ACTA
PROTOZOOLOGICA

Acta Protozool. (2015) 54: 107–122
www.ejournals.eu/Acta-Protozoologica
doi:10.4467/16890027AP.15.009.2734

Two Oxytrichids from the Ancient Lake Biwa, Japan, with Notes on Morphogenesis of *Notohymena australis* (Ciliophora, Sporadotrichida)

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Abstract. Two oxytrichid freshwater ciliates, *Apoamphisiella tihanyiensis* (Gellért and Tamás, 1958) Foissner, 1997 and *Notohymena australis* (Foissner and O'Donoghue, 1990) Berger, 1999, were recorded for the first time in Lake Biwa, a 4-million-year-old lake located at the Shiga Prefecture in Japan. Their morphology was investigated based on observations of live and protargol-impregnated material. Based on the present observation and previous descriptions, *A. tihanyiensis* is characterized by having an elliptical body shape, yellowish cortical granules, two long frontoventral rows, enlarged frontal and transverse cirri, highly variable numbers of frontoventral, and postoral ventral cirri, and six to 11 caudal cirri arranged in three short rows. New data confirm the presence of pretransverse ventral cirri in this species. Morphologically, *N. australis* differs from its congeners in having the following combination of characters: greenish cortical granules, the cirrus V/2 located slight anterior to the leftmost transverse cirrus, dorsal kinety 3 almost as long as body, and seven to 10 caudal cirri arranged in three short rows. Morphogenesis in *N. australis* shows the same pattern as in *N. apoaustralis* but differs from that of other congeners in the origin of oral primordium and the formation of more than just three caudal cirri.

Key words: *Apoamphisiella*, freshwater ciliate, infraciliature, *Notohymena*, ontogenesis, Stichotrichia.

INTRODUCTION

The oxytrichids *s.l.* are a group of hypotrichs distributed in various biotopes worldwide (e.g. Kahl 1932; Berger 1999, 2006; Küppers and Claps 2013; Tirjaková and Vd'ačný 2013). Most species have a characteristic 18 frontal-ventral-transverse cirral pattern (Stiller 1974; Berger and Foissner 1997; Weisse *et al.* 2013). A recent comprehensive guide to this group of ciliates was provided by Berger (1999). According to this revision, the Oxytrichidae includes ca. 32 valid genera and 170 valid species. However, many of them were described on only a few occasions and from a limited geographical range, and intraspecific morphological variability remains unknown. In the last two decades, several new species were added to this family and ten more genera were reported or re-established, which suggests that the diversity of this group of ciliates is under-estimated (e.g. Foissner *et al.* 2002; Paiva *et al.* 2003, 2004; Küppers *et al.* 2007, 2011; Berger 2008, 2011; Li *et al.* 2010; Shao *et al.* 2011, 2013a, b; Lv *et al.* 2013; Singh and Kamra 2013).

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The genus *Apoamphisiella* has an unclear position within the family Oxytrichidae (Berger 1999). It was erected by Foissner (1997) and comprises five species, i.e., *Apoamphisiella foissneri* Paiva *et al.*, 2004, *A. hymenophora* (Stokes, 1886) Berger, 1999, *A. jurubatiba* Paiva *et al.*, 2003, *A. tihanyiensis* (Gellért and Tamás, 1958) Foissner, 1997, and *A. vernalis* (Stokes, 1887) Berger, 2006. They can be clearly recognized by having undulating membranes in the *Cyrtohymena* pattern, two frontoventral rows, distinct differentiation of frontal, frontoventral, postoral ventral, transverse and caudal cirri, one marginal row on each side of the cell. *Apoamphisiella tihanyiensis*, the type of the genus, was described for three times, but there are several differences in the cirral patterns of three described populations, which implies that detailed descriptions of additional populations are needed (Berger 1999; Paiva *et al.* 2004).

The shape and the arrangement of the paroral and endoral membranes are useful features for separating oxytrichids (Blatterer and Foissner 1988; Foissner 1989; Voss and Foissner 1996; Berger 1999). Blatterer and Foissner (1988) established the genus *Notohymena* for species having a hooked-shaped distal end of paroral membrane, which is sometimes inconspicuous. Seven valid species have been included in this genus so far, i.e. *Notohymena rubescens* Blatterer and Foissner, 1988 (the type by original designation), *N. antarctica* Foissner, 1996, *N. apoaustralis* Lv *et al.*, 2013, *N. australis* (Foissner and O'Donoghue, 1990) Berger, 1999, *N. pampasica* Küppers *et al.*, 2007, *N. saprai* Kamra and Kumar, 2010, and *N. selvatica* (Hemberger, 1985) Blatterer and Foissner, 1988 (Berger 1999; Küppers *et al.* 2007; Kamra and Kumar 2010; Lv *et al.* 2013). Divisional morphogenesis has been described only for *N. rubescens, N. saprai*, and *N. apoaustralis* (Voss 1991; Kamra and Kumar 2010; Lv *et al.* 2013). Of these seven species, only *N. australis* and *N. rubescens* have an unusually high number of caudal cirri, which are arranged in three short rows. The others each have just 3 invididual caudal cirri. *Notohymena australis* was described from two geographically distant locations, i.e. Perth, Australia and Bavaria, Germany, indicating that this species possibly is cosmopolitan. It has not been found elsewhere since then.

Lake Biwa is located in Shiga Prefecture, Japan. It is the largest freshwater lake of Japan and an ancient lake, with a history of over 4 million years. More than 60 endemic species of freshwater fish, invertebrates, water plants and algae have been recognized there (Rossiter 2000). As a result of a recent comprehensive faunistic survey on aquatic organisms conducted from 2006 to 2010 by the Lake Biwa Museum, a few ciliates were newly described or redescribed (Foissner *et al.* 2008, 2009; Ji and Kusuoka 2009). We here present morphological data on two species from Lake Biwa, along with the description of divisional morphogenesis of *N. australis*.

