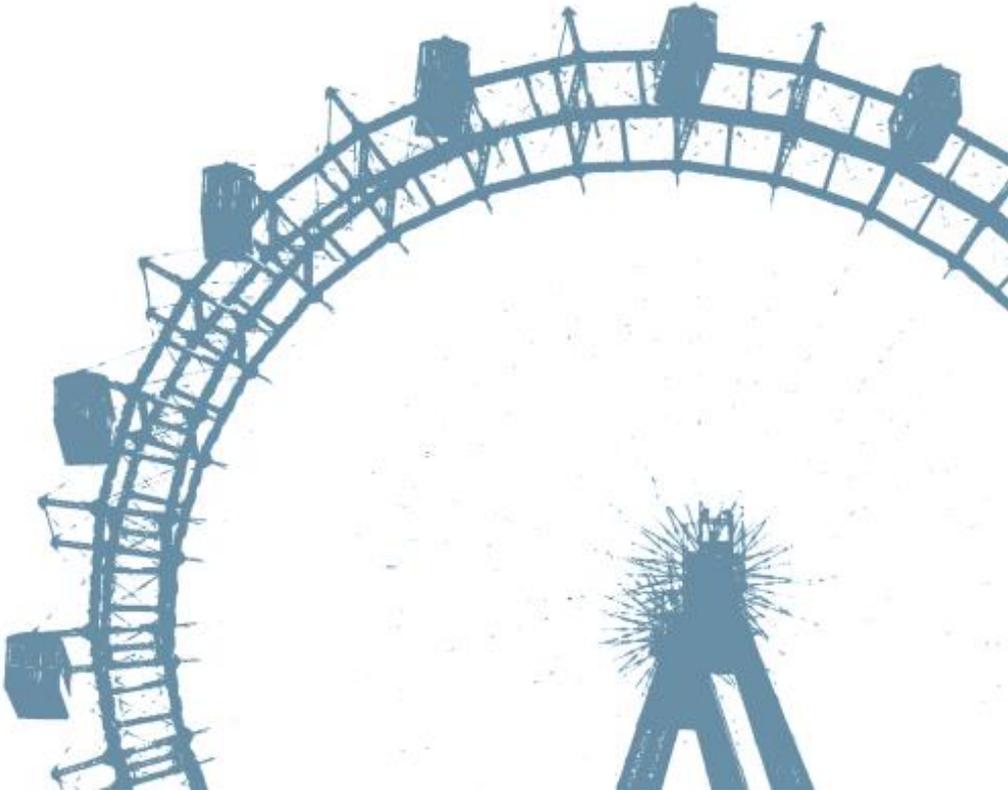




**38th Meeting of the German Society  
for Protozoology (DGP)**



# **Abstract booklet**

**“Life-forms of Protists”**

**February 20<sup>th</sup> to 22<sup>nd</sup>, 2019**

Vienna, Austria

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Thomas Posch	Markus Weitere
Renate Radek	Claudia Wylezich

# Contents

<b>Program overview.....</b>	<b>4</b>
<b>Program details.....</b>	<b>5</b>
<b>Abstracts: Keynote lectures.....</b>	<b>15</b>
<b>Abstracts: Oral presentations.....</b>	<b>23</b>
<b>Abstracts: Posters.....</b>	<b>113</b>
<b>List of participants.....</b>	<b>146</b>

Mon	Tuesday	Wednesday	Thursday	Friday	Sat	Sun
	<b>19.2.2019</b>	<b>20.2.2019</b>	<b>21.2.2019</b>	<b>22.2.2019</b>		
		<u>9:00-9:15</u> <b>Welcome</b> <u>9:15-10:30</u> <b>Evolution</b> <b>9:15-10:00 key note</b> Jürgen Strassert "A phylogenomic approach to resolve the eukaryotic tree of life"	<u>9:00-10:30</u> <b>Ecology</b> <b>9:00-9:45 key note</b> Markus Weitere "The role of protozoans within environmental biofilms" <b>9:45-10:15</b> <b>Grell Award</b> (Alexandra Schoenle) "The unique protozoan communities of the abyssal sea floors"	<u>9:00-10:30</u> <b>Microbial Interactions</b> <b>9:00-9:30 review</b> Christian Bardele "Giant Viruses and their Protistan Hosts"		
		<b>10:30-11:00 coffee</b>	<b>10:30-11:00 coffee</b>	<b>10:30-11:00 coffee</b>		
	<u>12:00-17:00</u> <b>Workshop for Students</b> <b>(Kursraum)</b> Kinderspitalgasse 15)	<u>11:00-12:30</u> <b>Phylogeny</b> <b>11:00-11:45 key note</b> Sabine Agatha "Tricky Trees – Problematic Inference of Relationships in Oligotrichea (Alveolata, Ciliophora)"	<u>11:00-13:00</u> <b>Cell biology</b> <b>11:00-11:45 key note</b> Sutherland Maciver "Meiosis in Asexual Organisms"	<u>11:00-13:00</u> <b>Closing Session</b>		
		<b>12:30-13:30 lunch</b>	<b>13:00-14:00 lunch</b>	<b>13:00-14:00 lunch</b>		
		<u>Diversity</u> <u>13:30-15:00</u>	<b>FOTO</b> <u>14:00-14:30</u> <b>poster slide show</b> <u>14:30-15:30</u> poster session + <b>coffee</b>			
		<b>coffee</b>				
		<u>Young Scientists</u> <u>15:30-17:30</u>	<u>16:00-18:00</u> <u>Tour NHM</u>			
	<b>18:00 welcome</b>	<u>17:30-19:00</u> <b>General Assembly</b>				
		<b>19:00 dinner</b>	<b>19:00 congress dinner + awards</b>			

# **PROGRAM DETAILS**

**Wednesday, February 20<sup>th</sup>**

**9:00-9:15 Welcome**

**9:15-10:30 Evolution**

Chairs: Renate Radek, Jens Boenigk

**9:15-10:00 Key note lecture I**

**A phylogenomic approach to resolve the eukaryotic tree of life**

Jürgen F. H. Strassert, Mahwash Jamy, Denis V. Tikhonenkov, Fabien Burki

**Evolution of heterotrophy in chrysophytes**

Jens Boenigk, Nadine Graupner, Jana Olefeld, Daniela Beisser

**Selection and paucity of phylogenetic signal challenge the utility of alpha-tubulin in reconstruction of evolutionary history of free-living litostomateans (Protista, Ciliophora)**

Łubomír Rajter, Peter Vd'áčný

## **Coffee**

**11:00-12:30 Phylogeny**

Chairs: Edward Mitchell, Martin Schlegel

**11:00-11:45 Key note lecture II**

**Tricky Trees – Problematic Inference of Relationships in Oligotrichea (Alveolata, Ciliophora)**

Sabine Agatha

**Re-analysis of the 18 rDNA gene phylogeny of the class Colpodea (Ciliophora)**

Wilhelm Foissner, Peter Vd'áčný

## **Mechanisms of speciation and diversification in Chrysophyceae**

Stephan Majda, Jens Boenigk, Daniela Beisser

## **Taxonomic revision of *Nebela militaris*, based on morphology, molecules...and history!**

Clément Duckert, Quentin Blandenier, Fanny A.L. Kupferschmid, Anush Kosakyan, Edward A.D. Mitchell, Enrique Lara, David Singer

# **Lunch**

## **13:30-15:00 Diversity**

Chairs: Claudia Wylezich, Markus Weitere

## **Biodiversity and adaptation of protists to extreme aquatic environments in the Atacama Desert**

Alexandra Rybarski, Frank Nitsche, Paulina Filz, Manon Hohlfeld, Hartmut Arndt

## **Checklist of peatlands testate amoebae diversity in Tierra del Fuego (Argentina)**

Burdman L, Mataloni G, Lara E

## **Microeukaryotic gene expression profiling along a pronounced salt gradient**

Sabine Filker, Steffen Kühner, Carolin Schöbel

## **Learning from social networks: be connected, be discovered, be in the trophic web**

Dominik Forster, Zishuai Qu, Gianna Pitsch, Estelle Bruni, Barbara Kammerlander, Thomas Pröschold, Bettina Sonntag, Thomas Posch, Thorsten Stoeck

## **Comparative analyses of protistan diversity in soil and corresponding freshwater lakes**

Guido Sieber, Jens Boenigk

**Small- and large-scale patterns of protists from the abyssal sea floors in the Caribbean Sea and the southern North Atlantic Ocean**

Manon Hohlfeld, Alexandra Schönle, Karoline Hermanns and Hartmut Arndt

## **Coffee**

### **15:30-17:30 Young Scientists**

Chairs: Ulrike Berninger, Thorsten Stöck

**Numerical and functional response of two contrasting freshwater ciliates in relation to temperature**

Lu, Xiaoteng , Weisse, Thomas

**High Throughput Sequencing approaches focusing specifically on genus *Nebela* allow testing the influence of the altitude on the community composition.**

Quentin Blandenier, David Singer, Raphael Jauslin, Clément Duckert, Amandine Pillonel, Molly Baur, Edward A.D Mitchell, and Enrique Lara

**Hidden diversity of choanoflagellates from extreme saline environments – Investigation of new species originating from the Atacama Desert**

Sabine Schiwitza, Hartmut Arndt, Frank Nitsche

**Paramecium bursaria or not Paramecium bursaria - that is the question**

Christian Spanner, Tatyana Darienko, Ulrike Scheffel, Bettina Sonntag, Thomas Proeschold

**The effects of direction of flow on the diversity of a model metacommunity**

Baierl, Vera, Wickham, Stephen

**Better together – old school morphological identification and modern PCR-based phylogeny for analysing indicator ciliates in freshwater ecosystems**

Anna Binder, Thomas Proeschold, Barbara Kammerlander, Thomas Posch, Thorsten Stoeck, Bettina Sonntag

**Inferring ecological status of coastal environments from eDNA metabarcoding**

Larissa Frühe, Guillaume Lentendu, Dominik Forster, Tristan Cordier, Thomas A. Wilding, Jan Pawlowski, Thorsten Stoeck

**The efficacy of silver-copper ions on the viability of biofilm-associated free-living amoebae**

Miray Üstüntürk-Onan, Tuba Ünsal, Esra Ilhan-Sungur

**17:30-19:00    General Assembly**

**19:00    Dinner**

**Thursday, February 21<sup>st</sup>**

**9:00-10:30 Ecology**

Chair: Hartmut Arndt

**9:00-9:45 Key note lecture III**

**The role of protozoans within environmental biofilms**

Markus Weitere

**9:45-10:15 Grell Award**

**The unique protozoan communities of the abyssal sea floors**

Alexandra Schoenle

**Direct and indirect effects of krill versus salps on plankton community structure and nutrient dynamics at the Western Antarctic Peninsula**

Stefanie Moorthi, Dominik Bahlburg, Philipp Wenta and Christoph Plum

**Coffee**

**11:00-13:00 Cell Biology**

Chairs: Sabine Agatha, Julia Walochnik

**11:00-11:45 Key note lecture IV**

**Meiosis in Asexual Organisms**

Sutherland Maciver, Zisis Koutsogiannis, Alvaro de Obeso Fernández del Valle

**Enhanced plasticity of programmed DNA elimination boosts adaptive potential in suboptimal environments**

Valerio Vitali, Rebecca Hagen, Francesco Catania

**Capsules – Unique Organelles Characterising Specific Tintinnid Ciliate Clades (Alveolata, Ciliophora)**

Ganser Maximillian, Agatha Sabine

**Tintinnid's First Top Model: Ultrastructural Insights into the Ciliature of *Schmidingerella meunieri* (Alveolata, Ciliophora)**

Gruber Michael, Agatha Sabine

**Conjugation and morphogenesis of metopids elucidate phylogenetic relationships within the SAL supercluster**

Vdacny Peter, Foissner Wilhelm

**Understanding phytomyxid-host interactions by combining transcriptomics with in-situ transcript visualisation.**

Neuhauser Sigrid, Kirchmair Martin, Stefan Ciaghi, Julia Badstöber, Arne Schwelm, Jutta Ludwig Müller, Adolf M. Sandbichler, Claire Gachon

**Lunch**

**14:00-14:30 Poster Slide Show**

**14:30-15:30 Poster Session + Coffee**

**16:00-18:00 Tour: Natural History Museum**

**19:00 Congress Dinner**

# Friday, February 22<sup>nd</sup>

## 9:00-10:30 Protist Interactions

Chairs: Bettina Sonntag, Thomas Posch

### 9:00-9:30 Review: Giant Viruses and their Protistan Hosts

Christian Bardele

#### **Euplotes daidaleos - a model ciliate for studying endosymbiotic events**

Proeschold Thomas, DarienkoTatyana, Schmidt Johanna, Pitt Alexandra, Hahn Martin, Sonntag Bettina

#### **“To eat or not to eat”: Which freshwater protists are major bacterivores during spring?**

Vesna Grujić, Indrani Mukherjee, Michaela M Salcher, Tanja Shabarova, Jiří Nedoma and Karel Šimek

#### **Developing to ‘nucleomorphs’ in dinoflagellates: the short motile period advanced the endosymbionts to tertiary plastids**

Norico Yamada, Hiroto Sakai, Ryo Onuma, Peter G. Kroth, Takeo Horiguchi

#### **Microbial food webs in hypertrophic fishponds: omnivorous ciliate taxa are major protistan bacterivores**

Šimek K, Grujić V, Nedoma J, Jezberová J, Šorf M, Matoušů A, Posch T, Bruni EP, Vrba J

## Coffee

## 11:00-13:00 Closing Session

Chairs: Anja Scherwass, Martina Schrällhammer

#### **Metagenomics for parasite detection: A proof-of-concept study using swine faeces samples**

Claudia Wylezich, Ariane Belka, Dennis Hanke, Martin Beer, Sandra Blome, Dirk Höper

## **Contrasted effects of cadavers, blood, urine and faeces on soil micro-eukaryotic communities – implications for biodiversity exploration and forensic application**

David Singer, Petr Hedě́nec, Raphael Jauslin, Thierry Heger, Ildikò Szelezcz, Enrique Lara, Clément Duckert, Edward A. D. Mitchell

## **Interaction of Apicomplexa parasites and their potential invertebrate hosts revealed by eDNA metabarcoding**

Petr Hedě́nec, David Singer, Enrique Lara, Clement Duckert, Erika Hiltbrunner and Edward A. D. Mitchell

## **Effects of light pollution on mixotrophic protists**

Schlager Elisabeth, Berninger Ulrike-G.

## **Performance and competitive ability of heterotrophic and mixotrophic ciliates under different regimes of light intensity and prey concentration**

Sabine Flöder, Lukas Ross, Lara Bromann, Laura Hennings, Jarrit van der Sluis, Stefanie Moorthi

## **Towards an ecological understanding of the killer trait - A reproducible protocol for testing its impact on freshwater ciliates**

Koehler Lars, Flemming Felicitas E., Schrollhammer Martina

## **Flow variability affects multi-trophic levels of fluvial biofilms: Within and across microbial groups effects**

Ute Risse-Buhl, Christine Anlanger, Antonis Chatzinotas, Christian Noss, Andreas Lorke, Markus Weitere

## **Assessing the plant-associated protist microbiome**

Kenneth Dumack, Sebastian Flues, Kai Feng, Susanne Schreiter, Rita Grosch, Ye Deng, Kornelia Smalla, Michael Bonkowski

## **Lunch and Farewell**

# **Abstracts**

## **Keynote lectures**

(in alphabetical order)

**Notes:**

## **Tricky Trees – Problematic Inference of Relationships in Oligotrichea (Alveolata, Ciliophora)**

**Agatha S<sup>1</sup>**

<sup>1</sup>Department of Biosciences, University of Salzburg, Hellbrunnerstraße 34, 5020 Salzburg, Austria  
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The vast majority of Oligotrichea are planktonic ciliates inhabiting marine habitats. They comprise the Oligotrichida and the Choreotrichida with their aloricate forms and the tintinnids. At a steady rate, new species and genera of oligotrichids and aloricate choreotrichids are described, which is in congruence with molecular findings indicating a considerable diversity of yet undescribed taxa. Unfortunately, the addition of the new taxa and new gene sequences does not considerably contribute to solve the problem of non-monophyletic genera and families and to settle the tree topology. Particularly, the evolutions of the somatic ciliary patterns and kinetid ultrastructures are promising character complexes providing apomorphies to explain the taxon clustering and to revise the classification. Without knowledge about kinetid homologies inferred from cell division, however, some of the newly discovered somatic ciliary patterns are difficult to integrate into the existing hypothesis on pattern evolution. While ultrastructural data on a freshwater oligotrichid perfectly support the previous cladistic analyses, incorrectly described somatic ciliary patterns and questionable identifications of taxa sequenced hamper phylogenetic analyses. Crypticity and phenotypic plasticity characterise especially tintinnid ciliates as indicated by molecular approaches. Although the probability for discovering divergent clades is lower in tintinnids than in oligotrichids and aloricate choreotrichids, new species and genera can be found as demonstrated by recent studies. Beyond the cytological features recognizable in the light microscope, tintinnids possess many apparently phylogenetically relevant characters accessible only by transmission electron microscopy. One of the most important issues impairing progress in tintinnid phylogeny, systematics, and ecology is the application of inadequate literature for identification as demonstrated based on material from the North Pacific. Likewise, synonymizations merely based on sequence identity of marker genes are counterproductive and should await support by morphological and/or ultrastructural features.

