

# UNKNOWN PROTIST SPECIES DISCOVERY

## Introduction:

Protists are eukaryotic organisms that are not plants, animals, or fungi. They exist in different forms and live in different places all over the world.

For this project, we sequenced 18S rRNA gene regions of the protists from several cultures collected at the Bowness-on-Solway sampling site in Wigton, UK. We selected the 18S regions for the analysis since this particular gene is present in all eukaryotes. These fragments were then inserted into plasmids, which we then introduced into *E. coli* by subjecting the bacteria to heat shock. We then extracted the DNA fragments of interest from the bacteria and sequenced them.

Thus, using bioinformatics methods, we were able to identify known and unknown protists from our culture. We then observed the cultures under the microscope, which subsequently helped us to link the data obtained from the sequencing to whatever we managed to see under the microscope.

## Methods:

### PCR amplification

We amplified the DNA extracted from the samples using universal eukaryotic primers for 18S (18S\_42F - Forward primer, and 18S\_1747R - Reverse primer).

### Gel electrophoresis

Then we checked if the 18S rRNA genes, present in all eukaryotes in our cultures, were amplified, with agarose gel electrophoresis.

### Purification

Using the Monarch DNA & PCR cleanup kit, we removed the PCR by-products from the amplified samples.

### Ligation

After that, we inserted the purified DNA fragments of interest into plasmids using the pCR 2.1 Vector TA Cloning Kit, by the process of ligation.

### Transformation

The plasmids were put into TOP10 competent *E. coli* cells. These bacteria were then spread on Petri dishes containing LB+Ampicillin and Xgal stain.

### Colony PCR

Successfully transformed colonies turned out being white in color, meaning that the plasmid contained the 18S insert, allowing it to digest Xgal. We picked the white colonies and amplified them by Colony PCR.

