

# New record of two spirotrichous ciliate species (Protozoa, Ciliophora) collected from coasts of South Korea

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Two newly recorded ciliates, *Aspidisca major* (Madsen, 1931) Kahl, 1932 and *Metaurostylopsis rubra* Song and Wilbert, 2002, were collected from eastern and southern coasts of South Korea, respectively. The morphology of these two species were studied based on observations of protargol-stained specimens. The morphological characteristics of the two species are as follows: (1) *Aspidisca major* is characterized by a size of  $76-78 \times 42-49 \,\mu\text{m}$  after protargol impregnation, two distinct macronuclei, seven frontoventral cirri in "*polystyla*-arrangement", and dorsal kinety 1 extends to posterior end of dorsal kinety 2; and (2) *Metaurostylopsis rubra* is characterized by a size of  $68-77 \times 49-58 \,\mu\text{m}$  size after protargol impregnation, brick-reddish body color, and large number of marginal rows. In the present study, we provide a brief diagnosis, remarks, and photomicrographs.

Keywords: Aspidisca major, marine ciliates, Metaurostylopsis rubra, taxonomy

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## INTRODUCTION

The genera Aspidisca Ehrenberg, 1830 and Metaurostylopsis Song et al., 2001 are widely distributed globally in marine ecosystems. Up to date, about 60 nominal Aspidisca species and eight Metaurostylopsis species have been recorded (Wu and Curds, 1979; Fernandez-Leborans de Zaldumbide, 1987; Valbonesi, 1996; Berger, 2001; Song and Wilbert, 2002; Lei et al., 2005; Shao et al., 2008a; 2008b; Jankowski, 2009; Shen et al., 2010; Chen et al., 2011; Jung et al., 2011; Jiang et al., 2013; Lu et al., 2016; Song et al., 2020; Zheng et al., 2022; Choi et al., 2024). According to the National Institution of Biological Resources (2023), only 11 Aspidisca species (A. aculeata (Ehrenberg, 1838) Kahl, 1932; A. cicada (Müller, 1786) Claparède and Lachmann, 1858; A. dentata Kahl, 1928; A. hexeris Quennerstedt, 1869; A. leptaspis Fresenius, 1865; A. lynceus (Müller, 1773) Ehrenberg, 1830; A. orthopogon Deroux and Tuffrau, 1965; A. polypoda (Dujardin, 1841) Kahl, 1932; A. polystyla Stein, 1859; A. steini (Buddenbrock, 1920) Kahl, 1932; and A. turrita (Ehrenberg, 1838) Claparède and Lachmann, 1858) and only 4 Metaurostylopsis species (M. cheni Chen et al., 2011; M. marina (Kahl, 1932) Song et al., 2001; M. salina Lei et al., 2005; and M. struederkypkeae Shao et al., 2008) have been recorded in South Korea, indicating undersampling of the Korean ciliate fauna (Shin and Kim, 1988; Lei *et al.*, 2005; Li *et al.*, 2010; Park *et al.*, 2012; Kim *et al.*, 2016; Kim and Jung, 2018; Kim *et al.*, 2018; 2020; Choi *et al.*, 2020; Choi *et al.*, 2023). Therefore, this study aims to further explore the diversity and distribution of these genera in Korean marine environments.

During a field survey, we identified two species, 1) Aspidisca major (Madsen, 1931) Kahl, 1932; and 2) Metaurostylopsis rubra Song and Wilbert, 2002, previously unrecorded in Korea. In this paper, we provide brief diagnosis, remarks, photomicrographs, and information about collection sites and conditions.

