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Reconciling classical and molecular phylogenies in the stichotrichines (Ciliophora, Spirotrichea), including new sequences from some rare species

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Abstract

We performed a comparative morphological and molecular study on oxytrichid and urostylid stichotrichs (=part of the former hypotrichs). Included are new small subunit (18S) ribosomal RNA (rRNA) gene sequences from five rare oxytrichids (*Gonostomum namibiense*, *Cyrtohymena citrina*, *Hemiurosoma terricola*, *Onychodromopsis flexilis*, *Orthoamphisiella breviseries*) and published sequences, based on cultures provided by the senior author, of two key stichotrichid genera, viz., *Gastrostyla* and *Engelmanniella*. These and other sequences, altogether 27 species representing 23 genera, were used to analyze how 18S rRNA-based phylogenetic trees can be reconciled with the morphological and ontogenetical data. In 18S rRNA trees, the oligotrichine family Halteriidae invariably clusters within the oxytrichid clade, usually near *Oxytricha granulifera*, type species of the genus. This position is hardly supported by morphological and ecological evidence and, especially, it contradicts the current ontogenetic findings; possibly, it is an artifact caused by taxa undersampling and/or special molecular evolutionary events. In contrast, most morphological and DNA sequence data of the stichotrichs can be harmonized with the CEUU (*C*onvergent *E*volution of *U*rostylids and *U*roleptids) hypothesis which suggests that the urostylid midventral pattern evolved from an oxytrichine ancestor, developing a second time within the Oxytrichidae. The systematic position of one of the two key genera could be clarified with the 18S rRNA sequences: *Gastrostyla* is a stylonychine oxytrichid. Based on the molecular data and a reassessment of ontogenesis, a new genus, *Styxophrya* nov. gen., is established for *Onychodromus quadricornutus* Foissner, Schlegel & Prescott, 1987.

Keywords: *Gastrostyla*; *Halteria* classification; Hypotrichs; Morphologic vs. gene sequence classification; Stichotrichia; *Styxophrya* nov. gen.

Introduction

The spirotrichs are a large group of ciliates consisting of about 2000 described species. The majority of these species have recently been classified into two main lineages, i.e., the oligotrichs and hypotrichs s.l. (Petz and Foissner 1992; Lynn and Small 1997). The heterotrichs, which were considered as typical spirotrichs for a long time (Bütschli 1889; Kahl 1932; Corliss 1979), are now regarded as a separate class within the subphylum Postciliodesmatophora due to their special somatic ultrastructure (postciliodesmata) and the macronucleus, which divides by extramacronuclear microtubules (Lynn and Small 1997). A large part of the remaining spirotrichs are hypotrichs

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s.l., which were split, mainly according to DNA sequence data (Lynn and Small 1997, 2002), into hypotrichs s.str. (the former euplotids) and stichotrichs (all other former hypotrichs). However, this classification is still in discussion (Chen and Song 2002).

Our paper has three main aims. First, we will discuss the classification of the oligotrichine halteriids which molecular phylogenies (Hoffman and Prescott 1997; Strüder-Kypke and Lynn 2003) assign to the stichotrichine hypotrichs, neglecting the highly conflicting ontogenetic evidence (Petz and Foissner 1992). The second aim of our study concerns the classification of some groups of stichotrichs for which sufficient morphological, ontogenetical, and DNA sequence data are available to investigate as to whether morphological and sequence data can be reconciled. We shall pay special attention to the oxytrichids and urostylids, two distinct lineages recognized already by Bütschli (1889), Kahl (1932) and Borror (1972). The former are characterized by the 18 fronto-ventral-transverse (FVT) cirral pattern (Berger 1999), while the latter have a so-called midventral row composed of two rows of cirri arranged in a highly characteristic zigzag (=midventral) pattern (Borror 1972). We will show that this pattern probably evolved twice from the 18 FVT cirral pattern by anlagen multiplication.

Finally, we will investigate the subfamilial and generic classification of the oxytrichid stichotrichs, a difficult enterprise not only for morphologists (see the opposing views of Berger and Foissner 1997 and Eigner 1997), but also for molecular systematists, who usually discuss this matter only marginally. For this reason, we sequenced 18S rRNA genes of several uncommon stichotrichs and evaluated published sequences. The majority of these sequences had been retrieved from rare and interesting species sent to or identified for Prof. Prescott by the senior author. The sequences of these species, especially *Gastrostyla steinii* and *Engelmanniella mobilis*, were published recently, but not discussed from a morphological point of view (Croft et al. 2003; Hewitt et al. 2003).

Our study tries to reconcile classical and molecular taxonomy of the stichotrichs by reconsidering the morphological axioms and emphasizing the frequently ignored hiatus between various molecular classifications. A detailed discussion of this matter is urgently needed because the increasing complexity of the data makes it difficult to discuss them properly. It is likely that most problems are caused by undersampling of taxa and parallel evolution, which is much more common than usually assumed and difficult to recognize with any method.

Materials and methods, terminology

Species investigated: Relevant data for the species investigated are compiled in Table 1. Most of the new sequences are from terrestrial stichotrichs cultivated with the non-flooded Petri dish method, as described in Foissner et al. (2002). About 50 specimens from each culture were collected with a fine pipette, washed in Eau de Volvic (French table water), immersed in a solution of 8 M guanidine chloride, and stored at room temperature until DNA extraction.

All other sequences are retrieved from GenBank. However, stichotrichs are not easily identified correctly. Several of the sequences deposited at GenBank are possibly from misidentified taxa. Thus, we used mainly those species which were identified or checked by ourselves or whose identity was verified by protargol impregnation (for details, see Table 1).

Extraction of DNA, isolation of 18S rRNA genes, and DNA sequencing: Cells were dissolved in 8 M guanidine chloride. Undissolved material was spun down at 10,000 g for 5 min. The supernatant was diluted with the same amount of water and precipitated with one volume of isopropanol and 0.1 volume of 3 M sodium acetate (pH 5.2). The pellet was washed twice with 70% ethanol, air-dried and dissolved in water. The 18S rRNA genes were amplified by PCR with the oligonucleotide primers targeting the conserved sequences close to the 5′ and 3′ termini of the gene (Moon-van der Staay et al. 2001). The PCR products were purified, inserted into the pGEM-T Easy vector (Promega), and transformed into *E. coli* XL1 blue cells. Plasmid DNA containing the 18S rRNA gene was purified with the FlexiPrep kit (Pharmacia). The 18S rDNA genes of clones were completely sequenced. Accession numbers of species used in analyses are given in Fig. 5.

Phylogenetic analyses: Berger and Foissner (1997) and/or Berger (1999) should be consulted for a detailed explanation of the morphologic features and their states (apomorphic, plesiomorphic). Morphologic analysis was performed with Hennig's cladistic method, both manually and by using a computer program (HENNIG). Results were identical.

18S rRNA gene sequences were aligned using Clustal X 1.81 (Jeanmougin et al. 1998), and refined manually using BioEdit 5.0.6 (Hall 1999). The complete alignment, excluding the PCR primer sites, was used for the phylogenetic analyses. Neighbour-joining (NJ) trees (Saitou and Nei 1987) were constructed with the PHYLIP package version 3.6 (Felsenstein 2002). Evolutionary distances were calculated with the Kimura two-parameter model, using a transition/transversion ratio of 2.0. The taxon addition order was randomized. Maximum parsimony analyses were performed with PAUP* 4.0 (Swofford 2002), using tree-bisection-reconnection branch swapping and random stepwise addition in a heuristic search. The monophyly of the clusters was assessed by bootstrap replicates. A maximum likelihood tree estimation was carried out with the PUZZLE 5.0 program (Strimmer and von Haeseler

Table 1. Origin and identification of the species (alphabetically arranged) investigated and used for the phylogenetic analysis of the gene sequences and tree construction

