

Phylogenetic Position of *Sorogena stoianovitchae* and Relationships within the Class Colpodea (Ciliophora) Based on SSU rDNA Sequences

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ABSTRACT. The ciliate *Sorogena stoianovitchae*, which can form a multicellular fruiting body, has been classified based upon its ultrastructure and morphology: the oral and somatic infraciliature of *S. stoianovitchae* most closely resemble those of members of the order Cyrtolophosidida in the class Colpodea. We characterized the small subunit ribosomal DNA (SSU rDNA) gene sequence from *S. stoianovitchae* and compared this sequence with those from representatives of all ciliate classes. These analyses placed *S. stoianovitchae* as either sister to members of the class Nassophorea or Colpodea. In an in-group analysis, including all SSU rDNA sequences from members of the classes Nassophorea and Colpodea and representatives of appropriate outgroups, *S. stoianovitchae* was always sister to *Platyophrya vorax* (class Colpodea, order Cyrtolophosidida). However, our analyses failed to support the monophyly of the class Colpodea. Instead, our data suggest that there are essentially three unresolved clades: (1) the class Nassophorea; (2) *Bresslaia vorax*, *Colpoda inflata*, *Pseudoplatyophrya nana*, and *Bursaria truncatella* (class Colpodea); and (3) *P. vorax* and *S. stoianovitchae* (class Colpodea).

Key Words. Bursariomorphida, ciliate phylogeny, Colpodida, Cyrtolophosidida, molecular systematics, Nassophorea, Sorogenida.

SOROGENA stoianovitchae is a unique ciliate that aggregates to produce an aerial fruiting body when cells are starved. During the unicellular stage, *S. stoianovitchae* feeds on ciliates, such as *Colpoda inflata* (Bradbury and Olive 1980; Olive 1978). Upon depleting the food supply, individual cells of *S. stoianovitchae* aggregate and form a fruiting body composed of a furrowed stalk and a sorocarp with encysted cells (Olive and Blanton 1980). The apical mouth and relatively simple longitudinal kineties of *S. stoianovitchae* were originally thought to resemble those of a gymnostome, possibly within the Haptorida (Olive 1978; Olive and Blanton 1980). However, analysis of somatic kinetid patterns using a combination of light and electron microscopy placed *S. stoianovitchae* within the class Colpodea (Bardele et al. 1991).

The sister class to the Colpodea is unclear. Analysis of ontogenetic, somatic, and oral ultrastructural data suggested that the Colpodea evolved from a haptorid or nassulid ancestor (Aescht et al. 1991; Foissner 1993). In contrast, independent analyses of stomatogenesis and kinetid features grouped the Prostomatea and Colpodea as sister classes (Hiller 1992, 1993). Previous molecular phylogenetic analyses including only a single colpodean, *C. inflata*, placed this taxon sister to the class Nassophorea (SSU rDNA: Bernhard et al. 1995; Hammerschmidt et al. 1996; Hirt et al. 1998; Wright et al. 1997; Wright and Lynn 1997b; large subunit (LSU) rDNA: Baroin Tourancheau et al. 1992), the class Oligohymenophorea (alpha tubulin: Baroin Tourancheau et al. 1998), or a clade containing both Oligohymenophorea and Nassophorea (histone H4: Bernhard and Schlegel 1998; LSU rDNA: Baroin Tourancheau et al. 1998). Analyses including two (Stechmann et al. 1998) and six (Lynn et al. 1999) colpodeans place these sequences sister to those of the Prostomatea. However, bootstrap support for interclass relationships in all analyses is low. Finally, the monophyly of the Colpodea is supported from analyses of SSU rDNA sequences from *C. inflata*, *Bresslaia vorax*, *Pseudoplatyophrya nana*, *Bryometopus sphagni*, *Bursaria truncatella*, and *Platyophrya vorax* (Lynn et al. 1999). Bootstrap support for the class Colpodea ranged from 61% estimated by maximum parsimony analysis to 92–98% estimated by distance methods (Lynn et al. 1999).

Our analyses of DNA polymorphisms at the SSU rDNA locus, using an inclusive sample of ciliates as well as an in-group sample of potential sister lineages to *S. stoianovitchae* (including all colpodeans used by Lynn et al. (1999) except for the

partial *B. sphagni* sequence), provide the first molecular hypothesis of the phylogenetic position of this organism. In addition, our results address (1) the relationships among the colpodeans, (2) the monophyly of the class Colpodea, (3) the sister taxon to the colpodeans, and (4) ordinal designations within the class.