#### MATERIALS AND METHODS

The detailed information about collection sites and conditions are provided in 'Material examined' section for each species. After gently stirring up surface marine water, samples were collected with bottom sediments. All samples were transferred to the laboratory and kept in Plant culture dishes at room temperature (ca. 20°C) with 1–3 autoclaved rice grains. The morphology of each species was studied using a stereomicroscope (SZ11; Olympus, Tokyo, Japan), and photomicrographs were captured

using a digital camera (DP74; Olympus). The protargol powder was synthesized using the method of Pan *et al.* (2013) and Kim and Jung (2017). The protargol-impregnated specimens were prepared using the 'procedure A' method of Foissner (2014). The differential through-focal images of the protargol-impregnated specimens were merged using Helicon Focus ver. 8.1.0 software (Helicon-Soft Ltd, Kharkiv, Ukraine). The basic terminology and taxonomic classification mainly followed Lynn (2008), Song *et al.* (2001), and Wu and Curds (1979).

## **RESULTS AND DISCUSSION**

Phylum Ciliophora Doflein, 1901 Class Spirotrichea Bütschli, 1889 Order Euplotida Small and Lynn, 1985 Suborder Euplotina Jankowski, 1979 Family Aspidiscidae Ehrenberg, 1830 Genus *Aspidisca* Ehrenberg, 1830

#### 1. Aspidisca major (Madsen, 1931) Kahl, 1932 (Fig. 1)

**Material examined.** Marine water (salinity 37.8‰, temperature 18.3°C) collected from Sodol beach, Jumun-ri, Jumunjin-eup, Gangneung-si, Gangwon-do, Korea (37° 54′24.6″N, 128°49′41.7″E) on May 15, 2023. **Diagnosis.** Size 76–78 × 42–49 µm after protargol im-

g, 1830special species among the congeners in having two mac-<br/>ronuclei (Fig. 1D). Because of this feature, A. major, A.<br/>binucleata Kahl, 1932, A. fusca Kahl, 1928, and A. mu-<br/>tans Kahl, 1932 can be easily distinguished from other<br/>members of the genus Aspidisca. However, A. major dif-

members of the genus *Aspidisca*. However, *A. major* differs from *A. binucleata* in the number of frontoventral cirri (7 vs. 9). *Aspidisca mutans* differs from *A. major* by the number of frontoventral cirri (12 vs. 7) and the number of transverse cirri (7 vs. 5) (Kahl, 1932). *Aspidisca fusca* differs from *A. major* by the presence of a peristomial spur

pregnation (n=3); body bean-shaped; cortex rigid; peri-

stomial spur and projections along left margin on ventral

side lacking (Fig. 1A); anterior adoral zone of mem-

branelles (AZM1) about 4 µm long with 4 membranelles,

posterior adoral zone of membranelles (AZM2) about 24 um long after protargol impregnation and with 15-

18 membranelles; invariably seven frontoventral cirri in

"polystyla-arrangement"; five transverse cirri, cilia of

each transverse cirrus not separated (Fig. 1A); four dorsal

kineties with 25–28, 18–22, 19–23, and 22–24 dikinetids in dorsal kineties 1–4, respectively (Fig. 1B); dorsal kinety 1 distinctly curved to right posteriorly and ends near posterior end of dorsal kinety 2 (Fig. 1C); two ellipsoid

**Remarks.** The Korean population of Aspidisca major

corresponds with the German population redescribed by

Kahl (1932) in all available aspects. Aspidisca major is a

macronuclei, micronucleus not observed (Fig. 1D).

Distribution. Germany, South Korea



**Fig. 1.** *Aspidisca major* after protargol impregnation (A–D). A, B. Ventral (A) and dorsal (B) view, showing the body shape, anterior and posterior portion of adoral zone of membranelles, the transverse cirri, the frontoventral cirri in "*polystyla*-arrangement", and dorsal kineties 1–4. C. Dorsal view showing the arrangement of dorsal kineties. Dorsal kinety 1 curved posteriorly and extends to posterior end of dorsal kinety 2. D. Two ellipsoidal macronuclei (arrowheads). AZM1 and 2, anterior and posterior adoral zone of membranelles; DK1–4, dorsal kineties 1–4; FVC, frontoventral cirri; TC, transverse cirri. Scale bars =  $20 \,\mu$ m (A, B);  $10 \,\mu$ m (C, D).



