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Rigidothrix goiseri nov. gen., nov. spec. (Rigidotrichidae nov. fam.), a new "flagship" ciliate from the Niger floodplain breaks the flexibility-dogma in the classification of stichotrichine spirotrichs (Ciliophora, Spirotrichea)

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Abstract

Rigidothrix goiseri nov. gen., nov. spec. was discovered in soil from the Niger floodplain near to the town of Timbuktu, Republic of Mali. Its morphology, ontogenesis, and 18S rDNA gene sequence were studied with standard methods. *Rigidothrix goiseri* is very conspicuous in vivo because of its average size of 230 × 70 µm and a distinct tail. Further main characteristics include the rigid body; the conspicuous, stylonychid frontal area; the undulating membranes in *Oxytricha* pattern; a mighty adoral zone of membranelles not reorganized during ontogenesis; distinct midventral rows of which those of the opisthe develop right of the parental ones; and eight dorsal kineties, of which three develop intrakinetally and five are generated dorsomarginally. *Rigidothrix goiseri* possesses main characteristics of oxytrichine, stylonychine, and urostyline stichotrichs, specifically, it is the first rigid stichotrich with midventral rows, and thus it breaks the flexibility dogma. Distinct similarities with the genus *Uroleptus* and the CEUU hypothesis suggest that *R. goiseri* is more closely related to the oxytrichine than urostyline stichotrichs, in spite of the conspicuous midventral rows. This is also supported by the sequence data which show that *R. goiseri* is almost equally similar to both *Oxytricha granulifera* (95.88%) and *Uroleptus gallina* (94.93%), but fairly different from *Urostyla grandis* (92.7%). The main morphological peculiarities of *R. goiseri* show that it represents a new genus which should be classified into a new family, the Rigidotrichidae, together with the genera *Uroleptus*, *Territricha*, and *Afrophrya* nov. gen., the latter comprising *Rigidothrix*-like stichotrichs with cyrtohymenid oral apparatus. The conspicuous size and shape make *R. goiseri* a biogeographic flagship likely confined to Africa.

Keywords: *Afrophrya* nov. gen.; Biogeography; CEUU hypothesis; Subtropical Africa; *Rigidothrix goiseri* nov. gen., nov. spec.; Stichotrich ciliate

Introduction

Diversity of microorganisms is one of the hot issues in current ecology (for a review, see Foissner 2006). Within protist diversity, the ciliates occupy a special position in this discussion due to their comparatively complex

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morphology, which facilitates morphospecies recognition, and the very different conclusions reached by some ecologists and taxonomists about their diversity and distribution.

Finlay et al. (1996) and Finlay (2001) concluded that ciliate morphospecies are cosmopolitan and the main habitats of the Earth have been sufficiently studied for ciliate diversity. They underpinned their diversity estimation with ecological theory and concluded that there are about 3000 free-living ciliate morphospecies. In contrast, Foissner et al. (2002) and Foissner (2006) estimated global diversity of free-living ciliates to be near 30,000 species. They based the estimation on detailed investigations of certain ecosystems globally (Chao et al. 2006; Foissner 1997; Foissner et al. 2002, 2005). These studies invariably revealed hundreds of undescribed ciliate morphospecies, including several "flagships" with restricted geographic distribution.

One of the highly diverse, yet poorly investigated, major habitats of our planet is the floodplain of large rivers. A single soil sample usually provides about 100 ciliate species, of which 5–15% are undescribed. If several samples of the same area are investigated, species numbers increase to 200–400, and about one third of these are undescribed (Chao et al. 2006; Foissner 1997; Foissner et al. 2002, 2005; W. Foissner, unpubl. data). The ciliate reported in the present study is from soil of the Niger floodplain in the Republic of Mali, West Africa. The two samples obtained contained one new ciliate family, three new genera, and about 15 new species! *Rigidothrix goiseri* is not only a "flagship" showing the restricted distribution of certain ciliate species (Foissner 2006; Tyler 1996), but also a new organization type revealing our ignorance of ciliate diversity.

Material and methods, terminology

Rigidothrix goiseri was discovered in a soil sample from a small island in the Niger River near the town of Timbuktu, Republic of Mali, West Africa, W3°N16°30′. The island was used as a plantation for mangos and cashew-nuts. The brown soil was collected from the upper 5 cm and contained little litter. Thus, it was mixed with some pieces of sterilized wheat straw to increase organic matter content and growth of bacteria and fungi. The sample was collected on January 13, 2005 and air-dried for 3 weeks. On April 8, 2005, the sample was rewetted with distilled water to obtain a non-flooded Petri dish culture, as described in Foissner et al. (2002). On April 13th, several new species were recognized. Accordingly, the soil percolate was collected for preparations and replaced by Eau de Volvic (French Table water). A day later, on April 14th, *R. goiseri* was present in considerable numbers, obviously from resting cysts.

Field material as obtained with the non-flooded Petri dish method was used for all investigations because several attempts to obtain pure cultures failed; we were even not successful in obtaining resting cysts because the isolated specimens died, irrespective of whether they were kept in centrifuged soil percolate or fresh Eau de Volvic. Fortunately, *R. goiseri* grew well in the non-flooded Petri dish culture. Thus, several samples could be collected over a period of 5 weeks to obtain the preparations needed.

Living cells were studied using a high-power oil immersion objective and differential interference contrast. Protargol impregnation and scanning electron microscopy (SEM) were performed as described by Foissner (1991). Counts and measurements on silvered specimens were performed at a magnification of × 1000. In vivo measurements were conducted at magnifications of × 40–1000. Drawings of live specimens were based on free-hand sketches and micrographs; those of impregnated cells were made with a drawing device. In the drawings of ontogenetic stages, parental structures are shown by contour, while newly formed structures are shaded black.

For analysis of the 18S rDNA sequence, 10 individual cells were isolated with a micropipette from the non-flooded Petri dish culture and transferred into 180 µl ATL buffer (Qiagen) and 20 µl Proteinase K (20 mg/ml). Subsequently, genomic DNA was extracted using the protocol for cultured animal cells of the DNEasy Tissue kit (Qiagen) according to the manufacturer's instructions. We used standard isopropanol precipitation to concentrate the extracted nucleic acids. Amplification of the 18S rDNA fragment was performed via PCR using the universal eukaryotic primers EukA and EukB (Medlin et al. 1988), and cloning was performed as described by Stoeck and Epstein (2003). Three positively screened (M13 reamplification) plasmids were sequenced bidirectionally with MWG (Ebersheim, Germany).

We aligned the 18S rDNA sequence to available Oxytrichinae, Stylonychinae and Urostylidae sequences using Clustal X (Thompson et al. 1997). The alignments were manually refined in MacClade (Maddison and Maddison 2003) according to conserved regions. We applied the program Modeltest (Posada and Crandall 1998) to choose the model of DNA substitution that best fit our data sets from among 56 possible models. We calculated a maximum parsimony tree, an evolutionary distance tree, and a maximum likelihood tree, using the PAUP software package 4.0b10 PAUP (Swofford 2001), and a Bayesian inference tree using Mr. Bayes (Ronquist and Huelsenbeck 2003). The DNA substitution model as well as the parameter settings for the trees constructed are described in the legend to Fig. 33. We assessed the relative stability of tree topologies using 1000 bootstrap replicates and posterior probabilities of 2862 Bayesian trees. Heuristic searches

for bootstrap analyses employed stepwise addition, starting trees with simple addition of sequences and TBR branch-swapping. Bootstrap analysis settings were chosen according to the Modeltest output. Further, rooted and unrooted trees were calculated. All these methods provided trees similar to that shown in Fig. 33, that is, none assigned *Rigidothrix* to the Stylonychinae, as suggested by the morphological and ontogenetic data. All alignments and trees are available from the authors upon request.

Terminology is according to Corliss (1979) and Foissner and Al-Rasheid (2006). The term "midventral rows" is used as defined by Berger (2004), that is, it designates a longitudinal series of zigzaging cirri in two rows near to the ventral midline.

Results

Family Rigidotrichidae nov. fam.

Diagnosis: Rigid or flexible, oxytrichid (?) Stichotrichia with oxytrichid frontal ciliature and dorsomarginal kineties; midventral complex in *Uroleptus* pattern.

Type genus: *Rigidothrix* nov. gen.

Genera assignable: *Rigidothrix* nov. gen., *Afrophrya* nov. gen., *Uroleptus* Ehrenberg, 1831; *Territricha* Berger and Foissner, 1988.

Genus *Rigidothrix* nov. gen.

Diagnosis: Rigid Rigidotrichidae with many narrowly spaced pairs of midventral cirri orally and postorally; frontal area stylonychid, undulating membranes in *Oxytricha* pattern; and with caudal cirri.

Type species: *Rigidothrix goiseri* nov. spec.

Etymology: Composite of the Latin adjective *rigidus* (rigid) and the Greek noun *thrix* (hair ~ciliate), meaning a "rigid ciliate". Feminine gender.

Description of *Rigidothrix goiseri* nov. spec.

Diagnosis: Size about 230 × 70 μm in vivo; clavate, i.e., with distinct tail. Two macronucleus nodules connected by a fine thread; two micronuclei. Midventral rows slightly shortened apically and terminally, composed of an average of 17 cirral pairs, cirri of right row distinctly larger than those of left. On average 40 adoral membranelles, 4 frontal cirri, 1 buccal cirrus, 2 transverse cirri, 27 right and 25 left marginal cirri, 8 dorsal kineties, and 3 caudal cirri.

Type locality: Floodplain soil from the Niger River near to the town of Timbuktu, Republic of Mali, West Africa, W3°N16°30′.

Type material: One holotype slide and 9 paratype slides with protargol-impregnated morphostatic and dividing specimens have been deposited in the Biology Centre of the Museum of Upper Austria, Linz (LI). Relevant specimens are marked by black ink circles on the coverslip. The 18S rDNA sequence of *R. goiseri* is deposited in GenBank (accession number DQ490236).

Dedication: The epithet "goiseri" is to acknowledge "Hubert von Goisern" (vulgo Hubert Achleitner), an Austrian entertainer who collected the sample on the occasion of his concert in Mali.

