Available online at www.sciencedirect.com

ScienceDirect

European Journal of Protistology 57 (2017) 61–72

European Journal of PROTISTOLOGY

www.elsevier.com/locate/ejop

Morphology and ontogenesis of *Stylonychia* (*Metastylonychia*) *nodulinucleata* nov. subgen. (Ciliophora, Hypotricha) from Australia

Santosh Kumar, Wilhelm Foissner*

Universität Salzburg, FB Organismische Biologie, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria

Received 20 July 2016; received in revised form 1 September 2016; accepted 2 September 2016
Available online 10 September 2016

Abstract

Using standard methods, we describe the morphology and ontogenesis of a possibly very rare ciliate, *Stylonychia* (*Metastylonychia*) *nodulinucleata* nov. subgen (basionym: *Stylonychia nodulinucleata* Shi and Li, 1993), isolated from soil of the Murray River floodplain in Australia. Further, we provide an English translation of the Chinese original description. Whether the Australian population is conspecific with the Chinese *Stylonychia nodulinucleata* requires a careful redescription of the latter. The new subgenus is characterized by a moniliform macronuclear strand and an ontogenetic feature, viz., that all cirri remain intact during anlagen formation. The moniliform macronuclear strand and the curved dorsal kineties resemble the stylonychid genus *Coniculostomum*. The species is easily recognized by the large size of the body and oral apparatus, the moniliform macronucleus, and the enormous caudal cirri reaching half of body length. Very likely, *Stylonychia* (*Metastylonychia*) *nodulinucleata* has a restricted distribution.

Keywords: Biogeography; Anlagen formation; Redescription; *Coniculostomum*; *Tetmemena*

Introduction

The genus *Stylonychia* Ehrenberg, 1830 is confined to freshwater with species of the *S. mytilus* complex being the most common ones. Berger (1999), in his detailed revision of the oxytrichids, recognized eleven *Stylonychia* species. However, several of them possibly belong to the genus *Tetmemena* Eigner, 1999. Since Berger's revision, *Stylonychia ammermanni* Gupta et al., 2001 and *S. harbinensis* Shi and Ammermann, 2004 have been added. Nevertheless, Foissner (2016), after reinvestigation of the type slides of *S. ammermanni*, proposed that *S. harbinensis* is very likely a synonym of *S. ammermanni*. Very recently, Foissner (2016) added two new species, namely *S. gibbera* Foissner and Heber in Foissner, 2016 and *S. notophorides* Foissner, 2016 from South America. Here, we describe a curious *Stylonychid* not reported since the original description by Shi and Li (1993), viz., *S. nodulinucleata*.

Material and Methods

Cells of *Stylonychia* (*Metastylonychia*) *nodulinucleata* were activated in December 1998 from resting cysts in an air-dried soil sample from Australia, using the non-flooded Petri dish method (Foissner 2016). For details on location and sample, see species description. Briefly, the non-flooded Petri dish method involves placing 50–500 g litter and soil in

*Corresponding author. Fax: +43 662 8044 5698.
E-mail address: wilhelm.foissner@sbg.ac.at (W. Foissner).

http://dx.doi.org/10.1016/j.ejop.2016.09.001
0932-4739/

a Petri dish and saturating, but not flooding it, with distilled water. Such a culture is analysed for ciliates by inspecting about 2 ml of the run-off on days 2, 7, 14, 21, and 28. For a detailed description, see Foissner et al. (2002).

Raw cultures could be established in Eau de Volvic containing some squashed wheat kernels and some millilitres of the run-off from the non-flooded Petri dish culture. Live observation, protargol impregnation and scanning electron microscopy (SEM) were performed according to Foissner (1991, 2014). Counts and measurements on silver-impregnated specimens were conducted at a magnification of 1000×. In vivo measurements were performed at magnifications of 40–1000×. Illustrations of live specimens were based on free-hand sketches and micrographs while those of impregnated cells were made with a drawing device. We did not sequence the species because this was not very common when it was rediscovered in 1998. Classification is according to Berger (1999) and Eigner (1997, 1999). Terminology is according to Berger (1999), Foissner and Al-Rasheid (2006), and Wallengren (1900).

Results

Stylonychia Ehrenberg, 1830

Improved diagnosis (includes original data): middle- to large-sized, rigid oxytrichids with two separate macronuclear nodules or a moniliform strand. On average 18 fronto-ventral-transverse cirri. One right and one left marginal cirral row. Undulating membranes in *Stylonychia* pattern. Six dorsal kineties with kinety 4 originating from kinety 3; caudal cirri narrowly or widely spaced and of same length as marginal cirri or distinctly longer. Proter anlage II originates from oral primordium or de novo; anlagen V and VI originate from or close to postoral cirrus V/4. Anlagen IV–VI of the opisthe originate from or close to cirrus V/4. Cirri IV/3 and V/4 not or partially involved in anlagen formation.

Type species: *Stylonychia mytilus* (Mueller, 1773) Ehrenberg, 1830.

Stylonychia (*Stylonychia*)

Diagnosis: macronuclear nodules separate. Cirrus IV/3 and V/4 partially involved in anlagen formation. Proter anlage II develops from oral primordium.

Species included: *Stylonychia* (*Stylonychia*) *mytilus* (type of genus), *S.* (*S.*) *lemnae*, *S.* (*S.*) *ammermanni*, *S.* (*S.*) *curvata*, *S.* (*S.*) *gibbera*, *S.* (*S.*) *notophora*, *S.* (*S.*) *notophorides*, *S.* (*S.*) *pseudograndis*, *S.* (*S.*) *putrina*, *S.* (*S.*) *pusilla*, *S.* (*S.*) *stylomuscorum* (author names and dates, see Berger 1999; Foissner 2016). Some of these very likely belong to the genus *Tetmemena* Eigner, 1999.

Stylonychia (*Metastylonychia*) nov. subgen.

Diagnosis: macronuclear nodules form a moniliform strand. All cirri remain intact during anlagen formation. Proter anlage II possibly originates de novo.

Type species: *Stylonychia nodulinucleata* Shi and Li, 1993.

Species included: *Stylonychia* (*Metastylonychia*) *nodulinucleata* Shi and Li, 1993.

Etymology: composite of the Greek prefix *meta* (associated with, next to, substituted for) and the genus-group name *Stylonychia*, referring to the similarity with *Stylonychia*. Feminine gender.

Description of an Australian *Stylonychia* (*Metastylonychia*) *nodulinucleata* Shi and Li, 1993. (Figs 1A–J, 2A–K, 3A–G, 4A–C, 5A–D, 6A–D; Table 1)

Improved diagnosis (averages are given): size in vivo about 230 × 105 μm; elongate obovate. Macronuclear strand composed of seven nodules; four micronuclei. Cirrus V/4 slightly anterior or at same level as cirrus IV/2; transverse cirri in two groups of three and two. Left marginal row composed of 26 cirri, right of 40. Adoral zone extends about 56% of body length, composed of 59 membranelles. Six dorsal kineties; kineties 1–3 distinctly curved towards right body margin anteriorly; caudal cirri widely spaced, about half of body length, right caudal cirrus optically upon sixth-last cirrus of right marginal row.

Material deposited: eight voucher slides with protargol-impregnated specimens have been deposited in the Biologiezentrum of the Oberösterreichische Landesmuseum in Linz (LI), reg. no. 2015/855–862. Relevant specimens have been marked with black ink circles on the coverslip.

Description: most important features of *S.* (*Metastylonychia*) *nodulinucleata* have an ordinary variability (CV ≤ 15%; Table 1), except for the number of macronuclear nodules (CV = 18.4%), the distance between marginal cirral rows and body end (CV = 23.2%), the location of caudal cirri 1 and 2 (CV > 37%), and the gap between caudal cirri 1 and 2 (CV = 23.4%).

