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Morphology and molecular phylogeny of a new freshwater ciliate *Urosomoida sejongensis* n. sp. (Ciliophora, Sporadotrichida, Oxytrichidae) from King George Island, Antarctica

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Abstract

In this study, a new "non-oxytrichid Dorsomarginalia" ciliate, *Urosomoida sejongensis* **n. sp.** discovered from freshwater of the King George Island, Antarctica, was investigated using morphological, morphometrical, and molecular methods. Morphology of *U. sejongensis* is characterized as follows: body shape slender to elongated; cortical granules spherical and colorless, groups of granules formed patchy distribution; ring-shaped structures scattered in cytoplasm; 27–30 adoral membranelles with undulating membranes in *Oxytricha* pattern; usually 17 frontal-ventral-transverse (FVT) cirri composed of 3 frontal, 1 buccal, 4 frontoventral, 3 postoral ventral, 2 pretransverse ventral and 4 transverse cirri; 1 right and 1 left marginal rows; 3 dorsal kineties with 1 dorsomarginal row, 3 caudal cirri; 1 micronucleus between 2 macronuclear nodules. This new species mainly differs from other congeners by the combination of following morphological features: a micronucleus, cortical granules, and ciliatures (e.g., adoral membranelles, FVT cirri). *Urosomoida sejongensis* shows a nucleotide similarity of 97.3% with *U. agilis*, type of this genus, using the SSU rDNA sequence. Molecular phylogeny shows a non-monophyletic relationship among *Urosomoida* species and emphasizes the need for further morphogenetic studies of this genus and other related species to resolve morphological convergences.

Key words: *Urosomoida sejongensis*, new freshwater species, SSU rDNA, Antarctica

Introduction

Urosomoida Hemberger in Foissner, 1982 is a benthic ciliate and consists of 13 species to date (Shao *et al.* 2011; Singh & Kamra 2015). Shao and colleagues provided an improved diagnosis of the genus *Urosomoida* and Singh & Kamra combined a species belonging to the genus *Urosomoida* to a new genus (see below). They usually occur in terrestrial habitats including freshwater ecosystem but some species are reported from highly saline soil or a lagoon (Berger 1999; Foissner *et al.* 2002; Paiva & Silva-Neto 2004). They usually feed on bacteria or small protists (e.g., algae, amoebae, flagellates, and ciliates).

Of the thirteen species, two are known to inhabit in Antarctica and they are as follows: *U. antarctica* and *U. granulifera* (Foissner 1998; Berger 1999). Type population of the two species was discovered from Antarctica (=locus classicus) and their type localities are South Victoria Land and South Shetland Islands, respectively.

Urosomoida was previously assigned to the family Oxytrichidae whose type genus *Oxytricha* has 18 frontal-ventral-transverse (FVT) cirri with dorsal kinety fragmentation. However, *Urosomoida* has reduced FVT cirri than the typical *Oxytricha* and lacks a dorsal kinety 3 fragmentation so that Berger (2006, 2008) placed the genus *Urosomoida* in the non-monophyletic assemblage "non-oxytrichid Dorsomarginalia". The group name Dorsomarginalia denotes the species which have dorsomarginal kinety originated from right marginal cirral anlage during morphogenesis.

The phylogenetic relationship of *Urosomoida agilis* and *U. longa* was recently investigated using the SSU rDNA sequences to represent a generic standard of this genus as the type species (Singh & Kamra 2015). Based on morphogenetic features and the molecular phylogenetic results, a new genus *Hemiurosomoida* was established and

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U. longa was combined as the type species of this genus. Two genera *Hemiurosomoida* and *Urosomoida* are not clearly distinguished by morphology of the non-dividing cell (=mature form), supporting a convergence on the cirral pattern as shown in *Anteholosticha*, one of the well-known non-monophyletic groups in family Urostylidae (Park *et al.* 2013).

In this study, we describe a new freshwater ciliate *Urosomoida sejongensis* **n. sp.** collected from King George Island, Antarctica. Its morphology and SSU rDNA sequence were analyzed and compared to those of other congeners.

Material and methods

Sampling site and morphological identification. New species was discovered from the freshwater near the King Sejong Station on King George Island, Antarctica (62°14′22.8″S, 58°44′40.1″W) in February 2013. The sample was collected from stirred-up sediment using a plankton net (20 μm mesh size). Cultures were maintained both in Petri dishes and 50 mL tissue culture flasks at 4–8°C (SPL, SPL Life sciences, Korea). Rice grains were used to enrich bacterial growth for a food source of ciliates. Living specimens were observed under a light microscope (Axio Imager.A2, Carl Zeiss, Germany) at magnifications ranging from 50 to 1,000 times. Protargol impregnation was performed in order to reveal the infraciliature (Foissner 1991). Terminology and classification are mainly according to Berger (1999) and Lynn (2008).

