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New record of three oxytrichid ciliates (Ciliophora: Oxytrichidae) from South Korea

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Three oxytrichid ciliates, *Oxytricha lithofera* Foissner, 2016, *Pleurotricha curdsi* (Shi *et al*., 2002) Gupta *et al*., 2003 and *Sterkiella tetracirrata* Kumar *et al*., 2015, were isolated from soils and confirmed as new to South Korea. *Oxytricha lithofera* was identified based on lithosomes, cortical granules, 21−33 adoral zone membranelles, one left (14−21 cirri) and one right (15−18) marginal rows and 5 transverse cirri. *Pleurotricha curdsi* was identified based on the possession of 2 macronuclear nodules, 2−3 micronuclei, 46−53 adoral zone membranelles, 3 frontal cirri, 5 frontoventral cirri, 5−7 postoral ventral cirri, 2−3 right marginal rows and 5 transverse cirri. *Sterkiella tetracirrata* was identified with respect 4 macronuclear nodules, 3−6 micronuclei, 25−40 adoral zone membranelles, 3 frontal cirri, 3 postoral ventral cirri, 2 pretransverse cirri, one left (21−30 cirri) and one right (24−30) marginal row and 4 transverse cirri. On the basis of 18S rDNA sequence analyses, we describe the phylogenetic positions of the three species.

Keywords: 18S rDNA gene, protargol impregnation, redescription, soil, taxonomy

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Introduction

The family Oxytrichidae Ehrenberg, 1830 is the largest family in the order Sporadotrichida, which currently includes at least nearly 200 valid morphospecies (Berger, 1999; Shao *et al*., 2015; Shao *et al*., 2019). Oxytrichids are characterized by 18 frontal-ventral-transverse cirri clustered to six distinct groups which typically originate from six longitudinal primordia segregating 1, 3, 3, 3, 4, 4 cirri (Berger, 1999; Shao *et al*., 2015). Oxytrichids in South Korea were reported firstly by Shin and Kim (1988), and over 80 species have been recorded since then (Jung *et al*., 2017; Kwon *et al*., 2019). In this study, we isolated three oxytrichid species from soil samples, which were identified as members of the three genera *Oxytricha* Bory de St. Vincent, 1824, *Pleurotricha* Stein, 1859 and *Sterkiella* Foissner *et al*., 1991. Three species were identified based on live and protargol preparation. In addition, we obtained their 18S rDNA gene sequences and then compared with those of oxytrichids retrieved from GenBank.

Materials and Methods

The soil samples were placed in Petri dishes, mixed with mineral water, and then incubated at room temperature (Foissner *et al*., 2002). Raw cultures were microscopically observed *in vivo* (Leica DM2500; Wetzlar, Germany) from ×50 to ×1000 magnification. Cell staining was performed to use Procedure A method described by Foissner (2014). Classification and terminology are according to Berger (1999) and Foissner (2016).

For amplifying the 18S rDNA, DNA extract, PCR amplification, and sequencing were performed according to the methods of Jung *et al*. (2012). The newly obtained sequences were aligned using BioEdit (Hall, 1999) and then were compared with those of 47 oxytrichid and four urostylid (outgroup) species retrieved from the GenBank. To determine the appropriate DNA substitution model for Maximum Likelihood (ML) and Bayesian Inference (BI) analyses, we used jModelTest 2.1.7 (Darriba *et al*., 2012). The model selected was TIM2 + I (0.5560) + G (0.5420). The ML analysis was conducted using PhyML version 3.1 (Guindon *et al*., 2010) and BI assessment was performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Pairwise distances were calculated using Mega 7.0.26 (Kumar *et al*., 2016).

Systematic Accounts

Phylum Ciliophora Doflein, 1901
Class Spirotrichea Bütschli, 1889
Order Sporadotrichida Fauré-Fremiet, 1961
Family Oxytrichidae Ehrenberg, 1830
Genus *Oxytricha* Bory de St. Vincent, 1824

1. *Oxytricha lithofera* Foissner, 2016 (Table 1, Figs. 1 and 2)

Oxytricha lithofera Foissner, 2016: p. 699; Luo *et al*., 2021: p. 1.

Korean name. 리소좀첨모하모충

Material examined. Slightly saline soil (1‰), Sacheon-si, Gyeongsangnam-do, South Korea (34°57′42.6″N, 128°02′15.2″E), collected by Kyu-Seok Chae in June 2020.

Diagnosis of the Korean population. Size *in vivo* about 100×45 μm, ellipsoidal body shape; 2 macronuclear nodules and 1–3 micronuclei; contractile vacuole at the left of mid-body; 21–28 adoral membranelles; cortical granules present; 3 frontal cirri; 4 frontoventral cirri; one buccal cirrus; 3 postoral-ventral cirri; 2 pretransverse and 5 transverse cirri; one left and one right marginal cirral row; 4 dorsal and 2 dorsomarginal kineties; 3 caudal cirri.

Description. Body size 90–110×40–50 μm *in vivo*, 67–88×25–40 μm after protargol preparations (Table 1). Flexible body outline usually elongated elliptical, both ends rounded (Fig. 1A). Two macronuclear nodules, with a size of 11–16×5–8 μm after protargol preparation (Fig. 1C). One to three micronuclei, size about 2 μm diameter after protargol preparation (Fig. 1C). Contractile vacuole in left margin of mid-body, about 11 μm in diameter (Fig. 2A, arrowhead). Colorless cortical granules usually arranged along and in between dorsal kineties (Fig. 2B). One or two ring-shaped lithosomes, 4–6 μm in diameter, with wall 0.8–1.2 μm thick, located subapically and/or subterminally (Figs. 1A, 2A). Cytoplasm colorless (Fig. 2C).

