Journal of Species Research 12(1):90-94, 2023

New record of three *Aspidisca* species (Protozoa, Ciliophora) from South Korea

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The morphology and infraciliature of three newly recorded *Aspidisca* species in Korea, two collected from the eastern coast and one collected from Jeju Island, were investigated in vivo and after protargol impregnation. The three species are as follows: *A. dentata* Kahl, 1928, *A. hexeris* Quennerstedt, 1869, and *A. polystyla* Stein, 1859. The three species are characterized by having a "*polystyla*-arrangement" of frontoventral cirri: 1) *A. dentata* is characterized by having a broadly rotund body shape, a distinct peristomial spur, and a dorsal thorn; 2) *A. hexeris* is characterized by a broadly oval body shape, four projections along the left margin of body, and the single peristomial spur; and 3) *A. polystyla* has the broadly rotund body shape, transverse cirri each split into several parts (especially in vivo), and lacking of the peristomial spur. Among them, *A. dentata* and *A. polystyla* are poorly known and lack morphological description based on silver staining. In the present study, we provide a brief diagnosis, remarks, and photomicrographs.

Keywords: *Aspidisca*, brackish, Ciliophora, marine, protargol impregnation, taxonomy

DOI:10.12651/JSR.2023.12.1.090

Introduction

Up to date, 662 ciliate species have been described in Korea (National Institute of Biological Resources, 2022; Jung *et al.*, 2017). Of the 62 known *Aspidisca* species, only eight species have been recorded in Korean habitats: *A. aculeata* (Ehrenberg, 1982) Kahl, 1932; *A. cicada* (Müller, 1786) Claparède & Lachmann, 1858; *A. leptaspis* Fresenius, 1865; *A. lynceus* (Müller, 1773) Ehrenberg, 1830; *A. orthopogon* Deroux & Tuffrau, 1965; *A. polypoda* (Dujardin, 1841) Kahl, 1932; *A. steini* (Buddenbrock, 1920) Kahl, 1932; and *A. turrita* (Ehrenberg, 1831) Claparède & Lachmann, 1858 (Shin and Kim, 1988; Li *et al.*, 2010; Kim and Jung, 2018; Kim *et al.*, 2018; 2020; Choi *et al.*, 2020). During a field survey of marine and brackish water in Korean habitats, we identified three *Aspidisca* species previously unrecorded in Korea. Here, we provide a brief diagnosis, remarks, and photomicrographs in vivo and after protargol impregnation for each species. The three congeners have a similar body size, the same arrangement of frontoventral cirri, namely "*polystyla*-arrangement", and the same number of frontoventral and transverse cirri and dorsal kineties, however, they differ from each other mainly by: 1) the number of projections, 2) the absence/presence of the peristomial spur, and 3) the absence/presence of the dorsal thorn.

Materials and Methods

The three *Aspidisca* species were collected from marine water from the eastern coast of Korea and a brackish water puddle on Jeju Island, Korea. Details about sample locality are described in the 'Material examined' section for each species. Samples were collected by gently stirring up bottom sediments. The brackish water sample was transferred to the laboratory within a few days. The marine samples were immediately transferred to the laboratory. All cultures were kept in Plant culture dishes at room temperature (ca. 18°C) for about three months. Also, 1–3 wheat grains were supplied for each culture to increase the number of the bacteria as a food resource. The morphology of each species was studied using a stereomicroscope (Olympus SZ11, Japan), a light microscope (Olympus BX53) with differential interference contrast at magnifications of 40–1000×, and photomicrographs were captured using a digital camera (Olympus DP74). 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 the software of Helicon Focus 8.1.0 (HeliconSoft Ltd, Ukraine). The basic terminology and taxonomic classification mainly followed Lynn (2008) and Wu and Curds (1979).

