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# Morphology and Molecular Phylogeny of *Pseudouroleptus jejuensis* nov. spec., a New Soil Ciliate (Ciliophora, Spirotrichea) from South Korea

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**Abstract.** A new soil ciliate, *Pseudouroleptus jejuensis*, was discovered from Jeju Island, South Korea and described based on live observation, protargol impregnation, and SSU rRNA gene sequence analyses. *Pseudouroleptus jejuensis* differs from other congeneric species mainly by number of dorsal kineties (5 vs. 4). Based on our observation of late dividers, we confirm that the dorsal kinety anlage 3 forms 3 kineties (i.e., dorsal kineties 3–5), and the dorsal kinety anlagen 1–3 form 3–5/1–2/0 caudal cirri, respectively. Our gene trees support the assignment of this new species in *Pseudouroleptus* to full supporting values.

**Key words:** *Pseudouroleptus jejuensis*, soil ciliate, morphology, small subunit ribosomal RNA gene, oxytrichid, South Korea.

## INTRODUCTION

According to previous outstanding studies (Foissner 1998; Foissner *et al.* 2002a, b, 2005, 2008; Chao *et al.* 2006), soil ciliates follow the "moderate endemicity model" (Foissner *et al.* 2008) and include many undescribed species (Chao *et al.* 2006). However, no new soil ciliate has been reported in South Korea because of a scarcity of Korean ciliatologists specializing in terrestrial ciliates. In the present study, we discovered and described a new soil ciliate collected from Jeju Island, South Korea using the modern techniques, including silver impregnation and molecular phylogeny (Foissner 1991, Jung *et al.* 2011, Vd'ačný and Foissner 2012).

The new species belongs to *Pseudouroleptus* Hemberger, 1985 which has a tailed posterior body and fragmented dorsal kinety (Berger 1999, 2008). *Pseudouroleptus jejuensis* nov. spec. was discovered on the volcanic Jeju Island, and has a similar morphology to the type species *P. caudatus* Hemberger, 1985. To clarify the Jeju population as a new species, we observed protargol-impregnated specimens including interphasic and some dividing individuals. In addition, we sequenced the small subunit ribosomal RNA (SSU rRNA) gene and compared the sequence with related oxytrichids by using phylogenetic analysis.

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## MATERIALS AND METHODS

### Sample collection and identification

*Pseudouroleptus jejuensis* was discovered in a soil sample of Jeju Island, South Korea (N33°18′22″ E126°15′42″) in December 2012. The new species was cultured by using the non-flooded Petri dish method (see Foissner *et al*. 2002a, b). Briefly, the soil sample was filled with distilled water to excyst soil ciliates in the dried samples and inspected after 2 days. We attempted to set up pure cultures of the species in the mineral water with wheat grains but were unsuccessful. Thus, all of the data presented here are based on raw culture specimens.

Live specimens were observed under a light microscope (Leica DM2500, Wetzlar, Germany) at magnifications ranging from 50 to 1,000. Protargol impregnation was performed to reveal the infraciliature (Foissner 1991, Vd'ačný and Foissner 2012) which included the interphase and some ontogenetic stages.

Terminology and classification are according to Berger (2008) and Lynn (2008).

### SSU rRNA gene sequence

Each individual of the new species was repeatedly washed with distilled water. Extraction of genomic DNAs from single specimens was performed according to the manufacturer's protocol, using a RED-Extract-N-Amp Tissue PCR Kit (Sigma, St. Louis, MO, USA). New EukA (5'-CTG GTT GAT YCT GCC AGT-3') forward primer modified from Medlin *et al.* (1988) and LSU rev3 (Sonnenberg *et al.* 2007) reverse primer were used for PCR amplification of the nearly complete SSU rRNA gene. The optimized PCR conditions were as follows: denaturation at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 4 min, and a final extension step at 72°C for 7 min. The QIAquick® PCR Purification Kit (QIAGEN, Hilden, Germany) was used for purification of the PCR products. Two internal primers (18S+810 [5'-GCC GGA ATA CAT TAG CAT GG-3'] and 18S-300 [5'-CAT GGT AGT CCA ATA CAC TAC-3']) were used for sequencing, which was performed with the ABI 3700 sequencer (Applied Biosystems, Foster City, CA, USA).

### Molecular analysis

Sequenced fragments of the SSU rRNA gene were assembled using the BioEdit program (Hall 1999) and aligned by using the Clustal X 1.81 (Jeanmougin *et al.* 1998). The Mega 4.0.2 (Tamura *et al.* 2007) was used to calculate genetic distances and to construct the neighbor joining (NJ) tree with 1,000 replications, which employed the Kimura 2-parameter (K2P) distance option (Kimura 1980). To determine the appropriate DNA substitution model for the phylogenetic analysis, we used the Akaike information criterion (AIC) to identify the best model of evolution that fitted our data, by conducting the jModelTest 2.1.1 (Darriba *et al.* 2012). The model selected for the data set was TIM2 + I (0.7660) + G (0.5970). Phylogenetic analyses were performed by using the maximum likelihood (ML) and the Bayesian inference (BI) methods. The PhyML version 3.0 (Guindon and Gascuel 2003) was used for the maximum likelihood analysis. Confidence in the resulting relationship was assessed by using the bootstrap procedure with 1,000 replications for the ML. The BI assessment was performed with the MrBayes 3.1.2, by simulating the Markov chain Monte Carlo (MCMC) algorithm for 1,000,000 generations. Trees were sampled every 100 generations and 300,000 were discarded as the burn-in (Ronquist and Huelsenbeck 2003). *Gonostomum strenuum* was selected as an outgroup for the trees.

