



27 Feb.-2 March 2013
Kartause Ittingen (CH)

The 32nd Meeting of the German Society for Protozoology ABSTRACTS

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Abstracts

**32nd Meeting of the
German Society for Protozoology (DGP)
27 February – 2 March 2013
Kartause Ittingen
CH-8532 Warth-Weiningen
Switzerland**

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Contributions to Workshop

This discussion group is focussed on a **special aspect of protistology**, which is of **increasing relevance** in the view of the organizers (limnologists). The two suggested topics and, hopefully, intensive discussions, should reveal to young and also experienced protistologists the prior knowledge (that got neglected!), the needs for future research, and problems concerning freshwater ecosystems health we may face. As limnologists, we selected two major topics relevant to many temperate freshwater ecosystems:

Nutrient overloading: The fertilization of lakes and rivers is problematic, especially in waters used as drinking water source or for recreation. Self-purification of waters is mainly based on pro- and eukaryotic microbes. The saprobic system based on protists is one of the most reliable indicator tools for detecting pollution. However, it seems that many scientists and politicians are **not aware of the saprobic system anymore**. Protists are also key-organisms to define a **reference status of freshwater systems** in palaeolimnological studies that aim to characterize the natural community composition before, during, and after pollution. It will be of interest to discuss **how and if molecular methods can support** and widen the existing knowledge about indicator-protists.

Micropollutants: Almost all wastewater treatment plants were primarily constructed to limit the input of major nutrients (C, P, N) in lakes and rivers. However, it was not foreseen that urban wastewaters would be a **cocktail of thousands of micropollutants** (hormones, pharmaceuticals, additives, etc.) in the meantime. We would like to discuss the importance of protists for **ecotoxicological tests**. Is there a need for a **new saprobic system** including the **aspects of micropollutants?**

This workshop is sponsored by the

'Hydrobiology-Limnology Foundation for Water Research'.

KEYNOTE LECTURE – 27.2.2013 – WORKSHOP

Protists as Indicators of Ecosystem Health: Quo Vadis?**Foissner Wilhelm***Dept. Organismic Biology, University of Salzburg, A-5020 Salzburg, Austria**wilhelm.foissner@sbg.ac.at*

Bioindication is a valuable tool for estimating ecosystem health and stimulating students to become interested in applied environmental matters. A great variety of autotrophic and heterotrophic organisms has been used as indicators for, e.g., water and soil quality, the influence of toxic substances, and the effects of various fertilizers. Protists played a rather prominent role in this scenario between the years 1980 and 2000, for instance, in the "Saprobiensystem" for the assessment of water quality. Then, the number of papers decreased from year to year, and presently there are very few protistologists working in this field. There are three main reasons for this: (1) The advent of fast and cheap chemical and molecular methods generally decreased bioindication. (2) The number of well-trained taxonomists and identifiers decreased, and it is increasingly difficult to interest students for protists in general and as bioindicators in particular. This results from changed sociological paradigms, forcing youngsters to do "meaningful" science and to make much money as early and fast as possible. (3) The indolence of university and government biologists to identify bioindicators. Often, this is left for technicians. This not only takes jobs from academic biologists but also makes it difficult to build a strong lobby.

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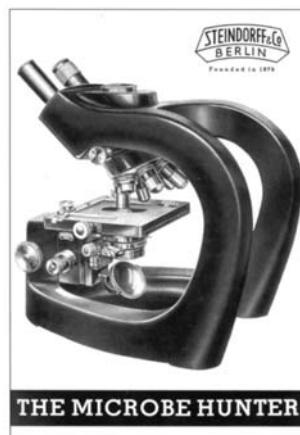


Abb. 1: Mikrobenjäger-Reklameblatt für den US-amerikanischen Markt.

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Talk – 27.2.2013 – WORKSHOP

An Arcellacea (testate lobose amoebae/thecamoebian) based transfer function for phosphorus in lakes**Patterson R.Timothy¹, Roe Helen M.² & Swindles Graeme T.³**

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Arcellacea communities were assessed from 73-sediment/water interface samples collected from lakes in urban and rural settings within the Greater Toronto Area (GTA), Ontario, Canada, as well as from forested control areas to the north. The results were used to: (1) develop a statistically rigorous arcellacean-based training set for sedimentary phosphorus (Olsen P (OP)) loading; and (2) derive a transfer function to reconstruct OP levels during the European settlement era using a chronologically well-constrained core from Haynes Lake on the environmentally sensitive Oak Ridges Moraine, within the GTA. Ordination analysis indicated that OP most influenced arcellacean assemblages, explaining 6.5% ($p<0.005$) of total variance. An improved training set where the influence of other important environmental variables (e.g. total organic carbon, total nitrogen, Mg) was reduced, comprised 40 samples from 31 lakes, and was used to construct the first published transfer function for lacustrine arcellaceans using tolerance down-weighted weighted averaging (WA-Tol) with inverse deshrinking (RMSEP Jack-77 pp ; $r^2\text{Jack}=0.68$). The reconstruction indicates that OP levels remained near pre-settlement background levels from settlement in the late 1870's through to the early 1970's. A significant increase in inferred OP concentration beginning ~1972 may have been related to a change in crops (e.g corn production) in the catchment resulting in more runoff, and the introduction of chemical fertilizers. A dramatic decline in OP after ~1985 probably corresponds to a reduction in chemical fertilizer use related to advances in agronomy, which permitted a more precise control over required fertilizer application. Another significant increase in OP levels after ~1995 may have been related to the construction of a large golf course upslope of Haynes Lake in 1993, where significant fertilizer use is required to maintain the fairways. These results demonstrate that arcellaceans have great potential for reconstructing lake water geochemistry and will complement other proxies in paleolimnological research.

KEYNOTE LECTURE – 27.2.2013 – WORKSHOP

Phenotypic and genotypic profiling of chemicals and particulate matter in *Tetrahymena* spec.

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Tetrahymena spp. combine traits of (1) a single cell, thus might replace or complement specific cell-line testing approaches, with traits of (2) a whole organism and population, thus allowing to study complete metabolic pathways and its consequences on population growth and genetic adaptation. Assays involving *Tetrahymena* spp. might easily be adapted for a rapid multi-level *in situ* or *ex situ* toxicity biosensor test system for ecologically relevant risk assessment.

Tetrahymena pyriformis, strain GL was used for the determination of catecholamines toxicity and their metabolism. Catecholamines exhibited acute toxicity to *Tetrahymena* cells where dopamine and L-DOPA showed higher toxic potential at EC₁₀ (0.39 ppm and 0.63 ppm respectively) and EC₂₀ (1.1 ppm and 1.0 ppm respectively) after 48 h exposure.

The growth inhibition test of fluorotelomeralcohols was made both in open system (96-well microplates) and in closed system (closed flasks). After treatment, cells were stained by acridine orange and observed under fluorescence microscopy at 488 nm. Direct membrane damage was detected by measuring LDH leakage. For 8:2 FTOH and 10:2 FTOH no growth inhibition was found in both systems. 4:2 FTOH was found inhibiting population growth in closed system (EC₅₀=276.1 mg/L), whereas, 6:2 FTOH affect population growth both in closed system (EC₅₀=64.3 mg/L) and in open system. Macronucleus destruction was observed with 6:2 FTOH. No direct membrane damage was detectable. Comparing the results from two test systems, tests in closed system are more reliable for testing these volatile compounds with *T. thermophila* than in open system.

Tetrahymena cell immediately endocytose TiO₂ nanoparticles and store them in food vacuoles. Then TiO₂ particles undergo exocytosis as larger aggregates after 18-24 h of ingestion. However, during the process of endocytosis and exocytosis of TiO₂ particles affects the cell growth and consequently induces acute toxicity. TiO₂ particles exerted higher cell growth inhibition after 20 h incubation. Surprisingly the effect of TiO₂ decreases after 40 h incubation owing to the recovery of cell growth. In addition, TiO₂ particle induces about 20% cytotoxic effect after 6 h exposure. Similar results were obtained for fullerene and fullerenol targeting selected genotypes of *T. thermophila*.

Gerhardt, A., Ud-Daula, A., Schramm, K.-W.: *Tetrahymena* spp. (Protista, Ciliophora) as test species in rapid multilevel ecotoxicity tests. Acta Protozool. 49, 271-280 (2010)

Wang, Z., Ud-Daula, A., Fiedler, S., Schramm, K.-W.: Impact of fluorotelomer alcohols (FTOH) on the molecular and macroscopic phenotype of *Tetrahymena thermophila*. Environ. Sci. Pollut. Res. 17, 154–164 (2010)

Keynote lectures

KEYNOTE LECTURE – 28.2.2013 – morning

**'May You Live in Interesting Times':
The Intersection of Classical and Modern Molecular Approaches for
Studying Protistan Diversity and Community Structure**

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Ecological studies of natural protistan communities have traditionally been constrained because of the incredible diversity of single-celled eukaryotic species that exists within natural ecosystems. For centuries, taxonomies of these species have been defined, and their ecological activities characterized, based on the application of microscopy and culture. Within approximately the last decade, however, molecular approaches and DNA sequence information on microbial eukaryotes have exploded onto the scene, adding tremendously to the methodologies available for characterizing the presence, abundances and ecological roles of microbial species including protists. While these new approaches have greatly improved our arsenal of tools used to study protists, they are still very much in their infancy, resulting in considerable discussion and debate regarding how this wealth of genetic information can be exploited and integrated with more-traditional ecological approaches and concepts. Indeed, this process has left some organismal biologists and taxonomists concerned about a wholesale loss of species morphotypes, behaviors and physiologies, and replacement with ‘nameless sequences subjected to complex and confusing statistical analyses that provide little ecological information’. While there is presently a reasonable amount of truth embedded in this anxiety, and it is unreasonable to expect a quick and easy resolution to this integration, it is also important to realize that these approaches have already enriched our ability to begin to answer some long-standing questions relating to organismal biology, community ecology and the evolution of protists. This talk will recount some of the most significant breakthroughs in protistan ecology resulting from the application of molecular methods, identify the most vexing issues preventing better and more rapid integration of these approaches, and those aspects of ecology that still remain unimproved by their incorporation.



KEYNOTE LECTURE – 28.2.2013 – afternoon

**Convergences and divergences in the evolution of
marine plankton ciliates (Protista, Ciliophora)**

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The Oligotrichida and Choreotrichida (Spirotricha) are the dominating ciliate taxa in the sea concerning diversity (> 1000 species) and abundances. The cladistic analysis of oligotrichids (“the sparsely ciliated ones”) and choreotrichids (“the dancing hairs”) considers more genera than gene sequence analysis, except for the loricate tintinnids. Nevertheless, the cladogram and molecular phylogeny match rather well, e.g., in the monophyly of the Oligotrichida, Tontoniidae, Choreotrichida, and Tintinnina, and both demonstrate the importance of features of the somatic ciliature for inferring evolutionary relationships. On the other hand, the trees deviate distinctly in the position of the genus *Lynnelia*. The non-monophyly of *Strombidium* in the gene tree and of the Strombidiidae in the cladogram are probably due to our insufficient knowledge about their morphologies. In the oligotrichids, some somatic ciliary patterns developed convergently in tailed and tailless taxa, while the ancestral kinetid structure has been retained. At the beginning of the choreotrichid evolution, however, dramatic changes took place in the kinetid structure of the alorate taxa and continued in the freshwater tintinnids, which otherwise have a comparatively simple somatic ciliature. In the lineage of marine tintinnids, no further kinetid changes occurred, but the ciliary patterns became more complex by the successive introduction of specialized kineties and ciliary fields. Since cytological characters are known in only 2% of the species, tintinnid taxonomy and systematics are exclusively based on lorica features despite unknown species limits. However, the preliminary data indicate that somatic ciliary pattern and lorica structure are not correlated. Although the lorica sac turned out to be of taxonomic significance and the capsules are promising features, a far-reaching revision of the tintinnid systematics requires the intensive co-operation of taxonomists and molecular biologists.

KEYNOTE LECTURE – 1.3.2013 – morning

**Extinctions, warming, & food webs linked using microbial
microcosm experiments**

Petchey Owen

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Experimental microbial communities containing protists have for many years provided important insights into the processes that pattern ecological communities. Experiments with protists are currently being used to research the links between environmental change, extinctions, food web structure, and ecosystem processes. One intriguing finding is that warming of relatively complex experimental food webs causes the extinction of herbivorous and predatory protist species, but not bacterivores or algae. The multiple candidate hypotheses for such interspecific variability in extinction risk can be grouped into two categories: direct effects of environmental conditions and effects of biotic interactions. I will talk through some of the evidence we have gathered from experimentation with microbial communities about these effects. Results indicate that modelling the future of biodiversity will require consideration of both environmental changes and the biotic interactions a species participates in. Furthermore, results show how experiments with model communities of bacteria and protists can lead to a deeper and more accurate understanding of how environmental change affects biodiversity.

KEYNOTE LECTURE – 1.3.2013 – afternoon

**Protist metabarcoding:
A new tool for environmental biomonitoring?**

Pawlowski Jan Wojciech

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Metabarcoding consists in using short DNA fragments to identify species in environmental samples. Recent development of metabarcoding was fostered by the emergence of next-gen sequencing (NGS) technologies that allow obtaining millions of DNA sequences relatively rapidly and cost effectively. The application of NGS to study protist diversity revealed a huge cryptic diversity at all taxonomic levels, from new phylogenetic lineages to high genetic variations within known morphospecies. The development of NGS-based metabarcoding also opened new perspectives for the wider use of protists in environmental biomonitoring. Although, there are still some problems with interpretation of NGS data, such as the taxonomic assignment of tag sequences, the distinction between active and dormant species, or the correlation between number of sequences and species abundance, some of these problems can be easily overcome, as shown by recent studies. The future of protist genetic monitoring depends mainly on the expansion of the protist barcoding database and better knowledge of the environmental impact on global protist community.

Talks

In alphabetical order by first author

Talk

**Spatial clustering of habitat structure effects
patterns of community composition and diversity**

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Natural ecosystems often show highly productive habitats that are clustered in space. Environmental disturbances are also often non-randomly distributed in space, and are either intrinsically linked to habitat quality or independent in occurrence. Theoretical studies predict that configuration and aggregation of habitat patch quality, directionally biased dispersal and disturbances can affect metacommunity composition and diversity, but experimental evidence is largely lacking. In a metacommunity experiment with protists kept in microcosms we tested the effects of spatially-autocorrelated disturbance and spatial aggregation of patch quality on regional and local richness, among-community dissimilarity, and community composition. We found that spatial aggregation of patch quality generally increased among-community dissimilarity (based on different measures of β -diversity) of the experimental protist communities. There were significant interacting effects of landscape structure and location of disturbances on β -diversity, which depended in part on the specific β -diversity measures used. Effects of disturbance on composition and richness in aggregated landscapes were generally dependent on distance and connectivity among habitat patches of different types. Our results also show that effects of disturbances in single patches cannot directly be extrapolated to the landscape scale: the predictions may be correct when only species richness is considered, but important changes in β -diversity may be overlooked. There is a need for biodiversity and conservation studies to consider the spatial aggregation of habitat quality and disturbance, as well as connectivity among spatial aggregations.

Talk

Non-linearity in the protistan world

Arndt Hartmut, Nomdedeu Mar Monsonis, Becks Lutz & Schieffer André

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A phenomenon seldom addressed regarding the life of protists is the fact that the abiotic and biotic environment of protists is characterized by non-linear processes. The contribution will give a short overview about the different facets of this statement. Finding out why natural population densities change over time and vary depending on location is one of the central goals of ecology. The recognition that even simple ecological systems have the potential for chaotic behaviour has made chaos a topic of considerable interest among theoretical ecologists. Protists and bacteria as their potential food organisms are useful model organisms in experiments due to their short generation times of only a few hours. Studies of dynamics defined predator-prey systems will be demonstrated. The observed dynamics will be characterized by their corresponding Lyapunov exponents. Apart from the intuitive understanding that external (extrinsic) stimuli (such as temperatures) influence the variability of abundances, we have to consider that the internal (intrinsic) qualities of a population give rise to population dynamics with large and (at certain parameter ranges) even chaotic fluctuations of abundances, even under wholly constant and predictable conditions. We are just at the beginning to understand these phenomena in nature.