RESULTS AND DISCUSSION

Phylum Ciliophora Doflein, 1901
Class Spirotrichea Bütschli, 1889
Subclass Hypotrichia Stein, 1859
Order Euplotida Small & Lynn, 1985
Suborder Euplotina Jankowksi, 1979
Family Aspidiscidae Ehrenberg, 1830
Genus *Aspidisca* Ehrenberg, 1830

1. *Aspidisca dentata* Kahl, 1928 (Fig. 1)

Material examined. Marine water (salinity 34.8‰, temperature 9.7°C) collected from Anin Beach, Gangdong-myeon, Gangneung-si, Gangwon-do, Korea (37°44′2″N, 128°59′26″E) on January 3, 2022.
Diagnosis. Size 33–39×29–34 μm in vivo and 26–28×21–27 μm after protargol impregnation (n=3); body broadly rotund; cortex rigid, with a single conspicuous peristomial spur along left margin, dorsal side ornamented with distinct ridges along dorsal kineties and a single thorn about 18 μm in vivo and about 10 μm after protargol impregnation (Fig. 1B, D); anterior adoral zone of membranelles (AZM1) about 4 μm long with 4 membranelles, posterior adoral zone of membranelles (AZM2) about 10 μm long after protargol impregnation and with 10–12 membranelles; 7 frontoventral cirri in "*polystyla*-arrangement"; 5 transverse cirri, only the leftmost cirrus splits into 2 parts (Fig. 1A, C); 4 dorsal kineties with 4–6, 6–9, 5–8, and 6 dikinetids in dorsal kineties 1–4, respectively; cytoplasm colorless; 1 horseshoe-shaped macronucleus, micronucleus not observed.
Distribution. Germany, North Sea, South Korea.
Remarks. *Aspidisca dentata* is a poorly known species and lacks sufficient morphological data. The Korean population of *A. dentata* is similar to the type population described by Kahl (1928) in all available aspects except the number of transverse cirri. Kahl (1928) reported that *A. dentata* has six transverse cirri, however, the Korean population usually has five transverse cirri, of which two parts are distinguishable in vivo and after protargol impregnation from the leftmost cirrus (Fig. 1A, C), giving the impression that it has six transverse cirri. Considering the presence of a distinct thorn on the dorsal side, three species namely *A. aculeata*, *A. herbicola* Kahl, 1932, and *A. turrita* should be compared to *A. dentata*. Both *A. herbicola* and *A. turrita* differ from *A. dentata* in having '*lynceus*-arrangement' (vs. '*polystyla*-arrangement') of frontoventral cirri (Wu and Curds, 1979; Foissner, 1994). Also, *A. herbicola*, which has both peristomial spur and dorsal thorn, is a freshwater species, while *A. dentata* is a marine species (Wu and Curds, 1979). *Aspidisca dentata* can be easily distinguished from both *A. aculeata* and *A. turrita* by the presence (vs. absence) of a peristomial spur (Kahl, 1928; 1932; Wu and Curds, 1979; Li *et al*., 2008).
Voucher slides. One slide with protargol-impregnated specimens was deposited at the National Marine Biodiversity Institute of Korean (MABIK PR00044190).

2. *Aspidisca hexeris* Quennerstedt, 1869 (Fig. 2)

Material examined. Brackish water (salinity 12.8‰, temperature 33.5°C) collected from Yongdumbeong, Sinyang-ri, Chuja-myeon, Jeju-si, Jeju-do, Korea (33°57′38.90″N, 126°17′7.30″E) on August 19, 2021.
Diagnosis. Size 32–33×26–29 μm in vivo and 23–30×21–25 μm after protargol impregnation (n=7); body shape broadly oval; cortex rigid with a single peristomial spur and 4 projections along left margin (Fig. 2A–C); AZM1 about 7 μm long with 7–8 membranelles, AZM2 about 10 μm long after protargol impregnation and with 10–12 membranelles; 7 frontoventral cirri in "*polystyla*-arrangement"; 5 transverse cirri, each transverse cirrus not separated except the leftmost one, which splits into 2 parts; 4 dorsal kineties with 8–9, 8–10, 8–12, 9–10 dikinetids in dorsal kineties 1–4, respectively; cytoplasm colorless; 1 macronucleus horseshoe-shaped, micronucleus not observed (Fig. 2D, E).
Distribution. China, South Korea.
Remarks. The Korean population of *Aspidisca hexeris* is similar to the Chinese population in all features except the body size (23–30×21–25 μm vs. 34–43×25–36 μm after protargol impregnation) (Jiang *et al*., 2013). *Aspidisca hexeris* is unique among the genus *Aspidisca* in having one conspicuous peristomial spur and four small projections along the left margin. Although Wu and Curds (1979) mentioned that the number of projections is highly variable among populations and Jiang *et al*. (2013) argued that the number of projections is an intraspecific difference, nonetheless, all specimens observed in this study have invariably four projections. Considering the presence of a peristomial spur and the "*polystyla*-arrangement" of frontoventral cirri, *A. leptaspis* and *A. magna* Kahl, 1932 are very similar to *A. hexeris*. However, *A. leptaspis* has eight (vs. 7) frontoventral cirri and has more membranelles in the AZM2 (14–21 vs. 10–12) (Li *et al*., 2010). *Aspidisca magna* is larger than *A. hexeris* (50–160×40–115 in vivo vs. 32–33×26–29), has

