UNIVERSITÄT DUISBURG ESSEN

Open-Minded

43rd Annual Meeting

of

The German Society of Protozoology

27 February 2024 – 1 March 2024

Haltern am See

Organizers: Jens Boenigk & Sonja Rückert

Program, Abstracts & Participants

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Welcome to the 43rd Annual Meeting of the German Society of Protozoology!

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University of the West of Scotland

Dr. hab. Anna Karnkowska
University of Warsaw

Dr. Stefan Geisen
Wageningen University

Dr. Susanne Wilken
University of Amsterdam

Grell Prize Lecture: Manon Dünn, University of Cologne

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Program

Tuesday 27.02.2024

High Throughput Analysis (Highlights) Workshop

08:00-09:00	**Breakfast**	
09:00-09:30	**Welcome**	
09:30-10:00	Semiautomatic analysis of food vacuole contents by digital fluorescence microscopy	Lisa Boden
10:00-10:30	**Vitamin Break**	
10:30-12:30	Digital light microscopic analysis of diatoms: from high throughput microscopy through image annotation to deep learning	Michael Kloster/ Andrea Burfeid Castellanos
12:30-13:30	**Lunch**	
13:30-14:15	Non-target analysis of organic micropollutants in the environment: Perspectives in integrating chemical data and biological indicators in aquatic ecosystems	Maryam Vosough
14:15-15:00	A brief introduction to OTU/ASV profile analysis	Daniela Beisser
15:00-15:30	Exploring the efficacy of metabarcoding and non-target screening for detecting treated wastewater	Guido Sieber
15:30-16:00	**Coffee Break**	
16:00-17:00	Questions and Discussion	
19:00-20:00	**Dinner**	
from 20:00	**Get together**	

Wednesday 28.02.2024

07:30-08:30	**Breakfast**	
08:30-08:45	**Welcome**	**Jens Boenigk Sonja Rückert**
Session 1	**Amoeboid protists and symbiosis**	**Chair: Kenneth Dumack**
08:45-09:30	**Keynote Lecture The role of amoebae in the emergence of bacterial antimicrobial resistance in the environment and considerations for One Health**	**Fiona Henriquez**
09:30-09:45	Hydrogenotrophic methanogenesis is the key process in the obligately syntrophic consortium of the anaerobic ameba *Pelomyxa schiedti*	Sebastian Christian Treitli
09:45-10:00	Species confusion in *Acanthamoeba*: redescription of *Acanthamoeba terricola* Pussard, 1964 (Amoebozoa: Acanthamoebidae)	Julia Walochnik
10:00-10:15	Phagocytosis underpins the biotrophic lifestyle of intracellular parasites in the class Phytomyxea (Rhizaria)	Andrea Garvetto
10:15-10:30	Local endoreduplication of the host is a conserved process during Phytomyxea-host interaction	Michaela Hittorf
10:30-11:00	**Vitamin Break**	
Session 2	**Symbionts in communities**	**Chair: Julia Walochnik**
11:00-11:15	Plant diversity, plant history, and soil history effects on community composition of Cercozoa, Oomycota, and bacteria	Marcel Dominik Solbach
11:15-11:30	Plant associated protists as plant parasites and symbionts	Sigrid Neuhauser
11:30-11:45	The diversity and ecological importance of holocarpic oomycetes - overlooked parasites in marine, limnic, and terrestrial ecosystems	Marco Thines
11:45-12:00	High throughput cross kingdom diversity analysis in multiple environments	Louis Weisse
12:00-12:15	Exploring the commonly assessed "microscopic picture" in wastewater treatment	Fabienne Baltes
12:15-12:30	Microeukaryotic diversity in anaerobic digesters	Maria Badra
12:30-13:30	**Lunch**	
13:45-14:00	Announcements	
Session 3	**Symbiosis, parasitism and predation**	**Chair: Hartmut Arndt**
14:00-14:45	**Keynote Lecture Exploring protist endosymbioses: Insights into diversity, ecology, and evolution**	**Anna Karnkowska**

14:45-15:00	Transitioning from endosymbiont to organelle: A proposal addressing the inability in attaining full organellar status	Lawrence Rudy Cadena
15:00-15:15	Life on nitrate: Ecophysiology of plagiopylean ciliates with nitrate-respiring endosymbionts in freshwater lakes	Linus Matz Zeller
15:15-15:30	Shape-transition of R-bodies isolated from the Paramecium endosymbiont *Caedimonas varicaedens*	Lennart Dörr
15:30-15:45	*Malpighivinco* and *Nephridiochytrium* are new fungal genera (Nephridiophagaceae) parasitizing insects	Renate Radek
15:45-16:00	The diversity of diatom predators (Protaspidae, Cercozoa) in the polar oceans	Nele-Joelle Maria Engelmann
16:00-16:30	**Coffee Break**	
16:30-17:15	**Grell Prize Lecture:** **Biogeography and its impact on benthic heterotrophic protists – Investigations from the littoral to the deep sea**	**Manon Dünn**
17:15-19:00	**Poster Session**	
19:00-20:00	**Dinner**	

Thursday 29.02.2024

07:30-08:30	**Breakfast**	
Session 4	**Ecology and function**	**Chair: Edward Mitchell**
08:30-09:15	**Keynote Lecture** **Mixotrophic protists in changing waters**	**Susanne Wilken**
09:15-09:30	Soil biodiversity ecosystem functioning: Using protists as a model	Alejandro Berlinches de Gea
09:30-09:45	A metatranscriptomic approach to study the effect of multiple stressors on the microbial community involved in CPOM degradation in freshwater habitats	Aman Deep
09:45-10:00	Genome-resolved metagenomics reveals effect of nutrient availability on bacterial genomic properties across 44 European freshwater lakes	Manan Shah
10:00-10:15	Exploring protist community interactions in polar biocrusts	Cristina Martinez Rendon
10:15-10:30	Spatial ecology on a chip exemplified by single-species experiments with *Tetrahymena*	Johannes Werner
10:30- 11:00	**Vitamin Break**	
Session 5	**From communities to species**	**Chair: Frank Nitsche**
11:00-11:15	Deep molecular characterization of microorganisms´ diversity and community composition in the tree canopies	Jule Freudenthal
11:15-11:30	High-up across the Andes: Protist communities of microbial mats from five Andean lagoons in the Atacama Desert	Eduardo Acosta
11:30-11:45	Amplification of exogenous RNAi trigger by RNA dependent RNA polymerases	Marcello Pirritano
11:45-12:00	Next generation taxonomy on three known and one new *Frontonia* species (Ciliophora, Peniculida) from aquatic ecosystems in Kenya	Maxwell Juti Owuor
12:00-12:15	The ciliate *Balanion planctonicum* from Lake Zurich –cryptic species or one complex species?	Martina Schalch-Schuler
12:15-12:30	**Announcement**	
12:30-13:30	**Lunch**	
13:30-16:30	**Excursions**	
17:30-18:45	**DGP Membership Meeting**	
19:00-01:00	**Conference Dinner & DGP Party**	

Friday 01.03.2024

08:00-09:00	**Breakfast**	
Session 6	**Terrestrial protist diversity**	**Chair: Renate Radek**
09:00-09:45	**Keynote Lecture** **Egotism and symbiosis: the story behind soil protist diversity**	**Stefan Geisen**
09:45-10:00	Biogeography of free-living terrestrial protists – lessons learned from phylogeography, climate niche distribution and atmospheric circulation modelling	Edward Mitchell
10:00-10:15	It's time to consider the Arcellinida shell as a weapon	Kenneth Dumack
10:15-10:45	**Vitamin Break**	
Session 7	**Ciliates: Aspects and perspectives**	**Chair: Sabine Agatha**
10:45-11:00	Proteins during evolution: Change of function (repurposing) and relocation	Helmut Plattner
11:00-11:15	How to drive gene expression along an epigenome with unique global properties: Supervision of transcriptional processes by a divergent Polymerase II complex in *Paramecium tetraurelia*	Franziska Drews
11:15-11:30	History and molecular biology of Cluster22: non-canonical sRNAs from a lncRNA precursor	Martin Simon
11:30-11:45	Thermal response of freshwater ciliates	Thomas Weisse
11:45-12:00	Ciliates involved in citizen science and science communication: *Stentor* as a role model	Bettina Sonntag
12:00-12:15	**Closing Ceremony**	
12:15-13:15	**Lunch & Farewell**	

Abstracts for talks

in order of appearance

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Workshop Talks

Semiautomatic analysis of food vacuole contents by digital fluorescence microscopy

Lisa Boden

Biodiversity, University of Duisburg-Essen, Germany

The quantification of cells via manual counting is a standard practice in numerous laboratories. However, this approach is not only time-consuming and labour-intensive but also susceptible to subjective counting. Despite these drawbacks, cell quantification remains a significant tool in environmental science. Therefore, the automation of cell counting is crucial, as it not only accelerates the counting process but also enables objective counting, leading to more accurate and reproducible results. Here, we employ a semi-automated workflow using 3D microscopy to monitor the uptake of preselected target bacteria by heterotrophic nanoflagellates under different abiotic conditions.

Digital light microscopic analysis of diatoms: from high throughput microscopy through image annotation to deep learning

Michael Kloster & Andrea Burfeid Castellanos

Phycology, University of Duisburg-Essen, Germany

Centre for Water and Environmental Research, University of Duisburg-Essen, Germany

Taxonomic identification of cleaned diatom frustules is broadly used in water quality assessment and ecological research. We have developed a completely digital workflow covering automated high-resolution imaging, manual taxonomic identification and generation of count reports. For several years now we are honing this workflow and apply it in our daily routine work. As a by-product of this work, we are producing huge amounts of taxonomically labelled image data, which are the essential requirement for training AI methods on automated diatom identification. In this workshop, we will present options for high-throughput imaging, paint a picture of the not too far future of automated diatom analysis, and provide some practical hands-on experience to our digital annotation workflow.

Non-target analysis of organic micropollutants in the environment: Perspectives in integrating chemical data and biological indicators in aquatic ecosystems

Maryam Vosough [1,2], Guido Sieber [1,3], Torsten C. Schmidt [1,2], Jens Boenigk [1,3]

1. Centre for Water and Environmental Research, University of Duisburg-Essen, Germany
2. Instrumental Analytical Chemistry, University of Duisburg-Essen, Germany
3. Biodiversity, University of Duisburg-Essen, Germany

Aquatic ecosystems are critical to all forms of life, yet they face a myriad of human-induced challenges, including pollution and overexploitation. In this context, the tools for evaluating water quality are of paramount importance. Non-target analysis (NTA) represents a significant departure from conventional methods of detecting specific pollutants, as it seeks to identify a broad spectrum of both known and unknown contaminants. The application of high-resolution mass spectrometry (HRMS) for NTA of organic micropollutants in complex water samples has seen rapid expansion in recent years, particularly in environmental monitoring research. HRMS-based NTA produces extensive datasets, necessitating advanced chemometrics/machine learning tools for the trend identification, discrimination and prioritization tasks. This presentation will focus on these methods, aiming to provide a comprehensive overview of recent advancements in NTA methodologies within aquatic environments and the critical role of sophisticated data processing, illustrated through specific case studies.