## RESULTS

### Description of *Pseudouroleptus jejuensis* (Figs 1–6; Table 1)

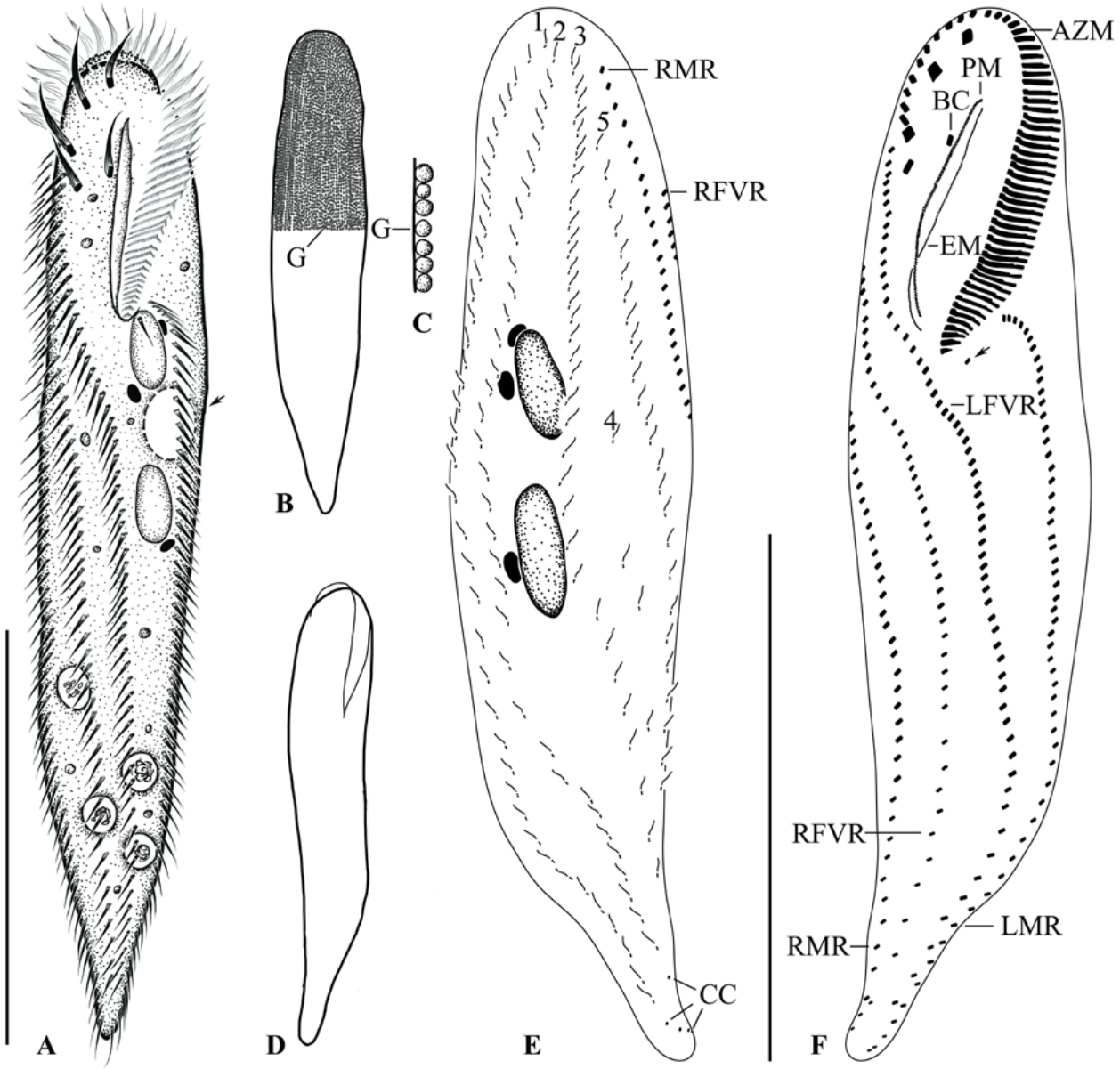
**Diagnosis:** Size *in vivo* about 260 × 45 μm. Cylindrical to elliptical with tail-like posterior portion. Two macronuclear nodules and 3 micronuclei on average. Contractile vacuole slightly above mid-body. Cortical granules densely arranged in longitudinal stripes, colourless, spherical to globular, about 1.3 μm in diameter. On average 56 adoral membranelles, 59 cirri each in right and left marginal row, 52 cirri in right frontoventral row, 63 cirri in left frontoventral row, 1 buccal cirrus, 1 cirrus behind right frontal cirrus, 1 postperistomial cirrus, and 5 dorsal kineties. Dorsal kinety 3 multiple-fragmented without forming caudal cirri.

**Type locality:** Soil with the litter of tree, *Celtis* sp., from Jeju Island, South Korea, N33°18′22″ E126°15′42″.

**Type material:** One holotype slide (NIBRPR-0000104240) and seven paratype slides (NIBRPR-0000104241–NIBRPR0000104247) with protargol-impregnated specimens including some dividers have been deposited in the National Institute of Biological Resources (NIBR), South Korea. Relevant specimens have been marked by circles on the bottom of the slides.

**Etymology:** Named after the island on which the specimens were discovered.

**Description (Figs 1–3):** Size *in vivo* 220–300 × 35–55 μm in raw cultures, usually about 260 × 45 μm *in vivo* (Table 1); posterior body portion usually slightly curved rightwards in protargol preparations. Length:width ratio *in vivo* moderately variable ([5.4–6.3]:1); body length slightly shorter in protargol-impregnated specimens because of curved body shape (on average 4.8:1). Shape cylindrical to elliptical with tail-like posterior portion, i.e., left and right margin almost parallel, and posterior body part at three-quarters of cell distinctly narrower than mid-body part that forms a tail-like body shape (Figs 1A, B, D, 2A, H); body shape straight in fast-moving behavior and slight-sigmoidal when gliding for feeding (Fig. 2H). Invariably 2 macronuclear nodules on left of midline behind buccal vertex; ellipsoidal, on average 25 × 8 μm in protargol-im-




**Figs 1A–F.** *Pseudouroleptus jejuensis* from life (A–D) and after protargol impregnation (E, F). **A** – ventral view of a representative specimen, arrow indicates contractile vacuole; **B, C** – arrangement of cortical granules on dorsal side (B) and optical section (C); **D** – ventral view of a specimen gliding for feed, showing a slightly curved body shape; **E, F** – dorsal (E) and ventral views (F) of the holotype specimen. Arrow in **F** denotes postperistomial ventral cirrus. AZM – adoral zone of membranelles, BC – buccal cirrus, CC – caudal cirri, 1–5 – dorsal kineties 1–5, EM – endoral membrane, G – cortical granules, LFVR – left frontoventral row, LMR – left marginal row, PM – paroral membrane, RFVR – right frontoventral row, RMR – right marginal row. Scale bars: 100 μm.