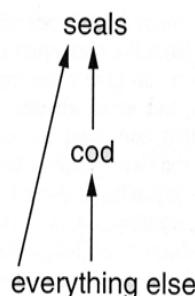


Figure 18.3. A food web for the northwest Atlantic.

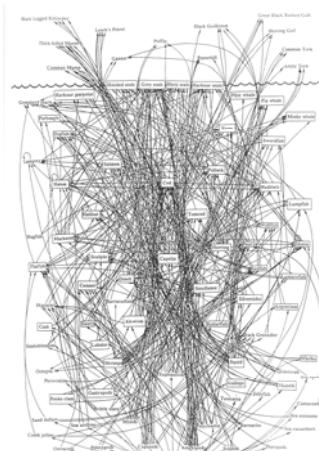


Figure 18.4. Another food web for the northwest Atlantic (reprinted with permission from Lavigne (1992)).

Talk

Classification of crucigenoid algae (Scenedesmaceae, Chlorophyta)

Bock Christina¹, Kusber Wolf-Henning² & Krienitz Lothar³

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The Crucigenioideae belong traditionally to the family Scenedesmaceae, and comprises green algae which exhibit flat, curved or three-dimensional coenobia and syncoenobia with ovate to spindle shaped cells. Despite a straightforward morphological characterization of this subfamily, the clear distinctive features used for discrimination at the genus level were often vague and doubtful, resulting in a high number of synonymous genera over time. Furthermore, questionable taxonomic changes hampered the correct classification of crucigenoid species. Here we present data of a combined morphological and molecular approach, focusing on different *Tetrastrum* and *Crucigenia* species. It became obvious, that the subfamily Crucigenioideae includes members from a vast range of different phylogenetic lineages. The spine-bearing species of *Tetrastrum*, including the type species *T. heteracanthum* form a new phylogenetic lineage within the *Oocystis*-clade (Trebouxiophyceae). Other strains of *Crucigenia* and *Tetrastrum* form a lineage near the *Botryococcus*-clade. This lineage was classified to the reinstated genus *Lemmermannia*. Other lineages were discovered next to *Scenedesmus* (Chlorophyceae) and *Chlorella* (Trebouxiophyceae). Our results are a first step to resolve the diversity and the taxonomic problems of this challenging group of green algae.

Talk

Effects of nutrient limitation and prey availability on feeding of potentially harmful mixotrophic dinoflagellates

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Many potentially harmful bloom-forming dinoflagellates are mixotrophic, i.e. are able to gain energy by ingesting prey organisms in addition to photosynthesis. However, the relevance of this nutritional strategy for bloom dynamics has been largely neglected in experimental studies so far, especially with regard to the availability of dissolved nutrients and different prey organisms. In the present study two microcosm experiments were conducted to investigate the impact of nutrient limitation (nitrogen and phosphorus) and the availability of differently sized prey organisms, including the Prasinophyte *Ostreococcus* sp. (0.8 - 2 µm) and the Raphidophyte *Heterosigma akashiwo* (10 – 25 µm), on growth and grazing characteristics of the two mixotrophic dinoflagellates *Lingulodinium polyedrum* and *Alexandrium catenella*. Prey availability increased growth in both dinoflagellates under nutrient limitation. In this context, both dinoflagellates showed higher grazing rates on *Ostreococcus* than on *Heterosigma*. At high nutrient concentrations, however, neither of the prey organisms was beneficial to the dinoflagellates. They rather acted as competitors, monopolizing dissolved nutrients, which strongly enhanced their production. These high prey abundances could not be controlled by consumer feeding anymore, resulting in a negative prey effect on the productivity of the dinoflagellate consumers. Overall, these results emphasize the relevance of mixotrophy for the bloom persistence of potentially harmful dinoflagellates especially when nutrients become deplete, making them superior competitors compared to purely photosynthetic phytoplankton.

Talk

Dynamic chromatin remodeling at HSP70 promoters enables fast transcriptional activation after heat shocks in *Paramecium tetraurelia*

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The chromatin state consists of many hidden information, e.g. about the possibility to prime transcription, enabled by the accessibility of the wrapped DNA region. Chromatin immunoprecipitation (ChIP) is a powerful technique, allowing insights into the epigenetic state of the chromatin by crosslinking DNA and associated proteins with subsequent analysis of isolated DNA fragments. Here, we demonstrate the optimization of ChIP for *Paramecium* macronuclear DNA, determining the crucial parameters to perform successful ChIP, e.g. crosslinking-conditions, optimization of shearing efficiency, antibody titration and optimization crosslink-reversal. Using this protocol, we characterized the chromatin at HSP70 promoters (PtHSP70-03 and PtHSP70-05) of cytosolic chaperones before and after transcriptional activation by heat shocks in comparison to constitutively transcribed housekeeping genes. Our results demonstrate that heat shocks are accompanied with strongly decreased nucleosome occupancy and increased levels of histone H3 lysine 9 acetylation at HSP70-03 and HSP70-05 promoters even 20 minutes after heat shocks. Both parameters indicate fast and dynamic chromatin remodelling towards an open chromatin structures which appears to be a prerequisite for transcriptional activation by the heat shock transcription factor.

Talk

Protists: key factor for energy fluxes in microbial food webs?

Corno Gianluca (IL Professore)

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A number of laboratory based experiments using artificially designed microbial communities show how direct and indirect effects of protistan grazing on bacterial communities play a fundamental role in boosting the efficiency and the productivity of aquatic microbial systems. In general, it is known that prey removal by predation tends to select for resistant and less efficient forms of bacteria. Our findings suggest that in the first days after a heavy grazing event, indirect ecological effects of predation (e.g. the recirculation of nutrients caused by bacterial digestion by protists) are in fact stimulating the activity of less resistant and very efficient bacteria. Efficiency and productivity of resource uptake in these conditions can raise up to 20-30 fold. Moreover, by promoting coaggregation, protistan grazing could be considered one of the key factors promoting the formation of flocks of microorganisms in waters, hot spots for microbial production in lakes and oceans.



© Sherman's Lagoon

Talk

Mitochondrial protein import in parasitic protists

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Mitochondrial protein import (MPI) is essential for the biogenesis of mitochondria in all eukaryotes. Prevalent models of MPI are predominantly based on experiments with one group of eukaryotes: the opistokonts. Although fascinating genome database-driven hypotheses on the evolution of MPI machineries have been published, previous experimental research on non-opistokonts usually focused on the analysis of single pathways or components. We therefore established the parasitic protist *Leishmania tarentolae* as a model organism for the comprehensive analysis of MPI into all four mitochondrial compartments. Our studies demonstrate that MPI pathways are functionally conserved among eukaryotes despite differences of the MPI machineries between eukaryotic lineages. Such differences include significantly altered and novel MPI components, for example, in kinetoplastid and apicomplexan parasites. In summary, the current knowledge on MPI in yeast and other opistokonts cannot be generally transferred to all eukaryotes and the corresponding pathways, components, and mechanisms remain to be analyzed [1-3].

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[2] Deponte M, Hoppe HC, Lee M, Maier AG, Richard D, Rug M, Spielmann T, and Przyborski JM (2012) Wherever I may roam: Protein and membrane trafficking in *P. falciparum*-infected red blood cells. *Mol Biochem Parasitol* 186:95-116.

[3] Eckers E, Petrungaro C, Groß D, Riemer J, Hell K, and Deponte M (2012) Divergent molecular evolution of the mitochondrial sulfhydryl:cytochrome c oxidoreductase Erv in opistokonts and parasitic protists. *J Biol Chem* (in press).

Talk

Ménage-à-trois: The amoeba *Nuclearia* sp. from Lake Zurich with its ecto- and endosymbiotic partners

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Symbiosis is an abundant phenomenon. Essentially, the observed complexity of life would not be imaginable without the benefits provided by symbiosis. Culture independent molecular methods like fluorescence *in situ* hybridisation (FISH) and sequencing of the small subunit of the ribosomal RNA (SSU rRNA) gene nowadays allow for an accurate characterisation of microbial symbionts. Here we present the value of these two techniques for the characterization of a nucleariid amoeba isolated twice (strain N and strain A) from Lake Zurich and its ecto- as well as endosymbiotic bacteria. The two isolates showed the same morphological characteristics and their SSU rDNA sequence was identical. Regarding phylogenetic analyses the affiliation to the genus *Nuclearia* was clear. However, for the species identification we obtained an ambiguous image. Sequence comparisons of the SSU rRNA genes (99.6% sequence similarity calculated by BLASTN) led to the affiliation of our isolates to the species *Nuclearia thermophila* (isolated by Yoshida from a 30 °C warm Japanese lake) but several morphological features of our isolates are in contradiction to the original description. The most striking difference between the isolates is the absence of symbionts that was originally reported for *N. thermophila*. In contrast, *Nuclearia* sp. strain N kept in monoclonal cultures was permanently associated with ecto- and endosymbionts (for almost 1.5 years). Beside the characterisation of the symbioses based on growth experiments and transmission electron microscopy the full SSU rRNA cycle approach was fulfilled for the identification of the two different symbionts. The ectosymbiont was identified as *Paucibacter toxinivorans* (a betaproteobacterium originally isolated through enrichment with the cyanobacterial toxin microcystin) and the symbiosis was characterized as not obligate. Our results suggest that *P. toxinivorans* might confer a gain of fitness to the nucleariid amoeba being involved in the detoxification of microcystins stored in the cyanobacterium *Planktothrix rubescens*, which was the sole food source for the amoeba. The SSU rDNA sequence obtained from endosymbionts affiliated to the gamma subdivision of the proteobacteria lineage but could not be grouped into any established genus. Therefore, we propose the provisional name *Candidatus Companero nuclearis* for these bacteria that have until today never been found neither free-living nor in a symbiotic association. The function of this symbionts harboured in symbiosoms randomly distributed inside the cytoplasm is still unknown. We never observed endosymbiont-free amoebae of our isolates during almost two years. Thus, we suggest that this endosymbiosis is obligate.

Talk

Evaluation of sequencing strategies to capture protistan diversity along a salt gradient

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Since the rapid advancements in next-generation sequencing (NGS), involving the creation of large amounts of read data coupled with decreasing sequencing costs, NGS techniques become more popular in diversity analyses. Due to the high throughput it is now even better possible to outline the extent of biodiversity and to broaden the understanding of the microbial world. But still, little is known about the interpretation and meaning of the sequencing data, as well as the ecological relevance. Also, since these novel techniques are not error-free, sequencing artifacts and other biases can distort the actual picture of species diversity and therefore can lead to false conclusions. Despite the intensive usage of next-generation sequencing platforms only few studies have been conducted to directly compare the different strategies. In this study, the traditional cloning approach and the two next-generation sequencing strategies, 454 and Illumina, were compared and evaluated by investigating protistan community shifts along a salt gradient in the Ria Formosa lagoon, Portugal. The goals of the study were i) to investigate if the results of the different methods lead to the same conclusions, ii) to compile a guideline how sequencing data should be interpreted and iii) to evaluate the ecological significance of the respective sequencing strategies.

Talk

A Natural Monument for Ciliated Protozoa: The Ephemeral Krauthügel Pond in the Town of Salzburg

Foissner Wilhelm¹, Medicus Reinhard², Augustin Hannes³ & Cotterill F.P.D.⁴

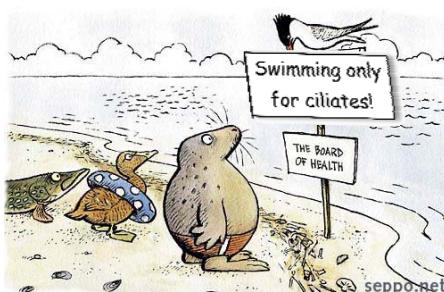
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On 19.01.2012, the mayor of the town of Salzburg declared the “Ephemeral Pond on the Krauthügel” as a natural monument for ciliates. The pond is on the foot of the castle of Hohensalzburg, i.e., near the city centre, in a natural depression. When filled, the pond is claviform with a size of about 30 × 15 m and a depth of about 30 cm. The pond has water for some days or weeks only after heavy rains or after longer periods of rain. The pond area was used for several hundred years as a field for root and leafy vegetables (e.g. cabbage). In the sixties of the past century, the field turned to grassland fertilized organically. Up to the eighties, the area was used as grassland and as autumn pasture. This turned the pond to a paradise for ciliates because the water became highly eutrophic by the excrements of the cows. We investigated the ciliates of the Krauthügel pond during the past 40 years, recognizing about 150 species of which 10 were undescribed, i.e., new to science. This was a main argument for our effort to conserve this pond. Further, we used ciliates from this pond as neotypes for another 10 species. Types and neotypes cause that the locality becomes the “type locality”. The type locality is a very important site because the type or neotype population is a measure for all future identifications, especially when not all characteristics can be seen in prepared specimens, as it is usual in protists. As far as we know, this is the first official conservation of a certain site for ciliates. (Supported by the FWF, Project number P22846-B17.)



Talk

Analyses of V4-SSU rDNA reveal distribution patterns of ciliate communities in European coastal waters

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In 2009, the BioMarkS (Biodiversity on Marine Eukaryotes) program started as a consortium of 8 European research institutes. The interdisciplinary approach of the program brought scientists from different fields of microbial eukaryotic research together; such as taxonomy, ecology, marine biology, evolution, genomics and bioinformatics. In one of the most extensive co-operations of European microbial biologists, aquatic and benthic samples at 8 distinct coastal sites from the Skagerrak to the Black Sea have been collected. The idea of the program was to gain new insights into the microbial life of the oceans, especially under the light of climate change and ocean acidification. With the help of next generation sequencing we obtained more than 1,400,000 sequences of the eukaryotic V4 small subunit (SSU) rDNA, of which nearly 90,000 could be assigned to ciliates. Further steps in the working pipeline included bioinformatical processing of the ciliate sequences which implied both, qualitative and quantitative analyses of the data. Thus, we did not only analyze the distribution of ciliates, but also the composition of whole ciliate communities at different locations. Taking the biological and physicochemical parameters of the habitats at the sampling sites into consideration, we could test three hypotheses which are consistent with the moderate endemicity model. In particular we focused on the question, whether a location specific distribution or a habitat specific distribution of ciliates is more likely. By answering this question we also revealed distribution patterns of ciliate communities in European coastal waters. All data which were retrieved in the BioMarkS program will be compiled to one of the largest databases on marine eukaryotic organisms. We believe that this database will become an important reference platform for future projects dealing with marine protistan biodiversity.

Talk

Soil naked amoeba in rare environments: Enormous diversity of mostly unknown species

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Naked amoeba (*Gymnamoeba*) occupy key positions in soil food webs; they are the major consumers of bacterial production in soil due to their amoeboid morphology, small size, high abundance and fast turnover rates. Despite their important functions for nutrient cycling as the base of the heterotrophic eukaryotic food web in soil we only have a vague idea on the identity of the dominant taxa. This lack of knowledge is caused by methodological difficulties in cultivation and quantification in the opaque soil environment as well as a lack of taxonomic expertise. However, recent developments in molecular techniques now promise to reduce this methodological gap on this largely unknown trophic link in the soil food web. We aim to combine morphological and molecular methods to increase the knowledge on *Gymnamoeba* in soils as part of the EU-project EcoFINDERS. We investigated the diversity of *Gymnamoeba* in high altitude soils from Tibet, provided by the University of Beijing as a satellite partner. We established parallel enrichment cultures of four different soils to cultivate and enumerate *Gymnamoeba*. Cultures were microscopically grouped into morphotypes supplemented by more detailed molecular information, i.e. sequences of the 18s rDNA. Conditions in the soils investigated were extreme in many respects; first, soils were investigated only after one year; second, soil water contents were very low, generally below 10 percent; third, samples were taken from very high altitudes of up to 5033 m. Consequently, the abundance of *Gymnamoeba* was low with a general average of 1407 amoeba g⁻¹ dry soil. However, the diversity of *Gymnamoeba* extracted was enormous: Most known morphotypes of soil *Gymnamoeba*, roughly representing phylogenetically related organisms, were recovered. However, sequences revealed that almost all cultures represent species new to science. This study suggests that a plethora of new amoeba still await discovery, not even mentioning distinct functional differences.