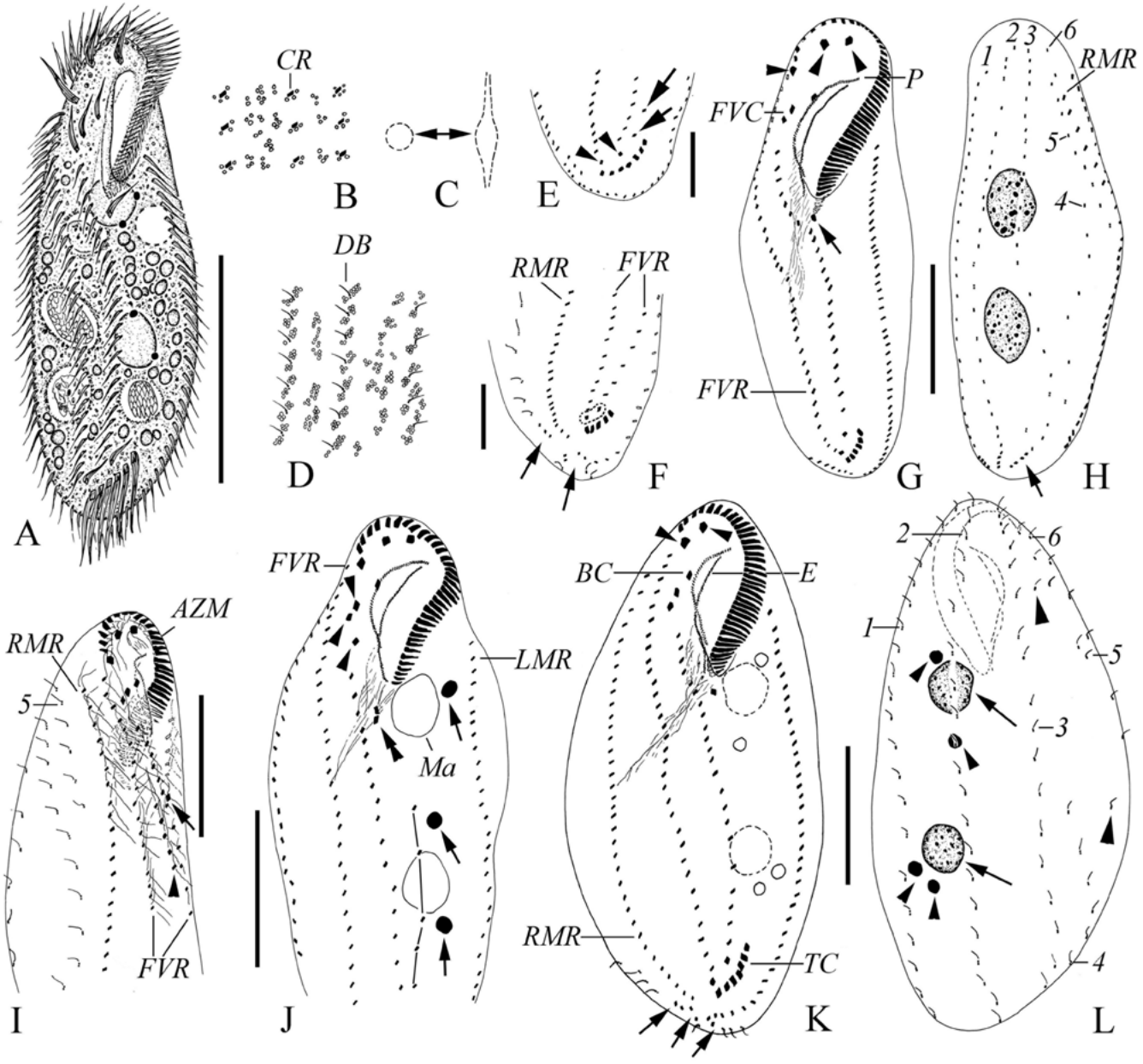
MATERIAL AND METHODS

Samples were collected from the inshore littoral zone of Lake Biwa near the Lake Biwa Museum, Shiga, Japan on 28 October 2007 using the Polyurethane Foam Unit (PFU) method (Cairns *et al.* 1973). Isolated specimens were kept as uniprotistan and raw cultures in Petri dishes for a few days at room temperature with rice grains added to stimulate enrichment of bacterial food. Live cells were observed using bright field and differential interference contrast microscopy (Nikon 80i, Tokyo, Japan) at 100–1000 × magnifications. Protargol impregnation was used to reveal the infraciliature and the nuclear apparatus (Wilbert 1975). Impregnated specimens were measured at a magnification of × 1250. Drawings were made with the aid of a camera lucida. Terminology and systematics are mainly according to Berger (1999).

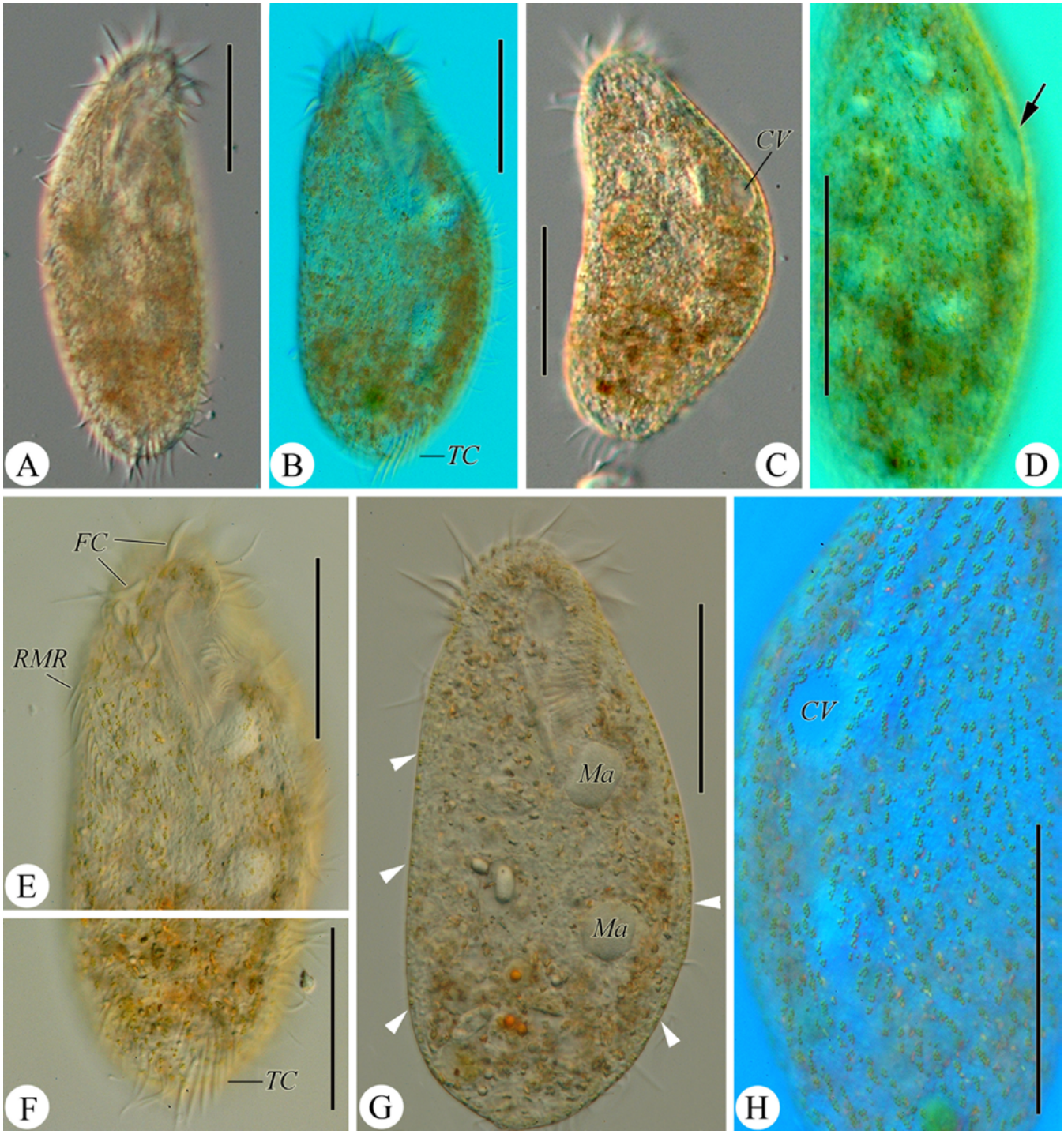
RESULTS

Morphology of Japan population of *Apoamphisiella tihanyiensis* (Figs 1–3; Table 1)

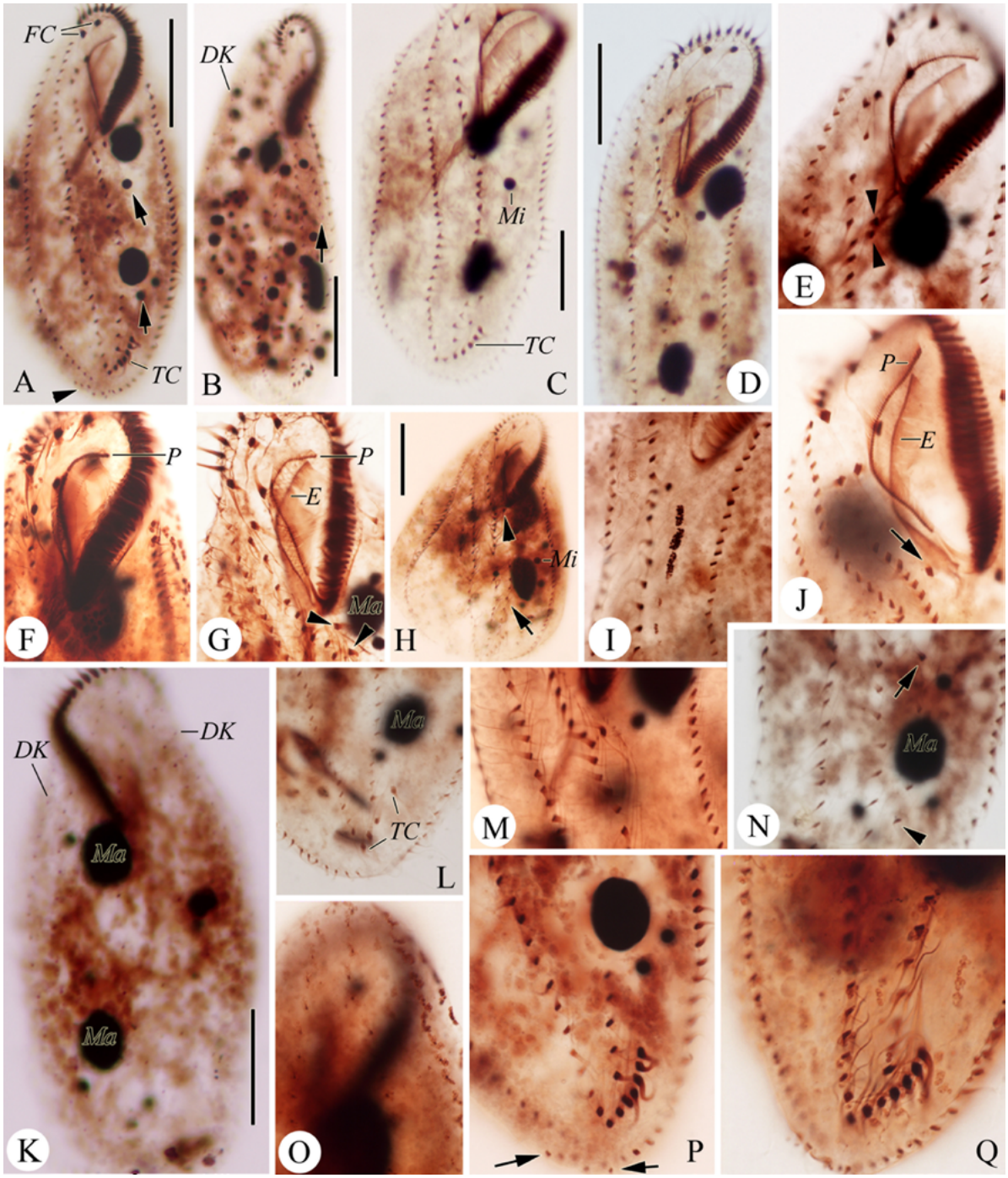
Size *in vivo* about 130–200 × 50–75 μm, dorsoventrally flattened up to 2:1; cell long elliptical, ratio of length to width 2.5–3.5:1, both ends rounded, anterior end narrower than posterior one, left and right margins straight to convex, widest area just behind mid-body (Figs 1A; 2A, B). Cell dark at low magnification in fresh sample but yellow-greenish after starvation due to cortical granules. Pellicle very soft and flexible and slightly contractile. Cortical granules yellow-greenish, 0.5 μm across, arranged in rather widely spaced, longitudinal stripes, including cirral and dorsal ciliary rows (Figs 1B, D; 2B, D, E, H; 3O). Endoplasm colorless, containing some food vacuoles. Invariably two spherical to ellipsoidal macronuclear nodules apart in central third of body slightly left of mid-line, both nodules about 20–25 μm long *in vivo* (Figs 1H, J, L; 2G; 3K). Three to ten spherical to slightly ellipsoidal micronuclei close to macronuclear nodules (Figs 1J, L; 3A, H). Contractile vacuole in front of mid-body, with anterior and posterior collecting canals, pulsating at intervals of



