37th Annual Meeting of the German Society for Protozoology

Programme & Abstracts

Protozoology – Diversity of Life in a Micrometer Scale

Cologne I Germany
27th February – 2nd March 2018

Organizers:

University of Cologne
Biocenter
Institute of Zoology
General Ecology
Zülpicher Str. 47b
D-50674 Cologne
Germany

DEAR PARTICIPANTS OF THE DGP 2018 IN COLOGNE!

Welcome to the official programme of the 37th Annual Meeting of the German Society for Protozoology hosted by the Institute for Zoology at the University of Cologne. The meeting will take place from February 27th to March 2nd 2018 in Cologne.

The motto of this year's meeting is "Protozoology - Diversity of Life in a Micrometer Scale". We have invited speakers covering the different dimensions of protozoan life and we are looking very much forward to your own contributions for our annual meeting in Cologne.

Since most of us will be accommodated at only two places, we expect lively discussions during the conference and evenings. Young scientists are invited to join a pre-conference workshop on the identification of protists on Tuesday. The workshop will take place at the Biocenter of the University of Cologne.

The official conference language will be English. This year we will have the ZEISS-Best-Talk-Award for students awarded with a Zeiss-Stemi 305 and prize for the best poster. They will be awarded at the social evening. For your entertainment, short excursions to the town center are planned on Wednesday late evening.

We are very much looking forward to your participation and scientific contribution, and welcome you heartly to Cologne!

ZEISS-Stemi 305

OUR THANKS GOES TO THE FOLLOWING

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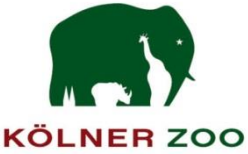

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1 General information

Venue - St. Georg Tagungs- und Gästehaus

Cologne, named in 50 AD after Emperor Claudius as „Colonia Claudia Ara Agrippinensium“ or Colony of Claudius and Altar of the Agrippinians was always a globally oriented city due to its strategically and economically valuable location at the River Rhine. Its late Roman and early medieval heritage is clearly visible in the cities architecture and the various findings of historians still enrich the millennia old city's history to this day. Cologne is most commonly known for its fifth season, the Carnival, and the Cologne Cathedral. The highly diverse and pulsing city is located in southern Northrhine-Westfalia and is an ideal hosting location with many cultural, social and scientific sights for a most pleasant DGP-meeting.

The conference venue will be the **“St. Georg Tagungs- und Gästehaus”** in Cologne. It serves as a conference and meeting center with included accommodation. It is located south to the city center and about 1 km west of the River Rhine and offers comfortable single, double and multi-bed rooms (up to four persons). Single and double rooms are equipped with private shower and toilet, multi-bed rooms (preferably occupied by students) have shower/WC on the floor. Meals are included in the conference fee and will be served in the guesthouse.

The address of the venue is

Rolandstraße 61, 50677 Cologne

How to get to the accommodation

Cologne is accessible by many different options. The Cologne Main Station can be reached by train. The Cologne-Bonn-Airport is located in south-eastern direction between Cologne and Bonn and provides a direct connection to Cologne Main Station via train. Cologne is also easily accessible by highways A1, A3, and A4 for travel by car or bus.

By public transport: Cologne main station is easy and fast accessible via the IC, ICE (Deutsche Bahn AG) and Thalys Network. For traveling by bus you have the possibility to travel to Köln Nord (Leverkusen Bf. Mitte) or Cologne-Bonn Airport (CGN). From there you can take a train to the Cologne Main Station. From Cologne main Station there are several possibilities to travel to “St. Georg Tagungshaus” (tram “16”, direction Bonn (get out at station Chlodwigplatz) or buses “132”, direction Zollstock-Südfriedhof and “133”, direction Rondorf/Meschenich).

By car: From Venlo or North-west via highway A61, exit on Kreuz Kerpen following highway A4. From Hannover or North-east via highway A2, exit on Kamener Kreuz following highway A1. From Aachen or South-west via highway A4. From Frankfurt or South-east via highway A3.

By plane: From Cologne-Bonn Airport (CGN): Take Railway “S13”, “S19”, “RE6” or “RE10816” to Cologne Main Station. From there, take the tram “16” or the bus “133” or “132” to “Chlodwigplatz”

By foot: If you arrive at Cologne Main Station, you can walk in about 40 minutes to the venue while being rewarded with an scenic tour through the city centre, or a beautiful walk along the River Rhine. If you want to go through the city centre keep yourself left when leaving the main station across the "Bahnhofsvorplatz" and follow the way to the "Wallrafplatz" with a wonderful view on the Cologne Cathedral. Go straight down the "Hohe Straße" pass the "Hohe Pforte" and the "Waidmarkt". There you turn slightly right into “Severinstr." and you will be at “Chlodwigplatz”, where you can see the medieval city wall. Walk straight to the end of "Chlodwigplatz" to reach the tram and bus stop. If you arrive at the tram station "Chlodwigplatz", the walk to the venue takes about 6 minutes. From Chlodwigplatz you have to walk down the "Bonner Street" till "Zugweg". There you have to turn right into Rolandstrasse, where the venue is located (Nr. 61).

Registration

Registration-desk will be located in the "St. Georg Tagungs- und Gästehaus". Registration will be open on:

- Monday, 26th February: 6 – 7.30 pm
- Tuesday, 27th February: 8 – 9 am and 5 – 7.30 pm
- Wednesday, 28th February: 8 – 9 am

Dinner & Get Together

When: 27th February (Tuesday)
Where: St. Georg Tagungs- und Gästehaus
Time: 7.30 pm

Guided City Tour

When: 28th February (Wednesday)
Time: 7.30 pm St. Georg Tagungs- und Gästehaus. We will travel together to the main station by train.

Social Evening in the Cologne Zoo

When: 1st March (Thursday)
Time: starting at 7.30 pm
Costs: included in conference fee
Where: Zoo Event, Riehler Str. 173, 50735 Cologne

The social evening will take place at the Restaurant of the Cologne Zoo at Cologne Niehl (Zoologischer Garten Köln in Köln Niehl).

2 Overview of the meeting

Time	Monday, February 26th	Tuesday, February 27th	Wednesday, February 28th	Thursday, March 1st	Friday, March 2nd
8:00		Breakfast & Registration	Breakfast & Registration	Breakfast	Breakfast
9:00		Workshop	Welcome Keynote Talk by SONJA RUECKERT	Keynote Talk by JOHN DOLAN Contributed Talks	Keynote Talk by POSCH, THOMAS
10:00			Contributed Talks	Coffee Break/ Poster Session	Contributed Talks
11:00			Coffee Break Contributed Talks	Contributed Talks	Coffee Break
12:00		Lunch	Poster Presentation	Lunch	Contributed Talks Farewell
13:00		Workshop	Lunch	Grell Award Ceremony and Presentation	Lunch
14:00			Keynote Talk by DANIEL RICHTER	Coffee Break/ Poster Session	End of the Meeting
15:00			Contributed Talks Poster Session & Drinks	Contributed Talks	
16:00			General Meeting of the DGP	Coffee Break	
17:00		Registration for the DGP 2018		Contributed Talks	
18:00	Registration for the Workshop		Dinner		
19:00	Dinner	Dinner & Get Together	Guided City Tour	Dinner & Social Eveningin the Cologne Zoo	
Evening					

3 Programme

Pre-Conference Workshop on the Identification of Protists

Monday, 26th February 2018	**St. Georg Tagungs- und Gästehaus**
18:00 - 19:30	Registration for the Pre-Conference Workshop
19:30 - 21:00	**Dinner**

Tuesday, 27th February 2018	**Biocenter, University of Cologne**
8:00 - 9:00	Breakfast & Registration for the Pre-Conference Workshop
9:00 - 12:00	Workshop on the Identification of Protists
12:00 - 13:00	**Lunch (Mensa, University of Cologne)**
13:00 - 17:00	Workshop on the Identification of Protists
17:00 - 19:30	Check in and Registration for DGP Conference 2018
From 19:30	**Dinner & Informal Get-Together**

Wednesday, 28th February 2018	**St. Georg Tagungs- und Gästehaus**
8:00 - 9:00	Breakfast & Registration for the DGP Conference 2018
9:00 – 09:15	Welcome addresses
Session 1: Protists as Endobionts **Chair: Julia Walochnik**	
09:15 – 10:00	Keynote Talk by **RUECKERT, SONJA** (Edinburgh) The secret world of gregarine apicomplexan parasites
10:00 - 10:15	**Radek, Renate** Serritermitid termite flagellate composition is shaped by horizontal transmission
10:15 - 10:30	**Carduck, Sarah** First record of gregarines (Apicomplexa) from the Atacama Desert: Two new species associated with *Scotobius* and *Psectrascelis* (Coleoptera, Tenebrionidae)
10:30 - 10:45	**Zinßmeister, Carmen** Life history and possible phylogenetic position of the parasitic dinoflagellate *Syltodinium listii* Drebes (Dinophyceae, Gymnodiniales).
10:45 – 11:15	**Coffee Break**

Session 2: Protists in Extreme Environments Chair: Thorsten Stoeck	
11:15 – 11:30	**Kühner, Steffen** Unravelling haloadaptation strategies of the heterotroph ciliate *Schmidingerotrhix salinarum* using transcriptomics
11:30 - 11:45	**Živaljić, Suzana** Studies on the survival and behavior analysis of deep sea protists
11:45 - 13:00	**Poster Presentations** Two minute presentations (max. 1-2 overheads) of all posters in the order of poster numbers (posters will be shown during the whole meeting in the upper floor)
13:00 - 14:00	**Lunch**

Session 3: Protist Environmental Genomics Chair: Frank Nitsche	
14:00 - 14:45	Keynote Talk by **RICHTER, DANIEL** (Barcelona) Ocean metagenomes and metatranscriptomes: which plankton live where and what are they doing?
14:45 – 15:00	**Schoenle, Alexandra** Underestimated diversity of microbial protists in the dark ocean
15:00 – 15:15	**Boenigk, Jens** Geographic distance and mountain ranges structure freshwater protist communities on a European scale

15:15 – 15:30	**Lentendu, Guillaume** Testing the effects of environmental filtering and competitive exclusion on protist communities
15:30 – 16:30	**Poster Session & Drinks**
15:30 – 18:00	**General Meeting of the DGP**
18:30 – 19:30	**Dinner**
19:30 – 21:30	Guided City Tour

Thursday, 1st March 2018	**St. Georg Tagungs- und Gästehaus**
8:00 - 9:00	Breakfast
	Session 4: Ciliated Protists **Chair: Anja Scherwaß**
09:00 – 09:45	Keynote Talk by **DOLAN, JOHN** (Villefranche-sur-Mer) Dynamics in the Deep Sea: The Protist Fauna of the Mesopelagic Mediterranean Sea
09:45 – 10:00	**Stoeck, Thorsten** Environmental DNA metabarcoding of ciliates indicates the benthic footprint of salmon farming
10:00 - 10:15	**Wilson, Monica** Extensive macronuclear fragmentation in *Didinium* sp. (Litostomatea)
10:15 - 10:45	**Coffee Break & Poster Session**

Session 5: Protist Evolution Chair: Sascha Krenek	
10:45 – 11:00	**Plattner, Helmut** Conserved signaling pathways link protozoa to mammalian nervous system
11:00 - 11:15	**Nitsche, Frank** Influence of bacteria on the morphology and life cycle of choanoflagellates and other eukaryotes
11:15 – 11:30	**Catania, Francesco** *Paramecium* genome evolution in a selection-free environment
11:30 - 11:45	**Majda, Stephan** Genome comparison of three *Poteriospumella lacustris* strains
11:45 – 12:45	**Lunch**

Grell Award Chair: Julia Walochnik	
13:00 – 14:30	**Singer, David** (Neuchâtel) A molecular approach to microeukaryotic diversity, ecology and biogeography associated with *Sphagnum* mosses **Dumack, Kenneth** (Cologne) Novel lineages in Cercozoa and their feeding strategies
14:30 - 15:00	**Coffee Break & Poster Session**

Session 6: Soil Protists I Chair: Quentin Blandenier	
15:00 – 15:15	**Foissner, Wilhelm** Two new monotypic, hypotrich families (Ciliophora, Hypotricha) from floodplain soil in Australia and Botswana, respectively
15:15 – 15:30	**Geisen, Stefan** Chances and issues for soil protistology: A methodological summary with comparisons to other biota
15:30 – 15:45	**Fiore-Donno, Anna Maria** Worldwide diversity of Cercozoa: summary of our current studies
15:45 – 16:00	**Flues, Sebastian** Diversity and functional roles of Cercozoa in the phyllosphere of plants
16:00 – 16:30	**Coffee Break**

