Algae Isolation – *Gomphonema sp.*

Diversity and Evolution of Microbial Eukaryotes
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Introduction

The aim of this report is to an algal species through the use of isolation, DNA extraction and sequencing. A phylogenetic tree was constructed with the sequencing result and the isolate was tried to be identified. The isolate is part of the genus *Gomphonema*, a genus containing diatoms.

Sample collection

The sample was collected on the twelfth of November 2024. The sample was taken from a water tank found in the tropical greenhouse of botaniska trädgården, Villavägen 6-8, 752 36 Uppsala (59.850503°N, 17.627763°E). The sample consists of water with some small water plants. The exact water tank used for sample collection can be seen in figure 1.

Figure 1: Water tank used for sample collection: Various waterplants can be found within, as well as some fish and frogs.

Methods

Isolation:

Enrichment cultures for the algae were grown on Z8 and MWC. A dilution to extinction method was used in order to obtain pure cultures. This dilution series was made in a 48-well plate. In the first column, 1 ml of the sample was pipetted in every row. Row A & D received 1 ml from the top of the tube, row B & E from the middle of the tube and row C & F from the bottom. In the following columns a 10-fold dilution was made with the use of either MWC (row A-C) or Z8 (row D-F) as medium. The 10 fold dilution was done in every column, resulting in a dilution series as can be seen in figure 2. Cultures were incubated at 18 ◦C, under a 12 hour light cycle.

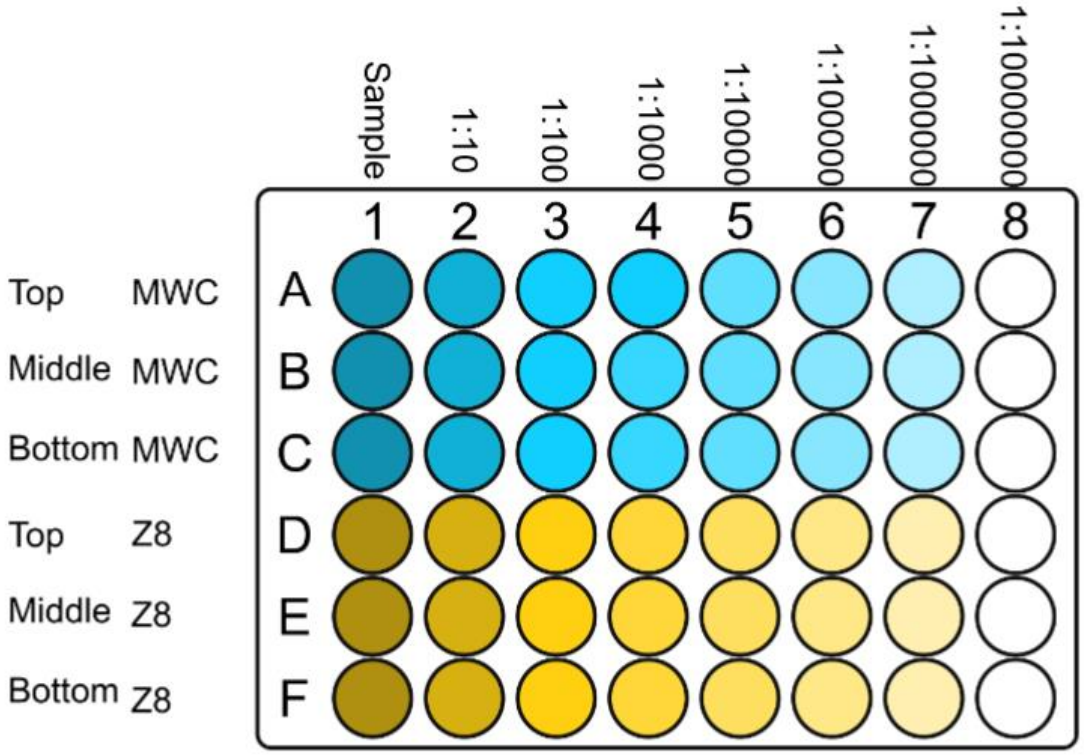

Figure 2: Dilution series for algae enrichment

Observation:

After a week of growth, the different cells were looked at to identify the algae within. The first column showed the most diversity, with copepods, rotifers, diatoms, green algae and ciliates. Diatoms were also predominately observed in the following columns 2-4. Additionally, an unknown species of green algae could be observed in some wells. From the fifth column onwards no algae were observed. In this experiment, the green algae was tried to be extracted. Well D4 showed virtually only the green algae and was therefore used for DNA extraction (figure 3). This cell contained a 1:1000 dilution and had Z8 as media.

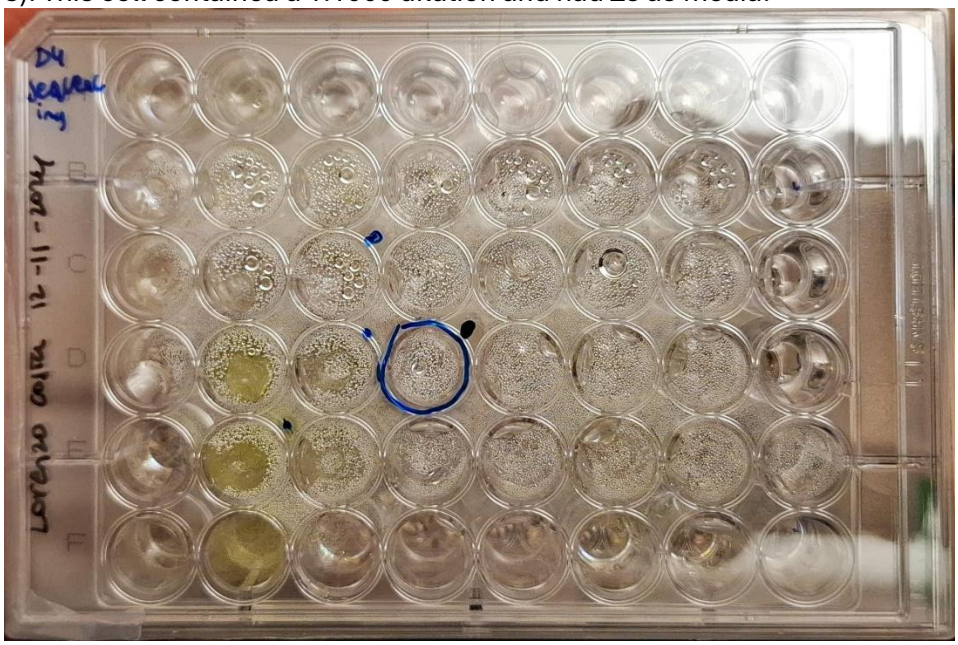

Figure 3: 48-well plate containing protist. Well D4, which is marked by the blue circle, with a 1:1000 dilution and Z8 for media, was used for DNA extraction.

Additionally, the algae was observed under the microscope (figure 4). The cells are oval in shape and can cluster together or are singular. The length of an individual cell is approximately 10 µm. No flagella can be observed.

