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Phylogenetic Relationships within the Class Oligohymenophorea, Phylum Ciliophora, Inferred from the Complete Small Subunit rRNA Gene Sequences of *Colpidium campylum, Glaucoma chattoni,* and *Opisthonecta henneguyi*

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Summary. Phylogenetic relationships within the class Oligohymenophorea, phylum Ciliophora, were investigated by determining the complete small subunit rRNA (SSrRNA) gene sequences for the hymenostomes *Colpidium campylum, Glaucoma chattoni,* and the peritrich *Opisthonecta henneguyi.* The affiliations of the oligohymenophoreans were assessed using both distance matrix (DM) and maximum parsimony (MP) analyses. Variations do exist in the phylogenies created by the two methods. However, the basic tree topologies are consistent. In both the DM and MP analyses the hymenostomes (*C. campylum, G. chattoni,* and the tetrahymenas) all form a very tight group associated with the peritrich *O. henneguyi.* The *Tetrahymena* lineage was monophyletic whereas *Colpidium* and *Glaucoma* were more closely related to each other than either was to the tetrahymenas. The monophyly of the genus *Tetrahymena* in the present analysis supports the phylogenies determined from morphological data and molecular sequence data from the histone H3II/H4II region of the genome. The perplexing and controversial phylogenetic position of the peritrichs is once again depicted in the present analysis. The distinctiveness of the peritrich *Opisthonecta* from both hymenostome and nassophorean ciliates based on evolutionary distances suggests that the elevation of the peritrichs to a higher taxonomic rank should be reconsidered.

Key words: Small subunit rRNA — Ciliophora — Phylogeny — *Colpidium* — *Glaucoma* — *Opisthonecta* — Maximum parsimony — Distance matrix

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Introduction

Corliss (1974) divided the history of ciliate systematics into four distinct periods: (1) the Age of Discovery (1880–1930); (2) the Age of Exploitation (1930–1950); (3) the Age of Infraciliature (1950–1970); and (4) the Age of Ultrastructure (1970–present). In all these periods there was both an increase in the number of species being discovered and also advancements and improvements in techniques. In each period this new information was assessed and interpreted in light of the past classification schemes, which were altered to accommodate the new data. The refinement in ultrastructure studies (e.g., Bardele 1980; Eisler 1988, 1989) and the use of molecular techniques to acquire genome information on ciliates (Allen and Li 1974; Elwood et al. 1985; Van Bell 1985; Sogin et al. 1986a,c; Baroin et al. 1988; Lynn and Sogin 1988; Nanney et al. 1989a,b; Preparata et al. 1989; Brunk et al. 1990; Greenwood et al. 1991) have allowed us to question relationships proposed using data acquired from earlier techniques. This may have already lead us into a new period of ciliate systematics, the fifth period, the Age of Refinement in which we will solidly establish phylogenetic relationships within the phylum Ciliophora.

The use of the polymerase chain reaction (PCR) and its application to phylogenetic studies of protists (Medlin et al. 1988; Brunk et al. 1990) finally provides us the opportunity to acquire molecular sequence information from species that are not easily cultured in the lab. However, prior to pursuing these more enigmatic species it is logical to construct a phylogenetic framework in which to evaluate these oddities. The significance and effectiveness of se-

quence data at elucidating relationships should first be evaluated through analysis of taxa in which morphological phylogenetic information abounds, for example, the oligohymenophorean ciliates. The greatest concentration of effort on taxa in the class Oligohymenophorea has been placed on the relationships within the genus *Tetrahymena,* which have been explored recently using various molecular techniques ranging from DNA/DNA hybridization to the evaluation of relationships based on nucleic acid sequence data (Allen and Li 1974; Van Bell 1985; Sogin et al. 1986a; Baroin et al. 1988; Nanney et al. 1989a,b; Preparata et al. 1989; Brunk et al. 1990).

The phylogenetic relationships of other members of the class Oligohymenophorea to the genus *Tetrahymena,* although investigated through classical techniques (Lynn and Didier 1978; Peck 1978), have only recently been initiated using molecular methods. Molecular sequence information from the complete 5S rRNA, 5.8S rRNA (Van Bell 1985; Nanney et al. 1989a), the partial small subunit rRNA (Lynn and Sogin 1988), and the partial large subunit rRNA (Nanney et al. 1989a,b; Preparata et al. 1989) of the hymenostome genera *Colpidium* and *Glaucoma* has been collected, while additional information for the genus *Glaucoma* exists from PCR-amplified segments near the histone H3II/H4II regions of the genome (Brunk et al. 1990). This new information should enable us to test ideas of affinities between taxa and may have direct impact on changing the classification.

The phylogenetic relationships within the class Oligohymenophorea are explored here by sequencing the complete SSrRNA genes from the hymenostomes *Colpidium campylum* and *Glaucoma chattoni,* and the peritrich *Opisthonecta henneguyi.* The affiliation of the oligohymenophoreans will be discussed by comparing phylogenies of rRNA sequence data using both distance matrix (DM) and maximum parsimony (MP) analyses, with three contemporary classification schemes (Corliss 1979; Small and Lynn 1981, 1985; de Puytorac et al. 1987).

Materials and Methods

Extraction and purification of nucleic acids from cultures of the following species, *C. campylum, G. chattoni* (original culture obtained from G.G. Holz, Jr.), and *O. henneguyi* [American Type Culture Collection (ATCC) 30600] were performed as previously described (Elwood et al. 1985; Lynn and Sogin 1988).

PCR gene amplification of purified ciliate DNA was accomplished using a Perkin-Elmer/Cetus DNA thermal cycler as previously described (Medlin et al. 1988; Greenwood et al. 1991). After 30 cycles of amplification, the DNA products were checked for the proper size by electrophoresing a sample [5 μl of sample + 5 μl of LT buffer (10mM Tris pH 7.5, 10 mM NaC, 0.5 mM EDTA) + 5 μl of stop dye marker] on a 2% Agarose gel against a 1-kb BRL (Bethesda Research Labs) marker and a sample of *Tetrahymena tropicalis* PCR-amplified ribosomal DNA (rDNA: size 1.8 kb). PCR-amplified rDNA samples were digested with the restriction enzymes Sal I and Bam HI (Bethesda Research Labs and/or Promega) to facilitate forced orientation cloning into M13. The rDNA was then ligated using T_4 ligase (Bethesda Research Labs) into the RF of M13mp18 or M13mp19 (Maniatis et al. 1982). Recombinant M13 vectors containing the SSrRNA gene were then used to transform *Escherichia coli* JM109. Single-stranded templates for directing DNA synthesis in the sequencing protocol were then prepared (Messing 1983). The SSrRNA genes were sequenced using the Sanger dideoxynucleotide chain termination sequencing protocol (Sanger et al. 1977) modified for use with the M13 vector system by Messing (1983).

Phylogeny Construction. Sequences were aligned using a procedure that considers the conservation of both the primary and secondary structure for phylogenetic reconstruction (Elwood et al. 1985). In the distance matrix analysis, sequence information was reduced to approximately 1400 unambiguously aligned positions for comparisons of the phylum Ciliophora in relation to other eukaryotes. In the maximum parsimony analysis only 15 sequences were used in the analysis (14 ciliates and the dinoflagellate *Prorocentrum micans*). For this reason an attempt was made to align all unambiguous nucleotide positions for the data set. Sequences were aligned using the Eyeball Sequence Editor (ESEE) version 1.09d (Cabot and Beckenbach 1989).

Sequence data used in the alignments were from: rat, *Rattus norvegicus* (Chan et al. 1984); brine shrimp, *Artemia salina* (Nelles et al. 1984); corn, *Zea mays* (Messing et al. 1984); the chlorophyte, *Chlamydomonas reinhardtii* (Gunderson et al. 1987); the chytridiomycete, *Blastocladiella emersonii* (Sogin, unpublished); yeast, *Saccharomyces cerevisiae* (Rubtsov et al. 1980); the bread mold, *Neurospora crassa* (Sogin et al. 1986b); the oomycete, *Achlya bisexualis* (Gunderson et al. 1987); the chrysophyte, *Ochromonas danica* (Gunderson et al. 1987); the dinoflagellate, *P. micans* (Herzog and Maroteaux 1986); the ciliate, *Blepharisma americanum* (Greenwood et al. 1991); the ciliate, *Colpoda inflata* (Greenwood et al. 1991), the ciliate, *Euplotes aediculatus* (Sogin et al. 1986c); the ciliate, *Oxytricha nova* (Elwood et al. 1985); the ciliate, *Oxytricha granulifera* (Schlegel et al. 1991); the ciliate, *Onychodromus quadricornutus* (Schlegel et al. 1991); the ciliate, *Stylonychia pustulata* (Elwood et al. 1985); the ciliate, *Paramecium tetraurelia* (Sogin and Elwood 1986); the ciliate, *Tetrahymena hegewischi* (Sogin et al. 1986a); the ciliate, *Tetrahymena thermophila* (Spangler and Blackburn 1985); the ciliate, *Tetrahymena pyriformis* (Sogin et al. 1986a); the slime mold, *Dictyostelium discoideum* (McCarroll et al. 1983).

Distance Matrix. Aligned sequences were analyzed using a DM (least-squares) method in which calculations of structural (sequence) similarity and evolutionary distance incorporate the Jukes and Cantor (1969) correction to estimate unobserved nucleotide substitutions. Phylogenetic trees were constructed by an additive tree method that determines the tree geometry and the branch lengths that best fit the evolutionary distance data (Olsen 1988).

Maximum Parsimony. The MP algorithm employed for the present analysis was developed by Moore et al. (1973) for amino acid sequences. The program was modified by Czelusniak et al. (1982) for nucleotide sequence data.