A brief introduction to OTU/ASV profile analysis

Daniela Beisser

Westphalian University of Applied Sciences, Germany

There are many ways to process and analyse amplicon sequencing data to study community structure. In the workshop we will go through the key steps of most processing workflows and discuss the traditional OTU (Operational Taxonomic Units) approach and the single-nucleotide-resolving methods that generate ASVs (amplicon sequence variants). In both cases, the output will be a count table with representative sequences and assigned taxonomic information. We will look at ways to examine these profiles and statistical methods for summarizing, filtering, visualizing and interpreting the data.

Exploring the efficacy of metabarcoding and non-target screening for detecting treated wastewater.

Guido Sieber [1,2], Maryam Vosough [2,3], Daniela Beisser [4]

1. Biodiversity, University of Duisburg-Essen, Germany
2. Centre for Water and Environmental Research, University of Duisburg-Essen, Germany
3. Instrumental Analytical Chemistry, University of Duisburg-Essen, Germany
4. Department of Engineering and Natural Sciences, Westphalian University of Applied Sciences, Germany

Wastewater treatment processes eliminate many pollutants; however, some remain, resulting in the introduction of organic compounds and microorganisms from treated wastewater into receiving waters. Nevertheless, neither organic pollutants nor microorganisms are currently monitored. Our objective is to compare the efficacy and sensitivity of two previously introduced methods: non-target analysis and metabarcoding of 18S V9 rRNA, and full-length 16S rRNA, in detecting treated wastewater. Through this integration, we aim to illustrate how these comprehensive approaches enhance our understanding of the environment and the impact of anthropogenic contaminants on ecosystem health, ultimately facilitating efforts to protect ecosystems.

Presentation Abstracts

Keynote Talk:

The role of amoebae in the emergence of bacterial antimicrobial resistance in the environment and considerations for One Health

Fiona Henriquez

University of the West of Scotland (UWS), Scotland

Free-living amoebae (FLA) represent a diverse group of predatory protists that play a major role in the health of their ecosystem by regulating bacteria via phagocytosis and promoting soil health. The co-evolution of many bacteria with FLA has resulted the development of symbiosis, allowing intracellular survival of bacteria. This has the potential to contribute to the survival of clinically important bacteria such as *Pseudomonas* and *Legionella* species. In our work, we aim to elucidate how the symbiotic relationship can protect bacteria in FLA from antimicrobials and allow survival during disinfection or treatment processes in the environment and within highly polluted areas. We explore the role of predator-prey interactions within these ecosystems and hypothesise how the close relationships between bacteria and predatory protists may contribute to the survival and the emergence of bacterial antimicrobial resistance within the context of ‘One Health'.

Hydrogenotrophic methanogenesis is the key process in the obligately syntrophic consortium of the anaerobic amoeba *Pelomyxa schiedti*

Sebastian C Treitli [1]*, Pavla Hanousková [2], Vladimír Beneš [3], Andreas Brune [4],

Ivan Čepička [2], Vladimír Hampl [1]

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2. Department of Zoology, Faculty of Science, Charles University, Czech Republic
3. Genome Biology Unit, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany
4. RG Insect Gut Microbiology and Symbiosis, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

* Present address: RG Insect Gut Microbiology and Symbiosis, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

Pelomyxa is a genus of anaerobic amoebae that harbour several prokaryotic endosymbionts. Although the symbionts represent a large fraction of the cellular biomass, their metabolic roles have not been investigated. Using single-cell genomics and transcriptomics, we characterized the prokaryotic community associated with *P. schiedti*, which consists of two bacteria and a merthanogenic archaeon. Fluorescence in situ hybridization and electron microscopy revealed that the bacterium *Candidatus Vesiculincola pelomyxae* is localized inside vesicles, whereas the other endosymbionts occur freely in the cytosol, with the metanogen *Candidatus Methanoregula pelomyxae* enriched around the nucleus. Genome and transcriptome-based reconstructions of the metabolism suggests that the cellulolytic activity of *P. schiedti* produces simple sugars that fuel its own metabolism and the metabolism of a *Ca. Vesiculincola pelomyxae*. On the other hand, *Candidatus Syntrophus pelomyxae* relies on the degradation of butyrate and isovalerate from the environment to fuel its metabolism. Both bacteria and the amoeba use hydrogenases to transfer electrons from reduced equivalents to hydrogen, a process that requires a low hydrogen partial pressure. This is achieved by the third endosymbiont, *Ca. Methanoregula pelomyxae*, which consumes H2 and formate for methanogenesis. While the bacterial symbionts can be successfully eliminated by vancomycin treatment without affecting the viability of the amoebae, treatment with 2-bromoethanesulfonate, a specific inhibitor of methanogenesis, killed the amoebae, indicating the essentiality of methanogenesis for this consortium.

Species confusion in *Acanthamoeba*: redescription of *Acanthamoeba terricola* Pussard, 1964 (Amoebozoa: Acanthamoebidae)

Daniele Corsaro [1], Martin Mrva [2], Philippe Colson [3], Julia Walochnik [4]

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2. Department of Zoology, Faculty of Natural Sciences, Comenius University in Bratislava, Slovak Republic
3. Institut de Recherche Pour le Développement (IRD), Microbes Evolution Phylogeny and Infections (MEPHI), Aix-Marseille Université, France
4. Molecular Parasitology, Institute of Specific Prophylaxis and Tropical Medicine, Medical University of Vienna, Austria

Acanthamoeba castellanii (Douglas) Page, 1967 is the type species of a widespread genus of free-living amoebae, potentially pathogenic for humans and animals. The Neff strain is one of the most widely used in biological research, serving as a model for both *A. castellanii* and the whole genus in general. The Neff strain, isolated in 1957 from soil in California, closely resembles another strain found in France and originally described as a separate species, *Acanthamoeba terricola* Pussard, 1964, but both were successively synonymized with *A. castellanii*. Molecular sequence analysis has largely replaced morphological diagnosis for species identification in Acanthamoeba, and rDNA phylogenies show that the Neff strain forms a distinct lineage from that of the type strain of *A. castellanii*. In this study, we compared the type strain of *A. terricola* with the Neff strain and *A. castellanii*, and analysed the available molecular data including new sequences obtained from *A. terricola*. We provide molecular evidence to validate the species *A. terricola*. The Neff strain is therefore transferred to *A. terricola* and should no longer be considered as belonging to *A. castellanii*.

Phagocytosis underpins the biotrophic lifestyle of intracellular parasites in the class Phytomyxea (Rhizaria)

Andrea Garvetto [1], Pedro Murúa [2], Martin Kirchmair [1], Willibald Salvenmoser [3], Michaela Hittorf [1], Stefan Ciaghi [1], Srilakshmy Harikrishnan [4], Claire Gachon M. M.[5], John Burns [6]

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4. VIB, Centre for Plant Systems Biology & Department of Plant Biotechnology and Bioinformatics, Ghent University, Ghent, Belgium
5. Muséum National d'Histoire Naturelle, Paris, France
6. Bigelow Laboratory for Ocean Sciences, East Boothbay, USA

Phytomyxea are intracellular biotrophic parasites infecting plants and stramenopiles and they include agriculturally impactful pathogens such as *Plasmodiophora brassicae*. Recent molecular investigations assigned them to the clade Rhizaria, mainly composed of free-living amoebae and amoeboflagellates where phagotrophy is a widespread mode of nutrition. Phagocytosis is a complex multigene trait specific to eukaryotes, well documented in free-living unicellular eukaryotes and specialised cellular types of animals. Studies on phagocytosis in intracellular biotrophic parasites are scant, since the direct consumption of host organelles and cellular components is seemingly at odds with the biotrophic requirement of keeping the invaded cell alive. Here we provide evidence that phagotrophy is part of the nutritional strategy of phytomyxea, using morphological and genetic data (including a novel transcriptome of the brown algae parasite *Maullinia ectocarpii*). We document intracellular phagocytosis in *P. brassicae* and *M. ectocarpii* by transmission electron microscopy and fluorescent in situ hybridization. Our investigations confirm the presence of molecular signatures of phagocytosis in Phytomyxea and hint at a small, specialised subset of genes used for intracellular phagocytosis. Microscopic evidence confirms the existence of intracellular phagocytosis, which in Phytomyxea targets primarily host organelles. Phagocytosis seems to coexist with the manipulation of host physiology typical of "traditional" biotrophic interactions. Our findings resolve long debated questions on the feeding behaviour of Phytomyxea, suggesting an unrecognised role for phagocytosis in biotrophic interactions.

Local endoreduplication of the host is a conserved process during Phytomyxea-host interaction

Michaela Hittorf [1], Andrea Garvetto [1], Magauer M. [2], Martin Kirchmair [1], Willibald Salvenmoser [3], Pedro Murúa [4], Sigrid Neuhauser [1]

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2. Department of Botany, Universität Innsbruck, Innsbruck, Austria
3. Department of Zoology, Universität Innsbruck, Innsbruck, Austria
4. Laboratorio de Macroalgas, Instituto de Acuicultura, Universidad Austral de Chile, Puerto Montt, Chile

Endoreduplication is a modified cell cycle in which cells duplicate their DNA without subsequent mitosis. This process is common in plants and can also be found in other organisms like algae and animals. Biotrophic plant pathogens have been shown to induce endoreduplication in their host to gain space and/or nutrients. Phytomyxea (divided into the Plasmodiophorida, the Phagomyxida, and the Marinomyxa clade) are obligate biotrophic parasites of plants, diatoms, brown algae, and oomycetes. Here, we tested if phytomyxids induce local endoreduplication in two distant hosts (plants and brown algae). By combining fluorescent in situ hybridisation (FISH) coupled with nuclear area measurements and flow cytometry, we confirmed that endoreduplication is induced by *Plasmodiophora brassicae* (Plasmodiophorida) in infected plants and demonstrate this process in combination with *Maullinia ectocarpii* and *Maullinia braseltonii* (Phagomyxida) in brown algae. We identified molecular signatures of endoreduplication in RNA-seq datasets of *P. brassicae*-infected *Brassica oleraceae* and *M. ectocarpii*-infected *Ectocarpus siliculosus*. Cell cycle switch proteins (CCS52A1 and B in plants and CCS52 in algae) as well as the protein kinase WEE1 (in plants) were identified as genes potentially important for the phytomyxean-induced switch from the mitotic cell cycle to the endocycle. Their expression pattern changed in infected plants and brown algae accordingly. In this study we expand the knowledge on Phytomyxea-host interactions by showing that induced endoreduplication in the host is a conserved feature in phytomyxid infections. The induction of this cellular mechanism by phytomyxid parasites in phylogenetically distant hosts further points at a fundamental importance of endoreduplication in these biotrophic interactions.