Session 7: Soil Protists II Chair: Anna Maria Fiore Donno	
16:30 – 16:45	**Blandenier, Quentin** Exploring the phylogeography of hyalospheniid testate amoebae (Amoebozoa) from regional to sub-continental scales
16:45 – 17:00	**Khanipour Roshan, Samira** What do protists feed on? A cloning approach exemplified on the shelled amoeba *Amphizonella* sp**.**

17:00 – 17:15	**Kahlich, Christopher** Shattered glass; the specialized predator *Phryganella paradoxa* (Arcellinida, Amoebozoa) ruptures frustules of diatoms by force
17:15 – 17:30	**Gao, Zhilei** Soil protists as drivers for soil disease suppressiveness by shifting bacterial community composition
19:30	**Dinner & Social Evening**

Friday, 2nd March 2018	**St. Georg Tagungs- und Gästehaus**
8:00 - 9:30	Breakfast
Session 8: Protist Ecology I **Chair: Hartmut Arndt**	
09:30 – 09:45	Keynote Talk by **POSCH, THOMAS** (Zürich) The effects of lake warming on protists and microbial food webs
10:15 – 10:30	**Trogant, Sandra** Clonal variability of predation induced phenotypic plasticity in ciliates
10:30 - 10:45	**Hess, Sebastian** Diversity of algivorous protoplast feeders and first insights into the molecular mechanisms underlying the perforation of algal cell walls
10:45 – 11:00	**Dirren, Sebastian** Nucleariid amoebae and their association with bacterial symbionts
11:00 - 11:30	**Coffee Break**

Session 9: Protist Ecology II Chair: Alexandra Rybarski	
11:30 – 11:45	**Rieser, Daniel** Systematic, molecular phylogeny and ecology of the genus *Coleps* (Prostomatida, Ciliophora)
11:00 - 11:15	**Macumber, Andrew L** Assessing the links between test morphology and the ecology of freshwater *Arcellinida* (testate lobose amoebae)
12:00 – 12:15	**Vitali, Valerio** Sub-optimal growth temperatures impact the fidelity of Programmed DNA elimination in *Paramecium tetraurelia*
12:15 – 12:30	**Singer, David** Temporal patterns of soil micro-eukaryotic diversity beneath pig cadavers decomposing on the ground or suspended
12:30 – 12:45	**Wanzenböck, Sabine** How to wow people by using ciliates for science transfer and public relations activities
12:45 – 13:00	**Farewell**
13:00 – 14:00	**Lunch**

4 Abstracts of Invited Speakers

The secret world of gregarine apicomplexan parasites

Sonja Rueckert

School of Applied Sciences, Edinburgh Napier University, Edinburgh, UK

Gregarine apicomplexans are a diverse group of parasites that inhabit the intestines, coeloms and reproductive vesicles of marine, terrestrial and freshwater invertebrates. Around 1600 species are known and described so far, but perhaps millions more have yet to be discovered. Gregarines have been conveniently lumped into three albeit poorly defined groups: archigregarines, eugregarines, and neogregarines. They have in most cases monoxenous (single host) life cycles with extracellular trophozoite stages that are very diverse in morphology and behaviour. Traditionally, gregarines have been thought to represent the most ancestral and therefore primitive lineages within the Apicomplexa. However, molecular phylogenetic and comparative ultrastructural data demonstrate that most gregarines are much more evolutionary derived than is usually assumed. An improved knowledge of these lineages will help elucidate the overall diversity and early evolutionary history of apicomplexans as a whole. Gregarine research is gaining a momentum, as they represent an important stepping-stone in the intriguing evolutionary transition from closely related free-living phototrophic/predatory organisms to obligate, intracellular parasites. I will introduce the diversity of the different gregarine lineages and present some of the on-going research as well as future directions.

Ocean metagenomes and metatranscriptomes: which plankton live where, and what are they doing?

Daniel J. Richter

Institut de Biologia Evolutiva (CSIC-UPF), Barcelona, Spain

The Tara Oceans expedition circumnavigated the globe over a multi-year period, collecting samples in the sunlit ocean from over 150 individual stations. For eukaryotic plankton ranging from 0.8 µm-2 mm, we produced three types of sequencing data for each sample, from the following three sources: (1) metagenomes: whole-sample genomic DNA extraction; (2) metabarcodes: PCR amplification of the V9 hypervariable region of the 18S ribosomal locus; (3) metatranscriptomes: polyadenylated mRNA selection of whole-sample RNA extraction. We present analyses that compare and contrast each data set. First, based on sequence similarity among metagenomes, we partition the oceans into regions inhabited by similar plankton communities. For smaller organisms, these regions are consistent with the provinces of Longhurst based on biogeochemical data, but this relationship breaks down above 20 µm, likely reflecting the contrast between shorter-lived, smaller primary producers and longer-lived, larger heterotrophs in the ocean. Next, we analyze taxonomically-identified metabarcode data, as a complement to largely anonymous metagenomes, to study the patterns of change within communities across an example region, the North Atlantic. For plankton sized 180 µm-2 mm, we demonstrate a transition from Collodaria-characterized communities to Metazoa-characterized communities along the Gulf Stream. Finally, to measure the activity of plankton communities, we apply a phylogenetic read placement approach to map metatranscriptomic sequences onto a set of 250 conserved eukaryotic gene trees. We find that, globally, metabarcodes and metatranscriptomes show a similar representation of major eukaryotic lineages. We close with an example of a specific gene, SIT, which transports extracellular silicon into organisms that construct silica-based structures, such as the frustules of diatoms. Diatoms are responsible for the majority of global SIT transcription, especially in the colder waters of the Southern Ocean, but their dominance is supplanted by choanoflagellates and animals in the Mediterranean and the Indian Ocean.

Dynamics in the Deep Sea: The Protist Fauna of the Mesopelagic Mediterranean Sea

John R. Dolan, Maria Ciobanu, Sophie Marro, and Laurent Coppola

Marine Microbial Ecology & Biogeochemistry Group, Laboratoire d'Oceanographie de Villefranche, France

We found a mesopelagic protist fauna composed of species different from that of the overlying surface community. These mesopelagic communities show remarkable seasonal changes in abundances and species composition corresponding with changes in water column structure. We focused on three distinct groups in which species identification is relatively unambiguous using light microscopy: tintinnid ciliates, phaeodarian radiolarians, and amphisolenid dinoflagellates. We found a deep-water community of tintinnid ciliates comprised of forms apparently restricted to deep waters (deep water natives) and species also found in the surface layer (invasives from the surface layer). This latter group of invasive species dominated during the winter mixis period when tintinnid concentrations were highest and subsequently declined with water column stratification. The community structures of the two tintinnid assemblages, deep water natives and invasives from the surface layer, showed distinct population structures. Phaeodarian radiolarians and the amphisolenid dinoflagellates were regularly found in deep samples but were largely absent from surface water samples and showed distinct patterns in the mesopelagic. Phaeodarian radiolarians declined with water column mixing and then increased in concentration with water column stratification whilst amphisolenid dinoflagellates concentrations showed no large shifts in abundance pattern but a distinct change in species composition. We conclude that for all three protists groups there are distinct mesopelagic forms and they show seasonal dynamics much like surface layer assemblages.

The effects of lake warming on protists and microbial food webs

Thomas Posch

Limnological Station, Institute of Plant and Microbial Biology, University of Zurich

Although some people (with an interesting hairstyle) argue that 'it is only weather', global warming has striking and well documented effects on the biosphere. Inland surface waters are immediately affected by warming due to the strong correlation between air and surface water temperatures and the impact of rising solar radiation. Increasing water temperatures may directly alter growth and population dynamics of planktonic protists, as documented by numerous lab studies. However, field studies show that the effects of lake warming on protistan communities reflect a complex interplay of limnological processes. Indeed, constant and rapid warming also changes physical and chemical properties of lakes and catchments within a few years. The average warming per annum is often attributed to striking temperature increases during cold seasons across the Northern Hemisphere. Nevertheless, recent studies for the pre-alpine region show the strongest warming during spring periods. This causes an earlier onset and prolongation of thermal stabilization in large deep lakes. In addition, stronger thermal stratification of lakes hampers complete water turnovers (holomixis), happening once or twice a year in the temperate zone. Holomixis is not only fundamental for the replenishment of deep layers with oxygen but also for the transfer of nutrients from the deep to the surface. These phenomena lead to an uncoupling or even to a loss of typical annual successions of protists within food webs. For example, phototrophic spring bloom communities, commonly formed by diatoms and cryptophytes, are cut off from essential nutrients such as phosphorus and nitrogen. This reduction in primary production affects the entire food web as algae are the major source of substrates for bacteria and of food for protistan (e.g., ciliates) and metazoan consumers. Ultimately, this phenomenon may propagate up to the level of top predators, causing drastic decreases in fish stocks. At the end of my talk, I will present some facts why harmful cyanobacteria apparently benefit from this drastic and rapid changes within microbial food webs.

5 Abstracts of Talks:

Grell Award Presentation

A molecular approach to microeukaryotic diversity, ecology and biogeography associated with *Sphagnum* mosses

David Singer

Laboratory of Soil Biodiversity, University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland

Despite the fact that free-living microeukaryotes compose the major part of Earth's biodiversity and play numerous essential roles in ecosystems, knowledge on their true diversity, ecology and their global patterns of distribution remain limited. In this sense, the objectives of this thesis are 1) to increase the knowledge on the diversity of microeukaryotes 2) characterize the ecological preferences and determine which are the main variables that influence community composition, and finally 3) to understand the rules that shape the communities at both local and global scales. To meet these objectives a specific component of the earth surface was selected: the "Sphagnosphere" i.e. the interstitial water directly influenced by *Sphagnum* mosses. This understudied but unique microenvironment is characterized by low nutrient contents, low pH, and high amounts of organic acids produced by the mosses. It is also very stable over time.

We first explored the diversity of two groups of protists in *Sphagnum* peatlands. The first group was genus *Nebela* (Arcellinida, Hyalospheniidae), a common testate amoeba taxon in acidic soils. We formally described the most abundant one and named it *Nebela gimllii* due to the small and stout shells. The different community profiles revealed that species are not randomly distributed among microhabitats in peatlands. Instead, we observed a strong phylogenetic clustering in nitrogen-poor areas suggesting that little amounts of nitrogen exerted strong environmental filtering. We also surveyed the molecular diversity of Oomycota, a clade of fungi- like stramenopiles which enclose many animal, fungi and plant parasites, as well as saprotrophic species. We revealed a high diversity, which was unexpected for osmotrophic organisms in nutrient-poor habitats unless most are parasitic. Moreover, most phylotypes found were not recorded in previous studies, which suggest the existence of highly specialized organisms.

We also surveyed the diversity of microbial eukaryotes along altitudinal gradients in three different climatic zones, temperate (western Alps), subtropical (Japan) and tropical (Costa Rica). We showed that 25 percent of phylotypes were shared in the three climatic zones. We found also a significant negative correlation between the proportion of phylotypes related to mixotrophic organisms and temperature. This, in line with other lines of evidence in the literature corroborates the idea that mixotrophy is disadvantageous under warm climates. Finally, we studied the spatial distribution of an emblematic morphospecies of testate amoeba found in the northern hemisphere peatlands: *Hyalosphenia papilio*. A total of 13 lineages were found, from which nine showed narrowly restricted distributions, and four were well distributed across the Holarctic realm. We showed, based on phylogenetic analyses and ancestral character reconstructions that *H. papilio* most probably appeared somewhere in the West Coast of North America.

In summary, my PhD revealed that the Sphagnosphere environment hosts high and unique diversity. This diversity is driven by physicochemical factors at the local scale and by climate and geographical distance at the global scale. We identified and quantified the main local abiotic variables, amongst which micro-topography and nitrogen content appeared to be the most significant in shaping micro-eukaryotic diversity within the same climate zone. These variables exerted strong environmental filtering, which appeared to be fundamental process of community assembly. On the other hand, at a global scale, we demonstrated that temperature was the factor that best explain community composition, and notably the abundance of mixotrophs (and hence a different functioning). At both scales, community composition, and therefore biotic interactions (and most probably ecosystem functioning) change drastically.