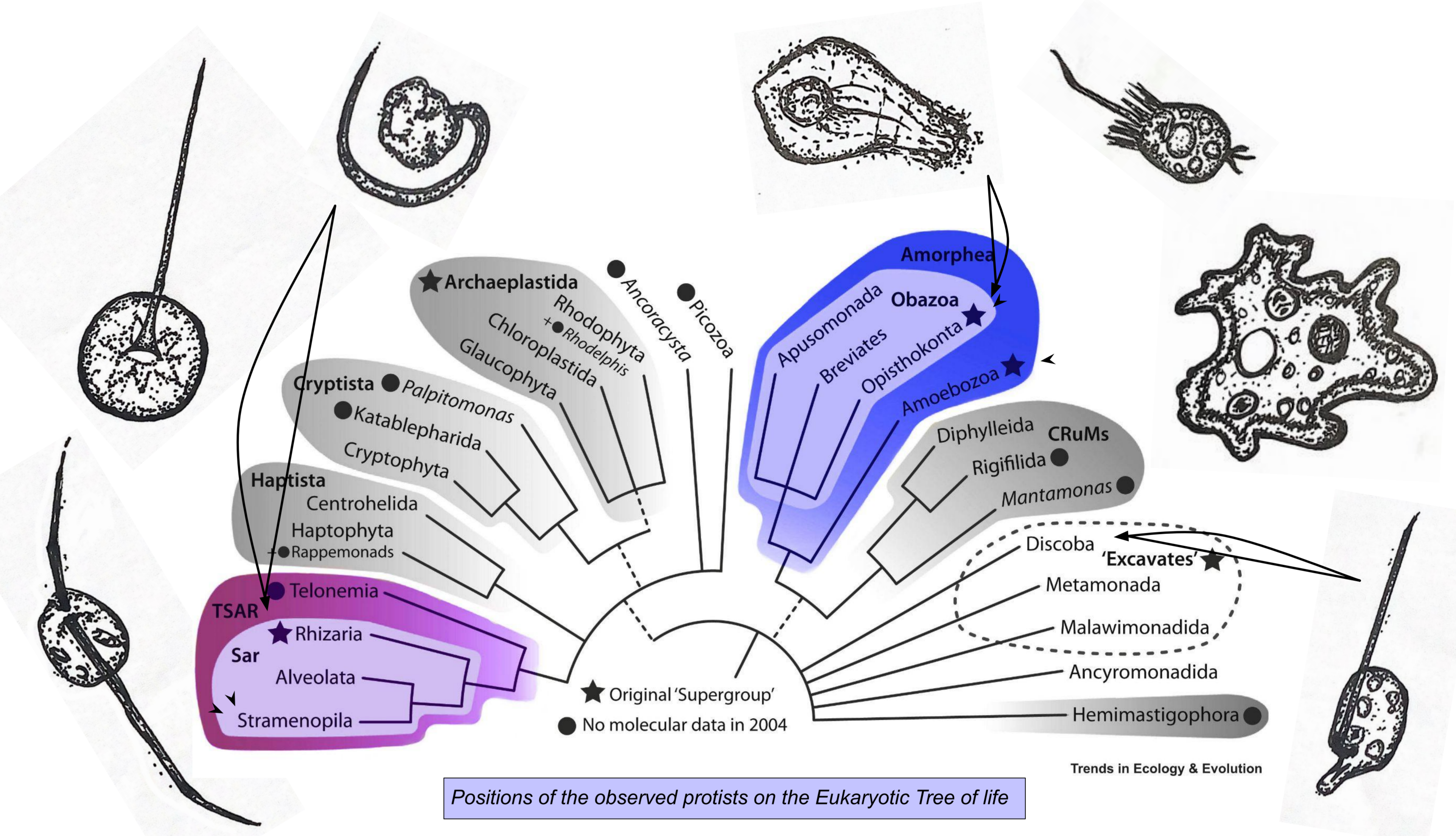