Species[a]	Origin	Identification	Main references[b]
Cyrtohymena citrina (Berger and Foissner, 1987)	Namibia, Etosha Pan; soil from Ghost Forest	W. Foissner	1
Cyrtohymena citrina[c] (Berger and Foissner, 1987)	Colorado, USA; freshwater; coll: D. Prescott	W. Foissner	1
Engelmanniella mobilis (Engelmann, 1862)	Turkey; rhizosphere soil from marsh plants in Anatolia	W. Foissner	2
Euplotoides eurystomus (Wrzesniowski, 1870)	Marl, Germany; freshwater	K. Heckmann	3
Gastrostyla steinii Engelmann, 1862	Austria; soil	W. Foissner	1
Gonostomum namibiense Foissner et al., 2002	Namibia, Etosha Pan; mud and soil from water hole Riedfontein	W. Foissner	4
Gonostomum strenuum (Engelmann, 1862)	Albury, Australia; soil from River Murray floodplain	W. Foissner	1, 5
Halteria grandinella[d] (Mueller, 1773)	Boulder, USA; freshwater; coll. D. Prescott	D. Prescott W. Foissner	6
Hemiurosoma terricola Foissner et al., 2002	Botswana; soil from floodplain of Zambezi River	W. Foissner	4
Holosticha multistilata Kahl, 1928	Korea; mosses in Seoul	M. K. Shin	7
Laurentiella strenua (Dingfelder, 1962)	Darwin, Australia; soil from dried dam	W. Foissner	1
Moneuplotes minuta (Yocum, 1930)	Villefranche sur Mer, France; seawater	K. Heckmann	3
Onychodromopsis flexilis[e] Stokes, 1887	Antarctica; Watts Lake (obtained from J. Laybourn-Parry)	W. Foissner	1, 8
Onychodromus grandis Stein, 1859	Pet shop Aquarium, Boulder, USA	W. Foissner	1
Onychodromus (now *Styxophrya*) *quadricornutus* Foissner et al., 1987	Pet shop Aquarium, Boulder, USA	W. Foissner	1
Orthoamphisiella breviseries Foissner et al., 2002	Namibia, Etosha (Fischer) Pan; mud and soil from water hole	W. Foissner	4
Oxytricha granulifera Foissner and Adam, 1983	Lower Austria; soil from deciduous forest	W. Foissner	1
Paraurostyla weissei (Stein, 1859)	Austria, freshwater	W. Foissner	1
Pattersoniella vitiphila Foissner, 1987	João Pessoa, Brazil; freshwater (obtained from T. Cordeiro)	W. Foissner	1
Phacodinium metchnikoffi (Certes, 1891)	Korea; mosses in Seoul	M. K. Shin D. Lynn	9
Pleurotricha lanceolata (Ehrenberg, 1835)	Colorado, USA; freshwater; coll: D. Prescott	W. Foissner	1
Steinia sphagnicola Foissner, 1989	Albury, Australia; soil from River Murray floodplain	W. Foissner	1
Sterkiella histriomuscorum[f] (Foissner et al., 1991)	Indiana, USA, Jordan River	W. Foissner D. Prescott	1,10
Sterkiella nova[f] Foissner and Berger, 1999	North Carolina, USA; freshwater (obtained from D. Prescott)	W. Foissner	10
Strombidium purpureum[g] Kahl, 1932	Denmark; marine sand	T. Fenchel C. Bernard	11
Stylonychia lemnae Ammermann and Schlegel, 1983	Harbin, China; freshwater	D. Ammermann M. Schlegel	1
Stylonychia mytilus (Mueller, 1773)	Entringen, Germany; freshwater	D. Ammermann M. Schlegel	1
Tetmemena pustulata[h] (Mueller, 1786)	China; likely freshwater	F. Zhu	1,12
Uroleptus gallina[h] (Mueller, 1786)	Boulder, USA; freshwater	D. Prescott	13
Uroleptus lepisma[i] (Wenzel, 1953)	Colorado, USA; freshwater; coll: D. Prescott	W. Foissner	15

Table 1 (*continued*)

Species[a]	Origin	Identification	Main
Uroleptus piscis[h] (Mueller, 1773)	Boulder, USA; freshwater	D. Prescott	13
Urostyla grandis[h] (Ehrenberg, 1830)	Boulder, USA; freshwater	D. Prescott	13

[a] Great care was taken with the correctness of names, authors, and dates. For literature, see Berger (2001).

[b] Literature (as found in the reference section): 1 = Berger (1999), 2 = Wirnsberger-Aescht et al. (1989), 3 = Curds (1975), 4 = Foissner et al. (2002), 5 = Foissner et al. (2001), 6 = Foissner et al. (1999), 7 = Shin et al. (2000) and Shin and Kim (1993), 8 = Petz and Foissner (1996), 9 = Dragesco and Dragesco-Kernéis (1986), 10 = Foissner and Berger (1999), 11 = Fenchel and Bernard (1993), 12 = Eigner (1999), 13 = Foissner et al. (1990, 1991), 14 = Prescott and Greslin (1992), 15 = Berger and Foissner (1989).

[c] Identification doubtful, see chapters on "material and methods" and "classification of lower categories".

[d] Prescott population; identification checked by W. Foissner.

[e] Berger (1999) overlooked neotypification by Petz and Foissner (1996) and thus wrongly assigned this species to *Allotricha*, a doubtful genus whose type species was never illustrated.

[f] Formerly named "*Oxytricha trifallax*" and "*Oxytricha nova*", see Foissner and Berger (1999). As concerns *S. histriomuscorum*, we suppose that Prescott used the strain we obtained from S. M. Adl, who got it from G. Herrick, one of the founders of "*O. trifallax*".

[g] We assume that Hirt et al. (1995) used the population discovered by Fenchel and Bernard (1993).

[h] Identification uncertain because probably not checked by silver impregnation. Hoffman and Prescott (1997) refer to Prescott and Greslin (1992) for the "cultivation of the various hypotrich species". However, this paper contains data only for *Oxytricha nova* (now *Sterkiella nova*) and *O. trifallax* (now *Sterkiella histriomuscorum*-complex). Thus, the source and identification of the other species contained in Hoffman and Prescott (1997) remain obscure.

[i] Formerly *Paruroleptus*. Likely this is the "*Holosticha* sp." in Hoffman and Prescott (1997).

1996) applying the HKY substitution model. The transition/transversion ratio and the base frequencies were estimated from the data.

Terminology: Basically, we use the terminology as explained in Corliss (1979). However, the higher systematic classification changed greatly since Corliss' review and is still in discussion. Thus, we use mainly a vernacular terminology (e.g., stichotrichs, oligotrichs) or add sensu stricto (s.str.)/lato (s.l.) to the taxa. The former hypotrichs are now considered to be composed of only two distantly related groups, viz., the hypotrichs s.str. (=euplotine hypotrichs) and the stichotrichs (all other former hypotrichs). Accordingly, our hypotrichs s.l. comprise euplotine and stichotrichine spirotrichs. Our oligotrichs comprise halteriids, strombidiids, tintinnids and strobilidiids (choreotrichs). The lower categories (families, genera) are treated in the same way. For instance, "urostylids" comprise urostylid and holostichid spirotrichs.

Results and discussion

The puzzling classification of halteriids

Halteria has been considered as a typical oligotrich ciliate for a long time (Kahl 1932; Corliss 1979). However, in 1988 doubts arose when Lynn and Sogin (1988) showed that 18S rRNA sequences locate *Halteria* within the stichotrichine spirotrichs (formerly, hypotrichs). Subsequently, this classification has been confirmed by other working groups and for several *Halteria* populations (Hoffman and Prescott 1997; Snoeyenbos-West et al. 2002; Hewitt et al. 2003; Strüder-Kypke and Lynn 2003) and even for a related genus, i.e., *Meseres* (Katz and Foissner, unpubl.): invariably, 18S rRNA sequences and the internal transcribed spacer of the rDNA sequences place the halteriids within the stichotrichine spirotrichs, usually within the Oxytrichidae, often as a near relative of *Oxytricha granulifera* (Fig. 5), type species of the genus *Oxytricha* (Berger 1999). This localization is stable and nearly independent of the algorithms used for tree construction. Notably, α-tubulin sequences assign *Halteria* to the oligotrichs, but at an unusual position, viz., as a sister group of the tintinnids (Snoeyenbos-West et al. 2002). In contrast, Petz and Foissner (1992) concluded from a detailed cladistic analysis of morphological and ontogenetical features that halteriids were the closest relatives of the hypotrichs s.l., albeit oligotrichs due to their enantiotropic division mode. This conclusion was confirmed by a parsimony analysis of mainly ultrastructural features (Puytorac et al. 1994). Figure 1 summarizes the various classifications of the Halteriidae.

Non-tintinnid oligotrichs have a rather uniform overall organization, viz., a globular to obconical, sparsely ciliated body and a mighty frontal adoral zone of membranelles (AZM) used to propel the cell and collect food. It is likely that these features of the oligotrichs are adaptations to the planktonic mode of life because they are found in various groups of planktonic organisms. In contrast, there is no evidence that the development of the AZM in a subsurface pouch and the enantiotropic division mode are adaptations to the planktonic lifestyle, although Kahl (1932) suggests that the first mentioned feature became necessary when the adoral zone took over locomotion. However,

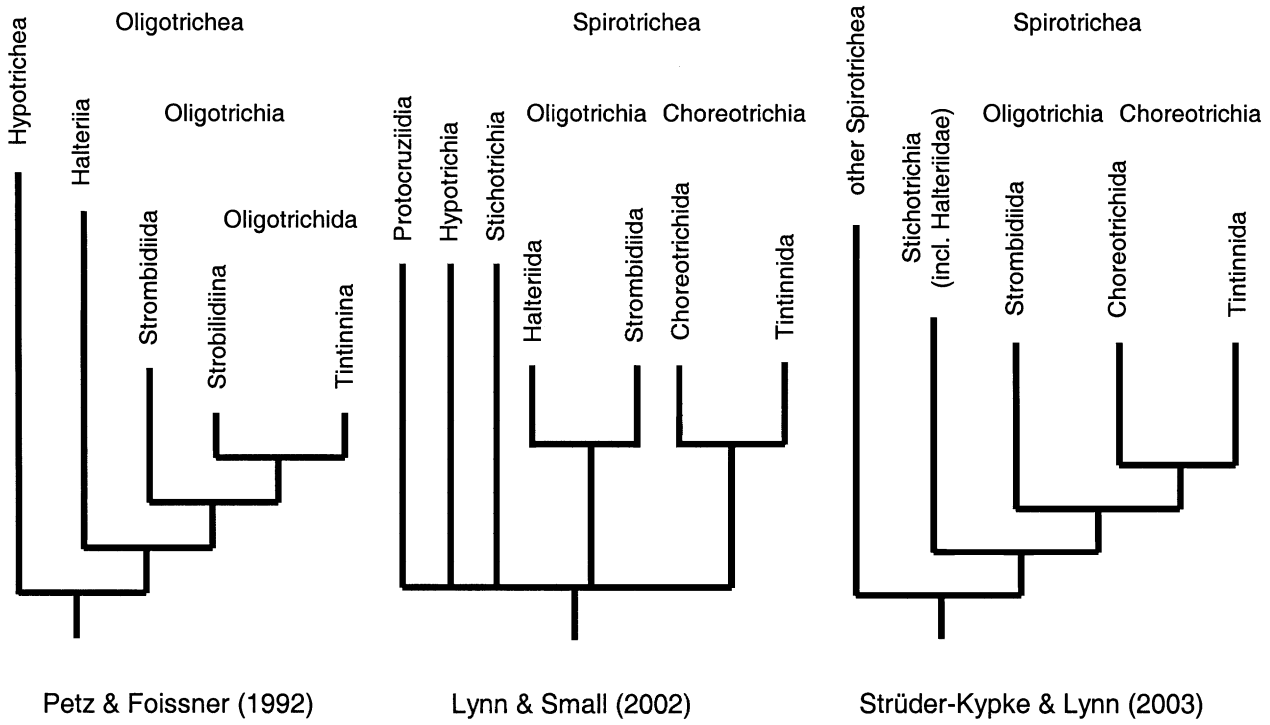


Fig. 1. Classification of the halteriids based on cladistic analysis of morphologic and ontogenetic features (Petz and Foissner 1992), on morphologic and gene sequence data (Lynn and Small 2002), and on ribosomal gene sequences (Strüder-Kypke and Lynn 2003).

