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Phylogenetic Relationships of Orders Within the Class Colpodea (Phylum Ciliophora) Inferred from Small Subunit rRNA Gene Sequences

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Abstract. Molecular analyses have been used recently to refine our knowledge of phylogenetic relationships within the ciliated protozoa (phylum Ciliophora). A current Hennigian phylogeny of the orders in the class Colpodea, based on light and electron microscopic analyses, makes three important assumptions with regard to apomorphic character states, namely, (1) that the kreyellid silver line evolved early in colpodean phylogeny, separating bryometopids, such as *Bryometopus,* from all other colpodeans; (2) that the macro–micronuclear complex is an autapomorphy of the cyrtolophosidids, such as *Platyophrya;* and (3) that merotelokinetal stomatogenesis is an apomorphic character of colpodids, such as *Colpoda*, *Bresslaua,* and *Pseudoplatyophrya.* These predictions of relationships within the class Colpodea were investigated by determining the complete small subunit rRNA gene sequences for the colpodid *Bresslaua vorax*, the grossglockneriid *Pseudoplatyophrya nana,* and the cyrtolophosidid *Platyophrya vorax* and a partial sequence for the bryometopid *Bryometopus sphagni.* These sequences were combined with the previously published complete SSrRNA sequences for the colpodid *Colpoda inflata* and the bursariomorphid *Bursaria truncatella.* The affiliations were assessed using both distance matrix and maximum-parsimony analyses. The tree topologies for the class Colpodea were identical in all analyses, with bootstrap support for bifurcations always exceeding 60%. The results suggest the following. (1) Since the clade including *Bryometopus* and its sister taxon, *Bursaria,* is never basal, the kreyellid silver-line system evolved later in colpodean phylogeny and does not separate bryometopids from all other colpodeans. (2) Since *Platyophrya* is always the sister taxon to the other five genera, there is a fundamental phylogenetic significance for its macro–micronuclear complex. (3) Since the colpodids, *Colpoda*, *Bresslaua*, and *Pseudoplatyophrya*, always group in one clade, merotelokinetal stomatogenesis appears to be a derived character state.

Key words: Ciliophora — Phylogeny — *Bresslaua* — *Colpoda* — *Bryometopus* — *Bursaria* — *Pseudoplatyophrya* — *Platyophrya* — Small subunit rRNA

Introduction

The colpodid ciliates have been recognized as a distinctive group since Bütschli (1889) placed the genus *Colpoda* in his order Trichostomatida. Kahl's treatment (1931–1935) recognized the family Colpodidae Poche, 1913, which included six genera: *Colpoda* Müller, 1786; *Tillina* Gruber, 1880; *Bresslaua* Kahl, 1931; *Bryophrya* Kahl, 1931; *Woodruffia* Kahl, 1931; and *Rhyposophrya* Kahl, 1933. de Puytorac et al. (1974) further distinguished the colpodids within their class Kinetofragminophorea by elevating them to an ordinal level, based on their telokinetal type of stomatogenesis (Fig. 1:3–5, 12,

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13). Lynn (1976a, 1981) reviewed the cortical ultrastructure of ciliates and argued that the structure of the somatic ciliary apparatus or somatic kinetid of ciliates was a primary indicator of common ancestry and an indicator of major clades. Based on this, Small and Lynn (1981) established several new classes of ciliates in their presentation of a new macrosystem for the phylum Ciliophora. They elevated the colpodid ciliates as the class Colpodea Small and Lynn, 1981, characterizing this taxon by the possession of a somatic kinetid with two ciliated kinetosomes and a transverse ribbon associated with the posterior kinetosome that extended posteriorly to overlap other ribbons, the so-called transversodesma (Small and Lynn 1981, 1985). Foissner's (1993) monographic treatment of the class has increased by an order of magnitude the number of genera assigned to the class Colpodea. There are two factors responsible for this growth: (a) Foissner (1993) has discovered new colpodean genera and species by intensive sampling of a variety of habitats around the globe; and (b) a number of genera have been transferred to the class on the basis of ultrastructural investigations.