Size in ordinary cultures 210–250 × 90–115 μm, usually about 230 × 105 μm in vivo (Table 1); about 340 × 200 μm in exponentially growing cultures used for studying the ontogenesis (Table 1). Body moderately to elongate obovate and slightly to distinctly curved, widest in mid of adoral zone of membranelles; dorsoventrally flattened 2–3:1, anterior and posterior quarter leaf-like and thus hyaline, dorsal central quarters more or less convex, depending on nutrition state, and thus dark and opaque (Figs 1A–C, G, 2A–E, I, J, 3A–D; Table 1). Nuclear apparatus in central quarters of cell, in or slightly left of body's midline; macronucleus about 118 μm long in protargol preparations; on average composed of seven nodules forming a straight or sigmoid moniliform strand with

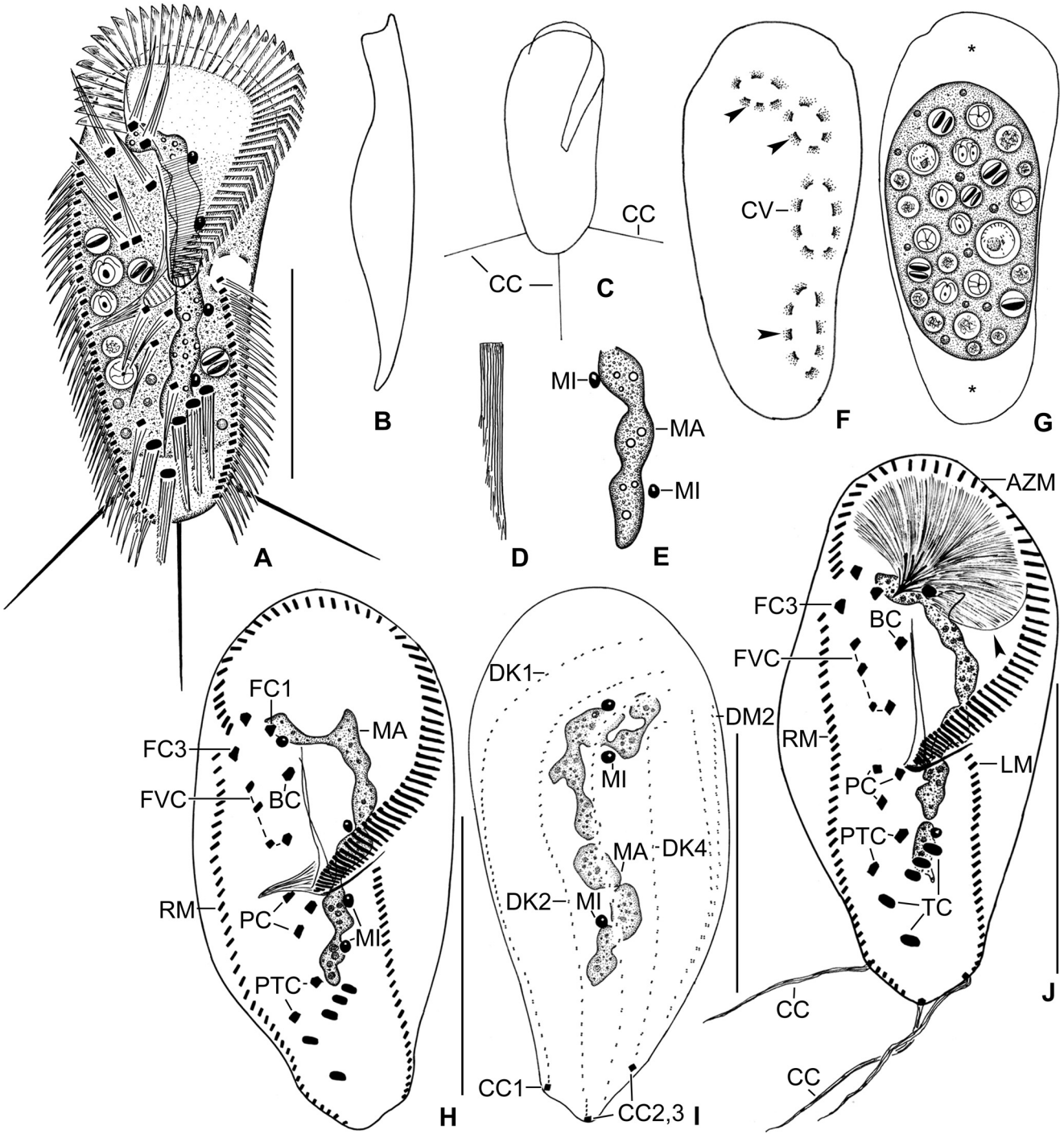

Fig. 1. *Stylonychia* (*Metastylonychia*) *nodulinucleata* from life (A–G) and after protargol impregnation (H–J). **A:** ventral view of a representative specimen, length 230 μm. **B:** lateral view. **C:** a specimen with almost rectangularly spread caudal cirri. **D:** optical section, showing the fringed portion of a transverse cirrus. **E:** optical section, showing part of the moniliform macronuclear strand with ring-shaped nucleoli (?). **F:** arrowheads mark some adventive bubbles anterior and posterior of the contractile vacuole. **G:** the central quarters are studded with food vacuoles and lipid droplets while the hyaline anterior and posterior quarters are marked by asterisks. **H–J:** ventral (H, J) and dorsal (I) view of main voucher specimens, showing the infraciliature and the nuclear apparatus. Arrowhead in (J) points to the stylonychid frontal area containing numerous fibres forming a fan-like pattern. Note the strongly curved dorsal kineties 1–3 (I). AZM – adoral zone of membranelles, BC – buccal cirrus, CC – caudal cirri, CV – contractile vacuole, DK1,2,4 – dorsal kineties, DM2 – dorsomarginal kinety, FC1, 3 – frontal cirri, FVC – frontoventral cirri, LM – left marginal cirral row, MA – macronuclear nodules, MI – micronuclei, PC – postoral cirri, PTC – pretransverse cirri, RM – right marginal cirral row, TC – transverse cirri. Scale bars 80 μm (I) and 100 μm (A, H, J).