Adoral zone membranelles along about 38% of body length, comprising of 21–33 membranelles (Figs. 1B, 2C, Table 1). Three frontal cirri, four frontoventral cirri, one buccal cirrus, and three postoral-ventral cirri (Figs. 1B, 2C, Table 1). Two pretransverse and five transverse cirri (Figs. 1B, 2C, Table 1). One left (14–21 cirri) and right (15–18 cirri) marginal row, both marginal rows non-confluent posteriorly (Fig. 1B, Table 1). Four dorsal and two dorsomarginal kineties (Figs. 1C, 2D, Table 1). Three caudal cirri located at ends of dorsal kineties 1, 2, 4 on posterior end of cell (Figs. 1C, 2D).

Distribution. Austria, China, Venezuela, and South Korea (This study).

Remarks. Cell body outline and distributional pattern

Table 1. Morphometric data for *Oxytricha lithofera* (upper line), *Pleurotricha curdsi* (middle line) and *Sterkiella tetracirrata* (lower line).

Characteristic[a]	Mean	SD	SE	CV	Min	Max	n
Body length, μm	75.8	6.5	1.4	8.5	67	88	20
	149.4	9.9	2.2	6.6	130	165	20
	101.8	10.5	2.3	10.3	85	122	20
Body width, μm	31.3	4.0	0.9	12.8	25	40	20
	74.4	14.7	3.3	19.8	55	100	20
	53.6	7.5	1.7	14.0	40	67	20
Adoral zone length, μm	28.7	2.7	0.6	9.5	21	33	20
	68	5.6	1.3	8.3	59	79	20
	42	6.4	1.4	15.3	32	58	20
AM, number	24.8	1.7	0.4	6.7	21	28	20
	50	2.2	0.5	4.4	46	53	20
	32	3.4	0.8	10.5	25	40	20
BC, number	1	0.0	0.0	0.0	1	1	20
	1	0.0	0.0	0.0	1	1	20
	1	0.0	0.0	0.0	1	1	20
FC, number	3	0.0	0.0	0.0	3	3	20
	3	0.0	0.0	0.0	3	3	20
	3	0.0	0.0	0.0	3	3	20
FVC, number	4	0.0	0.0	0.0	4	4	20
	5	0.0	0.0	0.0	5	5	20
	4	0.0	0.0	0.0	4	4	20
PVC, number	3	0.0	0.0	0.0	3	3	20
	6	0.3	0.1	5.4	5	7	20
	3	0.0	0.0	0.0	3	3	20
PTC, number	2	0.0	0.0	0.0	2	2	20
	2	0.0	0.0	0.0	2	2	20
	2	0.0	0.0	0.0	2	2	20
TC, number	5	0.0	0.0	0.0	5	5	20
	5	0.0	0.0	0.0	5	5	20
	4	0.0	0.0	0.0	4	4	20
LMC, number	16	1.6	0.2	10.1	14	21	20
	25	2.4	0.5	6.9	22	28	20
	24	2.0	0.5	8.4	21	30	20
RMC1, number	16	0.8	0.2	4.8	15	18	20
	30	1.3	0.3	4.1	28	34	20
	26	1.7	0.4	6.7	24	30	20
RMC2, number	–	–	–	–	–	–	–
	19	2.4	0.5	12.4	14	25	20
	–	–	–	–	–	–	–
RMC3, number	–	–	–	–	–	–	–
	5	2.4	0.5	42.1	1	9	7
	–	–	–	–	–	–	–
DK, number	4	0.0	0.0	0.0	4	4	20
	4	0.0	0.0	0.0	4	4	20
	4	0.0	0.0	0.0	4	4	20
DM, number	2	0.0	0.0	0.0	2	2	20
	2	0.0	0.0	0.0	2	2	20
	2	0.0	0.0	0.0	2	2	20
Ma, number	2	0.0	0.0	0.0	2	2	20
	2	0.0	0.0	0.0	2	2	20
	4	0.0	0.0	0.0	4	4	20
Mi, number	2	0.4	0.1	20.2	1	3	20
	2	0.4	0.1	19.7	2	3	20
	4.7	1.0	1.0	22.4	3	6	20

AM, adoral membranelles; BC, buccal cirrus; CV, coefficient of variation in %; DK, dorsal kineties; DM, dorsomarginal row; FC, frontal cirri; FVC, frontoventral cirri; LMC, left marginal cirri; Ma, macronuclear nodules; Max, maximum; Mi, micronuclei; Min, minimum; n, number of specimens examined; PTC, pretransverse cirrus; PVC, postoral ventral cirri; RMC, right marginal cirri; SD, standard deviation; SE, standard error of the arithmetic mean; TC, transverse cirri.

[a]Data based on randomly selected, protargol-stained specimens.