MATERIALS AND METHODS

Sorogena stoianovitchae (ATCC #50031) were fed *C. inflata* and cultured according to protocols outlined by Olive (1978) and Olive and Blanton (1980). After several weeks in culture, 10 sorocarps were hand-picked for DNA analysis. DNA extractions were performed following standard protocols (Ausubel et al. 1993). We amplified the SSU rDNA genes using primers from Medlin et al. (1988). PCR products were cleaned with the Quiaquick PCR Purification system (Qiagen Inc, Valencia, CA, USA #28104), cloned into pAMP1 (Life Technologies Inc., Gaithersburg, MD, USA #19627–017) and transformed in JM109 competent cells (Promega Inc., Madison, WI, USA, #L2001). Miniprep (Promega #A1460) DNA was amplified using M13 primers and sequenced with gene-specific primers using the BigDye terminator kit (Perkin Elmer Co., Wellesley, MA, USA, #430315). Sequences were run on either an ABI 310 or ABI 377 automated sequencer. Contigs were edited in Seqman (DNASTar Inc., Madison, WI, USA). Multiple sequence alignments were generated by the Dedicated Comparative Sequence Editor (DCSE) software (De Rijk and De Wachter 1993) and adjusted by eye to account for SSU rDNA secondary structure. To assess the impact of alignment on analyses, an alignment was also generated by the ClustalW (Thompson et al. 1994) algorithm as implemented by Megalign (DNASTar Inc.).

Genealogies were constructed using several methodologies in order to assess the impact of different evolutionary models on relationships among sequences. Analyses were performed using maximum parsimony (MP), maximum likelihood (ML), neighbor-joining (NJ), and LogDet (LD) distance settings as implemented in PAUP* 4.0 (Swofford 1999). MP analyses used a heuristic search with ten random addition sequences and a 2/1 weighting of transversions to transitions to account for the relative frequencies of these events in the data. The parameters for the ML tree were estimated by the hierarchical likelihood ratio tests implemented in ModelTest 3.0 (Posada and Crandall 1998), which selected the TrN (Tamura and Nei 1993) model with invariant sites and gamma distribution shape parameters of 0.4270 and 0.4752, respectively. ML genealogies were generated with heuristic searches using these parameters, ten ran-

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dom addition sequences, and six rate categories to estimate among site variation. Neighbor-Joining (NJ) analyses used a Kimura two-parameter (K2P) correction, gamma distribution with $\alpha = 0.5$, and inverse squared objective weighting. Heuristic searches were also performed using LogDet (LD) distances to account for compositional biases. Bootstrap support was calculated using 100 replicates for each model.

