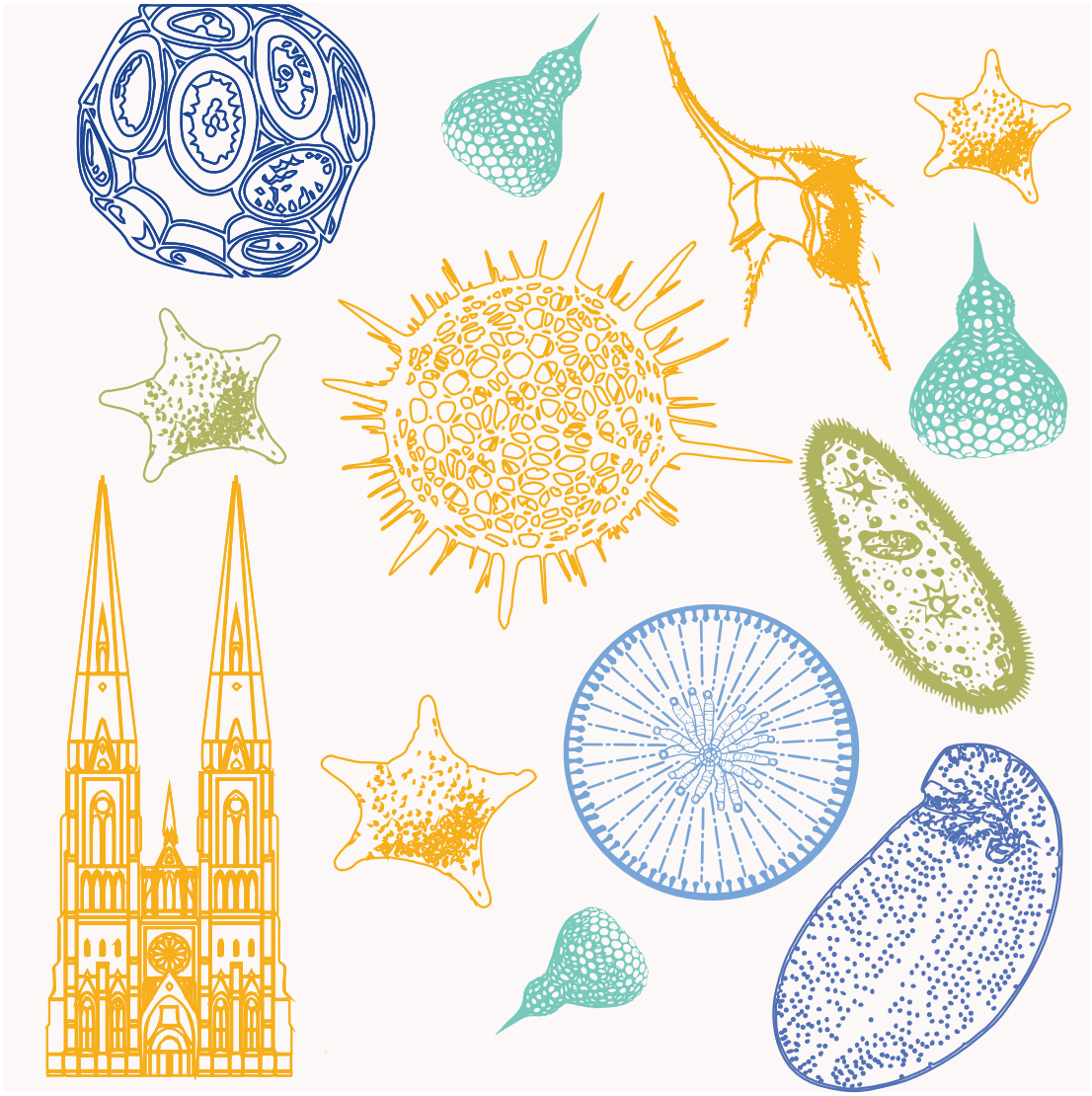

PROTISTOLOGY NORDICS UPPSALA 2024

Programme & Abstracts

2–3 May 2024

Dear Colleagues,

We are delighted to announce that Protistology Nordics 2024 will be held in Uppsala, providing an opportunity for scientists in Nordic countries to come together and showcase their research on the genetics, cell biology, ecology, and evolution of protists. Our aim for this meeting is to continue promoting a supportive environment for both early-career and advanced researchers to present their work and connect with others in the field.

A dedicated session on metagenomics will feature an invited talk by Ramiro Logares (Institut de Ciències del Mar, CSIC), and a mini-hands on workshop led by Jennah Dharamshi (Uppsala University & Institute of Evolutionary Biology, CSIC-UPF).

We are delighted to announce that Elisabeth Hehenberger (Biology Centre, Czech Academy of Sciences) will deliver the plenary talk. Fourteen talks, and twenty-seven flash talks will be delivered across three sessions.

SciLifeLab Planetary Biology is supporting Protistology Nordics 2024 by providing funds for general running costs, and organisational support. UiO:Life Science (University of Oslo) is supporting Protistology Nordics 2024 by providing funds for general running costs.

We look forward to seeing you all at Protistology Nordics 2024.

Kind regards,
The organising committee

Mahwash Jamy, Jennah Dharamshi, Annabella Aguilera, Fabien Burki, Micah Dunthorn, Courtney Stairs

UNIVERSITY OF OSLO

Contents

Organisers

Mahwash Jamy
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Swedish University of Agricultural Sciences
Uppsala
&
Natural History Museum
University of Oslo
Norway

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Travel to Uppsala

There are several ways to come to Uppsala. There is an airport and a train station in town.

Arriving by train

If your train brings you into Stockholm City station, it's easy to change to a commuter (SJ or Mälartåg) train and continue to Uppsala. They depart every 30 minutes throughout the day; check times and book your ticket at SJ.se or Malartag.se.

Arriving by air

Public transport runs between Uppsala and Arlanda airport frequently, and includes buses, trains and taxis. Depending on the mode of transport you use, it will take between 20 and 45 minutes to travel from Arlanda to Uppsala. You can also consult the Arlanda airport homepage for more information.

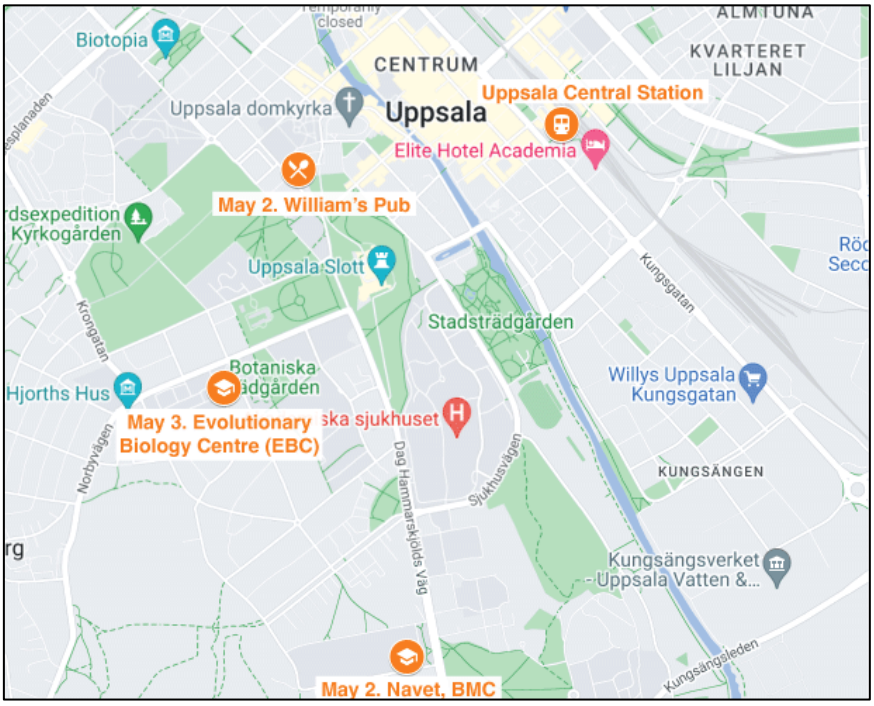
If you would like to take the train from Arlanda to Uppsala, you can purchase tickets at the tourist information and train ticket office at the airport. Taking the train will likely be the fastest way for you to travel. Tickets will cost approximately SEK 175. For more information, please the Uppsala local public traffic company UL's website.

You can also take bus number 801 from Arlanda to Uppsala, and will cost you approximately SEK 117.

You'll find taxis outside the arrival hall at Terminal 5 and outside Terminal 2. To pre-book your taxi, visit the website of Uppsala Taxi.

Premises

An overview of important locations for Protistology Nordics, Uppsala is shown below.



2 May

Workshop on metagenomics and methods in protistology
Navet, BMC, Uppsala University
Husargatan 3, Uppsala

A 35-minute walk from Uppsala Central Station or an 8-minute bus ride.

Directions: Google Maps

Pub Dinner
William's Pub
Åsgränd 5c, Uppsala

Directions: Google Maps

3 May

Symposium on Protist Cell Biology, Ecology, and Evolution
Friessalen, Evolutionary Biology Centre (EBC), Uppsala University
Norbyvägen 16, Uppsala

A 25-minute walk from Uppsala Central Station or a 15-minute bus ride.

Directions: Google Maps

Practical Information

Lunch and coffee and snacks will be provided on both days of the meeting.

In order to reduce plastic waste, we kindly ask all participants to bring their reusable water bottles or cups to fill with water at the venue.

