

# New record of three hypotrich soil ciliates (Ciliophora: Hypotricha) from South Korea: *Oxytricha multilineata*, *Mixophrya pantanalensis pantanalensis* and *Caudiurostyla sinensis*

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*Oxytricha multilineata*, *Mixophrya pantanalensis pantanalensis*, and *Caudiurostyla sinensis* were isolated from soil samples collected from Cheongju-si and Yeosu-si, confirmed as new to South Korea. *Oxytricha multilineata* was distinguished from other congeners by seven dorsal kineties and dorsal bristles about 15 µm long. *Mixophrya pantanalensis pantanalensis* was characterized by five to seven lithosomes and six dorsal kineties. *Caudiurostyla sinensis* was characterized by colorless cortical granules present, 10–14 midventral pairs, 7–9 left and 6–9 right marginal rows and four or five dorsal kineties. We determined the ribosomal DNA sequences (including 18S rDNA, ITS1, 5.8S rDNA, ITS2, and partial 28S rDNA) from above three species. And the genetic distances were compared with their congeners.

Keywords: 18S rDNA gene, Hemicycliostylidae, Oxytrichidae, protargol staining, terrestrial habitat

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## INTRODUCTION

Recent studies on the taxonomy, morphogenesis, and molecular phylogeny of hypotrichs have led to increased knowledge and understanding of ciliate systematics (Berger, 1999; 2006; 2008; 2011; Chen *et al.*, 2017; Song and Shao, 2017; Zhang *et al.*, 2020). This is due to the variety of their ciliary patterns and nuclear apparatus, which offer important insights for understanding the evolutionary relationships among them species (Zhang *et al.*, 2020). A recent faunistic studies showed that Hypotricha Stein, 1859 is a morphologically diverse group, inhabiting a wider biotope than previously thought (Li *et al.*, 2021).

In the present study, we have reported unrecorded three unrecord soil ciliate species belonging to the subclass Hypotricha in South Korea. We have provided morphological data for each species, including information on their 18S rDNA sequences. Three species assigned to the three genera *Oxytricha* Bory, 1824; *Mixophrya* Foissner and Berger, 2021; and *Caudiurostyla* Lyu *et al.*, 2018 have been identified, and two genera *Mixophrya* and *Caudiurostyla* are first reported in Korea.

## MATERIALS AND METHODS

Three ciliate species were isolated from surface layers (0–5 cm) of soil. The soil samples were dried for two weeks, transferred to Petri dishes, mixed with mineral water, and incubated at room temperature (18–20°C) (Foissner *et al.*, 2002).

Living and protargol impregnation specimens were observed using a stereomicroscope (SZH10; Olympus, Tokyo, Japan) and an optical microscope (Leica DM2500; Wetzlar, Germany) at ×50 to ×1000 magnification. Protargol staining was performed as described by Foissner (2014) (Procedure A). Terminology and classification were performed as described by Berger (1999), Foissner (2016), Foissner and Berger (2021) and Lynn (2008).

DNA extraction, PCR amplification, and sequencing were conducted following Jung *et al.* (2012) methods to amplify the rDNA sequences. The sequences obtained were aligned using BioEdit (Hall, 1999) and compared with seven Oxytrichidae and five Hemicycliostylidae species retrieved from GenBank. Pairwise distances were calculated using Mega X (Kumar *et al.*, 2018).

## RESULTS AND DISCUSSION

Phylum Ciliophora Doflein, 1901  
 Class Spirotrichea Bütschli, 1889  
 Subclass Hypotrichia Stein, 1859  
 Order Sporadotrichida Fauré-Fremiet, 1961  
 Family Oxytrichidae Ehrenberg, 1830  
 Genus *Oxytricha* Bory, 1824

### *Oxytricha multilineata* Jin *et al.*, 2022 (Fig. 1)

다줄첨모하모충 (신칭)

*Oxytricha multilineata* Jin *et al.*, 2022: p. 4, 5, Figs. 2, 3, Table 1.

**Material examined.** Soil sample from Odong Reservoir, Cheongju-si, Chungcheongbuk-do, Korea (36°41'19.6"N, 127°28'26.0"E), collected by Kyu-Seok Chae on 5 May 2021.

**Diagnosis.** Size about 60–85 × 15–29 μm in protargol preparation; flexible body elliptical shape; contractile vacuole positioned at left side of the center of the body; 21–24 adoral zone of membranelles; paroral bipartite; 2 macronuclear nodules with 2 or 3 micronuclei; 3 frontal cirri; 4 frontoventral cirri; 1 buccal cirrus; 3 postoral cirri; 1 left (12–15 cirri) and 1 right (9–12 cirri) marginal row; 2 pretransverse cirri; 5 transverse cirri; 7 dorsal kineties, dorsal bristles about 15 μm long; 3 caudal cirri.

