Journal of Species Research 9(1):68-76, 2020

New record of five ciliate species from temporary ponds on a grass lawn

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We identified 22 ciliate species, including five unrecorded ciliate species, from temporary ponds on a grass lawn. The five unrecorded species are as follows: class Nassophorea - *Pseudomicrothorax agilis* Mermod, 1914, *Nassula exigua* Kahl, 1931, class Colpodea - *Cyrtolophosis mucicola* Stokes, 1885, *Maryna ovata* (Gelei, 1950) Foissner, 1993, and class Spirotrichea - *Meseres corlissi* Petz & Foissner, 1992. Most of these 22 ciliate species disappeared from a raw culture within a few days (probably encystment), and a few cells were available from some species that resulted in incomplete identifications (e.g., genus-level). About the unrecorded five ciliate species, they are small in size (< 60 μm in vivo), and two of them live in a hyaline dwelling-tube, which is easily deserted by a cell with a stress. Their taxonomic classification is summarized as three classes, five orders, five families, and five genera. Here, we provide brief descriptions, micrographs of their morphology, and some remarks.

Keywords: infraciliature, protargol impregnation, redescription, taxonomy

DOI:10.12651/JSR.2020.9.1.068

Introduction

The diversity of ciliate species in Korea has been studied intensely for the last three decades and has revealed more than 450 species (Kwon *et al*., 2019). Since the checklist by Jung *et al*. (2017), about 100 species have been included to date. However, most of the species are concentrated in the class Spirotrichea Bütschli, 1889 (Jung *et al*., 2017). To understand the Korean ciliate diversity, it is necessary to study non-spritrotrichous ciliates. For instance, a monograph of colpodid ciliates by Foissner (1993) includes 170 species, but about 10 species have been reported in Korea (Kwon *et al*., 2019). Considering that colpodids are very common ciliates in terrestrial habitats, many Korean species are likely awaiting discovery.

Here we focus on ciliates inhabiting temporary ponds to reveal Korean terrestrial ciliate diversity. We collected 22 ciliate species including five unrecorded species in Korea belonging to three classes, five orders, five families, and five genera. In the present study, the five unrecorded ciliates are reported with brief descriptions, micrographs, and remarks.

Materials and Methods

Water samples including some soil debris were collected from temporary ponds on a lawn (100 × 85 m, Fig. 1) in the Gangneung-Wonju National University (37°46′12.4″N, 128°52′16.5″E) after heavy rainfall (about 50−100 mm/day) at 24 July, 2019. These ponds disappeared within a few hours after the rainfall. Raw cultures were maintained for morphometry; however, most species disappeared within a few days. With several repeated sampling attempts, we could not find any ponds after rainfall of 0−50 mm per day. So, rather than isolating each species for culture/impregnation, about 10 mL of the water samples for each impregnation procedure was fixed using concentrated Bouin's fluid (Coats and Heinbokel, 1982) to make protargol preparations. So, each preparation includes several species together with some of the five unrecorded species (Table 1).

Living cells were observed under a stereomicroscope (SZ11, Olympus, Japan) and light microscopes (BX53, Olympus; inverted microscope Eclipse Ti-U, Nikon, Japan) using bright/dark field and differential interference contrast (DIC) at magnifications of 50−1000 ×. The Bouin's fixed cells were protargol-impregnated with acetone developer ('Procedure A') (Foissner, 2014) and laboratory-

Fig. 1. Sampling site. Temporary ponds on a lawn.

synthesized protargol (Kim and Jung, 2017). Usually five protargol-impregnated specimens were examined for the morphology. In some cases, a silver carbonate impregnation method was conducted (Foissner, 2014). General terminology follows Lynn (2008), and specific terms for each taxon follow Petz and Foissner (1992), Foissner (1993), Foissner *et al*. (1994), and Foissner *et al*. (2002).

Results and Discussion

Class Nassophorea Small & Lynn, 1981
Order Microthoracida Jankowski, 1967
Family Pseudomicrothoracidae Jankowski, 1967
Genus *Pseudomicrothorax* Mermod, 1914