Genomic DNA extraction, amplification and sequencing. Each individual was washed repeatedly with distilled water. Extraction of genomic DNA from a single specimen was performed according to the manufacturer's protocol, using a RED-Extract-N-Amp Tissue PCR Kit (Sigma, St. Louis, USA). The New EukA primer modified from Medlin *et al.* (1988) and LSU rev3 primer (Sonnenberg *et al.* 2007) were used for PCR amplification of the nearly complete SSU rDNA sequence. The optimized PCR condition was as follows: denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 95°C for 15 s, annealing at 56°C for 30 s, extension at 72°C for 4 min, and then 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 and 18S-300 (Jung *et al.* 2014), were used for sequencing with ABI 3700 sequencer (Applied Biosystems, Foster City, Ca, USA).

Molecular analysis. Sequenced fragments of the SSU RNA gene were assembled using the BioEdit program (Hall 1999) and were aligned using Clustal X 1.81 (Jeanmougin *et al.* 1998). MEGA 5.2.2 (Tamura *et al.* 2011) was used to calculate pairwise genetic distances. The alignment was then refined using Gblocks v.0.91b (Talavera & Castresana 2007) and finally 1,479 bp was used to reconstruct the phylogenetic trees. To confirm the systematic position of the new species, the sequences of all the known *Urosomoida* and each representative species of the genera in the family Oxytrichidae, Neokeronopsidae, Amphisiellidae, and Uroleptidae were retrieved from the GenBank databases.

We evaluated phylogenetic relationships using maximum likelihood (ML) and Bayesian inference (BI) analyses. To determine the appropriate DNA substitution model for ML and BI, we used the Akaike information criterion (AIC) to identify the best-fit model using the jModelTest 2.1.1 (Darriba *et al.* 2012). The model selected was TIM2 +I (0.7640) +G (0.4840). The ML analysis was conducted using PhyML version 3.1 (Guindon *et al.* 2010) with 1,000 bootstrap replicates. BI assessment was performed using MrBayes 3.2.2 (Ronquist & Huelsenbeck 2003) by simulating a Markov chain Monte Carlo (MCMC) for 1,000,000 generations. Trees were sampled every 100 generations, from which the first 30% were discarded as burn-in.

Results

We analyzed morphological and molecular attributes of the Antarctic soil ciliate. The results are obtained from the individuals of a raw culture. Morphology of the ciliate including living cells and stained specimens is presented usually based on the descriptive statistics, and a gene tree using the nuclear SSU rDNA sequences is shown below.

