Andreas Andersson
Diversity and Evolution of Microbial Eukaryotes
HT2024

UPPSALA UNIVERSITET

An Aggregated Algae á Flogsta - *Synura sp.*

Summary
In this course, environmental samples were taken of both fungi and algae. This report will follow the algae. The algae samples were diluted in a well plate and studied under the microscope. Following PCR, the samples were sent for sequencing and the sequenced genomes were used to identify the species and build a phylogenetic tree.

Collecting the sample

On the 12th of november 2024 at 09:59, a water sample was taken from Ekebydammen. The exact location is 59.845738°N, 17.599607°E in Ekeby, Uppsala. The water sample was taken by lowering a 50 mL falcon tube into the surface and letting it fill up.

Figure 1: Ekebydammen, where the algae sample was taken.

Isolating a single species from the sample

The algae sample was cultivated in two different media, MWC and Z8. The sample was diluted into a 48-well plate (6*8). The top three rows were using MWC as medium and the bottom three rows used Z8. The dilution series were made with a 1:10 dilution, resulting in the last well having a dilution of $1.0*10^{-7}$. After a week of incubation the cultures in the wells were observed under a light microscope. A well with a pure culture was chosen for DNA extraction and PCR. The algae in this well was also photographed. To extract the DNA, 2µL of the chosen colony was pipetted into a PCR tube and exposed to three rounds of cold shock, using liquid nitrogen. GoTaq, primers and nuclease free water was then added to the PCR

tube. For algae, the primers 3NDf and V4 euk R1 were used. These primers amplify the v4 region on the small-subunit ribosomal RNA gene, which is a general eukaryotic marker. After running the PCR, Agarose gel electrophoresis was done to ensure a clean band and successful PCR. The sample was then sent for gene sequencing.

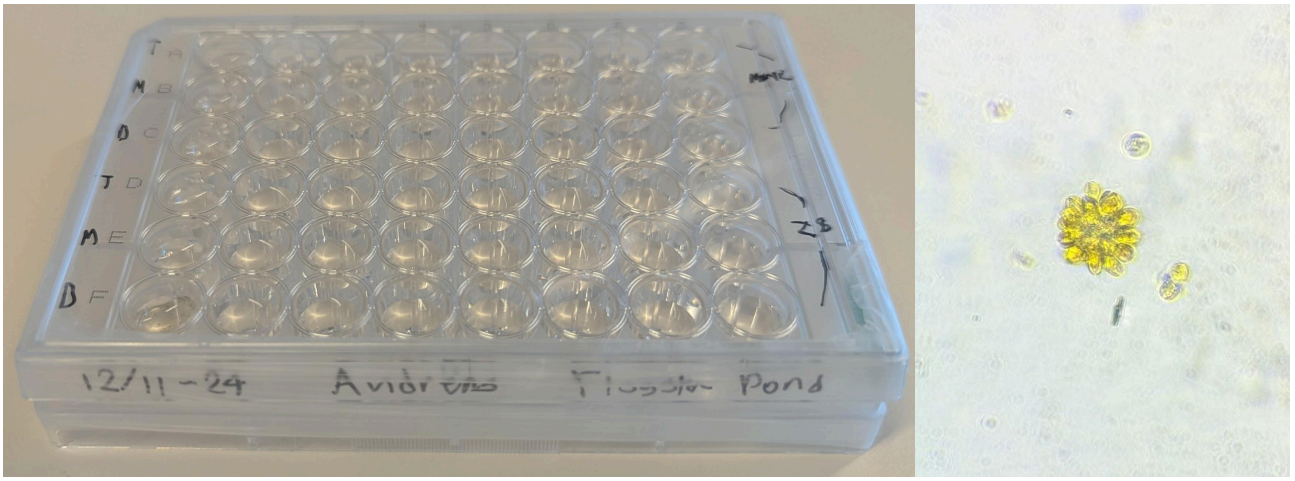

Figure 2: Left: The 48-well plate with MWC and Z8 medium. 1:10 dilution for every column. Right: A microscopic image of the chosen algae.

Identifying the algae

When the sequences came back, they were analyzed as a chromatogram in SnapGene. Bad ends were cut and the forward and reverse sequence were aligned to create a reliable sequence which was then used with BLAST. The species with the most identical sequence were *Synura truttae*, being 99% identical. The top 25 matches were then downloaded as a .FASTA file and aligned using MAFFT. A new .FASTA file was downloaded from MAFFT and put into AliView to see that all the sequences had aligned correctly. The .FASTA file was uploaded to IQTREE to generate a phylogenetic tree. The tree was later visualized with iTOL. The species belongs to the genus *Synura*. In the phylogenetic tree below it is labeled as *Synura sp.* As the highest percent identity was lower than 100% (99), the sample algae will not be recognized as a specific species but instead a part of the genus *Synura*. On the next page the phylogenetic tree is shown and the sample algae is labelled in red.