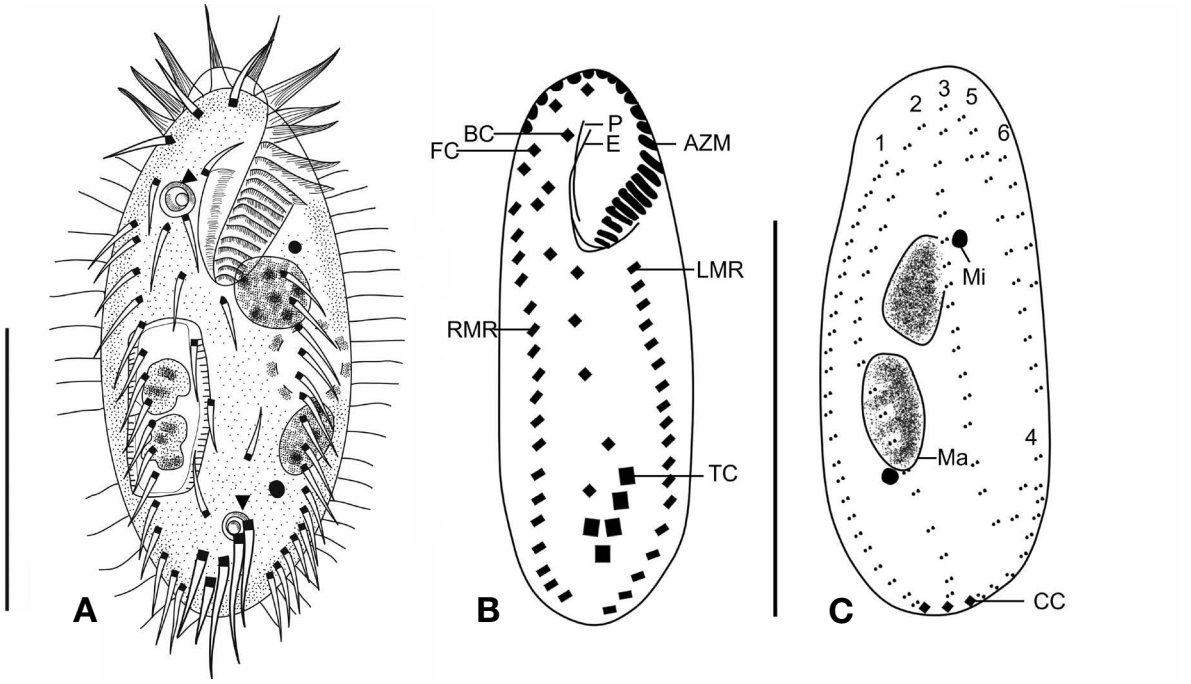

Fig. 1. Morphology of *Oxytricha lithofera* from live (A) and protargol-impregnated (B, C) specimens. A, Ventral view arrowheads denote lithosomes; B, C, Ventral and dorsal view of typical specimen; AZM, adoral zone of membranelles; BC, buccal cirrus; CC, caudal cirri; E, endoral membrane; FC, frontal cirri; LMR, left marginal cirral row; Ma, macronuclear nodules; Mi, micronuclei; P, paroral membrane; RMR, right marginal cirral row; TC, transverse cirri; 1−6, dorsal kineties 1−6; Scale bars: 50 μm (A, C).

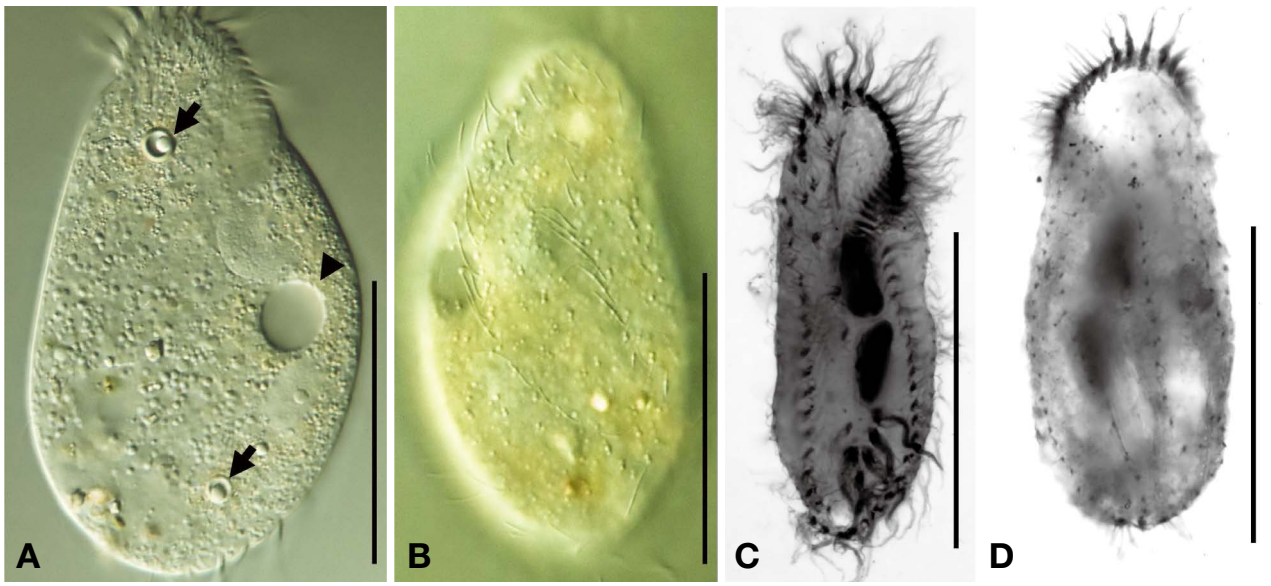

Fig. 2. Photomicrographs of *Oxytricha lithofera* from live (A, B) and after protargol impregnation (C, D). A, Ventral view showing lithosomes (arrows) and contractile vacuole (arrowhead); B, Dorsal view showing dorsal bristles and cortical granules; C, D, Ventral and dorsal view of typical specimen; Scale bars: 50 μm (A−D).

of cirri in *O. lithofera* are similar to those of *O. siseris* Vuxanovici, 1963. However, *O. lithofera* differs from *O. siseris* in the number of right marginal cirri (15−18 vs. 10−14) and lithosomes (present vs. absent) (Foissner, 2016; Vuxanovici, 1963). Apart from the similarity between the Chinese population and most samples of the