The parsimony algorithm required an initial tree input that can be any branch arrangement linking the contemporary sequences (species). From the initial tree branching order the number of fixed mutations or nucleotide replacements (NR) separating any two contemporary sequences was calculated. The algorithm then proceeded from the contemporary species at the exterior nodes of the tree and descended down the tree toward the root,

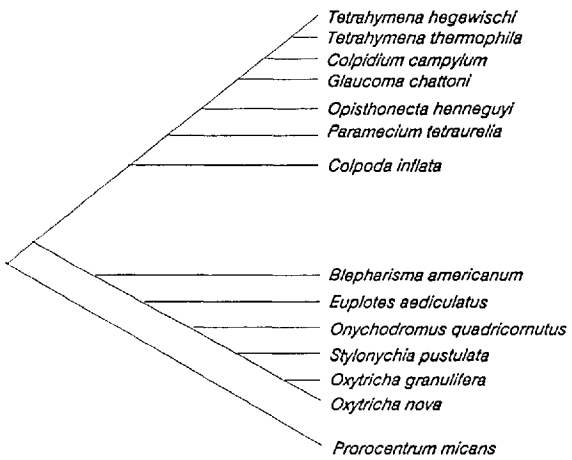

Fig. 1. The classical ciliate tree of Corliss (1979). No significance is placed on the lengths of the branches connecting species.

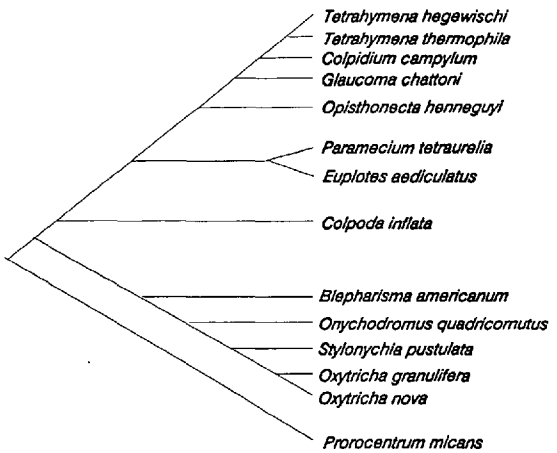

Fig. 2. The Small and Lynn (1981, 1985) ciliate tree indicating a proposed close relationship of the hypotrich *Euplotes* to the nassophorean *Paramecium*. No significance is placed on the lengths of the branches connecting species.

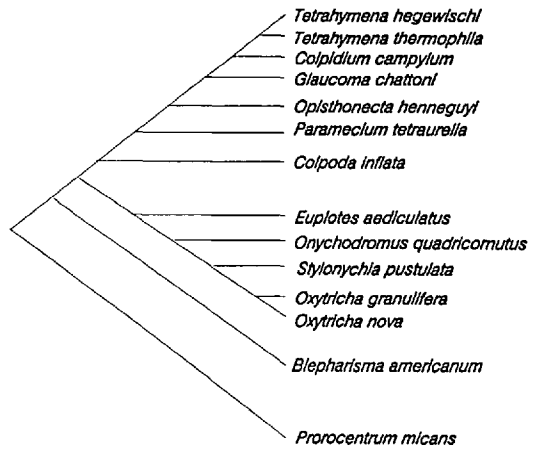

Fig. 3. The Lynn and Sogin (1988) ciliate tree constructed from partial and complete SSrRNA sequence comparisons. No significance is placed on the lengths of the branches connecting species.

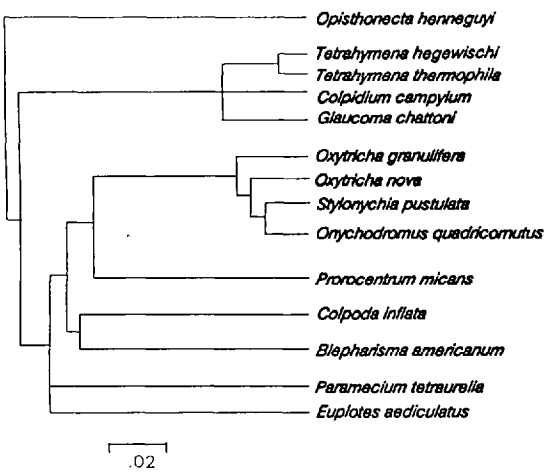

Fig. 4 Ciliate phylogeny inferred from 14 complete SSrRNA sequences employing UPGMA. The evolutionary distances separating species on the tree were determined from unweighted sequence similarities using the Jukes and Cantor (1969) correction. The horizontal distance separating species represents the evolutionary distance.

calculating the NR score separating adjacent nodes. The sum of all the NR scores for the individual links separating nodes on the tree was the total tree length. The maximum parsimony algorithm used an iterative approach in which local branch swapping or nearest-neighbor single step changes (NNSSC) were made on the initial tree in order to determine the lowest NR score (Goodman et al. 1979; Czelusniak et al. 1990). The algorithm terminated when it failed to find a tree with a lower NR score.

The procedure involved constructing starting trees with different phylogenetic possibilities and therefore different NR scores (Goodman 1981). The SSrRNA sequence data permitted testing of previous morphological and molecular phylogenies by using the MP analysis. Four initial starting trees were used. The first tree (Fig. 1) used the classical ciliate phylogeny of Corliss (1979), in which *Colpidium, Glaucoma, Opisthonecta, Paramecium,* and *Tetrahymena* are grouped within the class Oligohymenophora (sensu Corliss 1979). In Corliss' scheme the hypotrich *Euplotes,* the heterotrich *Blepharisma,* and stichotrichs, *Onychodromus, Oxytricha,* and *Stylonychia* are grouped in the class Polyhymenophora. The only variation to the Corlissian scheme was the placement of *Colpoda* in close relationship to the oligohymenophoreans rather than in a separate class Kinetofragminophora (Fig. 1). The second tree used a branching order similar to the above tree except that the hypotrich *Euplotes* was grouped with *Paramecium* in the class Nassophorea (Small and Lynn 1981, 1985) (Fig. 2). The third tree used the branching order determined by Lynn and Sogin (1988) from partial and complete ciliate SSrRNA sequences (Fig. 3). The heterotrich *Blepharisma* was placed as a separate deeper lineage within the tree in order to test the relationship between the hypotrichs and heterotrichs, which were proposed by Small and Lynn (1981, 1985) to be sister groups within the class Spirotrichea. The fourth tree (Fig. 4) was constructed by an unweighted pair group method using arithmetic averages (UPGMA), which incorporates the Jukes and Cantor (1969) correction for evolutionary distances calculated from the SSrRNA sequence data set used in the parsimony analysis. The UPGMA method is constrained by the requirement that the pairwise distances be ultrametric. Therefore, an ultrametric tree relating the taxa assumes that all descendants be equidistant from the root of the tree (or common ancestor). Because genes do not diverge uniformly in all lineages, the evolutionary distance estimates cannot be ultrametric. This results in systematic errors introduced into the phylogenetic tree based on the sensitivity of the UPGMA method to differences in evolutionary rates between

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O.henneguyi     aacctggttg atcctgccag tAGTCATATG CTTGTCTTAA AGATTAATCC ATGCATGTGT AAGTATAAGT ATTGTACAGC GAAACTGCGA ATGGCTCATT  100
G.chattoni      aacctggttg atcctgccag tTA-CATATG CTTGTCTTAA ATATTAACCC ATGCATGTGC CAGT-TCAGT ATTGAACAGC GAAACTGAGA ATGGCTCATT  98
C.campylum      aacctggttg atcctgccag tTA-CATATG CTTGTCTTAA ATATTAACCC ATGCATGTGC CAGT-TCAGT ATTGAACAGC GAAACTGCGA ATGGCTCATT  98
T.thermophila   AACCTGGTTG ATCCTGCCAG TTA-CATATG CTTGTCTTAA ATATTAACCC ATGCATGTGC CAGT-TCAGT ATTGAACAGC GAAACTGCGA ATGGCTCATT  98

O.henneguyi     ACATCAGTTA TAATTTATTT GATAATCGAA AGTTACATGG ATAACCGTGA CAAATT--AC AGCTAATACA TGCAGTC-AG ACCTGGTCCA AGGGT-----  192
G.chattoni      AAAACAGTTA TAGTTTATTT GATAATTAAA GATTACATGG ATAACCGAGC T-AATTGTTG GGCTAATACA TGCTTAAAAT TCCGTGTCCT GCGACCGGAA  197
C.campylum      AAAACAGTTA TAGTTTATTT GATAATTAAA GATTACATGG ATAACCGAGC T-AATTGTTG GGCTAATACA TGCTTAAAAT TCCGTGTCCC GTGACCGGAA  197
T.thermophila   AAAACAGTTA TAGTTTATTT GATAATTAAA GATTACATGG ATAACCGAGC T-AATTGTTG GGCTAATACA TGCTTAAAAT TCCGTGTCCT GCGACCGGAA  197

O.henneguyi     CGTAATTATT AG-TATTAAA CCATTT-CCG CAAGGAG--T GTGATGAATC ATAATAATCG AACGAATCGC CTGTTGTG-C GATAAATCAT TCAAGTTTCT  287
G.chattoni      CGTATTTATT AGATATTAAA CCAATCGCAG CAATGTGATT GAGATGAATC AAAGTAACTG ATCGAATCGT AGCTTGCTAC GATAAATCAT CTAAGTTTCT  297
C.campylum      CGTATTTATT AGATATTAGA CCAATCGCAG CGATGTGATT GAGATGATTC AAAGTAACTG ATCGAATCGT AGCTTGCTAC GATAAATCAT CTAAGTTTCT  297
T.thermophila   CGTATTTATT AGATATTAGA CCAATCGCAG CAATGTGATT GAGATGAATC AAAGTAACTG ATCGGATCGA GGTTTACCTC GATAAATCAT CTAAGTTTCT  297