**Distribution.** China and South Korea.

**Remarks.** The Korean population of *O. multilineata* is consistent with the type population (Chinese population) described by Jin *et al.* (2022). However, the Korean population differs from the Chinese population in terms of body size in vivo (60–90 × 30–40 vs. 85–110 × 30–40) (Jin *et al.*, 2022). *Oxytricha multilineata* differs from *O. siseris* in the number of dorsal kineties (7 vs. 5) and dorsal kineties 3 and 4 (distinctly separated vs. not distinctly separated) (Berger, 1999).

The 18S rDNA sequence of *O. multilineata* was 3032 bp in length (GenBank accession number: OQ919810). The 18S rDNA gene sequences of the Korean and Chinese populations were 100% identical (Table 1).

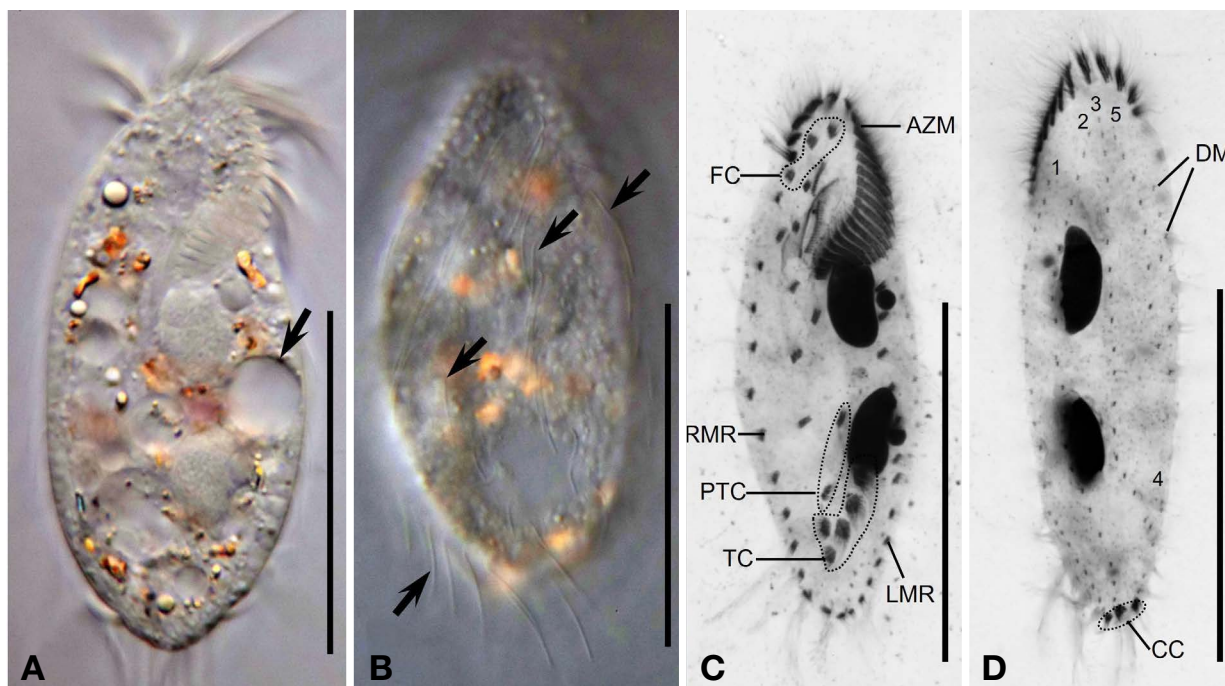
**Voucher slides.** One slide with protargol-impregnated specimens was deposited at the National Institute of Biological Resources (NIBRPR0000111056).

Genus *Mixophrya* Foissner and Berger, 2021

### *Mixophrya pantanalensis pantanalensis* Foissner and Berger, 2021 (Fig. 2) 눈썹첨모하모충 (신칭)

*Mixophrya pantanalensis pantanalensis* Foissner and Berger, 2021: p. 248, Figs. 76–78, Table 32.