Figs 1A–L. *Apoamphisiella tihanyiensis* from life (**A–D**) and after protargol impregnation (**E–L**). **A** – ventral view of a representative individual; **B** – arrangement of cortical granules on ventral side; **C** – shape of contractile vacuole; **D** – arrangement of cortical granules on dorsal side; **E** – posterior part of cell in ventral view, showing 2 pretransverse ventral cirri (arrowheads) and two additional ventral cirri ahead of transverse cirri (arrows); **F** – posterior part of cell in ventral view, showing 2 pretransverse ventral cirri (encircled by broken line) and caudal cirri (arrows); **G, H** – ventral (G) and dorsal (H) infraciliature of the same specimen, showing single postoral ventral cirrus (arrow in G), caudal cirri at the posterior end of dorsal kinety 4 (arrow in H), and frontal cirri (arrowheads in G); **I** – anterior half of cell in ventral view, showing single postoral ventral cirrus (arrow) and several parental ventral cirri (arrowhead); **J** – part of ventral infraciliature, showing micronuclei (arrows), frontoventral cirri (arrowheads), 2 postoral ventral cirri (double-arrowhead), and several parental ventral cirri (connected by broken line); **K, L** – ventral (K) and dorsal (L) infraciliature of the same specimen, showing caudal cirri (arrows in K), macronuclear nodules (arrows in L), frontal cirri (arrowheads in K), and micronuclei (arrowheads in L). 1–6 – dorsal kinety 1–6, AZM, adoral zone of membranelles; BC – buccal cirrus, CR – cirral row, DB – dorsal bristle, E – endoral membrane, FVC – frontoventral cirri, FVR – frontoventral row, LMR – left marginal row, Ma – macronuclear nodule, P – paroral membrane, RMR – right marginal row, TC – transverse cirri. Scale bars: 80 μm (A), 40 μm (I, G, H, J–L), 20 μm (E, F).



Figs 2A–H. *Apoamphisiella tihanyiensis* from life. **A, B** – ventral view of different cells; **C** – a contracted cell, showing contractile vacuole; **D** – part of cell in dorsal view, showing contractile vacuole with collecting canals (arrow); **E, F** – ventral views of anterior (E) and posterior (F) parts of the same cell, showing arrangement of cortical granules; **G** – plumper individual, showing cortical granules (arrowheads) and macronuclear nodules; **H** – part of cell in dorsal view showing spherical contractile vacuole and arrangement of cortical granules. CV – contractile vacuole, FC – frontal cirri, Ma – macronuclear nodule, RMR – right marginal row, TC – transverse cirri. Scale bars: 60 µm.



Figs 3A–Q. *Apoamphisiella tihanyiensis* after protargol impregnation. **A** – ventral view of specimen with two frontal cirri, showing micronuclei (arrows) and caudal cirri (arrowhead); **B** – right lateral view showing several parental cirri (arrow); **C** – ventral view of a specimen with two additional ventral cirri ahead of transverse cirri; **D, F** – ventral view of anterior part of an individual with two frontoventral cirri; **E, G** – ventral view of anterior part of a cell with three frontoventral and two postoral ventral cirri (arrowheads); **H** – ventral view of cell with several parental cirri (arrow), and single postoral ventral cirrus (arrowhead); **I** – ventral view of middle part of cell, showing oral primordium; **J** – ventral view of a cell just after division, showing anteriorly placed postoral ventral cirrus (arrow); **K** – dorsal view of a cell, showing dorsal kineties and macronuclear nodules; **L** – ventral view of posterior part of specimen with four transverse cirri; **M** – ventral view of middle part of cell, showing cirri associated with fibres; **N** – ventral view of middle part of a cell, showing single postoral ventral cirrus (arrow) and parental cirri (arrowhead); **O** – dorsal view of anterior part of a cell, showing dorsal kineties and associated cortical granules; **P, Q** – ventral views of posterior part of cell, showing caudal cirri (arrows). DK – dorsal kinety, E – endoral membrane, FC – frontal cirri, Ma – macronuclear nodule, Mi – micronucleus, P – paroral membrane, TC – transverse cirri. Scale bars: 40 μm.

Table 1. Morphometric data of *Apoamphisiella tihanyiensis* (At) and *Notohymena australis* (Na). Data based on protargol-impregnated specimens. CV – coefficient of variance (%), Max – maximum, Mean – arithmetic mean, Min – minimum, n – number of specimens examined, SD – standard deviation, – data unavailable.

Character	Species	Min	Max	Mean	SD	CV	n
Body length (μm)	At	128	196	155.8	18.84	12.1	18
	Na	120	160	142.0	13.20	9.3	20
Body width (μm)	At	53	82	70.9	9.38	13.2	12
	Na	45	76	63.3	8.46	13.4	19
Adoral zone of membranelles, length (μm)	At	41	70	52.7	7.25	13.8	18
	Na	34	62	54.4	6.67	12.3	20
Adoral membranelles, number	At	36	55	44.4	4.90	11.0	18
	Na	35	42	38.3	1.74	4.5	20
Frontal cirri, number	At	2	3	2.9	0.24	8.3	18
	Na	2	3	3.0	0.22	7.3	20
Frontoventral cirri, number	At	1	3	2.1	0.42	20.0	18
	Na	4	4	4	0	0	20
Buccal cirrus, number	At	1	1	1	0	0	18
	Na	1	1	1	0	0	20
Postoral ventral cirri, number	At	1	3	1.1	0.51	46.4	18
	Na	3	3	3	0	0	20
Pretransverse ventral cirri, number	At	2	2	0	0	0	18
	Na	2	2	2	0	0	20
Ventral cirri, number	At	3	4	3.3	0.67	20.3	18
	Na	5	6	5.1	0.22	4.3	20
Cirri in left frontoventral row, number	At	16	27	22.6	2.91	12.9	18
	Na	–	–	–	–	–	–
Cirri in right frontoventral row, number	At	30	43	35.9	3.14	8.7	17
	Na	–	–	–	–	–	–
Transverse cirri, number	At	4	7	6.1	0.94	15.4	18
	Na	4	6	5.0	0.32	6.4	20
Dorsal kineties, number	At	6	7	6.1	0.35	5.7	15
	Na	6	6	6	0	0	18
Caudal cirri, number	At	6	11	8.6	1.33	15.5	13
	Na	7	10	8	1.13	14.1	12
Caudal cirri at end of dorsal kinety 1, number	At	2	5	3.1	0.86	27.7	13
	Na	2	4	2.4	0.67	27.9	12
Caudal cirri at end of dorsal kinety 2, number	At	1	2	1.7	0.49	28.8	12
	Na	2	3	2.2	0.39	17.7	12
Caudal cirri at end of dorsal kinety 4, number	At	3	5	3.9	0.49	12.6	13
	Na	3	4	3.4	0.51	15.0	12
Macronuclear nodules, number	At	2	2	2	0	0	18
	Na	2	2	2	0	0	19
Micronuclei, number	At	3	10	4.1	1.65	40.2	16
	Na	3	5	–	–	–	3
Cirri in left marginal row, number	At	26	46	38.9	5.16	13.3	17
	Na	30	38	33.4	2.09	6.3	19
Cirri in right marginal row, number	At	33	46	39.6	3.66	9.2	17
	Na	30	50	34.4	4.10	11.9	19
Macronuclear nodule, length (μm)	At	12	26	18.4	3.61	19.6	36
	Na	13	28	21.6	3.47	16.1	38
Macronuclear nodule, width (μm)	At	10	21	13.6	2.89	21.3	36
	Na	9	18	13.9	2.24	16.1	38

12 seconds (Figs 1C; 2C, D, H). Locomotion by crawling slowly on substrates, but moving quickly when disturbed; more or less thigmotactic.

