

MORPHOLOGICAL & PHYLOGENETIC ANALYSIS OF TWO
SPECIES OF HETEROKONT ALAGE

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ABSTRACT

The freshwater heterokont algae *Anthophysa vegetans* and *Botrydiopsis pyrenoidosa* (strain SAG 31.83) were reexamined in the light microscope and their phylogenetic positions were investigated using DNA sequence analyses. Although differences in numbers of cells per colony and cell sizes were observed, these morphological data are consistent with previously published information for both species. In particular, the highly multinucleate nature of *B. pyrenoidosa* cells was confirmed by fluorescence microscopy using the DNA-binding fluorochrome DAPI. Parsimony and maximum likelihood analyses of nuclear small subunit ribosomal RNA (18S rRNA) gene sequences confirm placement of the colorless, colonial flagellate *A. vegetans* within the Chrysophyceae and imply that *A. vegetans* is most closely related to *Poterioochromonas* spp. and *Ochromonas sphaerocystis*. Evidence is presented for four independent losses of photosynthetic capacity within the Chrysophyceae. *Botrydiopsis pyrenoidosa* was first described by Treknwalder, classified in the Xanthophyceae, and deposited in the Sammlung von Algenkulturen (strain SAG 31.83). Analyses of 18S rRNA gene sequence(s) derived from an authentic isolate indicate that *B. pyrenoidosa* is not closely related to *Botrydiopsis intercedens* and probably does not belong in the Xanthophyceae. The alga cannot be confidently placed in any known class of heterokont algae and a new genus, *Polykaryon*, is erected to include *P. pyrenoidosum* (Treknwalder) Bailey *et* Misner. *Polykaryon* is treated as *incertae sedis* within the heterokont algal lineage. In a further investigation, coccoid vegetative cells of the heterokont alga *Polykaryon pyrenoidosum* (Treknwalder) Bailey *et* Misner were examined by transmission electron microscopy. All cells are characterized by the presence of cell walls (80-740 nm) with an

extracellular ‘fringe’ and most contain multiple nuclei that are spherical or elongate. One Golgi apparatus is present in each cell and this organelle is always associated with a nucleus. *P. pyrenidosum* cells contain multiple chloroplasts lacking eyespots that have bulging pyrenoids not traversed by thylakoids, photosynthetic membranes in groups of three, and girdle lamellae. Flagellar hairs (FHs) *ca.* 16 nm in cross sectional diameter are found within the endomembrane system of many vegetative cells and vacuoles containing FHs are observed in the cytosol and associated with chloroplasts and nuclei. Although the taxonomic affinities of the species remains obscure, the combination of ultrastructural features observed in this study serves to exclude the alga from many classes of heterokont algae. Although very unlikely, our results do not rule out the possibility that the species is an unusual member of the Chrysophyceae. Total evidence implies that *P. pyrenidosum* is most similar to species placed in the Phaeothamniophyceae and Xanthophyceae.

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DEDICATION

This thesis is dedicated to _____ (your name here) _____.

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