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Diversity and Evolution of Microbial Eukaryotes
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Algal identification: *Mayamaea sp.*

Introduction

The course Diversity and Evolution of Microbial Eukaryotes highlights the outstanding diversity of protists and fungi. These organisms can be found in various environments and play key roles in countless ecosystems. This study gives a glimpse of the existing algal diversity in freshwater habitats. Here, an algal specimen was isolated from a water sample and cultured in laboratory conditions. Genetic analysis permitted the identification of the organism at the genus level and the description of its phylogeny.

Sampling

On 7 November 2024, a water sample was collected from a puddle on a walking path at the foot of the Håga mound (Hågahögen, also known as Kung Björns hög) in Uppsala, Sweden (Figure 1). More precisely, the sampling was done at location 59.8373042, 17.5862199. The puddle water was collected after slightly disturbing the sediment at the bottom.

Figure 1: Sampling location.

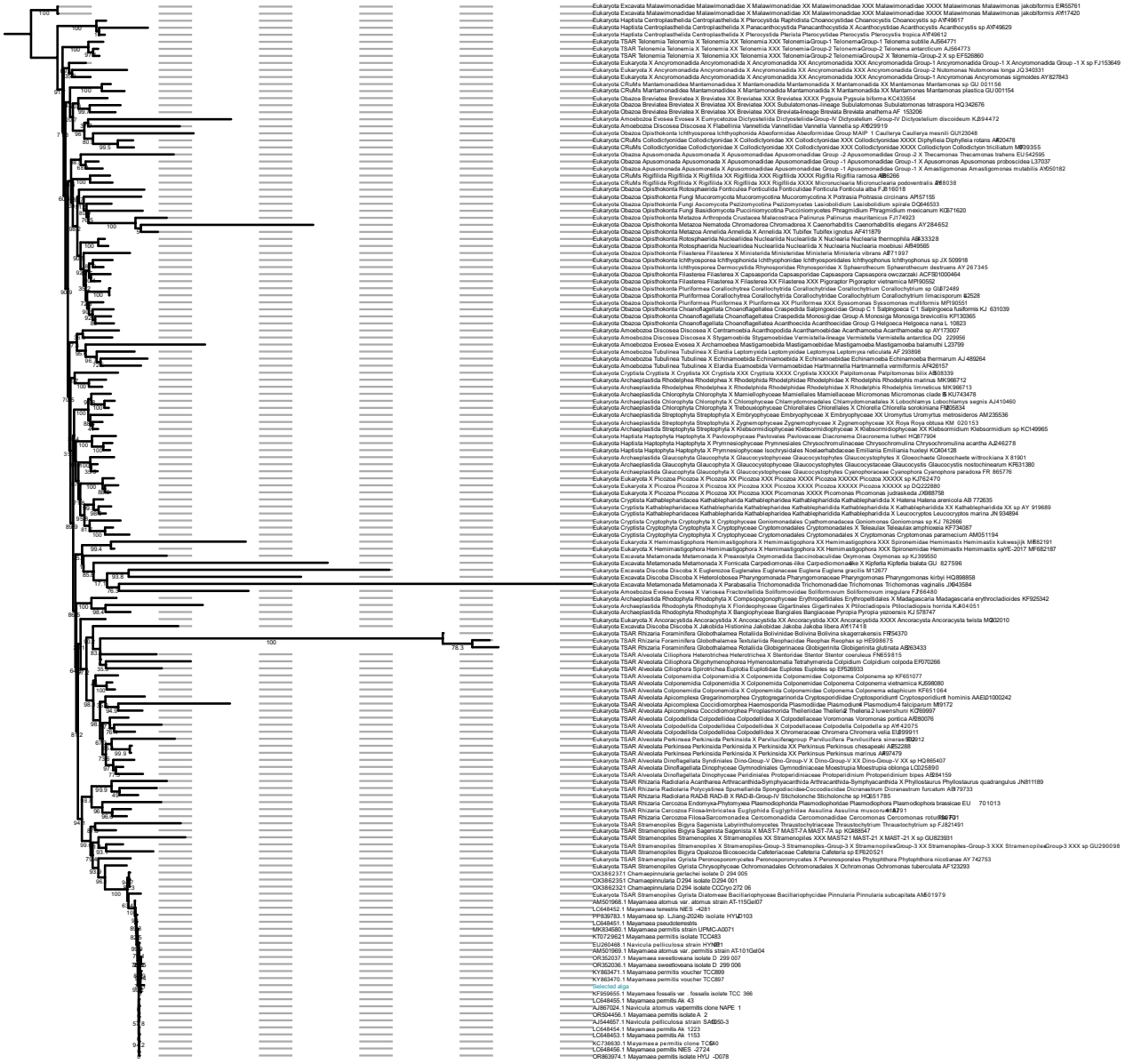
Culturing

Before inoculation into the culture media, the water sample was allowed to settle. 1 mL of sample was then taken from different levels of the sampling tube and pipetted into each well in the first column of a 48-well plate. Two types of diluted culture media were selected: MWC (generic algal medium) and Z8 (suitable for golden algae). 0.9 mL of MWC media was pipetted into the remaining wells in the first three rows of the well plate. 