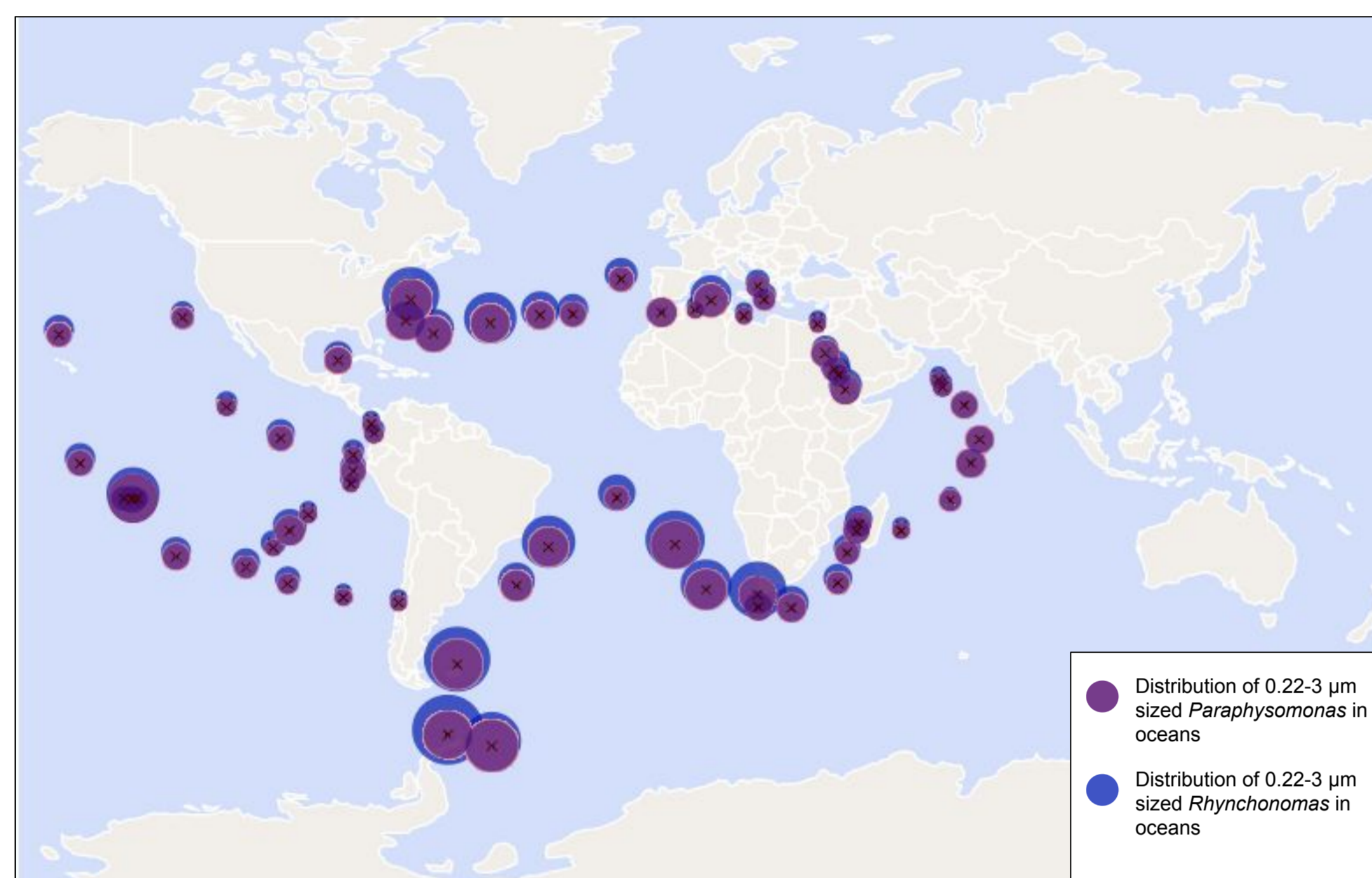
### Sequencing