Description: Size 180–260 × 50–80 μm, usually about 230 × 70 μm in vivo; length:width ratio near 3:1 both in vivo and in preparations (Table 1). Body shape and rigidity similar to those of *Stylonychia mytilus*, except for the highly characteristic tail. Oblong triangular, widest near obliquely truncated anterior end, gradually narrowing posteriorly with tail-like rear quarter frequently curved rightwards (Figs 1, 3, 8, 9, 14, 32 and 36). Dorsoventrally flattened about 2:1, ventral side flat, dorsal middle third distinctly convex, oblique anterior end curved dorsally by about 45°, frontal portion of adoral zone of membranelles thus hardly recognizable in cells viewed ventrally (Figs 9, 11 and 13). Shape details usually not preserved in protargol preparations, especially the oblique anterior truncation (Figs 3, 8 and 14). Nuclear apparatus usually in anterior body half, that is, in second quarter of the cell left of body's midline (Figs 1, 5, 14 and 32). Macronucleus nodules close together, average distance in between only 4 μm (Table 1), connected by a fine argyrophilic strand, anterior nodule slightly shorter than posterior; individual nodules broadly to elongate ellipsoidal, on average about 30 × 15 μm in vivo, studded with ordinary-sized nucleoli. Usually a micronucleus each attached to left side of macronucleus nodules, individual micronuclei broadly ellipsoidal to spherical, about 5 μm across (Figs 1, 14 and 32). Contractile vacuole distinctly above mid-body at left margin of cell, with lacunar collecting canals (Fig. 1). Cortex without specific granules, rigid and glossy as in *Stylonychia*, usually strongly wrinkled in cells prepared for scanning electron microscopy (Fig. 10). Cytoplasm colourless, granular, usually contains many food vacuoles up to 20 μm across and, mainly in tail region, some colourless and orange (from algal food) lipid droplets 1–4 μm across and up to 8 μm long crystals of usual shape. Feeds on bacteria, colourless (*Polytomella*) and green (*Chlorogonium*) flagellates as well as on various small ciliates, such as *Protocyclidium muscicola*, *Sathrophilus muscorum*, *Leptopharynx costatus*, and *Drepanomonas* sp. Glides and swims rapidly on microscope slides, never rests. When kept in small Petri dishes, specimens usually swim in the free water, suggesting that *R. goiseri* is a planktonic species.

Cirral pattern and number of cirri rather constant, that is, variability coefficients < 10% (Figs 1–15, Table 1). Cirri conspicuous because up to 40 μm long and comparatively thick in vivo, become thinner and

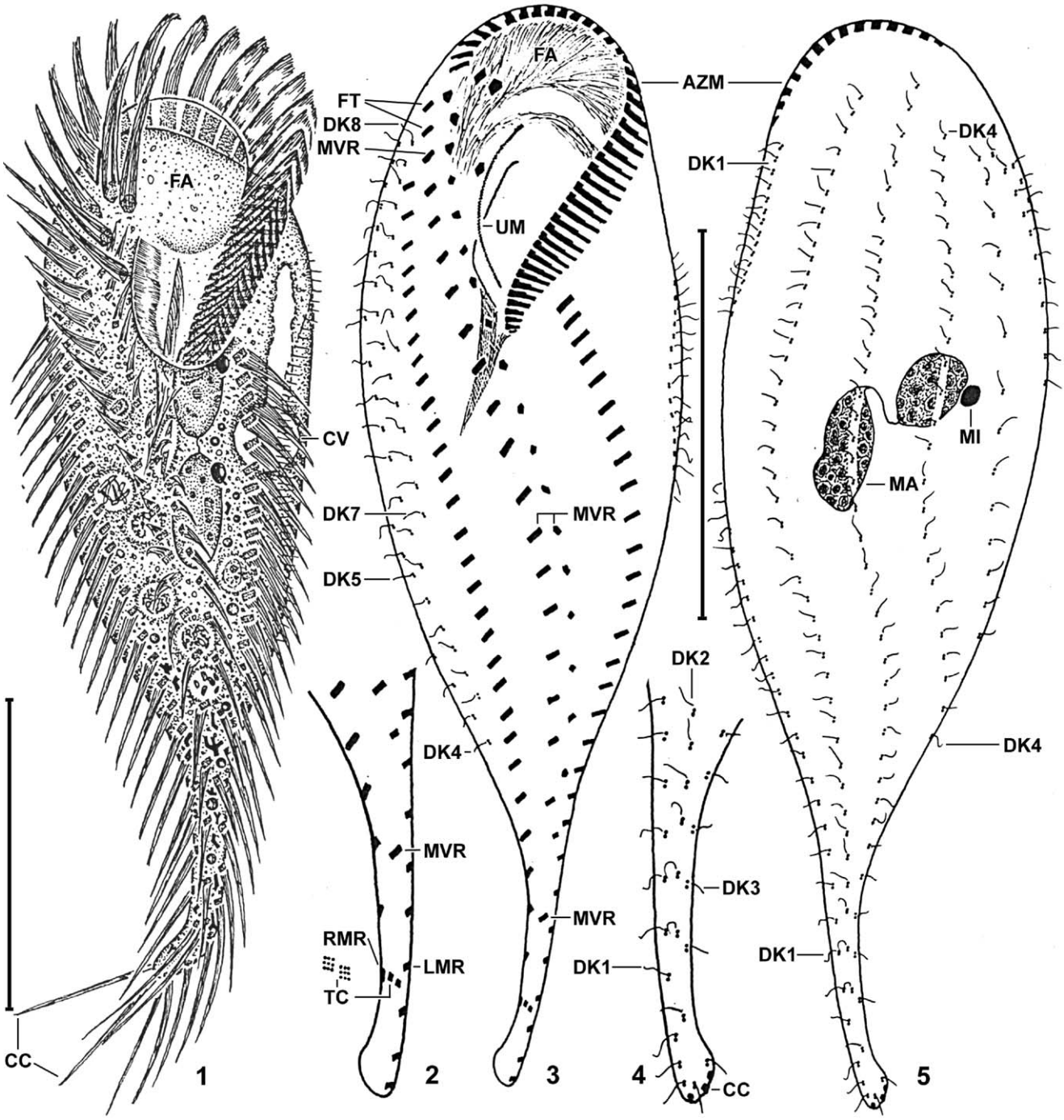
Table 1. Morphometric data on *Rigidothrix goiseri*

Characteristics[a]	$\bar{x}$	*M*	SD	SE	CV	Min	Max	*n*
Body, length	201.8	202	12.0	2.6	5.9	180	240	21
Body, width	63.2	62	7.3	1.6	11.6	51	76	21
Anterior end to proximal end of AZM	62.6	62	3.7	0.8	6.0	58	73	21
Anterior end to begin of paroral membrane	21.1	21	1.8	0.4	8.5	18	25	21
Anterior end to end of paroral membrane	54.2	54	3.9	0.9	7.2	48	64	21
Anterior end to begin of endoral membrane	30.2	30	1.8	0.4	6.0	27	35	21
Anterior end to end of endoral membrane	56.5	56	3.8	0.8	6.7	50	65	21
Anterior end to buccal cirrus	28.0	27	3.1	0.7	10.9	23	34	21
Anterior end to right marginal row	35.2	35	5.1	1.1	14.6	22	43	21
Anterior end to right midventral row[b]	17.4	18	3.7	0.8	21.2	9	25	21
Posterior end to right midventral row	31.8	32	2.9	0.6	9.1	27	39	21
Anterior end to left midventral row	27.0	27	3.5	0.8	13.1	18	34	21
Posterior end to left midventral row	49.0	49	4.0	0.9	8.1	40	57	21
Posterior end to transverse cirri	12.4	13	1.8	0.4	14.7	9	15	21
Left body margin to summit of paroral	41.5	41	5.5	1.2	13.3	32	50	21
Anterior body end to macronucleus figure	44.1	44	4.5	1.0	10.1	36	53	21
Macronucleus figure, length	51.1	50	4.7	1.0	9.2	42	60	21
Macronucleus nodules, distance in between	4.0	4	1.6	0.3	40.4	1	7	21
Anterior macronucleus nodule, length	22.8	23	2.2	0.5	9.6	18	27	21
Anterior macronucleus nodule, width	10.5	11	1.1	0.2	10.2	9	13	21
Posterior macronucleus nodule, length	24.9	25	1.8	0.4	7.1	21	28	21
Posterior macronucleus nodule, width	10.6	11	1.1	0.2	10.5	9	12	21
Posterior micronucleus, length	5.5	5	0.4	0.1	7.6	4	6	21
Posterior micronucleus, width	4.8	5	–	–	–	4	5	21
Macronucleus nodules, number	2.0	2	0.0	0.0	0.0	2	2	21
Micronuclei, number	2.0	2	–	–	–	1	2	21
Adoral membranelles, number	40.5	40	1.7	0.4	4.1	37	44	21
Frontal cirri, number	4.0	4	0.0	0.0	0.0	4	4	21
Buccal cirri, number	1.0	1	0.0	0.0	0.0	1	1	21
Transverse cirri, number	2.0	2	0.0	0.0	0.0	2	2	21
Caudal cirri, number	3.0	3	0.0	0.0	0.0	3	3	21
Right marginal cirri, number	27.0	27	1.5	0.3	5.5	24	30	21
Left marginal cirri, number	25.7	25	1.4	0.3	5.4	24	30	21
Right midventral cirri, number[b]	20.5	20	1.4	0.3	6.7	18	23	21
Left midventral cirri, number	16.4	17	1.5	0.3	9.1	14	20	21
Dorsal kineties, number	7.8	8	–	–	–	7	8	21
Bristles in dorsal kinety 1, number	49.0	50	3.0	0.7	6.1	43	53	21
Bristles in rightmost dorsal kinety, number	9.3	8	3.8	0.8	41.5	3	16	21