Halteria grandinella, one of the most common and thus ecologically successful ciliates, generates the AZM on the surface, suggesting that subsurface development has little or no adaptive value.

Recently, Shin et al. (2000) and Strüder-Kypke and Lynn (2003) revived not only Kahl's idea, but in some way also the hypothesis of Lepsi (1929) that euplotid ciliates like *Gastrocirrhus* Lepsi, 1928 could be predecessors of the oligotrichs, that is, they consider "halteriids as secondarily evolved planktonic stichotrichs" and argue that such changes "certainly occurred among the euplotine hypotrichs: *Cytharoides* Tuffrau, 1974 and *Gastrocirrhus* Lepsi, 1928 are planktonic-like forms". However, all *Cytharoides* and *Gastrocirrhus* species studied so far are not planktonic but inhabit the Antarctic sea-ice or thrive as semi-sessile bottom and Aufwuchs dwellers (for literature on genera and species, see Berger 2001). Indeed, euplanktonic hypotrichs and stichotrichs are rare and basically organized as ordinary stichotrichine spirotrichs, including a homothetogenic division mode (for reviews, see Foissner 1996 and Foissner et al. 1999). Thus, there is no firm evidence that the planktonic lifestyle causes a halteriid organization in hypotrichs and stichotrichine spirotrichs. Nonetheless, one can imagine halteriids as "dorsalized" stichotrichs, assuming that the body involuted ventrally, resorbed the cirri, and modified the dorsal bristles to halteriid jumping bristles (Fig. 2). Unfortunately, this attractive hypothesis does not explain the enantiotropic division mode of *Halteria* and its relatives.

We have tabulated the oligotrichine and stichotrichine features found in halteriids (Table 2 Of these, the most important oligotrichine features are the frontal adoral zone of membranelles, the specific resting cysts, and the enantiotropic cell division. While the first feature can possibly be interpreted as an adaptation to the planktonic lifestyle, the specific resting cysts and the enantiotropic cell division can hardly be related to ecological constraints or convergent evolution. More likely, they are unique inventions of oligotrichine spirotrichs. The most important stichotrichine features of halteriids are the two undulating membranes (paroral and endoral) and the development of the oral primordium on the surface, i.e., not in a subsurface pouch as in all other oligotrichs. While the last mentioned feature developed convergently in various ciliate lineages (Foissner 1996), the two undulating membranes are a specific feature of stichotrichine spirotrichs. A paroral and endoral membrane are present in *Halteria* spp. and the related genus *Meseres* (Szabó 1935; Petz and Foissner 1992). The endoral membrane is rather long. The paroral consists of two short cilia in *H. grandinella* (Petz and Foissner 1992); of four to six basal bodies, of which only two to three are ciliated in a new *Halteria* species from the Dominican Republic (Foissner, unpubl.); and of six cilia in *H. geleiana* (Szabó 1935), where

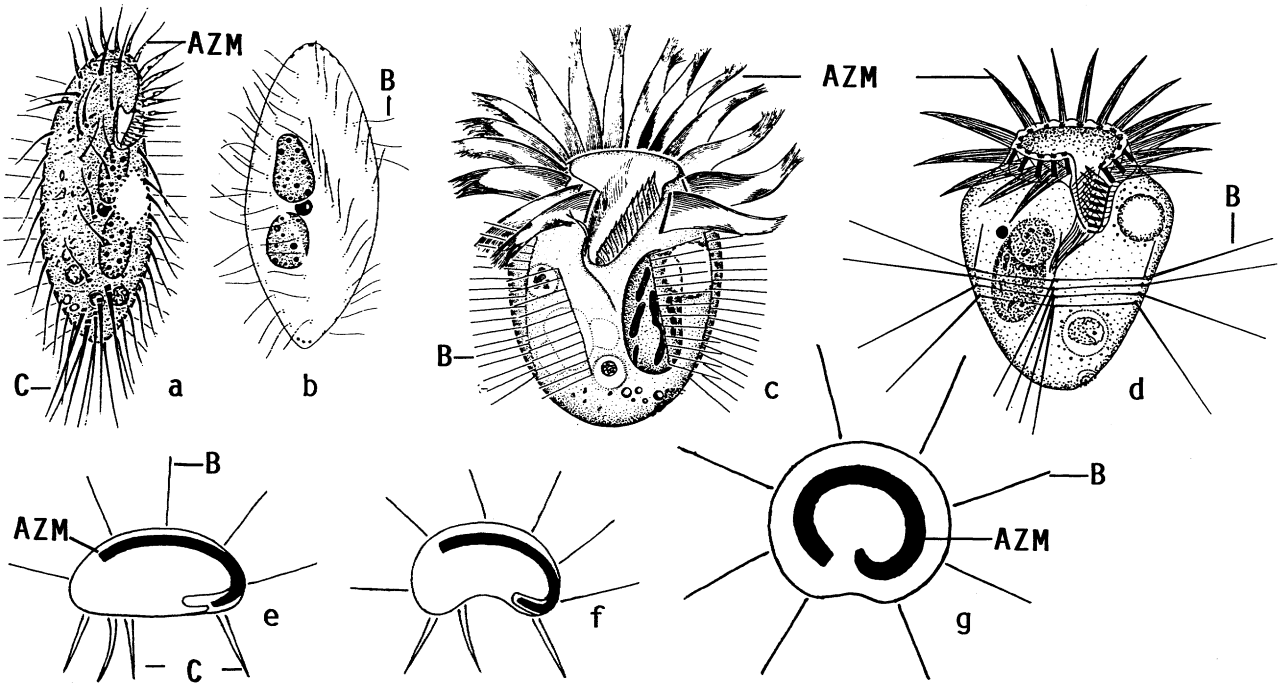


Fig. 2. a–g. Origin of halteriids from flexible stichotrich oxytrichids by "dorsalization", that is, involution of the ventral side and complete reduction of the cirri. The stichotrich dorsal bristles become the halteriid jumping bristles. Unfortunately, this attractive hypothesis cannot explain the enantiotropic cell division of halteriids. **(a, b)** Ventral and dorsal view of an *Oxytricha*. **(c, d)** Ventral views of the halteriids *Meseres* and *Halteria*. **(e)** Frontal view of *Oxytricha* in oral region. **(f)** Hypothetical transition stage. **(g)** Frontal view of *Meseres* and *Halteria*. AZM—adoral zone of membranelles, B—bristles, C—cirri.

it is thus as conspicuous as in several stichotrichs, for instance, *Paragonostomum* and *Wallackia* (Foissner et al. 2002).

Very recently, we discovered another important feature, viz., the wall structure of the resting cysts. These data will be presented in a separate paper. Here, we give a very brief summary. The surface of the resting cyst wall of *Halteria* and *Meseres* consists of fine fibres forming a three-dimensional, reticular structure. In the new *Halteria* species mentioned above, this reticulum forms 1–3 μm long, thorn-like scales or, in *Meseres corlissi*, conspicuous, faceted spheres up to 10 μm across. These scales can be removed mechanically without destroying the cysts. Similar cysts occur in marine and limnetic strombidiids (Reid 1987; Müller 1996) and tintinnids (Reid and John 1978). In contrast, resting cysts of stichotrichs are like those of many other ciliates, that is, have a compact surface never covered by scales; if the surface is spiny, the spines are part of the cyst wall and thus cannot be removed (Berger 1999; unpubl. data of Foissner on cysts of about 30 stichotrichs). These observations strongly support an oligotrichine classification of *Halteria* because the resting cysts of *Halteria* and oligotrichs have very special features in common, definitely different from those found in the stichotrichs.

Although we tend to consider the curious resting cysts and the enantiotropic cell division as "master features", the stichotrichine characteristics of halteriids cannot be denied. Thus, the available morphological and ontogenetical features cannot unambiguously answer the question whether halteriids are oligotrichine or stichotrichine spirotrichs. Principally and in agreement with Petz and Foissner (1992), we are not opposed to consider halteriids as nearest relatives of the stichotrichine spirotrichs. However, 18S rDNA data locate the halteriids within the oxytrichid clade, viz., usually near or very near to *Oxytricha granulifera*, type species of the genus (Fig. 5). Such a position is not supported by any specific morphological or ontogenetical features, suggesting that the rDNA trees either reflect an undersampling of stichotrichine taxa or are influenced by other phenomena not yet known or considered, for instance mutational saturation (Germot and Philippe 1999; Hackstein 2002).