Novel Lineages in Cercozoa and Their Feeding Strategies

Kenneth Dumack

Department of Terrestrial Ecology, Institute for Zoology, University of Cologne, Cologne, 50674, Germany

Environmental sampling surveys revealed not only a large diversity of cercozoan clades in soil, freshwater and saline habitats, but also that Cercozoa (together with Amoebozoa) represent most often the dominant protist group with the largest biomass in terrestrial habitats (Bass and Cavalier-Smith 2004; Fiore Donno et al. 2017, Geisen et al. 2015; Grossmann et al. 2016; Howe et al. 2009; Lentendu et al. 2014, Sierra et al. 2016). However these environmental sequencing studies, although giving overview of dispersal and hidden diversity cannot be thoroughly interpreted by ecological means if genetic data is not linked to morphological and experimental data. Still, there is a high discrepancy between the number of described cercozoan species (about 600; Pawlowski et al. 2012) and the suspected thousands of lineages that are retrieved by environmental sequencing (Burki & Keeling 2014, Fiore-Donno et al. 2017). Most strikingly in cercozoan research is the neglect of the Thecofilosea, Cercozoa. Although there have been numerous publications on Cercozoa in the recent 10-15 years, before I started my work, there were only two publications dealing (partially only superficially) with the molecular and morphological identification of thecofilosean amoebae (Howe et al. 2011; Wylezich et al. 2002). The neglect of thecofilosean amoeba diversity is nicely illustrated by a joint publication of leading testate amoeba experts that contains a full page long paragraph with the title: “Filose testate amoebae [of which by far most belong the the Cercozoa] are still (almost) uncharted territory” (Kosakyan et al. 2016).

The major aim of these studies was to obtain cultures of the Thecofilosea (Cercozoa) that enable the connection of morphological and genetic data, facilitating the analyses of environmental sequencing data in the future. For this we cultured numerous thecofilosean (or thecofilosean-like) species and studied their cell morphology, feeding processes and life history stages (Dumack et al. 2016a,b,c; 2017a,b,c,d,e,f; 2018). This was achieved by using mainly light microscopy and time-lapse photography but also ultra structure data were obtained. Genetic markers, e.g. SSU rDNA and LSU rDNA were subjected to

phylogenetic analyses to draw conclusions on cercozoan evolution. The phenotypic, phylogenetic and ecologic data of the investigated cercozoan amoebae resulted in a comprehensive characterization of thecofilosean amoebae and the novel lineage Krakenidae. Based on intensive literature research and a critical evaluation of it, a stable phylogeny-based taxonomic framework of these cercozoan lineages was obtained. The talk will also refer to recently obtained results of one environmental sequencing study to illustrate the value of the outcome of this thesis.

6 Abstracts: Talks

Session 1:

PROTISTS AS ENDOBIONTS

Serritermitid termite flagellate composition is shaped by horizontal transmission

Renate Radek, Katja Meuser, Jürgen F. H. Strassert, Oguzhan Arslan, Anika Teßmer, Jan Šobotník, David Sillam-Dussèz, Ricardo Augusto Nink and Andreas Brune

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The guts of lower termites are inhabited by host-specific consortia of cellulose-digesting flagellates. We investigated the symbionts of the serritermitid termites *Glossotermes oculatus* and *Serritermes serrifer*. Serritermitids cluster within the Rhinotermitidae but the composition of their symbiotic flagellates differs considerably. We found that each serritermitid genus harbors similar parabasalid morphotypes: large *Pseudotrichonympha*-like cells, medium-sized *Leptospironympha*-like cells with spiraled bands of flagella, and small *Hexamastix*-like cells; oxymonadid flagellates were absent. Despite the morphological resemblance of the large- and medium-sized trichonymphid flagellates to *Pseudotrichonympha* and *Leptospironympha*, a SSU rRNA-based phylogenetic analysis showed that they are only distantly related to them. Only the *Hexamastix*-like flagellates are closely related to trichomonadid flagellates from Rhinotermitidae. There was a progressive loss of flagellates during the evolution of the rhinotermitids but our data suggest occasional transfaunation events occurred, whereby flagellates were transferred horizontally between members of different termite families. The *Pseudotrichonympha*-like and *Leptospironympha*-like cells were probably acquired from stolotermitid termites. In addition to the molecular phylogenetic analyses, we present a light and electron microscopic characterization of the *Leptospironympha*-like species, which has been described as a new spirotrichosomid genus, *Heliconympha* (Radek et al. 2017).

First record of gregarines (Apicomplexa) from the Atacama Desert: Two new species associated with *Scotobius* and *Psectrascelis* (Coleoptera, Tenebrionidae)

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Gregarines inhabit the intestines, coeloms and reproductive vesicles of aquatic as well as terrestrial invertebrates. The infection rates of gregarines can vary significantly between individuals and species, though most invertebrate groups which have been investigated intensively possess their own specific gregarines. Due to their widespread presence in animals, gregarine-host relationships may form a suitable system for evolutionary and co-evolutionary studies. We investigated the occurrence of gregarines in the gut of three wingless darkling beetles endemic to the Atacama Desert (Northern Chile, South America). For several beetles speciation has occurred already in the Atacama Desert. Thus, gregarines living as specific endobionts in beetles, which themselves may have been co-evolved with endemic plant communities, are assumed to show already a clear speciation. Even local differences might be traced. The molecular diversity of gregarines associated to beetles is not well understood. Here, we describe two novel gregarine species inhabiting the midgut of *Scotobius brevipes* and *Psectrascelis intricaticollis* (Coleoptera: Tenebrionidae) from the Atacama region. *Atacamagregarina paposa* gen. nov., sp. nov. and *Atacamagregarina psectrascelii* gen. nov., sp. nov. (Apicomplexa: Eugregarinoridea, Stylocephalidae) cluster with gregarines inhabiting other terrestrial invertebrates and form a clade with gregarines living in association with North American darkling beetles. In addition, phylogenetic analyses revealed that hosts, which are closely related to each other, contain also phylogenetically related gregarines, though further taxon sampling is necessary to better understand the co-evolutionary processes.

Life history and possible phylogenetic position of the parasitic dinoflagellate *Syltodinium listii* Drebes (Dinophyceae, Gymnodiniales).

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Parasitic dinoflagellates developed different life styles and ways of sufficient dietary intake. Some are host specific others infecting a wide range of hosts such as other protists up to different kinds of metazoan taxa. *Syltodinium listii* and *Gyrodinium undulans* are two parasitic dinoflagellates with identical morphologies, only distinguished through their seasonal occurrence, which is during summertime for *S. listii* and wintertime for *G. undulans*, and their host spectrum. Both are parasitizing on copepod and rotifers eggs. Only *G. undulans* is predominantly parasitizing on diatoms. Although, these parasitic dinoflagellates have been documented before, feeding experiments with different hosts and molecular phylogenetic analyses of *S. listii* didn´t take place yet.

For this study *S. listii* cells have been isolated and cultivated from plankton net samples of the inner harbor of Wilhelmshaven, Germany during summertime. Cultivated cells have been offered fresh eggs of rotifers, copepods and nauplia stages collected from plankton samples about every day a week. Additionally, feeding experiments have been performed with different kinds of diatoms and at different temperatures. The morphological changes during life history have been documented with an inverted light microscope equipped with a camera. Also some cells have been isolated for molecular analysis inferred form ribosomal DNA (ITS1, 5.8S, ITS2 and partial LSU).

Molecular studies have shown before, that a dinoflagellate determined as cf. *G. undulans* belongs to Gymnodiniales *(Gymnodinium* s.s. clade). Our sequences of *S. listii* have been compared with cf. *G. undulans* and our results are that they are identical. New morphological results and video sequences on *S. listii* feeding behavior showed that the parasite preferred copepod eggs and rotifer eggs depending on their sessional occurrence. The color of trophonts has been host dependent and size of dinospores was variable. *S. listii* cells have never feed on any of the provided diatoms and couldn't be found during wintertime.

This still leads to the question, if we are dealing with only one instead of two species. For verification both *S. listii* and *G. undulans* have to be cultivated and molecularly analyzed from their type locality in North Sea or nearby. Nevertheless, if we are dealing with one or two species, a taxonomic revision of the parasitic genus will be necessary.

Session 2:

PROTISTS IN EXTREME ENVIRONMENTS

Unravelling haloadaptation strategies of the heterotroph ciliate *Schmidingerotrhix salinarum* using transcriptomics

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Salt is one of the major environmental determinants of microbial community composition, exerting a high evolutionary selection pressure on life. The main challenges for microorganisms to cope with high-salt conditions are most likely the extreme energetic costs associated with osmoregulation and the evolution of cellular haloadaptive strategies. While haloadaptation is a focus in prokaryote research, protists remain a largely neglected group of microorganisms in this respect. For heterotrophic protists in specific, almost nothing is known about their haloadaptations. Only two recent studies on three heterotroph protists reported that the organisms use the compatible solutes glycine betaine, myo-inositol, hydroxyectoine and ectoine to counteract osmotic stress. The latter two compatible solutes come as a surprise since ectoines have thus far been considered exclusive to prokaryotes. Until now, there is no information about the exact metabolic pathways of the compatible solutes used by heterotroph protists. In addition, regulatory mechanisms to maintain ion homeostasis still remain in the dark. We, therefore, exposed the halophile, heterotroph ciliate *Schmidingerothrix salinarum* (Hypotricha) to a salt shock and monitored physiological changes through differential transcriptome analysis. We will present data, which allow us to unravel the transcriptional response and the regulatory mechanisms of the heterotroph ciliate to external salinity change.

Studies on the survival and behavior analysis of deep-sea protists

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Deep-sea organisms have to cope with extreme environmental conditions including low food resources, low temperatures, darkness and high pressure, making life more challenging in the deep sea in comparison to surface waters. Despite the vastness of this biotope, the most recent studies are based on protists inhabiting the euphotic zone, while deep-sea protistan assemblages remain largely uncharacterized. There are only very few cultures available from protists originating from the deep sea (>3000 m). Protist genotypes determined from deep-sea samples using next generation sequencing might originate from vital deep-sea populations or from cysts of organisms sedimented down from surface waters. The latter one may have never been active under deep-sea conditions. Here we will report on survival ability of several protists and behavior analysis of one ciliate exposed to high hydrostatic pressure. All experiments were done using a modified high-pressure system with the ability of direct observation. Survival experiments were carried out with protists isolated and cultivated during a deep-sea expedition with R/V Meteor. Strains were exposed to a maximum pressure up to 500 bar (in steps of 50 bar) or to pressures at which they were still active. Recovery experiments were carried out in the opposite way (pressure was decreased from maximum exposed pressure to 1 bar). Also, we analyzed the moving behavior of the deep-sea ciliate *Euplotes* sp. at different pressure levels (up to 500 bar). All strains were shown to be barotolerant. Some strains might even be able to populate surface as well as deep-sea waters.

Session 3:

PROTISTS ENVIRONMENTAL GENOMICS

Underestimated diversity of microbial protists in the dark ocean

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Although protists are assumed to be the major regulators of deep-sea sediment bacterial communities, information on eukaryotic microbial diversity in deep-sea surface sediments is scarce, especially in the abyss as the largest part of Earth´ surface. We collected sediment from 17 deep-sea basins (bathyal, abyssal and hadal regions) at the Atlantic and Pacific Ocean to analyze the genotype diversity of benthic protist communities. We found benthic deep-sea assemblages especially in size classes smaller than 10 µm surprisingly diverse and significantly different from littoral and water column protist communities. Kinetoplastid flagellates and ciliates had much higher genotype diversity in the abyssal deep sea, while Dinophyceae had the highest diversity in surface water communities. Many genotypes had no close representatives in genetic databases suggesting the presence of many novel taxa in deep-sea sediments. A high percentage of our retrieved deep-sea sequences was affiliated with parasitic or symbiotic species.

Geographic distance and mountain ranges structure freshwater protist communities on a European scale

Jens Boenigk, Sabina Wodniok, Christina Bock, Daniela Beisser, Christopher Hempel, Lars Grossmann, Anja Lange, Manfred Jensen

University Duisburg-Essen

Protists influence ecosystems by modulating microbial population size, diversity, metabolic outputs and gene flow. In this study we used eukaryotic ribosomal amplicon diversity from 218 European freshwater lakes sampled in August 2012 to assess the effect of mountain ranges as biogeographic barriers on spatial patterns and microbial community structure in European freshwaters. The diversity of microbial communities as reflected by amplicon clusters suggested that the eukaryotic microbial inventory of lakes was well-sampled at the European and at the local scale. Our pan-European diversity analysis indicated that biodiversity and richness of high mountain lakes differed from that of lowland lakes. Further, the taxon inventory of high-mountain lakes strongly contributed to beta-diversity despite a low taxon inventory. Even though ecological factors, in general, strongly affect protist community pattern, we show that geographic distance and geographic barriers significantly contribute to community composition particularly for high mountain regions which presumably act as biogeographic islands. However, community composition in lowland lakes was also affected by geographic distance but less pronounced as in high mountain regions. In consequence protist populations are locally structured into distinct biogeographic provinces and community analyses revealed biogeographic patterns also for lowland lakes whereby European mountain ranges act as dispersal barriers in particular for short to intermediate distances whereas the effect of mountain ranges levels off on larger scale.

