The Search Finds an End: Colpodidiids Belong to the Class Nassophorea (Ciliophora)

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ABSTRACT.   At its discovery in 1982, the ciliate genus *Colpodidium* was assigned to the Class Colpodea. Redescriptions of the type species *Colpodidium caudatum* caused the establishment of a new family (Colpodidiidae). Based on ontogenetic data, eventually a new order—Colpodidiida—was established and hypothesized to belong to the Class Nassophorea. Despite a remarkable increase in the number of colpodidiid species, no sequence data were available to confirm or reject either class assignment or to assess the phylogenetic validity of the Colpodidiidae and the Colpodidiida. We here retrieved and phylogenetically analyzed the SSrDNA sequences of *C. caudatum* from a Namibian soil and an as-yet undescribed colpodidiid ciliate from the Chobe River floodplain, Botswana. Bayesian inference methods and evolutionary distance analyses confirmed the assignment of these taxa to the class Nassophorea.

Key Words. Classification, Colpodea, Colpodidiida, Colpodidiidae, *Colpodidium*, Nassophorea, phylogeny, SSrDNA.

IN 1978, Wilbert investigated an undescribed ciliate from herbaceous soil-covering of a steppe in Ningerhar, Afghanistan. Four years later, the author (Wilbert 1982) described the new species as *Colpodidium caudatum* nov. gen., nov. spec. Based on live observation and silver impregnation, the new genus was assigned to the family Colpodidae Ehrenberg, 1838 (order Colpodida Puytorac et al., 1974, class Colpodea Small and Lynn, 1981). The main reasoning for this classification was the ciliate's somatic dikinetids and two ciliary fields in an inconspicuous vestibulum. However, Wilbert (1982) was unable to visualize the ciliate's silverline pattern and argued that its absence is a peculiarity of *C. caudatum*. Hence, as the specific colpodid silverline pattern is a diagnostic character for the order Colpodida (Foissner 1993), he failed to provide strong support for this classification.

Foissner (1990) re-investigated the type species, and identified not only somatic monokinetids accompanied by an argyrophilic granule and an alveolocyst, but also a tightly meshed, irregular silverline pattern (Klein's dry silver nitrate impregnation). Based on these observations, the idea was born that *C. caudatum* may belong to the class Nassophorea Small and Lynn, 1981. Specifically, Foissner (1990) assumed an affinity to the family Furgasoniidae Corliss, 1979, which found support in the location of the contractile vacuole and the cytopyge as well as in the silverline pattern, in the somatic monokinetids, and the paroral dikinetids. A redescription of *C. caudatum* isolated from tropical dry forest soil in Costa Rica (Foissner 1995) confirmed high similarities between this species and members of the family Furgasoniidae. However, as the organism showed some distinct apomorphies, Foissner (1995) established the novel family Colpodidiidae with the type genus *Colpodidium*. Eventually, Foissner, Agatha, and Berger (2002) erected the new order Colpodidiida (type family: Colpodidiidae) which, based on ontogenetic data, very likely belongs to the class Nassophorea (Foissner et al. 2002).

In the past few years, the small subunit ribosomal DNA (SSrDNA) has proved to be a helpful tool in elucidating the phylogenetic position of taxonomic groups in many kinds of organisms (Cracraft and Donoghue 2004), including protists (Berney and Pawlowski 2003; Hoppenrath and Leander 2006; Lopez-Garcia, Rodriguez-Valera, and Moreira 2002; Lynn et al. 1999; Stoeck, Foissner, and Lynn 2007). However, even though the family Colpodidiidae, which has been greatly enlarged since 1982 to now include nine described species, three genera, and four subgenera,

there are still no sequence data to confirm or reject the proposed assignment of the Colpodidiidae to the Nassophorea. Therefore, we set out to obtain the SSrDNA sequences of the type species, *C. caudatum*, and of a novel colpodidiid genus.