Talk

Tara Oceans: Exploring ciliate diversity in the world's oceans

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Marine ecosystems are essential for the regulation of the biogeochemical cycles and climate on Earth. Nevertheless, the organization, dynamics and evolution of these ecosystems are still poorly understood. Furthermore, we still do not know the impacts of climate change on these ecosystems and the microorganisms which are living there. For this reason, the Tara Oceans project was launched in September 2009 to study the structure of plankton ecosystems in the oceans for three years. The project implemented a unique sampling program including optical and genomic methods to describe viruses, bacteria, archaea, protists and planktonic metazoans in their physicochemical environment. More than 30,000 samples of 153 sampling stations around the world have been collected by an international team of scientists. For a first overview, samples of different depths and size fractions of 36 sampling stations have been sequenced using next generation sequencing methods. The targeted gene region was the hypervariable V9 region of the small ribosomal subunit. Using high-throughput sequencing techniques (Illumina) we obtained more than 4 million sequences which could be assigned to the phylum Ciliophora. Based on these data we analyzed the biogeographical distribution of ciliates and compared the biodiversity of ciliate communities in the world's oceans.

Talk

Scaling body size fluctuations

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Size of an organism matters for its metabolic, growth, mortality and other vital rates. Scale-free community size spectra (i.e., size distributions regardless of species) are routinely observed in natural ecosystems and are the product of intra- and inter-species regulation of the relative abundance of organisms of different sizes. Intra- and inter-species distributions of body sizes are thus major determinants of ecosystem structure and function. We show experimentally that single-species mass distributions of unicellular eukaryotes covering different phyla exhibit both characteristic sizes and universal features over four orders of magnitude in mass. Remarkably, we find that the mean size of a species is sufficient to fully characterize its size distribution and that the latter has a universal form across all species. We show that an analytical physiological model accounts for the observed universality, which can be synthesized in a log-normal form for the intra-species size distributions. We propose how ecological and physiological processes should interact to produce scale-invariant community size spectra and discuss the implications of our results on biological power-law relationships.

Talk

Small but manifold - hidden diversity in the colourless chrysophycean 'genus' *Spumella*

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The colourless chrysophycean genus *Spumella* has been identified as a major phagotroph and feeder on bacteria in freshwater and soil food webs. The group appears as the clustered 'Spumella-like flagellates' in applied studies (concerned with questions of biodiversity and ecology). We assume, however, that the picture drawn is inexact blurring diversity and leaving the distinct ecology unanswered. Here, we present results of multigene analyses as well as morphological data showing the polyphyly of *Spumella* and calling for an altered database reference thus gaining a deeper insight into the biodiversity and ecology of the group.

Talk

Molecular analysis of trans acting siRNAs in *Paramecium tetraurelia***Günzler Ulrike, Kraushaar Katja & Simon Martin**

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Regulatory events which are mediated by small RNAs are widespread mechanisms throughout species. RNA-Interference (RNAi) in *Paramecium* can be triggered by the injection of a truncated transgene into the macronucleus. The mechanism of RNAi triggered by a transgene is hardly understood. Here, we describe the molecular analysis of the ~22nt siRNAs which are responsible for transgene induced silencing. Their synthesis depends on the RNA dependent RNA-polymerase Rdr3 and Dicer (Dcr1) because silencing of these RNAi factors inhibits siRNA accumulation and as a consequence transgene induced silencing. Biochemical analysis in combination with northern blot analysis revealed that these siRNAs show a modification of their 3'-end. In context with a loss of transgene induced silencing during silencing of Hen1, a 2'-O-methyltransferase, this modification seems likely to be a methyl group. Silencing of Hen1 leading to decreased levels of RNA 2'-O-methylation seems also to destabilize transgene induced RNAs, however the stabilizing effect seems not to be required for the small RNAs to induce silencing. As also three Piwi proteins are involved in the transgene silencing mechanisms, our analysis of all the involved components (RdRP, Dicer, Hen, Piwi) allows for a very precise characterization of the subsequent steps of trans acting siRNAs in *Paramecium*. Our experiments enable us to propose a step by step model for the transgene induced silencing machinery which serves as an easy to follow model for several trans acting mechanisms of gene expression e.g. Antigenic Variation.

Talk

Two highly specialised flagellates devouring freshwater algae**Hess Sebastian & Melkonian Michael**

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Several eukaryotic micro-organisms have a parasitic or parasitoid lifestyle and infect microalgae. In freshwater systems Chytridiomycota are well known to infest certain microalgae, but also unicellular protists are able to attack freshwater microalgae and are - similar to parasites - dependent on specific host algae. Since the 19th century scientists have described these protists and discussed their position in the system of life. Nowadays we don't know much more about such organisms, because they are difficult to cultivate, and many species have never been found again since their original description. Furthermore, some descriptions are doubtful, because the criteria for species definition used in the past differed from those we use today. Using modern techniques of isolation and culturing several strains of parasitoid flagellates devouring conjugating green algae have been established. Nine isolates of rhizarian affinities have been studied regarding morphology and ultrastructure, life history as well as their phylogenetic position using the nuclear-encoded SSU rDNA. Based on molecular phylogenies and feeding experiments it seems appropriate to create two new genera within a new family. These and their fascinating mechanisms of food acquisition will be presented in the talk.



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Talk

Studies on the phylogenetic relationships, morphology and autecology of non-acanthoecid choanoflagellates (Craspedida)

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Choanoflagellates are small heterotrophic flagellates with a ubiquitous distribution in marine and freshwater. They possess a single apical flagellum surrounded by a collar with microvilli. Choanoflagellates are of great evolutionary interest because of their close relationship to Metazoa and of great ecological importance due to their global distribution, their small cell size and significant impact on the food web as filter feeders on suspended bacteria. Currently choanoflagellates are classified into three families - Salpingoecidae (non-loricates), Acanthoecidae (nudiform loricates) and Stephanoecidae (tectiform loricates). Molecular data, mainly based on SSU rDNA, shows that on the one hand the phylogeny of loricate species is well defined and monophyletic families exist. On the other hand the former families of Salpingoecidae and Codosigidae, based on morphologic characters only, were abandoned as they were clearly not monophyletic. Here, we will present non-acanthoecid species mainly isolated from the River Rhine and in the course of worldwide sampling procedures. Their phylogeny, autecology and morphology have been studied to get new insights into their relationships.

Talk

Protistan plankton communities in transparent and glacier-fed turbid high mountain lakes (Alps and Himalaya)

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High mountain lakes, whether transparent or turbid glacier-fed ones, are well known extreme aquatic ecosystems where planktonic organisms live under harsh environmental conditions. Despite the ecological importance of protists as link to higher trophic levels, knowledge about their diversity in high mountain lakes is poor. In this study, we compared the protistan planktonic community structure (including Fungi) of two transparent and two glacier-fed turbid lakes from distinct geographical regions (Faselfad, Austria, Europe and Khumbu Valley, Nepal, Asia) using next generation sequencing approaches. First results show that the main protistan groups belong to the phyla Alveolata (53.9 %) and Stramenopila (35.5 %) followed by Cryptophyta (4.0 %), Viridiplantae (3.7 %), and Fungi (1.7 %). The representation of the phyla Centroheliozoa, Choanoflagellida, Rhizaria, Rhodophyta, and Telonemida were below 1%. A network analysis showed that the protistan diversity was 2.2-fold higher in the Himalayan lakes (HL) than in the Faselfad lakes (FAS) (number of taxa: FAS vs. HL: 81 vs. 118). Further, important differences in taxon composition were found between turbid and transparent lakes in both regions. In addition, taxon richness was higher in turbid than in transparent lakes. These results suggest that environmental conditions found in glacier-fed turbid lakes favor colonization by protistan species and that receding glaciers together with the resulting connectivity loss to lakes will cause a decrease in protistan biodiversity.

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Talk

The cascading effects of predation by planktivorous juvenile fish on the microbial loop

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In freshwater ecosystems the trophic interactions between meta- and protozooplankton can form an important linkage between the microbial and classical food webs. How this is regulated in shallow and turbid lake ecosystems is still poorly understood. Furthermore there is still lack of studies that consider the cascading effect of planktivorous fish to the microbial loop. The role of fish is often neglected because of difficulties of assessing their abundance and feeding rate. Our goal was therefore to study simultaneously planktivorous fish feeding and the feeding of metazooplankters and protozoans on bacteria. Concurrently we followed the seasonal dynamics of bacteria, protozoans, phytoplankton and metazooplankton. Our study was carried out in two natural fishponds: one was stocked with planktivorous whitefish while the other had no planktivorous fish at all. We hypothesized that in the ponds with planktivorous fish, there would be less metazooplankton, microbial loop is more active and bacterial numbers suppressed. Our results showed that the amount of metazooplankton eaten by the fish was significantly correlated with the metazooplankton biomass and the cascading effects were visible in the whole food web. In the fishless pond the role of the top predator shifted to the predaceous zooplankter *Leptodora kindtii*, which controlled the abundance of smaller metazooplankters. Opposite to our expectations the grazing on bacteria was more active in the absence of planktivorous fish, leading also to much lower bacterial abundances. The interactions between bacteria, microbial loop, metazooplankton and planktivorous fish will be discussed in more details.

Talk

Distribution of *Sphagnum*- and soil-dwelling testate amoebae in taiga (Russia)

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Distribution and ecological characteristics of protozoa in terrestrial ecosystems are important for a better understanding of ecosystem functioning and bioindication values of protozoan species. This is especially important for taiga ecosystems as they are expected to be among the most sensitive to climate variations. The aim of this work is to study the distribution and species composition of testate amoeba assemblages in *Sphagnum*-dominated and soil biotopes in the taiga of the European side of the Ural Mountains (Komi Republic, Russia). Samples for the analysis were collected in nine peatland ecosystems in the Pechora-Ilych Biosphere Reserve. The analysis of the samples revealed 123 testate amoebae taxa belonging to 23 genera. The number of taxa per sample varied from 2 to 28 (mean value 16.5 taxa). The most abundant species were *Hyalosphenia papilio* (11.1% of the total counts), *Trinema lineare* (7.7%), *Nebela tincta* (7.5%), *Acherella flavum* (5.8%), *Nebela militaris* (5.3%) and *Assulina muscorum* (4.9%). Some of these taxa (*A. muscorum*, *N. militaris*, *T. lineare*) and *Corythion dubium*, *Nebela collaris*, *Nebela parvula* were also characterized by high occurrence and were encountered in more than 50% of samples. Many taxa were rare (found in one sample only). The total density of testate amoebae varied from 0.3 to 52×10^3 ind. g⁻¹ of absolutely dry substrate (mean value 8.0×10^3 ind. g⁻¹). The results of a multivariate analysis indicate that most of the variation in the testate amoeba community structure was related to the hydrological gradient. Hydrophilous species *A. flavum*, *Physochilla tenella* were mostly found in *Sphagnum*-dominated biotopes, whereas xerophilous and ubiquitous species *T. lineare*, *Trinema complanatum*, *N. tincta* were encountered in wet forest biotopes. The study demonstrates that *Sphagnum*-dominated and soil biotopes in taiga are characterized by rich and diverse testate amoeba assemblages which are strongly regulated by substrate moisture.

Talk

Linkages between testate amoebae and vegetation communities along a poor- to extremely rich-fen gradient

Lamentowicz Łukasz^{1,2}, Jassey Vincent E. J.^{3,4}, Gąbka Maciej², Rusińska Anna⁵
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Ecology of testate amoebae in *Sphagnum* peatlands is actually well understood in comparison to those inhabiting minerotrophic fens. We studied the vegetation, testate amoebae and 14 physical-chemical variables of water (depth of the water table, pH, electrical conductivity, %O₂, O₂ mg l⁻¹, concentrations of ions Ca²⁺, Mg²⁺, Na⁺, K⁺, Fe, N-NH₄, N-NO₃, PO₄, SO₄ mg l⁻¹) along full poor to extremely rich gradient in western Poland fens. In this study we wanted to answer several questions: (i) how diversity of amoebae, mosses and vascular plants changes along full poor-rich gradient? (ii) does vegetation determine testate amoeba communities? (iii) how are testate amoebae, mosses and vascular plants related to measured physical-chemical properties of fen's water? We assessed relationships among biotic communities and abiotic variables along full poor-rich gradient using redundancy analysis (RDA), moving window analysis and multiple factor analysis (MFA). Abiotic variables explained a higher percentage of the species data for testate amoebae (20.45%) than for mosses (15.62%) and vascular plants (17.36%). MFA showed that mosses and environmental parameters have the most important influence on testate amoebae communities whereas vascular plants play the minor role. The moving-window indicated that variation explained by the physical-chemical variables and mosses tended to decrease along the poor-rich gradient for testate amoebae. The variation of the mosses and vascular plants explained by the environmental factors was very high at the beginning of the gradient, decreased in the middle and became higher at the end of the gradient. Diversity patterns differed between groups and the highest value of Shannon diversity was observed in moderate rich fens. Results of this study suggest that testate amoebae, vascular plants and mosses respond similarly to site conditions. Biotic groups are strictly dependent on content of minerals in the water. Macronutrients such as sulphur nitrate, ammonium and phosphorous have a minor importance.

Talk

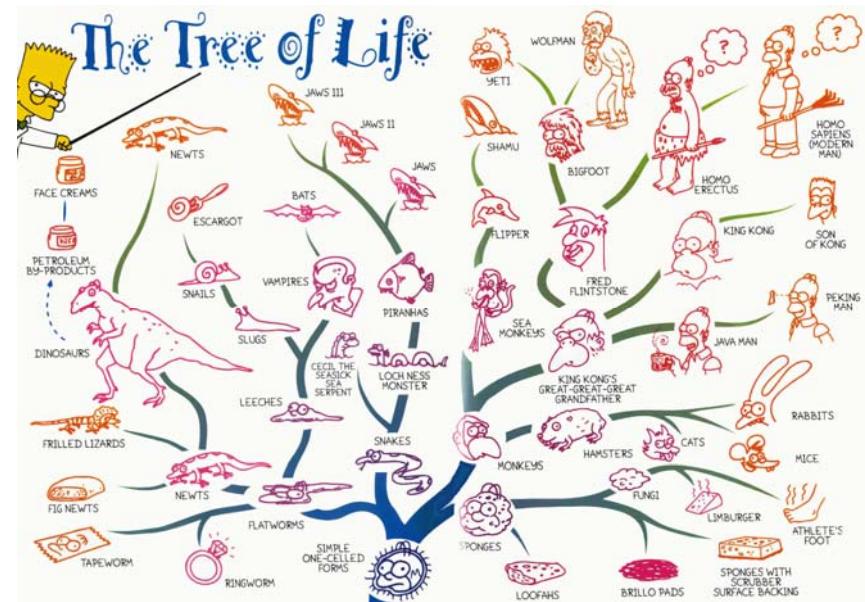
A new perspective on testate amoebae taxonomy and phylogeny

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Testate amoebae are conspicuous protists that were already known by naturalists more than two hundred years ago. However, their placement in the eukaryotic tree has only recently been clarified, and their true diversity remains unknown, largely because of the difficulties inherent to their culturing and molecular characterisation. Taxonomic uncertainty makes it impossible to estimate reliably local as well as global diversity of testate amoebae and to assess their biogeographic patterns. It also potentially undermines their use as bioindicators of past and present environmental conditions. Here, I present the latest results in testate amoebae phylogeny of both filose (i.e. mainly Euglyphida) and lobose (Arcellinida) testate amoebae. I also introduce the Amphitremidae, the first group of heterokont testate amoebae revealed.