0.9 mL of Z8 media was pipetted into the remaining wells in the last three rows. 0.1 mL of the sample from the first well in each row was then pipetted into the next well in the same row. This step was repeated until all of the wells were inoculated, resulting in a dilution series. The cultures were incubated under a 12-hour light cycle at 18°C. After three weeks, a pure culture established from a 1:100000 dilution in MWC media was selected for further study. DNA extraction from the algal cells was performed by rapidly freezing them in liquid nitrogen several times. The sample was then prepared for PCR. The 3NDf (forward) and V4_euk_R1 (reverse) primers were used: they amplify the V4 region of the small-subunit ribosomal RNA gene common to all eukaryotes. Gel electrophoresis of the PCR product showed that the PCR was successful. The PCR product was then purified using ExoSap and prepared for sequencing.

Bioinformatic analysis

After sequencing, the resulting chromatograms were examined using SnapGene. The forward and reverse sequences were trimmed and aligned. The consensus sequence was then input into BLAST to search for similar database entries, excluding “Uncultured/environmental sample sequences”. The isolated algal sample had a 97.70% similarity to several species in the genus *Mayamaea*. 25 sequences with the highest

level of similarity were downloaded and aligned with sequences from a reference data set and the consensus sequence. The alignment was completed with the help of MAFFT software. IQTREE was then used to generate a phylogenetic tree, which was visualized using iTOL. The tree was rerooted with Malawimonadida as the outgroup. The phylogeny suggests that the selected alga is closely related to species in the genera *Mayamaea* and *Navicula* and belongs to the genus *Mayamaea* (Figure 2).



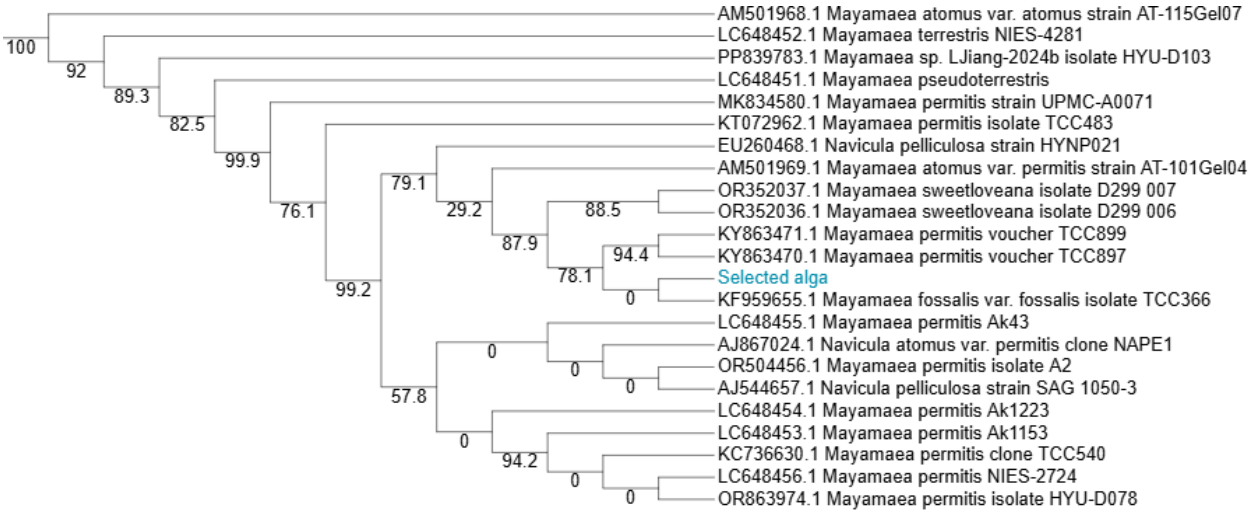


Figure 2: Phylogenetic tree and its close-up containing the selected alga (marked in blue). The tree is based on 25 highly similar sequences obtained from BLAST and 124 sequences from a reference data set.