Programme Overview

2 May

Workshop on metagenomics and methods in protistology

Navet, BMC, Uppsala University
Husargatan 3, Uppsala

8:45-9:00	Registration Early-career researchers only
9:00-10:30	Introductions by early-career researchers Early-career researchers only
10:30-11:00	Fika (coffee + snacks)
11:00-12:00	Invited talk on metagenomics
12:00-13:00	Lunch
13:00-14:30	Mini-workshop on metagenomics
14:30-15:00	Fika (coffee + snacks)
15:00-17:00	A presentation of infrastructure in Sweden for genomics, proteomics, and imaging

Pub Dinner

William's Pub
Åsgränd 5c, Uppsala

18:00-	Pub Dinner

3 May

Symposium on Protist Cell Biology, Ecology, and Evolution

Friessalen, EBC, Uppsala University
Norbyvägen 16, Uppsala

8:30-8:50	Registration
8:50-9:00	Welcome address
9:00-9:45	Plenary talk
9:45-10:15	Fika (coffee + snacks)
10:15-11:55	Contributed talks and flash talks
11:55-12:00	Group photo
12:00-13:00	Lunch
13:00-15:00	Contributed talks and flash talks
15:00-15:30	Fika (coffee + snacks)
15:30-17:35	Contributed talks and flash talks
17:35-17:45	Short break
17:45-18:00	Concluding remarks and best student talk awards

Detailed Programme

2 May

Workshop on metagenomics and methods in protistology

Navet, BMC, Uppsala University
Husargatan 3, Uppsala

The workshop will be open to all following the first session (i.e., 10:30 onwards).

8:45-9:00	Registration *Early-career researchers only*
9:00-10:30	Introductions by early-career researchers *Early-career researchers only*
10:30-11:00	Fika (coffee + snacks)

Invited talk on Metagenomics

11:00-12:00	**Ramiro Logares** (Institute of Marine Sciences, CSIC) *Population genomics of protists: insights from metagenomics*
12:00-13:00	Lunch

Mini-workshop on Metagenomics

13:00-14:30	**Jennah Dharamshi** (Uppsala University & IBE, CSIC) *Metagenomics is for eukaryotes too!*
14:30-15:00	**Fika** (coffee + snacks)

Research Infrastructure for Protistology

15:00-15:20	**Anabella Aguilera** (Planetary Biology at SciLifeLab) *Brief intro to SciLifeLab & Planetary Biology Capability*
15:20-15:50	**Charlotte Stadler** (Spatial Biology Platform) *Opportunities and services available*
15:50-16:20	**Sara Henriksson** (Molecular and Cellular Imaging Platform) *Opportunities and services available*
16:20-16:50	**Henrik Lantz** (National Bioinformatics Infrastructure) *Opportunities and services available*
16:50-17:00	**Anabella Aguilera** (Planetary Biology at SciLifeLab) **Mahwash Jamy** (Swedish University of Agricultural Sciences & University of Oslo) *Concluding remarks*

Pub Dinner

William's Pub
Åsgränd 5c, Uppsala

18:00-	**Pub Dinner**

3 May

Symposium on Protist Cell Biology, Ecology, and Evolution

Friessalen, Evolutionary Biology Centre (EBC), Uppsala University
Norbyvägen 16, Uppsala

8:30-8:50	Registration
8:50-9:00	Welcome address **Mahwash Jamy** (Swedish University of Agricultural Sciences, University of Oslo)

Plenary Talk

Chair: Fabien Burki

9:00-9:45	**Elisabeth Hehenberger** (Biology Centre, Czech Academy of Sciences) *Dinoflagellates - Never stop shopping for plastids*
9:45-10:15	**Fika** (coffee + snacks)

Contributed and Flash Talks 1

Chair: Courtney Stairs

10:15-10:30	**Anne Walraven** (Uppsala University) *Exploring the plastid symbiosis in the marine centrohelid Meringosphaera: a novel model system for plastid integration*
10:30-10:45	**Daniel Méndez-Sánchez** (Charles University) *Insights into the specificity of methanogenic archaeal symbionts of anaerobic ciliates (Ciliophora: Metopida)*
10:45-11:00	**Ondřej Pomahač** (Charles University) *The first case of intranuclear methanogenic symbiont in a eukaryote*

11:00-11:15 **Clarence Sim** (Nanyang Technological University)
Functional interactions of protists in the under-ice Arctic phytoplankton spring bloom

11:15-11:30 **Neea Hanström** (Stockholm University)
Hidden interactions of protist parasites in plankton food webs

11:30-11:35 **Hanna Strindberg** (University of Oslo)
Investigating the Nature of Interaction between Labyrinthulomycetes and Diatoms in Co-culture systems

11:35-11:40 **Isabelle Ewers** (University of Oslo)
A review of protistan parasites in & around Norwegian marine fish farms

11:40-11:45 **Zélia Bontemps** (Uppsala University)
Genome analysis of the novel Legionellales 'Ca. Pokemonas kadabra' and 'Ca. Fiscibacter pecunius' suggests an obligate relationship with their amoebal hosts, the rhizarian Fisculla

11:45-11:50 **Anders Alfjjorden** (Uppsala University)
Description of Hexamita nelsoni, a cryptic and neglected oyster and blue mussel parasite.

11:50-11:55 **Jon Jerlström Hultqvist** (Uppsala University)
A unique symbiosome in an anaerobic single-celled eukaryote

11:55-12:00 **Anna-Lotta Hiillos** (University of Oslo)
A prologue of a tripartite symbiosis: describing the diversity of gregarine-microsporidian symbiosis within polychaetes

12:00-12:05 **Emily Knott** (University of Jyväskylä)
Temporal shifts in abundance allow co-infection of three apicomplexan symbionts in a shared polychaete host

12:05-12:10 **Ioana Onut Brännström** (Uppsala University)
'Travelers in a suitcase'. Molecular perspectives from photobionts to understand lichens adaptation to environment

12:10-12:15 **Anne Winding** (Aarhus University)
Plant root compartment impacts bacterial food source of predatory soil protists

12:15-13:15 **Lunch**

Contributed and Flash Talks 2

Chair: Anna-Lotta Hiillos

13:15-13:30 **Paulina Arancibia** (University of Jyväskylä)
High-throughput experimental ecology: combining robotics with machine-learning to study protist communities

13:30-13:45 **Adrià Antich** (NORCE)
Teach me how and have it now: Prediction of the ecological impacts of Norwegian fish farms with Machine Learning approaches

13:45-14:00 **Julie Egelund Andersen** (Aarhus University)
Tillage regime affects the functional community structure of soil protists

14:00-14:15 **Nina Pohl** (Uppsala University)
Insights into Picozoa diversity through a metabarcoding and phylogenomic-resolved tree and associated cell morphology

14:15-14:30 **Anders K. Krabberød** (University of Oslo)
Identification of chimeric sequences in long-read amplicon data

14:30-14:35 **Daniel Vaulot** (University of Oslo)
Protist community structure in Singapore Strait based on full rRNA operon metabarcodes

14:35-14:40 **David Singer** (Changins)
Improving Soil Health and Plant Protection through Microbial Monitoring using Nanopore MinION sequencing

14:40-14:45 **Olav Myrann** (University of Oslo)
The effects of light and salinity on growth of spring bloom diatoms in the Oslofjord

14:45-14:50 **David U. Hernández-Becerril** (Universidad Nacional Autónoma de México)
Diversity and distribution of the eukaryotic picoplankton in the oxygen minimum zone of the tropical Mexican Pacific

14:50-14:55 **Marit Bjorbækmo** (Norwegian Institute for Water Research)
Characterising Oomycetes in Brown Algae along the Norwegian Coast (OOMYCOAST): An NBIC funded project

14:55-15:00 **Sandra Irén Bongo** (University of Oslo)
Labyrinthulomycetes; defining the taxonomy and ecology of a ubiquitous marine protist

15:00-15:05 **Sigurd Eliassen** (University of Oslo)
Investigating the phytoplankton spring bloom dynamics in the Oslofjord by single-species and common garden growth experiments

15:05-15:10 **Elise Nygård** (University of Oslo)
Exploration of community dynamics of protists during the autumn season through metabarcoding and intensive sampling

15:10-15:15 **Simon Kline** (University of Oslo)
Seasonal dynamics of the protist community in the Oslofjord and Skagerrak under change

15:15-15:20 **Yash Pardasani** (Uppsala University)

Unraveling novel Paulinella diversity to investigate the second primary endosymbiosis event

15:20-15:25 **Group Photo**

15:25-16:00 **Fika** (coffee + snacks)

Contributed and Flash Talks 3

Chair: Micah Dunthorn

16:00-16:15 **Allen Williamson** (Lund University)
Visualization of RNA transcripts within live eukaryotic cells

16:15-16:30 **Yevgeniya Wochinger** (Uppsala University)
CRISPR/Cas9 mutagenesis and encystation in Giardia intestinalis

16:30-16:45 **Adriana Lopes dos Santos** (University of Oslo)
Diversity of protists in the southwest Pacific Ocean: a synopsis from carbon production to export

16:45-17:00 **Sandra Baldauf** (Uppsala University)
Evidence for, and implications of, an excavate root for eukaryotes

17:00-17:05 **Ingrid Sætersdal** (University of Oslo)
Investigating how niches evolve in ciliates

17:05-17:10 **Zeynep Akdeniz** (Uppsala University)
The expanded genome of Hexamita inflata, a free-living diplomonad

17:10-17:15 **Alejandro Jiménez-González** (Uppsala University)
Metabolic analysis of Hexamita inflata, a free-living diplomonad

17:15-17:20 **Anaisa Moreno** (University of Zürich)
High-efficiency transfection of Acanthamoeba castellanii using a cationic polymer

17:20-17:25	**Ana Gomes** (NORCE) *Reconstructing the past functioning of the biological carbon pump: a sedimentary ancient DNA approach*
17:25-17:30	**Fredrik Klinghammer** (Lund University) *Taking the microfluidics approach to soil protistology*
17:30-17:35	**David Calvo Mora** (Uppsala University) *A three-dimensional assessment of chemical properties and microbial diversity in pore and ditch waters of three 20-year restored rich fens*
17:35-17:40	**Stefan Bertilsson** (Swedish University of Agricultural Sciences) *De novo assembled single-cell transcriptomes from aquatic phytoflagellates reveal a metabolically distinct cell population*
17:40-17:50	**Short Break**
17:50-18:00	**Concluding Remarks and Best Student Talk Prizes**

Invited Talks

Population Genomics of Protists: Insights from Metagenomics

Ramiro Logares[1]

[1]*Institute of Marine Sciences (ICM), CSIC, Barcelona, Spain*

Understanding the characteristics and structure of populations is fundamental to comprehending ecosystem processes and evolutionary adaptations. While the study of animal and plant populations has spanned a few centuries, microbial populations (including protists) have been under scientific scrutiny for a considerably shorter period. In the ocean, analyzing the genetic composition of protist populations and their adaptations to multiple niches can yield important insights into ecosystem function and the community response to global change. However, protist populations have remained elusive to the scientific community due to the challenges associated with culturing microbes in the laboratory. Today, advancements in metagenomics facilitate the investigation of protist populations. As an example, I will present our work on the population structure of a widespread, uncultured marine picoeukaryotic flagellate: MAST-4. We investigated the population structure and differentiation of four MAST-4 species across the global surface ocean. We identified distinct patterns of population-level genomic divergence by calling and analyzing Single Nucleotide Variants (SNVs). Some species exhibited differentiated clusters (potential populations) due to possible limitations in gene flow or adaptation to different niches. Temperature and salinity emerged as the primary factors structuring MAST-4 populations. We calculated the dN/dS ratio for MAST-4 genes to identify those that may represent the basis of differential adaptation between populations. We detected positively selected gene clusters in all MAST-4 species associated with genomic populations and oceanic basins. The analysis of the functional role of the genes under positive selection provided insights into the metabolic functions that have been the focus of selection.