The studies are financially supported by the FWF projects P28790 and I3268.

**Notes:**

## Meiosis in Asexual Organisms

**Sutherland Maciver<sup>1</sup>, Zisis Koutsogiannis<sup>1</sup>, Alvaro de Obeso Fernández del Valle<sup>1</sup>**

<sup>1</sup> Centre for Discovery Brain Sciences, Edinburgh Medical School, Biomedical Sciences, University of Edinburgh, Hugh Robson Building, George Square. Edinburgh. EH8 9XD. Scotland. UK

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The amoebae (and many other protists) have traditionally been viewed as being asexual organisms. However, it has been hypothesized that asexual reproduction cannot sustain populations indefinitely due to the build up of mutations. This is known as “Muller’s ratchet” and one of the functions of sexual reproduction is to avoid this mutational build up. Over the last decade opinion has changed and these asexual organisms are now believed to be cryptically sexual, or are recently related to sexual lineages, and that sexual reproduction is the ancestral state. This idea is mainly based on many protistan genome studies in which the presence of “meiotic genes” has been discovered in these previously postulated asexual lineages. The *Acanthamoeba* genome too contains the core set of meiotic genes. Using RNA-seq, we have found that the entire repertoire of these meiotic genes is expressed (in many cases producing large amounts of mRNA species) in exponentially growing *Acanthamoeba* with no other indication of meiosis being visible (cell or nuclear fusion). We argue that these so called meiotic genes are involved in the related process of homologous recombination (HR) in this amoeba. We hypothesise that they are only involved in meiosis in other organisms that indulge in sex, and that in asexual organisms such as *Acanthamoeba*, HR is important as a guard against the accumulation of mutations. *Acanthamoeba* and asexual amoebae in general tend to be highly polyploid, meaning that any spontaneous mutation is corrected by HR using the other gene copies as templates. We also suggest that asexual reproduction is the ancestral state.

**Notes:**

## The unique protozoan communities of the abyssal sea floors

### **Schoenle A**

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Although the abyssal seafloor (3-6 km depths) is the largest benthic habitat on this planet covering around 54% of the Earth's surface, knowledge about deep-sea protists, their ecological function, species-level distribution and diversity in these depths is scarce. This is in striking contrast to their potential importance regarding the material flux and bacteria consumption in the deep sea. We established a protocol combining several available qualitative and quantitative methods to receive detailed information on abundances, biodiversity and biogeography of benthic deep-sea protist communities. For a global comparison we sampled sediment from 20 deep-sea basins located in the Pacific and Atlantic Ocean and extracted the DNA for next-generation sequencing (NGS). Comparing our deep-sea OTUs to reference sequences indicated the existence of a specific deep-sea protist fauna. The majority of our total OTUs could be assigned to protists. Dominant protist OTUs belonged to Discoba (Diplonemea and Kinetoplastea), Alveolata (Dinophyceae, MALV-I, MALV-II, Ciliophora) and Rhizaria (Foraminifera and Endomyxa). The different deep-sea basins had a specific protist community with less than 1% of protist OTUs occurring at all sampled stations. The most abundant OTU (55% of total reads) belonged to the bicosoecid *Cafeteria burkhardi* sp. nov. and occurred in all investigated deep-sea stations. In addition, we could cultivate and isolate *C. burkhardi* from the North Atlantic, South East Atlantic, Pacific, Mediterranean Sea and Baltic Sea. Cultured isolates, including *C. burkhardi*, were further used to verify the potential deep-sea origin of the protists recovered by the NGS technique with the aid of pressure and temperature experiments. In case of *C. burkhardi* several strains, despite their isolation of depths ranging from surface waters down to 8000m depths, survived high hydrostatic pressure indicating their potential to live and reproduce in the abyss. For the southern North Atlantic Ocean we could show that *C. burkhardi* was associated to the brown algae *Sargassum*, which has been reported with large biomasses from the deep-sea floor. Thus, there might be a possible exchange of several protist groups between surface waters and the deep sea via aggregations such as sinking detritus or *Sargassum*. The enhanced cultivation of deep-sea and surface water protists led to a deeper evaluation of the phylogenetic relationship within the Cafeteriaceae. Based on multigene analysis, we regrouped the Cafeteriaceae into eight species of which six were newly described. While *Cafeteria burkhardi* seems to have a cosmopolitan distribution as indicated by the NGS and cultivation results, the distribution of the other *Cafeteria* species seems to be more restricted. We showed that protist diversity in the dark ocean differs on a local and global scale and are distinct from surface water communities, while several genotypes occur in cultures from surface waters and the deep sea. Multiple processes might operate at the same time structuring communities of deep-sea protists including bathymetric features, depths and biotic environmental factors may all play a role.

**Notes:**

## **A phylogenomic approach to resolve the eukaryotic tree of life**

**Jürgen F. H. Strassert<sup>1</sup>, Mahwash Jamy<sup>2</sup>, Denis V. Tikhonenkov<sup>3</sup>, Fabien Burki<sup>4</sup>**

<sup>1</sup>Uppsala University, Department of Organismal Biology, Systematic Biology

<sup>2</sup>Uppsala University, Department of Organismal Biology, Systematic Biology

<sup>3</sup>Institute for Biology of Inland Waters, Russian Academy of Sciences

<sup>4</sup>Uppsala University, Department of Organismal Biology, Systematic Biology  
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The eukaryotic tree of life is constantly improving, but the phylogenetic relationships between the major supergroups as well as the evolutionary origin of several other deeply diverging clades remain unknown. Resolving the phylogenetic position of these 'orphan' lineages is important, because they represent missing evolutionary links between established groups. *Telonemia* is one such orphan taxon for which little is known. The group is composed of molecularly diverse biflagellated protists, often prevalent although not abundant in aquatic environments. Following a phylogenomic approach, we extracted 321 genes from transcriptomic/genomic data from each of more than 740 taxa representing all eukaryotic supergroups. This so far largest taxon sampling enabled us to build an exceptional clean dataset by detecting contaminants, endosymbiotic gene transfers, and paralogs, which can easily be overlooked in smaller-scale analyses. Our phylogenetic analysis of this dataset not only revealed the evolutionary origin of *Telonemia* but also resolved most of the deep evolutionary relationships among eukaryotes.

**Notes:**

# The role of protozoans within environmental biofilms

## Markus Weitere

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Protozoans play important roles within microbial food webs, e.g. by mediating the trophic transfer from bacteria and algae to higher trophic levels. In this position, they stimulate the overall flux of matter within food webs. While this role is well investigated in suspended communities (plankton) far less is known about protozoans within surface-associated communities, so called biofilms. Biofilms are complex communities of microbiota (including archaea, bacteria, heterotrophic and autotrophic protists, small metazoans) and their extracellular material that occur in association with aquatic interfaces. The densities of those organism groups, including protozoans, are usually strongly enhanced in comparison to those in plankton environments. Especially in environments which are dominated by substratum (i.e., aquifers, streams and rivers, shallow lakes, etc.), biofilms mostly dominate microbial whole-ecosystem processes in comparison to the plankton. However, despite this high numerical dominance, protozoan-mediated processes are rarely considered within biofilms and bacterial biofilms are often considered grazing resistant towards micro-grazers, thus neglecting significant roles of protozoans for the within-biofilm flux of matter. This presentation challenges this common view. It reviews the current knowledge on the occurrence, control and role of protozoans and micro-metazoans within surface-associated communities in aquatic ecosystems and critically evaluate related concepts, e.g. on the grazing resistance of bacterial biofilms. On this basis, a food web concept for biofilms with focus on the role of protozoans will be presented with three major elements: (i) Source of matter and energy for biofilms. Biofilms receive matter and energy from plankton environments especially by dissolved organic carbon or dissolved inorganic nutrients as resources for heterotrophic bacteria and photoautotrophs, respectively. Micrograzer (both protozoans and small metazoans) offer another, as yet hardly considered, pathway of matter flux from the plankton to the biofilm by the presence of suspension feeders. This pathway can be similarly important as the pathway of dissolved matter. (ii) Utilization of basal resources (prokaryotes and algae) within biofilms. In contrast to the common view of grazing resistance of biofilms, several specialized protozoans are able to consume significant amounts of biofilm biomass. However, the accessibility and food quality, especially of prokaryotes, decreases with biofilm succession. Hence, the basal resources of food webs show pronounced food quality gradients, which has implications for the transfer efficiency between basal resource and consumers. (iii) Trophic complexity of biofilm food webs. Besides consumers, several predators exist among protozoans and small metazoans. It was shown with the help of video microscopy that several trophic levels exist within microbial biofilm food webs. This suggests strong flux and turnover of matter within biofilm communities. Overall, protozoans play a significant role within biofilms affecting both their dynamics and the functioning.



# **Abstracts**

## **Oral Presentations**

(in alphabetical order)

**Notes:**

## THE EFFECTS OF DIRECTION OF FLOW ON THE DIVERSITY OF A MODEL METACOMMUNITY

**Baierl, Vera, Wickham, Stephen**

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The metacommunity concept is an important way to think about linkages between different spatial scales in ecology. A metacommunity is defined as a set of local communities that are linked by the dispersal of potentially interacting species. There has already been a lot of research about metacommunities where organisms can migrate equally in different directions but there is a lack of studies about the influence of a defined flow. In many aquatic ecosystems, however (e.g. lakes or ponds linked by), flow has the potential to skew migration in a downstream direction.

In this experiment the effects of different flow rates on the diversity of an aquatic model metacommunity has been tested, with the goal to determine the relationship of flow rate and diversity.

The experimental design consisted of bottles filled with water and connected with tubes. Pumps were used to simulate the flow. For six weeks we tested the effects on the organisms in a system without flow, one with a low flow rate (30% exchange per day) and one with a high flow rate (60% flow rate per day). As model organisms we used heterotrophic and mixotrophic ciliates, one species of rotifers and as prey, algae.

Preliminary results show that algal biomass was highest the first bottles in the chained metacommunity. Algal biomass was highest in the system with the high flow rate and the lowest in the system without flow.

**Notes:**

# Giant Viruses and their Protistan Hosts

## **Christian F. Bardele**

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Discovery, morphology and replication cycle of Mimivirus. In 1992 a British microbiologist was looking for the causative agent of a local pneumonia outbreak. In the cooling tower of the local power plant he found an *Acanthamoeba* with a hitherto unknown gram-positive microorganism, which withstood all molecular assays to detect 16S RNA to clarify its systematic position. It took 11 years until French scientists around La Scola, B. and D. Raoult in Marseille discovered that it was a giant virus (GV), which shook the foundations of our knowledge of viruses.

Mimicking a microbe, this giant virus was called Mimivirus. Together with the peripheral fibers and icosahedral capsid this virus measures 750 nm in diameter. Mimivirus and GVs are classified as Nucleocytoplasmic large DNA viruses (NCLDV), which have a double-stranded DNA genome. With a 1200 kb genome encoding almost 1000 predicted proteins Mimivirus surpassed all other viruses known in 2003. It even surpasses small intracellular eubacteria like *Mycoplasma genitalium* whose 580 kb genome encodes 475 open reading frames. For comparison: HIV measures 120 nm across and its 10 kilobases genome encodes 10 proteins.

GVs were first detected in *Acanthamoeba* species (*A. castellanii* and *A. polyphaga*), both known as an “evolutionary melting pot”. Eubacteria, fungi, phages and other viruses seem to have exchanged genes thanks to horizontal gene transfer (HGT). Mimivirus and other GVs are often isolated with *Acanthamoeba*-cultures as bait. Mimivirus enters the amoeba by phagocytosis, stimulated by the peripheral fibers. DNA replication, mRNA transcription, assembly of viral proteins into empty capsids, and filling with DNA all takes place in and at the periphery of a special cytoplasmic organelle, the “virus factory” (sometimes regarded as the living part of a virus). At the end of this process over 100 mature virions leave the dying cell within 24 h of the initial infection. Mimivirus has a stargate structure, never seen before in viruses, that is used to deliver viral DNA once it has entered the host cell. On the opposite side the virus has a second opening. This is used to fill in a large amount of DNA into the small space of the prefabricated capsid with the help of an A32-like DNA packing ATPase.

The host cell *Acanthamoeba* with two stages, the trophozoite and the cyst, is a common soil amoeba. It can be the causative agent of two human diseases, the Granulomatous Amoebic

Encephalitis and Keratitis. Contact lens wearers may catch an *Acanthamoeba* cell infected with Mimivirus from warm-water supply systems.

### Other protistan giant viruses, their worldwide distribution and their ecological impact.

GVs, now also called Megavirales, occur worldwide in freshwater and marine biota. They have been detected in the deserts, in deep ocean sediments at - 3000 m, in 30000 years old Siberian permafrost, in Antarctica, in tropical forests, in all kinds of biofilms, and in sewage treatment plants. GVs are particularly widespread among protists. Heterotrophic nanoflagellates (*Cafeteria roenbergensis*), coccolithophorids (*Emiliana huxleyi*), *Ostreococcus tauri* (the smallest eukaryote ever seen), *Bodo saltans*, *Acanthocystis turfacea*, *Paramecium bursaria* and many other protists are the natural hosts of GVs.

The impact of GVs to marine ecosystems is best illustrated by the coccolithovirus, which regulates the largest algal bloom caused by the haptophyte *Emiliana huxleyi*, thereby influencing the large-scale turnover of carbon and sulfur. Moreover, it had and still has an impact on

geological phenomena, like the formation of White Cliffs of Dover, and recent marine sediments from coccoliths of *Emiliania huxleyi*. Moreover, GV-killed diploid *Emiliania* cells release a fluid gas (dimethylsulfide) that has an important effect on the composition of the earth atmosphere and thus on earth climate in general. *Emiliania* cells avoid being totally killed by coccolithoviruses when they undergo meiosis. The resulting motile haploid stages, which carry no carbonate scales, are resistant to the virus and thus survive. When the haploid gametes mate they become sensitive once more.

There is another way to fight against GVs, seen e.g. in the marine nanoflagellate *Cafeteria roenbergensis*. It shows that GVs too can fall ill. A virophage, called Mavirus impairs morphogenesis of the Cafeteriavirus and thus prolongs the life of its protistan host. A virus parasitizes a virus — again, never seen before. Similar to the prophages in bacteria, Mavirus can integrate into the genome of the flagellate, “waiting” until the flagellate becomes infected by its GV before it leaves its hiding place to fight against the enemy of its protistan host. Such an arms race is also performed by other virophages like Sputnik and Zamilon. Twenty virophages are currently listed and sequenced. They have mini-genomes with about 20 genes.

A totally new morphology - some even found in 30000 years old permafrost. Certain GVs display morphology never seen before in viruses: the amphora-shaped capsid seen in Pithovirus (from pithos amphora in Greek), Pandoravirus, Cedratvirus and Mollivirus. Some of these viruses have one or two distinctive “corks” or poreplates at one or both of their poles. Pithovirus sibericum and Mollivirus sibericum from Siberian permafrost or melting ice have been “grown” in *Acanthamoeba* cells. These GVs thus raise the question what will happen if climate change continues to progress and larger areas of permafrost melt, thus releasing not only large amounts of methane, but perhaps also new germs.

The genomic complements of giant viruses and the hypotheses of their origin. Pandoravirus salinus from the coastal waters of Chile has the largest viral genome at 2,473,870 bp, which encodes 2541 proteins. Several GVs code for an almost complete translation machinery. The Tupanvirus, discovered in 2018, is the current record holder for viral particle size (2,4 µm). It has genes for 20 aminoacyl-tRNA synthetases, 70 tRNAs, many transcription factors and genes involved in maturation and modification of tRNA and mRNA. It seems that this GV cooperates with (or partly escapes from) the control of basic molecular steps of its host cell. Tupanvirus just lacks ribosomes and depends on ATP from its host. Other GVs code for DNA repair, histone-like proteins and parts of certain metabolic pathways (carbohydrate, lipid synthesis). Beyond this, GVs may code for a huge number of proteins, often with no known function (with up to 90 % of them not possessing homolog in protein databases). Mining metagenome databases is now the quickest way to find new GVs. Klosneuviruses are an example of GVs discovered by this approach. A trade-off of these searches is that neither the morphology nor the natural host of these GVs is known for the moment. The universally conserved NCLDV proteins like DNA polymerase, major capsid protein, packaging ATPase, A18-like helicase, and late transcription factor VLTf3 have been used to reconstruct the phylogeny of the GVs with no convincing results. Thus, GV taxonomy remains to be settled.

HGT is proposed to play a significant role in the evolution of GVs. GV genomes are often characterized by gene duplications. These surplus genes may mutate rapidly and result in orphan genes of unknown function. Robbed genes from their eukaryote hosts or caught from eubacteria are also found in most GVs. Repeated gain of genes could have led to their enormous size. Loss of genes seems to have happened likewise. But where did they originate from in the first place? Did they appear before the diversification of the three domains of life, the eubacteria, archaeobacteria and eukaryotes?