Description

The genus *Mayamaea* belongs to the stramenopile clade in the SAR supergroup. Members of the genus are diatoms (Vijver & Cox, 2013). The isolated *Mayamaea sp.* is a non-motile, oval-shaped protist and approximately 10µm in length. In line with the characteristics of the selected specimens, which were isolated from puddle water, members of *Mayamaea* are often abundant in ephemeral habitats (Spaulding et al., 2021). The isolated algae are thus likely to be tolerant to high levels of pollution, which is confirmed by existing literature (Spaulding et al., 2021).

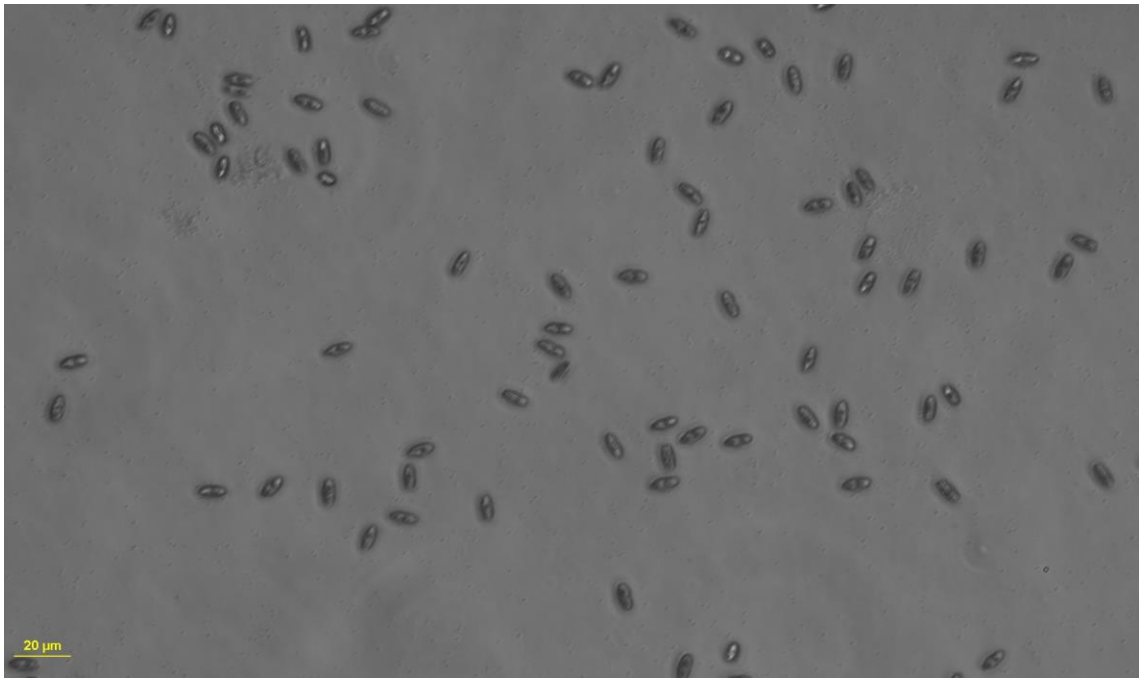


Figure 3: Pure culture of the selected algal specimen.

References

Spaulding, S., Edlund, M. (2008). Mayamaea. Diatoms.org. https://diatoms.org/genera/mayamaea

Vijver, B. V. D., & Cox, E. J. (2013). New and Interesting Small-Celled Naviculoid Diatoms (Bacillariophyceae) from a Lava Tube Cave on Île Amsterdam (TAAF, Southern Indian Ocean). *Cryptogamie, Algologie*, *34*(1), 37–47. https://doi.org/10.7872/crya.v34.iss1.2013.37