Unknown protist species discovery

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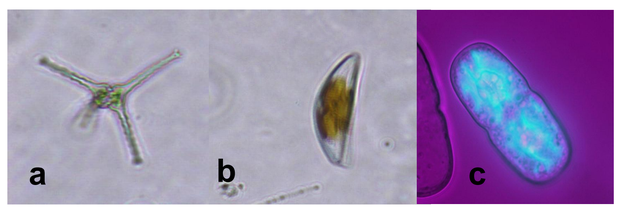
Introduction

Protists are eukaryotes that are not plants, animals, or fungi.
They exhibit many different phenotypes and can be found all over the world's oceans. They can also occupy different ecological niches within these oceans. Many species of protists have not yet been discovered, and others have been sequenced but not yet identified and morpohologically described.



Protists found in freshwater samples under 40x brightfield microscopy (a and b) and photosynthetic protist with chlorophyll autofluorescence (100x, Oil)

What did we do?

We sequenced several cultures by isolating the 18S regions of the rRNA and amplifying them.

Then, we inserted these fragments into plasmids which we propagated in E. Coli. Then, we extracted the DNA and sequenced it.

We also observed the protist cultures under microscope to then be able to connect the sequences to morphology and behavior.

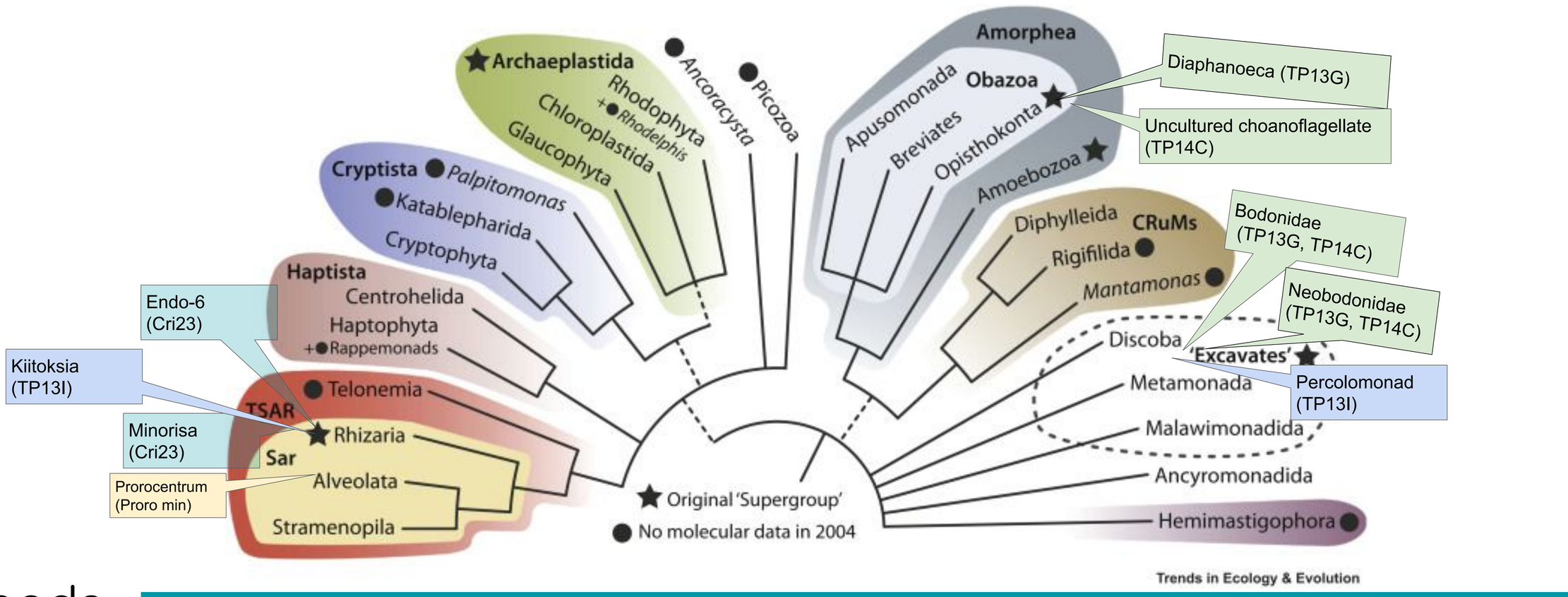
Why?

Our goal was to sequence and describe new species of protists. We used microscopy to gain an understanding of their morphology. Then we placed them on the eukaryotic tree of life using both their morphologies and sequences.

We have many protists' sequences from previous studies but know very little about what these actually mean for the protists themselves and their morphology.

Results

Protists from 5 different cultures ("TP13I, TP13G, TP14C, Cri23 and Proromin") were identified and described.