Bart Simpson's Tree of Life, © Matt Groening

Talk

Response of soil testate amoebae to the presence of a decomposing pig cadaver – a study using morphological and molecular approaches

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Forensic estimation of post-mortem interval (PMI) the time since death is first based on medical and after ca. 2-3 days mainly entomological methods, which become less accurate over time and additional methods are required for periods of months to years. Cadaveric fluids represent a strong nutrient pulse to the soil environment. Soil physico-chemical characteristics and soil organisms therefore represent potentially useful indicators of PMI over long periods of time (months-years). We studied the response of testate amoebae to decomposing cadavers in two field experiments in deciduous forests, in Neuchâtel (Switzerland) and the Bavarian National Park (Germany), using pigs and roe deer, respectively with three treatments: 1) cadavers, 2) fake cadavers (bags simulating cadavers) and 3) control. We analysed litter/soil samples by 1) direct microscopy count (for both experiments – to 22 days and 280 days, respectively) and 2) high throughput sequencing: Illumina, V9-SSUrRNA of Eukaryotes, analysis for Euglyphida (Neuchâtel, to day 1051). The morphological studies showed that in both experiments testate amoebae were strongly affected by the cadavers. The percentage of dead shells increased from 16/65% (in CH/D experiments) to 70/90% at day 8 and 100%/100% at day 22/20 and until 119 days (D) where after 280 days ca. 5% encysted amoebae were found. The molecular study shows that cadaver effect on community composition increased until day 309 and after which (day 1051) the trend reversed. Ca. 90% of phylotypes responded negatively to cadavers but seven responded slightly to strongly positively. Soil testate amoebae are highly sensitive to decomposing cadavers over at least 2.5 years, well beyond the time when only bones remain and could be useful to estimate the PMI but also to indicate past presence of cadavers even without any remaining evidence. e.g. in cases where attempts are made to remove evidence of past crimes (e.g. in war zones).



Talk

Competition and nutrient dynamics among mixotrophic and heterotrophic ciliates

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Mixotrophic protists are relevant primary producers and consumers in planktonic food webs. So far, little is known about their stoichiometric requirements in terms of major nutrients, and whether these depend on their degree of mixotrophy. The present study investigated competition and nutrient dynamics in assemblages of a purely heterotrophic and two mixotrophic ciliates, differing in their degree of mixotrophy (i.e. one more heterotrophic, the other one more phototrophic). Ciliates were grown on microalgae reared under different nitrogen- and phosphorus-concentrations and ratios to investigate stoichiometric requirements of mixotrophs compared to heterotrophs, effects of food-quality on their performance and competitive behavior, and finally dynamics of dissolved nutrients in heterotrophic and mixotrophic assemblages. Depending on their primary nutritional mode, mixotrophs showed differences in their stoichiometry and their effects on nutrient turnover, among each other and compared to heterotrophs. One of the mixotrophs substantially hampered regeneration of dissolved nutrients and altered N:P ratios. Under nutrient depletion, its growth was enhanced by the presence of the heterotroph, presumably due to nutrient regeneration through heterotrophic feeding. This study provides first insights into the stoichiometric traits of mixotrophic microzooplankton and advances our understanding of mixotrophy and nutrient dynamics in planktonic food webs.

Talk

Comparison of similar Arctic and Antarctic morphotypes of heterotrophic protists regarding its genotypes and ecotypes

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The polar regions offer the opportunity to study possible diversification processes of spatially and temporally separated populations. We focused our study on sibling morphotypes/species of heterotrophic flagellates and ciliates originating from both, Antarctic and Arctic waters: 38 choanoflagellate morphotypes (*Acanthocorbis*, *Helgoeca*, *Diaphanoeca*, *Savillea*, *Stephanoeca*), four other flagellate morphotypes (*Cafeteria*, *Podomonas*, *Procryptobia*, *Protaspis*) and six ciliate taxa (*Holosticha*, *Uronema*, *Pseudocohnilembus*). We analysed similarities and differences regarding their genotypes (rDNA SSU) and for several species regarding morphotypes (light and/or SEM) and autecology (temperature and salinity tolerance). Most of the investigated polar isolates of protists were psychrophilic, adapted to coldwater, and showed a high salinity tolerance. Morphologically well defined acanthoecid choanoflagellates isolated from both poles showed the lowest intraspecific diversity (< 0.5% p-distance). No intragenomic polymorphism of SSU rDNA within one individual and among clones from one population occurred. The way of dispersal, especially for non-cyst forming species like acanthoecid choanoflagellates still remains unclear. Even under extreme stress of high temperatures and low salinity, none of the examined strains formed cysts. Single cell PCR appeared to be an appropriate method to investigate species not available as monoclonal cultures. As a prerequisite for barcoding, acanthoecid choanoflagellate species have a very low intraspecific variability regarding SSU rDNA. There was a clear correlation between autecological, morphological and molecular data sets, which may help interpreting molecular data from clone libraries or next generation sequencing.

Protistan predation on prokaryotic population in a meromictic lake

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Anoxic habitats can be found worldwide with a typical example being the deeper waters of stratified lakes. In such anoxic water bodies important biogeochemical processes are performed by prokaryotes. Protists are known to be important consumers of bacterioplankton affecting indirectly prokaryote-mediated processes. However, due to methodological difficulties, trophic interactions between prokaryotes and protists in anoxic water bodies are largely obscured. Therefore, to gain insights into the lacustrine microbial food web, we estimated phagotrophy of microbial eukaryotes in a meromictic lake (Alatsee, Germany). Short term grazing experiments using Fluorescently Labeled Prokaryotes (FLP) were conducted in the anoxic monimolimnion, the interface and the oxygenated mixolimnion. Subsamples were fixed at selected time points after the addition of FLP. Phagotrophy estimators were calculated in order to quantify total protistan grazing and to estimate the grazing impact of different groups. Bacterial removal rate was higher in the oxygenated mixolimnion, compared to the anoxic monimolimnion. Pigmented flagellates in the oxic layer showed the lowest grazing rate of all phagotropic protists but they accounted for ca. 70 % of total phagotrophy. The importance of mixotrophy as a survival strategy in the mixolimnion is indicated. In the aerobic microbial community predation seemed to play an important role on the ecosystem functioning. Bacterial removal rate for heterotrophic flagellates in the anoxic compartment was found to be as low as 0.37% out of the standing bacterial stock. Our results support the assumption that the impact of phagotrophic protists on prokaryote prey populations in anoxic water compartments is lower than in oxygenated ones, possibly due to their less efficient metabolism or alternative trophic modes.

Talk

An Arcellacea (testate lobose amoebae/thecamoebian) based transfer function for phosphorus in lakes

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Arcellacea communities were assessed from 73-sediment/water interface samples collected from lakes in urban and rural settings within the Greater Toronto Area (GTA), Ontario, Canada, as well as from forested control areas to the north. The results were used to: (1) develop a statistically rigorous arcellacean-based training set for sedimentary phosphorus (Olsen P (OP)) loading; and (2) derive a transfer function to reconstruct OP levels during the European settlement era using a chronologically well-constrained core from Haynes Lake on the environmentally sensitive Oak Ridges Moraine, within the GTA. Ordination analysis indicated that OP most influenced arcellacean assemblages, explaining 6.5% ($p<0.005$) of total variance. An improved training set where the influence of other important environmental variables (e.g. total organic carbon, total nitrogen, Mg) was reduced, comprised 40 samples from 31 lakes, and was used to construct the first published transfer function for lacustrine arcellaceans using tolerance down-weighted weighted averaging (WA-Tol) with inverse deshrinking (RMSEP_{jack}-77 pp; $r^2_{jack}=0.68$). The reconstruction indicates that OP levels remained near pre-settlement background levels from settlement in the late 1870's through to the early 1970's. A significant increase in inferred OP concentration beginning ~1972 may have been related to a change in crops (e.g corn production) in the catchment resulting in more runoff, and the introduction of chemical fertilizers. A dramatic decline in OP after ~1985 probably corresponds to a reduction in chemical fertilizer use related to advances in agronomy, which permitted a more precise control over required fertilizer application. Another significant increase in OP levels after ~1995 may have been related to the construction of a large golf course upslope of Haynes Lake in 1993, where significant fertilizer use is required to maintain the fairways. These results demonstrate that arcellaceans have great potential for reconstructing lake water geochemistry and will complement other proxies in paleolimnological research.

Talk

Mesoscale distribution and functional diversity of picoeukaryotes in the sea ice of the Canadian Arctic Archipelago during spring

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Sea ice, a characteristic feature of polar waters, is home to diverse microbial communities. Sea ice picoeukaryotes (unicellular eukaryotes with cell size $< 3 \mu\text{m}$) have received little attention compared to diatoms that dominate the spring bloom in Arctic sea ice. Here, we investigated the abundance of 12 picoeukaryotic groups at 13 sea ice stations distributed in Barrow Strait and in the vicinity of Cornwallis Island (McDougall Sound and Resolute Passage), Canadian Arctic Archipelago. We applied Catalysed Reporter Deposition - Fluorescence *in situ* Hybridization and specific oligonucleotide probes to identify selected groups at a single cell level. Marine stramenopiles from groups MAST-2 and MAST-6 were, for the first time, detected and enumerated in sea ice. Total numbers of picoeukaryotes, identified with probe Euk516, were significantly more abundant in the vicinity of Cornwallis Island than in Barrow Strait. Chlorophytes and cryptophytes were the dominant plastidic groups at all stations. Numbers of total picoeukaryotes, chlorophytes and MAST-2 stramenopiles were positively correlated with snow cover thickness. We found picoeukaryotes from various trophic groups: putative mixotrophs (chlorophytes, bolidophytes), mixotrophs (cryptophytes, haptophytes, pedinellids), phagotrophic heterotrophs (MAST-1, MAST-2 and MAST-6 stramenopiles) and parasitic heterotrophs (Amoebophryidae), showing the diverse ecological roles of picoeukaryotes in the sea ice. Yet, $> 50\%$ of total sea ice picoeukaryote cells remained unidentified with the applied probes, highlighting the need for further study of functional and phylogenetic sea ice diversity to elucidate the risks posed by on-going Arctic changes.

Talk

More proteins for a dynamic organelle – The contractile vacuole complex

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The contractile vacuole complex (CVC) of freshwater protists serves the osmotic equilibration of water and ions, notably Ca^{2+} , by chemiosmotic exploitation of a H^+ -gradient that is generated by an organelle-resident V-type H^+ -ATPase. Some molecular aspects are analyzed in more detail either in *Paramecium*, others in *Dictyostelium*, each of them contributing almost half of the CVC proteins reported, with a few additional data from other systems. In *Paramecium*, the major parts of the CVC contain v-/R-SNAREs (Synaptobrevins) and t-/Q-SNAREs (Syntaxins), in sum seven types. SNAREs are also reported anecdotally from the CVC of other systems. This reflects a multitude of membrane interactions and fusion processes in the organelle, complemented by Rab-type GTPases (shown in *Tetrahymena*) and exocyst components (*Chlamydomonas*). $\text{Ca}^{2+}/\text{H}^+$ -and other exchangers are to be postulated, as are aquaporins and mechanosensitive Ca^{2+} -channels. Some other CVC membrane proteins are characterized specifically in *Dictyostelium*. Altogether almost 100 CVC proteins are currently reported from all systems, but from the complexity of the organelle many more may be expected. Currently aspects of ongoing biogenetic processes, including vesicle delivery and epigenetic organelle positioning, are a particular challenge. The manifold regulators found in the CVC suggest the cooperation of different delivery mechanisms to maintain and replicate this complex organelle.

Talk

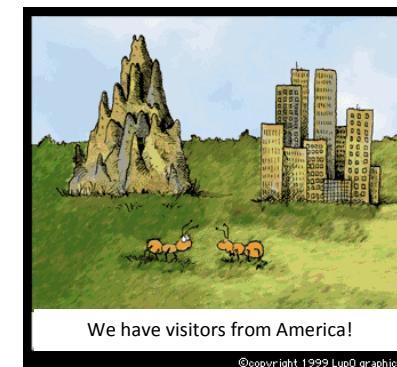
Hidden diversity of oxymonads in dry wood termites

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The hindgut of lower termites is inhabited by great numbers of symbiotic gut flagellates, which are indispensable for the digestion of cellulose and hemicellulose. A diverse community of prokaryotes contributes other physiological functions. Each termite species possesses a typical consortium of flagellate species. Most flagellates have only been described by light microscopy based upon a restricted number of visible characters. Especially small cells are very difficult to differentiate and are easily overlooked, particularly when they are attached to the gut wall. We analyzed the biodiversity of oxymonads from different dry wood termites and compared them to the known symbionts in these hosts by using light, scanning and transmission electron microscopy. Sequence analyses are in progress and can hopefully supplement the morphologic results. We most intensively analyzed the oxymonads from *Neotermes jouteli*. The only known oxymonad *Oxymonas jouteli* is described morphologically in detail and a second yet undescribed oxymonad from this host is a member of the genus *Opisthomitus*. The affiliation of *Opisthomitus* with the oxymonads was uncertain for a long time. We can clearly show oxymonad features. Light and scanning electron micrographs of flagellates from some further dry wood termites depict more hitherto unknown oxymonad morphotypes. In *Incisitermes tabogae*, where recently three small morphotypes of oxymonads (including an amoeboid form) were described, also three phylotypes clustering with the genus *Oxymonas* can be identified by sequence analysis of the SSU rDNA. Probably, many new species of termite flagellates await their detection.



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Talk

Can phagotrophic protists enhance microbial leaf litter processing at critical oxygen levels?

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In pools of fragmented streams, which receive high quantities of leaf litter due to increasing frequency and duration of drought periods, oxygen concentration drop to hypoxia repressing the performance of fungi involved in the decomposition of leaf litter. Since aquatic phagotrophic protists can stimulate organic matter processing under normoxic conditions we hypothesized that protists enhance the activity of leaf-associated microbial communities and the leaf decomposition also at critical oxygen levels. In microcosms, leaf discs (*Alnus glutinosa*) colonized by a multispecies bacterial community (enriched from a low order forested stream), a fungi (*Heliscus lugdunensis*) and a ciliate (*Glaucoma scintillans*) were exposed to normoxic and low oxic conditions during 105 days. Under both oxygen conditions, leaf mass loss was 1.75 times higher in treatments with ciliates (0.14 mg AFDM d⁻¹) compared to treatments without ciliates (0.08 mg AFDM d⁻¹). The leaf-associated community respiration was stimulated by the ciliate under normoxic conditions only. Similar as under normoxic conditions, our data indicate that phagotrophic protists stimulated the litter processing and energy flow under critical oxygen levels.

Talk

Arcellaceans (testate lobose amoebae) as bio-indicators of salt contamination in lakes: Insights from the Greater Toronto Region, Canada

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Arcellacea, or testate lobose amoebae, are unicellular protozoans that occur in fresh and brackish water environments. They are useful for palaeoenvironmental reconstruction because they are sensitive to a wide variety of environmental variables and because their tests are generally resistant to dissolution. In lakes, faunal assemblages are sensitive to many parameters, including substrate changes, nutrient loading, temperature change, salinity, pH and metal contamination. Few studies have, however, attempted to quantify the relationships which this predominantly benthic group of organisms have with specific environmental controls, or studied intra-lake variations in assemblage composition. We examined modern lake arcellacean faunas from lakes with a significant history of winter de-icing salt contamination in the Greater Toronto Area, together with lakes with reduced or no de-icing salt inputs from more rural settings, to: i) examine spatial responses to salt loading; and ii) develop an arcellacean-derived 'Salt Contamination Index' for assessing lake community response to salt contamination over several seasons. Key road-salt related water property (e.g. conductivity, Na, Mg and Cl ions) and sediment-based geochemical variables were measured. Detrended Correspondence Analysis (DCA) and Q-R-mode cluster analysis of the assemblages from 50 stations in 15 lakes resulted in recognition of four community groupings. The findings show that the group has considerable potential for elucidating the timing, scale and impacts of salt contamination in lakes and for delimiting pre-disturbance conditions, which are integral for remedial efforts.