Given the GV discoveries over the past years, some French scientists have even postulated a “fourth domain” of life. One idea is that they are descendants from “ribocells”, inhabitants of the former RNA-world. Finally, the old discussion whether viruses are alive or not still remains. What is an appropriate definition for viruses, we must still ask. There is a tremendous gap between classical viruses and the GVs. No doubt, the discovery of the GVs has hit virologists like a major earthquake.

Colson, P. et al. (2017) Mimivirus: leading the way in the discovery of giant viruses of amoebae. *Nat. Rev. Microbiol.* 15, 243-254.

**Notes:**

## **Effects of light pollution on mixotrophic protists**

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A multitude of field and laboratory studies have shown that light strongly affects mixotrophic protists. Their abundance, their biodiversity and their relative contribution to primary production and consumption of organic material depend on the individual species' requirements for the light climate in their environment, varying for example with season, water depth and turbidity of the water column.

In the summer of 2018, a large-scale mesocosm experiment was conducted in "Lake Stechlin" (Brandenburg, Germany) to test the possible effect of light pollution ("skyglow" during night time) and brownification (due to increased concentrations of humic substances) on the plankton community. Within these experimental manipulations, we conducted tracer-uptake experiments to investigate the possible direct and indirect effects on the abundance of small (2-20  $\mu\text{m}$ ) mixotrophic protists and their relative contribution to bacterivory and/or primary production in the system.

Our results imply that brownification affected the abundance of mixotrophs more strongly than artificial illumination during night time, and that the contribution of mixotrophs to the plankton community was relatively low but undergoes strong fluctuations. In the context of our data we will discuss possible caveats of tracer experiments.

**Notes:**

## **Better together – old school morphological identification and modern PCR-based phylogeny for analysing indicator ciliates in freshwater ecosystems**

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Ciliates – very common and abundant protists in every freshwater system – feed mainly on bacteria and phytoplankton. Depending on the availability and composition of the food source and abiotic factors, the species assemblage varies in quality and quantity. As classified by many researchers (e.g. W. Foissner or H. Berger), some ciliates are suitable indicators to describe the trophic status of a freshwater ecosystem. Therefore, an identification at species level is necessary. Up to now, ciliates were solely identified from morphology. Following the recent trend on using molecular methods and bioinformatics tools, we combined both approaches. We investigated the ciliate assemblages of different habitats along a longitudinal gradient including the shores of the two lakes Mondsee and Irrsee in Austria that are connected by the river Zeller Ache. Our sampling strategy for ciliates followed the standard DIN 38410 (Berger et al. 1997). We collected submersed stones, plants or wood debris and biofilm thereof as well as sediment. By microscopy, we (i) identified living ciliates on species level, (ii) noticed their behaviour in respect to food vacuole content, movement, interactions etc. and, (iii) documented observed ciliates with an image analysis system. After proper identification of a species, single cells were washed in sterile filtrated lake water and starved overnight, before we conducted single cell PCR followed by a nested PCR. The edited sequences (SSU and ITS rDNA sequences) of the morphologically identified ciliates were finally deposited in public databases (UniEuk, NCBI). This integrative approach will form a basis for future molecular water quality assessment tools applied in freshwater ecosystems. The study is funded by the Austrian Science Fund (FWF): I2238-B25.

Reference: Berger, H., Foissner, W., & Kohmann, F. (1997). *Bestimmung und Ökologie der Mikrosaprobien nach DIN 38410*. Fischer.

**Notes:**

## High Throughput Sequencing approaches focusing specifically on genus *Nebela* allow testing the influence of the altitude on the community composition.

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Testing ecological hypotheses on the field often need large experimental designs that are difficult to implement, especially in protistology where organisms cannot be directly observed. The application of molecular approaches, and especially High Throughput Sequencing allows the retrieval of large amounts of genetic data in a short time and within reasonable costs.

Variation of diversity across an altitudinal gradient is a textbook example in classical ecology, but to date, was typically difficult to visualize in protists. Here, we designed an altitudinal gradient across peatlands located in the Swiss Pre-Alps comprising a total of 12 altitudinal steps ranging from 600 to 1800 m asl, taking 8 replicates per samples at each site. We based our survey on genus *Nebela* (Amoebozoa; Arcellinida) which comprises 8 similar-looking species whose ecological preferences have been well documented.

Our aim was to test to which extent climatic variables (related to altitude) or soil parameters (pH and N values) influenced community composition. We sequenced environmental amplicons with Illumina MiSeq with a specific PCR protocol adapted to *Nebela* mitochondrial Cytochrome Oxidase subunit I (COI). We analyzed the ~26 million sequence reads obtained with a bioinformatics pipeline that we adapted specifically. We obtained 14 operational taxonomic units (OTUs) including: 6 already described species, 2 different haplotypes from described species and 6 Unknown Phylotype (UP). Three OTUs largely dominate the communities with 94% of all sequences (i.e. *N. rotunda*, *N. gimlii* and *N. collaris* with 45%, 30 % and 19% respectively), whereas five of them (all undescribed) appeared in low numbers (i.e. < 0.1% of the community).

This large amount of data are the prerequisite for obtaining the statistical power to resolve macro-ecological questions in protistology and enable comparing protist and “macro-organism” data towards a generalization of the ecological theory to microbes.

**Notes :**

## Evolution of heterotrophy in chrysophytes

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Endosymbiosis has played a major role for the evolution of all eukaryotic forms of life. More than 1.4 billion years ago an alpha-proteobacterium was engulfed by an ancestor of all known living eukaryotes to create mitochondria. The photosynthetic organelles (plastids) derived from a later uptake of a photosynthetic cyanobacterium by a eukaryotic host. During coevolution of the host and the photosynthetic endosymbiont, the cyanobacterium reduced its genome and a large amount of genes were lost or transferred to the nucleus of the host by a process termed endosymbiotic gene transfer (EGT), a special case of horizontal gene transfer (HGT). The primary endosymbiosis was followed by events of secondary endosymbiosis of red or green algae taken up by other eukaryotes. Once gained plastids seem to get seldomly lost completely. Apart from beneficial newly acquired functions from the plastid, the presence of the plastid led to a reduction of duplicated original host-cell based cytosolic pathways and some essential pathways now take place in the organelle. They include the synthesis of amino acids, isoprenoids, fatty acids, heme/tetrapyrroles and iron sulphur clusters. Understanding the evolution of plastid reduction is central to understanding evolution and present day diversity of eukaryotes. In chrysophytes the transition from phototrophy to heterotrophy occurred surprisingly often. I will present our current knowledge on trends in genome evolution in chrysophytes during the course of switching the nutritional mode.

**Notes:**

## Checklist of peatlands testate amoebae diversity in Tierra del Fuego (Argentina)

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Although most studies on testate amoebae diversity have focused on boreal peatlands, large extensions of peatlands are found in Southern Hemisphere under comparable climates. Tierra del Fuego encompasses the southernmost extensive area of peatland development.

Most of our knowledge on testate amoebae diversity in the area comes from Maria Cristina Vucetich's work during the 1956-1980 period. She reported a total richness of 44 species in a complex of six peat bogs near Ushuaia. More recent work from van Bellen et al. (2014) reported 32 species in five fuegian peat bogs.

Rancho Hambre and Andorra are two peatland complexes dominated by a *Sphagnum magellanicum* moss matrix, containing both clear and vegetated ponds. With the aim to characterizing species environmental preferences, during the summer of 2016 at each peatland we sampled periphyton, plankton and sediments from 9 water bodies and 4 matrix sites, differing in their characteristics (vegetation, physical and chemical features, morphometric).

A total of 146 morphotypes belonging to 23 genera were observed, including 40 potentially new species. This diversity greatly exceeds that found previously in peatlands from Tierra del Fuego. Typical South American species such as *Certesella australis* were found alongside with Southern Hemisphere taxa such as *Apodera*, *Alocodera* and *Amphitrema paparoensis*, described from New Zealand but reported for the first time in South America. Other morphotypes (from genera *Diffflugia* and *Padaungiella*) could not be assigned to any known species. Molecular approaches will be needed however to disentangle the true diversity of the highly plastic genera *Heleopera*, *Centropyxis* and *Netzelia*.

**Notes:**

## Enhanced plasticity of programmed DNA elimination boosts adaptive potential in suboptimal environments

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Germ-soma differentiation is integral to the life of sexually reproducing organisms.

Although the molecular dynamics of this process have received considerable attention, the impact that environmental changes have on the development of a new somatic genome is surprisingly neglected, yielding an incomplete understanding of the mechanisms that underlie adaptation to new environments. Here, we leverage the biological properties of the ciliate *Paramecium* to examine this question. When it reproduces sexually, *P. tetraurelia*'s polyploid somatic genome is regenerated from the co-cytoplasmic germline genome via a developmental process that is central to preserving somatic integrity and involves the removal of intervening sequences termed Internal Eliminated Sequences (IESs). We examined the effects of temperature changes on this process of Programmed DNA Elimination (PDE). We uncover a remarkable increase in PDE-associated variation in genomes rearranged at sub-optimal growth temperatures (18°C and 32°C) relative to 25°C, both in terms of loci number and magnitude of IES retention. Hundreds of the IESs that are partly retained in the somatic genome are epigenetically regulated. They lie within genes—where they either disrupt open reading frames or diversify protein sequences—and are most likely inherited trans-somatically. These results demonstrate that temperature changes can substantially impact somatic development in *Paramecium*, facilitating the emergence of heritable alternative DNA splicing variants, which dually embody cryptic (germline) variation and *de novo* induced (somatic) mutations. Our observations advance current understanding of the link between genetic and phenotypic variation via environmental stress. They provide support for the role of developmental plasticity in evolutionary innovation.

**Notes:**

## Taxonomic revision of *Nebela militaris*, based on morphology, molecules...and history!

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Molecular data have considerably contributed to building the taxonomy of protists. Recently, the systematics of Hyalospheniidae (Amoebozoa; Tubulinea; Arcellinida) has been widely revised, with implications extending to ecological, biogeographical and evolutionary investigations. Certain taxa, however, still have an uncertain phylogenetic position, including the common and conspicuous species *Nebela militaris*. A phylogenetic reconstruction of the Hyalospheniidae using partial sequences of the mitochondrial Cytochrome Oxidase Subunit 1 (COI) shows that *N. militaris* does not belong to the genus *Nebela*, but should be placed in its own genus. The morphological singularities (strongly curved pseudostome and a marked notch in lateral view) and phylogenetic placement of our isolates motivated the creation of a new genus: *Alabasta* gen. nov. Based on an extensive search of the literature, we also show that old descriptions must be interpreted with caution and discuss why historical work is an important part of taxonomic revisions.

**Notes:**

## Assessing the plant-associated protist microbiome

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Plants recruit subsets of the soil microbial community termed the “plant microbiome”. Although plant-associated bacteria and fungi have been well characterized, knowledge on plant-associated protist communities is scant.

We aimed to disentangle the contribution of soil type and plant species to the assembly of plant species-associated communities of protists. We used a fully factorial field experiment including three different soil types (sand, loam, loess) and the two different plant species, lettuce (*Lactuca sativa*) and potato (*Solanum tuberosum*). We identified the root-associated cercozoan communities using SSU rDNA metagenomics. The plant species harboured highly specific protist communities. Plant species explained three times more variation of cercozoan beta diversity than soil types. The results give clear evidence that different plant species in natural habitats assemble their own specific protist communities.

**Notes:**

## **Microeukaryotic gene expression profiling along a pronounced salt gradient**

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Salinity is one of the major environmental factors shaping microbial community composition. Increasing salinity leads to pronounced microeukaryotic diversity changes in terms of species richness decline and taxonomic composition shifts. Even more, specific salt transition boundaries for microeukaryotes were detected that cause significant species turnover. Different cellular responses to this environmental factor, i. e. haloadaptation strategies, were assumed to be the most likely explanation for this. We, therefore, conducted a metatranscriptome analysis targeting the functional capacities of microeukaryotic communities along a salt gradient covering salinities between 4 and 38 ‰. Putative metabolic patterns could be linked to the dominant microeukaryotic lineages in high-salt environments. Eukaryotic gene expression in lower salinities (4 and 11 ‰) was dominated by dinoflagellates, followed by ciliates rising in intermediate salinities (11 and 15 ‰), whereas higher salinities (20 – 38 ‰) exhibited strongest contributions from chlorophytes. Comparison of relative transcript abundances revealed salinity-related shifts in the functional modes of the different communities, which match metagenomic microeukaryotic community shift patterns. Distinct haloadaptation-associated functional groups, such as for example osmolyte biosynthetic pathways, were found to be responsible for the partitioning of the communities. Our study, thus, identifies metabolic constraints that are responsible for the dispersal capabilities of microeukaryotes in high-salt environments.

**Notes:**

# Performance and competitive ability of heterotrophic and mixotrophic ciliates under different regimes of light intensity and prey concentration

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Consumer diversity effects on food web dynamics are context-dependent, and determined by species-specific traits and associated trade-offs. Our project investigates the role of trait variation on two interacting trophic levels, freshwater ciliate consumers and microalgal prey. The present study focusses on modes of nutrition and describes a short-term experiment (9 days) on the competitive ability of heterotrophic and mixotrophic ciliates. We used four species belonging to the genera *Euplotes* and *Coleps*. *Euplotes daidaleos* ( $E_{\text{mix}}$ ) and *Coleps* sp. ( $C_{\text{mix}}$ ) are mixotrophic, *Euplotes octocarinatus* ( $E_{\text{het}}$ ) and *Coleps hirtus* ( $C_{\text{het}}$ ) are heterotrophic. The microalgae *Cryptomonas* sp. (*Cry*) served as prey. Ciliate species were set up as monocultures, two-genera mixtures ( $E_{\text{mix}} + C_{\text{mix}}$ ,  $E_{\text{het}} + C_{\text{mix}}$ ,  $E_{\text{mix}} + C_{\text{het}}$ ,  $E_{\text{het}} + C_{\text{het}}$ ) and control (no ciliates). These were exposed to two prey concentrations (low: 1000 cells ml<sup>-1</sup>, high: 10000 cells ml<sup>-1</sup>) and two light intensities (low: 10 μmol PPF m<sup>-2</sup> s<sup>-2</sup>, high: 60 μmol PPF m<sup>-2</sup> s<sup>-2</sup>) in a fully factorial design.

Ciliate biovolume accumulation was affected by the interaction of species combination, light intensity and prey concentration. With prey concentration being the most important factor. Trait variation within and between mixotrophic and heterotrophic modes of nutrition affected competitive dynamics in two genera mixtures. In the  $E_{\text{mix}} + C_{\text{mix}}$  treatment  $E_{\text{mix}}$  was a strong competitor and dominated at high prey concentration. In contrast,  $E_{\text{mix}}$  was inferior in the  $E_{\text{mix}} + C_{\text{het}}$  treatment where  $C_{\text{het}}$  gained dominance.  $C_{\text{mix}}$  dominated in the  $E_{\text{het}} + C_{\text{mix}}$  treatment at high prey concentration, whereas species biovolume was evenly distributed in the  $E_{\text{het}} + C_{\text{het}}$  treatment. Our results suggest that performance and competitive ability of heterotrophic and mixotrophic ciliates depend on functional traits and on food web configuration.

**Notes:**

## Re-analysis of the 18 rDNA gene phylogeny of the class Colpodea (Ciliophora)

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The ciliate class Colpodea is characterized by a specific somatic fibrillar system. As yet, 80 colpodid genera with 218 species have been described. The senior author has about 30 new taxa in his laboratory notebook. The small subunit ribosomal DNA is available for 35 genera and 58 species.

We critically review gene phylogeny, using several molecular methods. As in previous studies, four main lineages are recognizable: (1) Bursariomorphida including bryometopids, (2) Platyophryida including sorogetenids, (3) Cyrtolophosidida, and (4) Colpodida including bryophryids and grossglockneriids. The Platyophryida branch off first and the Cyrtolophosidida and Colpodida are classified as sister groups. On basis of multiple statistical tests, we unravel three problematic issues in colpodean phylogenies: the positions of the Bursariomorphida and Platyophryida are unstable and depend on alignment masking; a sister relationship of the Platyophryida and Cyrtolophosidida cannot be excluded by any statistical tree topology test; and clustering of bryophryids and grossglockneriids outside the Colpodida are also statistically valid possibilities. Natural classification of the highly diverse order Colpodida remains puzzling, possibly due to the lack of a phylogenetic signal and morphostasis of the oral ciliature in several *Colpoda*-like lineages. According to the "Ur-*Colpoda*" hypothesis, *Colpoda* represents the stem lineage from which both *Colpoda*-like and morphologically more derived taxa might have branched off. This evolutionary concept preserves not only information on morphology, ecology, and evolutionary processes of colpodid ciliates, but also aids practicability because the connection to the traditional literature is optimally maintained. There is a main difference in the gene phylogeny 2011 and 2018: the core colpodids form a "good" (dichotomous) tree in the former while a massive paraphyly in the latter in spite of the advanced methods used.