Noteably, a similar case exists in molecular phylogenies of the angiosperms where monocots are paraphyletic with dicots. Duvall and Ervin (2004) discuss six possible reasons: (i) the published 18S sequence of *Acorus calamus* is, in some way, erroneous; (ii) monocots are truly paraphyletic with selected dicot paleoherbs; (iii) insufficient taxon density; (iv) long branch attraction; (v) the previously determined 18S sequences of *Acorus* actually represent aberrant paralogues; and (vi) lineage sorting or other molecular evolutionary events. They could exclude possibilities (i) to (v), while differential lineage sorting from a poly-

Table 2. Features classifying halteriids as oligotrich or stichotrichine spirotrich ciliates

Oligotrich features	Stichotrich features
Morphological features	**Morphological features**
(1) Frontal adoral zone of membranelles (AZM), as typical for oligotrichs. Stichotrichs have a ventral AZM, and among the hypotrichs only *Gastrocirrhus* has a frontal AZM similar to that of halteriids.	(1) Halteriid bristles look like modified cirri or stichotrich dorsal bristles. However, homology has not yet been scrutinized.
(2) Globular to obconical body shape, as typical for oligotrichs. Body usually ellipsoidal and flattened in stichotrichs and hypotrichs with conspicuous ventral (cirri and AZM)—dorsal (rows of dikinetidal bristles) differentiation.	(2) Halteriids have two undulating membranes, like typical stichotrichs. However, one of the membranes is very small (reduced?) and homology uncertain for both because they originate de novo, in contrast to the stichotrichs, where they originate, with few exceptions from parental structures (proter) or from cirral and/or AZM anlagen (opisthe).
(3) Strongly reduced somatic ciliature (less than 10 bristle rows) only partially used for locomotion, as typical for oligotrichs. Stichotrichs and hypotrichs use the ventral cirri for locomotion.	**Ontogenetic features**
(4) Single macronucleus, as most oligotrichs. Stichotrichs have at least two macronucleus nodules. Many hypotrichs have, however, a single macronucleus, as halteriids.	(3) The oral primordium develops on body surface, as in most stichotrichs (with few exceptions, e.g. *Psilotricha*). In oligotrichs, the oral primordium invariably develops in a subsurface pouch, as in hypotrichs s.str.
(5) Pelagic life style, as typical for oligotrichs. There are very few euplanktonic stichotrichs and hypotrichs.	(4) The developing oral primordium curves right anteriorly in halteriids and stichotrichs, while right posteriorly in typical oligotrichs.
Ontogenetic features	(5) The halteriid somatic ciliature originates de novo by separate anlagen each in proter and opisthe, similar to stichotrichs, where, however, the anlagen usually develop from parental cirri or bristles. In oligotrichs, the somatic ciliature doubles by intrakinetal proliferation of basal bodies.
(6) Enantiotropic division, as highly characteristic for all oligotrichs. All stichotrichs and most ciliates in general, divide homothetogenically.	**Resting cysts**
(7) De novo origin of the undulating membranes, as in all oligotrichs investigated so far. In stichotrichs, they originate from the cirral and/or AZM anlagen. In the proter, the paroral may develop de novo.	(6) Resting cysts of stichotrichs are never covered by scales; if they are spiny, the spines are part of the cyst wall (Berger 1999 and unpubl. data of Foissner on cysts of about 30 hypotrichs).
Resting cysts	
(8) The resting cysts of a new *Halteria* species from the Dom. Rep. are covered by thorn–like scales; and *Meseres corlissi* has large, spherical scales (Foissner et al., manuscript submitted)	

For literature, see Berger (1999), Corliss (1979), Foissner (1996), Foissner et al. (1999, 2002), Lynn and Small (2002), Petz and Foissner (1992), and Strüder-Kypke and Lynn (2003).

morphic ancestral population remains a possibility, particularly for a family of multiple paralogues such as nuclear ribosomal loci. In the case of *Halteria*, we can exclude only possibilities (i) and (iv).

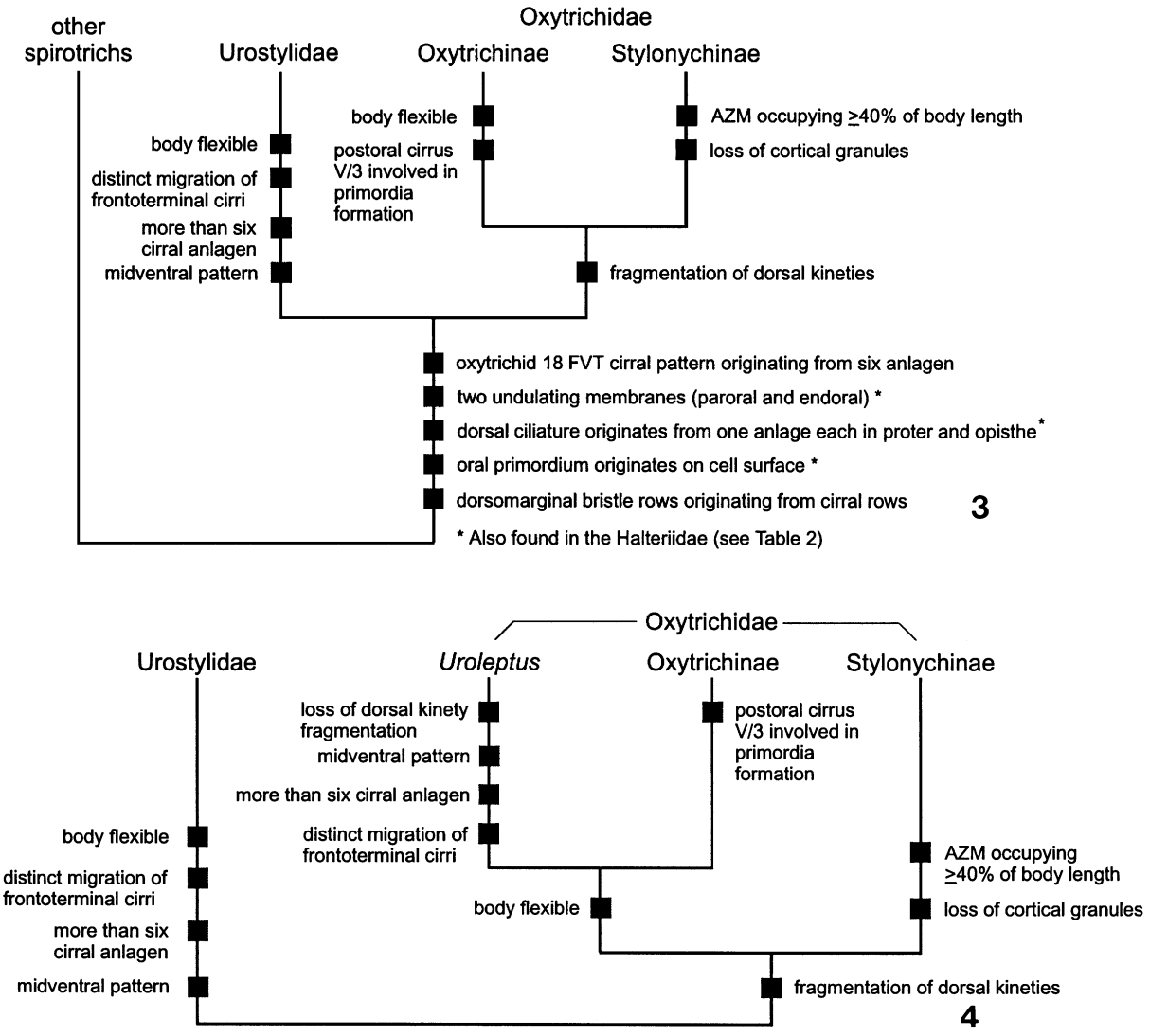
As discussed above, there is also no convincing argument that halteriids are secondarily evolved planktonic hypotrichs. Thus, we consider the conclusion of Strüder-Kypke and Lynn (2003) as premature that "there is now no doubt that halteriid ciliates are derived from a strichotrich clade that became secondarily adapted to the planktonic habitat". Potentially, broader taxa sampling, the analysis of the other questions raised above, and a phylogenetic analysis of the large subunit rRNA together with a consideration of the secondary structure will be able to reconcile the morphological and molecular data.

Contrasting morphological and molecular classifications of two major stichotrichine lineages: oxytrichids and urostylids/holostichids

Berger and Foissner (1997) and/or Berger (1999) should be consulted for terminology and synonymy and for a detailed discussion of oxytrichid morphology and characters.

The distinction of oxytrichids from urostylids (composed of urostylid and holostichid stichotrichs) dates back to Bütschli (1889). Later, Borror (1972) refined terminology by introducing the term midventral row, that is, two narrowly spaced, median cirral rows, whose paired cirri form a highly characteristic zigzag (= midventral) pattern (Fig. 6(b)). He also recognized the special origin of this pattern by rather many oblique cirral anlagen. The oxytrichids, on the other hand, were characterized by their typical fronto-ventral-transverse (FVT) cirral pattern, usually comprising 18 cirri (3 frontal, 4 frontoventral, 1 buccal, 3 postoral, 5 transverse, and 2 pretransverse cirri; Fig. 6(a)). Later authors, although often considerably differing in the overall classification of the hypotrichs s.l., maintained these two groups at family level, at least (e.g. Corliss 1979; Lynn and Small 1997). We restrict the phylogenetic analyses to just these two highly characteristic groups, using all other spirotrichs as an outgroup (Figs. 3-5). Thus, several of the features characterizing oxytrichids and urostylids are found also in the euplotine hypotrichs, for instance, the basic cirral pattern, whose homology with the oxytrichid pattern was already supposed by Wallengren (1900). We shall not discuss as to whether the oligotrichs or the euplotid hypotrichs are the sister group of the stichotrichs (Petz and Foissner 1992; Chen and Song 2002; Strüder-Kypke and Lynn 2003).

Most morphologists agree that oxytrichids and urostylids/holostichids are two monophyletic lineages (Fauré-Fremiet 1961; Borror 1972; Corliss 1979; Borror and Wicklow 1983; Lynn and Small 1997, 2002; Shi et al.