Order Sporadotrichida Fauré-Fremiet, 1961

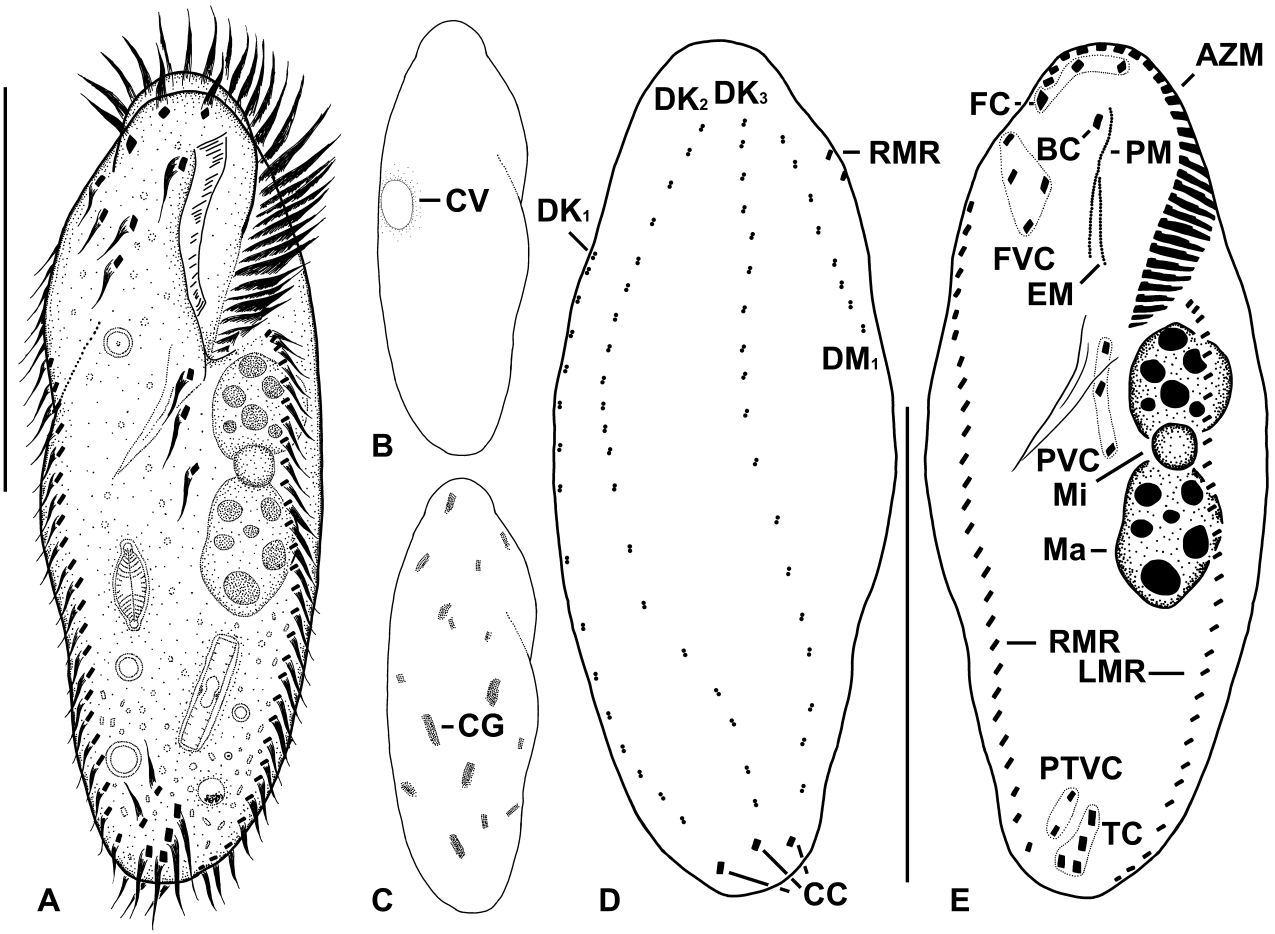
Family Oxytrichidae Ehrenberg, 1838

Genus *Urosomoida* Hemberger in Foissner, 1982

***Urosomoida sejongensis* n. sp.**
(Fig. 1–2; Table 1)

Diagnosis. Size in vivo 75–130 × 20–35 μm; slender to elongated shape; flexible but not contractile; grayish under low magnification. Contractile vacuole slightly above left of mid-body. 1 micronucleus between two macronuclear nodules. Groups of cortical granules sparsely distributed. Ring-shaped structures in cytoplasm. 17 frontal-ventral-transverse cirri composed of 3 frontal, 1 buccal, 4 frontoventral, 3 postoral ventral, 2 pretransverse ventral, and 4 transverse cirri. 1 left and 1 right marginal cirral row. On average 28 adoral membranelles with undulating membranes in *Oxytricha* pattern. 4 kineties composed of 3 dorsal and 1 dorsomarginal kineties. 3 caudal cirri.

Type locality. Freshwater near the King Sejong Station, King George Island, Antarctica (62°14′22.8″S, 58°44′40.1″W).



FIGUIRE 1. Morphology of *Urosomoida sejongensis* **n. sp.** in vivo (A–C) and after protargol impregnation (D, E). A. Ventral view of representative specimen. B, C. Dorsal views showing contractile vacuole (CV) and cortical granules (CG), respectively. Dorsal (D) and ventral (E) view of holotype specimen. AZM—adoral zone of membranelles; BC—buccal cirrus; DK—dorsal kineties; DM—dorsomarginal kinety; EM—endoral membrane; FC—frontal cirri; FVC—frontoventral cirri; LMR—left marginal cirral row; Ma—macronuclear nodules; Mi—micronucleus; PM—paroral membrane; PTVC—pretransverse ventral cirri; PVC—postoral ventral cirri; RMR—right marginal cirral row; TC—transverse cirri. Scale bars: 50 μm.