Methods

DNA Isolation

DNA was extracted by Quigaen Power Soil DNA isolation kit according to manufacturer's protocol.

Polymerase Chain Reaction

Once the sample is purified and extracted, a Mastermix(MM) is added. MM consists of DNA universal eukaryotic primers for 18S (18S_42F - Forward primer, and 18S_1747R - Reverse primer), DNA polymerase, and a nucleotide solution mix. The solution has been placed into a thermal cycler to begin the replication/amplification process. This initial DNA sample is replicated into millions of duplicate DNA segments.

Gel Electrophoresis

The success of the PCR reaction is checked using 1% agarose gel electrophoresis of the DNA samples. Samples containing the fragments of ~1500-2000bp length are expected to be the 18S amplicons which are aimed to obtain

Amplicon Purification

The purpose of purification is removing the PCR by-products. To conduct that operation the Monarch DNA&PCR cleanup kit was used.

DNA Ligation

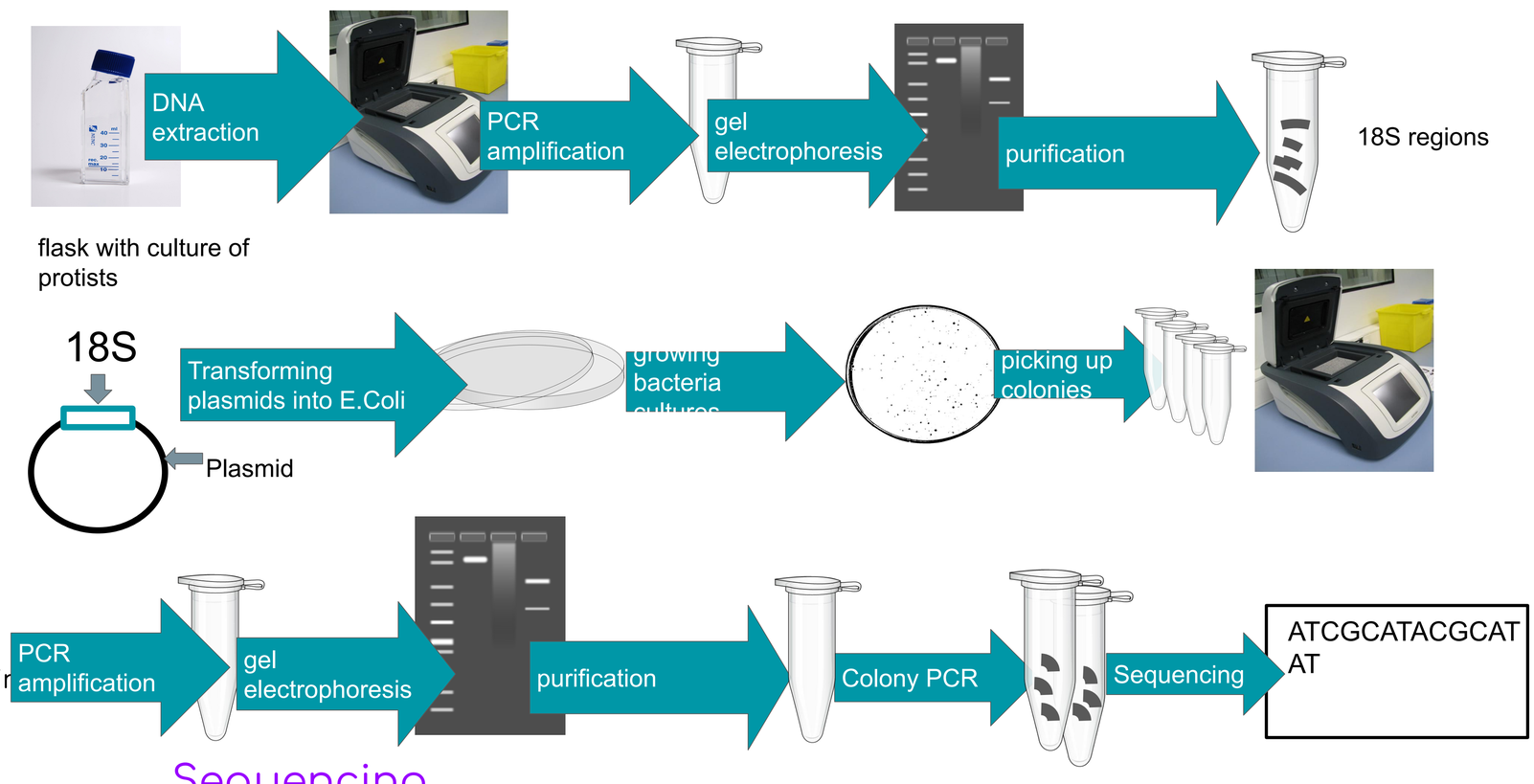
DNA fragments were ligated into plasmids using the TA Cloning™ Kit, with pCR™2.1 Vector.

