ACTA
PROTOZOOLOGICA

Acta Protozool. (2011) 50: 289–300
http://www.eko.uj.edu.pl/ap
doi: 10.4467/16890027AP.11.026.0063

A New Marine Ciliate, *Metaurostylopsis antarctica* nov. spec. (Ciliophora, Urostylida) from the Antarctic Ocean

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Summary. In this study, a new marine urostylid ciliate, *Metaurostylopsis antarctica* nov. spec. collected from the Antarctic Ocean was investigated using morphological, morphometrical, and molecular methods. *Metaurostylopsis antarctica* nov. spec. is characterized as follows: slender to ellipsoid form in body shape; two types of cortical granules, ellipsoid large one (type I, yellow-green, 1.5 × 1 μm) in rows along dorsal kineties and cirri, circular small one (type II, colourless, 0.3 μm in diameter) scattered throughout whole body; 19–24 adoral membranelles, 4 frontal cirri, 2–5 frontoterminal cirri, 1 buccal and 2 transverse cirri; 3–5 midventral pairs, 10–15 cirri of midventral row; 1 right and 2 left marginal rows; 3 dorsal kineties; about 43 macronuclear nodules. This new species mainly differs from the congeners by the number of marginal rows (1 vs. 3 or more on right side; 2 vs. 3 or more on left side). In addition, proter's oral primordium developed on the right side of the oral cavity (vs. in center of oral cavity), and the rightmost anlage splits into two parts, namely, the frontoterminal cirri and a transverse cirrus (vs. only frontoterminal cirri). Inter-specific dissimilarities of the SSU rRNA gene between the congeners range from 3.3 to 4.4%.

Key words: Antarctic Ocean, marine ciliate, *Metaurostylopsis antarctica*, infraciliature, morphogenesis, SSU rRNA.

INTRODUCTION

The Antarctic Ocean contains many ciliates that have not yet been described, and especially benthic ciliates remain largely unknown when compared with planktonics (Petz *et al.* 1995, Song and Wilbert 2002, Wilbert and Song 2008). According to Petz (2005), at least 161 species have been recorded in the Antarctic Ocean, and Wilbert and Song (2008) reported that at least more than half of the ciliates that they have identified and described in the Antarctic Ocean were new species (Song and Wilbert 2002, Wilbert and Song 2005).

Six species belonging to the family Urostylidae have been reported from the Antarctic Ocean (Petz 2005), including *Metaurostylopsis rubra* Song and Wilbert, 2002. The genus *Metaurostylopsis* in the Urostylidae was established by Song *et al.* (2001) and, to date, six species have been described worldwide (Song *et al.* 2001, Song and Wilbert 2002, Lei *et al.* 2005, Shao *et al.* 2008, Chen *et al.* 2011). The genus *Metaurostylopsis* has the following characteristics: 3–5 frontal cirri;

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buccal and transverse cirri present; midventral complex composed of midventral pairs and/or one midventral row; one or more rows of marginal cirri on each side; no caudal cirri. Recently, *Apourostylopsis* was split from *Metaurostylopsis* and *A. sinica* was fixed as type species (Song *et al.* 2011). *Apourostylopsis* has specific characteristics that are different from *Metaurostylopsis*, such as no midventral row (vs. present), presence of pretransverse cirri (vs. absent), and midventral pairs extending to the posterior cell end (vs. conspicuously shortened at the same level as the cytostome).

In this study, we described a new Antarctic marine ciliate species, *Metaurostylopsis antarctica* nov. spec. collected from the littoral zone of King George Island, based on live and protargol-impregnated specimens. In addition, for precise assignment of this new species, phylogenetic analysis based on SSU rRNA gene and comparison of the morphogenesis among species in the genus *Metaurostylopsis* was performed.

MATERIALS AND METHODS

Sample site and morphological identification

Specimens of the new species were collected from the seashore near the King Sejong Station on King George Island, Antarctica (62°13′S, 58°42′W) in January 2011. The sampling site had the following environmental factors: salinity, 34.9‰; and temperature, 4.6°C. The sample was collected from stirred up sediment on the seashore using a plankton net (20 μm mesh size).

Cultures were maintained in both Petri dishes and 50 mL tissue culture flasks at 4°C (Greiner Bio-one, Frickenhausen, Germany). Rice grains were used to enrich bacterial growth in the culture, and the enriched bacteria were grazed on ciliates as a food source. Living specimens were observed under a light microscope (Leica DM2500, Wetzlar, Germany) at magnifications ranging from 50 to 1,000. Protargol impregnation was performed in order to reveal the infraciliature, which included both the interphase and morphogenetic stage (Foissner 1991). In the morphogenetic stages, parental structures are shown by contour, while newly formed structures are shaded black.

Terminology and classification are mainly according to Berger (2006), Lynn (2008), and Song *et al.* (2011).

SSU rRNA gene sequence

Each individual was washed repeatedly 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, USA). New EukA (5'-CTG GTT GAT YCT GCC AGT-3') modified from Medlin *et al*. (1988) and LSU rev3 (Sonnenberg *et al.* 2007) primers were used for PCR amplification of the nearly complete SSU rRNA gene. The optimized PCR condition was as follow: 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 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 were used for sequencing: 18S+810, 5'-GCC GGA ATA CAT TAG CAT GG-3' and 18S-300, 5'-CAT GGT AGT CCA ATA CAC TAC-3'. An ABI 3700 sequencer (Applied Biosystems, Foster City, CA, USA) was used for sequencing.

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 3.1 (Kumar *et al.* 2004) was used to calculate genetic distance, which employed the Kimura two-parameter distance option (Kimura 1980).

To confirm the systematic position of the new species, the sequences of all the known *Metaurostylopsis* and each representative species of the genera in the family Urostylidae were retrieved from the GenBank database. The list of species is provided in the Supplementary material.

RESULTS

Description of *Metaurostylopsis antarctica* nov. spec.

Diagnosis: Size about 70–110 × 20–30 μm *in vivo* with slender to ellipsoid body form; cell grayish to colourless under low magnification. Two types of cortical granules, one large ellipsoid granule (type I, yellow-green, 1.5 × 1 μm) in rows along dorsal kineties and cirri, and one small circular granule (type II, colourless, 0.3 μm in diameter) scattered throughout the whole body surface. 19–24 adoral membranelles, 4 frontal, 2–5 frontoterminal, 1 buccal, and 2 transverse cirri; 3–5 midventral pairs, 10–15 cirri in midventral row; 1 right, 2 left marginal cirral rows; 3 dorsal kineties; about 43 macronuclear nodules.