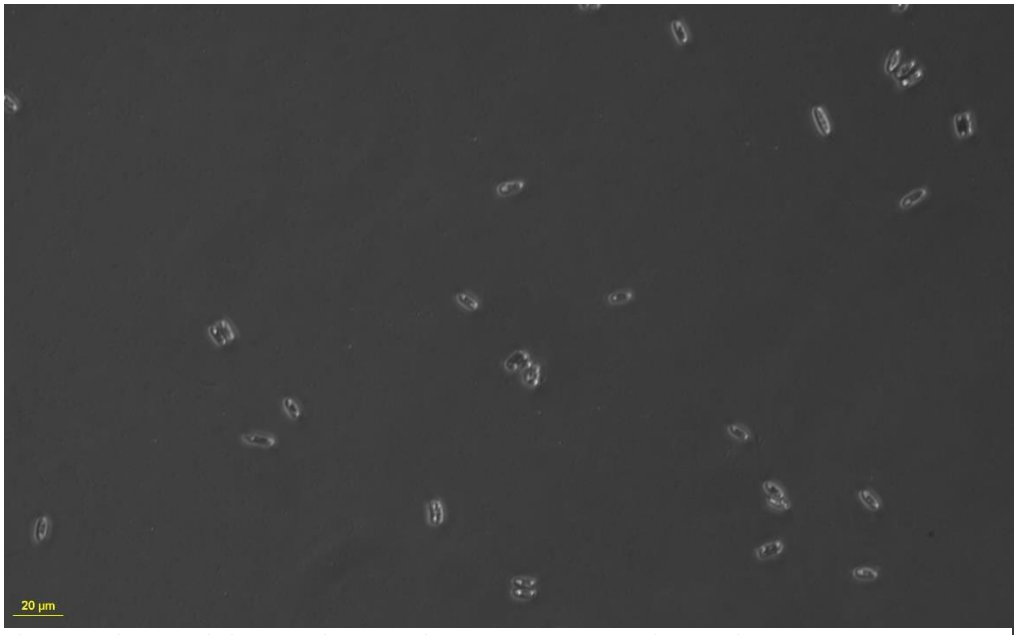

Figure 4: Microscopic image of isolated diatoms. The cells are either solitary or cluster together. No flagella can be observed.

DNA extraction, amplification and quality assessment:

For the DNA extraction, 2 µl of the well of interest (D4) was pipetted into a PCR tube. The tube was quickly spun down using a centrifuge and was subjected to three rounds of coldshock using liquid nitrogen. The PCR mastermix was directly added to the PCR tube. The primers used for the PCR were 3NDf and V4_euk_R1. These are general eukaryote primers and amplify the V4 region of the 18S rDNA. The resulting PCR products were loaded on to an agarose gel for gel electrophoresis in order to assess quality. A faint band could be observed in the gel. The product was prepared and sent for sequencing.

Algae identification

After receiving the sequences, the forward and the reverse sequence were used to create a consensus sequence. The consensus sequence was trimmed to preserve the highest quality possible. The program SnapGene (Llc, n.d.) was used for these steps. The trimmed consensus sequence was then used in a nucleotide BLAST (Sayers et al., 2021) search, where the top 25 results were downloaded to be used in the following steps. The result with the highest percent identity was the diatom *Gomphonema gracile* with 97.86%. The top 25 results were exclusively various species within the *Gomphonema* genus. The consensus sequence and the top 25 BLAST results were added to a provided database with V4 regions of the main lineages within the protists. These sequences were aligned using the program MAFFT (Rozewicki et al., 2019), after which the resulting alignments were used to construct a phylogenetic tree with the help of a program called IQTREE (Nguyen et al., 2015). The resulting tree was given shape by the software iTOL (Letunic et al,. 2006), which can be seen in figures 5 and 6.