Transformation

The ligated plasmid was transformed into TOP10 competent E. coli cells. Cells were plated in petri dishes with LB+Ampicillin+Xgal.

Colony PCR

To prepare the material for the sequencing, several operations were conducted (colony PCR, electrophoresis and amplicon purification).



Sequencing

Samples were sent to Sanger sequencing to Eesti Biokeskuse tuumiklabor (Riia 23b-302, 51010 Tartu, ESTONIA).

Sequence Analysis

BLAST software (blast.ncbi.nlm.nih.gov) was used to compare to known nucleotide sequences against the NCBI nucleotide database. Ocean Gene Atlas webportal was used to (https://tara-oceans.mio.osupytheas.fr/ocean-gene-atlas/) visualize geographic distributions of the sequences we obtained.

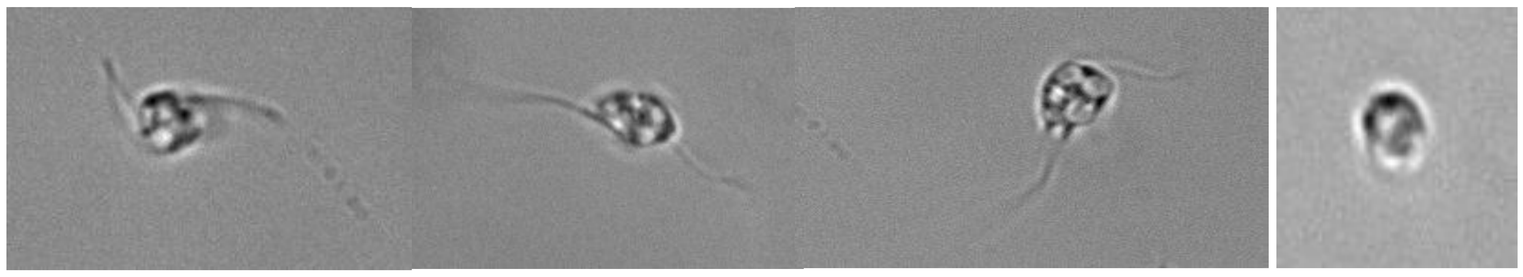
Microscopy

We microscoped the samples in Bright-field with Nikon Eclipse TS1200 (camera: Axiocam 506) on magnifications 40X and 100X (Oill) and Zeiss Axio Observer.Z1 (camera: DXM 1200C) on magnification 100X(Oil). Obtained images were processed with Fiji.

“Cri23” culture =Minorisa+Endo_6 representative

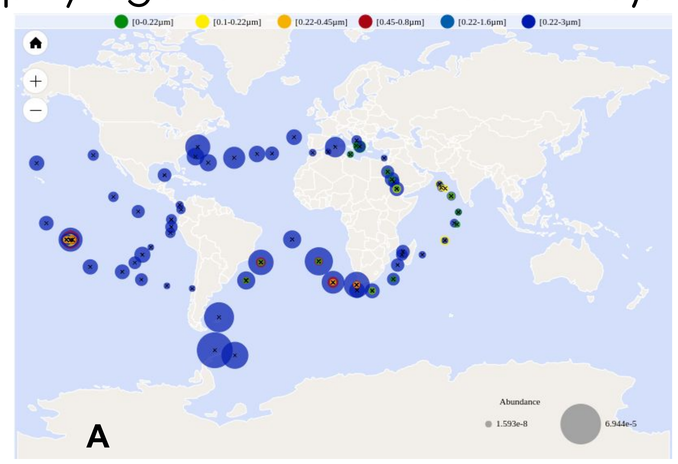
From the sequencing data 3 contigs representing the *Minorisa minuta* species were found. *Minorisa minuta* is the marine protist, also known as the smallest predator of the oceans. It belongs to Chlorarachniophyceae, Cercozoa. *Minorisa minuta*, which were sequenced in this sample, account for nearly 5% of coastal heterotrophic protists. They are bacteriovores and are presumed to be important players in their ecosystems because they can regulate bacteria levels and affect carbon levels. They are both widely distributed and abundant, despite their small size (1.6 μm).

The other contig of sequences revealed a presence of an unknown eukaryote with extremely low affinity to the Endo-6 lineage (Endomyxa, Rhizaria). Sequences of the Endo-6 were published, though they are not characterized morphologically yet (there are no relevant morphological and phylogenetic data observed).



Brightfield microphotograph of Endo-6(Endomyxa) representative on 100X magnification. *We* can see the representative of the group Endo-6, which have visible 2 flagella. This could mean whether that it is the certain development stage of life cycle of Endo-6, which present in other species of Endomyxa as well, or other life form that also belongs to the Endomyxa.

