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New Record of Two Oligohymenophorean Ciliates (Protozoa: Ciliophora) from Korea

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ABSTRACT

During a field survey on Korean coastal marine waters, two ciliates belonging to the genera *Pseudovorticella* Foissner & Schiffmann, 1975 and *Frontonia* Ehrenberg, 1838 in the class Oligohymenophorea de Puytorac et al., 1974, were collected. Even though the two genera are very common in the aquatic ecosystems, they had been superficially studied in Korean habitats. Using the observation of living cells and specimens impregnated by protargol, silver carbonate, and silver nitrate, two newly recorded ciliate are identified as *P. paramarina* Sun, Ji, Warren & Song, 2009 and *F. ocularis* Bullington, 1939. A brief diagnosis, remark, and comprehensive micrographs are provided for each species.

Keywords: diversity, free-living eukaryote, marine ciliates, Oligohymenophorea, taxonomy

INTRODUCTION

The genera *Pseudovorticella* and *Frontonia* are common taxa in aquatic environments, sometimes even in terrestrial habitats, and contain more than sixty and thirty species, respectively (Warren, 1987; Foissner et al., 2002; Berger and Foissner, 2003; Song et al., 2009). According to the 'National Species List of KOREA' (Kwon et al., 2019) and resent studies since then (Park and Min, 2019; Kim et al., 2020a, 2020b; Omar and Jung, 2021), only eight species have been reported in Korea, that is their diversity is highly overlooked.

In comparison with spirotrichean ciliates (i.e., about three hundred species have been reported in Korea), oligohymenophorean ciliates have been poorly investigated (Kwon et al., 2019; Jung, 2021). In this study, we intended to collect Korean oligohymenophoreans and two oligohymenophorean ciliates from coastal water samples were identified based on the observation of living cells and stained specimens. Brief diagnosis, remark, and micrographs are provided for each species. The voucher slides have been deposited in the National Marine Biodiversity Institute of Korea.

MATERIALS AND METHODS

Oligohymenophorean ciliates were collected from coastal water samples from Gangneung-si, Korea. The information of sampling sites is provided in the 'Material examined' section for each species. The samples were transferred to the laboratory and cultured in plant culture dishes (SPL Life Science, Korea) at room temperature of about 20−25°C. In each dish, two or three sterilized wheat grains were added to stimulate the growth of bacteria as food source.

Living cells and stained specimens were examined using a stereomicroscope (Olympus SZ11, Japan), an inverted microscope (Olympus IX73) at a magnification of ×40−400, and an optical microscope (Olympus BX53) at a magnification of ×40−1,000, and photomicrographs were taken using a digital camera (Olympus DP74). Protargol was synthesized using the method described by Pan et al. (2013a) with slight modification (Kim and Jung, 2017). The 'procedure A' method and acetone developer were used for protargol impregnation (Foissner, 2014). For wet silver nitrate preparation of *Pseudovorticella paramarina* and silver carbonate preparation of *Frontonia ocularis*, I followed the methods of Foissner (2014). The differential through-focal micrographs of the stained cells were merged using the software of Helicon Focus 7.6.4 (HeliconSoft Ltd., Ukraine). The general classification follows Lynn (2008). Types of micronuclei in *Frontonia* follows Fokin (2010).