Type locality: Littoral zone on King George Island, Antarctica, 62°13′S, 58°42′W.

Type specimen: One holotype slide (NIBRPR0000103109) and four paratype slides (NIBRPR0000103110–NIBRPR0000103113) with protargol-impregnated specimens including dividing individuals are deposited in the National Institute of Biological Resources, South Korea. Five additional paratype slides are deposited in the National Fisheries Research and Development Institute (NFRDI) of Korea. Relevant specimens have been marked by black circles on the bottom of the slides.

Etymology: Region where the species was discovered, the Antarctic Ocean.

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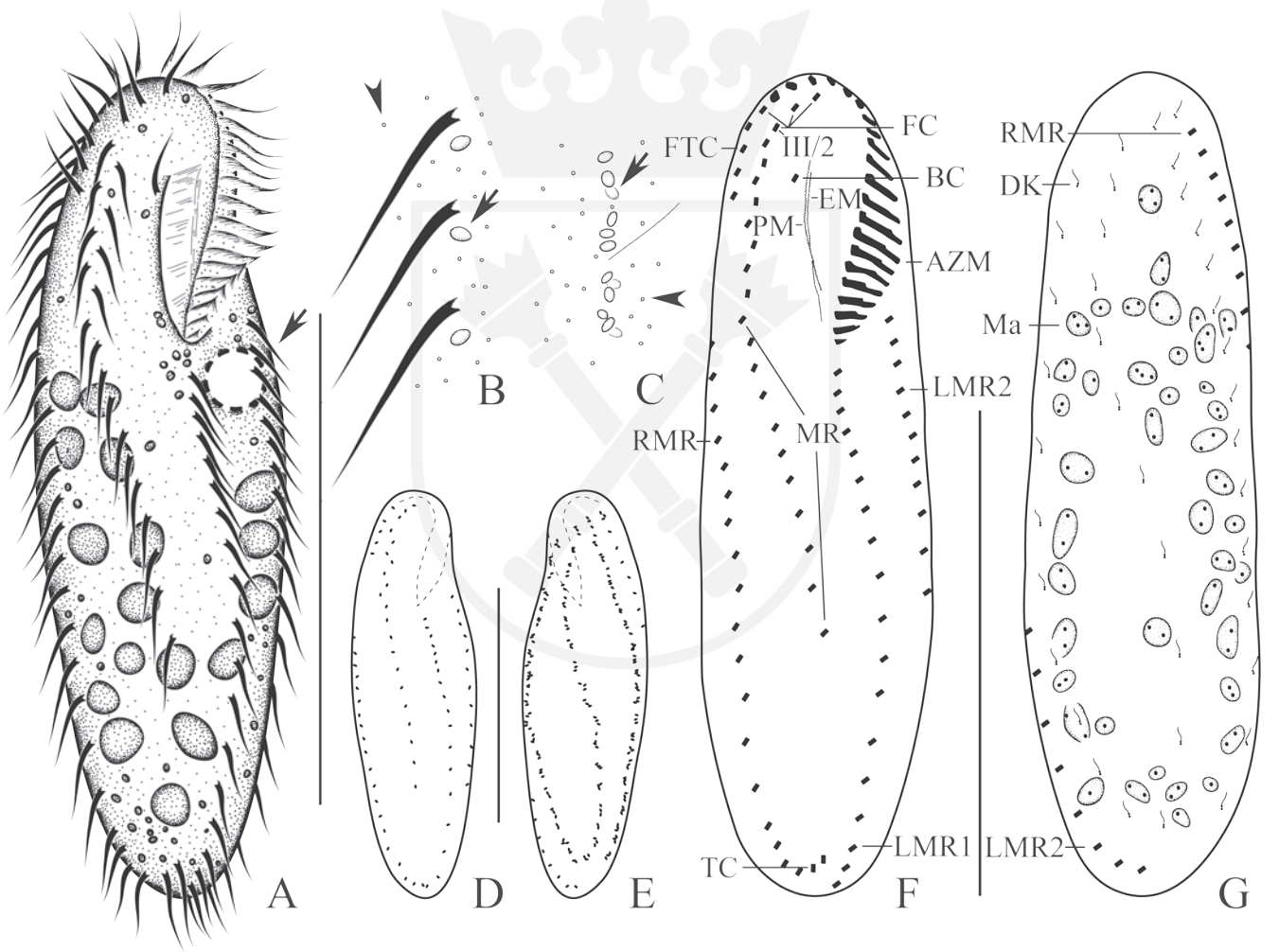
Description: Size 70–110 × 20–30 μm *in vivo* (Figs 1A, D, E, 4A–E), on average 80 × 26 μm in protargol preparations (Figs 1F, G). Body flexible and slightly contractile; cell colour grayish to colourless. Two types of cortical granules; larger type I granules yellow-greenish colour, size about 1.5 × 1 μm, that is, of ellipsoidal shape, usually one granule along one cirrus and several granules between dorsal dikinetids arranged in rows along dorsal kineties and cirral rows (Figs 1B, C, 4F, J, arrows; 1D, E); smaller type II granules colourless, size about 0.3 μm in diameter, densely scattered throughout the whole body (Figs 1B, C, arrowhead; 4I, J, double-arrowhead). On average 43 macronuclear nodules distributed mainly along the cell margin; individual nodules ellipsoid (Figs 1G, 5E). Contractile vacuole located on left side of 1/3 body, about 10 μm in diameter (Figs 1A, 4D, arrow). Crawling moderately fast on the bottom of Petri dish.

All cirri relatively fine, mostly 10–12 μm long, four frontal cirri including parabuccal cirrus (III/3) and two transverse cirri ca. 12 μm long (Figs 1A, 4G, H, 5A). Invariably four frontal cirri continued with midventral complex, which is composed of 3–5 midventral pairs and one midventral row composed of 10–15 cirri; 'zigzag' midventral pairs shortened and located at the level of posterior curve of adoral zone, and continuously midventral row terminated at about 2/3 of the body length (Fig. 1F). 2–5 frontoterminal cirri in a short line located near midventral pairs (Figs 1F, 5C, arrows). Buccal cir-



Figs 1A–G. Morphology of *Metaurostylopsis antarctica* nov. spec., from life (A–E) and after protargol impregnation (F, G). **A** – ventral view of a representative individual, arrow indicates contractile vacuole; **B**–**E** – ventral (B, D) and dorsal (C, E) views, showing the arrangement of type I (large; arrow) and II (small; arrowhead) cortical granules; **F**, **G** – ventral and dorsal view of the holotype specimen. AZM – adoral zone of membranelles, BC – buccal cirrus, DK – dorsal kineties, EM – endoral membrane, FC – frontal cirri (III/2 parabuccal cirrus included), FTC – frontoterminal cirri, LMR1, 2 – left marginal rows 1, 2, Ma – macronulcear nodules, MR – midventral row, PM – paroral membrane, RMR – right marginal row, TC – transverse cirri. Scale bars: 50 μm.