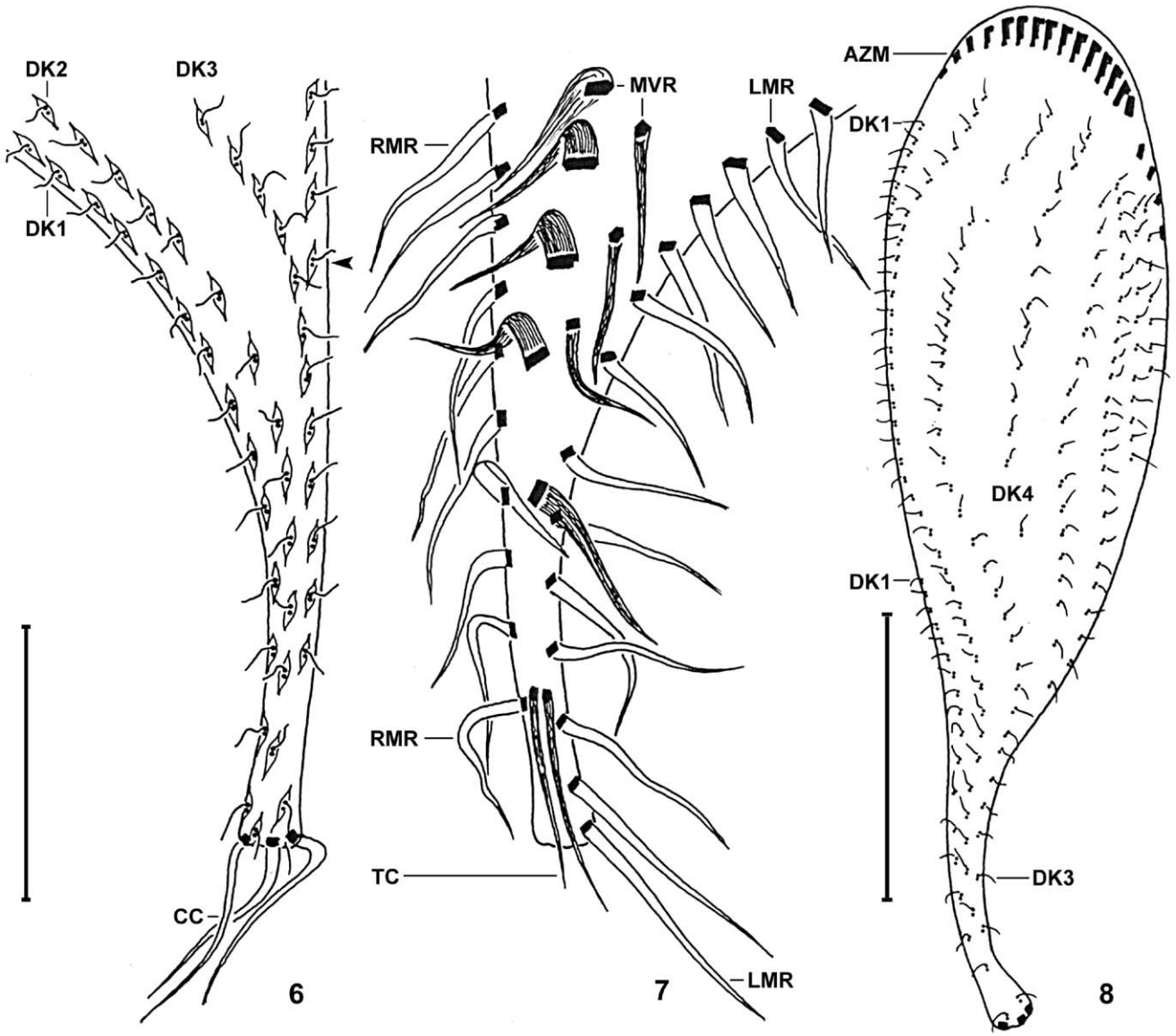
[a]Data based on mounted, protargol-impregnated, randomly selected specimens from a non-flooded Petri dish culture. Measurements in μm. AZM, adoral zone of membranelles; CV, coefficient of variation in %; *M*, median; Max, maximum; Min, minimum; *n*, number of specimens investigated; SD, standard deviation; SE, standard error of mean; $\bar{x}$, arithmetic mean.

[b]Including the two frontoterminal cirri.

more widely spaced in tail region; post-dividers with rather irregular cirral bases ciliated only in right half. Marginal cirri large, i.e., with wide base and about 25 μm long, those of left row gradually lengthen to 40 μm in distal region of tail; right row distinctly shortened anteriorly, that is, commences at or underneath level of buccal cirrus, ends subterminally at level of transverse cirri. Cirri of midventral rows about 25 μm long and ordinarily spaced both within and between rows, cirri of right row of similar size to those of marginal rows, left row cirri only half as thick as those of right. Right midventral row anteriorly lengthened by two frontoterminal cirri (Figs 22, 27, 30 and 31), commences underneath distal end of adoral zone of membranelles and ends subterminally of level of transverse cirri; left row terminates near base of tail. Frontal cirri arranged in quadrangular pattern near distal end of adoral zone of membranelles, that is, right of body midline, cirri 1 and 2 thick and 40 μm long, cirri 3 and 4 slightly thinner and shorter. Buccal cirrus slightly above summit of paroral membrane, of similar size to frontal cirri 3 and 4. Transverse cirri close



Figs 1–5. *Rigidothrix goiseri* from life (1) and after protargol impregnation (2–5). **1–3.** Ventral views of representative specimens, showing the main features of *Rigidothrix*, viz., the tailed body; the stylonychid general organization, especially the large, roundish frontal area (FA), where countless fibres extend; the intersecting undulating membranes (*Oxytricha* pattern); the urostylid midventral rows (last cirrus of right row marked by MVR in Fig. 2); and the inconspicuous transverse cirri. The holotype specimen has only one micronucleus (5). **3–5.** Ventral and dorsal views of holotype specimen, showing eight bristle rows, of which only rows 1–3 extend to posterior body end, where three 40 μm long caudal cirri insert. AZM–adoral zone of membranelles, CC–caudal cirri, CV–contractile vacuole, DK1–8–dorsal kineties, FA–frontal area, FT–frontoterminal cirri, LMR–left marginal row, MA–macronucleus nodules, MI–micronucleus, MVR–midventral rows, RMR–right marginal row, TC–transverse cirri, UM–undulating membranes. Scale bars 80 μm.

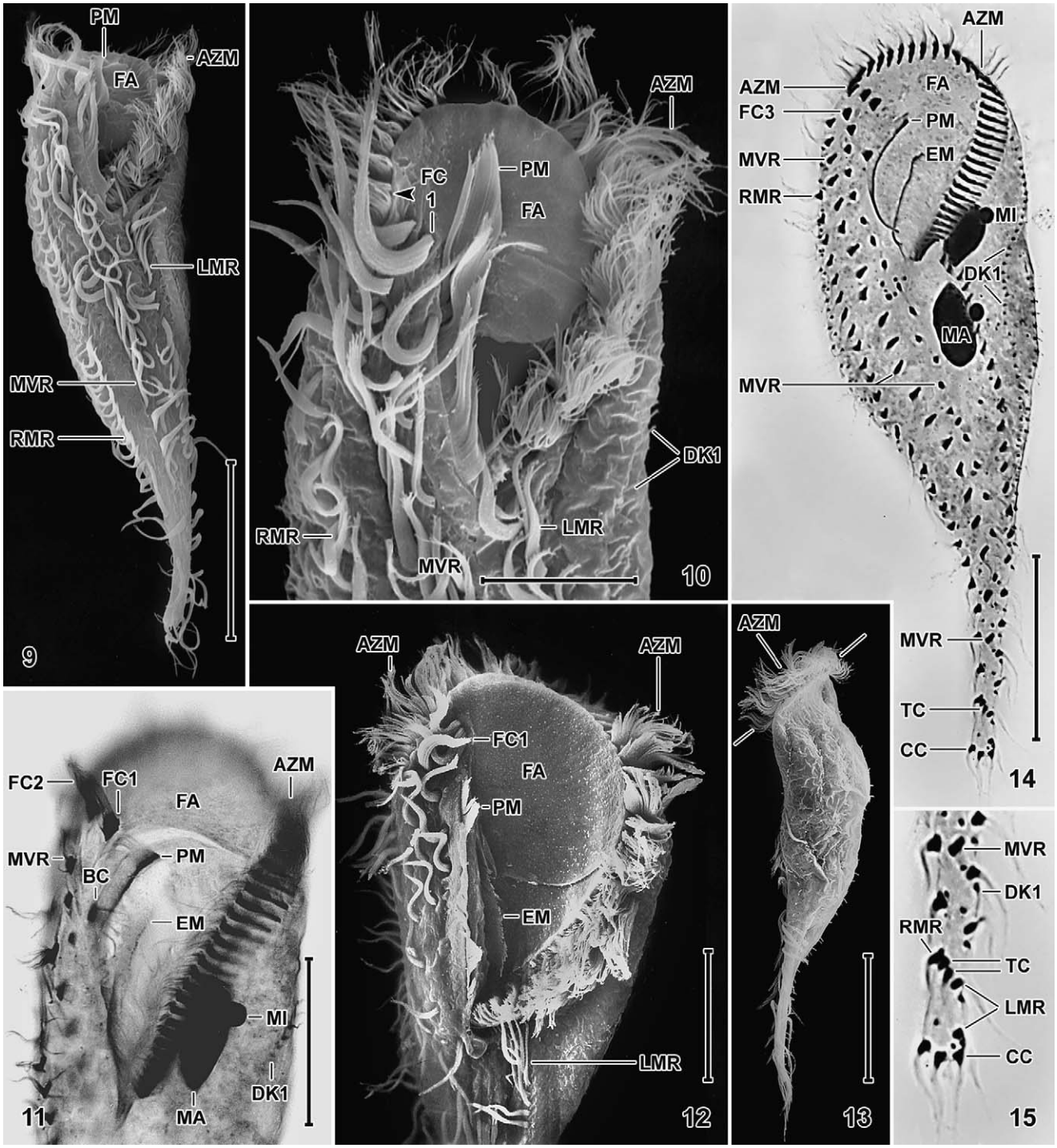


Figs 6–8. *Rigidothrix goiseri* after protargol impregnation. **6, 7.** Dorsal and ventral views of posterior body region, showing the fusiform, fibrous basket surrounding the dorsal dikinetids, a stylonychid feature; the two last left marginal cirri which are elongated to 30 μm (40 μm in vivo); the right marginal cirri row which ends subterminally; and the two minute transverse cirri. The cirri of the left midventral row are much smaller than those of the right row. Arrowhead marks end of dorsal kinety 4. **8.** Dorsal view of a paratype specimen. Bristle rows 1–3 extend whole body length, while rows 4–8 are more or less distinctly shortened because they are dorsomarginal kineties. Dorsal kinety 1 curves ventrally in middle body third (cp. Fig. 3). AZM–adoral zone of membranelles, CC–caudal cirri, DK1–4–dorsal kineties, LMR–left marginal row, MVR–midventral rows, RMR–right marginal row, TC–transverse cirri. Scale bars 30 μm (6, 7) and 50 μm (8).

together, insert subterminally with distal half projecting from body proper (Figs 1–15, Table 1).

Dorsal bristles about 5 μm long and surrounded by a fibrous, fusiform cage, as in *Stylonychia mytilus* (Fig. 6); arranged in eight, rarely in seven slightly curved rows (Figs 1, 3–6, 8, 10, 14 and 36, Table 1). Rows 1–3 ontogenetically active (Fig. 28) and almost as long as body, i.e., commence subapically, each terminating with a conspicuous caudal cirrus about 35 μm long at distal end of tail; row 1 usually with a distinct convexity in mid-body and thus recognizable in cells viewed ventrally, composed of an average of 49 bristles (Figs 3, 5, 10, 14, 31, 32 and 36, Table 1). Rows 4–8 generated dorsomarginally and gradually decreasing in length with last row composed of an average of nine bristles (Fig. 8 and 24, Table 1).