Buccal field broad, ca. half of body width and one third of body length, its proximal end covered by buccal lip (Fig. 2E). Adoral zone composed of 36–55 membranelles. Undulating membranes distinctly curved, especially paroral membrane; endoral membrane optically intersecting paroral one (Figs 1G, K; 3E–G, J). Pharyngeal fibres conspicuously recognizable in protargol-impregnated specimens (Fig. 1G, J, K).

Almost always three frontal cirri (Figs 1G; 3D), one buccal cirrus (Figs 1K; 3F), two frontoventral cirri (Figs 1G, I, K; 3D, F), one postoral ventral cirrus (Figs 1G, I, K; 3D, H, J, N) and two pretransverse ventral cirri (Figs 1F, G, K; 3A, P, Q). A few specimens with one or three frontoventral cirri (Figs 1J; 3E, G) or two postoral ventral cirri (Figs 1J; 3E, G). Frontal cirri distinctly enlarged, in life about 15 μm long, rightmost cirrus very close to distal end of adoral zone of membranelles. Frontoventral cirri to left of anterior portion of right frontoventral row. Postoral ventral cirrus/cirri near buccal vertex. Right frontoventral row long, commencing near distal end of adoral zone of membranelles and ending in front of rightmost pretransverse ventral cirrus (Figs 1G, H, K; 3A, C). Left frontoventral row parallel to and widely separated from right one, beginning behind frontoventral cirri and extending to transverse cirri. An additional short row of five or more than five cirri – possible reminiscent of parental cirri – present behind buccal vertex between cirral rows in three of 18 specimens (Figs 1I, J; 3B, H, N). Pretransverse ventral cirri slightly enlarged, usually between frontoventral rows and transverse cirri. Two specimens with one or two additional ventral cirri ahead of transverse cirri, respectively (Figs 1E; 3C). Four to seven transverse cirri distinctly enlarged, in life about 20 μm long, projecting beyond posterior body margin, arranged in curved oblique row (Figs 2B, F; 3C, L, P, Q). Marginal rows nonconfluent posteriorly, separated by narrow gap (Fig. 1G); left one J-shaped, terminating at rear end of the cell, its posterior-most cirri thus easily confused with caudal cirri at rear end of first dorsal kinety (Figs 1K; 3A); right one starting near anterior end of right frontoventral row, terminating posteriorly. All other cirri on ventral side relatively fine, ca. 12–14 μm long in life.

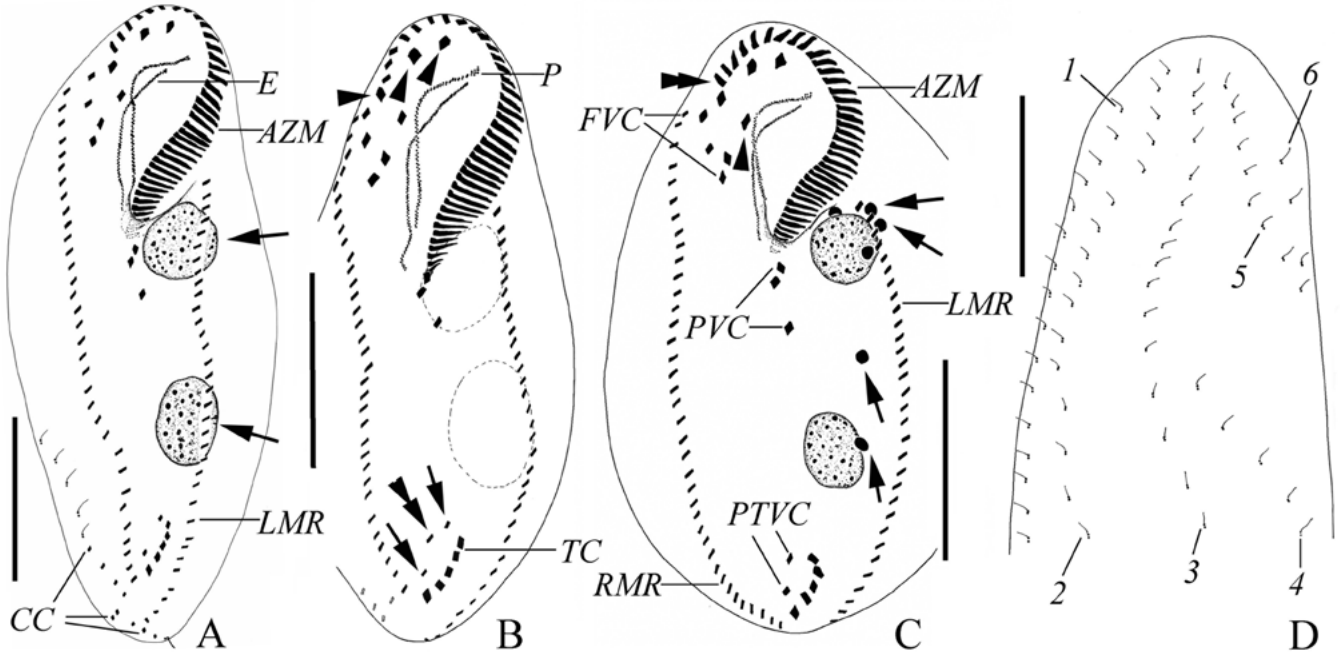
Dorsal cilia in life about 3 μm long, usually arranged in six kineties. Dorsal kineties 1–3 almost as long as body; kinety 4 anteriorly commencing ahead of mid-body, posteriorly ending with caudal cirri; kineties 5 and 6 posteriorly distinctly shortened, terminating anterior to mid-body (Figs 1H, L; 3K). Six to 11 caudal cirri in three groups, located at posterior ends of dorsal kineties 1, 2 and 4 (Figs 1H, K; 3P, Q).

Only one specimen at very early stage of divisional morphogenesis was available (Fig. 3I). On its ventral side, oral primordium composed of several groups of densely arranged kinetosomes was formed *de novo* just behind the postoral ventral cirrus and near the left frontoventral row.

Interphase morphology of Japan population of *Notohymena australis* (Figs 4, 7, 8; Table 1)

Size *in vivo* about 120–180 × 40–60 μm, usually 150 × 50 μm, ratio of length to width ca. 3–4:1. Cell elliptical in outline, anterior end narrowly rounded, posterior end broadly rounded, left margin straight or slightly convex, right margin almost straight (Figs 7B, C). Cell grayish or slightly greenish at low magnification. Body flexible and slightly contractile (Figs 7A, G), dorsoventrally flattened about 2:1. Cortical granules ca. 0.8 μm across, yellow-greenish, grouped around cirri and dorsal cilia or aligned between cirral rows or dorsal kineties (Figs 7E, H, I). Endoplasm full of many spherical granules (2–5 μm across; Figs 7D, G). One contractile vacuole about 12 μm across, located at about 40% of body length near left margin, pulsating at 11-second intervals (Fig. 7G). Always two macronuclear nodules located far apart in middle third of cell left of median line (Figs 7G; 8A–C, F, G); in life a few micronuclei sometimes recognizable close to macronuclear nodules (Fig. 7F), ellipsoid or spherical in outline in impregnated specimens (Figs 4A, C; 8B, I). Movement mostly by slow crawling on substrates, occasionally swimming in water body; sensitive to disturbance. Feeding on debris, bacteria, flagellates, and other ciliates (Figs 7D, G; 8G).

Adoral zone of membranelles about 28–48% (on average 37%) of body length in protargol-impregnated specimens (n = 20), composed of 35–42 membranelles. Cilia and bases of membranelles up to 15 μm long and 8 μm wide, respectively. Distal end of adoral zone extending far onto right side of cell, with a DE-value 0.18–0.28 (Figs 4C; 8A, M). Paroral and endoral membrane almost equal in length, intersecting when viewed ventrally (Figs 4A–C; 8A, C, F, M); the former distinctly curved with its distal end hooked, located slightly anterior to the latter (Figs 8E, J, M). Almost always three enlarged frontal cirri at anterior end of cell, rightmost one close to distal end of adoral zone (Figs 4A, B; 8A,



