (2) a swollen thoracic region with modified chaetiger 10, and (3) an abdominal region bearing the ventral groove and branchiae (Blake, 2011; Law et al., 2013; Parapar et al., 2021). Several *Thoracophelia* species were previously described as members of the genera *Euzonus* Grube, 1866, *Pectinophelia* Hartman, 1938, and *Lobochesis* Hutchings and Murray, 1984; however, these genera have since been synonymized with *Thoracophelia* because they share a similar body morphology composed of three distinct regions. Additionally, the pinnules on the branchiae of *Pectinophelia* were interpreted as species-level adaptations to habitat, and *Lobochesis* was nested within a monophyletic clade of *Euzonus*, which has been synonymized with *Thoracophelia* (Parke, 1973; Santos et al., 2004; Sene Silva, 2007; Blake, 2011; Brewer et al., 2011).

To date, 19 species have been described worldwide, and seven species have been recorded in Northeast Asia (Jimi et al., 2021; WoRMS Editorial Board, 2025). Despite the species diversity in Northeast Asia, two species, *T. foliformis* Lee, Lee and Min, 2024 and *T. williamsi* (Hartman, 1938), have been recorded in South Korea (Song et al., 2017; Jimi et al., 2021; Lee et al., 2024). Therefore, we conducted a taxonomic investigation on Korean *Thoracophelia* specimens.

In the present study, we examined bulk samples deposited at the National Institute of Biological Resources (NIBR), South Korea, and discovered *Thoracophelia japonica*, previously unrecorded in South Korea. Morphological observations were made using a stereomicroscope. Photographs of the specimens were captured using a digital camera (Dhyana 400DC; Tucsen, China) with the Mosaic capture program (Mosaic version 15; Tucsen). Staining patterns were investigated using a methyl green solution following Mackie and Gobin (1993). The specimens were re-deposited at the NIBR in Incheon, South Korea, with newly assigned voucher numbers.

## SYSTEMATIC ACCOUNTS

Phylum Annelida Lamarck, 1809
Class Polychaeta Grube, 1850
Family Opheliidae Malmgren, 1867
Genus *Thoracophelia* Ehlers, 1897

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: 82-32-860-7692, Fax: 82-32-874-6737
E-mail: mingisik@inha.ac.kr (G.-S. Min)
Tel: 82-51-720-2534, Fax: 82-51-720-2515
E-mail: tjftk145@daum.net (G.H. Lee)

eISSN 2234-8190

Copyright © The Korean Society of Systematic Zoology

[1]***Thoracophelia japonica* (Misaka and Sato, 2003) (Fig. 1)**

*Euzonus japonicus* Misaka and Sato, 2003: 1172, figs. 1–4.
*Euzonus ezoensis*: Yokoyama and Hayashi, 1980: 46.
*Euzonus* sp. 1: Nishi et al., 2001: 256.

**Material examined.** South Korea: 25 specimens, Gyeongsangbuk-do, Uljin-gun, Jukbyeon-myeon, 1 Jul 2002, Jae-Sang Hong, NIBRIV0000929320, NIBRIV0000929322, NIBRIV0000929323.

**Diagnosis.** Fifteen pairs of branchiae present on chaetiger 12–26. Branchiae palmatifid with 4–6 filaments. Chaetiger 10 with a pair of lateral transverse rows of 8–10 dorsal and 2–3 ventral conical cirri. Pygidium with lateral lobes fringed with 6–8 cirri on each side, lacking midventral plate. A tiny midventral anal cirrus present between lateral lobes, often obscure.

**Description.** Longest complete specimens with 32 chaetigers, 17.2 mm long, 1.8–2.1 mm wide in thoracic region and 1.5 mm wide in abdominal region.

Body distinctly divided into three regions by weak constrictions: cephalic, thoracic, and abdominal regions (Fig. 1A). The cephalic region consisting of a pointed prostomium and two setigers. A pair of nuchal organs present in cephalic region dorsolaterally. Proboscis eversible from mouth region. The thoracic region inflated and consisting of eight chaetigers (Fig. 1A). Chaetiger 10 with a pair of lateral transverse rows of 8–10 dorsal and 2–3 ventral conical cirri (Fig. 1B, C). The abdominal region includes 22 chaetigers and pygidium. Last 4 chaetigers decreasing in size (Fig. 1E, F). Longitudinal mid-ventral groove extending from about chaetiger 10 to pygidium (Fig. 1A). A pair of longitudinal lateral grooves present in the abdominal region, one on each side of body (Fig. 1A). Body formula 11a (abranchiate) + 15b (branchiate) + 6a (posterior abranchiate) = 32 chaetigers. Black rod-shaped spicules in coelom cavity corrugate or curve, up to 133 μm in length (Fig. 1B).

Branchiae palmatifid, wrinkled, occurring from chaetigers 12 to 26 (Fig. 1B, D, E). Branchiae with wrinkled finger-shaped branchial filaments; the well-developed ones bear up to 6 filaments.

Parapodia biramous, with low pre- and postchaetal lobes and postchaetal lobes slightly larger than prechaetal lobes (Fig. 1C). Neuropodia with short and rounded postchaetal lobes (Fig. 1C). Neuropodial ventral conical cirri first present from chaetiger 9 to chaetigers 20–25 (Fig. 1B–E, H). The number of ventral conical cirri varies; 2 or 3 on chaetigers 9–12 and then decrease to single cirrus from around chaetigers 13. Lateral organs present in between noto- and neuropodial (Fig. 1C). Chaetae distinctly longer in chaetigers 3–5 and posterior 6 chaetigers (Fig. 1A, E–G).

Pygidium with a pair of lateral lobes bearing 6–8 anal cirri on each side and a small midventral anal cirrus (Fig. 1E, F). Midventral plate absent.

**Methyl green staining pattern.** The annulations of each segment showed faint line-shaped stains (Fig. 1G, J). The last annulation of each segment showed a more intense line-shaped staining (Fig. 1G, J). The distal ends of the dorsal and ventral conical cirri of chaetiger 9 and 10 were intensely stained (Fig. 1H). The prechaetal lobes of the abdominal neuropodia were also stained, and the base of each branchial stem showed a circular stain (Fig. 1I).

**Distribution.** Japan and Korea (present study).

**Remarks.** The Korean specimens of *Thoracophelia japonica* correspond well with the original description of *Euzonus japonicus* by Misaka and Sato (2003) in having the following features: (1) 15 pairs of palmatifid branchiae, starting from chaetiger 12, (2) a pair of lateral transverse rows of conical cirri present on chaetiger 10, with 8–10 dorsal and 2–3 ventral conical cirri, (3) ventral conical cirri present from chaetiger 9, (4) pygidium with fringed lateral lobes and a tiny midventral anal cirrus, and (5) absence of triangular midventral plate in pygidium. Since the generic name *Euzonus* was synonymized with *Thoracophelia*, *Euzonus japonicus* was subsequently revised to *Thoracophelia japonica* by Blake (2011).

In terms of the presence of a transverse row of conical cirri on chaetiger 10, the Korean specimens are also similar to *T. flabellifera* Ziegelmeier, 1955, *T. mammillata* (Santos et al., 2004), and *T. papillata* (Santos et al., 2004). However, the former is distinguished from the latter species based on the type of branchiae (palmatifid vs. bifid or trifid). In addition, the Korean specimens are also clearly distinguished from other *Thoracophelia* species based on the shape of the branchiae. *Thoracophelia japonica* is the only species known to possess palmatifid branchiae, whereas other *Thoracophelia* species have been described to have simple, bifid, trifid, pectinate, or dichotomously branched branchiae.

In South Korea, *T. foliformis* and *T. williamsi* have been recorded in the western and eastern Korean waters, respectively. The present Korean specimens differ from *T. williamsi*, collected from Uljin-gun, South Korea, in the number of pairs of branchiae (15 pairs vs. 16 [or 19] pairs), the shape of branchia (palmatifid branchiae vs. bifid or trifid branchiae), and the shape of chaetiger 10 (with transverse row of dorsal and ventral conical cirri vs. smooth lateral ridge) (Hartman, 1938; Probert, 1976; Misaka and Sato, 2003; Santos et al., 2004). The Korean specimens also differ from *T. foliformis* in the shape of the branchia (palmatifid vs. pectinate), the shape

Korean name: [1]*고깔큰요정갯지렁이 (신칭)

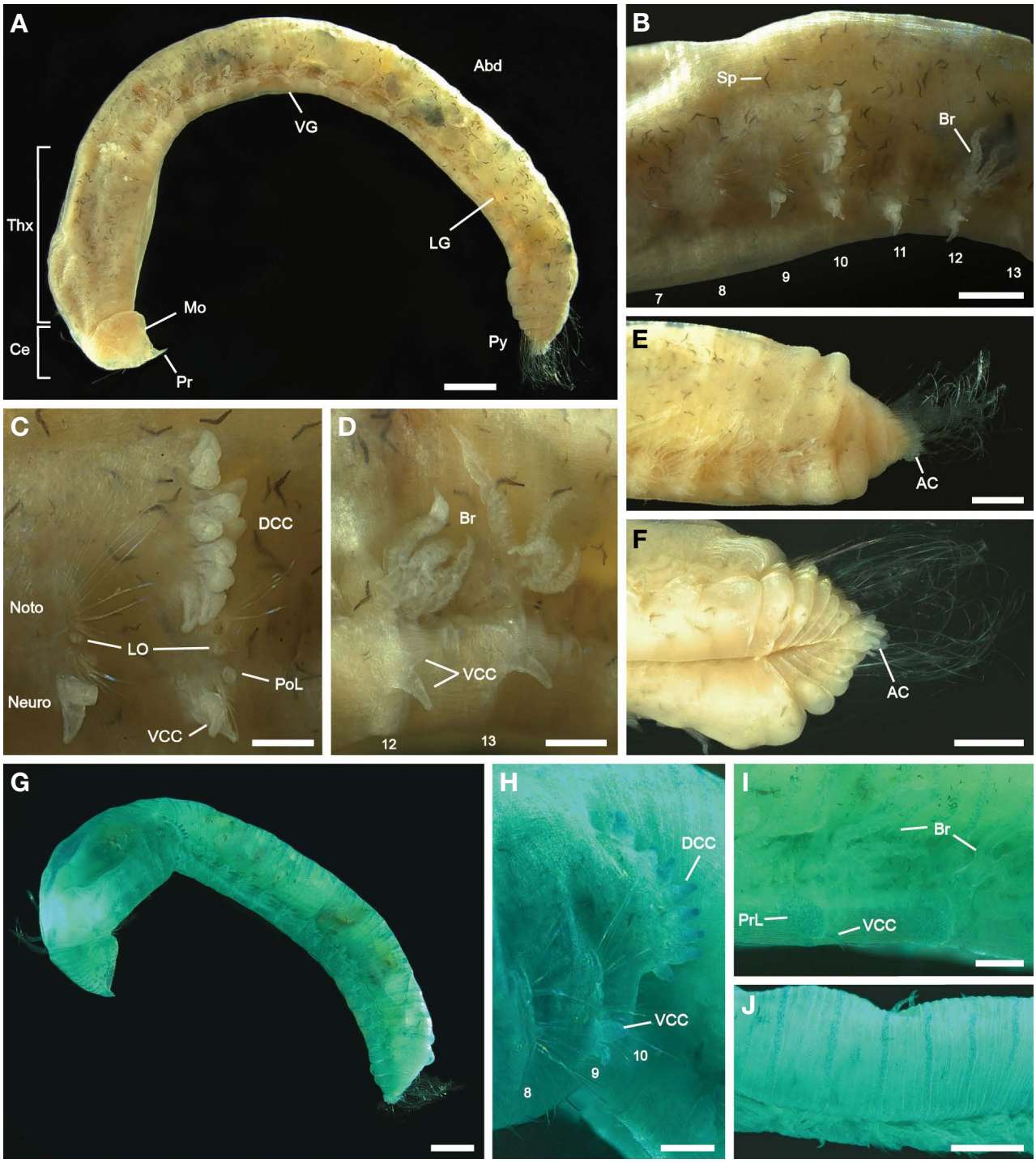


**Fig. 1.** Photographs of Korean *Thoracophelia japonica*. A, Whole body; B, Transition of thorax and abdomen; C, Parapodia and lateral transverse rows of dorsal and ventral conical cirri on chaetigers 9–10; D, Anterior branchiate parapodia; E, Posterior abdominal region, lateral view; F, Pygidium, ventral view; G, Whole body, stained with methyl green staining solution; H, Stained dorsal and conical cirri; I, Stained abdominal parapodia; J, Stained abdominal region, dorsolateral view. Abd, abdomen; AC, anal cirri; Br, branchia; Ce, cephalic region; DCC, dorsal conical cirri; LG, lateral groove; LO, lateral organ; Mo, mouth; Neuro, neuropodium; Noto, notopodium; PoL, postchaetal lobe; Pr, prostomium; PrL, prechaetal lobe; Py, pygidium; Sp, spicule; Thx, thorax; VCC, ventral conical cirri; VG, ventral groove. Scale bars: A, G = 1 mm, B, E, F, J = 0.5 mm, C, D, H, I = 0.2 mm.

of chaetiger 10 (with a transverse row of dorsal and ventral conical cirri vs. a smooth lateral ridge), and the presence of a midventral plate (absent vs. present) (Misaka and Sato, 2003; Lee et al., 2024). Furthermore, circular stains on the base of the branchial stem were more distinct and intense in *T. foliformis*, and the line-shaped stains were significantly thicker in the middorsal region of *T. foliformis*, whereas those in the Korean *T. japonica* were relatively constant (see fig. 4D, E in Lee et al., 2024).

## ORCID

Ha-Eun Lee: https://orcid.org/0000-0002-4694-4889
Gi-Sik Min: https://orcid.org/0000-0003-2739-3978
Geon Hyeok Lee: https://orcid.org/0000-0001-7517-3086

## CONFLICTS OF INTEREST

Gi-Sik Min, a contributing editor of the Animal Systematics, Evolution and Diversity, was not involved in the editorial evaluation or decision to publish this article. Remaining authors have declared no conflicts of interest.

## ACKNOWLEDGMENTS

This study was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR 202502201). This study was also supported by Inha University.

## REFERENCES

Blake JA, 2011. Revalidation of the genus *Thoracophelia* Ehlers, 1897, replacing *Euzonus* Grube, 1866 (Polychaeta: Opheliidae), junior homonym of *Euzonus* Menge, 1854 (Arthropoda: Diplopoda), together with a literature summary and updated listing of *Thoracophelia* species. Zootaxa, 2807:65-68. https://doi.org/10.11646/zootaxa.2807.1.5

Brewer MS, Sierwald P, Bond JE, 2011. A generic homonym concerning chordeumatid millipedes (Arthropoda: Diplopoda) and ophellid worms (Annelida: Polycheata). Zootaxa, 2744:65-68. https://doi.org/10.11646/zootaxa.2744.1.6

Ehlers E, 1897. Polychaeten. Ergebnisse der Hamburger Magalhaensischen Sammelreise 1892/93. III. Band. Bryozoen und Würmer. Friederichsen & Co., Hamburg, pp. 1-148 (in German). https://doi.org/10.5962/bhl.title.63772

Hartman O, 1938. Descriptions of new species and new generic records of polychaetous annelids from California of the families Glyceridae, Eunicidae, Stauronereidae, and Opheliidae. University of California Publications in Zoology, 43:93-111.

Jimi N, Fujimoto S, Takehara M, Imura S, 2021. Black spicules from a new interstitial opheliid polychaete *Thoracophelia minuta* sp. nov. (Annelida: Opheliidae). Scientific Reports, 11:1557. https://doi.org/10.1038/s41598-020-80702-6

Law CJ, Dorgan KM, Rouse GW, 2013. Validation of three sympatric *Thoracophelia* species (Annelida: Opheliidae) from Dillon Beach, California using mitochondrial and nuclear DNA sequence data. Zootaxa, 3608:67-74. https://doi.org/10.11646/zootaxa.3608.1.4

Lee HE, Lee GH, Min GS, 2024. A new species of *Thoracophelia* (Annelida, Opheliidae) from the Yellow Sea of South Korea. Biodiversity Data Journal, 12:e129526. https://doi.org/10.3897/BDJ.12.e129526

Mackie ASY, Gobin J, 1993. A review of the genus *Johnstonia* Quatrefages, 1866 (Polychaeta, Maldanidae), with a description of a new species from Trinidad, West Indies. Zoologica Scripta, 22:229-241. https://doi.org/10.1111/j.1463-6409.1993.tb00354.x

Malmgren AJ, 1867. Annulata Polychaeta Spetsbergiae, Groenlandiae, Islandiae et Scandinaviae hactenus cognita. Hactenus Cognita. Ex Officina Frenckelliana, Helsingforslæ, pp. 1-127 (in Finnish). https://doi.org/10.5962/bhl.title.13358

Misaka T, Sato M, 2003. A new species of *Euzonus* (Polychaeta: Opheliidae) from subtidal zones in Japan. Zoological Science, 20:1171-1177. https://doi.org/10.2108/zsj.20.1171

Nishi E, Kato T, Ueshima R, 2001. Polychaetous annelids from off Shimoda Port, Sagami Bay, Japan. Memoirs of the National Science Museum, Tokyo, 37:251-259 (in Japanese).

