

Apogastrostyla rigescens (Kahl, 1932) gen. nov., comb. nov. (Ciliophora, Hypotricha): Morphology, Notes on Cell Division, SSU rRNA Gene Sequence Data, and Neotypification

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Summary. The morphology, the infraciliature, some stages of cell division and physiological reorganization, and the SSU rRNA gene sequence of the little-known marine 18-cirri hypotrich *Tachysoma rigescens* (Kahl, 1932) Borror, 1972 [basonym *Oxytricha* (*Tachysoma*) *rigescens*], isolated from mariculture waters near Qingdao, China, were investigated. This rare species is characterized, inter alia, by narrowly spaced, small, colourless cortical granules and several conspicuous ring-shaped structures in the cytoplasm. The caudal cirri and the simple dorsal kinety pattern (three bipolar kineties) are probably plesiomorphic traits within the Hypotricha, the composition of the adoral zone of the proter from new and parental membranelles, as well as the presence of two ‘extra’ cirri behind the right marginal row strongly suggest a misclassification in *Tachysoma*. The SSU rRNA gene sequence data indicate that *T. rigescens* branches off rather basally in the Hypotricha tree, which supports the hypothesis that the 18-cirri pattern occurred very early, probably already in the last common ancestor of the Hypotricha. A detailed survey of the early branching 18-cirri hypotrichs and similar taxa (e.g. *Trachelostyla pediculiformis*, *Hemigastrostyla enigmatica*, *Protogastrostyla pulchra*) reveals that for *T. rigescens* a new genus (*Apogastrostyla* gen. nov.) has to be established, because there are important differences, inter alia, in the dorsal infraciliature. Besides the type species, *A. rigescens* comb. nov., which seems to be confined to the northern hemisphere according to the sparse faunistic data, a second marine species, *A. szaboi* comb. nov. (basonym *Hemigastrostyla szaboi*), so far only twice recorded from the Antarctic region, can be included. The Chinese population is fixed as neotype to define the species objectively, because no type material of *A. rigescens* is present and the original type locality is not known. The species name *Tachysoma multinucleate* is emended: *Tachysoma multinucleatum* nom. corr.

Key words: China, ecology, *Hemigastrostyla*, infraciliature, ontogenesis, Spirotricha, Stichotrichia, Yellow Sea.

INTRODUCTION

Tachysoma rigescens (Kahl, 1932) Borror, 1972 is a very little known marine hypotrichous ciliate. Kahl (1932) made rather detailed live observations, but could not recognize the exact cirral pattern of the rear body portion, that is, he did not unambiguously see whether caudal cirri are lacking ('marginal rows open posteriorly') or present ('marginal rows closed'). He preliminarily classified it in the subgenus *Oxytricha* (*Tachysoma*) Stokes, 1887 because he supposed that the marginal rows are separated (Kahl 1932, 1933). Aladro Lubel and López-Ochoterena (1967) and Aladro Lubel *et al.* (1990) described a brackish water population from Mexico, however, without providing new details about the infraciliature. Since then, no redescription has been published according to the review by Berger (1999).

During a faunal survey of coastal waters of northern China, we found this rare species and could study its morphology and cell division and analyse the SSU rRNA. The results largely confirm the sparse observations of the original description, but the new data on the dorsal infraciliature and the morphogenesis clearly demonstrate that it is misclassified in *Tachysoma* and in all other genera established so far. Thus, we fix it as type species of the new genus *Apogastrostyla*. The molecular data allow a rough estimation of the phylogenetic position, and since no type material is available and the type locality is not known, we fix the Chinese population as neotype.

MATERIALS AND METHODS

Sample site and cultivation

For details on the collecting site of the Chinese population, see section occurrence and ecology. The ciliates were collected via an artificial substrate, that is, slides were fixed to a frame and immersed to a depth of 1 m for about 10 d to allow colonization (Gong *et al.* 2005). Following retrieval of the slides, ciliates were isolated and cultures were established at room temperature (20°C) in Petri dishes containing filtered seawater with squeezed rice grains to enrich the bacterial food.

Morphology and morphogenesis

Ciliates were examined using bright field and differential interference contrast microscopy. The protargol silver staining method according to Wilbert (1975) was used to reveal the infraciliature. All drawings of the infraciliature were performed at a magnification of 1250 × with the aid of a drawing tube. To demonstrate the changes

occurring during morphogenesis and reorganisation, parental structures are depicted by contour whereas new ones are shaded black.

General terminology is mainly according to Lynn (2008); for explanation of group-specific terms, see Berger (1999, 2006, 2008) and Foissner and Al-Rasheid (2006); for the designation of the frontal-ventral-transverse cirri and anlagen, the numbering system by Wallengren (1900) is used (details see Berger 1999: 16). The term 18-cirri hypotrich means a hypotrich with 18 frontal-ventral-transverse cirri (e.g. Berger 2008: 27). The mixed row (Berger 2008: 2) formed by the three frontoventral and all postoral ventral cirri is termed median cirral row because it originates from more than one anlage (Eigner & Foissner 1994, Berger 2008). For authorship and date of scientific names, see Berger (2001, 2006, 2008: 24).

SSU rRNA gene sequence, sequence availability, and phylogenetic analyses

Genomic DNA extraction, PCR amplification, and SSU rRNA gene cloning and sequencing were performed according to the method described by Yi *et al.* (2009).

The SSU rRNA gene sequences used for the analyses were obtained from the GenBank database (accession numbers see Fig. 6). The "euplotids" comprise the following species: *Aspidisa steini* (AF305625); *Certesita quadrinucleata* (DQ059581); *Euplotes aediculatus* (AF164136); *Euplotes vannus* (AY361856). The sequences were aligned with Clustal W implemented in Bioedit 7.0 (Hall 1999). The alignment was then modified by removing both termini of the alignment and highly variable regions. *Protocruzia contrax* was used as outgroup. A maximum parsimony (MP) tree was obtained via random addition and swapped using the tree-bisection-reconnection (TBR) algorithm. Parameters for the MP tree were the following: heuristic search, 100 random-addition sequences, and tree-bisection-reconnection (TBR) branch swapping. Gaps were treated as missing data. MP analysis was performed with the software package PAUP* 4.0b10 (Swofford 2002), and the support for the internal branches was estimated using the bootstrap method with 1000 replicates. The program MrModeltest v.2.0 (Nylander 2004) selected the GTR+I+G as the best model according to the AIC criterion, which was then used for Bayesian (BI) analysis. BI analysis was performed with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) using the Markov chain Monte Carlo algorithm. The program was run for 1,000,000 generations with a sample frequency of 100 and a burn in of 2500. Neighbor-joining (NJ) analysis was conducted using the program package MEGA 4 (Tamura *et al.* 2007). Distances were estimated by the NJ method using the Tamura-Nei (TrN) model of substitution and support was assessed using 1000 bootstrap replicates (Tamura and Nei 1993).

RESULTS AND DISCUSSION

Apogastrostyla gen. nov.

Diagnosis: Marine 18-cirri hypotrichs with more or less distinctly narrowed (cephalized) anterior body portion and undulating membranes roughly in *Stylonychia* pattern. Distal portion of adoral zone far

extending posteriorly. Most frontoventral cirri and postoral ventral cirri form median cirral row. One left and one right marginal row. Two 'extra' cirri behind right marginal row. Three bipolar dorsal kineties. Caudal cirri present. Adoral zone of proter composed of newly formed proximal and central portion and parental distal portion. Frontal-ventral-transverse cirri originate from anlagen I–VI. Dorsal morphogenesis in *Gonostomum*-pattern, that is, intrakinetal proliferation within all parental rows; kinety fragmentation and dorsomarginal kineties lacking.

Type species: *Oxytricha (Tachysoma) rigescens* Kahl, 1932.

Nomenclature: Composite of the Greek prefix *apo-* (from, off, away, after, without, separate; Brown 1954) and the genus-group name *Gastrostyla* Engelmann, 1862 (for derivation see Berger 2008: 136). It alludes to the fact that the frontoventral and postoral ventral cirri form a row similar to that in *Gastrostyla*. Like *Gastrostyla* feminine gender (Aescht 2001: 283).

Species assignable: (1) *Apogastrostyla rigescens* (Kahl, 1932) comb. nov. [type; basionym *Oxytricha (Tachysoma) rigescens* Kahl, 1932]; (2) *Apogastrostyla szaboi* (Wilbert and Song, 2005) comb. nov. (basionym *Hemigastrostyla szaboi* Wilbert and Song, 2005).

Remarks: For separation from related and similar genera and phylogenetic position, see below.

***Apogastrostyla rigescens* (Kahl, 1932) comb. nov. (Figs 1a–f, 2a–k, 3a–d, 4a–c, 5a–m, 6; Table 1)**

- 1932 *Tachysoma*? (ev. *Oxytricha*) *rigescens* spec. n. – Kahl, *Tierwelt Dtl.* **25**: 605, Fig. 116₁₂ (original description, no type material available; correct basionym, see nomenclature).
- 1933 *Tachysoma rigescens* Kahl 1932 – Kahl, *Tierwelt N. -u. Ostsee* **23**: 113, Fig. 17.19 (guide to marine ciliates).
- 1967 *Tachysoma rigescens* Kahl, 1932 – Aladro Lubel and López-Ochoterena, *Revta Soc. mex. Hist. nat.* **28**: 63, Fig. 15 (description of Mexican population).
- 1972 *Tachysoma rigescens* Kahl, 1932 – Borror, *J. Protozool.* **19**: 15 (classification of hypotrichs; combination with *Tachysoma*, see nomenclature).
- 1990 *Tachysoma rigescens* Kahl, 1932 – Aladro Lubel, Martínez Murillo and Mayén Estrada, *Manual de ciliados*, p. 139, figure on page 139 (review of marine ciliates from Mexico).
- 1999 *Tachysoma rigescens* (Kahl, 1932) Borror, 1972 – Berger, *Monographiae Biol.* **78**: 461, Fig. 141a–c (detailed review).