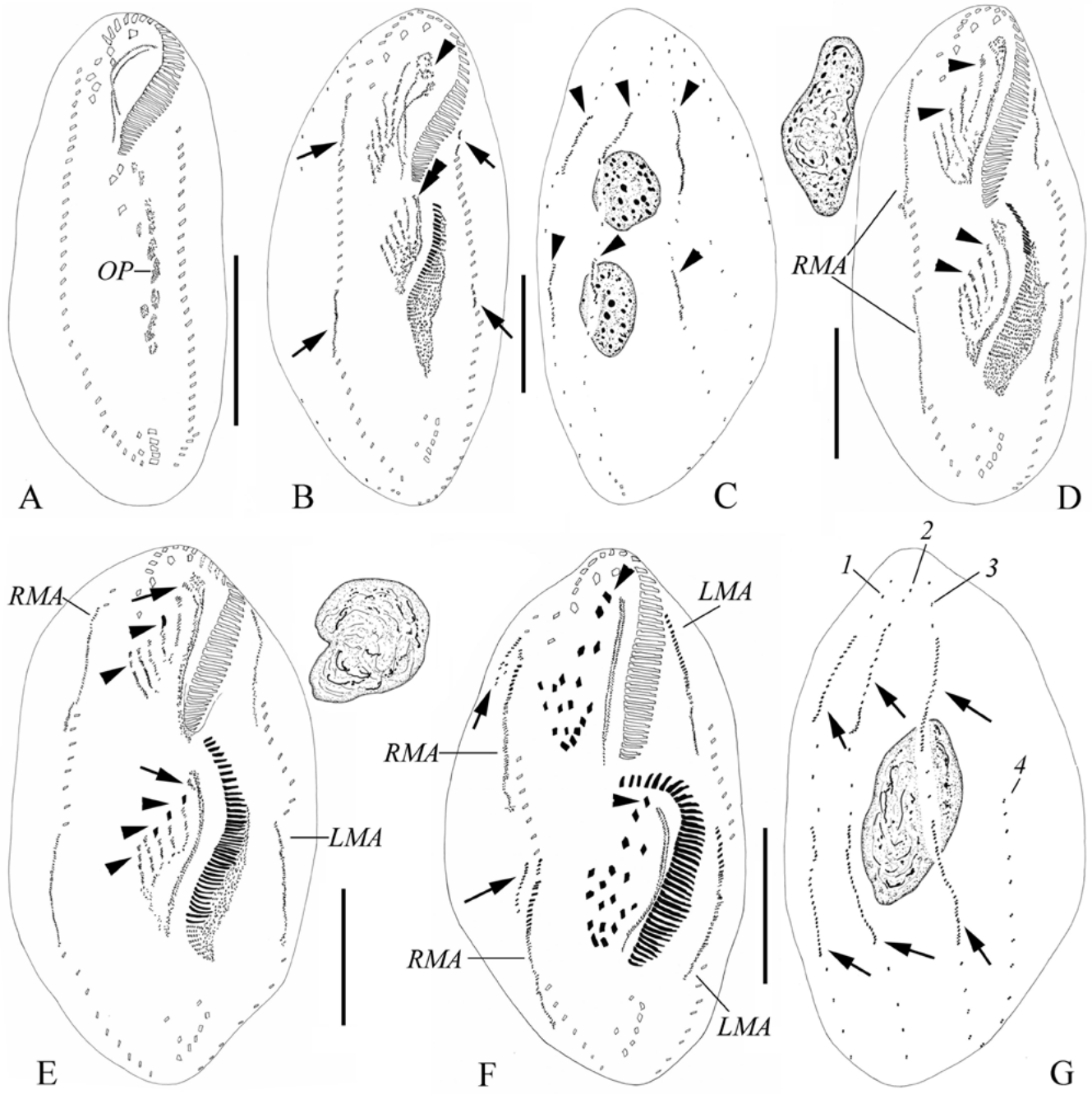
Figs 4A–D. *Notohymena australis* after protargol impregnation. **A** – ventral view of a representative specimen showing infraciliature and macronuclear nodules (arrows); **B** – ventral view of cell with two pretransverse ventral cirri (arrows), one additional ventral cirrus (double-arrowhead), and three frontal cirri (arrowheads); **C** – ventral view of cell, showing infraciliature, macronuclear nodules and micronuclei (arrows), buccal cirrus (arrowhead), and distal end of adoral zone (double-arrowhead); **D** – anterior part of specimen in dorsal view, showing dorsal kineties. AZM – adoral zone of membranelles, CC – caudal cirri, E – endoral membrane, FVC – frontoventral cirri, 1–6 – dorsal kinety 1–6, LMR – left marginal row, P – paroral membrane, PTVC– pretransverse ventral cirri, PVC– postoral ventral cirri, RMR – right marginal row, TC – transverse cirri. Scale bars: 40 μm.

E, J, M; only two observed in one specimen at early stage of morphogenesis, Fig. 5A); one buccal cirrus near anterior third of paroral membrane (Figs 4C; 8A, C, M); four frontoventral cirri between anterior part of right marginal row and paroral membrane, arranged in V-shape (Figs 4A–C; 8C, J, M); usually three (rarely four) postoral ventral cirri forming group close to buccal vertex (Figs 4A–C; 8A, D, J, M); two pretransverse ventral cirri (Figs 4B; 8A, K, L) near five transverse cirri, these latter in a line (Figs 4B; 8C, K, L). Cirrus V/2 positioned slightly in front of leftmost transverse cirrus. Only one specimen observed with one additional ventral cirrus nearby pretransverse ventral cirri (Figs 4B; 8L). Distance between cirri V/4 and I/1 0.68 and 1.0 (0.81 on average) of that between cirri V/4 and VI/1. Left and right marginal row separated posteriorly, composed of about 33 and 34 cirri, respectively; left row terminating at posterior end of cell, right one to right of and at level of rightmost transverse cirrus (Figs 4A, B; 8K).

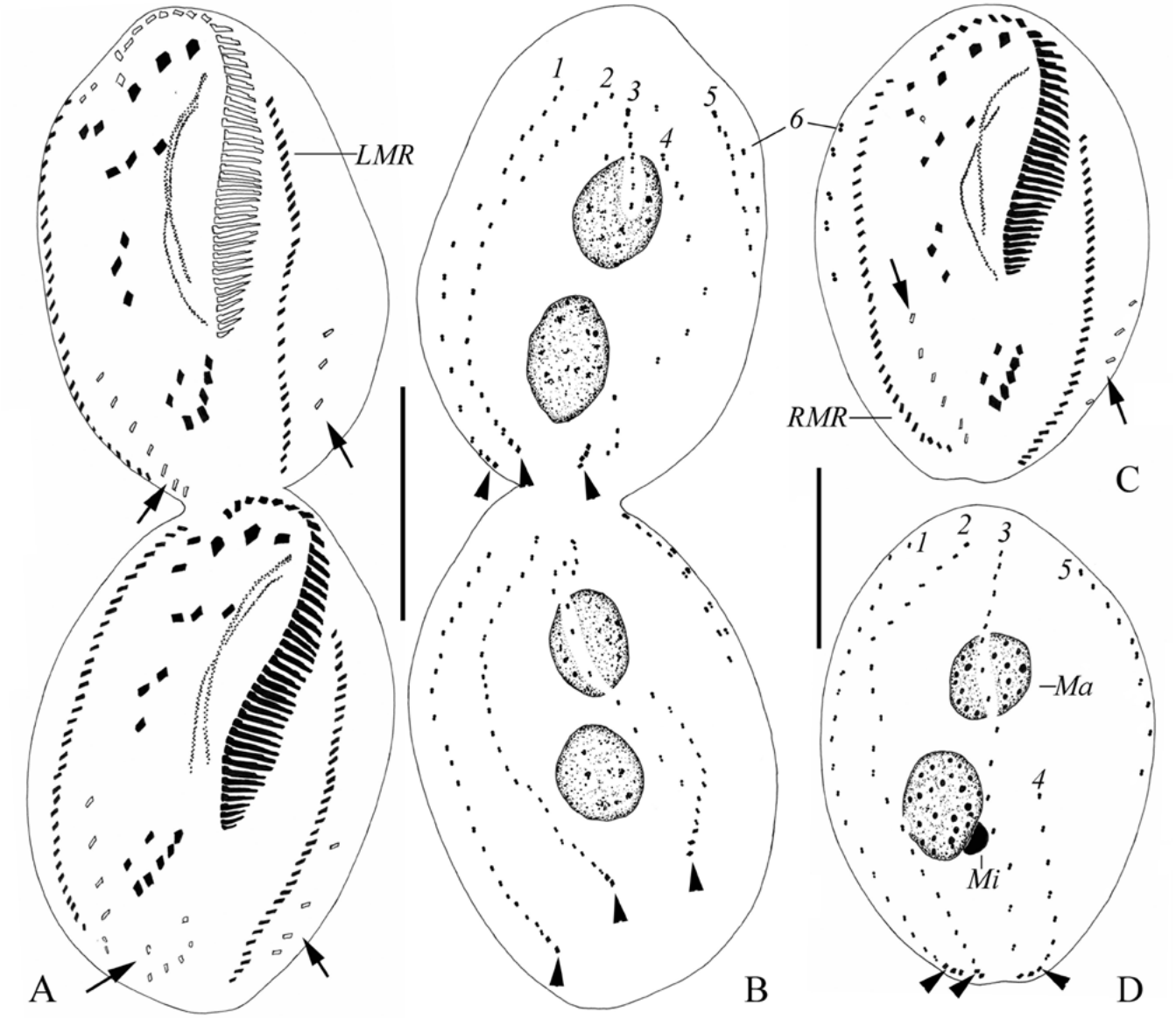
Dorsal ciliature invariably composed of six kineties (Fig. 4D). Kineties 1, 2, and 3 almost bipolar; kinety 4 anteriorly commencing anterior to mid-body, posteriorly ending with caudal cirri; kinety 5 posteriorly terminating ahead of mid-body; kinety 6 consisting of three to five dikinetids, parallel to kinety 5. Seven to 10 caudal cirri arranged in three rows, located respectively at posterior ends of dorsal kineties 1, 2, and 4.