Bright-field micrographs of *Minorisa minuta* on 100X(Oil) magnification. *M. minuta* has the round shape and one flagellum, that is visible here.

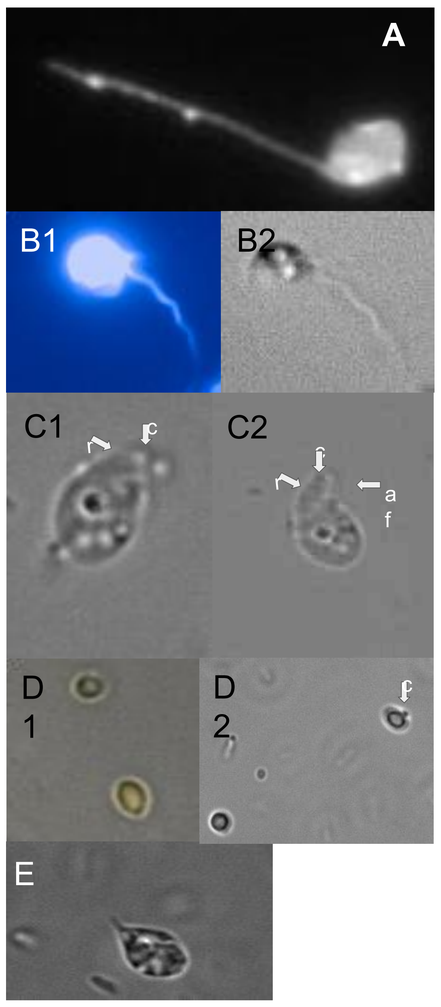


Geographical distribution and abundance of the sequences matching *Minorisa minuta* representative: of Cri23 culture A)-for the Surface layer of water; B) -for the Deep Chlorophyll Maximum layer. Circle sizes correspond to abundance values, and color shows size fraction.

% identity	evalue	coverage	Accession number	Taxonomic group name (if known)
92.91	0.0	99%	KU587848.1	*Minorisa sp.*
97.21	0.0	81%	MT355137.1	Endomyxan amoeba or incertae sedis
99.84	0.0	92%	MT355131.1	*Minorisa sp.*
99.56	99.56	100%		*Minorisa sp.*

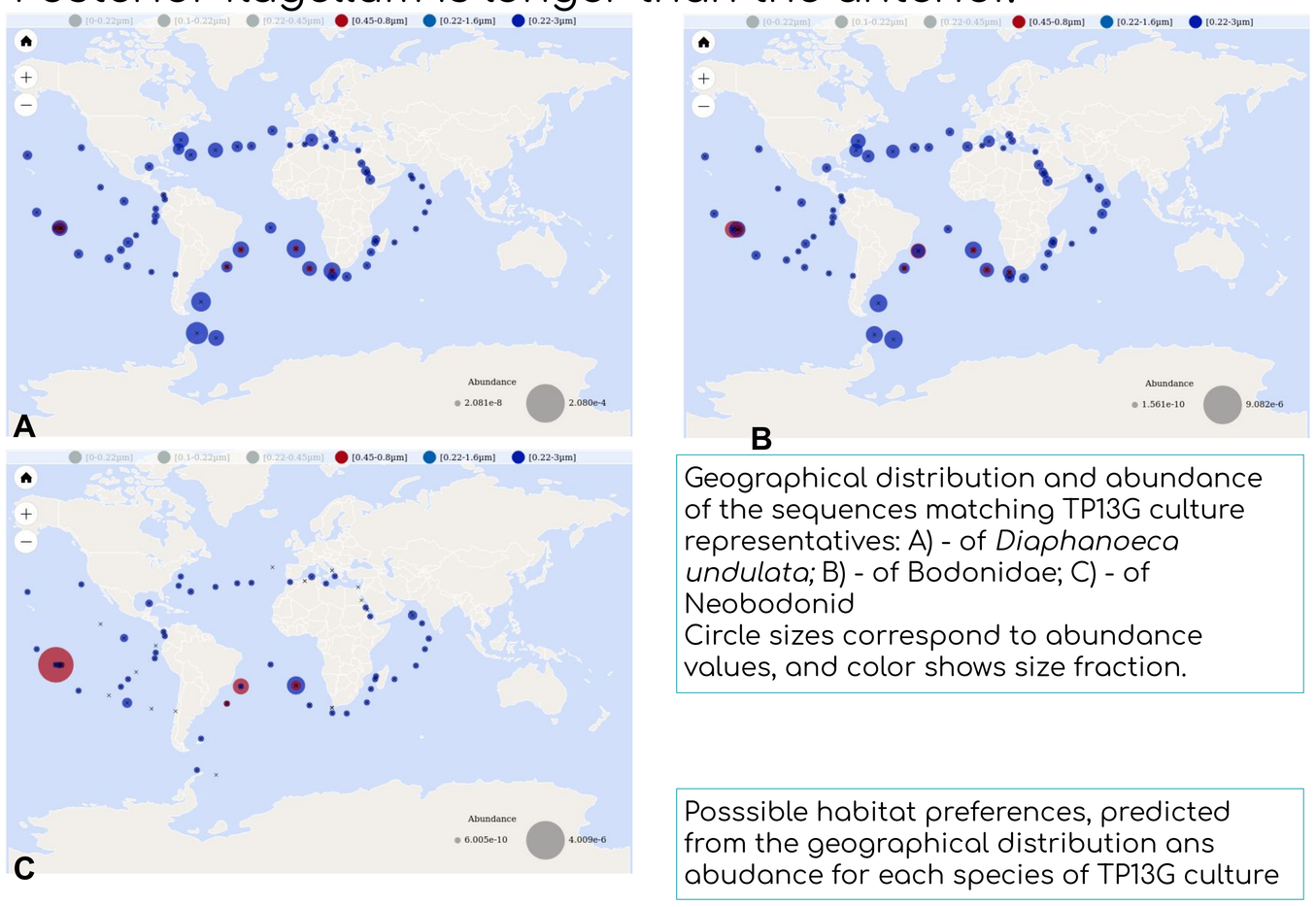
BLAST statistics for each contig obtained from “Cri23” culture sequences.