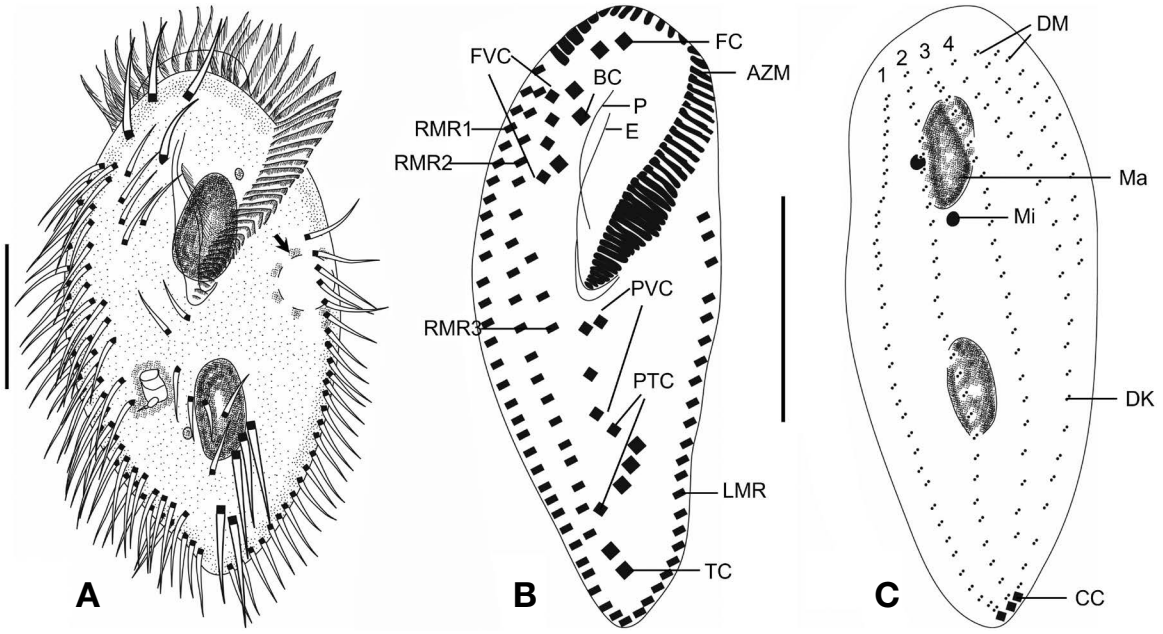

Fig. 3. Morphology of *Pleurotricha curdsi* from live (A) and protargol-impregnated (B, C) specimens. A, Ventral view of typical specimen live arrow denotes contractile vacuole (arrow); B, C, Ventral and dorsal view of typical specimen; AZM, adoral zone of membranelles; BC, buccal cirri; CC, caudal cirri; DM, dorsomarginal row; DK, dorsal kinety; E, endoral membrane; FC, frontal cirri; FVC, frontoventral cirri; LMR, left marginal cirral row; Ma, macronuclear nodules; Mi, micronuclei; P, paroral membrane; PTC, pretransverse cirri; PVC, postoral ventral cirri; RMR, right marginal cirral row; TC, transverse cirri; 1–4, dorsal kineties 1–4; Scale bars: 50 μm (A–C).

Korean population by the one-bristle-wide gap in dorsal kinety 1, this character generally serves as an important feature that can be used to distinguish *O. lithofera* from other *Oxytricha* species (Foissner, 2016).

The Korean population of *O. lithofera* corresponds to the type population from Venezuela based on the body size, cirri pattern, number of macronuclear nodules, and micronucleus, up to 10 μm long dorsal bristles and mitochondria-like granules present. However, they differ in the number of adoral zone of membranelles (21–33 vs. 19–21) and the number of left and right marginal cirri (14–21 and 15–18 vs. 12–15 and 9–12) (Foissner, 2016). However, the Korean population differs from the type population with regards to the absence of a one-bristle-wide gap in dorsal kinety 1 in most samples, which is present in the latter population. In addition, the Korean population differs with respect to the size of micronucleus (about 2 μm in diameter vs. about 4 μm in diameter) and habitats (saline soil, 1‰ vs. brackish water, 3‰) of the Chinese population (Luo *et al*., 2021). In addition, the Korean and Chinese population differ from the type population with regards to the absence of a one-bristle-wide gap in dorsal kinety 1 in most samples, which is present in the latter population.

The 18S rDNA gene sequence of the Korean *O. lithofera* was 1770 bp in length (GenBank accession number: OM212987). The Korean and Chinese populations formed a clade with high support (100% ML, 0.99 BI) in the phylogenetic tree (arrow in Fig. 8), with 0.12% genetic divergence (2 nt difference).

Deposition. The voucher slide with protargol-impregnated specimens is deposited in the National Institute of Biological Resources in Korea (NIBRPR0000110853).

Genus *Pleurotricha* Stein, 1859

2. *Pleurotricha curdsi* (Shi *et al*., 2002) Gupta *et al*., 2003 (Table 1, Figs. 3–5)

Allotricha curdsi Shi *et al*., 2002: p. 397.

Pleurotricha curdsi Gupta *et al*., 2003: p. 275; Xu *et al*., 2015: p. 190, figs. 5A–N, table 2; Lu *et al*., 2015: p. 3216, fig. 2, table 1.

Korean name. 분열횡극모하모충

Material examined. Soil, Gimhae-si, Gyeongsangnam-do, South Korea (35°19′1″ N 128°48′11″ E, 1), collected by Kyu-Seok Chae in May 2020.