Oral area and oral apparatus conspicuously stylonychid in vivo and in the scanning electron microscope due



Figs 9–15. *Rigidothrix goiseri* (9–11, 13–15) and *Laurentiella strenua* (12) in the SEM (9, 10, 12, 13) and after protargol impregnation (11, 14, 15). **9, 13–15.** Ventral (9, 14, 15) and lateral (13) views, showing the tailed body, the cirral pattern with the urostylid midventral rows (MVR), and the oral and nuclear apparatus (9, 14). The anterior body region is so strongly curved dorsally (13, lines) that the anterior body margin appears transversely truncate in ventral view (9). **10–12.** *Rigidothrix goiseri* has a stylonychid frontal area (10), just as the stylonychine *L. strenua* (12). The arrowhead in Fig. 10 marks the distal end of the adoral zone of membranelles. The cortex of the frontal area contains countless fine fibres (11). AZM–adoral zone of membranelles, BC–buccal cirrus, CC–caudal cirri, DK1–dorsal kinety 1, EM–endoral membrane, FA–frontal area, FC1-3–frontal cirri, LMR, RMR–left and right marginal row, MA–macronucleus nodules, MI–micronuclei, MVR–midventral rows, PM–paroral membrane, TC–transverse cirri. Scale bars 50 μm (9, 12–14) and 25 μm (10, 11).

to the triangular overall shape; the high, dorsally curved frontal scutum; and the large, almost circular frontal area highly similar to that found in *Laurentiella strenua* (Fig. 12) and *Stylonychia mytilus* (Berger 1999) and containing numerous very fine fibres (Figs 1, 3, 9–14, 32 and 36). Adoral zone occupies 31% of body length on average, of usual shape and structure, composed of an average of 40 membranelles with long lateral membranellar cilia strongly beating posteriorly; bases of largest membranelles up to 12 µm wide in vivo. Buccal cavity of ordinary width and depth, right margin with inconspicuous lip. Paroral membrane slightly to rather distinctly curved, only half as long as ventral portion of adoral zone of membranelles, thus producing the large frontal area; composed of zigzagging dikinetids, in distal portion usually some short, oblique kineties each comprising three to four cilia. Endoral membrane slightly shorter and less distinctly curved than paroral, both intersect optically in posterior half of buccal cavity. Pharyngeal fibres short, extend slightly obliquely backwards (Figs 1, 3, 9–14, 31, 32 and 36, Table 1).

Ontogenesis of *Rigidothrix goiseri*

About 25 well-impregnated dividers were found in the protargol slides. They show the whole process, but each stage is represented by only one to four individuals. Thus, quantitative features cannot be underpinned by statistics.

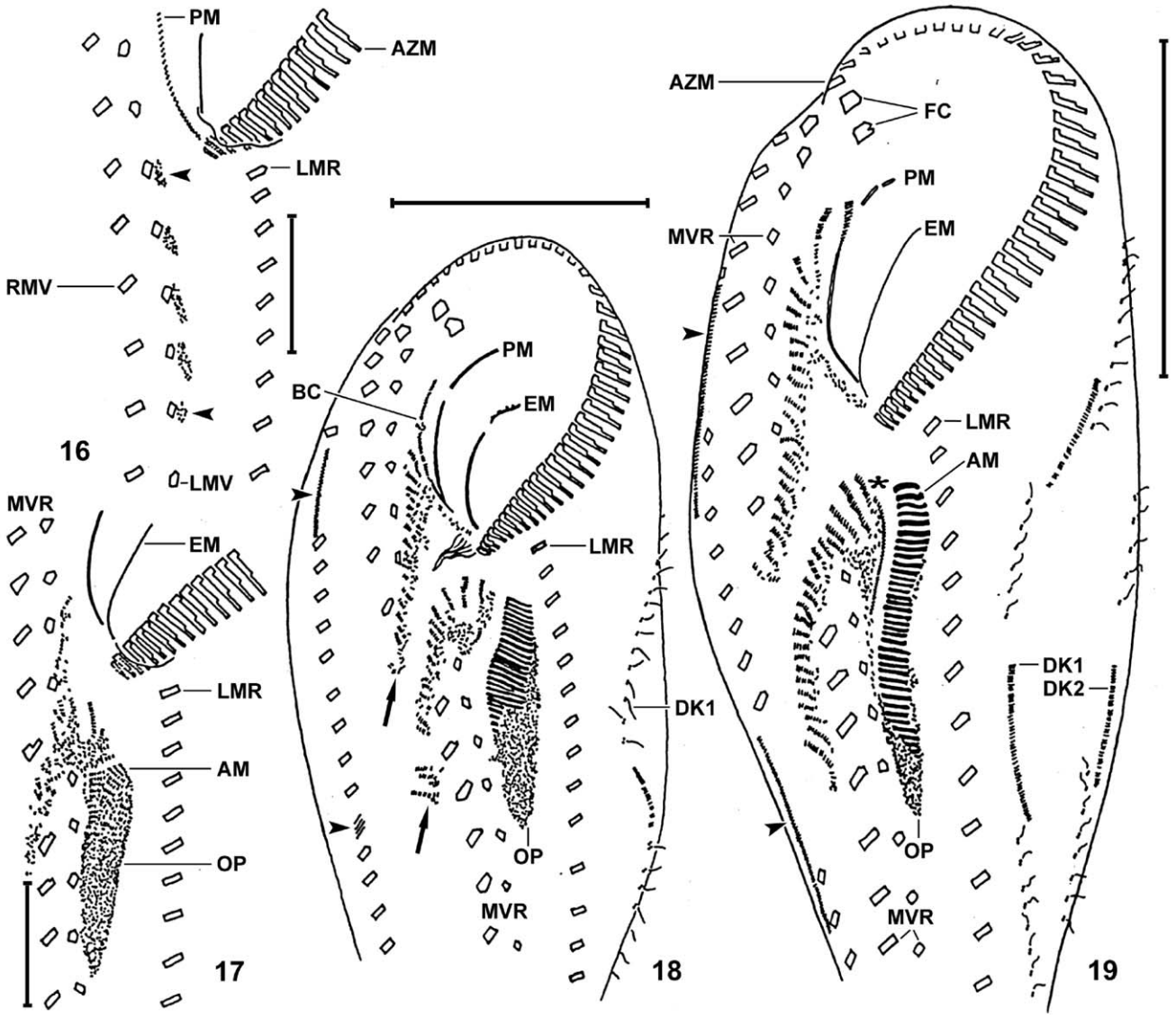
Oral apparatus: Ontogenesis commences with the formation of an oral primordium underneath the buccal vertex, where about five consecutive cirri of the left midventral row produce small accumulations of basal bodies at their left sides (Figs 16 and 23). By further growth, the basal body fields fuse, forming an oblanceolate oral primordium (Fig. 17). Anteriorly, some scattered basal bodies remain and reproduce, generating a strongly oblique anlage which will produce the new midventral rows. Next, adoral membranelles develop within the oral primordium from anterior to posterior and from right to left (Fig. 18). The oral primordium becomes fusiform because it narrows anteriorly when the protomembranelles, which consist of two rows of basal bodies, assemble. At right anterior end of the oral primordium, some scattered basal body pairs remain and become the anlage for the opithe's undulating membranes (Figs 18 and 19). The parental undulating membranes begin to disintegrate and the buccal cavity flattens, drifting apart the membranes (Figs 18 and 19).

In early dividers (Fig. 19) and early mid-dividers (Fig. 20), the oral primordium develops to a long ribbon of protomembranelles each composed of two long and one short ciliary row. Disintegration and resorption of the parental undulating membranes proceed (Fig. 19), and an anlage for the new undulating membranes appears at right anterior end of the flattened buccal cavity, that is, left of the new frontal cirrus 1 (Fig. 20). The origin of these basal bodies could be not clarified; likely, they are remnants from the midventral anlagen. In mid-dividers, the opisthe's adoral zone of membranelles is almost complete, and the anlagen of the undulating membranes appear as long streaks of very narrowly spaced, scattered basal bodies in both proter and opisthe (Figs 22 and 26). In late dividers, a fourth, very short row of basal bodies is added to the new adoral membranelles, and the anterior half of the membranellar zone curves to the right (Fig. 27). The anlagen for the undulating membranes split into a paroral and an endoral membrane, both likely consisting of basal body pairs (Figs 27 and 30). In very late dividers and in post-dividers, the buccal cavity deepens and shapes in such a way that the undulating membranes become arranged in an *Oxytricha* pattern, that is, they intersect optically in the posterior half (Figs 31, 32).

The parental adoral zone of membranelles remains unchanged, while the pharyngeal fibres are reorganized and form a short funnel in early post-dividers (Figs 29 and 32).

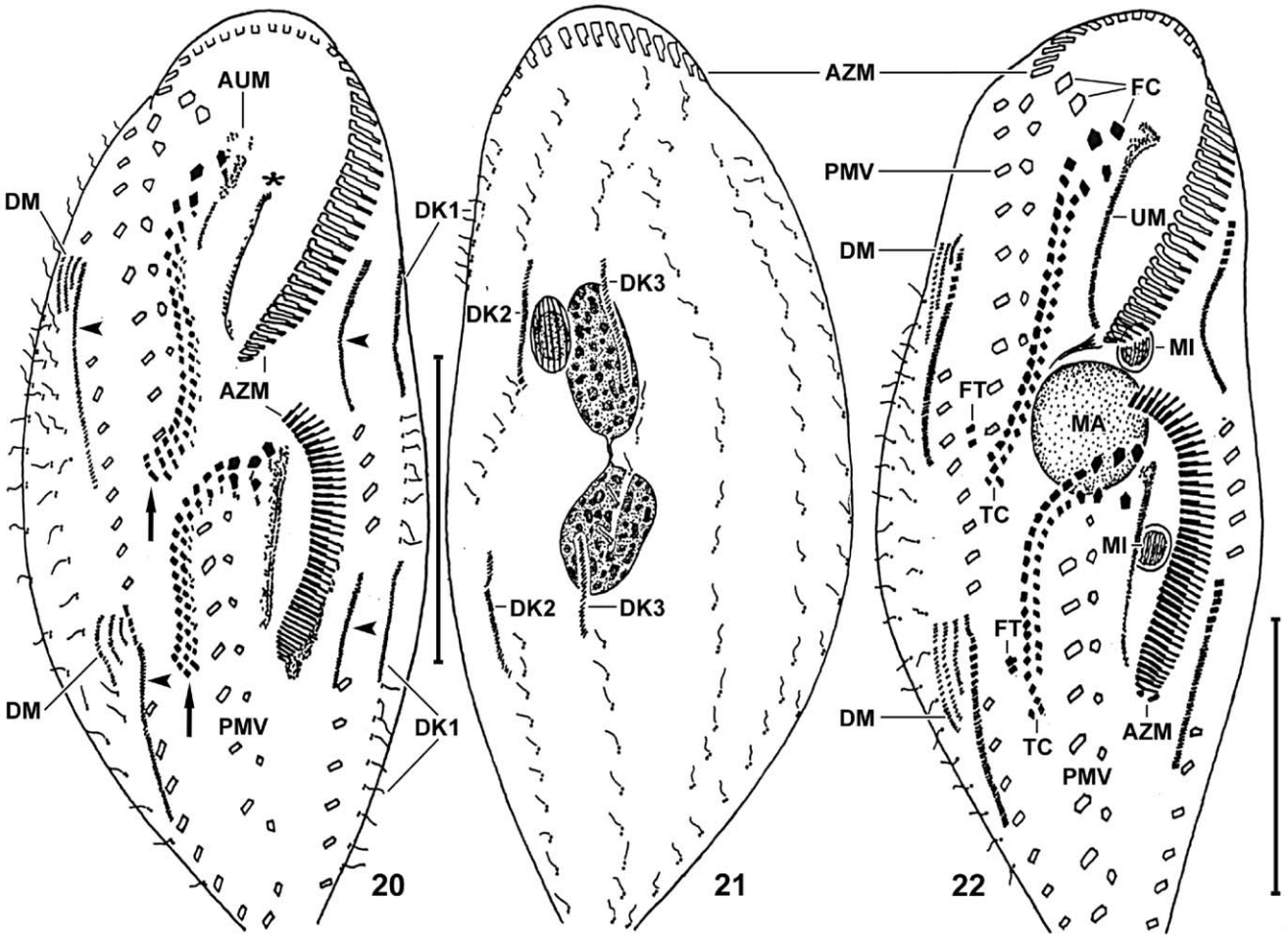
Ventral cirral pattern (midventral rows): The proter and opisthe midventral rows are produced from the same anlage as the opisthe's oral primordium. When the first protomembranelles develop, the midventral anlagen for the proter and opisthe form a strongly oblique, irregular stripe of paired basal bodies at the right anterior corner of the oral primordium (Fig. 17). Anteriorly, this stripe extends along the posterior third of the parental oral apparatus; posteriorly, the stripe grows into the parental midventral rows, which are thus split in such a way that the anterior half of the anlage extends left of the parental midventral rows, while the posterior half extends right of the parental midventral rows. Likely, the space required for anlagen ingrowth is not obtained by resorption of midventral cirri, but by increasing the distance between the first pair of postoral midventral cirri.

