

Phylogenetic relationships among free-living litostomateans in the light of 18S rRNA gene-ITS region sequences and secondary structure of the ITS2 molecule

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Interrelationships between four free-living litostomatean lineages were investigated, using 18S rRNA gene and ITS region sequences as well as the secondary structure of the ITS2 molecules. Our phylogenetic analyses confirmed the deep split of free-living litostomateans into Rhynchostomatia and Haptoria represented here by Haptorida, Pleurostomatida, and Spathidiida. This bifurcation is also corroborated by the signature of the rhynchostomatian and haptorian ITS2 molecules. Specifically, the consensus stems of helices II and III are longer by one base pair in Rhynchostomatia, while the terminal loops of both helices are longer by one or two nucleotide/-s in Haptoria. A close relationship of Pleurostomatida and Haptorida is favored by quartet likelihood-mapping and supported by a 5'-AG vs. CU-3' motif in the variable part of helix II and by two morphological apomorphies, i.e., meridionally extending somatic kineties and a non-three-rowed dorsal brush. Although monophyletic origin of Spathidiida is poorly supported in phylogenetic trees, the unique motif 5'-GA vs. UC-3' present in the consensus helix II stem could be an important molecular synapomorphy of spathidiids, apart from the ancestrally anteriorly curved somatic kineties and the three-rowed dorsal brush. The peculiar family Pseudoholophryidae has very likely found its phylogenetic home among spathidiids, as an early branching lineage. Utilization of the 18S rRNA gene along with the ITS region was thus revealed to be beneficial for phylogenetic inferences in ciliates. Moreover, the conservative parts of helix II and III of the ITS2 molecule might serve as a good proxy for reconstruction of not only recent but also deep evolutionary events. Finally, it was shown that molecular phylogenetic analyses of the 18S rRNA gene and the ITS region harmonize well with assumptions based on the structural conservatism hypothesis, postulating that somatic ciliary structures are phylogenetically more informative than oral patterns.