Parapar J, Martínez A, Moreira J, 2021. On the systematics and biodiversity of the Opheliidae and Scalibregmatidae. Diversity, 13:87. https://doi.org/10.3390/d13020087

Parke SR, 1973. Biological aspects of speciation in three sympatric *Euzonus* species at Dillon Beach, California (Polychaeta: Opheliidae). Unpublished Master of Science Thesis, University of the Pacific, Stockton, CA, pp. 1-69.

Probert PK, 1976. New species of *Euzonus* (Polychaeta: Opheliidae) from a New Zealand sandy beach. New Zealand Journal of Marine and Freshwater Research, 10:375-379. https://doi.org/10.1080/00288330.1976.9515621

Santos CSG, Nonato EF, Petersen ME, 2004. Two new species of Opheliidae (Annelida: Polychaeta): *Euzonus papillatus* sp. n. from a northeastern Brazilian sandy beach and *Euzonus mammillatus* sp. n. from the continental shelf of southeastern Brazil. Zootaxa, 478:1-12. https://doi.org/10.11646/zootaxa.478.1.1

Sene Silva G, 2007. Filogenia de Opheliidae (Annelida: Polychaeta). Unpublished Thesis presented for the degree, Doctor of Sciences, in Zoology, Universidade Federal do Paraná, Curitiba, pp. 1-95.

Song JH, Han YD, Lee WK, Kim IH, Paik SG, Lee J, Soh HY, Lee W, Jung J, Kim SH, Lee JS, Kim JP, Park T, Yoo JS, Kil HJ, Nam E, Min GS, 2017. Unrecorded species of Ko-

rean metazoans discovered through the project of "Discovery of Korean Indigenous Species" (2006-2010). Journal of Species Research, 6:164-171. http://doi.org/10.12651/JSR.2017.6(S).164

WoRMS Editorial Board, 2025. World Register of Marine Species [Internet]. World Register of Marine Species, Flemish Marine Institute (VLIZ), Ostend, Accessed 24 Oct 2025, <http://www.marinespecies.org>.

Yokoyama H, Hayashi I, 1980. Zonation and species diversity of smaller macrobenthos in the westernmost part of Wakasa Bay (the Sea of Tango). Journal of the Oceanographical Society of Japan, 36:46-58. https://doi.org/10.1007/BF02209355

Ziegelmeier E, 1955. *Thoracophelia flabellifera* n. sp., a new sedentary polychaete (family Opheliidae) in the German Bight. Helgoländer Wissenschaftliche Meeresuntersuchungen, 5:251-257 (in German).

Received November 6, 2025
First Revised December 23, 2025
Second Revised December 24, 2025
Accepted January 8, 2026

ASED http://e-ASED.org
Animal Systematics, Evolution and Diversity

Anim. Syst. Evol. Divers. Vol. 42, No. 1: 93-99, January 2026
https://doi.org/10.5635/ASED.2026.42.1.019

Short communication

# Mitogenomic Resources of *Tanakia latimarginata* and *T. koreensis* with Implications for Taxonomic Accuracy in Acheilognathidae

Hyung-Bae Jeon[1], Hari Won[2], Ho Young Suk[3,*]

[1]National Institute of Biological Resources, Incheon 22689, Korea
[2]Honam National Institute of Biological Resources, Mokpo 59235, Korea
[3]Department of Life Sciences, Yeungnam University, Gyeongsan 38541, Korea

## ABSTRACT

Accurate mitochondrial genome sequences are essential for reliable species identification and phylogenetic inference, particularly in morphologically cryptic taxa. In the genus *Tanakia*, however, several mitochondrial sequences deposited in public repositories have been suggested to be erroneous, potentially obscuring taxonomic boundaries. In this study, we comparatively analyzed the complete mitochondrial genomes of *Tanakia latimarginata* and *T. koreensis*, two closely related bitterling species, to establish validated reference sequences and clarify their phylogenetic relationships within Acheilognathidae. Both mitogenomes display the typical vertebrate mitochondrial gene complement and identical gene order. Phylogenetic analyses resolved *T. latimarginata* as sister to the clade comprising *T. koreensis* and *T. signifer*, in agreement with previous morphological and molecular studies. In addition, several publicly available mitogenomes were placed incongruently with current taxonomy, highlighting persistent issues in reference database reliability. Our results provide robust comparative mitogenomic data for *Tanakia* and support the construction of a curated mitochondrial genome repository for Acheilognathidae.

**Keywords:** mitochondrial genome, Acheilognathidae, *Tanakia*, phylogeny, cryptic species

## INTRODUCTION

The bitterling genus *Tanakia* (Cypriniformes: Acheilognathidae) currently comprises six valid species distributed across East Asia, ranging from northeastern China through the Korean Peninsula to western Japan (Chang et al., 2014; Jeon et al., 2018; Choi and Lee, 2024, 2025). Under recent environmental threats such as exotic species and habitat destruction, the Korean Ministry of Environment has designated *T. signifer* and *T. somjinensis* as legally endangered given their limited habitats and small population sizes on the Korean Peninsula (Jeon et al., 2018). *T. latimarginata* (Kim et al., 2014) and *T. koreensis* (Kim and Kim, 1990) represent a particularly challenging taxonomic pair because of their close morphological similarity and overlapping geographic ranges (Jeon et al., 2018). Accurate species identification within this genus therefore requires robust molecular reference data.

Although species-level identification of *Tanakia* is feasible for taxonomic experts based on morphology (Jeon et al., 2018), this approach is often impractical in ecological surveys, conservation planning, and environmental DNA (eDNA) applications. Consequently, mitochondrial DNA markers have been increasingly used for species discrimination (Li et al., 2018; Janzen et al., 2022), and previous studies have demonstrated their utility in resolving relationships within Acheilognathidae (Jeon et al., 2018). However, several mitochondrial sequences currently deposited in GenBank for *Tanakia* species have been flagged as potentially erroneous (EF483932 in *T. koreensis*, and EF483930 in *T. signifer*), raising concerns regarding the reliability of publicly available reference data (Jeon et al., 2018; Li et al., 2018).

In this context, direct comparative mitogenomic analyses of closely related species are critical not only for refining phylogenetic relationships but also for identifying inconsistencies in existing databases. Here, we present the complete mitochondrial genomes of *T. latimarginata* and *T. koreensis* ob-

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: 82-53-810-2379, Fax: 82-53-810-4613
E-mail: hsuk@ynu.ac.kr

eISSN 2234-8190

Copyright © The Korean Society of Systematic Zoology

tained from confidently identified individuals collected from non-overlapping habitats. By comparing their genomic structures and phylogenetic placements within Acheilognathidae, we aim to (1) provide validated reference mitogenomes for these two species and (2) evaluate the extent to which erroneous sequences may obscure taxonomic boundaries in this group.

The samples were collected from the natural habitats of *Tanakia latimarginata* (type locality; 35°57′55″N, 128°57′32″E; in 2014) and *T. koreensis* (35°39′16″N, 128°48′04’″E; in 2017) in the Nakdong River. The collection of *T. koreensis* samples was performed from the Nakdong River where only *T. koreensis* existed to completely exclude individuals with mitochondrial introgression from *T. latimarginata*. Likewise, *T. latimarginata* samples were collected from a tributary of the Nakdong River where the holotype specimen was collected and *T. koreensis* did not exist. The individual samples were stored in the Conservation Genome Resource Bank for Korean Wildlife (CGRB: http://www.cgrb.org/) and accessible as CGRB16201 (*T. koreensis*) and CGRB16202 (*T. latimarginata*).

The DNA was extracted using Wizard Genomic DNA purification kit (Promega, USA) in accordance with the protocol. To avoid the possibility of interspecific contamination, extraction was performed for the two species on completely separate days. Fourteen pairs of primers were designed from the mitochondrial genome of *Tanakia* species retrieved from National Center for Biotechnology Information GenBank (JQ714034.1, Hwang et al., 2012; EF483933.1, Hwang et al., 2013; FJ515921, Hwang et al., 2014) using Primer3 (Untergasser et al., 2012) implemented in software Geneious 9.1.8 (Table 1) (Kearse et al., 2012). Polymerase chain reaction (PCR) was carried out using 50 μL mixture containing ca. 100 ng genomic DNA, 1× PCR buffer, 3 mM $MgCl_2$, and 0.25 units of *Taq* polymerase (Solgent, Korea). GenePro (BIOER, China) was used for the thermal cycling procedure under the setup of 94°C for 10 min, 30 cycles of 30 s at 94°C, 45 s at 56°C and 45 s at 72°C and final elongation at 72°C for 15 min. The PCR products were purified using Solg PCR purification kit (Solgent) and were sent for the commercial sequenc-

**Table 1.** List of primers used for the assembly of the mitogenomes from *Tanakia latimarginata* and *T. koreensis*

| Primer | Position | Sequence (5′-3′) | $T_m$ | Length |
|---|---|---|---|---|
| TK01F | 286F | GCTAACAGGGCCGGTAAAAC | 59.2 | 20 |
| TK01R | 1885R | CCCTCTTGATTTGGGGTCGT | 59.7 | 20 |
| TK02F | 1425F | GGCAAAAGAGTGGGACGAAC | 59.4 | 20 |
| TK02R | 3026R | GTCTGCGATCGGTTGTAGGA | 59.5 | 20 |
| TK03F | 2708F | GCCCCGCCCCTAATTAATGA | 59.9 | 20 |
| TK03R | 4308R | CGGCTATTTCTCATGTGCCC | 59.1 | 20 |
| TK04F | 3817F | CTCCGCAGCTATAACCCAGG | 60.0 | 20 |
| TK04R | 5417R | CCTCTCCCTATCAAGTCCCG | 58.7 | 20 |
| TK05F | 4857F | ACAAAACAAGACCTCCCCACA | 59.7 | 21 |
| TK05R | 6457R | CAGTGTGGCCAGTCAGCTAA | 60.0 | 20 |
| TK06F | 6369F | ATGTAGACACTCGCGCCTAT | 58.7 | 20 |
| TK06R | 7968R | GGTTGGGGTTCAATTGTGGC | 60.0 | 20 |
| TK07F | 7243F | ACTTCCACGACCATGCCCTA | 60.9 | 20 |
| TK07R | 8842R | TTAGTGGTCATGGGCTTGGG | 59.7 | 20 |
| TK08F | 8210F | GCCCGATGAGTAAACAACCG | 59.3 | 20 |
| TK08R | 9810R | AATCGCAGGGAGAAAGGCAA | 60.0 | 20 |
| TK09F | 9247F | GGCGAACGAAAACAAGCCAT | 60.0 | 20 |
| TK09R | 10847R | TAGGGCAACTAAGAGGGGCA | 60.3 | 20 |
| TK10F | 10438F | CACACAGCCTCCTAATCGCT | 59.8 | 20 |
| TK10R | 12038R | GGTTTAGGGCTTAGCGTGGT | 60.0 | 20 |
| TK11F | 11519F | ATCCCCAACGACCATTGCAC | 61.0 | 20 |
| TK11R | 13118R | AATGGGGTGCCTGTAAGAGC | 60.0 | 20 |
| TK12F | 13017F | TGACGAGCAAGATATCCGGA | 58.3 | 20 |
| TK12R | 14619R | AAGCCTCGGATTAACCAGCC | 60.1 | 20 |
| TK13F | 14434F | CCTACCAACACCCTCCAACA | 59.2 | 20 |
| TK13R | 16034R | CACCGACTGTCCATGAGATGT | 59.8 | 21 |
| TK14F | 15947F | CCATGATGGAATCAGGGACAC | 61.0 | 21 |
| TK14R | 382R | GTTGCAGCCCTTGACCACC | 62.4 | 19 |

ing to LabGenomics (Korea), where sequencing was conducted in an ABI3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA) with the same primer sets used in our PCR reactions.

Obtained sequences were mapped against the reference sequences of *Tanakia* species and assembled to construct the genomic structure using Geneious. The mitochondrial genomes constructed and annotated using Geneious under default options were aligned with those of acheilognathids. A Bayesian inference tree was reconstructed to examine the phylogenetic structure with the new *Tanakia* genome sequences obtained and the divergence time among clades using BEAST2 (Bouckaert et al., 2019). Two independent runs of Markov Chain Monte Carlo (MCMC) were performed on 60 million generations, with sampling every 1,000 steps, using strict clock model (clock.rate = 0.1) and gamma site model (general time reversible). We discarded the first 1 million generations as burn-in. Calibration of the molecular clock was

**Table 2.** The structure and gene arrangement of the mitogenome from *Tanakia latimarginata*

| Name | Type | Position | | Length (bp) | Start codon | Stop codon | Direction |
|---|---|---|---|---|---|---|---|
| | | From | To | | | | |
| tRNA-Phe | tRNA | 1 | 68 | 68 | – | – | + |
| 12S rRNA | rRNA | 69 | 1,028 | 960 | – | – | + |
| tRNA-Val | tRNA | 1,029 | 1,100 | 72 | – | – | + |
| 16S rRNA | rRNA | 1,101 | 2,782 | 1,682 | – | – | + |
| tRNA-Leu | tRNA | 2,783 | 2,858 | 76 | – | – | + |
| ND1 | gene | 2,859 | 3,830 | 972 | ATG | UAG | + |
| tRNA-Ile | tRNA | 3,835 | 3,906 | 72 | – | – | + |
| tRNA-Gln | tRNA | 3,905 | 3,975 | 71 | – | – | – |
| tRNA-Met | tRNA | 3,977 | 4,045 | 69 | – | – | + |
| ND2 | gene | 4,046 | 5,090 | 1,045 | ATG | U | + |
| tRNA-Trp | tRNA | 5,091 | 5,161 | 71 | – | – | + |
| tRNA-Ala | tRNA | 5,163 | 5,231 | 69 | – | – | – |
| tRNA-Asn | tRNA | 5,233 | 5,305 | 73 | – | – | – |
| tRNA-Cys | tRNA | 5,337 | 5,403 | 67 | – | – | – |
| tRNA-Tyr | tRNA | 5,404 | 5,474 | 71 | – | – | – |
| COX1 | gene | 5,476 | 7,026 | 1,551 | GTG | UAA | + |
| tRNA-Ser | tRNA | 7,027 | 7,097 | 71 | – | – | – |
| tRNA-Asp | tRNA | 7,100 | 7,169 | 70 | – | – | + |
| COX2 | gene | 7,178 | 7,868 | 691 | ATG | U | + |
| tRNA-Lys | tRNA | 7,869 | 7,944 | 76 | – | – | + |
| ATP8 | gene | 7,946 | 8,110 | 165 | ATG | UAA | + |
| ATP6 | gene | 8,104 | 8,787 | 684 | ATG | UAA | + |
| COX3 | gene | 8,787 | 9,570 | 784 | ATG | U | + |
| tRNA-Gly | tRNA | 9,571 | 9,641 | 71 | – | – | + |
| ND3 | gene | 9,642 | 9,990 | 349 | ATG | U | + |
| tRNA-Arg | tRNA | 9,991 | 10,060 | 70 | – | – | + |
| ND4L | gene | 10,061 | 10,357 | 297 | ATG | UAA | + |
| ND4 | gene | 10,351 | 11,732 | 1,382 | ATG | UA | + |
| tRNA-His | tRNA | 11,733 | 11,801 | 69 | – | – | + |
| tRNA-Ser | tRNA | 11,802 | 11,870 | 69 | – | – | + |
| tRNA-Leu | tRNA | 11,872 | 11,944 | 73 | – | – | + |
| ND5 | gene | 11,945 | 13,780 | 1,836 | ATG | UAA | + |
| ND6 | gene | 13,777 | 14,298 | 522 | ATG | UAA | – |
| tRNA-Glu | tRNA | 14,299 | 14,367 | 69 | – | – | – |
| CYTB | gene | 14,373 | 15,513 | 1,141 | ATG | U | + |
| tRNA-Thr | tRNA | 15,514 | 15,587 | 74 | – | – | + |
| tRNA-Pro | tRNA | 15,587 | 15,656 | 70 | – | – | – |
| Control region | | 15,659 | 16,568 | 910 | – | – | + |