Next, the midventral anlage splits obliquely (Figs 17, 18 and 25). The anterior portion becomes the proter's midventral rows, while the posterior portion generates the opisthe's midventral rows. The split of the anlage is connected with an intense proliferation of basal bodies which arrange to short, oblique streaks, forming a distinct ribbon each in proter and opisthe (Fig. 19). When cirri segregate within the streaks, three regions become recognizable within each ribbon (Figs 19, 20, 25 and 26): anteriorly, two long and thick streaks and the anlage (I) for the undulating membranes develop three enlarged frontal cirri and the buccal cirrus, while the fourth enlarged frontal cirrus (III/2) is produced by anlage III; the long mid-region, which is composed of



Figs 16–19. *Rigidothrix goiseri*, ventral views of early dividers after protargol impregnation. **16.** Very early divider, showing anlagen formation (arrowheads) left of some postoral cirri of the left midventral row. **17.** Very early stage showing the development of the oral primordium along the left midventral row. Adoral membranelles form in the anterior portion of the oral primordium. Basal bodies from the anterior end of the oral primordium develop to an oblique cirral anlage which splits the parental midventral rows in such a way that the proter cirral anlagen develop left of the left midventral row, while the opisthe cirral anlagen develop right of the right midventral row. **18.** Early divider with anlagen in the right marginal row (arrowheads) and dorsal kinety 1. Adoral membranelles develop in the oral primordium from anterior to posterior, and the parental undulating membranes begin to dissolve. The anlage for the frontal and midventral cirri splits obliquely, producing a field of cirral anlagen each in proter and opisthe (arrows). Some scattered basal bodies, which will grow to the opisthe's undulating membranes (Fig. 19, asterisk), remain at anterior right end of the oral primordium. The buccal cirrus (BC) forms a long anlage right of the fragmenting paroral membrane. **19.** Early mid-divider showing the midventral and frontal cirri developing from many short, oblique anlagen both in proter and opisthe. The anlagen (arrowheads) in the right marginal row and dorsal kineties 1–3 grow to long steaks. The parental undulating membranes disintegrate, while the new opisthe's undulating membranes form right of the oral primordium (asterisk). AM–adoral membranelles, AZM–adoral zone of membranelles, BC–buccal cirrus, DK1, 2–dorsal kineties, EM–endoral membrane, FC–frontal cirri, LMR–left marginal row, LMV–left midventral row, MVR–midventral rows, OP–oral primordium, PM–paroral membrane, RMV–right midventral row. Scale bars 20 μm (Figs 16 and 17) and 50 μm (Figs 18 and 19).

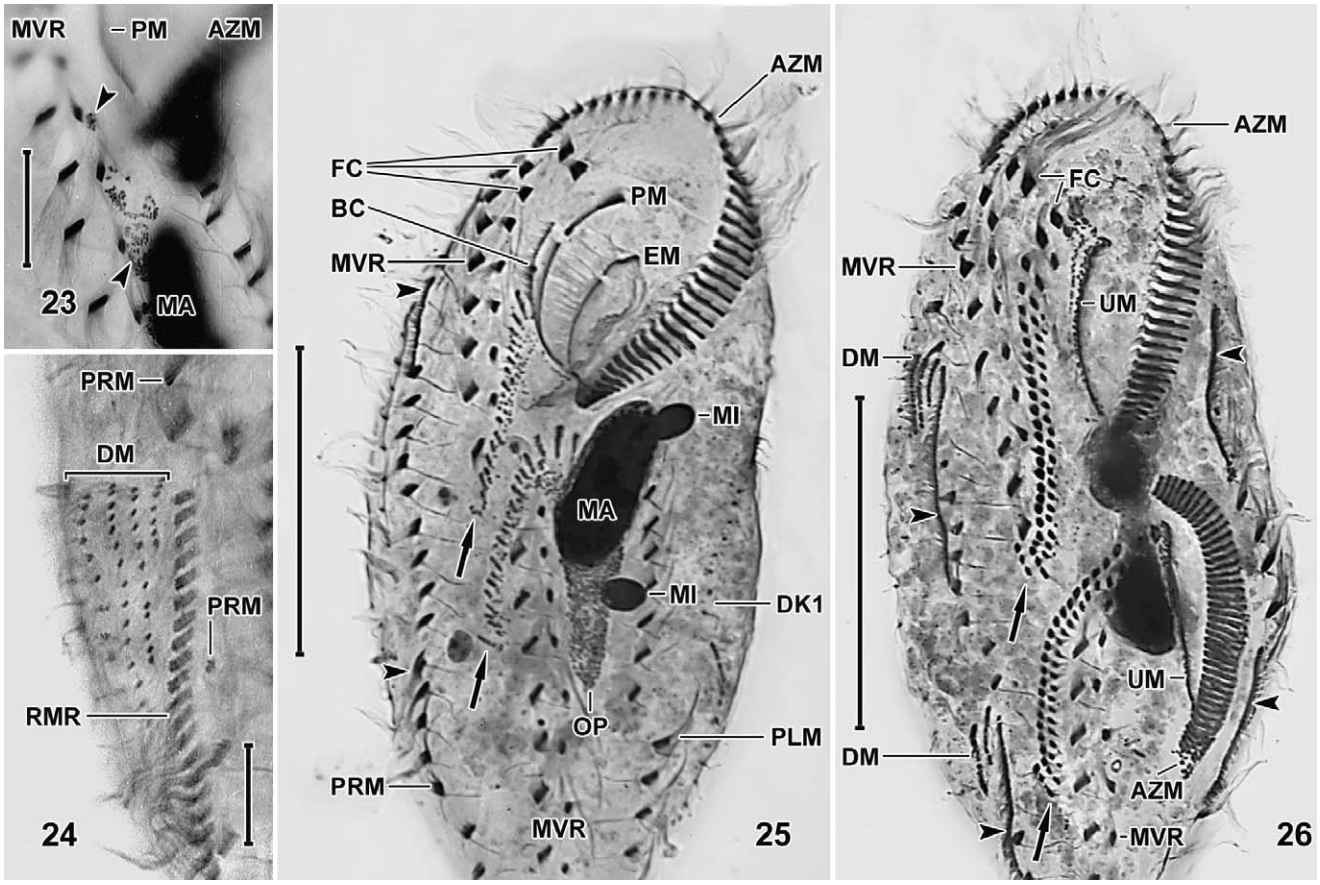
short, thin streaks, produces many pairs of midventral cirri; in the posterior region, some elongated streaks generate two frontoterminal cirri, two transverse cirri, and two pairs of midventral cirri. Some scattered basal body pairs remain at the left side of the ribbons and are likely resorbed (Figs 19, 20, 25 and 26).



Figs 20–22. *Rigidothrix goiseri*, dividers after protargol impregnation. **20, 21.** Ventral and dorsal views of an early mid-divider, showing the developing dorsomarginal kineties (DM) and the anlagen in dorsal kineties 1–3. Arrowheads mark anlagen for the new marginal cirral rows. The new adoral zone of membranelles is almost finished, and most of the parental undulating membranes has been resorbed (asterisk). Cirri are formed within the ribbon of oblique ventral anlagen, where three regions are recognizable: in the anterior region, two long and thick streaks and the anlage (I) for the undulating membranes develop three enlarged frontal cirri and the buccal cirrus, while the fourth enlarged frontal cirrus (III/2) is produced by streak III; in the long mid-region, many pairs of midventral cirri are generated; and in the broadened posterior region (arrows), some streaks with three cirri each develop two frontoterminal cirri, two transverse cirri, and two pairs of midventral cirri (see also next figure). **22.** Mid-divider with fused macronucleus nodules and dividing micronuclei. The number of dorsomarginal kineties is increasing (cp. Fig. 20), the frontoterminal cirri commence to move anteriorly, while the transverse cirri migrate posteriorly. Both, proter and opisthe generate new undulating membranes. AUM–anlage for the new undulating membranes, AZM–adoral zone of membranelles, DK1-3–dorsal kineties, DM–dorsomarginal kineties, FC–frontal cirri, FT–frontoterminal cirri, MA–fused macronucleus nodules, MI–micronuclei, PMV–parental midventral rows, TC–transverse cirri, UM–undulating membranes. Scale bars 50 μm.

In mid-dividers, the new midventral rows are fully developed, and the frontoterminal cirri begin to migrate anteriorly (Fig. 22). In late dividers, the frontoterminal cirri have migrated to about mid-body (Figs 27 and 30). They reach the final position only in very late dividers and in post-dividers, where they arrange on top of the right midventral row, making it difficult to recognize them in morphostatic specimens (Figs 3, 10 and 36). The transverse cirri migrate posteriad only in post-dividers, i.e., when the tail is forming and the parental ciliature has been resorbed (Figs 29–31).