**Material examined.** Soil sample from Yeosu-si, Gyeonggi-do, Korea (37°18'26.8"N, 127°41'02.4"E), collect-

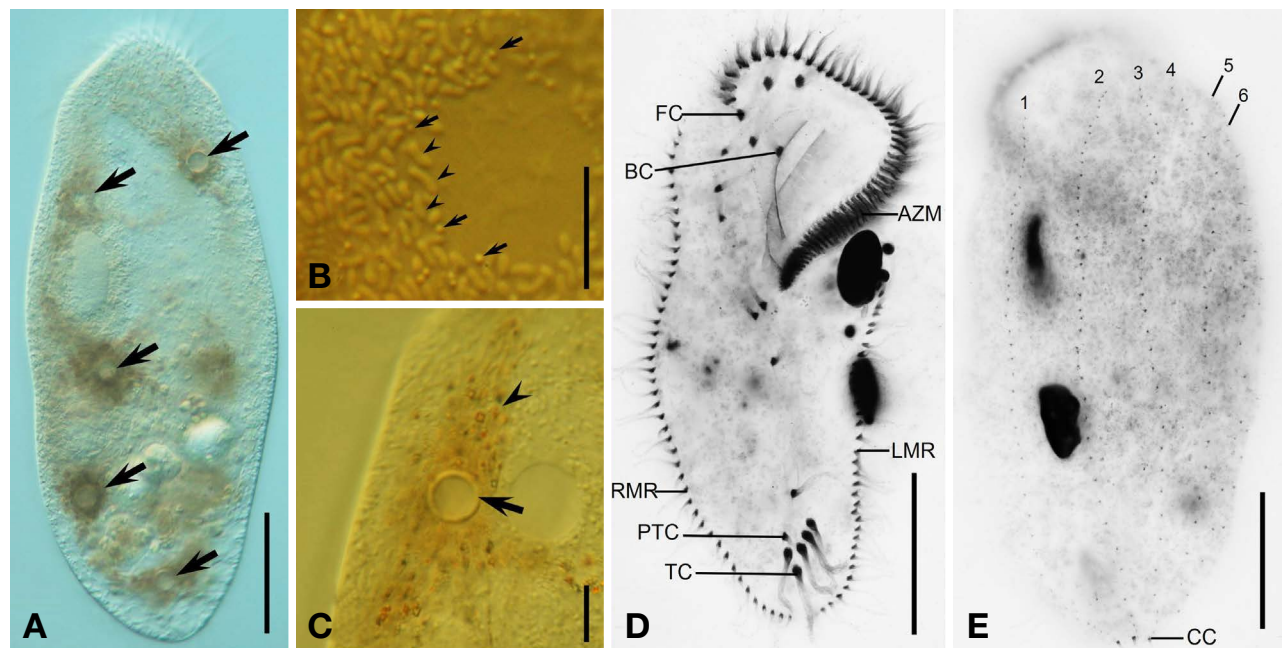


**Fig. 1.** *Oxytricha multilineata* from life (A, B) and after protargol impregnation (C, D). A, Ventral view showing cytoplasm and arrow indicating contractile vacuole; B, Dorsal view showing dorsal bristles (arrows); C, D, Ventral and dorsal view of typical specimen; AZM, adoral zone of membranelles; CC, caudal cirri; DM, dorsomarginal row; FC, frontal cirri; LMR, left marginal cirral row; PTC, pretransverse cirri; RMR, right marginal cirral row; TC, transverse cirri; 1–5, dorsal kineties 1–5. Scale bars = 50 μm.

**Table 1.** The number of nucleotides differences (above the diagonal) and pairwise distances (below the diagonal) between selected Oxytrichidae 18S rDNA sequences.

	1	2	3	4	5	6	7	8	9
<b>1. <i>Oxytricha multilineata</i> OQ919810</b>		0	8	16	18	20	20	23	25
2. <i>Oxytricha multilineata</i> OK299176	0.000		8	16	18	20	20	23	25
3. <i>Quadristicha setigera</i> MG603606	0.004	0.004		16	19	26	22	22	27
4. <i>Urosoma emarginata</i> MH393886	0.009	0.009	0.010		21	21	25	38	19
5. <i>Oxytricha longigranulosa</i> JX899420	0.010	0.010	0.011	0.012		26	24	34	27
6. <i>Urosoma salmastra</i> MH393884	0.012	0.012	0.015	0.012	0.015		29	25	31
7. <i>Oxytricha seokmoensis</i> MK749436	0.012	0.012	0.013	0.015	0.014	0.017		35	30
8. <i>Oxytricha lithofer</i> MT364897	0.013	0.013	0.013	0.016	0.020	0.015	0.021		38
<b>9. <i>Mixophrya pantanalensis pantanalensis</i> OQ919808</b>	0.015	0.015	0.016	0.011	0.016	0.018	0.018	0.022	

\*Bold is this study.