**Table 3.** The structure and gene arrangement of the mitogenome from *Tanakia koreensis*

| Name | Type | Position | | Length (bp) | Start codon | Stop codon | Direction |
|---|---|---|---|---|---|---|---|
| | | From | To | | | | |
| tRNA-Phe | tRNA | 1 | 68 | 68 | – | – | + |
| 12S rRNA | rRNA | 69 | 1,027 | 959 | – | – | + |
| tRNA-Val | tRNA | 1,028 | 1,099 | 72 | – | – | + |
| 16S rRNA | rRNA | 1,100 | 2,780 | 1,681 | – | – | + |
| tRNA-Leu | tRNA | 2,781 | 2,856 | 76 | – | – | + |
| ND1 | gene | 2,857 | 3,828 | 972 | ATG | UAG | + |
| tRNA-Ile | tRNA | 3,833 | 3,904 | 72 | – | – | + |
| tRNA-Gln | tRNA | 3,903 | 3,973 | 71 | – | – | – |
| tRNA-Met | tRNA | 3,975 | 4,043 | 69 | – | – | + |
| ND2 | gene | 4,044 | 5,088 | 1,045 | ATG | U | + |
| tRNA-Trp | tRNA | 5,089 | 5,169 | 81 | – | – | + |
| tRNA-Ala | tRNA | 5,161 | 5,229 | 69 | – | – | – |
| tRNA-Asn | tRNA | 5,231 | 5,303 | 73 | – | – | – |
| tRNA-Cys | tRNA | 5,335 | 5,401 | 67 | – | – | – |
| tRNA-Tyr | tRNA | 5,402 | 5,471 | 70 | – | – | – |
| COX1 | gene | 5,473 | 7,023 | 1,551 | GTG | UAA | + |
| tRNA-Ser | tRNA | 7,024 | 7,094 | 71 | – | – | – |
| tRNA-Asp | tRNA | 7,097 | 7,166 | 70 | – | – | + |
| COX2 | gene | 7,175 | 7,865 | 691 | ATG | U | + |
| tRNA-Lys | tRNA | 7,866 | 7, 941 | 76 | – | – | + |
| ATP8 | gene | 7,943 | 8,107 | 165 | ATG | UAG | + |
| ATP6 | gene | 8,101 | 8,784 | 684 | ATG | UAA | + |
| COX3 | gene | 8,784 | 9,567 | 784 | ATG | U | + |
| tRNA-Gly | tRNA | 9,568 | 9,638 | 71 | – | – | + |
| ND3 | gene | 9,639 | 9,987 | 349 | ATG | U | + |
| tRNA-Arg | tRNA | 9,988 | 10,057 | 70 | – | – | + |
| ND4L | gene | 10,058 | 10,354 | 275 | ATG | UAA | + |
| ND4 | gene | 10,348 | 11,729 | 1,382 | ATG | UA | + |
| tRNA-His | tRNA | 11,730 | 11,798 | 69 | – | – | + |
| tRNA-Ser | tRNA | 11,799 | 11,867 | 69 | – | – | + |
| tRNA-Leu | tRNA | 11,869 | 11,941 | 73 | – | – | + |
| ND5 | gene | 11,942 | 13,774 | 1,833 | ATG | UAA | + |
| ND6 | gene | 13,771 | 14,292 | 522 | ATG | UAG | – |
| tRNA-Glu | tRNA | 14,293 | 14,361 | 69 | – | – | – |
| CYTB | gene | 14,367 | 15,507 | 1,141 | ATG | U | + |
| tRNA-Thr | tRNA | 15,508 | 15,581 | 74 | – | – | + |
| tRNA-Pro | tRNA | 15,581 | 15,650 | 70 | – | – | – |
| Control region | | 15,651 | 16,562 | 912 | – | – | + |

conducted by using the earliest fossil record data of *Tanakia* lanceolata (5 million years ago [MYA]) and Acheilognathidae (25 MYA) (Tomoda et al., 1977; Yasuno, 1984). The node supporting values of the consensus tree were estimated based on the posterior probabilities. Effective sample sizes was checked to determine if exceeded 200 and the MCMC parameters joined on the equivalent stationary point under a unimodal distribution.

The entire procedure of this study was approved by the Yeungnam University Institutional Animal Care and Use Committee (Protocol # 2020-035). Since the two species used here have not been assigned as legally protected species, no permits were required to collect them.

## RESULTS AND DISCUSSION

The complete mitochondrial genomes of *T. latimarginata*

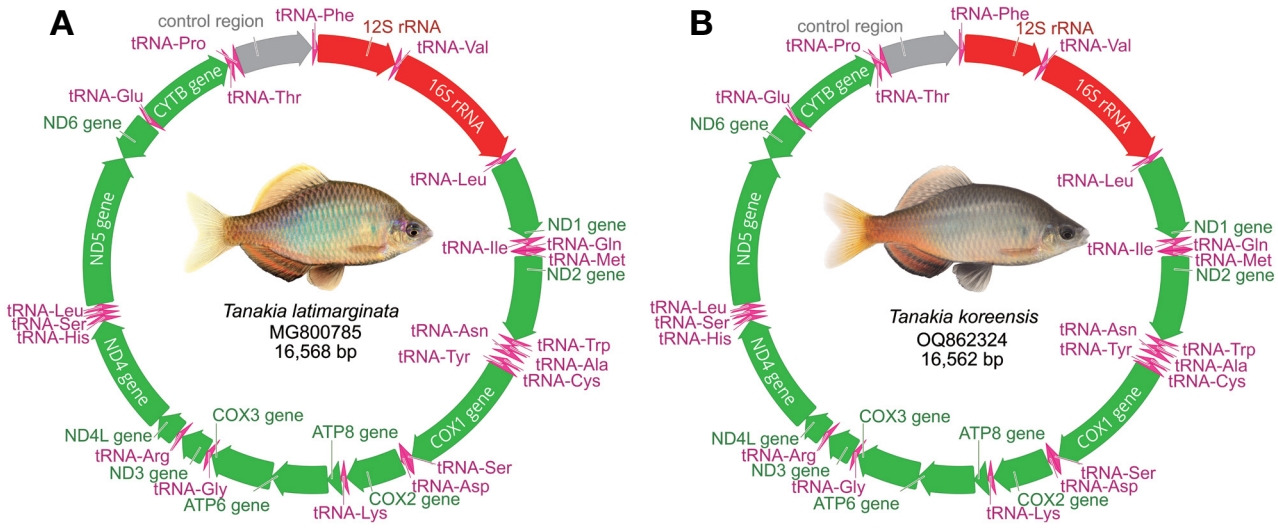


**Fig. 1.** The mitochondrial genomic arrangement of *Tanakia latimarginata* (A) and *T. koreensis* (B). Please refer to Table 2 and Table 3 for the detailed structure of the genome and locations of genes. The photos of the two species were taken by Hyung-Bae Jeon, the first author.

(16,568 bp) and *T. koreensis* (16,562 bp) were successfully characterized and deposited in GenBank under the accession numbers of MG800785 (BioProject: PRJNA1005220; BioSample: SAMN38816162) and OQ862324 (BioProject: PRJNA1005218; BioSample: SAMN38816157), respectively. Both genomes exhibit the canonical vertebrate mitochondrial gene complement, with 13 protein-coding genes (ATP6, ATP8, COX1−3, CYTB, ND1−6, and ND4L), two ribosomal RNA (12S and 16S), 22 tRNA and one control region (Tables 2, 3, Fig. 1). The gene arrangement of these two genomes (Fig. 1) was identical to those observed in other acheilognathid species (Jeon et al., 2016). The overall genome organization indicates a high degree of structural conservation between the two species, reflecting their close evolutionary relationship (Fig. 1). Single nucleotide polymorphisms between these two species were found in the 12S, 16S, ND2, COX1, COX2, CYTB, and control region. InDel sites were found in the 16S and control region.

Despite this structural similarity, phylogenetic analyses based on complete mitogenomes clearly distinguished the two species (Fig. 2). *Tanakia latimarginata* was recovered as sister to the clade comprising *T. koreensis* and *T. signifer*, a relationship congruent with previous molecular studies and general patterns of morphological similarity within *Tanakia* (Jeon et al., 2018). This result contrasts with earlier reports suggesting a closer affinity between *T. latimarginata* and *T. lanceolata* (Kim et al., 2014), highlighting the importance of complete and accurately identified mitogenomes for resolving such discrepancies.

Notably, our phylogenetic reconstruction revealed multiple cases in which previously deposited mitogenomic sequences were placed in positions incompatible with current taxonomic classifications (see also Jeon et al., 2018; Li et al., 2018) (Fig. 2). These inconsistencies were observed not only within *Tanakia* but also across other acheilognathid genera (Fig. 2), indicating that erroneous or misidentified sequences remain prevalent in public databases. For example, a mitogenome of *Rhodeus sinensis* (KF533721) was embedded within the clade *R. ocellatus* complex, while being separated from the clade of other *R. sinensis* (DQ155662 and EF483937) (Fig. 2). A mitogenome of *R. ocellatus* (KT004415) was embedded in the clade of different genus, *Acheilognathus*, not in the clade of congeneric species (Fig. 2). Such errors are particularly problematic for comparative studies and eDNA-based monitoring, where reference accuracy is paramount.

In addition, putative heteroplasmic sites were detected in the ND2 and ND6 regions of the *T. latimarginata* mitogenome (data not shown). While these variants were consistently observed across repeated sequencing, further population-level sampling will be required to determine whether heteroplasmy is a common feature of this species or a population-specific phenomenon.

Overall, the comparative mitogenomic analysis of *T. latimarginata* and *T. koreensis* presented here reinforces their close yet distinct evolutionary identities and underscores the necessity of curated reference genomes. By anchoring taxonomic inference to accurately identified and complete mitochondrial sequences, this study provides a more reliable framework for future phylogenetic, taxonomic, and conservation research on Acheilognathidae.

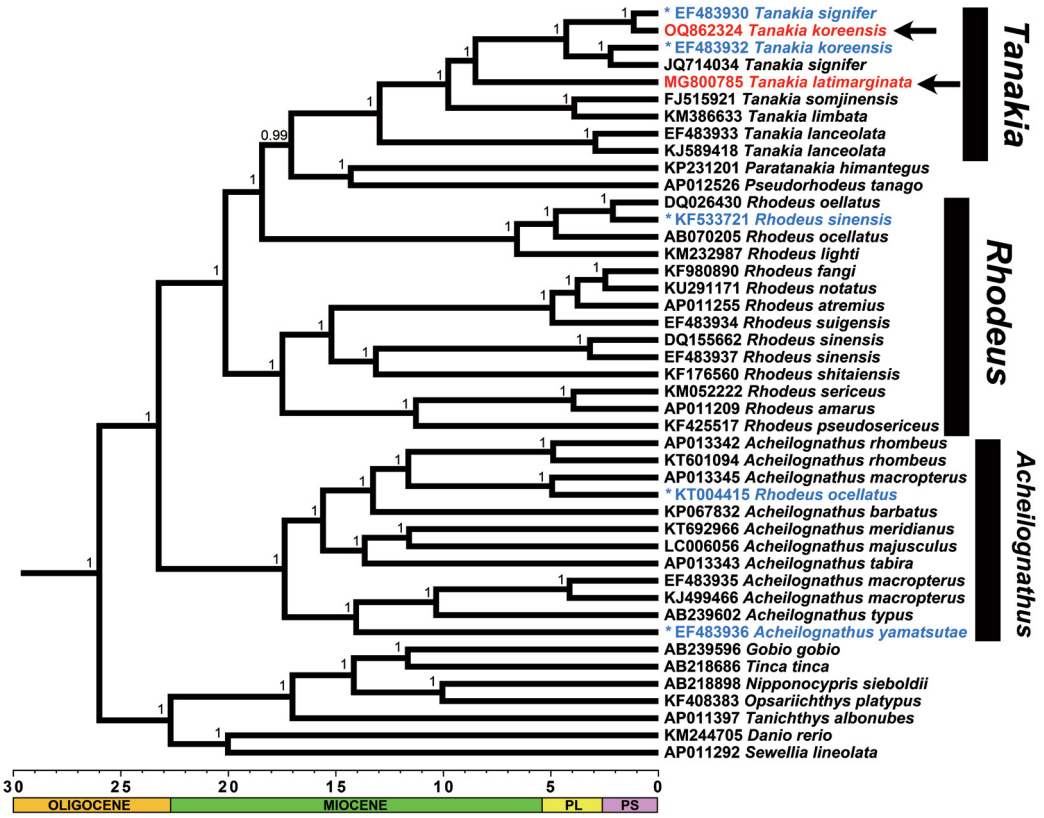


**Fig. 2.** The phylogenetic relationship and the divergence time estimation of acheilognathid species. Arrows indicate the mitogenome of *Tanakia koreensis* and *T. latimarginata* derived from the present study. The taxa with an asterisk (in blue) denote the putative erroneous mitochondrial genome, based on the phylogenetic clustering pattern and current classification system. Integers on the scale bar indicate million years.

## ORCID

Hyung-Bae Jeon: https://orcid.org/0000-0002-0819-5004
Hari Won: https://orcid.org/0009-0002-3453-9242
Ho Young Suk: https://orcid.org/0000-0002-5338-3362

## CONFLICTS OF INTEREST

Ho Young Suk, a contributing editor of the Animal Systematics, Evolution and Diversity, was not involved in the editorial evaluation or decision to publish this article. All remaining authors have declared no conflicts of interest.

## ACKNOWLEDGMENTS

This research was supported by the Regional Innovation System & Education (RISE) program through the Gyeongbuk RISE CENTER, funded by the Ministry of Education (MOE) and the Gyeongsangbuk-do, Republic of Korea (2025-RISE-15-115). We would like to express our deepest gratitude to Hyunji Kim, who played a significant practical role in the experimental process of this study.

## REFERENCES

Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A, Heled J, Jones G, Kühnert D, De Maio N, Matschiner M, Mendes FK, Müller NF, Ogilvie HA, du Plessis L, Popinga A, Rambaut A, Rasmussen D, Siveroni I, Suchard MA, Wu CH, Xie D, Zhang C, Stadler T, Drummond AJ, 2019. BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis. PLoS Computational Biology, 15:e1006650. https://doi.org/10.1371/journal.pcbi.1006650

Chang CH, Li F, Shao KT, Lin YS, Morosawa T, Kim S, Koo H, Kim W, Lee JS, He S, Smith C, Reichard M, Miya M, Sado T, Uehara K, Lavoué S, Chen WJ, Mayden RL, 2014. Phylogenetic relationships of Acheilognathidae (Cypriniformes: Cyprinoidea) as revealed from evidence of both nuclear and mitochondrial gene sequence variation: evidence for neces-

sary taxonomic revision in the family and the identification of cryptic species. Molecular Phylogenetics and Evolution, 81:182-194. https://doi.org/10.1016/j.ympev.2014.08.026

Choi HK, Lee HJ, 2024. Host size matters for reproduction: evolution of spawning preference and female reproductive phenotypes in mussel-symbiotic freshwater bitterling fishes. Ecology and Evolution, 14:e11142. https://doi.org/10.1002/ece3.11142

Choi HK, Lee HJ, 2025. Genetic diversity and phylogeographic structure of the endangered Korean Bitterling (*Tanakia signifer*) and its symbiotic mussel (*Nodularia breviconcha*), based on mitochondrial DNA variation: implications for conservation. Conservation Genetics, 26:513-528. https://doi.org/10.1007/s10592-025-01685-3

Hwang DS, Byeon HK, Lee JS, 2014. Complete mitochondrial genome of the freshwater fish, *Acheilognathus somjinensis* (Cypriniformes, Cyprinidae). Mitochondrial DNA, 25:13-14. https://doi.org/10.3109/19401736.2013.775266

Hwang DS, Lee WO, Lee JS, 2013. Complete mitochondrial genome of the Korean bitterling *Acheilognathus koreensis* (Cypriniformes; Cyprinidae). Mitochondrial DNA, 24:414-415. https://doi.org/10.3109/19401736.2013.766178

Hwang DY, Kim SK, Ryu SH, Hwang UW, 2012. Complete mitochondrial genome of *Acheilognathus* signifer (Cypriniformes, Cyprinidae): comparison of light-strand replication origins from six *Acheilognathus* species. Mitochondrial DNA, 23:306-307. https://doi.org/10.3109/19401736.2012.683180

Janzen FH, Crampton WGR, Lovejoy NR, 2022. A new taxonomist-curated reference library of DNA barcodes for Neotropical electric fish (Teleostei: Gymnotiformes). Zoological Journal of the Linnean Society, 196:1718-1742. https://doi.org/10.1093/zoolinnean/zlac039

Jeon HB, Anderson D, Won H, Lim H, Suk HY, 2018. Taxonomic characterization of *Tanakia* species (Acheilognathidae) using DNA barcoding analyses. Mitochondrial DNA Part A, 29: 964-973. https://doi.org/10.1080/24701394.2017.1398746

Jeon HB, Lee YJ, Lim HJ, Cha SH, Suk HY, 2016. Complete mitochondrial genome of endangered *Rhodeus pseudosericeus* and its implications for the reconstruction of phylogenetic relationship among Acheilognathinae species. Mitochondrial DNA Part B, 1:312-314. https://doi.org/10.1080/23802359.2016.1172036

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A, 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28:1647-1649. https://doi.org/10.1093/bioinformatics/bts199

Kim D, Jeon HB, Suk HY, 2014. *Tanakia latimarginata*, a new species of bitterling from the Nakdong River, South Korea (Teleostei: Cyprinidae). Ichthyological Exploration of Freshwaters, 25:59-68.

Kim IS, Kim CH, 1990. A new acheilognathine fish *Acheilognathus koreensis* (Pisces, Cyprinidae) from Korea. Korean Journal of Ichthyology, 2:47-52.

Li X, Shen X, Chen X, Xiang D, Murphy RW, Shen Y, 2018. Detection of potential problematic *Cytb* gene sequences of fishes in GenBank. Frontiers in Genetics, 9:30. https://doi.org/10.3389/fgene.2018.00030

Tomoda Y, Kodera H, Nakajima T, Yasuno T, 1977. Fossil freshwater fishes from Japan. Memoirs of the Geological Society of Japan, 14:221-243.

Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG, 2012. Primer3: new capabilities and interfaces. Nucleic Acids Research, 40:e115. https://doi.org/10.1093/nar/gks596

Yasuno K, 1984. Fossil pharyngeal teeth of the Rhodeinae fish from the Miocene Katabira formation of the Kani Group, Gifu Prefecture, Japan. Bulletin of the Mizunami Fossil Museum, 11:101-105.

Received December 28, 2025
First revised January 8, 2026
Second revised January 13, 2026
Accepted January 13, 2026

ASED http://e-ASED.org
Animal Systematics, Evolution and Diversity

Anim. Syst. Evol. Divers. Vol. 42, No. 1: 100-110, January 2026
https://doi.org/10.5635/ASED.2026.42.1.004

Special issue

# Characterization of Two *Urostomides* Species (Ciliophora, Armophorea, Metopida) Newly Recorded from Jindo Island, Korea

Nanda Dwi Kristanti†, Novia Cahyani†, Quoc Dung Nguyen, Mann Kyoon Shin*

Department of Biological Sciences, University of Ulsan, Ulsan 44610, Korea

## ABSTRACT

Research on anaerobic ciliates has expanded significantly around the world in recent decades, and Korea is no exception. Recent investigations on the Korean Peninsula have revealed previously undocumented diversity within this group. This study reports *Urostomides bacillatus* (Levander, 1894) Bourland et al., 2017 from Jindo Island, and *Urostomides striatus* (McMurrich, 1884) Jankowski, 1964 from Jindo Island and Sinan-gun, South Korea. *Urostomides bacillatus* has an obpyriform body shape with a rounded or obliquely truncated posterior end. Its adoral zone forms an angle of less than 180°. Notably, the Korean population of *U. bacillatus* displays a diplostichomonad paroral membrane, a characteristic that has not previously been reported for this species. *Urostomides striatus*, the type species of the genus, is primarily distinguished by its obpyriform body that tapers into a distinct, tail-like posterior end. It possesses prominent extrusomes and a paroral membrane of either stichomonad or diplostichomonad type, and an adoral zone forming an angle of approximately 180°. Morphological characteristics and small subunit ribosomal DNA analyses reveal population-level variation in both Korean populations of *U. bacillatus* and *U. striatus*, providing insight into their morphological and genetic diversity.

**Keywords:** anaerobes, Apometopidae, freshwater, morphology, phylogeny

## INTRODUCTION

Over the past few decades, research on anaerobic ciliates has made remarkable progress, driven by the continual discoveries of new taxa and advances in both morphological and molecular understanding. Major progress includes integrative studies combining morphological and phylogenetic analyses, investigations of morphogenesis, phylogenomic reconstructions, and explorations of symbiotic relationships (Bourland et al., 2014, 2017a, 2017b, 2018, 2020; Vd'ačný and Foissner, 2017, 2019; Campello-Nunes et al., 2020; Rotterová et al., 2020, 2022, 2024; Chen et al., 2022; Méndez-Sánchez et al., 2022, 2024; Fokin et al., 2023; Li et al., 2023; Pomahač et al. 2023, 2024; Schrecengost et al., 2024; Zhang et al., 2024; Feng et al., 2025a, 2025b).

In Korea, research on anaerobic ciliates has also expanded in recent years, leading to several new records and descriptions (He and Choi, 2015; Omar and Jung, 2022; Nguyen and Shin, 2024; Nguyen et al., 2024, 2026; Cahyani et al., 2025; Kristanti et al., 2025a, 2025b). However, studies focusing on metopid ciliates, a taxonomically diverse and morphologically intricate lineage, remain limited. Although over 90 species have been described globally, only 14 have been recorded from Korea to date, including two *Brachonella* Jankowski, 1964, four *Bothrostoma* Stokes, 1887, six *Metopus* Clarépede and Lachmann, 1858, and two *Tropidoatractus* Levander, 1894 (Bourland and Wendell, 2014; Bourland et al., 2014, 2017a, 2017b, 2018, 2020, 2022, 2024; He and Choi, 2015; Rotterová et al., 2018, 2020; Zhuang et al., 2021, 2022, 2025; Méndez-Sánchez et al., 2022; Omar and Jung, 2022; Feng et al., 2024, 2025b; Nguyen and Shin, 2024; Nguyen et al., 2024, 2026; Pomahač et al., 2024; Kristanti et al., 2025a, 2025b). This knowledge gap indicates metopid diversity in Korean remains underexplored, especially within lineages recently undergone major systematic revision, such as the genus *Urostomides*.

The genus *Urostomides* was established by Jankowski (1964), with *U. striatus* (McMurrich, 1994) Jankowski, 1964

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.

Nanda Dwi Kristanti and Novia Cahyani contributed equally to this work.
*To whom correspondence should be addressed
Tel: 82-52-259-2396, Fax: 82-52-259-1694
E-mail: mkshin@ulsan.ac.kr

eISSN 2234-8190

Copyright © The Korean Society of Systematic Zoology

designated as the type species. Subsequent studies have revealed that taxonomy of this group is more complex than previously assumed. Bourland et al. (2017a) provided the most comprehensive morphological and molecular investigation of seven *Urostomides* species, clarified the systematic position of the genus by recognizing it as the senior synonym of *Apometopus* Foissner, 2016, and transferring it to the family Apometopidae Foissner, 2016. This reassignment is supported by distinct morphological characteristics, particularly the presence of four rows of perizonal stripes (PS), and robust phylogenetic relationships (Jankowski, 2007; Foissner, 2016; Bourland et al., 2017a; Zhuang et al., 2021; Feng et al., 2025b). Furthermore, the recent discovery of a diplostichomonad within this lineage suggests that higher-level systematic relationship among metopids require reevaluation, although detailed explanations for this affiliation are still lacking (Bourland et al., 2024; Feng et al., 2025a, 2025b; Kristanti et al., 2025a, 2025b).

This study reports the first occurrence of two species of *Urostomides* from Korea, thereby expanding the known geographical distribution of the genus. Comprehensive morphological characterization, supported by small subunit ribosomal DNA (SSU rDNA) analyses, provides integrative evidence for their identification and phylogenetic position within Metopida. These findings contribute to a broader understanding of metopid ciliate diversity and advance the taxonomic framework for this group.

## MATERIALS AND METHODS

### Sample collection and morphological observations

*Urostomides bacillatus* was collected from a reservoir in Yeonju-ri, Uisin-myeon, Jindo-gun, Jeollanam-do, Korea (34°25′30.6″N, 126°19′06.5″E), in April 2024. *Urostomides striatus* was collected from a reservoir in Sinjang-ri, Aphae-eup, Sinan-gun, Jeollanam-do, Korea (34°50′09.2″N, 126°20′59.6″E), in October 2021 and a reservoir in Sopo-ri, Jisan-myeon, Jindo-gun, Jeollanam-do, Korea (34°28′52.8″N, 126°11′39.5″E), in July 2024. Both samples were treated using the same protocol. Samples containing sediments and water were collected in 1 L bottles, transferred to the laboratory, and supplemented with several rice grains to promote bacterial growth. The bottles were then tightly sealed and incubated at room temperature for one to two weeks.

Live ciliates were observed using bright field (40–200×) and differential interference contrast (400–1,000×) Axio Imager A1 microscope (Carl Zeiss, Oberkochen, Germany). The ciliature and nuclear apparatus were revealed using the protargol impregnation method following Wilbert (1975), with protargol powder made in-house (Pan et al., 2013). In vivo measurements were performed at 40–1,000× magnification, while counts and measurements on protargol-impregnated specimens were conducted at 1,000× magnification. Quantifications and measurements were performed using the calibrated Carl Zeiss Axiovision 4.8 software package (Carl Zeiss Microscopy, LLC). Photomicrographs were captured using a CCD camera (AxioCam MRc, Carl Zeiss), and line drawings were prepared using Adobe Illustrator 2022.

### DNA extraction, polymerase chain reaction amplification, and sequencing

Three cells of each species were isolated using a glass micropipette and washed three times with distilled water. Each cell was then transferred to an EP tube containing the minimum amount of water necessary. Genomic DNA was extracted using a RED Extract-N-Amp Tissue PCR Kit (Sigma, St. Louis, MO, USA) according to the manufacturer's protocol. Polymerase chain reaction (PCR) amplification was performed using TaKaRa ExTaq polymerase (TaKaRa Biomedicals, Japan). Additional PCR parameters and sequencing primers were referenced from Kristanti et al. (2025a). Sequence assembly and contig generation were performed using Geneious Prime (ver. 2019.0.4).

### Phylogenetic analyses

A dataset comprising two newly obtained *Urostomides* and 77 SSU rDNA sequences retrieved from the GenBank database was assembled. Seven clevelandellids species were included as an outgroup. Sequence alignment was conducted using the G-INS-i algorithm on the MAFFT server (https://mafft.cbrc.jp/alignment/server/index.html) (Katoh et al., 2019). These alignments were then manually refined in MEGA11 (Tamura et al., 2021). The final dataset contained 1,676 unambiguously aligned nucleotide positions and is available upon request. Phylogenetic analyses were inferred using maximum likelihood (ML) and Bayesian inference (BI). The ML analysis was performed in IQ-TREE v.2.4.0. (Minh et al., 2020), using the GTR + F + I + R4 model, selected by ModelFinder implemented in IQ-TREE according to the Akaike information criterion (AIC) (Kalyaanamoorthy et al., 2017). Node support was assessed using 1,000 ultrafast bootstrap replicates. BI analysis was performed in MrBayes 3.2.7a (Ronquist et al., 2012), using the GTR + I + G model, which was selected using the AIC in MrModeltest ver. 2.4 (Nylander, 2004). Markov chain Monte Carlo simulations were run for five million generations, sampling every 100 generations, until the mean standard deviation of split frequencies was less than 0.01. The first 25% of the sampled trees were discarded as burn-in. Phylogenetic tree topologies were visualized using FigTree v.1.4.4 (Rambaut, 2018). Genetic distances were estimated using the Kimura 2-parameter model implemented in MEGA11 (Kimura, 1980; Tamura et al., 2021).

## RESULTS

Class Armophorea Lynn, 2004
Order Metopida Jankowski, 1980
Family Apometopidae Foissner, 2016
[1]*Genus *Urostomides* Jankowski, 1964

[2]****Urostomides bacillatus*** **(Levander, 1894) Bourland et al., 2017 (Table 1, Fig. 1)**

*Metopus bacillatus* Levander, 1894: figs. 12–13; Kahl, 1932: 420–421, fig. 70 (21–22).

*Urostomides bacillatus* (Levander, 1894), Bourland et al., 2017: figs. 7–8, table 5.

**Material examined.** Sediments collected from the reservoir in Uisin-myeon, Jindo-gun, Jeollanam-do, Korea (34°25′30.6″N, 126°19′06.5″E) in April 2024.

**Voucher specimens.** Two slides of protargol-stained voucher specimens (registration number: UBL20251001001, UBL 20251001002) are deposited in the Biodiversity Laboratory, University of Ulsan, Ulsan, Korea.

**Descriptions.** Body size *in vivo* ranges from 65–95 × 50–65 μm, with body length-to-width ratio about 1.5 : 1, and 70–105 × 50–80 μm, body length-to-width ratio about 1.4 : 1 after protargol impregnation (Fig. 1A–D, J–L). Body obpyriform with a broadly rounded preoral dome occupying 60–70% of body length (Fig. 1A–D, J–L). Right margin convex; left anterior margin slightly overhangs left side of the body (Fig. 1A, B, D, J, K). Dome brims broad, overhanging entire adoral zone (Fig. 1A–D, K, L). Posterior region triangular in cross section, end rounded or obliquely truncated (Fig. 1A–D, J). Macronucleus mostly globular, 16–20 μm across *in vivo*; 20–39 × 16–33 μm after protargol impregnation (Fig. 1A, B, D, I, K, L). Micronucleus broadly ellipsoidal, 5–7 μm in both conditions (Fig. 1A, I). Contractile vacuole subterminal, 18–24 μm in diameter when fully dilated (Fig. 1A, D, J). Extrusomes narrow, rod-shaped, 2.2–2.8 × 0.5–0.9 μm, colorless, and form a refractive fringe along the cell periphery or 7–8 discontinuous interkinetal rows on the cell surface (Fig. 1E, G). Cytoplasm transparent and contains scattered granules approximately 3 μm in diameter (Fig. 1E). Food vacuoles spherical, 10–15 μm across (Fig. 1D, E).

Somatic kineties arranged in 20–25 longitudinal rows; 7–8 of these extending anteriorly into preoral dome as dome kineties (DK) (Fig. 1B, C, K, L). Perizonal stripe of four rows (PS1–PS4); PS1–PS3 closely spaced and PS4 separated by a distinct gap; PS not forming false kineties (Fig. 1B, C, K, L). Somatic cilia 10–12 μm long; perizonal stripe cilia slightly longer (12–15 μm) (Fig. 1A). Caudal cilia 3–5 in number, elongated, 25–30 μm in length (Fig. 1A, J).

**Table 1.** Morphometric characteristics of *Urostomides bacillatus* based on protargol-impregnated specimens

| Characters[a] | Mean | M | SD | CV | Min | Max | n |
|---|---|---|---|---|---|---|---|
| Body, length | 88 | 86 | 10 | 11 | 70 | 105 | 14 |
| Body, width | 64 | 63 | 8 | 12 | 50 | 80 | 14 |
| Body, length-width, ratio | 1.4 | 1.4 | 0.1 | 4.2 | 1.3 | 1.5 | 14 |
| Anterior cell end to posterior end of adoral zone, distance | 63 | 63 | 7 | 11 | 50 | 73 | 14 |
| Anterior cell end to posterior end of adoral zone, % body length | 71 | 72 | 2 | 3 | 67 | 74 | 14 |
| Anterior cell end to anterior end of adoral zone, distance | 43 | 42 | 6 | 15 | 34 | 55 | 14 |
| Anterior cell end to anterior end of adoral zone, % body length | 49 | 49 | 5 | 10 | 40 | 55 | 14 |
| Anterior cell end to posterior end to macronucleus, distance | 54 | 53 | 7 | 12 | 42 | 66 | 14 |
| Anterior cell end to posterior end of macronucleus, % body length | 61 | 63 | 4 | 7 | 50 | 65 | 14 |
| Macronucleus, length | 27 | 26 | 5 | 18 | 20 | 39 | 14 |
| Macronucleus, width | 25 | 26 | 5 | 19 | 16 | 33 | 14 |
| Micronucleus, diameter | 6 | 6 | 1 | 14 | 5 | 7 | 3 |
| Adoral membranelles, number | 37 | 36 | 2 | 7 | 33 | 42 | 14 |
| Longest adoral membranelles, width | 5 | 5 | 0 | 9 | 5 | 6 | 14 |
| Somatic kineties, number | 23 | 22 | 1 | 7 | 20 | 25 | 14 |
| Preoral dome kinety, number | 8 | 8 | 0 | 3 | 7 | 8 | 14 |
| Perizonal stripe rows, number | 4 | 4 | 0 | 0 | 4 | 4 | 14 |
| Paroral membrane 1, length | 23 | 24 | 3 | 11 | 17 | 26 | 14 |
| Paroral membrane 2, length | 15 | 16 | 2 | 14 | 12 | 19 | 14 |

Mean, arithmetic mean; M, median; SD, standard deviation; CV, coefficient of variation (%); Min, minimum value; Max, maximum value; n, number of individuals investigated.
[a]All measurements of size, length, and width in μm. All data are based on the protargol-impregnated specimens.