O.henneguyi     GCCCTATCAG CTTTGGATGG TAGTGTATTG GACTACCA-T GGCAGTCACG GGTAACGGAG AATTAGGGTT C-GATTCCGG AGAGGGAGCC TGAGAAACGG  385
G.chattoni      GCCCTATCAG CTCTCGATGG TAGTGTATTG GACTACCA-T GGCAGTCACG GGTAACGGAG AATTAGGGTT C-GATTCCGG AGAAGGAGCC TGAGAAACGG  395
C.campylum      GCCCTATCAG CTCTCGATGG TAGTGTATTG GACTACCAGT GGCAGTCACG GGTAACGGAG AATTAGGGTT CCGATTCCGG AGAAGGAGCC TGAGAAACGG  397
T.thermophila   GCCCTATCAG CTCTCGATGG TAGTGTATTG GACTACCA-T GGCAGTCACG GGTAACGGAG AATTAGGGTT C-GATTCCGG AGAAGGAGCC TGAGAAACGG  395

O.henneguyi     CTACCACATC TACGGAA-GG CAGCAGGAGC GAAAATTGCC CAATCCCGAC ACGGGGAGGC AGTGACGAGA AATAACAACT CTTGGTTATT CTAAGAAGT-  483
G.chattoni      CTACTACAAC TACGGTTTGG CAGCAGGGAA GAAAATTGGC CAATCCTAAT TCAGGGAGCC AGTGACAAGA AATAGCAGAC C-GGGAAACT -ACGTTTCTA  493
C.campylum      CTACTACAAC TACGGTTTGG CAGCAGGGAA GAAAATTGGC CAATCCTAAT TCAGGGAGCC AGTGACAAGA AATAGCAAAC C-GGGAAACT -CAGTTTCTA  495
T.thermophila   CTACTACAAC TACGGTTCGG CAGCAGGGAA GAAAATTGGC CAATCCTAAT TCAGGGAGCC AGTGACAAGA AATAGCAAGC T-GGGAAACT TACGTTTCTA  494

O.henneguyi     -------GTA ATGAGGATAA TTTAAAACCC TTACCGAAAG -CAATTGGAG GGCAAGTC-T GGTGCCAGCA GCCGCGGTAA TTCCAGCTCC AATAGCGTAT  574
G.chattoni      CGGTACTGAA ATGAGAACAG TGTTAATCTC TTAGCGAGAA ACAATT-GAG G-CAAG-CCT GCTGCCAGCA GCCGCGGTAA TTCCAGCTCC AATAGCGTAC  590
C.campylum      CGGTATTGAA ATGAGAACAG TGTTAATCTC TTAGCGAGGA ACAATTGGAG GGCAAG-CCT GGTGCCAGCA GCCGCGGTAA TTCCAGCTCC AATAGCGTAT  594
T.thermophila   CGGCATTGAA ATGAGAACAG TGTAAATCTC TTAGCGAGGA ACAATTGGAG GGCAAGTCAT GGTGCCAGCA GCCGCGGTAA TTCCAGCTCC AATAGCGTAT  594

O.henneguyi     ATTAAAGTTG TTGCAGTTAA AAAGCTCGTA GTTGAAGTTC TGGCTGTTCG ATCCTCGAGC TCTGTAGCCG AGGA-CTCGA CAGTCATCCG CTTGCAAATA  673
G.chattoni      ATTAAAG-NG N-GCAGTTAA AAAGCTCGTA GTTGAACTTC AGTGTCCAGG TTCGTCTCGG CTCGCCGAGG CAACTCTGGA CATACGTCTG CAAGCTAAAA  688
C.campylum      ATTAAAGTTG TTGCAGTTAA AAAGCTCGTA GTTGAACTTC TGCC--CAGG ACTATTTCGA TTCGTCGGGT AGTG-CTGGG CATACGTCTG CAAGCTAAAA  691
T.thermophila   ATTAAAGTTG TTGCAGTTAA AAAGCTCGTA GTTGAACTTC TGT-T-CAGG TTCATTTCGA TTCGTCGTGT GAAA-CTGGA CATACGTTTG CAAACTAAAA  691

O.henneguyi     TATGTTCGCC -TTTAACCGG GTGGCTATAT GAGTAAGCAA TTTACCTTGA GAAAAA-CAG AGTGTTCCAG GCAGGTTTGT -CCGGAATGC ATTAGCATGG  770
G.chattoni      TCGGCCTTTA CTGGTTCGAC TTAGTGAGTA GA-----CAT TTTACTGTGC AAAAAATTAG AGTGTTTCAG GCAGGTTTTA GCCCGTATAC ATTAGCATGG  783
C.campylum      TCGGCCTTTA CTGGTTCGAC TTAGTAAGTA GA-----CAT TTTACTGTGA AAAAA-TTAG AGTGTTTCAG GCAGGTTTTA GCCCGTATAC ATTAGCATGG  785
T.thermophila   TCGGCCTTCA CTGGTTCGAC TTAGGGAGTA AA-----CAT TTTACTGTGA AAAAA-TTAG AGTGTTCCAG GCAGGTTTTA GCCCGAATAC ATTAGCATGG  785

O.henneguyi     AATAATAGAA TATGACTGAA GTCGATTTAT TGGTTTGAGG CTTTAGTAAT GATTAATAGG AACAGTCGGG GGCATTGGTA CTTGACAGTC AGAGGTGAAA  870
G.chattoni      AATAATGGAA TAGGACTAAG TCCATTTTAT TGGTTATTGG ATTTGGTAAT GATTAATAGG GACAATTGGG GGCATTAGTA TTTAATAGTC AGAGGTGAAA  883
C.campylum      AATAATGGAA TAGGACTGAG TCCATTTTAT TGGTTCTTGG ATTTAGTAAT GATTAATAGG GACAGTTGGG GGCATTAGTA TTTAATAGTC AGAGGTGAAA  885
T.thermophila   AATAATGGAA TAGGACTAAG TCCATTTTAT TGGTTCTTGG ATTTGGTAAT GATTAATAGG GACAGTTGGG GGCATTAGTA TTTAATAGTC AGAGGTGAAA  885

O.henneguyi     TTCTAGGATT TGTCGAAGAC TAACAAATGC GAAAGCATCT GCCAAGGA-T GTTTTCATTA ATCAAGAACG AAAGTTAGGG GATCAAAGAC GATCAGATAC  969
G.chattoni      TTCTTGGATT TATTAAGGN- -AACTAATGC GAAAGCATTT GCCAAAGA-T GTTTTCATTA ATCAAGAACG AAAGTTAGGG GATCAAAGAC GATCAGATAC  980
C.campylum      TTCTTGGATT TATTAAGGAC TAACTAATGC GAAAGCATTT GCCAAAGACT GTTTTCATTA ATCAAGAACG AAAGTTAGGG GATCAAAGAC GATCAGATAC  985
T.thermophila   TTCTTGGATT TATTAAGGAC TAACTAATGC GAAAGCATTT GCCAAAGA-T GTTTTCATTA ATCAAGAACG AAAGTTAGGG GATCAAAGAC GATCAGATAC  984
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Fig. 5. Small subunit rRNA gene sequences of the ciliates *Colpidium campylum* (1754 nucleotides), *Glaucoma chattoni* (1743 nucleotides), and *Opisthonecta henneguyi* (1731 nucleotides) aligned with the sequence from *Tetrahymena thermophila* (1753 nucleotides). Lowercase letters indicate positions that correspond to the amplification primers. Numbers at the ends of lines indicate the number of nucleotides. The differences in sequence length were accounted for by introducing alignment gaps (–) in the sequences. Ambiguities in nucleotide positions are indicated in the figure by the letter N.

lineages (Swofford and Olsen 1990). Although having this pitfall the UPGMA method constructed a phylogenetic hypothesis (initial input tree) to use in the MP analysis that avoids any preconceived ideas of tree branching order.

The dinoflagellates represented by *P. micans* are considered by many to be the sister group of the ciliates (Gunderson et al. 1987) and were therefore used as an outgroup in all trees.

Results

The complete SSrRNA sequences were determined for *C. campylum* (1754 nucleotides), *G. chattoni* (1743 nucleotides), and *O. henneguyi* (1731 nucleotides) (Fig. 5). These sequences have been deposited with the EMBL data library under accession numbers X56532, X56533, and X56531, respectively.

Distance Matrix Analysis

The DM tree (Fig. 6 and Table 1) indicated that the ciliates diverged as a monophyletic group, and that the dinoflagellates represented by *P. micans* were the sister group to the ciliates as shown previously by Gunderson et al. (1987). The branching between some of the major ciliate groups was deeper than the separation between animals and plants: the peritrich *Opisthonecta* and the hypotrich *Euplotes* were

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O.henneguyi     CGTCCTAGTC TTAACTATAA ACTATACCGA CTCGGATTCA GATGAATCAT AAAGTTCATT T-GGGACCGT AGGAGAAATC AAAGTTTTTG GGTTCTGGGG  1068
G.chattoni      CGTCGTAGTC TTAACTATAA ACTATACCGA CTCGGGATCG GCTGGAATAT A--TGTCCAG TCGGCACCGT ATGAGAAATC AAAGTCTTTG GGTTCTGGGG  1078
C.campylum      CGTCGTAGTC TTAACTATAA ACTATACCGA CTCGGGATCG GCTGGAACAC ACGTGTCCAG TCGGCACCGT ATGAGAAATC AAAGTCTTTG GGTTCTGGGG  1085
T.thermophila   CGTCGTAGTC TTAACTATAA ACTATACCGA CTCGGGATCG GCTGGAATAA A--TGTCCAG TCGGCACCGT ATGAGAAATC AAAGTCTTTG GGTTCTGGGG  1082