“TP13G” culture = Kinetoplastids+Choanozoa

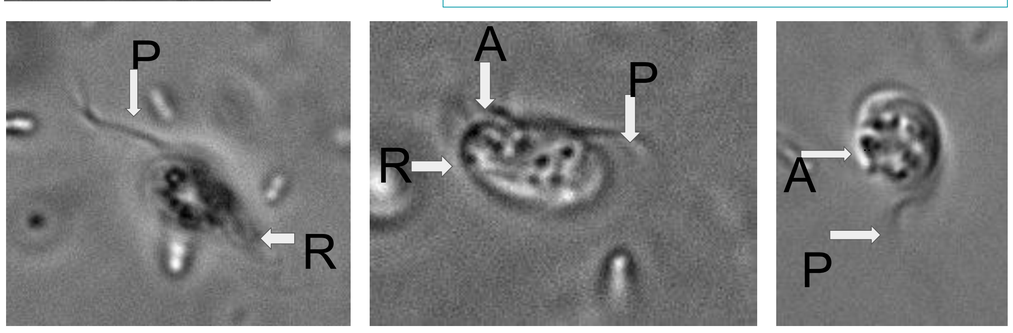


A,B1 - images were made in fluorescent microscope under 100x Oil, fixed with Lugol's solution and dyed with DiOC6 in 1:200. C,B2(bright-light), D,E(phase-contrast) images were also made under 100x oil microscopy. A)-*Neobodo*, has a flagellum(f) B)-*Neobodo*(differs from A)has a flagellum(f). Two spots are probably belong to the nucleus and kinetoplast. C)- This is a fast-moving protist *Bodonidae* with a shape of rounded drop, we could determine its nucleus(n), located in the centre of the cell, it also has a flagellum which makes waving and wrapping half-circular movements.The anterior flagellum(af), cytostome(c) and rostrum(r) are visible. D) *Diaphanoecas'* flagellum is not visible, the organism is egg-shaped, moves slowly, not linearly, the nucleus(n) can be seen by one side of the cell, the collar(c) is also seen. E) *Bodonidae*. Drop-shaped cell is visible.

Sequencing revealed the presence of two different representatives of Bodonid flagellates (Kineplatids, Excavates) and a single, yet already known, Choanoflagellate species. Bodonid flagellates are heterotrophic protists that belong to the class Kinetoplastea, phylum Euglenozoa inside Discoba group. They possess some characteristic morphological features, such as elongated body with roundish posterior end and a rostrum with cytostome on anterior end, and two flagella. Posterior flagellum is longer than the anterior.



Geographical distribution and abundance of the sequences matching TP13G culture representatives: A) - of *Diaphanoeca undulata;* B) - of Bodonidae; C) - of Neobodonid
Circle sizes correspond to abundance values, and color shows size fraction.



Brightfield microphotograph of bodonid flagellates on 100X magnification. Two flagella (arrows; A - anterior flagellum, P - posterior flagellum) and rostrum (R) with cytostome are visible.