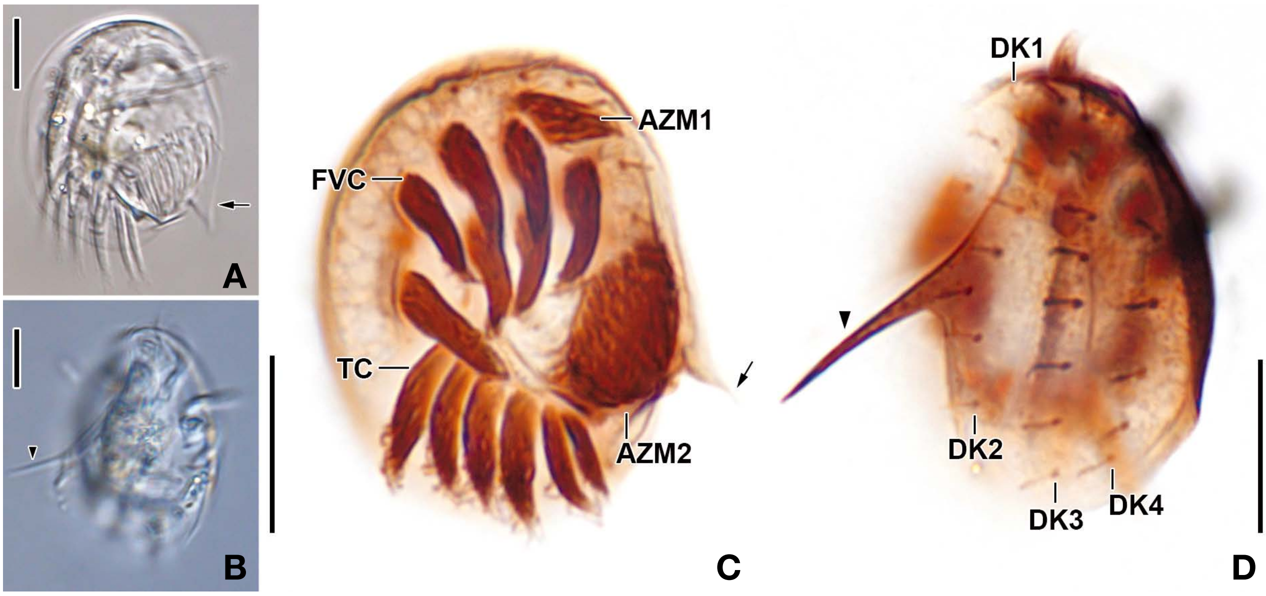

Fig. 1. *Aspidisca dentata* in life (A, B) and after protargol impregnation (C, D). A, B, Ventral (A) and dorsal (B) view, showing the body shape, the prominent peristomial spur (arrow), and the dorsal thorn (arrowhead). C, D, Ventral (C) and dorsal (D) view, showing the anterior and posterior portion of adoral zone of membranelles, the transverse cirri, and the frontoventral cirri in "*polystyla*-arrangement". The arrow marks the peristomial spur and the arrowhead denotes the dorsal thorn. AZM1, adoral zone of membranells 1; AZM2, adoral zone of membranelles 2; DK1-4, dorsal kineties; FVC, frontoventral cirri; TC, transverse cirri;. Scale bars = 10 μm.