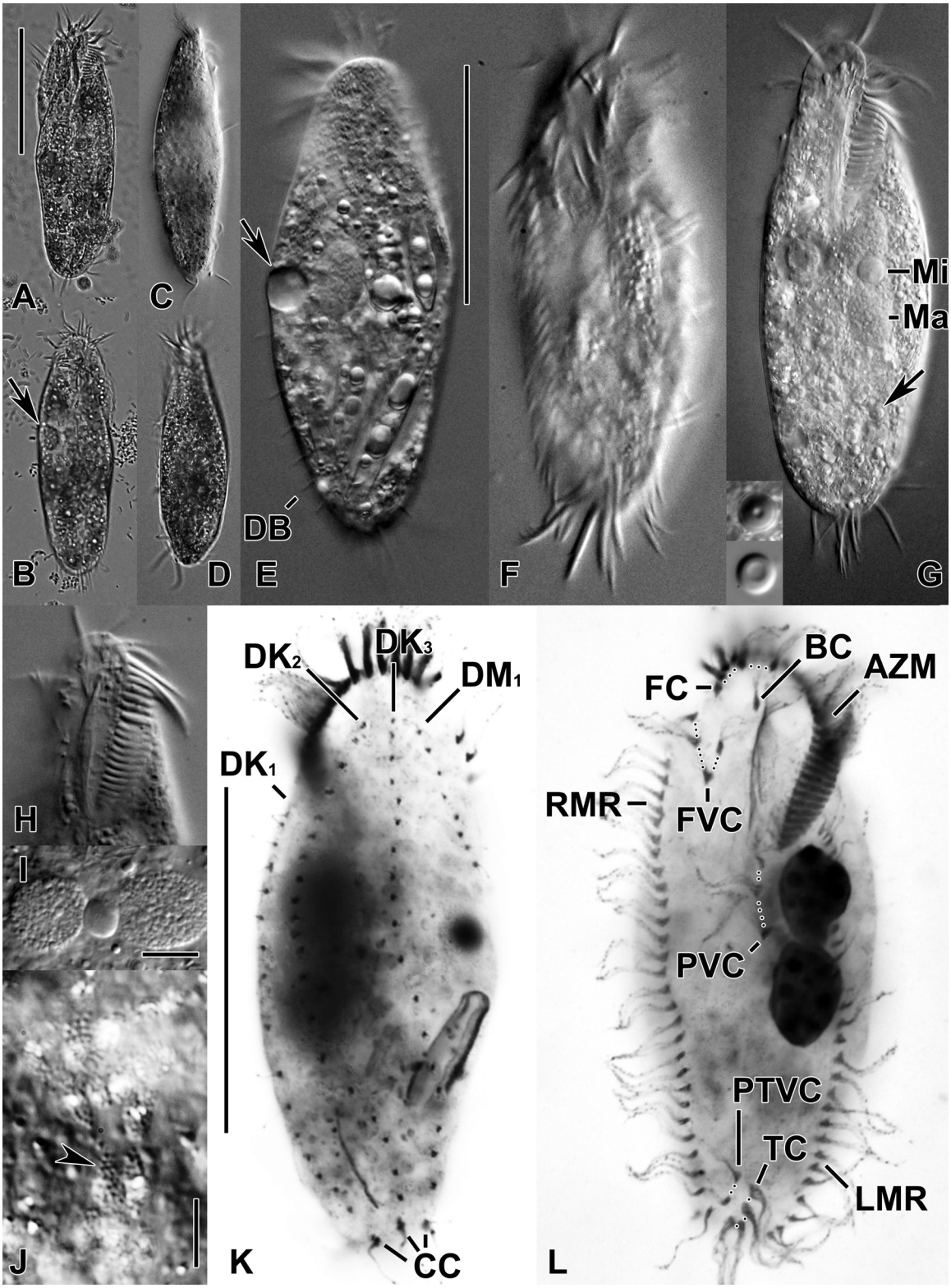


FIGURE 2. Photomicrograph of *Urosomoida sejongensis* **n. sp.** in vivo (A–J) and after protargol impregnation (K, L). A, B. Ventral and dorsal view. Arrow denotes contractile vacuole. C, D. Lateral views. E. Dorsal view showing contractile vacuole (arrow) with dorsal bristles (DB). F. Ventral view with overall cirral arrangement. G. Ventral view with nuclear apparatus and ring-shaped structures (inserts). H. Ventral view of oral apparatus. I. Nuclear apparatus showing 1 micronucleus between 2 macronuclear nodules. J. Cortical granules (arrowhead). K, L. Dorsal and ventral view of holotype specimen, respectively. AZM—adoral zone of membranelles; BC—buccal cirrus; CC—caudal cirri; DK—dorsal kineties; DM—dorsomarginal kinety; FC—frontal cirri; FVC—frontoventral cirri; LMR—left marginal cirral row; Ma—macronuclear nodules; Mi—micronucleus; PTVC—pretransverse ventral cirri; PVC—postoral ventral cirri; RMR—right marginal cirral row; TC—transverse cirri. Scale bars: 50 μm (in A, E, K), 5 μm (in I, J).