Divisional morphogenesis of *Notohymena australis* (Figs 5, 6, 9, 10)

Stomatogenesis: Division commences with the apokinetal proliferation of groups of closely spaced kinetosomes between the left marginal row and the postoral ventral cirri, far from the anterior-most transverse cirri, which is oral primordum of the opisthe (Figs 5A; 9A). With rapid kinetosomal proliferation in it, oral primordium widens and lengthens, and then adoral membranelles become organized posteriad; simultaneously, anlagen of undulating membranes are formed

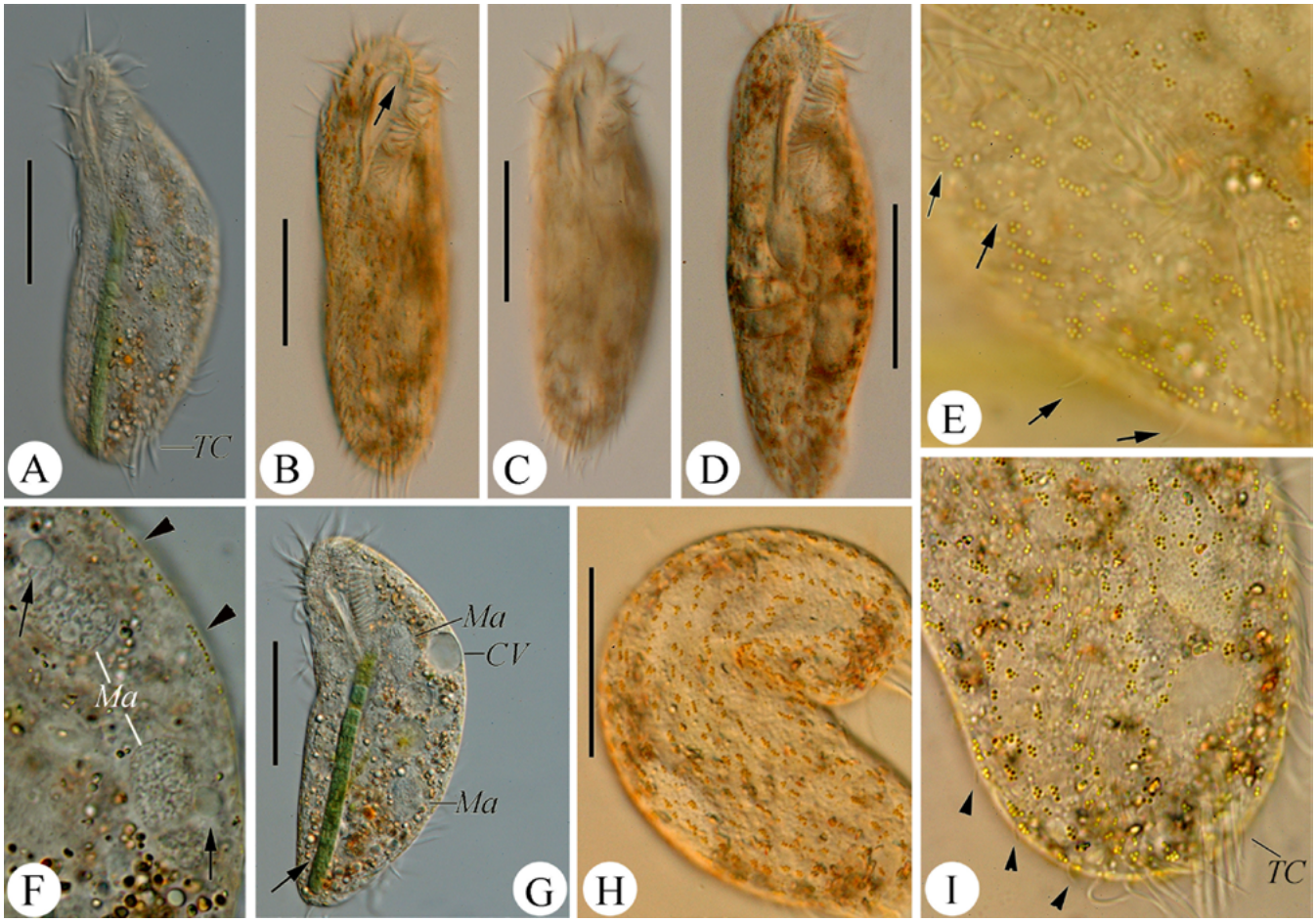


Figs 5A–G. *Notohymena australis* after protargol impregnation. **A** – ventral view of an early divider, showing oral primordium; **B, C** – ventral (B) and dorsal (C) view of the same middle divider, showing the formation of frontal-ventral-transverse cirral anlagen in proter and opisthe, and anlagen of marginal rows (arrows), dedifferentiation of anterior end of undulating membranes (arrowheads), and undulating membrane anlagen in opisthe (double-arrowhead); **D, E** – ventral infraciliature of two middle dividers, showing frontal-ventral-transverse cirral anlagen beginning to organize into new cirri from anterior (arrowheads) and a small anlage separating from anterior right end of undulating membrane anlagen (arrows), insets: fused macronucleus; **F, G** – ventral (B) and dorsal (C) view of the same late divider, showing the formation of anlgen of dorsomarginal kineties at anterior right end of right marginal rows anlagen (arrows in F), frontal-ventral-transverse cirral anlagen II – VI segregating into 3, 3, 3, 4 and 4 cirri, the leftmost frontal cirrus originated from undulating membranes anlagen (arrowheads), and development of dorsal kinety anlagen (arrows in G). 1–4 – dorsal kinety 1–4, LMA – anlage of left marginal row, OP – oral primordium, RMA – anlagen of right marginal row. Scale bars: 40 μm.



Figs 6A–D. *Notohymena australis* after protargol impregnation. **A, B** – ventral (A) and dorsal (B) infraciliature of the same very late divider, showing parental cirri (arrows) and newly formed caudal cirri at posterior ends of first, second and fourth dorsal kineties (arrowheads); **C, D** – ventral (C) and dorsal (D) infraciliature of the proter just after division showing two macronuclear nodules, one micronucleus, parental cirri (arrows), and newly formed caudal cirri at the posterior ends of first, second and fourth dorsal kineties (arrowheads). 1–6 – dorsal kinety 1–6, LMR – left marginal row, Ma – macronuclear nodule, Mi – micronucleus, RMR – right marginal row. Scale bars: 40 µm.

to right of oral primordium as a long streak of kinetosomes (Fig. 5B). In the proter, the parental adoral zone of membranelles remains unchanged, while anterior ends of paroral and endoral membranes begin to dedifferentiate (Figs 5B; 9B). Oral primordium develops further and adoral membranelles increase in number (Fig. 5D). Anlagen of undulating membranes are also formed in the proter (Fig. 5D). Then, a small anlage becomes detached from the anterior end of the undulating membranes anlagen (Figs 5E; 9D, F, G), which finally generate the leftmost frontal cirrus (Figs 5F; 9H). Later, development of oral primordium is complete in the opisthe, and anterior part of the adoral zone of membranelles curves rightwards (Figs 5F; 8H). The paren-