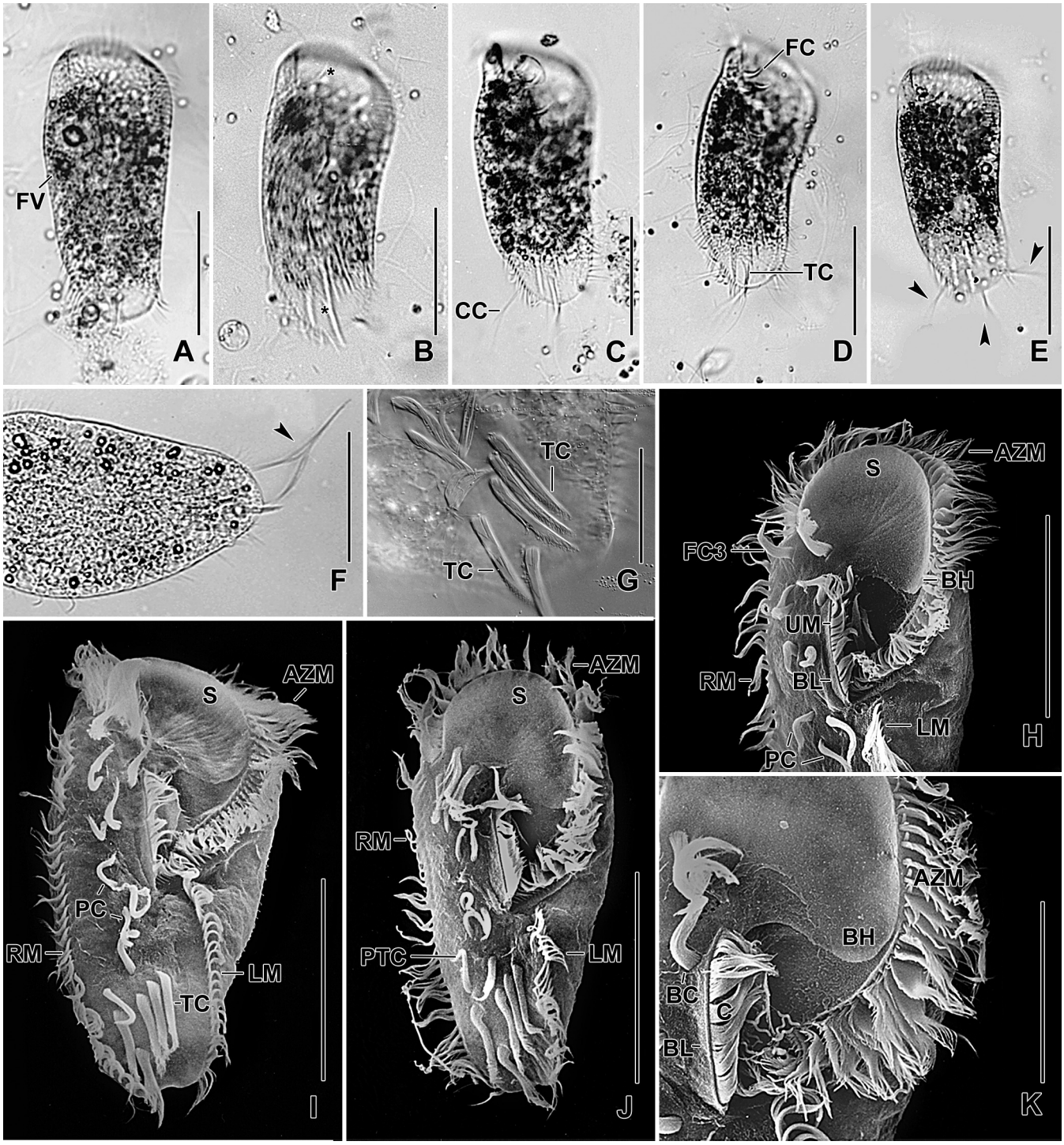

Fig. 2. *Stylonychia* (*Metastylonychia*) *nodulinucleata* from life (A–G) and in the scanning electron microscope (H–K). **A–E:** body shape is quite similar to that of *S. mytilus*. Asterisks in (B) mark the hyaline anterior and posterior quarter. Arrowheads in (E) point to the cone produced by the vibrating caudal cirri. **F:** the strongly vibrating caudal cirri (arrowhead) appears split in this micrograph. **G:** the posterior right margin of the transverse cirri is fringed, the two rightmost project slightly from body proper. **I, J:** ventral view of ordinary specimens, showing the ciliature, the large convex frontal area, and the dorsally curved scutum. **H, K:** oral area, showing the prominent buccal horn and the narrow buccal lip with a distal cleft from which the paroral cilia emerge. AZM – adoral zone of membranelles, BC – buccal cirrus, BH – buccal horn, BL – buccal lip, C – paroral cilia, FC – frontal cirrus, FV – food vacuole, LM – left marginal cirral row, PC – postoral cirri, PTC – pretransverse cirri, RM – right marginal cirral row, S – scutum, TC – transverse cirri, UM – undulating membranes. Scale bars 40 μm (F, K), 50 μm (G), 80 μm (H–J), and 100 μm (A–E).

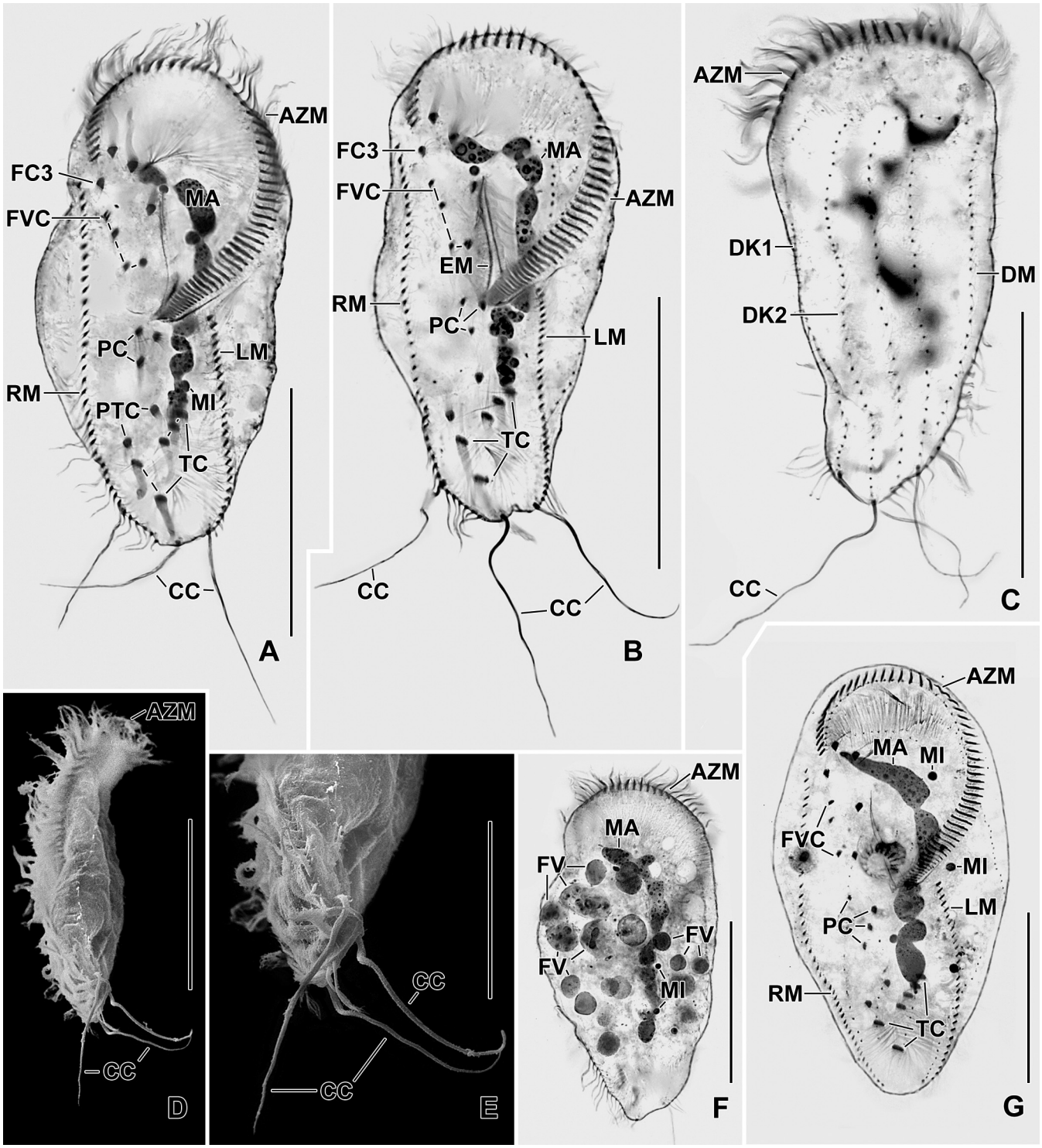

Fig. 3. *Stylonychia* (*Metastylonychia*) *nodulinucleata* after protargol impregnation (A–C, F, G) and in the scanning electron microscope (D, E). **A–C:** ventral (A, B) and dorsal (C) views of ordinary specimens, showing infraciliature and nuclear apparatus. Note the enormous caudal cirri, the fan-like spread fibres in the frontal area (A), the macronuclear strand, and dorsal kineties 1–4 distinctly curved towards right body margin anteriorly. **D, E:** lateral views, showing the long caudal cirri. **F:** a specimen studded with food vacuoles containing ciliates (*Tetrahymena mobilis*, *Halteria grandinella*) and heterotrophic flagellates. **G:** a specimen with increased number of postoral and transverse cirri. AZM – adoral zone of membranelles, CC – caudal cirri, DK1,2 – dorsal kineties, DM – dorsomarginal kinety, EM – endoral membrane, FC3 – frontal cirrus 3, FV – food vacuoles, FVC – frontoventral cirri, LM – left marginal cirral row, MA – macronuclear nodules, MI – micronuclei, PC – postoral cirri, PTC – pretransverse cirri, RM – right marginal cirral row, TC – transverse cirri. Scale bars 40 μm (E), 80 μm (C, D), and 100 μm (A, B, F, G).

Table 1. Morphometric data on *Stylonychia* (*Metastylonychia*) *nodulinucleata*.