1. *Pseudomicrothorax agilis* Mermod, 1914

Diagnosis. Body size in vivo about 52 × 36 μm (n = 1), 43–57 × 26–37 μm (on average 50.5 × 32.3 μm) after protargol impregnation; elliptical to oval shape; densely filled with green algae. Longitudinal furrows along somatic kineties; extrusomes arranged along the kineties, spindle shape; anchor-shaped when extruded. Nuclear apparatus composed of a single macronucleus (5.5–8.7 × 5.2–5.8 μm) and a micronucleus (1.5–1.9 × 1.1–1.7 μm); both nuclei spherical to ellipsoidal; micronucleus attached to macronucleus. Three adoral membranelles obliquely arranged to left of oral opening; 15 or 16 nasse kinetosomes; paroral membrane lacking; about 9 basal bodies of oral primordium at left posterior third of kinety 1, always more faint than somatic kineties. 12 somatic kineties.
Distribution. Europe, USA, India, China, and Korea.
Remarks. The Korean population corresponds with a redescription by Foissner *et al*. (1994), except for the absence of a paroral membrane (see Fig. 6. on p. 468). Bussers (1976) also reported the paroral in a trophont of *P. agilis* using the silver nitrate method; however it disappeared or reduced to a few disordered kinetosomes in an encysted cell. From the additional 12 protargol-impregnated specimens examined to confirm the presence/absence of the paroral, we did not find the paroral and any cyst wall. Peck (1974) also did not identify this structure from the protargol preparation of *P. dubius*, but observed using the Chatton-Lwoff technique supporting that this species/genus lacks the paroral which is usually confused with the nasse kinetosomes. *Pseudomicrothorax agilis* can be distinguished from its congeners by the number of somatic kineties (12 vs. 13 or 14 in *P. dubius*) or the body shape (rounded vs. truncated posterior body end in *P. foliformis*) (Foissner *et al*., 1994).
Voucher slides. Two slides of protargol impregnated specimens were deposited at National Institute of Biological Resources, Korea (NIBRPR0000110175, NIBRPR0000110176).

Order Nassulida Jankowski, 1967
Family Nassulidae de Fromentel, 1874

Table 1. List of species with the accession number of protargol preparations deposited at National Institute of Biological Resources (NIBR). The five unrecorded species are in bold.

Species	Slide accession numbers and the date of fixation for impregnation									
	24 July, 2019						25 July, 2019			
	NIBRPR 0000110175	NIBRPR 0000110176	NIBRPR 0000110179	NIBRPR 0000110180	NIBRPR 0000110181	NIBRPR 0000110183	NIBRPR 0000110177	NIBRPR 0000110178	NIBRPR 0000110182	NIBRPR 0000110184
Apogonostomum sp.							+			
Blepharisma sp.	+	+		+		+		+	+	+
Bryometopus sp.	+	+						+		
Coleps hirtus hirtus	+		+	+	+	+		+		+
Cyrtolophosis mucicola	+	+	+	+	+	+	+	+	+	+
Frontonia sp.									+	
Furgasonia sp.		+							+	+
Gonostomum affine					?	+				
Halteria grandinella							+	+	+	+
Holostichides cf. *chardezi*							+			+
Leptopharynx costatus	+		+	+	+				+	+
Maryna ovata	+	+	+	+	+	+	+	+	+	+
Meseres corlissi	+	+				+	+	+	+	+
Nassula exigua	+	+		+			+	+	+	
Notohymena sp.				+		+	+	+		+
Platyophrya sp.	+					+	+	+		+
Pseudomicrothorax agilis	+	+		+	+	+	+	+	+	+
Uroleptus sp.			+							
Urotricha furcata?	+			+						
unidentified colpodid (s)*	+	+	+	+	+	+	+	+	+	+
unidentified oxytrichid (s)*				+			+	+	+	+
unidentified peritrich (s)*	+	+	+	+	+	+	+	+	+	+

*might be more than one species based on different body size of cells.