MATERIALS AND METHODS

Colpodidium caudatum was collected from soil of the Etosha Pan, Namibia (Foissner et al. 2002), and a novel colpodidiid species, probably a new genus, was discovered in soil from the Chobe River floodplain, Botswana. DNA was extracted, and full SSrDNA sequences were obtained as described previously (Stoeck et al. 2007). Sequence alignments and phylogenetic analyses, both Bayesian inference and evolutionary distance, were performed as outlined in Stoeck et al. (2007) using the programs ClustalX 2.0 (Thompson et al. 1997), MacClade 4.06 (Maddison and Maddison 2003), PAUP 4.0b10 (Swofford 2002) and MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The manually refined alignment consisted of 1,419 unambiguously aligned positions and is available upon request by the corresponding author. The evolutionary distance tree was calculated under ML criteria (GTR+I+G) with base frequencies, proportion of invariable sites, gamma distribution shape parameter, and a substitution model rate matrix determined as determined by Modeltest 3.7 (Posada and Crandall 1998). We assessed the relative stability of tree topologies using 1,000 bootstrap replicates. Heuristic searches for bootstrap analyses employed stepwise addition, starting trees with simple addition of sequences and followed by TBR branch-swapping. Settings for bootstrap analyses were chosen according to the Modeltest output as described above. For the Bayesian tree we ran two simultaneous, completely independent analyses starting from different random trees. The analysis also employed GTR+I+G as the DNA substitution model with the gamma distribution shape parameter, the proportion of invariable sites, base frequencies and a rate matrix for the substitution model as assessed by MrBayes. Metropolis coupling with 3 heated chains and one ''cold'' chain was employed to improve the Markov Chain Monte Carlo sampling of the target distribution. We ran 1,000,000 generations and sampled every 1,000th generation, resulting in 1,001 samples from the posterior probability distribution. From the GenBank data base, we chose SSrDNA sequences representing the eleven (ribo)classes recognized by Lynn and Small (2002). The two new colpodidiid sequences were deposited in the GenBank database and have the Accession No. EU264560 (*Colpodidium caudatum*) and EU264561 (undescribed Colpodidiidae nov. gen. nov. spec.).

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RESULTS AND DISCUSSION

The lengths and GC-contents of the PCR-amplified SSrDNA retrieved in this study are 1,748 nucleotides and 44% for *C. caudatum* and 1,766 nucleotides and 45% for the undescribed Colpodiidae nov. gen. nov. spec., respectively.