Improved diagnosis: The following diagnosis is solely based on the neotype material, which agrees very well with the population described by Kahl (1932): Body size 130–180 × 30–60 μm *in vivo*.

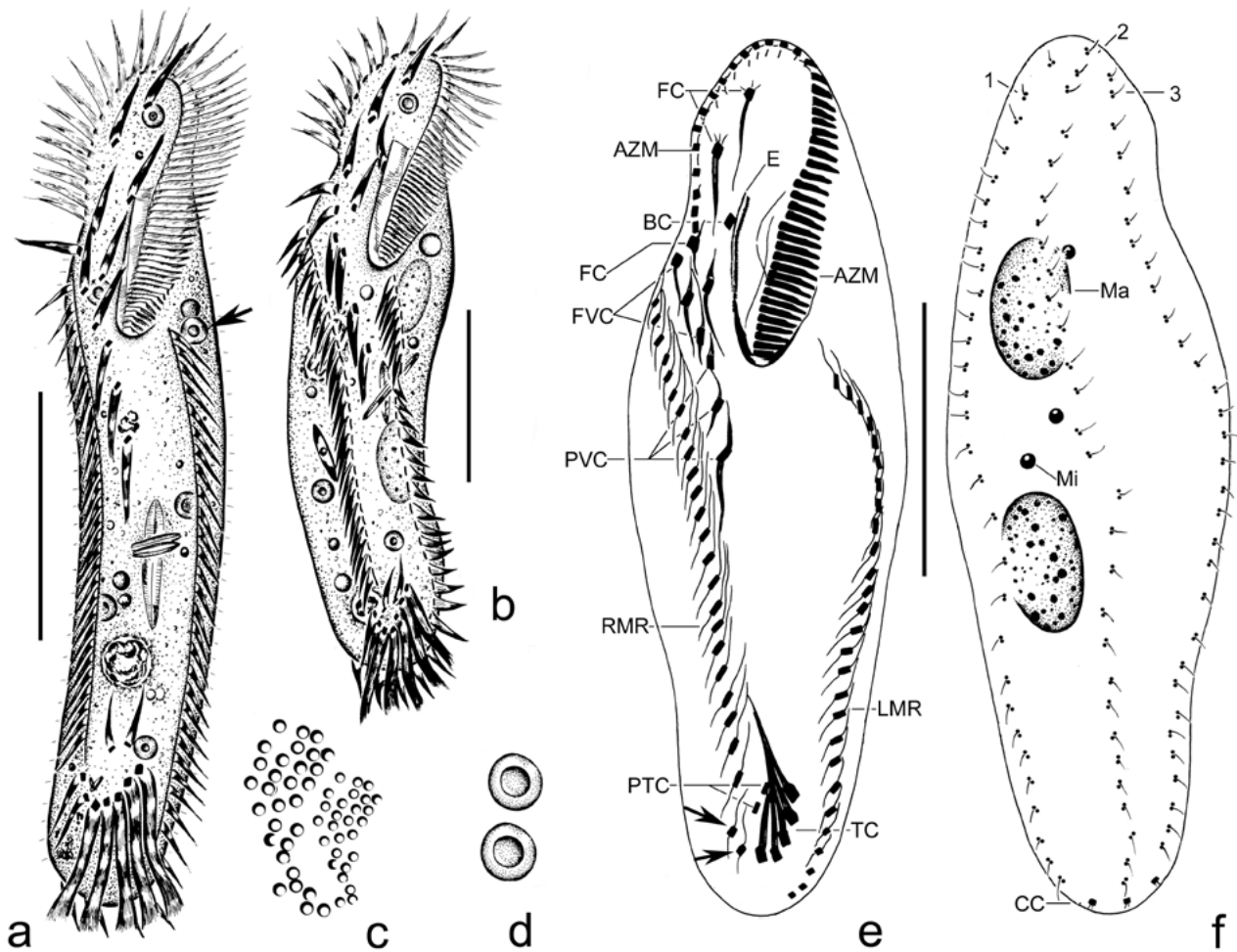
Body elongate-rectangular, slightly brownish at low magnification. Cortex only slightly flexible, packed with 0.3–0.5 μm-sized, colourless cortical granules. Several ring-shaped cytoplasmic inclusions. On average 43 adoral membranelles and 23 right and 27 left marginal cirri. Each dorsal kinety with one caudal cirrus. Two macronuclear nodules and 1–4 micronuclei. Contractile vacuole lacking.

Nomenclature: Kahl (1932: 601) classified *Tachysoma* as subgenus of *Oxytricha*. Thus, the correct basionym is *Oxytricha (Tachysoma) rigescens* Kahl, 1932. The somewhat confusing name "*Tachysoma?* (ev. *Oxytricha*) *rigescens*" in Kahl (1932: 605) should simply indicate that he was uncertain about the subgeneric assignment. Later workers (see list of synonyms) overlooked the fact that *Tachysoma* had subgenus rank in Kahl (1932) and therefore incorrectly assumed that Kahl has established this species in the genus *Tachysoma*. Berger (1999) fixed Borror (1972) as combining author, although Borror did not formally transfer it to *Tachysoma*. Borror (1972) – and not Aladro Lubel and López-Ochoterena (1967) – was selected because he was the first reviser.

No derivation of the species-group name was given in the original description and the review by Berger (1999). The name *rigescens* (Latin active verb participle; stem: *rigescent*; stiff) likely alludes to the fact that this species rests motionless ('stiff') on the debris for a rather long time (Kahl 1932). *Tachysoma rigescens* in Chen *et al.* (2009) is an incorrect subsequent spelling.

Deposition of neotype material: The slide containing the protargol-impregnated neotype specimen (Fig. 1e, f) has been deposited in the Natural History Museum, London, UK, with registration number 2010:1:30:5. Two further protargol-impregnated slides (LLQ-2007-09-24-01-1, LLQ-2007-09-24-01-2) of the neotype population have been deposited in the Laboratory of Protozoology, Ocean University of China, Qingdao 266003, China. Details on neotypification see below. The SSU rRNA gene sequence has been submitted to the GenBank database (accession number FJ870089).

Morphology of neotype population (Figs 1a–f, 2a–k, 5a–h; Table 1): Body size *in vivo* about 155 × 45 μm on average, length:width ratio 3.4–5.5:1 *in vivo* (Fig. 2a, f), on average 3.5:1 in protargol preparations (Table 1). Body outline elongate-rectangular to elliptical with both ends broadly rounded; both margins almost straight and parallel; frontal portion more or less distinctly narrowed causing



Figs 1a–f. Morphology of neotype population of *Apogastrostyla rigescens* (a–d, from life; e, f, protargol impregnation). **a, b** – ventral views of representative individuals. Arrow in (a) indicates a ring-shaped structure; **c** – colorless, globular cortical granules 0.3–0.5 μm across; **d** – ring-shaped structures about 6 μm in diameter; **e, f** – infraciliature of ventral and dorsal side and nuclear apparatus of neotype specimen. Arrows mark the ‘extra’ cirri behind the right marginal row. Note the median cirral row which is formed by three frontoventral cirri and all postoral ventral cirri. AZM – adoral zone of membranelles, BC – buccal cirrus, CC – caudal cirri, E – endoral, FC – frontal cirri, FVC – frontoventral cirri, LMR – left marginal row, Ma – anterior macronuclear nodule, Mi – micronucleus, PTC – pretransverse ventral cirri, PVC – postoral ventral cirri, RMR – right marginal row, TC – transverse cirri, 1–3 – dorsal kineties. Scale bars: 50 μm .

a cephalization. Body shape slightly variable due to different nutritional status (Figs 1a, b, 2a–e). Body about 2:1 flattened dorsoventrally; moderately rigid, that is, only slightly flexible and contractile. Invariably two ellipsoidal macronuclear nodules in central body portion slightly left of midline (Figs 1b, f, 2j, 5a, b; Table 1). 1–4 spherical micronuclei arranged close to and/or between macronuclear nodules (Fig. 1f). No contractile vacuole recognizable. Cortex coarse and relatively thick. Cells appear somewhat ferruginous under low magnification and slightly brownish when observed under high magnification; reason for

color not known in detail, perhaps due to food (e.g. diatoms) and/or some light refraction of the cortical granules, although they are more or less colourless (Fig. 2a–e, j); individual granules globular (0.3–0.5 μm across), tightly packed all over the cell in more or less distinct short rows except along cirral rows (Figs 1c, 2g, k); do not impregnate with the protargol method used; stainability with methyl-green pyronin not checked. Cytoplasm transparent to opaque, contains numerous food vacuoles, other globules, and several (usually less than 10) ring-shaped structures (‘hollow’ spheres, lithosomes?) about 6 μm in diameter (Figs 1a, d, 2b,

Table 1. Morphometric data on neotype population of *Apogastrostyla rigescens*.^a

Characteristics	Min	Max	Mean	SD	SE	CV	n
Body, length	124	180	148.6	13.9	2.8	9.4	25
Body, width	30	56	42.1	7.0	1.4	16.6	25
Adoral zone of membranelles, length	48	78	60.6	6.7	1.3	11.1	25
Adoral membranelles, number	37	51	42.9	3.2	0.6	7.5	25
Buccal cirri, number	1	1	1.0	0.0	0.0	0.0	25
Frontal cirri, number	3	3	3.0	0.0	0.0	0.0	25
Frontoventral cirri, number	4	4	4.0	0.0	0.0	0.0	25
Postoral ventral cirri, number	3	3	3.0	0.0	0.0	0.0	25
Pretransverse ventral cirri, number	2	2	2.0	0.0	0.0	0.0	25
Transverse cirri, number	5	5	5.0	0.0	0.0	0.0	25
Left marginal cirri, number	23	31	27.2	2.1	0.4	7.7	25
Right marginal cirri, number	17	27	22.0	2.3	0.5	10.5	25
'Extra' cirri, number	2	2	2.0	0.0	0.0	0.0	25
Dorsal kineties, number	3	3	3.0	0.0	0.0	0.0	25
Macronuclear nodules, number	2	2	2.0	0.0	0.0	0.0	25
Micronuclei, number	1	4	2.4	0.8	0.0	33.0	25
Macronuclear nodules, length	14	22	17.1	2.4	0.5	14.0	25
Macronuclear nodules, width	6	11	8.5	1.6	0.3	18.8	25