Enjoyed this talk? Find the speaker here: ramiro.logares@icm.csic.es

Dinoflagellates - Never Stop Shopping for Plastids

Elisabeth Hehenberger[1]

[1]Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic

Dinoflagellates are a highly diverse, abundant and widespread group of unicellular eukaryotes with a number of fascinating characteristics, including for example the evolution of eye-like structures, elaborate feeding techniques or genomes so gigantic they are packed into permanently condensed, liquid-crystalline chromosomes. Also in regard to their plastids dinoflagellates follow a uncommon trajectory – their plastid evolution is likely the most dynamic of all plastid-bearing eukaryotes. Several dinoflagellate lineages have replaced their ancestral dinoflagellate plastid (the "peridinin" plastid) with new permanent plastids from various algal donors and the mechanism of kleptoplastidy – the stealing and transient retention of plastids from algal prey – is comparably frequent in dinoflagellates. One dinoflagellate family, the Kareniaceae, harbors permanent plastids derived from haptophytes and these plastids were initially considered to have originated once, in the ancestor of the family. Descriptions of new lineages, including kleptoplastidic taxa, and deep transcriptomic analysis of known lineages are beginning to contradict this idea, suggesting instead the Kareniaceae as a hub for plastid exchange. Here I will present our analyses of several members of this family and discuss how these results impact our view of plastid evolution.

Enjoyed this talk? Find the speaker here: elisabeth.hehenberger@paru.cas.cz

Contributed Talks and Flash Talks

(In alphabetical order. Speaker's name highlighted in bold)

The Expanded Genome of *Hexamita inflata*, a Free-living Diplomonad

Zeynep Akdeniz[1], Michal Havelka[2], Feifei Xu[1], Courtney W. Stairs[3], Alejandro Jiménez-González[1], Jon Jerlström-Hultqvist[1], Martin Kolisko[4], Jan O. Andersson[1], Jan Tachezy[2] and Staffan Svärd[1]

[1]*Department of Cell and Molecular Biology, Uppsala University, Uppsala, Sweden*
[2]*Department of Parasitology, Charles University in Prague, Czech Republic*
[3]*Department of Biology, Lund University, Lund, Sweden*
[4]*Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic*

Diplomonadida is a lineage of microaerophilic, flagellated protists with two nuclei, being part of the Fornicata group of Eukaryotes. Diplomonads either live as parasites of animals or free-living in low-oxygen environments. Genomic information has been generated from parasitic diplomonads like *Giardia intestinalis* and *Spironucleus salmonicida* but little is known about genomes of free-living diplomonads. We have generated the first reference genome of a free-living diplomonad, *Hexamita inflata*. The final version of the genome is fragmented (1241 contigs) but substantially larger (142 Mbp) than the parasitic diplomonad genomes. It encodes 79,341 proteins and many proteins are part of large gene families (e.g. leucine-rich repeat proteins, homeobox proteins and papain-like cysteine proteases). Interspersed repeats make up 34% of the genome (6083 LINEs, 2686 DNA transposons, and 59 LTR elements). The large expansion of protein-encoding capacity and the repetitive sequences are the major reasons to the large genome size. This well-annotated genome from a free-living diplomonad will be the basis for further comparative analyses of the Diplomonadida lineage.

Enjoyed this talk? Find the speaker here: zeynep.hitay.akdeniz@gmail.com

Description of *Hexamita nelsoni*, a Cryptic and Neglected Oyster and Blue Mussel Parasite

Anders Alfjorden[1], Jon Jerlström Hultqvist[2], Staffan Svärd[2], Fabien Burki[1]

[1]*Department of Organismal Biology (IOB), Uppsala University, Uppsala, Sweden*
[2]*Department of Cell and Molecular Biology (ICM), Uppsala University, Uppsala, Sweden*

Marine bivalves are filter feeders that constantly consume and filter a vast array microorganism, including protists. However, some protists can survive within the host's intestinal system, utilizing released resources or other microorganisms, and occasionally turn parasitic.

During histopathological surveillance of blue mussels (Mytilus edulis) and flat oyster (Ostrea edulis) in Sweden, we have recorded cryptic lesions over several years and in high prevalence. These lesions were widespread and possibly threatening the hosts health and defense against predation. Our investigations, based on histology, molecular phylogeny, fluorescence in situ hybridization, and cell cultivation revealed that the metamonad flagellate Hexamita nelsoniis likely responsible for the observed phenotype. The establishment of a culture of these flagellates has enabled a better understanding of the cell biology, life cycle, host interaction and potential host impact.

While Hexamita spp.was first detected in oyster over 100 years ago, its lifestyle in this host has remained unclear and no detection in blue mussels has been reported prior to our work. Earlier studies of this protist, mainly between 1950-1980, have failed to provide a clear picture of this organisms as parasite or commensal, leaving little or no interest thereafter for further investigations in relation to its oyster host. At this meeting, we will argue that H. nelsoniis a neglected parasite affecting both blue mussels and flat oysters, both commercially important in aquaculture. Further on our findings indicate that this parasite might be horizontally transmitted from adult to larvae via infections within gonad ovaries.

Enjoyed this talk? Find the speaker here: anders.alfjorden@ebc.uu.se

Tillage Regime Affects the Functional Community Structure of Soil Protists

Julie Egelund Andersen[1], Athanasios Zervas[1], Rumakanta Sapkota[1], Anne Winding[1]

[1]Department of Environmental Science, Aarhus University, Frederiksborgvej 399, 4000 Roskilde, Denmark

Protist community assembly and distribution in soil are mainly structured by deterministic environmental processes such as soil moisture, pH and nutrient availability rather than through stochastic events and passive dispersal. Nevertheless, the main drivers of the protist community structure remain poorly understood. Hence, we conducted a large-scale field experiment examining the diversity of soil protists to gain further insight into the impact of agricultural practices i.e. tillage regime (conventional tillage vs. no-tillage), use of different types of fertilizer (pig slurry vs. chemical fertilizers), and three different nitrification inhibitors on protist community composition. The experiments were carried out at a field location in Foulum, Viborg, Jutland, Denmark, with sampling in April, 2021. The genetic diversity of the protist community in 60 soil samples was assessed by metabarcoding of DNA using protist specific primers targeting the V9 region of the 18S rRNA gene. In an attempt to understand protists not just as species but as organisms with different properties, we assigned trait-based functional diversity to our taxonomic data by applying the framework by Giachello et al. 2019.

Considering all 60 samples we found diverse protist communities within the phyla of *Cercozoa*, *Amoebazoa* and *Cillophora* with a dominance of *Cercozoa*. Using PERMANOVA tests tillage, regime and choice of fertilizer were found to be vital factors in shaping the protist community structure and composition at genus level. Organically fertilized soil exhibited a distinct protist community signature compared to chemically fertilized soil. The trait-based approach revealed the functional diversity to be affected mainly by tillage regime. The relative abundance of endoparasitic protists was found to be increased in soil subjected to direct seeding. Consequently, an increase in the relative abundance of free-living protist consumers was observed in ploughed soil. This knowledge will help unravel the factors that shape the functional community structure of protists and will lead to a better understanding of the soil food web.

Enjoyed this talk? Find the speaker here: au730682@uni.au.dk

Teach Me How and Have It Now: Prediction of the Ecological Impacts of Norwegian Fish Farms with Machine Learning Approaches

Adrià Antich[1], Kristina Cermakova[2], Jan Pawlowski[2,3], Tristan Cordier[3]

[1]NORCE Climate and Environment, NORCE Norwegian Research Centre AS
[2]ID-Gene Ecodiagnostics, Plan-les-Ouates, Switzerland
[3]Institute of Oceanology, Polish Academy of Sciences, Sopot, Poland

Salmon aquaculture is a pillar industry in the present and future of Norway. However, the concerns over its ecological impacts, tied to the organic matter enrichment of fjord seafloor, must be addressed.

To comply with the objectives set by the Marine Strategy Framework Directive, Biotic Indices (BI) have been proposed (i.e. AMBI index, Borja et al., 2000). Such BI can be used to assess these impacts based on bio-indicator species and their ecological niche. However, this requires taxonomic expertise that is scarce, and samples processing time is very time demanding.

During the last decade, DNA metabarcoding has revolutionized biodiversity assessment by providing fast and efficient high-resolution data. Moreover, in recent years, machine learning algorithms have become increasingly popular for extracting ecological insights from massive eDNA datasets.

In our study, we examined sediment samples collected along gradients of impact in 28 farms spanning the entire Norwegian coast. Using the AMBI index as labels and DNA metabarcoding data as features, we trained Random Forest models to predict the AMBI-derived quality status based solely on the metabarcoding data. To assess the predictive performance, we employed a Leave -One-Out Cross- Validation strategy (i.e. by permuting all the samples of a given farm). Our results indicate a strong correlation between predicted and observed AMBI values. This demonstrates the effectiveness of machine learning models trained on DNA metabarcoding data in predicting environmental quality. Additionally, the analysis of DNA metabarcoding data enables us to pinpoint sequences of greater importance within the models, unveiling new DNA-based ecological indicators.