Plant diversity, plant history, and soil history effects on community composition of Cercozoa, Oomycota, and bacteria

Marcel Dominik Solbach [1], Justus Hennecke [2], Leonardo Bassi [2], Cynthia Albracht [3], Akanksha Rai [4], Aaron Fox [5], Katalin Wagner [1], Yan Chen [6], Markus Lange [4], Alexandra Weigelt [2], Nico Eisenhauer [7], Michael Bonkowski [1]

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2. Leipzig University, Leipzig, Germany
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6. Chinese Academy of Science, Institute of Soil Science, Nanjing City, Jiangsu, China
7. German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

The "Jena Experiment" is a long-term field experiment established in 2002, consisting of experimental plots with varying plant communities ranging from 1 to 60 species. In 2016, a sub-experiment, the "ΔBEF Experiment" (DELTa-BEF; short for DEterminants of Long-Term Biodiversity Effects on Ecosystem Functioning), was set up. On all plots of the Jena Experiment, three subplots with differing soil and plant history were established. Bulk soil was sampled in 2021. We investigated by amplicon sequencing how plant species richness and the history treatments influenced the diversity of Bacteria and two protistan taxa, Cercozoa (Rhizaria), and Oomycota (Stramenopila). These two protistan groups are functionally diverse and include a wide range of plant parasites. Our results did show that plant diversity and the history treatments had a significant effect on the diversity and composition of those microbial communities. We observed that bacterial alpha diversity increased with plant diversity. In contrast, the alpha diversity of Oomycota decreased, and the alpha diversity of Cercozoa was similar across all plant diversity levels. However, the alpha diversity of Phytomyxea (subset of Cercozoa, plant parasites) also increased with plant diversity, potentially indicating "pathogen dilution" along the plant diversity gradient. Our results indicate that not all microbial groups react equally to plant diversity. Community compositions of all three microbial groups were driven by the plant diversity gradient and by the history treatments, while the soil history effects were much stronger than plant history effects.

Plant associated protists as plant parasites and symbionts

Sigrid Neuhauser, Martin Kirchmair, Michaela Hittorf, Andrea Garvetto, Sabine Oberhofer

Department of Microbiology, University of Innsbruck, Austria

In phytopathology, researchers study the interactions between viruses, bacteria, fungi, or oomycetes and their plant hosts, focusing on the intricate relationships between these microorganisms and plants. But there is a growing number of eukaryote microorganisms not belonging to fungi and oomycetes - often also referred to by the term protists - which form intricate biotic interactions with photosynthetic hosts, and which are abundant and impact environments in diverse ways. As we just start to understand the diversity of these parasites, the ways in which they interact with their hosts are not yet understood. Using the example of phytomyxea, gaps in biodiversity will be highlighted. Phytomyxea have developed a unique way of interacting with their host, which makes them difficult to control using approaches designed for different microorganisms because of the way they feed on their host and the way they interact with their host.

The diversity and ecological importance of holocarpic oomycetes - overlooked parasites in marine, limnic, and terrestrial ecosystems

Marco Thines, Anthony Buaya

Goethe University, Frankfurt am Main, Germany
Senckenberg Biodiversity and Climate Research Centre, Frankfurt am Main, Germany

Oomycetes are fungus-like eukaryotes belonging to the kingdom Straminipila. Their filamentous plant and animal pathogens are widely known, such as *Phytophthora infestans*, which has triggered the Great Irish Famine of the mid-19th century or *Aphanomyces astaci*, the causal agent of the crayfish plague. In contrast, little is known about oomycetes not forming big thalli – those that are holocarpic, converting their entire cytoplasm into zoospores. These organisms mostly diverged earlier than the crown group and account for the majority of the order-level clades of the oomycetes. They can be found in all terrestrial, limnic, and marine environments. Most species of holocarpic oomycetes cannot be cultivated and are obligate biotrophic, as they rely on living host cells for their development. Holocarpic oomycetes parasitise a wide range of eukaryotes and prokaryotes, among others red, green, and brown algae, diatoms, other oomycetes, dinoflagellates, as well as cyanobacteria. During the past ten years, we have been able to rediscover many species before known only from decades-old records and found several new species in both arctic and temperate regions. A summary of recent discoveries and an outline on future research directions will be given.

High throughput cross kingdom diversity analysis in multiple environments

Louis Weisse, Bouziane Moumen, Yann Hechard, Vincent Delafont

Lab. Ecologie et Biologie des Interactions - UMR CNRS, France

Despite widespread use of high throughput sequencing, the diversity of protists, remains rarely investigated in the environment - even if they may represent diverse communities, important for ecosystem functioning. Thus, our understanding of protists community composition and dynamics remain to this day very scarce. Added to that, symbiotic associations of protists with various bacteria are known to be frequent, though the way such associations shape protist community composition is poorly studied. Strictly intracellular bacteria such as Chlamydiae and recently described Dependentiae are often found infecting protists, resulting in symbioses ranging from mutualism to parasitism.

Exploring the commonly assessed "microscopic picture" in wastewater treatment

Fabienne Baltes, Kenneth Dumack

Terrestrial Ecology, Institute of Zoology, University of Cologne, Germany

Understanding microbial dynamics in wastewater treatment plants (WWTPs) is crucial for treatment efficiency. Extensive research has been conducted to study prokaryotes in wastewater and comparatively little efforts have been done to study wastewater protists. We examined the influence of different physicochemical measures on microbial communities within WWTP aeration tanks, i.e. the main bioreactor during treatment. Analyzing microscopic data from various German WWTPs collected over several years, we observed temperature's dominant role in shaping the wastewater community composition next to nitrogen and pH. I will present current methodology in the generation, and use of microscopic data in wastewater treatment operation and will discuss its strengths and limitations for research.

Microeukaryotic diversity in anaerobic digesters

Maria Badra, Kenneth Dumack

Terrestrial Ecology, Institute of Zoology, University of Cologne, Germany

Wastewater treatment plants (WWTPs; "Kläranlagen") remove excess nutrients and parasites before the treated water is returned to the environment. Within WWTPs the sludge, i.e. the remaining organic, particular matter is collected and further processed in anaerobic digesters ("Faultürme"). During anaerobic digestion, diverse microorganisms break down the collected sludge into biogas and digestate under anoxic conditions (anaerobic digestion). In this anoxic environment, not only prokaryotes are important, but microeukaryotes, i.e. protists and fungi, are often neglected.

We used publicly available metatranscriptomic (RNA) data from nine different anaerobic digesters located in Japan and the USA to investigate the microeukaryotic community in anaerobic digesters. As expected, the eukaryotic species richness was low, with a surprisingly high abundance of a specific ciliate.

A holistic view of the entire microbial community involved in anaerobic digestion is desired for understanding microbial interactions. With this, wastewater treatment plants can be understood better and run more efficiently for biogas and fertilizer production.

Keynote Lecture:

Exploring protist endosymbioses: Insights into diversity, ecology, and evolution

Anna Karnkowska

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The phenomenon of endosymbiosis - in which one organism lives within another - led to major evolutionary transitions such as the origin of mitochondria and plastids. We currently know that there have been several independent events of endosymbiosis in early eukaryotic evolution, giving rise in some cases to major eukaryotic superclades. However, despite its significance, we still do not know the understand the mechanism of endosymbiotic integration. Only by examining more recent endosymbioses in various phases of integration can we make progress in our understanding of the host-endosymbiont integration process.

The endosymbioses of microbial eukaryotes, due to the unicellular nature of the host, provide the most suitable systems for studying the establishment of endosymbiosis. Recent advances in diversity research suggest that intracellular symbioses are common among microbial eukaryotes, however systematic studies focused on certain groups of protists or environments are still quite limited. New models are, however, essential to answer questions on the early stages of endosymbiosis.

I will emphasise the importance of systematic studies on the endosymbiotic relationships between protists and prokaryotes, focusing on their diversity and occurrence in different ecosystems and their potential ecological role. I will also introduce emerging protistan models, such as the euglenid *Rapaza viridis*, that give us insights into the processes that accompany the early stages of endosymbiosis.

Transitioning from endosymbiont to organelle: A proposal addressing the inability in attaining full organellar status

Lawrence Rudy Cadena

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The prevailing consensus in the scientific community supports the view that the genesis of energy-producing organelles, specifically mitochondria and primary plastids, derived from the symbiotic incorporation of an alphaproteobacterial endosymbiont two billion years ago and the assimilation of a cyanobacterial endosymbiont 1.5 billion years ago, respectively. The comprehensive integration of these energy-producing organelles is substantiated by evidence of near-complete endosymbiont gene transfer (EGT) to the nucleus and the presence of intricate protein import machineries. An intriguing exception to this paradigm is the cyanobacterial-derived photosynthetic organelles observed in *Paulinella chromatophora* termed chromatophores, which have manifested a remarkable level of integration (gene loss and the emergence of a sophisticated import machinery) within the last 100 million years.

The last two decades of advancements in protistology has revealed a ubiquity and diversity of endosymbionts across eukaryote clades, with evidence of many endosymbionts having laterally evolved with their hosts for millions of years and playing supportive roles in specific metabolic pathways (e.g., amino acid/vitamin synthesis, nitrogen fixation). However, among these endosymbionts, only three cases – mitochondria, plastids, and chromatophores – have demonstrated extensive integration of the original endosymbiont to their respective host, establishing a bottleneck in the transition from endosymbiont to organelle.

In light of these observations, I propose that the near-complete transition from endosymbiont to organelle hinges fundamentally on the endosymbiont's capacity to supply energy to its host. This proposition is substantiated by two key arguments: firstly, the exclusive instance of primary energy-producing organelles in eukaryotes trace their origin to endosymbionts, and secondly, that "long-term and permanent" endosymbionts, prevalent in diverse organisms, exhibit minimal levels of integration and correlated with the absence to confer energy to their hosts, irrespective of their evolutionary age.