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Table 1. Morphometric data on *Metaurostylopsis antarctica* n. sp.

Characteristics [a]	Min	Max	Mean	SD	CV	n
Length of body	68	92	79.6	7.0	8.8	25
Width of body	20	32	25.8	3.5	13.4	25
Length of buccal field	22	32	25.5	2.2	8.7	25
No., adoral membranelles	19	24	21.6	1.4	6.3	25
No., frontal cirri [b]	4	4	4.0	0.0	0.0	25
No., buccal cirri	1	1	1.0	0.0	0.0	25
No., frontoterminal cirri	2	5	3.6	0.6	17.8	24
No., transverse cirri	2	2	2.0	0.0	0.0	25
No., midventral pairs	3	5	4.1	0.6	14.6	25
No., cirri of midventral row	10	15	12.2	1.4	11.6	25
No., left marginal row	2	2	2.0	0.0	0.0	25
No., cirri in left marginal row 1 (inner)	14	20	16.9	1.3	7.7	25
No., cirri in left marginal row 2 (outer)	17	23	18.9	1.5	7.9	25
No., right marginal row	1	1	1.0	0.0	0.0	25
No., cirri in right marginal row	20	30	25.4	2.5	9.9	25
No., macronuclear nodules	38	50	42.6	3.2	7.6	25
No., dorsal kineties	3	3	3.0	0.0	0.0	25

[a] Data based on protargol-impregnated specimens. Measurements in µm. CV – coefficient of variation in %; Max – maximum; Mean – arithmetic mean; Min – minimum; n – number of specimens investigated; SD – standard deviation.
[b] Frontal cirri including parabuccal cirrus (III/2).

rus right of anterior portion of paroral membrane (Figs 1F, 5B). One right and two left marginal cirral rows; anterior right marginal cirri and posterior cirri of outer left marginal row located on dorsal side (Figs 1F, G).

Adoral zone of membranelles about 1/3 of body length in fixed specimens, base of largest membranelles about 6 µm long, cilia of membranelles about 15 µm long. Distal end of adoral zone slightly bending to the anterior right side of body (Figs 1F, 5C). Paroral and endoral membrane long and slightly curved, almost parallel and crossing mid-point (Fig. 1F).

Three complete dorsal kineties (Figs 1G, 5D, arrows). Usually one or two basal bodies ahead of right marginal row (Figs 1G, 5D, 6C). Dorsal cilia 2–3 µm long (Fig. 4J).

Morphogenesis of *Metaurostylopsis antarctica*

Stomatogenesis and cirral streaks: Division commences with the apokinetal appearance of groups of closely spaced basal bodies adjacent to the left of the midventral row (Fig. 2A, arrowheads). Subsequently, the groups merge and form an anarchic field that become longitudinally wider (Fig. 2B, arrowhead). Simultaneously, a small elliptical field of basal bodies appears apokinetally to right of the endoral membrane (Figs 2B, arrow; 5F). Soon, oral primordia of both dividers continue to grow and differentiate into new membranelles in a posterior direction (Fig. 2C). While the new membranelles formed, in the opisthe, frontoventral-transverse anlagen appears as a group of basal bodies to the right of the membranelles without resorbing the midventral cirri (Fig. 2C).

In the next middle stage, the differentiation of the membranelles is almost complete and the frontoventral--transverse cirral anlagen (FVT-anlagen) begin to differentiate in each divider (Figs 2D, F). A single cirrus, the left frontal cirrus, develops from the anterior end of the undulating membranes anlage (Fig. 2D).

Later, the anterior ends of the two new adoral zone of membranelles form a curved shape and all cirri appeared in both dividers (Figs 2H, 3A). Usually two cirri originate from the FVT-anlagen with the exception of streak n-1 and n (numbered from left to right) which form the midventral row (Fig. 3A, ca. 12 cirri), the fron-

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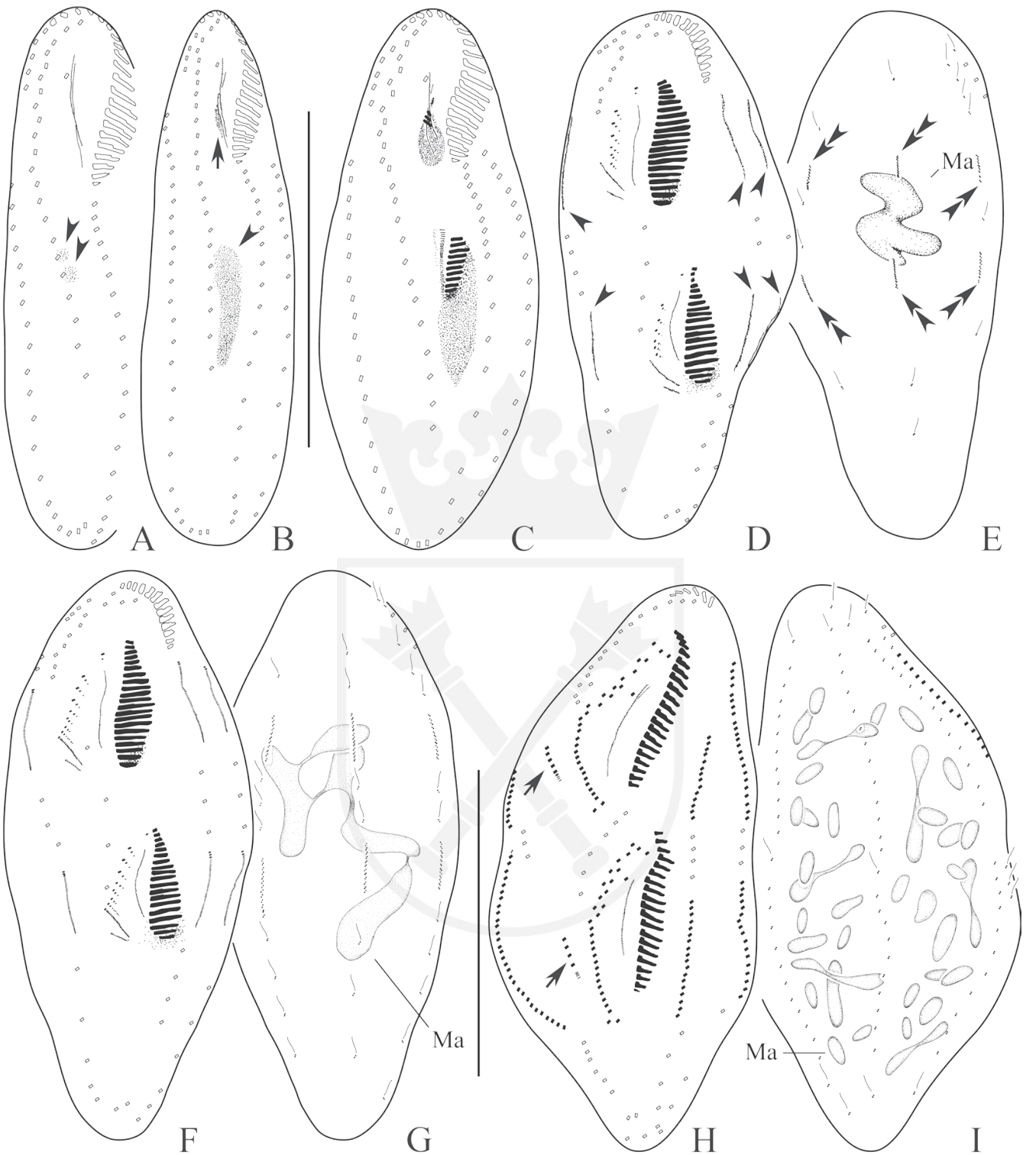
Table 2. Comparison of seven species of *Metaurostylopsis*. The data was cited and modified from Chen *et al*., 2011.