pregnated specimens. Two to four micronuclei near to or overlapped with macronuclear nodules, ellipsoidal, on average 6 × 4 μm in silver preparations (Figs 1A, 2D, 3I). Contractile vacuole slightly above mid-body, without distinct collecting canals, on average 18 × 13 μm when fully extended (Figs 1A, 2A). Cortex flexible; cortical granules densely arranged in narrow vertical stripes, colourless, about 1.3 μm in diameter (Figs

**Table 1.** Morphometric data from *Pseudouroleptus jejuensis*.

| Characteristics[a] | mean | M | SD | SE | CV | Min | Max | *n* |
|---|---|---|---|---|---|---|---|---|
| Body, length | 217 | 220 | 17.5 | 3.9 | 8.1 | 185 | 250 | 20 |
| Body, width | 45.3 | 45 | 5.5 | 1.2 | 12.1 | 36.3 | 55 | 20 |
| Body, length:width ratio | 4.8 | 4.8 | 0.6 | 0.1 | 11.8 | 3.5 | 5.6 | 20 |
| Anterior body end to end of adoral zone, distance | 65 | 65 | 4.4 | 1.0 | 6.7 | 55 | 72.5 | 20 |
| Body length:distance from anterior body end to end of adoral zone, ratio | 0.30 | 0.30 | 0.03 | 0.01 | 9.1 | 0.22 | 0.35 | 20 |
| Adoral membranelles, number | 56.4 | 56 | 2.7 | 0.6 | 4.7 | 52 | 62 | 19 |
| Anterior macronuclear nodule, length | 24.6 | 24.4 | 3.8 | 0.8 | 15.3 | 16 | 32 | 20 |
| Anterior macronuclear nodule, width | 8.2 | 8 | 0.9 | 0.2 | 11.3 | 6.4 | 9.6 | 20 |
| Macronuclear nodules, number | 2 | 2 | 0 | 0 | 0 | 2 | 2 | 19 |
| Micronuclei, length | 5.8 | 5.6 | 0.9 | 0.2 | 15.3 | 4.8 | 8 | 20 |
| Micronuclei, width | 4 | 4 | 0.7 | 0.2 | 16.9 | 3.2 | 4.8 | 20 |
| Micronuclei, number | 2.6 | 2 | 0.7 | 0.2 | 26.9 | 2 | 4 | 19 |
| Frontal cirri, number | 3 | 3 | 0 | 0 | 0 | 3 | 3 | 19 |
| Buccal cirri, number | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 19 |
| Cirri behind right frontal cirrus, number | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 19 |
| Postperistomial cirri, number | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 19 |
| Left frontoventral row, number of cirri | 62.5 | 63 | 4.2 | 1.0 | 6.8 | 55 | 72 | 19 |
| Right frontoventral row, number of cirri | 52 | 52 | 3.7 | 0.9 | 7.1 | 46 | 58 | 19 |
| Left marginal cirri, number | 59.2 | 60 | 3.3 | 0.8 | 5.6 | 53 | 66 | 19 |
| Right marginal cirri, number | 59.1 | 59 | 3.1 | 0.7 | 5.3 | 53 | 66 | 19 |
| Dorsal kineties, number[b] | 5 | 5 | 0 | 0 | 0 | 5 | 5 | 19 |
| Dorsal kinety 1, number of bristles | 37 | 38 | 4.1 | 1.0 | 11.2 | 29 | 44 | 19 |
| Dorsal kinety 2, number of bristles | 35.3 | 35 | 3.1 | 0.7 | 8.9 | 29 | 40 | 19 |
| Dorsal kinety 3, number of bristles | 31.1 | 30 | 4.4 | 1.0 | 14.2 | 25 | 40 | 19 |
| Dorsal kinety 4, number of bristles[b] | 6.1 | 6 | 1.6 | 0.4 | 26.1 | 4 | 9 | 19 |
| Dorsal kinety 5, number of bristles | 27.8 | 28 | 3.1 | 0.7 | 11.0 | 24 | 37 | 19 |
| Caudal cirri, total number | 5.4 | 5 | 0.9 | 0.2 | 16.6 | 4 | 7 | 19 |
| Caudal cirri in dorsal kinety 1, number | 3.9 | 4 | 0.8 | 0.2 | 19.8 | 3 | 5 | 19 |
| Caudal cirri in dorsal kinety 2, number | 1.5 | 1 | 0.5 | 0.1 | 34.8 | 1 | 2 | 19 |

[a] Data based on protargol-impregnated specimens. Measurements in µm. CV – coefficient of variation in %; M – median; Max – maximum; mean – arithmetic mean; Min – minimum; *n* – number of specimens investigated; SD – standard deviation; SE – standard error of arithmetic mean.

[b] The basal bodies between dorsal kineties 3 and 5 are slightly sparsely located and these basal bodies are considered as a single row, namely dorsal kinety 4.