Talk

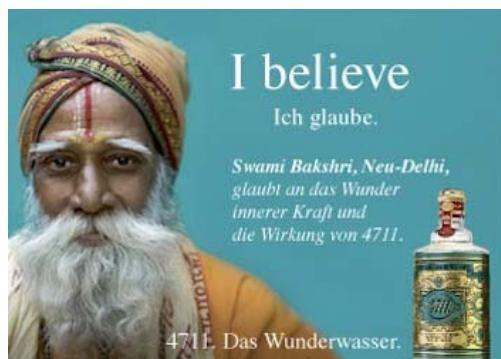
Long-term dynamics of ciliates in the plankton of River Rhine at Cologne

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During the last two decades, several investigations were run to assess the role of protozoans in the plankton of rivers (e.g. Lair et al. 1999, Weitere & Arndt 2004, Scherwass & Arndt 2005, Bergfeld et al. 2008). These studies pointed to an important role of protozoans in the food web of large rivers. However, less is known about long-term dynamics of protozoans in rivers. In the present study, dynamics of the abundance and taxonomic composition of ciliates in the plankton of the River Rhine at Cologne have been analyzed between 1998 and 2005 and compared to results of younger investigations (2010-2012). This comparison showed that the structure and abundance of ciliates partly differed between the years. Multivariate and correlation analysis were used to focus on the main influences affecting planktonic ciliates which will be presented and discussed in the talk.



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Talk

Caedibacter and Holospora: *Similarities and differences of Paramecium endosymbionts*

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Paramecium is well known for its ability to harbor many bacteria, in terms of total numbers as well as in bacterial diversity. Several *Paramecium* endosymbionts have been characterized, but *Caedibacter* and *Holospora* are the best studied. Both are Proteobacteria and, being obligate intracellular bacteria, completely dependent on their *Paramecium* host for reproduction. During the complex lifecycle of *Holospora*, the rod-shaped reproductive form differentiates into the infectious form with its peculiar morphology. In the case of *Caedibacter*, two different morphologies connected with different physiological functions are present as well. But whereas *Holospora* is generally considered a parasite, *Caedibacter* is associated with the killer trait of *Paramecium* and therefore often regarded as mutualist. During the past years, we studied different aspects of both endosymbionts including their effect on *Paramecium*, host range, intra-species variability, and evolutionary history. Although *Caedibacter* and *Holospora* are often considered as sister taxa, they co-evolved diverging with *Paramecium* and developed unique ways to ensure their maintenance within the host populations.

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Talk

Tetrahymena and Escherichia coli in interaction: A model for endosymbiosis in ciliates

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Endosymbiosis is a common phenomenon in nature, also among single celled organisms. Because of its diversity and its evolutionary importance it has been of great interest for many decades of research. In order to understand the mechanisms and conditions for a novel endosymbiosis in protists, laboratory models are required, because existing interactions show only the state and not the occurrence. Thus, we chose two experimentally convenient model organisms for interaction studies: *Tetrahymena pyriformis* and *Escherichia coli*. By microscopic analyses with a transformed fluorescent strain of *Escherichia coli*, containing the plasmid pSK-dsred, we detected single bacteria leaving food vacuoles and – in consequence – escaped digestion, which is an important requirement for further experiments. Under conditions, providing a selective advantage, *Tetrahymena pyriformis* was shown to internalize *Escherichia coli* cells for longer duration. Bacteria, transformed with the plasmids pBS-neoTet or pNeo4 provide *Tetrahymena pyriformis* with the ability to handle toxic conditions, caused by the amino-glycoside antibiotic Paromomycin in comparison with axenic cultures or cocultures with untransformed wild type bacteria after approximately one week of cocultivation. After exposure to Paromomycin containing media *Tetrahymena pyriformis* cocultured with transformant strains, show higher survival rates and grow to higher cell densities. The bacteria in the surrounding medium of cocultures die within one day after exposure to Paromomycin. PCR analysis reveals bacterial DNA even after two weeks of cocultivation in the presence of Paromomycin. The target DNA for these PCR experiments was shown to reside inside *Tetrahymena pyriformis*. Fluorescence microscopical analysis of selectively grown cocultures revealed particles in the cytoplasm of *Tetrahymena pyriformis* containing DNA as well as lipids, corresponding in size to *Escherichia coli* cells. In summary, the interaction of *Tetrahymena pyriformis* and *Escherichia coli* under conditions providing selective advantages for both partners, may serve as laboratory model for developing endosymbiosis in ciliates that offers good options for mechanistic investigations at genetic and biochemical levels.

Acknowledgement: We thank Dr. Kazufumi Mochizuki from Vienna for providing the parental plasmids containing the codon-optimized Paromomycin-resistant gene.

Talk

Planktonic ciliate ecology: Photoprotection and recovery strategies from damages induced by ultraviolet radiation

Sonntag Bettina (talking on Samstag)

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Three common mixotrophic *Chlorella*-bearing ciliates, i.e., *Pelagodileptus tracheliooides*, *Stokesia vernalis* and *Vorticella chlorellata* were investigated for their survival strategies under natural and increased ultraviolet radiation (UVR) intensities. For the three planktonic freshwater species, recently, natural sunscreen compounds synthesized by their algal symbionts were detected. These so-called mycosporine-like amino acids (MAAs) are colorless water-soluble compounds that absorb in the UV-B (280 – 320 nm) and the UV-A range (320 – 400 nm) and can protect an organism from severe (DNA) damages. Against this background, for the first time in ciliates, the assumed photoprotective function of MAAs was investigated in a series of laboratory experiments. First, ciliates survival was monitored after exposure to the full solar radiation spectrum and to photosynthetically active radiation (PAR) only against a dark control. Second, potential DNA-recovery strategies from UVR induced damages (dark repair, photoenzymatic repair) were tested. Third, to identify UVR wavelengths responsible for potential damages and mortality, a set of long-pass cut-off filters excluding specific wavelengths of the UV-B and UV-A spectrum were applied in experiments with *P. tracheliooides*. During all experiments, subsequently, the individual ciliates were monitored for changes in their behavior, shape and mortality. Although all ciliates seemed to be adapted to their natural UVR conditions they could not survive higher UVR doses and responses were species-specific. For example, *P. tracheliooides* was seriously damaged even during exposure to natural UVR conditions but was able to recover under PAR and in the dark. *Stokesia vernalis* and *V. chlorellata* were obviously not directly affected even by increased UVR levels but did not survive in the long-term or recover in the dark. Species-specific responses of the ciliates tested will be discussed and their photoprotective strategies elucidated.

Supported by the Austrian Science Fund, FWF project P21013-B03.



Talk

Immunolocalization of phospholipase C multigene family members in *Paramecium tetraurelia* indicates differential cellular localization and secretion of individual isoforms

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Phospholipase C (PLC) enzymes cleave phospholipids and are essential for eukaryotic cell physiology, especially by their role in the formation of the second messenger inositol 1,4,5-trisphosphate (IP_3) by cleavage of phosphatidylinositol 4,5-bisphosphate (PIP₂). To gain insight in which pathways PLCs are involved and to understand the diversification process which led to evolution of two subfamilies within ciliate PLCs, we describe here the immunolocalisation of four isoforms of phospholipase C in the ciliate *P. tetraurelia* by immunofluorescence, immuno-gold labeling and Western blots. Our results indicate differential localization of different isoforms. Immune fluorescence with antibodies against PLC2 and PLC6 indicate localization beneath the cell surface and electron microscopy verifies their localization close to alveolar sacs. Both isoforms as well as PLC3 also show localization close to the spongiome of the contractile vacuole complex (CVC). As both, alveolar sacs and the contractile vacuoles represent intracellular calcium stores, an involvement of the PLC isoforms in different calcium dependent signaling pathways appears possible. Moreover, localization of PLC2 and 6 appear not to be restricted to intracellular compartments, as immunofluorescence and electron microscopy also exhibit signals on the ciliary and non-ciliary cell membrane. Finally, the concentration of these isoforms in the culture medium suggests a secretory pathway. Surface localization and secretion fits to the phenotypes observed after RNAi analysis of these enzymes, as this results in reduced release of GPI-anchored surface proteins, thus providing a suitable explanation for their cleavage activity on surface exposed GPI-anchors. Our current results provide new insight into intracellular signaling in *Paramecium* as well as into cell-cell communication as we describe for the first time (i) localization of some PLCs to the CVC (known to harbor IP_3 -receptors) and (ii) surface localization and secretion of GPI-specific PLCs.

Talk

Isolated evolution of ciliate communities through environmental selection and geological chronology in deep-sea basins

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Deep hypersaline anoxic basins (DHABs) are isolated salt lakes at the bottom of the eastern Mediterranean Sea, which originate from the ancient dissolution of Messinian evaporites. The different basins share communities from the same source, but their geological evolution eventually constituted sharp environmental barriers, restricting genetic exchange between the individual basins. Therefore, DHABs are unique model systems to assess the effect of historical geological events and environmental conditions on the evolution and diversification of protistan plankton. We here examine evidence for isolated evolution of microeukaryote protistan plankton communities driven by geological and environmental selection. We pyrosequenced the hypervariable V4 fragment of the small subunit ribosomal RNA of ciliated protists as a major component of protistan DHAB plankton. Ciliate communities in the brines of four investigated DHABs are distinctively different from ciliate communities in the interfaces (haloclines) above the brines. The interface ciliate communities are relatively similar to each other. The brine ciliate communities are significantly different from each other. As expected, we found no distance-decay relationship, and canonical correspondence analyses identified oxygen and sodium as most important hydrochemical parameters explaining the partitioning of diversity between interface and brine ciliate communities. However, none of the analyzed hydrochemical parameters explained the significant differences between the individual brine ciliate communities. Our data indicate a frequent genetic exchange in the deep-sea water above the brines. The “isolated island character” of the different brines resulting from historical geological events and contemporary environmental conditions, build selective pressures driving evolutionary processes, and over time, support speciation and shape protistan community composition.

Talk

Ecology of *Sphagnum*-dwelling testate amoebae in the forest-steppe zone and development of a local transfer function for palaeohydrological reconstruction

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Quantitative palaeoreconstructions of hydrological regime in *Sphagnum*-dominated peatlands at local level can significantly contribute to a better understanding of climate dynamic at a bigger scale and ecosystem responses to climatic variations. The reconstructions can be achieved by using transfer functions based on ecological preferences of testate amoebae. The aims of this work are (i) to study ecology of *Sphagnum*-dwelling testate amoebae in peatlands located in the forest-steppe zone and (ii) to model the relationships between testate amoebae and hydrological conditions in order to build a transfer function which can be used for palaeohydrological reconstructions in this area. Samples for the testate amoeba analysis were collected in eleven *Sphagnum*-dominated bogs located in the forest-steppe zone (Tula and Orel regions, Russia). At each sampling site, we measured the depth to the water table, pH, and mineralization of water. The analysis of the samples revealed 58 testate amoeba taxa belonging to 16 genera. Most of the changes in the species composition of the testate amoeba assemblages could be explained by the depth to the water table and pH. To model the relationships between these environmental variables and testate amoebae we calculated optimum and tolerance values for each species. These data can be further used for palaeoreconstructions of hydrological regimes in peatlands located in the forest-steppe zone.

Talk

Contrasting life strategies of two common ciliates from tank bromeliads

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This study investigates the ecology and life strategy of two recently described ciliate species from tank bromeliads. We conducted laboratory experiments to assess the response of the ciliates to food, pH, and competition. *Glaucostoma bromelicola* (family Bromeliophryidae) is the most common ciliate in the reservoirs (tanks) of bromeliads. In contrast to the coexisting species *Bromeliothrix metopoides* and many other colpodean ciliates, *G. bromelicola* does not form resting cysts, which jeopardizes this ciliate when its small aquatic habitats dry out. Both ciliates form bacterivorous microstomes and flagellate feeding macrostomes. However, only *G. bromelicola* has a low feeding threshold and is able to adapt to different protist food. The higher affinity to the local bacterial and flagellate food renders it the superior competitor relative to *B. metopoides*. Continuous encystment and excystment of the latter enable stable coexistence of both species in their natural habitat. Both species are tolerant to a wide range of pH (4-9). These ciliates appear to be limited to tank bromeliads because they either lack resting cysts (*G. bromelicola*) or have highly specific food requirements (*B. metopoides*).

Talk

**The relative importance of metazooplankters and planktivorous fish
in regulating the community of planktonic ciliates
in a shallow eutrophic lake**

Zingel Priit, Agasild Helen, Karus Katrit, Kangro Kersti, Salujõe Jaana & Nõges

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In freshwater ecosystems the trophic interactions between metazooplankton and ciliates can form an important linkage between the microbial and classical food webs. How this is regulated in shallow and turbid lake ecosystems is still poorly understood. In large, shallow and turbid eutrophic Lake Võrtsjärv (270 km², 2.8 m) the ciliate community is characterized by high abundances, species richness and with biomasses that usually exceed that of metazooplankton. Previous research has demonstrated the prevalence of microbial loop in food web of Lake Võrtsjärv and the great importance of planktonic ciliates in it. In current study we investigated the effect of small-sized crustacean zooplankton on ciliate abundance and community structure and on microbial loop organisms. Our hypothesis was that ciliate abundance is not directly controlled by metazooplankton grazing in a shallow eutrophic lake. We used both enclosure (2009) and mesocosm experiments (2010). We removed larger crustaceans in enclosure experiments and enriched mesocosms contrariwise with copepods and cladocerans. Both sets of experiments revealed only modest crustacean predatory impact on planktonic ciliate abundances and the dominant role of interactions within the ciliate community. We also assessed feeding of planktivorous fish larvae on ciliate protozooplankton (2005). Our study revealed that fish consume only minor part of planktonic ciliate abundances and cannot control their numbers. The relative importance of metazooplankters and fish in predation on planktonic ciliates and the relationships between different ciliate groups will be discussed in more details.

Posters

In alphabetical order by first author

Poster P1

Live labelling of natural planktonic ciliates – a method for studying the ciliate-crustacean trophic interactions in aquatic systems

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Planktonic ciliates form an abundant group of organisms in aquatic systems and constitute an important link between lower and higher trophic levels in pelagic food webs. Studying of feeding interactions and predation rates between planktonic ciliates and their potential predators, such as metazoan zooplankton, is however, often problematic. Various types of food tracers, such as radioactively and fluorescently labelled cells and artificial fluorescent particles have been used to directly estimate zooplankton grazing on phytoplankton and bacteria. Planktonic ciliates are actively moving organisms that make the common application of food tracer techniques complicated. Here the live labelling technique with fluorescent microparticles offers an advantageous solution to directly measure the feeding rates on planktonic ciliates without changing the characteristics of their motile living cells in grazing experiment. We used the live labelled natural assemblage of microciliates to measure the crustacean feeding rates on planktonic ciliates in two shallow eutrophic lakes in Estonia. The method revealed that the ciliates were ingested by all dominant cladoceran and copepod species in the studied lakes. The ciliate – crustacean trophic link formed a significant carbon flow from microbial loop to higher trophic level in food webs in these lakes.