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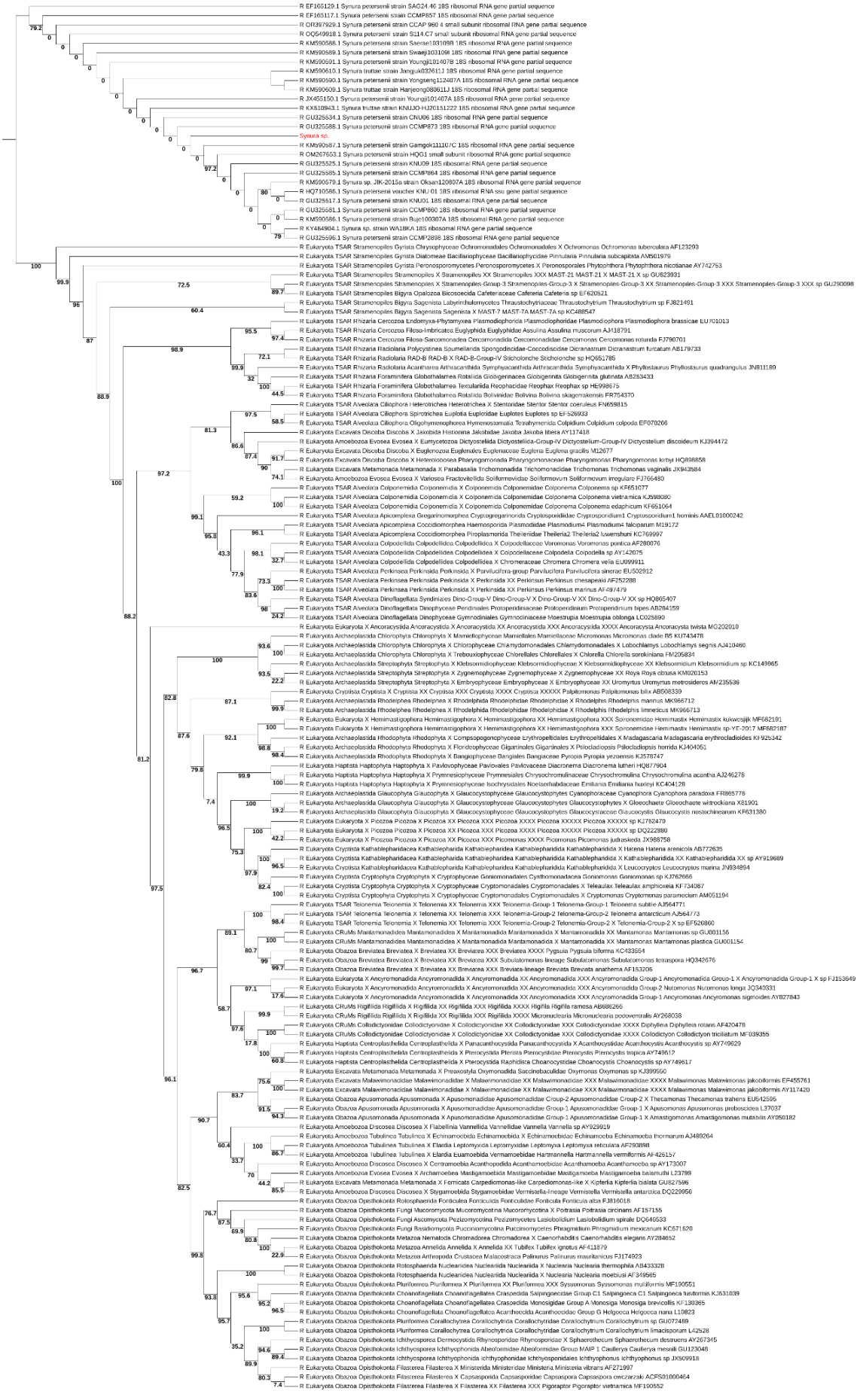
(Below) Figure 3: A phylogenetic tree of large eukaryotic reference groups, the top 25 identity matches from BLAST and the algae sample species, labelled in red. The numbers are showing the probability of relation.



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The genus Synura

The species in the genus Synura are motile flagellated algae. They usually form colonies. The colonies of Synura are usually round, but bigger colonies with more cells tend to get more club-shaped. The colonies vary in sizes, but can be anywhere from 20µm - 400µm. They have a distinct golden colour and the individual cells are coated in silica scales. They are a heterokont euplanktonic algae and exist mostly in freshwater environments, most commonly found in ponds and lakes.

References

1. Aaron A, Vogan J, Mahwash, Javier F G. (2024). *Diversity and Evolution of Microbial Eukaryotes - 1BG235 Lab 1 - Enviromental Isolation.* Uppsala University
2. De Hoog, G.S., *Geotrichum and Its Teleomorphs*. Available at: https://www.sciencedirect.com/science/article/pii/B9780127415505500155 (Accessed: 18 June 2024)