28. Wissenschaftliche Tagung der

Deutschen Gesellschaft für Protozoologie

25. bis 28. Februar 2009
in Naumburg (Saale)

Programm & Abstracts

Organisation
Universität Leipzig
Institut für Biologie II
AG Molekulare Evolution
und Systematik der Tiere
Talstraße 33
04103 Leipzig

HELMHOLTZ CENTRE FOR ENVIRONMENTAL RESEARCH – UFZ

600 JAHRE
UNIVERSITÄT LEIPZIG

28. Wissenschaftliche Tagung der

Deutschen Gesellschaft für Protozoologie

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Naumburg (Saale)

Präsident der Deutschen Gesellschaft für Protozoologie

Prof. Dr. Thomas Weisse

Institut für Limnologie

Österreichische Akademie der Wissenschaften

Mondseestrasse 9

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Tagungsort:

Euroville: Jugend- und Sporthotel

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Mittwoch, 25. Februar 2009

Anreise

nachmittags Registrierung im Euroville: Jugend- und Sporthotel in Naumburg

Ab 18.30 Gemeinsames Abendessen

Donnerstag, 26. Februar 2009

Ab 7.30 Frühstück

Eröffnung der Tagung

9.00 – 9.30 Begrüßung durch den Präsidenten der DGP
Thomas Weisse

Grußwort des Prorektors für Forschung und wissenschaftlichen Nachwuchs der Universität Leipzig
Martin Schlegel

Evolution / Phylogenie / Taxonomie
Vorsitz: Martin Simon / Renate Radek

9.30 – 10.15	PV	**Nowacki, Mariusz** An RNA-Mediated Epigenetic Mechanism for Inheritance of Acquired Mutations
10.15 – 10.30	KV	**Pfabel, Cornelia**; Nebel, Markus; Stock, Alexandra; Stoeck, Thorsten Delineation of Taxonomic Units in the Phylum Ciliophora Using 18S rDNA Sequence Similarities
10.30 – 10.45	KV	**Burkart, Corinna**; Barth, Dana; Berendonk, Thomas U. Genetic Variation in a Mitochondrial Minisatellite of *Paramecium caudatum*
10.45 – 11.00	KV	**Krenek, Sascha**; Berendonk, Thomas U. The Hsp70 Multigene Family of *Paramecium caudatum*: Phylogenetics and Differential Gene Expression
11.00 – 11.30		Kaffeepause
11.30 – 11.45	KV	**Kudryavtsev, Alexander** SSU rDNA and Ultrastructure of Cochliopodiidae, *Thecamoeba* and *Pellita*: Implications for Phylogeny of Discosean Amoebae
11.45 – 12.00	KV	**Schmidt, Maria** Zellbiologische Charakterisierung von Isolaten der heterokonten Algenklasse Synchromophyceae
12.00 – 12.15	KV	**Strassert, Jürgen F. H.**; Desai, Mahesh S.; Radek, Renate; Brune, Andreas *Joenia annectens*: Ein Termitenflagellat mit einer hohen Diversität an bakteriellen Symbionten
12.15 – 12.30	KV	**Schrallhammer, Martina**; Schweikert, Michael; Altenbuchner, Josef; Petroni, Giulio; Görtz, Hans-Dieter Characterization of R-Bodies, Inclusion Bodies Produced by *Paramecium* Endosymbionts *Caedibacter* Species
12.30 – 13.30		Mittagspause

13.30 – 13.45	KV	**Fokin, Sergei**; Ferrantini, Filippo; Modeo, Letizia; Verni, Franco; Petroni, Giulio *Copemetopus subsalsus*, *Parablepharisma bacteriophora*, and a New *Parablepharisma*-like Organism from Habitats with Oxygen Deficiency – are they Heterotrichids?
13.45 – 14.00	KV	**Vďačný, Peter**; Foissner, Wilhelm Morphological Taxonomy of Dileptids (Litostomatea, Haptoria): the Quest of Species Characters
14.00 – 14.15	KV	**Foissner, Wilhelm**; Strüder-Kypke, Michaela A Chain Forming Ciliate from Tank Bromeliads
14.15 – 14.30	KV	**Norf, Helge**; Foissner, Wilhelm A New, Stunning Peritrich Ciliate from the Rhine River (Germany)
14.30 – 15.00		Kaffeepause