TABLE 1. Morphometric data on protargol-impregnated specimens of *Urosomoida sejongensis* **n. sp.**

Characteristics	N	Mean	SD	SE	CV	Min	M	Max
Body, length	25	92.5	8.8	1.8	9.5	71.5	95.0	110.5
Body, width	25	32.3	3.8	0.8	11.8	22.6	32.4	38.1
Adoral zone, length	25	29.8	2.4	0.5	8.2	25.7	30.3	34.3
Adoral membranelles, number	21	28.0	1.1	0.2	3.9	27	28	30
Longest adoral membranelles, length	25	5.5	0.3	0.1	5.0	5.1	5.4	6.2
Ratio of body length : adoral zone length	25	3.1	0.3	0.1	10.3	2.6	3.1	3.8
Macronucleus, length	25	14.3	1.9	0.4	13.4	10.0	14.7	17.5
Macronucleus, width	25	9.0	1.6	0.3	17.4	6.0	8.8	12.1
Macronuclear nodules, number	25	2.0	0.0	0.0	0.0	2	2	2
Micronuclei, length	21	3.4	0.2	0.0	6.6	3.0	3.4	3.8
Micronuclei, width	21	3.2	0.3	0.1	9.1	2.5	3.2	3.5
Micronucleus, number	21	1.0	0.0	0.0	0.0	1	1	1
Frontal cirri, number	21	8.0	0.0	0.0	0.0	8	8	8
Postoral ventral cirri, number	21	3.0	0.0	0.0	0.0	3	3	3
Pretransverse ventral and transverse cirri, number	21	5.9	0.3	0.1	5.1	5	6	6
Dorsal kineties, number	21	3.0	0.0	0.0	0.0	3	3	3
Dorsal bristles in dorsal kinety 1, number	21	16.2	1.5	0.3	9.5	14	16	19
Dorsal bristles in dorsal kinety 2, number	21	19.2	1.1	0.2	5.9	16	19	21
Dorsal bristles in dorsal kinety 3, number	21	17.3	1.6	0.3	9.0	14	17	21
Dorsomarginal kinety, number	21	1.0	0.0	0.0	0.0	1	1	1
Dorsal bristles in dorsomarginal kinety, number	21	8.4	1.1	0.2	12.8	7	8	10
Caudal cirri, number	21	3.0	0.0	0.0	0.0	3	3	3
Left marginal cirri, number	21	29.0	1.4	0.3	4.9	26	29	32
Right marginal cirri, number	21	31.2	1.5	0.3	4.9	28	32	33

All measurements in μm. CV—coefficient of variation (%); Max—maximum; Min—minimum; N—number of specimens investigated; SD—standard deviation; SE—standard error of arithmetic mean.

Type slides. One holotype slide (NIBRPR0000106568) and one paratype slide (ACNS000272) of protargol-impregnated specimens have been deposited in the National Institute of Biological Resources and the Korea Polar Research Institute (KOPRI) in South Korea, respectively. Relevant specimens including holotype have been marked with circles on the bottom of the slides.

Etymology. The name "*sejongensis*" is derived from the name of the King Sejong Station (Korea Antarctic Research station) because the species was discovered near the station.

Gene sequence. SSU rDNA sequence was deposited in the GenBank under the accession number KT723011.

Morphological description. Size 75–130 × 20–35 μm in vivo (Fig. 1A; 2A–G), on average 95 × 32 μm in protargol preparation (Fig. 1D, E; 2K, L). Body slender to elongated shape, slightly concave on right margin of mid-body (Fig. 1A; 2A, B), dorsoventrally flattened (width:thickness = 1:0.75); flexible but not contractile; cell color grayish under low magnification. Invariably, on left of mid-body, two macronuclear nodules and one micronucleus (Fig. 1A, E; 2G, I, L). Macronuclear nodules ellipsoid, 10–17.5 × 6–12.1 μm (stained); spherical micronucleus located between the nodules, 3.0–3.8 × 2.5–3.5 μm (stained). One contractile vacuole slightly above left of mid-body, 10 μm in diameter, lacking conspicuous collecting canals (Fig. 1B; 2B, E). Cortical granules spherical and colorless, 0.4 μm in diameter; groups of granules formed patchy distribution on dorsal side (Fig. 1C; 2J). Spherical ring-shaped structures distributed in cytoplasm, 3–7 μm in diameter (Fig. 1A; 2G); the shape left intact from burst cell. Feed on bacteria and diatoms.