O.henneguyi     GAAGTATGGT -CGCAAGGCT GAAACTTAAA GGAATTGACG GTTTTGCACC ACCATGGAGT GGAGTCTGCG GCTTAATTTG ACTCAACACT GGGAAACTCA  1167
G.chattoni      GAAGTATGGT ACGCAAGTCT GAAACTTAAA GGAATTGACG GAAGAGCA-C ACCAGAAGTG GAACC-TGCG GCTTAATTTG ACTCAACACG GGGAAACTCA  1176
C.campylum      GAAGTATGGT ACGCAAGTCT GAAACTTAAA GGAATTGACG GAACAGCA-C ACCAGAAGTG GAACC-TGCG GCTTAATTTG ACTCAACACG GGGAAACTCA  1183
T.thermophila   GAAGTATGGT ACGCAAGTCT GAAACTTAAA GGAATTGACG GAACAGCA-C ACCAGAAGTG GAACC-TGCG GCTTAATTTG ACTCAACACG GGGAAACTCA  1180

O.henneguyi     TCAGGGCAAG AAGATTGTAG GATTGACAGA TTGAGAGTTC TTTCTTGATT GGTCTAGTGG TGGTGCATGG CCGTTCTTAG TTGGTGGAGT GATTTGTCTG  1267
G.chattoni      CGAGCGCAAG ACAGAGAAGG GATTGACAGA TTGAGAGCTC TTTCTTGATT CTTTGGGTGG TGGTGCATGG CCGTTCTTAG TTGGTGGAGT GATTTGTCTG  1276
C.campylum      CGAGCGCAAG ACAGAGAAGG GATTGACAGA TTGAGAGCTC TTTCTTGATT CTTTGGGTGG TGGTGCATGG CCGTTCTTAG TTGGTGGAGT GATTTGTCTG  1283
T.thermophila   CGAGCGCAAG ACAGAGAAGG GATTGACAGA TTGAGAGCTC TTTCTTGATT CTTTGGGTGG TGGTGCATGG CCGTTCTTAG TTGGTGGAGT GATTTGTCTG  1280

O.henneguyi     GTTAATTCCG TTAACGAACG AGACCTTAAC CTGCTAACTA GTACACAGAT GACAAATCTG TGATACTTCT TAGAGGGACT ATGTGATGTA -ATCACATGG  1366
G.chattoni      GTTAATTCCG TTAACGAACG AGACCTTAAC CTGCTAACTA GTCTCCCTGT GAACAACGGG GTGTACTTCT TAGAGGGACT ATTGTGCAAG AAGCCAATGG  1376
C.campylum      GTTAATTCCG TTAACGAACG AGACCTTAAC CTGCTAACTA GTCGCCCTGT GAACAACGGG ATGTACTTCT TAGAGGGACT ATTGTGCAAG AAGCCAATGG  1383
T.thermophila   GTTAATTCCG TTAACGAACG AGACCTTAAC CTGCTAACTA GTCTGCTTGT AAATAACAGG TTGTACTTCT TAGAGGGACT ATTGTGCAAT AAGCCAATGG  1380

O.henneguyi     AAGTTTGAGG CAATAACAGG TCTGTGATGC CCTTAGATGT CCTGAGCTGC ACGCGTACTA CAATGGTGCT TTCAACGAGC TTTTCCTGAT CC-GAAAGGA  1465
G.chattoni      AAGTTTAAGG CAATAACAGG TCTGTGATGC CCCTAGAC-T GCTCGGCCGC ACGCGCGTTA CAATGACTGG CGCAGAAAGT TTTTCCTG-G CCTGGGAAGG  1474
C.campylum      AAGTTTAAGG CAATAACAGG TCTGTGATGC CCCTAGACGT GCTCGGCCGC ACGCGCGTTA CAATGACTGG CGCAGAAAGC ATTTCCTG-T CCTGGGAAGG  1482
T.thermophila   AAGTTTAAGG CAATAACAGG TCTGTGATGC CCCTAGACGT GCTCGGCCGC ACGCGCGTTA CAATGACTGG CGCAAAAAGT ATTTCCTG-T CCTGGGAAGG  1479

O.henneguyi     TTTGGGTAAT CTTTTTAGTG AGCACCGTGC TT-GGGATAG ATCTTTGTAA TTATGGATCT TGAACTAGGA ATTCCTAGTA ACGACGCGTC ATCAGCGCGT  1564
G.chattoni      TTCGGGTAAT CTTATTAATA CCAGTCGTG- TTAGGAATAG TTCTTTGGAA TTTTGGATCT TGAACGAGGA ATTTCTAGTA AGTGCAAGTC ATCAGCTTGC  1573
C.campylum      TACGGGTAAT CTTATTAATA CCAGTCGTG- TTAGGGATAG TTCTTTGGAA TTTTGGATCT TGAACGAGGA ATTTCTAGTA AGTGCAAGTC ATCAGCTTGC  1581
T.thermophila   TACGGGTAAT CTTATTAATA CCAGTCGTG- TTAGGGATAG TTCTTTGGAA TTGTGGATCT TGAACGAGGA ATTTCTAGTA AGTGCAAGTC ATCAGCTTGC  1578

O.henneguyi     GCTGATTACG TCCCTGCAAA ATGTACACAC CGCCCGTCGC TATTACCGAT TGAGTGTAAA GGTGAACCTT CTCGA-TAGA CGCAAGTCCA -----GAAA-  1657
G.chattoni      GTTGATTATG TCCCTGCCGT TTGTACACAC CGCCCGTCGC TTGTAGTAAC -GAATGGTCT GGTGAACCTT CTGGACTGCT CAGCAATAA- --GCGGAAAA  1669
C.campylum      GTTGATTATG TCCCTGCCGT TTGTACACAC CGCCCGTCGC TTGTAGTAAC -GAATGGTCT GGTGAACCTT CTGGACTGCT TCTGAATAAG GAGCGGGAAA  1680
T.thermophila   GTTGATTATG TCCCTGCCGT TTGTACACAC CGCCCGTCGC TTGTAGTAAC -GAATGGTCT GGTGAACCTT CTGGACTGCG ACAGCAATGT T-GCGGAAAA  1676

O.henneguyi     TTAAGTAAAC CTTTGCACTT AGAGGAAATA AAAGTCGTAG CAAGGTTTCC gtaggtgaac ctgcagaagg atca                            1731
G.chattoni      ATAAGTAAAC CCATCCATTT GGAACAACAA GAAGTCGTAA CAAGGTATCT gtaggtgaac ctgcagaagg atca                            1743
C.campylum      ATAAGTAAAC CCTACCATTT GGAACAACAA GAAGTCGTAA CAAGGTATCT gtaggtgaac ctgcagaagg atca                            1754
T.thermophila   ATAAGTAAAC CCTACCATTT GGAACAACAA GAAGTCGTAA CAAGGTATCT GTAGGTGAAC CTGCAGATGG ATCATTA                          1753
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Fig. 5. Continued.

separated by an evolutionary distance of 0.245, while the rat *Rattus* and corn *Zea* were separated by an evolutionary distance of 0.196, indicating a rapid divergence of the ciliate ancestral stock after its separation from the main eukaryote line (Table 1).

In the DM tree the subclass Hymenostomatia (*Colpidium, Glaucoma, T. hegewischi, T. pyriformis,* and *T. thermophila*) was maintained as a sister group of the subclass Peritrichia (*Opisthonecta*), all within the class Oligohymenophorea (sensu Small and Lynn, 1985). The tetrahymenas were more closely related to each other than they were to *Glaucoma* or *Colpidium* (Table 1). Note that the evolutionary distance separating the peritrich *Opisthonecta* from the hymenostomes (≈0.200) was greater than the distance separating *Opisthonecta* from the nassophorean *Parameciu*m (0.176) (Table 1).

The hypotrichs (*Euplotes*) and the stichotrichs (*Onychodromus, Oxytricha,* and *Stylonychia*) remained sister groups within the class Spirotrichea. The stichotrich, *O. nova* was more closely related by sequence similarity (0.993) to *S. pustulata* than to *O. granulifera* (0.989) as shown previously (Schlegel et al. 1991).

The classes Nassophorea and Colpodea (sensu Small and Lynn 1981, 1985), represented by *Paramecium* and *Colpoda* respectively, tree more closely with the oligohymenophoreans than the spirotrichs. The heterotrich *Belpharisma* was placed as the earliest branching ciliate as shown previously (Greenwood et al. 1991) (Fig. 6).

Maximum Parsimony Analysis

The MP analysis of ciliate SSrRNA sequences using the four different input trees generated two alternative trees that differed in their NR scores by 20 (Figs. 7 and 8). The classical ciliate tree of Corliss (1979) and the UPGMA tree produced the same final tree having the lowest NR score of 2332 (Fig. 7). The input tree derived from the reverse transcripts of SSrRNA (Lynn and Sogin 1988) and the Small and Lynn (1981, 1985) tree produced the same final tree having an NR score of 2352 (Fig. 8). The branching order of the two alternative trees depicted a similar phylogeny. In both trees the ciliates of the subclass Hymenostomatia (*Colpidium, Glaucoma,* and *Tetrahymena*) and the subclass Peritrichia (*Opisthonecta*) of the class Oligohymenophorea clustered together, whereas *Paramecium* of the class

Table 1. Structural similarity and evolutionary distance data for eukaryote small subunit rRNA gene sequences