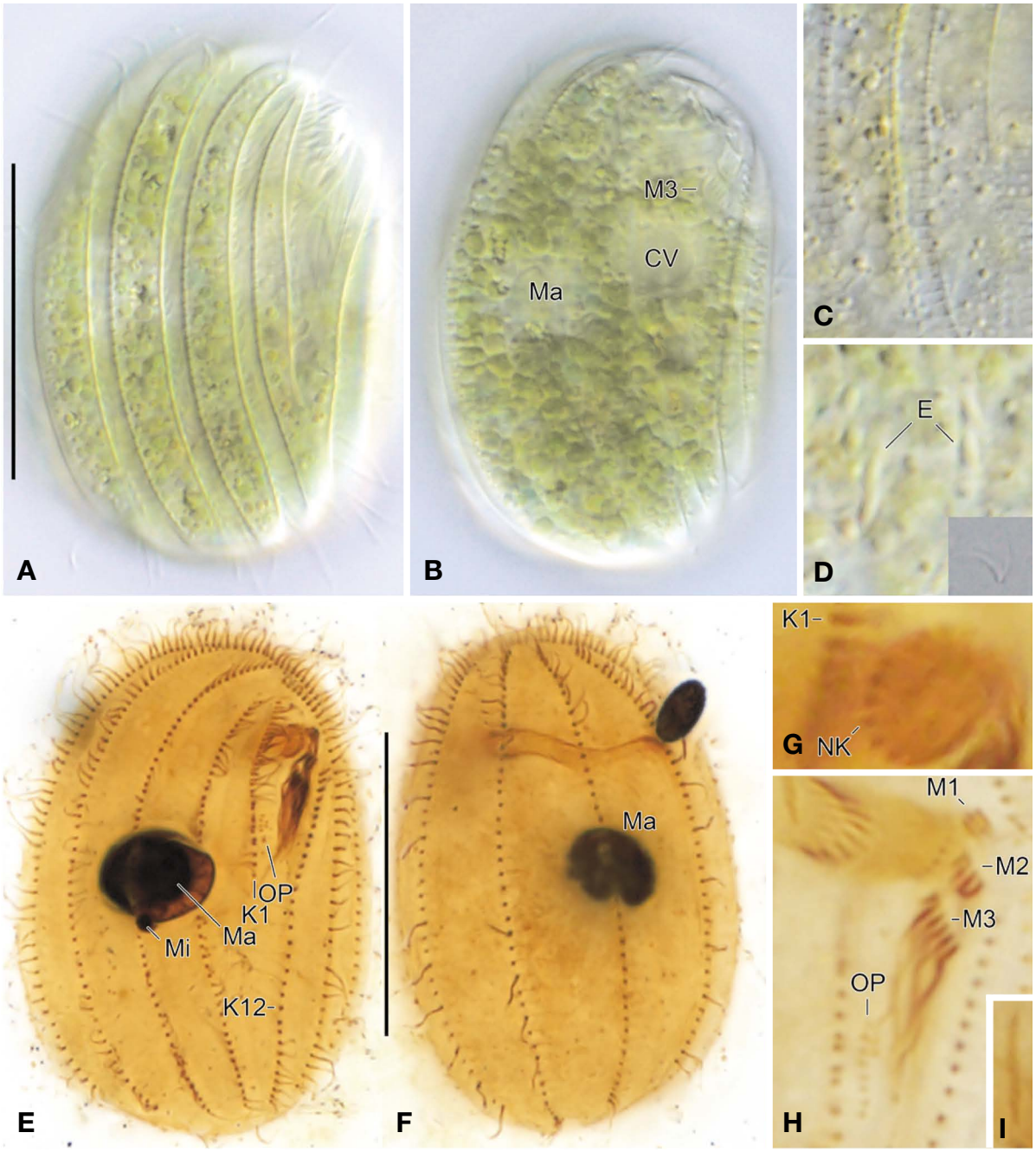

Fig. 2. *Pseudomicrothorax agilis* in life (A–D) and after protargol impregnation (E–I). A, B. Right side view of the same specimen showing conspicuous cortical ridges, contractile vacuole, macronucleus, and adoral membranelle 3. C. Ladder/web structure on furrow. D, I. Extrusomes, insert in D shows extruded form; non-extruded ones in D and I. E, F. Right and left side view of a representative specimen. G. Nasse kinetosomes and somatic kinety 1. H. Adoral membranelles 1–3 and oral primordium. CV, contractile vacuole; E, extrusomes; K1, K12, somatic kinety 1, 12; Ma, macronucleus; Mi, micronucleus; M1–3, adoral membranelles 1–3; NK, nasse kinetosomes; OP, oral primordium. Scale bars: 30 μm.

Genus *Nassula* Ehrenberg, 1834

2. *Nassula exigua* Kahl, 1931

Diagnosis. Body size 32–39 × 14–25 μm, on average 35.3 × 17.5 μm, after protargol impregnation; body shape ellipsoidal to oval. Nuclear apparatus composed of a single spherical to ellipsoidal macronucleus (7.9–9.5 × 7.3–8.1 μm) and one micronucleus (2.2 × 2.1 μm, n = 1) at variable positions, from anterior to posterior body end.