Korean name: [1]*미구(尾口)섬모충속, [2]*막대미구섬모충

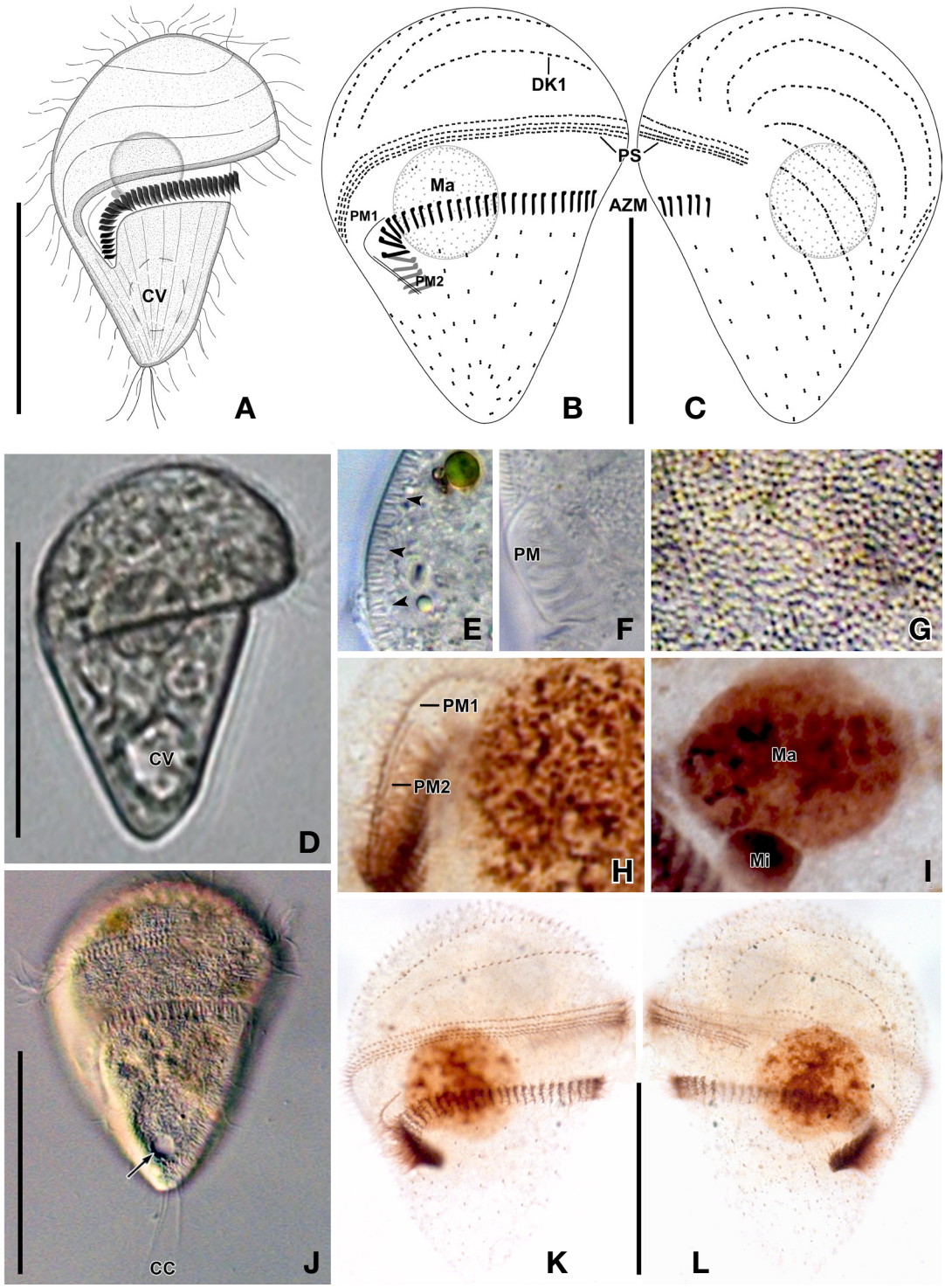


**Fig. 1.** *Urostomides bacillatus*. Line drawings (A–C) and micrographs from live (D–G, J) and protargol-impregnated (H, I, K, L) specimens. A, D, Ventral view of a representative specimen, showing a conspicuous preoral dome, a rounded posterior end, a subterminal contractile vacuole, and four elongated caudal cilia; B, C, Ventral and dorsal views of the holotype specimen detailing the somatic ciliatures, the adoral zone, and the nuclear apparatus; E, G, Extrusomes form a refractile fringe along the cell periphery (black arrowheads) (E) and interkinetal rows on the cell surface (G); F, H, Paroral membrane from a live (F) and a protargol-impregnated (H) specimen; I, Macronucleus and micronucleus; J, Elongated caudal cilia at the posterior end of the cell, black arrow denotes the contractile vacuole; K, L, Ventral and dorsal views of the holotype specimen. AZM, adoral zone of membranelles; CC, caudal cilia; CV, contractile vacuole; DK, preoral dome kinety; Ma, macronucleus; Mi, micronucleus; PM, paroral membrane; PS, perizonal stripe. Scale bars: A–D, J–L = 50 μm.

Adoral zone consist of 33–42 membranelles, originating at right ventral margin, extending obliquely to left of the dorsal surface (Fig. 1A–C, K, L). Longest adoral membranelles measure 5–7 μm (Fig. 1B, K). Proximal portion of adoral zone performs <180° anticlockwise turn as it descends into the buccal cavity (Fig. 1B, K). Paroral membrane diplostichomonad; longer PM (PM1) 17–26 μm long, shorter PM (PM2) 12–19 μm long (Fig. 1F, H).

**Distribution.** Canada, Germany, USA, Korea (present study).

**Remarks.** The Korean population of *U. bacillatus* is unequivocally identified based on the morphological diagnoses of previous studies (Levander, 1894; Kahl, 1932; Bourland et al., 2017a). The present population displays a broadly ellipsoidal body in cross-section with a distinctly rounded preoral dome; a posterior portion roughly triangular, notably rounded or truncated terminus does not coalesce into tail-like form, from which extend elongated caudal cilia; about 21 somatic ciliary rows; perizonal stripe not forming false kineties; adoral zone encircle <180° turn around long axis. Collectively, these morphological characters affirm the identity of Korean population as *U. bacillatus* (Levander, 1894) Bourland et al., 2017 (Levander, 1894; Kahl, 1932; Bourland et al., 2017a). Nonetheless, the Korean population exhibits a diplostichomonad paroral membrane, in contrast to the stichomonad pattern reported by Bourland et al. (2017a). This variation is plausibly attributable to methodological artifacts arising from the staining procedure, as the narrow gap between PM1 and PM2 often results in PM2 being obscured under conditions of either excessive or insufficient impregnation.

*Urostomides bacillatus* closely resembles *U. minimus* (Kahl, 1927) Feng et al., 2025 and *U. pullus* (Kahl, 1927) Bourland et al., 2017 in that it possesses an adoral zone that encircles less than 180°. However, *U. bacillatus* can be distinctly distinguished by its larger body size, higher density of adoral membranelles, and the absence of false kineties formed by perizonal stripes (Bourland et al., 2017a; Feng et al., 2025b). Moreover, *U. bacillatus* resembles *U. denarius* (Kahl, 1927) Bourland et al., 2017, *U. campanula* (Kahl, 1932) Bourland et al., 2017, and *U. caducus* (Kahl, 1927) Bourland et al., 2017 due to the absence of tail-like structure or spine-like projection at the posterior end. However, *U. bacillatus* can be distinguished by its adoral zone, which encircles less than 180° (Kahl, 1932; Bourland et al., 2017a). Lastly, *U. bacillatus* is distinguished by its rounded or truncated posterior end, which lacks tail-like structure observed in *U. darwini* (Kahl, 1927) Bourland et al., 2017 and the spine-like projection characteristic of *U. spinosus* Zhuang et al., 2021 (Kahl, 1932; Bourland et al., 2017a; Zhuang et al., 2021).

### [1]*_Urostomides striatus_ (McMurrich, 1884) Jankowski, 1964 (Table 2, Fig. 2)

*Metopus striatus*, McMurrich, 1884: 830; Kahl, 1927: 121–203, fig. 17i; 1932: 421, fig. 70 (24); Foissner, 1980: 5, fig. 10a, b; Foissner et al., 1992: figs. 45–62, 88–93, 97, 98.

**Table 2.** Morphometric characteristics of *Urostomides striatus* based on protargol-impregnated specimens

| Characters[a] | Mean | M | SD | CV | Min | Max | n |
|---|---|---|---|---|---|---|---|
| Body, length | 65 | 60 | 14.1 | 21.8 | 50 | 110 | 22 |
| Body, width | 45 | 40 | 10.4 | 23.2 | 35 | 75 | 22 |
| Body, length-width, ratio | 1.4 | 1.5 | 0.1 | 7.2 | 1.3 | 1.6 | 22 |
| Anterior cell end to posterior end of adoral zone, distance | 44 | 42 | 10.6 | 24.3 | 35 | 75 | 22 |
| Anterior cell end to posterior end of adoral zone, % body length | 68 | 68 | 3.4 | 5.1 | 63 | 75 | 22 |
| Anterior cell end to anterior end of adoral zone, distance | 23 | 21 | 7.6 | 33.2 | 16 | 45 | 22 |
| Anterior cell end to anterior end of adoral zone, % body length | 35 | 33 | 4.3 | 12.2 | 29 | 45 | 22 |
| Anterior cell end to posterior end to macronucleus, distance | 30 | 28 | 11.0 | 36.4 | 21 | 70 | 22 |
| Anterior cell end to posterior end of macronucleus, % body length | 46 | 45 | 5.5 | 11.9 | 40 | 64 | 22 |
| Macronucleus, diameter | 17 | 15 | 8.3 | 47.7 | 13 | 35 | 22 |
| Micronucleus, diameter | 5 | 5 | 0.5 | 10.4 | 4 | 6 | 22 |
| Adoral membranelles, number | 35 | 34 | 4.9 | 14.3 | 29 | 52 | 22 |
| Longest adoral membranelles, width | 6 | 6 | 0.6 | 10.6 | 5 | 7 | 22 |
| Somatic kineties, number | 20 | 20 | 2.9 | 14.5 | 17 | 30 | 22 |
| Preoral dome kinety, number | 8 | 8 | 0.6 | 7.7 | 7 | 10 | 22 |
| Perizonal stripe rows, number | 4 | 4 | 0.0 | 0.0 | 4 | 4 | 22 |
| Paroral membrane, length | 19 | 19 | 2.0 | 10.4 | 17 | 25 | 22 |

Mean, arithmetic mean; M, median; SD, standard deviation; CV, coefficient of variation (%); Min, minimum value; Max, maximum value; n, number of individuals investigated.

[a]All measurements of size, length, and width in μm. All data are based on the protargol-impregnated specimens.

Korean name: [1]*무늬미구섬모충

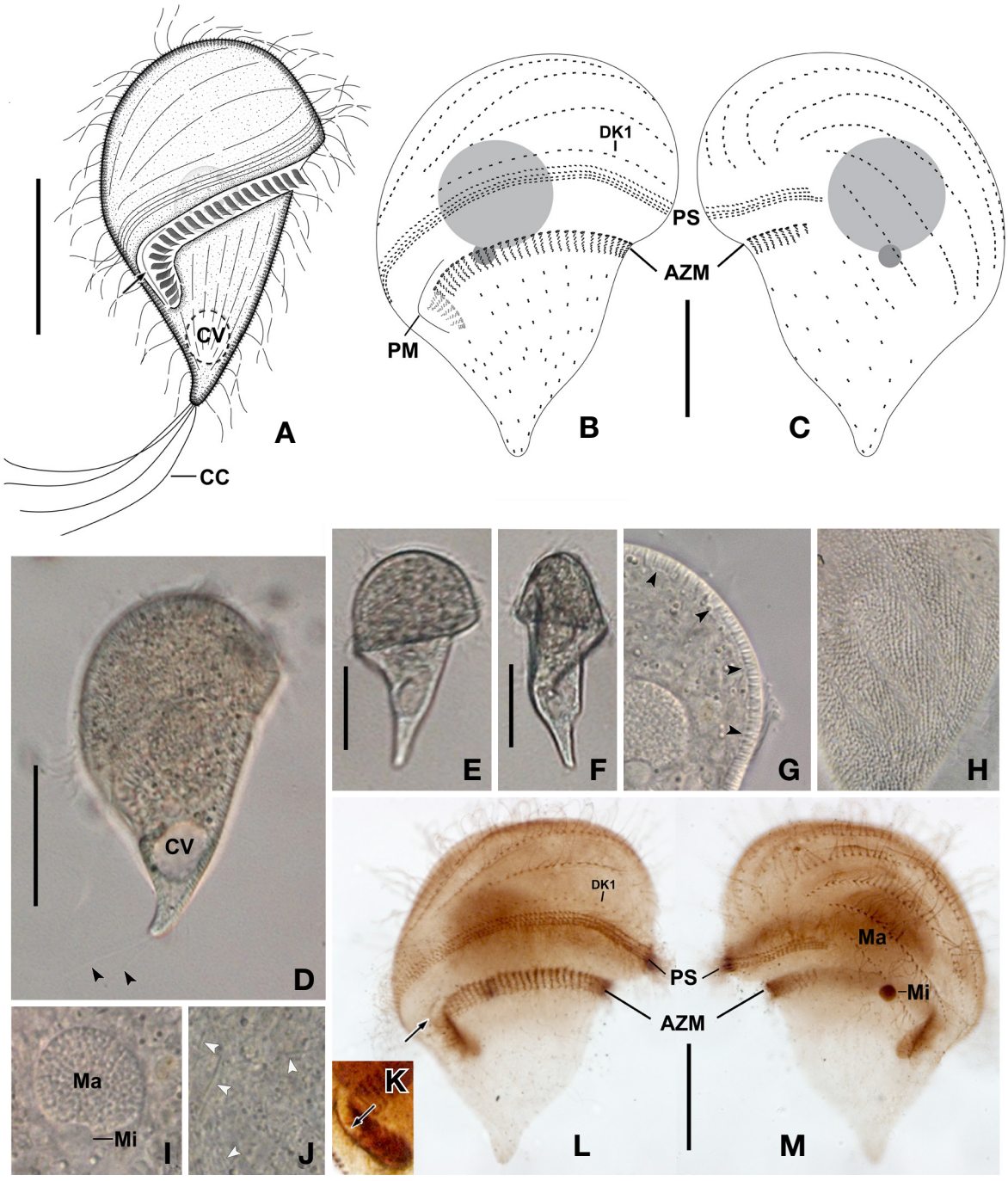


**Fig. 2.** *Urostomides striatus*. Line drawings (A–C) and micrographs from live (D–J), and protargol-impregnated (K–M) specimens. A, Ventral view of a representative specimen showing overall body shape, tail-like posterior end, subterminal contractile vacuole, and four elongated caudal cilia; the arrow denotes the paroral membrane; B, C, Ciliary pattern on the ventral and dorsal side as well as the nuclear apparatus of the holotype specimen; D, Typical body shape *in vivo*, arrowheads indicate elongated caudal cilia; E, F, Images of the same specimen captured at low magnification: ventral (E) and right lateral (F) views showing the preoral dome overhanging the left cell margin, the tail-like posterior end, and the dorsoventral flattening; G, An optical section showing extrusomes forming a peripheral fringe (black arrowheads); H, Extrusomes are densely packed between adjacent kineties on the cell surface; I, Macronucleus and micronucleus *in vivo*; J, Endosymbiotic bacteria scattered throughout the cytoplasm (white arrowheads); K, A detailed view of the paroral membrane (black arrow); L, M, Ciliary pattern on the ventral and dorsal sides of the holotype specimen; black arrow indicates the paroral membrane. AZM, adoral zone of membranelles; CC, caudal cilia; CV, contractile vacuole; DK, preoral dome kinety; Ma, macronucleus; Mi, micronucleus; PM, paroral membrane; PS, perizonal stripe. Scale bars: A–F, L, M = 30 μm.

*Metopus striata*, Stokes, 1888: 1, fig. 11.
*Urostomides striatus* (McMurrich, 1884), Jankowski, 1964: 185–294, figs. 11, 12; Bourland et al., 2017a: 61, fig. 9; Feng et al., 2025b: 203, fig. 7.

**Material examined.** Sediments collected from a reservoir in Sinan-gun, Jeollanam-do, Korea (34°50′09.2″N, 126° 20′59.6″E) in October 2021 and a reservoir in Sopo-ri, Jisan-myeon, Jindo-gun, Jeollanam-do, Korea (34°28′52.8″N, 126° 11′39.5″E), in July 2024.
**Voucher specimens.** Two slides of protargol-stained voucher specimens (registration number: HNIBRPR159, HNIBIPR 160) are deposited in the Honam Institute of Biological Resources, Mokpo, Korea.
**Descriptions.** Body size 60–90 × 40–55 μm (73 × 47 μm on average) *in vivo*, 50–110 × 35–75 μm (60 × 42 μm on average) after protargol impregnation; body length-to-width ratio about 1.6 : 1 and 1.4 : 1, respectively (Fig. 2A–E, L, M). Body semi deltoid to clavate, dorso-ventrally flattened; preoral dome broadly rounded, overhanging left margin, occupying 30–35% of the body length; posterior end tapers into a narrow tail (Fig. 2A, D–F). Single spherical macronucleus located centrally with micronucleus adjacent to it, 13–35 μm and 4–6 μm across, *in vivo*, respectively (Fig. 2A–D, I, M). Contractile vacuole subterminal (Fig. 2A, D, E). Two to four caudal cirri, 35–30 μm long (Fig. 2A, D). Extrusomes highly prominent, oblong shape, 2.4–3 × 0.5–1 μm, form a dense refractive fringe; arranged obliquely on cell surface and scattered in cytoplasm (Fig. 2G, H). Rod-shaped endosymbiont scattered throughout cytoplasm (Fig. 2J).

Somatic ciliature comprises 17–30 rows, including 7–10 preoral DK; DK1 loosely arranged; posterior kineties disorganized (Fig. 2B, C, L, M). Four perizonal stripes rows, equal in length to adoral zone of membranelles, never arranged in a false kinety; PS1–PS3 closely spaced, PS4 more distant; both somatic kineties and PS composed of dikinetids (Fig. 2A–C, L, M). Adoral zone comprises 29–52 membranelles, 35 membranelles on average (Fig. 2B, C, L, M). Paroral membrane stichomonad, 17–25 μm long (Fig. 2B, K).
**Distribution.** Canada, Czech Republic, Chile, China, Germany, Russia, USA, Korea (present study).
**Remarks.** Our *Urostomides striatus* mostly conform to previous descriptions, with an obpyriform body tapering posteriorly into a narrow tail, conspicuous elongated caudal cilia, and dense, prominent extrusomes (McMurrich, 1884; Stokes, 1888; Kahl, 1927, 1932; Jankowski, 1964; Foissner et al., 1992). The Chinese population exhibits variation at the rear, ranging from a sharp point to a tail-like extension, which is likely reflect intraspecific phenotypic plasticity (Feng et al., 2025b). The Korean population differs from the Russian and Chinese populations in that it has fewer caudal cilia (2–4 vs. 5 and 4–5, respectively) (Jankowski, 1964; Feng et al., 2025b), whereas the American and Czech Republic populations have only inconspicuous caudal cilia (Bourland et al., 2017a). Feng et al. (2025b) also reported a diplostichomonad paroral membrane in the Chinese population. However, our protargol-impregnated specimens displayed a stichomonad type, which is consistent with earlier reports. This discrepancy is likely due to the strong impregnation obscuring finer structures.