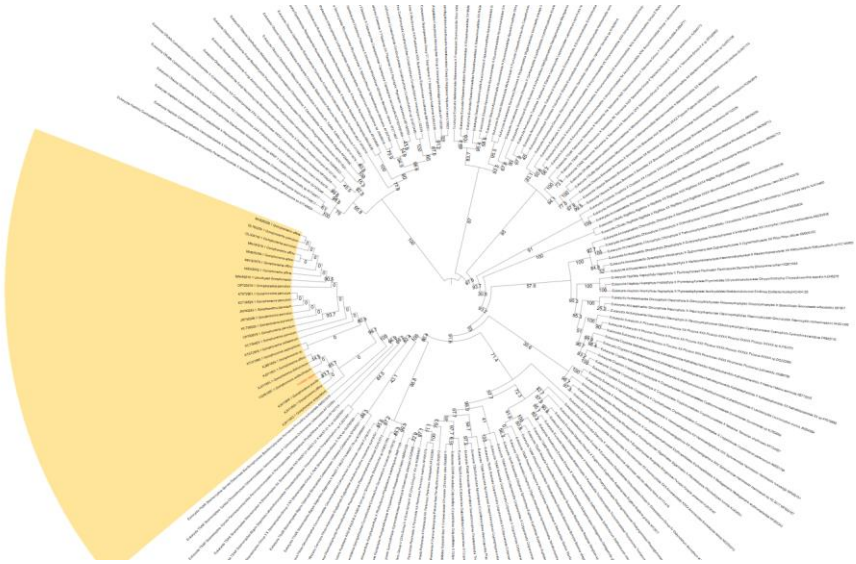

Figure 5: Algal phylogenetic tree. Yellow area contains all Gomphonema species and also contains the isolated diatom.

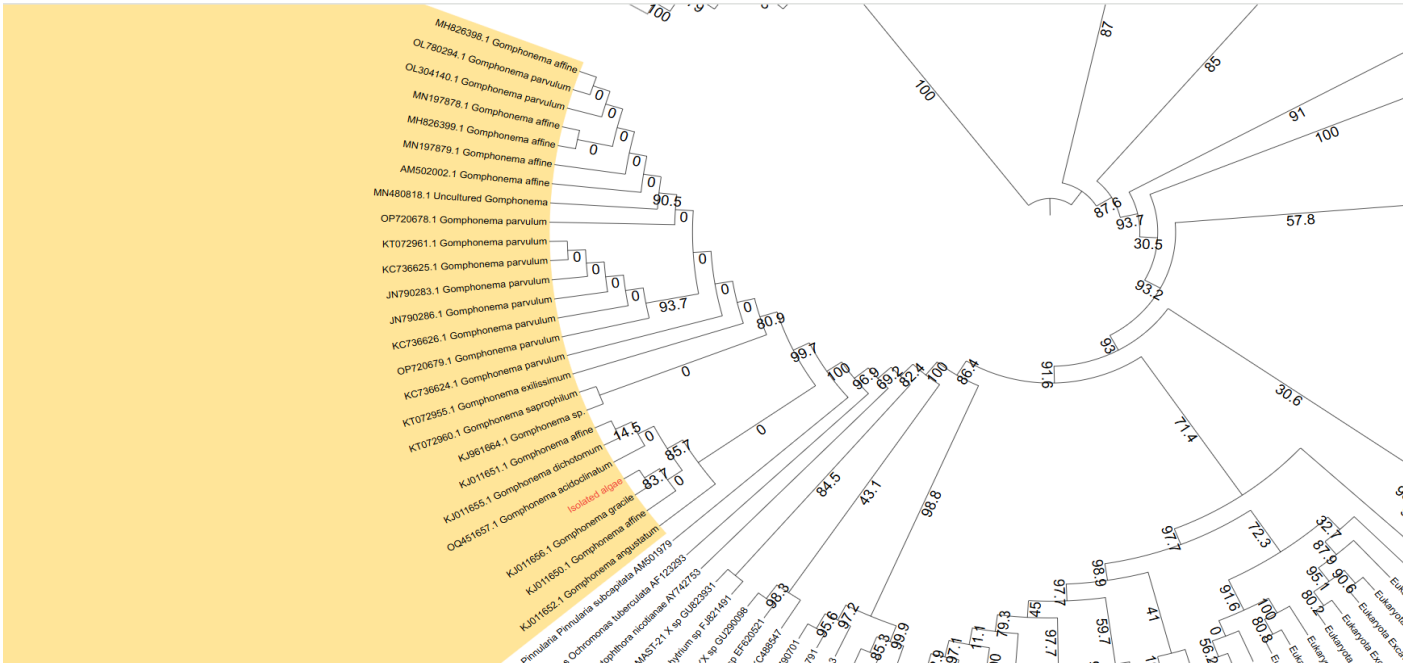

Figure 6: Zoomed in algal phylogenetic tree. The isolated algae is marked in red. It can be found withing a clade containing Gomphonema species (yellow). Bootstrap values are displayed on the lines.