Testing the effects of environmental filtering and competitive exclusion on protist communities

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Multiple mechanisms give rise to why species co-occur or not in the same community. Species sharing the same ecological niche tend to co-occur due to environmental filtering, but this process is balanced by competitive exclusion when they use the same resource. Which of these two processes primarily drives the assembly of natural protist communities has not been previously tested. Here we developed a novel analytical approach to link evolutionary distance with OTU co-occurrence, in two large environmental sequencing datasets from Neotropical rainforest soils and global marine waters. Using co-occurrence networks and evolutionary distance estimated by pairwise-sequence dissimilarities and phylogenetic distances, we found that evolutionary close OTUs systematically and significantly co-occurred more often than expected by chance, while OTUs with intermediate evolutionary distances co-occurred less often than expected by chance. These patterns support environmental filtering as the main driver of protist community assembly.

Session 4:

CILIATED PROTISTS

Environmental DNA metabarcoding of ciliates indicates the benthic footprint of salmon farming

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Traditionally, environmental impact assessments (EIA) in finfish aquaculture are based on the collection and identification of macrofaunal indicator organisms. However, this monitoring strategy is very tedious and expensive. Therefore, concerted efforts are in order to develop alternative monitoring systems. One very promising approach is environmental DNA metabarcoding, which relies on the identification of short DNA tags with indicator qualities for organic pollution. In a pilot project conducted along an organic enrichment gradient of a Scottish salmon farm, we have evaluated the potential indicator powers of benthic ciliate communities for EIA. We found a significant change and species turnover in ciliate community composition along this transect, corroborating well with the benchmark data obtained from traditional macrofauna monitoring of the same sampling sites. Our study revealed high variations between ciliate communities collected in the vicinity of fish farms and at distant locations. We found decreased species richness at impacted sites and species turnover presented itself as a function of organic input to the sediment. In conclusion, ciliate metabarcoding appears as a rapid and accurate tool for the evaluation of the quality of aquatic ecosystems. Hence, we propose the integration of protist metabarcoding in future biomonitoring projects as a complement of traditional methods and a source of new biosensors for environmental impact assessment in aquaculture.

Extensive macronuclear fragmentation in *Didinium* sp. (Litostomatea)

Monica Wilson, Xyrus Maurer-Alcalá, Ying Yan, Laura Katz

Smith College und Kaiserslautern

Genome architecture is incredibly diverse among eukaryotes and can even differ greatly among closely related taxa. In the classes Armophorea, Spirotrichea and Phyllopharyngea, some lineages have been known to extensively fragment their somatic genome, a feature associated with a suite of characteristics including differential amplification of genes and higher rates of protein evolution. Identifying and studying these cases not only expands knowledge of ciliate evolution, but provides key insights into parallels across the entire eukaryotic tree of life. This study combines molecular tools and bioinformatics to characterize the genome structure of *Didinium* (Litostomatea), for which extensive somatic fragmentation has not previously been reported. The first component uses a PCR-based approach to assess chromosome size in the somatic genome. The second component uses single cell 'omics to analyze the structure of the chromosomes and their putative function using reference databases. The results of this thesis suggest extensive macronuclear fragmentation in the class Litostomatea. Using genome and transcriptome data for *Didinium*, we identified 69 contigs with two telomeres at both ends of the chromosome, 42 of which mapped to the transcriptome. An A-T rich motif that appeared in all 42 gene-sized chromosomes was found at the terminal ends of the chromosomes and may signal chromosome breakage.

Session 5:

PROTIST EVOLUTION

Conserved signaling pathways link protozoa to mammalian nervous system

Helmut Plattner

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The aim of the present article is to analyze the evolutionary links between protozoa and neuronal and neurosecretory cells. To this effect we employ functional and topological data available for ciliates, in particular for Paramecium. Of note, much less data are available for choanoflagellates, the progenitors of metazoans, which currently are in the focus of metazoan genomic data mining. Key molecular players are found from the base to the highest levels of eukaryote evolution, including neurons and neurosecretory cells. Several common fundamental mechanisms, such as SNARE proteins and assembly of exocytosis sites, GTPases, Ca2+-sensors, voltage-dependent Ca2+-influx channels and their inhibition by the forming Ca2+/calmodulin complex are conserved, albeit with different subcellular channel localization, from protozoans to man. Similarly, Ca2+-release channels represented by InsP3 receptors and precursors of ryanodine receptors, which emerged in protozoa, serve for focal intracellular Ca2+ signaling from ciliates to mammalian neuronal cells. Restriction of Ca2+ signals by high capacity/low affinity Ca2+-binding proteins is maintained throughout the evolutionary tree although the proteins involved differ between the taxa. Phosphatase 2B/calcineurin appears to be involved signaling and in in vesicle recycling throughout evolution. Some of the proteins and mechanisms addressed here are crucial for long term potentiation (learning). Most impressive example of evolutionary conservation is the sub-second dynamics of exocytosis-endocytosis coupling in Paramecium cells, with similar kinetics in neuronal and neurosecretory systems, respectively. Intercellular communication of different kind, as well as intracellular communication through formation of a variety of

second messengers, also occurs in protozoa. Numerous cell surface receptors and channels that emerge in protozoa operate in the human nervous system. Finally, additional requirements for increasing neuronal complexity during evolution, such as cell adhesion molecules, become of increasing importance for neuronal evolution

Influence of bacteria on the morphology and life cycle of choanoflagellates and other eukaryotes

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In this study we present preliminary results on the influence of a bacteria strain on choanoflagellates. When incubated with this prokaryote, the eukaryotic cells do not complete their mitosis (or meiosis) but cells stay attached, forming one huge cell. These multi-collared cells are fully viable and give offspring after some time. We applied the bacteria culture and also the supernatant to 3 different craspedid choanoflagellate species with the same, replicable result. This is a further proof for the importance of bacteria regulating the live-cycle of choanoflagellates and hence playing an important part in the evolution of multicellularity.

Paramecium genome evolution in a selection-free environment

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Thousands of Internal Eliminated Sequences (IESs) populate the germline genome of the ciliate *Paramecium*. At each event of sexual reproduction, these intervening and largely intragenic DNA sequences must be accurately removed to regenerate a functional somatic nucleus. IES excision is often imperfect, however. More than that, its inaccuracies can impact organismal fitness and are thus counteracted by natural selection. Here, we examine IES excision accuracy in Paramecium lines reared for tens of sexual generations in an environment where selection intensity was deliberately relaxed. A number of observations, which bear on the evolutionary significance of DNA-level splicing as well as the mechanisms of IES excision will be discussed.

Genome comparison of three *Poteriospumella lacustris* strains

Stephan Majda, Daniela Beisser, Jens Boenigk

University Duisburg-Essen

The genome comparison of *Poteriospumella lacustris* emphasises exemplary the intraspecific variation of Chrysophyceae. The non-scaled, colorless, flagellated, heterotrophic *Poteriospumella lacustris* belongs to the Chrysophyceae class. The investigated strains JBC07, JBM10 and JBNZ41 originate from fresh water habitats of different geographical locations (China, Austria, New Zealand). *P. lacustris* evolved from a phototrophic ancestor and reduced its genome size. With the comparative genome analysis in conjunction with transcriptome data we want to find out which essential genes are still remaining and which are optional.

Here we present the specifics of *Poteriospumella lacustris* with insights into the metabolic pathways and gene settings like core/pan genes, gene density and gene order. Based on nuclear staining the estimated genome size ranges between 50 and 80 Mbp.. After the de novo assembly, the sum of the contig lengths of each strain is in the order of 50 Mbp. Therein we identify around 20,000 genes, of which half can be assigned to KEGG Orthology (KO) identifiers. KO identifiers function as unique flags for a functional ortholog group of genes which enables a gene identification and comparison across species. Most annotated genes are related to the genetic information processing (~ 1000 genes) or nucleotide & amino acid metabolism (~ 700 - 1000 genes).While the gene sets of JBC07 and JBM10 are almost identical, JBNZ41 differs particularly in the higher amount of genes belonging to environmental information processing and cofactor and vitamin biosynthesis. In contrast, the number of genes related to cellular processes is nearly constant in each strain (120 – 133 genes).

To automate the analyses for further chrysophytes a bioinformatic pipeline was designed. The workflow comprises the processing of whole genome sequencing data, including steps for the de-novo assembly, gene prediction, gene annotation, analysis and visualization. It applies the snakemake workflow engine, which combines the use of executable tools and scripts.

The automated analyses enable further comparisons within the Chrysophyceae, especially with regard to nutritional mode or evolution.

Session 6:

SOIL PROTISTS I

Two new monotypic, hypotrich families (Ciliophora, Hypotricha) from floodplain soil in Australia and Botswana, respectively

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Both species – not named here for nomenclatural reasons – are possibly endemics. Both have a unique combination of features suggesting them as new genera and families. The first species was discovered in soil and litter from the floodplain of the Murray River in Australia.

Morphostatic characteristics of Australian organism. Species 1

- size about 90 × 40 µm, conical/triangular
- a conical body shape as, e.g., *Psilotrichides*;
- a frontal plate as, e.g., *Stylonychia*;
- a minute dorsomarginal kinety on ventral side;
- a ridge at right margin of buccal cavity, as in the Psilotrichidae

Ontogenetic characteristics of species 1

- the oral primordium develops in a flat pouch covered by the cortex, similar to euplotids and oligotrichs;
- proter and opisthe develop independently;
- the proter endoral membrane produces a new paroral by lateral proliferation of basal bodies, similar to nassulids;
- the new opisthe adoral zone of membranelles curves so strongly to the right that the anterior half becomes oriented horizontally, as in oligotrichs;

- there are four ventral cirral anlagen in proter and opisthe. They produce two ventral cirral rows, three frontal cirri, and one buccal cirrus;

- the minute dorsomarginal kinety and the right row of marginal cirri originate de novo;
- each of the three dorsal kineties produces one long kinety and two short dorsal kineties of which the leftmost produces a caudal cirrus, a very unusual pattern not known from other hypotrichs but similar to the multifragmentation in several hypotrich families.

Species 2 was discovered in Botswana, i.e., in soil from a green part ("green river bed") of the Chobe river. It is ellipsoid and about 120 × 50 µm in size and has up to 15 µm long dorsal bristles; it resembles the oxytrichids, especially *Territricha* BERGER and FOISSNER, 1988 because the two rows of ventral cirri form an indistinct midventral pattern, and dorsal kinety 4 is produced by a split of kinety 3. This ciliate has a unique feature each in morphostatic and dividing specimens: first, the (oral) primordium is located and formed as in *Oxytricha*, viz., originates left and slightly anteriorly to the parental transverse cirri and produces the new opisthe oral apparatus and – via a short streak – the proter undulating membranes. Additionally, the buccal cirrus consists of six individual, minute rows of basal bodies. A second primordium, unique to the hypotrichs so far described, develops slightly anterior to mid-body between the right row of parental proter marginal cirri and the right row of ventral cirri. The eight cirral anlagen become primary primordia which organize new frontal and transverse cirri as well as new ventral cirral rows for both, proter and opisthe. (Supported by the Austrian Science Fund, Project number P26325-B16.)

Chances and issues for soil protistology: A methodological summary with comparisons to other biota

Stefan Geisen

Netherlands Institute of Ecology

Protists are dominant members of soil biota and key for major ecosystem processes. Yet, research on soil protists is lacking behind that on other soil organisms. Here I will show that this bias favouring studies on other microorganisms or animals rather than protists in soils has a largely methodological background which is unrelated to scientific significance.

I will show that there are many methodological overlaps with other scientific fields. Especially sequencing techniques are now increasingly being used to study the composition of soil microbial diversity, including bacteria and fungi, but also protists. However, I will highlight that sequencing technologies alone provide only relative abundances and fail at providing absolute abundance information, which we need to understand ecosystem processes.

Last I will highlight with case studies that experimental approaches are needed to better understand the functioning of soil protists including (1) mechanistic laboratory experiments, e.g. to study interactions of protists with bacteria, (2) greenhouse experiments, e.g. to investigate the influence of protists on microbiome and eventually plant performance and (3) field experiments, e.g. to investigate the importance of protists as biofertilizers for application in agricultural settings.

This talk will show that a combined methodology targeting the diversity of (soil) protists will be an essential component of basic and applied research in advancing soil science!