When Foissner started with colpodids in 1985, he was convinced to clarify their phylogeny if he could get good data from further 10 genera and 30 new species. But the trouble increased, and now the same happens with the molecular phylogenies. For instance, our most recent and painstakingly tested molecular tree shows: (1) a massive polyphyly in the core colpodids, (2) taxa whose classification is difficult morphologically are also difficult to classify with molecular technics, for instance the genera *Bardeliella*, *Ilsiella*, and *Kalometopia*, (3) the family Sandmanniellidae is not "basal" to the class though it has a long cytopharynx highly resembling the outgroup viz., the order Nassulida, and (4) the largest species of the class, i.e., the Bursariomorphida give rise to all other colpodids contrasting the "rule" that large groups begin with small species.

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**Notes:**

## **Learning from social networks: be connected, be discovered, be in the trophic web**

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The complex interactions of microbial organisms (eukaryotic and prokaryotic) and their relevance for aquatic ecosystems are major research subjects in limnology and protistology. Although several studies have successfully highlighted different important functions of protists in planktonic food webs, we still lack a thorough understanding of the microbial interaction network in lake ecosystems as a whole. The current study aimed at filling this void by conducting a multidisciplinary approach over three years in Lake Zurich, Switzerland. The heart of the study was a parallel collection of samples for molecular analyses via high-throughput sequencing (HTS) of the V9 18S rDNA and for morphospecies cell counts. In addition, several more biotic parameters (e.g. bacteria cell counts) and abiotic, physico-chemical parameters were measured. All of the data were integrated into one statistical analysis and culminated in co-occurrence networks from which we inferred interactions between microbial organisms and their biotic and abiotic partners. From the results we hypothesize that seasonal succession leads to fundamentally different interaction networks in the Lake Zurich ecosystem: one more complex network in the cold season dominated by phototrophs (and possibly mixotrophs) and one less complex network in the warm season dominated by heterotrophs. Of all organisms, ciliates emerged as key players that mediated the most processes with interaction partners of different taxonomic groups. Therefore, we focused in a second step on twelve ciliate species that were also discovered in morphospecies samples of lake Zurich and evaluated their positions in the networks. By comparing their biotic and abiotic interaction partners with literature data, we could test the validity of our approach while drawing novel conclusions about the ecology of the species. These species-specific subnetworks will serve as a starting point for designing future experiments on living specimen.

**Notes:**

## **Inferring ecological status of coastal environments from eDNA metabarcoding**

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Finfish aquaculture is one of the fastest growing sectors of food industry. To maintain a mutually satisfying equilibrium between economic efficiency and ecological tenability constant monitoring is necessary. Traditional methods using incidence and abundance of macrofauna run on high costs, are very time consuming and require a lot of time and taxonomic expertise. Accelerating the process of environmental assessment enables faster responses to changes of the ecological status. Using ciliate eDNA-metabarcodes instead of macrofaunal species shows a high potential to be forthcoming used in standard ecological status assessment. With less expenses and time ciliates measure up with the macrofauna benchmarks. Our new approach is trying to infer the ecological status of sediments underneath salmon farms using eDNA metabarcodes of benthic ciliate communities. Sediment samples from seven farms located in Norway were analysed. eDNA was extracted and ecological status groups (ESG) following the traditional macrofaunal based index AMBI were assigned to the samples. In parallel, we explored different methods to infer the ecological status using the obtained molecular metabarcodes. One method employed indicator value inference from specific ciliate metabarcodes. As a second method, we used Supervised Machine Learning (SML) with random forest algorithm to create prediction models. In a third method, we calculated co-occurrence networks to directly link specific ciliate eDNA indicator metabarcodes to macrofauna indicator species, which are already assigned indicator values. Finally, we use MVabund to conduct multivariate abundance analyses and fit statistical models to our sequence data to identify key taxa for individual ESG. The outcome of these different methods will be compared in our presentation to determine the most promising methods which can possibly be implemented in routine biomonitoring processes in the field of aquafarming.

**Notes:**

## **Capsules – Unique Organelles Characterising Specific Tintinnid Ciliate Clades (Alveolata, Ciliophora)**

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The main feature of tintinnid ciliates is their lorica, which was used to describe and classify the more than 1,000 species. Current phylogenies demonstrate, however, that the present classification does not reflect the evolutionary relationships inferred from gene sequences in this monophyletic group. Pivotal for understanding the genealogies of tintinnid taxa as revealed by SSU rDNA gene trees is the cell morphology, for which the body of literature is comparatively scarce. The somatic ciliary pattern is of particular importance. Its development from a simple to a complex pattern by the successive addition of ciliary fields and specialised kineties generally reflects the evolutionary inferences derived from the molecular data. Nevertheless, many tintinnid species can currently not properly be assigned to particular families and/or their evolutionary relationships remain unresolved. A promising feature complex for supporting molecular evolutionary hypotheses is the capsule. These nanoscale organelles are primarily located directly underneath the cell membrane in cytoplasmic extensions that form beaded strands (striae) and/or pin-shaped organelles (tentaculoids) between or closely attached to the adoral membranelles of the tintinnids. The ultrastructure of the capsules suggests a new type of extrusive organelle restricted to the tintinnid ciliates. Various capsule morphotypes possessing a similar basic structure are known. Different capsule types are supposed to be characteristic for certain genera or even families. In the present study, ultrastructural investigations on capsules from seven tintinnid families and clades allowed for verifying previous data and for expanding the taxon coverage. Taxa clustering together despite different lorica structures (hyaline, agglutinated), thus, often share the same capsule type. These preliminary data have to be confirmed by a broader taxon sampling, but might finally provide characters for diagnosing particular branches in the tintinnid phylogeny.

This study is supported by the Austrian Science Fund (FWF Project I 3268).

**Notes:**

# Tintinnid's First Top Model: Ultrastructural Insights into the Ciliature of *Schmidingerella meunieri* (Alveolata, Ciliophora)

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The Oligotricha are mainly marine planktonic ciliates comprising the oligotrichids and the choreotrichids, including the about 1,000 tintinnid species. Molecular phylogenies of the tintinnids are incongruent with the lorica-based classification, revealing several unresolved branches, unexpected relationships, and non-monophylies. Only integrative approaches combining taxonomic, molecular, and ultrastructural methods might allow for reconciling the systematics. Members of the genus *Schmidingerella*, particularly *S. meunieri* (Kofoid & Campbell, 1929) Agatha & Strüder-Kypke, 2014, have been investigated in various, primarily ecological respects, making them a suitable tintinnid model. Thus, this species was used in the present ultrastructural study of the ciliature. The data obtained are cladistically analysed with the hypotrich ciliates as outgroup. Compared to most ciliate groups, the structure of the somatic kinetids as inferred from protargol-stains is less conserved in choreotrichids and might be useful for explaining the topology of gene trees. The ultrastructural data not only confirmed the protargol-based "Kinetid Transformation Hypothesis" for a certain group of tintinnids, but also displayed unique fibrillar associates of the somatic kinetids that form an exceptional network in the anterior cell portion. The oral ciliature of ciliates is more constrained by fitness owing to its involvement in nutrition and is thus supposed to reveal an even higher morphological variability than somatic kinetids. In contrast to the hypotrich outgroup and the oligotrichid sistergroup, the choreotrichids possess a circular arrangement of the adoral membranelles. Despite this difference, the links between the basal bodies of a membranelle are similar in *S. meunieri* and the hypotrichs. Deviating from the microtubules connecting the membranelles with each other in hypotrichs, two circular microtubular bundles are horizontally orientated in the peristomial rim of *S. meunieri* and further bundles extend between these rings and the posterior end of cell proper on the ventral and dorsal sides. Ultrastructural data on these features in oligotrichids are generally not available. Accordingly, the anterior cell portion of *S. meunieri* is stabilised by various microtubular networks. TEM studies on further tintinnids and especially aloricate choreotrichids are required to hypothesise, for instance, a correlation between these strong networks and the possession of a bulky lorica. The study was financially supported by the Austrian Science Fund (FWF Project P 28790).

**Notes:**

## **“TO EAT OR NOT TO EAT”: WHICH FRESHWATER PROTISTS ARE MAJOR BACTERIVORES DURING SPRING?**

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Protists are fundamental for channeling dissolved organic matter to the microbial food webs via grazing on bacteria. However, knowledge about the community dynamics, trophic interactions and core taxa of bacterivorous protists *in situ* is still quite rudimentary. Deep freshwater reservoirs provide an ideal site to examine contrasting trophic interactions of protists at different depths. Thus we performed high frequency sampling of three depths (0.5m, 10m and 30m) in mesoeutrophic Řimov reservoir (CZ) during spring. To access protistan composition, dynamics and interactions with bacteria we combined three methods: amplicon sequencing of 18S rRNA genes, CARD-FISH and double hybridization of flagellate–predator and bacterial–prey. To accurately estimate abundances and to visualize the morphology of specific groups, we used CARD-FISH probes targeting different protistan groups. Amplicon sequencing revealed differences in community composition at different depths. For example, some groups dominated in epilimnion (Cryptophyta, Ciliophora, Synurophyceae) while others dominated in hypolimnion (Perkinsozoa, Cercozoa, Kinetoplastida). The abundance of different groups changed temporally, e.g., Kinetoplastids were increasing towards the end of spring bloom (in deeper strata) with the onset of pronounced stratification. Furthermore, by using double hybridization technique we could visualize particular groups of flagellates with bacteria in their food vacuoles. Overall, Cryptophytes were major bacterivores in the epilimnion since their food vacuoles were often filled with bacteria.

**Notes:**

## Interaction of Apicomplexa parasites and their potential invertebrate hosts revealed by eDNA metabarcoding

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Phylum Apicomplexa represents host-specific parasites of many invertebrate groups, such as arthropods or annelids as well as vertebrates including livestock and humans. Recent studies based on high-throughput sequencing of environmental DNA (eDNA) revealed a high diversity of Apicomplexa in tropical soils, which is believed to reflect the diversity of their potential invertebrate hosts, but such studies have not yet been done in cold environments. The Furka pass region in the central Swiss Alps offers a high heterogeneity of habitats, topography, bedrock and soil types resulting in high plant and animal diversity. However, the diversity of soil organisms is not well documented. We sampled litter and moss samples from various contrasted habitats in the Furka pass region. We assessed the phylotype richness and abundance of soil eukaryotes using Illumina sequencing of the v9 region of the 18S rRNA gene and specifically explored the diversity patterns of Apicomplexa and their potential hosts. Linear regression models revealed positive correlations of overall the phylotype richness of Annelida, Hymenoptera or Coleoptera and that of Apicomplexa. Phylogenetic reconstruction clustered Apicomplexa phylotypes into three main groups of phylotypes. The largest group contained mostly parasites of earthworms and insects. A second largest group included mostly parasites of insects and other terrestrial arthropods. The third group consisted of parasites of marine polychaetes such as *Polyplicarium*. We hypothesize that phylotypes affiliated with *Polyplicarium* indicate similar host-specific relationships in terrestrial habitats and possibly a novel clade of apicomplexan parasites of elusive terrestrial polychaetes such as *Hrabeiella periglandulata* or *Parergodrilus heideri*. Inferred host-parasite relationships illustrate the potential of eDNA metabarcoding approach to explore unknown diversity and complex host-parasites interaction. These finding could be further explored by combining traditional soil fauna analysis with metabarcoding of eDNA.

**Notes:**

## **Small- and large-scale patterns of protists from the abyssal sea floors in the Caribbean Sea and the southern North Atlantic Ocean**

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The deep-sea floor represents the most common habitat on earth. In the past, it was assumed to be a contiguous and desert-like habitat with relatively constant environmental conditions and lacking physical barriers. This led to the assumption that species have vast distribution ranges. Nowadays, it is known that deep-sea ecosystems are extremely heterogeneous at all spatial scales and frequently characterized by sudden changes. Bathymetric features such as mid-ocean ridges and fracture zones form a highly complex landscape. Canyons, seamounts, deep-water coral reefs, pockmarks or faults shape the habitat at a local scale. However, there is still a great lack of knowledge concerning patterns of species diversity and distribution in this vast environment.

In this study, three abyssal basins in the southern North Atlantic Ocean and the Caribbean Sea were analysed by a metabarcoding study of the V9-region on the SSU rDNA. Differences in the protist community composition of the three deep-sea stations, revealed large-scale patterns of protist diversity which might be shaped by environmental gradients such as bathymetric features. The analysis of sediment samples taken from two cores of the same Multi-Corer deployment, being separated by ~1m, showed differences in the community structure, indicating a distinct microbial eukaryotic diversity even at a local spatial scale. A high number of reads had no close representatives in the reference database, suggesting a potentially great number of so far undescribed species in the deep, dark ocean.

**Notes:**

# Numerical and functional response of two contrasting freshwater ciliates in relation to temperature

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Only few numerical response (NR) and functional response (FR) data are available for freshwater ciliates, and the temperature response is virtually unknown for NR and FR of planktonic ciliates. Therefore, we investigated NR and FR of two contrasting freshwater ciliates, *Vorticella natans* and *Histiobalantium bodamicum*, over temperature ranging from 5°C to 20°C and food levels ranging from 0.02 to 7.0 mgC L<sup>-1</sup>. The ciliates were fed the small cryptophyte *Cryptomonas* sp. Both ciliates are common planktonic species but their abundance as well as swimming behavior are clearly different. Both species are well adapted to cold conditions, tolerating 5°C, but *H. bodamicum* cannot survive at temperature >18.5°C. In contrast, *Vorticella natans* thrived at 20°C. Three main alterations were observed in the shape of NR and FR with temperature: change in maximum growth rate ( $\mu_{max}$ ), in the threshold level ( $V'$ ) needed to sustain the population, and in maximum ingestion rate ( $I_{max}$ ). *Vorticella natans* reached highest  $\mu_{max}$  at 20°C (0.83 d<sup>-1</sup>), almost three-fold higher than at 5°C. Its  $V'$  peaked at 10°C and was lower and almost constant ( $\sim 0.10$  mgC L<sup>-1</sup>) at higher temperatures (15-20°C). *Histiobalantium bodamicum* reached highest  $\mu_{max}$  at 18°C (0.44 d<sup>-1</sup>); its  $V'$  was generally higher than that of *V. natans*. Our FR data did not fit to Holling's type II curvilinear response but rather to Holling's type I rectilinear response. The relation of FR to temperature differed between the two species:  $I_{max}$  of *V. natans* increased with temperature, whereas  $I_{max}$  of *H. bodamicum* decreased with temperature. The result suggests that *V. natans* can outcompete *H. bodamicum* at low to moderate temperatures and, in contrast to the latter species, is able to survive in warmer waters (>20°C). However, the abundance of *V. natans* in lakes is usually low compared to *H. bodamicum* and other common freshwater ciliates. A study conducted in parallel to the present one (Lu et al, unpubl.; see also Poster by Beckmann, Lu & Weisse) revealed that microcrustaceans (*Daphnia*, calanoid and cyclopoid copepods etc.) feed differentially upon these two ciliates, suggesting that *V. natans* is more strongly top-down controlled than *H. bodamicum*.

**Notes:**

## Mechanisms of speciation and diversification in Chrysophyceae

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We examined 16 genomes of chrysophytes with a special focus on *Poteriospumella lacustris* to gain insights into the diversification mechanism, genome structure and genome variation.

The strains were sequenced with the Illumina and PacBio platforms and subsequently assembled to draft genomes with a size of 50-110 Mb. We could show that gene mutations occur in different functional groups with varying frequencies. Most surprisingly the ploidy differs between the three strains of *P. lacustris* (JBM10: diploid, JBC07: triploid, JBNZ41: tetraploid). The ploidy in the other species ranged from diploidy to tetraploidy. Intraspecific genetic variation occurs predominantly in non-coding regions or genes belonging to ecological niche adaptation. The amount of mutations indicates which pathways are more sensitive to mutation and therefore conserved (e.g. energy metabolism) and which are faster evolving (e.g. genetic information processing).

Intraspecific variation indicates several mechanisms of diversification. Furthermore, the interspecific variation reveals genome changes induced by nutritional shift like genome reduction.

**Notes:**

# **Direct and indirect effects of krill versus salps on plankton community structure and nutrient dynamics at the Western Antarctic Peninsula**

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In the Southern Ocean around the Western Antarctic Peninsula (WAP), krill and salps are the most important macrozooplankton grazers. However, these grazers occupy different ecological niches, e.g. regarding their feeding mode and nutrient recycling, and thus their potential impact on the lower plankton food web. Recent observations have indicated a shift from krill to salps in that area, which has partly been attributed to changes in phytoplankton composition towards small-sized cells and flagellates rather than large diatoms due to warming. The consequences of such a shift for the plankton food web, however, remain largely unknown. In the present study we conducted on-board experiments north of the WAP, where we incubated krill and salps with a natural plankton community to investigate their functional role in controlling phytoplankton productivity, microbial food web composition as well as nutrient fluxes and stoichiometry. Both grazers strongly reduced phytoplankton biomass; salps, however, seemed to have a stronger grazing impact on nanophytoplankton and bacteria compared to krill. After an incubation time of 5 days, dissolved molar nitrogen:phosphorus ratios (N:P) were significantly higher in salp treatments compared to krill treatments and this effect was consistent across two different experiments. In follow-up experiments we investigated the effects of this salp- and krill-preconditioned seawater (0.2  $\mu\text{m}$  filtered) on an ambient phytoplankton community to assess indirect effects of krill and salps via nutrient recycling. Bacterial and nanophytoplankton growth were more enhanced in krill-conditioned seawater compared to salp-conditioned water. Overall, our data suggest that a shift from krill to salps would indeed alter nutrient stoichiometry as well as plankton community structure and dynamics. Comparing our experimental results to observed large-scale patterns in the field will further enhance our understanding of the direct and indirect consequences of a potential grazer shift in the area of the WAP.