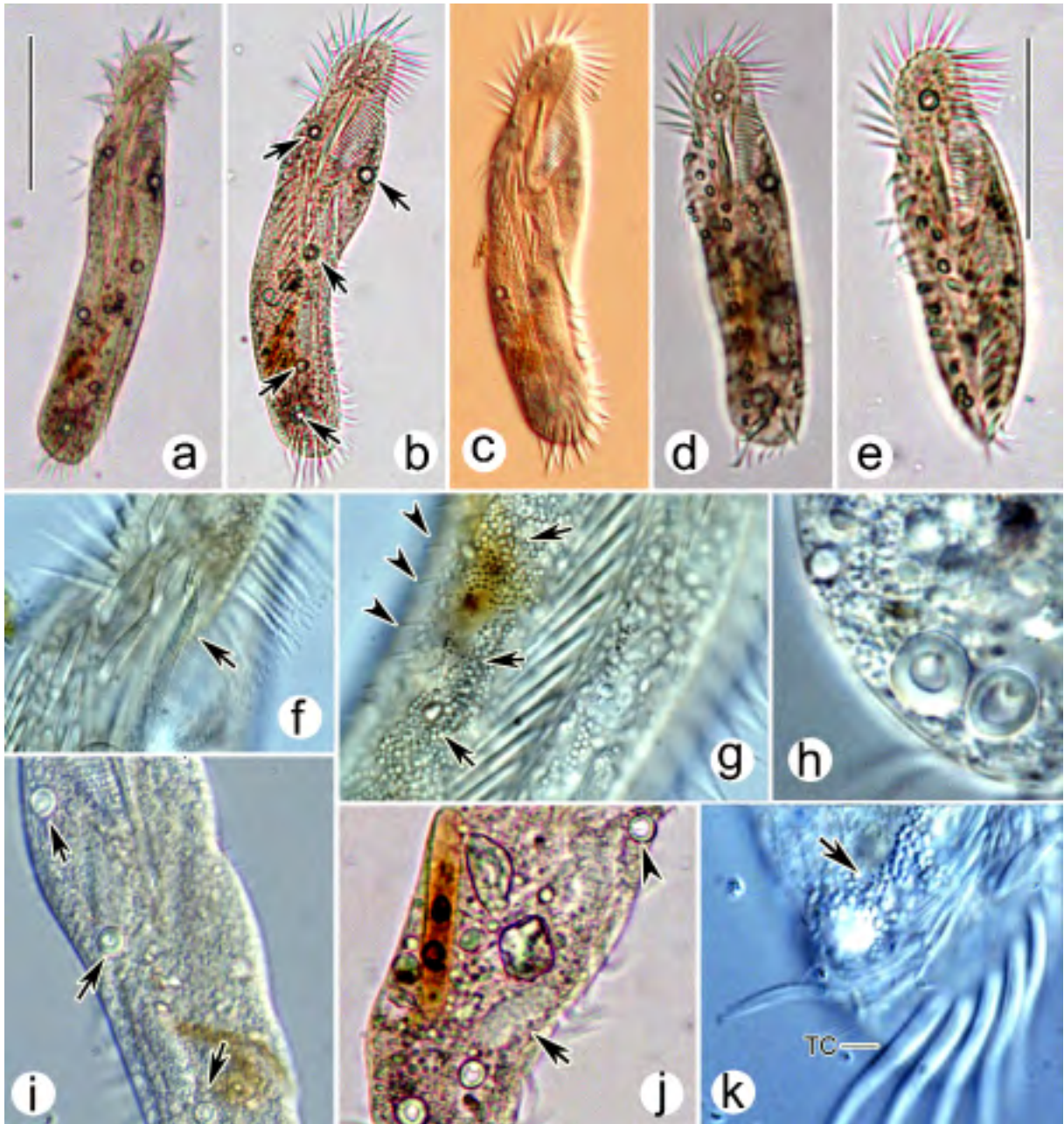
^a All data are based on protargol-impregnated specimens. Measurements in μm . CV – coefficient of variation in %; Max – maximum; Mean – arithmetic mean; Min – minimum; n – number of specimens investigated; SD – standard deviation; SE – standard error of arithmetic mean.

h–j). Crawls moderately fast on debris or on bottom of Petri dish, sometimes standing still for a while.

Adoral zone occupies about 30–40% of body length in life, 40% on average in protargol preparations, composed on average of 43 membranelles of ordinary fine structure; distal portion of zone extends far posteriorly on right side, that is, DE-value (Berger 2006: 18) in neotype specimen 0.59 (Fig. 1e). Central and proximal portion of adoral zone extend obliquely backwards towards cell midline (Fig. 1a, b, e). Bases of largest membranelles about 10 μm long, those of distal membranelles distinctly shorter (Figs 1a, b, e, 2b, c, 5a–e). Buccal field wedge-shaped, that is, rather narrow in life (Figs 1a, b, 2a–e); in specimens impregnated with Wilbert-method relatively wide due to inflation (Figs 1e, 5a–e). Paroral and endoral straight to slightly curved, arranged in parallel or slightly intersecting optically, almost of equal length, that is, undulating membranes roughly in *Stylonychia*-pattern; paroral somewhat thicker than endoral (Figs 1e, 2f). Pharyngeal fibers extend backwards (Fig. 5e, f).

Eighteen frontal-ventral-transverse cirri pattern very stable (Figs 1e, 2c, 5a–e; Table 1). Frontal cirri only

slightly enlarged, arranged along posteriorly extending distal portion of adoral zone with right cirrus (= cirrus III/3) at distal end of zone (Figs 1e, 2c, 5e). Buccal cirrus slightly behind anterior end of paroral (Figs 1e, 5e). Frontoventral cirrus III/2 (= parabuccal cirrus) behind right frontal cirrus about at level of rear frontoterminal cirrus. Frontoventral cirri VI/4, VI/3, and IV/3 and postoral ventral cirri (IV/2, V/4, V/3) form rather distinct median row extending to 48% of body length in neotype specimen (Fig. 1e). Pretransverse ventral cirri almost longitudinally arranged right of anteriormost transverse cirri, relatively small compared to remaining cirri. Transverse cirri rather prominent, slightly subterminally arranged in J-shaped pattern, protrude distinctly (30–50%) beyond rear end of cell *in vivo* (Figs 1a, b, e, 2b, c, k, 5a). Cirri on frontoventral area about 12–15 μm long *in vivo*, transverse cirri about 18–20 μm . Right marginal row shortened anteriorly, that is, commencing about at level of cirrus III/2, terminates about at level of anteriormost transverse cirrus. Invariably two 'extra' cirri distinctly off-set from rear end of right marginal row and to the right of rearmost transverse cirri (Figs 1e, 5h; see also cell division). Left marginal row begins



Figs 2a–k. Photomicrographs of neotype population of *Apogastrostyla rigescens* (a, b, d, e, j, bright field; c, f–i, k, differential interference contrast). **a–d** – ventral views of different individuals, showing general body shape, parts of cirral pattern (c), and ring-shaped structures (arrows in b) within cytoplasm. Note that some specimens (b, c) are distinctly twisted about main body axis; **e** – ventral view of a stout specimen; **f** – ventral view of buccal field, showing, inter alia, the paroral (arrow) and the far posteriorly extending distal end of the adoral zone; **g** – ventrolateral view of middle portion showing dorsal bristles (arrowheads) and cortical granules (arrows); **h** – rear body portion with two ring-shaped structures; **i** – dorsal view demonstrating the distribution of the ring-shaped structures (arrows); **j** – ventral view of a slightly squeezed specimen. Arrow marks macronuclear nodule, arrowhead denotes a ring-shaped structure; **k** – ventral view of posterior body portion. Arrow marks cortical granules. The cirrus right of the transverse cirri is one of the two ‘extra’ cirri. TC – transverse cirri. Scale bars: 50 μm .