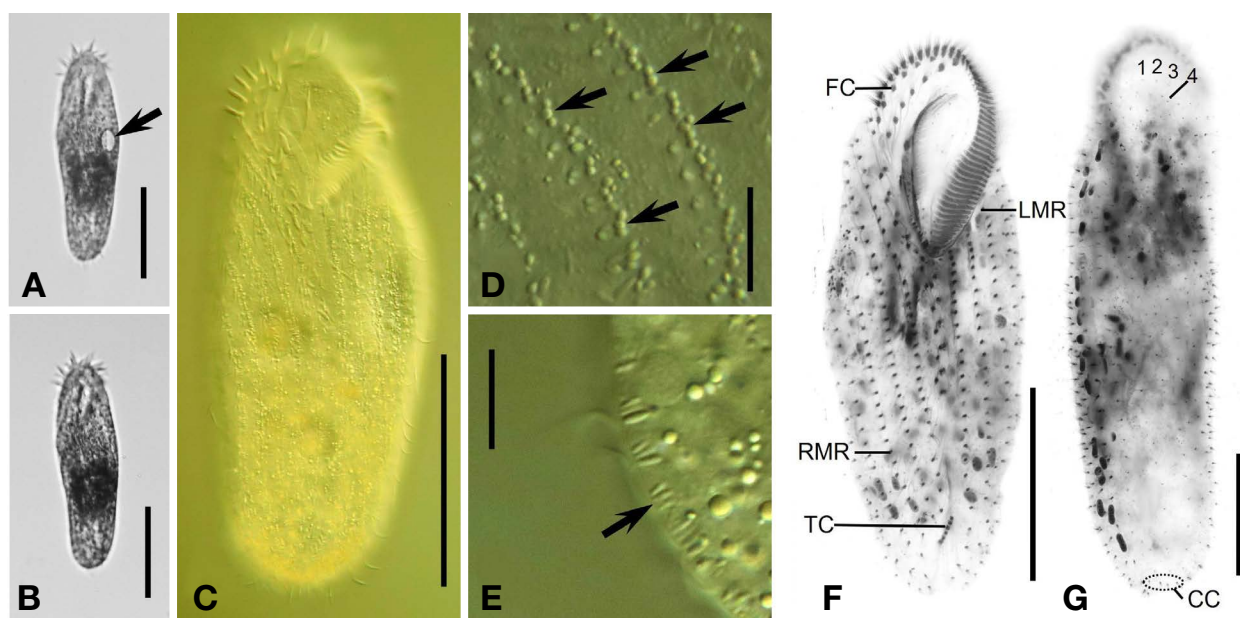
**Fig. 2.** *Mixophrya pantanalensis pantanalensis* from life (A–C) and after protargol impregnation (D, E). A, Ventral view showing lithosomes (arrows); B, Cytoplasm showing cortical granule (arrows) and mitochondria (arrowheads); C, Cytoplasm showing surrounded lithosome (arrow) from crystalline structures and masses of granules (arrowhead); D, E, Ventral and dorsal view of typical specimen; AZM, adoral zone of membranelles; BC, buccal cirrus; CC, caudal cirri; FC, frontal cirri; LMR, left marginal cirral row; PTC, pretransverse cirri; RMR, right marginal cirral row; TC, transverse cirri; 1–6, dorsal kineties 1–6. Scale bars = 10 µm (B, C); 50 µm (A, D, E).

ed by Kyu-Seok Chae on 22 September 2020.

**Diagnosis.** Cell size about 140–245 × 70–126 µm in protargol preparations; flexible body; 48–62 adoral zone of membranelles; 2 macronuclear nodules with about 1–9 micronuclei; contractile vacuole at the left of mid-body; 5–7 lithosomes; 3 frontal cirri; 1 buccal cirrus; 4 cirri in frontoventral cirri; 3 postoral ventral cirri; 2 pre-transverse and 5 transverse cirri; 1 left (35–54 cirri) and 1 right (40–53 cirri) marginal cirral row; 6 dorsal kineties; 3 caudal cirri.