Worldwide diversity of Cercozoa: summary of our current studies

Anna Maria Fiore-Donno, Kenneth Dumack, Michael Bonkowski

University of Cologne, Zoology Institute, Terrestrial Ecology Group

We are currently using Cercozoa as model to unveil local and global patterns driving the protistan distribution in soils. Cercozoa, according to both microscopical observation and molecular environmental sampling, is one of the major protistan groups in soils. This huge phylum includes a vast array of forms - naked and testate amoebae as well as flagellates - and nutrition modes - free-living bacterivores, algivores, fungivores or predators, free-living autotrophs or myxotrophs and parasites. This variety of forms and nutrition modes will be exploited to disentangle their role in the soil food-web and how they are differentially influenced by biotic and abiotic edaphic factors. We developed specific primers targeting the group and we are using them in several collaborative projects. We also classified the Cercozoa in functional groups according to morphology, nutrition modes and locomotion. I will summarize and discuss our latest findings on cercozoan diversity and functional diversity in varied envirnoments, such as drylands, temperate grassland, biological crusts and agricultural soils.

Diversity and functional roles of cercozoa in the phyllosphere of plants

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The phyllosphere comprises the aboveground compartments of plants and is dominated by leaves. Plant leaves are forming the largest biological surface on Earth with 10^8 km² globally, an area approximately twice as large as the global land surface. Bacteria are by far the most numerous colonizers of leaves and research on bacterial assemblages in the phyllosphere has gained much interest in recent years. However, microbial communities on leaves are taxonomically more diverse including also fungi, yeasts, algae and protists; and complex interactions within the phyllosphere microbiome are expected to occur. Although the occurrence of protists on plant leaves has long been recognized, a comprehensive understanding about the diversity and functions of phyllosphere protists is lacking.

In frame of my PhD-thesis, we studied the diversity and functional roles of plant-associated Cercozoa. In this talk, we will provide a comparative analysis of the diversity of plant-associated cercomonad Cercozoa from the rhizosphere and phyllosphere of different plant species and further present how grazing of leaf-associated cercomonads structures bacterial community composition and function. Subsequently we will highlight the ecology and function of plant-associated testate amoebae from the family Rhogostomidae (Cercozoa) and provide indications how the Rhogostomidae also prey on other (co-isolated) members of the phyllosphere microbiome.

Session 7:

SOIL PROTISTS II

Exploring the phylogeography of hyalospheniid testate amoebae (Amoebozoa) from regional to sub-continental scales

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Molecular techniques are revealing a considerable cryptic diversity in many protist groups. These organisms, which are morphologically similar but genetically distinct, have been traditionally pooled together within single species, leading to erroneous diversity estimates, and wrong conclusions on their ecology (e.g. habitat specificity) and geographical distributions. Testate amoebae are a group of ecologically specialized protists easy to handle individually which represent a good model to test ecological, biogeographical and phylogeographical hypotheses. In this study, we surveyed the diversity of family Hyalospheniidae (Arcellinida, Amoebozoa) with focus on genera *Padaungiella, Alocodera* and *Apodera* (hereafter referred to as subfamily Apoderinae) and genus Nebela, based on single-cell COI barcoding and environmental DNA survey. Previous studies showed that the diversity of genus Nebela was underestimated, even in well surveyed environments (such as Swiss peatlands), and hosted a large

diversity of species differing in their ecological optima. Here, we expanded our survey to different geographical locations and/or environment types (Europe, Zealandia, North and South America). We discovered a novel diversity in genus *Nebela*, including new clades discriminated chiefly by environment type rather than geographic origin, suggesting phylogenetic niche conservatism. Most diversity of Apoderinae was found in Southern Hemisphere locations, suggesting a Gondwanaland origin for the group. Here, a new form was found, diverging clearly in its COI sequences, which lead us to propose a new genus (*Paulistella*). Furthermore, morphometric and genetic data allowed us to split morphospecies of supposedly pan-gondwanian distribution into several units with geographically limited distributions. These examples call for a re-evaluation of Southern Hemisphere protists diversity, which is too often disregarded due to taxonomic overfitting.

What do protists feed on? A cloning approach exemplified on the shelled amoeba *Amphizonella* sp.

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Heterotrophic protists represent an important link in food webs all over the world. Still today most often food spectra of protists are determined by artificial laboratory experiments, which often enable only poorly connection to natural conditions. Thus, for many protists little is known whether and how they select food sources, what they really feed on in the natural habitats and how they by that influence community composition. Due to the opaque nature of soil particles, food preferences of terrestrial heterotrophic protists cannot easily be determined by microscopy. In the present study, the testate amoeba *Amphizonella* sp. was chosen as a test organism. *Amphizonella*, a genus of shelled amoebae in the Amoebozoa, is known to be algivorous and bears a transparent theca which enables microscopy of the cell content. It was picked from fresh biological soil crusts (BSCs), a complex mosaic of soil particles and organisms and without further incubation used for food content analyses. In this analysis a novel method of single-cell PCRs combined with mismatching primers and a subsequent cloning step was developed. With this method, omnivorous feeding behavior of *Amphizonella* sp., exceeding previously known food preferences, could be recognized. However more studies on protist trophic interactions and comparison of different testate amoebae are needed.

Shattered glass; the specialized predator *Phryganella paradoxa* (Arcellinida, Amoebozoa) ruptures frustules of diatoms by force.

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A major drawback in testate amoeba research is a general lack of scientific studies combining molecular approaches and classical laboratory experiments. We isolated five yet uncultured testate amoebae of the genus *Phryganella*, Penard 1902 from three different rivers and one pond in Germany. Based on established cultures we show their morphology, which we studied by light and electron microscopy, and present their unique feeding mode on abundant and common pennate diatoms like *Nitzschia* spp. and *Synedra* spp., whose frustules are bent and frequently, but not always, broken during the feeding process. We further obtained the first SSU rDNA sequences of the family Phryganellidae, all of which contain introns. We used the sequences to confirm the taxonomic placement of the Phryganellidae in the Arcellinida (Amoebozoa), branching as a sister group to the Cryptodifflugiidae.

Soil protists as drivers for soil disease suppressiveness by shifting bacterial community composition

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Soil-borne disease is the causes of worldwide yield losses and there is an urgent need to develop environmentally sound disease control strategies. Plant health greatly benefits from mutualistic interactions with beneficial soil microorganisms that improve plant growth and suppress diseases. However, our current knowledge does not allow for predicting or controlling the mutualistic activity of free-living soil microbes in the rhizosphere in order to serve to enhance disease suppression. Here we show predation by soil protists shift soil bacterial community which enables to suppress plant pathogen. We hypothesized plant probiotic traits can also confer a fitness advantage in the rhizosphere as predators selectively feeding on microbes lacking probiotic activity, thereby promoting disease suppression. We inoculated plants with protists-free bacterial communities and three protists isolates. We then assessed the rhizosphere bacterial community structure and bacterial community diversity. Protists cause the different bacterial composition in the soil when harvesting at 3 weeks, especially *Acanthamoeba* sp. induces functional-associated bacterial species. However, the changes of rhizosphere bacterial community evenness were not found. Further experiments and analysis are needed to investigate the protists beneficial impacts on various plant pathogens.

Session 8:

PROTIST ECOLOGY I

Clonal variability of predation induced phenotypic plasticity in ciliates

M.Sc. Sandra Trogant, Dr. Linda C. Weiss, Prof. Dr. Ralph Tollrian

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Predation is a major factor driving adaptation. Prey species have evolved defensive strategies that reduce predation risk. Prey species may show adaptive morphological features, behavioural traits or shifts of life history parameters. Some predators can counter prey plasticity, by being plastic themselves.
This has been described for ciliate species. The prey protist *Euplotes* spp is known to develop protective lateral "wings" in the presence of the predatory ciliate *Lembadion* spp. In turn *Lembadion* is able to gradually adjust its size to the size of its prey, facilitating ingestion of defended prey. This kind of prey induced counter offence strategy is reversed when prey species are undefended and therefore another form of plasticity.
We studied the reaction norms of morphological defences and offences in both, *Euplotes* and *Lembadion*. We find differences in reaction norms of plasticity between clonal strains in prey and predator. This high degree of trait variability, complicates trophic system interactions.

Diversity of algivorous protoplast feeders and first insights into the molecular mechanisms underlying the perforation of algal cell walls

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Two distantly related groups of amoeboid protists, namely the vampyrellid amoebae (Vampyrellida, Rhizaria) and the viridiraptorid amoeboflagellates (Viridiraptoridae, Rhizaria), display the ability to perforate the cell walls of green algae and to feed on protoplast material. I will provide an overview about known and novel rhizarian protoplast feeders as well as the cell wall characteristics of their algal prey. The viridiraptorid amoeboflagellates, which consume zygnematophycean green algae and grow to high cell densities under laboratory conditions, are excellent model organisms to study the molecular mechanisms underlying the perforation of prey cell walls. We used comparative transcriptomics on synchronised, bacteria-free cultures of *Orciraptor agilis* and identified carbohydrate-active enzymes (CAZymes) that are differentially expressed upon contact with the prey alga (*Mougeotia* sp.). Whereas pectate lyase and endo-β-1,4-mannanase (GH5_10) may be involved in the degradation of gel-like cell wall components, a highly up-regulated, plasma membrane-anchored cellulase (GH5_5) with endo-β-1,4-glucanase activity seems to be the key player in the degradation of the tough cellulosic cell wall layer of zygnematophycean prey. Future plans to study these enzymes in detail (e.g. substrate specificity) and to localise them in attacking cells of *Orciraptor* will be presented.

Nucleariid amoebae and their association with bacterial symbionts

Sebastian Dirren, Estelle Bruni and Thomas Posch

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Nucleariid amoebae (Nucleariidae, Opisthokonta) have been observed and described infrequently during the last 150 years. In fact, there are only about two handful of publications dealing specifically with species belonging to *Nucleraria*; i.e. the single genus inside the family nucleariidae. These sporadic reports have drawn the picture of a rare group of protists. Here we now present nucleariid isolates from 9 Swiss and 17 Austrian lakes and ponds. In other words, we were able to isolate representatives of the genus from every so far sampled prealpine and alpine lake/pond. Although nucleariids were usually not abundant and had to be enriched prior to isolation, this hints to a ubiquitous occurrence of nucleariid amoebae in these ecosystems. Based on the 18S rRNA genes the 57 sequenced isolates were affiliated to four established species and three novel phylogenetic clusters. In previous studies we could show, that nucleariids are frequently associated with endo- as well as ectosymbiotic bacteria. By highlighting in the phylogenetic tree the isolates acting as hosts, an uneven distribution of symbiotic associations inside the genus became evident. While the two species *N. delicatula* and *N. thermophila* are most frequently associated with bacterial symbionts, other phylogenetic clusters contain only few or no isolates with symbionts. This points to a species-specific tendency of nucleariid amoebae to establish symbiotic relationships. By focusing on already known bacterial symbionts, we were able to address their host specificities. While Ca. *Endonucleariobacter rarus* could be detected exclusively in *N. thermophila* isolates, Ca. *Turbabacter delicatus* was found beyond the species level in *N. thermophila* as well as in *N. delicatula*. Thus, regarding their nature the symbiotic interactions with these two symbionts seem to be fundamentally different. In order to further characterize the infection cycles of the symbionts and their impact on the host's fitness, we designed growth experiments. In my talk I will present results from these experiments and discuss the relevance of the two endosymbionts with contrasting live styles.

Session 9:

PROTIST ECOLOGY II

Chaos in *Coleps*? – morphologic and phylogenetic approaches to identify these common ciliates

Daniel Rieser, Bettina Sonntag and Thomas Pröschold

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Heterotrophic and algal-bearing ciliate species of the genus *Coleps* are frequently detected in the plankton of Lake Mondsee, Austria. Based on microscopic investigations, three mixotrophic species were identified so far: *Coleps hirtus viridis*, *Coleps spetai* and another morphotype matching characteristic features of both. To figure out a possible phenotypic plasticity in the morphotype, we additionally investigated the genetic variability of other *Coleps* strains that were either already available from culture collections or that we isolated from different habitats including heterotrophic *Coleps* species. Overall, we applied an integrative approach: (i) cultivation of strain-specific clones, (ii) morphological investigation from living and silver-stained specimens, and (iii) sequencing of the SSU and ITS rDNA including analysis of the secondary structure. First results reveal that our investigated *Coleps* strains show a high phenotypic plasticity in contrast to a low genetic variability. The study is funded by the Austrian Science Fund (FWF): project P28333-B25.

Assessing the links between test morphology and the ecology of freshwater Arcellinida (testate lobose amoebae)

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For centuries researchers have illustrated Arcellinida (testate lobose amoebae) tests – defining their place on the tree of life based on such attributes as size, aperture position, spines, wall composition and internal structures. Recently, however, genetic barcoding techniques have demonstrated significant issues with morphology-based taxonomies of the group. We review the current understanding of the links between test morphology and ecology in freshwater testate lobose amoebae.