Characters	*M. marina*	*M. rubra*	*M. songi*[a]	*M. salina*	*M. struederkypkeae*	*M. cheni*	*M. antarctica* nov. spec.
Body size *in vivo*	80–120 × 50–80	150–300 × 50–90	90–150 × 20–35	40–80 × 15–30	90–120 × 20–30	100–140 × 40–60	70–110 × 20–30
Cell colour	colourless to grayish	brick reddish	colourless	somewhat russet	rose-reddish	colourless	colourless to grayish
Colour of cortical granules	colourless	colourless	colourless	colourless	type I: yellow-green type II: wine reddish	type I: yellow-green type II: colourless	type I: yellow-green type II: colourless
Position of CV	above mid-body	40% of body length	anterior mid-body	equatorial level	40% of body length	40% of body length	33% of body length
No., adoral membranelles	ca. 28 (27–30)	ca. 40 (35–46)	ca. 34 (28–47)	ca. 20 (18–23)	ca. 23 (20–25)	ca. 23 (21–26)	ca. 22 (19–24)
No., FC[b]	4	4	5	3	4	4	4
No., FTC	ca. 4 (3–6)	ca. 6 (5–8)	ca. 2 (2–3)	ca. 4 (3–5)	ca. 5 (4–6)	4	ca. 4 (2–5)
No., MP	ca. 9 (7–11)	ca. 10 (8–11)	ca. 11 (9–12)	ca. 5 (4–5)	4–7	ca. 7 (5–9)	ca. 4 (3–5)
No., MR	4–7	8–13	–	5–7	4–8	4–5	ca. 12 (10–15)
No., LMR	ca. 4 (3–5)	ca. 8 (6–9)	3	3	ca. 4 (4–5)	ca. 3 (3–4)	2
No., RMR	ca. 4 (3–5)	ca. 6 (6–7)	3	3	3	3	1
No., TC	ca. 7 (5–9)	ca. 5 (4–6)	ca. 7 (6–7)	ca. 4 (2–5)	ca. 3 (2–5)	ca. 6 (5–8)	2
Data source	Song *et al*. (2001)	Song and Wilbert (2002)	Lei *et al*. (2005)	Lei *et al*. (2005)	Shao *et al.* (2008)	Chen *et al.* (2011)	original

Measurements in μm. CV – contractile vacuole; FC – frontal cirri; FTC – frontoterminal cirri; LMR – left marginal row; MP – midventral pairs; MR – midventral row; RMR – right marginal row; TC – transverse cirri.

[a] Species considered *incertae sedis* (Song *et al.* 2011).

[b] Frontal cirri including parabuccal cirrus (III/2) except *M. salina.*



Figs 2A–E. Morphogenesis of *Metaurostylopsis antarctica* nov. spec. at early to late stages after protargol impregnation. **A**, **B** – ventral view of early dividers, arrowheads indicate the oral primordium and the arrow marks the proter's oral primordium; **C** – ventral view of a slightly later divider, to show the formation of oral primordia in the proter and the opisthe; **D**–**E** – ventral (D) and dorsal (E) view of the same specimen, arrowheads show the marginal anlagen and double-arrowheads mark the dorsal kineties anlagen. **F**, **G** – ventral (F) and dorsal (G) views of a divider in middle stage; **H**, **I** – ventral (H) and dorsal (I) views of a late divider, arrows indicate frontoterminal cirri migrating anteriad. Ma – macronuclear nodules. Scale bars: 50 μm.