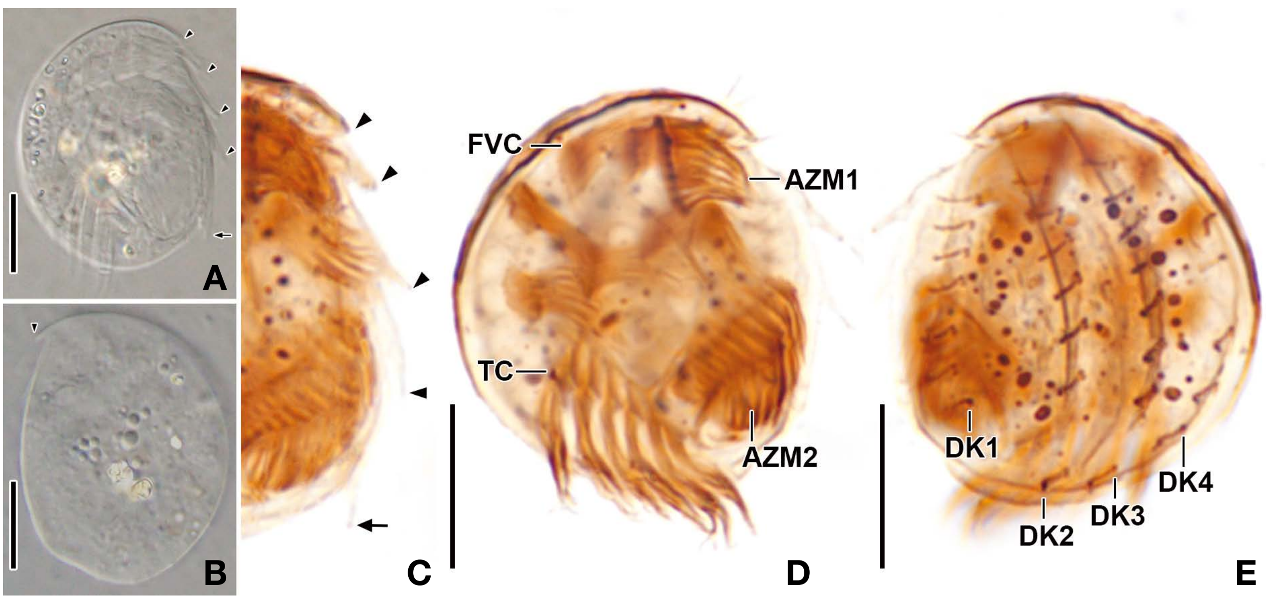

Fig. 2. *Aspidisca hexeris* in life (A, B) and after protargol impregnation (C-E). A, B, Ventral (A) and dorsal (B) view, showing the body shape, the peristomial spur (arrow), and the four small projections (arrowheads) along the left margin. C, Ventral view showing the projections (arrowheads) and the peristomial spur (arrow) along the left margin. D, E, Ventral (D) and dorsal (E) view, showing the anterior and posterior portion of adoral zone of membranelles, the frontoventral cirri, the five transverse cirri, and the projections along left margin of body. AZM1, adoral zone of membranells 1; AZM2, adoral zone of membranelles 2; DK1-4, dorsal kineties; FVC, frontoventral cirri; TC, transverse cirri. Scale bars = 10 μm.

more frontoventral cirri (7-9 vs. invariably 7), and also has more membranelles in the AZM2 (15-18 vs. 10-12) (Li *et al*., 2010; Huang *et al*., 2011; Jiang *et al*., 2013). Furthermore, both *A. dentata* and *A. hexeris* possess