	Structural similarity/evolutionary distance to										
Organisms	Rat	*A.s.*	*Z.m.*	*C.r.*	*B.e.*	*S.c.*	*N.c.*	*A.b.*	*O.d.*	*P.m.*	*E.a.*
Rat		0.873	0.827	0.817	0.820	0.819	0.800	0.798	0.796	0.796	0.751
A. salina	0.139		0.814	0.807	0.811	0.817	0.816	0.797	0.805	0.798	0.750
Z. mays	0.196	0.213		0.900	0.852	0.859	0.844	0.854	0.852	0.853	0.787
C. reinhardtii	0.210	0.223	0.107		0.867	0.860	0.853	0.855	0.859	0.853	0.786
B. emersonii	0.205	0.217	0.165	0.146		0.880	0.859	0.854	0.847	0.846	0.792
S. cerevisiae	0.207	0.209	0.156	0.155	0.130		0.907	0.852	0.853	0.852	0.801
N. crassa	0.232	0.211	0.175	0.163	0.166	0.099		0.834	0.847	0.845	0.786
A. bisexualis	0.235	0.236	0.163	0.161	0.162	0.165	0.188		0.899	0.861	0.811
O. danica	0.238	0.226	0.165	0.167	0.170	0.164	0.171	0.109		0.864	0.813
P. micans	0.237	0.236	0.164	0.163	0.173	0.165	0.174	0.154	0.150		0.828
E. aediculatus	0.302	0.304	0.250	0.251	0.244	0.232	0.253	0.218	0.215	0.196	
B. americanum	0.274	0.266	0.202	0.197	0.205	0.195	0.208	0.209	0.179	0.164	0.213
O. nova	0.256	0.256	0.187	0.188	0.186	0.165	0.184	0.171	0.155	0.130	0.145
O. granulifera	0.270	0.271	0.198	0.201	0.196	0.182	0.197	0.184	0.169	0.152	0.159
O. quadricornutus	0.255	0.262	0.183	0.188	0.184	0.168	0.184	0.170	0.161	0.129	0.149
S. pustulata	0.260	0.259	0.183	0.192	0.190	0.171	0.189	0.173	0.161	0.135	0.147
C. inflata	0.262	0.258	0.195	0.201	0.187	0.179	0.192	0.168	0.154	0.153	0.190
P. tetraurelia	0.266	0.277	0.204	0.198	0.189	0.185	0.197	0.186	0.183	0.162	0.195
O. henneguyi	0.318	0.317	0.253	0.248	0.244	0.227	0.244	0.235	0.227	0.201	0.245
G. chattoni	0.321	0.334	0.265	0.265	0.250	0.222	0.245	0.240	0.236	0.225	0.249
C. campylum	0.314	0.329	0.251	0.255	0.241	0.212	0.238	0.233	0.229	0.216	0.240
T. hegewischi	0.313	0.325	0.256	0.256	0.245	0.215	0.238	0.232	0.226	0.216	0.242
T. thermophila	0.307	0.323	0.250	0.251	0.244	0.216	0.238	0.229	0.221	0.218	0.242
T. pyriformis	0.309	0.326	0.252	0.253	0.243	0.213	0.236	0.230	0.223	0.217	0.242
D. discoideum	0.336	0.318	0.287	0.294	0.280	0.279	0.310	0.255	0.262	0.291	0.324

The upper half of the table gives the structural similarity (S) values for all pairs of aligned small subunit rRNA sequences. The lower half of the table gives the evolutionary distance values (average number of nucleotide substitutions per sequence position) determined by the Jukes and Cantor (1969) formula for conversion of structural similarity. Sequence data for the aligned eukaryote small subunit rRNA sequences are listed in Materials and Methods

Nassophorea and *Colpoda* of the class Colpodea were separate lineages on the same main branch. Also, in both trees the subclass Hypotrichia (*Euplotes*) and the subclass Stichotrichia (*Onychodromus, Oxytricha,* and *Stylonychia*) of the class Spirotrichea clustered together. The only difference between the two MP trees was in the placement of the heterotrich *Blepharisma.* The tree with the lowest NR score 2332 placed *Blepharisma* as a separate lineage on the branch leading to the hymenostomes (Fig. 7) whereas the alternative tree with an NR score of 2352 placed *Blepharisma* at a deeper branch position prior to the branching of all other ciliates (Fig. 8).

Discussion

Distance Matrix vs Maximum Parsimony

The literature teems with arguments regarding which method is the most appropriate for revealing true phylogeny (Farris 1981, 1985, 1986; Felsenstein 1981, 1984, 1986; Penny and Hendy 1986; Sourdis and Nei 1988). The justification of any method is always countered by its inappropriateness in a specific instance. Rothschild et al. (1986) appropriately stated that no character should be considered the Rosetta stone of phylogeny, and this philosophy might easily be extended to methods. The goal of phylogenetic analyses is to get the best estimate of relatedness. Estimates are best evaluated through comparisons of phylogenies created by different characters (morphological and molecular) and by different analytical methods (parsimony and DM). Thus, when congruence of phylogenies produced by different characters and methods occurs, more confidence can be placed in these as best estimates.

In the present analyses, both the DM and MP methods produced similar phylogenies that depicted the ciliates as a monophyletic group (Figs. 6–8). However, differences in topology existed. In both the DM and MP analyses the hymenostomes (*Colpidium, Glaucoma,* and the tetrahymenas) all form a very tight group with the peritrich *Opisthonecta.* The stichotrichs *Onychodromus, Stylonychia,* and the two oxytrichs clustered together while the hypotrich *Euplotes* was more distantly related to this group (Figs. 6–8). Both DM and MP trees indicate that *O. nova* was more closely related to *S. pustulata* than to *O. granulifera.* It has recently been argued

Table 1. Extended

Structural similarity/evolutionary distance to													
B.a.	*O.n.*	*O.g.*	*O.q.*	*S.p.*	*C.i.*	*P.t.*	*O.h.*	*G.c.*	*C.c.*	*T.h.*	*T.t.*	*T.p.*	*D.d.*
0.770	0.783	0.773	0.784	0.781	0.779	0.776	0.741	0.739	0.744	0.744	0.748	0.747	0.729
0.776	0.783	0.773	0.779	0.781	0.782	0.768	0.741	0.731	0.734	0.736	0.738	0.735	0.744
0.823	0.835	0.826	0.838	0.838	0.828	0.822	0.785	0.777	0.787	0.783	0.787	0.786	0.761
0.827	0.834	0.823	0.834	0.831	0.823	0.826	0.789	0.777	0.784	0.783	0.787	0.785	0.756
0.821	0.835	0.827	0.837	0.832	0.834	0.833	0.791	0.788	0.794	0.791	0.792	0.792	0.766
0.828	0.582	0.839	0.849	0.847	0.841	0.836	0.804	0.808	0.815	0.813	0.812	0.814	0.767
0.818	0.837	0.826	0.837	0.833	0.830	0.827	0.792	0.791	0.796	0.796	0.796	0.797	0.746
0.817	0.847	0.837	0.848	0.845	0.849	0.836	0.798	0.795	0.799	0.800	0.803	0.802	0.784
0.841	0.860	0.849	0.855	0.855	0.860	0.837	0.804	0.797	0.802	0.805	0.808	0.807	0.779
0.852	0.880	0.862	0.881	0.877	0.861	0.855	0.824	0.806	0.813	0.812	0.810	0.812	0.759
0.814	0.868	0.857	0.865	0.866	0.832	0.829	0.791	0.788	0.795	0.793	0.793	0.793	0.737
	0.868	0.858	0.865	0.863	0.859	0.855	0.805	0.803	0.812	0.810	0.809	0.813	0.748
0.145		0.972	0.989	0.993	0.895	0.879	0.839	0.830	0.837	0.839	0.836	0.838	0.770
0.157	0.029		0.968	0.969	0.884	0.865	0.828	0.819	0.826	0.829	0.826	0.827	0.760
0.149	0.011	0.032		0.986	0.894	0.880	0.839	0.831	0.837	0.839	0.837	0.839	0.768
0.151	0.007	0.032	0.013		0.892	0.878	0.839	0.829	0.834	0.837	0.833	0.835	0.768
0.156	0.113	0.126	0.115	0.122		0.881	0.836	0.835	0.844	0.843	0.843	0.842	0.778
0.161	0.132	0.149	0.130	0.133	0.129		0.843	0.841	0.849	0.851	0.854	0.855	0.765
0.226	0.181	0.195	0.181	0.182	0.186	0.176		0.821	0.826	0.825	0.826	0.828	0.744
0.228	0.192	0.207	0.192	0.194	0.187	0.178	0.204		0.979	0.978	0.971	0.974	0.739
0.217	0.184	0.197	0.184	0.188	0.175	0.168	0.198	0.021		0.984	0.978	0.983	0.740
0.219	0.181	0.194	0.180	0.184	0.177	0.166	0.199	0.023	0.016		0.986	0.991	0.741
0.220	0.184	0.198	0.184	0.189	0.177	0.162	0.198	0.030	0.023	0.014		0.994	0.746
0.215	0.182	0.197	0.181	0.187	0.177	0.161	0.196	0.026	0.017	0.009	0.006		0.741
0.308	0.290	0.290	0.278	0.278	0.263	0.282	0.313	0.321	0.319	0.318	0.311	0.317	

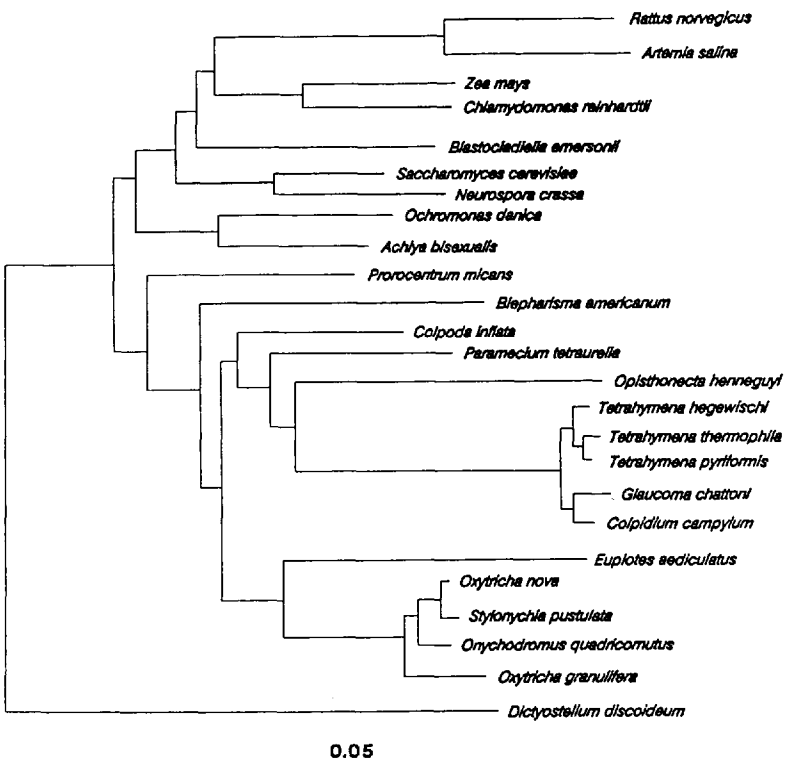

Fig. 6. Ciliate phylogeny inferred from complete SSrRNA sequence similarities using a DM method. The analysis was limited to approximately 1400 positions that could be unambiguously aligned in all of the rRNA sequences. The evolutionary distance (average number of nucleotide replacements per sequence position) is represented by the horizontal component separating species in the figure. The scale bar equals 0.05 nucleotide replacements per sequence position. Data are given in Table 1.