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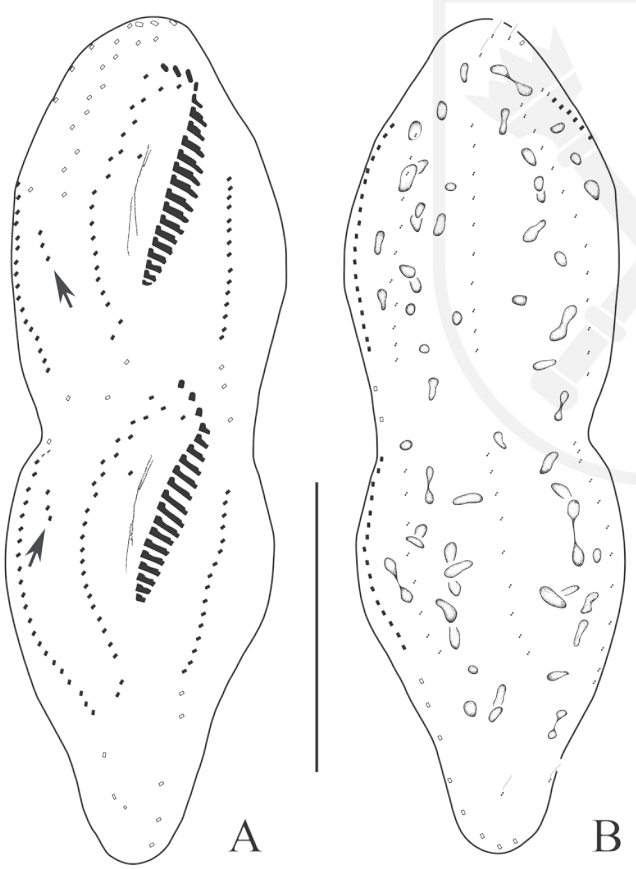
toterminal cirri (Fig. 3A, ca. 4 cirri), and the transverse cirri. The streak n-1 develops to the midventral row with one transverse cirrus, and the streak n is differentiated to the frontoterminal cirri with one transverse cirrus (Figs 3A; 6D, arrow); streak II differentiates to the second frontal and buccal cirri; streak III forms the right frontal and the parabuccal cirrus; streak IV to n-2 develop midventral pairs. Most of the parental cirri are resorbed in late dividers (Figs 3A, 6E).

Marginal and dorsal anlagen: After the beginning of morphogenesis, a few cirri near the anterior end and below the mid-body within each parental marginal row develop to form two anlagen in each kinety (Figs 2D, 5G). These anlagen stretch to posterior direction and gradually replace the parental cirri in each row (Figs 3A, 6A, B).

Two dorsal kineties anlagen formed in each parental row. The anlagen elongate within the parental rows, replace or incorporate with parental structure and no caudal cirri are formed (Figs 2E, G, I, 3B, 5I).



Figs 3A, B. Ventral and dorsal view of a late divider of *Metaurostylopsis antarctica* nov. spec. after protargol impregnation. Arrows mark the new frontoterminal cirri. Scale bar: 50 μm.

Division of nuclear apparatus: The nuclear apparatus divides in an unusual way of urostylids. Briefly, at the early-divisional stage, each macronuclear nodules fuse together and become slightly bigger in size (Figs 5F, H). All macronuclear nodules fuse into a single mass as a slightly branched mass at the mid-divisional stage (Figs 2E, 5J). Division of micronuclei in the later stage was not observed.

Molecular analysis

The SSU rRNA gene sequence of *Metaurostylopsis antarctica* has 1,732 bp in length (JF906730) and inter-specific dissimilarities with the congeners are as follows: 4.4% with *M. salina* (EU220229), 4.3% with *M. cheni* (GU170204), and 3.5% with *M. struederkypkeae* (EU220228). In the phylogenetic analysis (Suppl. Fig. 1), the monophyly of *Metaurostylopsis* was supported and the new species, *M. antarctica,* was positioned at the basal with confidences of 95% from the maximum likelihood bootstrap value and 1.00 from the Bayesian inference posterior probability value.

DISCUSSION

Comparison with congeners

Morphology: The genus *Metaurostylopsis* can be briefly characterized using the following characteristics: several marginal rows; urostylid-type midventral complex (midventral pairs with one midventral row); and a marine habitat (Song *et al.* 2001). To date, seven species have been reported including *M. antarctica* nov. spec.: *M. marina* (type species), *M. rubra*, *M. songi*, *M. salina*, *M. struederkypkeae*, and *M. cheni* (Song *et al.* 2001, Song and Wilbert 2002, Lei *et al.* 2005, Shao *et al.* 2008, Chen *et al.* 2011). Their characteristics are shown in Table 2.

Metaurostylopsis antarctica differs from *M. marina* (Kahl, 1932) Song *et al*., 2001 by body shape (elliptical vs. oval form), types of cortical granules (two vs. one), adoral membranelles (19–24 vs. 27–30), midventral pairs (3–5 vs. 7–11), cirri of ventral row (10–15 vs. 4–7), transverse cirri (2 vs. 5–9), left and right marginal rows (2 vs. 3–5, 1 vs. 3–5, respectively).

Metaurostylopsis rubra Song and Wilbert, 2002 has the largest body size (150–300 × 50–90 μm vs. 70–110 × 20–30 μm in *M. antarctica*) and the highest number of macronuclear nodules (ca. 100 vs. ca. 43 in *M. antarctica*) among the congeners. Furthermore, *M. rubra*

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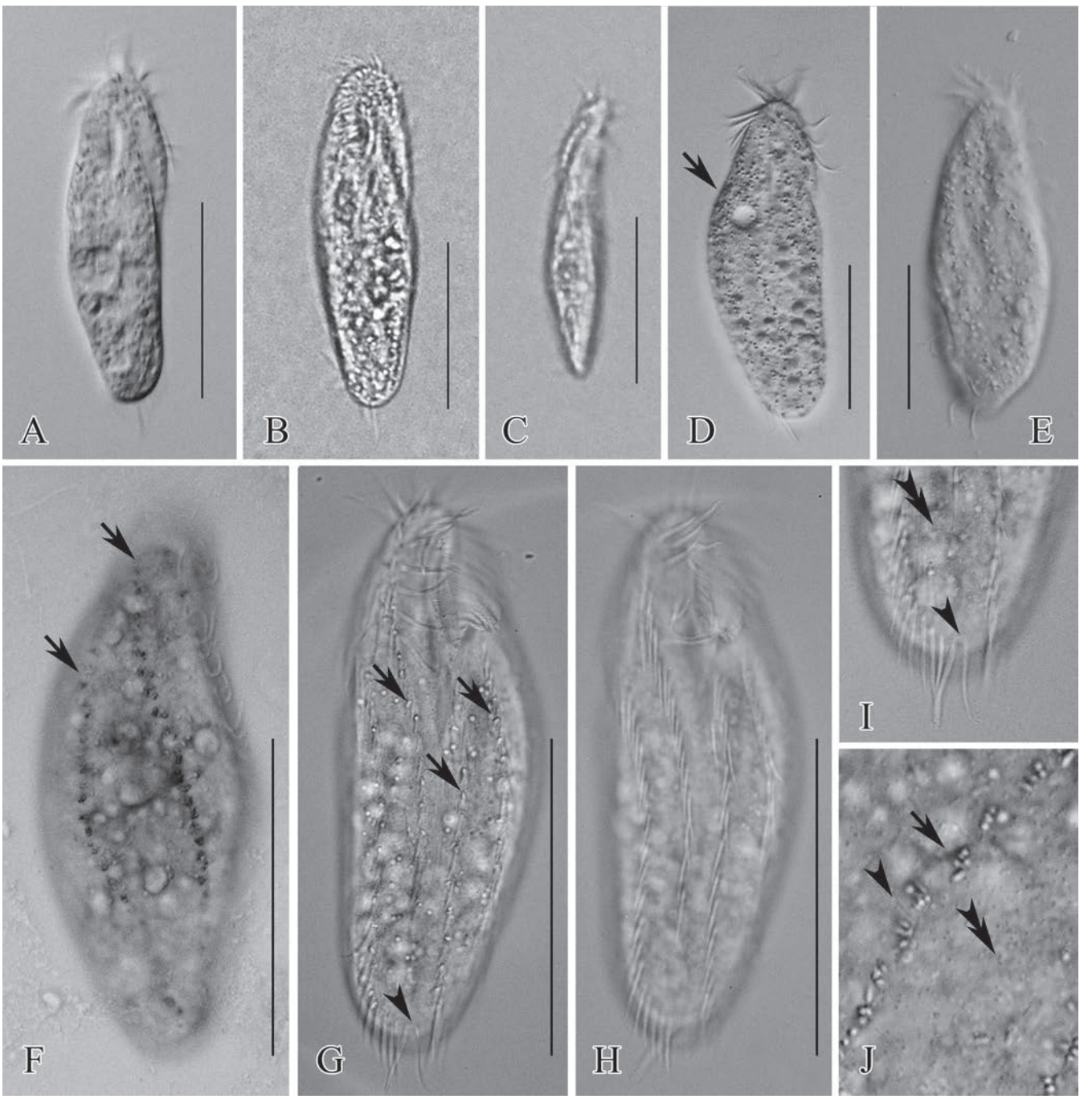