The 1970s and 1980s marked the Age of Ultrastructure in the history of ciliate systematics (Corliss 1974). It was during this period that ultrastructural descriptions of recognized colpodids occurred: for example, *Bryophrya* (Grain et al. 1979), *Colpoda* (Didier and Chessa 1970; Gerassimova 1976; Lynn 1976a–c, 1977, 1978; Perez-Paniagua et al. 1979), *Bresslaua* (Garcia-Rodriguez et al. 1981; Lynn 1979), *Platyophrya* (Dragesco et al. 1977), *Tillina* (Gerassimova 1976; Lynn 1977, 1978), and *Woodruffia* (Golder and Lynn 1980; de Puytorac et al. 1979). Ultrastructural study of the somatic kinetids as a complement to light microscopy corroborated newly discovered genera as colpodean: *Bresslauides* (Platt and Hausmann 1993), *Cosmocolpoda* (Foissner and Foissner 1994), *Grossglockneria* (Aescht et al. 1991), *Kuklikophrya* (Njiné 1979), and *Pseudoplatyophrya* (de Puytorac et al. 1983). Finally, ultrastructural data have provided evidence of colpodean somatic kinetids in ciliates previously placed in different classes but now considered to be colpodean: *Cyrtolophosis* (Detcheva 1976; Didier et al. 1980), formerly an oligohymenophorean; *Sorogena* (Bardele et al. 1991), formerly a kinetofragminophorean (Bradbury and Olive 1980); and *Bursaria* (Gerassimova et al. 1979; Lynn 1980; Perez-Paniagua et al. 1980; de Puytorac and Perez-Paniagua 1979) and *Bryometopus* (Wirnsberger et al. 1985), formerly heterotrichid spirotricheans. Bardele (1989) expressed doubts about the monophyly of the class Colpodea because this assemblage of species showed diversity in the kinds of intramembranous particle arrays in their ciliary membranes, features Bardele (1989) considered strongly indicative of common ancestry. Thus, the characterization of the small subunit rRNA (SSrRNA) genes of additional genera, presented below, tests the monophyly suggested by the studies on somatic kinetid ultrastructure reported above and by the phylogenetic analysis of the SSrRNA gene sequences of *Colpoda* and *Bursaria* (Stechmann et al. 1998).

Foissner (1993) has presented the first hypothesis for evolution of the orders within the class, using an Hennigian argumentation. His phylogeny made three predictions: (a) that the kreyellid silver-line system evolved early in colpodean phylogeny, separating bryometopids, such as *Bryometopus*, from all other colpodeans; (b) that the macro–micronuclear complex is an autapomorphy of the cyrtolophosidids, such as *Platyophrya;* and (c) that the merotelokinetal stomatogenesis of colpodids is derived from the ancestral pleurotelokinetal stomatogenesis typical of all other colpodeans (Fig. 1:1–13).

We have tested the monophyly of the class Colpodea and Foissner's (1993) phylogeny using the published complete sequences of the SSrRNA genes of the colpodid *Colpoda inflata* (Greenwood et al. 1991b) and the bursariomorphid *Bursaria truncatella* (Stechmann et al. 1998) and new SSrRNA sequences of the colpodid *Bresslaua vorax,* the grossglockneriid *Pseudoplatyophrya nana,* and the cyrtolophosidid *Platyophrya vorax* and a partial sequence from the bryometopid *Bryometopus sphagni.*

Materials and Methods

Sources of Organisms

Bresslaua vorax was cultured in Volvic springwater and fed *Chlorogonium.* Ciliates were able virtually to eliminate prey over several days of feeding as they increased in numbers. At this time, *B. vorax* cells were carefully removed from the culture fluid by pipette and gently concentrated by filtering the culture fluid through Whatman No. 1 filter paper.

Pseudoplatyophrya nana was cultured in Volvic springwater and fed baker's yeast (Foissner and Didier 1983). Ciliates would rapidly deplete the yeast prior to encysting. Cultures at this stage were harvested, with care taken to reduce contamination by yeast cells as far as possible.

Platyophrya vorax was cultured in Volvic springwater and fed *Colpoda* species. Ciliates were carefully isolated from cultures that had almost completely depleted their prey.

Bryometopus sphagni was collected from cultures established from field samples of mosses collected in the Salzburg area of Austria. Individual ciliates were micropipetted from these cultures, washed carefully, and examined for food vacuoles containing *Colpoda* species. Ciliates without food vacuoles or food vacuoles in which prey were not identifiable were retained.

The concentrated ciliates of each species were then fixed in 70% ethanol and accumulated until numbers were sufficient for extraction of DNA.

Extraction of DNA, Isolation of SSrRNA Genes, and DNA Sequencing

Extraction of DNA and isolation of the SSrRNA genes followed procedures published elsewhere (Stechmann et al. 1998).

The SSrRNA genes were sequenced in both directions. The Sanger dideoxy-chain termination sequencing protocol (Sanger et al. 1977)