Life on nitrate: Ecophysiology of plagiopylean ciliates with nitrate-respiring endosymbionts in freshwater lakes

Linus Matz Zeller, Sina Schorn, Louison Nicolas-Asselineau, Jon S. Graf, Jana Milucka,

Max Plank Institute for Marine Microbiology, Bremen, Germany

The acquisition of bacterial endosymbionts was a fundamental event in the evolution of eukaryotes, giving rise to mitochondria and consequently higher forms of life capable of oxygen respiration. The discovery of *'Candidatus Azoamicus ciliaticola*', a gamma-proteobacterial endosymbiont of an anaerobic plagiopylean ciliate, shows that some extant eukaryotes may also host and possess other 'respiratory' endosymbionts to supplement or even replace their mitochondria. *'Ca. A. ciliaticola'*, like most obligate endosymbionts, has a strongly reduced genome, but has retained key genes for anaerobic nitrate respiration and exchange of energy in the form of ATP. It likely supplies its host with energy derived from nitrate respiration, thus fulfilling a role analogous to mitochondria.

Our bioinformatic analyses suggest a broad distribution of *'Ca. A. ciliaticola'* in freshwater ecosystems; however, at this point it is unclear what factors control the distribution of this symbiosis in the environment. Using endosymbiont-specific oligonucleotide probes and amplicon data we now show that the host distribution in two meromictic lakes (Lake Zug and Lake Lugano) was consistently constrained to anoxic but nitrate-replete waters. Unlike many obligately anaerobic ciliates, the *'Ca. A. ciliaticola'* host is typically absent from waters containing free sulfide. Interestingly, in our samples collected from anoxic depths, we often detected ciliate hosts that appeared to be in the process of division, with *'Ca. A. ciliaticola'* present inside the host cells at all observed division stages. These results confirm the hypothesized anaerobic lifestyle of the ciliate and suggests that vertical transmission of the endosymbiont may be coordinated with, and possibly controlled by, its host.

Our combined data reveal for the first time the presence of the nitrate-respiring endosymbiont *'Ca. A. ciliaticola'* and its plagiopylean host beyond Lake Zug, and offers insights into the ecophysiology of this to-date uncultured symbiosis.

Shape-transition of R-bodies isolated from the *Paramecium* endosymbiont *Caedimonas varicaedens*

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R-bodies – coiled proteinaceous ribbon structures, produced by a variety of gram-negative bacteria – are responsible for the so-called 'killer-effect' in *Paramecia*. They are about 500 nm in width and can reversibly transition from their coiled resting state into an around 10 µm long rod under specific conditions like a decrease in pH. While the well-characterized R-bodies of *Paramecium* endosymbiont *Caedibacter taeniospiralis* respond consistently to pH-changes, R-bodies of *Caedimonas varicaedens* respond to various triggers and within different pH ranges. To improve understanding of the shape-transition mechanism and how the R-bodies' response to various triggers differs, we captured high-speed high-resolution phase-contrast light microscopic videos of isolated R-bodies undergoing shape-transition and complement these with electron microscopic observations.

Malpighivinco and *Nephridiochytrium* are new fungal genera (Nephridiophagaceae) parasitizing insects

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Nephridiophagids are unicellular fungi (Chytridiomycota) that infect the Malpighian tubules of insects. An infection is not fatal but reduces the fitness of its host, which is noticeable e.g. by reduced mobility, fat reserves, and offspring. Most nephridiophagid species have been found in cockroach hosts and belong to the genus *Nephridiophaga*. Three additional genera have been described from beetles and an earwig. Here, we characterize morphologically and molecular phylogenetically the nephridiophagids of the European earwig *Forficula auricularia* and the mallow beetle *Podagrica malvae*. Their morphology and life cycle stages resemble those of other nephridiophagids, but their rRNA gene sequences support the existence of two additional genera. Whereas the earwig nephridiophagid (*Nephridiochytrium forficulae*) forms a sister lineage of the *Nephridiophaga* cluster, the mallow beetle nephridiophagid (*Malpighivinco podagricae*) represents the earliest divergent lineage within the nephridiophagids, being sister to all other species. Our results corroborate the hypothesis that different insect groups harbor distinct nephridiophagid lineages.

Radek R et al. (2023). New nephridiophagid genera (Fungi, Chytridiomycota) in a mallow beetle and an earwig. MycoKeys 100: 245–260 DOI: 10.3897/mycokeys.100.111298.

The diversity of diatom predators (Protaspidae, Cercozoa) in the polar oceans

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Sea ice is an important habitat in both polar regions and microorganisms within build the foundation for marine food webs. The role of diatoms as major primary producers of sea ice communities and their impact on the global ecosystem has been thoroughly studied. Yet, the role of their protistan consumers in the ice remains almost unexplored. This project focuses on the genetic diversity of polar Cryomonadida, a group of specialized predators of diatoms. We established cultures from Antarctica and the Arctic, which were studied morphologically and genetically and compared with yet described species within the Cryomonadida. Since the Arctic is particularly affected by climate change-induced sea ice melting, a better understanding of the ecological role of micro-heterotrophs is essential for assessing the impacts of global warming. As Cryomonadida function as predators of diatoms, directly impacting the population of algae, their biological role in the marine ecosystem becomes particularly interesting. We will discuss challenges and perspectives in working with the cold-adapted, marine Cryomonadida.

Grell Prize Lecture:

Biogeography and its impact on benthic heterotrophic protists – Investigations from the littoral to the deep sea

Manon Dünn

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Knowledge on the biogeography of organisms, their abundances, distribution and diversity across temporal and spatial scales, is fundamental as it is linked to the function of ecosystems and gives important information on evolutionary mechanisms regulating diversity. But, if biogeographical concepts, established for macroorganisms, also apply for microbial communities is not yet clarified. Circumglobal expeditions like Tara Ocean and Malaspina have discovered that heterotrophic protists are the most diverse group of organisms in surface and bathypelagic waters in the oceans. However, much less is known on their diversity and distribution in marine sediments, especially in the deep sea. The overall aim of this study was to extend the knowledge on biogeographical distribution patterns of protist communities at the ocean floor considering different spatial scales to understand ecological and evolutionary processes within this vast environment.

We investigated benthic protist communities in different regions of the Caribbean Sea, the Atlantic and Pacific Ocean in bathyal, abyssal and hadal depths, showing that different ocean basins harbour unique protist communities with high rates of endemism, being distinct from surface water communities. Our results showed high variations in the structure of protist communities at large, but also at local spatial scales. In addition, benthic protist communities along depth transects around multiple islands and seamounts of the Azores archipelago were investigated, showing strong differences between communities from different depths and islands. This indicates a separation of communities on island shelfs/seamounts from communities inhabiting the surrounding deep-sea areas, favouring speciation processes. Using two protist groups as model organisms (Cafeteriaceae and Percolomonadidae), we investigated the distribution of benthic protist species more in detail. Within both groups, single species seemed to be globally distributed in marine surface waters and the deep sea and were able to survive varying environmental conditions, while most species seemed to be locally restricted in their distribution.

Keynote Lecture:

Mixotrophic protists in changing waters

Susanne Wilken

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The recognition of mixotrophic protists that combine photosynthesis with a heterotrophic nutrition as important microbial predators in lakes and oceans changes traditional paradigms. For example, nutrients contained in prey can directly fuel primary production by mixotrophic predators, and feeding rates by mixotrophs can be regulated by the availability of light. The relative importance of feeding versus photosynthesis determines the role of mixotrophs in the carbon cycle and depends on environmental conditions, yet little is known about how this role might change in a future climate. Here we present a range of approaches exploring the physiology and ecological roles of mixotrophs across changing environmental conditions, such as increasing temperature, altered nutrient and light availability, and ocean acidification. While first experiments suggested that mixotrophs might become more heterotrophic with increasing temperature, their functional and phylogenetic diversity is likely to complicate the picture. For instance, we find closely related mixotrophic chrysophytes to differ in their responses to ocean acidification, with some shifting their nutritional balance towards autotrophy under high CO_2 availability, while others do not. Similarly, laboratory experiments with cultured strains, as well as microcosm and mesocosm experiments with natural communities reveal functional diversity among mixotrophs regarding their responses to altered light and nutrient availability. These differences are further reflected in distinct population dynamics both over the course of a mesocosm experiment manipulating light availability, and over the seasonal cycle as revealed by a 4-year time series from an oligotrophic lake. While predicting the future role of mixotrophic protists in the carbon cycle thus remains challenging, the functional diversity among them will likely need to be considered to solve this challenge.

Soil biodiversity ecosystem functioning: Using protists as a Model

Alejandro Berlinches de Gea [1], Guixin Li [2], Wenjia Wu [3], Aarzoo Kohra [4], Jingxuan Olivia Chen [5], Semih Karst Aslan [1], Joshua Both [1], Nina Haas [1], Stefan Geisen [1]

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The relationship between biodiversity and ecosystem functioning (BEF) is well-established for plants with positive common links shown for plant diversity and ecosystem functions. These BEF relationships have been shown under ambient conditions and different global change factors such as in distinct climate conditions (e.g. drought) and management forms (e.g. nitrogen addition). However, the BEF relationship is barely known in soils (sBEF), the world's most diverse ecosystems hosting ≈59% of the total known diversity on Earth, including protist. The impact of cooccurring global change drivers (GCDs) on this sBEF relationship is even less known. In two greenhouse experiments, we used protists as models for soil biodiversity to investigate the effect of increasing protist diversity (from 0 to 30 species) on plant biomass (*Solanum lycopersicum* and *Cannabis sativa*) and nutrient cycling. We showed that sBEF patterns were not only positive (up to 23% biomass increase under nematode infection) but could also be negative (up to 39% biomass loss under drought). Similarly, increasing biodiversity did not consistently increase plant biomass, but led to a similar plant biomass in 25, 100 and 150 kg N ha-1 year-1 treatments. Combined stressors exhibited additive patterns, cancelling out individual GCD effects. These results challenge existing claims about (s)BEF relationships, emphasizing that (s)BEF patterns might be biased as they depend on external conditions, the organismal group studied, or even the GCD(s) tested.