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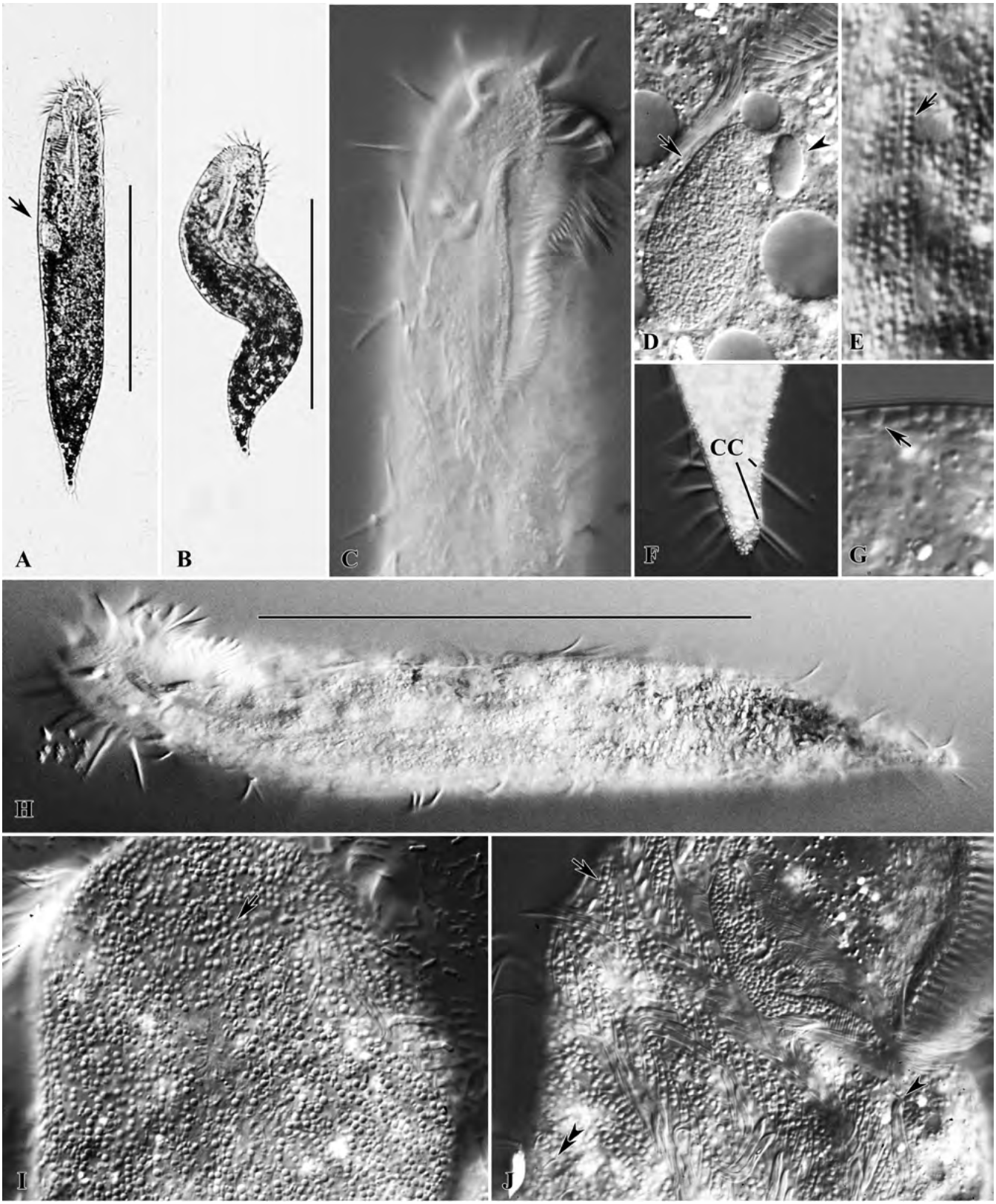
**Figs 2A–J.** *Pseudouroleptus jejuensis* from life. **A–C, H** – dorsal (A, B) and ventral (C, H) views, arrow marks contractile vacuole; **D** – ventral view showing macronuclear nodule (arrow) and micronucleus (arrowhead); **E, G, I, J** – cortical granules (arrows) of dorsal views (E, I), ventral view (J), and optical section (G); dorsal bristles (double arrowhead) and postperistomial cirrus (arrowhead) denoted in **J**; **F** – caudal cirri in dorsal view. CC – caudal cirri. Scale bars: 130 µm.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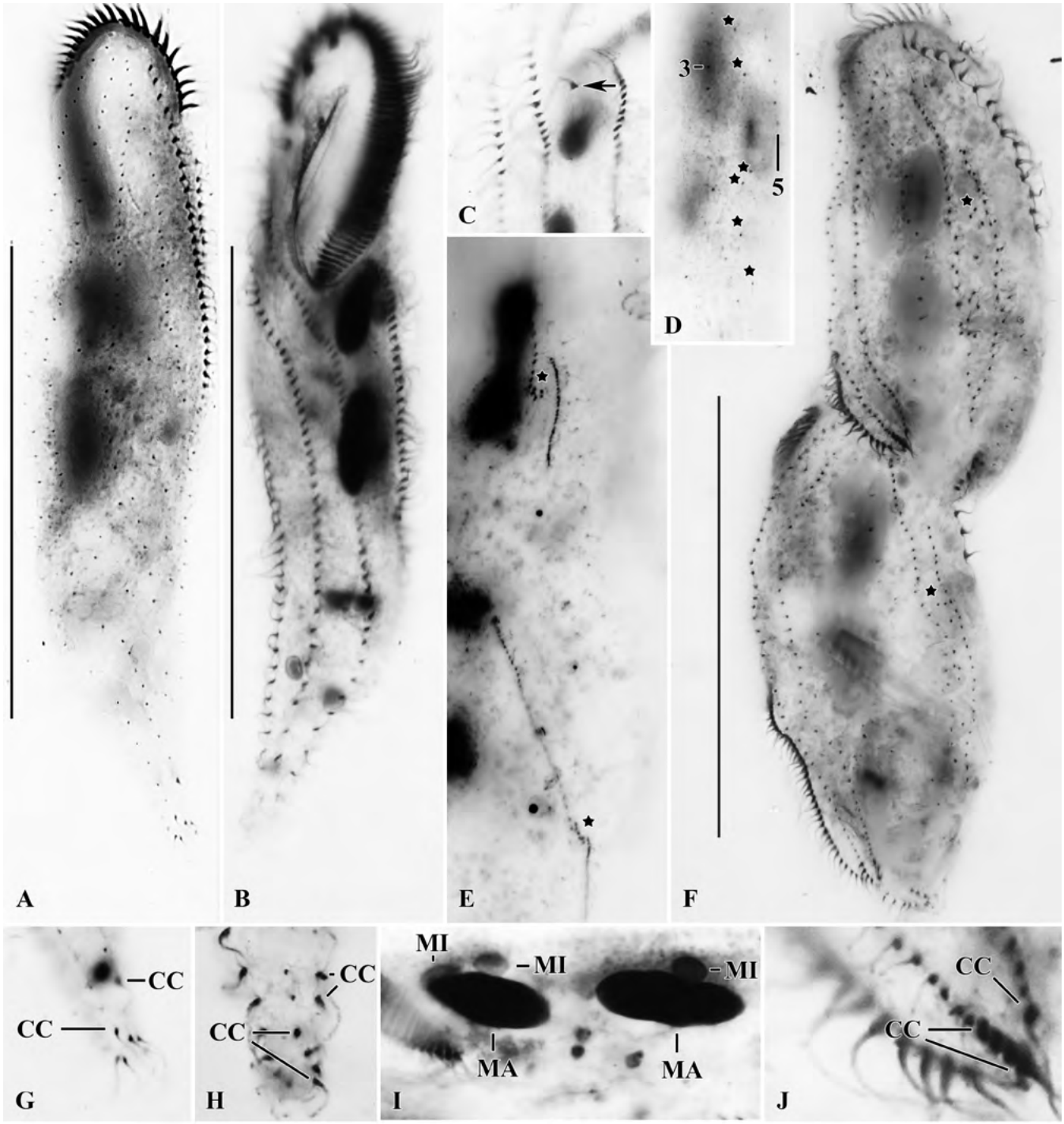