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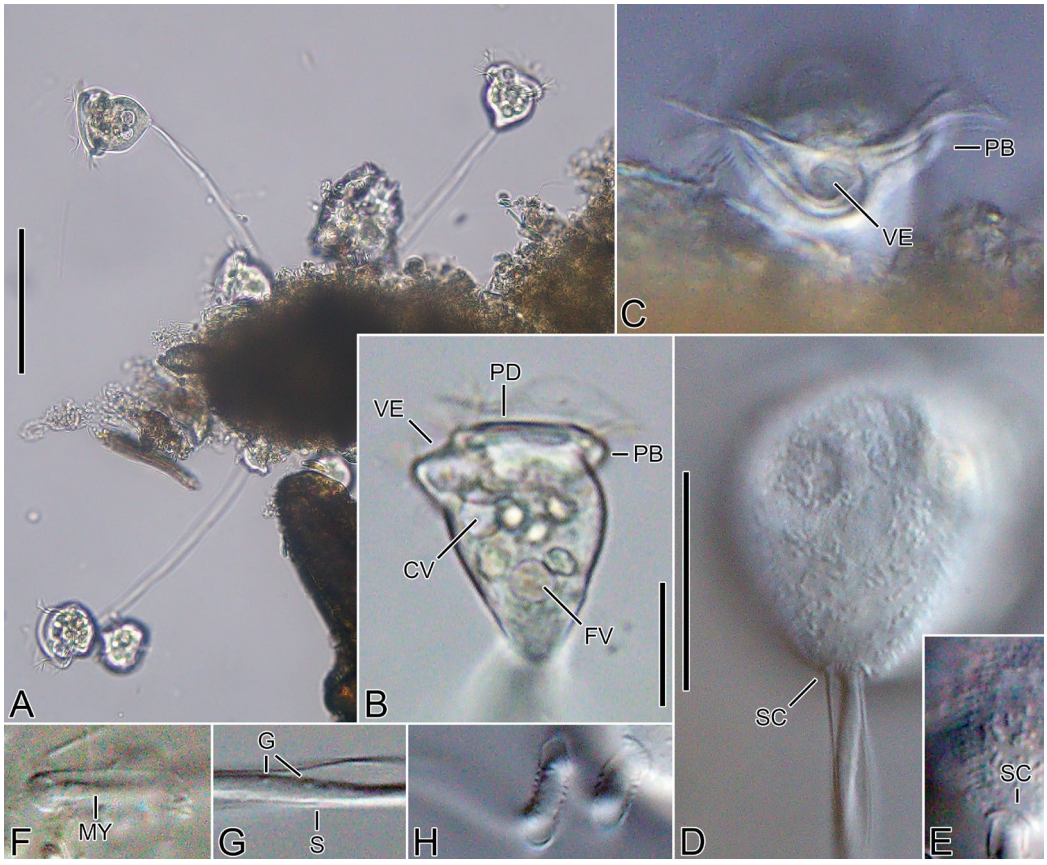

Fig. 1. *Pseudovorticella paramarina* from life. A, Representative specimens showing the body shape and stalks; B, Zooid showing the body outline and the position of the contractile vacuole; C, Top-lateral view of a zooid showing vestibular entrance; D, E, Bottom-lateral views showing scopula and transverse striations; F–H, Stalks showing stalk myoneme and colorless granules. CV, contractile vacuole; FV, food vacuole; G, granules; MY, myoneme; PB, peristomial bulge; PD, peristomial disc; S, stalk; SC, scopula; VE, vestibular entrance. Scale bars: A = 100 μm, B, D = 25 μm.

RESULTS AND DISCUSSION

Phylum Ciliophora Doflein, 1901
Class Oligohymenophorea de Puytorac et al., 1974
Subclass Peritrichia Stein, 1859
Order Sessilida Kahl, 1933
Family Vorticellidae Ehrenberg, 1838
[1]*Genus *Pseudovorticella* Foissner and Schiffmann, 1975

[2]****Pseudovorticella paramarina*** **Sun, Ji, Warren and Song, 2009 (Figs. 1A–H, 2A–E)**

Pseudovorticella paramarina Sun, Ji, Warren and Song, 2009: 236, figs. 7.6F, 7.7H–J; Mayén-Estrada and Utz, 2018: 322.

Material examined. Coastal water sample (temperature: 18.2°C; salinity: 36.1‰) from Gangdong-myeon, Gangneung-si, Gangwon-do, Republic of Korea (37°44′4″N, 128°59′24″E) in Jun 2021.

Diagnosis. Zooid 26–57 × 26–46 μm, 46.9 × 39.4 μm on average, *in vivo*; zooid bell-shaped with peristomial bulge (lip), stalk 80–180 μm long and about 5 μm wide. Macronucleus J-shaped. Single contractile vacuole at ventral wall of vestibulum. Pellicle smooth and cytoplasm colorless; 21–27 transverse silverlines between peristome and aboral trochal band, 8–14 silverlines between aboral trochal band and scopula. Adoral ciliary spiral about 1.3 turns around peristomial disc; peniculi 1–3 composed of 3 kineties each; kinety 1 of peniculus 3 distinctly proximally shortened than kineties 2 and 3 which are closely spaced together.