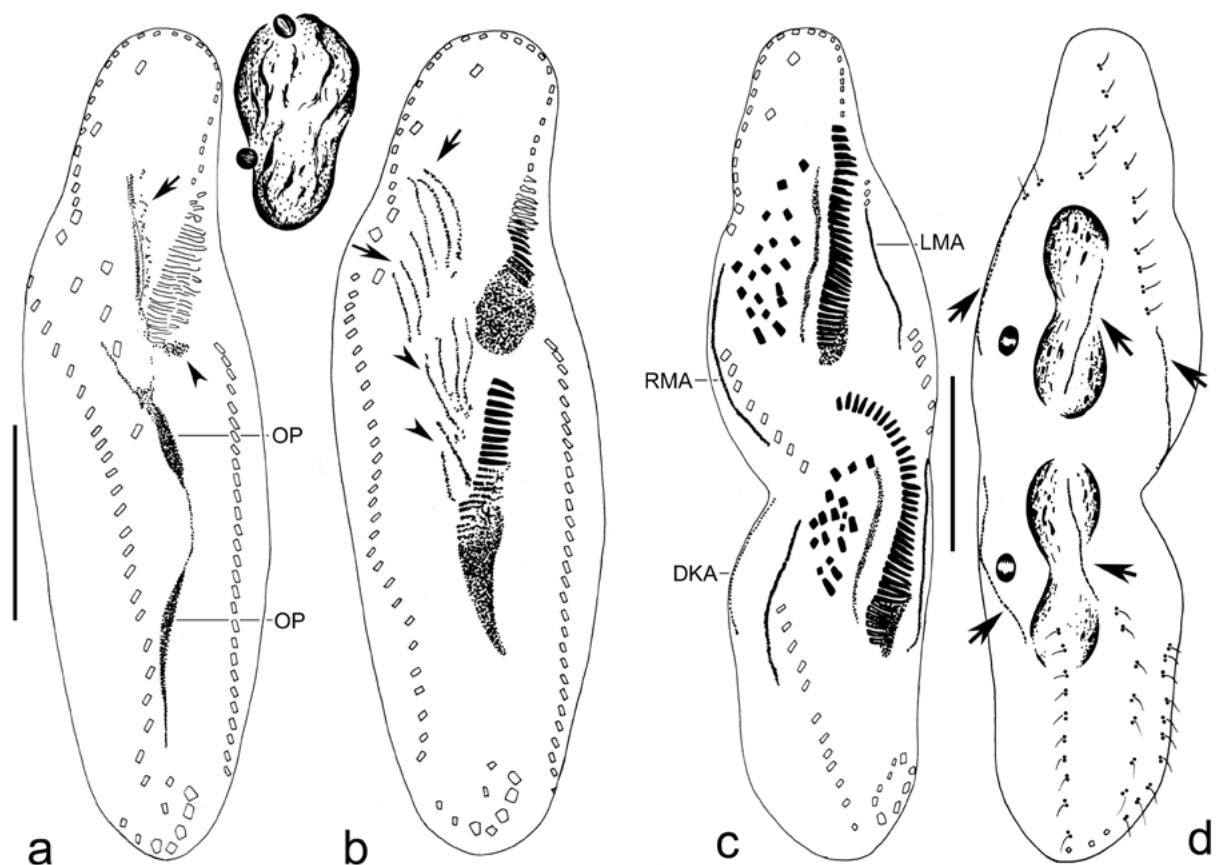
near to buccal vertex, terminates near rear cell end and thus optically confluent with caudal cirri (Figs 1a, b, e, 2c, 5a, h). Marginal cirri strong, but only about 10 μm long in life and therefore almost not protruding beyond body margin; densely arranged in crenellated furrow and associated with prominent fibers (Figs 1e, 5a–d).

Dorsal bristles about 3–4 μm long, invariably arranged in three bipolar kineties (Figs 1f, 2g, 5g; Table 1); each kinety with one relatively fine caudal cirrus terminating about at same level as transverse cirri and therefore caudal cirri not easily recognizable *in vivo* (Figs 1e, 2c, 5g).

Notes on cell division (Figs 3a–d, 4a, b, 5i–l): We found only few dividers showing four different stages, which are, however, almost evenly distributed about the whole morphogenetic sequence. Thus, some interesting features of this part of the life cycle can be described.

The earliest stage observed shows a long, roughly dumbbell-shaped oral primordium extending from near the left pretransverse ventral cirrus to the postoral ventral cirri (Fig. 3a). One postoral ventral cirrus (V/4?) obviously contributes to primordium formation. A long, fine streak extends anteriorly into the area right of the anteriormost postoral ventral cirrus. The central and proximal portions of the parental adoral zone become disintegrated, and in the buccal cavity at the proximal end of the zone, a small patch of basal bodies appears, obviously the oral primordium of the proter. A further anlage occurs in the buccal cavity left of the parental undulating membranes, which dissolve in the next stages (Fig. 3b). The macronuclear nodules are not yet fused in this stage.

In a middle divider, six roughly longitudinally arranged frontal-ventral-transverse cirri anlagen are recog-

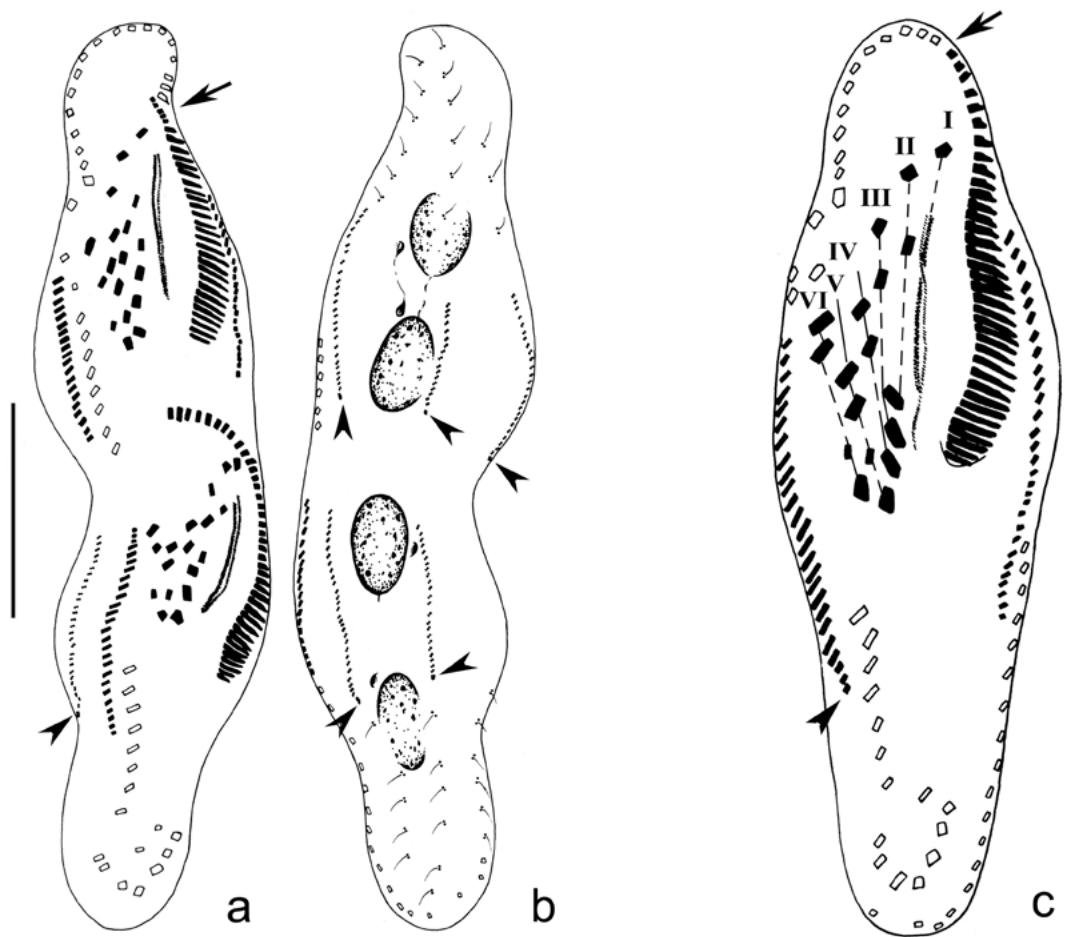


Figs 3a–d. Morphogenesis of neotype population of *Apogastrostyla rigescens* (protargol impregnation). **a** – ventral view of an early divider. Arrow marks the dedifferentiated parental undulating membranes; arrowhead indicates the oral primordium of the proter at the proximal end of the partly disorganized parental adoral zone; **b** – ventral view of a middle divider. Arrows and arrowheads mark the anlagen for both dividers. Inset: the fused macronucleus and two micronuclei; **c**, **d** – ventral and dorsal view of late divider. Arrows in (d) mark the anlagen for the dorsal kineties. DKA – dorsal kinety 3 anlage of opisthe, LMA – left marginal row anlage of proter, OP – oral primordium, RMA – right marginal row anlage of proter. Scale bars: 40 μm (a), 30 μm (c, d).

nizable in both the proter and the opisthe (Figs 3b, 5i). We do not know whether these anlagen are formed independently in the proter and the opisthe or via so-called primary primordia, that is, from common anlagen which later divide into an anterior and posterior portion. The parental frontoventral cirri (except frontoterminal cirri), the buccal cirrus, and the postoral ventral cirri have been modified to anlagen or are already dissolved. The formation of new adoral membranelles proceeds from the front backwards in both oral primordia. The two macronuclear nodules are fused to a single mass (Fig. 3b; inset).

Later, the adoral zone of the opisthe bends to the right anteriorly, while the new zone of the proter is still stretched (Fig. 3c, d). The frontal-ventral-transverse

cirri anlagen I–VI segregate the ordinary number of 18 cirri, that is, from left (anlage I) to right (anlage VI) one, three, three, three, four, and four cirri are formed (some small basal body patches are later reabsorbed; see Fig. 4a). The undulating membrane anlagen (= anlage I), which form – as is usual – the left frontal cirrus, are long and obviously start to separate longitudinally into the paroral and the endoral. Two dorsal kineties anlagen have developed within each of the parental rows (Fig. 3d). Whether the dorsal kinety anlagen of the proter and the opisthe are connected in early stages (primary primordia) or separated since the very beginning is not known. Each one dumbbell-shaped macronucleus is present both in the proter and the opisthe (Fig. 3d).



Figs 4a–c. Morphogenesis and reorganization of neotype population of *Apogastrostyla rigescens* (protargol impregnation). **a, b** – ventral and dorsal view of a late divider. Arrow in (a) marks the transition zone between new and parental portions of the adoral zone of the proter. A caudal cirrus (arrowheads) is formed at the rear end of each kinety, which is a major difference to *Tachysoma*, which lacks caudal cirri; **c** – ventral view of a late reorganizer. The 18 frontal-ventral-transverse cirri originate in the ordinary way, that is, 1, 3, 3, 3, 4, and 4 cirri are formed from the anlagen I–VI. Arrowhead marks rear end of new right marginal row; perhaps the rearmost two cirri become the ‘extra’ cirri. Arrow denotes distal end of new portion of adoral zone. I–VI – frontal-ventral-transverse cirri anlagen. Scale bars: 40 μ m (a, b), 30 μ m (c).