Lake sediments are important archives of environmental change, recording both natural fluctuations and anthropogenic disturbances on scales ranging from decades to millennia. The excellent preservation potential of Arcellinida tests provides researchers with an effective tool to characterize a range of environmental changes (e.g., nutrient enrichment, salinity change). Traditionally, researchers have drawn inferences from changes in species composition, diversity and abundance. Issues associated with the established taxonomic subdivision of testate lobose amoebae now raise questions about the accuracy of inferences based on species defined by test morphology alone.

Biometric tools (e.g., geometric morphometrics) can be used to quantify morphological variability independent of size variability, whilst multivariate statistical techniques can be used to test for relationships between morphology and ecological change. In spite of their significant potential, these approaches are rarely combined. We discuss the relationship between Arcellinida test morphology and lake nutrient enrichment, drawing on results from the 'ECOTRAIT' project – an EU funded project that is examining the association between testate lobose amoebae ecology and morphology on both temporal and spatial scales.

Sub-optimal growth temperatures impact the fidelity of Programmed DNA elimination in *Paramecium tetraurelia*

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Although ciliates Internal Eliminated Sequences (IESs) have decayed from transposons into non-autonomous genetic elements, the extent to which they continue to play an active role in shaping genomes structure is not known. At each event of sexual generation, IESs must be precisely eliminated from the germline so that a functional somatic genome may be reconstituted via Programmed DNA elimination (PDE). Although organismal survival requires the high fidelity of PDE, evidence suggests that IES excision is not foolproof. Moreover, PDE-mediated somatic variability may be inherited across sexual generations as a consequence of epigenetic mechanisms. The extent to which the efficiency of PDE is influenced by different environmental conditions is yet to be established. To address this gap, we reared clonal *Paramecium tetraurelia* lines at suboptimal temperatures, allowed autogamy to proceed at these different temperatures, and subjected the resulting somatic genomes to ultra deep Illumina-sequencing. We find that departures from the optimal growth temperature during autogamy can significantly affect the efficiency and specificity of PDE.

Temporal patterns of soil micro-eukaryotic diversity beneath pig cadavers decomposing on the ground or suspended

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Cadavers represent a natural perturbation that abruptly influences environmental parameters in a soil for up to several years. A major objective in forensic sciences is dating the time of death, which is relatively straightforward when less than one month has elapsed, but still very challenging for longer periods. Also as hanging is a common suicide method it is important to compare decomposition patterns of cadavers decomposing on the soil and suspended in the air as the timing and pattern may differ. We conducted a field experiment in a spruce forest above Neuchâtel, Switzerland, using decomposing pig cadavers placed in scavenger-proof cages on the soil and suspended 1m above ground (5 each). We investigated the changes in the soil micro-eukaryotic communities by Illumina sequencing of the V4 region of the rDNA SSU gene. We identified indicator OTUs responding positively or negatively to the presence of cadavers using an indicator species approach. Decomposition patterns differed between ground and suspended cadavers and this was reflected in the soil micro-eukaryotic communities. Cadavers caused a decrease in diversity but cadaver indicators included rarely reported taxa, many parasites of other protists such as basal Alveolates (e.g. Colpodellids) and unknown organisms, with match (< 80% identity) to any GenBank sequence. This study confirms that cadaver-impacted soils are home to a highly specific community of mostly unknown protists. Furthermore, we witnessed a clear succession between early colonizers (small bacterivores, characteristic of the early decomposition stages) and late settlers

arriving after about two months. These results pave the way for developing new tools in criminal investigations and discovering unknown soil eukaryotic diversity.

How to wow people by using ciliates for science transfer and public relations activities

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Publishing research data by writing publications and giving talks is researchers' daily work. However, to present easy-to-digest research data to fascinate the public including children and adults of all ages for scientific work and to promote its benefits is still a challenge for the scientific community. At the Research Department for Limnology in Mondsee (University of Innsbruck, Austria), we are regularly releasing 'Newsletters', media articles, summer workshops for the local community, and specific science transfer projects. Our recent project entitled "Wasserleben" ('Life in Water') will be presented in this talk as an example for the successful implementation of knowledge transfer to young people. Ciliates and other planktonic organisms were in the spotlight of this project and one of the major goals was to create awareness for an intact aquatic ecosystem. More than 780 children and teenagers aged between 5 and 18 years were involved in this 18-months-project. During research days and excursions assisted by experts in the field, the children took water samples from two lakes (Lake Mondsee and Lake Attersee) in the Salzkammergut area, analyzed the samples under the microscope and identified the organisms. To draw the attention of children to this field of research, we created a set of playful elements including ciliate pictures to be colored, a ciliate game ("Memory") and a high-score computer game ("Ciliatenjagd"='Ciliate hunt'). Finally, the children's favorite topics were the huge plankton diversity in the lake samples and the handling of a microscope. The project was funded by the Austrian Research Promotion Agency (FFG) and the Austrian Ministry for Transport, Innovation and Technology: project 849550. The Austrian Science Fund (FWF) is involved with project I2238-B25.

7 Abstracts: Poster

PROTIST ENVIRONMENTAL GENOMICS

P1 - Responses of peatland micro-eukaryotic community structure and diversity to warming - a field experiment

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Peatlands store approximately one third of the global terrestrial carbon stock on 3% of the land surface. On-going climate change threatens peatlands in their structure (biotic communities) and function (Carbon sink). Micro-eukaryotes including protists and fungi are key actors of soil carbon cycling but their diversity and response to climate change are still not well known.

We assessed the influence of warming and reduction of precipitation on the structure and diversity of micro-eukaryotic communities using a high-throughput sequencing (HTS) of the V4 region of the 18S ribosomal RNA gene.

We identified 754 different Operational Taxonomic Units (OTUs) related to micro-eukaryotes, among which Rhizarians (189 OTUs) and Alveolates (180 OTUs) were dominant. Community structure and inferred function changed significantly in

response to the manipulations as shown by constrained analysis of principal coordinates. Potential indicators of peatland warming identified by IndVal analysis, include an OTU related to the desmid genus *Actinotaenium*.

By identifying indicators of environmental changes and inferring the functional significance of these changes, HTS of micro-eukaryotes is a useful approach to better understand how the functioning of peatland ecosystems changes in response to ongoing climate change.

P2 - Deep molecular characterization of eukaryotic microorganisms´ diversity and community composition in forest soils and the canopy region across biomes using a multiple barcoding approach

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Soil 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. Accordingly, the structure of natural protist communities and taxa-specific ecological functions are largely unknown. We apply advanced cultivation-independent high throughput sequencing methods using group-specific primers 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. Here, we report our first results of the analyses of three different autochthonous tree species in in a temperate floodplain forest (Leipzig, Canopy Crane Facility).

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

P3 - Comparative analysis of protistan diversity in soil and freshwater lakes

Sieber G., Bock C., Jensen M., Grossmann L., Boenigk J.

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There is no clear boundary between freshwater lakes and the surrounding soil, but nutrients and organisms are exchanged between the compartments. We took samples from 56 freshwater lakes and the surrounding soils from sites across Germany and analyzed protistan diversity using Illumina sequencing. The analysis was based on the V9 and ITS1 region of the SSU rDNA. Furthermore we categorized the landscape into different vegetation types, particularly woodland or grassland. We aimed at corresponding geographic patterns of protistan communities between soil and water, presumably due to either environmental pressures or the exchange of organisms via the land-water-interface. Furthermore, we aimed at corresponding patterns between vegetation and protist communities.

P4 – Analysis of evolutionary processes within the placidid stramenopile flagellates

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Evolution at the basis of the tree of life has long been and is still fascinating scientists. Reconstructing the phylogenetic tree that unites all lineages of eukaryotes is still a grand challenge. The difficulty to define homologous characters across the very different lineages makes it extremely difficult to resolve evolutionary processes. The incompleteness of consistent paleontological records of the delicate single cell organisms at the basis of the tree of life makes calibration of evolutionary time scales imprecise. To get more insights into evolutionary processes of heterotrophic flagellates, we use Placididea. This recently discovered class within the stramenopile flagellates has been isolated from nearly all continents, originating from locally separated and extreme conditions, as well as coastal regions. Placidids are potentially fast evolving organisms due to their small size and their adaptability to live at isolated and extreme conditions. The first described species was Wobblia lunata Moriya et al., 2000 obtained from marine coastal waters of Japan. Two years later, they discovered an additional species in Japan, Placidia cafeteriopsis. Until the recent discovery of Suigetsumonas clinomigrationis Okamura and Kondo, 2015, no further Placididea were described and only partial sequences of 18S rDNA of strains from Canada, Poland, South Africa, Kenya and the English Channel have been published. We succeeded in isolating nine novel strains from Chile, two from Germany, one from the Galápagos Islands and Kenya. 14 placidids have been used for multigene analysis to obtain information about the mutation rates of geographically very distant organisms. The investigation of multiple genes increase the possibility for the differentiation between species and populations and for a higher robustness of the phylogenetic tree. We contribute first insights into evolutionary processes, mutation rates, and the molecular clock within the class of Placididea combining morphological-, ecological- and phylogenetical approaches.

CILIATED PROTISTS

P5 - Testing the Kinetid Transformation Hypothesis in Choreotrichid Ciliates: First Ultrastructural Data on Dikinetids in a Tintinnid (Alveolata, Ciliophora, Spirotricha)

Michael S. Gruber, Alexandra Mühlthaler, Sabine Agatha

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The structural variation of somatic kinetids in choreotrichid ciliates contradicts the structural conservation postulated by Lynn in the nineteen eighties and used to revise the ciliate classification. The dorsal kineties of euplotids and hypotrichs are exclusively composed of dikinetids having associated a cilium only with the anterior basal body. The same kinetid type occurs in the two ciliary rows of the oligotrichids, the sistergroup of the choreotrichids. Choreotrichids, however, might possess monokinetids, diciliated dikinetids or dikinetids with a cilium only at the anterior or posterior basal body. Since the somatic dikinetids of the taxa mentioned above are considered homologous, differences in the associated kinetodesmal fibres as well as transverse and postciliary microtubular ribbons and further structures are used to infer phylogenetic relationships. Yet, transmission electron microscopic data on somatic dikinetids are not available for oligotrichids, and here the first data for choreotrichids, namely from a tintinnid (loricate choreotrichids), are presented. The dikinetids of the dorsal kinety in *Schmidingerella meunieri* (culture material was kindly provided by Kelley Bright, Shannon Point Marine Centre, Western Washington University, U.S.A.) have a cilium associated with the posterior basal body and possess beyond the commonly associated structures three additional microtubular ribbons originating at the left side of the dikinetid. All kinetid components have phylogenetically been analysed, detecting some apomorphies. The new findings are also important for testing the hypothesized transformation of the somatic kinetids from a dikinetid with an anterior cilium only, via a diciliated dikinetid to a dikinetid with a posterior cilium only and finally to a ciliated monokinetid. However, ultrastructural data on the dikinetids in oligotrichids as well as on the other kinetid types in choreotrichids are required. Finally, the discovered

synapomorphies shall be mapped on the molecular tree, providing features for a (more detailed) morphological characterization of the particular branches. Additionally, transformations of the kinetid ultrastructure might be related to changes in the function of the somatic cilia, providing insights into general evolutionary mechanisms on level of cell organelles. The study was financially supported by the Austrian Science Fund (Project P28790).

P6 - "Back to the roots" – An Inventory of Tintinnid species (Alveolata, Ciliophora) in the North-Eastern Pacific based on Original Descriptions

Margit Kagerer, Carmen Zinßmeister, Sabine Agatha

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Among planktonic ciliates, only tintinnids build a solid shell (lorica), which is the only feature complex for taxonomy and classification in the about 1,000 species. Identification keys to the species do not exist. Hence, monographs compiling the highly scattered literature have to be used, specifically the outstanding books of Kofoid and Campbell published in 1929 and 1939 as well as the more recent Chinese compilation by Zhang and collaborator published in 2012. Species identification by means of Kofoid's and Campbell's comprehensive monograph published in 1929 is hampered by (i) the lack of measurements and descriptions for most species, (ii) the numerous subjective synonymizations (usually without any discussion), (iii) the broadening of the species limitations by the synonymizations, and (iv) illustrations that are not from the original species descriptions, but often show subjective synonyms. Likewise, the Chinese book – although being more complete – can hardly be applied as sole source for identification, because it provides measurements without critical verification of the species identifications and often does not consider the original descriptions. Therefore, it is strongly recommended to go "back to the roots", i.e., to use exclusively the original species descriptions for determination. This approach was applied in the present study, establishing an inventory of tintinnid species in the North-Eastern Pacific. The preliminary analyses revealed 107 morphospecies and 30 genera occurring at three stations on a transect from oceanic to coastal waters; however, these numbers merely represent conservative estimates of the real diversity. The most common genera at the three sampling sites are *Steenstrupiella, Epiplocylis*, and *Eutintinnus*; they possess hyaline loricae. In the light of climate change, these preliminary data shall be compared to the tintinnid diversity in the eastern tropical Pacific assessed by Kofoid and Campbell more than a century ago. The project was funded by the Federal Institute for Geosciences and Natural Resources of Germany.