**Fig. 2.** *Metaurostylopsis rubra* after protargol impregnation (A, B). A. Ventral view showing the body shape, adoral zone of membranelles, frontal and transverse cirri, midventral pairs and row, and left and right marginal rows. Arrowhead denotes one buccal cirrus. B. Dorsal view showing the dorsal kineties 1–3 and macronuclear nodules. Double arrowhead denotes fragment-like kineties anterior to the leftmost right marginal row. AZM, adoral zone of membranelles; DK1–3, dorsal kineties 1–3; FC, frontal cirri; LMR, left marginal rows; MA, macronuclear nodules, MP, midventral pairs; MR, midventral row; RMR, right marginal rows; TC, transverse cirri. Scale bar = 30 µm.

(present vs. absent) (Wu and Curds, 1979). Considering the arrangement of frontoventral cirri, number of membranelles in AZM1 and the presence of peristomial spur, the Korean population of *A. major* is similar to *A. steini* however, they differ mainly in the number of macronuclei (two vs. one) and larger body size (76–78 × 42–49  $\mu$ m vs. 20–35 × 15–27  $\mu$ m) (Wu and Curds, 1979; Song and Wilbert, 1997).

**Voucher slides.** Two slides with protargol-impregnated specimens were deposited at the National Marine Biodiversity Institute of Korea (MABIK PR00045067, MA-BIK PR00045068).

Order Urostylida Jankowski, 1979 Family Urostylidae Bütschli, 1889 Genus *Metaurostylopsis* Song, Petz and Warren, 2001

## 2. Metaurostylopsis rubra Song and Wilbert, 2002 (Fig. 2)

**Material examined.** Marine water (salinity 36.1‰, temperature 16.8°C) collected from Sanyang-eup, Tong-

yeong-si, Gyeongsangnam-do, South Korea (34°49'16" N, 128°26'27"E) on April 17, 2023.

**Diagnosis.** Size  $68-77 \times 49-58 \ \mu\text{m}$  after protargol impregnation (n = 3); body oval to ellipsoidal with brick-reddish color; body flexible and slightly contractile; adoral zone of membranelles occupies about 50% of body length; 36-44 adoral membranelles; 1 buccal cirrus; 3 frontal, 8-9 frontoterminal, and 5-6 transverse cirri; 13-16 midventral pairs and a midventral row with about 8-12 cirri, extending to the midline of body; 10-12 left and 6-7 right marginal rows (Fig. 2A); three dorsal kineties (Fig. 2B); and about 70 macronuclear nodules.

Distribution. Antarctic, South Korea

**Remarks.** The Korean population of *Metaurostylopsis rubra* is similar to the type population of *M. rubra* described by Song and Wilbert (2002) in most features except for the slightly non-overlapping number of midventral pairs (13–16 vs. 8–11) and left marginal rows (10–12 vs. 6–9) (Song and Wilbert, 2002). *Metaurostylopsis rubra* can be easily distinguished from other congeners by the cell color and the number of midventral pairs and left marginal rows (Fig. 2A). Since the number of living

cells were not enough to examine in detail, we cannot determine whether the reddish color of the cells is due to the reddish cytoplasm or the cortical granules. Among the congeners, there is no species with more than six right marginal rows (Song *et al.*, 2020). Also, only two species, *M. struederkypkeae* and *M. parastruederkypkeae* Lu *et al.*, 2016, have reddish body color like *M. rubra*, but they differ from the present Korean population by the number of right marginal rows (3 vs. 3–5 vs. 6–7) (Shao *et al.*, 2008b; Lu *et al.*, 2016; Song *et al.*, 2020). **Voucher slides.** Two slides with protargol-impregnated specimens were deposited at the National Marine Biodiversity Institute of Korea (MABIK PR00045065, MA-BIK PR00045066).

## **CONFLICTS OF INTEREST**

The author of this paper has no affiliation with any interests and is solely responsible for the paper.