Characteristics[a]	Mean	M	SD	SE	CV	Min	Max	n
Body, length (in vivo, ordinary culture)	230.0	230.0	14.1	4.3	6.1	210.0	250.0	11
Body, width (in vivo, ordinary culture)	105.5	105.0	7.6	2.3	7.2	90.0	115.0	11
Body, length (non-flooded Petri dish culture)	186.0	187.0	14.2	2.8	7.6	160.0	210.0	25
Body, width (non-flooded Petri dish culture)	90.3	91.0	9.2	1.8	10.2	72.0	110.0	25
Body, length (culture in exponential growth phase)	345.6	335.0	41.3	9.0	12.0	285.0	432.0	21
Body, width (culture in exponential growth phase)	194.0	185.0	29.4	6.4	15.2	145.0	245.0	21
Body length:width, ratio (non-flooded Petri dish culture)	2.1	2.0	0.1	0.0	6.0	1.9	2.4	25
Body width:length, percentage	48.8	49.4	2.6	0.5	5.4	42.4	52.4	25
Anterior body end to proximal end of adoral zone, distance	102.9	101	7.2	1.4	7.0	88.0	117.0	25
Anterior body end to distal end of adoral zone, distance	38.4	38.0	6.7	1.3	17.4	27.0	52.0	25
Body length:AZM length, ratio	1.8	1.8	0.1	0.0	4.2	1.7	2.0	21
Anterior body end to proximal end of adoral zone, % of body length	55.8	55.9	2.2	0.4	4.0	50.5	60.1	25
Anterior body end to distal end of adoral zone, % of body length	20.8	20.8	3.2	0.6	15.6	12.9	27.3	25
DE-value (see text)	0.4	0.4	0.0	0.0	13.2	0.3	0.5	25
Adoral membranelles, number	59.4	59.0	4.1	0.9	6.8	52.0	70.0	21
Adoral membranelles, width of longest base	11.6	12.0	0.7	0.1	5.8	10.0	13.0	21
Gap between AZM and paroral membrane (width of buccal cavity)	36.7	36.0	3.0	0.7	8.2	32.0	43.0	21
Anterior body end to paroral membrane, distance	51.8	52.0	4.4	1.0	8.6	43.0	58.0	21
Paroral cilia, length	13.8	14.0	2.0	0.5	14.6	10.0	17.0	17
Paroral membrane, length	46.5	46.0	3.5	0.8	7.5	40.0	53.0	19
Anterior body end to endoral membrane, distance	55.8	56.0	5.0	1.1	8.9	47.0	63.0	21
Endoral membrane, length	45.0	45.0	3.9	0.9	8.6	37.0	52.0	19
Anterior body end to anterior macronuclear nodule, distance	40.7	41.0	4.1	0.9	10.1	30.0	48.0	21
Posterior body end to posteriormost macronuclear nodule, distance	43.7	43.0	5.4	1.2	12.3	34.0	53.0	21
Macronuclear figure, length	101.6	102.0	11.2	2.5	11.1	80.0	122.0	21
Macronuclear strand, length (~stretched)	117.8	120.0	17.9	3.9	15.2	80.0	145.0	21
Anteriormost macronuclear nodule, length	16.3	16.0	3.4	0.8	21.0	9.0	22.0	19
Anteriormost macronuclear nodule, width	10.0	10.0	1.8	0.4	18.0	7.0	13.0	19
Macronuclear nodules, number	6.7	6.0	1.2	0.3	18.4	5.0	10.0	19
Anterior body end to anteriormost micronucleus, distance	50.3	50.0	11.9	2.6	23.8	32.0	92.0	21
Anteriormost micronucleus, length	4.3	4.5	0.4	0.1	9.9	3.5	5.0	21
Anteriormost micronucleus, width	3.8	4.0	–	–	–	3.5	4.0	21
Micronuclei, number	3.8	4.0	0.8	0.2	20.4	3.0	6.0	21
Anterior body end to right marginal row, distance	48.2	48.0	7.3	1.6	15.2	37.0	63.0	21
Posterior body end to right marginal row, distance	3.7	4.0	1.2	0.3	33.2	2.0	6.0	19
Right marginal row, number of cirri	40.1	40.0	3.0	0.7	7.5	35.0	46.0	21
Anterior body end to left marginal row, distance	99.2	100.0	7.1	1.6	7.2	85.0	112.0	21
Posterior body end to left marginal row, distance	8.4	8.0	2.0	0.4	23.2	5.0	13.0	19
Left marginal row, number of cirri	26.4	26.0	1.6	0.3	5.9	24.0	31.0	21
Gap between last cirrus of marginal rows	22.8	22.0	3.1	0.7	13.7	18.0	29.0	21
Frontal cirri, number	3.0	3.0	0.0	0.0	0.0	3.0	3.0	21
Anterior body end to buccal cirrus, distance	58.0	58.0	6.0	1.3	10.3	48.0	67.0	21
Buccal cirrus, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	21
Anterior body end to posteriormost frontoventral cirrus, distance	83.0	83.0	7.1	1.5	8.5	72.0	97.0	21
Frontoventral cirri, number	4.0	4.0	0.0	0.0	0.0	4.0	4.0	21
Anterior body end to posteriormost postoral cirrus, distance	115.9	117.0	9.6	2.1	8.3	98.0	135.0	21
Postoral cirri, number	3.0	3.0	0.0	0.0	0.0	3.0	3.0	21
Posterior body end to anterior pretransverse cirrus, distance	51.7	52.0	3.0	0.6	5.7	47.0	59.0	21
Posterior body end to rear pretransverse cirrus, distance	41.6	42.0	3.9	0.8	9.3	35.0	49.0	21
Pretransverse cirri, number	2.0	2.0	0.0	0.0	0.0	2.0	2.0	21
Posterior body end to rear transverse cirrus, distance	18.2	18.0	2.0	0.5	10.9	15.0	22.0	19
Transverse cirri, number	5.0	5.0	0.0	0.0	0.0	5.0	5.0	21
Dorsal kineties, number	6.0	6.0	0.0	0.0	0.0	6.0	6.0	21
Dorsal kinety 1, number of bristles	57.9	58.0	5.6	1.6	9.7	48.0	68.0	13
Dorsal kinety 2, number of bristles	52.2	53.0	2.2	0.6	4.1	48.0	55.0	13
Dorsal kinety 3, number of bristles[b]	39.1	40.0	3.5	0.9	9.1	32.0	45.0	17
Dorsal kinety 4, number of bristles[b]	31.5	31.0	2.6	0.7	8.1	27.0	36.0	15

Table 1 (*Continued*)

Characteristics[a]	Mean	M	SD	SE	CV	Min	Max	n
Dorsomarginal row 1, number of bristles[b]	38.7	40.0	4.2	1.0	10.8	27.0	43.0	17
Dorsomarginal row 2, number of bristles[b]	24.7	25.0	2.2	0.5	8.7	20.0	28.0	16
Posterior body end to caudal cirrus 1, distance (for number see Fig. 1I)	8.1	9.0	3.0	0.7	37.1	4.0	13.0	17
Posterior body end to caudal cirrus 2, distance (for number see Fig. 1I)	1.4	1.0	0.6	0.2	45.2	1.0	3.0	15
Posterior body end to caudal cirrus 3, distance (for number see Fig. 1I)	16.4	17.0	2.0	0.5	12.2	13.0	20.0	17
Gap between caudal cirrus 1 and 2 (for number see Fig. 1I)	14.0	13.5	3.3	0.8	23.4	10.0	20.0	16
Gap between caudal cirrus 2 and 3 (for number see Fig. 1I)	22.8	22.5	2.8	0.7	12.2	18.0	28.0	16
Marginal cirri posterior of caudal cirrus 3, number (for number see Fig. 1I)	6.4	6.0	0.9	0.2	13.3	5.0	8.0	18
Caudal cirri, number	2.9	3.0	–	–	–	2.0	3.0	19

[a] Data based, if not mentioned otherwise, on mounted, protargol-impregnated, and randomly selected specimens from a non-flooded Petri dish culture. Measurements in μm. CV – coefficient of variation in %, M – median, Max – maximum, Mean – arithmetic mean, Min – minimum, n – number of individuals investigated, SD – standard deviation, SE – standard error of arithmetic mean.

[b] Data obtained from vegetative cells and late dividers (opisthe).

anterior nodule usually directed to right body margin; individual nodules globular to ellipsoid, contain many granular and ring-shaped structures (nucleoli?) 0.5 μm and 2–5 μm across in vivo, respectively (Figs 1A, E, H–J, 3A, B, F, G, 4A; Table 1). On average four globular to ellipsoid micronuclei near to or attached to various sites of macronuclear strand, about 5–6 μm across in vivo (Figs 1A, E, H–J, 3A, B, F, G, 4C; Table 1). A typical contractile vacuole in mid-body close to left body margin, anterior and posterior of vacuole some adventive bubbles (Fig. 1A, F). Cortex rigid, stiff like a plank when swimming and not disturbed by a coverslip; specific cortical granules absent. Cells hyaline and slightly vacuolated in specimens from the non-flooded Petri dish culture, while dark in raw cultures because studded with food vacuoles up to 40 μm across and containing heterotrophic flagellates (*Polytoma* sp.), starch grains (which later transform into lipid droplets), small ciliates (*Tetrahymena mobilis*, *Halteria* sp.), and bacteria (Figs 1A, G, 3F, 4A, C). When feeding slightly tilted, cirri immobile and spread obliquely, frontal part of adoral zone of membranelles curved dorsally; almost immobile on surface and bottom of cultures where they can stand for a while swirling food into the mouth. Prefers living in upper water layers indicating a periphytic lifestyle. Swims moderately fast in wide spirals.