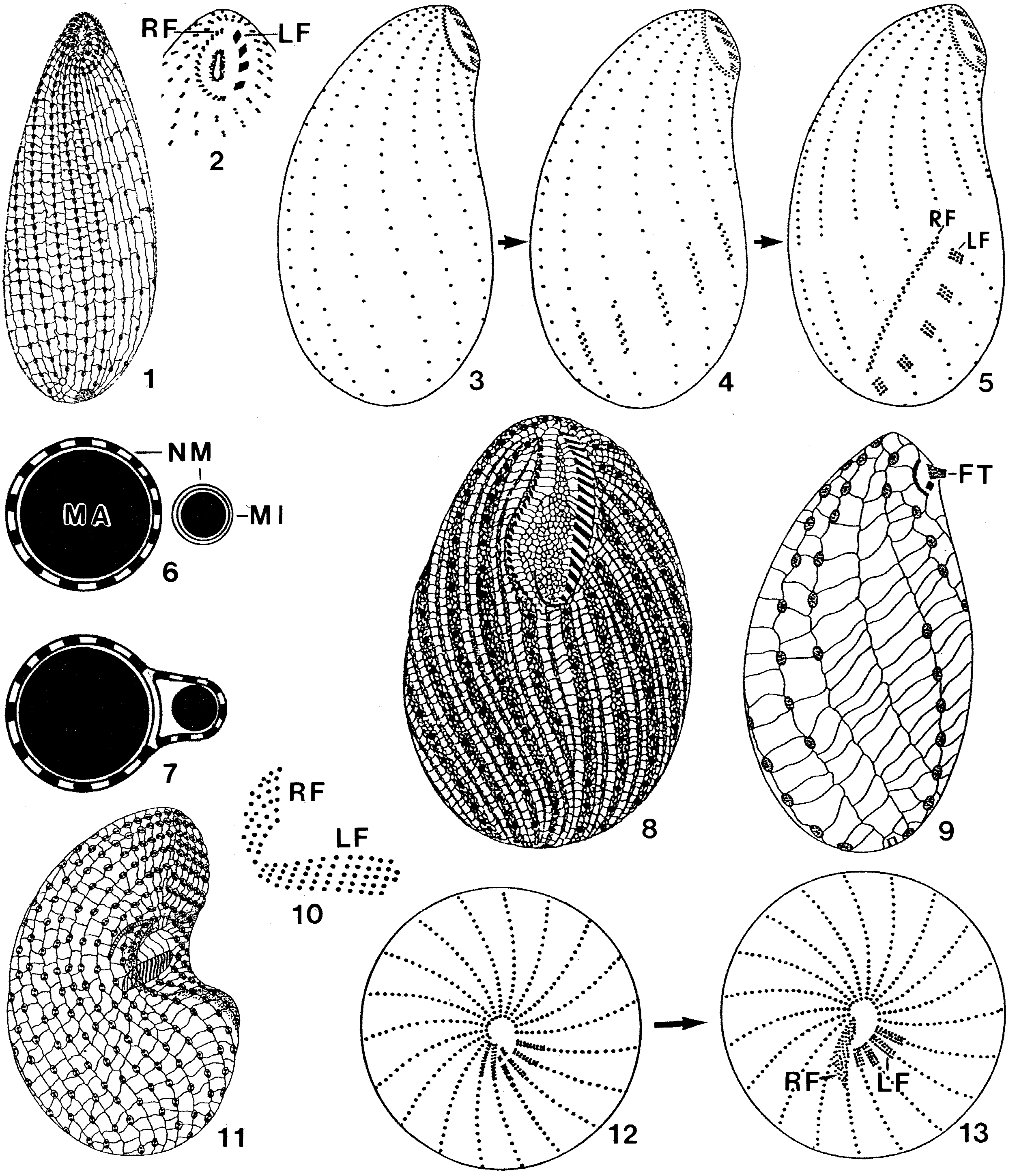

Fig. 1. Morphological and ontogenetic features used by Foissner (1993) to reconstruct evolution of orders within the class Colpodea. Foissner (1993) split the class into the subclasses Colpodia and Bryometopia, using the silver-line pattern. This pattern, which is a system of cortical fibers recognizable after silver nitrate impregnation, can be of the cyrtolophosidid pattern **(1)**, the kreyellid pattern **(8)**, or the colpodid pattern **(9, 11)**. Within the subclass Colpodia, the order Cyrtolophosidida has a distinct nuclear apparatus: the micronucleus is within the perinuclear space of the macronucleus **(7)** rather than separated from it as is typical of most ciliates **(6)**. Furthermore, Foissner (1993) suggested that the merotelokinetal stomatogenesis **(12, 13)** of the orders Colpodida and Grossglockneriida is derived from the ancestral pleurotelokinetal stomatogenesis typical of all other colpodeans **(3–5)**. 1–5: *Platyophrya,* a cyrtolophosidid colpodid, has a longitudinal silver line between two kineties **(1)**. Its oral apparatus consists of a right (RF) and a left (LF) ciliary field **(2)**, which originate pleurotelokinetally, that is, by proliferation of kinetosomes at the anterior end of lateral somatic kineties that have split **(3–5)**. 6 and 7: Macro–micronuclear arrangements in ciliates. Usually the macronucleus (MA) and micronucleus (MI) each have a nuclear membrane (NM) **(6)**. In cyrtolophosidids, however, the micronucleus is in the perinuclear space of the macronucleus **(7)**. 8: *Bryometopus* has a kreyellid silver-line system composed of many small, irregular meshes. 9: *Pseudoplatyophrya* is a highly specialized grossglockneriid colpodean with a minute feeding tube (FT) used to penetrate and feed on fungal hyphae. 10–13: *Colpoda* **(11)** and its relatives have a distinct oral cavity containing a right (RF) and a left (LF) ciliary field **(10)**. These oral ciliary fields originate merotelokinetally, that is, by proliferation of kinetosomes at the anterior end of a few kineties **(12, 13)**. The silver-line system is widely and regularly meshed (9, 11). All drawings from Foissner (1993) except 6 and 7.

was used to sequence pUC18 clones of *B. vorax* and *P. nana,* following the methods of Stechmann et al. (1998). The pUC18 clones of the partial gene of *B. sphagni* and the complete gene of *P. vorax* were sequenced with an ABI Prism 377 Automated DNA Sequencer (Applied Biosystems Inc.) using dye terminator and Taq FS with three forward and three reverse internal universal 18S primers (Elwood et al. 1985) and the M13 vector forward and reverse primers.