Figs. 3 and 4. Cladistic (Hennigian) systems of the Oxytrichidae and Urostylidae, two main lineages of the stichotrichine spirotrichs. See Fig. 6 for evolution of the midventral pattern. **3:** Scheme of argumentation if assumed that the Oxytrichidae and Urostylidae are monophyletic lineages. **4:** Scheme of argumentation if the midventral pattern evolved twice convergently, as proposed by the CEUU hypothesis.

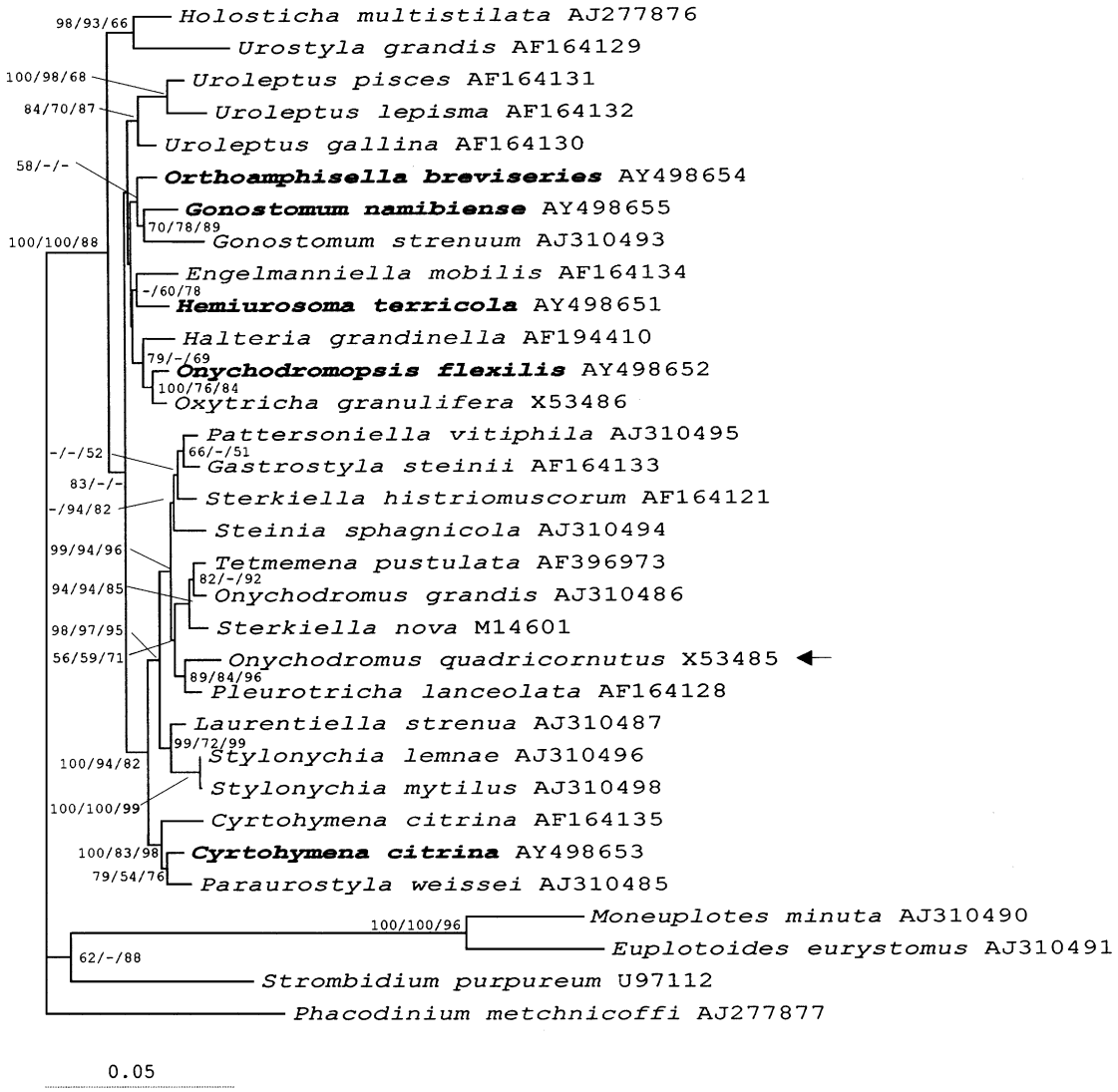


Fig. 5. Neighbour-joining tree (NJ) of oxytrichid and urostylid stichotrichs based on 18S rRNA gene sequences. The codes following the names are the GenBank Accession Numbers. The numbers at nodes represent the neighbour-joining and maximum-parsimony bootstrap percentages from 100 replicates (values below 50% not shown.) and the quartet puzzle support values obtained with 10000 puzzling steps, respectively. The scale bar corresponds to a distance of 5 substitutions per 100 nucleotide positions. The type species of *Styxophrya* nov. gen. is indicated by an arrow.

1999). However, Eigner (1997) proposes a polyphyly of the oxytrichids. He assumes that the highly characteristic 18 FVT cirral pattern of the oxytrichids and the specific ontogenetic processes producing this pattern evolved independently several times. On the other hand, he agrees about the monophyly of the urostylids/holostichids (Eigner 2001). Eigner's polyphyly of the oxytrichids is, inter alia, based on incompletely understood ontogenetic details, paraphylies, and a too rigid interpretation of the computer-generated trees (Berger 1999). We base the monophyly of the oxytrichids on their highly characteristic 18 FVT cirral pattern and its origin from basically six anlagen; further, they have fragmentising dorsal kineties, a specific feature which occurs only rarely and probably convergently in other stichotrichs, for instance, in the amphisiellid (?) *Pseudouroleptus* (Berger 1999) and the urostylid *Neokeronopsis* (Warren et al. 2002). The urostylids have only one, but very strong apomorphy, viz., the midventral row and its origin from few to many cirral anlagen. There is no indication in the literature that the midventral pattern evolved convergently in several stichotrichs. This results in a cladistic classification as shown in Fig. 3.

Our NJ and parsimony analyses support with 100% bootstrap values the morphological and ontogenetical data on the monophyly of the *Urostyla*/*Holosticha* clade (Fig. 5). However, the three *Uroleptus* species cluster with the oxytrichids, although they are quite typical urostylids/holostichids differing from *Holosticha* only in

body shape (tailed vs. untailed) and the presence/absence of dorsomarginal kineties (Borror 1972; Eigner 2001). This suggests a biphyletic origin of the urostylid stichotrichs, which is supported by the rather similar molecular trees of Hewitt et al. (2003) and Snoeyenbos-West et al. (2002). In contrast, the trees of Bernhard et al. (2001), Chen and Song (2002) and Strüder-Kypke and Lynn (2003) cluster *Holosticha* very near to *Oxytricha granulifera*, although they contain genera, such as *Stylonychia* and *Onychodromus*, which are morphologically much nearer to *Oxytricha* than *Holosticha*. Probably, this is because they do not include *Urostyla*. This indicates that such profound differences are caused by insufficient taxa sampling. Sequences are available from only about 40 of the 300 oxytrichids and urostylids known (Berger 1999, 2001). Further, more than 50% of the ciliate morphospecies are probably undescribed (for a review, see Foissner et al. 2002). Accordingly, our and other trees contain less than 5% of the stichotrich taxa that probably exist! Nonetheless, differences in alignment, outgroup, phylogenetic algorithm, and clustering method may also contribute to the differences in the molecular trees available. Lastly, the low sequence divergence among the stichotrichine spirotrichs hampers phylogenetic analysis and makes it very sensitive to undersampling.

The CEUU hypothesis: an attempt to reconcile morphological and molecular classifications of oxytrichid and urostylid stichotrichs

There is increasing evidence for convergent evolution of many of the characteristic stichotrichine cirral patterns (Berger and Foissner 1997; Eigner 1994, 1997; Berger 1999; Shi et al. 1999; Foissner et al. 2002). The CEUU (*C*onvergent *E*volution of *U*rostylids and *U*roleptids) hypothesis, which tries to combine classical and molecular data, proposes that even one of the most distinct features, viz., the midventral pattern, evolved convergently in at least two lineages (Fig. 4). The CEUU hypothesis is based on several assumptions and/or lines of evidence, which can be partially verified by further investigations. See the previous section for a general overview and details of present classifications.

Traditionally, oxytrichid stichotrichs are regarded as derived from ancestors with many cirral rows, such as *Urostyla* (Kahl 1932; Borror 1972). However, the CEUU hypothesis proposes the opposite way because the euplotine hypotrichs have an *Oxytricha*-like cirral pattern (Wallengren 1900) and are basal to the stichotrichs in gene sequence trees (Bernhard et al. 2001; Strüder-Kypke and Lynn 2003). Further, the CEUU hypothesis proposes that cirri and anlagen multiplication are not necessarily correlated with the production of a midventral pattern and occurred frequently in oxytrichid stichotrichs, producing many patterns (see the oxytrichids s.l., for instance, *Paraurostyla*, *Laurentiella*, and *Styxophrya*), including midventral-like arrangements in, e.g., *Pattersoniella* and *Territricha* (Figs. 7 and 8). Indeed, the last mentioned genera are considered as a distinct urostylid family (Pattersoniellidae) by Shi et al. (1999). Although we do not agree with this classification, because the cirral pattern can be derived from that of typical oxytrichids (Fig. 8) and *Pattersoniella* is found in the Stylonychinae sequence cluster (Fig. 5), it shows the narrowness of the gap between oxytrichids and urostylids! This is emphasized by genera like *Vermioxytricha* and *Eschaneustyla* (Fig. 7(e)) which have reduced the number of cirri so strongly that the oxytrichid, and/or urostylid patterns are recognizable only during ontogenesis (Eigner 1994; Foissner et al. 2002).