Cirral pattern as typical for genus (Berger 1999), i.e., usually 18 fronto-ventral-transverse cirri; number increased in four out of 30 specimens (Figs 1A, H, J, 2I, J, 3A, B, G; Table 1). Three enlarged frontal cirri about 45 μm long in vivo, right cirrus posterior of distalmost adoral membranelle. Invariably one slightly thickened buccal cirrus about 6 μm posterior of distal end of paroral membrane in protargol preparations (Figs 1A, H, J, 2H–K, 3A, B, G; Table 1). Four frontoventral cirri arranged in L-shaped pattern; last two cirri usually anterior of proximal end of undulating membranes. Three, very rarely four postoral cirri (Figs 1A, H, J, 2H–J, 3A, B, G; Table 1). Two slightly obliquely arranged pretransverse cirri, anterior cirrus on average 52 μm distant from posterior body end. Usually five transverse cirri in two groups of three and two, distinctly subterminal and thus only the two posteriormost cirri project slightly from body proper, about 45 μm long in vivo, posterior right side fringed (Figs 1A, D, H, J, 2G, I, J, 3A, B, G; Table 1). Marginal cirri fine and short (23 μm long in vivo) when compared to body size, arranged in two non-confluent rows extending to near posterior body margin; left row on average composed of 26 cirri, right of 40 (Figs 1A, H, J, 2I, J, 3A, B, G; Table 1).

Six dorsal kineties with bristles 3–4 μm long in protargol preparations (Figs Fig. 1I, 3C, 4A–C; Table 1): kineties 1–3 strongly curved towards right body margin anteriorly, curved portion often disconnected (7 out of 20 specimens analysed) indicating parental retention (Fig. 4A, B); kinety 4 slightly curved and distinctly shortened anteriorly; rows 5 and 6 slightly to distinctly shortened anteriorly and posteriorly. Three widely spaced caudal cirri about 80 μm long in vivo, the lateral ones can be rectangularly spread, distinctly separate from marginal rows, caudal cirrus 3 about 16 μm distant from posterior body end and optically at level of sixth-last cirrus of right marginal row; individual cirri about 3 μm thick at base while very thin and motile in distal half, forming a vibrating cone which appears as split caudal cirrus (Figs 1A, C, I, J, 2E, F, 3A–E, 4A; Table 1).

Adoral zone very conspicuous because extending over 56% of body length, commences far subapically on right margin of cell (about 21% of body length, DE-value 0.4), on average composed of 59 ordinary membranelles with up to 25 μm long cilia, bases of largest membranelles on average 15 μm wide in vivo and 12 μm in protargol preparations; frontal scutum high and dorsally curved; buccal horn conspicuously projecting from large, cap-shaped frontal area, underlaid by numerous fine fibres commencing (originating from?) at adoral membranelles and meeting slightly left of frontal cirrus 1 thus forming a fan-shaped pattern (Figs 1A, H, J, 2H–K, 3A, B, G; Table 1). Buccal cavity wide, flat anteriorly and deepening posteriorly; buccal lip narrow and hyaline, about 5 μm wide in vivo, covers proximal membranelles, with distal cleft containing basal bodies of paroral cilia (Figs 1A, 2H–K; Table 1). Undulating membranes in or slightly right of body's midline, cilia about 20 μm long

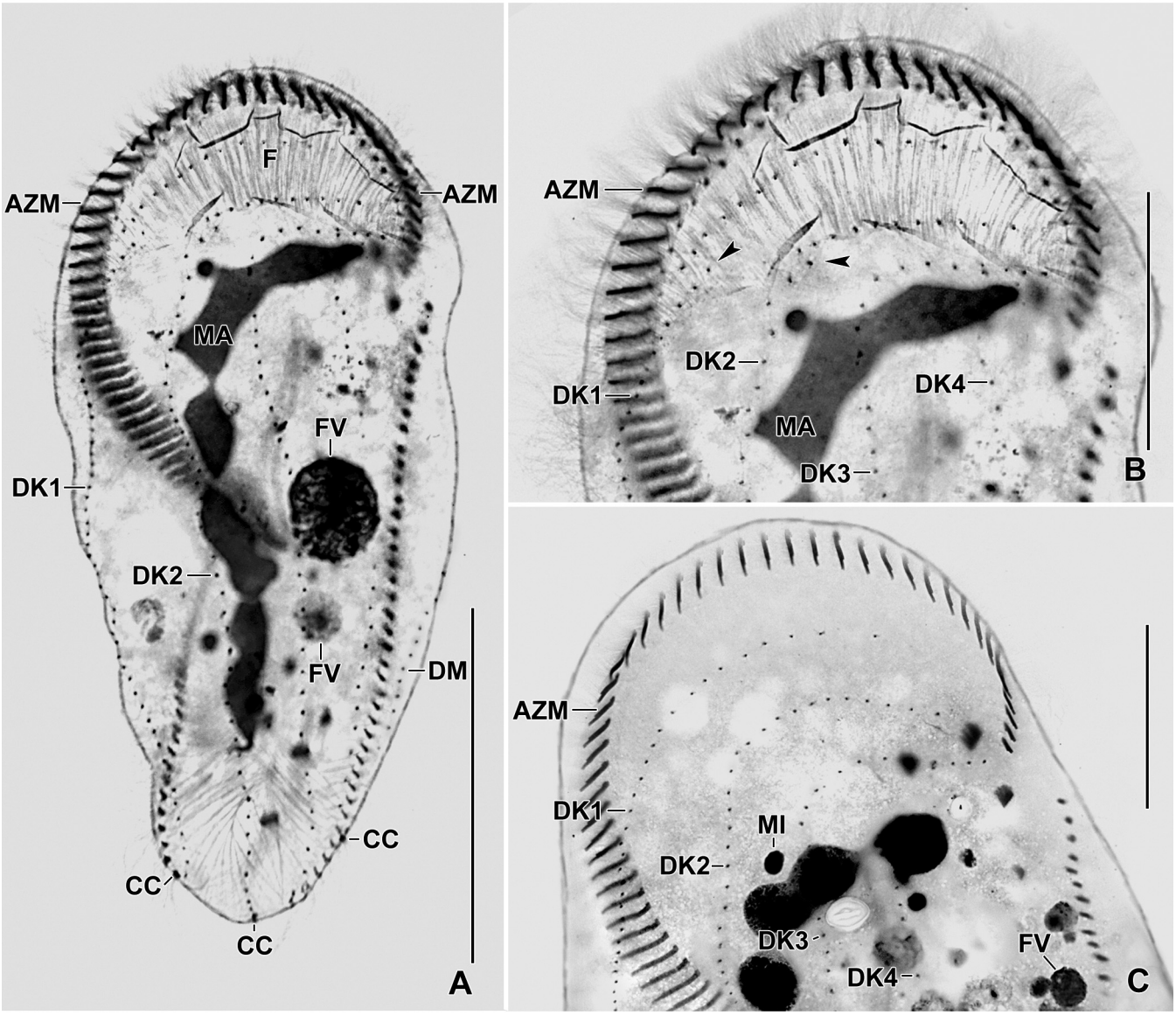

Fig. 4. *Stylonychia* (*Metastylonychia*) *nodulinucleata*, dorsal views after protargol impregnation, showing the infraciliature and the nuclear apparatus. Note the strongly curved dorsal kineties 1–3 and the curved anterior portion which is frequently disconnected (B, arrowheads). The adoral zone of membranelles is conspicuous and extends far proximally at the distal end. The caudal cirri are widely spaced with the right one optically upon the sixth-last right marginal cirrus (A). Note the fan-like spread fibres in the frontal field. AZM – adoral zone of membranelles, CC – caudal cirri, DK1,4 – dorsal kineties, DM – dorsomarginal kinety, F – fibres, FV – food vacuoles, MA – macronuclear nodules, MI – micronuclei. Scale bars 50 μm (B, C) and 100 μm (A).

in vivo and 14 μm in protargol preparations, membranes side by side or optically one upon the other, straight or slightly curved. Paroral membrane commences about 52 μm posterior to anterior body end; endoral membrane commences about 4 μm posterior to paroral membrane and extends to buccal vertex (Figs 1A, H, J, 2H–K, 3A, B, G; Table 1). Pharyngeal fibres of ordinary shape and structure, extend transversely to right body margin (Fig. 1A, H).