Enjoyed this talk? Find the speaker here: adan@norceresearch.no

High-Throughput Experimental Ecology: Combining Robotics with Machine-Learning to Study Protist Communities

Paulina A. Arancibia[1] & Otso Ovaskainen[1,2,3]

[1]Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland
[2]Organismal and Evolutionary Biology Research Programme, University of Helsinki, Helsinki, Finland
[3]Centre for Biodiversity Dynamics, Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway

Drivers behind community ecology are complex and thus difficult to fully understand. Though observational data describing ecological patterns is ample, experimental data lags behind since the high cost of experiments restricts their scale of inquiry and/or level of replication.

Current technological advances allow using robotics to run high-throughput experiments (HTE) with low manual effort, as well as automating pipelines to process data without major human effort. Despite this promise we show that automated HTE are still rare in community ecology, even if some automated methods are part of a study. We present a case study with aquatic protists where we showthe feasibility of running HTE coupling robotic microscopy and machine-learning to count and identify species. Albeit HTE are rarely used in community ecology, they present great potential, stilltheir use requires effort and the methods cannot be directly generalized among study systems.

Enjoyed this talk? Find the speaker here: paulina.a.arancibia@jyu.fi

Evidence For, and Implications of, an Excavate Root for Eukaryotes

Caesar Al Jewari[1] and **Sandra L. Baldauf**[1]

[1]*Department of Organismal Biology (Systematic Biology), Uppsala University*

Phylogenetic analyses of 183 eukaryotic proteins of archaeal ancestry place four major lineages of excavate taxa (formerly "Metamonada") at the base of the eukaryote tree - Parabasalia, Fornicata, Preaxostyla and Discoba (Al Jewari & Baldauf 2023). All four excavate branch points receive 100% support from analyses of the full data set, including a new protein structure-based phylogenetic model, and various controls for deep phylogeny artifacts. I will review these findings, present additional supporting data from outgroup-free rooting using random concatenation of paralogs (Al Jewari & Baldauf, unpub), and discuss some of the more problematic implications of these results.

Enjoyed this talk? Find the speaker here: sandra.baldauf@ebc.uu.se

De novo Assembled Single-Cell Transcriptomes from Aquatic Phytoflagellates Reveal A Metabolically Distinct Cell Population

Javier Florenza[1†], Aditya Jeevannavar[2], Anna-Maria Divne[3], Manu Tamminen[2], **Stefan Bertilsson**[4]

[1]*Department of Ecology and Genetics, Uppsala University, Uppsala, Sweden*
[2]*Department of Biology, University of Turku, Turku, Finland*
[3]*Science for Life Laboratory, Department of Cell and Molecular Biology, Uppsala University, Uppsala, Sweden*
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Single-cell transcriptomics has rapidly become a standard tool for decoding cell identity, fate and interactions in mammalian model organisms, but evidence of applicability to non-model, poorly understood microeukaryotes remains limited. In the present study, live *Ochromonas triangulata* cells from two distinct growth phases were FACS-sorted based on food vacuole and chlorophyll fluorescence, resulting in 744 full-length transcript single-cell libraries. Lacking a reference genome, an *ad-hoc* global transcriptome was assembled *de novo* and used as reference for read mapping. Clustering the expression profiles confirmed the two expected transcriptional states corresponding to each growth phase and revealed a third distinct expression cluster present in both growth phases. Combining sequence similarity and structural prediction strategies for annotation, this group showed increased response to oxidative stress and extensive downregulation of pathways associated with ribosome- functioning, CO_2 fixation and core carbohydrate catabolism, such as glycolysis, β oxidation of fatty acids and tricarboxylic acid cycle. However, further biological underpinnings could not be fully resolved since a major proportion of transcripts remained unannotated, highlighting poor microeukaryote representation in current annotation databases. As a supplementary feature, the identity of 88% of the cells could be verified using carry-over rRNA reads, demonstrating the value of rRNA spillover to assign taxonomy to expression clusters. In conclusion, we showcase the power of single cell transcriptomics for metabolic mapping of microeukaryotes for which reference resources might be limited and thereby highlight its potential as a tool to gain access to microeukaryote dynamics in natural communities.

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Characterising Oomycetes in Brown Algae Along the Norwegian Coast (OOMYCOAST): An NBIC Funded Project

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Oomycetes (Stramenopiles) are important pathogens and saprotrophs of a range of organisms, including seaweeds, both kelp and rockweed.

Despite their ubiquity and ecological importance, the species diversity and distribution patterns of marine oomycetes are largely unknown. DNA-based studies have revealed that oomycetes are an abundant and species-rich group of eukaryotes. Currently, there are >1200 named oomycete species, but it has been estimated that only around 10% of the global oomycete species diversity is known. New species are being discovered at a high pace, and the pathogenicity and host range of these novel taxa are often unknown. So far, we have very limited knowledge about marine oomycetes in Norway (zero marine coastal species registered in the Norwegian Biodiversity Information Centre Species Map), and we lack basic information about taxonomy and epidemiology of marine oomycete seaweed pathogens.

In the OOMYCOAST project we will provide a first assessment of the distribution and diversity of oomycetes associated with three common species of rockweed in the intertidal and upper subtidal along the Norwegian coast: Fucus vesiculosus, F. serratus and Ascophyllum nodosum.

We will use modern molecular methods (high-throughput short- and long-read DNA sequencing techniques) in conjunction with cultivation experiments and microscopy techniques to provide solid documentation of the morphology, physiology, taxonomy, and phylogenetic placement of novel rockweed-associated oomycetes. Through strategic field sampling along the Norwegian coast, we will cover the mainecoregions and environmental gradients, allowing us to obtain a comprehensiveoverview of the distribution and diversity of oomycetes associated with ecologicallyimportant rockweeds.

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Labyrinthulomycetes; Defining the Taxonomy and Ecology of a Ubiquitous Marine Protist

Sandra Irén Bongo[1], Daniel Vaulot[1,2], Bente Edvardsen[1], Adriana Lopes dos Santos[1]

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Marine protists constitute the bulk of eukaryotic diversity and play a crucial role in the marine ecosystem. However, they have been historically disregarded. One such group is the Labyrinthulomycetes, a class of heterotrophic stramenopiles that are ubiquitous in aquatic habitats. While most of them feed saprotrophically and serve as important decomposers of organic matter, they are mostly known for being parasites as they were responsible for the 1930s seagrass wasting disease outbreak.

To date, the diversity and taxonomy of Labyrinthulomycetes is still not well understood, despite a growing repertoire of publicly available datasets from metabarcoding. My research involves revising the taxonomic diversity within Labyrinthulomycetes by using over 1200 18S ribosomal RNA sequences available in the Protist Ribosomal (PR2) reference sequence database, together with around 1000 additional sequences available in GenBank. With the new reference, we will explore the global distribution of this group by re-assigning 1052 ASVs gathered from 6000 samples collected in the frame of the metaPR2 sequence database. This database consists of processed 18S rRNA metabarcodes annotated with the PR2 reference sequence database allowing the direct comparison of ASVs (Amplicon Sequence Variants).

By employing this new classification and incorporating metabarcodes from various regions, the geographical distribution of the different taxonomic groups can be investigated. This insight into the distribution of genetically similar clades and their respective habitats is vital for the understanding of their functional role in the oceanic ecosystem and is the first step in creating monitoring programs to detect invasive species and early outbreaks.

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Genome Analysis of the Novel Legionellales 'Ca. *Pokemonas kadabra*' and 'Ca. *Fiscibacter pecunius*' Suggests an Obligate Relationship with Their Amoebal Hosts, the Rhizarian *Fisculla*

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Host-symbiont associations have evolved many times and cover the entire mutualism-parasitism continuum. The order Legionellales (Gammaproteobacteria) is particularly remarkable because all its known species have an intracellular lifestyle. Amoebae of the eukaryotic supergroup Amoebozoa are their most common hosts. Recently, it was discovered that unrelated amoebal taxa such as the genus Fisculla (supergroup Rhizaria) also host novel species of Legionellales closely related to genera like Ca. Berkiella and Aquicella. The nature of the interaction between these Legionellales species and their rhizarian hosts remains unknown. To understand the nature of these interactions, the amoeba Fisculla terrestris was sampled, enriched by cell sorting, and subsequently, we established two novel genomes ('Ca. Pokemonas kadabra' and 'Ca. Fiscibacter pecunius'). Compared to other Legionellales species, these new genomes are relatively small (1.65 and 2.45 Mbp, respectively) with a low GC content (38.9% and 43.6%, respectively). Only a few flagellar-related protein homologs were found, however host-symbiont interaction genes were found in both species, with a near-complete type IVB secretion system and effector protein homologs. Taken together, this study suggests that these novel genomes encode for a likely functional infection machinery and belong to non-motile, obligate intracellular symbionts.

Enjoyed this talk? Find the speaker here: zelia.bontemps@imbim.uu.se

A Three-Dimensional Assessment of Chemical Properties and Microbial Diversity In Pore and Ditch Waters of Three 20-Year Restored Rich Fens

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Peatlands sequester and store significant amounts of carbon, given their relatively small coverage of only 3% of the global land surface. Their drainage results in increased greenhouse gas emissions, nutrient leaching and downstream export, and a homogenization of the microbial community composition and their functions. Although the popularity peatland rewetting is recently gaining, there is still much to be done to assess its effects.

This project focuses on the chemical and microbial characterization of pore and ditch waters from three rich fens that were partially restored 20 years ago. Preliminary results discard a hypothetical rewetted-drained continuum, and reflect significant site and seasonal variations. The distance from the ditch did not affect the chemical properties, contrary to what was expected. However, rewetting does have an impact on water chemistry, as does the clear-cut treatment, although the results cannot be directly compared to the natural control. An expected chemical gradient in depth was found, for instance, regarding dissolved organic and inorganic carbon.

