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Multigene Evidence for the Placement of a Heterotrophic Amoeboid Lineage *Leukarachnion* sp. among Photosynthetic Stramenopiles

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The colorless amoeboid eukaryote genus *Leukarachnion* represents one of a long list of microbial lineages for which there have been few taxonomic studies. In this study, we analyze molecular data to assess the placement of a species of *Leukarachnion* on the eukaryotic tree of life and we report fine structural data to provide additional information on the identity of this taxon. Our multigene analyses indicate that *Leukarachnion* sp. (ATCC® PRA-24) is a member of the stramenopiles, sister to the Chrysophyceae/Synurophyceae clade. It also forms a sister group relationship to the clade containing *Chlamydomyxa labyrinthuloides* and *Synchroma grande*, both of which are characterized by net-like amoeboid phases. *Leukarachnion* sp. and *Chlamydomyxa labyrinthuloides* also share fine structural cyst morphology such as bilayered structure of the cyst wall. The amoeboid form and heterotrophic habit of *Leukarachnion* sp. highlight the multiple origins of diverse body forms and multiple plastid losses within the stramenopiles.

Key words: stramenopiles; *Leukarachnion*; multigene analysis; heterotrophic; SSU-rDNA; phylogeny; Heterokontophyta; Chrysophyceae; Synurophyceae; amoeboid.

Introduction

The colorless amoeboid eukaryote *Leukarachnion* Geitler is a large multinucleate amoeba with branching 'net-like' pseudopodia (Geitler 1942). *Leukarachnion* grows to several millimeters in size, forming anastomosing networks (Geitler 1942). The genus *Leukarachnion* has one described species, *Leukarachnion batrachospermi*, which was originally found in close association with the freshwater red alga, *Batrachospermum boryanum* (Geitler 1942).

The taxonomic affinity of *Leukarachnion* is uncertain partly due to lack of detailed morphological data (Geitler 1942; Patterson 2002). *Leukarachnion* has been superficially compared to other large plasmodial amoebae such as *Thallasomyxa*, *Stereomyxa*, and *Cenetidomyxa* (Geitler 1942; Patterson 2002) as well as to lineages that create net-like structures such as *Chrysarachnion*

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insidians (Geitler 1942). In this study we characterize multiple gene sequences from the *Leukarachnion* sp. obtained from American Type Culture Collection (ATCC® PRA-24) and present fine structural analyses to enhance the identification of this strain.

To assess the phylogenetic position of *Leukarachnion* sp., we analyze three different data sets that include up to four nuclear encoded genes: SSU-rDNA, actin, alpha- and beta-tubulin. Our preliminary multigene analysis, which included a broad sampling of representative eukaryotes, placed *Leukarachnion* sp. ATCC® PRA-24 within the stramenopiles, a diverse assemblage of organisms that include a wide array of forms, many of which are photosynthetic. We assess the support for the placement of *Leukarachnion* sp. within the stramenopiles and investigate its relationship with other members of this clade.

Stramenopiles form one of the robust eukaryotic groups that have been recovered consistently in a number of molecular studies (Ben Ali et al. 2002; Bhattacharya et al. 1992; Cavalier-Smith et al. 1994; Cavalier-Smith and Chao 2006; Leipe et al. 1994; Van de Peer and De Wachter 1997; Van der Auwera and De Wachter 1997; Yoon et al. 2008) and share an ultrastructural synapomorphy, tripartite tubular hairs attached to one of their flagella (Patterson 1989). Inclusion of the colorless amoeboid *Leukarachnion* sp. within this clade enhances the diversity of body forms among stramenopiles and suggests that additional amoeboid lineages of stramenopiles await further study.

Results

Light Microscopic Observations

Leukarachnion sp. ATCC® PRA-24 formed a large delicate network with enlarged cell bodies (2—10 μm) and fine cytoplasmic extensions (Fig. 1A). The network was dynamic as ingested bacteria and other cytoplasmic contents moved through the thin strands of the network to the core cell bodies (Fig. 1A). When the network was disturbed it rolled back to form blobby cells, reminiscent of how cysts form during unfavorable conditions. Younger cultures contained cysts of varying sizes, ranging from 5 to 15 μm (Fig. 1B), and as it aged the cysts became smaller in size until the culture was filled with uniform 5 μm cysts. A flagellate was observed in the first culture worked on (used to generate tube 1 DNA) but not in any subsequent cultures (used to generate tubes 2 and 3 DNA). We did not have an opportunity to observe the flagellate in close detail within the first culture so the morphology of the flagella was not determined. Future examination is required to elucidate additional details of the life cycle of *Leukarachnion* sp. ATCC® PRA-24.

TEM Observations

The overall fine structure of *Leukarachnion* cells (Fig. 2A, B) exhibited a finely granular, relatively hyaline, cytoplasm surrounded by an uncoated plasma membrane. Slender pseudopodial projections were commonly observed projecting from

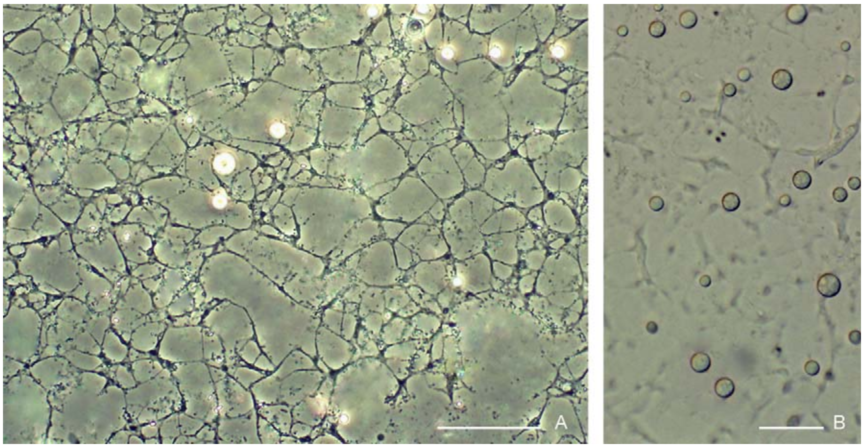

Figure 1. Light micrograph of *Leukarachnion* sp. ATCC®-PRA-24. **A**. Image of a living organism showing an extensively branched network. Scale bar = 50 μm. **B**. Cysts at different maturation stage. Mature cysts are smaller in size. Scale bar = 25 μm.