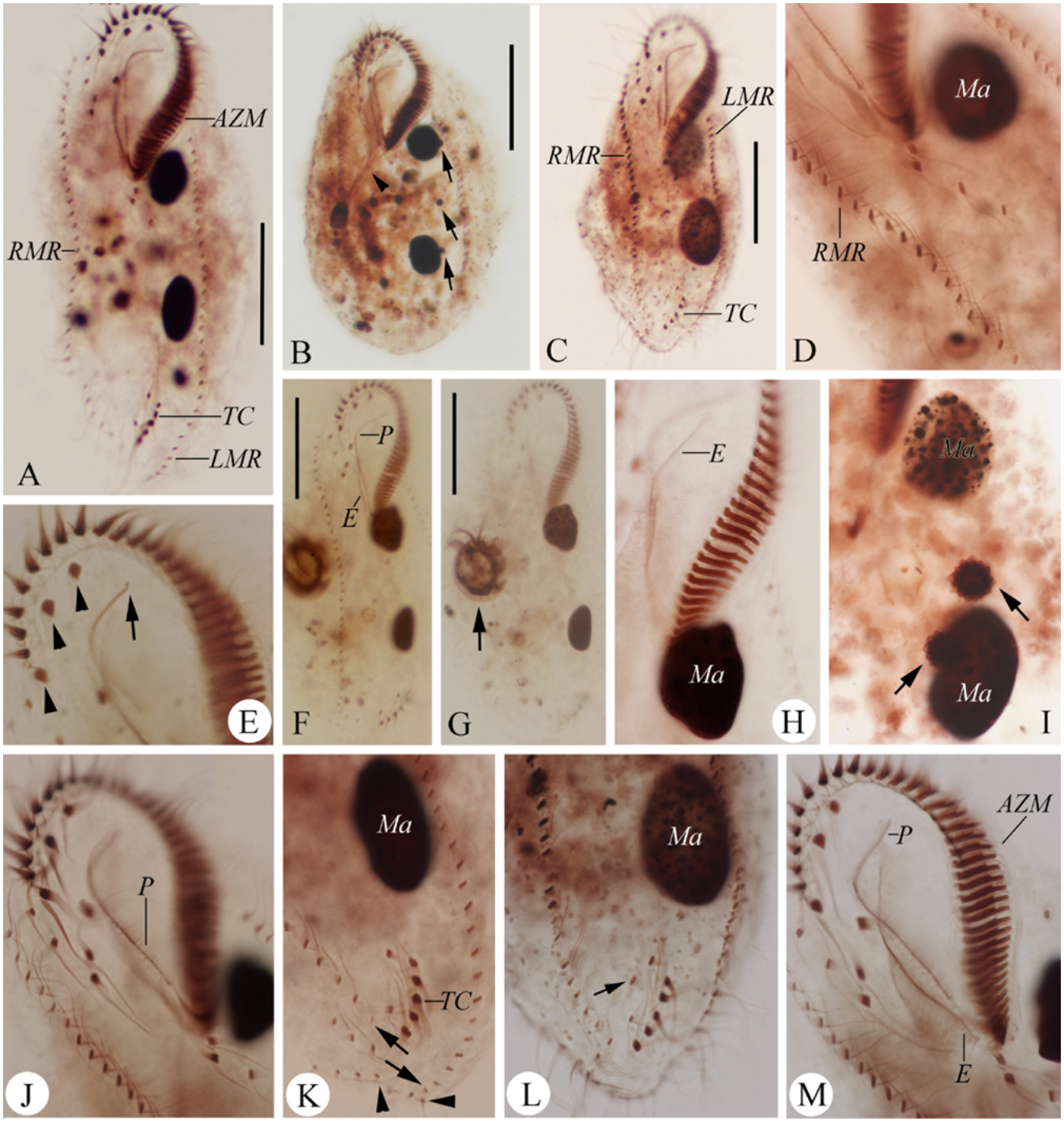
Figs 7A–I. *Notohymena australis* from life. **A, G** – ventral view of the same cell, showing flexible body, contractile vacuole, ingested algae (arrow) and macronuclear nodules; **B, C, D** – ventral view of individuals in different states, showing hooked anterior end of peristomial lip (arrow); **E, I** – view of portion of cell, showing the arrangement of yellow-greenish cortical granules, and dorsal cilia (arrows in E and arrowheads in I); **F** – macronuclear nodules, micronuclei (arrows) and cortical granules (arrowheads); **H** – dorsal view of a curved cell, showing cortical granules grouped in lines. CV – contractile vacuole, Ma – macronuclear nodule, TC – transverse cirri. Scale bars: 40 µm.

tal adoral zone of membranelles is wholly inherited by the proter. The anlagen of undulating membranes begin to split longitudinally into two streaks from which the paroral and endoral membranes are formed (Figs 5F; 6A, C; 9H; 10A, B).

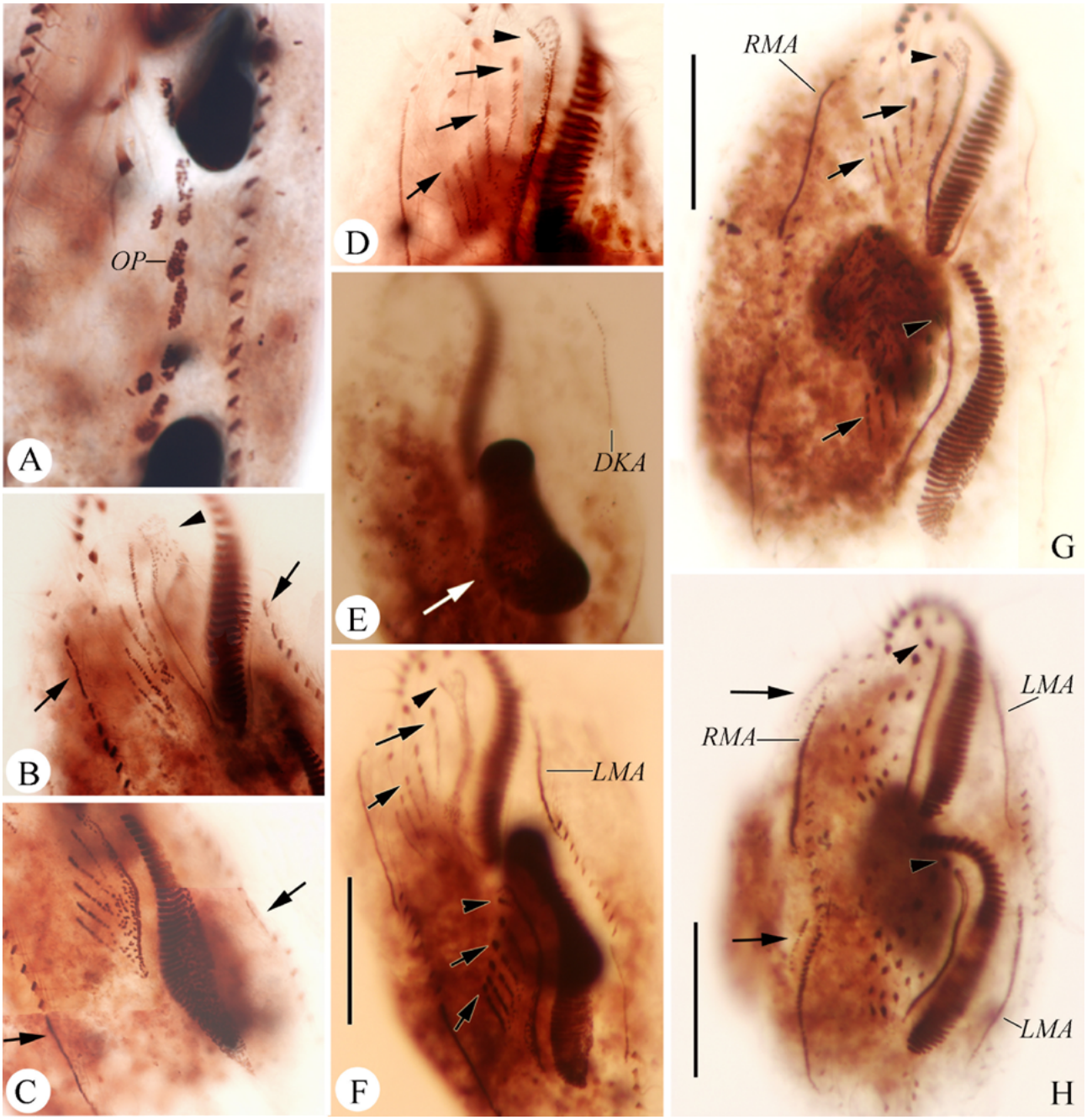
Development of frontoventral transverse cirri: At the same time as the development of oral primordium, two sets of frontal-ventral-transverse cirral anlagen (FVT-anlagen), each with five streaks, appear in the anterior and posterior halves (Figs 5B; 9B, C). The parental buccal, frontoventral (III/2, IV/3) and three postoral ventral cirri very likely disorganize and join in the formation of these anlagen, whereas the frontal cirri, rest frontoventral and pretransverse ventral cirri as well transverse cirri remain unchanged. Quickly, streaks of FVT-anlagen segregate into cirri from anterior to posterior (Figs 5D, E; 9D, F, G). These streaks of FVT-anlagen give rise to the following numbers of cirri in both dividers: 3, 3, 3, 4, 4 (Figs 5F; 9H). Newly formed cirri move to their right position (Figs 6A, C; 10A, B).

Development of marginal cirri: Anlagen of marginal rows originate intrakinetally; those in the left row are distinctly shorter than those in the right row (Figs 5B; 9B, C). With kinetosomal proliferation, these anlagen lengthen and develop into new cirri from anterior to posterior (Figs 5D–F; 6A; 9F–H; 10A).