In the latest stage which we found, the newly formed portions of the adoral zone of the proter joins with the parental distal membranelles to form the adoral zone of the proter (Fig. 4a). All adoral membranelles of the opisthe are recognisable. The frontal-ventral-transverse cirri start to migrate to their final positions (Figs 4a, 5j–l) to form the characteristic pattern also known from many other 18-cirri hypotrichs (Fig. 1e; e.g. Fig. 6a in Berger 1999). At this stage, all new marginal cirri are present, but no cirri are off-set from the rear end of the right marginal rows. Thus, we do not know the origin of the ‘extra’ cirri. Basically, only two possibilities exist, namely (i) two parental marginal cirri are retained both in the proter and the opisthe; or (ii) the ‘extra’ cirri are the two rearmost new right marginal cirri. Later stages have to be investigated to answer this question. At the end of each new dorsal kinety one caudal cirrus is formed (Fig. 4b). Both the proter and the opisthe contain two macronuclear nodules.

Notes on physiological reorganization (Figs 4c, 5m): A late reorganizer shows, as expected, basically the same features as a late divider (Fig. 4a), namely, (i) the proximal and central portion of the adoral zone are newly formed and fuse with the distal parental portion; (ii) the anlagen I–VI form the ordinary number/pattern of 18 frontal-ventral-transverse cirri; and (iii) anlage I generates, beside frontal cirrus I/1, the undulating membranes. No cirri are detached from the rear end of the new right marginal row at this state.

Estimation of phylogenetic position based on SSU rRNA gene sequence (Fig. 6): The SSU rRNA gene sequence of *Apogastrostyla rigescens* is 1770 bp long, including the primers.

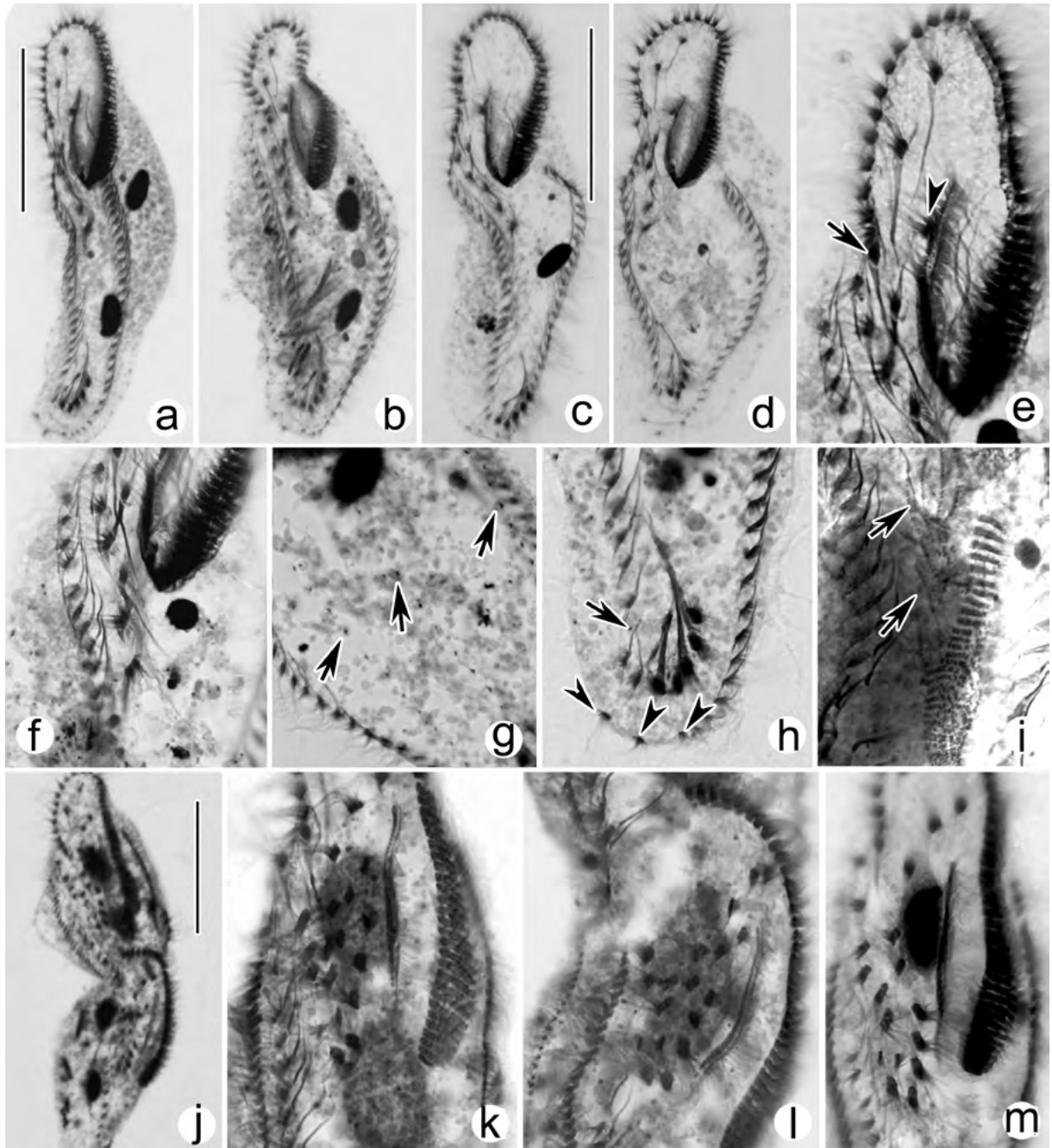
Apogastrostyla rigescens clusters with *Hemigastrostyla enigmatica* with low support in MP and NJ trees (43% MP, 50% NJ), in spite of high support in Bayesian analysis (0.94). This cluster forms a polytomy together with *Amphisiella annulata*, a large group from *Pleurotricha lanceolata* to *Protogastrostyla pulchra* (containing, inter alia, the stylonychines and other oxytrichids, and the core-urostyloids), *Trachelostyla pediculiformis* + *Parabirojimia similis*, and *Pseudoamphisiella alveolata* + *Prodiscocephalus borrori*. The sistergroup to this polytomy are the oligotrichs + *Amphisiella milnei* followed by the holostichids *Holosticha* + *Psammomitra* (Fig. 6). The most relevant information of the analyses is that *Apogastrostyla rigescens* branches off near/at the base of the Hypotricha-tree.

Morphology of previously described populations: Since *Apogastrostyla rigescens* is neotypified

(see below), the available morphological data have to be reviewed in detail (illustrations see Berger 1999: 462).

The original description is rather brief. The following characterization is a composite of the description and the illustration provided by Kahl (1932), both based on live observation only. Body length 150–180 µm, length:width ratio of specimen illustrated about 4.5:1. Body slender elliptical with anterior portion narrowed, only moderately flattened dorsoventrally. Two ellipsoidal macronuclear nodules in left body portion behind buccal vertex. Probably two micronuclei (not illustrated). Contractile vacuole and cortical granules neither mentioned nor illustrated. Specimen illustrated with six ‘ring-shaped reserve bodies.’ Adoral zone prominent, occupies about one third of body length and composed of many membranelles, extending far posteriorly on right side. Marginal cirri short and wide, projecting beyond cell margin only posteriorly. Kahl (1932) mentioned that his illustrations differ from each other concerning the gap between the rear end of the marginal rows. The sole illustration published shows that the marginal rows are distinctly separated, that is, he supposed that caudal cirri are lacking (Kahl 1932: 601; key). Simultaneously, he stated that *Oxytricha (Tachysoma) rigescens* has to be assigned to *Oxytricha (Oxytricha)*, when the marginal rows are ‘closed,’ that is, when caudal cirri are present. Five solid transverse cirri, 30–50% of their length projecting beyond cell end. Dorsal bristles about 3–4 µm long (estimated from illustration). Kahl did not provide further details about the cirral pattern, but the illustration and the classification in *Oxytricha* demonstrate that it has 18 frontal-ventral-transverse cirri. The arrangement of the transverse cirri is somewhat unusual, because the rightmost cirrus is more anterior than the leftmost cirrus; perhaps he noticed the pattern when he observed a specimen from the dorsal side. According to Kahl (1932), the present species attracts attention because it often rests (‘almost as dead’) on the debris for a long time. For problems with the type locality, see section occurrence and ecology. Kahl (1933) in his review on the ciliates from the North Sea and the Baltic Sea provided actually only a simplified redrawing of the original illustration, and he was still uncertain about the subgeneric assignment.

Aladro Lubel and López-Ochoterena (1967) described a population from brackish water in Mexico. In the material and method section they write that permanent preparations using haematoxylin staining, a method previously used to reveal the nuclear apparatus,



Figs 5a–m. Photomicrographs of non-dividers (a–h), dividers (i–l), and a reorganizer (m) of the neotype population of *Apogastrostyla rigescens* (protargol impregnation). **a–d** – ventral views showing infraciliature and nuclear apparatus. Note that the cells are inflated more or less distinctly due to the protargol impregnation method used (for normal body shape, see Figs 1a, b, 2a–d); **e** – ventral view of anterior body portion. Arrowhead marks buccal cirrus, arrow denotes right frontal cirrus (= cirrus III/3) at distal end of adoral zone. Note the fibers emerging from the cirral bases; **f** – ventral view of middle portion. Note that three of the four frontoventral cirri and the postoral ventral cirri from a more or less distinct row; **g** – dorsal view showing the three dorsal kineties (arrows); **h** – ventral view of rear body portion showing the gap (arrow) between the right marginal row and the ‘extra’ cirri; arrowheads mark the fine caudal cirri; **i** – ventral view of central portion of an early divider showing the frontal-ventral-transverse cirri anlagen of the opisthe (arrows); **j** – ventral view of a very late divider; **k, l** – proter (k) and opisthe (l) of same very late divider showing differentiation of new cirri. The new undulating membranes are arranged in parallel; **m** – ventral view of a late reorganizer. Scale bars: 50 μ m.