Cirri 10–15 µm long in vivo; usually 17 cirri, on ventral side, composed of 3 frontal, 1 buccal, 4 frontoventral, 3 postoral ventral, 2 pretransverse ventral, and 4 transverse cirri (Fig. 1A; 2F, L); very rarely 1 pretransverse ventral cirri (1 of 21 cells). Two marginal cirral rows composed of one left and one right row, terminated at level of posteriormost transverse cirrus, marginal rows never connected at posterior body end. Anterior part of right marginal row commenced on dorsal side (Fig. 1D; 2K). Invariably four dorsal kinety rows composed of 3 dorsal and 1 dorsomarginal kineties without any fragmentations. Dorsal bristles about 4 µm in vivo. One caudal cirrus at the end of each of dorsal kineties, resultant invariably 3 caudal cirri. Leftmost dorsal kinety anteriorly shortened; dorsomarginal row about 1/3 of cell length.

Adoral zone composed of 27–30 membranelles with continuous arrangement; about 1/3 of cell length. Undulating membranes nearly parallel, slightly curved (=*Oxytricha* pattern); one buccal cirrus located anterior right end of paroral membrane (Fig. 1E; 2L).

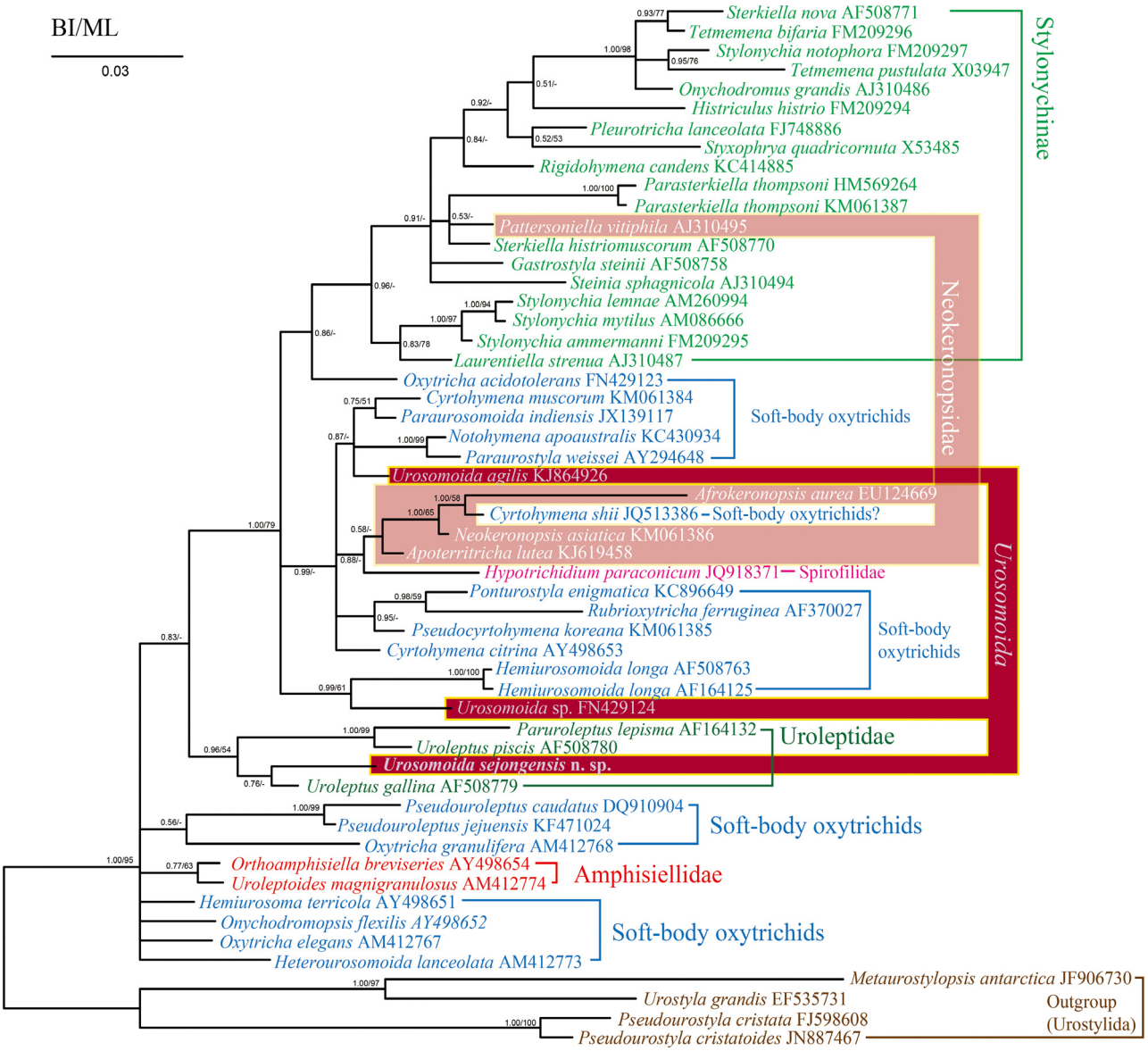


FIGURE 3. Majority consensus tree of Bayesian inference (BI) using SSU rDNA sequences. On interior branches, posterior probabilities of BI and bootstrap values of maximum likelihood (ML) are represented, respectively.

Molecular analysis of *Urosomoida sejongensis* n. sp. (Fig. 3). The SSU rDNA sequence of *U. sejongensis* is 1,578 bp in length and had 97.3% and 96.4% nucleotide similarity with *U. agilis* (type of this genus) and *U.* sp. (FN429124), respectively. In Fig. 3, *Urosomoida sejongensis* clustered with family Uroleptidae, not with the type

species *U. agilis* or *U.* sp. The three SSU rDNA sequences of *Urosomoida* were split into each clade in the tree showing a non-monophyletic relationship. *Urosomoida agilis* clustered with the species having the undulating membranes in *Cyrtohymena* pattern. *Urosomoida* sp. showed a sister relationship with the clade *Hemiurosomoida longa* (formerly *U. longa*) and had a SSU rDNA sequence similarity of 96.9%.

