

**Phylogenetic analysis of the “*Nannochloris*-like” algae and diagnoses of *Picochlorum*
oklahomensis gen. et sp. nov. (Trebouxiophyceae, Chlorophyta)**

William J. Henley, Janice L. Hironaka, Laure Guillou, Mark A. Buchheim, Julie A. Buchheim,
Marvin W. Fawley and Karen P. Fawley

A broadly halotolerant new isolate of a small asexual coccoid chlorophyte and six new, related freshwater isolates provided the impetus for a phylogenetic analysis of the so-called “*Nannochloris*-like” algae within the Trebouxiophyceae. Previous taxonomic disagreements concerning this group had not been rigorously tested with molecular phylogenetic analyses. We show with 18S rDNA sequence phylogeny that nineteen of twenty-two isolates previously assigned to either *Nannochloris* or *Nanochlorum* fall within a diverse sister clade to a clade including the four “true” *Chlorella* species *sensu lato*. In addition, *Marvania geminata*, *Gloeotila contorta*, *Chlorella* sp. Yanaqocha RA1, *Koliella spiculiformis*, “*Chlorella minutissima*” C-1.1.9, and new *Koliella*, *Gloeotila* and *Marvania* isolates were included in the “*Nannochloris*-like” clade. Distinct freshwater and marine/saline lineages comprise at least three major subclades, generally corresponding to cell division pattern. Seven of fourteen marine/saline isolates are known (and others presumed) to divide by autospore formation. Eight freshwater isolates divide by binary fission, including two *Koliella*, two *Gloeotila*, *N. bacillaris*, *Chlorella* sp. Yanaqocha RA1, and two new unassigned isolates. Four freshwater isolates divide by budding or autospore formation (three *Marvania*, including CCAP 251/1b, previously assigned to *N. coccoides*). The autospore taxa *N. eucaryotum* UTEX 2502 (marine) and “*C. minutissima*” C-1.1.9 (freshwater), which have nearly identical 18S rDNA sequences, are deeper-branching than the freshwater and marine/saline lineage. We propose including the thirteen marine/saline, autospore taxa (excluding *N. eucaryotum* UTEX 2502) in the genus *Picochlorum* until distinctive morphological or biochemical characters are identified that would indicate multiple genera corresponding to subclades. Such characters exist in the freshwater lineages, supporting retention of *Koliella*, *Gloeotila*, *Marvania*, and *Nannochloris* as distinct genera, although each is currently represented by few isolates. *Nannochloris* at this time may be restricted to *N. bacillaris* and *C. sp.* Yanaqocha RA1. We also describe halotolerant *Picochlorum oklahomensis* Hironaka sp. nov. Based on 18S rDNA sequence and lack of chlorophyll *b.*, *N. sp.* UTEX 2379 should be reassigned to the Eustigmatophyceae.

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