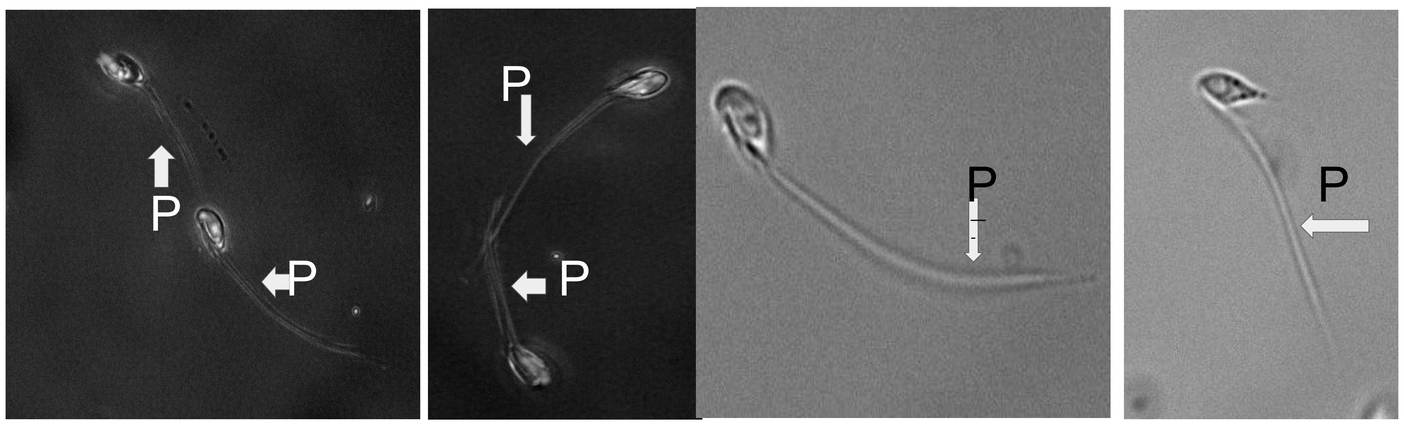
% identity	evalue	coverage	Accession number	Taxonomic group name (if known)
97.920	0.0	98%	KU587848.1	*Diaphanoeca undulata*
98.813	0.0	95%	MT355137.1	Bodonidae sp.
97.047	0.0	99%	MT355131.1	Neobodo sp.

BLAST statistics for each contig obtained from “TP13G” culture sequences.

Possible habitat preferences, predicted from the geographical distribution ans abudance for each species of TP13G culture

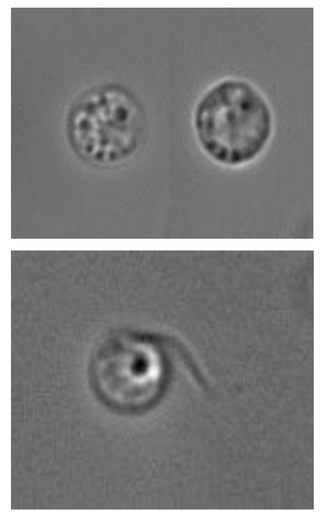
	Temperature	Salniity	Seafloor
Diaphanoeca	Smaller ones require higher ambient temperatures.	prefers water with great solity, relatively tolerant to the changes of it between 33-40 psu	smaller organisms tend to live in smaller depths
Bodonidae	Such organisms are adapted to a wide range of temperatures.	prefers water with great solity, relatively tolerant to the changes of it between 33-39 psu	Tend to live mainly at medium depths.
Neobodo	Larger organisms can survive at lower temperatures	great sality, conservative with the 34-36 psu	The organism lives in great depths

“TP13I” culture = Kiitoksia+Percolomonad(?)



Brightfield microphotograph on 100X(Oil) magnification of *Percolomonads*, as identified based on morphological features. Long posterior flagellum (P) is visible (arrows).

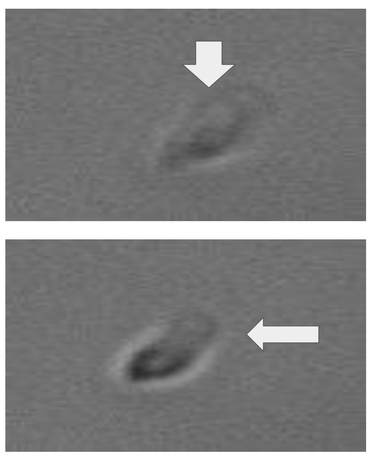
Although sequencing data from this culture was incomplete, two distinctive morphotypes, likely corresponding to Precolomonad (Heterolobosea, Excavata) and *Kiitoksia sp.* could be identified clearly.