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### REFERENCES

- Berger, H. 2001. Catalogue of ciliate names 1. Hypotrichs. Verlag Helmet Berger, Salzburg.
- Chen, X., J. Huang and W. Song. 2011. Ontogeny and phylogeny of *Metaurostylopsis cheni* sp. n. (Protozoa, Ciliophora), with estimating the sytematic position of *Metaurostylopsis*. Zoologica Scripta 40(1):99-111.
- Choi, J.H., A. Omar and J.-H. Jung. 2023. New record of three Aspidisca species (Protozoa, Ciliophora) from South Korea. Journal of Species Research 12(1):90-94.
- Choi, J.H., A. Omar and J.-H. Jung. 2024. Morphology, morphogenesis, and molecular phylogeny of *Aspidisca koreana* n. sp. (Ciliophora, Euplotida) from South Korea. European Journal of Protistology 92:126037.
- Choi, J.M., J.-H. Jung, J.-H. Kang and Y.-O. Kim. 2020. New record of four ciliates (Protozoa, Ciliophora) collected from rocky intertidal pools of South Korea. Journal of Species Research 9:455-461.
- Fernandez-Leborans, G. and M.C. de Zaldumbide. 1987. A new species of the genus *Aspidisca* (Ciliophora, Hypo-

trichida). Journal of National History 21:1293-1301.

- 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.
- Jankowski, A.W. 2009. Aspidisca beringiana sp. nov. and Simbiodisca subgen. nov. (Ciliophora, Euplotida), a symbiont of terebellid polychaetes in the Bering Sea. Zoosystematica Rossica 18:184-190.
- Jiang, J., J. Huang, L. Li, C. Shao, K.A.S. Al-Rasheid, S.A. Al-Farraj and Z. Chen. 2013. Morphology of two marine euplotids (Ciliophora: Euplotida), *Aspidisca fusca* Kahl, 1928 and *A. hexeris* Quennerstedt, 1869, with notes on their small subunit rRNA gene sequences. European Journal of Protistology 49:634-643.
- Jung, J-H., Y.-S. Park, S.H. Kim, H.-G. Choi and G.-S. Min. 2011. A new marine ciliate, *Metaurostylopsis antarctica* nov. spec. (Ciliophora, Urostylida) from the Antarctic ocean. Acta Protozoologica 50:289-300.
- Kahl, A. 1932. Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 3. Spirotricha. Tierwelt Deutschlands 25:299-650.
- Kim, J.H. and J.-H. Jung. 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.
- Kim, J.H. and J.-H. Jung. 2018. New record of five ciliates (Protozoa, Ciliophora) collected in eastern areas of Gangwon-do province, Korea. Journal of Species Research 7:181-186.
- Kim, J.H., J.H. Moon and J.-H. Jung. 2018. New records of 24 ciliate species (Protozoa, Ciliophora) collected in South Korea. Journal of Species Research 7:291-314.
- Kim, J.H., A. Omar, J.H. Moon and J.-H. Jung. 2020. Taxonomy of 16 indigenous ciliate species (Protozoa, Ciliophora) from South Korea. Journal of Species Research 9:427-442.
- Kim, K.-S., K.-M. Park, J.-H. Jung and G.-S. Min. 2016. First description of three ciliates (Ciliophora: Stichotrichia) from Korea. Journal of Species Research 5(3):468-476.
- Lei, Y., J.K. Choi, K. Xu and W. Petz. 2005. Morphology and infraciliature of three species of *Metaurostylopsis* (Ciliophora, Stichotrichia): *M. songi* n. sp., *M. salina* n. sp., and *M. marina* (Kahl 1932) from sediments, saline ponds, and coastal waters. Journal of Eukaryotic Microbiology 52:1-10.
- Li, L., Q. Zhang, K.A.S. Al-Rasheid, C.B. Kwon and M.K. Shin. 2010. Morphological redescriptions of *Aspidisca magna* Kahl, 1932 and *A. leptaspis* Fresenius, 1865 (Ciliophora, Euplotida), with notes on ontogenesis in *A. magna*. Acta Protozoologica 49:327-337.
- Lu, B., C. Wang, J. Huang, Y. Shi and X. Chen. 2016. Morphology and SSU rDNA sequence analysis of two hypotrichous ciliates (Protozoa, Ciliophora, Hypotrichia)

including the new species *Metaurostylopsis parastruederkypkeae* n. sp. Journal of Ocean University of China 15(5):866-878.