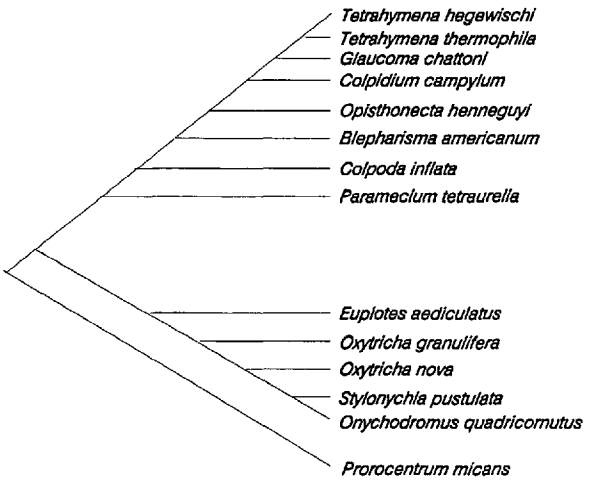

Fig. 7. Ciliate phylogeny inferred from 14 complete SSrRNA sequences using MP analysis. The same tree was derived from both the UPGMA tree and Corliss' (1979) classical ciliate input trees. The NR score for this tree is 2332. The dinoflagellate *Prorocentrum micans* was chosen as the outgroup for the ciliates (Gunderson et al. 1987).

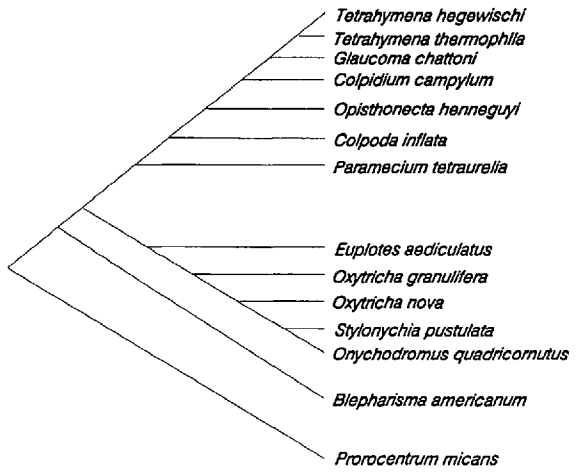

Fig. 8. Ciliate phylogeny inferred from 14 complete SSrRNA sequences using MP analysis. The NR score for this tree is 2352. The same tree was derived from both the Lynn and Sogin (1988) RT tree and Small and Lynn (1981, 1985) input trees. The dinoflagellate *Prorocentrum micans* was chosen as the outgroup for the ciliates (Gunderson et al. 1987).

that *O. nova* is actually a *Stylonychia* species (M. Schlegel, personal communication).

In the DM and MP trees the placement of the colpodean *Colpoda* and the nassophorean *Paramecium* are reversed. However, both methods depicted them as being more closely related to the oligohymenophoreans than to the spirotrichs. The only other difference between the two MP trees was in the placement of the heterotrich *Blepharisma.* The tree with the lowest NR score 2332 places *Blepharisma* as a separate lineage on the branch leading to the hymenostomes (Fig. 7) whereas the alternative tree with an NR score of 2352 places *Blepharisma* at a deeper position prior to all other ciliates (Fig. 8). The latter MP tree agrees with the placement of *Blepharisma* in the DM tree (Fig. 6). The concordance of the MP tree (NR score of 2352) with the DM tree further suggests that the heterotrichs exemplified by *Blepharisma* are distinct from the hypotrichs.

Although variations in the phylogenies do exist between the two methods employed to analyze the SSrRNA sequence data, the basic tree topologies are consistent, indicating at least with this data set that either method produces a similar estimate of phylogenetic relatedness.

Molecular vs Morphological Phylogenies

The phylogenies determined from the complete SSrRNA sequences from *C. campylum, G. chattoni,* and *O. henneguyi* enable us to examine affinities between taxa and to critically examine classificatory hypotheses that have been constructed based on previous morphological and molecular evidence.

The Hymenostome Lineage

The analysis of complete SSrRNA shows the *Tetrahymena* lineage as monophyletic whereas *Colpidium* and *Glaucoma* were more closely related to each other than either was to the tetrahymenas (Fig. 8 and Table 1). This agrees with the placement of *Colpidium* and *Glaucoma* determined by Lynn and Sogin (1988) from partial SSrRNA sequences derived from reverse transcripts.

However, a different relationship has been determined by Nanney's group, which has been concentrating on the nucleotide sequences from domain 2 (D2) of the large subunit rRNA (LSrRNA) and pooled information from 5S and 5.8S rRNA sequences (Nanney et al. 1989a,b; Preparata et al. 1989). Their most comprehensive phylogeny included 14 ciliates (*Colpidium striatum, Colpoda maupasi, Colpoda steini, G. chattoni, Didinium, Paramecium,* and 8 tetrahymenas) and 1 dinoflagellate (*Crypthecodinium cohnii*) (Preparata et al. 1989).

Both the present study and Nanney's group place *Colpidium* and *Glaucoma* as the closest relatives to the *Tetrahymena* lineage. However, their phylogeny (Preparata et al. 1989) illustrated the genus *Tetrahymena* as paraphyletic: *Glaucoma* was more closely related to some tetrahymenas than the tetrahymenas were to each other; and *Colpidium* was more closely related to some *Tetrahymena* groups than it was to *Glaucoma.* Preparata et al. (1989) tentatively interpreted their phylogeny as indicating that *Colpidium* and *Glaucoma* are more derived than the primitive tetrahymenas. However, because the tetrahymenas may be paraphyletic based on the partial

LSrRNA sequences, any general conclusions about which species are primitive or derived must be disregarded until more representatives of the genera *Glaucoma* and *Colpidium* can be sequenced.

The monophyly of the tetrahymenas illustrated in the present study is further supported by independent molecular studies. Katzen et al. (1981) determined that the tetrahymenas all have similar macronuclear DNA profiles whereas *Glaucoma* was shown to possess a different macronuclear DNA profile based on its shorter length macronuclear DNA molecules. Brunk et al. (1990) have recently analyzed the region between the histone H3II/H4II genes in the genome of 19 tetrahymenas and *G. chattoni*; the length of the region varied in different species from 519 nucleotides in *Glaucoma* to 563 nucleotides in *T. paravorax*. The Brunk et al. (1990) phylogeny supports the monophyly of the tetrahymenas by placing *Glaucoma* as their sister group.

Van Bell (1985) produced phylogenies based on 5S rRNA and 5.8S rRNA sequences that also showed the genus *Tetrahymena* to be monophyletic, with *Colpidium* more closely related to the tetrahymenas than *Glaucoma*. This is not congruent with the findings of the present study in which *Colpidium* and *Glaucoma* were more closely related to each other than either was to the tetrahymenas (Fig. 8).

The variation seen between the present study and both the Van Bell (1985) and Preparata et al. (1989) studies in determining the phylogenetic relationships of the hymenostome ciliates may have resulted from the small number of nucleotide positions (<200 nucleotides) used by Van Bell (1985) and Preparata et al. (1989). The 5S rRNA (120 nucleotides) and the 5.8S rRNA (160 nucleotides) are limited by the low number of independently variable nucleotide sites within the gene (Hori and Osawa 1987; Olsen 1988). It is also possible that the partial LSrRNA sequences from domain 2 (180 nucleotides) used by Preparata et al. (1989) may not contain enough variable sites to differentiate closely related phylogenetic assemblages.

The classification schemes of Corliss (1979), Small and Lynn (1981, 1985), and de Puytorac et al. (1987) all depict the hymenostomes (*Colpidium, Glaucoma,* and the tetrahymenas) as a monophyletic group. Both Corliss (1979) and de Puytorac et al. (1987) suggest a close relationship between *Colpidium* and the tetrahymenas and place them in the same family Tetrahymenidae whereas Small and Lynn (1981, 1985) place *Colpidium, Glaucoma,* and the tetrahymenas in separate families within the same order Hymenostomatida. The SSrRNA phylogenies indicate that *Colpidium* was more closely related to *Glaucoma* than either was to the tetrahymenas (Fig. 8). More SSrRNA sequences are required from species in the genera *Glaucoma* and *Colpidium* to firmly establish the sister group of the tetrahymenas. However, the SSrRNA phylogeny is more consistent with Small and Lynn's (1981, 1985) classification, as *Colpidium* and the tetrahymenas do not share a branch distinct from *Glaucoma* (Fig. 8).

The Peritrich Ciliates

At present the only molecular information on peritrich ciliates is the small subunit rRNA sequence from *Opisthonecta* in the present study and the partial SSrRNA sequence (Lynn and Sogin 1988).