**Figs 3A–J.** *Pseudouroleptus jejuensis* during interphase (A–D, G–I) and ontogenesis (E, F, J) after protargol impregnation. **A–C** – dorsal view (A) and ventral views (B, C), arrow indicates postperistomial cirrus; **D** – dorsal view showing basal bodies (asterisks) in dorsal kinety 4; **E**, **F** – dorsal views of late dividers, asterisks denote dorsal kinety 4 developed by multiple fragmentation of dorsal kinety anlage (DKA) 3; **G, H, J** – dorsal views showing caudal cirri developed from DKA 1, 2 while DKA 3 does not participate in the formation of these caudal cirri during ontogenesis; **I** – ventral view showing macronuclear nodules and micronuclei. CC – caudal cirri, MA – macronuclear nodules, MI – micronuclei. Scale bars: 100 μm.

1B, C, 2E, G, I, J). Cytoplasm colourless, with 8–16 µm-sized food vacuoles usually in posterior half of cell. Feeds on bacteria, testate amoebae, diatoms, and organic soil particles. Glides moderately fast on the bottom of Petri dish.

All cirri, except for frontal and buccal cirri, 15–20 µm long *in vivo*; frontal and buccal cirri 18–25 µm long *in vivo*, and composed of distinctly more basal bodies than other cirri (Figs 1F, 3B). Marginal and frontoventral cirri evenly spaced within the rows, and similar-sized, but intervals and size becoming slightly wider and smaller, respectively, at posterior body portion (Fig. 1F). Right marginal and right frontoterminal row commence on dorsal side. Cirral pattern highly similar to that of type species *P. caudatus* (for morphometric data of these cirri, see Table 1).

Dorsal bristles 3–4 µm long *in vivo*, 5 dorsal kineties; dorsal kinety 3 multiple-fragmented and not associated with forming caudal cirri; dikinetids of dorsal kinety 4 sparsely arranged in a row and rarely appearing in 2 rows, because of the scattered distribution pattern, but considered here as a single row (Figs 1E, 3D–F). Four to seven caudal cirri developed from dorsal kineties 1 and 2 (Figs 3G, H, J). Late dividers observed and support the above features (Figs 3E, F, J; see ontogenesis section).

Oral apparatus oxytrichid pattern (Berger 1999), i.e., undulating membranes *Oxytricha* pattern and adoral zone of membranelles 30% of body length on average (Table 1); undulating membranes optical crossing at posterior half; buccal lip distinct (Fig. 2C) and very likely angular (for review of oral apparatus, see Foissner and Al-Rasheid (2006)). Adoral zone of membranelles shaped like a question mark, largest membranelles about 10 µm width *in vivo* and in protargol impregnation; cilia about 15–20 µm long *in vivo*.

**Ontogenesis of *P. jejuensis* (Figs 4, 5)**

Some of dividers (i.e., middle, late, and post-dividers) were observed and described.

**Nuclear apparatus:** Division of the nuclear apparatus proceeds as in most oxytrichids and urostylids (Berger 1999, 2006). In mid-dividers, the macronuclear nodules fuse and become a single mass and micronuclei are inflated (Fig. 4A). Next, the macronuclear mass divides twice and the inflated micronuclei become split and finish the division. The micronuclei always lie on the left of the macronuclear mass during the ontogenesis.

**Oral apparatus:** During the ontogenesis, the parental adoral membranelles are very likely inherited unchanged. In mid-dividers, the adoral membranelles of the opisthe form their definite structure and shape. The undulating membranes are still not separated in both the proter and opisthe. Next, the distal ends of adoral zones form arches like question mark. The undulating membranes become split.

**Ventral cirral pattern:** In the mid-dividers, cirral anlagen I–VI are formed between right frontoventral row and adoral zone (Fig. 4B). Anterior portions of anlagen IV and VI are separated from the posterior portions. Left frontoventral row is very likely composed of these anterior portions of anlagen IV and VI, and entire anlage V. The rearmost cirrus of anlage IV migrates posteriorly below adoral zone (Figs 4D, 5B, D, arrowheads).

**Marginal cirral rows:** As usual the marginal cirral anlagen are developed within the parental rows and replace the parental structures. Dorsomarginal kineties are not found.