Amplicons were sent to Eesti Biokeskuse tuumiklabor (Riia 23b-302, Tartu) for Sanger sequencing. We used BLAST to compare the obtained sequences with the NCBI database.

### Microscopy

Finally, we performed microscopy and checked whether the resulting images of the cultures matched the sequences obtained from them.

## Results:



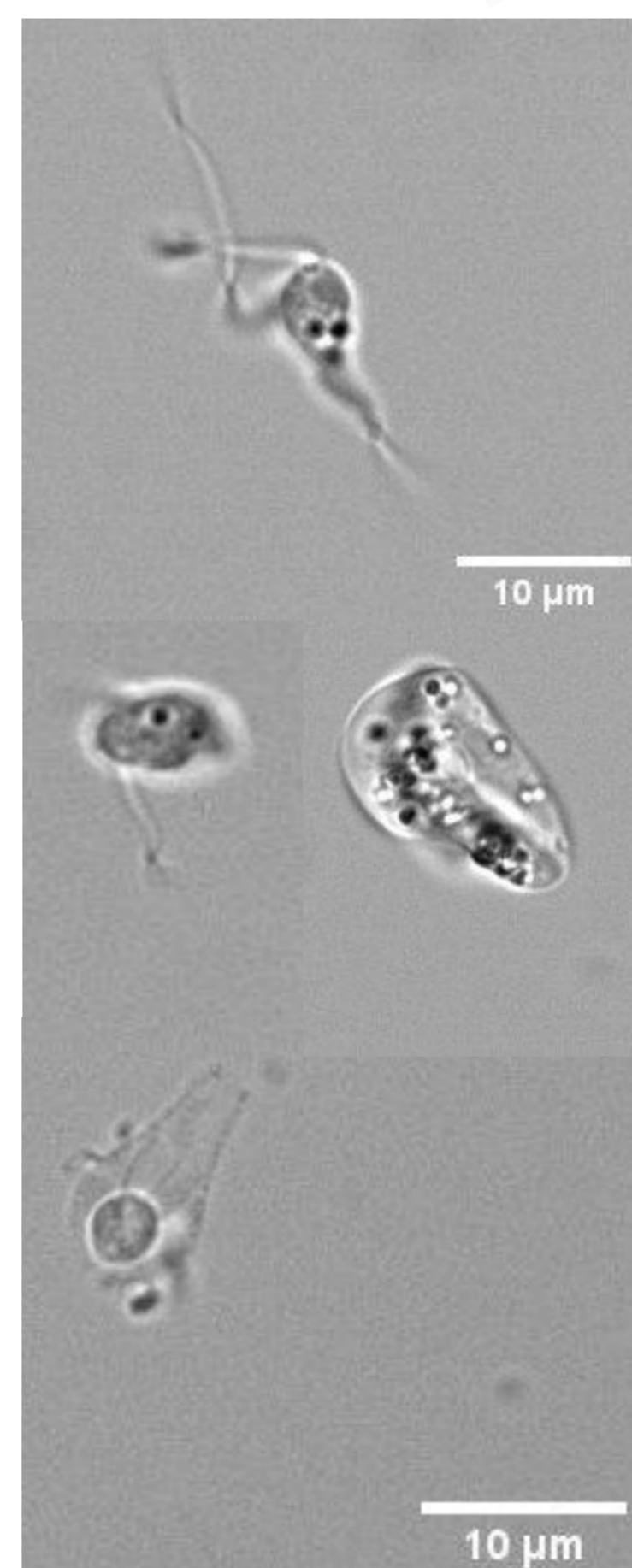
### Culture 3

Sequencing data from this culture revealed four different species of protists. One of them was *Caecitellus paraparvulus*, a member of the phylum Stramenopila, which we managed to capture during microscopy. Caecitellus is a genus of small bacterivorous zooflagellates in marine environments, distinguished because the cells typically move by gliding along a substrate rather than by swimming.

Another curious protist we sequenced was *Rhynchomonas nasuta*, a kinetoplastid of approximately 5 µm in size. It is easily recognized by the bulbous motile snout which contains its mouth and a trailing flagellum about 2-3 times its body length.

*Rhynchomonas* glides with a wobbling motion with snout moving from side to side. While observing the culture, we also discovered several beautiful choanoflagellates, which are considered to be among the closest living protozoan relatives of animals, including humans.

Scientific Name	Query Cover	E value	% identity	Accession
uncultured eukaryote	94%	0	99.09	KP404739.1
uncultured eukaryote	94%	0	99.09	KP404633.1
<i>Paraphysomonas imperforata</i>	94%	0	99.09	EF432518.1