Figs 4A–J. Photomicrographs of *Metaurostylopsis antarctica* nov. spec. from life. **A**, **B**, **D**, **E** – dorsal views, arrow in (D) marks contractile vacuole; **C** – left side view; **F**, **G**, **I**, **J** – cortical granules and transverse cirri on dorsal (F, J) and ventral (G, I) views, arrows denote large granules (type I), and small granules (double-arrowheads), which are distributed throughout the whole cell surface, arrowhead in (G, I) marks transverse cirri; **H** – ventral view represents cirral pattern. Scale bars: 50 μm.

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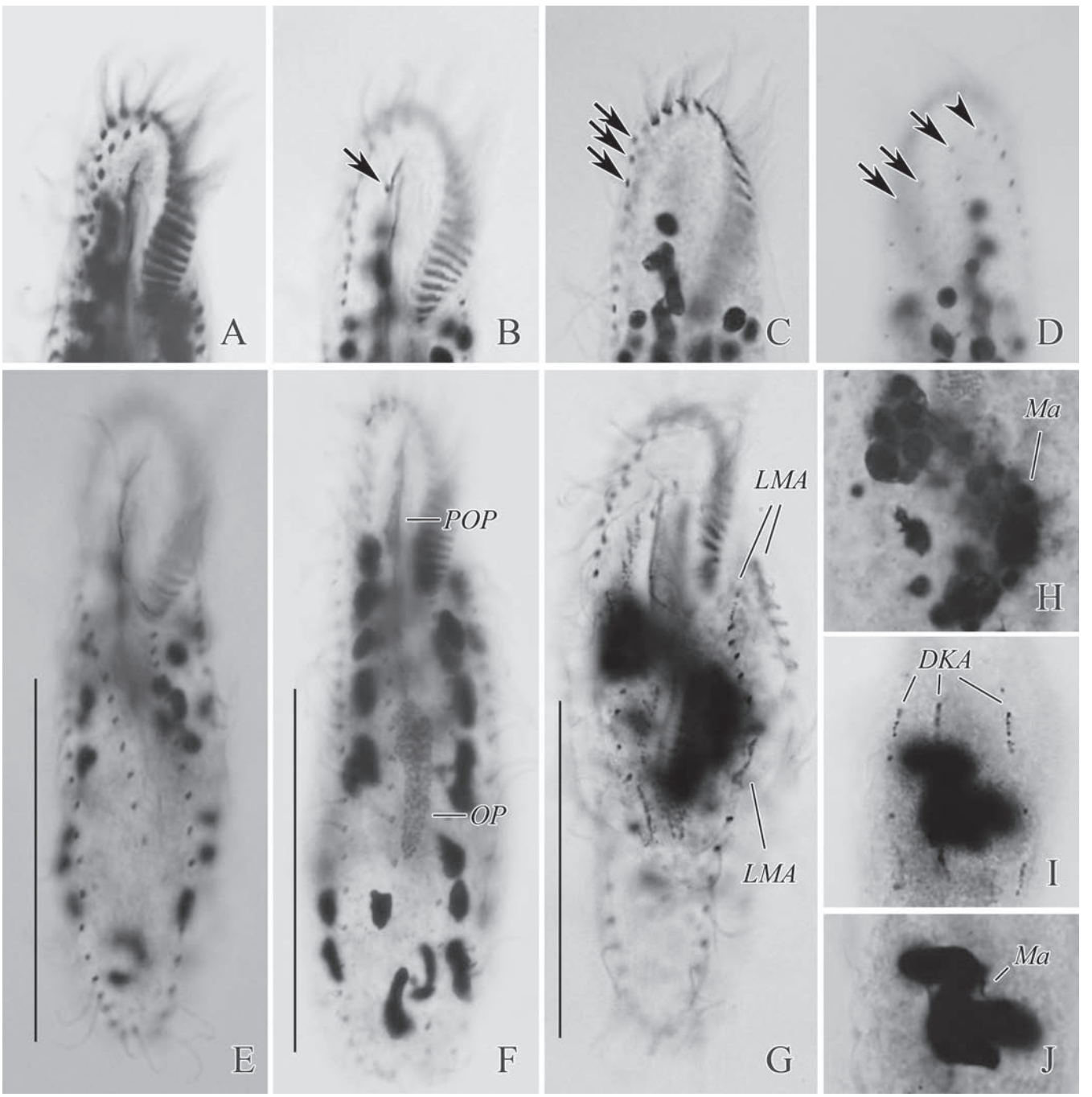

Figs 5A–J. Photomicrographs of *Metaurostylopsis antarctica* nov. spec. during interphase (A–E) and morphogenesis after protargol impregnation (F–J). **A–C** – ventral views of infraciliature of the anterior body portion, arrow in (B) denotes the buccal cirrus, arrows in (C) mark the frontoterminal cirri; **D** – complete dorsal kineties (arrows) and one basal body ahead of the right marginal row; **E** – ventral view of the holotype specimen; **F–J** – early dividing stage, oral primordia are developed apokinetally, and both dorsal kineties and marginal rows develop within the parental structure, macronuclear fuse to form a branched complex. DKA – dorsal kineties anlagen, LMA – left marginal anlagen, Ma – macronuclear nodules, OP – opisthe's oral primordium, POP – proter's oral primordium. Scale bars: 50 μm.