Sequence Availability and Phylogenetic Analysis

The nucleotide sequences in this paper are available from the GenBank/EMBL databases under the following accession numbers: *Climacostomum virens,* X65152 (Hammerschmidt et al. 1996); *Colpidium campylum,* X56532 (Greenwood et al. 1991a); *Colpoda inflata,* M97908 (Greenwood et al. 1991b); *Cyclidium glaucoma,* Z22879 (Embley et al. 1995); *Discophrya collini,* L26446 (Leipe et al. 1994a); *Entodinium caudatum,* U57765 (Wright et. al. 1997); *Eufolliculina uhligi,* U47620 (Hammerschmidt et al. 1996); *Euplotes aediculatus,* X03949 and M14590 (Sogin et al. 1986); *Furgasonia blochmanni,* X65150 (Bernhard et al. 1995); *Glaucoma chattoni,* X56533 (Greenwood et al. 1991a); *Homalozoon vermiculare,* L26447 (Leipe et al. 1994a); *Ichthyophthirius multifiliis,* U17354 (Wright and Lynn 1995); *Labyrinthuloides minuta,* L27634 (Leipe et al. 1994b); *Loxodes striatus,* U24248 (Hammerschmidt et al. 1996); *Loxophyllum utriculariae,* L26448 (Leipe et al. 1994a); *Metopus contortus,* Z29516 (Embley et al., unpublished data); *Metopus palaeformis,* M86385 (Embley et al. 1992); *Ophryoglena catenula,* U17355 (Wright and Lynn 1995); *Opisthonecta henneguyi,* X56531 (Greenwood et al. 1991a); *Oxytricha granulifera,* X53486 (Schlegel et al. 1991); *Paramecium tetraurelia,* X03772 (Sogin and Elwood 1986); *Plagiopyla nasuta,* Z29442 and Z29543 (Embley et al. 1995); *Polyplastron multivesiculatum,* U57767 (Wright et al. 1997); *Prorocentrum micans,* M14649 (Herzog and Maroteaux 1986); *Protocruzia* sp., X65153 (Hammerschmidt et al. 1996); *Pseudomicrothorax dubius,* X65151 (Bernhard et al. 1995); *Sarcocystis muris,* M64244 and M34846 (Gajadhar et al. 1991); *Spathidium* sp., Z22931 (Dyal and Embley, unpublished data); *Stylonychia pustulata,* M14600 and X03947 (Elwood et al. 1985); *Symbiodinium pilosum,* X62650 and S44661 (Sadler et al. 1992); *Tetrahymena corlissi,* U17356 (Wright and Lynn 1995); *Theileria buffeli,* Z15106 (Allsopp et al. 1994); *Tracheloraphis* sp., L31520 (Hirt et al. 1995); *Trimyema compressa,* Z29438 and Z29556 (Embley et al. 1995); *Trithigmostoma steini,* X71134 (Leipe et al. 1994a); and *Uronema marinum,* Z22881 (Dyal et al., unpublished data).

Two dinoflagellates (*Prorocentrum, Symbiodinium*), *Labyrinthuloides,* and two apicomplexans (*Theilera, Sarcocystis*) were used as the outgroups for the ciliates (Watrous and Wheeler 1981; Maddison et al. 1984; Smith 1994; Stackebrandt and Ludwig 1994). All sequences were globally aligned using the Dedicated Comparative Sequence Editor (DCSE) program (De Rijk and De Wachter 1993) and further refined by considering secondary structural features of the 18S molecule.

PHYLIP (Version 3.51C) (Felsenstein 1993) was used to calculate the sequence similarity and evolutionary distances between pairs of nucleotide sequences using the Kimura (1980) two-parameter model. Distance-matrix trees were then constructed using the Fitch and Margoliash (1967) least-squares (LS) method and the neighbor-joining (NJ) method (Saitou and Nei 1987). For the maximum-parsimony (MP) analysis, sequence data were reduced from 1834 sites to 828 phylogenetically informative sites. The most-parsimonious tree was found using the heuristic search option in the maximum-parsimony analysis of PAUP (Swofford 1993). Both parsimony and distance data were bootstrap resampled (Felsenstein 1985) 1000 times.

Results

The complete SSrRNA sequences were determined for *B. vorax* (1780 nucleotides; GenBank Accession No. AF060453), *P. nana* (1779 nucleotides; GenBank Accession No. AF060452), and *P. vorax* (1753 nucleotides; GenBank Accession No. AF060454). Only a partial sequence was obtained for *B. sphagni* (1202 nucleotides; GenBank Accession No. AF060455), beginning at the 5′ end at nucleotide 573 of the *C. inflata* sequence.