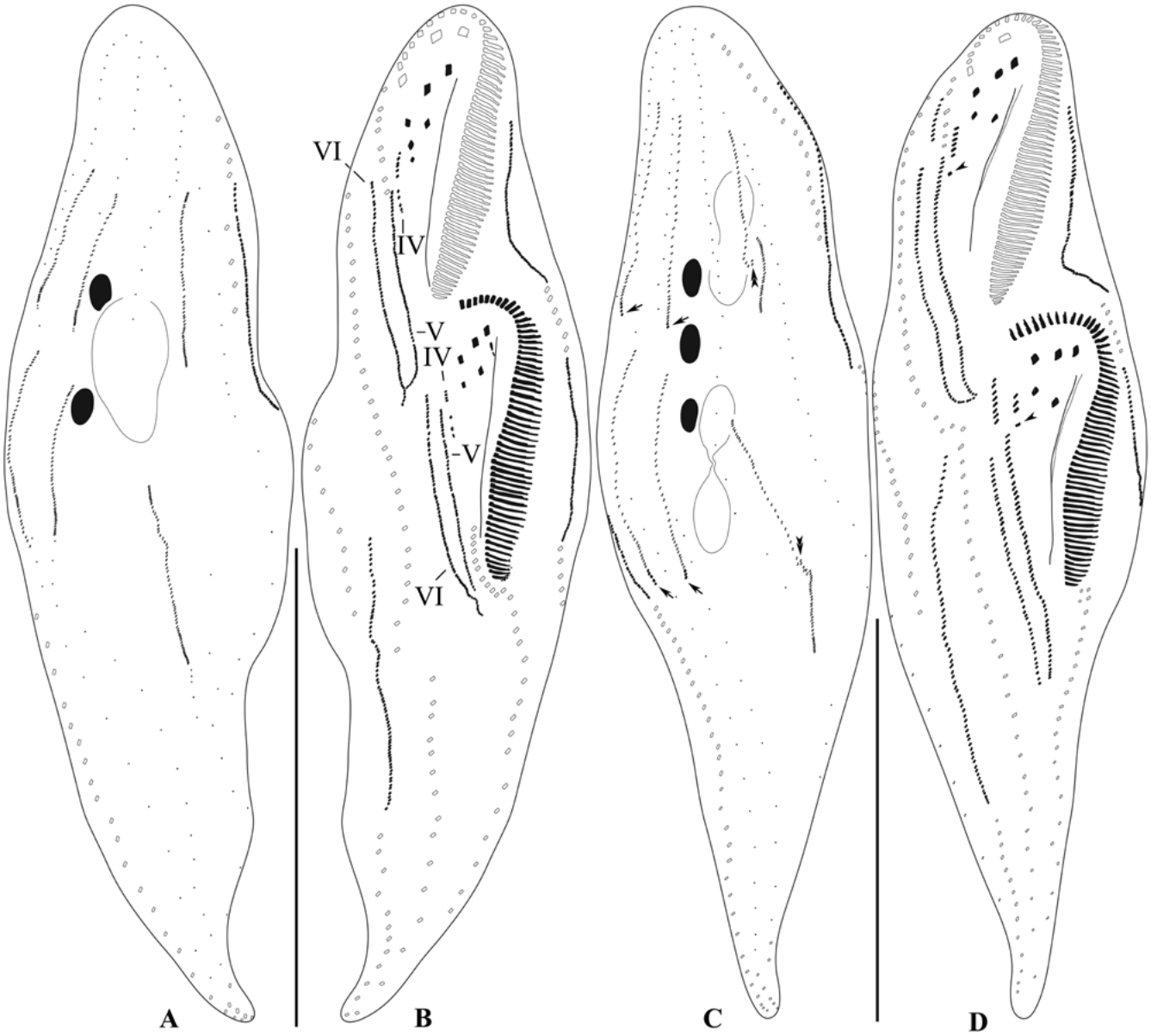
**Dorsal ciliature:** In the mid-dividers, the dorsal kineties 1–3 anlagen extend to the ends of the cell. Next, the kinety 3 anlage is split into three fragments, namely dorsal kineties 3–5 (Fig. 4C, double arrowheads). Caudal cirri are developed at the posterior portions of kineties 1, 2 anlagen only (Figs 4C, 5A, C, arrows). The caudal cirri are not found from kinety 3 anlage, even in late and post-dividers (Figs 5A, C).

**Sequence analysis:** The SSU rRNA gene sequence of *P. jejuensis* is 1,561 bp long and was deposited in the GenBank under accession number KF471024. *Pseudouroleptus jejuensis* was clustered with *P. caudatus* DQ910904 and this clade was highly supported by all trees (NJ/ML/BI, 100/100/1.00) (Fig. 4). The K2P distance between *P. jejuensis* and *P. caudatus* was 0.71%.