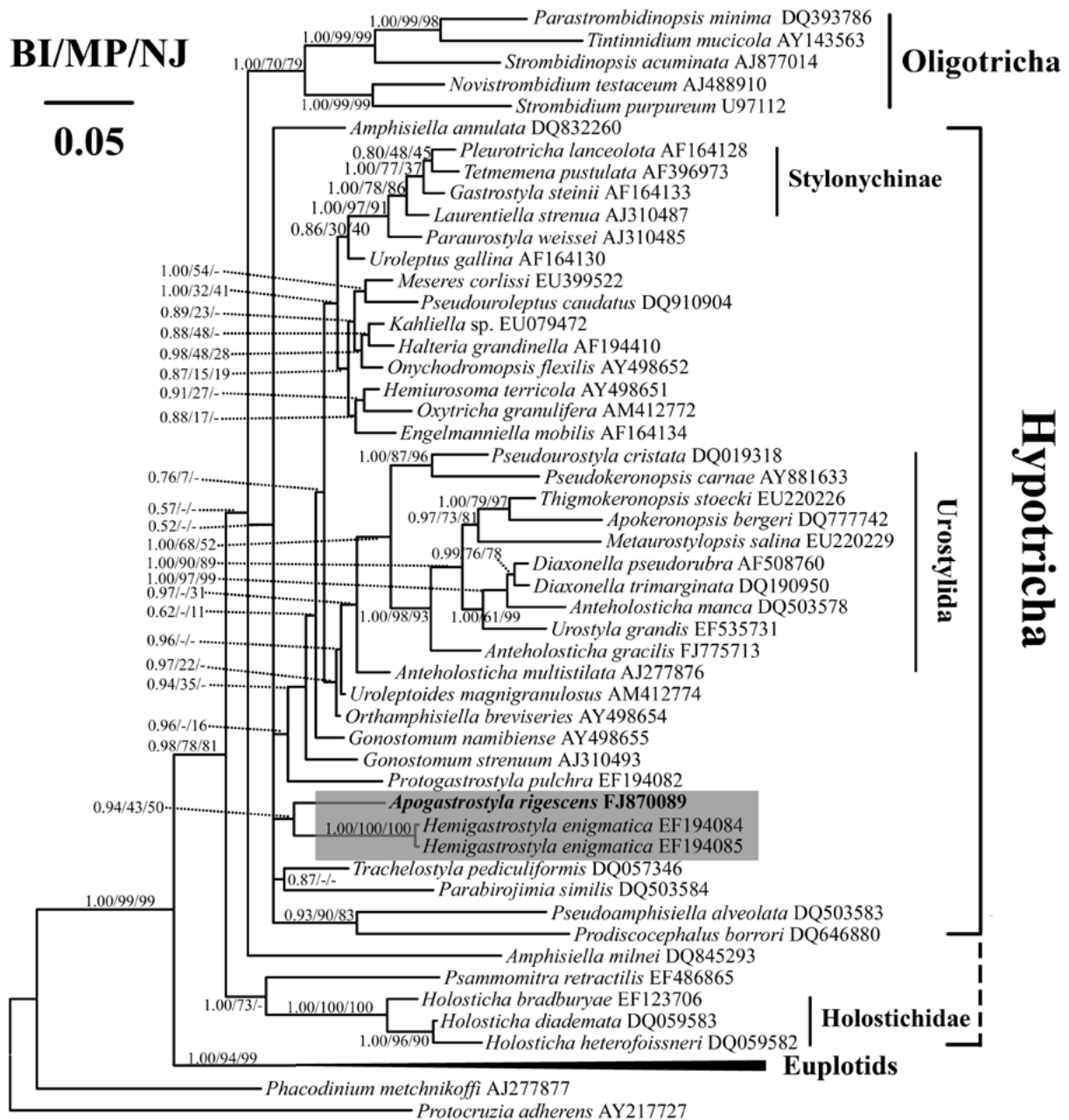


Fig. 6. Bayesian tree inferred from the small subunit ribosomal RNA (SSU rRNA) gene sequences of 54 spirotrichous taxa, showing the position of *Apogastrostyla rigescens* (bold). Nodal support for branches in the Bayesian inference (BI), Maximum parsimony (MP) and neighbor-joining (NJ) trees are marked in order. Clades with different topologies in the MP and NJ trees relative to the BI tree are indicated with “-”. The scale bar corresponds to five substitutions per 100 nucleotide positions. *Protocruzia* was taken as an out-group taxon. Since the position of many species is rather variable in molecular trees, only some stable higher taxa are labeled. The names are adapted to the monograph of hypotrichs (Berger 1999, 2006, 2008).

have been made. The slides have been deposited in the Laboratorio de Protozoología de la Facultad de Ciencias, U.N.A.M. [Universidad Nacional Autónoma de México]). However, neither the description nor the

figure legend of *A. rigescens* contain a hint that for *A. rigescens* such preparations have been made. Ma. Antonieta Aladro Lubel informed us that no useable permanent slides of the Mexican population exist.

Body (*in vivo?*) 179–260 μm (on average 200 μm) long and 49–55 μm (on average 50 μm) wide. Body outline fusiform (specimen illustrated, however, roughly with parallel margins); ventral side plane, dorsal side convex. Cytoplasm containing food vacuoles, content, however, not mentioned. Two macronuclear nodules, (*in vivo?*) 17–23 \times 9 μm . Micronuclei not observed. Adoral zone about one third of body length. Marginal cirri short, except anterior and posterior one which are long. The cirral pattern of the specimen illustrated basically agrees with that of the original description, except for pretransverse ventral cirri and transverse cirri, which are partially not illustrated (overlooked? really lacking?). Aladro Lubel and López-Ochoterena (1967) did not mention the ring-shaped structures described and illustrated by Kahl (1932), but there are some larger globules, which could be these structures, recognizable in the illustration. Aladro Lubel *et al.* (1990) obviously took over the description from Aladro Lubel and López-Ochoterena (1967), but provided a new illustration. Whether it is based on new observations or only a strongly modified redrawing of the 1967-figure is not indicated. Anyhow, the frontoventral cirri are localized very close to the frontal cirri indicating a misobservation. The same is obviously true for the up to 7 μm large contractile vacuole close to the transverse cirri. Dorsal bristles about 4 μm long, according to the illustration.

Occurrence and ecology: *Apogastrostyla rigescens* is a rare species living in marine and brackish waters. Kahl (1932) discovered it in aquaria from Heligoland (Helgoland), a German island in the North Sea. Whether the material in the tanks was from the surroundings of Heligoland or from any other marine site(s) is not mentioned (e.g. the tanks could have contained material from an expedition or the content could have been a mixture from various sample sites). Thus, the original type locality is, strictly speaking, not known (ICZN 1999, Article 76.1.1); Berger (1999) incorrectly assumed that Heligoland is the locus classicus. This is one reason to designate a neotype, and due to this act the place of origin of the neotype becomes the type locality of the species (ICZN 1999, Articles 75.3.1, 76.3). We found *Apogastrostyla rigescens* in open mussel-farming waters in the Jiaozhou Bay (Yellow Sea) about 1 km off the coast near Qingdao (Tsingtao; 36°08'N, 120°43'E), China, on September 24, 2007. The neotype material was collected at the following conditions: salinity 29–31‰, water temperature 10°C, pH 7.9. Chen *et al.* (2009) recorded *A. rigescens* in a constructed

mangrove wetland for treating municipal sewage in Shenzhen, southern China. For pre-1999 records, see Berger (1999: 462). The sparse faunistic and ecological data suggest that *A. rigescens* prefers eutrophic and/or slightly saprobic saltwaters. Records from limnetic and terrestrial habitats are very likely misidentifications (for review see Berger 1999). *Apogastrostyla rigescens* feeds mainly on diatoms, in cultures also on bacteria (own observations; Figs 1a, 2j).

Comparison of *Apogastrostyla rigescens* populations: The original description is – as is usual for the species described by Kahl (1932) – rather short, but expressive (see above). Our material agrees very well with the population studied by Kahl, mainly in the general habitus, body size, cirral pattern, oral apparatus, length of dorsal bristles, nuclear apparatus, ring-shaped structures, lack of contractile vacuole, and marine habitat. Three noteworthy differences exist, which however can be explained: (i) Kahl (1932) neither mentioned nor illustrated cortical granules which are present in our population. We suppose that Kahl overlooked them because they are colorless and small (0.3–0.5 μm across) and therefore difficult to recognize, especially with bright field microscopy. (ii) Kahl (1932) found that the marginal rows were distinctly separated posteriorly. Thus, he classified the species in the subgenus *Oxytricha* (*Tachysoma*), which is characterized by the lack of caudal cirri. By contrast, the Chinese population has caudal cirri, a feature sometimes unambiguously recognizable only in protargol-impregnated late dividers (Figs 1e, 4a, b). Kahl (1932, 1933) was very uncertain about this feature and he suggested himself that the species has to be transferred to *Oxytricha* (*Oxytricha*) when caudal cirri are traceable. We strongly suppose that Kahl has overlooked them; unfortunately, we will never know whether Kahl's population had caudal cirri or not. (iii) The Chinese population has two more or less distinctly off-set 'extra' cirri behind the right marginal row (Figs 1e, 5h). By contrast, Kahl (1932, 1933) neither mentioned nor illustrated this cirral group. However, this is unsurprisingly because such subtle details are clearly recognizable only in protargol preparations.

The following point is basically no difference between the original description and our population, but concerns a very important feature not described by Kahl (1932) because he did not have the advantage of protargol impregnation. The dorsal infraciliature (dorsal kineties, caudal cirri) is, besides the ventral cirral pattern, a key feature for hypotrich systematics. The

problem with the caudal cirri in Kahl's population is discussed above, while the arrangement, number, and formation of the kineties are not known from the original description. The length of the bristles (3–4 μm) can be estimated from Kahl's illustration (see above), and agrees with our observations.