Discussion

Morphological and molecular phylogeny of *U. sejongensis*. *Urosomoida* Hemberger in Foissner, 1982 belongs to the non-monophyletic assemblage "non-oxytrichid Dorsomarginalia", which has the dorsomarginal kinety but lacks a dorsal kinety 3 fragmentation (Berger 2006, 2008). Among them, the genus *Urosomoida* has the reduced frontal-ventral-transverse cirri than the typical oxytrichids, that is, less than 18 cirri. Up to date, thirteen species of *Urosomoida* have been described (Shao *et al.* 2011; Singh & Kamra 2015): *U. agiliformis* Foissner, 1982; *U. agilis* (Engelmann, 1862) Hemberger in Foissner, 1982 (type species); *U. antarctica* Foissner, 1996; *U. deserticola* Foissner, Agatha & Berger, 2002; *U. dorsiincisura* Foissner, 1982; *U. granulifera* Foissner, 1996; *U. marcili* (Paiva & Silva-Neto, 2004) Shao *et al.*, 2011; *U. minima* Hemberger, 1985; *U. monostyla* Foissner, Agatha & Berger, 2002; *U. namibiensis* Foissner, Agatha & Berger, 2002; *U. perthensis* Foissner & O'Donoghue, 1990; *U. pseudofurcata* (Berger, 1999) Shao *et al*., 2011; and *U. reticulata* Foissner, Agatha & Berger, 2002.

Of the thirteen species, three species have 1 micronucleus between 2 macronuclear nodules as in *U. sejongensis* **n. sp.** Their morphological features are summarized in Table 2 and clearly distinguished from *U. sejongensis* by cell size, cortical granules, and number of ciliatures (e.g. adoral membranelles, cirri, and dorsal bristles) because the new species has cortical granules (vs. absent), the largest body size and the most numerous ciliatures than the others (Table 2).

TABLE 2. Comparison of morphological features in *U. sejongensis* **n. sp.** with those of closely related species.