The phylogenetic position of the peritrichs has always been puzzling and controversial (Corliss 1979). During the Age of Discovery (1880–1930), microscopy suggested that the peritrichs were more closely allied with the spirotrich ciliates based on their reduced somatic ciliature and spiralled oral ciliature (Corliss 1974). During the Age of Exploitation (1930–1950), improvement of both microscopes and staining techniques led to the discovery of many new species and the recognition of great diversity within the phylum Ciliophora. Corliss (1974) ascribed the elevation of the peritrichs at this time to a taxonomic rank equivalent to spirotrichs and holotrichs because of Kahl's (1933) separation of the larger number of species within the order Peritricha into two suborders the Sessilia and Mobilia. The impact of the discovery of the infraciliature of ciliates on understanding the phylogenetic relationships among the Ciliophora was not fully appreciated until the Age of Infraciliature (1950–1970). During this period, detailed life history studies with emphasis on the infraciliature of the peritrich ciliates determined their affinities with such holotrichs as *Tetrahymena* and *Pleuronema* (Fauré-Fremiet 1965). The advent of the electron microscope led to the Age of Ultrastructure (1970–present). Corliss (1968) placed the peritrichs firmly within the class Oligohymenophora stating that, based on the ultrastructure of the buccal apparatus (Lom et al. 1968), the peritrichs showed considerable affinity with the hymenostomes and scuticociliates. However, based on their highly unique life history and great diversity, Corliss (1968) recognized the distinctness of the peritrichs by elevating them to a subclass.

SSrRNA sequence comparisons indicate that the evolutionary distance separating the peritrich *Opisthonecta* from the hymenostomes (0.200) is greater than the distance separating *Opisthonecta* from the nassophorean *Paramecium* (0.176) (Table 1). This is interesting since de Puytorac et al. (1984) determined from a phenetic analysis of 122 morphological characters that the peritrich *Trichodina* (Mobilina, Peritrichia) was more closely related to the nassophorean ciliates (*Furgasonia, Nassula, Paramecium*). However, this classification has since been abandoned by de Puytorac et al. (1987) who now

place the peritrichs within the subclass Peritrichia of the class Oligohymenophorea thereby agreeing with the classification schemes of Corliss (1979) and Small and Lynn (1981, 1985). The present study once again illustrates the distinctiveness of the peritrichs from both the hymenostomes and the nassophoreans, especially when one considers that the evolutionary distance separating the peritrichs from both these groups (0.200 and 0.176, respectively) is greater than the evolutionary distance separating rat and brine shrimp (*Artemia*) (0.139) (Table 1): the high taxonomic rank of the peritrichs suggested initially by Kahl (1933) and adamantly supported by peritrichologists (Rabbe 1964; Finley 1974) may be appropriate. Prior to doing this, more sequence information should be obtained from other peritrichs (e.g., *Vorticella*) and other oligohymenophoreans, especially the scuticociliates. This would allow us to assess the cohesiveness of the class Oligohymenophorea.

The impact of molecular information and its implications to our understanding of the systematic relationships within the phylum Ciliophora are only now being realized. As more sequence data become available they should allow us to assess the validity of our past classification schemes. The next few years will determine if sequence information will lead us into a new period of ciliate systematics.

Evolution of the Ciliate Genetic Code

The ciliates appear to deviate from the universal genetic code by using TAA and TAG as codons for glutamine or glutamic acid (Gln), thus leaving only TGA as a stop codon. This phenomenon was found in such divergent ciliate groups as the stichotrichs (*Stylonychia, Oxytricha*), nassophoreans (*Paramecium*), and the hymenostomes (*Tetrahymena*) (Martindale 1989, and references therein). Although only a relatively small number of genes have been sequenced, this feature was assumed to be a derived characteristic having arisen in the most recent common ancestor of the phylum (Hanyu et al. 1986). However, a differential use of stop codons was discovered in the hypotrich ciliate genus *Euplotes*. The actin, β-tubulin, and histone H4 gene of *Euplotes crassus* (Harper and Jahn 1989) and pheromone ER-1 gene of *Euplotes raikovi* (Miceli et al. 1989) were both found to use TAA as a stop codon. The exact function of TGA and TAG in the genus *Euplotes* is unknown. This dilemma leaves the question of antiquity of the altered genetic code in ciliates open for speculation.

A comparison of the genetic code information with the phylogeny generated from SSrRNA sequences indicates that the three groups (stichotrichs, nassophoreans, and hymenostomes) using TAA and TAG as codons for glutamine or glutamic acid (Gln) are on two different branches of the tree (Fig. 6). The nassophoreans (*Paramecium*) and hymenostomes (*Tetrahymena*) are found on the same branch, whereas the stichotrichs (*Stylonychia, Oxytricha*) are placed as the sister group to the hypotrichs (*Euplotes*) (Fig. 6). This suggests that two potential scenarios exist for the differences observed in the ciliate genetic code. The first hypothesis is that the most recent common ancestor to the ciliates possessed an altered genetic code and that the hypotrichs (*Euplotes*) have undergone a reversal to a condition similar to the universal genetic code. This would predict that all ciliates (other than possibly hypotrichs) should have the altered code (TAA and TAG coding for Gln). The alternative hypothesis, suggested by Harper and Jahn (1989), is that the most recent common ancestor to the ciliates possessed the universal genetic code and that the altered code arose later possibly in two (or more) lineages. This hypothesis takes into consideration the possibility that the genetic code is more flexible. This would account for the role of suppressor tRNAs in stop codon recognition in order to allow for the differential expression of specific proteins (Valle and Morch 1988; Harper and Jahn 1989). This suggestion is interesting in light of the fact that the genes coding for surface antigens (51A, 51C, 51H, and 156G) in *Paramecium*, which are highly expressed, and the genes coding for cnjB in *Tetrahymena thermophila*, which is briefly expressed, contain the highest number of the altered codons TAA and TAG (Martindale 1989). Therefore, the role of an altered genetic code in ciliates may be a method of gene regulation.

The SSrRNA phylogenetic tree offers some insight into which groups should be pursued in order to fully investigate the altered genetic code in ciliates. Sequencing of protein-coding genes from *Colpidium, Colpoda, Glaucoma,* and *Opisthonecta* would determine if the altered genetic code exhibited by *Oxytricha, Paramecium, Tetrahymena,* and *Stylonychia* is characteristic of the entire phylum or lineage dependent. The most intriguing candidate for exploring the origin of the genetic code within the phylum would be to acquire sequence information from the heterotrich *Blepharisma,* which branches before the hypotrichs, stichotrichs, nassophoreans, and hymenostomes diverge.

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References

Allen SL, Li CI (1974) Nucleotide sequence divergence among DNA fractions of different syngens of *Tetrahymena pyriformis*. Biochem Genet 12:213–233

Bardele CF (1980) The imprints of ciliate phylogeny revealed by comparative freeze–fracture study of the ciliary membrane. In: Schwemmler W, Schenk HEA (eds) Endocytobiology, endosymbiosis and cell biology. Walter de Gruyter, Berlin, pp 51–61

Baroin A, Perasso R, Qu LH, Brugerolle G, Bachellerie JP, Adoutte A (1988) Partial phylogeny of the unicellular eukaryotes based on rapid sequencing of a portion of 28S ribosomal RNA. Proc Natl Acad Sci USA 85:3474–3478

Brunk CF, Kahn RW, Sadler LA (1990) Phylogenetic relationships among *Tetrahymena* species determined using the polymerase chain reaction. J Mol Evol 30:290–297

Cabot EL, Beckenbach AT (1989) Simultaneous editing of multiple nucleic acids and protein sequences with ESEE. Comput Appl Biosci 5:233–234

Chan Y, Gutell R, Noller HF, Wool IG (1984) The nucleotide sequence of a rat 18S ribosomal ribonucleic acid gene and proposal for the secondary structure of 18S ribosomal ribonucleic acid. J Biol Chem 259:224–230

Corliss JO (1968) The value of ontogenetic data in reconstructing protozoan phylogenies. Trans Am Microsc Soc 87:1–20

Corliss JO (1974) The changing world of ciliate systematics: historical analysis of past efforts and a newly proposed phylogenetic scheme of classification for the protistan phylum Ciliophora. Syst Zool 23:91–138

Corliss JO (1979) The ciliated protozoa: characterization, classification, and guide to the literature, ed 2. Pergamon, New York

Czelusniak J, Goodman M, Hewett-Emmett D, Weiss ML, Venta PJ, Tashian RE (1982) Phylogenetic origins and adaptive evolution of avian and mammalian haemoglobin genes. Nature 298:297–300

Czelusniak J, Goodman M, Moncreif ND, Kehoe SM (1990) Maximum parsimony approach to construction of evolutionary trees from aligned homologous sequences. In: Doolittle RF (ed) Methods in enzymology: molecular evolution: computer analysis of protein and nucleic acid sequences, vol 183. Academic Press, New York, pp 601–615

Eisler K (1988) Electron microscopical observations on the ciliate *Furgasonia blochmanni* Fauré-Fremiet, 1967, Part 1: an update on morphology. Eur J Protistol 24:75–93

Eisler K (1989) Electron microscopical observations on the ciliate *Furgasonia blochmanni* Fauré-Fremiet, 1967, Part 2: morphogenesis and phylogenetic conclusions. Eur J Protistol 24:181–199

Elwood HJ, Olsen GJ, Sogin ML (1985) The small subunit ribosomal RNA gene sequences from the hypotrichous ciliates *Oxytricha nova* and *Stylonychia pustulata*. Mol Biol Evol 2: 399–410

Farris JS (1981) Distance data in phylogenetic analysis. In: Funk VA, Brooks DR (eds) Advances in cladistics: proceedings of the first Willi Hennig Society. New York Botanical Garden, Bronx, pp 3–23