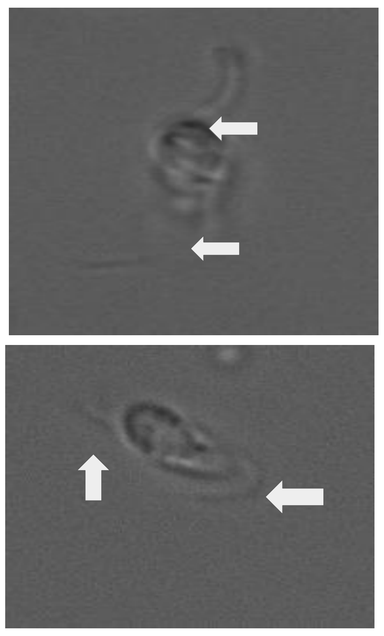
Brightfield microphotograph on 100X(Oil) magnification of *Kiitoksia*

“TP14C” culture = Kinetoplastids+Choanozoa

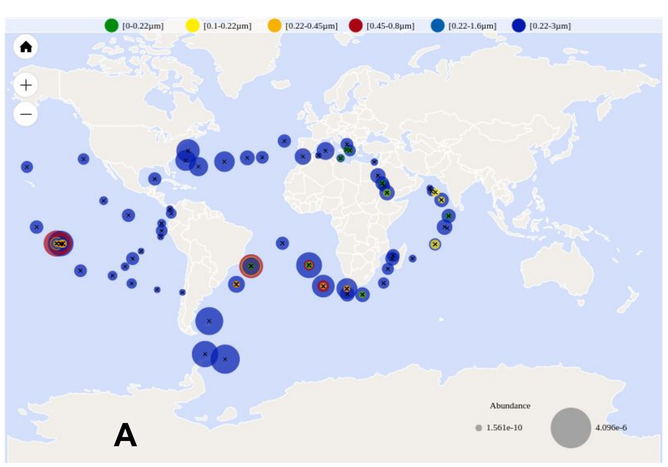
From sequencing results this species belongs to Bodonidae, which are free-living *Kinetoplastids*. It moves, holding on substrate with posterior flagellum, while making round moves with anterior. Mostly abundant in surface waters. Tolerates temperature range from -5 to 30 °C. Lives on Depth from 0 to 1000 m. Prefers salinity from 32 to 40 PSU. In lab conditions was kept in room temperature, in darkness.



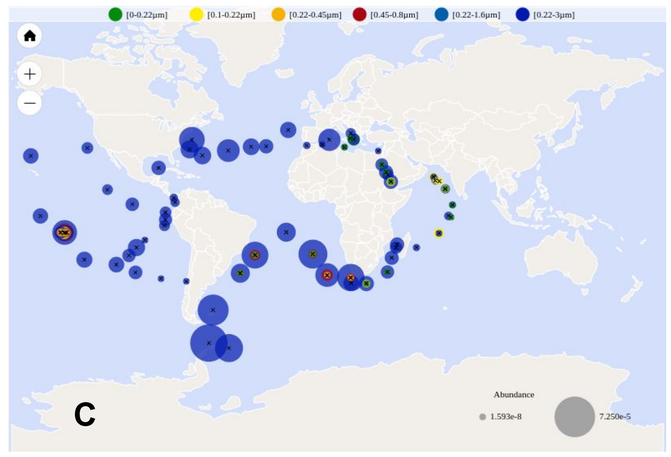
Brightfield microphotograph of a Choanoflagellate species from TP14C culture (magnicifation 100X).
Drop-shaped and collar is visible.
A single flagellum surrounded by a transparent collar, acts as a food catching net. When free swimming, It is used in locomotion, pushing the cell along.



Brightfield microphotograph of a Kinetoplastid species from TP14C culture (magnicifation 100X).
Drop-shaped body anterior and posterior flagella are visible.



Geographical distribution and abundance of the sequences matching TP14C culture representatives for the Surface layer of water: A)-Bodonidae; B) -unculture Choanoflagellate; C)-Neobodonid. Circle sizes correspond to abundance values, and color shows size fraction.

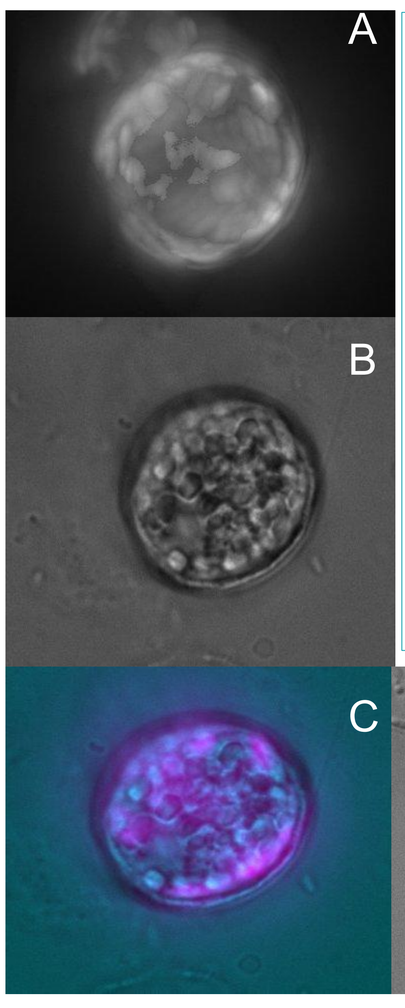


% identity	evalue	coverage	Accession number	Taxonomic group name (if known)
98.372	0.0	92%	MT355137.1	Bodonidae sp
98.596	0.0	100%	AY789788.1	.Uncultured Choanoflagellate
97.047	0.0	99%	MT355131.1	Neobodo sp.