*Urostomides striatus* resembles *U. bacillatus* in terms of body size and the extrusome prominence, but can be distinguished by its sharply tapered, tail-like posterior end (vs. rounded or obliquely truncated end). This trait also differentiates *U. striatus* from *U. caducus*, *U. campanula*, and *U. pullus*, all of which have rounded or truncated posterior ends. In addition, the adoral zone in *U. striatus* forms a smaller angle than its congeners (~180° vs. ≥360°, ~270°, and ~220°, respectively) (Kahl, 1927; Bourland et al., 2017a).

Compared to *U. denarius* and *U. spinosus*, *U. striatus* is larger in size (50–110 × 35–75 μm vs. 35–57 × 50–41 μm vs. 25–45 × 20–35 μm, respectively), has a differently shaped posterior (sharp to tail-like vs. rounded vs. tapered into three spines, respectively), and a smaller adoral zone configuration (Bourland et al., 2017a; Zhuang et al., 2021). Similarly, *U. striatus* can be distinguished from *U. darwini* by its sharp, tail-like posterior end (vs. narrow tail), fewer adoral membranelles (28–46 vs. 50–63), and a smaller adoral zone angle (~180° vs. ≥360°) (Bourland et al., 2017a). Finally, *U. striatus* differs from *U. minimus* by its tapered posterior end (vs. rounded) and the adoral zone angle (~180° vs. ≤180°) (Feng et al., 2025b). *U. striatus* is also distinct from *U. pelobius* based on both the posterior end morphology (tail-like vs. rounded) and the structure of paroral membrane (single or two rows of monokinetids vs. a single row of dikinetids) (Foissner, 2016).

## Phylogenetic relationships and genetic distinctiveness of Korean *Urostomides*

The 18S ribosomal DNA (rDNA) sequences of Korean populations of *U. bacillatus* (accession number: PX470666) and *U. striatus* (accession number: PX470667) comprise 1,696 bp and 1,712 bp in length, with GC contents of 42% and 43.1%, respectively. Phylogenetic analyses performed using the ML and BI methods yield congruent topologies; therefore, only the ML tree is presented, displaying nodal support values from both methods (Fig. 3). The family Apometopidae recovered as a clade alongside the family Tropidoatractidae, with low to moderate support values (80% ML, 0.62 BI). Apometopidae includes two recognized genera, *Urostomides* and *Cirranter* Jankowski, 1964, however, molecular data for *Cirranter* remain unavailable. All available SSU rDNA sequences of *Urostomides* form a fully supported monophyletic clade (100% ML, 1.00 BI). Within this group, the Korean population of *U.*

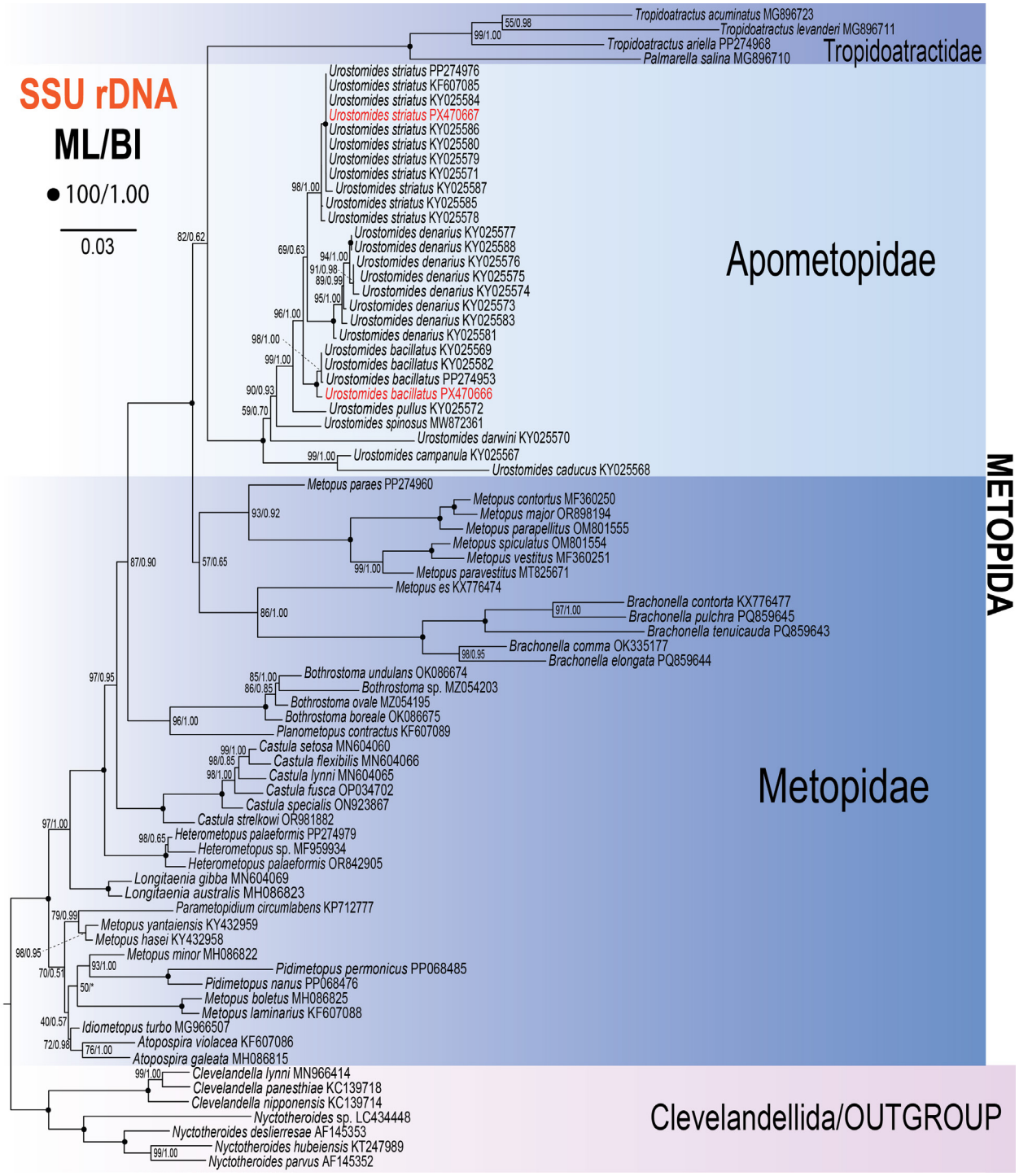


**Fig. 3.** Phylogenetic tree based on 18S ribosomal DNA sequences, reconstructed using the maximum likelihood (ML) method in IQ-TREE v.2.3.3. Node labels represent the bootstrap values of ML and the posterior probabilities of Bayesian inference (BI), shown as ML/BI. Sequences newly obtained for this study are marked in red. Asterisks (*) indicate topological discrepancies between the ML and BI trees. The scale bar represents three changes per 100 nucleotide positions.

*striatus* is confidently nested among conspecific sequences (98% ML, 1.00 BI), whereas *U. bacillatus* from Korea occupies the basal position in its respective clade with full support (100% ML, 1.00 BI).

To further evaluate the genetic divergence of the newly reported Korean populations of *Urostomides*, the Korean population of *U. bacillatus* shares 98.1% nucleotide identity or 31 nucleotide differences (nd) to the Korean population of *U. striatus*. Intergenic divergence is lowest between *Urostomides bacillatus* and *U. striatus* (0.0134–0.0163; 21–24 nd) and highest observed with *U. caducus* (0.0656–0.0719; 89–105 nd). A similar pattern also occurs in *U. striatus*, which shows maximum intergenic distinction from *U. caducus* (0.0671–0.0737; 90–107 nd).

Compared with sequences from other regions, Korean population of *U. bacillatus* displays 0.0036–0.0046 intragenic differences (6–7 nd) relative to sequences from Idaho, Canada, and the Czech Republic, whereas Korean population of *U. striatus* shows 0–0.0038 intragenic differences (0–4 nd) compared to sequences from Idaho, the Czech Republic, Canada, and Chile. Together, the genetic distinctiveness yet close affinity of the Korean population is consistent with previous studies (Bourland et al., 2017a; Zhuang et al., 2021; Feng et al., 2025b). Overall, this study enhances the morphological and molecular resolution of *Urostomides*, offering a valuable reference for future comparative and phylogenetic investigations within the family Apometopidae.

## ORCID

Nanda Dwi Kristanti: https://orcid.org/0009-0008-3822-2617
Novia Cahyani: https://orcid.org/0009-0005-8294-1182
Quoc Dung Nguyen: https://orcid.org/0009-0008-3104-0407
Mann Kyoon Shin: https://orcid.org/0000-0001-6592-1204

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

## ACKNOWLEDGMENTS

This work was supported by grants from the Honam National Institute of Biological Resources (HNIBR 202101120), and the NRF Korea funded by the Ministry of Education of the Republic of Korea (NRF- 2021R1I1A2048744).

## REFERENCES

Bourland W, Pomahač O, Čepička I, 2022. Morphology and phylogeny of two anaerobic freshwater ciliates: *Brachonella comma* sp. nov. and the widely distributed but little-known caenomorphid, *Ludio parvulus* Penard, 1922. Journal of Eukaryotic Microbiology, 69:e12892. https://doi.org/10.1111/jeu.12892

Bourland W, Pomahač O, Čepička I, 2024. Redescription and molecular phylogeny of the freshwater metopid, *Castula strelkowi* (Jankowski, 1964) from the Czech Republic and synonymization of *Pileometopus* with *Castula*. Protist, 175: 126034. https://doi.org/10.1016/j.protis.2024.126034

Bourland W, Rotterová J, Čepička I, 2017a. Morphologic and molecular characterization of seven species of the remarkably diverse and widely distributed metopid genus *Urostomides* Jankowski, 1964 (Armophorea, Ciliophora). European Journal of Protistology, 61:194-232. https://doi.org/10.1016/j.ejop.2017.07.003

Bourland W, Rotterová J, Čepička I, 2017b. Redescription and molecular phylogeny of the type species for two main metopid genera, *Metopus es* (Müller, 1776) Lauterborn, 1916 and *Brachonella contorta* (Levander, 1894) Jankowski, 1964 (Metopida, Ciliophora), based on broad geographic sampling. European Journal of Protistology, 59:133-154. https://doi.org/10.1016/j.ejop.2016.11.002

Bourland W, Rotterová J, Čepička I, 2020. Description of three new genera of Metopidae (Metopida, Ciliophora): *Pileometopus* gen. nov., *Castula* gen. nov., and *Longitaenia* gen. nov., with notes on the phylogeny and cryptic diversity of Metopid ciliates. Protist, 171:125740. https://doi.org/10.1016/j.protis.2020.125740

Bourland W, Rotterová J, Luo X, Čepička I, 2018. The little-known freshwater metopid ciliate, *Idiometopus turbo* (Dragesco and Dragesco-Kernéis, 1986) nov. gen., nov. comb., originally discovered in Africa, found on the Micronesian island of Guam. Protist, 169:494-506. https://doi.org/10.1016/j.protis.2018.05.004

Bourland WA, Wendell L, 2014. Redescription of *Atopospira galeata* (Kahl, 1927) nov. comb. and *A. violacea* (Kahl, 1926) nov. comb. with redefinition of *Atopospira* Jankowski, 1964 nov. stat. and *Brachonella* Jankowski, 1964 (Ciliophora, Armophorida). European Journal of Protistology, 50:356-372. https://doi.org/10.1016/j.ejop.2014.05.004

Bourland WA, Wendell L, Hampikian G, 2014. Morphologic and molecular description of *Metopus fuscus* Kahl from North America and new rDNA sequences from seven metopids (Armophorea, Metopidae). European Journal of Protistology, 50:213-230. https://doi.org/10.1016/j.ejop.2014.01.002

Cahyani N, Sahr U, Shin MK, 2025. Morphology and molecular phylogeny of two newly recorded plagiopylean ciliate species from South Korea. Animal Systematics, Evolution and Diversity, 41:109-120. https://doi.org/10.5635/ASED.2025.41.2.023

Campello-Nunes PH, Fernandes NM, Szokoli F, Fokin SI, Serra V, Modeo L, Petroni G, Soares CAG, Paiva TS, Silva-Neto ID, 2020. *Parablepharisma* (Ciliophora) is not a Heterotrich: a phylogenetic and morphological study with the proposal of new taxa. Protist, 171:125716. https://doi.org/10.1016/j.protis.

2020.125716

Chen Z, Li J, Salas-Leiva DE, Chen M, Chen S, Li S, Wu Y, Yi Z, 2022. Group-specific functional patterns of mitochondrion-related organelles shed light on their multiple transitions from mitochondria in ciliated protists. Marine Life Science and Technology, 4:609-623. https://doi.org/10.1007/s42995-022-00147-w

Feng X, Méndez-Sánchez D, Zhuang W, Li R, Pomahač O, Čepička I, Rotterová J, Hu X, 2024. Morphology, morphogenesis, and molecular characterization of *Castula specialis* sp. nov. (Ciliophora, Armophorea, Metopida). Journal of Eukaryotic Microbiology, 71:e13014. https://doi.org/10.1111/jeu.13014

Feng X, Zhuang W, Li R, Hu X, 2025a. Morphogenesis of the anaerobic ciliate *Tropidoatractus levanderi* (Ciliophora, Armophorea, Metopida), with notes on conjugation and molecular phylogeny. European Journal of Protistology, 101:126166. https://doi.org/10.1016/j.ejop.2025.126166

Feng X, Zhuang W, Li R, Hu X, 2025b. Ontogeny and phylogeny of the anaerobic genus *Urostomides* with supplementing morphological characterization of the type and one little-known species (Alveolata: Ciliophora: Armophorea). Zoological Journal of the Linnean Society, 203:zlae055. https://doi.org/10.1093/zoolinnean/zlae055

Foissner W, 1980. Taxonomische studien über die ciliaten des grossglocknergebietes IX. Ordnungen Heterotrichida und Hypotrichida. Berichte der Naturwissenschaftlich-Medizinischen Vereinigung in Slazburg, 5:71-117.

Foissner W, 2016. Terrestrial and semiterrestrial ciliates (Protozoa, Ciliophora) from Venezuela and Galápagos. Denisia, 35:1-912.

Foissner W, Berger H, Kohmann F, 1992. Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems - Band II: Peritrichia, Heterotrichida, Odontostomatida. Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft, 5:1-502.

Fokin SI, Serra V, Gammuto L, Allievi A, Petroni G, Modeo L, 2023. Multidisciplinary characterization of the new species *Copemetopus mystakophoros* and its symbionts with a proposal for the new class Copemetopea (Alveolata: Ciliophora). Zoological Journal of the Linnean Society, 198:1171-1200. https://doi.org/10.1093/zoolinnean/zlac114

He Y, Choi JK, 2015. First record of four soil ciliates from Korea. Korean Journal of Environmental Biology, 33:215-222. https://doi.org/10.11626/KJEB.2015.33.2.215

Jankowski AW, 1964. Morphology and evolution of Ciliophora. III. Diagnoses and phylogenesis of 53 sapropelebionts, mainly of the order Heterotrichida. Archiv für Protistenkunde, 107: 185-330.

Jankowski AW, 2007. Phylum Ciliophora Doflein, 1901. In: Protista. Part 2, Handbook on Zoology (Ed., Alimof AF). Russian Academy of Sciences, Zoological Institute, St. Petersburg, pp. 371-393.

Kahl A, 1927. Neue und ergänzende Beobachtungen heterotricher Ciliaten. Archiv für Protistenkunde, 57:121-203.

Kahl A, 1932. Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 3. Spirotricha. Die Tierwelt Deutschlands und der angrenzenden Meeresteile. Gustav Fischer Verlag, Jena, 21: 399-650.

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS, 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods, 14:587-589. https://doi.org/10.1038/nmeth.4285

Katoh K, Rozewicki J, Yamada KD, 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics, 20:1160-1166. https://doi.org/10.1093/bib/bbx108

Kimura M, 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 16: 111-120. https://doi.org/10.1007/BF01731581

Kristanti ND, Cahyani N, Nguyen QD, Shin MK, 2025a. Expanding the family Tropidoatractidae: characterization of two new *Tropidoatractus* species (Armophorea: Metopida) from South Korea based on morphology and molecular phylogeny. Journal of Eukaryotic Microbiology, 72:e70048. https://doi.org/10.1111/jeu.70048

Kristanti ND, Nguyen QD, Shin MK, 2025b. Exploring the diversity of *Brachonella*: morphological and molecular analysis with the discovery of a new species, *Brachonella tenuicauda* nov. spec. (Ciliophora, Armophorea, Metopida). European Journal of Protistology, 99:126142. https://doi.org/10.1016/j.ejop.2025.126142

Levander KM, 1894. Beiträge zur Kenntnis einiger Ciliaten. Acta Societatis Pro Fauna et Flora Fennica, 9:1-87.