Diagnosis of the Korean population. Size *in vivo* about 160 × 95 μm, broadly ellipsoid body shape; 2 macronuclear nodules and 2–3 micronuclei; contractile vacuole at left of mid-body; 46–53 adoral membranelles; cortical granules absent; 3 frontal cirri; 6 frontoventral cirri; 3–5 postoral ventral cirri; 2 pretransverse cirri; 5 transverse cirri; one buccal cirrus; one left and 2–3 right marginal

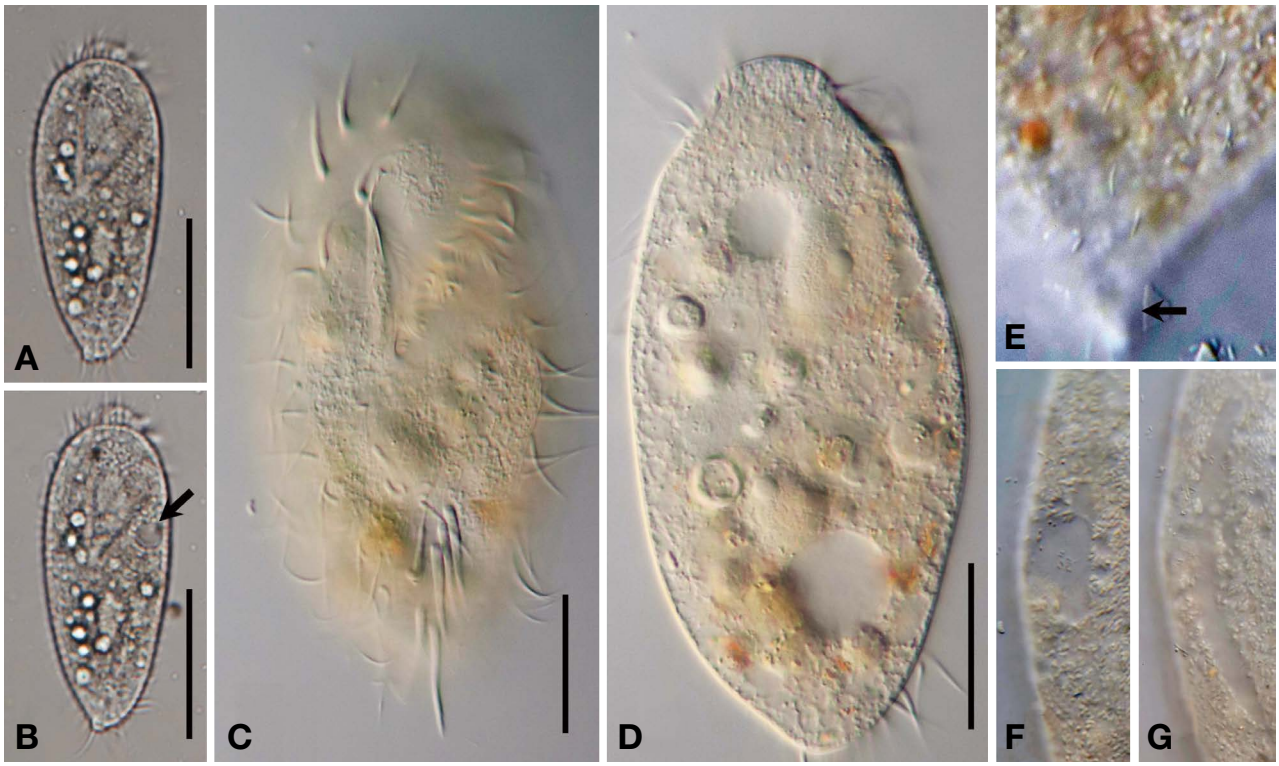

Fig. 4. Photomicrographs of *Pleurotricha curdsi* from live (A–G). A, B, Ventral view arrow denotes contractile vacuole; C, Ventral view from live; D, Dorsal view showing cytoplasm; E, Dorsal views of arrow mark the tapered posterior end; F, G, Extension of the contractile vacuole.; Scale bars: 50 μm (A–D).

cirral rows; 4 dorsal and 2 dorsomarginal kineties.

Description. Body size *in vivo* 110–200 × 50–105 μm, 130–165 × 55–100 μm after protargol impregnation (Table 1). Body shape broadly ellipsoid to obovate with rigid cortex (Figs. 3B, 4A). Length to width ratio about 2:1. Contractile vacuole on left side of mid-body, about 20 μm in diameter (Figs. 3A, 4B, arrow). Two macronuclear nodules, with a size of 17–35 × 10–15 μm after protargol preparation (Figs. 3C, 5C, arrow). Two to three micronuclei, 3–5 μm in diameter after protargol preparation (Figs. 3C, 5C, arrowhead). Cortical granules absent. Cytoplasm colorless (Fig. 4D).

Adoral zone of membranelles conspicuous, about 45% of the body length, and composed of 46–53 membranelles (Figs. 3B, 5A, Table 1). Both paroral and endoral membrane curved leftward anteriorly (Fig. 3B). 21–23 frontal-ventral-transverse cirri, including three frontal, one buccal, five frontoventral, three to five postoral ventral, two pretransverse, and five transverse cirri (Figs. 3B, 5A, Table 1). Marginal cirri arranged in one left and two to three right marginal rows, right marginal rows gradually shortened from right to left (Figs. 3B, 5A Table 1). Dorsal ciliature comprising four kineties and two dorsomarginal rows, three caudal cirri located at ends of dorsal kineties 1, 2, 4 on posterior end of cell (Figs. 3C, 5B).

Distribution. China, India, and South Korea (This study).

Remarks. *Pleurotricha curdsi* is most similar to *P. lanceolata* (Ehrenberg, 1835) Stein, 1859, the type species of the genus *Pleurotricha* based on their two or more right marginal rows, and three caudal cirri (Gupta *et al*., 2003). However, *P. curdsi* differs from *P. lanceolata* with respect to the number of frontoventral-transverse cirri (21–23 vs. 17 or 18) (Gupta *et al*., 2003; Xu *et al*., 2015).

The Korean population corresponds to the type population from China, but they are different in the number of adoral membranelles (46–53 vs. 53–61) (Shi *et al*., 2002). The Korean population is also similar to the Indian population, except for the number of right marginal rows (2 to 3 vs. 2) (Gupta *et al*., 2003).