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Poster P2

Predator-prey interactions induce rapid adaptation in a freshwater bacterial isolate

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The foraging of size-selective protistan grazers is an important factor in the aquatic environment that shifts bacterial community composition towards protected species and might select for specific adaptations in bacteria. As a result, flagellates continuously influence the evolution of bacteria, and bacterial genotypes with increased antipredator fitness should evolve. The formation of aggregates is one such strategy that increases the ability to escape flagellate predation. We investigated the adaptation of an aggregate forming bacterium isolated from Lake Zurich, *Sphingobium* sp. Z007, to constant grazing pressure by the bacterivorous flagellate *Poterioochromonas* sp. strain DS. To assess evolutionary changes of the bacterial strain we ran a long-term experiment with semi-continuous batch cocultures of predator and prey in oligotrophic medium for a period of several months. Phenotypic shifts, such as the ability of aggregate formation, biofilm development, and substrate degrading abilities of *Sphingobium* strains evolved with and without flagellates were examined every six weeks. Strains evolved under predation pressure showed a continuous increase in aggregate formation. This putative fitness gain in coexistence with *Poterioochromonas* was, however, accompanied by lower growth rates and cell densities compared to strains evolved without predation pressure. These results are pointing at the importance of protistan predation as a driver of evolutionary changes in aquatic bacteria.

Poster P3

Comparative analysis of direction dependent photokinesis in ciliates**Bechert Jan & Bräucker Richard***Deutsches Zentrum für Luft- und Raumfahrt / DLR_School_Lab Köln, 51147 Köln, Germany**jan.bechert@dlr.de*

It is obvious that even for unicellular organisms the perception of direction and intensity of a stimulus is a crucial phylogenetical and ecological factor. In ciliates, stimulus perception can be studied by analyzing the swimming behavior, due to the electromotor coupling (distinct relationship between membrane potential and ciliary movement). We examined four species to gain insight in their photobehavior: *Paramecium aurelia*, *Paramecium caudatum*, *Didinium nasutum* and *Ophryoglena flava*. The cells were illuminated unidirectional using different combinations of wave-length and light-intensity. All species showed photoreactions at every stimulus combinations applied (more than 1.2 million measurements). Commonly, a kinesis is defined as a change in locomotion velocity dependent on the intensity of a stimulus, but independent from its direction. So far, gravikinesis has been a generally accepted exception, because it is affected by the intensity as well as the direction of the acceleration vector. In this work we show that also photokinesis is dependent on stimulus direction. The cells showed a maximum photoreaction when the direction of light differed 40° from the cells' centerlines. In *Ophryoglena flava*, the organelle of Lieberkühn has been assumed to be involved in photoperception acting as a shading device. This is supported by our findings considering the position of Lieberkühn's organelle. We hypothesize that in the other species the nucleus functions as a structure shading the photoreceptor. The development of taxes (as a result of kinesis and orientation) seems to require asymmetric structures, such as shading devices or unequal distributions of masses or receptors. It would be interesting to investigate whether direction dependent kineses can be found for more stimuli in ciliates.

Poster P4

Underestimated diversity of *Symbiodinium* (Dinozoa) as zooxanthellae in *Phyllodesmium* (Gastropoda)**Bleidißel Sabrina & Preisfeld Gela***Institute of Biology - Zoology and Didactics of Biology, Bergische University Wuppertal, 42119 Wuppertal, Germany**bleidiss@uni-wuppertal.de*

The genus *Phyllodesmium* (Gastropoda, Aeolidida) is well known to host symbiotic photosynthetic dinoflagellates of the genus *Symbiodinium* (Alveolata, Dinozoa). While these dinoflagellates mostly occur free living in fresh water and marine habitats, some are known to exist as endosymbionts (zooxanthellae) in reef-building corals and in other invertebrates nutritionally reliant on corals, for example in the coral feeding sea slug *Phyllodesmium*. During the digestion of the corals by the slug *Phyllodesmium* the *Symbiodinium* cells nested as zooxanthellae within the corals remain embedded functionally intact in the cerata tissue of the slug. Even those incorporated *Symbiodinium* maintain fully functional plastids to perform photosynthesis that can be measured by PAM (pulse amplitude modulated fluorometry). Although distribution of *Symbiodinium* is well studied in corals, very little is known about the diversity within the genus *Phyllodesmium*. Due to extremely restricted morphological and ultrastructural synapomorphies the genus *Symbiodinium* is divided exclusively by molecular data into 9 clades named A to I. So far only *Symbiodinium* clade C and A have been isolated and molecularly identified as zooxanthellae in the genus *Phyllodesmium* and in the sea slug *Pteraeolidia ianthina*. To investigate whether more of the expected clades can be detected in *Phyllodesmium* different methods less likely to reveal only clades with a majority number of species have been employed. Our results show that implementation of new molecular methods like highly sensitive qPCR analysis in the genus *Phyllodesmium* can be used successfully to detect less abundant clades.

Poster P5

Is highly sensitive qPCR an adequate method to measure the purity of isolated chloroplast DNA from phototrophic euglenid cells?

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Chloroplast genomes of *Euglena gracilis*, a model organism for phototrophic euglenids, and *Euglena longa*, a secondary osmotrophic euglenid, have been published in 1993 and 2000, respectively. Recently, the complete chloroplast genome of *Eutreptiella gymnastica* and the nearly complete chloroplast genome of *Eutreptia viridis* sequenced by 454-sequencing were published. For phylogenetic implications of plastid genome evolution in euglenids, it seems vital to collect data from taxa of other clades, linking primordial species with derived taxa. This study aims to sequence complete plastid genomes of photosynthetic euglenids, in order to obtain pure plastid DNA, our first approach was to isolate intact chloroplasts from whole cells in contrast to recently published works where total DNA was used. Initially, chloroplasts have been separated from cells by ultrasonic waves, followed by density gradient centrifugation. Intact chloroplasts of different species could be eluted and rinsed for further DNA isolation. For subsequent next generation sequencing it was necessary to apply highly purified plastid DNA. Contamination with nuclear and/or mitochondrial DNA can lead to ambiguous data. To determine the purity of plastid DNA or – in lack of purity - the amount of chloroplast DNA relative to contamination by nuclear DNA and total DNA a quantitative real time PCR approach was used with specific primers for chloroplast rbcL genes and nuclear rbcS genes. Both genes encode for ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO). RbcL encodes the large subunits of the protein and is plastid located whereas rbcS encodes the small subunit and is located in the nucleus. Here we present our first results on the measurement of plastid DNA of two phototrophic euglenids by qPCR.

Poster P6

Free living amoebae as reservoirs and training grounds for *Legionellae* – Are VBNC *Legionellae* directly able to infect human host cells?

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Freeliving amoebae are known to be reservoirs and vehicles for a wide range of bacteria, amongst others, important human pathogens like *Legionella pneumophila*. Under poor nutrient conditions, *L. pneumophila* is not able to grow in the absence of amoebae. In some cases, like after disinfection, they can even enter the viable but non culturable (VBNC) state. If a formerly nonculturable *Legionella* strain enters a host cell, which is normally an amoeba, it may become resuscitated and culturable again. Furthermore it has been shown, that pathogenic legionellae become more invasive for human macrophages after intracellular replication in amoebae. Our aim is to provide a comprehensive overview over the VBNC-legionellae, investigating their infectious potential.

Poster P7

Next-Generation-Sequencing of Protist Community Structures

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To understand the relationship between biodiversity and ecosystem function is a key question to ecologists. Recent research was done to identify protozoan community structures of various ecosystems. Different methods lead to different results concerning the amount of data and the force of expression. Morphological determination of protists in combination with PCR techniques enables the identification and description of species on an elaborate level. Nevertheless, morphological techniques are time consuming and isolation of organisms is highly selective. Therefore, it is likely that the usage of only morphological approaches highly underestimated the diversity of protists so far. Classical determination of protist species however is the foundation for further genetic approaches and is therefore indispensable. Recently, more and more molecular techniques (e.g. clone libraries and Next-Generation-Sequencing) could also be established to identify community structures in environmental samples producing increasing data output. We analyzed community structures of globally distributed protists, using Next-Generation-Sequencing (NGS). NGS techniques deliver thousands of sequence reads in a single sequencing run. The resulting huge datasets should enable detailed studies of community structures including even rare organisms. Yet, sequence reads can differ in length and quality and several steps of quality control had to be included in every sequence analysis. This excludes low quality sequences and increases the reliability of the estimations. We were able to analyze all data at least to the super-group level. Additionally, we aligned the DNA sequences of rhizarians and kinetoplastids obtained by morphological studies combined with PCR techniques to the sequences obtained by NGS. Hereby we were able to compare the outcome of morphological and molecular/PCR techniques.

Poster P8

Feeding on their competitor's remains: Grazing resistant freshwater bacteria profit from organic carbon possibly released through protistan foraging

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The rise of grazing resistant planktonic bacteria in freshwater lakes during vernal phytoplankton blooms is favoured by the high predation pressure from heterotrophic nanoflagellates (HNF). At the same time, the spring period is also characterized by the availability of organic carbon species that are in parts derived from cellular debris generated during bacterivory or viral lysis, such as peptidoglycan, chitin and their subunit N-acetylglucosamine (NAG). We tested the hypothesis that two dominant groups of grazing resistant bacteria, ac1 tribe of *Actinobacteria* (ac1) and filamentous bacteria from the LD2 lineage (*Saprospiraceae*), would profit from such carbon sources during periods of intense HNF predation. The abundances of ac1 and LD2 rose in parallel with the HNF population, and disproportionately high fractions of cells from both bacterial lineages were involved in NAG uptake. Members of ac1 and LD2 were significantly (albeit transiently) more enriched after NAG addition than in control incubations. However, highest growth rates of both bacterial lineages were found on the amino sugar containing polymers chitin and peptidoglycan. Moreover, the direct or indirect transfer of organic carbon from peptidoglycan to LD2 filaments could be demonstrated. We thus provide evidence that these taxa may benefit twofold from HNF predation: by direct removal of their competitors, and by specific physiological adaptations to utilize carbon sources that are released during protistan grazing or viral lysis.

Poster P9

Cyst formation by Chrysophyceae**Fegers Kathrin, Jost Steffen & Boenigk Jens***University of Duisburg-Essen, Allgemeine Botanik, 45117 Essen, Germany**kathrin.fegers@gmx.de*

The formation of cysts of the heterotrophic chrysomonad strains AR4A6, JBC/S23, NIES-1846, *Spumella vulgaris* and *Pedospumella encystans* was investigated as a function of bacterial food concentration. Differences in encystment patterns were studied by determining abundances of vegetative cells, bacteria and cysts. No differences were observed with respect to growth and mortality rates of the vegetative cells. All investigated heterotrophic strains built cysts but patterns of cyst formation differed between strains. The strains JBC/S23 and *Spumella vulgaris* showed the highest gross cyst formation rates ($0.113 \text{ h}^{-1} \text{ ml}^{-1}$ and $0.109 \text{ h}^{-1} \text{ ml}^{-1}$) but it seems they started encystment only after the vegetative cells began to die back resulting in a moderate number of total cysts produced (719 ml^{-1} and 138 ml^{-1}). In contrast the strains *Pedospumella encystans* and NIES-1846 built cysts only at moderate maximal gross cyst formation rates ($0.047 \text{ h}^{-1} \text{ ml}^{-1}$ and $0.054 \text{ h}^{-1} \text{ ml}^{-1}$) but did so from early on therefore yielding at high numbers of cysts produced (1847 ml^{-1} and 2405 ml^{-1}). The strain AR4A6 did hardly build cysts. Cyst formation started in all strains at the calculated reference time. Whereas in JBC/S23 and 199hm the individual (per capita) rate of cyst formation became as low as approximately $-12 \text{ cell}^{-1} \text{ ml}^{-1}$ and $-15 \text{ cell}^{-1} \text{ ml}^{-1}$ while growing at satiating food conditions, the strains JBM/S11 and NIES-1846 increased the per capita rate continuously throughout the experiment from the beginning. This may indicate that the strains JBC/S23 and 199hm require more stable environments, while JBM/S11 and NIES-1846 are able to survive in habitats with strong and quick fluctuations of abiotic factors.

Poster P10

Effect of the bacterial parasite *Holospora caryophila* on the fitness of *Paramecium biaurelia***Grosser Katrin, Koehler Lars, Schrallhammer Martina & Berendonk Thomas U.***Institute of Hydrobiology, 01217 Dresden, Germany**katrin.tf@t-online.de*

The endosymbiont *Holospora* belongs to the subphylum Alphaproteobacteria and lives in the nucleus of *Paramecium*. Probably due to a reduced genome size, *Holospora* cannot live outside of its host and infects either the macro- or micronucleus. It prefers as obligate intracellular endosymbiont in general one *Paramecium* species and is therefore host and nucleus specific. In the present work, we analyzed the effect of *Holospora caryophila* on the fitness of different *Paramecium biaurelia* strains. The growth rate and carrying capacity were used as fitness factors. The effect of *H. caryophila* on *P. biaurelia* was tested in a growth experiment. We used chronically infected strains and cured lines received from these strains by antibiotic treatment. Additionally, we used not infected strains and, applying experimental infection, we obtained the corresponding lines carrying *H. caryophila*. Two out of four used *P. biaurelia* strains could be infected with a prevalence of approximately 100 %. The other two strains could not be infected or only with a very low prevalence. Chronically infected *Paramecium* cells show a statistically significant higher growth rate than without *H. caryophila*. The parasite's impact on growth rate is not unambiguous for acute infected paramecia. On the other hand, the carrying capacity from acute and chronically infected paramecia is lower than the carrying capacity from not infected *P. biaurelia*. Our results show that *P. biaurelia* is limited by *H. caryophila* in the carrying capacity. But during the exponential growth phase, the endosymbiont provides a statistically significant advantage for the host.

Poster P11

Metagenomic Analysis of Soil Microbial Communities

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The linking of microbial communities to function with particular reference to their environment is an unresolved interesting question. We analyse and compare different unassembled metagenomic sequences of terrestrial environments by using the database KEGG (Kyoto Encyclopedia of Genes and Genomics) and the published sequences of soil microbial communities on JGI (Joint Genome Institute) focusing on eukaryotic cells. The frequency of expressed genes reflects the characteristics of the environment and gives us new indications for future genome analyses and genome correlations. The focus is on functional genes with a high frequency. We search for a new indicator of relationships between microbial communities. Further by making a comparison between the published data and our own samples from Europe we analyse and interpret the differences of the habitats.

Poster P12

An automated water sampler, plankton communities, and long-term data comparison

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The **Continuous Plankton Recorder (CPR)** is a plankton sampling instrument that has been towed from ferries and merchant ships across the North Atlantic since the 1930s. From sub-surface waters the 270 µm mesh in the CPR collects organisms that are preserved in formalin and were, until recently, only visually identified by taxonomists of the Sir Alistair Hardy Foundation for Ocean Science (SAHFOS). This changed with the development of novel DNA extraction methods that enabled the formalin-preserved samples to be molecularly analysed. In addition a new instrument, the **Water and Microplankton Sampler (WaMS)**, has been engineered that can be mounted together with the CPR. WaMS is able to collect organisms <270 µm, thus adding to the data from CPR and thereby providing a more accurate picture of plankton diversity and abundance. We pyrosequenced (Roche 454) a fragment of the 18S rDNA from WaMS samples and compared the data to taxonomic identification from CPR samples. We expected some overlap in taxa distribution, but also differences in diversity according to size classes. The 454 investigation of WaMS samples revealed a striking variation in community composition, novel taxa were discovered, and previously unreported pathogenic organisms were identified. Unexpectedly, data from WaMS and CPR matched in 68% of taxonomic classes, independent of size classes ranging from picoplankton to zooplankton. We conclude that the CPR-WaMS is a versatile autonomous plankton sampler that can provide vital information on the distribution of very diverse planktonic organisms over spatial and temporal scales, and thus on changes in ecosystem health and functionality.