Li R, Zhuang W, Feng X, Al-Farraj SA, Schrecengost A, Rotterová J, Beinart RA, Hu X, 2023. Molecular phylogeny and taxonomy of three anaerobic plagiopyleans (Alveolata: Ciliophora), retrieved from two geographically distant localities in Asia and North America. Zoological Journal of the Linnean Society, 199:493-510. https://doi.org/10.1093/zoolinnean/zlad015

McMurrich JP, 1884. A new species of infusorian. The American Naturalist, 18:830-832.

Méndez-Sánchez D, Pomahač O, Rotterová J, Bourland W, Čepička I, 2022. Diversity and phylogenetic position of *Bothrostoma* Stokes, 1887 (Ciliophora: Metopida), with description of four new species. Protist, 173:125887. https://doi.org/10.1016/j.protis.2022.125887

Méndez-Sánchez D, Schrecengost, A, Rotterová J, Koštířová K, Beinart RA, Čepička I, 2024. Methanogenic symbionts of anaerobic ciliates are host and habitat specific. The ISME Journal, 18:wrae164. https://doi.org/10.1093/ismejo/wrae164

Minh BQ, Schimdt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R, 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Molecular Biology and Evolution, 37:1530-1534. https://doi.org/10.1093/molbev/msaa015

Nguyen QD, Cahyani N, Shin MK, 2024. Morphological description of three anaerobic ciliates unrecorded in Korea. Animal Systematics, Evolution and Diversity, 40:211-220. https://doi.

org/10.5635/ASED.2024.40.3.004

Nguyen QD, Kristanti ND, Cahyani N, Shin MK, 2026. Step-by-step exploration of the diversity of the genus *Bothrostoma* Stokes, 1887 (Ciliophora, Armophorea) in Korea using morphological and molecular analyses. European Journal of Protistology, 102:126174. https://doi.org/10.1016/j.ejop.2025.126174

Nguyen QD, Shin MK, 2024. First records of two *Bothrostoma* species (Ciliophora: Armophorea: Metopidae) from Korea. Animal Systematics, Evolution and Diversity, 40:323-331. https://doi.org/10.5635/ASED.2024.40.4.017

Nylander JAA, 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala.

Omar A, Jung JH, 2022. New record of five anaerobic ciliate species from South Korea. Journal of Species Research, 11:108-116. https://doi.org/10.12651/JSR.2022.11.2.108

Pan X, Bourland WA, Song W, 2013. Protargol synthesis: an in-house protocol. Journal of Eukaryotic Microbiology, 60:609-614. https://doi.org/10.1111/jeu.12067

Pomahač O, Méndez-Sánchez D, Čepička I, 2024. Bit by bit toward the diversity of metopids: description of the genus *Pidimetopus* n. gen. (Ciliophora: Armophorea). Journal of Eukaryotic Microbiology, 71:e13034. https://doi.org/10.1111/jeu.13034

Pomahač O, Méndez-Sánchez D, Poláková K, Müller M, Solito MM, Bourland WA, Čepička I, 2023. Rediscovery of remarkably rare anaerobic tentaculiferous ciliate genera *Legendrea* and *Dactylochlamys* (Ciliophora: Litostomatea). Biology, 12:707. https://doi.org/10.3390/biology12050707

Rambaut A, 2018. FigTree v 1.4.4. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh.

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

Rotterová J, Bourland W, Čepička I, 2018. Tropidoatractidae fam. nov., a deep branching lineage of Metopida (Armophorea, Ciliophora) found in diverse habitats and possessing prokaryotic symbionts. Protist, 169:362-405. https://doi.org/10.1016/j.protis.2018.04.003

Rotterová J, Edgcomb VP, Čepička I, Beinart R, 2022. Anaerobic ciliates as a model group for studying symbioses in oxygen-depleted environments. Journal of Eukaryotic Microbiology, 69:e12912. https://doi.org/10.1111/jeu.12912

Rotterová J, Pánek T, Salomaki ED, Kotyk M, Táborský P, Kolísko M, Čepička I, 2024. Single cell transcriptomics reveals UAR codon reassignment in *Palmarella salina* (Metopida, Armophorea) and confirms Armophorida belongs to APM clade. Molecular Phylogenetics and Evolution, 191:107991. https://doi.org/10.1016/j.ympev.2023.107991

Rotterová J, Salomaki ED, Pánek T, Bourland W, Žihala D, Táborský P, Edgcomb VP, Beinart RA, Kolísko M, Čepička I, 2020. Genomics of new ciliate lineages provides insight into the evolution of obligate anaerobiosis. Current Biology, 30:2037-2050. https://doi.org/10.1016/j.cub.2020.03.064

Schrecengost A, Rotterová J, Poláková K, Čepička I, Beinart RA, 2024. Divergent marine anaerobic ciliates harbor closely related *Methanocorpusculum* endosymbionts. The ISME Journal, 18:wrae125. https://doi.org/10.1093/ismejo/wrae125

Stokes AC, 1888. A preliminary contribution toward a history of the freshwater infusoria of the United States. Journal of the Trenton Natural History Society, 1:71-344.

Tamura K, Stecher G, Kumar S, 2021. MEGA11: molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution, 38:3022-3027. https://doi.org/10.1093/molbev/msab120

Vd'ačný P, Foissner W, 2017. A huge diversity of metopids (Ciliophora, Armophorea) in soil from the Murray River floodplain, Australia. II. Morphology and morphogenesis of *Lepidometopus platycephalus* nov. gen., nov. spec. Acta Protozoologica, 56:39-57. https://doi.org/10.4467/16890027AP.17.004.6968

Vd'ačný P, Foissner W, 2019. A huge diversity of metopids (Ciliophora, Armophorea) in soil from the Murray River floodplain, Australia. III. Morphology, ontogenesis and conjugation of *Metopus boletus* nov. spec., with implications for the phylogeny of the SAL supercluster. European Journal of Protistology, 69:117-137. https://doi.org/10.1016/j.ejop.2019.04.002

Wilbert N, 1975. Eine verbesserte Technik der Protargolimprägnation für Ciliaten. Mikrokosmos, 64:171-179.

Zhang Y, Liang F, Fu Y, Chi Y, Song W, Warren A, Li F, 2024. Comparative genomics provides insights into the evolutionary history of the phylum Ciliophora (Eukaryotic, Alveolata) and uncovers the adaptive evolution of anaerobic ciliate classes. Zoologica Scripta, 54:270-290. https://doi.org/10.1111/zsc.12700

Zhuang W, Feng X, Li R, Hu X, 2025. Molecular phylogeny and taxonomy of three anaerobic ciliates including *Bothrostoma aporobustum* nov. spec. (Ciliophora, Metopida). European Journal of Protistology, 100:126155. https://doi.org/10.1016/j.ejop.2025.126155

Zhuang W, Li R, Feng X, Al-Farraj SA, Hu X, 2022. New contribution to the diversity of the anaerobic genus *Metopus* (Ciliophora, Armophorea), with descriptions of three new marine species. Frontiers in Marine Science, 9:884834. https://doi.org/10.3389/fmars.2022.884834

Zhuang W, Li S, Bai Y, Zhang T, Al-Rashied KAS, Hu X, 2021. Morphology and molecular phylogeny of the anaerobic freshwater ciliates *Urostomides spinosus* nov. spec. (Ciliophora, Armophorea, Metopida) from China. European Journal of Protistology, 81:125823. https://doi.org/10.1016/j.ejop.2021.125823

Received September 2, 2025
First revised October 21, 2025
Second revised December 9, 2025
Accepted December 12, 2025

ASED
Animal Systematics, Evolution and Diversity

*Animal Systematics, Evolution and Diversity* (Anim Syst Evol Divers, ASED) is published quarterly (Jan 31st, Apr 30th, Jul 31st, and Oct 31st) by the Korean Society of Systematic Zoology. Papers submitted to the Journal should be original contributions on systematics, conservation biology, and evolutionary biology with a preference toward systematic works such as discovery of new taxa, taxonomic reviews and revisions, phylogenetics and population genetics, biogeography and phylogeography, and biodiversity studies mainly concerning animal taxa of the Korean peninsula and surrounding areas of East Asia. Only manuscripts written in English are accepted. ASED follows the Open Access Journal policy. All contents of ASED are freely available online. Digital files can be read, downloaded, and printed freely, at http://kjsz.kofst.or.kr/PublishedPaper/before_list.asp, http://www.kpubs.org/journal/journalArchive.kpubs?kojic=DMBRBT or http://central.oak.go.kr/journallist/journallist.do?journal=10013.

Manuscripts for submission to the *Animal Systematics, Evolution and Diversity* should be prepared according to the following instructions.

## EDITORIAL POLICY

### Creative Commons Attribution License

ASED follows the Creative Commons Attribution Non-Commercial License available from: http://creativecommons.org/licenses/by-nc/3.0/.

### Copyright

The regulations for acceptance of a manuscript for publication automatically include the consent of the author(s) to transfer the copyright and license to the *Korean Society of Systematic Zoology*. Authors will complete a Copyright Transfer Form at the time of proofreading. The corresponding author can sign on behalf of co-authors. The CAF can be downloaded from the online manuscript submission center at http://e-ASED.org, or obtained from the editorial office. Acceptance of the agreement will ensure full copyright protection and help to disseminate the article to the widest possible readership in print and electronic formats. Authors are responsible for obtaining permission to reproduce copyrighted material from other sources.

The authors assure that no substantial part of the work has been published or is being considered for publication elsewhere. When any of the results are to appear in another journal, details must be submitted to the Editor-in-Chief, together with a copy of the other paper(s) and the expected date(s) of publication.

### Disclaimer

The publisher, editors, and reviewers do not assume any legal responsibility for errors, omissions, or claims, nor do they provide any warranty, expressed or implied, with respect to information published in ASED.

## RESEARCH AND PUBLICATION ETHICS

The journal adheres to the ethical guidelines for research and publication described in Guidelines on Good Publication (http://publicationethics.org/resources/guidelines) and the ICMJE Guidelines (http://www.icmje.org).

### Authorship

Authorship credit should be based on 1) substantial contributions to conception and design, acquisition of data, and/or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; 3) final approval of the version to be published; and 4) agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Every author should meet all of these four conditions. After the initial submission of a manuscript, any changes whatsoever in authorship (adding author(s), deleting author(s), or re-arranging the order of authors) must be explained by a letter to the editor from the authors concerned. This letter must be signed by all authors of the paper.

- Corresponding author and first author: ASED does not allow multiple corresponding authors for one article. Only one author should correspond with the editorial office and readers for one article.
- Correction of authorship: Authorship may be changed before publication but after submission when an authorship correction is requested by all of the authors involved with the manuscript.

### Originality, Plagiarism, and Duplicate Publication

Submitted manuscripts must not have been previously published or be under consideration for publication elsewhere. No part of the accepted manuscript should be duplicated in any other scientific journal without the permission of the Editorial Board. Submitted manuscripts are screened for possible plagiarism or duplicate publication by CrossCheck upon arrival. If plagiarism or duplicate publication related to the papers of this journal is detected, the manuscripts may be rejected, the authors will be announced in the journal, and their institutions will be informed. There will also be penalties for the authors.

A letter of permission is required for any and all material that has been published previously. It is the responsibility of the author to request permission from the publisher for any material that is being reproduced. This requirement applies to text, figures, and tables.

### Secondary Publication

It is possible to republish manuscripts if the manuscripts satisfy

the conditions of secondary publication of the ICMJE Recommendations (http://www.icmje.org/urm_main.html).

### Conflict of Interest Statement

The corresponding author must inform the editor of any potential conflicts of interest that could influence the authors' interpretation of the data. Examples of potential conflicts of interest are financial support from or connections to companies, political pressure from interest groups, and academically related issues. In particular, all sources of funding applicable to the study should be explicitly stated.

### Process for Managing Research and Publication Misconduct

When the journal faces suspected cases of research and publication misconduct such as redundant (duplicate) publication, plagiarism, fraudulent or fabricated data, changes in authorship, an undisclosed conflict of interest, ethical problems with a submitted manuscript, a reviewer who has appropriated an author's idea or data, complaints against editors, and so on, the resolution process will follow the flowchart provided by the Committee on Publication Ethics (http://publicationethics.org/resources/flowcharts). The discussion and decision on the suspected cases are carried out by the Editorial Board.

### Editorial Responsibilities

The Editorial Board will continuously work to monitor and safeguard publication ethics: guidelines for retracting articles; maintenance of the integrity of the academic record; preclusion of business needs from compromising intellectual and ethical standards; publishing corrections, clarifications, retractions, and apologies when needed; and excluding plagiarism and fraudulent data. The editors maintain the following responsibilities: responsibility and authority to reject and accept articles; avoiding any conflict of interest with respect to articles they reject or accept; promoting publication of corrections or retractions when errors are found; and preservation of the anonymity of reviewers.

## SUBMISSION OF MANUSCRIPT

ASED has no article submission charges. Manuscript submission is only available through the online manuscript submission center at http://e-ASED.org. Any researcher anywhere in the world can submit a manuscript if the scope of the manuscript is appropriate. Manuscripts should be submitted in English. Authors are required to have their manuscripts edited by a native English speaker before submission. Authors have to upload a certificate or a document in the submission process that they have followed the requirement. All manuscripts should be submitted as MS Word files (.doc or .docx files). Figures should be saved as one of the following formats: TIFF (tagged image file format), PostScript or EPS (encapsulated PostScript), and should contain all the necessary font information and the source file of the application (e.g. CorelDraw/Mac, CorelDraw/PC).

Submission instructions are available at the website. All articles submitted to the ASED must comply with these instructions. Failure to do so will result in return of the manuscript and possible delay in publication. As you prepare to submit your manuscript, a checklist for author(s) should be completed in the online manuscript submission center at http://e-ASED.org. For assistance please contact us via e-mail (systzoology@hanmail.net), telephone (+82-2-569-0133).

## PEER REVIEW & REVISION FOR MANUSCRIPTS

### Peer Review

Peer review involves the following steps: 1) Editorial office checks formatting, 2) Editor-in-chief assigns a subject editor, 3) Subject editor assigns at least three peer reviewers, 4) Reviewers submit review reports, 5) Subject editor makes recommendations, 6) Editor-in-chief makes final decision, and 7) Author is informed of decision. An initial decision will normally be made within one month of receipt of a manuscript. However, the reviewing process will normally take longer, depending on the length of the manuscript and reviewer's responses. A manuscript which has been published or of which a substantial portion has been published elsewhere will not be accepted. The Editor-in-Chief is responsible for final decisions regarding the acceptance of a peer-reviewed paper.

### Revision for Manuscript

When a manuscript is returned to the corresponding author for revision, the reviewed manuscript must be re-submitted within one month. Failure to resubmit the revised manuscript within a month of the editorial decision is regarded as a withdrawal. The final version of accepted manuscript should be uploaded with a signed copyright transfer form available in the online manuscript submission center.

## PREPARATION OF MANUSCRIPTS

### General Requirements

Manuscripts should be prepared using MS Word, in an A4 (210 × 297 mm) page format. Extra space should not be left between paragraphs. Only a single font (e.g., Times New Roman) in 12 point or greater should be used. All manuscript sheets must be numbered successively at the middle of the bottom. The abstract, each table, and the set of figure legends should be on separate sheets. Normally all parts of a manuscript together should not exceed 40 printed pages. Captions of plates/figures should be inserted where the figures are intended to appear within the text, or listed at the end of the manuscript. Vector diagrams/charts generated in programs such as MS Excel can be embedded in the text file as well. Professional quality lettering should be used for drawings. Typewriter labels are unacceptable. The manuscript style should conform to that of recent issues.

### Original Article

Original research articles focus on systematic zoology and evolutionary biology concerning the Korean peninsula and surrounding areas. The most desirable organization of the manuscript is as follows: Title Page, Abstract & Keywords, Introduc-

tion, Materials and Methods, Results (or Systematic Accounts), Discussion (if applicable), Acknowledgments, References, Tables, and Figure Legends. Results and Discussion may be combined.

## Short Communication

Short reports of original studies are accepted for publication. The manuscript organization for a short communication basically follows that of an original article but some sections may be combined as necessary.

## Letters to the Editor

Critical comments are welcomed for correcting errors of published facts and for providing alternative interpretations of published data. The sequence for a Letter to the Editor is title page, text, references, and names and affiliations of authors. If needed, tables and figures can be included. The text should be written without subdivision and without extra spacing between paragraphs. A Letter to the Editor should not be longer than a printed page.

## Review Articles

Invited or submitted review papers are accepted. We prefer brief summaries of developments in fast moving areas or historical overviews of specific taxa in the Korean peninsula and surrounding areas of East Asia. Manuscripts include a title page (with a running head), abstract and keywords, text, references, tables, and figures.