Notes on ontogenesis: the ontogenesis is similar to that of *Stylonychia mytilus* (for a review, see Berger 1999) except that no parental cirri are involved in primordia formation and, therefore, mentioned only briefly. The oral primordium originates close to and left of the two uppermost transverse cirri (Fig. 5A–D). Basal body proliferation for anlagen formation occurs close to cirri III/2, IV/3, and V/4; proter anlage 2 possibly develops de novo (Figs 5A–D, 6A–D). Six anlagen streaks are formed each in proter and opisthe and all parental cirri are resorbed in late and very late dividers (Figs 5A–D, 6A–D). The dorsal ontogenesis is as in *Stylonychia mytilus*, i.e., shows simple fragmentation of kinety 3 and invariably two dorsomarginal kineties (Fig. 6B, D). Possibly, the curved anterior portion of kineties 1–3 is retained.

Occurrence and ecology: as yet found in China (Shi and Li 1993) and Australia, i.e., in marsh water of Morshan

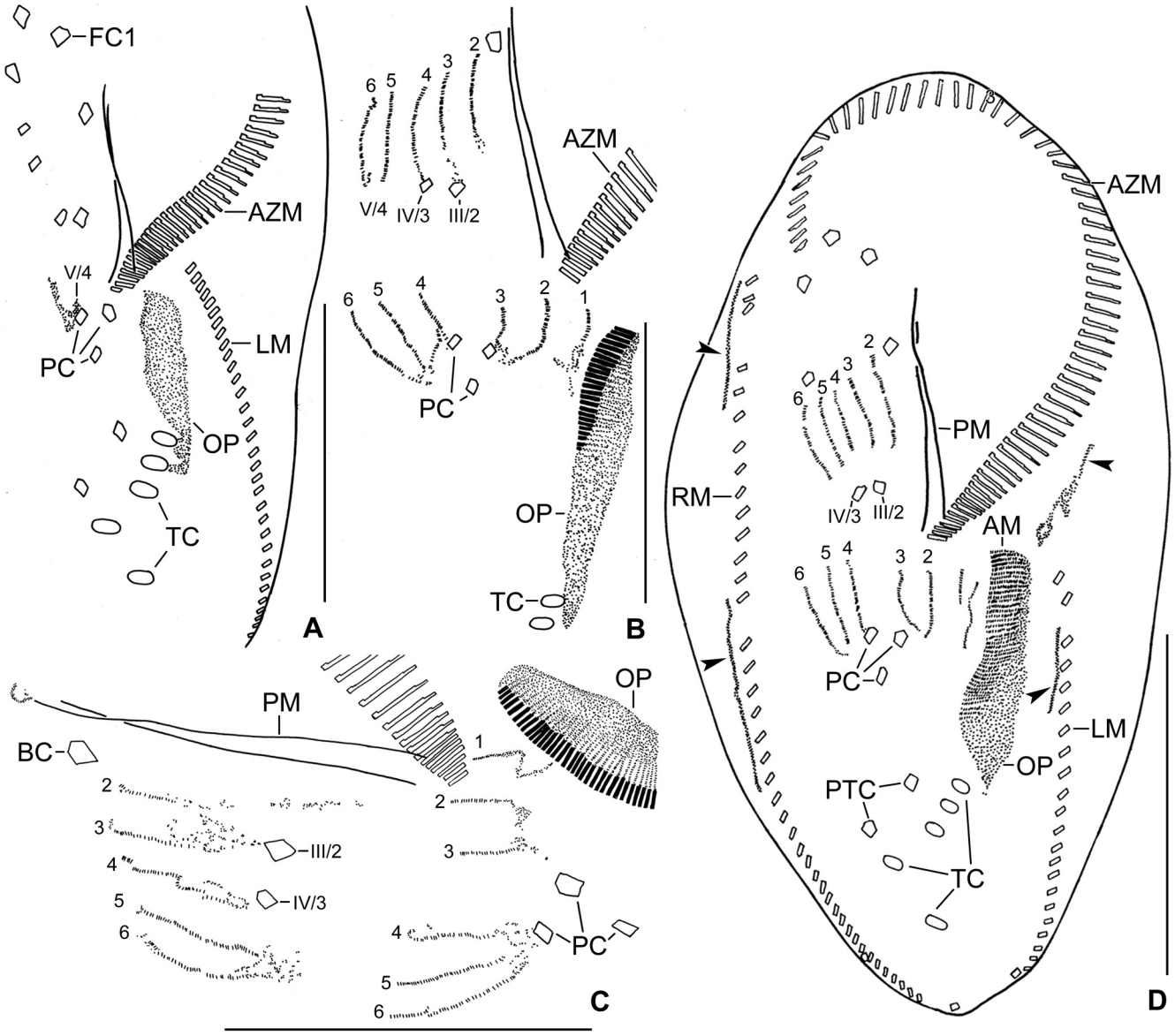

Fig. 5. *Stylonychia* (*Metastylonychia*) *nodulinucleata*, ventral view of early dividers after protargol impregnation; parental structures shown by contour, newly formed structures shaded black. **A:** early divider, showing the formation of the oral primordium close to the two uppermost transverse cirri, and a proliferation of basal bodies close to postoral cirrus V/4. **B:** the oral primordium and the proliferation of basal bodies close to the postoral cirri generate cirral anlagen for the opisthe. The buccal cirrus and the parental frontoventral cirri III/2 and IV/3 remain intact during division but are possibly involved in basal body proliferation. **C:** the oral primordium generates anlagen I–III for the opisthe and, possibly, anlage II of the proter. Anlagen IV–VI of the opisthe originate by proliferation of basal bodies close to cirrus V/4. Anlagen III and IV of the proter originate by proliferation of basal bodies close to cirrus III/2 and IV/3. The parental undulating membranes are reorganized anteriorly. **D:** six cirral anlagen are produced in each daughter cell, parental cirri are not involved in anlagen formation; arrowheads point to anlagen for marginal cirri. AM – adoral membranelles, AZM – adoral zone of membranelles, BC – buccal cirrus, FC1 – frontal cirrus 1, LM – left marginal cirral row, OP – oral primordium, PC – postoral cirri, PM – paroral membrane, PTC – pretransverse cirri, RM – right marginal cirral row, TC – transverse cirri. Roman numerals denote parental cirri, Latin numerals denote newly formed anlagen. Scale bars 100 μm (A–C) and 150 μm (D).

town, Heilongjiang Province and in almost black soil, pH 5.2, with many fine roots, from the floodplain of the Murray River near to the town of Albury, waterside of Ryans road, 36°04′50″S 146°54′57″E. Omnivorous, i.e., feeds on heterotrophic flagellates (*Polytoma* sp.), starch grains, small ciliates (*Tetrahymena mobilis*, *Halteria* sp.), and bacteria.

Discussion

Justification of the new subgenus

Stylonychia (*Metastylonychia*) *nodulinucleata* shows unique features with respect to the nuclear apparatus and the