Notes:

## **Understanding phytomyxid-host interactions by combining transcriptomics with in-situ transcript visualisation.**

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Phytophytes are obligate biotrophic protist belonging to the Rhizaria. They are parasites of land plants, diatoms, oomycetes and brown algae. Some members cause economically important diseases of land plants with the most prominent example of clubroot disease accounting for approximately 10% loss of the global brassica crop – and a disease which is also widespread in Tyrolean vegetable growing areas. Despite this economic importance, the genetic and physiological basis of the interaction are still not understood. This is because phytomyxids cannot be grown without a living host. To address this lack of knowledge we recently generate transcriptomes of the clubroot pathogen *Plasmodiophora brassicae* and the brown algal parasite *Maullinia ectocarpii*. Analysing these data we were able find first evidence for a pathogen induced systemic reaction which offers new approaches for research on host tolerance. We selected biologically interesting genes and developed single molecule FISH methods to validate (i) the expression of the gene in the parasite and (ii) to link the transcripts to specific stages of the life cycle. Using a Methyltransferase which is produced by *P. brassicae* (PbBSMT) and which is to date the best studied gene in *P. brassicae* we were able to demonstrate spatiotemporal patten. We were also able to provide proof for a fast and local pathogen response in brown algae. Overall we show that the combination of transcriptome analysis with single molecule transcript visualisation is a powerful tool to study the interaction of parasite and their host along both spatial and temporal gradients.

Notes:

## ***Euplotes daidaleos* - a model ciliate for studying endosymbiotic events**

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Among the more than hundred described species of *Euplotes* (Ciliophora, Spirotrichea), only *Euplotes daidaleos* bears green algal endosymbionts. This species belongs to the clade I (*Euplotoides* sensu Borror & Hill), which comprises almost all freshwater species of *Euplotes*. Interestingly, it is known that some of these taxa have endosymbiotic bacteria of the genus *Polynucleobacter*. We studied the phenotypic plasticity and the molecular variability of the ciliate as well as of bacterial and green algal symbionts. Several European populations of *E. daidaleos* and close relatives were investigated using an integrative approach (morphological and molecular characterization of all partners). All strains with green algal endosymbionts were identical in SSU and ITS rDNA sequences and showed only little variations in their morphology, which fits to the original description of *E. daidaleos*. All green algal endosymbionts belong to the genus *Meyerella*, a rare free-living planktonic *Chlorella*-like genus, which has so far only been recorded from North America and, recently, as endosymbiont of *Paramecium chlorelligerum* found in water bodies from Germany and the European part of Russia. Closely related to *E. daidaleos* were strains which were identified as *E. octocarinatus*. All investigated strains contained endosymbiotic bacteria of the genus *Polynucleobacter*, identified by specific Fluorescence *In-Situ* Hybridization (FISH) and by sequencing. One *Euplotes* strain had an additional bacterial endosymbiont, which represented a new genus.

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Notes:

# **Selection and paucity of phylogenetic signal challenge the utility of alpha-tubulin in reconstruction of evolutionary history of free-living litostomateans (Protista, Ciliophora)**

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The class Litostomatea represents a highly diverse but monophyletic group, uniting both free-living and endosymbiotic ciliates. Ribosomal RNA genes and ITS-region sequences helped to recognize and define the main litostomatean lineages, but did not provide enough phylogenetic signal to unambiguously resolve their interrelationships. In this study, we attempted to improve the resolution among main free-living predatory lineages by adding the gene coding for alpha-tubulin. However, our phylogenetic analyses challenged the performance of alpha-tubulin in reconstruction of evolutionary history of free-living litostomateans. We identified several mutually interconnected problems associated with the ciliate alpha-tubulin gene: the paucity of phylogenetic signal, molecular homoplasies and non-neutral evolution. Positive selection may generate molecular homoplasies (parallel evolution), while negative selection may cause a small number of changes and hence little phylogenetic informativeness. Both problems were encountered in nucleotide and amino acid alpha-tubulin alignments, indicating an action of various selective pressures. Taking into account the involvement of alpha-tubulin in many essential biological processes, this protein could be so strongly affected by purifying selection that it even might have become an inappropriate molecular marker for reconstruction of phylogenetic relationships. Therefore, a great caution should be paid when tubulin genes are included in phylogenetic and/or phylogenomic analyses.

**Notes:**

## **Flow variability affects multi-trophic levels of fluvial biofilms: Within and across microbial groups effects**

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Near bed flow is an important physical driver of microbial community structure in freshwater biofilms. However, most studies so far focused on single microbial groups in biofilms. Here we tested the effects of near bed flow on distinct microbial groups (i.e., autotrophic cyanobacteria and protists, bacteria and heterotrophic protists) of biofilm communities within and across trophic levels in two mountainous streams with different nutrient backgrounds. Our results demonstrate that near bed flow velocity ( $\bar{u}$ ) and turbulent kinetic energy (TKE) were important physical drivers for the abundance and diversity of microbial groups.  $\bar{u}$  or TKE significantly affected community structure of all microbial groups, although explaining only between 1.9% to 3.8% of the variability in the community structure. Seasonally varying physicochemical factors, including temperature, light, dissolved carbon and nutrient concentrations explained between 3% and 15% of the variability in the community structure of the studied biofilm groups. Changes in these physicochemical factors were identical in both streams suggesting that biofilm succession was driven by environmental boundary conditions at larger temporal scales (season), whereas locally the microbial diversity of fluvial biofilms was shaped by near bed flow. Near bed flow affected the abundance and diversity of larger sized primary producers and heterotrophic protists, but not that of the smaller sized bacteria. Abundance of autotrophs was in the majority of cases positively correlated with increasing  $\bar{u}$  and TKE indicating that biofilms shifted towards increasing autotrophy with increasing shear stresses. The contribution of filamentous traits of algae increased with  $\bar{u}$  and TKE providing a protected area from the physical shear forces for the smaller sized bacteria. The abundance of heterotrophic protists decreased with  $\bar{u}$  and TKE leading to decreasing grazer to prokaryote ratios. The control of bacteria thus shifted from a biological control (i.e. grazing by heterotrophic protists) at slowly-flowing and less turbulent sites to a physical control (i.e. shear stress) at fast-flowing more turbulent sites. In conclusion, our results suggest that near bed flow can affect the ecosystem function of fluvial biofilms and possibly impact the magnitude and the direction of matter fluxes through the microbial food web.

**Notes:**

# **Biodiversity and adaptation of protists to extreme aquatic environments in the Atacama Desert**

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Hypersaline environments tend to decrease the amount of species richness, but in contrast, various studies show a high degree of phylogenetic novelty under these extreme conditions. In addition to salinity, the variable chemical parameters of the environment greatly influence the eukaryotic composition. These findings call for a more thorough look into the species richness of hypersaline environments, which seems to be more diverse than previously thought.

In this study various hypersaline lakes of northern Chile were investigated regarding protistan species diversity and richness by sequencing and analysing the V9 region of the SSU rDNA of environmental samples. Together with the Institute of Geology in Cologne, the exact chemical composition of these lakes was determined via Inductively Coupled Plasma Optical Emission Spectrometry to get more information about the composition of elements. Moreover, heterotrophic flagellates from various inland water bodies were isolated for molecular, morphological and ecological investigations. The recently discovered class, the Placididea (Stramenopiles), was previously described as halophilic protists. Up to now, only few species from various aquatic environments across the globe were described, leaving the phylogeny broadly unresolved. In this study, we were able to isolate and cultivate 21 novel strains of Placididea: 15 from the Atacama in Chile and in addition, for deeper phylogenetic analysis, two from Germany, one from Kenya, one from the Atlantic Ocean and two from the abyssal zone of the Caribbean Sea. These strains resulted in the description of four new genera and eight new species, giving a more detailed phylogenetic insight into the systematic and distribution of this class. This data enlarges the knowledge on the biodiversity of protists from extreme habitats and the autecological traits which are necessary, to survive in these hypersaline inland waters. We compare the results of metabarcoding with our isolated strains, trying to recover them.

**Notes:**

## Hidden diversity of choanoflagellates from extreme saline environments – Investigation of new species originating from the Atacama Desert

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With this study we aim to extend the knowledge on the diversity and adaptation of protists and in particular choanoflagellates from extreme saline environments. Until now, very little is known about choanoflagellates from these challenging environmental conditions, except for their occurrence in high saline ice biota in the polar regions. Therefore, we investigated several hypersaline endorheic basins, so-called salt flats or salares within the Atacama Desert in Northern Chile, which provide an intense environment regarding fluctuating salinities combined with extreme temperature shifts and probably the world's highest UV radiation.

Within this study, we describe five new choanoflagellate species including one new genus, isolated from different salt flats, based on their morphological characteristics using light and electron microscopy, the salinity tolerance and a molecular analysis using transcriptomic data. All described species were able to tolerate a broad range of salinities as an adaptation to the fluctuating environmental conditions. In particular, the newly erected genus and species *Enibas tolerabilis* gen. et sp. nov., which could be described as the first euryoecious nudiform acanthoecid choanoflagellate, highlights the isolation-driven speciation within these habitats. Up to now, the common knowledge was that only six species from four genera exist in this well studied family, indicating that the diversity of choanoflagellates is broadly underestimated. In addition to morphological and phylogenetic data, we emphasize the need of adding additional data such as autecological characteristics, ways of reproduction and life cycle stages to amend species definitions, underlining the importance of cultivation.

**Notes:**

## **Towards an ecological understanding of the killer trait - A reproducible protocol for testing its impact on freshwater ciliates**

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*Paramecium* strains with the ability to kill other paramecia often harbor intracellular bacteria belonging to the genera *Caedibacter* or *Caedimonas*. Central structures of this killer trait are refractile bodies (R-bodies) produced by the endosymbionts. Once ingested by a sensitive *Paramecium*, R-bodies presumably act as delivery system for an unidentified toxin which causes the death of endosymbiont-free paramecia while those infected gain resistance from their symbionts. The killer trait is therefore considered as competitive advantage for the hosts of R-body producers. While its effectiveness against paramecia is well documented, the effects on other aquatic ciliates are much less studied.

In order to address the broadness of the killer trait, a reproducible killer test assay considering the effects on predatory ciliates (*Climacostomum virens* and *Dileptus jonesi*) as well as potential bacterivorous *Paramecium* competitors (*Dexiostoma campyla*, *Euplotes aediculatus*, *Euplotes woodruffi*, and *Spirostomum teres*) as possibly susceptible species was established. All used organisms were molecularly characterized to increase traceability and reproducibility. The absence of any lethal effects in both predators and competitors after exposure to killer paramecia strongly suggests a narrow action range for the killer trait. Thus, R-body producing bacteria provide their host with a complex, costly strategy to outcompete symbiont-free congeners only.

**Notes:**

## **Comparative analyses of protistan diversity in soil and corresponding freshwater lakes**

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Protistan communities provide multiple ecological services in soil and freshwater lakes a detailed knowledge of factors shaping and affecting communities is crucial. Protistan communities are affected by many different environmental factors which impact community composition. Despite their central role protists are still understudied compared with macroscopic investigations and many factors shaping the protistan community remain unknown. We analyzed the molecular diversity of protists and fungi in both, soil and freshwater lakes, by means of Next-Generation-Amplicon-Sequencing using the 18S SSU V9 rRNA gene fragment. We show that the soil protist community co-varies with the vegetation type and that the community composition of freshwater lakes is largely independent from the corresponding soil communities. We found that higher diversity indices in soil as compared to freshwater are largely due to fungi. Co-occurrence networks indicated that soil protists and fungi more connected as compared to freshwater lakes.

**Notes:**

## Microbial food webs in hypertrophic fishponds: omnivorous ciliate taxa are major protistan bacterivores

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Planktonic trophic structure, microbial food web interactions and protistan bacterivory (both of ciliates and heterotrophic nanoflagellates – HNF) were studied in ten shallow hypertrophic fishponds with different fish stock in South Bohemia (Czech Republic) during the growing season. We used fluorescently labeled bacteria as bacterivory tracers to estimate how abundant protistan populations in the fishponds, typically 4–25 × 10<sup>3</sup> HNF ml<sup>-1</sup> and 55–800 ciliates ml<sup>-1</sup>, contribute to total bacterial mortality. Fluorescence microscopy, innovative image processing tools, and quantitative protargol staining were combined to detect major bacterivorous and omnivorous ciliate taxa. Based on abundance, seasonal dynamics, and cell-specific uptake rates of different ciliates, as well as HNF grazing, we were able to quantify bacterivory by individual ciliate species or morphotypes, total ciliates, and aggregated protists in the fishponds. On average, ciliates were more efficient bacterivores than HNF, accounting for 56% and 44% of total protistan grazing, respectively. Total protistan bacterivory removed 45% of bacterial standing stock daily, which corresponded to 40% of total bacterial production and 71% of production in free-living bacterial (<1 μm) size fraction. Notably, genera *Halteria*/*Pelagohalteria* (Stichotrichia) were clearly the most important bacterivores in the fishponds and their proportions positively correlated with numbers of Cladocera, which indicates their lower vulnerability to zooplankton predation. In contrast, the total ciliate abundance was strongly negatively correlated with cladoceran abundance. *Rimostrombidium* spp. (Oligotrichia) contributed with lesser extent to bulk ciliate bacterivory rates, followed by Peritrichia and Scuticociliata. However, the highest cell-specific bacterivory (ca. 10,500–11,260 bacteria ciliate<sup>-1</sup> h<sup>-1</sup>) was shown by the peritrichous species - *Pelagovorticella natans* and *Epistylis procumbens*. Analyses of ciliate food vacuole contents revealed that omnivory was the most successful feeding strategy in hypertrophic fishponds, as the major fine-filter feeding bacterivorous ciliates were not specialized bacterivores, but omnivores with a marked contribution of small algae to their diet.

**Notes:**

## **Contrasted effects of cadavers, blood, urine and faeces on soil micro-eukaryotic communities – implications for biodiversity exploration and forensic application**

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Communities of soil micro-eukaryotes are known to vary in relation to local abiotic conditions and were recently shown to respond very clearly to decomposing cadavers. Such responses are the basis for the development of bioindication tools including forensic investigations. However, from a forensic perspective it is crucial to determine if the observed response patterns of soil microeukaryotes are specific to cadavers or if similar responses could be induced by other nutrient sources such as urine, faeces or blood.

We assessed the temporal changes (98 days) in soil micro-eukaryotic community composition in response to various sources of nutrients (pig carcass, blood, faeces and urine) by high throughput sequencing (HTS - Illumina) of eDNA targeting the v4 region of the 18S SSU rRNA gene. Diversity declined in all treatments as compared to the control. The temporal patterns of community composition (NMDS) in the urine and blood treatments followed were similar but differed from those of the pig treatment, which was the most divergent. Redundancy analysis (RDA) identified ammonium (NH<sub>4</sub><sup>+</sup>) as the main driver of community changes in the early stages of each treatment and nitrate (NO<sub>3</sub><sup>-</sup>) in later stages. The Indicator Value (IndVal) revealed significant indicator sequences for each sampling point and treatment. A high proportion of indicators for cadavers did not find any close match in reference databases. Micro-eukaryotes living in putrid environments present different responses and patterns to local stress factors. The existence of highly specific micro-eukaryotic communities in cadaver-impacted soils constitute a basis for developing reliable tools to estimate the Post-Mortem Interval (PMI). On a more fundamental side, this study confirms the existence of a high diversity of soil micro-eukaryotes and an especially high proportion of unknown taxa in cadaver-impacted soils, which represent opportunities for original taxonomic research. Such “unusual” or less-studied habitats clearly deserve more attention!

**Notes:**

## ***Paramecium bursaria* or not *Paramecium bursaria* - that is the question**

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*Paramecium bursaria* is a model ciliate, which is distributed worldwide and easy to cultivate. This species lives in symbiosis with endosymbiotic green algae, which were identified as species of the genera *Chlorella* and *Micractinium*. 'Green' *Paramecium* isolates from all over the world are frequently involved into various experimental approaches. So far, the ciliates were always assigned to the species *P. bursaria*. Although that *P. bursaria* is a model, the morphology and its phenotypic plasticity has never been investigated in a comparative study. Recently, *P. chlorelligerum* has been re-described and it cannot be excluded that there were some misidentifications in the laboratory strains before. The aim of this study was to reinvestigate the morphology of widespread laboratory strains of 'green' paramecia in detail as well as their SSU and the ITS rDNA phylogeny. From literature, six lineages which presumably represented different syngens are already known. From clonal cultures, we observed the morphology by light microscopy from living individuals and after silver staining. We assessed common features (size, macro-, micronucleus, caudal cilia, contractile vacuoles, excretory pores, and the nature of the collecting channels/vesicles, ciliary rows, extrusomes, movement). All strains showed a high phenotypic plasticity. Especially the size varied among the strains, even from the same sampling site isolated 15 years apart, i.e., mean length of  $130 \pm 17.3 \mu\text{m}$  vs.  $102 \pm 9.6 \mu\text{m}$ . Detailed morphology and phylogeny of the endosymbionts were investigated as well by applying a diagnostic PCR method that was developed due to difficulties in isolation and cultivation of these algae. *Chlorella variabilis* and *Micractinium conductrix* were identified as endosymbionts which showed no correlation to the syngens of *P. bursaria*. Finally, we will obtain a more detailed and complete characterization of the 'green' paramecia and their endosymbionts. The study is funded by the Austrian Science Fund (FWF): P28333-B25.