Farris JS (1985) Distance data revisited. Cladistics 1:67–85

Farris JS (1986) Distances and statistics. Cladistics 2:144–157

Fauré-Fremiet E (1965) Morphologie comparée des ciliés Peritrichida. Prog Protozool, pp 13–14

Felsenstein J (1981) A likelihood approach to character weighting and what it tells us about parsimony and compatibility. Biol J Linn Soc 16:183–196

Felsenstein J (1984) Distance methods for inferring phylogenies: a justification. Evolution 38:16–24

Felsenstein J (1986) Distance methods: a reply to Farris. Cladistics 2:130–143

Finley HE (1974) The peritrichs, now and then: 1676 to 1973. Trans Am Microsc Soc 93:307–313

Goodman M (1981) Decoding the pattern of protein evolution. Prog Biophys Mol Biol 37:105–164

Goodman M, Czelusniak J, Moore GW, Romero-Herrera AE, Matsuda G (1979) Fitting the gene lineage into its species lineage, a parsimony strategy illustrated by cladograms constructed from globin sequences. Syst Zool 28:132–163

Greenwood SJ, Schlegel M, Sogin ML, Lynn DH (1991) Phylogenetic relationships of *Blepharisma americanum* and *Colpoda inflata* within the phylum Ciliophora inferred from complete small subunit rRNA gene sequences. J Protozool 38: 1–6

Gunderson JH, Elwood HJ, Ingold A, Kindle K, Sogin ML (1987) Phylogenetic relationships between chlorophytes, chrysophytes, and oomycetes. Proc Natl Acad Sci USA 84: 5823–5827

Hanyu N, Kichino Y, Nishimura S, Beier H (1986) Dramatic events in ciliate evolution: alteration of UAA and UAG termination codons to glutamine codons due to anticodon mutations in two *Tetrahymena* $tRNAs^{Gln}$. EMBO J 5:1307–1311

Harper DS, Jahn, CL (1989) Differential use of termination codons in ciliated protozoa. Proc Natl Acad Sci USA 86: 3252–3256

Herzog M, Maroteaux L (1986) Dinoflagellate 17S rRNA sequence inferred from the gene sequence: evolutionary implications. Proc Natl Acad Sci USA 83:8644–8648

Hori H, Osawa S (1987) Origin and evolution of organisms as deduced from 5S ribosomal RNA sequences. Mol Biol Evol 9:191–201

Jukes TH, Cantor CR (1969) Evolution of protein molecules. In: Munro HN (ed) Mammalian protein metabolism. Academic Press, New York, pp 22–126

Kahl A (1933) Ciliata libera et ectocommensalia. In: Grimpe G, Wagler E (eds), Die Tierwelt der Nord-und Ostee, Lief 23. Leipzig, pp 29–146

Katzen AL, Cann GM, Blackburn EH (1981) Sequence-specific fragmentation of macronuclear DNA in a holotrichous ciliate. Cell 24:313–320

Lom J, Corliss JO, Noirot-Timothée C (1968) Observations on the ultrastructure of the buccal apparatus in thigmotrich ciliates and their bearing on thigmotrich–peritrich affinities. J Protozool 15:824–840

Lynn DH, Didier P (1978) Caractéristiques ultrastructurales du cortex somatique et buccal du cilié *Colpidium campylum* (Oligohymenophora, Tetrahymenina) quant à la position sytématique du *Turaniella*. Can J Zool 56:2336–2343

Lynn DH, Sogin ML (1988) Assessment of the phylogenetic relationships among ciliated protists using partial ribosomal RNA sequences derived from reverse transcripts. BioSystems 21:249–254

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor NY

Martindale DW (1989) Codon usage in *Tetrahymena* and other ciliates. J Protozool 36:29–34

McCarroll R, Olsen GJ, Stahl YD, Woese CR, Sogin ML (1983) Nucleotide sequence of *Dictyostelium discoideum* small subunit ribosomal ribonucleic acid inferred from the gene sequence: evolutionary implications. Biochemistry 22:5858–5868

Medlin L, Elwood HJ, Stickel S, Sogin ML (1988) The characterization of enzymatically amplified eukaryotic 16S-like rRNA coding regions. Gene 71:491–499

Messing J (1983) New M13 vectors for cloning. In: Wu R, Grossman LG, Moldave K (eds), Methods in enzymology:

recombinant DNA, vol 101. Academic Press, New York, pp 20–78

Messing J, Carlson J, Hagen G, Rubenstein I, Oleson A (1984) Cloning and sequencing of the ribosomal RNA genes in maize: the 17S region. DNA 3:31–40

Miceli C, La Terza A, Melli ML (1989) Isolation and structural characterization of cDNA clones encoding the mating pheromone Er-1 secreted by the ciliate *Euplotes raikovi.* Proc Natl Acad Sci USA 86:3016–3020

Moore GW, Barnabas J, Goodman M (1973) A method for constructing maximum parsimony ancestral amino acid sequences on a given network. J Theor Biol 38:459–485

Nanney DL, Meyer EB, Simon EM, Preparata RM (1989a) Comparison of ribosomal and isozymic phylogenies of tetrahymenine ciliates. J Protozool 36:1–8

Nanney DL, Preparata RM, Preparata FP, Meyer EB, Simon EM (1989b) Shifting ditypic site analysis: heuristics for extending the phylogenetic range of nucleotide sequences in Sankoff analyses. J Mol Evol 28:451–459

Nelles L, Fang B, Volckaert G, Vandenberghe A, DeWachter R (1984) Nucleotide sequence of a crustacean 18S ribosomal RNA gene and secondary structure of eukaryotic small subunit ribosomal RNAs. Nucleic Acids Res 12:8749–8768

Olsen GJ (1988) Phylogenetic analysis using ribosomal RNA. In: Wu R, Grossman LG (eds) Methods in enzymology: ribosomes, vol 164. Academic Press, New York, pp 793–812

Peck RK (1978) Ultrastructure of the somatic and buccal cortex of the tetrahymenine hymenostome *Glaucoma chattoni.* J Protozool 25:186–198

Penny D, Hendy M (1986) Estimating the reliability of evolutionary trees. Mol Biol Evol 3:403–417

Preparata RM, Meyer EB, Preparata FP, Simon EM, Vossbrinck CR, Nanney DL (1989) Ciliate evolution: the ribosomal phylogenies of the tetrahymenine ciliates. J Mol Evol 28:427–441

Puytorac P de, Grain J, Legendre P, Devaux J (1984) Essai d'application de l'analyse phénétique à la classification du phylum des Ciliophora. J Protozool 31:496–507

Puytorac P de, Grain J, Mignot J-P (1987) Précis de protistologie. Société Nouvelle des Éditions Boubée, Paris

Rabbe Z (1964) The taxonomic position and rank of the Peritricha. Acta Protozool 2:19–32

Rothschild LJ, Ragan MA, Coleman AW, Heywood P, Gerbi SA (1986) Are rRNA sequence comparisons the Rosetta stone of phylogenetics? Cell 47:640

Rubtsov PM, Musakhanov MM, Zakharyev VM, Krayev AS, Skryabin KG, Bayev AA (1980) The structure of the yeast ribosomal RNA genes. I. The complete nucleotide sequence of the 18S ribosomal RNA gene from *Saccharomyces cerevisiae.* Nucleic Acids Res 8:5779–5794

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74: 5463–5467

Schlegel M, Elwood HJ, Sogin ML (1991) Molecular evolution in hypotrichous ciliates: Sequence of the small subunit ribosomal RNA genes from *Onychodromus quadricornutus* and *Oxytricha granulifera* (Oxytrichidae, Hypotrichida, Ciliphora). J Mol Evol 32:64–69

Small EB, Lynn DH (1981) A new macrosystem for the phylum Ciliophora Doflein, 1901. BioSystems 14:387–401

Small EB, Lynn DH (1985) Phylum Ciliophora. In: Lee JJ, Hutner SH, Bovee EC (eds) An illustrated guide to the protozoa. Society of Protozoology, Lawrence KS, pp 393–575

Sogin ML, Elwood HJ (1986) Primary structure of the *Paramecium tetraurelia* small-subunit rRNA coding region: phylogenetic relationships within the Ciliophora. J Mol Evol 23: 53–60

Sogin ML, Ingold A, Karlok M, Nielsen H, Enberg J (1986a) Phylogenetic evidence for the acquisition of ribosomal RNA introns subsequent to the divergence of some of the major *Tetrahymena* groups. EMBO J 5:3625–3630

Sogin ML, Miotto K, Miller L (1986b) Primary structure of the *Neurospora crassa* small subunit ribosomal RNA coding region. Nucleic Acids Res 14:9540

Sogin ML, Swanton MT, Gunderson JH, Elwood HJ (1986c) Sequence for the small subunit ribosomal RNA gene from the hypotrichous ciliate *Euplotes aediculatus.* J Protozool 33:26–29

Sourdis J, Nei M (1988) Relative efficiencies of the maximum parsimony and distance matrix methods in obtaining the correct phylogenetic tree. Mol Biol Evol 5:298–311

Spangler EA, Blackburn EH (1985) The nucleotide sequence of the 17S ribosomal RNA gene of *Tetrahymena thermophila* and the identification of point mutations resulting in resistance to the antibiotics paromomycin and hygromycin. J Biol Chem 260:6334–6340

Swofford DL, Olsen GJ (1990) Phylogeny reconstruction. In: Hillis DM, Moritz C (eds) Molecular systematics. Sinauer, Sunderland MA, pp 411–500

Valle RPC, Morch M-D (1988) Stop making sense or regulation at the level of termination in eukaryotic protein synthesis. FEBS Lett 235:1–15

Van Bell CT (1985) 5S and 5.8S ribosomal RNA evolution in the suborder Tetrahymenina (Ciliophora: Hymenostomatida). J Mol Evol 22:231–236

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