Umwelt / Angewandte Protistologie
Vorsitz: Antonis Chatzinotas

15.00 – 15.45	PV	**Mitchell, Edward** Consolidating the Foundations of Ecology and Palaeoecology by Studying the Phylogeny, Taxonomy and Biogeography of Testate Amoebae
15.45 – 16.00	KV	**Stoeck, Thorsten**; Richards, Thomas A.; Bass, David; Nebel, Markus Exploring the "Rare Protistan Biosphere"
16.00 – 16.15	KV	**Behnke, Anke**; Edgcomb, Virginia; Christen, Richard; Stoeck, Thorsten The Effect of Geographical Distance and Environmental Conditions on Protistan Diversity in Two Permanently Anoxic Marine Basins
16.15 – 16.30	KV	**Müller, Helga**; Achilles-Day, Undine E. M.; Day, John G. Cryopreservation of the Ciliate *Meseres corlissi*
16.30 – 16.45	KV	**Schwarz, Julian** Strain deposition at the ATCC

16.45 – 17.15 **Posterpräsentation I**
Evolution / Phylogenie / Taxonomie
Umwelt / Angewandte Protistologie

Posterpräsentation I
Vorsitz: Antonis Chatzinotas

P1 **Kiss, Áron Keve**; Török, Júlia Katalin; Ács, Éva; Kiss, Keve Tihamér
New Testate Amoeba Species from the Plankton of the River Danube

P2 **Müller, Melanie**; Wylezich, Claudia; Nitsche, Frank; Nopper, Nicole; Arndt, Hartmut
A New Clade of Naked Bicosoecids from Different Freshwater Environments

P3 **Török, Júlia Katalin**
Phylogeny of *Arcella*: a Comparative Analysis of Different Morphological Species Groups Based on Partial SSU rRNA Gene Sequences

P4 **Berger, Helmut**
The Monograph of the Hypotricha (Ciliophora, Spirotricha): Three of Six Volumes Already Available

P5 **Berger, Helmut**; AL-Rasheid, Khaled A. S.; Foissner, Wilhelm
Two New Species of Hypotrichous Ciliates (Ciliophora, Spirotricha) from a Saline Soil in Saudi Arabia

P6 **Blake, Natalie**; Foissner, Wilhelm
Exploring a New Ciliate World: Two Peritrichs from Costa Rican Bromeliads

P7 **Gabilondo, Regina**; Foissner, Wilhelm
Four New Species of Haptorid Soil Ciliates from Four Different Biogeographic Regions

P8 **Çapar, Sirma**
Turkish Ciliate (Protozoa, Ciliophora) Fauna

P9 **Sonntag, Bettina**; Strüder-Kypke, Michaela; Summerer, Monika
Uroleptus willii: Morphology, Phylogeny and Ecology of a New Euplanktonic Freshwater Ciliate (Dorsomarginalia, Spirotrichea, Ciliophora)

P10 **Anderson, Ruth**; Weber, Felix; Jürgens, Klaus
Protist Diversity, Distribution and Bacterivory in Baltic Sea Pelagic Redoxclines

P11 **Glaser, Karin**; Solé, Magali; Harms, Hauke; Chatzinotas, Antonis
Identification of Potential Activity Markers in Protists by Molecular Techniques