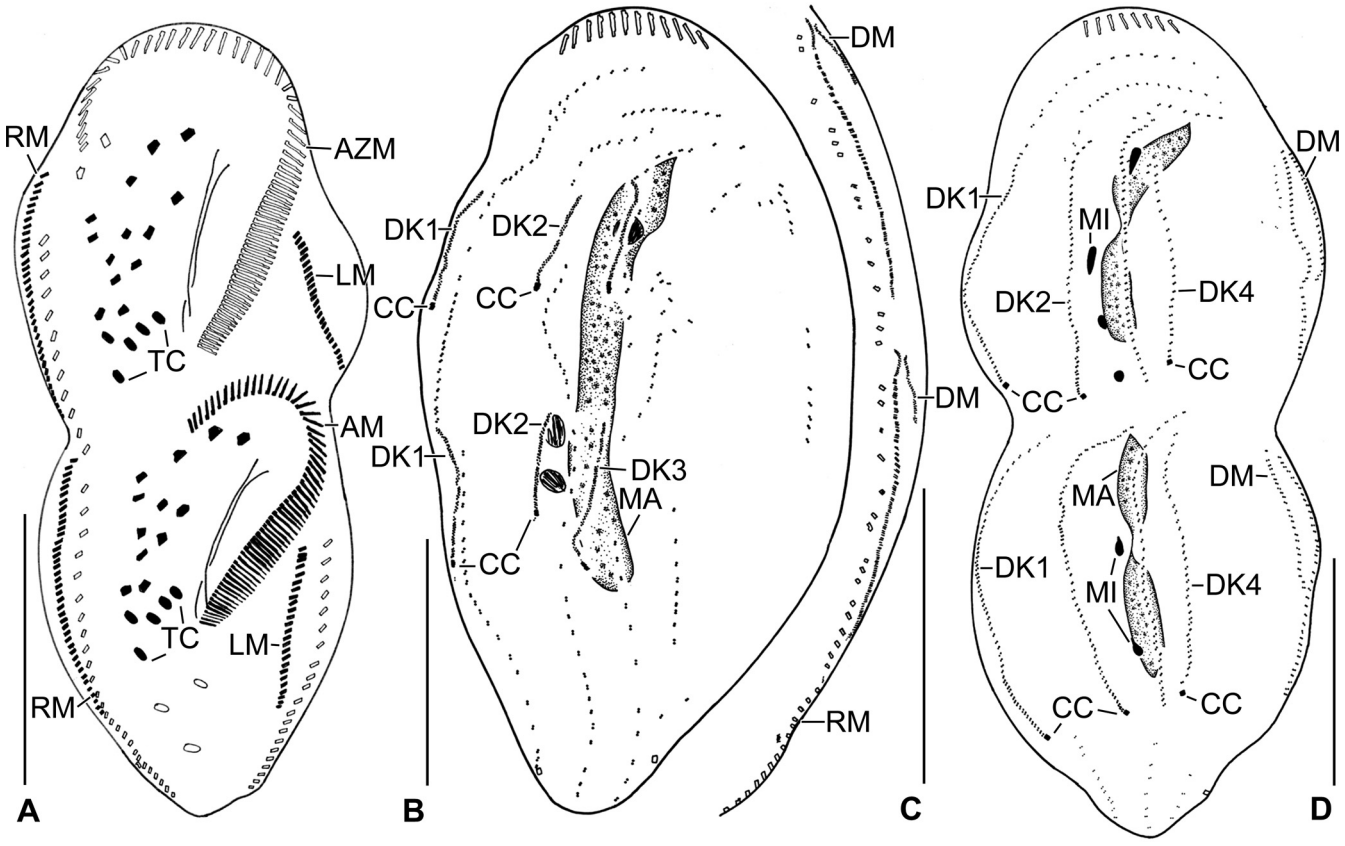

Fig. 6. *Stylonychia* (*Metastylonychia*) *nodulinucleata*, ventral view of middle and late dividers after protargol impregnation. Parental structures shown by contour, newly formed structures shaded black. **A:** the newly formed fronto-ventral-transverse cirri migrate to their specific sites. **B:** mid-divider, showing the formation of dorsal kineties. **C:** formation of dorsomarginal kineties close to the new right marginal cirral row. **D:** late divider, showing caudal cirrus 3 associated with kinety 4, indicating that kinety 4 originated by a split of kinety 3. The macronucleus completes the second division. AM – adoral membranelles, AZM – adoral zone of membranelles, CC – caudal cirri, DK1,4 – dorsal kineties, DM – dorsomarginal kineties, LM – left marginal cirral row, MA – macronucleus, MI – micronuclei, RM – right marginal cirral row, TC – transverse cirri. Scale bars 100 μm (B, C) and 150 μm (A, D).

ontogenesis. The moniliform macronuclear strand does not fit into the generic diagnosis of *Stylonychia*, as already mentioned by Shi and Li (1993). However, they did not classify *S.* (*M.*) *nodulinucleata* as a new genus because this feature occurs in other oxytrichid genera as well, e.g., *Oxytricha* and *Coniculostomum* (Berger 1999). However, ontogenesis shows also a peculiarity in that the 18 fronto-ventral-transverse cirri remain intact during anlagen formation, a feature never reported in any oxytrichid ciliate and very likely overlooked by Shi and Li (1993). This and the moniliform macronucleus justify a separation at subgenus level, at least.

Stylonychia nodulinucleata as described by Shi and Li, 1993 (Fig. 7A–C)

Shi and Li (1993) provided a rather brief description of *Stylonychia nodulinucleata* in Chinese language which few foreign people can read (Berger 1999). Thus, we asked Prof. Weibo Song for a translation; as concerns the photographs (Fig. 1: 1–6 in original description), we refer the reader to the original description while we include the line drawings (Fig. 7A–C; Fig. 1A–C in original description).

1. **Body shape and size.** Shape similar to *Stylonychia mytilus* and *S. lemnae* but anterior end less curved than in *S. mytilus* (Fig 1: 1). Size 270–310 × 95–110 μm, thickness 25–30 μm. Maximum body width at posterior third of adoral zone. One contractile vacuole posterior of adoral zone near left body margin (Fig 1: 1, CV). Compared with *S. mytilus* and *S. lemnae*, *S. nodulinucleata* has three very long caudal cirri exceeding 1/3 of body length (Fig 1: 1, C).
2. **Infraciliature.** Adoral zone shaped like a question mark, distal end distinctly extending posteriorly, i.e., DE value higher than in *S. mytilus* and *S. lemnae*. 70–75 adoral membranelles. Undulating membranes shorter than in *Stylonychia mytilus* and *S. lemnae*, i.e., about half length of adoral zone. One left and one right marginal row not confluent posteriorly; 23 or 24 left marginal cirri; 35 or 36 right marginal cirri. Eight frontal, five ventral and five transverse cirri; frontal cirri positioned more or less pos-