A metatranscriptomic approach to study the effect of multiple stressors on the microbial community involved in CPOM degradation in freshwater habitats

Aman Deep [1,6], Tom L. Stach [2], Guido Sieber [1], Iris Madge Pimentel [3], Lisa Boden [1], Daria Baikova [4], Una Hadziomerovic [4], Gwendoline David [5], Jens Boenigk [1], Daniela Beisser [6]

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The degradation of coarse particulate organic matter (CPOM) in streams is primarily influenced by microbial activity, specifically of fungi and bacteria. However, the impact of environmental factors such as temperature and salinity on the metabolic pathways and enzymatic reactions of these decomposers remains unclear. This study aims to elucidate the distinct roles played by various fungal and bacterial groups in the enzymatic decomposition of CPOM within the Emscher/Boye and Kinzig catchments, both in the presence and absence of stressors.

To assess functional and taxonomic diversity, we employed an enhanced organic matter approach, utilizing the AquaFlow mesocosm to observe changes in CPOM-related functional pathways over time. Metatranscriptomic sequences were analysed using a customized pipeline for preprocessing eukaryotic and prokaryotic mRNA, followed by mapping to databases such as Mycocosm, CAZy, and NCBI to extract taxonomic and functional information.

By investigating the impact of multiple stressors - temperature, salinity, and flow velocity - we are testing the hypothesis that function recovers more rapidly than community composition due to functional redundancy. Here we employ the experimental systems AquaFlow (indoors) and ExStream (outdoors). Our expectation aligns with this hypothesis, anticipating that fungi and bacteria share CPOM-degrading functions, resulting in a faster recovery of function compared to the restoration of microbial community composition following the release of stressors.

Genome-resolved metagenomics reveals effect of nutrient availability on bacterial genomic properties across 44 European freshwater lakes

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Understanding intricate microbial interactions in the environment, like the relationships between nutrients and bacteria are crucial, especially because phosphorus, nitrogen and organic carbon availability are known to influence bacterial populations dynamics. Low nutrient conditions have been suggested to prompt the evolutionary process of genome streamlining to conserve these scarce nutrients and proliferate. This associates with genomic properties like %GC content, genes encoding sigma factors, percent coding regions, gene redundancy, and functional shifts in processes like cell motility and ATP binding cassette transporters among others. The current study aims to unveil nutritional impact on genome size, GC content and functional properties of pelagic freshwater bacteria at finer taxonomic resolutions for many metagenomically characterized communities. Our study confirms the interplay of trophic level and genomic properties while highlighting that, different nutrient types, particularly phosphorus and nitrogen, differentially impact these properties. Covariation of functional traits with genome size was observed; larger genomes exhibit enriched pathways for motility, environmental interaction, and regulatory genes. ABC transporter genes mirror environmental conditions, with small genomes presumably relying more on metabolites from other organisms. Different phyla adopting distinct strategies to adapt to oligotrophic environments are also discussed. The findings contribute to understanding genomic adaptations within complex microbial communities.

Exploring protist community interactions in polar biocrusts

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Polar biocrusts, the dominant vegetation type in the Arctic and Antarctica, are also subject to changes attributed to global warming. Comprised of associated photoautotrophic and heterotrophic prokaryotes and microbial eukaryotes, mosses, lichens, and soil particles, biocrusts gradually colonize barren soils following glacier retreat in the polar regions. Here, as often happens in other nutrient-poor soils, terrestrial green algae and cyanobacteria frequently dominate the photoautotrophic component, fostering a largely undescribed microbial network. We explored the food web structure and function that arises within these systems.

We characterized the biodiversity of three prominent protists inhabiting biocrusts, namely, green algae, Bacillariophyta, and Cercozoa. Through a comprehensive approach involving amplicon-based high-throughput sequencing, co-occurrence network analyses, and classical culture-based food choice experiments, we elucidated potential food webs and delineated functional traits of various taxa. Consistently, our observations reaffirmed that several abundant Cercozoa heterotrophic taxa engage in algivory. Our findings form a baseline to study energy flow in Polar Region terrestrial biocrust food webs, shaping our understanding of these ecosystems.

Spatial ecology on a chip exemplified by single-species experiments with *Tetrahymena*

Johannes Werner, Hartmut Arndt

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Spatial distribution is fundamental for organisms in natural systems. An interesting feature lies within the inherent ability of individuals to autonomously create spatial patterns. These self-organized spatial patterns, for instance, can emerge due to nearby patches accumulating differences in abundances through short-range positive and negative feedback, thereby generating diverse and complex spatial distribution patterns. Here, we present results obtained with an adaptable microfluidic chip fabricated from Plexiglass, specifically designed to identify temporal changes in spatial distribution patterns of freely moving and growing protists. Through advanced image analysis, this technique provides high-resolution insights into cellular interactions and distribution ecology within confined environments. We show a self-organized pattern formation of the ciliate *Tetrahymena pyriformis* in proof-of-concept experiments, followed by a mathematical model incorporating experimentally determined parameters. These exemplary experiments revealed the possibility of the presence of chaotic distribution dynamics even within examinations of single-species populations. Studying these patterns and their nonlinear behaviour offers insights into ecosystem functionality and strategies for conservation and sustainability. Nonlinearity should be regarded as a crucial phenomenon in single-species dynamics, and as a prerequisite for maintaining high biodiversity in nature, essential for effective conservation.

Deep molecular characterization of microorganisms´ diversity and community composition in the tree canopies

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Forests cover nearly one-third of the Earth's terrestrial surface with more than 3 trillion trees worldwide. Their canopies form numerous diverse and dynamic habitats. In recent years, invasive pathogenic microorganisms have caused considerable damage to forest ecosystems. Thus, a deeper knowledge of the entire diversity and composition of what might be considered a 'healthy microbiome' of forest trees is urgently needed.

As part of the DFG-priority program 'Taxon-Omics - New approaches to discovering and naming biodiversity', we explore the microbial diversity and community composition in the canopy region in the Leipzig floodplain forest using a metatranscriptomics approach. This presentation is a continuation of last year's report on the entire active microbiome on the barc of tree canopies, the microbial food webs, and tree species-specific differences. Here, we will present further results on the diversity of canopy-associated protists.

High-up across the Andes: Protist communities of microbial mats from five Andean lagoons in the Atacama Desert

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Heterotrophic protists colonizing microbial mats have received little attention over the last years despite their important role in the microbial foodwebs, as those found in the remote Andean lagoons. We sampled these mats from different isolated aquatic systems distributed across the Altiplano, where extreme radiation and wide temperature changes prevail, potentially structuring the microbial communities. Here, we present the results of the metabarcoding from DNA and cDNA of protists (V9 18SSU rDNA) in microbial mats across high-altitude lagoons of different salinity (4.3-34 PSU), including insights into their vertical stratification. We assessed the DNA-based protists detected across all the study sites. However, for the cDNA-based analysis, amplification was achieved primarily in three study sites with lower salinity. Sequence variants classified as the amoeboid rhizarian *Rhogostoma* and the ciliate *Euplotes* were found to be common members of the heterotrophic protist community in both the DNA and cDNA- based analyses of the studied lagoons. They were accompanied by diatoms and kinetoplastids. As detected through DNA, different ASVs classified as *Rhogostoma* and *Neobodo* are the shared species between the most saline lagoons. Furthermore, our analyses point to salinity of the water column as a main driver influencing the structure of the protist communities at the five studied microbial mats. Interestingly, the sequence of ASVs classified as *Rhogostoma* are highly similar to the sequence of *R. olyaorum*, a species recently isolated from the Atacama Desert. Our results provide a first snapshot of the unculturable and active protist diversity thriving five athalossohaline lagoons from the Andean plateau.

Amplification of exogenous RNAi trigger by RNA dependent RNA polymerases

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RNAinterference (RNAi) by exogenous RNA is a widespread mechanism among many different species and involves the specific down-regulation of genes in a homology-dependent manner. The exogenous trigger RNA is usually double stranded (dsRNA) and is believed to be processed by Dicer into primary small interfering RNAs (1°siRNAs). Those siRNAs can attack transcripts, causing their degradation and hence, phenotypical down-regulation of the target gene.

In *Paramecium tetraurelia*, several of the key enzymes involved in this pathway have been identified. While the involvement of some enzymes in the biogenesis and function of the 1°siRNAs is not surprising, like Dicer1, the necessity of RNA-dependent-RNA-Polymerase (RdRP) activity for 1° siRNA production remains unknown.

The function of RdRPs in general is the synthesis of a complementary strand using a single-stranded RNA as a template. Due to the initial trigger RNA already being double-stranded, needing this function of RdRPs in this context seems counterintuitive at first.

In this work, we focus on the specific function of RdRPs in the biogenesis of 1°siRNAs. For this, we predicted the structure of RdRPs and potential interaction partners and analyzed the feeding of a single-stranded RNA as well as an "heteroduplex" dsRNA with mismatches to the endogenous targets and between strand.

Our data suggest initial activity of RdRPs on the trigger RNA due to the presence of heteroduplex-derived strand-specific mismatches on reads of the opposite strand as well as the presents of the complementary strand after single-stranded RNA feeding. This indicates that RdRPs process the initial trigger of exogenous RNA: this could be due to a mechanism of trigger amplification or may represent a mechanism that allows dissecting self and non-self RNAs preventing the immediate addition of exogenous RNA to the endogenous RNAi pool.

Next generation taxonomy on three known and one new *Frontonia* species (Ciliophora, Peniculida) from aquatic ecosystems in Kenya

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Frontonia is amongst the most diverse ciliate taxa, exhibiting several morphotypes of which some host green algal endosymbionts. Studies have revealed its ubiquity in almost all aquatic ecosystems; thus, its diversity remains subject of wider studies. Like for other tropical ecosystems, only scanty details are available on *Frontonia* species from varying aquatic ecosystems in Kenya. Consequently, the current study investigated the *Frontonia* diversity in soda lake (Lake Bogoria), freshwater (Lake Naivasha and a pond at Egerton) and marine (Indian Ocean) ecosystems in Kenya using a set of different methods. The morphology and infraciliature were based on living and silver carbonate-stained individuals, molecular methods were performed using the small subunit ribosomal rRNA gene (SSU rRNA). Four *Frontonia* species were found including *F. leucas*, *F. vernalis*, *F. minuta* and a new species. The novel *Frontonia* is similar to *F. leucas* except for the size, i.e., 300-400 µm vs. 120-360 µm and the postoral kineties which are 8 vs. 4 or 5. The study also revealed a new green algal endosymbiont in *F. vernalis*. These studies indicate the importance of applying an integrative taxonomic approach in the delineation of morphospecies especially on our novel species and *F. leucas*. It also revealed a lack of specificity of green algal endosymbiosis found in *F. vernalis*.

The ciliate *Balanion planctonicum* from Lake Zurich –cryptic species or one complex species?