Ab 17.15	Mitgliederversammlung der DGPF
Ab 19.00	Abendessen

Freitag, 27. Februar 2009

Ab 7.30 Frühstück

Zellbiologie / Genetik
Vorsitz: Christian Wilhelm

9.00 – 9.45	PV	**Steinberg, Christian E. W.** Natural Chemical Stress, Multiple Stress Resistance, and Longevity in Aquatic Organisms
9.45 – 10.00	KV	**Marker, Simone**; LeMouël, Anne; Meyer, Eric; Simon, Martin Functional Analysis of Putative RNA-dependent-RNA-Polymerases (RdRPs) in *Paramecium tetraurelia*: Distinct Pathways of dsRNA- and Transgene-induced Silencing
10.00 – 10.15	KV	Marker, Simone; LeMouël, Anne; Meyer, Eric; **Simon, Martin** Different Classes of Small RNAs in *Paramecium* Involved in RNAi and Regulation of Endogenous Gene Expression
10.15 – 10.45		Kaffeepause
10.45 – 11.00	KV	**Görtz, Hans-Dieter** How to Find and Describe Intracellular Bacteria in Ciliates - an Example

11.00 – 11.15	KV	**Ladenburger, Eva-Maria**; Sehring, Ivonne M.; Korn, Iris; Plattner, Helmut Intracellular Ca^{2+}-Release Channels in *Paramecium tetraurelia*
11.15 – 11.30	KV	**Sehring, Ivonne M.**; Reiner, Christoph; Schönemann, Barbara; Plattner, Helmut Gene Silencing Discloses Multiple, but Widely Specific Functional Roles of the Actin4 Subfamily in *Paramecium tetraurelia* Cells
11.30 – 11.45	KV	**Plattner, Helmut**; Sehring, Ivonne M.; Ladenburger, Eva-Maria Pharmacology of Ciliated Protozoa - Drug (In)Sensitivity and Experimental Drug (Ab)Use
11.45 – 12.00	KV	**Treuner, Theresa**; Berendonk, Thomas U.; Schlegel, Martin Increase of Tyrosinase Transcription after UV-B Irradiation in the Cellular Slime Mould *Dictyostelium discoideum*
12.00 – 13.00		Mittagspause

Ökologie
Vorsitz: Thomas U. Berendonk / Sascha Krenek

13.00 – 13.45	PV	**Matz, Carsten** Microbial Battlegrounds – The Multiple Facets of Protozoa-Bacteria Interactions in Aquatic Communities
13.45 – 14.00	KV	**Boenigk, Jens**; Findenig, Barbara Population Structure and Perennation Strategies of Chrysomonad Flagellates
14.00 – 14.15	KV	**Kiss, Áron Keve**; Ács, Éva; Kiss, Keve Tihamér The Local Diversity of Heterotrophic Nanoeukaryotes has been Underestimated: A Case Study in the River Danube with Emphasis on Heterotrophic Flagellates

14.15 – 14.30	KV	**Moser, Michael**; Scheffel, Ulrike; Stadler, Peter; Weisse, Thomas Flagellates at Extremely Low pH – Specialist vs. Generalist Life Strategies
14.30 – 15.00		Kaffeepause
15.00 – 15.15	KV	**Haentzsch, Madlen**; Andres, Claudia; Berendonk, Thomas U.; Schlegel, Martin; Bernhard, Detlef Analyses of Ciliate Diversity Using Single Strand Conformation Polymorphism Analyses (SSCP Analyses)
15.15 – 15.30	KV	**Weisse, Thomas**; Kamjunke, Norbert; Moser, Michael; Scheffel, Ulrike; Stadler, Peter; Weithoff, Guntram Significant Habitat Effects Influence Protist Fitness in Acid Mining Lakes
15.30 – 15.45	KV	Marcus, Hanna; **Wey, Jennifer**; Norf, Helge; Arndt, Hartmut; Weitere, Markus Effekte mechanischer und biologischer Störungen auf biofilmassoziierte Ciliatengemeinschaften
15.45 – 16.00	KV	**Stumm, Karen**; Weidhase, Germaine; Mencfeld, Björn; Buschner, Marcus Investigation on the Diversity of Prokaryotes in Tree Canopies of a Temperate Forest
16.00 – 16.15	KV	**Adolph, Stephanie**; Haentzsch, Madlen; Schlegel, Martin; Bernhard, Detlef The Hidden Life in Treetops – a Molecular Survey of Ciliates in the Canopy of a Temperate Floodplain Forest
16.15 – 16.45		**Posterpräsentation II** Zellbiologie / Genetik / Ökologie