Marginal cirral rows: The marginal cirral rows reproduce as usual, that is, a primordium each develops in the proter and opisthe of early dividers (Figs 18, 19 and 25). However, the anlagen of the left row develop much later than those of the right (Figs 18–20). About five dorsomarginal kineties are generated right of the anterior end of the right marginal primordia in early mid-dividers to late dividers. The dorsomarginal rows



Figs 23–26. *Rigidothrix goiseri*, ventral views of dividing specimens after protargol impregnation. **23.** Very early divider with anlagen (arrowheads) for the oral primordium left of the left midventral row. **24.** Opisthe of late divider with five fully developed dorsomarginal kineties (DM) near to the anterior end of the new right marginal row (cp. Figs 27 and 30). **25.** Early divider with anlagen (arrowheads) in the right marginal row and dorsal kinety 1. The parental undulating membranes begin to dissolve. The anlage for the frontal and midventral cirri splits obliquely, producing a field of cirral anlagen each in proter and opisthe (arrows). The buccal cirrus (BC) forms a long anlage right of the fragmenting paroral membrane. **26.** Mid-divider with fusing macronucleus nodules and developing dorsomarginal kineties. Arrowheads mark anlagen for the new marginal rows. Frontoterminal and transverse cirri (arrows) develop at the posterior end of the new midventral rows. Both, proter and opisthe generate new undulating membranes. AZM–adoral zone of membranelles, BC–buccal cirrus, DK 1–dorsal kinety 1, DM–dorsomarginal kineties, EM–endoral membrane, FC–frontal cirri, MA–macronucleus nodules, MI–micronuclei, MVR–parental midventral rows, OP–oral primordium, PLM–parental left marginal row, PM–paroral membrane, PRM–parental right marginal row, RMR–new right marginal row, UM–undulating membranes. Scale bars 10 μm (23, 24) and 50 μm (25, 26).

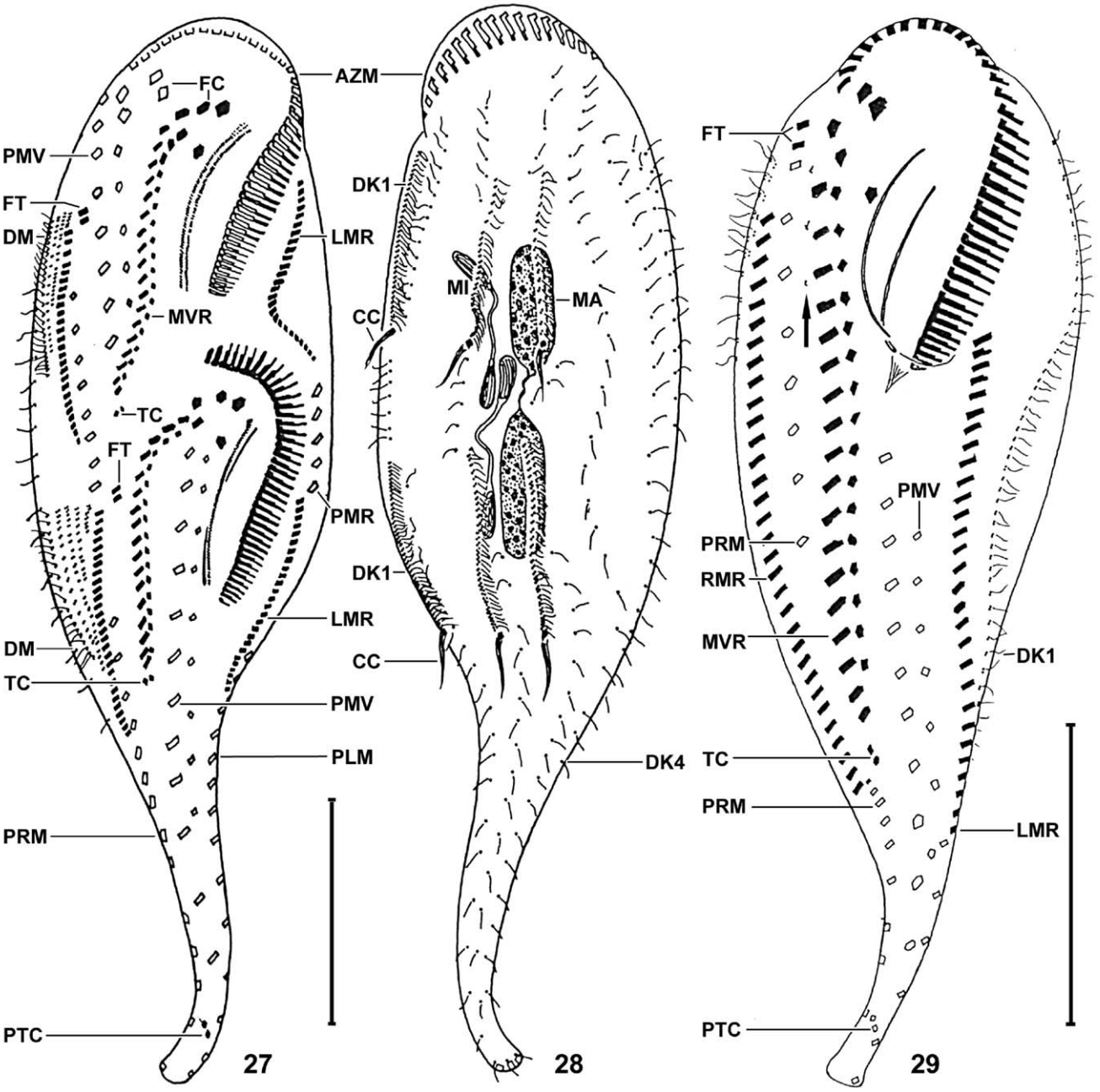
increase in length from left to right and migrate onto the dorsal side in late dividers to form dorsal bristle rows 4–8 (Figs 20, 22, 24, 26, 27 and 30).

Dorsal ciliature: The dorsal ciliature develops according to type II (Foissner and Adam 1983), that is, three rows are produced intrakinetally and five rows are generated dorsomarginally, as described above. Anlagen development commences in early dividers and is completed in late dividers, where a caudal cirrus each is generated at posterior end of rows 1–3 (Figs 18–22, 24, 26–28 and 30).

Nuclear apparatus and parental cirri: The nuclear apparatus divides as usual (Figs 21, 22, 26, 28 and 30). The parental cirri and dorsal bristles become resorbed in early to late post-dividers (Figs 29 and 31). No parental structures remain in ordinary morphostatic cells, except for the adoral zone of membranelles.

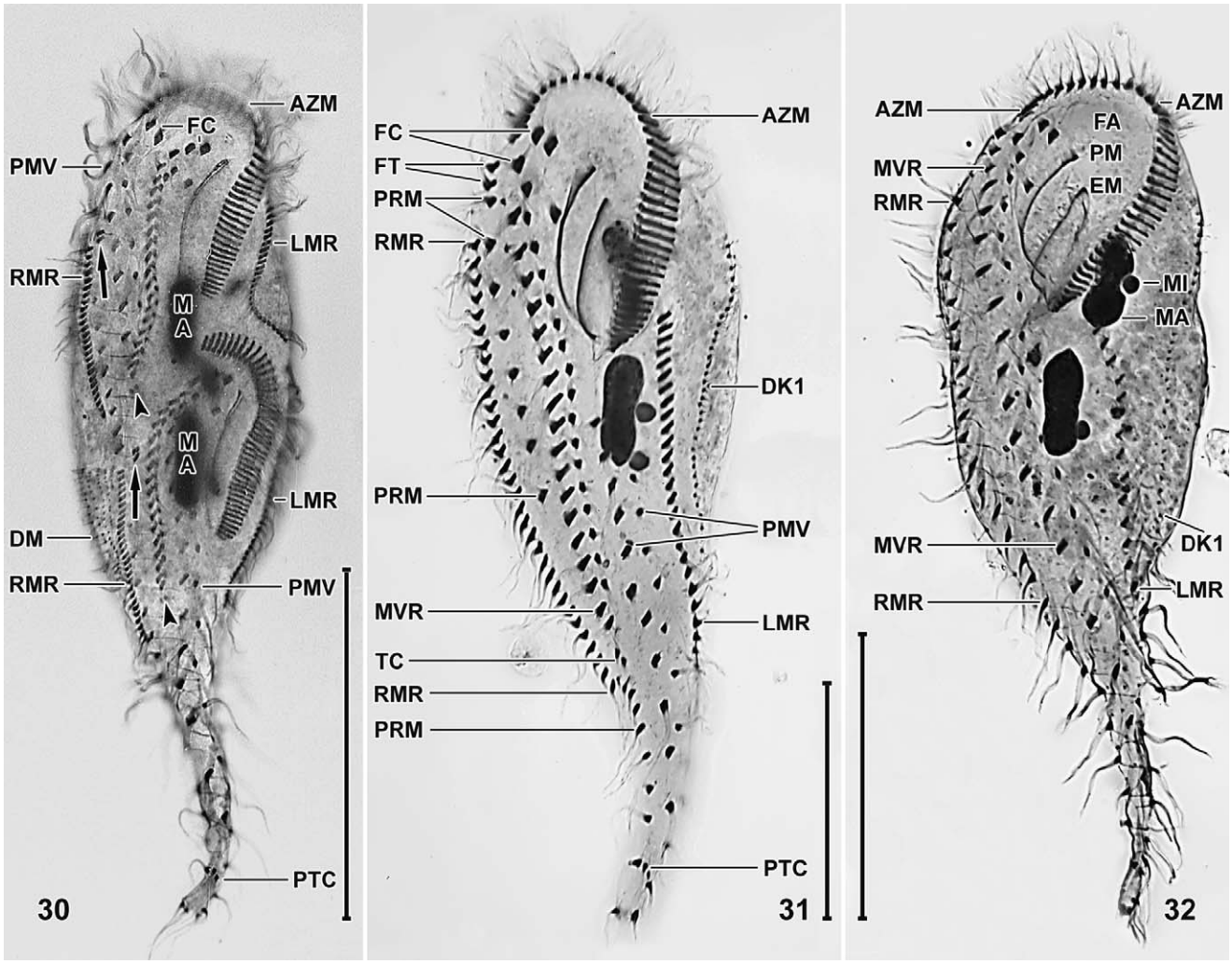
Sequence analysis

The 18S rDNA sequence of *R. goiseri* is 1771 bp long and available under GenBank accession number DQ490236. Comparing the *R. goiseri* sequence to the available ciliate 18S rDNA sequences identifies *Oxytricha granulifera* (accession number X53486) as the closest relative of *R. goiseri* in all phylogenetic analyses. We here only show the evolutionary distance and



Figs 27–29. *Rigidothrix goiseri*, dividing specimens after protargol impregnation. **27, 28.** Ventral and dorsal views of a late divider showing the new midventral rows (MVR), the dorsomarginal kineties (DM), the frontoterminal cirri (FT) migrating anteriorly, the new transverse cirri (TC), the new caudal cirri (CC) at the posterior end of the developing dorsal kineties 1–3, the dividing macronucleus mass (MA), and the dividing micronuclei (MI). The proter undulating membranes have been reorganized, while the adoral zone of membranelles has not. The posterior, barren basal body of the dorsal dikinetids has been resorbed. **29.** Ventral view of an opisthe post-divider. Part of the parental ciliature is still recognizable; arrow marks minute clusters of basal bodies, that is, likely remnants from the formation of the midventral cirri. AZM–adoral zone of membranelles, CC–new caudal cirri, DK1–new dorsal kinety 1, DK4–parental dorsal kinety 4, DM–dorsomarginal kineties, FC–frontal cirri, FT–frontoterminal cirri, LMR–new left marginal row, MA–macronucleus nodules, MI–dividing micronuclei, MVR–new midventral rows, PLM–parental left marginal row, PMV–parental midventral rows, PRM–parental right marginal row, PTC–parental transverse cirri, RMR–new right marginal row, TC–new transverse cirri. Scale bars 50 μm.