**Notes:**

## **The efficacy of silver-copper ions on the viability of biofilm-associated free-living amoebae**

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Biofilm formation is one of the most important problems frequently encountered in cooling towers. Free-living amoebae found in biofilm can interact with the bacteria in the same environment and may play a role in their multiplication. This situation leads to difficulty in the elimination of increased number of bacteria from the water systems. Today, silver-copper (Ag-Cu) ionization system which is cheaper, effective and environmentally friendly than biocides is preferred for the disinfection of water. The use of this system, which is recommended for water hygiene in United States and Europe, is limited in Turkey.

The aim of this study was to determine the efficacy of Ag-Cu ions (0.1-1.3 ppm) against free-living amoebae associated with biofilm formed on galvanized steel surfaces in a model water circulation system simulating cooling tower. For this purpose, the experiments were carried out in two separate systems, the model water circulation system and the beaker system containing Ag-Cu ions. The model water circulation system containing galvanized steel coupons was operated continuously over 6 months for biofilm formation and the beaker system containing Ag-Cu ions was set up regularly every month for disinfection assays. Coupons were removed regularly each month from the model water circulation system, and exposed to Ag-Cu ions for certain contact times (0, 6, 24, 168, 336, and 504 hours). After each contact time biofilm was collected from the surface of galvanized steel coupons, resuspended in sterile tap water, and the aliquots (1 ml) were inoculated on *Escherichia coli* seeded non-nutrient agar plates in triplicate. Plates were incubated at 30°C and checked daily under an inverted microscope for the viability of free-living amoebae.

In the one-month old biofilm, no free-living amoebae growth was detected from 24 hours of exposure to Ag-Cu ions. However, the tested concentration of Ag-Cu ions did not affect the growth of free-living amoebae after the fourth month, even after 504 hours of contact time. These results indicate that, the tested concentration of Ag-Cu ions is highly effective against free-living amoebae found in the early stage of biofilm. The inefficacy of Ag-Cu ions in killing free-living amoebae found in the developed biofilm may due to the decreased penetration of these ions into the mature biofilm.

According to the author's knowledge, this is the first study reporting the synergistic biocidal efficacy of Ag-Cu ions against free-living amoebae found in biofilm formed on galvanized steel surfaces of cooling tower.

This work was supported by Scientific Research Project Coordination Unit of Istanbul University. Project number: FBA-2018-30882.

**Notes:**

## **Conjugation and morphogenesis of metopids elucidate phylogenetic relationships within the SAL supercluster**

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Extensive transcriptome analyses unite the classes Spirotrichea, Armophorea and Litostomatea into the so-called SAL supercluster. Relationships among them remain, however, unresolved although over hundred genes are used to infer phylogeny. Interestingly, conjugation and morphogenesis data can elucidate the intricate sister-group relationships within the SAL supercluster. The oblique to ventral-to-dorsal pair formation and the strongly arched to almost rod-like arrangement of the conjugants might be synapomorphies corroborating the monophyletic origin of the armophoreans and litostomateans. This is further supported by the purely somatic and telokinetal stomatogenesis beginning in the dorsal and dorsolateral kineties as well as by the origin of the oral structures from migrating kinetofragments. On the other hand, the spirotricheans have a ventral-to-ventral and homopolar conjugation mode with the main body axes oriented in parallel. Their stomatogenesis is apokinetal, proliferation of basal bodies begins on the ventral side and the new oral structures differentiate from an oral primordium and are thus not migrating kinetofragments. In addition to conjugation and morphogenesis, the plate-like arranged postciliary microtubule ribbons, forming a layer right of and between the ciliary rows, also sustain a close relationship of the armophoreans and litostomateans within the SAL supercluster.

**Notes :**

## Metagenomics for parasite detection: A proof-of-concept study using swine faeces samples

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Diagnostic metagenomics with high-throughput sequencing techniques is an unspecific but sensitive method for the identification of pathogens in human, animal, and food samples. A great benefit of the generic metagenomics approach is its pathogen-independent nature, and thus the applicability for cases where the pathogen is hitherto unknown. Whereas it was frequently used for the detection of viruses and bacteria, it was rather seldom applied for parasite detection. Therefore, metagenomic datasets prepared from swine faeces using an unbiased sample processing approach were re-analyzed in the present study with respect to the detection of parasite-specific sequences.

We used the taxonomic identification tool RIEMS for initial detection and subsequently applied reference mapping analyses. The former tool provided basic hints on potential pathogens within the samples. The suspected pathogens (*Blastocystis*, *Entamoeba*, *Iodamoeba*, *Neobalantidium*, *Trichomonas*) could be verified using the latter analyses on the base of high-identity assessments of ribosomal RNA gene sequences. Nearly full-length gene sequences could be extracted. In case of *Blastocystis*, it was possible to assign the found sequences to 5 out of 17 previously defined subtypes. Some of the RIEMS-suspected candidates turned out to be false-positives caused by the poor status of reference sequences available in public databases. Altogether, ten different intestinal protist species/subtypes could be detected in 37 out of 41 metagenomics datasets.

In conclusion, the applied approach allows the taxonomic classification including subtyping of protist and metazoan endobionts (parasites, commensals or mutualists) based on an abundant biomarker, the 18S ribosomal RNA gene, without any primer bias that typically hampers the analysis of amplicon-based approaches. In addition, the extraction of 18S rRNA sequences from metagenomics datasets can circumvent the current shortcoming of missing reference sequences for many taxa.

**Notes:**

## Developing to 'nucleomorphs' in dinoflagellates: the short motile period advanced the endosymbionts to tertiary plastids

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Dinoflagellates called 'dinotoms' are known to possess endosymbiotic diatoms (ESDs) as their tertiary plastids. The ESDs are evolutionarily in intermediate plastids: the diatoms still retain the nucleus, the mitochondria, the ribosomes, in addition to the plastids, while it has been reported that host dinoflagellates can maintain their ESDs permanently by controlling the diatom cell division. Recently, we revealed that dinotoms utilise eleven diverse species of ESDs respectively depending on host species. The fact indicates that dinotoms have repeated to replace the ESDs to free-living diatoms.

In contrast to the majority of dinotoms, five species of benthic dinotoms, which make a clade with bootstrap 100% in 18S rDNA molecular phylogeny, maintain the genetically concordant ESDs despite of their geographically-diverse habitats. To clarify why they share identical diatoms, we microscopically observed four species in this clade: *Galeidinium rugatum*, *Dinotrix paradoxa* and two novel species. All of these dinotoms show an extremely short motile stage. Motile cells appear approximately once in three days after the cell division and the duration is from a few minutes to several hours, the rest of the time the cells are living non-motile cells. We hypothesise that the specific life style makes it less likely that the cells meet diatoms that they can take up. We also obtained an indication by CLSM that the nucleus of ESDs are extremely reduced the size compared to nuclei in ESDs of other dinotoms. This result implies that the ESD was acquired in the early history of dinotoms, and never replaced the ESD unlike other dinotoms. We started the transcriptomic analysis on one of these species to know what extent the diatom nucleus transcriptionally active.



# **Abstracts**

## **Posters**

(in alphabetical order)

# **P1 Top-down control of the planktonic peritrich ciliate *Vorticella natans***

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Planktonic ciliates are an important link between the microbial food web and the classical grazer food chain in aquatic ecosystems. Their population dynamics is controlled 'bottom-up' via food (algae, bacteria) and physical factors (e.g., temperature) and 'top-down' by grazing of mainly microcrustaceans. We investigated the relative significance of these drivers with the motile freshwater peritrich *Vorticella natans*, which is common in different freshwater ecosystems but less abundant than many other co-occurring ciliates.

Our former numerical and functional response experiments presented at last year's DGP meeting (Lu & Weisse, 2018; Lu et al., in prep.) showed that maximum growth rates, ingestion rates, and threshold food concentrations of *V. natans* are within the range of other freshwater ciliates (see Lu & Weisse, this meeting). Furthermore, these experiments revealed that *V. natans* survives at temperatures ranging from 5-22° C. We infer that bottom-up control does not explain why *V. natans* is less abundant than most sympatric ciliates.

Since *V. natans* is constantly and slowly moving and has no known morphological or chemical defence mechanisms, we assumed that this species is primarily top-down controlled in lakes. To test this hypothesis, we investigated the grazing impact on *V. natans* in microcosm experiments with three microcrustacean species (*Daphnia* sp., *Eudiaptomus* sp., *Mesocyclops* sp.) representing different feeding behaviour. All species were isolated from oligo-mesotrophic Lake Mondsee (Austria). Experiments were conducted at three different predator levels ranging from 1-100 animals L<sup>-1</sup>. Surprisingly, grazing rates, ingestion rates and clearance rates calculated for the three crustacean species yielded no species-specific significant differences. Predator-induced mortality rates of *V. natans* were close to ciliate growth rates measured at moderate food levels at the same experimental temperature (15°C). We conclude that *V. natans* is top-down controlled in L. Mondsee and similar lakes throughout most of the year.

## **P2 Biodiversity and biogeography of soil protists in continental and oceanic islands**

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Global patterns of plant and animal diversity have been known for more than two centuries, but the diversity and the distribution of soil organisms, especially protists, as well as the factors driving their biogeography, are still poorly understood. In addition, despite recent improvements of high throughput sequencing (HTS) methods for environmental samples, only a small proportion of the diversity of terrestrial protists is known and referenced in databases. Thus, information about their function is scarce.

This poster presents our new project "Biodiversity and biogeography of soil protists in continental and oceanic islands" and puts it in the context of the current state of knowledge. The project is funded for 4 years, 2019-22.

Besides improving reference databases by making our data fully accessible, the main goal of the project is to test how far basic biogeographical rules developed for large organisms like animals and plants apply to microbial eukaryotes with special focus on protists, and especially on testate amoebae. The key questions we address are: Do the taxonomic richness, the endemism, the endemic richness, the phylogenetic diversity and the age of endemic species (as inferred from phylogenetic distance) vary in relation to land surface area (small islands – large islands – continents), age of the island, distance to the nearest continent, elevation, land-use (e.g. pasture or tree plantation vs. native forest or grassland), and organism size within given taxonomic groups (i.e. size-decay pattern) ?

To answer these questions, soil samples in both hemispheres will be collected in four islands/archipelagos with increasing distance to continents (Canary < Réunion < Azores < Hawaii), two archipelagos of large islands/subcontinent with contrasted distance to the nearest continent (Japan < New-Zealand), and two distant continents of different paleogeographic origin (Laurasia: Western Europe, Gondwana: Chile).

By using different HTS methods (e.g. Illumina, PacBio, Sanger sequencing) for different gene regions, we will study the diversity of i) all soil micro-eukaryotes at coarse taxonomic level, ii) selected taxa of phylogenetic interest, iii) Hyalospheniidae and/or Euglyphida testate amoebae and Amobozoa at the species level, and iv) Hyalospheniidae and Euglyphida testate amoebae by microscopy and DNA barcoding.

We welcome applications from MSc students to do their thesis work on this project.

### **P3 Molecular taxonomy of *Spirochona gemmipara* (Ciliophora, Chonotrichia), an epibiont living on the gills of *Gammarus pulex*. First results.**

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The chonotrichs (Chonotrichia, Ciliophora) live settled on diverse appendices (antennas, pereopods, gills, etc. ) of several kinds of marine and freshwater crustaceans (Decapods, Nebaliaceas, Isopods, Amphipods, ... ). The chonotrichs reproduce principally by budding, and exhibit complex life cycles driven by the moulting of their host. The chonotrich taxonomy was established in the milestone book of Jankowski (1973) which described more than 40 genera and 100 species living all around the world. Ultrastructural data are known for some species, but molecular data exist for two species only. Here, we present the phylogenetic analysis of 18S rRNA gene of *Spirochona gemmipara* sampled in several localities in Switzerland.

## **P4 Illuminating the impact of algal endosymbionts on host behaviour**

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*Paramecium bursaria* lives in a facultative, mutualistic symbiosis with *Chlorella*-like algae ('green' paramecia). The ciliate profits from this interaction by gaining access to photosynthesis products and the algae benefit by increasing their motility. Green paramecia accumulate in the light. Studies exploring this photoaccumulative behaviour reveal contradicting results. While some report that algae-free *P. bursaria* ('white' paramecia) lose this ability indicating a crucial role of the symbiont, others still observe photoaccumulation even after elimination of the algae.

We combined quantitative photoaccumulation assays including green and white paramecia with molecular phylogeny of several *P. bursaria* strains and their symbionts to shed light on the impact of the algal symbionts on their host's behaviour. Specifically, we asked if there is a correlation between algal species identity and photoaccumulation of the host and to which extent the algae are responsible for *P. bursaria* behaviour.

We detect statistically significant accumulation of green paramecia in illuminated areas regardless of the endosymbiont species. Such clear pattern is missing for white paramecia. Still, more white cells accumulate in the light but to a lesser degree than green cells. Thus, our data extend previous results: the *Chlorella*-like endosymbionts influence *P. bursaria*'s photoaccumulative behaviour but they do not cause it. Currently, we are investigating a potential correlation between the duration of the symbiont-free cultivation and their affinity to accumulate in the light.

## **P5 Molecular and Morphological Analysis of Benthic Littoral Protists of the Central Azores**

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Protists are ubiquitous and play a crucial role in marine ecosystems, but the knowledge on their overall diversity and biogeography is still far from complete. Recent next-generation sequencing studies on protist diversity and distribution revealed new insights in various habitats but none of it focused islands. By combining morphological and molecular based methods we investigated benthic, heterotrophic protist diversity and their community structure of two Azorean islands. Samples originated from the littoral zone of Faial and Pico, islands belonging to the central group of the Azores in the central North Atlantic Ocean. The Azores rose *de novo* from the ocean by massive submarine volcanic eruptions in an until today active volcanic hot spot area near the Mid-Atlantic Ridge. Hence the islands were never connected to land masses they are an interesting site to study protist dispersal and diversity.

Our metabarcoding analysis, based on the V9 region of SSU rDNA, revealed differences in protist community composition between the sampling sites of the coastline around Faial, as well as differences in the community composition on a microscale within a sampling site. Morphological investigations revealed patterns in the community structure as well. The morphological data were additionally aligned to the global diversity study on flagellates from Lee and Patterson (1998). Several globally distributed species could be reported from the Azores beside unknown species and species with so far not reported ubiquitous distribution.

## **P6 Taxonomic Revision of Freshwater Foraminifera with the Description of Two New Agglutinated Species and Genera**

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Most foraminifera inhabit marine habitats, but some species of monothalamids have been described from freshwater environments, mainly from Swiss water bodies over 100 years ago. Recent environmental DNA surveys revealed the presence of four major phylogenetic clades of freshwater foraminifera. However, until now only one of them (clade 2) has been associated to a morphologically described taxon – the family Reticulomyxidae. Here, we present morphological and molecular data for the genera representing the three remaining clades. We describe two new agglutinated freshwater genera from China and the Netherlands, *Lacogromia* and *Limnogromia*, which represent clades 3 and 4, respectively. We also report the first ribosomal DNA sequences of the genus *Lieberkuehnia*, which place this genus within clade 1. Our study provides the first morphotaxonomic documentation of molecular clades of freshwater foraminifera, showing that the environmental DNA sequences correspond to the agglutinated monothalamous species, morphologically similar to those described 100 years ago.

## **P7 Deep Molecular Characterisation of Oomycete and Alveolate Diversity in Forest Soils and Tree Canopies**

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Protists occupy key nodes in terrestrial food webs due to their high abundance, fast turnover and functional importance as microbial grazers. However, methodological drawbacks in both culturing and molecular methods still strongly limit the knowledge of protist diversity, so that large groups remain virtually unknown.

Here, we apply advanced cultivation-independent high throughput sequencing methods using newly designed group-specific primers and reverse rRNA-transcriptomics for a comprehensive assessment of protist diversity across all ecological compartments from forest soils (litter layer & mineral soil) to the canopy region (bark, leaves, dead wood, branch forks, knotholes, epiphytes) in temperate and tropical biomes. With these results, we want to test to which extent diverse microhabitats influence species richness in eukaryotic microorganisms.