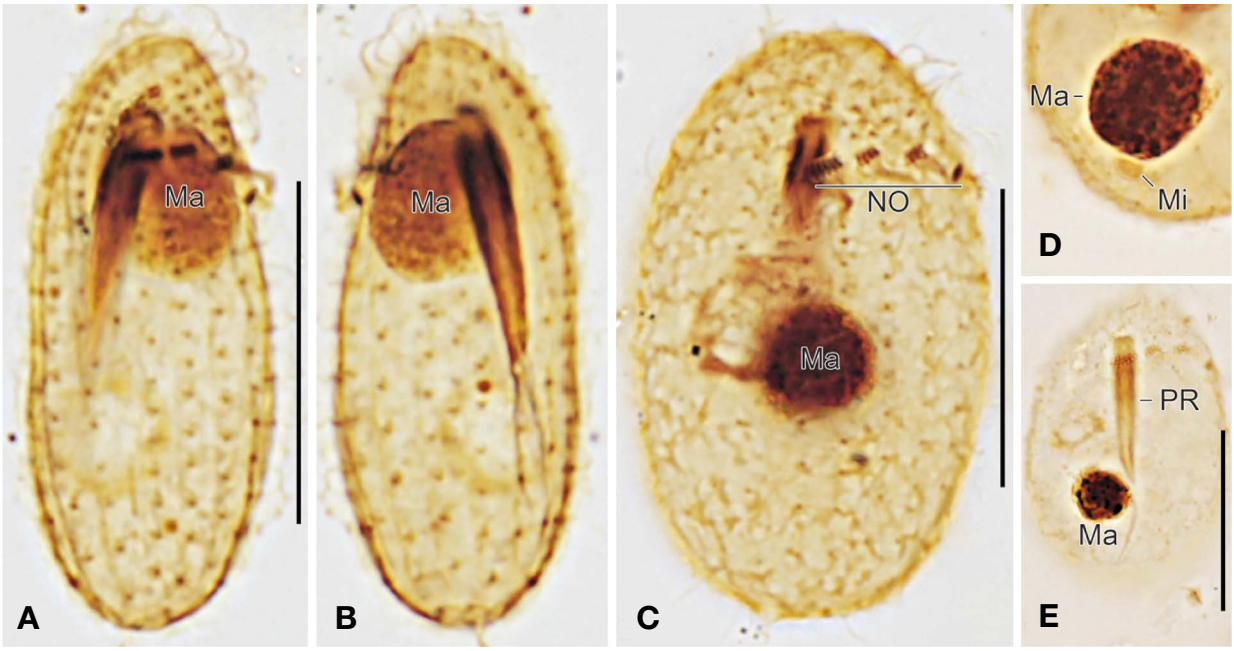

Fig. 3. *Nassula exigua* after protargol impregnation of ventral (A, C–E) and dorsal (B) views showing ciliary pattern, nassulid organelles, pharyngeal rods, and nuclear apparatus. A–D. Variable position of the nuclear apparatus. Ma, macronucleus; Mi, micronucleus; NO, nassulid organelles; PR, pharyngeal rods. Scale bars: 20 μm.

Extrusomes lacking. 14 pharyngeal rods; 4 nassulid organelles, leftmost one on lateral side. 14 somatic kineties.
Distribution. Europe, USA, Venezuela, and Korea.
Remarks. *Nassula exigua* highly resembles *N. parva* Kahl, 1928, but can be distinguished by the body size (in vivo 27–45 vs. 40–70), the number of micronuclei (two or three vs. one), pharyngeal rods (8–12 vs. 12–18), and nassulid organelles (three or four vs. four or five) (Foissner *et al*., 2002). However, the Korean population interestingly shows an intermediate morphology between the two species. In the present study, we found only a few protargol-impregnated specimens and, as mentioned by Foissner *et al*. (2002), further study, especially concerning the number of micronuclei, is necessary to determine whether *N. exigua* and *N. parva* are distinct species. In addition, they stated that this species might be assigned to *Naxella* Fryd-Versavel *et al*., 1980; however, for the generic assignment, further study on the pharyngeal basket is necessary .
Voucher slides. Two slides of protargol impregnated specimens were deposited at National Institute of Biological Resources, Korea (NIBRPR0000110177, NIBRPR 0000110178).