Bayesian results (Fig. 33). The MP and the ML trees as well as trees from the calculations mentioned in the method section are available from the authors upon request. The sequence similarity runs to 95.88% between *R. goiseri* and *O. granulifera*, which is similar to that between *R. goiseri* and *Uroleptus gallina* (94.93%;



Figs 30–32. *Rigidothrix goiseri*, ventral views of dividing specimens after protargol impregnation. **30.** Late divider after first round of macronucleus division (cp. Figs 27 and 28). Note the tailed body, the dorsomarginal kineties (DM), the migrating frontoterminal cirri (arrows), the new transverse cirri (arrowheads), and the L-shaped left marginal row of the proter (LMR). **31.** Opisthe post-divider with part of the parental ciliature still recognizable (cp. Fig. 29). Note dorsal kinety 1 which curves ventrally in mid third. **32.** A very late post-divider recognizable by the undulating membranes which do not intersect in the mid-region of the buccal cavity, as in morphostatic specimens (Fig. 3), but near the posterior end. Note the large frontal area (FA), the distinct tail, and the developing pharyngeal fibres. AZM–adoral zone of membranelles, DK1–new dorsal kinety 1, DM–dorsomarginal kineties, EM–endoral membrane, FA–frontal area, FC–frontal cirri, FT–frontoterminal cirri, LMR–new left marginal row, MA–macronucleus nodules, MI–micronucleus, MVR–new midventral rows, PM–paroral membrane, PMV–parental midventral rows, PRM–parental right marginal row, PTC–parental transverse cirri, RMR–new right marginal row, TC–new transverse cirri. Scale bars 100 μm (30) and 50 μm (31, 32).

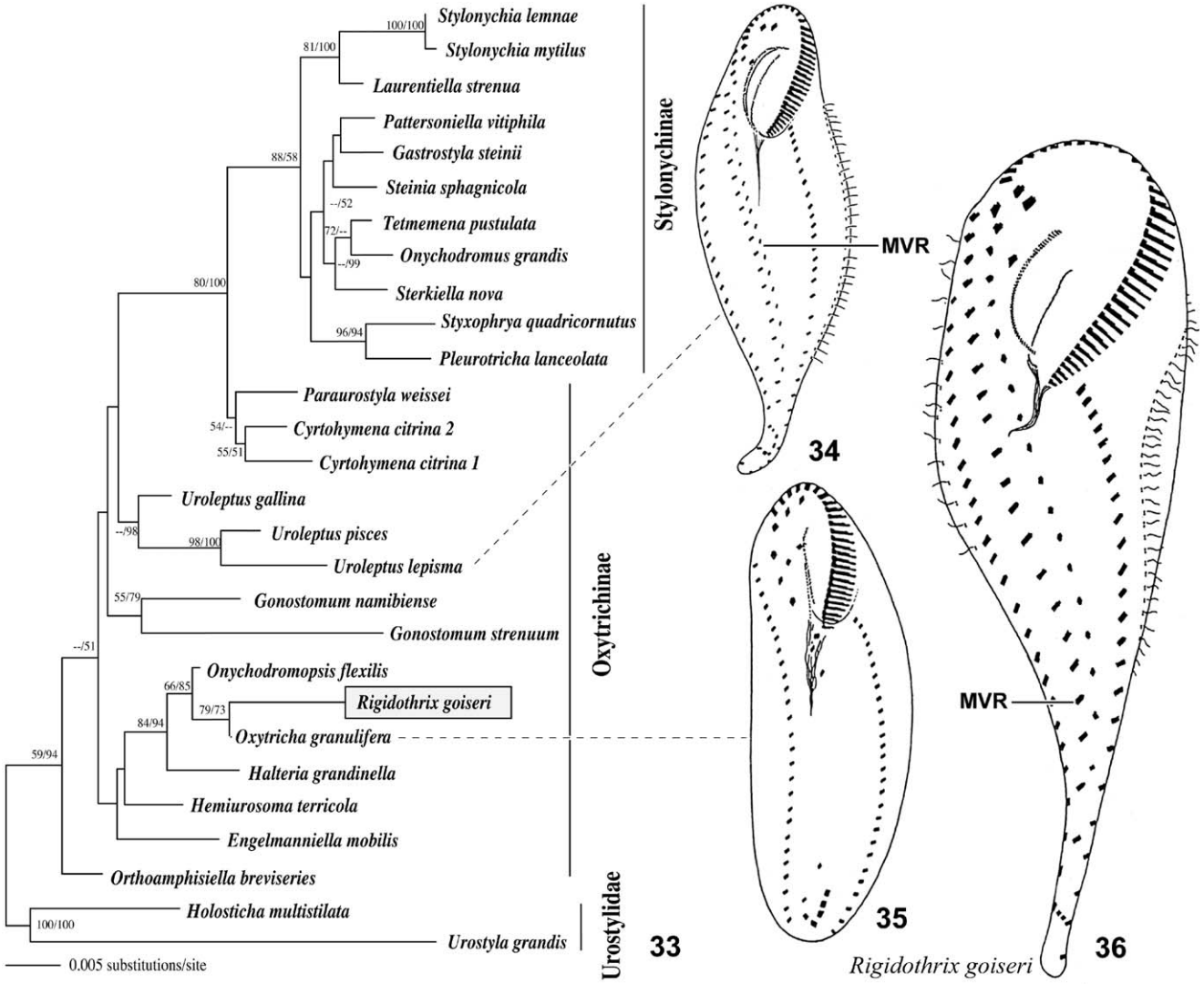
AF164130), while that of *Urostyla grandis* (AF164129) is different (92.7%).

Discussion

The flexibility dogma in the classification of stichotrichine spirotrichs

Kahl (1932), Berger and Foissner (1997), and Berger (1999, 2006) recognized two kinds of stichotrichine spirotrichs: those which have a flexible body and others which are rigid. The structural basis of this difference is not known, but likely it is related to the fine structure of the cortex (microtubule pattern? perilemma?). Most stichotrichs are flexible (e.g., all urostylids, amphisiellids), while members of the oxytrichid subfamily Stylonychinae (e.g., *Stylonychia*, *Pleurotricha*, *Laurentiella*) and some stichotrichs of doubtful systematic position are rigid (e.g., *Psilotricha*; Foissner 1983).

Conventional and computer-assisted phylogenetic analyses as well as molecular phylogenies showed that



Figs 33–36. Phylogenetic tree of 18S rDNA sequences showing the position of *Rigidothrix goiseri*. The tree was constructed under maximum-likelihood criteria by using a GTR + *I* + *G* DNA substitution model with the variable-site gamma distribution shape parameter (*G*) at 0.4694; the proportion of invariable sites at 0.8372; and base frequencies and a rate matrix for the substitution model as suggested by Modeltest, based on 1700 unambiguously aligned positions. Distance bootstrap values over 50% from an analysis of 1000 bootstrap replicates (first number) and posterior probabilities over 50% of 2862 Bayesian trees, are given at the respective nodes. *Rigidothrix goiseri* branches within the *Oxytricha* cluster. However, *Oxytricha* has a very different cirral pattern composed of 18 frontal, ventral, and transverse cirri (Fig. 35, *Oxytricha islandica*; from Berger and Foissner 1989). Thus, we propose that *R. goiseri* (Fig. 36) is related to the oxytrichine genus *Uroleptus* (Fig. 34, from Berger and Foissner 1989) which also possesses midventral rows (MVR).

body flexibility/rigidity is a main feature in the classification of stichotrichine spirotrichs (Berger 1999; Berger and Foissner 1997; Foissner et al. 2004): all rigid oxytrichid stichotrichs belong to the Stylonychinae. So far, no exception was known, specifically, all stichotrichs with midventral rows are flexible (Berger 1999, 2006). *Rigidothrix goiseri* breaks this "dogma": it is conspicuously rigid, although possessing midventral rows (Figs 3, 9, 10, 14 and 31).

Ontogenesis

Ontogenesis of *R. goiseri* follows the basic pattern known from stichotrichs with midventral rows (Berger 2006; Eigner 2001; Hu and Song 2001; Hu et al. 2000, 2002, 2003; Martin et al. 1981; Song 1990; Song et al. 1992; Warren et al. 2002; Wirnsberger 1987). However, when considered in detail, the ontogenetic patterns of the "midventral stichotrichs" are very different,

suggesting polyphyly of the group (Berger 2006; Hu and Song 2001). For instance, a *Bakuella* pattern is produced when the posterior-most midventral anlagen produce many cirri (Song et al. 1992); a *Periholosticha* pattern is generated when the penultimate anlage produces many more cirri than the other anlagen (Song 1990); and a *Neokeronopsis* pattern is created when the anterior midventral anlagen develop into a frontal bicorona (Warren et al. 2002).

The ontogenetic pattern of *R. goiseri* matches that of *Uroleptus caudatus*, as described by Eigner (2001), specifically, both produce dorsomarginal rows and locate the frontoterminal cirri in such a way that they become an elongation of the right midventral row. *Uroleptus* is now considered as a highly derived oxytrichine stichotrich (Foissner et al. 2004). The ontogenetic homologies suggest that *Rigidothrix* belongs to this group (see next chapter).

Classification of *Rigidothrix goiseri* into a new family

Classification of *R. goiseri* is difficult because it possesses main characteristics of several groups of stichotrichs (Table 2). Unfortunately, the phylogenetic analyses of the 18S rDNA do not solve the problem because they place the rigid, midventral rows-bearing *R. goiseri* very near to the flexible *Oxytricha granulifera*, which has a typical 18FVT cirral pattern (Figs 33, 35 and 36). Nonetheless, the molecular data agree with the morphological peculiarities in that they indicate a special position of *R. goiseri* within the stichotrichine spirotrichs (Figs 33–36).

Table 2 shows that *R. goiseri* has main characteristics of *Uroleptus* spp., the oxytrichine and stylonychine Oxytrichidae, and the Urostylidae. Thus the classification needs some assumptions, viz., the CEUU hypothesis which suggests that a midventral cirral pattern evolved convergently in uroleptid and urostylid stichotrichs (Foissner et al. 2004). Indeed, a rather close relationship of *Rigidothrix* and *Uroleptus* is indicated by body shape, the midventral cirral pattern, the location of the frontoterminal cirri, and the dorsomarginal kineties (Table 2). Further, *Uroleptus* differs from *Rigidothrix* only by the flexible body. On the other hand, the rigid body, the shape of the frontal field, and the structure of the dorsal bristles suggest a stylonychid relationship (Figs 1, 6 and 9–14; Table 2).