## **P8 *Let the show begin!* Ciliates in the spotlight of the aquatic food web of Lake Mondsee, Austria**

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### **Abstract**

Food webs are powerful consumer-resource systems driven by multiple interactions of their biotic components. Ciliates represent an inherent part of the plankton and an important link in the dynamics of energy flow from lower to higher trophic levels. These protists feed on hetero- and autotrophic pico/nanoplankton and are in turn a relevant food source for metazoans. Although their key role in lakes is realized, knowledge about their networking with food web players is minimal and only ‘the tip of an iceberg’ has been elucidated so far. To shed light on specific interactions of ciliates, we investigated their seasonal and spatial distribution and related these dynamics to phytoplankton, metazooplankton, bacteria and abiotic parameters throughout one year (June 2016 – May 2017) by monthly sampling of Lake Mondsee (0 - 65m). Based on morphotype counting, the ciliate assemblage (mean abundance: 6,200 cells L<sup>-1</sup>; ca. 70 morphospecies) was mainly dominated by Spirotrichea (43% of total abundance; *Halteria* spp., *Rimostrombidium* spp., *Tintinnidium* spp.), Prostomatea (31%; *Balanion planctonicum*, *Urotricha* spp.), Oligohymenophorea (19%; *Histiobalantium bodamicum*) and Litostomatea (8%; *Askenasia* spp.). Mainly algivorous and omnivorous species (ca. 80% on average) were predominant. Maximal total numbers were recorded during summer stratification (~150,000 cells L<sup>-1</sup>) mainly in the upper water layers whereas only a fifth (~27,000 cells L<sup>-1</sup>) was observed for the cold season evenly distributed over the water column. Haptorid, choreotrich, oligotrich, prostomatid and stichotrich ciliates were more abundant in the warm season (spring/ summer), while peritrichs and scuticociliates mainly appeared in the cold season (autumn/ winter). Beside the summer maximum, two smaller peaks were observed in spring and autumn. Phytoplankton and bacterial dynamics showed similar patterns, whereas zooplankton showed a divergent succession. These results form the basis for detailed food web experiments to understand species-specific relationships and cross-links among planktonic organisms.

Supported by the Austrian Science Fund (FWF): project I2238-B25.

## **P9 Synthesis and *in vitro* activity of new biguanide-containing dendrimers on pathogenic isolates of *Acanthamoeba polyphaga* and *Acanthamoeba griffini***

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The genus *Acanthamoeba* can cause *Acanthamoeba* keratitis (AK) and granulomatous amoebic encephalitis (GAE). The treatment of these illness is hampered by the existence of a resistance stage that many times causes infection relapses. In an attempt to add new agents to our chemotherapeutic arsenal against acanthamoebiosis, two *Acanthamoeba* isolates were treated *in vitro* with newly synthesized biguanide dendrimers. Trophozoite viability analysis and ultrastructural studies showed that dendrimers prevent encystment by lysing the cellular membrane of the amoebae. Moreover, one of the dendrimers showed low toxicity when tested on mammalian cell cultures, which suggest that it might be eventually used as an amoebicidal drug or as disinfection compound in contact lens solutions.

## **P10 Vertical distribution of particle-associated protists from marine plankton communities of the North Atlantic and the Caribbean Sea**

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Marine snow describes macroscopic aggregates consisting of material from a variety of sources, including inorganic material, detritus and living organisms. They form benthos-like habitats in the pelagic part of the open ocean and greatly influence the flux of carbon and nutrients within the ocean. Only few studies so far focused on the importance of these particles for protist biodiversity and their distribution along the water column. One approach to give some resolution to protist biodiversity is the use of cultivation-based studies, which were able to reveal numerous new species. One major advantage of cultivation based studies is that they also allow for morphological and ecological descriptions. However, cultivation-based studies are also subjected to a high selectivity since a lot of species and whole taxonomic groups are difficult or impossible to cultivate. Cultivation independent studies like those using next generation sequencing have focused on studying protist communities in the sunlit ocean. However, only little is known about the dark ocean, even though it comprises the majority of our world's biosphere. We sampled plankton, including marine snow, at different stations in the Caribbean Sea and the North Atlantic during the research cruises M139 and M150 with the RV Meteor in 2017 and 2018. Our results show a clear change in protist community structure with depth.

## P11 Predicting current, past and future distribution of *Apodera vas* s.l. (Amoebozoa; Arcellinida) from bioclimatic niche-based models

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Microbial biogeography is a dynamic field of research, yet global assessments of distribution patterns of individual taxa are very rare. A notable exception is the testate amoeba *Apodera vas* (Amoebozoa; Arcellinida), an iconic and highly conspicuous flagship taxon in this debate. The distribution of *A. vas* stands out as being mostly restricted to former Gondwana, suggesting an origin in this landmass, with only limited subsequent dispersal. This taxon should theoretically find appropriate environments in northern continental regions where protistologists have so far failed to find it, but its potential distribution has not yet been rigorously assessed. We compiled an extensive database of ca. 400 known records of *A. vas*. Based on the geographical coordinates, we built a bioclimatic niche-based distribution model and determined its potential distribution according to current climate, future climate scenarios (CMIP5 prediction for 2061–2080) and last glacial maximum (LGM - PMIP3) climatic conditions. The modelled potential distribution of *A. vas* clearly shows that this taxon could potentially occur in well-studied regions of the Holarctic; its absence can thus be interpreted as evidence for limited dispersal. Furthermore, LGM prediction shows a broader distribution suggesting substantial range contraction and expansion over glacial-interglacial cycles. Predictions of future distribution show drastic reduction in potential range. These modelling results are valuable to develop hypotheses on phylogeographical patterns to be tested using molecular methods.

## **P12 Transition from phototrophy to heterotrophy drives genome size evolution in chrysophytes**

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The cellular content of nuclear DNA varies up to 200,000-fold between eukaryotes. These differences can arise via different mechanisms. In particular, cell size and nutritional mode may influence evolution of the nuclear DNA content. Chrysophytes comprise organisms with different cell organizations and nutritional modes. Heterotrophic clades evolved independently several times from phototrophic or mixotrophic ancestors. Thus, chrysophytes are an ideal model taxon for investigating the effect of the nutritional mode on cellular DNA content. We investigated the genome size of heterotrophic, mixotrophic, and phototrophic chrysophytes. We demonstrate that cell sizes and genome sizes differ significantly between taxa with different nutritional modes. Phototrophic strains tend to have larger cell volumes and larger genomes than heterotrophic strains do. The investigated mixotrophic strains had intermediate cell volumes and small to intermediate genome sizes. Heterotrophic chrysophytes had the smallest genomes and cell volumes compared to other chrysophytes. In general, genome size increased with cell volume, but cell volume only partially explained the variation in genome size. In particular, genome sizes of mixotrophic strains were smaller than expected based on cell sizes.

## **P13 Through the magnifying glass – the global diversity of Rhogostomidae and their environmental drivers**

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Recent environmental studies consistently report a high abundance and diversity of Rhogostomidae (Thecofilosea, Cercozoa, Rhizaria) in various aquatic and terrestrial habitats. However, since the rise of protistology only a hand full of Rhogostomidae species have been described so far. *Rhogostoma schuessleri* and *R. minus* were first described by Belar in 1921 and only very recently three new *Rhogostoma* species have been described from soil (*R. cylindrica*), freshwater (*R. micra*) and plant leaves (*R. epiphylla*). We investigated the putative cryptic diversity of Rhogostomidae, by reanalysing environmental sequencing data from marine, freshwater and terrestrial habitats around the globe, isolating and characterizing new rhogostomid species via their SSU rDNA sequence and morphology. So far we were able to detect more than 10 major clades in a comprehensive analysis of SSU rDNA phylogeny of NCBI data and approx. 450 sequences lacking any assigned described species. During these studies the previously wrongly assigned species *Sacciforma* (= *Plagiophrys*) *sacciformis* was reevaluated and shifted into a new genus in the Rhogostomidae.

We hypothesise that Rhogostomidae contain various cryptic species and that geographic regions are not the main factor shaping their dispersal, since preliminary data indicate a distribution of phylotypes within specific habitat types.

We will show data on the relationship between species composition and their environments as well as on the morphology of different species in order to gain a better picture of the family Rhogostomidae.

## **P14 Seasonality of planktonic freshwater ciliates: Are analyses based on V9 regions of the 18S rRNA gene correlated with morphospecies counts?**

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Ciliates represent central nodes in freshwater planktonic food webs, and many species show pronounced seasonality, with short-lived maxima of a few dominant taxa while many being rare or ephemeral. These observations are primarily based on

morphospecies counting methods, which, however, have limitations concerning the amount and volume of samples that can be processed. For high sampling frequencies at large scales, high throughput sequencing (HTS) of freshwater ciliates seems to be a promising tool. However, several studies reported large discrepancy between species abundance determinations by molecular compared to morphological means. Therefore, we compared ciliate DNA metabarcodes (V9 regions of the 18S rRNA gene) with morphospecies counts for a three-year study (Lake Zurich, Switzerland; biweekly sampling, n = 74). In addition, we isolated, cultivated and sequenced the 18S rRNA gene of twelve selected ciliate species that served as seeds for HTS analyses. This workflow allowed for a detailed comparison of V9 data with microscopic analyses by quantitative protargol staining (QPS). The dynamics of V9 read abundances over the seasonal cycle corroborated well with morphospecies population patterns. Annual successions of rare and ephemeral species were more adequately characterized by V9 reads than by QPS. However, abundance values of species specific reads only partly reflected rank orders seen by counts. In contrast, biomass-based assemblage compositions showed higher similarity to V9 read abundances, probably indicating a relation between cell sizes and numbers / sizes of macronuclei (or 18S rRNA operons). Full-length 18S rRNA sequences of ciliates assigned to certain morphospecies are urgently needed for barcoding approaches as planktonic taxa are still poorly represented in public databases and the interpretation of HTS data depends on profound reference sequences. Through linking operational taxonomic units (OTUs) with known morphospecies, we can use the deep knowledge about the autecology of these species.

## **P15 Evidence for predation on protists >730 million years ago**

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Neoproterozoic (1000–541 million years ago, Ma) fossils record the taxonomic and ecological expansion of eukaryotes in a world previously dominated by prokaryotes. The early phase of this diversification (1000–720 Ma) is characterized by an increase in protistan microfossil diversity, the first appearance of eukaryotic biomineralization, and the appearance of simple multicellularity in several eukaryotic clades; the later phase, following ~85 million years of global glaciations, is characterized by the dramatic expansion of animals, culminating in the “Cambrian explosion”. It has been hypothesized that just as animal predation may have driven the Cambrian diversification of animals, the appearance of protists capable of preying on other protists may have driven the early diversification of eukaryotes. In this talk I will discuss evidence from my work on microfossils from the ~780–730 Ma Chuar Group, Grand Canyon, USA, consistent with this view. Upper Chuar rocks preserve an incredible abundance of diverse vase-shaped microfossils interpreted to be the remains of arcellinid testate amoebae; lower Chuar shales and siltstones host beautifully preserved organic-walled remains of eukaryotic cells and cysts. About 10% of both vase-shaped and organic-walled microfossils exhibit perfectly circular or half-moon shaped holes in their walls; these are interpreted to be perforations made by predatory protists. Together with hydrocarbon biomarker molecules from upper Chuar rocks that provide evidence for the presence of toxins capable of lysing eukaryotic cells, these fossils indicate the presence of a diversity of predators in the shallow tropical Chuar sea.

## **P16 Protist of the Year – A short summary of the last decade and *Nuclearia*, the star of 2019**

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In 2007, Hans-Dieter Görtz\* realized the brilliant idea that each year, the German Society for Protozoology (DGP) presents a protistan genus or species to a broader readership and the public. As a key issue, the 'Protist of the Year' is highlighted by a 'flyer' which can be downloaded from the DGP website and distributed as handout in lectures for scholars, students and any kind of dissemination. Long-established members of the DGP are well aware of this periodical, however, (young) students or 'guests' of the annual meetings may not know about this initiative. Until now, flyers were only written in German, but hopefully English versions will be soon available (volunteers are welcome). On our poster we recapitulate the meanwhile twelve 'Protist of the Year' candidates including their specific flyers as take-away. A phylogenetic tree of eukaryotes will show which lineages have been presented during the last decade. This overview may give first suggestions which protists could become valuable candidates for future presentations. Finally, we introduce the opisthokont genus *Nuclearia* as 'Protist of the Year 2019'. Nucleariids are unique due to their multiple symbioses ('living together') with endo- and ectosymbiotic bacteria. These amoebae are able to feed on filamentous harmful cyanobacteria and overcome their grazing protection. Nucleariids use different feeding strategies to break-down and digest long cyanobacterial filaments whereby the toxins contained in their prey get released. However, excreted toxins become degraded by accompanying bacteria living together with the amoebae in cultures. Thus, in addition to cell-associated bacteria, Nucleariids seem to have also close interactions with accompanying bacteria.

\* We dedicate this poster to Hans-Dieter Görtz who was an outstanding scientific mentor and real friend for many members of the society.

## **P17 It's lunchtime! 'drinks' and food selection in key ciliates from Lake Mondsee (Austria)**

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The cross-linking of ciliates among food web players including predators and algae is largely a black box, though ciliate grazing on phytoplankton contributes significantly to the carbon flow. Ciliates growth and grazing rates largely depend on the quantity and quality of available food and are species specific. For a better understanding of the role of ciliates within the aquatic food web, investigations on a large variety of predator-prey relationships are needed. Ciliates of the genera *Coleps* and *Urotricha* are dominant prostomatids in Lake Mondsee (Austria). On clonal cultures of two species, eight cultivation media (modified Woods Hole MBL medium - WC, WC:Volvic Mineral water at different ratios, Blue-Green medium - BG11, sterile-filtered lake water, Volvic, modified Bourrelly medium) were tested for the two ciliate species *Coleps* sp. and *Urotricha castalia*. These experimental approaches were combined with different food algae partly isolated from Lake Mondsee. We offered eight immobile and four mobile flagella-bearing species of planktonic algae. All cultures were kept at 15 °C and a 14:10 h light:dark cycle. Preliminary results revealed that the optimal medium for *Coleps* sp. was WC:Volvic 5:1, and for *U. castalia* also WC:Volvic 5:1 and WC:Volvic 1:1. The results revealed that *Coleps* sp. was feeding on mobile (*Chilomonas paramecium*, *Cryptomonas phaseolus* and *Cryptomonas* sp. SAG26.80) and on immobile algae (*Cosmarium* sp., *Choricystis* sp.) as well, however, *U. castalia* was feeding only on mobile algae (*C. phaseolus* and *Cryptomonas* sp.). Both ciliates did not feed on *Coelastrum* sp., *Scenedesmus obliquus*, *Chlorella* sp., 2 *Microcystis* spp., *Peridinium* sp. and *Stephanodiscus minutus*. These preliminary results provide knowledge on the autecology of these ciliates to understand their important role in aquatic food webs.

The study is funded by the Austrian Science Fund (FWF): I2238-B25.

## **P18 Novel oxymonads from the lower termite *Porotermes adamsoni***

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The symbiotic gut flagellates of lower termites form host-specific consortia, which are composed of members of the Parabasalia and Oxymonadida. The analysis of their coevolution with termites is so far hampered by a lack of information particularly on the species colonizing the basal host lineages. To date, there are no reports on the presence of oxymonads in termites of the family Stolotermitidae. We discovered three deep-branching lineages of oxymonads in the damp-wood termite *Porotermes adamsoni*. One tiny species (6–10 µm) morphologically closely resembles the genus *Monocercomonoides* but shows a high sequence dissimilarity to recently published sequences of Polymastigidae from cockroaches and vertebrates. A second small species (9–13 µm) has a slight affinity to members of the Saccinobaculidae, which are found exclusively in wood-feeding cockroaches of the genus *Cryptocercus*, the closest relatives of termites, but shows a combination of morphological features that is unprecedented among any oxymonad family. The third species is much larger (30–120 µm) and morphologically resembles members of the genus *Oxymonas*, its phylogenetic sister group. These findings significantly advance our understanding of the diversity of oxymonads in termite guts provide important cues that will help to reconstruct the evolutionary history of symbiotic digestion.

## **P19 Diversity and evolution of ciliates associated with freshwater turbellarians**

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Three freshwater turbellarian species (*Dugesia gonocephala*, *Girardia tigrina* and *Polycelis felina*), belonging to the order Tricladida, were examined for ciliate infections. Living morphology and phylogenetic position of the isolated ciliates were studied using light microscopy and molecular phylogenetic methods. Only three ciliate species, all from the highly diverse class Oligohymenophorea, were detected: *Haptophrya planariorum* from the subclass Astomata, *Urceolaria mitra* from the subclass Peritrichia, and *Tetrahymena* sp. from the subclass Hymenostomatia. Each of these ciliates became specialized on different parts of the turbellarian bodies. *Haptophrya planariorum* lives in the pharynx and rami of the intestine, *U. mitra* colonizes the body surface, and *Tetrahymena* sp. attacks open wounds and feeds on the mesenchyme. The phylogenetic framework of astome and peritrich ciliates mirrors the occurrence of their hosts in the geological history of earth, i.e., astomes and peritrichs isolated from turbellarians branch off before their relatives isolated from annelids and mollusks. On the other hand, *Tetrahymena* sp. isolated from turbellarians is classified comparatively deeply within the family Tetrahymenidae, suggesting that the phylogeny of tetrahymenids does not correlate with that of their obligate/facultative host groups. Nevertheless, the reconstruction of ancestral traits corroborated the hypothesis that histophagy was a life history trait already of the progenitor of the subclass Hymenostomatia where *Tetrahymena* belongs to.