In figure 6 the isolated algae can be seen in red. The bootstrap values support that it is closely related to the diatom *Pinnularia subcapitala* and that it is within the *Gomphonema* species. However, the bootstrap values do not support identification on species level. Some are even as low as 0. So it can be concluded that the algae is part of the genus *Gomphonema*, but nothing can be said about the species.

Gomphonema sp.

Gomphonema is a genus containing over 400 taxa. The 400 taxa consists of diatoms with different shapes, but most have an linear and elliptical shape, as can be seen in figure 7. They are autotrophic and play an important role in carbon fixation. The genus is ubiquitous in water environments around the world. The diversity within the genus is mostly undocumented, however some species have been well studied under the (electron) microscope. The more cosmopolitan taxa, such as *Gomphonema parvulum*, is primarily found in lakes and rivers. Cells

can be either free-living or form benthic colonies (Kociolek et al., 2003). Those species grow primarily on mucilaginous stalks (*Guide to Gomphonema | Genera - Diatoms of North America*, n.d.).

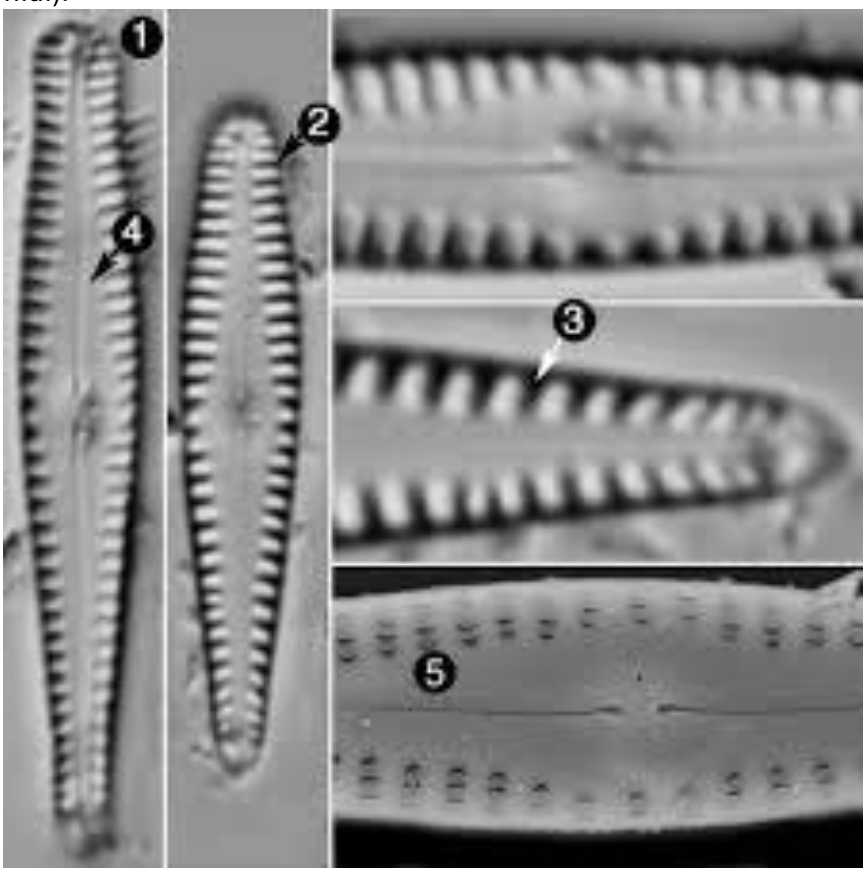

Figure 7: Microscopic image Gomphonema sierranum.

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