

UNKNOWN PROTIST





SMTB Introduction

Protists are eukaryotic, predominantly unicellular organisms, diverse from plants, animals and fungi. Protists come in many different shapes and forms, they vary greatly in cellular organisation and differ in motion patterns. This organisms are distributed worldwide, and can be found not only in any water, but also in the sands, muds, marshes and on some plants. Many species of protists remain undiscovered, or sequenced, but not yet identified or described.

Project goal

We assigned ourselves two tasks. First one was sequencing and describing the morphology of the protists species from the particular culture, taken from the Solway Coast, Scotland. The second goal was to find the most abundant protist in the culture and define its ecology. **Project overview**

Our work was divided into the wetlab part and the microscopy part. In the wetlab part, we multiplied the 18S ribosomal RNA regions of the DNA found in our culture, and inserted the 18S gene into plasmids, which were then propagated in bacteria. We have grown bacterial colonies, and picked ones that contained the inserted 18S sequences. Then we amplified the DNA once again and sequenced it. In the second part of the project, we observed our culture under the microscope to get more information about the protist species living in the culture and to be able to compare the microscopy images and sequence results.



igation

Inserting the 18S gene

from the

culture

into the plasmid

DNA extraction DNA of the protists living in

our culture was extracted by Daryna Zavadska in the Biology and Ecology of Abundant Protists lab



Heat shocking bacteria

can take in plasmids with 18S gene

Preparing agarose gel and running the

Stressing the bacteria with 42°C, so their cell membrane would be partially destroyed and they

Electrophoresis

in our PCR

Bacteria plating

Electrophoresis

gene in our PCR

Preparing agarose gel and running

electrophoresis to check if we have 18S

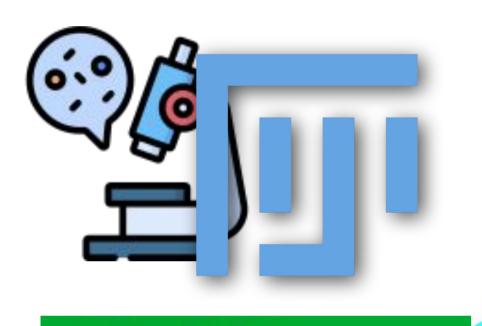


Putting bacteria on the agar plate, where only those which contain the plasmid with the insert can growth

Sequencing

Sequencing the obtained amplicons

Drylab methods



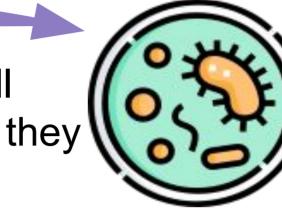
FPbase

We used the FPbase (<u>https://www.fpbase.org/</u>) to calculate the overlap ratio of the dyes fluorescence spectrums. We were choosing from the DAPI, DRAQ5, SYBR Green and Hoechst, and

We took pictures of the protists in the culture we investigated with the fluorescent microscope with 63x Oil objective and edited them in the Fiji.

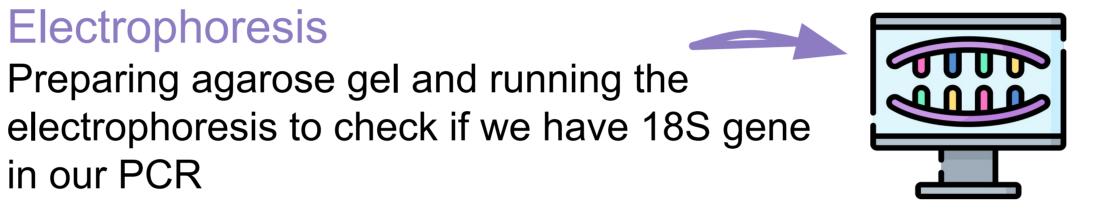
comparing their overlap ratio with DiO dye.