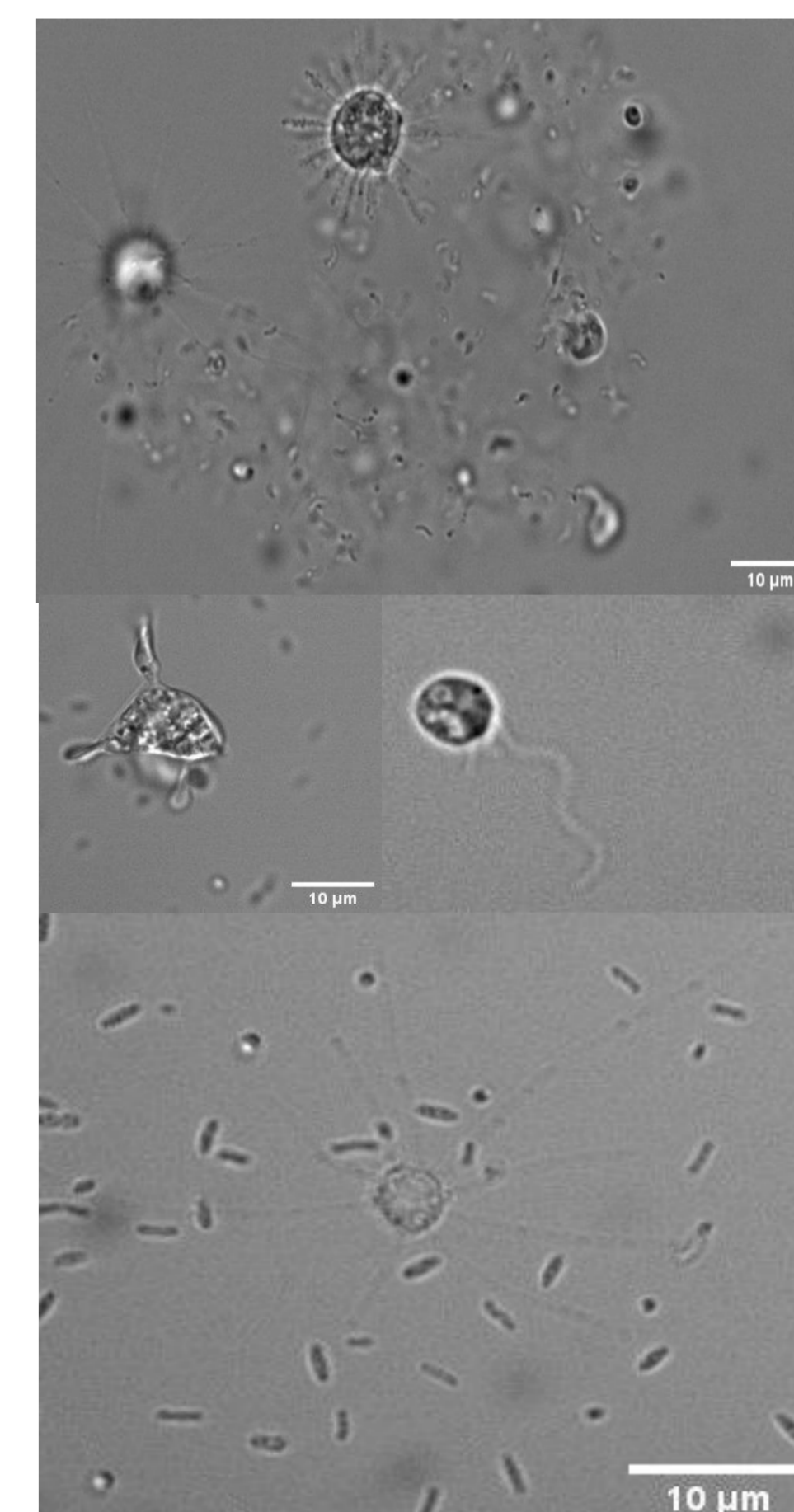


### Culture 4

According to the sequencing results, the most prevalent species in this culture turned out to be *Paraphysomonas imperforata*, a heterotrophic, free-living flagellate algae of the class Chrysophyceae, typically ranging in size from 4 to 8 µm in diameter. This species and other members of this genus are identified by the siliceous scales that cover their cells. *Paraphysomonas* is a cosmopolitan, as it is observed in a wide range of freshwater and marine environments.

In addition to *Paraphysomonas*, we also managed to see some fascinating granulofilose amoebae in this culture.

Scientific Name	Query Cover	E value	% identity	Accession
uncultured Bicosoecida	95%	0.0	99.51	EF620523.1
<i>Caecitellus paraparvulus</i>	95%	0.0	99.51	DQ220717.1
<i>Caecitellus paraparvulus</i>	95%	0.0	99.51	DQ220716.1
<i>Caecitellus paraparvulus</i>	95%	0.0	99.51	DQ220715.1