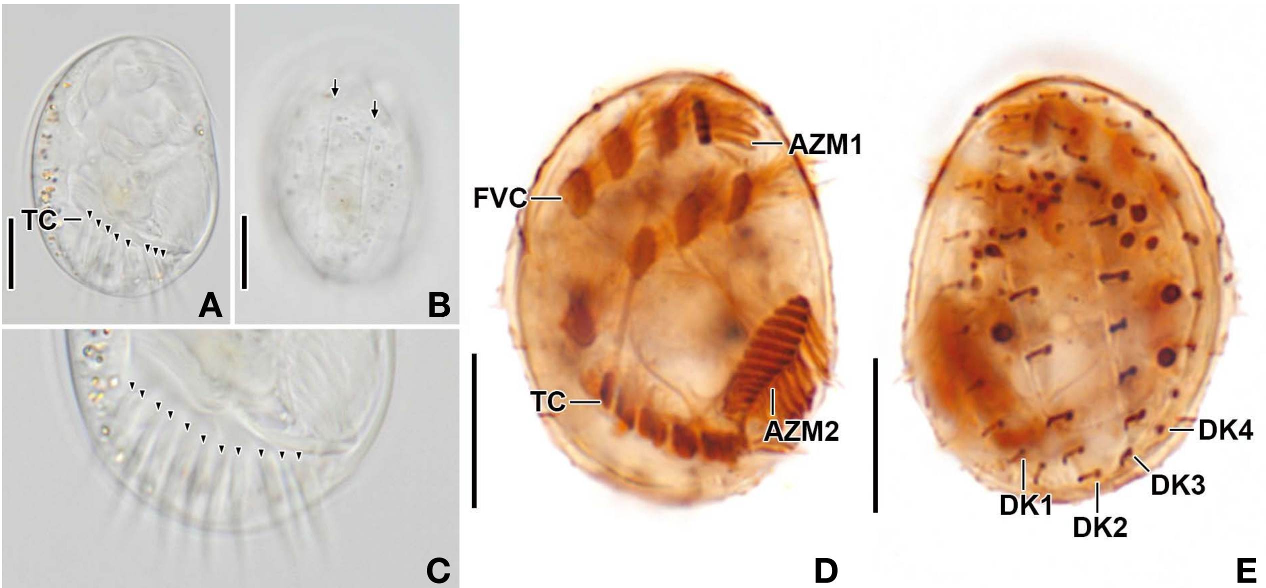

Fig. 3. *Aspidisca polystyla* in life (A–C) and after protargol impregnation (D, E). A, B, Ventral (A) and dorsal (B) view, showing the body shape, the transverse cirri (arrowheads), and the dorsal ridges (arrows). C, Ventral view showing the transverse cirri, each of which separated into two or three parts (arrowheads). D, E, Ventral (D) and dorsal (E) view, showing the anterior and posterior portion of adoral zone of membranelles and the five transverse cirri. Note the split occurs in the cilia of cirri while the bases are ordinary. AZM1, adoral zone of membranelles 1; AZM2, adoral zone of membranelles 2; DK1–4, dorsal kineties; FVC, frontoventral cirri; TC, transverse cirri. Scale bars = 10 μm.

the peristomial spur, however, they differ mainly in the absence (vs. presence) of the projections along the left margin.

Voucher slides. Three slides with protargol-impregnated specimens were deposited at the National Marine Biodiversity Institute of Korean (MABIK PR00044187, PR00044188, and PR00044189).

3. *Aspidisca polystyla* Stein, 1859 (Fig. 3)

Material examined. Marine water (salinity 40.0‰, temperature 11.1°C) collected from Anin Beach, Gangdong-myeon, Gangneung-si, Gangwon-do, Korea (37° 44′3″N, 128°59′25″E) on February 28, 2022.

Diagnosis. Size 31–38 × 26–28 μm in vivo and 28–37 × 22–29 μm after protargol impregnation (n = 8); body shape broadly rotund; cortex rigid, without the peristomial spur and projections along left margin; AZM1 about 2 μm long invariably with 4 membranelles, AZM2 about 10 μm long after protargol impregnation and with 11–14 membranelles; 7 frontoventral cirri in "*polystyla*-arrangement"; 5 transverse cirri, (Fig. 3A, C, D), transverse cirrus 1 (leftmost) splits into 2 or 3 parts, cirri 2, 3, and 5 usually split into 2 parts, transverse cirrus 4 consists of one or two parts; 4 dorsal kineties with 8–9, 8–10, 8–12, and 9–10 dikinetids in dorsal kineties 1–4, respectively (Fig. 3B, E); cytoplasm colorless; 1 horseshoe-shaped macronucleus, micronucleus not observed.

Distribution. Italy, Baltic Sea, South Korea.