The next phase of the project involves including the results from the Illumina MiSeq 16S rDNA amplicon sequencing of 212 samples. It is theorized that microbial communities will have responded to this variability in water quality, and the gradients will likely contribute to a higher microbial diversity in the rewetted transects. This project aims to view ditch water from a peatland's outlet as an indicator of the peatland's quality. In the future, this approach could provide a practical and efficient method for monitoring and assessing the rewetting process.

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Diversity of Protists In the Southwest Pacific Ocean: a Synopsis from Carbon Production to Export

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The biological carbon pump (BCP) is the transfer of photosynthetically fixed organic carbon from the surface to the deep ocean by particle sinking, vertical mixing, and active transport by larger organisms. The BCP is fundamental for the functioning of marine ecosystems. During this talk, we will discuss the link between the diversity of protists in the southwest Pacific Ocean and two main components of the BCP – the primary production and particle sinking. In the first study, we aimed to better understand the relationship between cell size and diversity of small phytoplankton and productivity. We directly quantified the cell-specific productivity of distinct small phytoplankton populations through 24-hour in-situ radiolabelled (^{14}C-sodium bicarbonate) incubations coupled to flow cytometry sorting and community analysis through DNA metabarcoding. In the second study, we characterised the diversity of microbial eukaryotes that is exported from the surface to the sediment via passive sinking of particulate organic matter (POM). Our results have contributed to increase the knowledge about the biological composition and ecological interactions among that produce and transform sinking POM throughout the water column.

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Investigating the Phytoplankton Spring Bloom Dynamics In the Oslofjord by Single-Species and Common Garden Growth Experiments

Sigurd Skaar Eliassen[1], Simon Hasselø Kline[1], Tom Andersen[1], Bente Edvardsen[1]

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During the last 15 years there has been a gradual decrease of diatom concentration during the spring bloom in the inner Oslofjord. A part of this decrease may be explained by decrease of the concentration of the genus *Skeletonema*, while other important diatom genera such as *Chaetoceros* and *Thalassiosira* have maintained a stable concentration since 2006. The reason for this is unclear.

Possible causes for these changes include changes of the physical or chemical conditions. One potential driver of the observed changes can be coastal darkening due to climate change leading to shifting phytoplankton dynamics in the inner Oslofjord. An increase of light absorbing molecules of dissolved coloured organic matter (cDOM) transferred to the fjord from river input could reduce the growth of phytoplankton and potentially also shift the spring bloom to a later date. Under conditions with limited light, the phytoplankton taxa with the ability to grow at the lowest light intensity may be able to outcompete other competitors for the available light.

The aim of this master's project is to get a better understanding of the dynamics of important spring bloom diatoms in the Oslofjord and possible explanations to the observed changes. By single-species growth experiments I will determine the compensation light intensity of strains of two diatom genera *Chaetoceros* and *Thalassiosira* grown at different salinities. In a common garden experiment strains of the two diatom genera will be grown together with a strain of *Skeletonema* under different light conditions, to determine their competitive ability to limiting irradiance.

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A Review of Protistan Parasites In & Around Norwegian Marine Fish Farms

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Marine fish farming is continually increasing along the Norwegian shores. While this industry has a socioeconomic benefit, it is significantly impacting environmental health. One aspect of this environmental health is the diversity and distribution of parasites. My project focuses on the different protistan parasites that occur in and around Norwegian fish farms and I am currently reviewing previous research. The different aspects of this review include the main protistan parasite taxa infecting farmed fish in Norway. The different lifestyles of theseparasite groups give insights into ecosystem status in that area, as the occurrences of internal or external parasites, monoxenous or heteroxenous parasites, and generalists or specialists are indications for healthy or stressed environments. Here I present the initial findings of my review, in which I found farmed fish to be infected by taxa of the Amoebozoa, Ciliophora, Diplomonadida, Kinetoplastida, Microsporidia, and Perkinsozoa. Feedback about potential protistan parasites that are missing is greatly appreciated.

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Reconstructing the Past Functioning of the Biological Carbon Pump: A Sedimentary Ancient DNA Approach

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The biological carbon pump (BCP) is a major component of the oceanic carbon cycle, contributing to the downward transport of organic matter and the sequestration of carbon dioxide (CO_2) in the deeper layers of the ocean for hundreds of years. The BCP plays a pivotal role in regulating Earth's climate system, yet uncertainties persist regarding the potential impact of ongoing climate changes on its strength. Filling this gap is crucial as a weakened BCP represents positive feedback on atmospheric CO_2 levels and amplification of global warming.

Understanding the functioning of the BCP during past warm and cold climates can provide valuable insights into its past dynamics, shedding light on its potential future behaviour. The efficiency of the BCP is closely tied to plankton community composition, with differences in size, morphology and elemental composition shaping the quality, quantity and speed of sinking particulate organic carbon (POC) reaching the seafloor. Recently, sinking plankton DNA signatures within deep-sea sediments were found to explain variations of POC flux reaching the seafloor in the modern ocean. This opened the possibility of using the plankton fraction of sedimentary ancient DNA (*seda*DNA) to reconstruct past POC flux from the geological record. I will present how we plan to develop *seda*DNA as a proxy for POC flux by using a global-scale surface-to-seafloor eukaryotic DNA dataset alongside *seda*DNA from sediment cores. Additionally, I will share preliminary results obtained with DNA metabarcoding (targeting the 18S-V9 region) that reveal shifts in plankton and benthic diversity in response to past climate variations.

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Hidden Interactions of Protist Parasites In Plankton Food Webs

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Plankton communities comprise a vast diversity of taxa, and their interactions in the aquatic food webs are immensely complex. Current research highlights that not only feeding interactions but also symbiotic ones are prominent in plankton communities. However, plankton parasites are often not included in current food web models and ecological studies. Parasites play a remarkable role in trophic transmission and contribute to global biogeochemical cycles. The role of plankton parasites in the food web interactions has been often overseen because the parasites are hiding inside the body cavities and guts of their hosts, and consequently, are often difficult to detect. We studied host-specific parasite infections in marine crustacean zooplankton, using DNA metabarcoding of 18S rRNA sequencing in different habitats along the Baltic Sea salinity gradient. The hypothesis was that the putative protist parasites in the order of Syndiniales have species-specific interactions with different zooplankton species. We discovered high Syndiniales infection rates in designated zooplankton taxa, which differ between hosts and habitats. To link the interactions with the environmental factors we test if eutrophication, oxygen concentration, salinity or host density affect these results. Further analysis using flow cytometry and fluorescent in situ hybridization (FISH) will provide more insight into the importance of the zooplankton-parasite interactions on the marine ecosystem functioning.

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Diversity and Distribution of the Eukaryotic Picoplankton In the Oxygen Minimum Zone of the Tropical Mexican Pacific

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The study of eukaryote picoplankton ecology in oxygen minimum zones (OMZ) is crucial to understand primary production and trophic dynamics of the plankton. We investigated the diversity and distribution of the eukaryote picoplankton in two locations in the oxygen minimum zone of the tropical Mexican Pacific using metabarcoding and flow cytometry. We discovered highly diverse and complex communities, including the finding of Chloropicophyceae and Prasinodermophyta, as well as expected groups of Chlorophytes (Mamiellophyceae) and Ochrophytes (Chrysophyceae, Dictyochophyceae and Pelagophyceae). Other non-photosynthetic groups were also relatively abundant such as Syndiniales, Radiolaria, and Sagenista. Differential distributions of picoeukaryotes were detected along the water column and almost exclusive communities were found at each depth. The photosynthetic Mamiellophyceae were abundant at the surface and subsurface layers, whereas other photosynthetic, mixotrophic and non-photosynthetic groups such as Syndiniales (parasitic dinoflagellates), Radiolaria, Ochrophyta and Sagenista (MAST's groups) dominated the oxyclines. The picoeukaryote diversity was higher at the oxycline layers than in the surface and subsurface layers. These results allow us to confirm that oxygen concentration is an important factor driving microbial distribution and, particularly the oxyclines act as specialized niches promoting high picoplankton diversity and various trophic strategies related to autotrophy, mixotrophy, heterotrophy (predation) and parasitism.

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A Prologue of a Tripartite Symbiosis: Describing the Diversity of Gregarine- Microsporidian Symbiosis Within Polychaetes

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Symbiotic interactions have played a significant role in ecological and evolutionary processes through the history of life and have ultimately shaped the biodiversity we witness today. Still many fundamental questions, particularly concerning interactions between micro-organisms (e.g., protists) and their hosts, remain unresolved. Marine polychaete worms (Annelida), key organisms in benthic ecosystems, are hosts to protistan symbionts (gregarines), which themselves are infected my microsporidian parasites, forming a tripartite symbiosis. However, it is unclear how common this tripartite interaction is in nature and its diversity and function in benthic ecosystems is still unknown. Using a metabarcoding approach targeting both symbiont taxa within two polychaete species, as well as environmental samples around the Kattegat-Skagerrak region of the Baltic Sea, we aim to unravel the diversity and occurrence of this tripartite symbiosis.

Gregarine and microsporidia species composition and diversity is described and compared between host species and environmental factors. Generally, because most of the gregarine and microsporidian diversity in marine hosts is still unknown, we expect new species in both groups to be discovered. This study is a first step towards disentangling what consequences the tripartite symbiosis has on the functioning of benthic ecosystems. Given the ecological importance of polychaetes, gregarines could potentially reduce polychaete populations size and therefore impact the functioning of benthic communities and other marine fauna that is dependent on these animals. Consequently, microsporidians could play an important role in controlling the possible antagonistic interaction between polychaetes and gregarines.