Poster P13

Morphology and morphogenesis of a *Psilotricha*-like ciliate from Hawaii

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The genus *Psilotricha* was described by Stein in 1859. Since then, there was some confusion about the species and its systematic position because the original description lacks information on the dorsal infraciliature and the ontogenetics. The present study describes a new *Psilotricha*-like species from Hawaii, using live observation, protargol impregnation and, for the first time, scanning electron microscopy of morphostatic and dividing specimens. The Hawaiian species has a size of about 65 × 45 µm in vivo and is broadly obovate. The most characteristic feature is an about 30 µm long left marginal cirrus on the acute posterior body end. The nuclear apparatus is composed of two macronuclear nodules and one micronucleus in between. The cortex is very rigid, as, for example, in *Euplotes*. There is an average of 23 cirri in four ventral rows, one postoral row, and one left and one right marginal row; frontal, buccal, and transverse cirri are absent. The adoral zone occupies about 43 % of body length and is on average composed of 21 ordinary, wide-spaced membranelles. The dorsal infraciliature is composed of about 33 bristles in three kinetics; caudal cirri are absent. The ontogenesis is hypoapokinetal, i. e., starts with the *de novo* formation of the oral primordium on the cell surface followed by an invagination into a subcortical pouch close to the buccal vertex. The proter undulating membranes reorganise but do not produce cirri. The postoral row is formed by terminal segregation of the second right ventral row as in *Psilotricha succisa* (Foissner 1983), indicating a close relationship. In spite of several attempts, the systematic position of the Psilotrichidae remains unknown. The rigid cortex and the hypoapokinetal stomatogenesis suggest some relationship with euplotids. Molecular data are needed!

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Poster P14

Ecology of vascular plant, bryophyte and testate amoeba communities in four *Sphagnum* peatlands along an altitudinal gradient in Switzerland

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Climate change is causing shifts in ecosystem structure (communities) and functioning (e.g. carbon cycling) that may directly feed back to warming in the case of carbon-rich ecosystems such as peatlands. Monitoring tools are therefore needed to assess these changes. Most research on global change impact on terrestrial communities focuses on vascular plants, but the responses of soil organisms and plants to ecological gradients and environmental change do not always correlate. Testate amoebae are important contributors to carbon and nitrogen cycling in soils and are useful bioindicators in ecology and paleoecology. Underlying assumptions for their use in bioindication are that their ecological optima are constant through space and time. However this has not been studied much. This study aims at 1) comparing the relationships of testate amoebae, bryophytes, and vascular plants with the main ecological drivers of community structure in four peatlands along an altitudinal gradient and thus their predictive power for these variables and 2) assessing how the structure of these three communities discriminate different micro-habitats along a humidity gradient. We studied the vascular plant, bryophyte and testate amoeba (selected taxa only) communities along the humidity gradient from hollow to hummock in four *Sphagnum*-dominated peatlands from 580 m to 1890 m a.s.l. in Switzerland in relation to soil temperature, water table depth and the carbon and nitrogen content of *Sphagnum* mosses. Indirect and direct gradient ordinations and multiple factor analysis showed that a selection of only ten testate amoeba taxa are more powerful (% variance explained) and accurate (discrimination among habitats) indicators of local conditions (micro-habitat type, moisture and *Sphagnum* elemental chemistry) than the vegetation (vascular plants and bryophytes individually or combined, even when the full vascular plant diversity was considered), and that they better reveal for the status (i.e. pristine vs. human-impacted) of bogs.

Poster P15

How does fertilization affect density, diversity and community structure of testate amoebae of montane tropical rainforests?

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Human activities increase the input of nutrients into natural ecosystems. Tropical montane rainforests are among the most species-rich ecosystems of the world and nutrient inputs threaten their diversity. Knowledge on the magnitude and direction of changes due N and P deposition is lacking. Therefore, we investigated the response of microorganisms and testate amoebae of old-growth montane rainforests of the Andes of southern Ecuador to moderate addition of N and P such as those predicted by anthropogenic change scenarios. Addition of P negatively affected live testate amoebae with the density and species number being reduced by 26% and 12%, respectively. In contrast to P, the addition of N increased the density of testate amoebae on average by 22%. However, the combined addition of P and N negated the positive effect of N but only at 1000 and 2000 m. At 1000 m testate amoebae already responded after one year of nutrient addition with changes in community structure to the addition of N, whereas at 2000 and 3000 m the response to nutrient addition was slower and community structure responded to P and N+P addition. The results suggest that even moderate and short-term addition of nutrients alters the density, species richness and community structure of testate amoebae.

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Poster P16

GBIF-D Algae & Protozoa: Portal for biodiversity data using BioCASE technology

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The Global Biodiversity Information Facility (<http://data.gbif.org>) is a global network of 59 countries and 47 organizations providing standardised observation and specimen data via the Internet to give open access to our knowledge of biodiversity. Currently more than 383 million records provided by 456 data publishers are available within the data network.

7.4 million records are attributed to algae and protozoa, thus GBIF Germany has set up a special interest portal filtering on these organism groups. GBIF-D Algae & Protozoa Portal (<http://protists.gbif.de/protists>) is using BioCASE technology (Biological Collection Access Service, www.biocase.org) to display GBIF data. Search functionality includes scientific names, higher ranks, images, types, collectors, and localities. Additional to the GBIF Data Portal (<http://data.gbif.org>) it displays partly live requested data from data providers including metadata, such as contact data for data sources, IPR statements, etc.

GBIF data are being used for teaching and research. Partly (e. g. Pangaea, www.pangaea.de/, AlgaTerra, www.algaterra.org) original data have been made available in GBIF and is cross referenced with scientific articles on environmental studies, taxonomy, and molecular barcoding. The vision is to present a complete overview of biodiversity, including data from citizen science to applied and basic research.

GBIF-D Plants, Algae and Protists (www.gbif.de) is funded by the Federal Ministry of Education and Research (BMBF, grant 01 LI 1001 A-F). The aim is to extend the quantity and quality of data as well as to raise the value of those data for re-usage.

Poster P17

Gold-FISH on protists: Combining *in situ* hybridization with scanning electron microscopy for identification

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The identification of unculturable microorganisms has always been a key point in characterising ecosystems and a challenge at the same time. With the rise of molecular methods, increasingly more studies use short molecular probes with a fluorescent tag targeting the small ribosomal subunit (SSU). With this fluorescent *in situ* hybridization (FISH), targeted prokaryotic and eukaryotic microbial cells can be quantified and characterised morphologically to some extent. While this technique in itself is very reliable, only basic information on cell morphology can be obtained – thus restricting identification. To circumvent this limitation, we applied FISH with deposition of a catalyzed reporter molecule and a nanogold-streptavidin-conjugate on cultures of protists and environmental samples (Gold-FISH). Using a nanogold-labeled marker enables us to detect the signal of our hybridized probe with EDX (energy dispersive X-ray spectroscopy) detectors in scanning electron microscopes. In combination with proper fixation and hybridization protocols, the surface morphology of targeted cells can be seen in high resolution and detailed descriptions can be made. This technique can help tremendously in the identification of protists in environmental samples so far only known from sequence data, such as the enigmatic MAST-clade of stramenopiles or other nanoflagellates. Furthermore, it can help in identifying and characterising cryptic species and species complexes. We present a working protocol for the identification of microbial eukaryotes in environmental samples, using a combination of Gold-FISH, proper fixation for scanning electron microscopy and subsequent detection of gold-signals.



Poster P18

Testing the response of peatland testate amoebae to in-situ water table changes

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Testate amoebae (TA) are commonly used as hydrological indicators in paleoecological studies of peatlands. However, their response to hydrological changes has never been tested experimentally. We tested the response of TA to changes in water table depth in a raised bog in the French Jura Mountains. Three trenches were dug out to create replicated hydrological gradients. Intact *Sphagnum* carpets of different origin (hummock, lawn, pool) were collected from the same peatland and placed at three different water table positions (dry, moist, wet) in August 2008. A second series of samples was seeded with microorganisms extracted from the three sampling situations to provide a complete community of all micro-sites. TA communities of all plots were analysed in August 2008, May 2009 and August 2009. We expected responses in terms of diversity and community composition to hydrological manipulations to be the strongest in the most contrasted combination – transplantation of *Sphagnum* from pool to dry situation and hummock to wet. The density and community structure of TA changed over time. The lowest density of living communities was observed in May 2009 (71.6 ind mg^{-1}) and highest in August 2009 ($712.1 \text{ ind mg}^{-1}$). Species richness and Shannon H significantly declined over time. However, for each contrasted combination these changes were not unequivocal. There were no significant changes in species richness, whereas in Shannon H we recorded significant drop of diversity for hummock in wet situation and no effect for pool in dry situation. These results suggest that pool TA species tolerate dry conditions better than hummock species tolerate wet conditions, which would be quite counter-intuitive. More studies are needed to investigate the response of TA to environmental variables, so they can be more efficiently used as bioindicators and proxy in palaeoecological studies.

Poster P19

Effect of experimental water table depth manipulation on peatland testate amoeba community structure and on pore number of *Hyalosphenia papilio* Leidy

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Testate amoebae are commonly used as a bioindicator in paleoecology, especially in peatland paleohydrology. However up to now this approach is mainly based on correlative studies, typically single sampling campaigns during which samples are collected and environmental variables of interest measured. Transfer functions are then built based on the testate amoeba community data and measured variables and used to reconstruct past environmental conditions. Species identification is mainly based on test morphology. However there is also considerable morphological variation within species/taxa, some of which may be due to environmental influences (phenotypic plasticity). Such an effect could potentially be used for developing bioindication approaches. The existence of a relationship between depth to water table (DWT) and the number of pores in *Hyalosphenia papilio* was recently reported. However this correlative study was based on single sampling and DWT measurements and other environmental influences were not studied. We set up a mesocosm experiment to test if the morphology of *H. papilio* indeed responds to DWT. We grew *Sphagnum fallax* over peat cores placed in water tanks. The mosses were seeded with an extract of microorganisms from dry, intermediate and wet habitats at the beginning of the experiment (August 2012), and were therefore identical at T₀. The level of the water was manipulated to control the DWT in the mesocosms and measured daily. Temperature (peat: -10 cm, *Sphagnum*: -5 cm, and air +150cm), moisture (*Sphagnum* and air), precipitation and solar radiation are also monitored. We collected *Sphagnum* samples at T₀ (02.08.2012), T₁ (04.10.2012), T₃ (29.11.2012) and analysed the density and morphology of *H. papilio* for three treatments: 1) -4 cm 2) -15 cm 3) -25 cm (15 replicates per treatment). Differences in community composition and morphology of *H. papilio* confirm the response of testate amoebae to water table changes both at community level and within-species morphology.

Poster P20

Updating the Microthoracids (Ciliophora, Microthoracida): Five new *Drepanomonas* taxa from soil

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Using standard methods, we describe five new *Drepanomonas* taxa. *Drepanomonas hymenofera* (Horváth, 1956) Omar and Foissner, 2012, which is composed of two subspecies, was discovered in Venezuela and Iceland, respectively. Both are comparatively large (~50 × 20 µm and ~40 × 18 µm), differing in the cortex pattern and the structure of kinetics 3 and 4. *Drepanomonas vasta* Foissner and Omar, 2012, which was discovered in the mud of a tree hole in Austria, is thick and middle-sized (~35 × 18 µm). It swims continuously and appears slightly helical at low magnification due to two ridges right of the distinctly converging kinetics 2 and 3 and the cuneate transverse shape. Species 3 and 4, both discovered in Venezuela and Germany, respectively, are spiny and large (~45 × 25 µm and ~50 × 25 µm). They have a crescentic body with an obfalcate cortical plate on the right side and differ mainly in the number of ventro-lateral spines (three vs. two) and the left side cortical pattern. Species 5, which was discovered in Australia, is rather large (~45 × 20 µm) and has an ellipsoidal body slightly tapered anteriorly and rounded posteriorly, resembling *D. obtusa* Penard, 1922. It has an obfalcate plate on the right body side and two ridges along kinetics 5 and 6. Ontogenesis shows that the ciliary pattern of *Drepanomonas* is homologous to that of *Leptopharynx*, specifically, the structure and origin of the so-called postoral complex. The main features for distinguishing *Drepanomonas* species are: detailed morphometrics; body size and shape; cortex pattern (ridges, furrows, and spines); arrangement and total number of basal bodies; position of oral apparatus; and movement.

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Poster P21

SSU rDNA phylogeny of the Discoba (Excavata), with emphasis on the Discicristata

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Molecular studies of the Excavata using small subunit ribosomal RNA gene (18S or SSU rDNA) sequences largely contributed to the understanding of phylogeny and inter-relationships of subordinate taxa. In 2009, Hampl et al. erected the taxon Discoba, which united the excavate taxa Jakobida and Discicristata, the latter comprising Heterolobosea and Euglenozoa. The Euglenozoa include four well characterized taxa, with inter-relationships unclear to date. Recently, discovery of new euglenozoan and heterolobosean species led to different tree topologies due to differing methods or problems like unbalanced taxon sampling. To investigate the impact of the new sequences on the phylogeny of the Discoba and to shed light on disputed inter-relationships, especially on euglenozoan phylogeny, a huge and well equilibrated SSU rDNA dataset was generated. Initially, we used jModeltest (Posada 2008) to check for the best likelihood model, subsequently, distance and likelihood analyses were performed and posterior probabilities estimated. Finally, we compared our diagnostic findings with existing morphological characters, which were mapped onto the obtained tree. Here we present our results of the phylogeny of the Discoba, with emphasis on the Discicristata.

Poster P22*

*This poster is excluded from the poster voting!

All you can eat – theoretical considerations and experiments about protists feeding on various toxic cyanobacteria

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Mass occurrences of harmful filamentous cyanobacteria are of increasing importance in lakes due to ongoing global warming and changing nutrient stoichiometry (Posch et al. 2012). Cyanobacteria may produce secondary metabolites being toxic for eukaryotes. Best known are the microcystins (MCs), cyclic heptapeptides, which are efficient inhibitors of eukaryotic protein phosphatases 1 and 2A. Potential consumers get in direct contact with endotoxins when filaments are digested. Thus, cyanobacteria are often regarded as toxic for all consumers; however various protists can be efficient predators of filaments. Up to now, most work focused on the ciliate *Pseudomicrothorax dubius* feeding on filamentous *Oscillatoria* spp. (Cyanophyceae). Here, we report on ciliate species from various taxonomic groups (*Obertrumia aurea*, *Trithigmostoma cucullulus*, *Paraurostyla weissei*, *Holosticha pullaster*) and some amoeboid genera feeding on the harmful cyanobacterium *Planktothrix rubescens*. Besides the documentation of their curious feeding behaviour we analyse how these protistan species are able to survive their toxic diet. For the amoeboid genera we have good indications that detoxifying mechanisms happen in symbiosis with bacteria. In the case of ciliates, it is still unknown if the mentioned species have other protein phosphatases which are not affected by microcystins or if ciliates metabolize toxins to less potent compounds via linkage to antioxidants.

Devotions of *Obertrumia aurea* before starting lunch:

Oh cyanobacterium you are so tasty but so toxic,

You live aerobic and sometimes even anoxic,

kill crustaceans feeding on you,

but, surprise, I am your end too!

Posch T, Köster O, Salcher MM, Pernthaler J (2012) Harmful filamentous cyanobacteria favoured by reduced water turnover with lake warming. Nature Climate Change 2:809-813

Poster P23

Biodiversity of testate amoebae in an Amazonian peatland

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Testate amoebae are protists that are increasingly used as palaeoenvironmental proxies as well as bioindicators in peatland monitoring. In the last decade the number of studies on these microorganisms has increased considerably. However, the majority of the research on testate amoebae has focused mostly on Europe, Asia and North America. Testate amoebae from South American peatlands have been much less investigated. This uneven distribution of sampling can strongly influence the interpretation of data on testate amoebae biogeography and global ecology. The aim of this study is to analyse the testate amoebae communities in relation to peatland vegetation and abiotic environment in Aucayacu which is a domed, forested peatland in Peruvian Amazonia. In order to investigate species composition, light microscopy and scanning electron microscopy was utilised. We recorded 40 species, including a new species of *Arcella*. The average species richness per sample is 14. The most common species are *Cryptodifflugia oviformis*, *Euglypha rotunda* type, *Phryganella acropodia*, *Pseudodifflugia* type and *Trinema enchelys*. Rare species include *Euglypha crenulata*, *Heleopera rosea*, *Lesqueresia spiralis*, *Physochila griseola* and *Trinema enchelys grandis*. One species typical of the southern hemisphere, *Argynnia spicata*, is present in the Aucayacu peatland. We plan to analyse the testate amoebae data using multivariate statistics to determine their potential as environmental and palaeoenvironmental indicators in Amazonian peatlands.