Distance Matrix Analysis

Both least-squares and neighbor-joining analyses provide strong bootstrap support for the following: (a) the monophyly of the phylum Ciliophora (100% LS, 100% NJ); (b) the monophyly of the subphyla Postciliodesmatophora (100% LS, 100% NJ) and Intramacronucleata (100% LS, 91% NJ); and (c) the monophyly of the classes Spirotrichea (92% LS, 91% NJ), Litostomatea (100% LS, 100% NJ), Phyllopharyngea (100% LS, 100% NJ), Oligohymenophorea excluding the plagiopylians *Plagiopyla* and *Trimyema* (92% LS, 80% NJ), Prostomatea (59% LS, 92% NJ), and Colpodea (92% LS, 98% NJ). The class Nassophorea is supported strongly only by neighbor-joining (79%). These analyses also distinguished several new clades: the plagiopylians, *Plagiopyla* and *Trimyema,* were separated from the oligohymenophoreans and the armophorids were separated from the spirotrichs (Fig. 2).

The class Colpodea is strongly supported by bootstrap analyses (92% LS, 98% NJ), although which of the related classes is its sister taxon is unresolved (Fig. 2). Within the class Colpodea, *Platyophrya* (order Cyrtolophosidida) is the sister taxon to the other genera, which are united by strong bootstrap support (96% LS, 97% NJ). Thus, the genus with an unusual macro–micronuclear complex is segregated from these other colpodean genera. The remaining five genera are representatives of four orders (i.e., Colpodida, Grossglockneriida, Bryometopida, Bursariomorphida) and two subclasses (i.e., Bryometopia, Colpodia). The two subclasses are not supported by the distance analyses of SSrRNA (Fig. 2), suggesting that the bryometopid “kreyellid” silver-line system evolved later in colpodean phylogeny. The order Colpodida is strongly supported by bootstrap analyses (100% LS, 100% NJ), and within it, the family Colpodidae, order Colpodida (i.e., *Colpoda, Bresslaua*), which is the sister taxon to the family Grossglockneriidae, order Grossglockneriida (i.e., *Pseudoplatyophrya*), is also strongly supported (98% LS, 98% NJ). The orders Bryometopida (i.e., *Bryometopus*) and Bursariomorphida (i.e., *Bursaria*) form a strongly supported sister clade (96% LS, 99% NJ) to the orders Colpodida and Grossglockneriida (Fig. 2). The incomplete sequence for *Bryometopus* did not influence topology within the class Colpodea: the same topology occurred when we repeated these analyses by eliminating 573 nucleotides from the 5′ end of the alignment for all other taxa (unpublished results).