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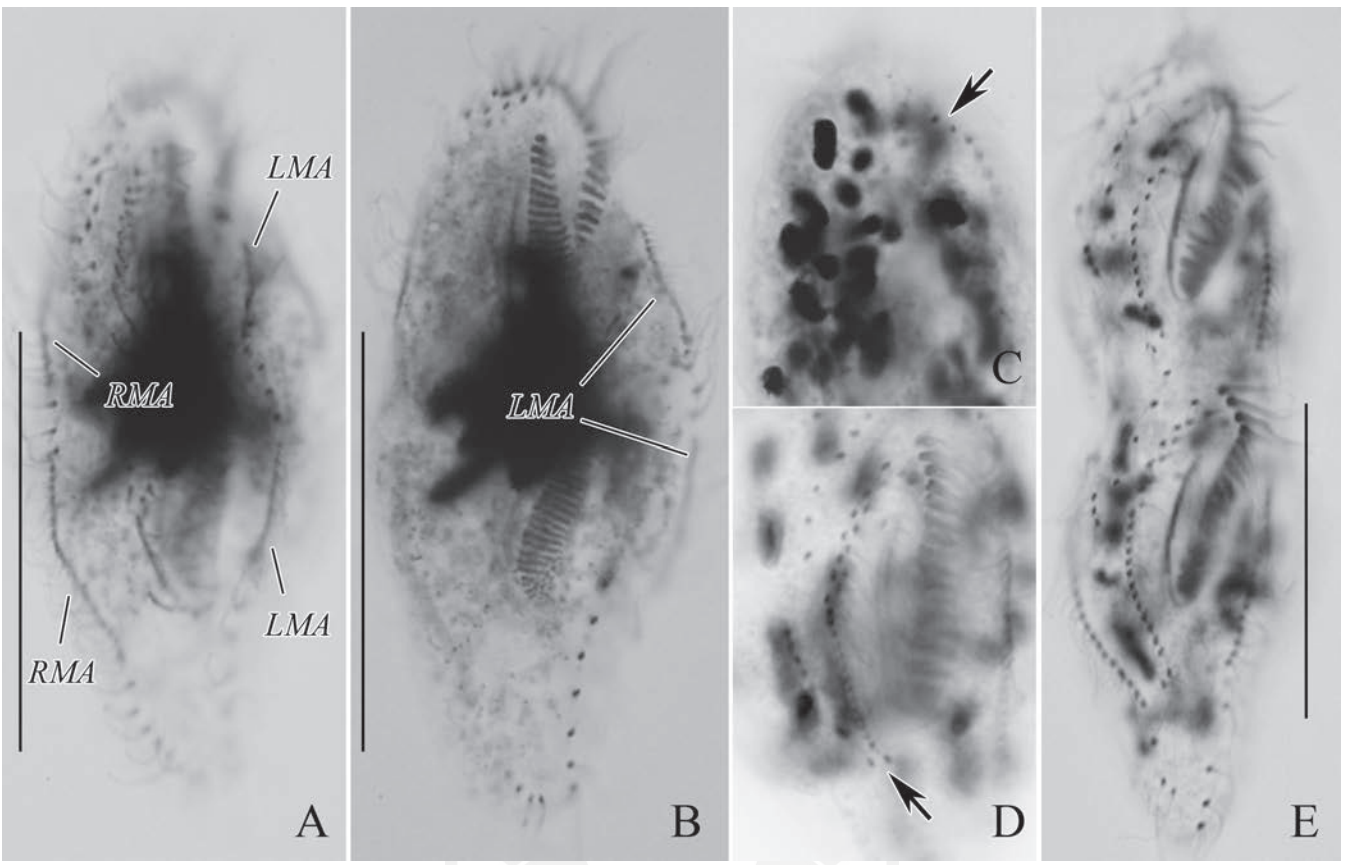

Figs 6A–E. Photomicrographs of dividing cells of *Metaurostylopsis antarctica* nov. spec. after protargol impregnation. **A**, **B** – middle divider, marginal anlagen replace parental rows; **C**–**E** – ventral and dorsal view of a late divider, arrow in (C) denote as two basal bodies ahead of the right marginal row; two transverse cirri (arrow, D); ventral view of the whole body of the divider (E). LMA – left marginal anlagen, RMA – right marginal anlagen. Scale bars: 50 μm.

can be distinguished from *M. antarctica* based on its cell colour (brick reddish vs. colourless), adoral membranelles (35–46 vs. 19–24), midventral pairs (8–11 vs. 3–5), and left and right marginal rows (6–9 vs. 2, 6–7 vs. 1, respectively).

Metaurostylopsis songi Lei *et al*., 2005 has distinct characteristics from *M. antarctica* by having one-type granules (vs. two), adoral membranelles (28–47 vs. 19–24), midventral pairs (9–12 vs. 3–5), transverse cirri (4–6 vs. 2), and left and right marginal rows (3 vs. 2, 3 vs. 1, respectively).

Metaurostylopsis salina Lei *et al*., 2005 can be separated from *M. antarctica* by cell colour (somewhat russet vs. colourless), cirri of midventral row (5–7 vs. 10–15), and left and right marginal rows (3 vs. 2, 3 vs. 1, respectively).

Metaurostylopsis struederkypkeae Shao *et al*., 2008 differs from *M. antarctica* by cell colour (rose-reddish vs. colourless), colour of type II granules (wine reddish vs. colourless), cirri of midventral row (4–8 vs. 10–15), and left and right marginal rows (4–5 vs. 2, 3 vs. 1, respectively).

Metaurostylopsis cheni Chen *et al*., 2011 can be distinguished from *M. antarctica* by midventral pairs (5–9 vs. 3–5), cirri of midventral row (4–5 vs. 10–15), transverse cirri (5–8 vs. 2), and left and right marginal rows (3–4 vs. 2, 3 vs. 1, respectively).