The 18S rDNA gene sequence of *P. curdsi* was 1770 bp in length (GenBank accession number: OM212985). The Korean population and others *Pleurotricha* congeners were monophyletic with high support (97% ML, 1.00 BI) in the phylogenetic tree (arrowhead in Fig. 8). Intraspecific genetic distances among *P. curdsi* populations were 0.06%–0.12% (1–2 nt difference), and interspecific genetic distances between *P. curdsi* and *P. lanceolata*, and between *P. curdsi* and *P. oligocirrata* were each 0.19% (3 nt difference) and 0.26% (4 nt difference), respectively.

Deposition. The voucher slide with protargol-impreg-

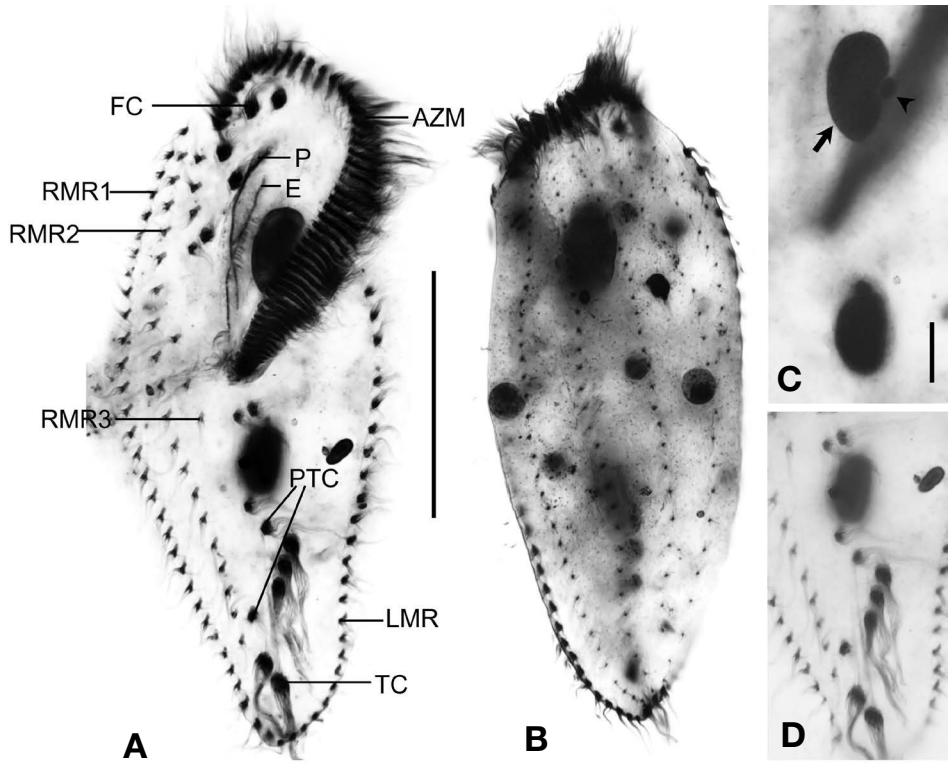

Fig. 5. Morphology of *Pleurotricha curdsi* from protargol-impregnated (A–D) specimens. A, B, Ventral and dorsal view of typical specimen; C, Ventral view showing macronuclear nodules (arrow) and micronuclei (arrowhead); D, Ventral view of posterior end; AZM, adoral zone of membranelles; E, endoral membrane; FC, frontal cirri; LMR, left marginal cirral row; P, paroral membrane; PTC, pretransverse cirri; RMR, right marginal cirral row; TC, transverse cirri; Scale bars: 50 μm (A), 10 μm (C).

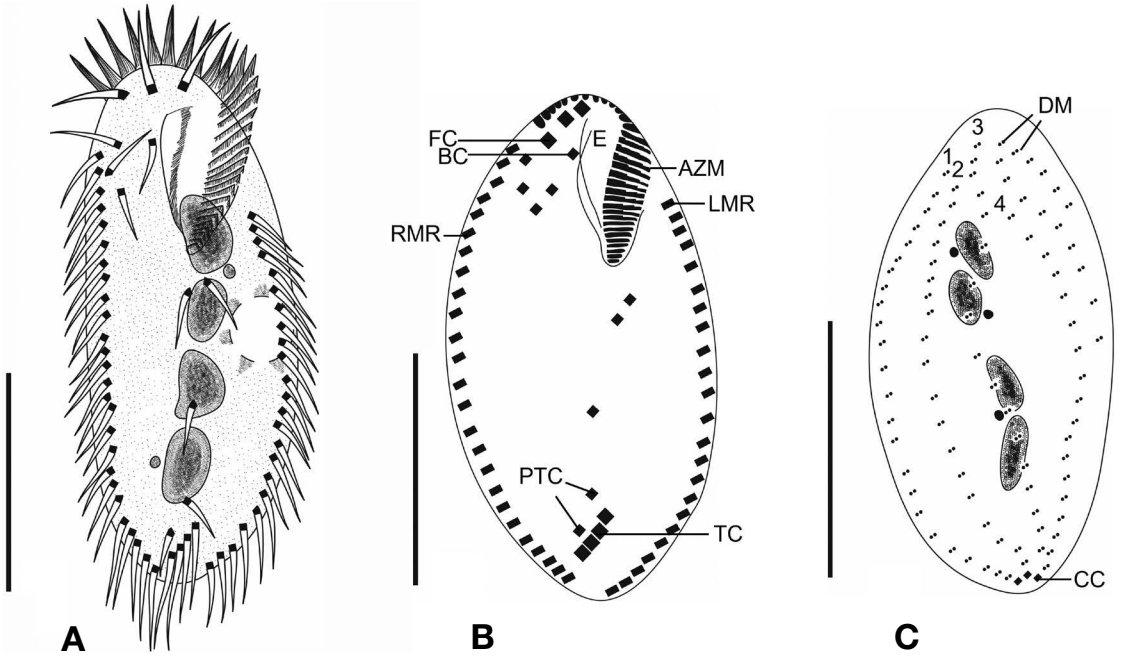

Fig. 6. Morphology of *Sterkiella tetracirrata* from live (A) and protargol-impregnated (B, C) specimens. A, Ventral view of typical specimen; B, C, Ventral and dorsal view of typical specimen; AZM, adoral zone of membranelles; BC, buccal cirri; CC, caudal cirri; DM, dorsomarginal row; E, endoral membrane; FC, frontal cirri; LMR, left marginal cirral row; PTC, pretransverse cirri; RMR, right marginal cirral row; TC, transverse cirri; 1–4, dorsal kineties 1–4; Scale bars: 50 μm (A–C).