Remarks. The Korean population of *P. paramarina* resembles the type population from China (Song et al., 2009), but the former has slightly more transverse silverlines between

Korean name: [1]*유사종벌레(신칭; *Pseudovorticella*속에 대한 기존 국명은 없었으나[국가생물종목록집, 2020] *Pseudovorticella difficilis*가 마디유사종벌레로 기록되어 있어 '유사종벌레'로 지칭함), [2]*긴바다유사종벌레(신칭)

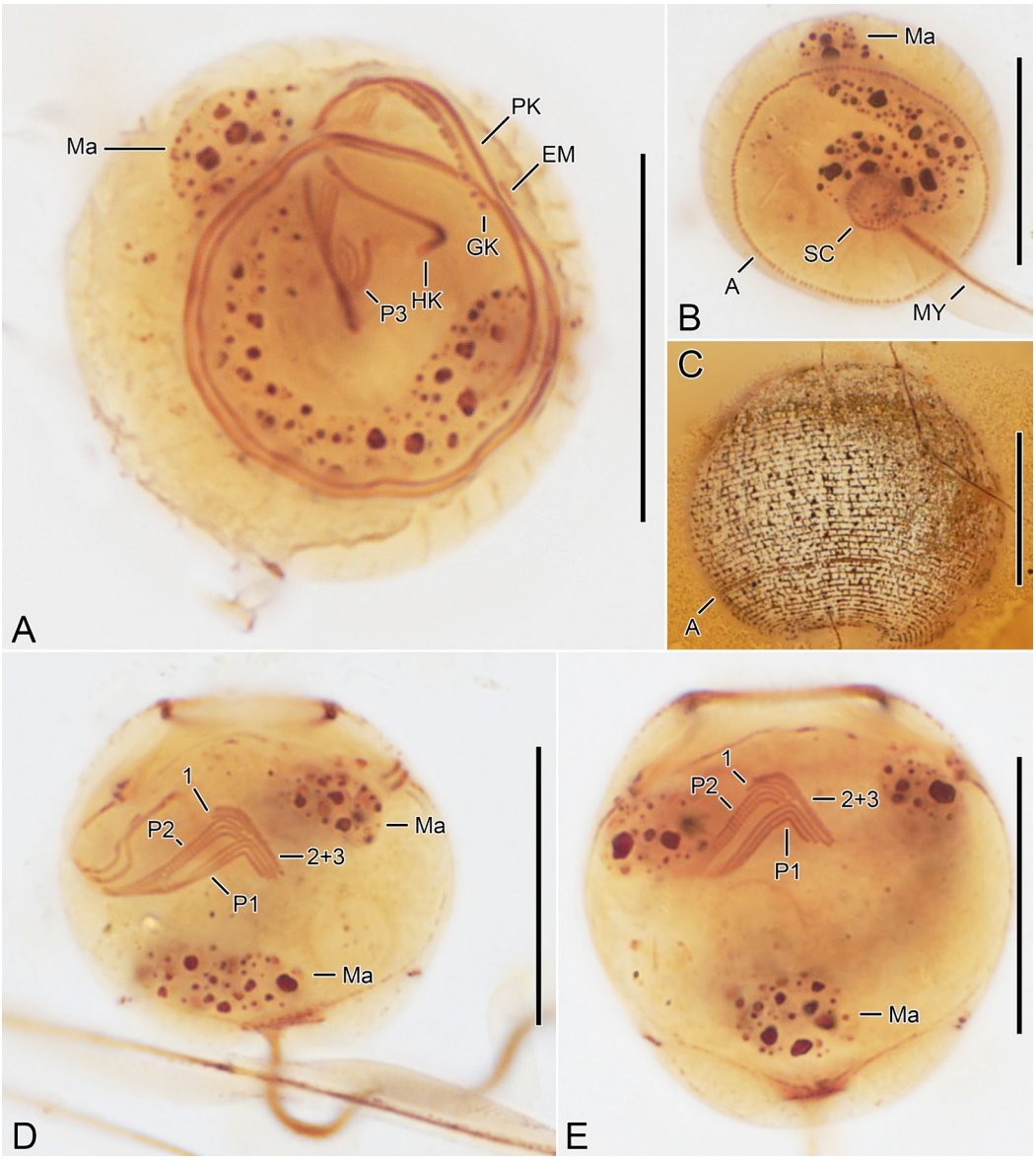

Fig. 2. *Pseudovorticella paramarina* after protargol (A, B, D, E) and wet silver nitrate (C) impregnation. A, Top view showing oral ciliary pattern and macronucleus; B, Bottom view showing aboral ciliary wreath, scopula, and stalk; C, Silverline system; D, E, Lateral views showing peniculi 1–3. A, anlage of aboral ciliary wreath; EM, epistomial membrane; GK, germinal kinety; HK, haplokinety; Ma, macronucleus; MY, myoneme; PK, polykinety; P1–3, peniculi 1–3; SC, scopula; 1–3, kineties 1–3 in peniculus 3. Scale bars: A–E = 20 μm.