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A Unique Symbiosome In an Anaerobic Single-Celled Eukaryote

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Symbiotic relationships between eukaryotes and prokaryotes played pivotal roles in the evolution of life and drove the emergence of specialized symbiotic structures in animals, plants and fungi. The host-evolved symbiotic structures of microbial eukaryotes – the vast majority of such hosts in nature – remain largely unstudied. Here we describe highly structured 'symbiosomes' within three free-living anaerobic protists (*Anaeramoeba* spp.). We dissected this symbiosis using complete genome sequencing and transcriptomics of host and symbiont cells coupled with fluorescent *in situ* hybridization, and 3D reconstruction using focused-ion-beam scanning electron microscopy. The emergence of the symbiosome was underpinned by expansion of gene families encoding regulators of membrane trafficking and phagosomal maturation and extensive bacteria-to-eukaryote lateral transfer. The symbionts reside deep within a symbiosomal membrane network that enables metabolic syntrophy by precisely positioning sulfate-reducing bacteria alongside host hydrogenosomes. Importantly, the symbionts maintain connections to the *Anaeramoeba* cell surface, blurring traditional boundaries between ecto- and endosymbiosis.

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Metabolic analysis of *Hexamita inflata*, a free-living diplomonad

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Parasites are viewed as organisms highly adapted to their specific niches due to the loss and gain of functions and modification of traits present in the free-living ancestor. As such, this adaptation is assumed to be a one-way process. However, this paradigm has been questioned because there are examples of free-living species with parasitic ancestors. In recent years, *Trepomonas* sp. PC1, a member of the diplomonads, has been described as one such example.

Here, we used genomic data to analyse the metabolism of *Hexamita inflata*, a free-living diplomonad closely related to *Trepomonas* sp. PC1. Our analysis shows glycolysis as the primary pathway to produce pyruvate and energy, as in all studied diplomonads. As a complement, *H. inflata* can also obtain energy from arginine through the arginine deiminase pathway. Unlike parasitic diplomonads, *H. inflata* can synthesise dNTP *de novo*. It encodes the most complex oxygen and arsenic detoxification systems in diplomonads because of the retention of ancestral reactions and the acquisition of new ones. It can also degrade complex carbohydrates that are inaccessible to other diplomonads. (e.g. cellulose or lichenin). The location prediction of many of the degradation enzymes (i.e. carbohydrate-active enzymes, peptidases and DNAses) shows signalling to be secreted or anchored in the cytoplasmic membrane.

We propose a feeding model in which food particles and bacteria prey are predigested before engulfment. This could represent an intermediate stage between the feeding mode of host-associated diplomonads in the animal intestine and the phagotrophy of whole cells usually observed in free-living protists.

Enjoyed this talk? Find the speaker here: alejandro.jimenez@icm.uu.se

Seasonal dynamics of the protist community in the Oslofjord and Skagerrak under change

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Aquatic protists and prokaryotes compose approximately 90% of the biomass in the oceans, and any changes within these communities are likely to have reverberating effects throughout marine ecosystems. Due to climate change, coastal marine systems are experiencing increased freshwater discharge from land and a darkening of the water column along with changes in nutrient concentration. The phenology is also undergoing changes, with the spring bloom of phytoplankton in the inner Oslofjord beginning on average two weeks later than 15 years ago. The cause of this delay is not currently known and is contrary to global trends of shifting dynamics leading to earlier spring blooms. In this study we examine the diversity andseasonal dynamics of the protist community through metabarcoding in the inner and outer Oslofjord, along with possible changes over the last 14 years. Monthly plankton samples were collected at three localities during 2023, similar to previously obtained samples from 2009-2011. These, along with samples collected through intensive sampling during the spring bloom of 2023 and 2024, will be sequenced by current high-throughput sequencing technology (Ilumina). Various environmental parameters have been measured, includingCDOM (colored dissolved organic matter). We ask whether browning or other other physico-chemical conditions can explain the delayed spring bloom and other observed changes in theprotist community in the Oslofjord and Skagerrak.

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Taking the Microfluidics Approach to Soil Protistology

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Protists play a key role in the functioning of terrestrial ecosystems, including soil carbon sequestration and plant productivity. In soils, protists indirectly drive carbon and nutrient cycling by consuming microbial heterotrophs, such as bacteria and fungi. Microbiology, including the isolation of protists and co-cultivation with their prey, has been historically used to explore protist ecology and physiology, but is inherently limited by the difficulty of cultivating protists *in vitro*. More recently, high-throughput amplicon sequencing of protist DNA barcodes has allowed for in-situ descriptions of soil protist community diversity and composition. Yet, inferring protist functions on sequencing-based approaches remains limited due to the lack of information on protist lifestyles and interactions. To tackle this challenge and bridge the gap between protist physiology and ecology, we propose the use of a microfluidics approach in the study of soil protists, enabling soil microscopy in complex environments. These microfluidic matrices, called "Soilchips", allow for the observation of protists under soil-like conditions along with their intra- and inter-microbial kingdom interactions in ecologically relevant systems. Here, we provide Soilchip-based documentation of protist behavior, involving protist-protist, fungal-protist, and protist-microfauna interactions. Ultimately, this will help in characterizing soil protist lifestyles and functional groups, connecting them with ecological data, and linking them to global processes.

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Temporal Shifts In Abundance Allow Co-Infection of Three Apicomplexan Symbionts In A Shared Polychaete Host

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Obligate symbionts are dependent upon resources provided by their host, raising the possibility of competition among species co-infecting the same host individual. We investigated dynamics of the apicomplexan community infecting the polychaete *Pygospio elegans* sampled from four sites at five different times of the year. The prevalence of infection in populations and infection loads per individual were determined for three targeted species: *Rhytidocystis* sp., *Selenidium pygospioins* and *Polyrhabdina pygospionis* using specific droplet digital PCR assays. To assess how different abiotic factors jointly determine the prevalence and infection load of the symbionts, we applied joint species distribution modelling with the framework Hierarchical Modelling of Species Communities (HMSC).

Host population was the most important variable explaining symbiont prevalence and associations between the different symbiont species. However, the variation in infection loadwas mostly captured at the level of hosts, so that some hosts had high loads and others not.Abiotic variables did not have a large role in explaining the variation. The species co-occurred negatively in time, so that if one of the species was abundant in one sampling month, it was likely that the other species would not simultaneously occur in high abundance.

Apicomplexan symbionts co-infecting *Pygospio elegans* are likely to be found from the same populations, but at different times, suggesting that temporal shifts in abundance can reduce competition for resources provided by the host. The role of the population (site) in explaining prevalence raises the question of possible vectors in the local benthic community.

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Identification of Chimeric Sequences In Long-Read Amplicon Data

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Environmental sequencing is a valuable tool for mapping and discovering protist diversity. Short-read Illumina sequencing, the standard approach for the past decade, has limited phylogenetic and taxonomic signals. Long-read sequencing techniques are gaining popularity as they allow the sequencing of extended regions (10-15 kilobases) with stronger phylogenetic signals.

Two types of errors are common in long-read amplicon data: PCR/sequencing mutations and chimeric sequences. Chimeric sequences occur when two or more DNA templates are fused into one during the PCR process. Longer-read amplicons will likely have a higher incidence of chimeric sequences due to the higher number of homologous regions where the templates can hybridize. While denoising or clustering methods similar to those used for shorter reads can correct point mutations, no good tools are explicitly designed for detecting chimeras in long-read amplicons.

We are developing new ways of detecting chimeras in long-read sequences by modifying the current algorithms implemented in VSEARCH, testing the effect of more than two parental sequences, and checking for a higher number of sequence partitions. As test data, we have amplicons from 144environmental soil samples, and two mock communities sequenced using PacBio Sequel II (HiFi-reads). With the new approach, we detected a significant proportion of chimeric sequences with multiple chimeric breakpoints in the mock community. The proportion of chimeric sequences was lower in the environmental samples. However, we could still identify a considerable portion of chimeras, with a noticeable effect of assuming more than two parental sequences.

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Insights Into the Specificity of Methanogenic Archaeal Symbionts of Anaerobic Ciliates (Ciliophora: Metopida)

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Symbioses between prokaryotes and protists are quite common and represent the key factor in the adaptation of some eukaryotic lineages to obligate anaerobic lifestyle. Anaerobic ciliates, like other anaerobic protists, have adapted to anoxia by transforming their mitochondria and making syntrophic relationships with methanogenic archaeal symbionts to yield an efficient energetic metabolism producing methane as a waste product, an important greenhouse gas. Although methanogenic archaea have been found in symbiotic interactions with various anaerobic ciliates, little is known about their diversity. We studied the methanogenic archaeal symbionts of 54 strains of ciliates belonging to 33 species, mainly metopids (Ciliophora, Armophorea, Metopida), using Sanger and Illumina amplicon sequencing of the 16S rRNA gene, as well as autofluorescence, FISH, and TEM. Analyses of the microbiome data demonstrated that these ciliates almost always harbor a single dominant methanogenic archaeon belonging to either genus Methanobacterium or Methanoregula in freshwater ciliate metopids, and Methanocorpusculum in marine strains. Furthermore, there is a degree of specificity at the host genus level, as well as at the ecology level. Additionally, co-cultivation experiments demonstrated that ciliates that harbor a particular methanogen do not exchange their symbionts over time when co-cultivated with another ciliate species that hosts a different methanogen. Altogether, our study suggests that anaerobic ciliates maintain strong specificity and fidelity for a particular methanogen genus via vertical transmission during cell division, but that horizontal replacements, potentially from the environment, also occur, suggesting a mixed transmission mode.

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High-Efficiency Transfection of *Acanthamoeba castellanii* Using a Cationic Polymer

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The free-living amoeba *Acanthamoeba castellanii* is an ecologically, clinically, and evolutionary significant microorganism ubiquitous in a variety of environments. *A. castellanii* can be pathogenic to humans causing a severe eye infection potentially resulting in visual impairments or the rare but lethal granulomatous amoebic encephalitis. In the environment, *A. castellanii* may act as a predator or host for many bacteria, fungi, and viruses. While it plays an important role in the regulation of these microorganisms, it can also serve as a reservoir and training ground for pathogens such as *Legionella*, *Mycobacterium*, and *Cryptococcus*.

Despite their importance, no reliable genetic manipulation system has been developed, hampering the use of *A. castellanii* and related species as model organisms. Transfecting *A. castellanii* is possible with commercial kits but is expensive, inefficient, and vulnerable to product discontinuation. In this contribution, we present a method for efficient transfection of *A. castellanii* with readily available and inexpensive cationic polymers – polyethylenimines. We systematically explore the parameters of the method, obtaining up to 100-fold higher transfection efficiency than currently used reagents. The method presented here provides a robust step towards a full genetic toolbox for *A. castellanii*, hence expanding its use as a model organism.