Morphogenesis: Using the previously investigated morphogenesis of the genus *Metaurostylopsis*, i.e. *M. cheni*, *M. marina*, *M. rubra*, and *M. struederkypkeae*, the morphogenetic process of *M. antarctica* was shown to resemble the congeners in the following aspects: entire parental ciliature, including oral primordia, is apokinetally replaced; intrakinetally occupied ciliature within the parental structures develops into marginal cirral rows and dorsal kineties; macronuclear nodules fuse into a branched structure during the middle stage of morphogenesis. Slight differences were observed at

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the early morphogenetic stage. Proter's oral primordium occupied the right side of the endoral membrane, whereas, according to the morphogenetic information of this genus (Song *et al.* 2001, Wilbert and Song 2005, Chen *et al.* 2011), the oral primordium appears between parental undulating membranes and adoral zone. In addition, streak n develops and splits into two parts, which are the frontoterminal cirri and transverse cirrus. With the exception of these slight differences, the other morphogenetic features of the new species support the hypothesis that this species more closely related to *Metaurostylopsis* than the genus *Apourostylopsis*, which has the following characteristics: (1) lacking the midventral row; (2) pretransverse cirri; and (3) midventral pairs extending to the posterior cell end (Song *et al*. 2011).

Molecular analysis

Based on the molecular analysis, the monophyly of *Metaurostylopsis* including *M. antarctica*, and the sister group relationship between *Metaurostylopsis* and *Apourostylopsis* are highly supported by both the maximum likelihood and Bayesian values, respectively (Suppl. Fig. 1). However, distinct morphogenetic features of *M. antarctica* (i.e. slightly different position of proter's oral primordium, and streak n splits into two parts) from the known morphogenetic process of the genus were observed and these distinct characteristics probably explain the intermediate position of the new species in the *Apourostylopsis-Metaurostylopsis* group from the inferred phylogenetic tree (Suppl. Fig. 1) and the relatively higher pairwise distances from the congeners, which range from 3.4 to 4.4%.

Acknowledgements. This work was supported by grants of the Korea Healthcare technology R & D Project, Ministry of Health, Welfare & Family Affairs, Republic of Korea (A090931), Korea Research Foundation Grant (KRF-2010-C00033), the Polar Academic Program (PAP) of KOPRI (G.-S. Min) and the Basic Research Program of KOPRI (PE11030) (H.-G. Choi).

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Received on 7th July, 2011; revised on 16th September, 2011; accepted on 28th September, 2011

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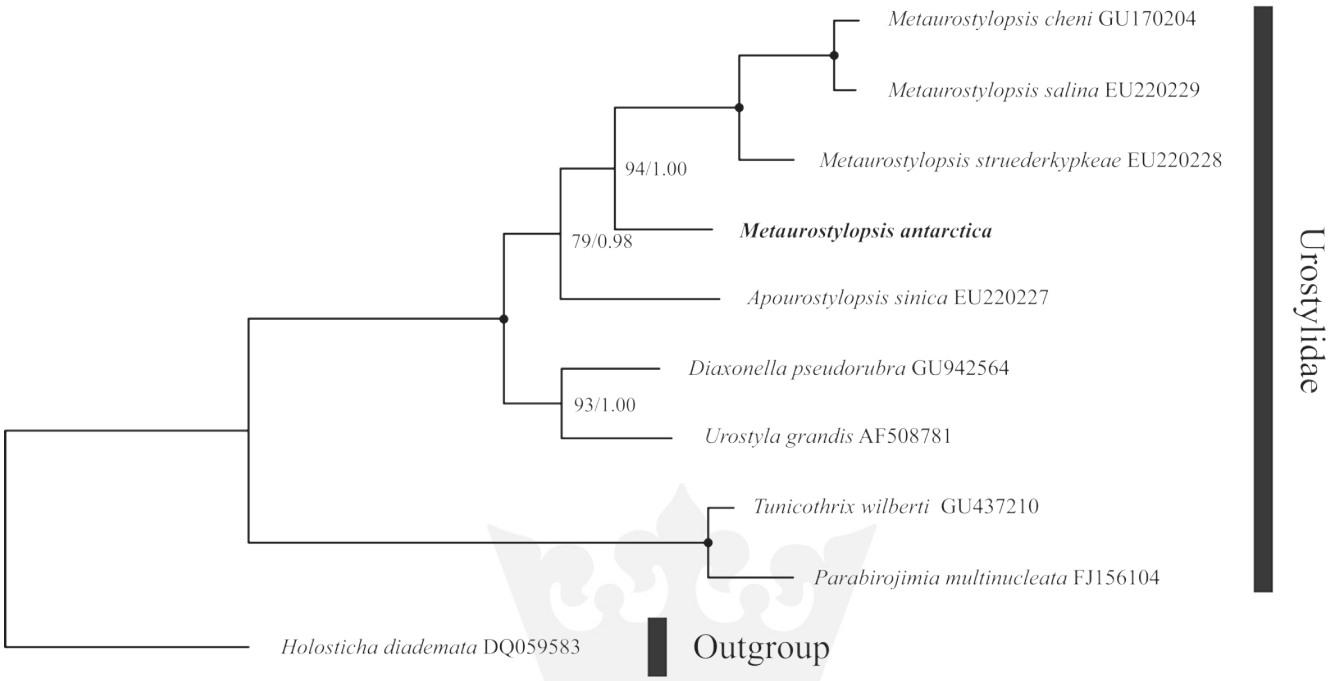

Suppl. Fig. 1. Phylogeny of *Metaurostylopsis antarctica* nov. spec. inferred from maximum likelihood (ML) and Bayesian inference (BI) based on SSU rRNA gene sequences. PhyML v. 3.0 and MrBayes v. 3.1.2 were used for ML and BI analyses, respectively. TIM2+G was selected as a best fit model from jModelTest. NCBI sequences are shown in the tree as the species name plus the GenBank accession number. Solid circles mark fully supported branches (100% ML, 1.00 BI). Numbers at the branches indicate the bootstrap values obtained from ML analysis (left) and Bayesian inference posterior probability (right). The taxa analyzed here for the phylogeny were mostly based on previous studies and core species belonging to the family Urostylidae were sorted and used in subsequent analysis (Chen *et al.* 2011, Yi and Song 2011). *Holosticha diademata* was selected as an outgroup which has a close relationship with Urostylidae (Berger 2006, Yi and Song 2011). See the references in the text.

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