RESULTS

We characterized four clones of SSU rDNA genes from *S. stoianovitchae* (GenBank: AF300285, AF300286, AF300287, AF300288) and observed between 0.233% and 0.292% differences among them using 1,714 base pairs unambiguously aligned by ClustalW. The average number of transitions to transversions between clones was four to 0.5. Additional sequences were from GenBank: *Anophyroides haemophila* U51554 (M.A. Ragan, unpubl. data), *Blepharisma americanum* M97909 (Greenwood et al. 1991), *Bresslaia vorax* AF060453 (Lynn et al. 1999), *Bursaria truncatella* U82204 (Stechmann, Schlegel, and Lynn 1998), *Caenomorphia uniserialis* U97108 (R. P. Hirt, P. L. Dyal, T. M. Embley, G. Esteban, and B. J. Finlay, unpubl. data), *Chilodonella uncinata* (consensus of AF300281, AF300282, AF300283, AF300284; Riley and Katz 2001), *Climacostomum virens* X65152 (Hammerschmidt et al. 1996), *Coleps hirtus* U97109 (R. P. Hirt, P. L. Dyal, T. M. Embley, G. Esteban, and B. J. Finlay, unpubl. data), *Coleps* sp. X76646 (Stechmann, Schlegel, and Lynn 1998), *Colpoda inflata* M97908 (Greenwood, Sogin, and Lynn 1991), *Didinium nasutum* U57771 (Wright and Lynn 1997a), *Diplodinium dentatum* U57764 (A.-D. Wright and D. Lynn, unpubl. data), *Discophrya collini* L26446 (Leipe et al. 1994), *Ephelota* sp. AF326357 (Riley and Katz 2001), *Epidinium caudatum* U57763 (Wright, Dehority, and Lynn 1997), *Eufolliculina uhligi* U47620 (Hammerschmidt et al. 1996), *Euplotes crassus* (consensus of AY007437, AY007438, AY007439, AY007440; Riley and Katz 2001), *Furgasonia blochmanni* X65150 (Bernhard et al. 1995), *Glaucoceria chattoni* X56533 (Greenwood, Sogin, and Lynn 1991), *Gruberia* sp. L31517 (Hirt et al. 1995), *Halteria grandinella* (consensus of AY007441, AY007442, AY007443, AY007444; Riley and Katz 2001), *Heliophrya erhardi* (consensus of AY007445, AY007446, AY007447, AY007448, AY007449; Riley and Katz 2001), *Isotricha intestinalis* U57770 (Wright and Lynn 1997a), *Loxodes magnus* L31519 (Hirt et al. 1995), *Loxodes striatus* U24248 (Hammerschmidt et al. 1996), *Metopus contortus* Z29516 (Hirt et al. 1995), *Metopus palaeformis* (consensus of AY007450, AY007451, AY007452, AY007453; Riley and Katz 2001), *Nyctotheroides desilerea* AF14535 (F.M. Affa'a and D.A. Hickey, unpubl. data), *Nyctotherus ovalis* (consensus of AY007454, AY007455, AY007456, AY007457; Riley and Katz 2001), *Obertruria georgiana* X65149 (Bernhard et al. 1995), *Ophryoglena catenula* U17355 (Wright and Lynn 1995), *Ophryoscolex purkynjei* U57768 (Wright and Lynn 1997b), *Oxytricha nova* X03948 (Elwood et al. 1985), *Paramecium tetraurelia* X03772 (Sogin and Elwood 1986), *Platyophrya vorax* AF060454 (Lynn et al. 1999), *Prorodon teres* X71140 (Bernhard et al. 1995), *Prorodon viridis* U97111 (R. P. Hirt, P. L. Dyal, T. M. Embley, G. Esteban, and B.J. Finlay, unpubl. data), *Pseudomicrothorax dubius* X65151 (Bernhard et al. 1995), *Pseudoplatyophrya nana* AF060452 (Lynn et al. 1999), *Spirostomum ambiguous* L31518 (Hirt et al. 1995), *Strombidium purpureum* U97112 (R. P. Hirt, P. L. Dyal, T. M. Embley, G. Esteban, and B. J. Finlay, unpubl. data), *Stylonychia lemnae* AF164124 (D. M. Prescott, E. A. Hewitt, L. F. Landweber, and V. Simon, unpubl. data), *Tetrahymena thermophila* X56165 (Sogin et al. 1986), *Tracheloraphis* sp. L31520 (Hirt et al.

1995), *Trithigmostoma steini* X71134 (Leipe et al. 1994). The alignment generated including all of these ciliates yielded 1,223 unambiguously aligned base pairs with no significant difference in compositional bias among sequences.

To determine the approximate phylogenetic position of *S. stoianovitchae*, we first included the *S. stoianovitchae* consensus sequence in a global alignment including up to five members of all ciliate classes, when available (see above). For outgroups, we included sequences from an apicomplexan and a dinoflagellate as analyses of both molecular (Lynn et al. 1999) and ultrastructural (Taylor 1999) characters indicate that these lineages are sister to the ciliates. These global analyses consistently placed the *S. stoianovitchae* sequence sister to either a colpodean or nassophorean sequence within a clade containing the classes Colpodea, Prostomatea, Oligohymenophorea, and Nassophorea (data not shown).

To accurately place the *S. stoianovitchae* SSU rDNA sequence, we regenerated alignments including representatives of the classes Colpodea, Prostomatea, Oligohymenophorea, and Nassophorea to yield 1,406 and 1,535 unambiguously aligned characters using DCSE and Clustal W algorithms, respectively. In all analyses of these alignments, *S. stoianovitchae* clustered with *P. vorax* with 100% bootstrap support (Fig. 1). In the DCSE alignment, the remaining members of the class Colpodea, *B. vorax*, *C. inflata*, *P. nana* (order Colpodida), and *B. truncatella* (order Bursariomorphida), also formed a monophyletic group (63%, 59%, 98%, and 99% for MP, ML, K2P, LD, respectively; Fig. 1).

No analysis of the DCSE alignment supported the monophyly of the class Colpodea. Moreover, no informative indels for the class Colpodea were observed in the excluded regions of the alignment. Instead, all genealogies grouped the clade containing *P. vorax* and *S. stoianovitchae* with the class Nassophorea, albeit with low bootstrap support (39%, 61%, 54%, and 29% for MP, ML, K2P, LD, respectively; Fig. 1). Similarly, only the LD genealogy generated from the Clustal W alignment supported the monophyly of the colpodeans with a bootstrap of 58% (data not shown).