P7 - Thermal Adaptation and Evolutionary Rescue in *Paramecium caudatum*

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Rapid environmental changes or environmental stress can result in a potential de-coupling ("mismatch") of an organism's physiology and the new environment, prospectively leading to population decline and extinction. Before this happens, resistant organisms already present in the population, or that appear sufficiently fast by mutation, may stop an initial population decline and restore positive population growth. These evolutionary adjustments occurring during such an Evolutionary Rescue (ER) scenario can happen very quickly within few generations. *Paramecium caudatum*, for example, seems to be able to adapt to different thermal niches, as we can find thermal generalists and specialized populations in natural habitats. Thus, *Paramecium* is a suitable model to test the theory of ER and concepts of thermal adaptation during experimental evolution. However, the success of ER and the evolutionary course of the population depends heavily on the speed of environmental change. Here, we investigated how different rates of temperature increase (from 23°C to 32°C) influence population persistence and evolutionary changes in experimental microcosms of *P. caudatum*. Consistent with theory, we found that those populations that experienced the slowest rate of temperature increase were the least likely to become extinct and adapted best to the new temperature environment. After about 200 asexual generations, all high-temperature populations were more tolerant to high heat exposure (35°C, 37°C), indicating a common heat protection mechanism. High-temperature populations also showed superior growth at optimal temperatures, but a reduced growth at low temperatures (5-9°C). This shift and alteration of the temperature niche indicates a trade-off between high and low temperature tolerances and can only be partially explained by selection from standing variation of six initial founder genotypes. While we have found complete genetic divergence between control and high-temperature populations using mitochondrial markers, the observed increased resistance to lethal heat

stress above the maximum selection temperature (32°C) is indicative for adaptive evolution. Our results support theories of thermal adaptation and confirm the basic predictions of ER by showing how adaptation to an extreme local environment can produce both positive and negative correlated responses to selection and lead to niche shifts.

P8 - Redescription of *Dexiotricha colpidiopsis* (Kahl, 1926) Jankowski, 1964 (Ciliophora, Oligohymenophorea) from a hot spring in Iceland

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We isolated and studied a ciliate, *Dexiotricha colpidiopsis* (Kahl, 1926) Jankowski, 1964, from a hot spring in Iceland with standard morphological and phylogenetic methods. It is the first report of the species from a hot spring habitat, which indicates its tough tolerance to the extreme environment. The present isolate of *Dexiotricha colpidiopsis* corresponds well with previous populations in all main characters, such as body shape and size in vivo, the number of somatic kineties, and the position of macronucleus and contractile vacuole. Additionally, the small-subunit ribosome gene (SSU rDNA) sequence of the species is provided. The phylogenetic analyses based on SSU rDNA sequences show that the genus *Dexiotricha* is monophyletic. We also briefly revised the genus *Dexiotricha* and provide a morphological key prior for species discrimination.

P9 - A Sleeping Beauty: Morphology and Ultrastructure of the Resting Cyst in the Marine Planktonic Tintinnid *Schmidingerella meunieri* (Alveolata, Ciliophora, Spirotricha)

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The ciliate *Schmidingerella meunieri* belongs to the tintinnids, a group of shell-building, mainly marine planktonic protists. Tintinnids can form resting cysts to overcome unsuitable environmental conditions. For the first time, morphology and ultrastructure not only of a tintinnid but also of a choreotrichid resting cyst are investigated for phylogenetically significant features. The present study focuses on the structure of the cyst wall, applying light microscopy on the living cyst and transmission electron microscopy on cryofixed material. Cultures of *S. meunieri*, originally collected in the northeast Pacific, were kindly provided by Kelley Bright (Shannon Point Marine Centre, Western Washington University, USA). The cysts formed under laboratory conditions after several months of cultivation. The resting cyst is roughly flask-shaped with two posterolateral horns and an emergence pore on the opposite side. Interestingly, this pore is directed to the bottom of the lorica and shows a cap-/plug-like structure. The cyst wall consists of three layers: the ectocyst, mesocyst, and endocyst. The layers differ in structure and variability of their thickness. The ectocyst is the thinnest and outermost layer of the cyst wall. It generally has a homogeneous appearance and is coated by two very thin sheets on both sides. The mesocyst is the thickest layer and is the main component of the horns; it shows the highest variability in thickness and is probably responsible for the uneven cyst surface. It is apparently composed of many small fibrils, whose density increases towards the endocyst. The endocyst is comparatively electron-dense and homogeneous. The ultrastructural features in resting cysts of *Schmidingerella meunieri* support the molecular phylogenies and provide new characters for cladistic analyses of the tintinnid ciliates. The chemical composition of the layers, which will be investigated in the future, represents another phylogenetically relevant feature. The study was financially supported by the Austrian Science Fund (FWF) Project I3268.

P10 - The 'blackbox' is open! Ciliates in the planktonic food web of Lake Mondsee, Austria

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Aquatic food webs are complex networks influenced by intra- and interspecific relationships among different organisms, challenging scientists in understanding their dynamics and interpretation of the effects on ecosystem functioning. We open the 'blackbox ciliate community' of Lake Mondsee (Austria) to reveal their co-occurrence patterns and interactions in the microbial food web in time and space. Ciliates and relevant environmental and biotic key parameters, including predatory zooplankton and possible food sources, e. g., bacteria, algae and heterotrophic nanoflagellates, were sampled monthly along vertical depth profiles from June 2016 to May 2017. Morphological data from six months show that the abundance and the composition of the ciliate assemblage (ca. 60 species; mean abundance ~8,500 Ind. L-1) changed seasonally (summer and autumn) and spatially (epi- and hypolimnion). The most abundant taxa were *Balanion planctonicum* > *Urotricha* spp. > *Histiobalantium bodamicum* and *Tintinnidium* spp./*Tintinnopsis* spp. During summer, mainly algi- and omnivorous ciliates (ca. 80% of average abundance) as well as mixotrophic species (ca. 10%) were recorded in surface layers, whereas bacterial feeders (ca. 10%) were more abundant in autumn. Exemplarily, co-occurrence network analyses (ELSA) for July and August were run to shed light on the ciliates' role in the aquatic food web, species-specific predator-prey relationships and food sources. Despite the complexity of the networks, they provide the basis for testing specific hypotheses fitted for dominant ciliate species in their environment.

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P11 - The story of a tiny Chlorella-bearing *Cyrtolophosis* (Ciliophora, Colpodea) living in lake plankton – a showcase of a facultative endosymbiosis with green algae?

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In plankton of several Austrian and Swiss lakes, we subsequently detect a symbiont-bearing *Cyrtolophosis* species of app. 20 x 10 µm in size. The morphological characters of the ciliate as well as the SSU rDNA sequence almost match with available descriptions and molecular sequences of *Cyrtolophosis mucicola*. The algae belong to the green algal genus *Chlorella* and only few (up to 15) are present in one ciliate cell simply because there is no space for more. Similar to *C. mucicola*, individual green *Cyrtolophosis* often live in *mucous colonies* consisting of long hyaline tubes. In culture, in the mucus of these tubes many green algae (former symbionts that escaped from dead ciliates?) are incorporated. Under unfavourable conditions, the green *Cyrtolophosis* form resting cysts still containing algal symbionts. In lakes, the green *Cyrtolophosis* occurs from the onset of the spring bloom through November and is one of the predominant ciliate species in the euphotic zone (up to several thousand individuals per litre). Overall, our results now raise the question if the green *Cyrtolophosis* is a new species or if the acquisition of green algal symbionts was an evolutionary adaptation to environmental habitat conditions.
The study is funded by the Austrian Science Fund (FWF): project P28333-B25 and the Swiss National Science Foundation (SNF): project SNF D-A-CH 310030E-160603/1 (2015-2018).

P12 - Who is who? Single cell PCR method establishing ciliate reference sequences for NGS approaches

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In the GenBank database more than 20,000 sequences of ciliates have already been deposited; however, only around 150 are assigned to species names validated by an expert. As ciliate taxonomists are rare and because the majority of ciliates cannot be grown in culture, sequence reference material remains unassigned in databases. Therefore, NGS approaches from lake plankton struggle when it comes to the interpretation of ecological questions underlying an exact identification of a species. To solve this problem, we developed a single cell PCR method to amplify the commonly used barcode markers V4- and V9- region of the nuclear SSU, the ITS-2, and, the D1/D2 region of the LSU. Single individuals of ciliates were isolated directly from a water body and morphologically characterized. After starvation of the single cells which is necessary in order to clear food vacuoles from other eukaryotes, the ciliates were directly processed by PCR reactions using universal eukaryotic primers for the SSU, ITS, and LSU regions. The resulting PCR products then served as templates for nested PCR amplifications. We already applied this newly developed method on a variety of different ciliates lineages providing validated sequences which can now readily be used for phylogenetic analyses, DNA barcoding and NGS approaches.

This study is funded by the Austrian Science Fund (FWF): projects P28333-B25 and I2238-B25.

P13 - Morphological and molecular characterization of *Euplotes daidaleos* and its green algal endosymbionts

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Among the more than hundred described species of *Euplotes* (Ciliophora, Spirotrichea), only *Euplotes daidaleos* bears green algal endosymbionts. Despite its worldwide distribution in freshwater habitats, only few studies investigated in detail the phenotypic plasticity and the molecular variability of the ciliate as well as of the symbionts. Moreover, the origin and phylogenetic placement of the endosymbiont are unknown. To shed light on the morphological and molecular characterization of both partners, we observed several European populations of *E. daidaleos*.

The ciliate as well as the algal strains were identical from morphology and from their nuclear SSU and ITS sequences. Phylogenetically, *E. daidaleos* belongs to clade I (Euplotoides sensu Borror & Hill). The algal endosymbiont belongs 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.

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P14 - Morphological and Molecular Based Analyses of Planktonic Ciliates in Lake Zurich

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The phylum Ciliophora (Alveolata) is a monophyletic group of complex and morphologically diverse protists. Ciliates are of great relevance in freshwater food webs where they act as link between lower and higher trophic levels.

Here we present the first morphological and molecular survey of pelagic ciliates in Lake Zurich, Switzerland. A unique dataset was collected bimonthly at 5 m depth in open waters from March 2014 to March 2017. Ciliate morphospecies were quantitatively and qualitatively analysed at high taxonomic resolution using quantitative protargol staining (QPS). The molecular characterisation was performed via next generation sequencing of the V9 18S rDNA fragment.

This three-year survey was the prerequisite for a comparison of morphological with molecular information, focusing on ciliate's total abundance, species diversity and taxonomic composition. It is well known that ciliates' abundance cannot be yet quantified based solely on molecular analyses. Nevertheless, the goal of this juxtaposition was to achieve at least a visualisation of succession and assemblage patterns also via next generation sequencing. This would allow for a rough and quick survey of pelagic ciliates with no need for time-consuming quantification. However, the study also shows that there is still an obvious discrepancy between the considerable knowledge on the diversity of freshwater ciliate morphotypes and the scarce information on their molecular taxonomy. Identification and isolation of ciliate and other protistan species is still needed to improve molecular analyses.

P15 - Similar but different: microbial co-occurrence networks of two alpine lakes

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The microbial species inventories of the two alpine lakes Mondsee (Austria) and Zürichsee (Switzerland) have been extensively studied in the last decades. Despite great efforts and the description of many formerly unknown organisms, we still have only a vague perception of how protists interact in these similar natural habitats. In the framework of a multinational project, we conducted a monthly sampling in both lakes from June 2016 to May 2017. For a comparison of co-occurrence networks between the lakes, protist communities were sampled from surface waters and deep waters in each lake. Although the obtained high-throughput sequencing data suggest a considerable overlap (up to 40,89 %) of microbial eukaryotes between the lakes, we scarcely observed the same co-occurrences of OTUs (less than 0,01%) in independent networks of both Mondsee and Zürichsee. Interestingly, we detected a higher congruency of communities and OTU co-occurrences in different depths of the same lake, than in same depths of different lakes. When having a closer look at ciliates as one of the most dominant heterotrophic groups among protists, we found that even though ciliates had different interaction partners in different habitats, these interaction partners belonged to similar taxonomic groups (on higher levels).