## **P20 Analysis of nonlinear dynamics in single-species systems**

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A famous paradox of real world ecosystems is the observed high diversity of species in plankton in the absence of a similarly high diversity of limiting conditions. Organisms are obviously faced with external variations of their environment, but also with intrinsic fluctuations governing their population dynamics. The interaction of nonlinear processes in Populations could lead to damped oscillation where abundances approach the capacity of the environment, stable limit cycles where abundances fluctuate around this capacity and deterministic chaos, where abundances fluctuate totally irregularly. Although the phenomenon of nonlinear dynamics has been mostly overlooked by ecologists, recent theoretical studies have highlighted its potential high influence on biodiversity. Chaotic fluctuations in population dynamics appear to favour the coexistence of species. However, mainly due to a lack of experimental evidences with real organisms, this phenomenon is not yet well accepted in the scientific community.

We try to answer the question whether chaotic dynamics can appear under stable conditions in flow-through systems with a single species. We reanalysed time series data from literature and conducted experiments with single-species systems. For the present study we used strains of the genus *Ochromonas* because they can grow easily under axenic and constant conditions in flow-through systems. The occurrence of deterministic chaos in a single species system may give a hint for nonlinear processes within the population, such as individual differences between single species.

## **P21 *Oophila* – an amphibian loving symbiont from northern Germany**

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The green algae *Oophila amblystomatis* is known to be associated with egg masses of several amphibian species. Since its first description several studies have been conducted to investigate the benefits of this relationship in detail. Being grazing protected in the egg jelly and benefitting from compounds of the nitrogenous (mainly ammonia) wastewaters of the unborn, the alga provides valuable oxygen to the embryo during daylight hours. The provided oxygen has been shown to increase the hatching success and general fitness of the embryos. Recent studies have proposed that different lineages of the algae may occur host specific or even form subclades regarding their geographical origin.

In this study, the phylogenetic position of algae collected in the egg masses of the agile frog, *Rana dalmatina* from an area near Brunswick, northern Germany was analyzed. By means of Sanger sequencing, sequence fragments of the small subunit rDNA were utilized to construct a phylogenetic tree. On basis of these results and the addition of morphological analysis, the alga was found to be genetically close to other symbiotic algae of the clade of Chlamydomonadales from northern USA and Japan.

## **P22 Results of monitoring Protozoa in seven different German rivers and the role in the microbial food web**

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### **Abstract**

The protozoan fauna (heterotrophic flagellates and ciliates) was investigated in seven German rivers (Spree, Havel, Rhine, Moselle, Ruhr, Isar and Ilz) during a one-year study with focus on the spring and summer season. Background of the study was the assessment of the elimination potential of the protozoan community regarding bacteria. This is especially important since the percentage of pathogenic and antibiotic resistant bacteria released from water treatment plants is increasing nowadays.

On the poster we will focus on the taxonomic and functional composition of the protozoan community in the different rivers based on morphological and molecular identification and the resulting interactions within the microbial loops and food webs, respectively. These were assessed by calculation of feeding rates on the basis of abundances and biovolumes of the protozoa found and by the consecutive calculation of grazing rates on the basis of literature data.

We could show that the rivers differ clearly in the structure of the microbial food webs which especially depends on different hydrological properties of each river. Moreover, the analysis of the protozoan fauna with molecular tools showed a high diversity of potentially bacterivorous protozoans.

The subsequent calculation of grazing rates on bacteria revealed that in some rivers (e.g. Rhine and Ruhr) protozoans may play an important role regarding the elimination of bacteria (and thereby also pathogenic bacteria). The occurrence of bacteria is presumed to be high during periods of high precipitation when wastewater might enter the rivers without having passed waste water treatment plants (storm water overflow discharge). We could prove that protozoan abundance increase rapidly after heavy rainfall and play an important role regarding the reduction of bacteria.

## **P23 Deep molecular characterization of cercozoan diversity and community composition in the canopy region of a floodplain forest using Illumina high-throughput sequencing**

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One of the most general patterns in community ecology is the increase in species richness with increasing habitat area. Forest canopies form the largest interface between earth and atmosphere, and a huge habitat area for colonizing organisms. However, our mechanistic understanding of how biodiversity is linked to canopy size and structure is still limited. Tree species differ strongly in surface topology and the quantity of microhabitats (e.g. leaves, bark, branches, knotholes, deadwood, epiphytes and arboreal soils) they provide. We analysed protist communities of Cercozoa in canopy samples of three different autochthonous tree species in a floodplain forest (Leipzig, Canopy Crane Facility) and tested to which degree a greater diversity of microhabitats influenced the species richness and abundance of microbial eukaryotes (habitat-heterogeneity hypothesis).

We acknowledge funding from the DFG within the Priority Program: Taxon-Omics: New Approaches for Discovering and Naming Biodiversity (SPP 1991).

## **P24 Inferring ecological information from complex plankton-networks using Augmented Reality**

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Network analyses are becoming increasingly popular in many biological disciplines. One major field of application is in ecological interaction or co-occurrence networks, which are usually constructed from high-throughput sequencing (HTS) data of environmental community samples. That is, millions of sequences are analyzed at the same time for statistically significant correlation patterns. Positive correlation may indicate trophic relationships between organisms, e.g. predator-prey, whereas negative correlation may indicate organisms competing for the same resources. Although graph theory provides a full toolkit for inspecting different ecological aspects, the most straightforward network inspection remains its visualization. But since networks of HTS data easily consist of hundreds of nodes and edges, static visualizations contain too much information to be easily processed by eye. Therefore, we propose Augmented Reality (AR) as an intuitive and innovative way of inferring information from ecological network visualizations. Thanks to AR, it becomes feasible to concentrate on certain nodes and investigate their effect on the network. This allows for answering specific questions about the biotic and abiotic factors that are important for an organisms' occurrence within an ecosystem, as well as about the consequences for the network if the organism would disappear. The information that can be visualized with AR is only limited by the database the researcher is using and may include pictures or seasonal abundance patterns. Given that such information is very tedious to find for standard network visualizations, we suggest that AR could be implemented in future network visualizations for easy and full access of all interesting information.

## P25 Marine protist diversity and community structure at the West Antarctic Peninsula

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The Western Antarctic Peninsula (WAP) is climatically extremely variable and belongs to the fastest warming regions on earth. It is also regularly exhibiting large phytoplankton blooms which provide the basis of a highly productive food web. Recent changes in the phytoplankton community composition from large diatoms to small flagellated cryptophytes have been associated with regional climate change and are potentially inducing further shifts in the dominance of major grazers in this region, i.e. Antarctic krill (*Euphausia superba*) and salps (*Salpa thompsoni*). Such a shift in grazer populations might again induce quantitative and qualitative changes in the planktonic food web in several ways, directly through differences in grazing selectivity and indirectly due to altered concentrations and ratios of excreted macronutrients. However, data on community composition encompassing the entire planktonic food web in this region are rare, especially outside the summer season. Within the project POSER we studied plankton community structure at ten different stations and four different depths during a cruise in late summer/autumn (March - May 2018) to the South Shetland Islands and the Antarctic Peninsula. In our analyses we focused on heterotrophic and phototrophic plankton of different size classes using a broad range of tools, e.g. microscopy, analyses of pigments and fatty acids, flow cytometry and DNA metabarcoding. Furthermore, particulate organic matter, dissolved organic carbon and dissolved inorganic nutrients were measured in addition to abiotic parameters (temperature, salinity, depth, O<sub>2</sub>, light). Here we present our first results with a main focus on 18S rDNA Illumina sequencing, describing protistan community composition and diversity in relation to potentially driving environmental parameters. The sampling locations allowed us to compare different water bodies as well as coastal and oceanic sites. These sequencing data provide a comprehensive picture of the protist distribution in this area over depth and in space, and will nicely complement further plankton community analyses to understand planktonic food web structure. Moreover, this work will serve as a basis for estimating the possible consequences of a shift from the currently still predominant krill population to an increasing occurrence of salps.

## **P26 Retention Effects of Biofilms of the River Rhine**

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The River Rhine is one of the most important navigation in Europe and although the quality of the water has improved within the last decades, the natural habitats have been greatly altered and reduced by construction activities. As a result the biodiversity is reduced, neozoans dominate the macrofauna and limited self-purification of the River Rhine is reduced.

The biofilms on stones of groyne fields in waters like the Rhine play a significant role for the functioning of the whole ecosystem. A variety of protozoans, micro- and macrozoobenthos can be found on biofilms, which influence each other and form food sources for higher trophic levels. In this study, the focus is mainly on the protozoans and the microzoobenthos and their effects on the reduction of bacteria including pathogens of the water of the river. The results of the existing study showed that the biofilm has a significant retention effect on flagellates and bacteria in the water column. Most important for the retention effect are ciliates of the biofilm. The results indicate that self-purification in rivers due to biofilms can be of great importance especially for the reduction of bacteria (including pathogens). At the moment the retention effect of the biofilm communities also regarding microplastics is investigated.

## **P27 A method for the direct measuring of protists in the flow**

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The necessity to determine the abundance of protists is obvious in many fields of research. But until now, there are only a few efficient and at the same time cost-effective methods to determine abundances with little effort over a long period of time. Continuous registration of abundances is essential for the analysis of population dynamics and its dynamic behavior. We have established an automatic abundance detection system working with video analysis, which reliably detects protists and allows a continuous abundance estimation. The advantages of modern techniques of flow cytometry to the classical methods of manually counting of organisms is the low amount of time needed for the analysis and the high number of samples that can be processed in a given time interval. However, flow cytometry systems are costly and an automatization for continuous registration within e.g. a flow through-system is difficult. The image and video analysis becomes easier and is gaining in importance, particularly as a result of advances and cheap production of microscope cameras. In our study, samples of *Ochromonas danica* were analyzed via an automatic registration. The main measurement takes place in a channel, in which an ocular camera takes video recordings. The examined videos are saved and the data are exported for analysis. A comparison of techniques revealed that our results of the image analysis are not significantly different from abundances measured through manual countings. The new method allows analyzing many samples simultaneously.



## **P28 First phylogenetic characterization of *Malpighamoeba mellificae* using gene sequences extracted from a metagenomic deep sequencing approach reveals affiliation to the Tubulinea**

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*Malpighamoeba mellificae* Prell, 1926, is an amoeba that infects the Malpighian tubules of honey bees. The amoebae, ingested as cysts, develop into trophozoites that feed upon tubule epithelia. The resulting damage of the Malpighian tubules can induce an imbalance of waste excretion and hemolymph exchange. This causes the so-called amoeba or amoebiasis disease in adult bees sometimes co-occurring with *Nosema apis* infections. The amoeba infection can weaken or kill bees and is discussed in relation to spring dwindling of beehives. Most reports of this amoeba come from the 1960s and earlier, and knowledge of the disease and its spreading is very poor. The lack of any genetic markers for the species hampers its sensitive identification using molecular tools and knowledge on its epidemiology.

In the present study, metagenomic sequencing was done with Malpighian tubules containing *Malpighamoeba mellificae* specimens (sample) and with Malpighian tubules not containing any *M. mellificae* specimens (control) as identified each under the light microscope. RNA was extracted, transcribed into DNA, transformed into sequencing libraries and sequenced using the Ion Torrent S5XL platform. Three candidate genes could be extracted from sequence datasets of the sample but not in the control dataset. Preliminary phylogenetic analyses allowed the phylogenetic characterization of this taxon for the first time. According to our analysis, *M. mellificae* branches off in the Tubulinea (Amoebozoa) with the closest relationship to *Micriamoeba tessaris* (Tubulinea incertae sedis, a genus newly described in 2012) based on actin and 18S rRNA, and *Micriamoeba* plus *Echinamoeba silvestris* based on 18S rRNA.

Next steps are to perform detailed microscopic investigations of the amoeba and the design of a sensitive diagnostic PCR tool to investigate the disease and the prevalence of *M. mellificae* in more detail.

## **P29 A new giant virus isolated from its natural host *Saccamoeba* sp. together with its virophage shows a narrow host range**

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During the last years, different giant viruses have been found in protists like *Cafeteria* and *Acanthamoeba*. The latter genus was often used as a bait to extract giant viruses from different habitats. Here, we present a natural host-parasite system isolated directly from the environment.

A *Saccamoeba* sp. was isolated from the bark of a sycamore tree and a culture was established (*Saccamoeba* strain SL-5). Inside the amoebae, a Mimivirus-like endoparasite (isolate KSL-5) with a virion size of 430-450 nm was found via transmission electron microscopic investigations. The replication of the virus has a long lag phase of at least 12 h. After 24 h, an additional small icosahedral virus (about 50-60 nm) became visible within the virus factory. This satellite virus / virophage seems to interfere with the replication of KSL-5. The giant virus could be re-isolated (isolate KSL-5x) from the same tree using a virus-free strain of *Saccamoeba*. KSL-5x did not show any affiliation with a satellite virus / virophage but possess a long tail. In contrast, the tail of KSL-5 is shorter and was therefore found rather seldom. All attempts to transfer KSL-5 and KSL-5x to other amoeba strains including *Acanthamoeba* sp. failed, indicating a rather narrow host range, whereas both virus isolates appeared to be susceptible for the virophage.

A first phylogenetic analysis of the Mimivirus shows that KSL-5x is related to the Mimiviridae sensu stricto rather than to other giant viruses and most likely forms an independent branch basal to the Mimiviridae lineages A, B, and C. The closest relationship was detected to the newly described Tupanvirus, a caudate virus related to Mimiviridae. The name *Platanovirus saccamoebae* for the new giant virus was proposed according to its locality and host. The preliminary analysis of the virophage revealed a "DNA polymerase type B, organellar and viral family protein [*Trichomonas vaginalis* G3]" indicating the virophage being derived from a transposon of the Polinton family.

## **P30 Use of intraspecific genetic variability in the internal transcribed spacer region of the rRNA operon to distinguish morphologically similar strains of the ciliate *Coleps hirtus***

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The present study aimed at developing a molecular method to distinguish three different *Coleps hirtus* clones in polyclonal assemblages. These clones are morphologically very similar, but vary substantially in their feeding and growth rates. We first tried to find sequence variabilities within the 18S rRNA gene before moving on to the internal transcribed spacer (ITS) regions of the rRNA operon of the clones. Although previous studies were able to distinguish closely-related *Paramecium* and *Tetrahymena* clones based on ITS sequences, no study has been conducted yet to distinguish *C. hirtus* clones using this marker. To address this problem, a specific primer pair 3770F/2104R (product: ~535 bps) was designed to bind in conserved sequences up- and downstream from the ITS-1 and -2 regions of the clones. Results after sequence analysis of the fragments showed that individual clones were clearly distinct from one another, indicating that the ITS regions were suitable for use as a nuclear marker for distinguishing our clones. PCR products were also analysed in a 15% (w/v) polyacrylamide gel using a modified denaturing gradient gel electrophoresis (DGGE) protocol. It was shown that PCR products of each clone stopped migrating at different positions in the gel, and thus allowed individual clones to be identified. The primer pair was also applied in a feeding experiment investigating trophic interactions of the predatory ciliate *Stylonychia* sp. and a polyclonal *C. hirtus* population. The presence and absence or decline of particular *C. hirtus* clones could be clearly detected in the gel. Hence, this DGGE approach proved to be a valuable tool to distinguish morphologically similar clones of the same ciliate species and to demonstrate preferential feeding behaviour of a predatory ciliate on these clones in a polyclonal population. This method has high potential to be applied in future experiments for estimating population dynamics of different *C. hirtus* clones in ecological experiments on food web interactions.

## **P31 Influence of hydrostatic pressure on the behavior of three deep-sea ciliates**

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Locomotion is considered as the main form of expression of ciliate behavior. This is already confirmed and described in many studies using different ciliate species. But how ciliates behave/move in extreme habitats such as the deep sea? This is still unclear, because data on ciliates isolated from the deep sea are very scarce and mostly based on molecular studies. So far, only a few ciliates have been collected alive from the deep sea and cultivated at laboratory conditions. To our knowledge, this was the first time that the ciliates were isolated from abyssal depth of the Atlantic Ocean (< 4000 m) and that their behavior was observed directly under high pressure. Experiments were done using a modified high-pressure system with the ability of direct observation. Here we will report about the behavior of three deep-sea ciliates, *Aristerostoma* sp., *Euplotes* sp. and *Pseudocohnilembus persalinus* under high hydrostatic pressures up to 500 bar. The typical behavior elements for a potentially new *Euplotes* species were observed at least up to 200 bar. For *Aristerostoma* sp. behavior elements were observed up to a maximum pressure of 400 bar, and for *P. persalinus* up to a maximum pressure of 300 bar. In addition, the long-term survival experiments were carried out with all three deep-sea ciliates exposed at 200, 350 and 430 bar. Some individuals showed an ability to survive high pressure and to recover (returning to their normal movement) indicating their barotolerance. Our results suggest that some ciliates might inhabit deep-sea waters and might be an active part of deep-sea protist communities. Additionally, we would like to present molecular and morphologic data of the ciliate *Euplotes* sp. isolated from abyssal deep-sea sediments.

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