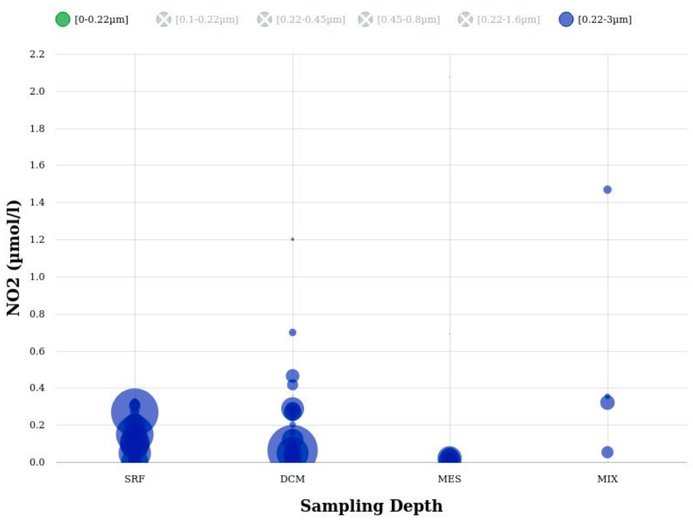
BLAST statistics for each contig obtained from “TP14C” culture sequences.

“*Prorocentrum minimum*” culture

Prorocentrum shikokuence may be one of the closest relatives of *Prorocentrum minimum*. They both may cause harmful water blooms in ocean.

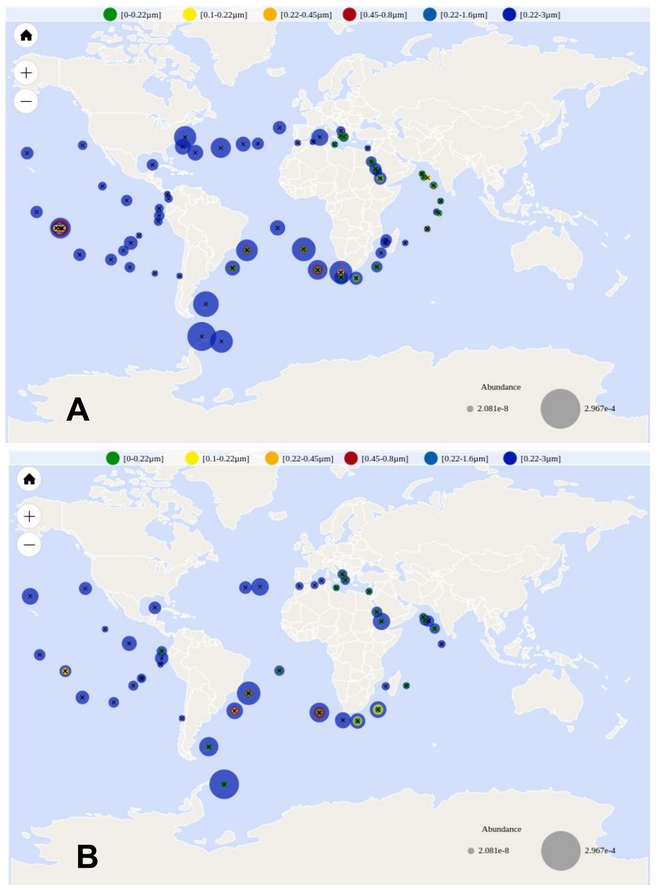


Brightfield microphotograph of *Prorocentrum minimum* - a very important player in ecology. **A)**-3D reconstruction shows its general shape at 100x magnification **B)**-*P. minimum* from above. Some organelles and membrane sacs can be seen. **C)**-The same image with the chlorophyl autofluorescence in pink; D)-A weird behavior of *Prorocentrum minimum*. This could be a reproductive or feeding behavior.



Prorocentrum Minimum requires Nitrogen for its growth. Correlation between available Nitrogen and the density of *P. minimum*. The less unavailable NO2, the higher density.

Geographical distribution and abundance of the sequences matching Proromin culture representative: A)-for the Surface layer of water; B) -for the Deep Chlorophyll Maximum layer. Circle sizes correspond to abundance values, and color shows size fraction.



% identity	evalue	coverage	Accession number	Taxonomic group name (if known)
99.83	100.0	0.00	FN598356.11	*Prorocentrum minimum*

Table 1. BLAST statistics for each contig obtained from “Prormin” culture sequences.

References and supplement available by the link