Remarks. Within the genus *Aspidisca*, *A. polystyla* seems to possess the highest number of transverse cirri. Stein (1859) reported that *A. polystyla* has 10–12 transverse cirri. Other investigations suggest that it has up to 15 transverse cirri (Plough, 1915; Tuffrau, 1964). However, Kahl (1932) reported that *A. polystyla* has 5–6 transverse cirri but it looks like it has higher number of transverse cirri because each transverse cirrus splits into two or more parts. According to our observations on the protargol-impregnated specimens of the Korean population (Fig. 3D), *A. polystyla* has only five transverse cirri, although it looks like it has eleven or more in vivo (Fig. 3C), suggesting that the split occurs only in the cilia while the bases of cirri are ordinary. Other members of genus *Aspidisca*, which have seven frontoventral cirri in "*polystyla*-arrangement" and without the peristomial spur (*A. major* (Madsen, 1931) Kahl, 1932, *A. steini*), can be easily separated from *A. polystyla*. *Aspidisca major* differs from *A. polystyla* by the larger body size (60–90 μm vs. 31–38 × 26–28 μm) (Kahl, 1932), the ordinary (vs. separated) transverse, and the two (vs. 1) macronuclear nodules (Wu and Curds, 1979). Also, the Chinese population of *A. steini* is most similar to *A. polystyla*, but *A. steini* differs from *A. polystyla* by the transverse cirri (the leftmost cirri separated into two parts vs. almost all cirri separated into 1–3 parts) (Wu and Curds, 1979; Song and Wilber, 1997).

Voucher slides. Three slides with protargol-impregnated specimens were deposited at the National Marine Biodiversity Institute of Korean (MABIK PR00044191, PR00044192, and PR00044193).

Acknowledgements

This work was supported by grants from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR202333201) and from National Marine Biodiversity Institute of Korea (2022M01100).

References

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.

Foissner, W. 1994. Die Chinesenmütze (*Aspidisca turrita*) - ein seltsames Wimpertierchen. Mikrokosmos 83:175-179.

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.

Huang, J., Z. Yi, M. Miao, K.A.S. Al-Rasheid and W. Song. 2011. Phylogenetic relationships within the genus *Aspidisca* (Protozoa, Ciliophora, Euplotida) revealed by ITS1-5.8S-ITS2 region sequences. Chinese Journal of Oceanology and Limnology 29:277-283.

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., M.-H. Park, S.Y. Kim, J.M. Choi, G.-S. Min and Y.-O. Kim. 2017. Checklist of Korean ciliates (Protozoa: Ciliophora). Journal of Species Research 6:241-257.

Kahl, A. 1928. Die Infusorien (Ciliata) der Oldesloer Salzwässerstellen. Archiv für Hydrobiologie 19:189-246.

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., 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, 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.

Li, L., C. Shao, Z. Yi, W. Song, A. Warren, K.A.S. Al-Rasheid, S.A. Al-Farraj, S.A. Al-Quraishy, Q. Zhang, X. Hu, M. Zhu and H. Ma. 2008. Redescriptions and SSrRNA gene sequence analyses of two marine species of *Aspidisca* (Ciliophora, Euplotida), with notes on the morphogenesis in *A. orthopogon*. Acta Protozoologica 47:83-94.

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.

Lynn, D.H. 2008. The ciliated protozoa: Characterization, classification, and guide to the literature. New York, Springer.

National Institute of Biological Resources (NIBR). 2022. National list of Korea. National Institute of Biological Resources. Incheon. Korea [Available from: http://kbr.go.kr/, accessed 27 Sep. 2022].

Pan, X., W.A. Bourland and W. Song. 2013. Protargol synthesis: An in-house protocol. Journal of Eukaryotic Microbiology 60:609-614.

Plough, H.H. 1916. The genus *Aspidisca* Ehrenberg. Transactions of the American Microscopical Society 35:233-244.

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. 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.

Stein, F. 1859. Der Organismus der Infusionsthiere nach eigenen Forschungen in systematischer Reihenfolge bearbeitet. I. Abtheilung. Allgemeiner Theil und Naturgeschichte der hypotrichen Infusionsthiere. Leipzig.

Tuffrau, M. 1964. La morphogenèse de bipartition et les structures neuromotriees dans le genre *Aspidisca* (ciliés hypotriehes). Revue de que1quelques espèces. Cahiers de Biologie Marine 5:173-199.

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.

Submitted: September 28, 2022
Accepted: November 23, 2022