Class Colpodea Small & Lynn, 1981
Order Cyrtolophosidida Foissner, 1978
Family Cyrtolophosididae Stokes, 1888
Genus *Cyrtolophosis* Stokes, 1885

3. *Cyrtolophosis mucicola* Stokes, 1885

Diagnosis. Body size in vivo 24–40 × 10–16 μm (n = 5), 18.4–28.0 × 9.9–12.3 μm (on average 23.4 × 11.3 μm) after protargol impregnation; body shape elliptical to spindle; it lives in a hyaline dwelling-tube, which adheres to each other or to debris. One spherical to ellipsoidal macronucleus (3.6–4.5 × 3.4–4.0 μm) and one micronucleus (1.1–1.7 × 1.1–1.4 μm), located together in body center. Contractile vacuole subterminal. Oral apparatus composed of 4 adoral organelles, one oblique kinety anterior to the adoral organelles, and paroral membrane; adoral organelle plate-like polykinetids; paroral membrane curved leftward at proximal end, but the length highly variable (i.e., anterior body end to proximal end of buccal cavity or to the level of second adoral organelle), rarely fragmented to two segments; elongated cilia at anterior body end presumably from anterior paroral membrane and somatic kineties. 10 somatic kineties.
Distribution. USA, Europe, China, New Zealand, Antarctica, Africa, India, Australia, Russia, Japan, Greenland, and Korea.
Remarks. According to Foissner (1993), *C. mucicola* can be distinguished from congeners by the combination of the following features: the smaller body size (vs. > 40 μm), the ellipsoid body shape (vs. ovoid), the elongated anterior cilia at anterior body end (vs. not elongated), the subterminal contractile vacuole (vs. terminal), and the