the peristome and aboral trochal band (21–27 vs. 19–25) and slightly longer kinety 1 in the peniculus 3 (1/2 vs. 1/3 of kinety 2 length). It might be a variation depending on populations and/or environments. As shown in the species-group name '*paramarina*', it is highly similar to *P. marina* (Greeff, 1870) Ji, Sun, Song and Warren in Sun, Ji, Warren and Song, 2009. However, *P. paramarina* can be easily distinguished from *P. marina* by the length of kinety 1 in the peniculus 3 (distinctly shorter than kinety 2 vs. as long as kinety 2) and the arrangement of the kineties 2 and 3 (closely spaced vs. separated) (Song et al., 2009).

Voucher slide. One slide with protargol-impregnated specimens was deposited at the National Marine Biodiversity Institute of Korea (MABIK PR00043270).

Order Peniculida Fauré-Fremiet in Corliss, 1956
Family Frontoniidae Kahl, 1926
Genus *Frontonia* Ehrenberg, 1838

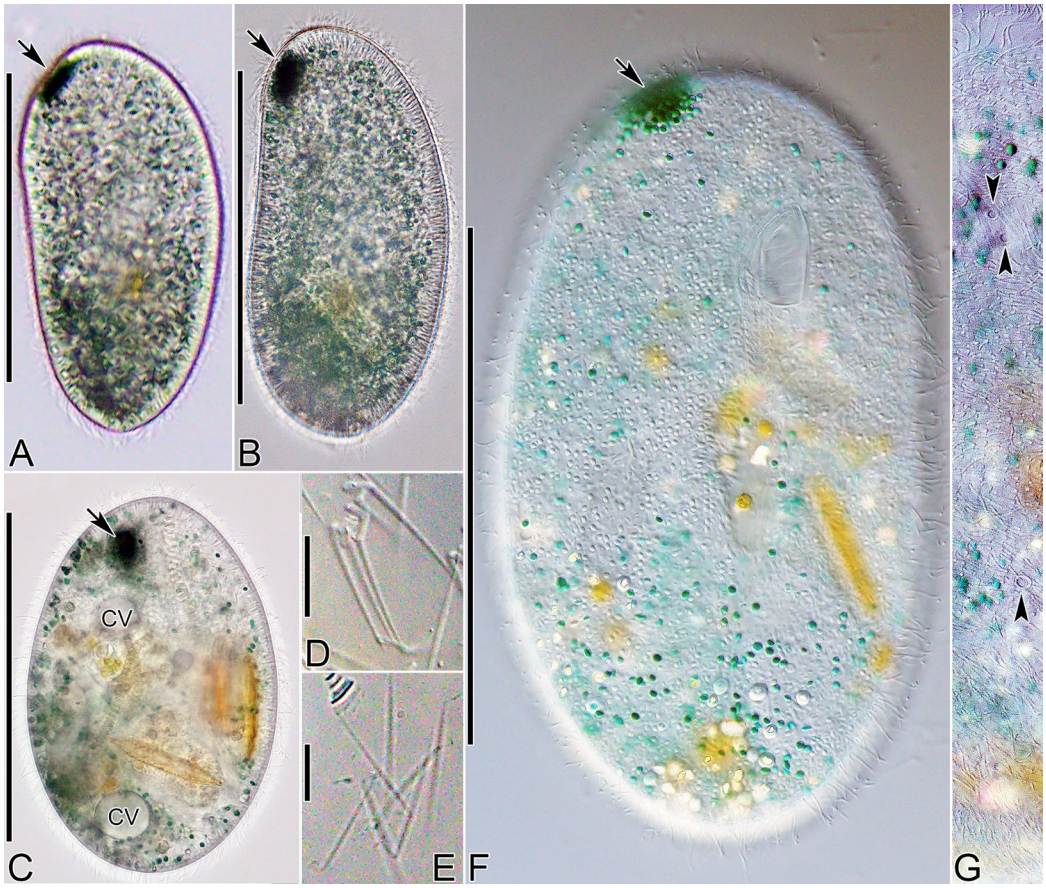

Fig. 3. *Frontonia ocularis* from life. A, B, Representative specimens showing body outline and spot of pigment granules (arrows); C, G, Pigment spot (arrow in C) and contractile vacuoles (arrowheads in G denote pores of contractile vacuoles); D, E, Extruded extrusomes; F, Ventral view showing the pigment granules scattered in entire cytoplasm and the spot on the anterior right body portion (arrow). CV, contractile vacuoles. Scale bars: A–C, F = 100 μm, D, E = 10 μm.