Posterpräsentation II
Vorsitz: Sascha Krenek

P12 Klöppel, Christine; Marker, Simone; Creutz, Jonathan; **Simon, Martin**
A Secreted, Cytosolic Phopshatidyl-Specific Phospholipase C Affecting Special GPI-Anchors on the Surface of *Paramecium*

P13 **Sterz, Angela**; Marker, Simone; Simon, Martin
Molecular Characterisation of the Four RdRP Genes in *Paramecium tetraurelia*

P14 **Treuner, Theresa**; Bernhard, Detlef; Burkart, Corinna; Weigert, Anne; Berendonk, Thomas U.
Enrichment of Micronuklei and Detection of Microsatellites in *Paramecium caudatum*

P15 **Behnke, Anke**; Stoeck, Thorsten
Factors Structuring Spatial and Temporal Patterns of Protistan Communities in an Anoxic Water Column

P16 Böhme, Anne; **Risse-Buhl, Ute**; Küsel, Kirsten
Grazing Activity of Ciliates and Flagellates with Different Feeding Modes Influence Bacterial Biofilm Morphology

P17 **Monsonís Nomdedeu, Mar**; Hünninghaus, Maike; Willen, Christine; Schieffer, Andre; Arndt, Hartmut
Determination and Influences of Variable Extrinsic and Intrinsic Parameters on Population Dynamics of the Ciliate *Tetrahymena pyriformis*. Model Analyses and Preliminary Results

P18 **Jost, Steffen**; Medinger, Ralph; Boenigk, Jens
Analysing Protist Population Structures by Multiplex Single-Cell PCR from Preserved Plankton Samples

P19 Barresi, Marta; **Schrallhammer, Martina**; Schweikert, Michael; Görtz, Hans-Dieter; Petroni, Giulio
First Description of *Euplotes raikovi* as Natural Reservoir of *Francisella philomiragia*

P20 **Schweikert, Michael**; Schrallhammer, Martina; Görtz, Hans-Dieter
High Resolution Electron Microscopy of R-Bodies in *Caedibacter* Species, Endosymbionts in Ciliates of the Genus *Paramecium*, Revealed by Electron Tomography

Ab 17.00	Mitgliederversammlung der DGP
Ab 19.30	Geselliger Abend mit Posterpreisverleihung

Samstag, 28. Februar 2009

Ab 7.30 Frühstück

Parasitologie
Vorsitz: Arwid Daugschies/Carlos Hermosilla

9.30 – 10.15	PV	**Carlos Hermosilla** Intriguing Parasite-Host of Apicomplexan *Eimeria boris*
10.15 – 10.30	KV	**Koethe, Martin**; Dr. Bangoura, Berit; Pott, Susan; Zöller, Birte; Dr. Ludewig, Martina; Prof. Dr. Fehlhaber, Karsten; Prof. Dr. Daugschies, Arwid; Dr. Mercier, Corinne; Prof. Dr. Straubinger, Reinhard Serological Detection of *Toxoplasma* Infection in Turkeys
10.30 – 10.45	KV	**Worliczek, Hanna Lucia**; Gerner, Wilhelm; Saalmüller, Armin; Joachim, Anja; Porcine Coccidiosis – Immune Response to *Isospora suis* in Piglets
10.45 – 11.15		Kaffeepause
11.15 – 11.30	KV	**Michel, Rolf**; Kurek, Rafael; Scheid, Patrik; Walochnik, Julia; Hauröder, Bärbel Isolierung von *Thecamoeba quadrilineata* mit pilzartigen intranukleären Parasiten und Beschreibung ihrer Entwicklung und des Wirtsspektums
11.30 – 11.45	KV	**Walochnik, Julia**; Lackner, Peter; Auer, Herbert; Schmutzhard, Erich Isolierung von *Acanthamoeba* Genotyp T5 aus dem ZNS
11.45 – 12.00	KV	**Etzold, Manja**; Prof. Dr. A. Daugschies, Dr. V. Dyachenko Stage-Specific Expression of Six Calcium-Dependent Protein Kinases in *Cryptosporidium parvum* In Vitro
12.00 – 13.00		Mittagspause