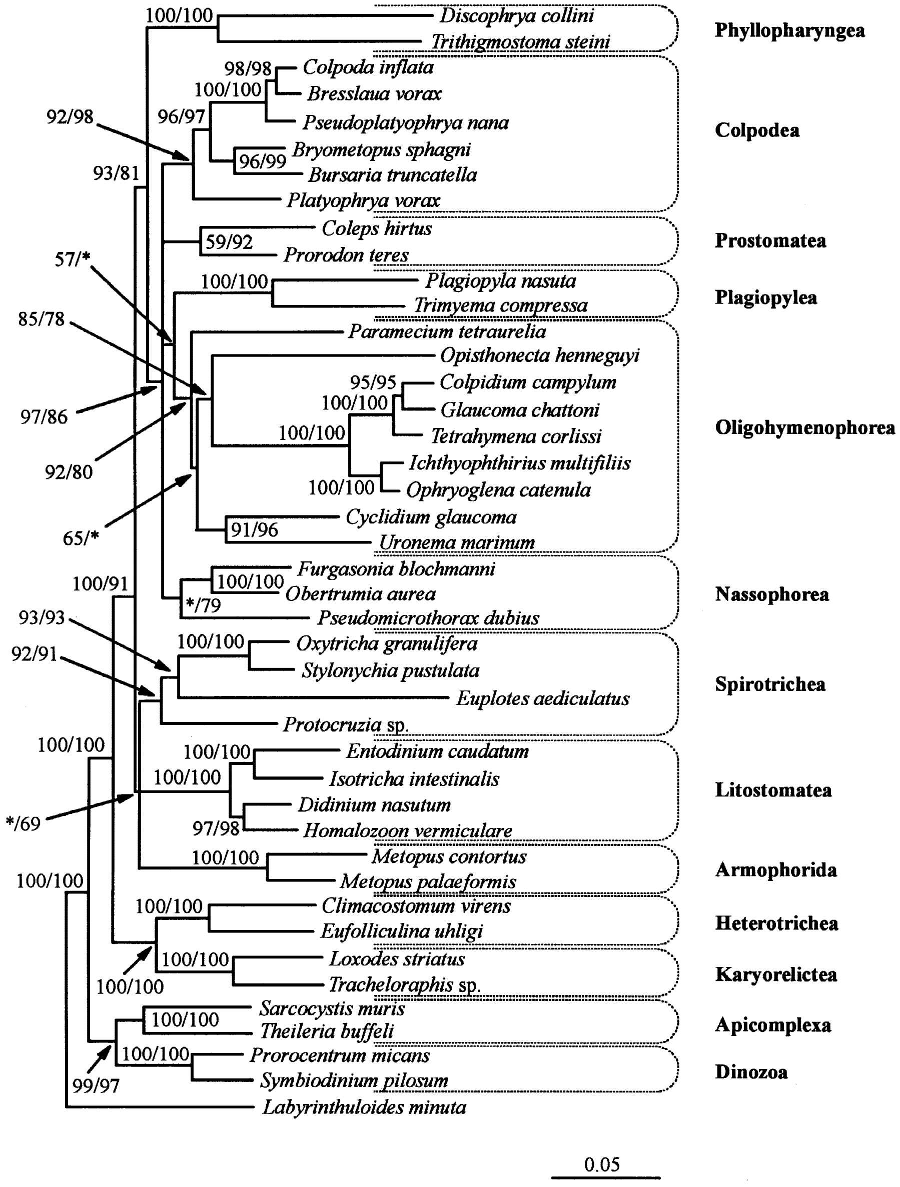

Fig. 2. A distance matrix tree of the ciliates derived from SSrRNA evolutionary distances produced by the Kimura (1980) two-parameter correction model and constructed using the Fitch and Margoliash (1967) least-squares (LS) method. The LS and the neighbor-joining (NJ) (Saitou and Nei 1987) consensus trees for 1000 bootstrap resamplings of the data set were computed independently and the bootstrap supports are indicated as a percentage at the *base* of each bifurcation. The LS bootstrap percentage is followed by the NJ bootstrap percentage. Bootstrap values less than 50% are indicated by an *asterisk*. Evolutionary distance is represented by the horizontal component separating species in the figure. The scale bar corresponds to 5 changes per 100 positions. Names for suprageneric taxa appear in *boldface.*

Maximum-Parsimony Analysis

Maximum-parsimony analysis provides strong bootstrap support for the phylum Ciliophora (85%) and the subphyla Postciliodesmatophora (100%) and Intramacronucleata (83%). Much of the “higher level” substructure of the distance matrix analyses was not corroborated by parsimony analysis. Nevertheless, one grouping common to both analyses included the classes Phyllopharyngea, Plagiopylea, Colpodea, Prostomatea, Oligohymenophorea, and Nassophorea (compare Figs. 2 and 3).

The class Colpodea is supported by bootstrap (61%). As in the distance matrix analyses, *Platyophrya* (order Cyrtolophosidida) is the sister taxon to the other colpodean genera, and the topology *within* the class is identical for both analyses (see above; compare Figs. 2 and 3).

Discussion

Phylogeny Within the Phylum Ciliophora

Molecular analyses of rRNA demonstrate a fundamental split among the ciliates (Baroin Tourancheau et al. 1995; Bernhard and Schlegel 1998; Hammerschmidt et al. 1996; Hirt et al. 1995). Lynn (1996) has now formalized this by suggesting the recognition of two subphyla: the Postciliodesmatophora, distinguished by having somatic kinetids whose postciliary microtubular ribbons overlap laterally to form the so-called postciliodesmata; and the Intramacronucleata, distinguished by macronuclei that divide using microtubules arrayed *within* the macronuclear envelope (Lynn 1996). There are only two clades consistently recognized within the subphylum Postcil-