[1]***Frontonia ocularis* Bullington, 1939**
(Table 1, Fig. 3A–G, 4A–F)

Frontonia ocularis Bullington, 1939: 42, Pl. 6 (figs. 1–5), fig. 8, Pl. 9 (fig. 6), fig. 11, tables 1–7; Pan, Liu, Yi, Fan, Al-Rasheid and Lin, 2013b: 40, figs. 2, 3, tables 1, 2.

Material examined. Coastal water sample (temperature: 22.0°C; salinity: 35.0‰) collected from Gangneung-si, Gangwon-do, Republic of Korea (37°47′51″N, 128°54′45″E) in Jun 2021.

Diagnosis. Size about 130 × 50 μm *in vivo*, 69.5–103.2 × 36.1–52.7 μm after protargol impregnation; body somewhat kidney-shaped. Single ellipsoidal macronucleus, 22.7–34.7 × 10.2–17.4 μm in size; 1–4 globular to ellipsoidal micronuclei, compact type, attached to macronucleus, 1.9–2.3 × 1.4–2.0 μm in size. 2 contractile vacuoles on dorsal side, without collecting canals, each with 1 or 2 pores. Extrusomes distinct and colorless, forming seam underneath pellicle. Blue-greenish pigment granules scattered throughout cytoplasm and gathered as a spot on anterior right body portion. 87–112 somatic kineties. Peniculi 1–3 composed of 4, 4, and 2 ciliary rows, respectively. 3 vestibular and 4 postoral kineties; paroral membrane double-rowed.

Remarks. The marine ciliate *Frontonia ocularis* has prominent pigment spot probably as shown in the species-group name (unfortunately its etymology is unavailable in the original description). Bullington (1939) mentioned that the color of the pigment spot is reddish brown, but Pan et al. (2013b) reported the Chinese population with brown-black pigment spot and considered it as a population-dependent variation. The color variation of the pigment spot is necessary to confirm whether it is population-dependent or not.

Serra et al. (2021) described an Italian population of *F. fusca* Quennerstedt, 1869 and they considered *F. ocularis* as a junior

Korean name: [1]*눈전구섬모충(신칭)