**Distribution.** Brazil and South Korea.

**Remarks.** The Korean population of *M. p. pantanalensis* morphologically corresponds to the type population (Brazilian population) in terms of ventral and dorsal ciliature (Foissner and Berger, 2021). However, the Korean population differs from the Brazilian population in terms of body size in vivo (about 250 × 130 vs. about 370 × 155 µm) (Foissner and Berger, 2021). *Mixophrya pantanalensis pantanalensis* differs from *M. gigantea* in the number of dorsal kineties (6 vs. 4) and the present of lithosomes



**Fig. 3.** *Caudiurostyla sinensis* from life (A–E) and after protargol impregnation (F, G). A, B, Ventral view showing contractile vacuole (arrow); C, Ventral view from live; D, Ventral view showing cortical granules (arrows) lying along with cirral; E, Side view of cortical granules (arrow); F, G, Ventral and dorsal view of typical specimen; FC, frontal cirri; LMR, left marginal cirral row; RMR, right marginal cirral row; TC, transverse cirri. Scale bars = 10  $\mu$ m (D, E); 50  $\mu$ m (A, B, C, F, G).

**Table 2.** The number of nucleotides differences (above the diagonal) and pairwise distances (below the diagonal) between selected Hemicyclostylidae 18S rDNA sequences.

	1	2	3	4	5	7
<b>1. <i>Caudiurostyla sinensis</i> OQ919809</b>		0	5	7	30	38
2. <i>Caudiurostyla sinensis</i> KY874004	0.000		5	7	30	38
3. <i>Hemicyclostyla sphagni</i> FJ361758	0.003	0.003		6	35	37
4. <i>Hemicyclostyla franzi</i> KY874008	0.004	0.004	0.003		37	35
5. <i>Hemicyclostyla franzi</i> MN160210	0.018	0.018	0.021	0.022		27
6. <i>Hemicyclostyla franzi</i> MN160324	0.023	0.023	0.022	0.021	0.016	

\*Bold is this study.

(present vs. absent). *Mixophrya pantanalensis pantanalensis* differs from *M. p. australiensis* by present of lithosomes (present vs. absent).

The 18S rDNA sequence of *M. p. pantanalensis* was 3028 bp in length (GenBank accession number: OQ919808). The 18S rDNA gene sequence of *M. p. pantanalensis* was compared with that of species within the Oxytrichidae family because no sequences were available for the same species or genus. The species with the lowest pairwise distance value was *Urosoma emarginata* (MH393886). The interspecific genetic distance between *M. p. pantanalensis* and *U. emarginata* was 0.011 (19 bp difference of 1660 bp) (Table 1).

**Voucher slides.** One slide with protargol-impregnated

specimens was deposited at the National Institute of Biological Resources (NIBRPR0000111059).

Order Urostylida Jankowski, 1979

Family Hemicyclostylidae Lyu *et al.*, 2018

Genus *Caudiurostyla* Lyu *et al.*, 2018

***Caudiurostyla sinensis* Lyu *et al.*, 2018 (Fig. 3)**

미극모미주하모충 (신칭)

*Caudiurostyla sinensis* Lyu *et al.*, 2018: p. 168–170, Figs. 1, 2, Table 1.

**Material examined.** Soil sample from Miho River, Cheongju-si, Chungcheongbuk-do, Korea (36°38'3.54"

N, 127°21'18.44"E), collected by Kyu-Seok Chae on 15 September 2020.

**Diagnosis.** Cell size 143–202 × 40–58 µm in protargol impregnation; body flexible with slender to elliptical shape; 46–60 adoral membranelles; 1 × 3–4 µm colorless cortical granules present; 100–177 macronuclear nodules with 3–6 micronuclei; 11–15 frontal cirri; 2 frontoterminal cirri; 11–15 midventral pair; 6–7 left and 5–7 right marginal row; 5 or 7 transverse cirri; 4 or 5 dorsal kineties; 3–5 caudal cirri.

**Distribution.** China and South Korea.

**Remarks.** The Korean population of *C. sinensis* is consistent with the type population (Chinese population) in terms of ventral and dorsal ciliature and the number of macronuclear nodules (Lyu *et al.*, 2018). *Caudiurostyla sinensis* resembles *Hemicycliostyla franzi* (Foissner, 1987) Paiva *et al.*, 2012, but two species can be distinguished by the number of caudal cirri (3 or 5 vs. absent) and buccal cirri (1 vs. 2–3) (Li *et al.*, 2018).

The 18S rDNA sequence of *C. sinensis* was 3026 bp in length (GenBank accession number: OQ919809). The 18S rDNA gene sequences of the Korean and Chinese populations are 100% identical (Table 2).

**Voucher slides.** One slide with protargol-impregnated specimens was deposited at the National Institute of Biological Resources (NIBRPR0000111060).

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