Poster P24

Grazing by the flagellate *Poterioochromonas* sp. Induces strain- and growth rate- specific defence mechanisms in different bacterial isolates

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The grazing vulnerability and competitiveness of four betaproteobacterial isolates was investigated in a chemostat experiment with the mixotrophic chrysophyte *Poterioochromonas* sp. strain DS as predator. At the same time, the impact of two different growth rates was investigated by using different dilution rates (fast variant: 1 day⁻¹, slow variant: 0.3 day⁻¹). Interestingly, contrasting grazing defence mechanisms were observed at different dilution rates. Flagellates were growing to higher densities in the slow variant, while bacteria were equally abundant at both dilution rates. Grazing pressure induced filamentation of the bacterium *Massilia* sp. 1-16A only in the slow variant, whereas *Acidovorax* sp. 1-28B bacteria were able to outgrow the predator, although they were also the most favourable prey, as assessed by short termed uptake experiments with fluorescently labelled bacteria. *Hydrogenophaga* sp. EEcy4 bacteria were not selectively ingested by *Poterioochromonas* sp., and could therefore retain high densities, while *Limnohabitans planktonicus* D5 was greatly reduced. Two of the strains (*Acidovorax* sp. 1-28B and *Hydrogenophaga* sp. EEcy4) were also able to form aggregates as a grazing defence; however, this was restricted to the fast variant. Consequently, they dominated the assemblage at the end of the experiment, while *Massilia* sp. 1-16A and *L. planktonicus* D5 were suffering from high grazing losses and were close to extinction. To sum up, *L. planktonicus* D5 was most vulnerable to grazing and lacked a successful defence strategy in both variants. Filamentation of *Massilia* sp. 1-16A was only successful in the slow variant, whereas *Acidovorax* sp. 1-28B and *Hydrogenophaga* sp. EEcy4 were able to choose between aggregation and enhancement of growth rate to escape predation. Such a linkage between growth rate and different types of grazing defence mechanisms has not been reported so far.

This study shows that microbial predator-prey interactions are highly species-specific and that bacteria should not be regarded as a homogenous food entity for bacterivorous protists.

Poster P25

Free-living amoebae (FLA) as vehicles for *Legionella pneumophila* and other bacteria – a pilot study

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Free-living amoebae (FLA) are well known as potential human pathogens. *Acanthamoeba* spp. and *Balamuthia mandrillaris* can cause granulomatous amoebic encephalitis (GAE) in immunocompromised humans. Additionally *Acanthamoeba* causes keratitis in contact lens wearers. *Naegleria fowleri* causes fatal primary amoebic meningoencephalitis (PAME) in completely healthy humans. Furthermore FLA may serve as vehicles of dispersal and replicative niches for bacterial pathogens in natural and man-made habitats. In particular, *Legionella pneumophila*, the causative agent of Legionnaires' disease, replicates in FLA. In addition, the amoebal cysts protect intracellular pathogens against disinfection measures. This can lead to colonization of air conditioning systems, cooling water devices and warm water preparation units, from where the bacteria spread via aerosols. Currently we are lacking suitable screening assays for fast and synchronous detection and identification of FLA. This is why, as part of an interdisciplinary project on the role of FLA as vehicles for bacteria in water systems, we aim to develop real-time PCR assays suitable for routine screening of water facilities and air-conditioning units in Austria. First step is the evaluation of the suitability of own and literature-based established probes and primers, specific for all amoebae relevant as hosts, like *Acanthamoeba*, *Balamuthia*, *Hartmannella*, *Sappinia* and *Vahlkampfiidae*, respectively *Naegleria*.

Poster P26

Changes in soil Euglyphida communities under pigs cadavers assessed by high throughput sequencing: A new indicator of the post mortem interval (PMI)?

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When a human cadaver is found, estimation of the post mortem interval (PMI) is an essential element in the criminal investigation. For now, PMI estimation is done mainly by medical and entomologic methods. These methods are accurate but limited to a period ranging from days to a few weeks after death. The establishment of reliable PMI bioindicators for time laps of month to years would therefore be useful. As cadavers are known to modify soil chemical conditions for more than two years, but not much is known on communities of micro-organisms as potential PMI bioindicators. We conducted an experiment in a deciduous forest in Neuchâtel. Soil samples were taken under pig cadavers, control plots and fake pigs (plastic bag of the same weight of the corresponding pig). The sampling was done over a 2.5 years period. Total soil DNA was extracted and the V9 region of the small sub-unit ribosomal DNA was amplified by PCR and sequenced by Illumina. The comparison of the Illumina sequences with the V9 *Euglyphida* database revealed seven known phylotypes and many new V9 *Euglyphida* phylotypes. For most observed phylotypes the number of sequences decreased beneath the pig cadavers between 0 and 64 days and then increased again from day 309 to 1050. However, some phylotypes showed a different pattern suggesting that some euglyphid species grow better under the influence of decomposing carcasses. These first results suggest that Euglyphid testate amoebae could potentially be useful as new PMI indicator.

Poster P27

Morphological and Molecular Description of a New Species in the Extraordinary Genus *Schmidingerothrix* (Ciliophora, Hypotricha)

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In 2012, Foissner described a very curious, new hypotrichous ciliate genus and species (Europ. J. Protistol. 48:237–251): *Schmidingerothrix extraordinaria*. This hypotrich, which was discovered in hypersaline soils (~ 100 %) from Namibia and Egypt, has an outstanding organization: it possesses a frayed buccal lip, three-rowed adoral membranelles, only one frontal cirrus, a distinct gap between frontal and ventral adoral membranelles, and a miniaturized first frontal membranelle, while a paroral membrane, dorsal bristle rows, and buccal, transverse, and caudal cirri are absent. Thus, Foissner put the new genus into a new family: the Schmidingerotrichidae. When Foissner's study became available, we discovered a very similar species in a marine saline in Portugal, differing from *S. extraordinaria* mainly by the number of frontal cirri (3 vs. 1), adoral membranelles (24 vs. 19), and ventral cirral rows (2 vs. 1). Foissner, not having the advantage of a molecular sequence, interpreted the specialities as a reduction caused by the extreme habitat. Thus, we were greatly surprised when the small subunit (SSU) rDNA placed *Schmidingerothrix*, with a bootstrap support of > 90%, at the base of all stichotrichine sequences, from, e.g., *Oxytricha*, *Stylonychia*, *Paraurostyia*, *Bistichella* and so on. Accordingly, *Schmidingerothrix* could be a living fossil, showing us how the stichotrichine hypotrichs evolved. This is corroborated by morphological investigations (Foissner, unpubl.) on *Cladotricha* spp. They have, like *Schmidingerothrix*, a frayed buccal lip but possess also dorsal bristle rows and caudal cirri both absent from *Schmidingerothrix*.

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Poster P28

Testate amoebae in soil – Their potential as an indicator of time since death

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The estimation of the post-mortem interval (PMI) - the time between death and recovery of a body - is crucial whenever events and circumstances of a death need to be reconstructed for legal investigations. In order to develop new and more precise forensic tools that can be used when medical and entomological methods become less and less applicable, we investigated the response of the testate amoeba community to cadaveric fluids in litter and soil. The release of cadaveric material forms a cadaver decomposition island (CDI), which provides a large amount of heterotrophic activity on a relatively small terrestrial area. In two experiments we therefore investigated the response of the testate amoeba community to cadaveric fluids released from pig (*Sus scrofa* – 20kg) and roe deer (*Capreolus capreolus* – 20kg) carcasses. The first experiment using pig cadavers took place in Neuchâtel, Switzerland starting in August 2009, the second one using roe deer in the Bavarian Forest National Park, Germany starting in August 2011. In both experiments samples were taken in defined intervals up to 309 days (experiment 1) and 280 days (experiment 2) underneath the decomposing cadavers, control plots and fake pig/roe deer plots (bags filled with soil). To observe possible changes in the abundance and community structure alive, dead and encysted testate amoebae were counted and identified morphologically. In both experiments the results indicate that live testate amoebae do respond to a decomposing cadaver in showing a significant decrease of living testate amoebae after 15/22 days (experiment 1) and 11 days (experiment 2) while the number of living specimen in the control and under the fake pig/roe deer seemed more or less constant over time.

Poster P29

Acanthamoeba invading human tissue

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Acanthamoebae have a worldwide distribution and can be found ubiquitously in water and soil, including man-made habitats such as tap water and swimming pools. Human contact with these amoebozoans is frequent and under certain circumstances the amoebae can invade human tissue. *Acanthamoeba* spp. are the causative agents of *Acanthamoeba* keratitis (AK) on one hand and of often fatal opportunistic infections such as skin lesions, pneumonitis and granulomatous amoebic encephalitis (GAE) on the other hand. Many aspects of *Acanthamoeba* infections have been elucidated in different animal models. The aim of our study was to establish *ex vivo* human infection models enabling in detail studies on the infection process on one hand, and on the suitability of potential drugs on the other hand. Two potential model systems were developed, a three-dimensional organotypic skin model and an *ex vivo* human cornea model using discarded corneas from the cornea bank. It was shown that acanthamoebae can invade artificial human skin as well as isolated human cornea within 48 hours. The amoebae can be detected within the respective tissue using specific immunofluorescence staining. Acanthamoebae topically applied onto the cornified envelope of the skin equivalent, readily penetrated and destroyed the cornified layers and migrated into the skin. Intercalation of acanthamoebae between corneocytes were detected after 6 h and first lesions were visible after 24 h. Later the amoebae were detectable also in deep layers of the stratum corneum and also within the layer of living, differentiating keratinocytes. The untreated skin equivalent behaved like "normal" skin, basal keratinocytes resided on the collagen matrix, differentiating keratinocytes and upper cornified layers were visible after 48 hours. Also, the skin equivalent grew normally and remained unaffected by the application of PBS. Similarly, *Acanthamoeba* trophozoites applied to isolated human cornea floating on nutrient medium in a microtiter plate, with and without contact lenses, invaded the cornea and were even able to form cysts within the cornea. Untreated corneas sitting on a grid with the site of inoculation exposed in a well of a microtiter plate and being nurtured by culture medium from below were viable in the microtiter wells for several days and remained unaffected by the application of PBS.

Poster P30

Structure analysis of an eukaryotic microbial community along a salinity gradient in the DHAB Discovery (Mediterranean Sea)

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Studies about deep hypersaline anoxic basins (DHABs) in the Mediterranean Sea promise new insights into microbial community structures in extreme habitats. This talk is about the DHAB "Discovery" which is located in a depth of 3580 m and is characterized by two unique geochemical values: a very high concentration of magnesium ions up to 5000 mM and a very low concentration of sodium ions (79 mM). At the present time, this magnesium ion concentration is the highest measured in marine environments and is considered hostile to life. Besides this, the increasing depth is accompanied by an increase in salt concentration and a loss of oxygen. These characteristic extremes only allow highly specialized halophilic organisms to survive in the brine. Those have to be adapted in various different ways to the high salt concentration. According to these adaptations they have a certain tolerance range with respect to the salt concentrations. This fact raises the question whether their adaptations follow a specific salt threshold, which can be determined through protist community analyses along a salinity gradient. The interphase of this DHAB is an ideal model for such analyses because of its steep salinity gradient on a very small spatial scale. To analyze the community structure along this salinity gradient we established a comparison of the V4-hypervariable region of SSU rDNA based on 454-sequencing in order to verify the following three hypotheses: (I) Because of the different environmental conditions the eukaryotic plankton communities differ in different salt concentrations. (II) The increase of salinity leads to a progressive reduction in the abundance and the number of different groups of eukaryotic microorganisms. (III) In the brine only a few highly adapted specialists can survive, wherefore only a few species with high abundances can be found.

Poster P31

Mixotrophy as an advantageous strategy: The role of food quality

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Although mixotrophy is a widespread nutritional mode among protists, it remains unclear when and how this strategy is advantageous. One possibility is that mixotrophy is advantageous when food quality is poor. Laboratory growth experiments were run using prey mixtures of varying quality to clarify if mixotrophic ciliates are more viable than heterotrophic ciliates when prey was P-deficient, HUFA(highly unsaturated fatty acids)-deficient, or both. The cyanobacterium *Synechococcus* sp. was used as poor-quality resource. Although it is P-rich, it only contains traces of HUFA's which, although essential for growth, cannot be synthesized by many heterotrophic ciliates. On the contrary *Cryptomonas* sp. (strain 26.80) has a broad spectrum of HUFA's and represented the high quality diet. The experiments compared growth rates over 5 to 8 day trials of a mixotrophic and a heterotrophic representative from three ciliate genera (*Coleps*, *Euplotes* and *Paramecium*) which were, for the first experiment run, diets of *Cryptomonas* strain 26.80. and *Synechococcus* sp. in 100:0, 80:20, 50:50 20:80 and 0:100 *Cryptomonas*:*Synechococcus* mixtures. The second series of experiments were similar but utilized P-deficient *Cryptomonas*. Food concentration was constant at 0.5 mg C L⁻¹. Preliminary results suggest that the mixotrophic *Euplotes daidaleos* grows better in a HUFA-poor environment, with higher growth rates in 100% *Synechococcus* than in 100% *Cryptomonas* whereas the other two mixotrophic ciliates have reduced growth. Two out of three heterotrophic ciliates showed zero or negative growth rates with the HUFA-poor resource whereas *Paramecium* sp. copes better with the cyanobacteria than *Paramecium bursaria*. The results of the experiments suggest clear differences in the food niches of the mixotrophic and heterotrophic pairs of species, but not necessarily an advantage for mixotrophy when food quality is poor.

Poster P32

Adaptation of V4 eukaryotic amplicon pyrosequencing for protists of oxic-anoxic boundary layers

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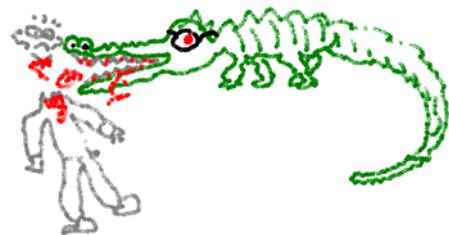
Next generation sequencing (NGS) has been increasingly used for biodiversity estimates and the detection of rare species within prokaryotic and eukaryotic assemblages in different habitats. Regarding protists, most studies used the V4 region of the 18S rRNA gene for NGS. However, established V4 primers from the literature show some mismatches to groups that seem to be important in hypoxic aquatic systems such as euglenozoans, some stramenopiles and ciliates (e.g. mesodiniids). As we were interested in the protist diversity of marine oxygen deficient zones, newly designed universal V4 primers in combination with different established primers were tested. A method using pre-amplification of the 18S rRNA was optimised and tested with a model community composed of six laboratory cultures (1 ciliate and 5 flagellates) as well as for environmental samples from the oxic-anoxic boundary layer (suboxic and sulfidic zone) of the Black Sea. The Black Sea samples have previously been investigated by 18S rRNA clone libraries and molecular fingerprints. All of these samples were analysed by means of their genomic DNA as well as ribosomal gene transcripts (cDNA) in order to include metabolically active protists. This NGS approach resulted in the detection of dominant taxa and, for the Black Sea, confirmed previously detected patterns in suboxic and sulfidic protist communities. That means for ciliates, sequences related to *Pleuronema* and *Strombidium* were mainly dominant in suboxic water layers whereas sequences affiliated with anaerobic plagiopylids and *Cyclidium* were detected only in the sulfidic zone. Among flagellates, a remarkable richness of bicosoecid taxa was confirmed for Black Sea oxygen depleted zones. The inclusion of an artificial model community allowed us to do quality estimations and OTU assessments for the data set.

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THIS IS THE END!



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