The redescrptions by Aladro Lubel and López-Ochoterena (1967) and Aladro Lubel *et al.* (1990) do not provide important additional information, for example, about details of the ventral and dorsal infraciliature. Actually, the descriptions (e.g. contractile vacuole in rear body portion) and the illustrations (frontoventral cirri and postoral ventral cirri very close to frontal cirri) are confusing rather than informative. However, at generous consideration the identifications can be accepted (Berger 1999).

Neotypification: In the present case it seems wise to designate a neotype because there are (i) discrepancies between the original description and our population in some important taxonomic features (cortical granulation, caudal cirri, 'extra' cirri; see previous section); (ii) the type locality of Kahl's population is unknown (see section occurrence and ecology); and (iii) the dorsal infraciliature, a key feature for hypotrich taxonomy, of Kahl's population is not known. According to Article 75.3 of the ICZN (1999), the valid designation of a neotype has to be accompanied by the publication of some particulars:

(i) The taxonomic status of *A. rigescens* is unclear because the original description and our redescription do not agree in some important features, and the dorsal infraciliature of the original type population is not known (see above).

(ii) For differentiation of *A. rigescens* from related species, see below.

(iii) The neotype (Fig. 1e, f) and its population are described in detail (see above), including some relevant morphogenetic stages and the SSU rRNA. Thus, recognition of the neotype is ensured.

(iv) It is generally known that no type material is available from species originally described by Kahl (1932, 1933). Further, there is no indication that Aladro Lubel and López-Ochoterena (1967) or Aladro Lubel *et al.* (1990) designated a neotype. Thus, the present species was not objectively defined via a type specimen so far.

(v) There is very strong evidence that the neotype (Fig. 1e, f) is identical with *Oxytricha (Tachysoma) rigescens* as originally described by Kahl (1932), although there are some (allegeable) differences (details, see above).

(vi) The original type locality is not known (details, see section occurrence and ecology). Thus, the neotype cannot come as nearly as practicable from the original type locality, as requested from Article 75.3.6 of the ICZN (1999). However, both the population studied by Kahl (1932) as well as our population from China are from marine habitats. A detailed description of the new type locality, that is, the sample site of the neotype population, is given in the section occurrence and ecology.

(vii) The protargol slide containing the neotype specimen is deposited in the Natural History Museum in London, UK. Two additional slides containing neotype material are deposited in the Ocean University of China (OUC). The gene sequence of the SSU rRNA has been submitted to the GenBank database (accession numbers see section deposition of neotype material).

Foundation for the establishment of *Apogastrostyla*: Kahl (1932) supposed that the present species, which is undoubtedly an 18-cirri hypotrich, lacks caudal cirri and therefore assigned it to the subgenus *Oxytricha (Tachysoma)*. Borror (1972) transferred it to the genus *Tachysoma* (see nomenclature above), a classification accepted in the review by Berger (1999: 433) through lack of new data. The limnetic *Tachysoma pellionellum*, type of *Tachysoma*, unequivocally lacks caudal cirri and has six dorsal kineties (e.g. Foissner and Didier 1981, Berger 1999), and according to Hemberger (1982), the anlagen of dorsal kineties 2 and 3 of both the proter and the opisthe fragment into two parts each so that in total five anlagen are formed per filial product. In addition, one dorsomarginal kinety originates from the anterior end of the new right marginal row (for review, see Berger 1999: 73). This rather complex dorsal morphogenesis (*Tachysoma*-pattern according to Berger and Foissner 1997) strongly indicates that *Tachysoma* is an oxytrichid hypotrich, at least, however, it very likely belongs to the so-called Dorsomarginalia, a group which comprises all hypotrichs producing one or more clearly detached dorsomarginal kineties (Berger 2006, 2008). By contrast, in '*T. rigescens*' the dorsal morphogenesis proceeds in the so-called *Gonostomum*-pattern (Berger and Foissner 1997), which is very probably part of the ground pattern of the Hypotricha (Berger 2008). Briefly, within each of the three parental kineties two anlagen occur, one for the proter and one for the opisthe. None of these anlagen disintegrates and no dorsomarginal kineties are formed. In addition, a caudal cirrus occurs at the end of each anlage (Figs 3d, 4a, b), and the adoral zone of the proter is a composite of parental and new membranelles, whereas the parental

adoral zone is retained in *T. pellionellum* (Hemberger 1982). These distinct morphological and morphogenetic differences clearly demonstrate that '*T. rigescens*' is misclassified in *Tachysoma*.

The SSU rRNA gene sequence of the type species *T. pellionellum* is not available at GenBank. Thus, we do not have molecular support for our assumption. The morphological identification of *T. pellionellum* sensu Shin (2005) is based on live observation only and therefore this sequence data have not been submitted to GenBank. We have tentatively reconstructed the phylogeny including Shin's (2005) population demonstrating that it is not closely related to *T. rigescens*.

Song and Wilbert (1997a) established *Hemigastrostyla* and included two marine species, namely, *H. stenocephala* (basonym *Gastrostyla stenocephala*; type species) and *H. enigmatica* (basonym *Oxytricha enigmatica*). Both species have, like '*T. rigescens*,' a cephalized body and two 'extra' cirri near the rear end of the right marginal row (for review see Berger 1999). Whether or not these 'extra' cirri are homologous is not known because their origin is still obscure and the species differ distinctly in some important features (for a comparison of '*T. rigescens*' with the third *Hemigastrostyla* species, *H. szaboi*, see below). *Hemigastrostyla stenocephala* has a gastrostylid cirral pattern (that is, a slightly increased number of frontoventral and postoral ventral cirri forms a distinct row), and 4–6 dorsal kineties and two fimbriate caudal cirri (Borror 1963). Unfortunately, the dorsal morphogenesis is not known in the type species of *Hemigastrostyla*. By contrast, *Hemigastrostyla enigmatica* is, like '*T. rigescens*,' an 18-cirri hypotrich which has, however, like the type species of *Hemigastrostyla*, 5–6 dorsal kineties (Dragesco and Dragesco-Kernéis 1986, Song and Wilbert 1997a, Gong *et al.* 2007). Caudal cirri are present, but the number ranges from constantly three (Song and Wilbert 1997a) to 5–7 (Dragesco and Dragesco-Kernéis 1986, Gong *et al.* 2007). Cell division, especially dorsal morphogenesis, proceeds rather peculiar (Song and Hu 1999). Dorsal kineties 1 and 2 fragment and form in total four kineties, kinety 3 divides without fragmentation, and the dorsomarginal kinety formed at the anterior end of the right marginal row does not detach. Further, the adoral zone of the proter is formed in the same way as in '*T. rigescens*,' the right marginal row anlage of the opisthe probably originates *de novo* (vs. distinctly intrakinetally in '*T. rigescens*'), and the two 'extra' cirri are very likely retained parental cirri

(origin not known in '*T. rigescens*,' perhaps from rear end of new right marginal row). The comparison shows that the situation is rather tricky. At the present state of knowledge it seems rational to retain the present classification of *H. enigmatica* in *Hemigastrostyla*, although it has, like '*T. rigescens*,' an 18-cirri pattern and forms a cluster with this species in the molecular tree (Fig. 6). Given that the tree is correct in this respect, they obviously have separated very early as documented by the deep branching, indicating that they should not be unified in a new genus, inasmuch as we do not yet know the position of the type species (*H. stenocephala*) in the tree. On the contrary, it also does not make sense to transfer '*T. rigescens*' to *Hemigastrostyla* although there are some interesting agreements (cephalization, two 'extra' cirri) because it differs from both *Hemigastrostyla* species in the number of dorsal kineties and their morphogenesis, and from the type species also in the number of frontoventral and postoral ventral cirri.

Wilbert and Song (2005) described the marine *Hemigastrostyla szaboi*. It lacks, according to the original description, the 'extra' cirri, but has, like '*T. rigescens*,' 18 frontal-ventral-transverse cirri, three bipolar dorsal kineties, and three caudal cirri. Wilbert and Song (2008) re-described *H. szaboi* and confirmed the lack of the 'extra' cirri in the description. However, their micrographs relatively clearly show that this cirral group is also present in *H. szaboi* (e.g. Fig. 12F in Wilbert and Song 2008). The anterior body portion is likely also somewhat cephalized and the undulating membranes are rather straight, as in '*T. rigescens*' (Fig. 12A, B in Wilbert and Song 2008). Thus, *Hemigastrostyla szaboi* should be removed from *Hemigastrostyla* and unified with '*T. rigescens*' in a new genus. We therefore establish *Apogastrostyla* gen. nov. and transfer both the type species, *Apogastrostyla rigescens* (Kahl, 1932) comb. nov., and *A. szaboi* (Wilbert and Song, 2005) comb. nov. to the new genus.

Some other genera should be briefly compared with *Apogastrostyla* because they have a similar ventral ciliature and emerge from the same polytomy as the cluster *Apogastrostyla rigescens* + *Hemigastrostyla enigmatica* (Fig. 6). *Trachelostyla pediculiformis* is the type of *Trachelostyla* and has, like *Apogastrostyla*, an 18-cirri pattern. However, the postoral ventral cirri are much more displaced anteriorly than in *Apogastrostyla*, the number of dorsal kineties is twice as high (6 vs. 3), and the dorsal kinety formation is much more complex (e.g. Gong *et al.* 2006, Shao *et al.* 2007; for review see Berger 2008: 474).