	U. monostyla	*U. perthensis*	*U. pseudofurcata*	*U. sejongensis* **n. sp.**
Body length (stained)	30–57	38–51	66 (in vivo)	72–111
Cortical granules	Absent	Absent	Absent	Present
Adoral membranelles, number	13–15	15–18	ca. 18	27–30
Right marginal cirri, number	12–16	17–22	ca. 13	28–33
Left marginal cirri, number	11–15	14–21	ca. 12	26–32
Postoral ventral cirri, number	1	3–4	3	3
Pretransverse ventral and transverse cirri, number	4–5	5	6	5–6
Dorsal kineties including dorsomarginal row	4	4	4 (rarely 5)	4
Caudal cirri, number	2	3	2	3
Dorsal bristles in kinety 1, number	5–7	5 (from illustrate)	4 (from illustrate)	14–19
Dorsal bristles in kinety 2, number	7 (from illustrate)	10 (from illustrate)	7 (from illustrate)	16–21
Dorsal bristles in kinety 3, number	6 (from illustrate)	8 (from illustrate)	7 (from illustrate)	14–21
Dorsal bristles in kinety 4[a], number	2–3	4 (from illustrate)	6 (from illustrate)	7–10
Data source	Foissner *et al.* (2002)	Berger (1999)	Berger (1999)	Original

[a] Dorsomarginal kinety.

With respect to the cortical granules, two of 13 species in *Urosomoida* are known to have the granules and only *U. granulifera* has the identical pattern to *U. sejongensis*. The former species can be separated from the new species by micronucleus (number—2–8 vs. 1; position—not between macronuclear nodules vs. between macronuclear nodules), adoral membranelles (21–27 vs. 27–30), right marginal cirri (16–30 vs. 28–33), and caudal cirri (2 vs. 3) (Berger 1999).

The genus *Hemiurosomoida* Singh & Kamra, 2015 includes one species *H. longa* (basionym *Oxytricha longa*) as monotypy. The type species was transferred from *Urosomoida* and the morphology of non-dividing cells (=mature form) between the genera *Hemiurosomoida* and *Urosomoida* is not conspicuous to identify at the genus level without the morphogenesis or gene sequences. However the non-dividing cells of *H. longa* and *U. sejongensis* can be easily distinguished at a species level by nuclear apparatus (number of micronuclei—1 or more vs. 1; position of micronuclei—not between macronuclear nodules vs. between macronuclear nodules), cortical granules (absent vs. present), adoral membranelles (15–22 vs. 27–30), right marginal cirri (14–23 vs. 28–33), left marginal cirri (13–23 vs. 26–32), caudal cirri (2 vs. 3), and a genetic distance (3.1%).

Non-monophyletic assemblage of *Urosomoida*. The autapomorphies of *Urosomoida* are the reduced number of postoral ventral cirri and/or pretransverse cirri and/or transverse cirri than the typical *Oxytricha*, and the lack of dorsal kinety 3 fragmentation (Berger 1999; Shao *et al.* 2011). Recently, Singh & Kamra (2015) acquired the SSU rDNA sequence of *U. agilis*, the type species of *Urosomoida*. Interestingly, the type species was not clustered with *U. longa*, which is the sole congeneric species available for the SSU rDNA sequence in the GenBank. In addition to the discrepancy of molecular data, the morphogenetic attributes also show some differences in these species (e.g., origination of anlagen V and VI—de novo in *U. agilis* vs. from V/4 and V/3 in *U. longa*). Based on the differences, Singh & Kamra (2015) established the new genus *Hemiurosomoida* and fixed *U. longa* as a type species. The morphology of non-dividing cells in these genera has a high similarity and is not conspicuous for the discrimination at a genus level supporting that the reduced number of FVT cirri might be convergently evolved. In addition to the morphology, the molecular phylogeny also showed the non-monophyletic relationship among the three sequences of *Urosomoida* (Fig. 3). Weisse *et al.* (2013) reported a population of *Urosomoida* sp. (FN429124) with a morphological description and SSU rDNA gene sequence. The gene sequence of the population showed a close relationship with *H. longa* as in our gene tree (Fig. 3).

In addition, some of other oxytrichids s. l. also showed non-monophyletic relationships (e.g., *Cyrtohymena*, Neokeronopsidae, *Oxytricha*, *Tetmemena* and *Uroleptus*). Hypotrichs are considered to be full of morphological convergence that hampers to infer their true evolutionary relationships and systematics. Of the convergent taxa, the family Neokeronopsidae is a distinct group showing a convergence feature to a different order Urostylida (Foissner & Stoeck 2008).

These results emphasize the need to further study on the morphogenesis and molecular analyses of the congeners including related other species to shed light on their true phylogenetic relationship.

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