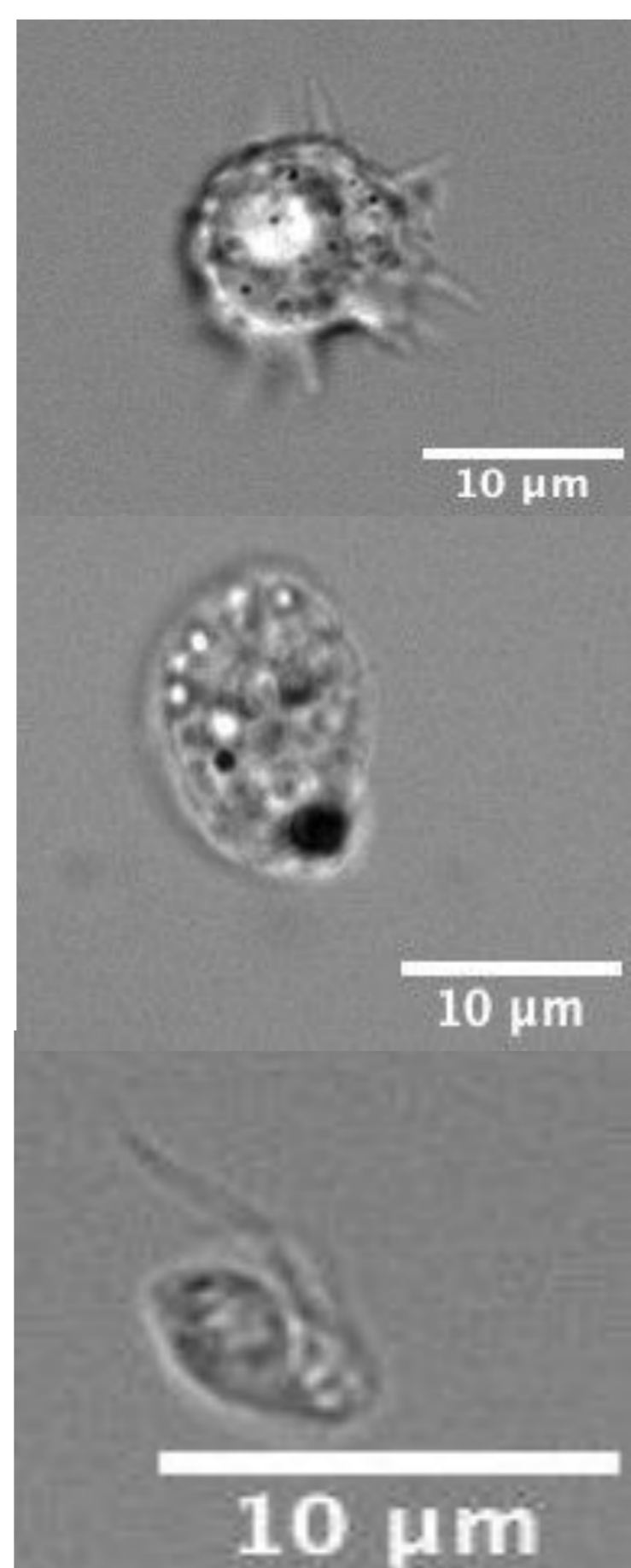


### Culture 6

When we observed culture number 6 under the microscope, we saw many different amoebae and choanoflagellates. We also noticed protists that morphologically resembled kinetoplastids, but the species to which they belonged remain to be identified.

However, according to the sequencing results, the most prominent species was once again *Paraphysomonas imperforata*, a species of Chrysophyceae (golden algae). It is a heterotrophic predatory algae that is being intensively studied as a model for the investigation of marine flagellates that feed on bacteria.

Scientific Name	Query Cover	E value	% identity	Accession
uncultured stramenopile	96%	0	98.48	1224
uncultured marine picoeukaryote	96%	0	98.38	1791
<i>Paraphysomonas imperforata</i>	96%	0	98.38	1793
<i>Paraphysomonas imperforata</i>	96%	0	98.38	1793
<i>Paraphysomonas foraminifera</i>	95%	0	98.47	1735
uncultured diatom	96%	0	98.19	1713



### Culture 8

Observing culture 8 under the microscope, a wide range of protists could be seen, including a variety of different amoebae and numerous flagellates.

After sequencing and blasting, just as in cultures 6 and 4, the 18S rRNA gene from *Paraphysomonas imperforata* was the most abundant in the sample.

Scientific Name	Query Cover	E value	% identity	Accession
uncultured marine picoeukaryote	94%	0	99.29	1791
uncultured eukaryote	94%	0	99.09	1775
uncultured eukaryote	94%	0	99.09	1774
<i>Paraphysomonas imperforata</i>	94%	0	99.09	1793
uncultured eukaryote	94%	0	98.99	1775
uncultured eukaryote	94%	0	98.99	1774