Our tree recovers the eleven ciliate (ribo)classes recognized by Lynn and Small (2002), most of which are fully supported in both the Bayesian and the evolutionary distance analyses (Fig. 1). The relationship between the classes is mostly unresolved (Lynn 2003), which may be due to insufficient resolving power of SSrDNA sequences and/or a fast radiation of ciliate evolution. Both phylogenetic analyses provided unambiguous support for placing the colpodidiids in the class Nassophorea. The bootstrap support in the evolutionary distance analysis and the posterior probability for the Bayesian analysis were 95% and 0.99, respectively (Fig. 1).
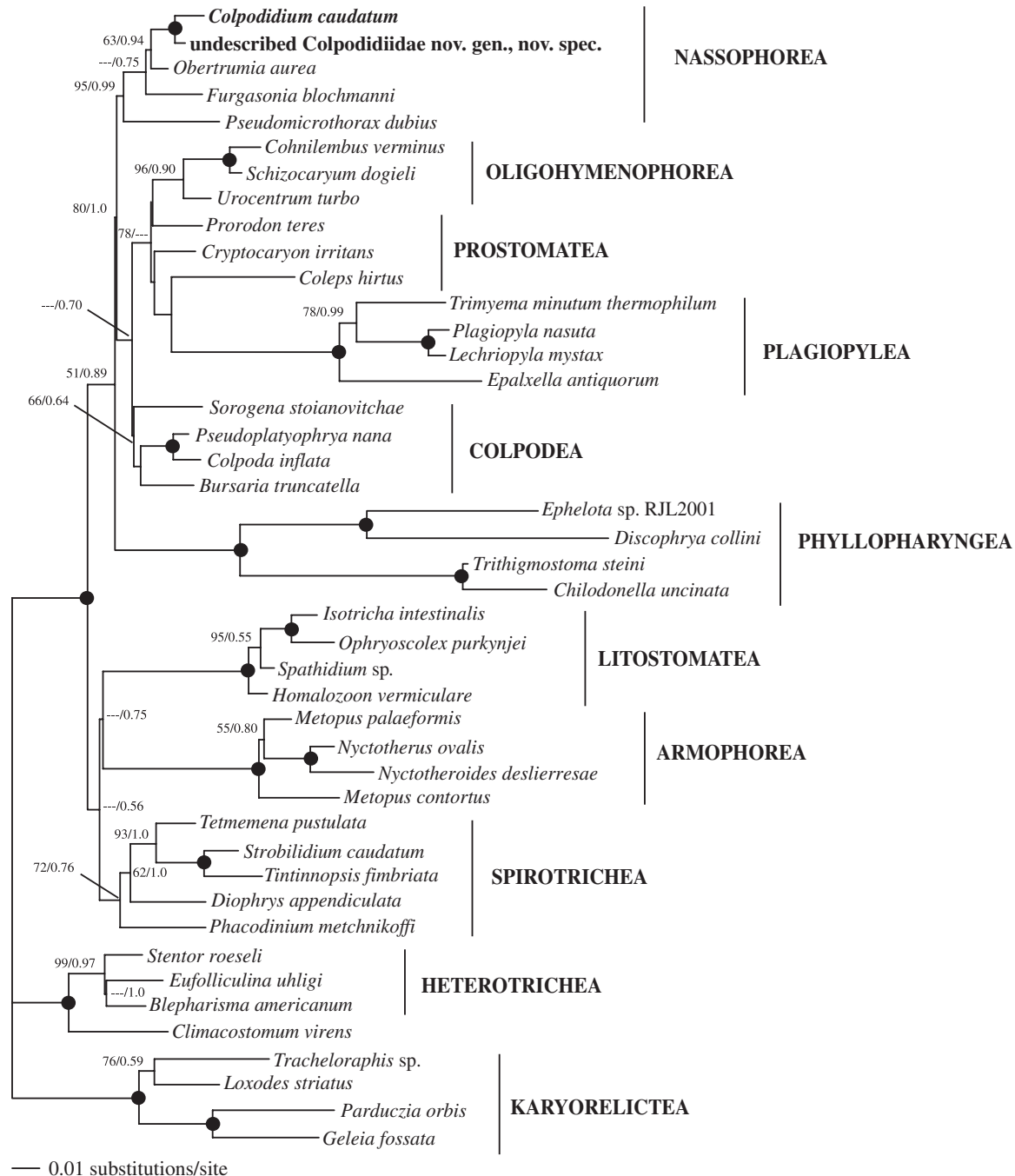

Fig. 1. Phylogenetic tree (evolutionary distance) based on small subunit rDNA sequences demonstrating the position of the colpodidiid ciliates *Colpodidium caudatum* Wilbert, 1982 and a novel, as yet undescribed Colpodidiidae (bold letters) among the 11 (ribo)classes of ciliated protozoa. The first number at the nodes is the bootstrap support based on 1,000 replicates. The second number shows posterior probabilities. Solid circles at nodes indicate full support in both analyses.

Even though ciliates belong to the best-studied protists, to date only four deposited SSrDNA sequences represent the class Nassophorea. Thus, at this time, it unfortunately is not possible to assess the phylogenetic validity of the order Colpodididiida Foissner, 2002 and the delineation of the family Colpodidiidae Foissner, 1995. Yet, based on the data available, we can make some speculation. Bootstrap and posterior probabilities provide full support for the relatedness of *C. caudatum* and the undescribed Colpodidiidae nov. gen., nov. spec. (Fig. 1), and both sequences have a primary structure similarity of 95.8%. At this point, no approximate benchmark values are available for the delineation of taxonomic entities within the ciliates. Yet, based on a sequence similarity of >95% it seems highly likely that both taxa may belong to the same family but different colpodidiid genera.

Once more, the necessity of an increased sequence sampling of described taxa becomes obvious; clearly, retrieving more SSrDNA sequences within the class Nassophorea is an essential task to elucidate phylogenetic affinities within this class. Nevertheless, regardless of the number of available nassophorean SSrDNA sequences, our phylogenetic analyses leave no doubt about the assignment of the Colpodidiidae Foissner, 1995 to the class Nassophorea.

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