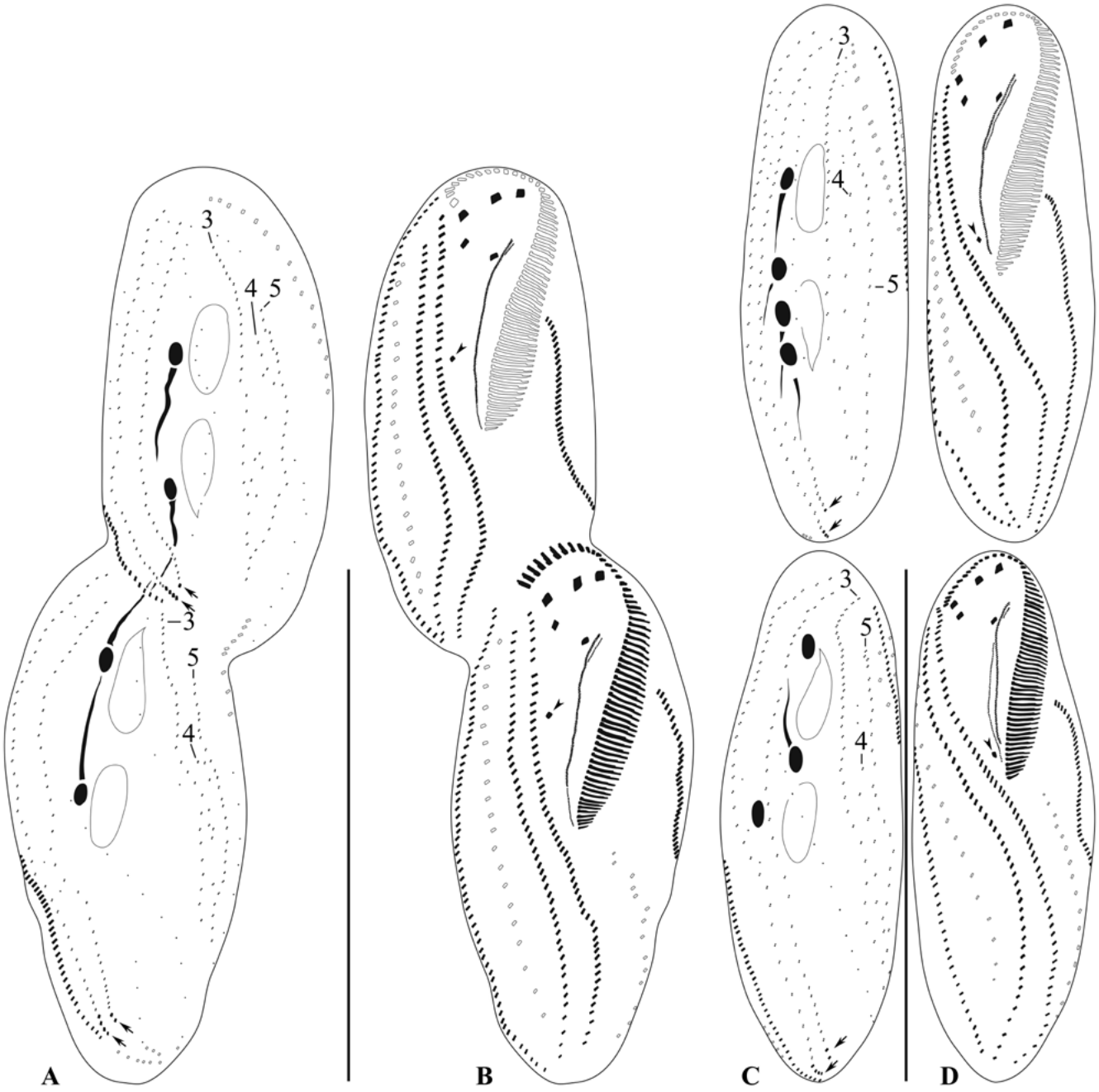
**Occurrence and ecology:** As yet found only at the type locality of Jeju Island. *Pseudouroleptus jejuensis* was isolated from a soil sample that was dark-brown coloured and covered with litter (*Celtis* sp.).

## DISCUSSION

**Comparison of *P. jejuensis* with similar species:** *Pseudouroleptus caudatus* is the sole species in the genus and consists of 2 subspecies (for review of the genus *Pseudouroleptus*, see Berger 2008): *Pseudouroleptus caudatus caudatus* Hemberger, 1985 and *P. caudatus na-*




**Figs 4A–D.** *Pseudouroleptus jejuensis*, middle (A, B) and late divider (C, D) after protargol impregnation. Note that the parental dorsal bristles are shown by single dots although still composed of dikinetids. The parental dorsal dikinetids become smaller and are less impregnated than newly developed one. **A, B** – dorsal (A) and ventral (B) views of middle divider showing dorsal kineties and cirral anlagen. **C, D** – dorsal (C) and ventral (D) views of late divider showing dorsal kinety 3 fragmentation (double arrowheads). Note that caudal cirri are developed at posterior end of kineties 1, 2 only (arrows). Postperistomial cirrus (arrowheads) is originated from the anlage IV and split from anterior part of the anlage. IV–VI – cirral anlagen IV–VI. Scale bars: 150 μm.




**Figs 5A–D.** *Pseudouroleptus jejuensis*, late (A, B) and post-dividers (C, D) after protargol impregnation. Note that the parental dorsal bristles are shown by single dots although they are still composed of dikinetids. **A, B** – dorsal (A) and ventral (B) views of late divider showing caudal cirri (arrows) and posteriorly migrating postperistomial cirrus (arrowheads). Note that the caudal cirri are not developed from dorsal kinety anlage 3. **C, D** – dorsal (C) and ventral (D) views of post-dividers. The two post-dividers were fixed from a single dividing cell immediately after the complete cell division. Some of parental dorsal bristles and cirri are still observed, and postperistomial (arrowheads) and caudal cirri (arrows) migrate forward to their final position. 3–5 – dorsal kineties 3–5. Scale bars: 150 μm.