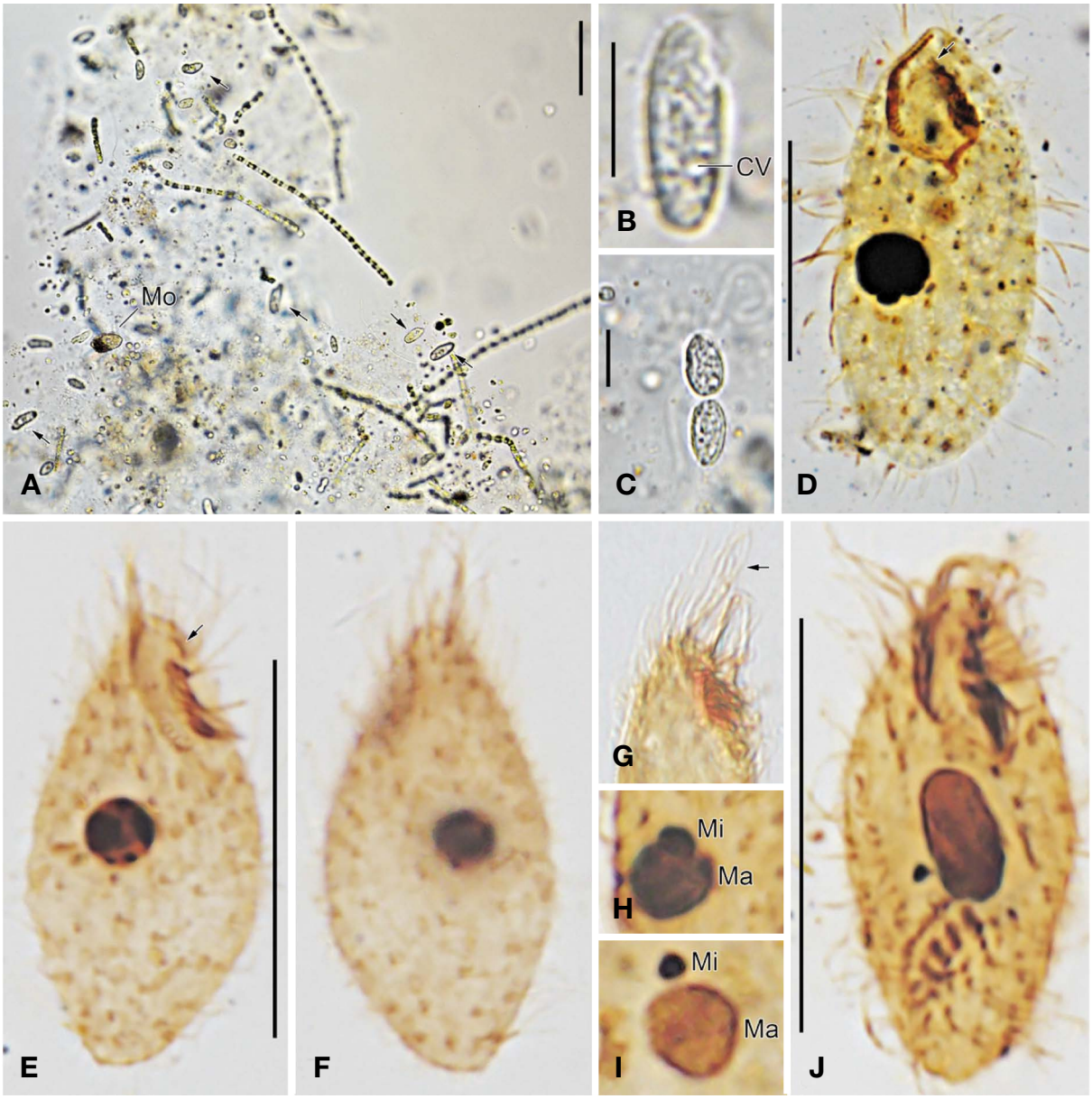

Fig. 4. *Cyrtolophosis mucicola* in life (A–C), after silver carbonate (D), and protargol impregnation (E–J). A. Cells with hyaline dwelling-tubes at low magnification; arrows indicate *C. mucicola*. B. Right side view shows the body shape and location of a contractile vacuole. C. A very late divider. D. Ventral view showing the paroral and the oblique kinety anterior to the adoral organelles (arrow). E, F. Ventral (E) and dorsal (F) view showing the somatic ciliature and nuclear apparatus, arrow denotes the oblique kinety anterior to the adoral organelles. G. Anterior body portion showing the elongated anterior cilia (arrow). H, I. Nuclear apparatus. J. Mid-divider with newly formed paroral and adoral organelles of the opisthe. CV, contractile vacuole; Ma, macronucleus; Mi, micronucleus; Mo, *Maryna ovata*. Scale bars: A = 100 μm; B–E, J = 20 μm.

presence of dwelling tube (vs. absence). *Cyrtolophosis bursaria* (Schewiakoff, 1892) Kahl, 1926 differs from *C. mucicola* by the body shape (ovoid vs. elliopsoid); however the former is poorly described and considered as a putative synonym of *C. mucicola* by Foissner (1993). Much care should be paid to confirm the presence/absence of the dwelling-tube because organisms usually desert the tube when transferring (i.e., from field) (Foissner, 1993).

Voucher slides. Two slides of protargol impregnated specimens were deposited at National Institute of Biological Resources, Korea (NIBRPR0000110181, NIBRPR 0000110182).

Order Colpodida de Puytorac *et al*., 1974
Family Marynidae Poche, 1913
Genus *Maryna* Gruber, 1879