## Book Reviews

Invited book reviews can be published. Manuscripts include the title of the book reviewed, author(s) and editor(s) of the book, printer and publisher (city, state and country), total pages, ISBN number, followed by the text and the reviewer with affiliation.

## Formatting

***Title page***: Provide the full title of the article, a short one for use as a running head, and full names and affiliations of all the authors. Titles should be short and descriptive. If some of the authors have different affiliations, place numbers as superscripts[1, 2, 3] ... after the surname of the authors and before the name of their affiliations. Place an asterisk (*) after the name of the corresponding author. At the bottom of the title page, give the name, telephone and fax numbers, and e-mail address of the corresponding author.

***Abstract and Keywords***: On a separate page, the abstract should provide a clear and succinct statement (at least 100 words, but not more than 250 words) and should be intelligible in itself. Authors should avoid nonstandard abbreviations, unfamiliar terms and symbols and references. Keywords should be given at the end of the abstract in 5 to 8 words or phrases. Keywords should ideally differ from the words used in the title.

***Introduction***: The introduction should supply sufficient background knowledge and information to allow the reader to understand and evaluate the value of the study. It must also provide a rationale for the study. Cite references to provide the most salient background rather than an exhaustive review of the topic.

***Materials and Methods***: All material examined should be listed in a similar format: Localities should be cited in order of increasing precision as in the examples, along with the collector. Sufficient information that will make it possible to repeat the work should provided. For modification of a published methodology, only the modification needs to be described with a reference to the source of the method. Information regarding statistical analysis of the data should be included. The source of special equipment or chemicals should also be given with the name and location of manufacturers, e.g., (Pharmacia, Uppsala, Sweden) or (Sigma, St. Louis, MO, USA). For "Short communications", the Materials and Methods section should be included at the end of the Introduction without a heading.

***Results***: The results should be presented succinctly and completely. New taxa or synonyms should be clearly marked as n. gen., n. sp., n. syn., or n. comb. Author has to announce Korean name about newly added species, genus or other taxa in Korean fauna trough the footnote and place an asterisk (*) at the front of name of the new species or taxon. If two or more taxon are newly reported, place numbers as superscripts beside asterisk. In a synonym list, the page number is noted after the colon (:), not the page abbreviation (p or pp): e.g., *Albus typical* Kim, 1991: 91(type locality: Korea, Gangwon-do, Mt. Baegunsan, holotype ♂, YSUW); Choi, 1993: 102. When necessary, appropriate type information (e.g., type locality, type specimen (s), type depository) is supplemented for an original description in the synonymy. For a new species description, complete collection and rearing data for the holotype and paratypes, and the depository must be specified as follows: Holotype, ♂, Korea: Gangwon-do, Jeongseon-gun, Yoryang-myeon, Mt. Panryun-san Forest, 22 Sep 1984, Hong GD (NSM). Paratypes, 1♂ 2♀, Taiwan: Chitou, 12 May 1985, Hardy J (USNM).

***Discussion***: The discussion should include a concise statement of the principal findings, discussion of the significance of the work, and appraisal of the findings in the light of other published works dealing with the same or a closely related organism. Redundant description of material in the introduction and results, and extensive discussion of literature are discouraged.

***Acknowledgments***: Contributions to the article that do not merit co-authorship, such as administrative support, technical assistance, critical reviews of the manuscript, and financial support, should be recognized here.

***References***: References should be cited in the text at the appropriate places. All references cited in the text should be listed at the end of the text, and arranged in alphabetical order according to the name of the first author. Journal titles should be provided in full. Text citations to references should be styled as, e.g., Kim and Park (2010); (Kim and Park, 2010); Davis et al. (1979). The use of "in preparation", "submitted for publication" or "personal communication" is not allowed in the reference list. "Unpublished data" and "personal communication" should appear parenthetically following the name(s) in the text.

## Journal article

Park E, Kim B, Won YJ, 2010. The complete mitochondrial

genome of *Dendronephthya gigantea* (Anthozoa: Octocorallia: Nephtheidae). Korean Journal of Systematic Zoology, 26:197-201.

Arakawa R, Nakamura M, Kanimus K, 1988. Laboratory colonization of *Anopheles omorii* (Diptera: Culicidae). Japanese Journal of Applied Zoology, 39:347-353 (in Japanese).

### Book

Cambell RC, 1989. Statistics for biologists. 3rd ed. Cambridge University Press, London, pp. 59-61.

### Book section

Davis BN, 1978. Urbanization and the diversity of insects. In: Diversity of insect faunas (Eds., Mound LA, Wjoloff N). Royal Entomological Society, London, pp. 126-138.

### Online document

International Commission on Zoological Nomenclature, 2011. About ZooBank [Internet]. International Commission on Zoological Nomenclature, London, Accessed 6 Feb 2011, <http://www.zoobank.org/About.aspx>.

### Conference

Conover JT, Sieburth JN, 1966. Effect of tannins excreted from Phaeophyta on planktonic animal survival in tide pools. In: Proceedings of the 5th International Seaweed Symposium (Eds., Young EG, McLachlan JL), Pergamon Press, Oxford, pp. 99-100.

### Dissertation/Thesis

Edwards MS, 2001. Scale-dependent patterns of community regulation in giant kelp forests. PhD dissertation, University of California, Santa Cruz, CA, USA, pp. 1-149.

Na KB, 2004. Taxonomy of the Chironomidae (Diptera, Insecta) in Seoul-Gyeonggi area, Korea. MS thesis, Seoul Women's University, Seoul, Korea, p. 100.

***Tables***: Tables should be as simple as possible, to be intelligible without requiring references to the text. Each table should have a concise heading, should be typed on a separate sheet of paper, and must have an explanatory title and sufficient explanatory material. All tables should be referred to in the text, and their approximate position indicated in the margin of the manuscript. Ruling in tables, especially vertical or oblique lines, should be avoided.

The table function in MS Word should be used to build tables so that the cells can be easily re-sized to fit the page by the typesetters. The Tab key should never be used to type tables, nor should the space bar be used to adjust spaces. This must be corrected before submission. It is recommended that a table not exceed one page when printed. Use lowercase letters in superscripts a, b, c ... for special remarks.

***Figures***: All figures should be termed "Figures" (not "Plates," although "Plates" are allowed in the supplements) and labeled with numbers. Within each figure, labels should begin from A, B, C, a, b, c,---. Lettering and symbols to appear on each drawing should be at the same points in each image. The conventions for abbreviations used in the journal should be noted so that the usage in illustrations and text are consistent. All figures should be cited in the text (e.g., Fig. 1, Fig. 1A-C, Figs. 1, 2, Figs. 1, 2A-D). The preferred location of the figures can be indicated in the text.

Photographs and illustrations should be prepared professionally Six hundred dpi or higher for photographs and illustrations are recommended. But, color and halftone photographs produced at 300 dpi or higher can be accepted. Line drawings must be produced at 600 dpi or higher resolution. Photographs must be of sufficient contrast to withstand the inevitable loss of contrast and detail during the printing process. If a figure is to be reduced, all elements, including labels, should be able to withstand reduction and remain legible. Electron and light microscopic figures must be original or scanned copies from the original. The magnification should be indicated on each micrograph with a scale bar.

## GENERAL TEXT STYLE

### Using Scientific Names of Genus and Species

Nomenclature must be in agreement with the International Code of Zoological Nomenclature (4th edition 1999; http://www.nhm.ac.uk/hosted-sites/iczn/code/), which came into force on 1 January 2000. When using a scientific name of a genus and species for the first time in a taxonomic paper, it should be written in full with author and year. Thereafter, the genus can be indicated by its initial letter except at the start of a sentence, and the author and date can be omitted. If a reference is placed immediately after a scientific name, it should be made clear in some way that the reference is not the author of the scientific name: e.g., *Albus typical* sensu Kim, 1991, or *Albus typical* (see Kim, 1991). Taxonomic descriptions should be in telegraphic style. When a new taxon is described, its detailed type data should be clearly stated.

### Synonym List

***Homidia social* Denis, 1929 (Figs. 1B, C5)**

*Homidia social* Denis, 1929: 310, figs. 1-4; Yosil, 1942: 487, fig. 10; Stach, 1965: 359, Pl. 32, figs. 1-6; Christiansen and Bellinger, 1980: 884, fig. 727.

If necessary, appropriate type data or additional information could be included, with the references in the synonym list (e.g., Albus typical Kim, 1991: 91 (type locality: Korea, Gangwondo, holotype ♂, YSUW); Choi, 1993 (subsequent description); Choi and Kim, 2000 (in Korean catalog)).

### English Transliteration of Localities

The Guidelines for the Romanization of Korean Localities should be followed (http://roman.cs.pusan.ac.kr/input.aspx?), but the most recent official English names should be used for non-Korean localities.

### Nucleotide and Amino Acid Sequences

Authors should explicitly mention the database abbreviation (as mentioned below) together with the actual database number, bearing in mind that an error in a letter or number can result in a dead link in the online version of the article. The following format should be used: Database accession no: xxxx. e.g., (GenBank accession no: AB123456).

### Units

Standard metric units are used for describing length, height, weight, and volume. The unit of temperature is given in degrees Celsius (°C). All others are in terms of the International System of Units (SI). All units must be preceded by one space except percentage (%) and temperature (°C).

**Numbers:**

In the text, numbers should be Arabic numerals, except when beginning a sentence. Numbers greater than 999 should have commas, e.g., 13,970. The 24-hour system is used to indicate time, e.g., 18:00 hr.

**Abbreviations:**

Abbreviations must be used as an aid to the reader, rather than as a convenience of the author, and therefore their use should be limited. Generally, abbreviations that are used less than 3 times in the text, including tables and figure legends, should be avoided. In addition to abbreviations for SI units, common molecular, chemical, immunological, and hematological terms can be used without definition in the title, abstract, text, tables, and figure legends, e.g., bp, kb, kDa, DNA, cDNA, RNA, mRNA, PCR. Other common abbreviations are as follows (the same abbreviations are used for plural forms): hr (hour; use 0-24:00 hr for time), sec (second), min (minute), day (not abbreviated), week (not abbreviated), month (not abbreviated), year (not abbreviated), L (liter), mL (milliliter), μL (microliter), g (gram), kg (kilogram), mg (milligram), μg (microgram), ng (nanogram), pg (picogram), *g* (gravity; not × *g*), n (sample size), SD (standard deviation of the mean), and SE (standard error of the mean).

## FINAL PREPARATION FOR PUBLICATION

### Final Version

After the paper has been accepted for publication, the author(s) should submit the final version of the manuscript. The names and affiliations of the authors should be double-checked, and if the originally submitted image files were of poor resolution, higher resolution image files should be submitted at this time. Color images must be created as CMYK files. The electronic original should be sent with appropriate labeling and arrows. The EPS, TIFF, Adobe Photoshop (PSD), JPEG, and PPT formats are preferred for submission of digital files of photographic images. Symbols (e.g., circles, triangles, squares), letters (e.g., words, abbreviations), and numbers should be large enough to be legible on reduction to the journal's column widths. All of the symbols must be defined in the figure caption. If the symbols are too complex to appear in the caption, they should appear on the illustration itself, within the area of the graph or diagram, not to the side. If references, tables, or figures are moved, added, or deleted during the revision process, they should be renumbered to reflect such changes so that all tables, references, and figures are cited in numeric order.

### Manuscript Corrections

Before publication, the manuscript editor may correct the manuscript such that it meets the standard publication format. The author(s) must respond within 2 days when the manuscript editor contacts the author for revisions. If the response is delayed, the manuscript's publication may be postponed to the next issue.

### Galley Proof

The author(s) will receive the final version of the manuscript as a PDF file. Upon receipt, within 3 days, the editorial office (or printing office) must be notified of any errors found in the file. Any errors found after this time are the responsibility of the author(s) and will have to be corrected as an erratum.

## PUBLICATION CHARGE

The page charge is US $40 per printed page. No off-prints will be provided, but authors who want off-prints can request them with their own expense. Waivers can be offered to students, retired scientists, and scientists working in low-income countries, if they are sole authors of a manuscript.

ASED http://e-ASED.org
Animal Systematics, Evolution and Diversity

# Checklists for Authors

This checklist must be completed, signed by the corresponding author, and submitted with the manuscript.
Please see Instructions for Authors for details related to this checklist.

*Please be sure to check over items from No. 1 to No. 12

☐ 1. Typed double-spaced in 12-point font on A4 sized paper and prepared with an MS-word file.

☐ 2. Cover page :
(1) complete title, (2) manuscript type, (3) name, (4) affiliation, (5) telephone, facsimle and E-mail address of corresponding author, (6) running title.

☐ 3. Abstract in unstructured format with 100-250 words.

☐ 4. Five to eight keywords, which differ from the words used in the title.

☐ 5. Main text is structured as follows :
Original article: Introduction, Materials and Methods, Results (or Systematic Account), and Discussion (if applicable).
Short communication: Introduction, Results (or Systematic Account), and Discussion (if applicable).

☐ 6. Main text is prepared using line numbers with continuous mode.

☐ 7. Reference in proper format.

☐ 8. All figures and tables referenced in the text and numbered in order of its appearance in the text.

☐ 9. Figures as a separate files, in TIFF or JPG format, minimum 300 DPI for photographs or 600 DPI for line drawings.

☐ 10. Elucidation of research or project support/funding.

☐ 11. Fully understand details in the Instruction for Authors, especially ethics.

☐ 12. Three non-Korean reviewers are recommended with full name, affiliation, phone numbers and email address.

ASED http://e-ASED.org
Animal Systematics, Evolution and Diversity

# Copyright Transfer Form

The authors hereby transfer all copyrights in and to the manuscript named above in all forms and media, now or hereafter known, to the Korean Society of Systematic Zoology effective if and when the paper is accepted for publication in the Animal Systematics, Evolution and Diversity. The authors reserve all proprietary right other than copyright, such as patent rights.

Everyone who is listed as an author in this article should have made a substantial, direct, intellectual contribution to the work and should take public responsibility for it.

All authors are responsible for recognizing any conflict of interest that could bias their work in the acknowledgments, disclosing all financial support and any other personal connections.

This paper contains works that have not previously published or not under consideration for publication in other journals.

Title of the paper: _____

Corresponding author: _____ Signature _____ Date _____

This form must be signed by all authors in an order as appeared in the article, and should be returned to the editorial office.

Korean Society of Systematic Zoology, Room 305-3, The Korea Science and Technology Center,
22, 7 gil, Teheran-ro, Gangnam-gu, Seoul, Korea
Tel: +82-2-569-0133 E-mail: systzoology@hanmail.net

ASED http://e-ASED.org
Animal Systematics, Evolution and Diversity

## CONTENTS Volume 42 Number 1 January 2026

### Original Articles

1 **New Record of *Eunice flavopicta* (Annelida: Eunicidae) from Korean Waters, with Notes on Korean *Eunice aphroditois***
Ha-Eun Lee, Taeseo Park, Gi-Sik Min

12 **Morphology and Phylogeny of Two Tintinnids (Ciliophora, Spirotrichea) from Korea**
Ji Hye Moon, Jae-Ho Jung

21 **A Contribution to Two Newly Recorded *Metopus* Species (Ciliophora: Metopida) in Korea**
Quoc Dung Nguyen, Sahr Uzma, Nanda Dwi Kristanti, Mann Kyoon Shin

34 **Two Newly Recorded Species of the Genus *Holothuria* (Holothuroidea: Holothuriida: Holothuriidae) from Jejudo Island, Korea**
Jinho Lee, Taekjun Lee

43 **New Record of *Euplotes corsica* from a Saltern in South Korea**
Sun Young Kim, Jeong Hyeon Yeo, Nam Seon Kang, Ga Young Kim, Seo Woo Son, Hyung June Kim, Jae-Ho Jung

49 **Two New Records of *Pycnoclavella* (Ascidiacea: Clavelinidae) in Korea**
Su Yuan Seo

54 ***Corhiza ulleungensis* sp. nov. (Hydrozoa: Leptothecata: Halopteridídae) from Korea, with the First Record of the Genus *Corhiza***
Hyo-Jin Yu, Sung-Jin Hwang

63 **Two New Species of Anenthemonae (Cnidaria: Anthozoa: Actiniaria) and a New Korean Record of *Metridium farcimen* (Enthemonae)**
Myeong Kyu Lim, Seung-Joon Lee, Sung-Jin Hwang

### Short Communications

82 **New Record of a Diogenid Species, *Diogenes pallescens* (Crustacea: Decapoda: Anomura: Diogenidae), from Korea**
Jibom Jung, Jin-Ho Park, Hee-seung Hwang

88 **New Record of *Thoracophelia japonica* (Annelida, Opheliidae) from South Korea**
Ha-Eun Lee, Gi-Sik Min, Geon Hyeok Lee

93 **Mitogenomic Resources of *Tanakia latimarginata* and *T. koreensis* with Implications for Taxonomic Accuracy in Acheilognathidae**
Hyung-Bae Jeon, Hari Won, Ho Young Suk

### Special Issue

100 **Characterization of Two *Urostomides* Species (Ciliophora, Armophorea, Metopida) Newly Recorded from Jindo Island, Korea**
Nanda Dwi Kristanti, Novia Cahyani, Quoc Dung Nguyen, Mann Kyoon Shin

The Korean Society of Systematic Zoology