Although support for relationships among colpodeans is only moderate (based on bootstrap support), our analyses provide evidence of the sister relationship between the classes Colpodea and Nassophorea (81%, 86%, 96%, and 79% bootstrap values for MP, ML, K2P, LD, respectively; Fig. 1). Analyses of the DCSE alignment also reconstructed the monophyly of the classes Oligohymenophorea and Nassophorea (Fig. 1). Only in the MP analysis did prostomatean sequences form a monophyletic group; all other topologies suggested a polyphyletic relationship among the prostomatean genera. However, bootstrap support is low at many deep nodes (Fig. 1) and additional taxa as well as molecular markers are necessary to better analyze these relationships.

DISCUSSION

Our phylogenetic analyses of SSU rDNA sequences strongly supported the sister relationship between *S. stoianovitchae* and *P. vorax*, consistent with morphological and ultrastructural data. Specifically, the oral and somatic infraciliature of *S. stoianovitchae* are almost identical to that of the cyrtolophosidid colpodeans, *Sagittaria* and *Platyophrya* (Bardele et al. 1991; Foissner 1993). Moreover, both *Sorogena* and the Cyrtolophosidida have parakinetal stomatogenesis and divide while free-swimming rather than as cysts (Bardele et al. 1991). In fact, the separation of *Sorogena* from the cyrtolophosidids at the ordinal level is largely due to *Sorogena*'s lack of one cyrtolophosidid's characteristic—the perinuclear membrane (Foissner 1993).