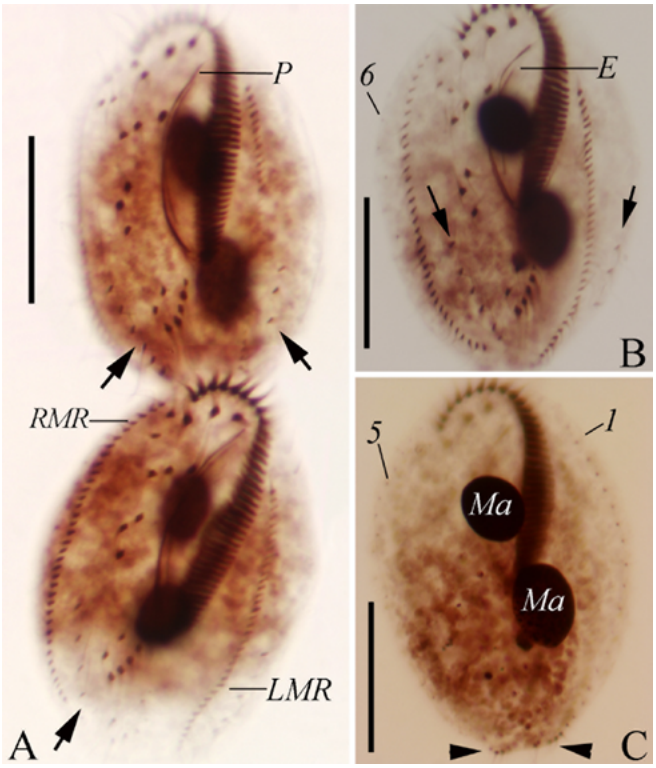
Development of dorsal ciliature: Dorsal kinety anlagen appear later than oral promordium, within the



Figs 8A–M. *Notohymena australis* after protargol impregnation. **A** – ventral view of a representative specimen showing infraciliature; **B** – plumper cell showing pharyngeal fibers (arrowhead), macronuclear nodules, and micronuclei (arrows); **C, L** – ventral infraciliature of the same cell with additional ventral cirrus (arrow); **D** –ventral view of middle part of a specimen with 4 postoral ventral cirri; **E** – anterior part of the same cell as in A, showing hooked anterior end of paroral membrane (arrow) and three enlarged frontal cirri (arrowheads); **F, G** – the same individual showing ventral ciliature and ingested ciliate (arrow, *Aspidisca* sp.); **H** – details of buccal apparatus showing endoral membrane and the structure of adoral membranelles; **I** – macronuclear nodules composed of many small nucleoi and micronuclei (arrows); **J, M** – ventral view of anterior part of cell, showing cirri associated with fibers; **K** – ventral view of posterior part of cell, showing left and right marginal rows terminating respectively at posterior end and at level of right transverse cirrus (arrows), and caudal cirri (arrowheads). AZM – adoral zone of membranelles; E – endoral membrane, LMR – left marginal row, Ma – macronuclear nodule, P – paroral membrane, RMR – right marginal row, TC – transverse cirri. Scale bars: 40 μm.



Figs 9A–H. *Notohymena australis* after protargol impregnation. **A** – ventral view of middle part of an early divider, showing oral primordium; **B, C** – ventral view of proter (B) and opisthe (C) of the same middle divider, showing the formation of frontal-ventral-transverse cirral anlagen in proter and opisthe, and anlagen of marginal rows (arrows), and dedifferentiation of anterior end of undulating membranes (arrowhead); **D** – ventral infraciliature of a proter of a middle divider, showing frontal-ventral-transverse cirral anlagen beginning to organize into new cirri from anterior (arrows) and a small anlage separating from anterior right end of undulating membranes anlagen (arrowhead); **E** – part of a middle divider in dorsal view, showing fused macronuclear mass (arrow); **F** – ventral infraciliature of the same divider as in E, showing development of anlagen of the leftmost frontal cirrus (arrowheads), and frontal-ventral-transverse cirral anlagen (arrows); **G** – ventral infraciliature of a middle-late divider, showing frontal-ventral-transverse cirral anlagen segregating into new cirri (arrows), the anlage of the leftmost frontal cirrus separating from undulating membrane anlagen (arrowheads), and fused macronuclear mass; **H** – ventral infraciliature of a late divider, showing the formation of anlagen of dorsomarginal kineties at anterior right end of right marginal rows anlagen (arrows) and anterior part of newly formed adoral zone of membranelles curving to right in the opisthe, frontal-ventral-transverse cirral anlagen II – VI segregating into 3, 3, 3, 4 and 4 cirri, the leftmost frontal cirrus originated from undulating membranes anlagen (arrowheads). DKA – dorsal kinety anlage, LMA – anlage of left marginal row, OP – oral primordium, RMA – anlagen of right marginal row. Scale bars: 40 μm.



Figs 10 A–C. *Notohymena australis* after protargol impregnation. **A** – venral view of a very late divider showing infraciliature and parental cirri (arrows); **B, C** – ventral (B) and dorsal (C) view of the proter just after division showing infraciliature and nuclear apparatus, parental cirri (arrows) and newly formed caudal cirri (arrowheads). 1, 5, 6 – dorsal kinety 1, 5, 6, E – endoral membrane, LMR – left marginal row, P – paroral membrane, RMR – right marginal row. Scale bars: 40 μm.

first, second, and third dorsal kineties; two anlagen per kinety are separately formed (Figs 5C, G). At very late stage, two anlagen of dorsomarginal kineties (one long, one short) appear close to the anterior parts of right marginal rows anlagen in both dividers (Figs 5F; 9H). The third dorsal kinety anlage fragments and forms two anlagen. The anlagen of dorsomarginal kineties migrate to dorsal side and develop into two kineties. Consequently six kineties in total are formed (Figs 6B–D). Two to four caudal cirri are evolved at posterior ends of the first, second, and fourth dorsal kinety (Figs 6B, D; 10C).

Division of macronuclear nodules: Macronuclear nodules fuse into a single mass at middle stage (Figs 5D, E, inset; 9E, G), than divide twice so that each filial cell has two macronuclear nodules (Figs 6B, D; 10A, C).

DISCUSSION

Comparison of *Apoamphiseilla tihanyiensis* with previous descriptions

The present species was identified as *Apoamphisiella tihanyiensis* because it fits well with previous descriptions (Berger 1999; Paiva *et al.* 2004). So far this species has been recorded from Europe, South America, and Asia, which might implies that it has a cosmopolitan distribution. Morphometric comparison can only be made among four populations (Gellért and Tamás 1958; Foissner 1997; Paiva *et al.* 2004). The Japanese population is smaller than the European one after fixation (ca. 156 μm long vs. ca. 220 μm long; Gellért and Tamás 1958), bigger than the Brazilian population (ca. 156 μm long vs. ca. 134 μm long; Paiva *et al.* 2004), but similar to the Peruvian population in cell length (vs. 147–190 μm; Foissner 1997). The present population has fewer adoral membranelles than earlier strains (mean of 44 vs. 50, 57 and 57), but values overlap to some extent. The numbers of buccal and frontal, and of cirri in the frontoventral and marginal rows are relatively stable among populations; by contrast, other morphometric features, for example the numbers of frontoventral, ventral, and caudal cirri, are highly variable in the present population, but within the range of the other three populations. All these justify conspecificity of these morphotypes.

Comparison of *Notohymena australis* with previous descriptions

Our form corresponds well with the type population of *Notohymena australis* in body shape, color and arrangement of cortical granules, ventral and dorsal ciliature, especially the number of adoral membranelles, and the presence of a bipolar dorsal kinety 3 and multiple caudal cirri in three rows; therefore, both are judged to be conspecific. Morphometric comparisons should be made between the present isolate and the other two populations. The body size of the present population of *Notohymena australis* is outside the range reported in the original description, which was 65–95 × 22–29 μm for fixed specimens (Foissner and O'Donoghue 1990; Berger 1999), but close to that of the German population studied by Foissner and Gschwind (1998), which measured 108–160 × 42–72 μm. The numbers of left and right marginal cirri of the current population are within the range of both earlier populations (24–44 LMC, 24–40 RMC). All these facts suggest a continuous variation in body size and number of marginal

cirri among populations. The Australian population has a variable number of postoral and pretransverse ventral cirri (Foissner and O'Donoghue 1990; Berger 1999), by contrast, this value varies less in the Japanese population (total number of ventral cirri: min = 5, max = 6, mean = 5.1, CV = 4.3%, n = 20).

Data on morphogenesis are available for four species in the genus, which confirms two differences among them. *Notohymena australis* is more similar to *N. apoaustralis* than to *N. rubescens* and *N. saprai* (Blatterer and Foissner 1988; Lv *et al.* 2013; Kamra and Kumar 2010). In the former two species, the new oral primordium originates to the left of the postoral ventral cirri and more than two caudal cirri are formed at posterior end of each anlage of dorsal kineties 1, 2, and 4. In the latter two species, however, oral primordium appears to the left of the leftmost transverse cirrus, and only one caudal cirrus is evolved from each of the mentioned dorsal kinety anlagen. Minor differences between *N. australis* and *N. apoaustralis* lie in the mentioned anlagen of marginal rows. As illustrated, these anlagen originate earlier in the former species than in the latter. The left anlagen very likely originate somewhat later than right ones in *N. australis*, but the same thing was not recorded in the original description of *N. apoaustralis*.

Acknowledgements. This work was supported by the Natural Science Foundation of China (Project number: 41176119), and by the Lake Biwa Museum's comprehensive research project S06–02, ''Biological exploration and systematics of the aquatic biota in and around Lake Biwa'', and a Marie Curie International Incoming Fellowship within the 7th European Community Framework Programme (return phase, 909381). Many thanks are due to Dr. Mark J. Grygier for improving the English style of the manuscript.

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Received on 23rd April, 2014; revised on 18th June, 2014; accepted on 2nd July, 2014