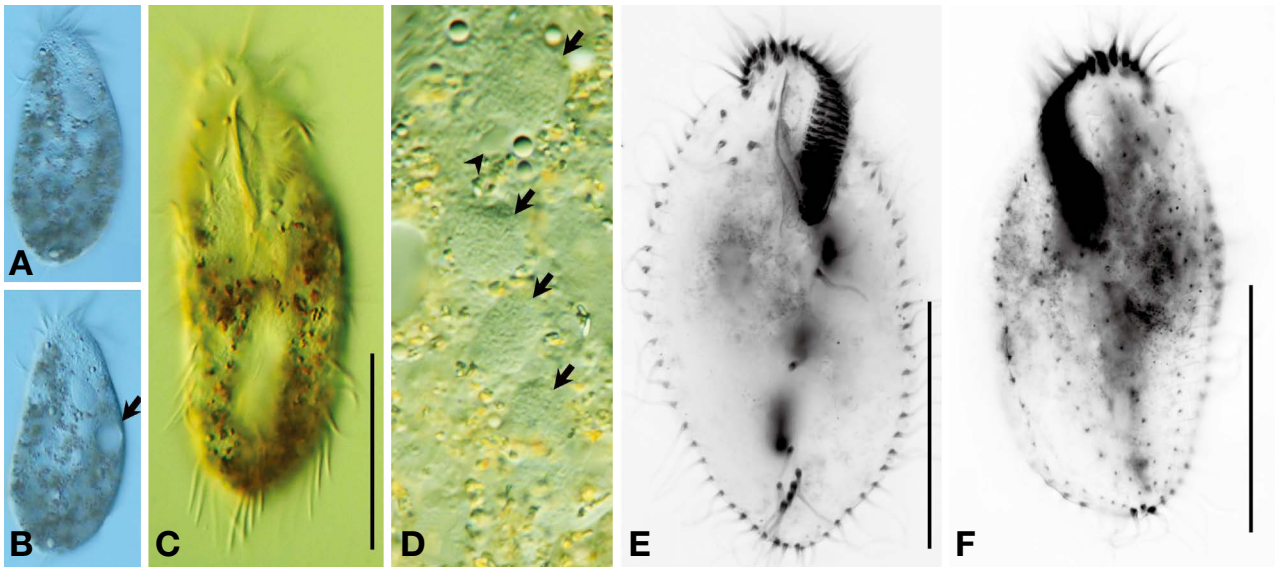

Fig. 7. Morphology of *Sterkiella tetracirrata* from live (A–D) and protargol-impregnated (E, F) specimens. A, B, Ventral view of typical specimen live arrow denotes contractile vacuole (arrow); C, Ventral view of typical specimen; D, Ventral view showing macronuclear nodules (arrow) and micronuclei (arrowhead); E, F, Ventral and dorsal view of typical specimen; Scale bars: 50 μm (C, E, F).

nated specimens is deposited in the National Institute of Biological Resources in Korea (NIBRPR0000110851).

Genus *Sterkiella* Foissner *et al*., 1991

3. *Sterkiella tetracirrata* Kumar *et al*., 2015 (Table 1, Figs. 6 and 7)

Sterkiella tetracirrata Kumar *et al*., 2015: p. 86.
Korean name. 사극모조직하모충

Material examined. Soil, Cheonan-si, Chungcheongnam-do, South Korea (36°54′03.9″ N 127°12′21.1″ E, 1), collected by Kyu-Seok Chae in June 2019.

Diagnosis of the Korean population. Size *in vivo* about 110×55 μm, ellipsoidal body shape; 4 macronuclear nodules; contractile vacuole at left of mid-body; 25–40 adoral membranelles; cortical granules absent; 3 frontal cirri; 4 frontoventral cirri; 3 postoral ventral cirri; 2 pretransverse cirri; 4 transverse cirri; one buccal cirrus; one left and one right marginal cirral row; 3 caudal cirri; 4 dorsal and 2 dorsomarginal kineties.

Description. Body size *in vivo* 100–120×50–60 μm, 85–122×40–67 μm after protargol impregnation (Table 1). Body shape elongated elliptical with rigid cortex (Figs. 6A, 7C, 7E, 7F). Length to width ratio about 2:1. Contractile vacuole on left side of mid-body, about 14 μm in diameter (Figs. 6A, 7B, arrow). Four macronuclear nodules and three to six micronuclei (Figs. 6C, 7D). Cortical granules absent. Cytoplasm colorless.

Adoral zone membranelles conspicuous, about 32% of the body length, comprising 25–40 membranelles (Fig. 6B, Table 1). Both paroral and endoral membranes curved leftward anteriorly (Fig. 6B). Three frontal, one buccal, three postoral-ventral cirri, two pretransverse and four transverse cirri (Figs. 6B, 7E, Table 1). One right (24–30 cirri) and left (21–30 cirri) marginal row (Figs. 6B, 7E, Table 1). Dorsal ciliature composed of four kineties and two dorsomarginal rows, three caudal cirri located at ends of dorsal kineties 1, 2, 4 on posterior end (Figs. 6C, 7F, Table 1).