Choanoflagellate and presumably Paraphysomonas (upper right on the right image); the yellow channel shows fluorescence of DRAQ5 (DNA-binding stain) and Chlorophyll A; The yellow channel shows DiO



jõ 🛏

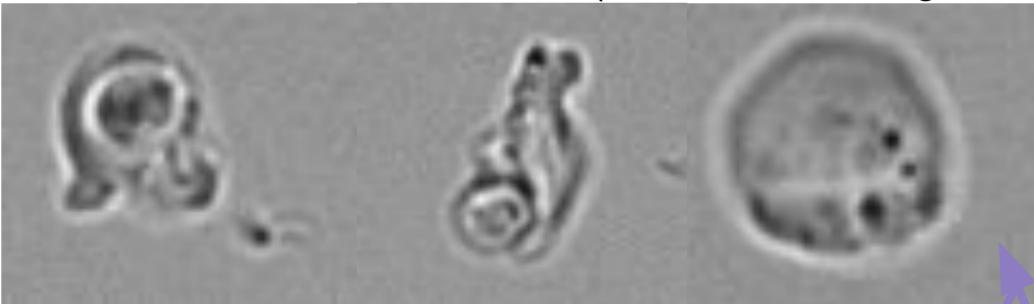
To amplify the DNA 00



fluorescence (membrane-binding stain)

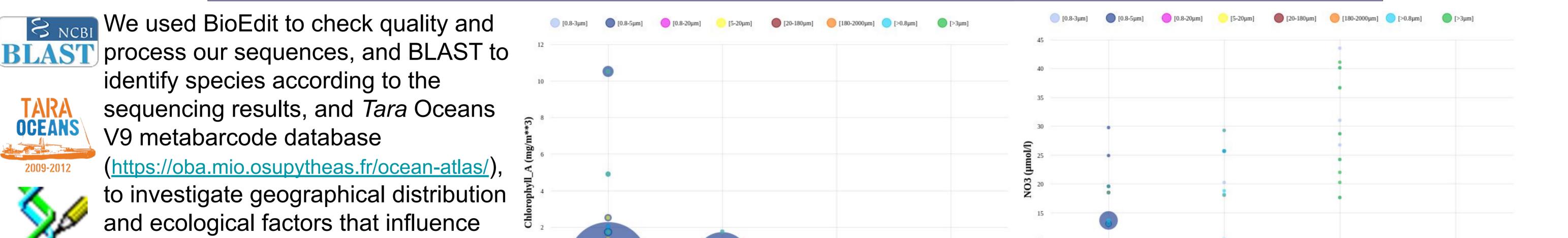


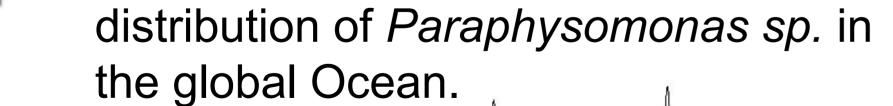
Heliozoans that were found in culture performing the contraction of axopodia (extended axopodia to the left, contracted - to the right)



A sequence of mysterious events, in which Vanellid amoeta attempts to consume an algae, presumably Paraphysomonas sp., but fails and crawls away in despair

Ciliate moving fast through the culture flask









The global distribution of *Paraphysomonas sp.* suggests a strong dependence on the Chlorophyll A and NO3 concentrations; *Paraphysomonas sp* occurs worldwide.

Conclusion

With the help of BLAST we processed the results of our sequences, and got the photosynthetic chrysophycean algae *Paraphysomonas*, which we could see everywhere in our culture microscopy as well. *Paraphysomonas* is globally distributed and inhabits the low chlorophyll and low nitrate waters, and it can tell us a lot about the water quality it lives in, in particular that the water is quite clean. This fact can help a lot in the ecological research. Extensive microscopy revealed the presence of many more protists that co-exist with *Paraphysomonas* in the culture; they were identified by morphology, and more sequencing will be done on the culture in the future.

NON CONTRACT ON CONTRACT ON CONTRACT ON CONTRACT