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The Effects of Light and Salinity on Growth of Spring Bloom Diatoms In the Oslofjord

Olav Myrann[1], Simon Kline[1], Tom Andersen[1] and Bente Edvardsen[1]

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Diatoms dominate during the spring bloom in the Oslofjord, with members of the genera Skeletonema, Thalassiosira and Chaetoceros being among the most abundant. During the last 15 years there has been a recorded reduction in Skeletonema abundance during the spring bloom in the Inner Oslofjord, that may explain an overall reduction of diatom abundance. In comparison the concentration of other diatoms such as Chaetoceros spp. has remained stable in the same period. The concentration of dinoflagellates has remained stable in contrast to the decrease of diatom concentration since 2006.

The main aim of this master's project is to obtain further knowledge on the abiotic factors affecting the important spring bloom diatom genera Skeletonema, Chaetoceros and Thalassiosira, and how environmental changes may affect their growth. To reach these aims, two different growth experiments using diatom cultures isolated from the spring bloom in the Oslofjord will be employed, measuring: 1) cell growth of Skeletonema and Chaetoceros under varying salinity and light intensity when held separately, and 2) cell growth in Skeletonema, Thalassiosira and two different species of Chaetoceros, conducting a common garden growth experiment. In the common garden growth experiment the abiotic factor that will be studied is salinity. The project will give insight into how salinity and light intensity affect the growth of specific spring blooming diatoms, and how salinity affects such diatoms when in a shared environment.

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Exploration of Community Dynamics of Protists During the Autumn Season Through Metabarcoding and Intensive Sampling

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Increasing anthropogenic stressors and climate change are contributing to shifts in phytoplankton communities, which has significant consequences for marine ecosystems and biogeochemical cycles. Temperate regions, like Southern Norway, have distinct spring and autumn phytoplankton blooms which often differ in species composition due to variations in temperature, salinity, and light conditions, as well as nutrient availability. However, the autumn bloom is less explored than the spring bloom. Traditionally, phytoplankton monitoring programs collect samples once a month in the production period, this frequency may miss important aspects of population dynamics and species composition, especially during periods with rapid environmental and biological changes.

This master's project aims to get a better understanding of the phytoplankton autumn bloom in the Oslofjord. We intend to see if there is a difference in the composition and timing of the autumn bloom between the inner and outer Oslofjord, and whether frequent sampling reveals more of the changes over time and locality than traditional monthly sampling. This will be done through water sampling twice a week for about a month (mid-September to mid-October) in 2023 and 2024 from two marine sampling stations. The community diversity and composition will be examined by high throughput Illumina sequencing (using the V4 region of the 18S rRNA gene). Environmental factors, such as chlorophyll-a concentrations, along with nutrient and cDOM will be measured.

Preliminary results from 2023 indicate differences in chlorophyll-levels between the stations, as well as temporal differences. Additionally, there appears to be weekly fluctuations in chlorophyll levels even with frequent sampling.

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‘Travelers In a Suitcase’: Molecular Perspectives From Photobionts to Understand Lichens Adaptation to Environment

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Lichens are complex and dynamic symbiotic entities formed by fungi, bacteria and algae (photobionts). They are key organisms for alpine, tundra, and taiga biomes and famous for their tremendous ability to adapt to the harshest conditions on Earth (intense UV light, extreme cold) or even extraterrestrial conditions. Yet, lichens are extremly vulnerable to pollution, while recent study indicates that cold adapted lichens are affected by the recent global warming.

The underlying mechanism of lichen adaptation, encompassing their broad distribution, resilience to extreme conditions, and simultaneous sensitivity to pollution and high temperatures, remains largely unknown but appears to be connected with the photobiont genotype. The project is built upon my conceptual model - the ‘Suitcase hypothesis’ - which suggests that lichen thalli can be viewed as ‘suitcasesä where photobiont genotypes with varying adaptive abilities coexist within the same thallus, with their frequencies changing in response to environmental conditions.

The aim of this project is to understand how photobiont communities living within lichens respond to different environmental conditions, such as seasonal changes or pollution. To address our questions, we utilize a combination of genetic data, digital PCR, and state-of-the-art microscopy. Preliminary results indicate that photobiont communities within lichens fluctuate with seasons and are highly affected by pollution.

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Unraveling Novel *Paulinella* Diversity to Investigate the Second Primary Endosymbiosis Event

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All plastids were once thought to derive directly or indirectly from a single primary endosymbiosis between cyanobacteria and a heterotrophic ancestor of Archaeplastida, a life-changing event that took place more than a billion years ago. We now know that primary plastids have also been established by a second, independent primary endosymbiosis in the rhizarian genus *Paulinella*. While much less consequential for the evolu'on of life on our planet, this second primary endosymbiosis represents a goldmine for our understanding of plastid evolution because it occurred much more recently than in Archaeplastida, around 100 million years ago. Thus, *Paulinella* potentially provides a unique opportunity to find and study close relatives of the lineages that engaged in primary plastid endosymbiosis. So far, however, only very few heterotrophic *Paulinella* have been studied, and almost no molecular data exist. We present here a survey of *Paulinella* diversity combining metagenomic datasets, as well as short and long-read metabarcoding, obtained using specific primers, from various locations worldwide. This targeted approach revealed important novel diversity and provides key information on geographic distribution and habitat preference of these amoebae. It also represents a first attempt to place the photosynthetic *Paulinella* into a more robust phylogenetic framework of environmental diversity. In the future, we will use this framework to target the isolation and culturing experiments of heterotrophic *Paulinella* to study their cell biology and genomes.

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Insights Into Picozoa Diversity Through a Metabarcoding and Phylogenomic-Resolved Tree and Associated Cell Morphology

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Archaeplastida encompasses a vast diversity of photosynthetic organisms, which acquired photosynthesis through the incorporation of a cyanobacterium into their metabolic processes. While it's widely believed that this occurred in a single event within their common ancestor, recent studies on a heterotrophic flagellate phylum belonging to Archaeplastida, the Picozoa, have complicated this assumption. These studies revealed a lack of any evidence of plastids within Picozoa, prompting questions regarding the evolution of plastids within Archaeplastida.

Despite the significance of Picozoa in addressing this question, there has been only one formal species description since its discovery in 2007, leaving the extent of Picozoa diversity unclear. Through a comprehensive phylogenetic analysis of metabarcoding data, we identified a substantial molecular diversity of Picozoa distributed across five well-defined environmental clades. Further phylogenomic analyses involving metagenome assembled genomes (MAGs) and single cell assembled genomes (SAGs) confirmed the higher level phylogeny of Picozoa observed in the metabarcoding data. To corroborate that the clades in the trees coincide, we employed a recently proposed approach linking MAGs to metabarcoding sequences based on their abundance variations across all samples.

Having gained insight into the molecular diversity, our next objective was to connect these sequences to cellular morphologies using clade-specific CARD-FISH (Catalyzed Reporter Deposition-Fluorescence in situ Hybridization) assays. In an initial experiment, we designed a probe spanning three clades and successfully captured the first images of a variety of Picozoa.

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The First Case of Intranuclear Methanogenic Symbiont In a Eukaryote

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Anaerobic freshwater ciliates represent an ecologically important and phylogenetically diverse guild among protists. They typically harbor archaeal methanogenic endosymbionts in their cytoplasm. This applies for the most studied freshwater anaerobic ciliate group belonging to the class Armophorea, with the typespecies *Metopus es*. In our research, we examined three strains of a newly discovered close relative of M. es focusing on their archaeal and other prokaryotic symbionts. Unexpectedly, we found the archaeal methanogen Methanobacteriuminside the macronucleus of all the strains. Although intramacronuclear bacterial symbionts have been documented in several unrelated ciliates, this is the first instance of intranuclear archaeal methanogen in any eukaryote. For detailed identification of the prokaryotic symbiont, we utilized Sanger and Illumina amplicon sequencing, fluorescence in situ hybridization with confocal imaging, transmission electron microscopy, and silver staining methods. Additionally, we documented the production of putative methane gas bubbles in the macronucleus of the host ciliatesand measured the presence of methane in the bubbles in an experimental assay.

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Investigating How Niches Evolve In Ciliates

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The niche is a central concept in ecology and evolution that can be defined as the set of biotic and abiotic conditions in which a species successfully grows and reproduces. Understanding how niches evolve is important to answer questions about diversification dynamics and community assembly. Microbial eukaryotes comprise most of the eukaryotic diversity and are important components of all aquatic and terrestrial ecosystems. However, we still lack basic knowledge on how their ecological niches have evolved. For instance, do closely related species occupy more similar niches, as is pre- dicted by the phylogenetic niche conservatism hypothesis? And do niches evolve in a directional, random, or some other manner? Here, we investigate niche evolution for ciliates, a diverse group of microbial eukaryotes, using a unique set of phylogenies inferred from environmental metabarcoding data spanning the small subunit ribosomal DNA. Analyzing topo-climatic and nutrient variables for ∼15 000 ciliate amplicon sequence variants (ASVs), we found a distinct phylogenetic signal associ- ated with climatic variables and pH, underlining their pronounced influence on niche evolution within ciliates. Conversely, nutrient and topographical variables exhibited minimal signal, suggesting their lesser role in shaping niche dynamics within this group. While the overall phylogenetic signal for climatic variables was strong for the ciliate phylogeny as a whole, examination of the main ciliate clades revealed variability in signal strength, reflecting nuanced evolutionary responses to environmental factors within distinct groups. Furthermore, comparing the fit of Brownian Motion, Ornstein Uhlenbeck and Early Burst models of evolution for our data and phylogeny identified a predominance of Ornstein Uhlenbeck as the optimal fit, implying evolutionary attraction towards the niche optimum. These novel results provides a greater understanding of niche evolution in microbial eukaryotes.