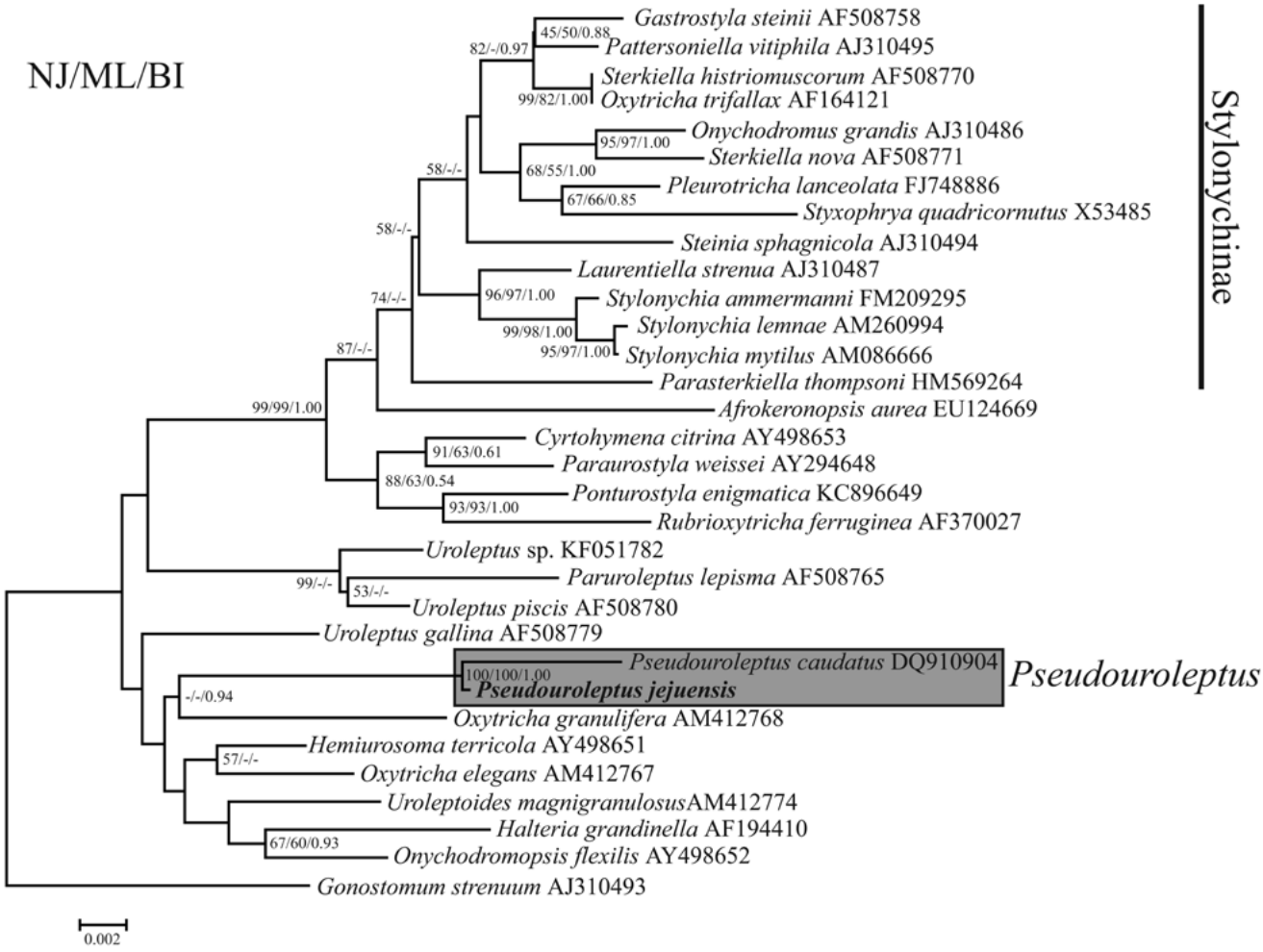



**Fig. 6.** Small subunit rRNA gene phylogeny of 31 oxytrichids based on 3 methods (NJ – Neighbor Joining; ML – Maximum Likelihood; BI – Bayesian Inference). Bootstrap values of the NJ and the ML are shown at each node with posterior probabilities of the BI; a dash denotes a value of below 0.50 (BI) or 50% (NJ and ML). *Pseudouroleptus jejuensis* is denoted in bold.

*mibiensis* Foissner, Agatha and Berger, 2002. *Pseudouroleptus jejuensis* differs from these subspecies mainly by the number of dorsal kineties, which include an additional dorsal kinety in comparison with the other 2 subspecies. The detailed comparison is discussed below.

*Pseudouroleptus caudatus caudatus* is the nominotypical subspecies and differs from *P. jejuensis* mainly by the dorsal kineties (4 vs. 5). However, the dorsal kineties are not clearly recognizable from previous reports (Hemberger 1982, Foissner *et al.* 2002). Thus, to establish a new species in *Pseudouroleptus* further investigation on the type species is necessary. Fortunately, Küppers and Claps (2013) reported the morphology of a Argentine population with a clear illustration of the dorsal kineties. The Argentine population has a consistent morphology with the type population and these two populations are located in the same continent, South America, so that conspecificity is beyond reasonable doubt. Based on a comparison with these populations of *P. caudatus caudatus*, we confirm that the Korean population is a new species in *Pseudouroleptus*. *Pseudouroleptus caudatus caudatus* can be separated from *P. jejuensis* by dorsal kineties (4 vs. 5), and development of caudal cirri from dorsal kinety 3 anlage (present vs. absent). Additionally, the former species forms a single caudal cirrus in dorsal kinety 1 (vs. 3–5 in

*P. jejuensis*) (Hemberger 1982, Berger 1999, Küppers and Claps 2013, Küppers pers. comm.).

*Pseudouroleptus caudatus namibiensis* can be distinguished from *P. jejuensis* by having fewer adoral membranelles (33–51 vs. 52–62), right frontoventral cirri (20–31 vs. 46–58), dorsal kineties (4 vs. 5), and caudal cirri (1–4 vs. 4–7). In *P. caudatus namibiensis*, the right frontoventral row is distinctly shorter than in *P. jejuensis*; this difference in length results in the different number of cirri.

In the ontogenesis, the processes are almost identical in *P. caudatus caudatus* and *P. jejuensis* with the exception of these characteristics: i) the number of caudal cirri developed from dorsal kineties 1, 3 anlagen; and ii) the number of dorsal kineties developed from dorsal kinety 3 anlage. Although Hemberger (1982) described these numbers of *P. caudatus caudatus* in the text, clear illustrations are not available to support the description in his paper. Recently, Küppers and Claps (2013) reported the morphology of the Argentine population of *P. caudatus caudatus*. According to Küppers and Claps (2013) and a personal communication to Küppers, the Argentine population differs from the Korean population by 4 dorsal kineties (vs. 5), 1 caudal cirrus at the end of dorsal kinety 1 (Küppers and Claps 2013, p. 70, Fig. 4C; vs. 3–5 caudal cirri), 1 caudal cirrus at the end of rightmost dorsal kinety (Küppers and Claps 2013, p. 70, Fig. 4C; vs. caudal cirri lacking), and number of dorsal bristles in each row (Küppers and Claps 2013, p. 70, Fig. 4C; DK1: 23 vs. 29–44; DK2: 23 vs. 29–40; DK3: 21 vs. 25–40; rightmost DK: 21 vs. 24–37). These comparisons confirm the Korean population is new species.

Our gene trees support the assignment of this new species as a distinct species in *Pseudouroleptus*, with full supporting values (Fig. 4). With the exception of Stylonychinae, other nodes presented low supporting values. Additional SSU rRNA gene sequences of representative oxytrichids are required to clarify these relationships, and these sequences should be combined with morphological data even for well-known species.

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