Martina Schalch-Schuler, Gianna Dirren-Pitsch, Barbara Bassin, Katherine Waller, Jakob Pernthaler, Thomas Posch

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Balanion planctonicum is a common, temporarily highly abundant ciliate species found worldwide in temperate lakes and ponds. In Lake Zurich, the tiny ciliate is omnipresent and particularly common during spring and early summer. Abundances of thousands of cells per liter are usually detected by morphology-based methods, e.g., by quantitative Protargol staining (QPS). In contrast, it seems difficult to detect this ciliate by molecular methods, as shown by a three-year study from Lake Zurich and results from a CARD-FISH approach (catalysed reporter deposition fluorescence in situ hybridization) using the 18S rDNA gene sequence. This hinted at variability within the 18S rDNA gene sequence of *B. planctonicum* from Lake Zurich. To shed more light on this topic, we isolated single cells of *B. planctonicum* from enrichment cultures, suspended with *Cryptomonas* sp. and bacteria, and investigated the genotypic and phenotypic variability as well as the phylogenetic position within the class Prostomatea. Using PCR and Sanger sequencing of around 150 isolates, we discovered four different *B. planctonicum* sequence types with variants in the 18S rDNA sequence (up to 28 different nucleotides) and in the ITS region. Three out of four *B. planctonicum* sequence types could be maintained in clonal cultures. In the phylogenetic tree, all sequence types clustered together with *B. masanensis* and build their own *Balanion* cluster, with the genus *Askenasia* as a potential sister group. Morphological comparisons via live measurements and qualitative Protargol staining demonstrated a slight difference in cell size between the sequence types. However, ciliates have a high number of 18S rDNA copies due to their nuclear dualism. To estimate if sequence variation is unique for a clonal *B. planctonicum* culture or if a clone harbors all four sequence variations but with different manifestations, we started to sequence full length 18S rDNA sequences using nanopore technology. In sum, ciliates classified as *B. planctonicum* by morphology-based methods might represent a morphotype harbouring cryptic taxa or one species with genotypic plasticity.

Keynote Lecture:

Egotism and symbiosis: the story behind soil protist diversity

Stefan Geisen

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Why is there so much biodiversity on the planet? Why isn't (functional) redundancy limiting this biodiversity to a minimum? These major ecological questions are also relevant for protists. Best known from the paradox of the plankton (also mostly about protists!), a huge range of protist species also live in soils. The most common of these soil protists virtually do the same thing: microbiome predation, i.e. preying on a wide range of microorganisms.

Here I will show some insights to explain the paradox of soil protist diversity. These include trait differences among protist species, which allow different species to co-exist in various spatial, environmental, and nutritional niches prevailing in the highly complex soil matrix. Changes in protist diversity have immediate impacts on the soil microbiome and thereby ecosystem functions such as on plant growth. Also, non-predatory symbiotic protists are diverse and functionally important members of soil life. I will show insights on the diversity aspects of those organisms as well and compare that to the more egotistic predatory protists.

While we are only beginning to understand the mechanisms and functioning of the immense biodiversity of soil protists, I believe that this research field holds great promises beyond basic research and also beyond the field of protistology. General ecological theories will be testable, results transferable to other organism groups and potential ways to practically enhance biodiversity will become available.

Biogeography of free-living terrestrial protists – lessons learned from phylogeography, climate niche distribution and atmospheric circulation modelling

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The biogeography of free-living soil protists has been a subject of heated debates in the late 20th century, partly because of conflicting views on taxonomy. While protist taxonomy remains a challenge and is sadly under-studied and under-funded, significant progress has been made thanks partly to the development of increasingly powerful and affordable molecular methods but also classical morphology-based work. This allowed clarifying the taxonomic status and geographical distribution of (in part cryptic) species and obtaining a better image of the diversity of soil protist. Modelling approaches and ambitious systematic and standardised sampling also increasingly help clarify the biogeographical patterns and their likely drivers. In this talk past and on-going work on testate amoebae as well as all protists ranging from phylogeography, climate niche modelling, atmospheric circulation modelling and soil DNA metabarcoding will be presented, illustrating the answer provided by each contributions and the new questions arising.

It's time to consider the Arcellinida shell as a weapon

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The shells of testate amoebae are morphologically diverse and persistent in the environment. Accordingly, the examination of the morphology and composition of shells became a standard tool in ecological, palaeoecological, and evolutionary studies. However, so far, the function of the shell remains poorly understood and, although based on limited evidence, the shell was considered as a defence mechanism. Based on recent evidence, we propose that the shell of arcellinid testate amoebae is a crucial component facilitating the amoebae's attack of large prey. Accordingly, the shell is not purely protective, but must be considered as a weapon. This change in perspective opens up numerous new avenues in protistology and will lead to a substantial change in ecological, paleoecological, and evolutionary research.

Proteins during evolution: Change of function (repurposing) and relocation

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During 1.5 x 109 years of eukaryote evolution, the number of genes increased only by a factor of <2 (disregarding formation of ohnologs), whereas the number of proteins increased due alternative splicing (rare in *Paramecium*, ~4 per protein, e.g. in mammals), covalent modifications, complex formation etc. Many proteins are found throughout evolution, e.g. ATPase-type H+-pumps, Ca2+-transporters, components of the membrane fusion machinery (including SNARE proteins). Two phenomena are less frequently addressed in the literature: (a) Relocation within cells and (b) repurposing for alternative functions. Ad (a): V-dependent Ca2+-influx channels established in the 1960ies in the cilia of ciliates are transferred to the non-ciliary membrane in cliated epithelia and exchanged for TRP-type channels in the primary cilium. They are also found in the cell membrane of many cells, e.g. of neurons. Ad (b) Repurposing in a larger sense concerns many proteins of neurons up to those dedicated to higher mental abilities (pyramidal cells), including many pumps and channels, receptors for signalling (Glu-, Rya- and InsP3), Ca2+-binding proteins, including a novel type, calsyntenin in the intracellular "spine apparatus" of nerve endings. Strict repurposing is most evident with calcineurin/protein phosphatase 2B (CaN/PP2B). In *Paramecium* CaN contributes to regulation of ciliary activity and trichocyst secretion, in mammalian cells it contributes to different processes.

In the mammalian immune systems CaN is essental for T-cell activation and transplant rejection; organ transplantations were enabled only after discovery of cyclosporin A as a CaN/PP2B inhibitor. In *Paramecium* the regulatory SU-B is much more conserved than the catalytic SU-A, with the exception of its SU-B binding site. The two genes for CaN-SU-B encode the same protein, due to the degenerated triplet code. Only a SU-B-like protein remains in plants for handling stress situations. Altogether CaN displays most flexibility with regard to structure, localization and function.

How to drive gene expression along an epigenome with unique global properties: Supervision of transcriptional processes by a divergent Polymerase II complex in *Paramecium tetraurelia*

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The regulation of genes is essential for cells to respond promptly to changing environmental conditions while maintaining cell homeostasis. This process involves rapid transcriptional activation or repression of genes and is regulated at the level of mRNA synthesis by the multi-subunit RNA Polymerase II complex. The carboxy terminus (CTD) of the largest PolII subunit Rpb1 is one of the most studied structures in terms of regulation during transcription initiation, pausing-release, elongation, and termination. The CTD consists of a flexible linker followed by a structure of seven repetitively organized amino acids of which almost all become phosphorylated during transcription, a process which is further guided by multiple different complexes that dynamically assemble along with the PolII complex.

In *Paramecium tetraurelia*, the PolII C-terminus lacks the heptad repeat structure and subunits of the complexes involved in transcriptional regulation are absent or highly divergent. Consistent with the rather unique features of the *Paramecium* MAC epigenome, such as high polyploidy and absence of heterochromatin, together with a high coding density, short intergenic regions, and tiny introns, we hypothesize that control of transcription must be performed in a fundamentally unique manner. To gain insight into the mechanism of regulation, we studied Paf1, a complex that controls transcription elongation. We were only able to identify three of five canonical subunits, Rtf1, Ctr9, and Cdc73, and initial silencing experiments against the subunits resulted in slower cell division rates while differential gene expression analysis identified chromatin organization and DNA repair as important biological processes. Since the complex also controls mRNA 3'-end processing, we further analysed total ribodepleted long RNA to detect accumulating aberrant transcripts, which are usually neglected in transcriptomic analyses. In conjunction with our studies on the MAC epigenome, we aim to identify mechanisms by which Paramecium controls the transcription cycle with a divergent machinery.

History and molecular biology of Cluster22: non-canonical sRNAs from a lncRNA precursor

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Paramecium produces many types of small RNAs, including vegetative siRNAs and the developmentally expressed scnRNAs and IES RNAs. All these classes have in common that they are RNAse III (Dicer and Dicer-like) dependent and are cleaved from dsRNA precursors. miRNAs cleaved from structured RNAs are thought to be absent in Paramecium.

Since the first sRNA sequencing of *Paramecium tetraurelia* strain 51, a class of sRNAs has been observed that map to a small locus on chromosome 22, and the most obvious feature of these small RNAs is that they are extremely abundant and often make up a large proportion of the total read count of a library.

We took a closer look at how these sRNAs are produced and what their function might be. Our data suggest that small RNAs are produced from the antisense strand of an lncRNA transcript: its sense transcript appears to be transcribed by RNA-dependent RNA polymerase 2 (RDR2). This transcript shows a high degree of backfolding capacity and its structure can be associated with stacks of sRNAs, similar to the behaviour of miRNAs. Surprisingly, we can observe variations in the size of these sRNAs. In contrast to the high dominance of 23nt for all siRNAs, cluster22 sRNAs show a variable proportion of 22nt reads. These are not degradation products, as our data show that 22nt reads of cluster22 are specifically enriched on Ptiwi12 and Ptiwi15, two Piwi proteins involved in the dsRNA feeding pathway.

The function of the cluster22 sRNAs is unknown. Their depletion leads to increased levels of cluster22 lncRNA, so one possibility is negative regulation of this lncRNAs. Our analysis of this locus shows that non-canonical, Dicer-independent sRNAs can be synthesized from structured loci in *Paramecium*, thus extending the genetic repertoire of sRNA accumulation pathways in this species. Although the vast majority of siRNAs in this species are loaded into Piwis with a size preference for 23nt, our data show that Ptiwi12 and 15 can also enrich for 22nt sRNAs.