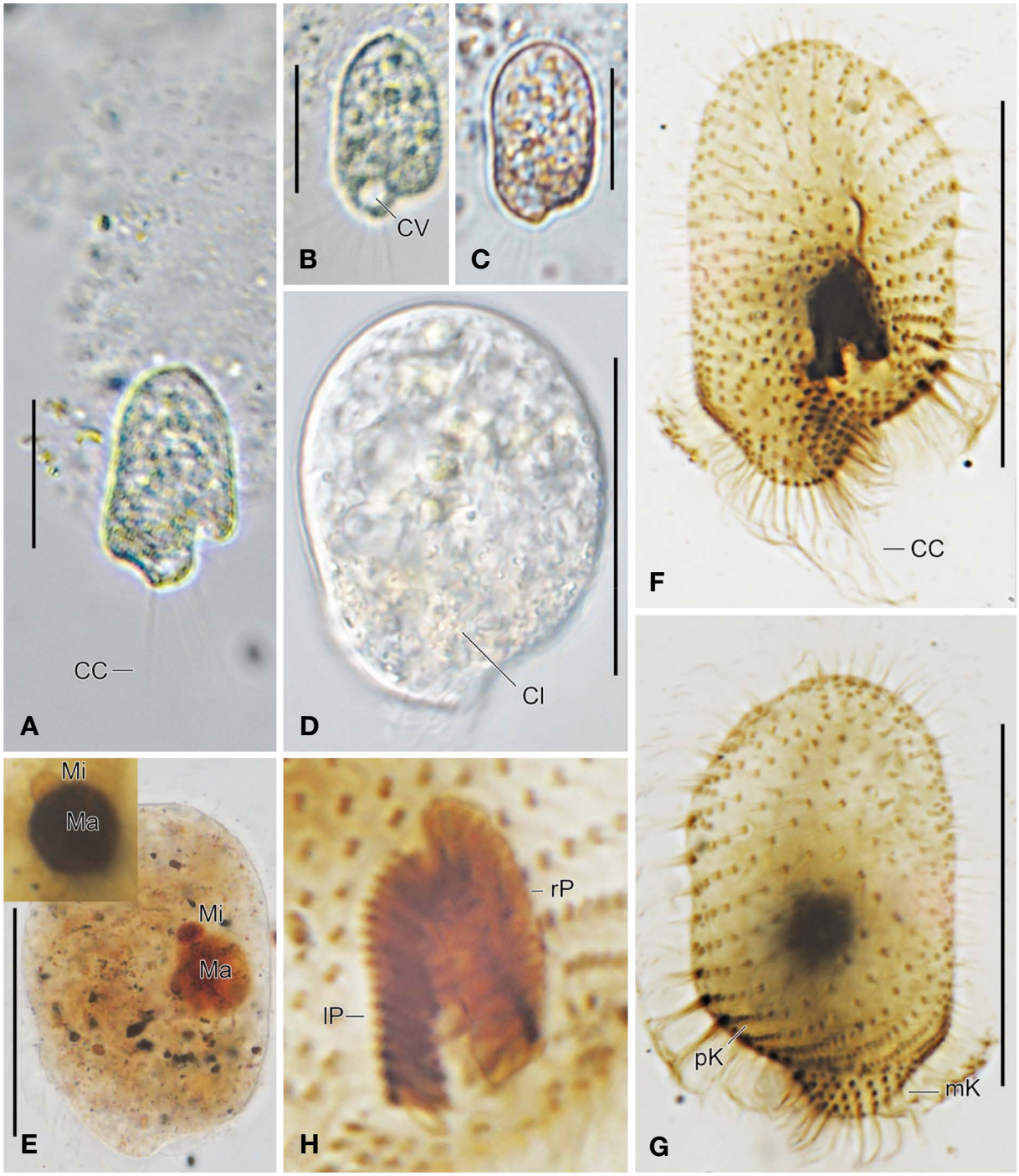

Fig. 5. *Maryna ovata* in life (A–D), after silver carbonate (E), and after protargol impregnation (F–H, insert in E). A–C. Ventral views showing body shape. D. Squeezed specimen showing food vacuoles and crystals in posterior body portion. E, insert in E. Nuclear apparatus. F, G. Ventral and dorsal view showing somatic ciliature (with elongated caudal cilia) and nuclear apparatus. H. Ventral view of oral polykinetids. CC, caudal cilia; Cl, crystals; CV, contractile vacuole; lP, left polykinetid; Ma, macronucleus; Mi, micronucleus; mK, mycteral kineties; pK, postoral kineties; rP, right polykinetid. Scale bars: 30 μm.

4. *Maryna ovata* (Gelei, 1950) Foissner, 1993

Diagnosis. Size in vivo 37–45 × 23–26 μm (on average 42 × 25 μm, n = 7), 31.1–36.3 × 22.2–23.5 μm (on average 34.1 × 22.9 μm) after protargol impregnation; it lives in a hyaline dwelling-tube, however, the tubes adhere to each other, to debris, or to the tubes of coexisting *C. mucicola*. Body reverse 'U' shape, anterior end rounded or slight-