Distribution. India and South Korea (This study).

Remarks. *Sterkiella tetracirrata* differs from *S. cavicola* (Kahl, 1935) Foissner *et al*., 1991 by the number of transverse cirri (4 vs. 5) (Foissner *et al*., 1991). *Sterkiella tetracirrata* can be distinguished from *S. quadrinucleatus* (Sick, 1933) Berger, 1999 by the number of transverse cirri (4 vs. 6–8) and habitat (soil vs. marine or brackish) (Berger, 1999). *Sterkiella tetracirrata* differs from *S. terricola* Berger, 1999 in the number of adoral membranelles (25–40 vs. 22–23), transverse cirri (4 vs. 3), and right and left marginal cirri (about 26 and 24 vs about 20 and 17) (Berger, 1999).

The morphology of the Korean population corresponds to the type population from India, but they are slightly different in the number of right and left marginal cirri (24–30 and 21–30 vs 21–25 and 20–23) (Kumar *et al*., 2015).

The 18S rDNA gene sequence of *S. tetracirrata* was 1770 bp in length (GenBank accession number: OM 212986). The Korean and Indian populations formed a highly supported clade (99% ML, 0.94 BI) in the phylogenetic tree (double-arrowhead in Fig. 8). The Korean and the Indian populations were identical, and had a se-

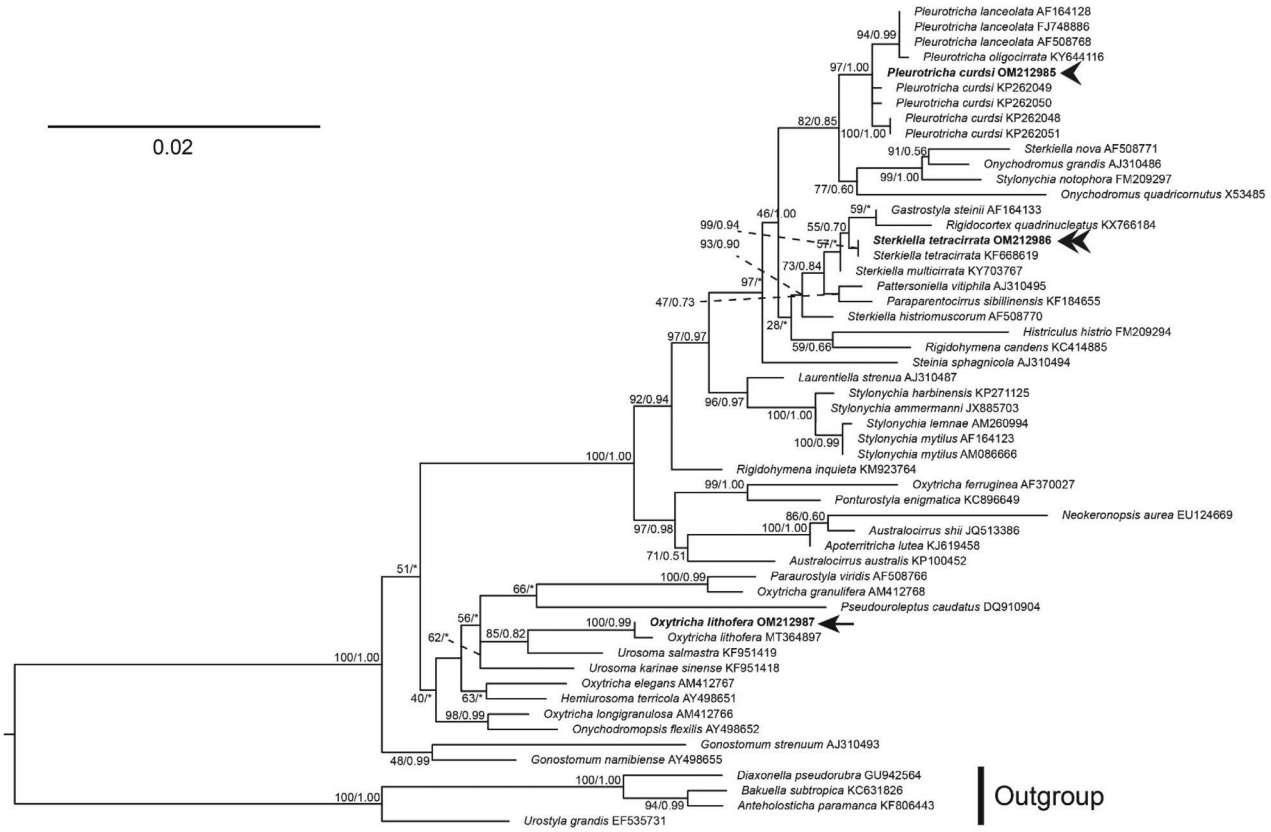

Fig. 8. Maximum likelihood (ML) phylogenetic tree inferred from 18S rDNA sequences of Oxytrichidae ciliates. The phylogenetic trees show the position of three soil ciliates of *Oxytricha lithofera* (arrow), *Pleurotricha curdsi* (arrowhead) and *Sterkiella tetracirrata* (double-arrowhead). New sequences are shown in bold text. Numbers at nodes indicate the bootstrap values of ML and the posterior probability of BI. Nodes designated by an asterisk (∗) correspond to the supports not recovered in the estimated BI tree. The scale bar corresponds to two substitutions per 100 nucleotide positions.

quence divergence of 0.12% to its congener *S. multicirrata* (2 nt difference).

Deposition. The voucher slide with protargol-impregnated specimens is deposited in the National Institute of Biological Resources in Korea (NIBRPR0000110852).

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