- Lynn, D.H. 2008. The ciliated protozoa: Characterization, classification, and guide to the literature. New York, Springer.
- Pan, X., W.A. Bourland and W. Song. 2013. Protargol synthesis: An in-house protocol. Journal of Eukaryotic Microbiology 60:609-614.
- Park, K.-M., J.-H. Jung and G.-S. Min. 2012. Redescription of two urostylid ciliates (Ciliophora: Urostylida), *Anteholosticha pulchra* and *Metaurostylopsis struederkypkeae* from Korea. Animal Systematics, Evolution and Diversity 28(1):20-28.
- Shao, C., M. Miao, S. Weibo, W. Alan, K.A.S. Al-Rasheid, S.A. Al-Quraishy and S.A. Al-Farraj. 2008a. Studies on two marine *Metaurostylopsis* spp. from China with notes on the morphogenesis of *M. sinica* nov. spec. (Ciliophora, Urostylida). Acta Protozoologica 47:959-112.
- Shao, C., W. Song, K.A.S. Al-Rasheid, Z. Yi, X. Chen, S.A. Al-Farraj and S.A. Al-Quraishy. 2008b. Morphology and infraciliature of two new marine urostylid ciliates: *Metaurostylopsis struederkypkeae* n. sp. and *Thigmokeronopsis stoecki* n. sp. (Ciliophora, Hypotrichida) from China. Journal of Eukaryotic Microbiology 55:289-296.
- Shen, Z., J. Huang, X. Lin, Z. Yi, J. Li and W. Song. 2010. Morphological and molecular characterization of *Aspidisca hongkongensis* spec. nov. (Ciliophora, Euplotida) from the South China Sea. European Journal of Protistology 46:204-211.
- Shin, M.K. and H.S. Kim. 1988. Several hypotrichous ciliates inbabiting the Han River in Seoul. Korean Journal of Systematic Zoology Special Issue No. 2:67-85.
- Song, W., W. Petz and A. Warren. 2001. Morphology and morphogenesis of the poorly-known marine urostylid

ciliate, *Metaurostylopsis marina* (Kahl, 1932) nov. gen., nov. comb. (Protozoa, Ciliophora, Hypotrichida). European Journal of Protistology 37:63-76.

- Song, W., Y. Qiao, J. Dong, W.A. Bourland, T. Zhang and X. Luo. 2020. Ontogeny and phylogeny of a new hypotrichous ciliate (Protista, Ciliophora), *Metaurostylopsis* alrasheidi n. sp., with establishment of a new genus *Monourostylopsis* n. gen. Frontiers in Marine Science 7:602317.
- Song, W. and N. Wilbert. 1997. Morphological investigations on some free living ciliates (Protozoa, Ciliophora) from China Sea with description of a new hypotrichous genus, *Hemigastrostyla* nov. gen. Archiv Für Protistenkunde 148: 413-444.
- Song, W. and N. Wilbert. 2002. Faunistic studies on marine ciliates from the Antarctic benthic area, including description of one epizoic form, 6 new species and, 2 new genera (Protozoa: Ciliophora). Acta Protozoologica 41:23-61.
- Valbonesi, A. 1996. Description of a new species of Aspidisca, Aspidisca terranovae sp. n., from Antarctica (Ciliophora, Hypotrichida). Italian Journal of Zoology 63 (4):377-380.
- Wu, I.C.H. and C.R. Curds. 1979. A guide to the species of the genus *Aspidisca*. Bull. Br. Mus. nat. Hist. (Zool.) 36:1-34.
- Zheng, B., X. Zhao, T. Ye, J. Huang, A. Vallesi, Y. Jiang and X. Chen. 2022. Morphology and molecular phylogeny of two new *Aspidisca* species (Ciliophora, Spirotrichea, Euplotida) collected from subtropical coastal waters in China. Frontiers Marine Science 9:970692.

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