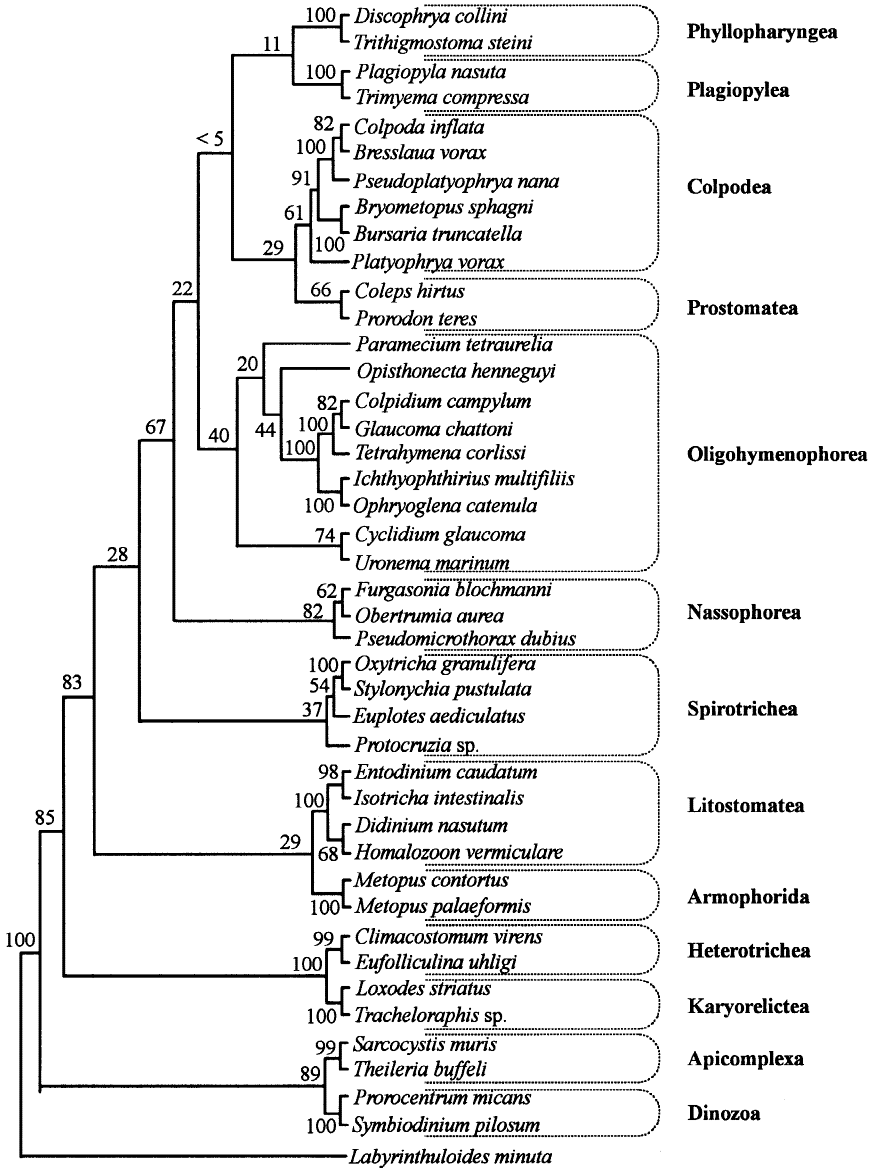

Fig. 3. A maximum-parsimony tree of the ciliates inferred from complete SSrRNA gene sequences using a bootstrap resampling of the data set. The *numbers* at the bifurcations represent the percentage of times the group occurred out of 1000 trees. No significance is placed on the lengths of the branches connecting the species. Names for suprageneric taxa appear in *boldface.*

iodesmatophora, and these have now been established as the classes Karyorelictea and Heterotrichea (Lynn and Small, 1997; de Puytorac et al. 1993). Relationships among classes in the subphylum Intramacronucleata are not yet resolved, although a grouping of six classes is supported by both distance and parsimony analyses.

Monophyly of the Class Colpodea

The structural conservatism hypothesis (Lynn 1976a) provided ciliate systematists with a rationale for placing taxonomic weight on the kinetid ultrastructure of ciliates to identify monophyletic groups. Lynn (1981, 1991) has characterized the colpodean somatic kinetid as follows: a dikinetid whose anterior ciliated kinetosome bears a tangential transverse ribbon at triplets 3, 4, and 5 and a single or several postciliary microtubules; and a posterior ciliated kinetosome that bears a divergent postciliary ribbon, a laterally directed, usually fan-shaped kinetodesmal fibril at triplets 5 and 6, and a long posteriorly directed transverse ribbon that arises obliquely from a desmose in the region of triplets 4 and 5. Platt and Hausmann (1993) have demonstrated using serial thin sections that the two kinetosomes of colpodeans are probably joined by one complex desmose, which extends from the basal to middle levels on the kinetosome shaft. As mentioned in the Introduction, this somatic kinetid has been found in taxa that were classically considered colpodids and has been identified in new taxa that are considered colpodean. This kinetid has also been found in other taxa classically not considered colpodid and has been used to justify their transfer to this class. However, Bardele (1989) expressed doubts regarding the monophyly of the class Colpodea, pointing to diversity that he observed in freeze–fracture analyses of the intramembranous particle arrays in the ciliary membranes of taxa assigned to this class. We have used SSrRNA gene sequences to test the monophyly of the class Colpodea. In both distance matrix and parsimony analyses, the six colpodean genera are strongly supported as a monophyletic group. Thus, the SSrRNA data favour a monophyletic origin for the class Colpodea (Foissner 1993; Lynn and Small 1997).

Phylogeny Within the Class Colpodea

The second major goal of our study was to test the phylogeny of the colpodean orders proposed by Foissner (1993). We have obtained SSrRNA sequences from representatives of five of the seven orders recognized by Foissner (1993): *Colpoda* and *Bresslaua* for the order Colpodida, *Pseudoplatyophrya* for the order Grossglockneriida, *Bryometopus* for the order Bryometopida, *Bursaria* for the order Bursariomorphida, and *Platyophrya* for the order Cyrtolophosidida. These genera permit a test of several conclusions deriving from Foissner's (1993) phylogeny.

Foissner (1993) argued that the merotelokinetal stomatogenesis displayed by genera that divide in cysts is apomorphous to the pleurotelokinetal stomatogenesis typical of those genera that divide while swimming free (Fig. 1:3–5, 12, 13). This synapomorphy unites the orders Colpodida and Grossglockneriida in Foissner's (1993) phylogeny. The SSrRNA gene sequences corroborated the relationship between these two groups: the clade represented by the colpodids *Colpoda* and *Bresslaua* is the sister taxon to the clade represented by the grossglockneriid *Pseudoplatyophrya* (Figs. 2 and 3). The magnitude of the genetic distance between representatives of these two groups suggests that separation at the ordinal level may be too high: for example, the suborder Tetrahymenina, which includes *Colpidium* and *Tetrahymena,* is separated by a greater genetic distance from members of its sister suborder Ophryoglenina, which includes *Ichthyophthirius* and *Ophryoglena* (Fig. 2).