SOIL PROTISTS

P16 - Microscopic Life in Soils – A School Course demonstrating Adaptations, Succession, and Food Web Structure

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Soil is an important ecosystem providing food for terrestrial life, including humans. However, this ecosystem is rarely treated in schools, or merely metazoans are presented. Hence, pupils have strongly limited information on the community of soil organisms and the food web structure. Therefore, we established a course unit for pupils from 15 years upwards, demonstrating (i) the microscopic life in soil, (ii) the organisms' adaptations to soil, especially its ephemeral character, (iii) succession, and (iv) the importance of organic material (e.g., litter and root remnants) for the soil food web. Particularly, protozoa are an important component of the soil community, distinctly affecting soil fertility indirectly by influencing the community of bacteria, the main decomposers of litter, and directly by excreting nitrogen compounds. Despite their microscopic size, soil ciliates are used as model in this course unit as they comprise morphologically rather distinct species groups that feed on bacteria (mainly colpodids), flagellates (mainly hypotrichs), or other ciliates (mainly litostomatids). Further, they show adaptations to soil in their life style and morphology and a succession after rewetting an air-dried soil sample. Protozoa are inexpensive model organisms as they are relatively easy to collect, handle, and cultivate, providing shortly after rewetting of the samples sufficient abundances for investigation. The pupils are encouraged to establish hypotheses and test them, using microscopes and different samples of soil treated by Foissner's non-flooded petri dish method. Covering the topics of succession, adaptation, and food web, the lesson not only perfectly fits into the biology curriculum of Austrian secondary schools, but also represents an opportunity for an active and vivid acquisition of knowledge and competences.

P17 - Understanding protozoa-bacteria interactions to support plant-beneficial traits

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A number of rhizospheric bacterial strains (e.g. *Bacillus* spp., *Pseudomonas* spp.) have been identified for fostering plant health and are seen as potential candidates for biocontrol application. These candidates, however, often fail to efficiently support plant health under field conditions. The rhizosphere being a nutrient rich habitat in the soil, introduced bacteria have to cope with high competition and predation to successfully colonize the roots. Protozoa are important predators of bacteria and are thus expected to influence establishment of introduced bacteria. Protozoa could support bacteria with plant-beneficial traits by 1) lowering the competition (e.g. consumption of competitors), and 2) inducing defence mechanism (e.g. production of antibiotics with wide-range action on plant-pathogens).

We performed an experiment to study the influence of protozoa on bacteria and inversely. We grew 9 *Pseudomonas* spp. (well-characterized and presenting a gradient in plant-pathogen inhibition) and 7 protozoan species (2 *Naegleria* spp., 2 *Cercomonas* spp., 1 *Vannella*, 2 *Acanthamoeba* spp.) in co-cultures for a duration of 4 days at 20°C and respectively monitored their growth. Subsequently, we studied the sterile supernatant toxicity of these co-cultures toward plant-pathogens of different kingdoms (*Fusarium oxysporum*, 2 *Ralstonia* spp.) to investigate if the toxicity was induced by the presence of predators and to assess the toxicity range of the supernatant.

Some of the bacterial isolates were able to inhibit the growth of all protozoan species while not being influenced in their growth, thus not suffering from any

predation pressure. Every protozoan species responded in a specific way depending of the bacterial strain, without clear link to their phylogenetical origin. The presence of the predator did not induce the production of bacterial toxic compounds, but the toxicity of the supernatant of some bacteria was observed to be specific against *Ralstonia* spp. or *Fusarium oxysporum*, without overlap. Our data suggest that some bacteria produce a set of compounds that can efficiently inhibit the growth of protozoa but also of some plant-pathogens, giving them a certain advantage in the rhizosphere compared to native bacteria unable to protect themselves against introduced protozoa.

P18 - Towards a methodology for removing and reconstructing soil protists with minimal disturbance of soil microbial communities

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Soil ecological theories on the role of fauna groups in soil functions are often tested in highly artificial conditions, i.e. on completely sterilized soils or pure quartz sand re-inoculated with a small selection of these fauna groups, which give inaccurate results as part of the interaction was missed. Due to the variable sensitivity of different soil biota groups to gamma irradiation and the relatively small disturbance of soil physical and chemical properties, gamma irradiation has been employed to selectively eliminate soil organisms. In recent research we managed to estimate the contribution of the entire nematode communities to C and N mineralization in a more realistic condition compared to completely sterilized soil, by selective removal of nematodes at 5 kGy gamma irradiation followed by re-inoculation. However, as a key component of soil food web, we still cannot quantify the ecological importance of protists in ecosystem functioning as the lack of methodology for selectively removing and reconstructing of soil protists with minimal disturbance of soil microflora. Accordingly, the objectives of this research are: 1) modifying the successful methodology of selective elimination of nematodes, to selectively eliminate soil fauna including nematodes and protists through optimizing gamma irradiation doses with minimal effects on the soil microbial community; and 2) reconstructing soil protists and microbial communities through adding multicellular fauna free pulverized soil. Agricultural soil was irradiated by different gamma ray doses (6, 8, 12, 16, and 25kGy) and two doses (16kGy and 25kGy) were re-inoculated by soil powder. Soil samples were incubated for 9 weeks under sterile condition at a constant moisture and temperature. Soil nematodes, microbial biomass and activity, mineral N dynamics were recorded by destructively sampling. Soil protists and PLFA signatures were compared after incubation. The results showed that irradiation significantly reduced the

abundance of soil protists. Flagellates and ciliates were totally removed at the lowest dose 6kGy, while amoebae still existed even at 16kGy (around 8% of the total protists from CTR). Re-inoculation successfully established protists population of a similar size and composition as in the control samples, showing the potential of soil powder on soil protists re-inoculation. At lower doses (6kGy and 8kGy), nematodes were removed after 2 weeks incubation while higher doses (12kGy and 16kGy) showed no nematodes at the beginning, and no nematodes were introduced into soil with re-inoculation. The microbial biomass C and dehydrogenase were strongly reduced by gamma irradiation, however, soil powder addition increased both parameters significantly at 25kGy, while none of them reached the same level of CTR. For PLFA signatures, both gamma irradiation and re-inoculation did not make significant change in total PLFA, G+ bacteria, G- bacteria, and AMF. However, gamma irradiation remarkably reduced G+/G+, fungi and F/B, while re-inoculation compensate these reductions, which shows the unavoidable disturbance and reshaping of soil microbial communities when removing and reconstructing soil protists. Mineral N was largely increased by gamma irradiation while slightly changed by soil powder addition, but re-inoculation significantly reduced NH_4^+ and increased NO_3^-, which indicates the soil protists probably enhanced nitrification.

PROTIST ECOLOGY

P19 - HPLC-based pigment analysis of Chrysophyceae

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The Chrysophyceae are eukaryotic microalgae belonging to the Stramenopiles. Within the chrysophytes there is a great variability of nutritional modes ranging from phototrophy to mixotrophy and further to heterotrophy. The mixotrophs differ in being predominantly either phototrophic or heterotrophic. All chrysophytes contain plastids derived from the endocytobiosis of red algae. Plastids in the heterotrophic taxa are secondarily reduced but nevertheless present. Their distinct pigment composition consists of chlorophyll a, chlorophyll c1 and c2, β-carotene as the major carotene and fucoxanthin as the main xanthophyll. These major pigments are accompanied by smaller amounts of accessory pigments contained in the xanthophyll cycles. There has only been a small amount of research covering the differences of pigment compositions within chrysophyte species or the change of pigment composition under varying conditions. Here we present the overall pigment composition of different chrysophyte species affiliated with different nutritional modes analyzed by High-Performance Liquid Chromatography (HPLC). Further, the change of pigment content and composition of different chrysophyte species under varying conditions (light, food availability) is analyzed.

P20 - An attempt to study the self-organized spatial patterns in the growth of a ciliate population

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Self-organized patterns play an important role in the field of population dynamics. We developed an experimental set-up that allows investigating the spatial pattern formation using *Tetrahymena pyriformis* as a model organism. The experimental set-up consists of a modified microscopic slide with 24 fields for measuring the abundance and movement of the model organism. The 24 fields are arranged in a honeycomb-like structure each connected to the three nearest adjacent fields via channels. A low-budget computerized microscope table was developed, that places each field into the view field of the microscope camera, whose pictures were analyzed by the computer program "ImageJ", thus having the possibility to accurately monitor pattern formations at a high frequency and over a longer period of time. The results indicate an active and collective movement and gathering through the microcosm, which was observed in both cases of a rise in growth rate and general movement towards the gradient of substrate in the culture medium.

P21 - Interactive effect of temperature and food concentration on numerical response: the first study on peritrich ciliate *Pelagovorticella natans*

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This is the first report on the numerical response of a motile peritrich ciliate. We studied the numerical response of *Pelagovorticella natan* at temperatures of 5°, 10°, 15° and 20 °C, with food levels ranging from 0.02 to 2.5 µg C ml-1. The ciliates were fed the small cryptophyte *Cryptomonas* sp. Three main alterations were observed in the shape of the numerical response of *P. natans* with temperature: change in the threshold level, in the initial slope of the numerical response curve, and in maximum growth rate (rmax). The rmax was highest at 20 °C (0.83 d-1), i.e. almost two-fold higher than at 10 °C (0.48). The initial slope were also shifted up from low to high temperatures. Similarly, the threshold food concentration peaked at 10 °C (0.17 µg C ml-1) and was lower (0.06-0.08 µg C ml-1) and almost constant at higher temperatures (15-20°C). The result suggests that temperature alters the numerical response in a way that the species is shifted from being adapted to low food concentrations at moderate temperatures to requiring, and potentially thriving at high food concentrations at the temperature extremes. Our findings support and extend conclusions previously obtained for metazooplankton and indicate that changes as small as 3°C could significantly affect the role of protozoa in planktonic food webs.

P22 - Rowdies on the biofilm: effect of gastropod grazing on peritrich ciliates

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As freshwater snails are distributed nearly around the globe and as they feed upon biofilms, they are very suitable for studying interactions between a grazer and biofilms. The prosobranchs *Bithynia tentaculata* and *Potamopyrgus antipodarum* were used in this investigation. As the sessile peritriche ciliate *Vorticella similis* is common in the River Rhines' biofilms, the effect of *B. tentaculata* and *P. antipodarum* on these ciliates was investigated. In these experiments, snails were added to cultures of *V. similis* and the changes in the ciliates abundance were counted. The experiments demonstrated that *P. antipodarum* could effectively feed on *V. similis* within 30 min, reducing the ciliates abundance significantly. *B. tentaculata* had no significant effect. In total, *P. antipoarum* consumed, relatively to its softbody mass, more ciliates than *B. tentaculata.*

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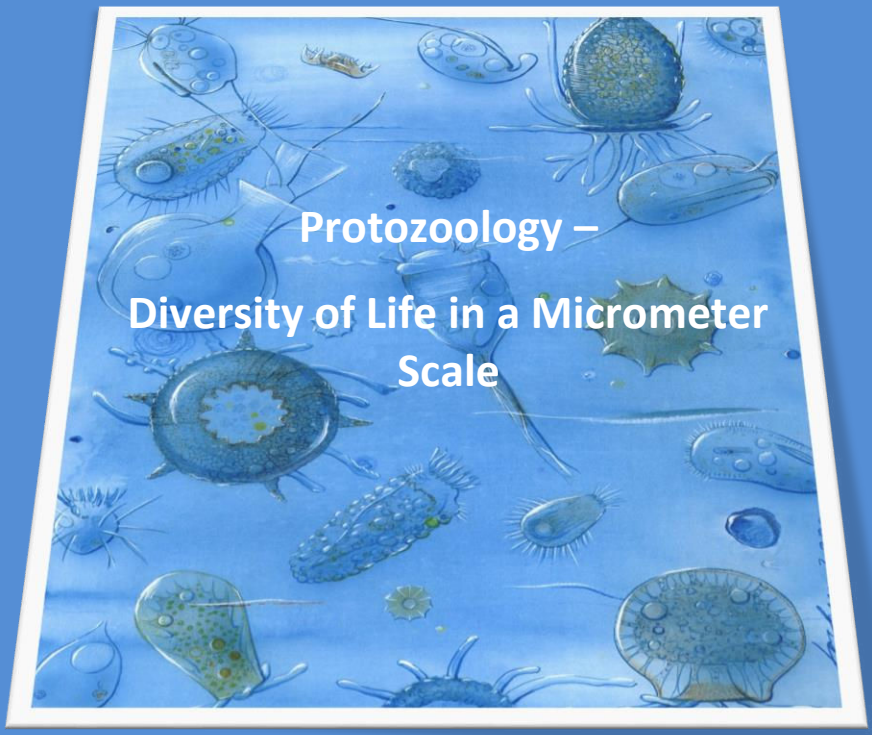

Protozoology –

Diversity of Life in a Micrometer Scale