Figure 6 shows the proposed homology of the oxytrichid and urostylid cirri as well as the origin of an urostylid stichotrich by inserting additional cirral anlagen, each producing a pair of cirri, among the basic six oxytrichid anlagen. This process must have occurred at least twice, according to the rDNA trees (Fig. 5). The first, older and more distinct event caused the common ancestor to split into an oxytrichid and a urostylid lineage. These are now the urostylids s.str. which cluster outside of the Oxytrichidae (Figs. 4 and 5). Later, a midventral pattern evolved again in the Oxytrichidae, from a different ancestor and possibly more gradually, forming the *Uroleptus* lineage, which is still in the oxytrichid cluster because it diverged less distinctly genetically than morphologically, if compared with the urostylids s.str. (Figs. 4, 5 and 7). Although cirri and anlagen multiplication are obviously not correlated with the generation of a midventral pattern, the scenario developed above is quite likely, considering the many cirral patterns found in the oxytrichids (for a review, see Berger 1999). It is likely that the second event was driven by ecological constraints because, by definition, all *Uroleptus* species are more or less distinctly tailed.

Although the scenario of the CEUU hypothesis is reasonable and in accordance with the molecular trees as well as with the high flexibility of the oxytrichid cirral pattern in general, we lack a specific morphological proof. Thus, the prostitution of a distinctive morphological and ontogenetic feature, viz., the midventral pattern, is probably too premature, especially when considering some rather "difficult" morphological features, e.g., body flexibility, which now obtains heavy weight (Fig. 4). Hopefully, morphological proof for the CEUU hypothesis can be obtained by refined ontogenetic studies (presence/absence of dorsomarginal kineties might be a key feature), detailed investigations of the fibrillar associates of the midventral cirri in *Urostyla*/*Holosticha* and *Uroleptus*, and molecular traits which show, for instance, that histone H4 is

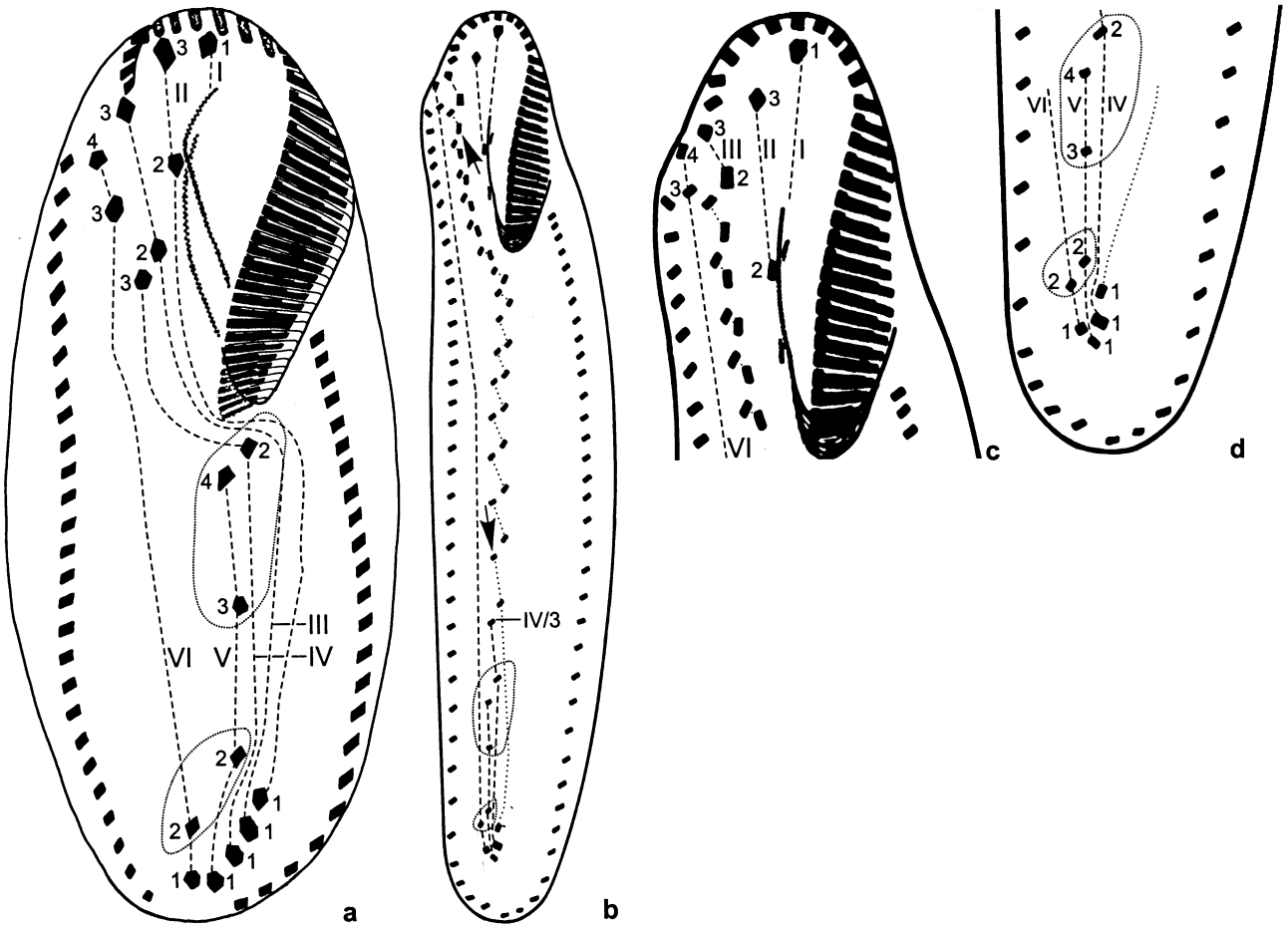


Fig. 6. a–d. Homology of cirri in an 18 FVT cirri oxytrichid (**a**; *Sterkiella histriomuscorum*) and an urostylid (**b–c**; *Holosticha australis*). Numbering of fronto-ventral-transverse cirral anlagen and cirri according to Wallengren (1900). Cirri originating from same anlagen (I–VI) connected by broken line. Postoral cirri (cirri IV/2, V/3, V/4) and pretransverse cirri (cirri V/2 and VI/2) encircled by dotted line. Urostylids probably evolved from an 18 cirri ancestor by inserting additional anlagen generating cirral pairs, which produce the highly characteristic zigzagging midventral pattern (first and last additional cirral pair marked by an arrow each in (b)). I–VI—fronto-ventral-transverse cirral anlagen, 1–4—cirri within anlage.

encoded by two or more macronuclear molecules in five oxytrichid genera (*Sterkiella*, *Stylonychia*, *Oxytricha*, *Pleurotricha*, *Paraurostyla*), while only one macronuclear molecule encodes histone H4 in *Uroleptus* sp. (Kelminson et al. 2002). Further, intensified research on alpha-diversity might discover true missing links and further midventral lineages, especially in combined morphological and gene sequence studies. Such investigations are pressingly needed because, if the CEUU hypothesis is correct, it will be impossible to assign genera with a midventral row unambiguously to either the urostylids or oxytrichids.

Classification of lower categories

Although 18S rRNA gene sequences are probably not the ideal marker for distinguishing ciliate genera and species, they are frequently used for this purpose, e.g., by Lynn et al. (2000), Strüder-Kypke et al. (2000), Modeo et al. (2003), and Shang et al. (2003). Indeed, morphologically related species often cluster together in 18S rRNA gene trees, as also evident from our data, for instance, the three *Uroleptus* species. However, in stichotrichs the matter is more complicated because the nucleotide sequences of their 18S rRNA gene are more similar to each other than in other phylogenetic groups (Bernhard et al. 2001; Snoeyenbos-West et al. 2002). Nevertheless, a more detailed interpretation appears valuable because (i) quite a lot of the molecular classifications match the morphological data rather well; (ii) the diverging cases might stimulate refined morphological and molecular work; (iii) molecular systematists usually do not discuss the morphological classification of genera and families; and (iv) the low genetic divergence of the stichotrichs contrasts with their