When the CEUU hypothesis is applied, *Rigidothrix* and *Uroleptus* form a distinct branch within the oxytrichine stichotrichs (Table 3). Thus, we unite both into a new family, the Rigidotrichidae, as defined in the results section. This does not contradict the sequence data because *R. goiseri* is almost equally similar to both *Oxytricha granulifera* (95.88%) and *Uroleptus gallina* (94.93%). Within this narrow range of sequence difference, it is hardly possible to distinguish different genera within the same family or different genera from different families. Concerted efforts are in order to retrieve sequences from *Territricha* and *Afrophrya*, the two other genera of the Rigidotrichidae.

Uniting *Rigidothrix* and *Uroleptus* into a distinct family has the advantage of clearing up the Oxytrichidae, especially when two other midventral rows-bearing genera, *Pattersoniella* and *Neokeronopsis*, are also removed and placed into the family Pattersoniellidae Shi et al., 1999. These authors assign to the Pattersoniellidae the genera *Pattersoniella* and *Territricha* (Table 3). However, *Territricha* lacks a frontal bicorona, suggesting that it is more closely related to *Rigidothrix* and *Uroleptus* than to *Pattersoniella*. Accordingly, *Territricha* is transferred from the Pattersoniellidae to the Rigidotrichidae.

As defined, the Rigidotrichidae contain rigid and flexible genera, just as do the Oxytrichidae where, however, the rigid and flexible genera are classified into distinct subfamilies (Fig. 33; Berger and Foissner 1997). Accordingly, the same should be done with the Rigidotrichidae, which are a subgroup of the Oxytrichidae in the phylogenetic tree (Fig. 33). However, such formal classification should be performed only when further data substantiate the distinctness of the family.

The genus *Territricha* Berger and Foissner and *Holosticha stueberi* Foissner

As discussed in the previous section, the genus *Territricha* Berger and Foissner (1988) should be transferred to the Rigidotrichidae. Thus, we provide an emended diagnosis: Flexible Rigidotrichidae with few, comparatively widely spaced pairs of midventral cirri postorally; frontal area and undulating membranes in *Oxytricha* pattern; and with caudal cirri.

Berger and Foissner (1988) discussed similarities of *T. stramenticola* with two African species, viz., *Holosticha camerounensis* and *H. macrostoma*, both described by Dragesco (1970). Indeed, these species resemble *Territricha* and *Uroleptus*, except for the cyrtohymenid oral apparatus. Thus, we assign them to a new genus, *Afrophrya*, diagnosed below. The classification into the Rigidotrichidae is tentative because the dorsal ciliature has been not described, that is, data on dorsomarginal kineties and caudal cirri are lacking.

Holosticha stueberi is another "difficult" species because it has dorsomarginal kineties, which are a typical oxytrichid feature (Berger 2006). Table 3 shows that the problem disappears when it is considered as an uroleptid and classified into the Rigidotrichidae. However, this poses another problem because Berger (2003) has fixed *H. stueberi* as type of the genus

Table 2. Comparison of vegetative and ontogenetic features in *Rigidothrix goiseri* and various stichotrichine genera and families

Characteristics[a]	Oxytrichidae		*R. goiseri*	*Uroleptus* spp.	Urostylidae[b]
	Oxytrichinae	Stylonychinae			
Vegetative specimens					
Body tailed	–	–	+	+	–
Body rigid	–	+	+	–	–
Frontal field stylonychid	–	+	+	–	–
Midventral cirral pattern	–	–	+	+	+
Dorsal bristles stylonychid	–	+	+	–	–
Ontogenesis					
Large field of anarchic basal bodies in proter oral area	–	–	–	–	+
The opisthe midventral rows develop right of the parental ones	–	–	+	+	–
Transverse cirri originate from the posteriormost midventral cirri	–	–	+	+	–
Parental adoral membranelles reorganized	–	–	–	–	+
Dorsomarginal kineties	+	+	+	+	–
Dorsal kinety fragmentation	+	+	–	–	–

The usual states are shown because there are deviating genera in all families, for instance, in the Oxytrichidae, where *Gonostomum* lacks dorsal kinety fragmentation. *Pseudokeronopsis* is considered to represent a family of its own. From various sources all cited in Berger (1999, 2001, 2006) and Eigner (2001).

[a] +, yes, present, –, no, not present, different.

[b] Including holostichids.

Caudiholosticha, a "weak" genus based on a combination of plesiomorphies. Unfortunately, all other species assigned to this genus by Berger (2003) likely lack dorsomarginal kineties, and thus cannot be classified into the Rigidotrichidae. Consequently, *H. stueberi* should be combined with *Uroleptus*; *Caudiholosticha* would fall into synonymy with *Uroleptus*; and a new genus would be required for the remaining *Caudiholosticha* species. However, we do not perform all these changes, but prefer to await further investigations of the species involved and of the stichotrichs in general.

Afrophrya nov. gen.

Diagnosis: Flexible (?) Rigidotrichidae with comparatively widely spaced pairs of midventral cirri orally and postorally; buccal cavity and undulating membranes in *Cyrtohymena* pattern.

Type species: *Afrophrya camerounensis* (Dragesco, 1970) nov. comb. (basionym: *Holosticha camerounensis* Dragesco, 1970). Further species assignable: *A. macrostoma* (Dragesco, 1970) nov. comb. (basionym: *Pleurotricha macrostoma* Dragesco, 1970).

Etymology: Composite of the Latin noun Africa (the continent in which the species was discovered) and the Greek noun ophrya (cilium). Feminine gender.

Rigidothrix goiseri as a new species

As concerns body shape and size, the stylonychid frontal area, and the cirral pattern, there are several species which resemble *R. goiseri*, especially *Uroleptus* spp. (for comprehensive reviews, see Berger (1999, 2006) and Kahl (1932)).

Uroleptus sphagni Stokes is flexible (vs. rigid) and the tail is contractile (vs. acontractile); further, it has only one micronucleus attached to the two macronucleus nodules (vs. a micronucleus attached to each of the macronucleus nodules). *Uroleptus novitas* Horvath has a flexible body (vs. rigid) and a very short (vs. rather long) tail. *Uroleptus magnificus* Kahl is 400–500 μm long (vs. up to 350 μm), lacks a pronounced (vs. distinct) tail, and has five conspicuous (vs. two inconspicuous) transverse cirri.

Among the Oxytrichidae, *Ancystropodium maupasi* Fauré-Fremiet, a very rare freshwater species from Europe, is similar to *R. goiseri*, but likely has a gastrostylid (vs. urostylid) cirral pattern, a stalk-like (vs. continuous) tail, a single micronucleus in between the two macronucleus nodules (vs. a micronucleus attached to each of the two macronucleus nodules, Figs 1, 14, 28 and 32). Further, some *Urosoma* species and amphisiellids resemble *R. goiseri*, but all are highly flexible (vs. conspicuously rigid) and are narrowed anteriorly (vs. widened). When the tail has been lost,

Table 3. Comparison of main features in stichotrichs with midventral rows

Taxa	Body	Undulating membrane pattern	Frontal cirral pattern	Postoral midventral pattern	Dorso-marginal kineties	Classification
Rigidothrix nov. gen.	Rigid	Oxytrichid	Oxytrichid	Distinct	Present	Rigidotrichidae nov. fam.
Territricha Berger and Foissner, 1988	Flexible	Oxytrichid	Oxytrichid	Indistinct	Present	
Uroleptus Ehrenberg, 1831	Flexible	Oxytrichid	Oxytrichid	Distinct	Present	
Holosticha stueberi Foissner, 1987a	Flexible[a]	Oxytrichid	Oxytrichid	Distinct	Present[a]	
Pattersoniella Foissner, 1987b	Rigid	Oxytrichid	Bicorona	Indistinct	Present	Pattersoniellidae Shi et al., 1999
Neokeronopsis Warren et al., 2002	Flexible	Cyrtohymenid	Bicorona	Distinct	Present	
Bicoronella Foissner, 1995	Flexible	Oxytrichid	Bicorona	Distinct	Lacking	Urostylidae[b] Bütschli, 1889
Holosticha Wrzesniowski, 1877	Flexible	Oxytrichid	Oxytrichid	Distinct	Lacking	
Urostyla Ehrenberg, 1830	Flexible	Oxytrichid	Bicorona	Distinct	Lacking	

[a]Foissner (unpublished data).
[b]The three genera mentioned are only examples (see Berger (2006) for a comprehensive review).

R. goiseri might resemble one of the large *Stylonychia* species, for instance, *S. mytilus*.

Rigidothrix goiseri as a biogeographic flagship

Species with conspicuous size, morphology and/or colour are called "flagship taxa". They are the elephants of the microscopic world. Tyler (1996) has summarized the reasons why such taxa have the greatest probability of real endemism: "Because they are so showy, or so novel, it is unlikely that such species would be overlooked if indeed they were widely distributed. If the Australian endemics occurred in Europe or North America then they would have been seen there, long ago".

Undoubtedly, *R. goiseri* is such a flagship species (Figs 1 and 9). We did not find it in over 1000 soil samples (Foissner 1998; Foissner et al. 2002), including about 40 samples from several large flood plains (Amazon in Brazil, Murray River in Australia, Danube River in Austria, Rio Corobici in Costa Rica, Bukaos River in Namibia, Chobe River in Botswana). Further, we did not observe it in over 1000 samples from stagnant and running waters of Austria and Germany. Thus, *R. goiseri* is probably an African or, at least Gondwanan endemic.

Considering the conspicuousness of *R. goiseri* and other stichotrichine flagships described recently, such as *Eschaneustyla lugeri* (Foissner et al. 2002), *Cyrtohymena* (*Cyrtohymenides*) *aspoecki* (Foissner 2004), and *Saudithrix terricola* (Berger et al. 2006), our ignorance about stichotrichine diversity becomes more and more obvious. Likely, there are thousands of undescribed stichotrichs, including even such conspicuous species as those mentioned above, considering the many new genera and species described in the past decade (for reviews, see Berger 1999, 2006; Foissner 1998; Foissner et al. 2002, 2005; Song and Wang 1999). This applies also to ciliates in general (Berger 1999, 2006; Chao et al. 2006; Foissner 1998; Foissner and Xu 2006; Song and Wang 1999). Thus, we disagree with the statement of Finlay et al. (1996) that "the majority of ciliate species in the more frequently studied habitats have probably already been discovered". There are no such habitats! Whether one looks in ponds of Germany (Kreutz and Foissner 2006; Song and Wilbert 1989), in ordinary soil globally (Foissner 1998; Foissner et al. 2002, 2005), in floodplain soils (Foissner et al. 2002), in the Chinese Sea (Song and Wang 1999), or the French mesopsammon (Dragesco 1999), all these habitats contain numerous undescribed ciliate species.

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