Amphisiella is, since the review by Berger (2008: 84), a relatively small group of marine hypotrichs, which have a rather long amphisiellid median cirral row formed by the many cirri of the two or three rightmost frontal-ventral-transverse cirri anlagen IV–VI (e.g. Eigner and Foissner 1994, Berger 2004, Hu *et al.* 2004, Li *et al.* 2007). Thus, the row is much more distinct in *Amphisiella* than in *Apogastrostyla*, although the rows themselves are undoubtedly homologous. Further differences between *Apogastrostyla* and *Amphisiella* concern the ‘extra’ cirri, which are lacking in *Amphisiella*, and the dorsal infraciliature (caudal cirri present vs. absent; three dorsal kineties vs. more than three).

Protogastrostyla was recently established for *Gastrostyla pulchra* by Gong *et al.* (2007). Both the monotypic *Protogastrostyla* as well as *Apogastrostyla* have a cephalized body, the distal portion of the adoral zone extends far posteriorly, most frontoventral and postoral ventral cirri form a distinct median row, dorsal kinety fragmentation and dorsomarginal kineties are lacking, caudal cirri are present, and the adoral zone of the proter is composed of new and parental membranelles. Important generic features (apomorphies?) of *Protogastrostyla* are the slightly increased number of cirri formed in the anlagen IV, V, and VI; the *de novo* formation of marginal row anlagen; the formation of the dorsal kinety anlagen via primary primordia and the retention of many parental kineties (Hu and Song 2000; for reviews see Berger 1999: 818, 2008: 137, 690). Further, they are not very closely related according to the molecular analyses (Fig. 6).

According to our molecular data, the small clusters containing *Parabirojimia* and *Pseudoamphisiella* emerge from the same polytomy as *Apogastrostyla* (Fig. 6). Both genera have – besides some other curious features (e.g. *Parabirojimia* has many marginal rows, *Pseudoamphisiella* has many dorsal kineties) – zigzagging frontoventral cirri, a pattern which is reminiscent of the urostyloids (e.g. Song *et al.* 1997, Hu *et al.* 2002, for review see Berger 2006). Consequently, an inclusion of *A. rigescens* in one of these genera is neither indicated from the morphological nor from the molecular point of view.

Gastrostyla (type *G. steinii*) is a stylonychine oxytrichid clearly separated from *Apogastrostyla* both morphologically (more frontoventral and postoral ventral cirri) and ontogenetically (oxytrichid dorsal kinety fragmentation and clearly detached dorsomarginal rows present) as well as by the gene sequence data (e.g. Berg-

er 1999: 789, 2008: 138; Foissner *et al.* 2002, 2004; Yi *et al.* 2008; Fig. 6). The more or less linear arrangement of most frontoventral and postoral ventral cirri in both taxa is therefore a convergence.

Comparison of *Apogastrostyla rigescens* with the congener and with similar species: The well-known 18-cirri pattern, also present in *Apogastrostyla*, is distributed all over the Hypotricha tree. The ‘main range’ of this pattern is in the oxytrichids, which have, however, a more complex dorsal kinety pattern and morphogenesis than *Apogastrostyla* (for review see Berger 1999; note that not all genera reviewed in this monograph, for example, *Gonostomum*, belong to the oxytrichids according to a more recent hypothesis postulated by Berger 2008). Thus, the comparison is confined to marine 18-cirri hypotrichs with a simpler dorsal kinety pattern.

Apogastrostyla szaboi differs from the type species in body length (on average 114 µm vs. 149 µm after protargol impregnation), the number of adoral membranelles (27 vs. 43), the cortical granulation (absent vs. present; however, feature not carefully checked in *A. szaboi*), and the ring-shaped structures (absent vs. present). Wilbert and Song (2005, 2008) wrote that *A. szaboi* lacks the two ‘extra’ cirri near the rear end of the right marginal row. However, at least according to Fig. 12F in Wilbert and Song (2008), two more or less distinctly off-set cirri are recognizable, indicating that this feature, but also the presence/absence of cortical granules (see above) have to be rechecked in *A. szaboi*, which is – so far at least – only known from the Antarctic region. By contrast, *A. rigescens* is recorded only from the northern hemisphere (Berger 1999), indicating that they are separated geographically. Of course further reliable records are needed to confirm or disprove these geographic ranges.

The review on *Tachysoma* by Berger (1999) comprises, besides *T. rigescens*, two further marine species, namely *T. dragescoi* and *T. ovata* (Song and Wilbert 1997b). Some years later, *Tachysoma multinucleatum* nom. corr. has been described by Gong and Choi (2007) from marine sands in Korea [Note: *Tachysoma* is neuter (Aesch 2001: 302); thus, the species-group name has to be changed from *multinucleata* (feminine) to *multinucleatum* (neuter; details see ICZN 1999)]. All of them have, like the present species, only three dorsal kineties and the distal portion of the adoral zone extends far posteriorly, except in *T. dragescoi*. However, in contrast to *A. rigescens*, they lack caudal cirri and the two ‘extra’ cirri, so that an inclusion of these species

in *Apogastrostyla* seems inappropriate. Probably they form a separate group outside the Dorsomarginalia, but relevant molecular data should be awaited to confirm their removal from *Tachysoma*.

Hemigastrostyla enigmatica is, like *A. rigescens*, a marine, cephalized 18-cirri hypotrich with two 'extra' cirri and an adoral zone the posterior portion of which extends far posteriorly (Dragesco and Dragesco-Kerné is 1986, Song and Wilbert 1997a, Berger 1999: 229, Song and Hu 1999, Gong *et al.* 2007). In addition, they form a cluster in our molecular tree (Fig. 6). The separation of these two species is difficult and requires detailed live observation to check the arrangement of the small cortical granules (mainly around dorsal bristles in *H. enigmatica* vs. narrowly spaced almost all over the cell in *A. rigescens*) as well as protargol impregnation to reveal the dorsal kinety pattern (five kineties vs. three). Because the species show distinct differences in the morphogenesis (marginal rows partly formed *de novo* vs. not; curious dorsal kinety fragmentation present vs. not), a unification of these two species in a single genus seems inappropriate (details, see section foundation of *Apogastrostyla*).

Trachelostyla pediculiformis, type of *Trachelostyla*, is a rather common marine 18-cirri hypotrich which has a strongly narrowed anterior body portion (e.g. Borror 1963, Maeda and Carey 1984, Gong *et al.* 2006, for review see Berger 2008: 478). It can be easily distinguished from *A. rigescens*, even in life, by the very narrow 'head,' the rapid movement, the far anteriorly displaced postoral ventral cirri, the long dorsal bristles (7–8 μm vs. 3–4 μm), and the many (usually around 9–17) macronuclear nodules. In addition, it has twice as many dorsal kineties, lacks cortical granules, and the distal portion of the adoral zone does not extend far posteriorly. The dorsal kinety formation proceeds very exceptional in *T. pediculiformis* because the six kineties originate only from anlagen formed in parental kineties 1 and 6 (Shao *et al.* 2007, Berger 2008: 687).

Protogastrostyla pulchra, the sole species of the genus, has in total three more frontoventral and postoral ventral cirri than *A. rigescens* because the anlagen IV–VI do not form three, four, and four cirri, but four, five, and five (e.g. Berger 1999: 818, 2008: 137; Hu and Song 2000; Gong *et al.* 2007). Thus, the median cirral row is somewhat more prominent. Although *P. pulchra* and *A. rigescens* have a very similar body shape and size and the same nuclear apparatus, they can be easily

distinguished in life by the position of the prominent transverse cirri (displaced very far anteriorly and not protruding beyond rear cell end vs. not anteriorly displaced and projecting).

Phylogenetic position of *Apogastrostyla* (Fig. 6): *Apogastrostyla* obviously has taken over most features from the supposed last common ancestor of the hypotrichs (Berger 2008: 23), for example, the 18 frontal-ventral-transverse cirri, two marginal rows, three bipolar dorsal kineties dividing in the *Gonostomum*-pattern, and caudal cirri. The simple dorsal kinety pattern and its morphogenesis (e.g. lack of kinety-3-fragmentation and dorsomarginal rows) place *Apogastrostyla* outside the Dorsomarginalia (Berger 2006: 38), that is, at or near the base of the Hypotricha tree. Other 'non-dorsomarginalian hypotrichs' are, for example, the urostyloids (Berger 2006), *Amphisiella*, *Trachelostyla* (with special type of fragmentation; Shao *et al.* 2007), and *Gonostomum* (Berger 2008: 46). The basal branching of these genera, deduced from morphological and ontogenetic data, is basically confirmed by gene sequence analyses (Fig. 6; e.g. Paiva *et al.* 2009, Yi *et al.* 2009).

Unfortunately, the many molecular Hypotricha trees published so far are not very coherent, making a serious discussion basically impossible. Thus, one should mainly concentrate on results which make sense in combination with morphological and ontogenetic data. Highly varying details should not be over-interpreted. The most important phylogenetic inference of our investigation is that the clade composed of the 18-cirri hypotrichs *A. rigescens* + *Hemigastrostyla enigmatica* branches off near the base of the Hypotricha tree. Its relative position to the main hypotrich cluster (from *Pleurotricha* to *Protogastrostyla*) and the other minor clusters at this level is unresolved in the presented tree. In addition, the results support the assumption that the 18-cirri pattern occurred very early in the Hypotricha tree, probably already in the last common ancestor of this group (Berger 2008: 23).

Acknowledgements. We thank Z. Yi and C. Shao (OUC) for reading the very first version of the manuscript, Q. Zhang and J. Ji-ang (OUC) for sampling and providing technical support, and Ma. Antonieta Aladro Lubel (Mexico) for information about the Mexican population of *Tachysoma rigescens*. This research was funded by the Natural Science Foundation of China (project numbers: 30870264, 40906065); the Center of Excellence in Biodiversity, King Saud University, Saudi Arabia (111 Project No. B08049); and the Austrian Science Fund (FWF; Project P20569-B17; H. Berger).

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Received on 17th February, 2010; revised on 17th May, 2010; accepted on 18th May, 2010