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Functional Interactions of Protists In the Under-Ice Arctic Phytoplankton Spring Bloom

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The Arctic Ocean harbours some of the most productive ecosystems in the world. Most of this marine primary production is carried out by single-celled phytoplankton from a wide range of evolutionary lineages. Several eukaryotic phytoplankton have been reported as phagotrophic mixotrophs, consuming prey while being able to photosynthesize. Additionally, heterotrophic flagellates, ciliates, and amoeboid protists are considered major micro-grazers of phytoplankton. The interplay of functional traits among single-cell microeukaryotes in structuring the under-ice food web remains unclear. To understand these functional interactions through the under-ice spring bloom, a time series was conducted from April to July 2016 on land-fast ice off the west coast of Baffin Bay. Sympagic and planktonic protist communities were characterised using 18S rRNA metabarcoding. Functional traits were assigned to all taxa, demonstrating the community succession of having high functional diversity (mixotrophy, eukaryvory, parasitism, saprotrophy, omnivory, bacterivory) in April to the dominance of phototrophs in July. Network analyses of RNA metabarcodes showed predation of phototrophs by eukaryvores as the most prevalent form of interaction in ice and water. Specifically, cercozoans and heterotrophic dinoflagellates have high co-occurrence with nano-sized (3-20 μm) and micro-sized (20-100 μm) phototrophs. In contrast, ciliates have high co-occurrence with pico-sized (0.2-3 μm) phototrophs and high co-exclusion from larger phototrophs. These suggests niche partitioning of eukaryvory based on prey size and energy transfer is not linear based on cell size. Uncovering these microbial interactions provide valuable insights into ecosystem productivity and functioning of the Arctic, especially how protist dynamics would change in the warming climate.

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Investigating the Nature of Interaction Between *Labyrinthulomycetes* And Diatoms In Co-Culture Systems

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Labyrinthulomycetes are saprophytic protist that play an important role in marine ecosystems as components of the microbial food web, contributing to nutrient cycling and carbon sequestration. Additionally, they are suggested to be the main cause of Eelgrass wasting disease, resulting in negative ecological impact along the Atlantic coast of North America and Europe. Recent studies propose a new perspective on Labyrinthulomycetes' interactions by proposing the concept of selective parasitism on diatoms, challenging the assumption that protist parasitism is always harmful to the host population.

Through experimental approaches, such as co-culturing and microscopic observations, we will explore the consequent effects of these interactions on the health and productivity of diatom populations. Additionally, we will explore the ultrastructure and morphological adaptations of Labyrinthulomycetes, providing detailed insights into their cellular features. Ultimately, our findings will expand our knowledge of the complex interaction between Labyrinthulomycetes and diatoms, shedding light on their ecological significance.

The limited availability of Labyrinthulomycetes strains in culture collections poses a barrier to further exploration of their diversity and ecological roles. Therefore, our research aims to expand our knowledge in this area. To achieve this, we will explore various isolation strategies, including dilution methods, enrichment cultures, baiting with different substrates and hand picking from environmental samples. By employing these isolation methods, we aim to obtain a more diverse collection of Labyrinthulomycetes strains, enabling us to characterize their molecular, physiological, and ecological features more accurately.

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Protist Community Structure In Singapore Strait Based on Full rRNA Operon Metabarcodes

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Metabarcoding of the rRNA operon has become the method of choice for investigating microbial eukaryotic communities. Most studies have used specific regions of the 18S rRNA gene (V4 or V9). While this approach yielded a wealth of data on the structure of eukaryotic microbial communities as well as on the biogeographic distribution of key groups and species, the relatively short length of these sequences often prevents assigning precisely the taxonomy of each amplicon. The development of long read sequencing with the PacBio and Nanopore technologies offers the possibility to use much longer region up to the full ribosomal RNA operon. We obtained data from Singapore Strait during a multi-year time-series for both full operon and V4 metabarcodes using the PacBio and Illumina technologies, respectively. We developed pipelines to process the full operon sequences using the cutadapt, dada2, barrnap and emu programs in order to obtain Amplicon Sequence Variants (ASVs) as well community composition. The overall structures of the protist community obtained with the V4 and the full operon metabarcodes are quite similar, at least at higher taxonomic levels (e.g. division). However, some groups such as Excavata and Amoebozoa are only obtained with the full operon. The use of the full operon also reveals the existence of novel cryptic species that share the same V4 or even the same 18S rRNA gene sequence but have marked differences in the ITS region. For example, we uncovered a novel species, belonging to the widespread picoplankton genus *Bathycoccus*, that could be characteristics of subtropical waters.

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Exploring the Plastid Symbiosis In the Marine Centrohelid *Meringosphaera*: A Novel Model System for Plastid Integration

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The evolution of plastids and their subsequent spread throughout the eukaryotic tree of life by primary and higher order endosymbiosis has transformed life on this planet. However, the ancient origin of most plastids makes it difficult to study how they were established. An interesting alternative to tracing back to origin of plastids to ancient times is the study of ongoing plastid symbiosis. *Meringosphaera* is an uncultured yet globally distributed marine centrohelid carrying plastids of Dictyochophyceae origin. While the exact nature of this plastid symbiosis is uncertain, horizontal gene transfers of plastid-associated genes to the *Meringosphaera* host genome indicates some level of genetic integration. This makes *Meringosphaera* an intriguing system to study the early stages of plastid integration. We will present data from 15 single-cell genomes of *Meringosphaera* isolated from the west coast of Sweden across multiple seasons. *Meringosphaera* is phylogenetically diverse, with several novel groups of both host and plastid that show strong evidence of co-speciation. To test the hypothesis of kleptoplastidy, we search for the potential source of *Meringosphaera*'s plastid in the environment and assess the plastid prevalence in different *Meringosphaera* groups by delploying a double catalysed reporter deposition – fluorescence in situ hybridization (CARD-FISH) protocol targeting both host and plastid simultaneously. Together, these results shed new light on a poorly known plastid symbiosis and contribute to our understanding of the mechanisms of plastid integration.

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Visualization of RNA Transcripts Within Live Eukaryotic Cells

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Fluorescent in situ hybridization (FISH) of transcript-annealing molecular beacons (FISH-TAMB) is a recently developed method to label intracellular RNA targets in prokaryotic cells without needing fixatives or surfactants. Traditional FISH relies on harsh fixation and permeabilization to deliver fluorescent oligonucleotide probes into cells for nucleic acid labeling. FISH-TAMB can deliver material into live cells without disrupting the membrane integrity. This is accomplished by coating the oligonucleotides with small cell-penetrating peptides (e.g., nine arginines, R9) that penetrate the cell membrane and release the material into the cytoplasm. These fluorescent probes or 'molecular beacons' (MBs) are hairpin oligonucleotides with sequences selected to target specific RNA transcripts. The MBs are equipped with a fluorophore and a fluorescence quencher. Recognition of the target sequence causes MB linearization which moves the fluorophore out of physical proximity of the quencher, resulting in fluorescence. The labeled cells can then be visualized via fluorescence microscopy. They can be used to differentiate transcriptional levels of target RNAs directly from environmental samples even for low-abundant community members and are effective for anaerobic samples.

In my thesis, I have worked to further develop this FISH-TAMB method to be used on unicellular eukaryotes. MBs targeting the 18S of Amoebozoa and Breviatea have been designed and tested in vitro as well as in traditional FISH. Testing of FISH-TAMB in eukaryotic cells is underway. If successful, this method can be used to fluorescently label transcripts of interest for downstream cell sorting.

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Plant Root Compartment Impacts Bacterial Food Source of Predatory Soil Protists

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Soil microbes interact directly and indirectly with other soil microbes forming complex microbe-microbe interactions. Among microbial interactions, the knowledge of associations between soil protists and bacteria is limited. We studied the differences in taxonomy of >150 cultured protists isolated from three different compartments of wheat roots and the relations to the protist-controlled food bacterial community. The bacteria of the protist cultures were compared to indigenous bacterial communities to understand the selection during protist feeding. Protist cultures were identified by Sanger sequencing and the diversity of the bacterial food communities was determined by 16S rRNA gene metabarcoding.

Overall, the bacterial communities of protists cultures were mainly shaped by the root compartment origin of the protist, followed by the protist genotype at genera level. Interestingly, protists of the genus Spumella isolated from rhizosphere and bulk soil were found to host similar bacterial food communities, indicating that Spumellacultures are associated to a specific microbiome. The Flectomonas, Neobodo, andSandona isolated from rhizosphere were found to harbor more distinct bacterial communities than those isolated from bulk soil. Only 6% of the bacterial ASVs from soil and rhizosphere were also identified among the food bacteria, indicating a high selection by the protist cultures. To our knowledge, this is the first study providing new insights into associated bacterial communities of protist cultures.

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CRISPR/Cas9 Mutagenesis and Encystation In *Giardia intestinalis*

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The microscopic world is one of the most fascinating in biology, and many microorganisms are being studied for their ability to combine relatively simple cellular morphology with a complexity of functions. Our research group has chosen *Giardia intestinalis*, an obligate, ectoparasitic unicellular organism adapted to many mammal host species (including *Homo sapiens*), thus being an essential part of human infection biology. *G. intestinalis* affects the gastrointestinal tract (mostly the small intestine), causing slight to severe giardiasis, a disease associated with acute to chronic diarrhoea, nausea, and weight loss.

The infection is waterborne, transmission being accomplished via cysts, which are, along with the trophozoites, one of the two life stages of *Giardia* and the object of our investigation. Cysts are formed under specific conditions (such as elevated pH level and cholesterol deprivation) and are the result of complex events on the molecular level, such as, *e.g.*, cascades of activated transcription factors (like Myb2), leading to expression of cell wall proteins and enzymes for building the β-1,3-linked N-acetylgalactosamine homopolymer of the cyst wall.

Attention was given to a yet unknown participant of these key events, gene ORF1470, the expression of which is highly elevated during early encystation, giving rise to a protein of unknown function. To study its function in *Giardia*, our first goal was to knock the gene out. To do this, a CRISPR/Cas9 technique new to this model organism was used. The obtained mutant trophozoites were then committed to encystation, and their cyst morphology and cyst abundance were studied.

Enjoyed this talk? Find the speaker here: sunnylightster@gmail.com