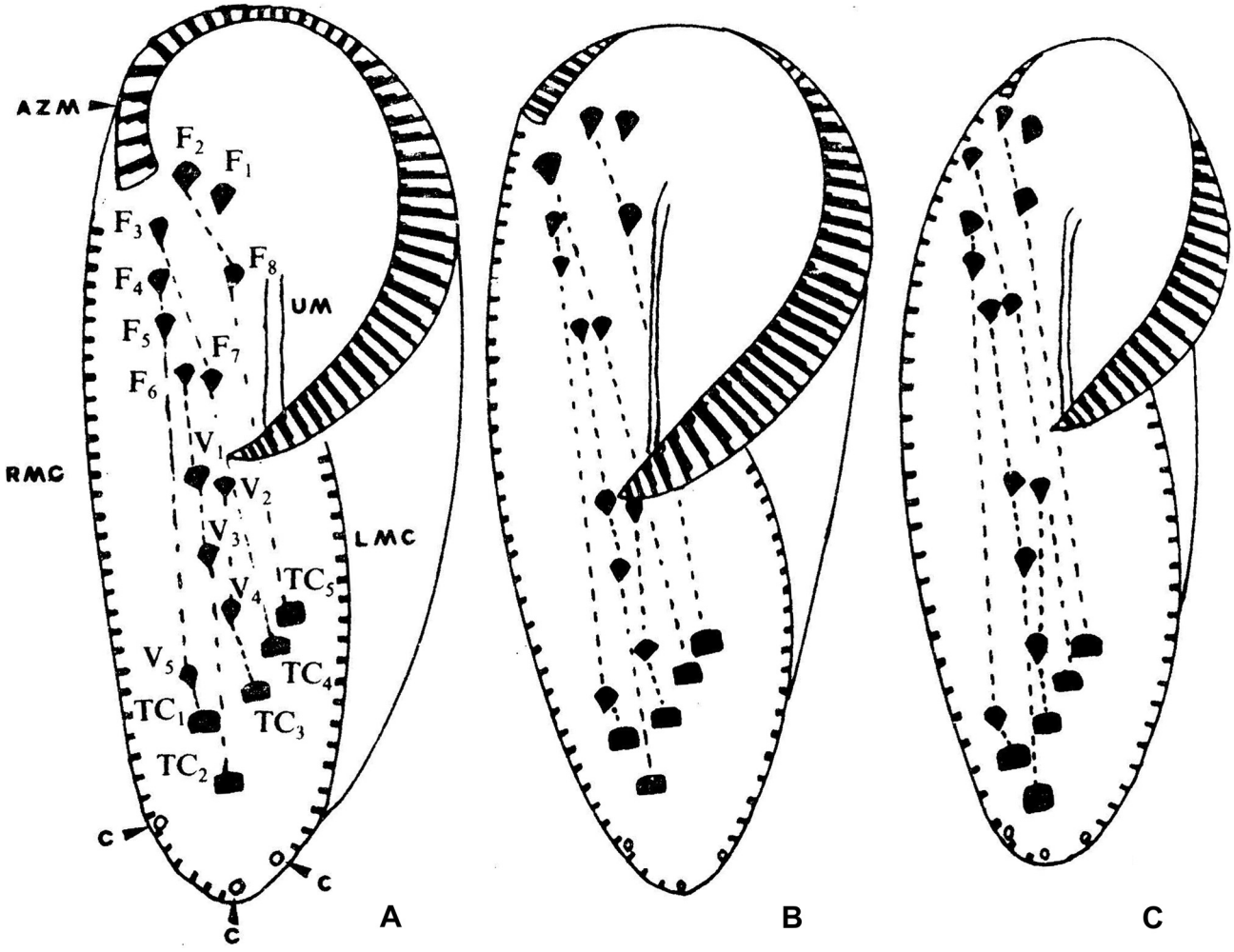

Fig. 7. *Stylonychia nodulinucleata* from Shi and Li (1993). The original English legend runs as follows: Morphological comparison of three species of *Stylonychia* with regard to the ventral cirral pattern, the adoral zone of membranelles, and the undulating membrane (drawn from protargol preparations). Translation of the original Chinese legend. **A:** *Stylonychia nodulinucleata*. AZM arrowhead depicts the adoral zone distally extending more posteriorly than in the congeners. Dotted lines indicate the origin of the fronto-ventral-transverse cirri. Arrowheads mark caudal cirri. F – frontal cirri, LMC – left marginal cirri, RMC – right marginal cirri, TC – transverse cirri, UM – undulating membranes, V – ventral cirri. **B:** *Stylonychia mytilus* (annotation omitted). **C:** *Stylonychia lemnae* (annotation omitted). The dotted lines show the cirri that originate from the same anlagen; however, it is incorrect (Berger 1999).

teriorly than in *S. mytilus* and *S. lemnae* (Fig. 7). Six dorsal kineties.

3. **Kinetodesmal and frontal fibres.** The pattern of the marginal kinetodesmal fibres is as in *Stylonychia mytilus* (Fig 1: 4, RMF). The kinetodesmal fibres of the frontal cirri are weaker than in *S. mytilus*. A most striking feature of *S. nodulinucleata* are the subpellicular frontal fibres which meet at a point left of frontal cirrus 1 (shown as F_1 in Fig. 7A), spreading fan-like (radial pattern) to the bases of the adoral membranelles (Fig 1: 4, FF).
4. **Nuclear apparatus.** Eight moniliform macronuclear nodules; 1–4 spherical micronuclei. Li and Shi (1993) reported amicronucleate cells.
5. **Ontogenesis and division of nuclear apparatus.** Stomatogenesis and development of the fronto-ventral-transverse cirri, marginal rows, and dorsal kineties as in *S. mytilus* (Shi and Frankel 1990). Macronuclear nodules divide amitotically; a replication band each appears at ends of the macronuclear strand during the S phase and moves towards the nuclear centre (Fig 1: 5, RB). Later, the replication bands fuse and the nucleus divides into two as cytokinesis proceeds. Micronuclei divide mitotically.

Based upon the observations, *Stylonychia nodulinucleata* should be classified as a new species. The type population has been kept in the Laboratory of Protozoa, Harbin Normal University.

Comparision with Chinese population

Obviously, the Australian specimens are quite similar to the Chinese ones, for instance, in the number of macronuclear

nodules (7 vs. 8), of left marginal cirri (24–31 vs. 23 or 24), and the number of adoral membranelles (52–70 vs. 70–75). However, Shi and Li (1993) possibly studied only two specimens, as indicated by the narrow range of, e.g., the number of marginal cirri. Shi and Li (1993) mentioned another peculiarity of *S. nodulinucleata*, viz., the short undulating membranes compared to *S. mytilus*. The same occurs in the Australian specimens: they extend ~20% of body length while ≥32% in *S. mytilus* as calculated from the morphometric data in Berger (1999).

In spite of these similarities, we cannot exclude that the Australian population represents a distinct species or subspecies because Shi and Li (1993) did not provide a morphometric analysis and detailed data on ontogenesis. Thus, the Chinese population should be redescribed.

Biogeographic considerations

With a size of up to 400 μm and a moniliform macronucleus, *Stylonychia* (*M.*) *nodulinucleata* is a flagship in the ciliate world (Foissner 2006). Thus, it is surprising that only two records are known, viz., Australia and China, i.e., from the transition zone of the Holarctic and the Australis. An unsubstantiated record is from a constructed mangrove wetland in China (Chen et al. 2009). This suggests a restricted distribution, especially because members of the *S. mytilus* group have been investigated intensively in central Europe and globally (Berger 1999).

Acknowledgements

Financial support was provided by the Austrian Science Fund (FWF Project P26325_B16). The technical assistance of Michael Gruber M.Sc., Robert Schörghofer, and Andreas Zankl is greatly acknowledged. We thank Dr. J. P. O'Donoghue (University of Adelaide, Australia) for help with sampling in Australia. We also thank Prof. Dr. Weibo Song and Mr. Xiaoteng Lu (Institute of Evolution and Marine Biodiversity, Laboratory of Protozoology, Ocean University of China) for translating the original description by Shi and Li (1993).

References

Berger, H., 1999. Monograph of the Oxytrichidae (Ciliophora, Hypotrichia). Monogr. Biol. 78, 1–1080.

Chen, Q.H., Tam, N.F., Shin, P.K.S., Cheung, S.G., Xu, R.L., 2009. Ciliate communities in a constructed mangrove wetland for wastewater treatment. Mar. Poll. Bull. 58, 711–719.

Eigner, P., 1997. Evolution of morphogenetic processes in the Orthoamphisiellidae n. fam., Oxytrichidae, and Parakahliellidae n. fam. and their depiction using a computer method (Ciliophora, Hypotrichida). J. Eukaryot. Microbiol. 44, 553–573.

Eigner, P., 1999. Comparison of divisional morphogenesis in four morphologically different clones of the genus *Gonostomum* and update of the natural hypotrich system (Ciliophora, Hypotrichida). Eur. J. Protistol. 35, 34–48.

Foissner, W., 1991. Basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa. Eur. J. Protistol. 27, 313–330.

Foissner, W., 2006. Biogeography and dispersal of microorganisms: a review emphasizing protists. Acta Protozool. 45, 111–136.

Foissner, W., 2014. An update of 'basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa'. Int. J. Syst. Evol. Microbiol. 64, 271–292.

Foissner, W., 2016. Terrestrial and semiterrestrial ciliates (Protozoa, Ciliophora) from Venezuela and Galápagos. Denisia 35, 1–912.

Foissner, W., Al-Rasheid, K., 2006. A unified organization of the stichotrichine oral apparatus, including a description of the buccal seal (Ciliophora, Spirotrichea). Acta Protozool. 45, 1–16.

Foissner, W., Agatha, S., Berger, H., 2002. Soil ciliates (Protozoa, Ciliophora) from Namibia (Southwest Africa), with emphasis on two contrasting environments, the Etosha Region and the Namib Desert. Denisia 5, 1–1459.

Gupta, R., Kamra, K., Arora, S., Sapra, G.R., 2001. *Stylonychia ammermanni* sp. n., a new oxytrichid (Ciliophora Hypotrichida) ciliate from the river Yamuna, Delhi, India. Acta Protozool. 40, 75–82.

Li, H., Shi, X., 1993. A preliminary study on amicronucleate individuals of *Stylonychia nodulinucleata*. Zool. Res. 14, 91–92 (in Chinese).

Shi, X., Frankel, J., 1990. Morphology and development of mirror-image doublets of *Stylonychia mytilus*. J. Protozool. 37, 1–13.

Shi, X., Li, H., 1993. Discovery of *Stylonychia nodulinucleata* sp. nov. (Ciliophora, Hypotrichida, Oxytrichidae) and comparison of its neighboring species. Zool. Res. 14, 10–14 (in Chinese with English summary).

Shi, X.B., Ammermann, D., 2004. *Stylonychia harbinensis* sp. n. a new oxytrichid ciliate (Ciliophora, Hypotrichia) from the Heilongjiang province, China. Protistology 3, 219–222.

Wallengren, H., 1900. Zur Kenntnis der vergleichenden Morphologie der hypotrichen Infusorien. Bih. K. svensk. VetenskAkad. Handl. 26, 1–31.