***Coelastrum Sp.***

Sampling:

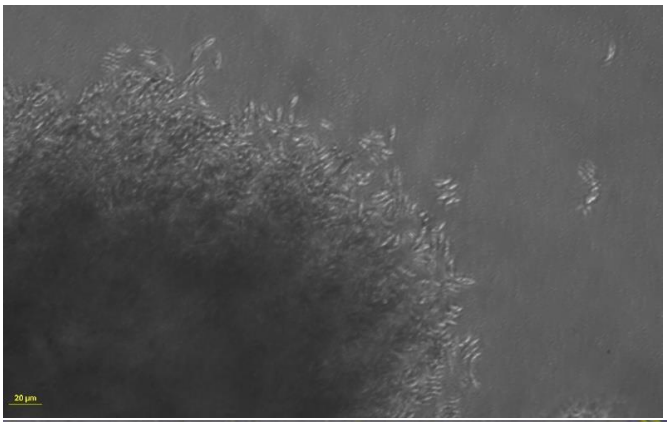
The Sample was a freshwater sample taken from a river at 60,2632456 N°, 16,8968872 O° on the 10th of November. The ground was stirred up a bit, therefor the water sample also included a small amount of sediment.

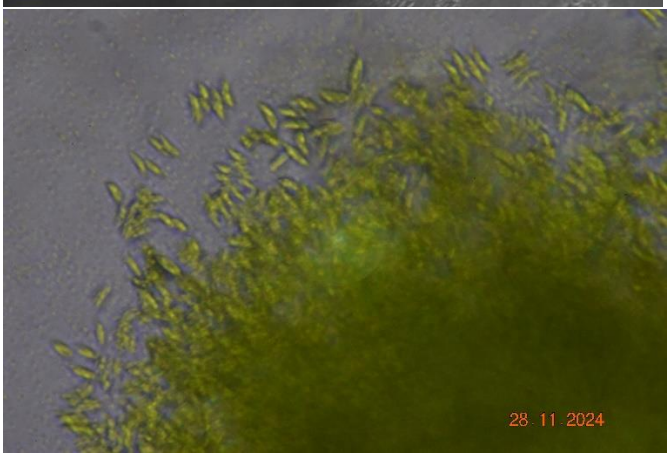
Culturing:

First, an enrichment culture in MWC medium with a 1:10 dilution series was established. Water was taken from the bottom of the sample, just over the sediment. MWC was diluted in a ratio of 1:5 before usage.

Observation:

The algae presented itself in the 1:1000 dilution. It was a clump of green, single cellular algae which grew close together, maybe as a colony. The single cells are long and slightly banana shaped.



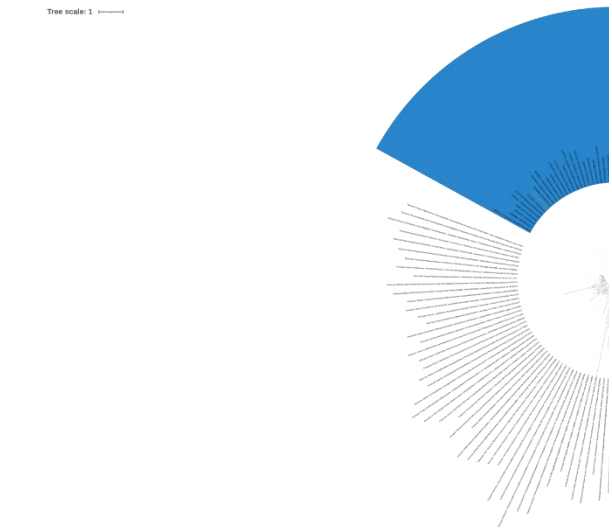

**Phylogeny:**

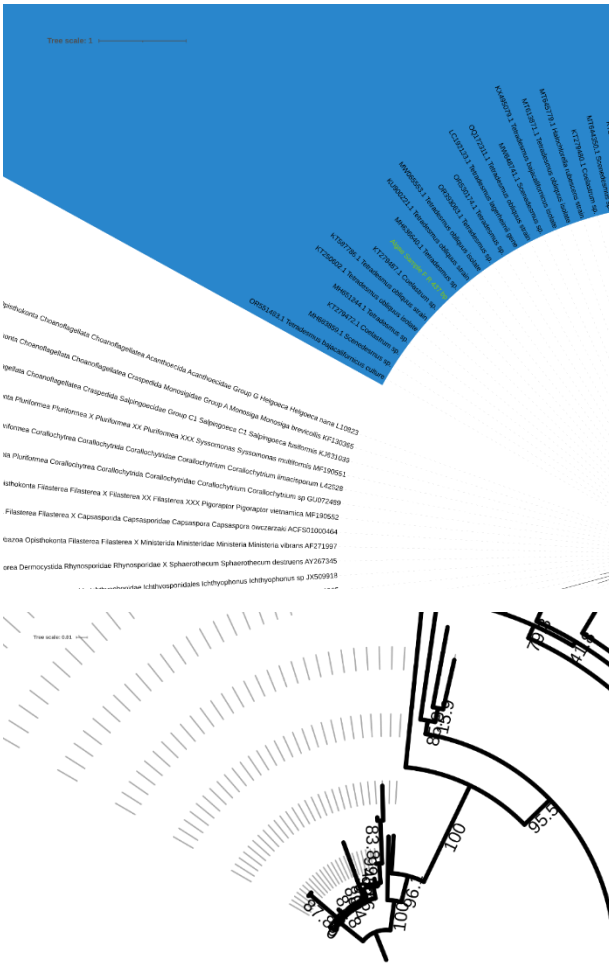
Methods:

2µl of the algal colony where pipetted from the well into a PCR tube and centrifuged. Cell lysis was achieved by cold shock in liquid nitrogen and reheating to 37 C° for three times. PCR was done with 1x GoTaq, 1µM 3NDf forward primer, 1µM V4_euk_R1 reverse Primer, 2µl Sample. Nuclease free water was added to reach a reaction volume of 25µl. After PCR gel electrophoresis was used to confirm successful DNA amplification of the V4 region of the 18S rRNA. The PCR product was purified with ExoSap at 37 C° for 15 minutes after which ExoSap was inactivated at 80 C° for 15 minutes. The purified PCR product was prepared for Sanger sequencing of the V4 region with the primers 3NDf and V4_euk_R1.

The sequence chromatogram was edited in SnapGene. The cleaned sequence was put into BLAST nucleotide search. The first 25 hits in the BLAST Databank, the sample sequence

and the Ref-database-ITS were combined and aligned using MAFFT online. The aligned sequences were put into IQTREE which created a phylogenetic tree which was edited in iTOL.





Discussion:

All of the first 25 BLAST results showed the same percentage identity of 99.77% and where either Coelastrum Sp., tetradesmus or Scenedesmus. All of them got clustered together in the tree as well. They branch of quite early from the rest of the reference Database and the branches also show quite good bootstrap values of over 80. A more thorough placement seems not possible with the limited Data and a very dense tree.

The sample does show the physical features of growing in a colony and the colony having horn shaped peripheral cells that come up when Coelastrum Sp. Is described.[1]

Sources:


1) https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/coelastrum