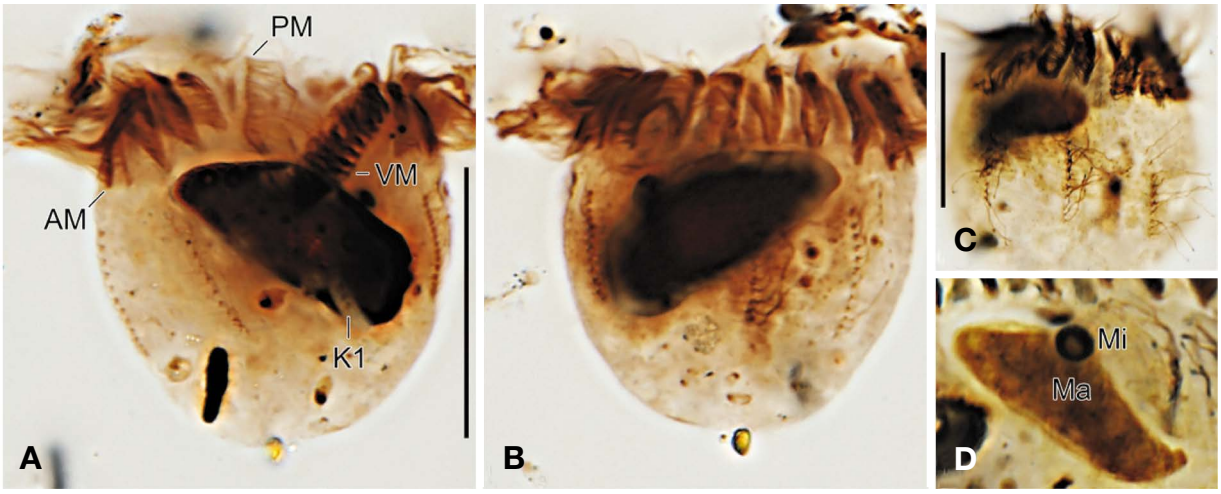

Fig. 6. *Meseres corlissi* after protargol impregnation. A, B. Ventral (A) and dorsal (B) view showing ciliary pattern, oral apparatus, and nuclear apparatus. C. Dorsal view showing elongated somatic cilia. D. Nuclear apparatus. AM, adoral membranelles; K1, somatic kinety 1; Ma, macronucleus; Mi, micronucleus; PM, paroral membrane; VM, ventral membranelles. Scale bars: 30 μm.

ly tapering, posterior end with distinct uvula, transverse section round to elliptical. Nuclear apparatus composed of a spherical to globular macronucleus (6.2–7.9 × 5.9–7.8 μm) and spherical to ellipsoidal micronucleus (2.8–3.3 × 2.4–3.1 μm). Contractile vacuole in uvula without collecting canals. Posterior third of body usually dark at low magnification due to dense yellowish crystals. 31–40 somatic kineties with spiral course; it should be noted that, as mentioned by Foissner (1993), the spiral course hampers to precisely count the number of kineties; caudal cilia more than two times longer than somatic ones. Oral apparatus above uvula; vestibulum funnel-shaped; left and right polykinetid with similar size.

Distribution. Europe and Korea.

Remarks. According to Foissner (1993), the small ciliate *M. ovata* can be identified by the elongated caudal cilia, globular macronucleus, contractile vacuole in uvula, and U-shaped body. This species resembles *M. rotunda* Dingfelder, 1962, but differs in having U-shaped body (vs. globular). However, the latter species was superficially described, and Foissner (1993) considered it being possibly synonymous with *M. ovata*. Dunthorn *et al.* (2012) used two molecular markers for understanding oral evolution of Marynidae *sensu lato* that resulted in non-monophyly of the group *Ilsiella* + *Maryna*, which are classified in the same family (Lynn, 2008).

Voucher slides. Two slides of protargol impregnated specimens were deposited at National Institute of Biological Resources, Korea (NIBRPR0000110179, NIBRPR 0000110180).

Class Spirotrichea Bütschli, 1889
Order Sporadotrichida Fauré-Fremiet, 1961
Family Halteriidae Claparède & Lachmann, 1858
Genus *Meseres* Schewiakoff, 1893

5. *Meseres corlissi* Petz & Foissner, 1992

Diagnosis. Body size 30–57 × 26–50 μm (on average 47.1 × 38.9 μm) after protargol impregnation. Body globular to slightly ellipsoidal, outline U-shaped with truncated anterior end, transverse section round. Macronucleus triangle to liver-shaped (18–27.2 × 8.3–12.8 μm) with one globular micronucleus (3.3–4.8 × 2.9–4.0 μm). Eight equatorial somatic kineties, with long cilia; 9–12 and 10–14 cilia in kineties 1 and 2, respectively. Oral apparatus composed of anterior (16–18) and ventral (11–14) membranelles, and paroral membrane.

Distribution. Africa, Australia, Europe, China, North America, and Korea.

Remarks. The Korean population corresponds well with the type population (Petz and Foissner, 1992); however, the Korean population is slightly smaller (vs. 53–78 × 47–66 μm in protargol preparations). *Meseres corlissi* is a widely distributed but rare planktonic ciliate. Weisse *et al.* (2008) reported that, of five clonal cultures collected from four continents, a Chinese culture significantly differed from the other cultures based on genetics, morphology, and ecology. Considering the number of kinetids in kinety 2, the Korean population differs from the Chinese (10–14 vs. 14–19); however, it should be noted that the numbering system on the somatic kineties from the original description differs from the system by Weisse *et al.* (2008).

Voucher slides. Two slides of protargol impregnated specimens were deposited at National Institute of Biological Resources, Korea (NIBRPR0000110183, NIBRPR 0000110184).

Acknowledgements

We are grateful to Dr. Atef Omar for improving this manuscript. This study was supported by grants from the National Institute of Biological Resources (NIBR) funded by the Ministry of Environment (ME) of the Republic of Korea (NIBR201902204).

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Submitted: October 15, 2019
Revised: November 8, 2019
Accepted: November 8, 2019