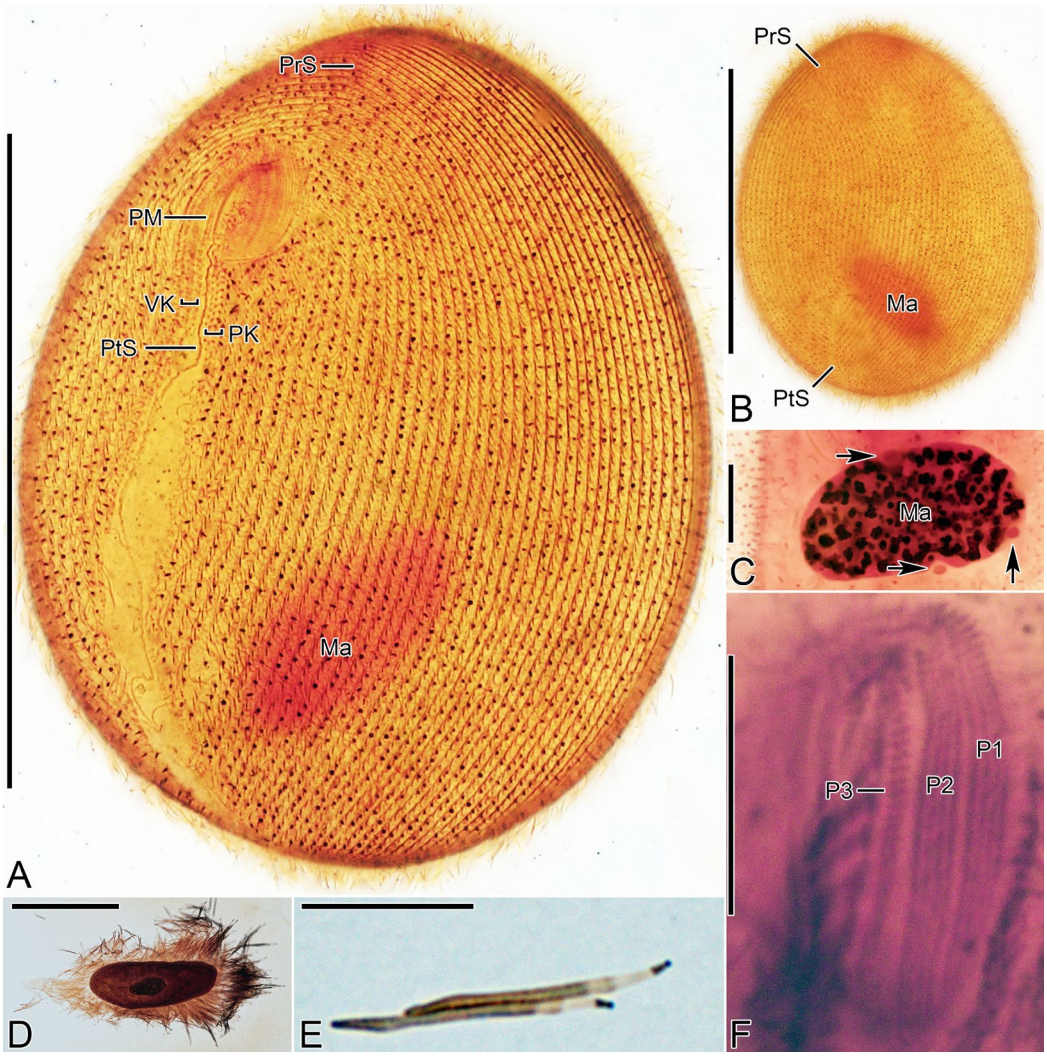

Fig. 4. *Frontonia ocularis* after silver carbonate (A, B) and protargol (C–F) impregnation. A, Ventral view showing ciliary pattern and macronucleus; B, Dorsal view showing somatic kineties and macronucleus; C, Nuclear apparatus, arrows denote micronuclei; D, E, Extruded extrusomes; F, Peniculi. Ma, macronucleus; PM, paroral membrane; PK, postoral kineties; PrS, preoral suture; PtS, postoral suture; P1–3, peniculi 1–3; VK, vestibular kineties. Scale bars: A, B, D = 100 μm, C, E, F = 10 μm.

Table 1. Comparison of morphological characteristics of *Frontonia ocularis* populations and the similar species *F. fusca*

Characteristics	*Frontonia ocularis* populations			*F. fusca*
	USA (type)	China	Korea	Italy
Body length *in vivo* (μm)	102–200	115–140	ca. 130	90–150 (stained)
Color of pigment spot	Reddish brown	Brown-black	Blue-greenish	Unavailable
Collecting canals of contractive vacuoles	Absent	Absent	Absent	Present
Pores on a contractile vacuole, number	Unavailable	1[a]	1 or 2	2 or 3
Micronuclei, type	Unavailable	Unavailable	Compact	Endosomal
Somatic kineties, number	Unavailable	93–107	87–112	75–92
Ciliary rows in peniculi 1–3, number	Unavailable	4, 4, 2	4, 4, 2	4, 4, 3
Data source	Bullington (1939)	Pan et al. (2013b)	This study	Serra et al. (2021)

[a]Data obtained from an illustration (fig. 2F; Pan et al., 2013b).

synonym of *F. fusca* based on morphological and gene sequence similarity. However, the contractile vacuoles of the Italian population have collecting canals. Also, Serra et al. (2021) did not mention the color of the pigment spot. Among the populations examined by stained specimens, the Italian population differs from the Chinese and Korean population by the number of pores on the contractile vacuoles (two or three vs. one or two in Korean pop.), the type of micronuclei (endosomal vs. compact in Korean pop.), the somatic kineties (75−92 vs. 87−112 in Korean pop. and 93−107 in Chinese pop.), and the ciliary rows in peniculi (4, 4, 3 vs. 4, 4, 2 in both Asian pops.) (Pan et al., 2013b; Serra et al., 2021). Even though the Italian and Chinese populations showed a high genetic similarity of 99.7% using nuclear small subunit ribosomal RNA gene, they show distinct morphological differences as mentioned above.