Ultrastructural studies revealed a highly unusual structural relationship between the macronucleus and the micronucleus of some colpodeans, although this is not unique among the ciliates (see Detcheva and de Puytorac 1979). The micronucleus resides in the perinuclear space of the macronucleus in *Woodruffia* (Golder 1976), *Cyrtolophosis* (Detcheva 1976; Didier et al. 1980), *Platyophrya* (Dragesco et al. 1977), and *Kuklikophrya* (Njiné 1979). Foissner (1993) recognized this as an autapomorphy for the order Cyrtolophosidida. Supporting this, our SSrRNA gene sequence data suggest that this macro–micronuclear complex separates the cyrtolophosidid *Platyophrya* from the other colpodeans (Figs. 2 and 3). Upon molecular analysis, we would expect the other cyrtolophosidid genera with this nuclear complex to cluster with *Platyophrya.* However, de Puytorac et al. (1992) have described the ultrastructure of a ciliate that they identified as the cyrtolophosidid *Platyophryides,* based on its somatic and oral cortical structures, but that lacks this kind of nuclear complex. This genus represents a serious systematic problem for defining the order Cyrtolophosidida, and it will be necessary to determine its SSrRNA molecular characters to test whether it represents a case of character convergence in somatic and oral cortical structures or whether it represents diversity in the macro–micronuclear complex.

Although admitting that it was a weak character, Foissner (1993) argued that the kreyellid silver-line system was an autopomorphy for the subclass Bryometopia, separating representatives of this class from all other colpodeans. Our SSrRNA data do not refute this conclusion. However, the cyrtolophosidid *Platyophrya* takes the "basal" position as the sister taxon to the other colpodean genera, rather than the bryometopid *Bryometopus* (Figs. 2 and 3). This suggests that the kreyellid silver-line system evolved later in colpodean phylogeny. While the bryometopid *Bryometopus* and the bursariomorphid *Bursaria* are sister taxa in all our molecular analyses (Figs. 2 and 3), significant differences in their silver-line systems (Foissner 1993) and their SSrRNA sequences support maintaining them as separate orders until we have data from either morphology or molecules of related genera that will more fully explain this relationship.

Foissner (1993) recognized seven orders within the class Colpodea. We have provided sequences of five of these seven: we have been unable to collect representatives of the orders Bryophryida and Sorogenida. Our molecular analyses do not support Foissner's (1993) subclass division within the class and favor suppression of the order Grossglockneriida. Lynn and Small (1997) have adopted this position, recognizing six orders within the class. It will now be necessary to obtain molecular data from bryophryids and sorogenids to test these viewpoints.

The Silver-Line System as a Character

The silver-line system is a pattern of lines and fibers that appears in the cortex of ciliates (Fig. 1:1, 8, 9, 11), particularly after the silver impregnation procedures devised by Klein (1933) and Chatton and Lwoff (1935). These lines indicate the pattern and presence of cortical fibers and filaments and the boundaries between alveolar membrane compartments in the cortex (Foissner 1976, 1977, 1981). The silver-line system can be quite different between related ciliates: for example, in the colpodean family Woodruffiidae, *Woodruffia* has a pattern typical of members of the family Colpodidae, while the closely related genus *Woodruffides* has a pattern typical of platyophryids; and members of the related family Reticulowoodruffiidae have a mixed silver-line system with either colpodid–platyophryid features or platyophryid–reticulate features in the cortex of a single species (Foissner 1993). In addition to this variability, the silver-line system may vary during the cell cycle of ciliates, changing during cell division and cystment (Foissner 1976). Given this variability, it is unlikely that the features of the silver-line system will provide strong and stable characters for establishing major clades within ciliate classes.

Within the class Colpodea, Foissner (1993) used the kreyellid silver-line system as the autapomorphy for the subclass Bryometopia. He admitted that this silver-line character is weak, since *Reticulowoodruffia* and *Pseudochlamydonella,* members of the order Cyrtolophosidida, have kreyellid regions in their silver-line system. Our results with SSrRNA sequences lead us to conclude that the silver-line system does not support subclass-level distinctions within this class. Foissner (1993), on the other hand, did note that bryometopid genera, such as *Bryometopus* and *Thylakidium,* are structurally similar to the bursariomorphid colpodids, such as *Bursaria.* The SSrRNA sequence data supported this conclusion: the bryometopid *Bryometopus* and the bursariomorphid *Bursaria* are always sister taxa (Figs. 2 and 3), although separated at a greater genetic distance from each other than the colpodids and grossglockneriids. Thus, the similarities in pattern of their somatic and oral infraciliature are phylogenetically more meaningful than differences in their silver-line systems, while placement in different orders is consistent with their genetic divergence in SSrRNA sequence and the differences in their silver-line systems.

In conclusion, the silver-line system is a weak character, showing homoplasy throughout members of this class—both in morphostatic stages of cyrtolophosidids and bryometopids and during morphogenesis of colpodids. We do not consider it a strong character for inferring deep phylogenetic relationships. It will still be useful, when it can be shown to be a stable character, to recognize diversity among genera and families, occasionally even orders, of ciliates.

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