Thermal response of freshwater ciliates

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I conducted a meta-analysis of the existing literature from field studies and experimental evidence to assess the parameters characterising the thermal response of freshwater ciliates. The statistical analysis revealed that the ciliates' thermal performance is affected by their planktonic lifestyle (euplanktonic vs tychoplanktonic), their ability to form cysts and their nutritional ecology. The shape of the thermal performance curve predicts the ciliates' survival at supraoptimal temperatures. One-quarter of the ciliate taxa may survive at temperatures above the temperatures currently encountered, i.e., they possess warming tolerance and a thermal safety margin. In contrast, cold-stenothermic species, which represent a significant fraction of euplanktonic ciliates, cannot survive by evolutionary adaptation to rapidly warming environments. Phenotypic plasticity and genetic variation, favouring the selection of pre-adapted species in a new environment, are widespread among freshwater ciliates. However, the present analysis is hampered by the lack of evidence for the temperature optima of most species. The extent of acclimation and adaptation requires further research with more ciliate species than the few chosen thus far. Significantly, thermal adaptation may conflict with adaptation to other stressors (predators, food availability, pH), making general predictions on the future role of freshwater ciliates in a warmer environment difficult.

Ciliates involved in citizen science and science communication: *Stentor* as a role model

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Protists are key organisms found in any ecosystem. Mainly because of their tininess and invisibility to the naked eye, most of them remain unknown to the public. To raise awareness for the hidden protist diversity, already many citizen science and science communication approaches have been developed. We will show some ideas how particularly ciliates can be presented, and curiosity of pupils and laypeople be attracted. Exemplarily, we focus on the aquatic protist diversity and how the significance of single species up to communities can be introduced to the public. By training citizen scientists on how to recognize protists, where they live, in which habitats they can be found, what they look like and why they are important, a general awareness for them can be raised. Especially ciliates of the genus *Stentor* are perfect role models because they are relatively huge (up to several millimetres) and colourful due to various pigmentation and endosymbionts.

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Posters

1. A first look into lineage-specific gene families in tintinnid ciliates (Alveolata, Ciliophora, Spirotrichea)

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Tintinnids have diversified into more than 1000 known species predominantly inhabiting the marine plankton. They are mostly distinguished by the morphology of their remarkable lorica – a robust shell formed from highly resistant probably proteinaceous material produced during cellular division. Despite over 250 years of research on tintinnids, the process of lorica formation and the underlying genetic mechanisms are unknown. Tintinnids are monophyletic and closely related to many taxa, which also share the planktonic lifestyle but do not form a lorica. We assume that genes associated with lorica formation are thus unique to tintinnids and can be considered lineage specific. In this study, we aim to establish the first baseline for lineage-specific gene families (LSGFs) in tintinnids, analysing single-cell transcriptomes obtained from culture material and field samples collected in the Atlantic and the Pacific. Potential LSGFs are identified utilizing state-of-the-art bioinformatic tools, including TIDeS, Orthofinder, and the phylogenomic pipeline PhyloToL. Our findings not only represent a significant step towards unravelling the genetic basis of lorica formation but also contribute to our understanding of the intricate relationship between gene evolution and emergent biological functions. Tintinnids, with their unique lorica, serve as a model for exploring these correlations and advancing our knowledge of the molecular underpinnings of lineage-specific features.

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2. Acanthoecid (Chaonoflagellatea) diversity in the Atlantic Ocean by group specific metabarcoding

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During two cruises on the MS Merian water samples were examined regarding acanthoecid protist communities. Applying group specific primers and metabarcoding, we studied the vertical and spatial distribution of acanthoecids in the Atlantic Ocean and found a clear pattern and indication, that the number of species might be highly underestimated.

3. Characterization and utilization of symbiosis model systems for studying the impact of modulated environmental conditions on freshwater invertebrate-gregarine relationships.

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Gregarines are an ancestral group of symbionts infecting invertebrates and are supposed to a have a key position for the understanding of the evolution of parasitism in the phylum Apicomplexa. However, due to their lack of direct clinical significance to humans, gregarines are extremely understudied. Ultrastructural or molecular data are still scarce, especially for species infecting freshwater hosts; they are e.g. underrepresented in metagenomic surveys for protists. Furthermore, for most gregarine species, it is unknown how they affect their hosts and how these effects might alter under different environmental conditions, though various studies have displayed that described effects span the whole spectrum of symbiosis.

To increase the pool of molecular and ultrastructure data available for freshwater gregarines, this project will screen environmental samples and common invertebrates from local freshwater systems and characterize (via SSU DNA sequencing and SEM) any potential gregarines. Molecular data will then be used for phylogenetic analysis to understand the relationships amongst the studied gregarines as well as gregarine sequences available on public genetic sequence repositories. To broaden our understanding of how gregarines impact their hosts this project will first evaluate identified gregarine hosts for their potential to be used in host/symbiont model systems. Infected and uninfected lab reared populations of suitable invertebrate hosts will then be generated and various metrics of fitness (e.g., fecundity, mortality, food consumption, etc.) will be measured for both populations. To understand how these impacts might change under differing environmental conditions, infected and uninfected host populations will be subjected to modulated environmental conditions (e.g., water temperature, water pH, pollutant levels, food type, food availability, etc.). Ultimately a generalized linear regression model will be applied to examine the relationship between infection status, environmental condition, and fitness metrics. The objectives of this project and some preliminary results will be presented.

4. First volumetric analyses of lorica forming material in the tintinnid model genus *Schmidingerella* (Alveolata, Ciliophora)

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Tintinnids are planktonic ciliates with a distinctive tubular or vase-shaped shell called lorica which is generally formed by material produced and secreted by the cell. These ciliates have an obconical cell attached by a contractile peduncle to the lorica bottom. The apical cell portion features a circular arrangement of long ciliary fans (membranelles), which are crucial for both movement and filter feeding on micro-/nanoplankton, making tintinnids integral to the microbial loop. During cell division, lorica forming material accumulates in the proter. After transverse fission, the proter creates a new lorica, while the opisthe retains the parental one. Previous studies only provided general observations of this process, leaving several questions unanswered, e.g., during which stage of cell division the material production starts and how much material is finally provided for the construction of a new lorica? The amount and the chemical composition of the material are important, considering its fate after cell death (nutrient recycling and fossilization). Our study focuses on *Schmidingerella*, a model genus with transparent, champagne flute-shaped loricae. We analysed monoclonal, Methyl Blue-Eosin-stained culture material from the Northeast Pacific and Protargol-stained field material from the Chesapeake Bay opening into the Northwest Atlantic. Utilising brightfield microscopy, image stacks of selected cells were captured, and the lorica forming material was individually marked in each image. Based on the marked areas, a 3D-model of the material was created for volumetric calculations. Additionally, we classified dividers into five stages according to their stomatogenesis, i.e., the different shapes of the opisthe's newly forming membranellar zone, a feature recognisable both in live and fixed material. Our preliminary results provide the first volumetric estimates of the lorica forming material in tintinnid ciliates, contributing to future volumetric comparisons of the intracellular material with the walls in fully formed loricae.

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5. Natrix2 – Improved amplicon workflow with novel Oxford Nanopore Technologies support and enhancements in clustering, classification and taxonomic databases

Aman Deep [1], Dana Bludau [1], Marius Welzel [2], Sandra Clemens [2], Dominik Heider [2], Jens Boenigk [1], Daniela Beisser [3]

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Sequencing of amplified DNA is the first step towards the generation of Amplicon Sequence Variants (ASVs) or Operational Taxonomic Units (OTUs) for biodiversity assessment and comparative analyses of environmental communities like protists. With Natrix, a user-friendly and reducible workflow solution, processing of prokaryotic and eukaryotic environmental Illumina sequences using 16S or 18S is possible. Here, we present an updated version of the pipeline, Natrix2, which incorporates VSEARCH as an alternative clustering method with better performance for 16S metabarcoding approaches and mothur for taxonomic classification on further databases, including PR2, UNITE and SILVA. Additionally, Natrix2 includes the handling of Nanopore reads, which entails initial error correction and refinement of reads using Medaka and Racon to subsequently determine their taxonomic classification.

6. Phylogenetic and functional diversity of Chrysophyceae in inland waters

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Despite the general importance and the central ecological role of Chrysophyceae in freshwaters, most molecular community analyses are limited to the class-level and fail to unravel questions regarding their phylogenetic and functional diversity. The diversity within the Chrysophyceae is exceptionally high with regard to phylogenetics, morphology and functions. They encompass a variety of orders, whereby heterotrophy has evolved independently in several phylogenetic lineages. Therefore, closely related taxa evolved that developed different feeding strategies (photo-, mixo-, heterotrophy). In our study, we analysed the distribution patterns of the different Chrysophyceae lineages on a European scale. Based on an extensive phylogenetic tree, we here affiliated 2370 environmental Chrysophyceae sequences originating from 218 European lakes to distinct phylogenetic lineages and orders as well as to distinct nutritional types. We thereby provide a basis for the linked analysis of the phylogenetic and functional diversity within Chrysophyceae. Our study demonstrates that Chrysophyceae are one of the most common groups in freshwaters. We found Chrysophyceae in 213 out of 218 sample sites across Europe. In several sites they belong to the most commonly retrieved taxa. Ochromonadales and a Chrysosacca-Apoikiida clade are the most widespread Chrysophyceae groups and show a high degree of OUT diversity. Most detected and assignable OTUs were affiliated with mixotrophic Chrysophyceae.

7. A phylogenetic revision of the widely distributed kinetoplastid flagellate morphospecies *Rhynchomonas nasuta*

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Rhynchomonas nasuta (Stokes) Klebs 1892 is a small heterotrophic flagellate with a long posterior flagellum and a pronounced proboscis, which it uses to search for food particles. Like the well-known genus *Neobodo*, it belongs to the order Neobodonida. The first described strain, originally named *Heteromita nasuta*, was found in a standing pond containing decaying *Sphagnum*. Since its initial discovery, *Rhynchomonas* strains have been found in freshwater, brackish and marine environments, in soils, sediments and in the water column, in surface waters as well as in the deep sea and in habitats with very different oxygen concentrations. Isolated and sequenced strains from such different environments often show a large genetic p-distance to the next known representative. These large genetic distances together with the occurrence of single strains in habitats with strongly diverging abiotic conditions raises the question, whether the taxonomic status of the morphospecies *Rhynchomonas nasuta* should be revised. In addition, the isolation and sequencing of many new strains from different regions (Madeira, River Rhine, Atlantic Ocean) offered the opportunity to conduct a phylogenetic revision of the Rhynchomonadidae. 18S and 28S SSU rDNA sequences were used to create phylogenetic trees utilizing different methods. The results reflect the wide diversity of strains agglomerated under the morphospecies *Rhynchomonas nasuta*. Further isolation and extensive sequencing of strains from different habitats, including less frequently sampled environments, such as hypoxic and anoxic zones, is crucial to solidify and complement this revised phylogeny. Additionally, elaborated microscopical techniques, like SEM, cLSM etc., and single strain experiments testing for tolerances regarding different abiotic parameters shall help to better understand the phylogenetic clustering of the genetically diverse genus *Rhynchomonas*.