Voucher slide. One slide with protargol-impregnated specimens was deposited at the National Marine Biodiversity Institute of Korea (MABIK PR00043271).

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CONFILICTS OF INTEREST

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

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REFERENCES

Berger H, Foissner W, 2003. Illustrated guide and ecological notes to ciliate indicator species (Protozoa, Ciliophora) in running waters, lakes, and sewage plants. In: Handbuch angewandte Limnologie, Vol. 17 (Eds., Steinberg C, Calmano W, Klapper W, Wilken RD). Ecomed Verlagsgesellschaft, Landsberg am Lech, pp. 1-160.

Bullington WE, 1939. A study of spiralling in the ciliate *Frontonia* with a review of the genus and description of two new species. Archiv Für Protistenkunde, 92:10-66.

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

Foissner W, Agatha S, Berger H, 2002. Soil ciliates (Protozoa, Ciliophora) from Namibia (Southwest Africa), with emphasis on two contrasting environments, the Etosha region and the Namib desert. Part I: Text and line drawings. Denisia, 5:1-1063.

Fokin SI, 2010. *Paramecium* genus: biodiversity, some morphological features and the key to the main morphospecies discrimination. Protistology, 6:227-235.

Jung JH, 2021. Taxonomy of four scuticociliates (Protozoa: Ciliophora) from coastal waters of South Korea. Journal of Species Research, 10:184-190.

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 JH, Omar A, Jung JH, 2020a. Brief description of 18 newly recorded ciliate species from soil and inland waters (Protozoa, Ciliophora) in South Korea. Journal of Species Research, 9:251-268. https://doi.org/10.12651/JSR.2020.9.3.251

Kim JH, Omar A, Moon JH, Jung JH, 2020b. Taxonomy of 16 indigenous ciliate species (Protozoa, Ciliophora) from South Korea. Journal of Species Research, 9:427-442. https://doi.org/10.12651/JSR.2020.9.4.427

Kwon CB, Shin MK, Kim SY, Kim SJ, Kim YO, Jung JH, 2019. Phylum CILIOPHORA. In: National species list of Korea. II. Vertebrates, invertebrates, protozoans (Ed., National Institute of Biological Resources). Designzip, Seoul, pp. 673-697 (in Korean).

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

Mayén-Estrada R, Utz LRP, 2018. A checklist of species of Vorticellidae (Ciliophora: Peritrichia) epibionts of crustaceans. Zootaxa, 4500:301-328. https://doi.org/10.11646/zootaxa.4500.3.1

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

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

Pan X, Liu W, Yi Z, Fan X, Al-Rasheid KAS, Lin X, 2013b. Studies on three diverse *Frontonia* species (Ciliophora, Peniculida), with brief notes on 14 marine or brackish congeners. Acta Protozoologica, 52:35-49. https://doi.org/10.4467/16890027AP.13.004.0832

Park MH, Min GS, 2019. New records of one marine and two soil ciliates (Ciliophora: Intramacronucleata) from Korea. Journal of Species Research, 8:113-115. https://doi.org/10.12651/JSR.2019.8.1.113

Quennerstedt A, 1869. Bidrag till Sveriges Infusorie-fauna III. Acta Universitatis Lundensis, 6:1-35.

Serra V, D'Alessandro A, Nitla V, Gammuto L, Modeo L, Petroni G, Fokin SI, 2021. The neotypification of *Frontonia vernalis* (Ehrenberg, 1833) Ehrenberg, 1838 and the description of *Frontonia paravernalis* sp. nov. trigger a critical revision of frontoniid systematics. BMC Zoology, 6:4.

Song W, Warren A, Hu X, 2009. Free-living ciliates in the Bohai and Yellow Seas, China. Science Press, Beijing, pp. 1-518.

Sun P, Ji D, Warren A, Song W, 2009. 7. Solitary sessilid peritrichs. In: Free-living ciliates in the Bohai and Yellow Seas, China (Eds., Song W, Warren A, Hu X). Science Press, Beijing, pp. 217-256.

Warren A, 1987. A revision of the genus *Pseudovorticella* Foissner & Schiffmann, 1974 (Ciliophora: Peritrichida). Bulletin of the British Museum (Natural History) Zoology, 52:1-12.

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