Our results are consistent with previous SSU rDNA analyses

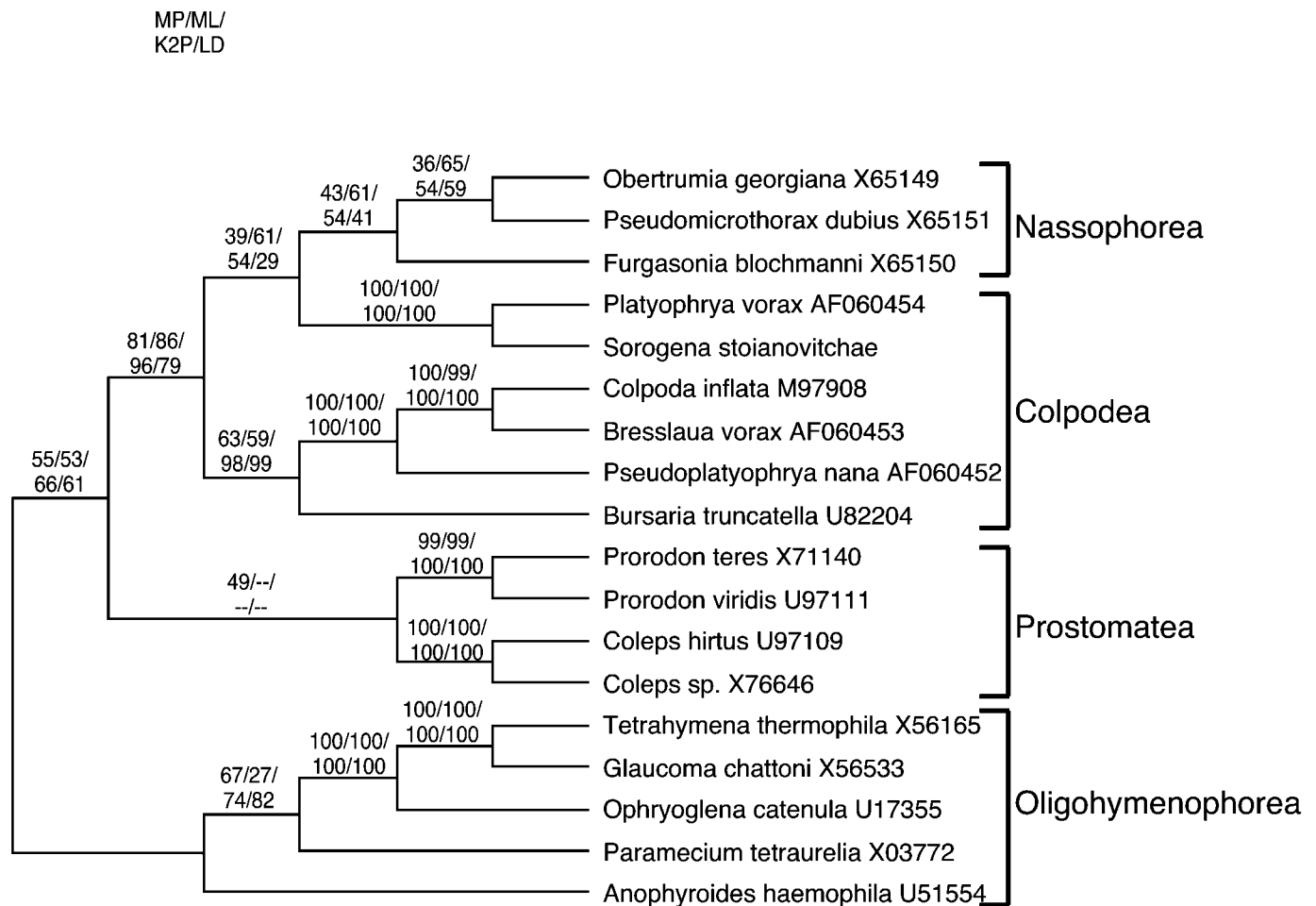


Fig. 1. Genealogy of small subunit rDNA sequences, which were aligned in DCSE to yield 1,406 unambiguously aligned characters, generated by MP analyses with transversions weighted 2/1 relative to transitions. The resulting genealogy is 1,075 steps long with a consistency index of 0.6288. Numbers on branches represent bootstrap support estimated by Maximum Parsimony, Maximum Likelihood, Kimura two-Parameter, and LogDet analyses, respectively. Branches that do not appear in analyses with other evolutionary models are indicated by dashes (-). The sequence for *Sorogena stoianovitchae* was a consensus of AF300285, AF300286, AF300287, and AF300288.

in showing that the order Colpodida is monophyletic (Fig. 1) and support the hypothesis that merotelokinetal stomatogenesis, division in cysts, and ciliary plaques in this lineage are derived characters (Lynn et al. 1999). However, in contrast to Lynn et al. (1999), only our LD analysis of one of our two alignments provided limited support for the monophyly of the class Colpodea. Instead of supporting the monophyly of this class, the low bootstrap support at relevant nodes of our genealogies suggest three unresolved clades: (1) the nassophoreans, (2) *Bresslaua vorax*, *Colpoda inflata*, *Pseudoplatyophrya nana*, and *Bursaria truncatella* (class Colpodea) and (3) *P. vorax* and *S. stoianovitchae* (class Colpodea). This is reflected by the high divergence between the Colpodida/Bursariomorphida and the *P. vorax*/*S. stoianovitchae* clades (average uncorrected pairwise distance = 5.2%) relative to the divergence between the colpodean clades and the nassophoreans (average uncorrected divergence between the Colpodida/Bursariomorphida and *P. vorax*/*S. stoianovitchae* clades and the nassophoreans is 6.2% and 5.7%, respectively).

Furthermore, our genealogies are discordant with those of Stechmann et al. (1998) and Lynn et al. (1999) in which the Prostomatea are sister to Colpodea. Instead, our analyses suggest that the classes Colpodea and Nassophorea are sister taxa

(Fig. 1), consistent with a hypothesis based on infraciliature in which the colpodeans evolved from a nassulid ancestor (Foisner 1993). These contradictory findings may be due to the different number of unambiguously aligned characters and/or taxa included in the analyses; both better sampling of ciliates and additional markers are needed to address the issue further.

Genetic divergence between taxa based on unambiguously aligned SSU rDNA sequences further questions the placement of *S. stoianovitchae* into its own order. The uncorrected pairwise distance between sequences from *S. stoianovitchae* (order Sorogenida) and *P. vorax* (order Cyrtolophosidida) is 1.7%, only slightly greater than the average value of divergence within the order Colpodida (1.5%). In contrast, the higher SSU rDNA divergence between the bursariomorphid, *B. truncatella*, and the colpodids is consistent with their ordinal designations (4.3%). This sequence divergence is correlated with morphological and behavioral differences: members of the order Bursariomorphida are the only colpodeans known to have sex and to replace their oral apparatus without cell division (Foisner 1993). Moreover, *Bursaria* lacks the ciliary plaques found in the colpodids and the perinuclear membrane found in virtually all cyrtolophosidids (Foisner 1993).

If the relatively high divergence between the Colpodida and

Bursariomorphida is indicative of ordinal level relationships, we must question if separating *Sorogena* from Cyrtolophosidida at the ordinal level is appropriate. Suppression of the order Sorogenida is also consistent with the fact that the perinuclear membrane, the major morphological difference between *S. stoianovitchae* and other cyrtophorids, may also be absent from other members of the order Cyrtolophosidida (Foissner 1993). It is more likely that the evolution of a multicellular fruiting body in *Sorogena* was a relatively recent innovation within the Cyrtolophosidida.

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