8. Exploring the spatial proteomics of *Paulinella* chromatophora: Insights into a nontraditional protein import machinery

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The chromatophores found in cercozoan amoebae of the genus *Paulinella* represent photosynthetic organelles derived from cyanobacteria via primary endosymbiosis. Despite their comparatively recent emergence, approximately 100 million years ago, these chromatophores have undergone extensive gene loss. The functional compensation for these losses is achieved through the import of nucleus-encoded proteins. The unique composition of the chromatophore envelope, consisting of two membranes separated by a peptidoglycan layer, offers an exceptional system for investigating the early stages of transitioning from a membrane system with bacterial identity to one with organellar identity.

While the chromatophore cytoplasmic membrane originates from cyanobacteria, the outer membrane is hypothesized to be host-derived. Notably, the absence of a protein translocon akin to the TIC/TOC complex in plastids suggests alternative protein import strategies. The precise mechanisms governing these import strategies remain elusive. Despite the absence of an N-terminal signal peptide, conventionally associated with protein sorting into the secretory pathway, immunogold analyses of a chromatophore-targeted protein (photosystem subunit PsaE) suggest the involvement of vesicular transport through the Golgi in translocating proteins across the outer chromatophore membrane.

Here, we aim to characterize the composition of Golgi-derived vesicles specifically targeted to chromatophores. Employing "Localization of Organelle Proteins by Isotope Tagging after Differential Ultracentrifugation (LOPIT-DC)" in *P. chromatophora*, an approach known for its informativeness in discerning the proteome composition of diverse compartments in protists, we anticipate identifying chromatophore-targeted proteins with multiple distribution peaks corresponding to fractions localized within the chromatophore, endoplasmic reticulum (ER), Golgi, and the vesicles bridging Golgi and chromatophore. Our investigation aims to unveil additional proteins associated with these vesicles, such as receptors, SNARE proteins, and coat proteins, shedding light on the molecular mechanisms establishing this nontraditional protein import pathway. Furthermore, the analysis has the potential to provide novel insights into the proteome composition of diverse subcellular compartments in *P. chromatophora*.

9. Growth rates calculated by two methods differ – effects of copper on the diatom *Cyclotella meneghiniana*

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Due to human activities, a high amount of copper enters the environment thus polluting aquatic habitats. Whereas it is an essential micronutrient used for example as co-factor in several enzymes, high intracellular copper concentrations result in the production of reactive oxygen species, reduced photosynthesis and cell division rates. In ecotoxicological experiments, growth rates are often estimated by following either cell counts or chlorophyll fluorescence over the exponential growth phase, and it is often assumed that the information about growth rates gained by either approach should be comparable. We tested this assumption by following growth of a strain of the planktonic diatom *Cyclotella meneghiniana* under different copper concentrations by manual cell counting, assessment of chlorophyll fluorescence with a plate reader, and an impedance-based cell counter. Whereas data raised through manual counting corresponded well with results of impedance measurements, cell division rates calculated based on chlorophyll fluorescence signal were found to significantly underestimate growth rates as compared to the two aforementioned methods.

10. Occurrence and distribution of ciliate taxa in the plankton of the Caribbean Sea and the Atlantic

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So far, the knowledge about the biodiversity of protozoan taxa in the ocean and their distribution between the upper ocean layer and the deep sea is still scarce. We conducted an investigation of ciliate taxa during a cruise with the Research vessel 'Maria Sybilla Merian' in November/December 2022 starting from Cartagena (Columbia) crossing the Caribbean Sea and the Atlantic and heading for the Canaries. Thus, throughout this journey, samples of different depths from 50 to more than 4000 m at different latitude stations in the Caribbean Sea and the Atlantic could be gained. At some sampling locations, we additionally isolated protozoa occurring on, or in association with, the floating macrophyte *Sargassum*. The taxa which could be cultivated were subsequently analysed with the use of molecular taxonomic methods (analysing sequences of the 18S rRNA) as well as morphologically characterized. Our hypothesis was that we might find the several species distributed between a wide range of distances and throughout the whole water column.

11. Toxicity of Nickel and Cobalt in *Paramecium*: uptake, transcriptomics, and epigenetic inheritance

Sara Homayooni Hamedan [1], Franziska Drews [1], Alicia Thiel [2], Diana Garza [1], Marcello Pirritano, Julia Bornhorst [2], Martin Simon [1]

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Cobalt and Nickel are increasingly present in our environment. Therefore, they have the potential to enter the food chain. We investigated the combined toxicity and uptake mechanisms into the food chain by studying bacteria and ciliates as the primary consumers. Element measurements of bacterial cell content after exposure showed increased nickel levels compared to cobalt after co-exposure. To mimic the first step in the food chain, we fed these bacteria to paramecia and measured the uptake of metals. The Nickel/Cobalt ratio is similar to that in the food organisms. This indicates that bacteria can specifically accumulate to introduce them into the food chain. When analysing the transcriptomics response of *Paramecium* to sublethal doses of Nickel and Cobalt, gene ontology (GO) analysis revealed common dysregulated signalling pathways such as ammonium transmembrane transport and ubiquitin-related protein degradation. Extensive dysregulation of gene expression was found in genes related to redox processes. This indicates that the cells adapt to increased ROS stress. The fact that both metals can also affect the same cell signalling pathways could explain the obtained increased toxicity when both metals are applied together.

Besides this analysis of acute toxicity, we are also interested in the effect of timely limited application of sublethal doses to cells and the persistence of cellular stress after trigger removal and in sexual offspring. For this, we investigated the effects of low Nickel concentrations on gene expression before and after removal of the metal. After induction of autogamy, we also analysed the F1 generation by transcriptomic analysis with the aim to see if alterations of the transcriptome could be transferred to progeny as an example of epigenetic inheritance of stress in the absence of the trigger.

12. Single-stranded or double-stranded RNA delivery, is that relevant at all?

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Targeted gene silencing by exogenous RNA interference (RNAi) is used for gene regulation in many eukaryotes. This post-transcriptional mechanism is induced by an exogenous RNA, which in most cases is a double-stranded RNA (dsRNA). The dsRNA is presumably converted into primary small interfering RNAs (1°siRNAs) by Dicer. The resulting siRNAs can then target transcripts and induce their degradation, resulting in the downregulation of the target gene.

In *Paramecium tetraurelia*, this mechanism is known to involve several key enzymes. However, it is not known what role the two RNA-dependent RNA polymerases (RdRPs) involved play in the production of siRNA and whether they are dependent on each other. Double-stranded RNA (double-stranded RNA, or dsRNA) delivery is the most commonly used method, which theoretically does not require the function of RdRP, since the primary function of RdRP is to produce dsRNA. Therefore, the question arises as to whether the delivery of single-stranded RNA in a sense or antisense direction to the target mRNA also leads to the production of 1° siRNAs due to the conversion of the single-stranded trigger into a double-stranded one by RdRP activity. For this study, different mutagenic strains of the two involved RdRPs and a wild type of *Paramecium tetraurelia* were used.

In each case, cells were cultivated under application of sense or antisense single-stranded RNA. This was done by feeding with *Escherichia coli*, transformed with an appropriate single-strand RNA producing plasmid.

These data can be used to determine whether *Paramecium tetraurelia* processes single-stranded RNA or only dsRNA during the RNAi-by-Feeding pathway. In addition, the dependence of the individual RdRPs on each other will be tested."

13. Effects of different types of tire wear particles on biofilm formation in the river Rhine

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Microplastic pollution is one of the most pressing ecological problems today. Rivers play a large role in transport and accumulation of such particles facilitating large amounts of plastic reaching the oceans or other remote areas. The largest source of microplastic pollution to European rivers are tire abrasion particles (TAP). Tires contain multiple ecotoxicologically relevant substances and might therefore impact biofilm formation and composition as has been shown for different types of microplastic particles before. How such an effect might express and differ between different types of tires is unknown to date. In order to gather information on differences in biofilm composition between different types of tires, we conducted an experiment in which tire particles were exposed to close to natural conditions in the river Rhine (on the ecological Rhine station in Cologne) for four weeks. The tested categories include different tire types (car summer tires, car all weather tires, truck front axle tires and truck driving axle tires), different wear conditions (used and unused) and two size categories (S = 200 630 µm, L = 1000 2000 µm). Effects were analysed using the ribosomal V4 region of both prokaryotes and eukaryotes. Here we report first results of TAP on microbial communities.

14. Central Collection of Algal Cultures (CCAC) – one of the largest collections of algal cultures worldwide

CCAC Team

University of Duisburg-Essen, Germany

The Central Collection of Algal Cultures (CCAC) is currently located at the University of Duisburg-Essen. Approximately 7500 clonal algal cultures are maintained at four different temperatures, two thirds of which are publicly available. The collection aims at the establishment and maintenance of underrepresented taxa and provides strains for teaching, research purposes or commercial uses.

15. Spatial and vertical distribution of heterotrophic nanoflagellates in relation to benthic and pelagic gene libraries in the Atlantic Ocean

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Although protists are the most common and most diverse eukaryotes in the oceans, knowledge concerning the vertical distribution of these unicellular organisms in the depth continuum of the oceans is limited. Throughout different ocean depths and even in the deep sea, they maintain a high level of genetic diversity despite demanding environmental conditions such as low temperatures, high pressure, the absence of light and low levels of nutrients. Recent studies have shown that benthic protist communities of different deep-sea basins vary significantly from each other, sometimes even endemic distribution patterns have been observed. However, it is still unknown whether deep-sea benthic communities are exchanged via the pelagial. The aim of the present study was to analyse if benthic-associated protists of the deep sea can also be found in the water column or whether a distribution via the pelagial is irrelevant.
Samples examined in this study were taken from the pelagial of the North Atlantic Ocean with special emphasis on the vertical and horizontal distribution of protist communities during the cruise of R/V Maria Sibylla Merian, MSM 112/2, from November to December 2022. Protist cultures were established from samples collected from the surface to depth down to 4000m. Single species were isolated and cultivated and their phylogeny and morphology was analysed. A comparison of taxa identified in the pelagic samples with those recorded in pelagic and deep-sea benthic genotype libraries was done to analyse their global distribution in the world oceans

List of Attendees

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