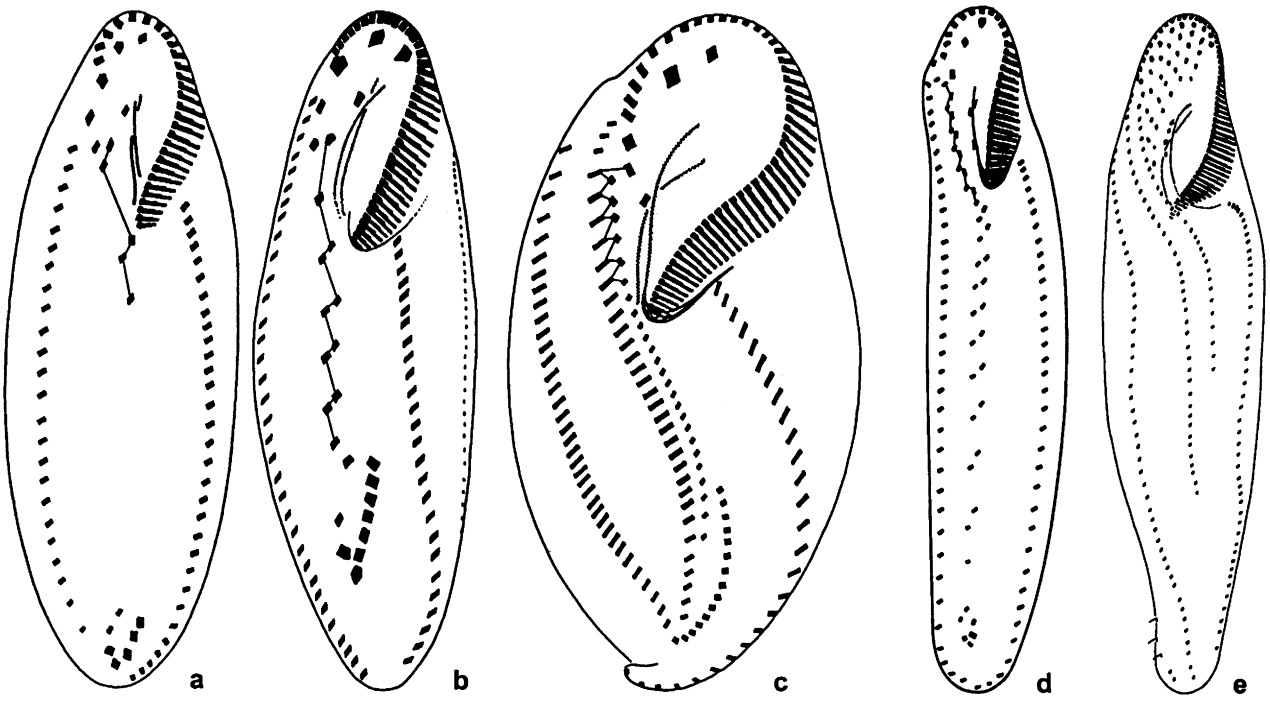


Fig. 7. a–e. Transition of the oxytrichid to the urostylid midventral cirral pattern in the genera *Oxytricha* (**a**), *Territricha* (**b**) and *Uroleptus* (**c**), and loss of the holostichid (**d**; *Holosticha*) cirral pattern in *Eschaneustyla* (**e**), where it is recognizable only during ontogenesis (Eigner 1994).

high morphologic diversity, which makes it easy to define genera and species (Berger 1999; Eigner and Foissner 1994), while familial and suprafamilial morphologic classification is highly controversial (Berger and Foissner 1997; Eigner and Foissner 1992, 1994; Eigner 1997; Berger 1999; Chen and Song 2002). We cannot comment on the latter because of the low bootstrap support in the available molecular phylogenetic trees and due to the lack of 18S rRNA sequence data from several of the main stichotrichid lineages, especially the Amphisiellidae and Kahliellidae.

Subfamiliar classification: Berger and Foissner (1997) and Berger (1999) divided the Oxytrichidae into two subfamilies, viz., the Oxytrichinae and Stylonychinae, differing in cortex structure (flexible vs. rigid), cortical granules (present vs. absent), and cirrus V/3, which is ontogenetically active only in the Oxytrichinae (Fig. 3). This classification, which differs markedly from that of Eigner mentioned above, has been supported by 18S rRNA phylogenies (Bernhard et al. 2001). Our molecular tree is ambiguous (Fig. 5): on the one hand, it still classifies all rigid species in the Stylonychinae cluster; on the other hand, not all flexible Oxytrichinae group together, leaving the *Paraurostyla-Cyrtohymena*-group as nearest relative of the rigid Stylonychinae with high bootstrap support (100/94/82%). Such division of the Oxytrichinae is not supported by any morphological evidence. In contrast, morphology and ontogenesis show that *Cyrtohymena* is a typical Oxytrichinae with cirrus V/3 involved in anlagen formation; and *C. citrina* has two other prominent features of the Oxytrichinae, viz., citrine cortical granules and high body flexibility (Berger 1999). The Stylonychinae consist of three sequence subclusters, viz., a basal cluster with *Laurentiella* and *Stylonychia*, and two sister clusters containing all other taxa. Basically, this matches the ontogenetic data in that only *Stylonychia*, *Steinia*, and probably also *Laurentiella* produce proter anlagen V and VI from cirrus V/4, while all other taxa generate these anlagen from cirrus IV/3. Unfortunately, *Steinia* remains an inexplicable exception.

Establishing Gastrostyla as a stichotrichine oxytrichid: *Gastrostyla* is a cosmopolitan genus with species occurring in limnetic, marine, and terrestrial habitats (for a review, see Berger 1999). Its cirral pattern is somehow in between those of the oxytrichids and amphisiellids, viz., there are rather many (>18) fronto-ventral-transverse (FVT) cirri arranged in an oblique row or stripe extending from anterior to posterior body end (Fig. 8). Over the years, *Gastrostyla* was assigned to the families Oxytrichidae, Amphisiellidae and Parakahliellidae, depending on the interpretation of the morphological and ontogenetical features; even a distinct family, Gastrostylidae, has been suggested (Shi et al. 2003). Recently, Foissner et al. (2002), who reinvestigated the morphology and ontogenesis of some species and discussed the data available, concluded: "None of the morphologic and ontogenetic

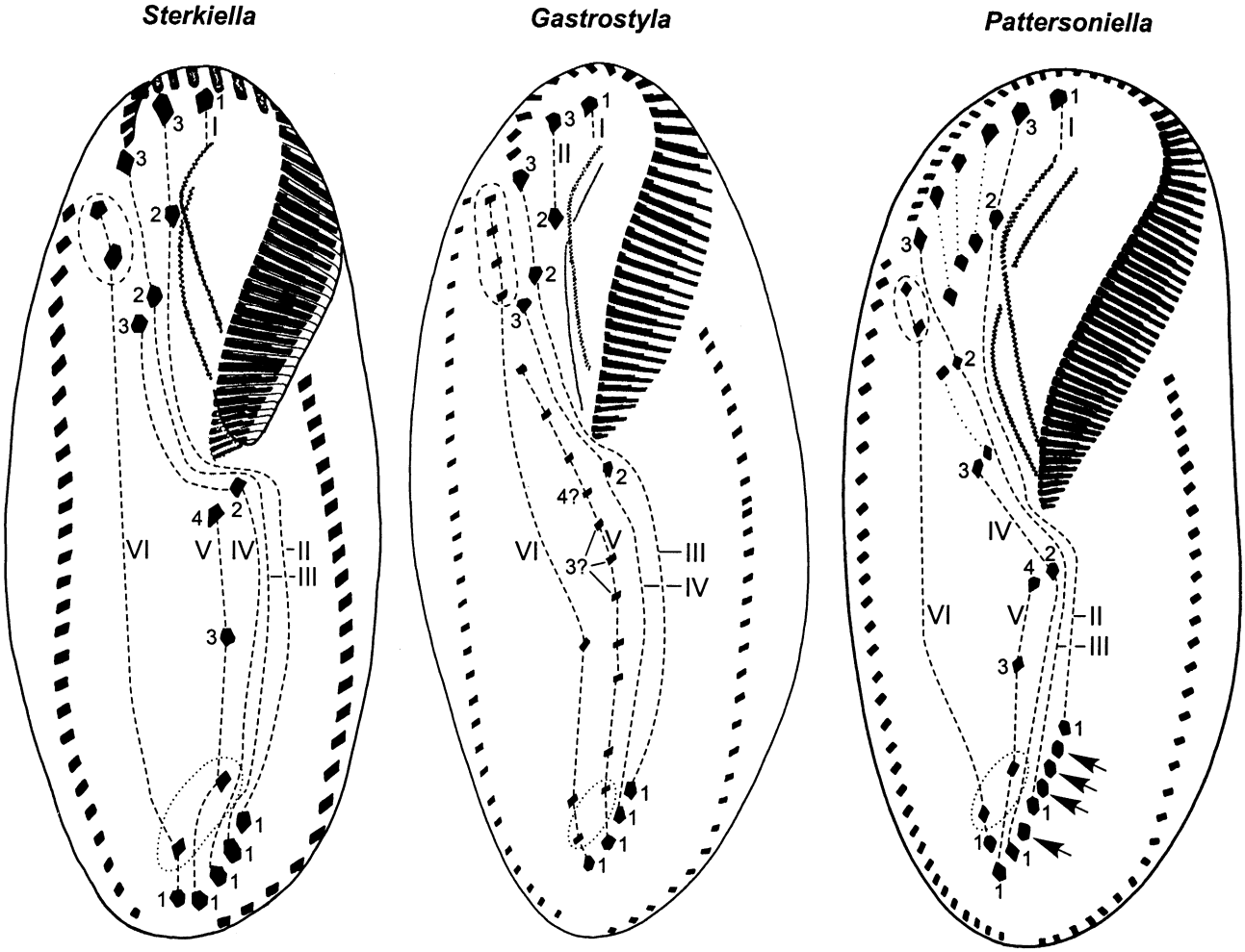


Fig. 8. Homology of cirri in *Sterkiella*, *Gastrostyla*, and *Pattersoniella*. Numbering of fronto-ventral-transverse cirral anlagen and cirri according to Wallengren (1900) and Berger (1999). Cirri originating from same anlage connected by broken line. Pretransverse cirri (cirri V/2 and VI/2) encircled by dotted line. Frontoterminal cirri (cirri VI/3 and VI/4; in *Gastrostyla* number slightly increased) encircled by broken line. *Sterkiella* has the plesiomorphic set of 18 fronto-ventral-transverse cirri. The anlagen V and VI of *Gastrostyla* produce an increased number of cirri which form the frontoventral row. The postoral cirri V/3 and V/4 cannot be homologized unequivocally and are thus marked by an "?". In *Gastrostyla steinii*, anlage II does not produce a transverse cirrus; thus, it has only four transverse cirri. *Pattersoniella* has some additional cirral anlagen whose cirri are connected by dotted lines (corresponding transverse cirri marked by arrows). I–VI—fronto-ventral-transverse cirral anlagen, 1–4—cirri within anlage.

features discussed unequivocally assigns *Gastrostyla* to the amphisiellids or oxytrichids, and thus we hope that gene sequence data will provide deeper insight into the proper classification".

