

# Novel insights into Ploetids, a non-monophyletic group of phagotrophic euglenids that are pivotal in understanding euglenid evolution

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Euglenids are a ubiquitous group of flagellates that includes phototrophs, osmotrophs and phagotrophs. The phylogenetics of phototrophic euglenids (and to lesser extent osmotrophs) is relatively well developed, but our basic understanding of deep euglenid evolution is severely hampered by a fragmentary knowledge of the biodiversity of phagotrophic euglenids, and their inter-relationships. The phagotrophic group of “ploetids” (including *Ploetia* and other associated genera) may be pivotal to understanding these deep relationships, since the scant molecular data available suggest that they gave rise to all other extant euglenid groups. Ploetids are rigid, biflagellated cells with around 10 pellicle strips that mostly feed on bacteria, although observations of eukaryovory exist. Using a combined culturing and single-cell approach, we acquired more than 35 novel SSU-rDNA sequences, linked with light microscopy imagery, capturing a large proportion of ploetid morphological diversity. Phylogenetic analyses show that “ploetids” are composed of even more distinct clades than previously recognised, and their likely relationships frequently conflict with current classification schemes, or the assumptions these are based on: e.g. *Ploetia vitrea* (the type species of *Ploetia*), is closely related to *Ploetia/Serpenomonas costata* (and to *P. oblonga*), but far removed from “*Ploetia* cf. *vitrea*”. Scanning electron microscopy data shows that the pellicle structure is different between those taxa, and a new genus and species for “*Ploetia* cf. *vitrea*” will be proposed. In addition we generated light microscopy and SSU-sequence data from several cells of *Anisonema trepidum* and *A. glaciale*. Our phylogenetic analyses show that these taxa are not closely related to other *Anisonema* strains at all, and clearly fall outside the Spirocuta clade among the “ploetids”; we will propose novel genus-level assignments for these forms. This work substantially clarifies the identities of the major clades within ploetids, and thus frames the questions to be resolved by future multigene phylogenetic studies.