

Title:

The planktonic freshwater ciliate *Balanion planctonicum* (Ciliophora, Prostomatea): a cryptic species complex or a 'complex species'?

Authors:

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Abstract (300 words maximum): :

Balanion planctonicum is a common, temporarily highly abundant ciliate species found worldwide in temperate lakes and ponds. In Lake Zurich, the tiny ciliate is omnipresent and particularly common during spring and early summer. Abundances of thousands of cells per liter are usually detected by morphology-based methods, e.g., by quantitative Protargol staining (QPS). In contrast, it seems difficult to detect this ciliate by molecular methods, as shown by results from a CARD-FISH approach (catalyzed reporter deposition fluorescence in situ hybridization) using the 18S rDNA gene sequence. This hinted at variability within the 18S rDNA gene sequence of *B. planctonicum* from Lake Zurich.

To shed more light on this topic, we isolated single cells of *B. planctonicum* from enrichment cultures, suspended with *Cryptomonas* sp. and flagellates, and investigated the genotypic and phenotypic variability as well as the phylogenetic position within the class Prostomatea. Using Sanger sequencing, we discovered four different *B. planctonicum* haplotypes with variants in the 18S rDNA sequence and in the ITS region. In the phylogenetic tree, all haplotypes clustered together with *B. masanensis* and build their own *Balanion* cluster, with the genus *Askenasia* as a potential sister group. Morphological comparisons via live measurements and qualitative Protargol staining demonstrated a slight difference in cell size between the haplotypes. However, ciliates have a high number of 18S rDNA copies due to their nuclear dualism. To estimate if sequence variation is unique for a clonal *B. planctonicum* culture or if a clone harbors all four sequence variations but with different manifestations, we sequenced full length 18S rDNA sequences using nanopore technology. The different haplotypes had no overlapping intra-clonal polymorphisms, providing evidence for a cryptic diversity of the species *B. planctonicum*. Additionally, CARD-FISH analyses indicated that the diversity of *B. planctonicum* is even more extensive with still unknown distinct clades.