Our phylogenetic analyses (Fig. 5) show that *Gastrostyla steinii*, type species of the genus, belongs to the rigid Stylonychinae, that is, forms a cluster with *Sterkiella histriomuscorum* (a classic oxytrichid), *Steinia sphagnicola* (also a typical oxytrichid) and, as nearest relative, *Pattersoniella vitiphila* (an oxytrichid sensu lato). This was already proposed by Berger (1999) and matches the morphological and ontogenetical data because *Pattersoniella*, like, *Gastrostyla*, has an increased number of FVT cirri and the individual cirral groups of *Sterkiella*, *Pattersoniella*, and *Gastrostyla* can be homologized (Fig. 8). Further, there are no morphological objections to derive the *Gastrostyla-Pattersoniella*-group from a *Sterkiella*-like ancestor because all have the same type of oral apparatus and dorsal kinety pattern, and contain species with more than the usual two macronucleus nodules; however, *Gastrostyla* may be paraphyletic because of rather conspicuous morphological and ontogenetical differences in the individual species (Foissner et al. 2002).

We consider the solution of the *Gastrostyla* problem, which teased morphologists for such a long time, as an excellent example for the usefulness of 18S rRNA gene sequences in generic taxonomy, provided they are

supplemented by detailed morphological and ontogenetical data to assess the uncertainties caused by general problems (undersampling, various algorithms etc., see above) and the high genetic similarity of the stichotrichine spirotrichs.

Engelmanniella, a touchstone for classification: The optimistic conclusion just drawn is not substantiated by *Engelmanniella mobilis*, a curious stichotrich, whose systematic position is still in discussion, in spite of the detailed data available on its morphology, ontogenesis, ultrastructure, resting cysts, and physiological reorganization (Wirnsberger-Aescht et al. 1989, 1990; Eigner 1997). Now, the 18S rRNA gene sequences place it in the oxytrichid clade (Fig. 5), albeit with insignificant bootstrap support, a classification never suggested by morphologists. However, the position is highly unstable because the maximum-likelihood tree of Snoeyenbos-West et al. (2002) classifies *Engelmanniella* with 92% bootstrap support as a close relative of *Urostyla*! Such a position is also unlikely because *Engelmanniella* lacks midventral cirri, the main diagnostic feature of the urostylids (see above). A position in the oxytrichid clade, as suggested by our NJ tree and the trees of Hewitt et al. (2003), is also difficult to believe because of the entirely different interphase morphology and ontogenesis, although the recently described *Vermioxytricha* has some similarity (Foissner et al. 2002). All these and some other discrepancies suggest overall misclassification of *Engelmanniella* by the 18S rRNA gene sequences, as in *Halteria*.

Matching generic classifications (Fig. 5): The morphological and the 18S rRNA gene phylogenies match in several cases which, however, need some explanations. The monophyly of the three *Uroleptus* species is supported by our analyses as well as by morphological studies (Berger and Foissner 1989; Foissner et al. 1991). *Gonostomum* clusters with *Orthoamphisiella breviseries* in the NJ tree, however, with only 58% bootstrap support. This rather unusual species, whose classification remained uncertain in the original description due to the lack of ontogenetic data, might be, indeed, related to *Gonostomum* because it has a gonostomoid oral apparatus, few frontoventral cirri, and lacks postoral cirri. The *Oxytricha granulifera* and *Onychodromopsis flexilis* cluster is also reasonable because no other *Oxytricha* species was included in the analysis and *O. flexilis* has a typical oxytrichid FVT cirral pattern and ontogenesis, differing from *Oxytricha* s.str. only by the increased number of *marginal cirral rows* (Petz and Foissner 1996). An analogous case represents the *Stylonychia-Laurentiella*-cluster from the rigid Stylonychinae subfamily, where *Laurentiella* has so strongly increased the *number of FVT* cirri that the plesiomorphic 18 FVT cirral pattern vanished. Unfortunately, detailed ontogenetic data are lacking for *Laurentiella*. However, a rather close relationship of *Stylonychia* and *Laurentiella* is supported by the very rigid body (a plesiomorphy) and distinct similarities in highly specific details of the oral apparatus (Berger 1999). The *Tetmemena*, *Onychodromus grandis*, and *Sterkiella* cluster also appears reasonable. *Tetmemena* and *O. grandis* agree in the oral apparatus (*Stylonychia* pattern) and differ mainly in the number of FVT cirri, which is slightly increased in *O. grandis*, while both still have the typical six oxytrichid FVT cirral anlagen. *Sterkiella nova* is basal to *Tetmemena* and *O. grandis*, which matches its different oral apparatus (*Oxytricha* pattern). Admittedly, the morphologic and ontogenetic features connecting these genera are of a rather general nature and do thus not strongly support, but also not refute, the sequence classification.

The two *Cyrtohymena citrina* populations and *Paraurostyla weissei* cluster with high bootstrap support (100/83/98%). This contradicts morphological and ontogenetical evidence which suggest a rather close relationship of *Cyrtohymena* and *Oxytricha*, although both taxa differ not only in the curvature of the paroral membrane but also in the origin of the proter's cirral primordia V and VI. Further, *Paraurostyla* does not have an 18 FVT cirral pattern but several rows of frontoventral cirri, similar to *Laurentiella* discussed above. On the other hand, there is convincing ontogenetic and gene sequence evidence that oxytrichids s.str. increased the number of frontoventral and marginal cirral rows several times independently, both in the Oxytrichinae (*Onychodromopsis*, *Paraurostyla*) and Stylonychinae (*Laurentiella*, *Pattersoniella*, *Onychodromus*, *Pleurotricha*). Thus, *Paraurostyla* might have evolved, indeed, from a *Cyrtohymena*-like ancestor, although morphologists never proposed such a relationship. A posteriori, we can find several specific similarities, viz., the highly flexible body, the strongly curved (cyrtohymenid) paroral membrane, the citrine cortical granules, and the spiny resting cysts (Foissner, unpubl.). That one of the two *C. citrina* populations clusters with *Paraurostyla* has little significance, because this has only up to 79% bootstrap support; it may indicate that *C. citrina* consists of several, not yet described species.

Mismatching generic classifications: We could not find reasonable explanations based on morphology or ontogeny for the following molecular classifications (Fig. 5): (i) that *Cyrtohymena* does not cluster with *Oxytricha*, as discussed in the previous section; (ii) that *Hemiurosoma* does not cluster with *Gonostomum*, as suggested by the morphological and ontogenetical data (Berger and Foissner 1997; Foissner et al. 2002); (iii) that *Steinia* does not cluster with *Stylonychia*, in spite of distinct ontogenetic similarities, as explained in the discussion on subfamiliar classification; (iv) that the two *Sterkiella* species are in different clusters, although they are sibling species indistinguishable morphologically and ontogenetically (Foissner and Berger 1999).

Styxophrya nov. gen.

Diagnosis: Oxytrichidae (Stylonychinae) with oral apparatus in *Stylonychia* pattern. Many (>30) fronto-ventral-transverse cirri in several distinct rows. More than 6 dorsal kineties. Caudal cirri present. Cytoplasmic processes (horns) on dorsal surface. Distinctly more than 6 fronto-ventral-transverse cirral primordia, each producing many cirri and originating independently in proter and opisthe. Dorsal kineties with multiple fragmentation, dorsomarginal kineties present.

Type species: *Onychodromus quadricornutus* Foissner, Schlegel and Prescott, 1987.

Etymology: Composite of the Latin noun *styx* (underworld) and the Greek noun *ophrya* (eyebrow ~ cilia ~ ciliate), meaning "a ciliate from the underworld". Feminine gender. *Styx* is the original "cavalier name" given this conspicuous species in Prescott's laboratory. Further nomenclatural details, see Foissner et al. (1987) and Berger (1999).

Species assignable: The type species mentioned above, needs to be combined with the new genus: *Styxophrya quadricornuta* (Foissner, Schlegel and Prescott, 1987) nov. comb. *Styxophrya* is monotypic because *Onychodromus indica* Kamra and Sapra is a junior synonym of *Styxophrya quadricornuta* (Berger 1999).

Comparison with related genera: The diagnosis of *Styxophrya* is adapted to fit the generic definitions of Berger (1999). *Onychodromus grandis*, type species of the genus *Onychodromus*, and *Onychodromus* (now *Styxophrya*) *quadricornutus* have a rather dissimilar morphology and ontogenesis (for a review, see Berger 1999). Briefly, *O. grandis* has slightly increased the number of FVT cirri, but the typical oxytrichid cirral pattern is still recognizable and originates, as in oxytrichids s.str., from six cirral anlagen streaks. In contrast, *Styxophrya quadricornuta* has so greatly increased the number of FVT cirri and cirral anlagen streaks that the oxytrichid pattern disappeared. Foissner et al. (1987) remarked that they classified *O. quadricornutus* conservatively, though two of the three reviewers suggested the establishment of a new genus for this spectacular ciliate. Since then, great progress occurred in the knowledge and classification of the Oxytrichidae (Berger 1999; Foissner et al. 2002), suggesting the placement of this ciliate in a distinct genus.

Separating *Styxophrya* from *Onychodromus* is also supported by the 18S rRNA gene data, where *S. quadricornuta* clusters with *Pleurotricha*, while *O. grandis* forms a group with *Tetmemena pustulata* and *Sterkiella nova*, as discussed above (Fig. 5). *Pleurotricha* and *Styxophrya* have the same type of oral apparatus, while the cirral pattern is rather different: *Pleurotricha* has increased the number of marginal rows, while *Styxophrya* has increased the number of fronto-ventral cirri. Thus, a reasonable missing link between the two genera is lacking. However, Foissner has an undescribed new genus which might be in between *Pleurotricha* and *Styxophrya*.

Acknowledgements

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