Plenarvortrag

An RNA-Mediated Epigenetic Mechanism for Inheritance of Acquired Mutations

Nowacki, Mariusz
Princeton University

RNA, normally thought of as a conduit in gene expression, has a novel mode of action in ciliates, where maternal RNA templates provide both an organizing guide for DNA rearrangements and a template that can transmit spontaneous point substitutions that may arise during somatic growth to the next generation (Nowacki et al. (2008) Nature 451:153-8). This opportunity for RNA-guided DNA repair is profound in its regulation of global DNA rearrangements in *Oxytricha*, involving loss of 95% of its germline genome, through a process that severely fragments its chromosomes and then sorts and reorders the hundreds of thousands of pieces remaining. Information for reordering comes from transiently-expressed maternal RNAs. Furthermore, the occasional transfer of point mutations in these RNA templates to the rearranged molecules provides a mechanism for stable inheritance of acquired, spontaneous somatic mutations (in either DNA sequence or alternative splicing pattern) without altering the germline genome. This mechanism for inheritance beyond the conventional DNA genome can epigenetically transfer information across multiple generations, hinting at the power of RNA molecules to shape genome information. The evolutionary consequences of a viable mechanism in ciliates to transmit acquired characters may contribute to their cosmopolitan success, as well as high substitution rates in somatic sequence comparisons.

Delineation of Taxonomic Units in the Phylum Ciliophora Using 18S rDNA Sequence Similarities

Pfabel, Cornelia[1]; Nebel, Markus[1]; Stock, Alexandra[1]; Stoeck, Thorsten[1]
[1]University of Kaiserslautern

The phylogenetic analyses of small subunit ribosomal RNA (SSU rRNA) genes emerged as a valuable tool in biodiversity studies and for placing (new) organisms in the phylogenetic tree of life. Even though controversial, in the domains Bacteria and Archaea sequences with greater than 97% identity are typically assigned to the same species, those with >95% identity are typically assigned to the same genus, and those with >80% identity are typically assigned to the same phylum. We here evaluate if such a concept can also be adopted for the domain Eukarya. The translation of sequence similarities into taxonomical hierarchies would greatly facilitate for example the phylogenetic assignment of incertae sedis taxa, the morphology-based classification of novel organisms and also the assignment of uncultivated sequences detected by environmental SSU rRNA sequencing to a defined taxonomic unit. In order to test if SSU rDNA sequence similarities may qualify to delineate taxonomic units in the domain Eukarya, we calculated the sequence similarities from pairwise alignments of all available ciliate sequences and correlated these data with the taxonomic relationships between all taxa. If a concept as applied for prokaryotes indeed holds true for eukaryotes, too, will be revealed in our talk.

Genetic Variation in a Mitochondrial Minisatellite of *Paramecium caudatum*

Burkart, Corinna[1]; Barth, Dana[1]; Berendonk, Thomas U.[1]
[1]Universität Leipzig; Institut für Biologie II

We present data on a large unknown mitochondrial gene within *Paramecium caudatum*. Within this ymf88-gene we detected an unusual feature, an intragenic minisatellite composed of 18 bp repeat units near the middle of the gene. We amplified and sequenced the respective gene region of 73 *P. caudatum* strains and found that the number of repeat units was highly variable, even between strains that contain identical Cytochrome *c* oxidase I sequences. Interestingly, this gene was not detected in the previously published mitochondrial genome of *Paramecium tetraurelia*.

Kurzvorträge

The Hsp70 Multigene Family of *Paramecium caudatum*: Phylogenetics and Differential Gene Expression

Krenek, Sascha[1]; Thomas U. Berendonk[1]
[1]Universität Leipzig, Institut für Biologie II

In Eukaryotes, members of the 70 kDa heat-shock protein family are subdivided into three subfamilies with respect to their functional activities in different subcellular compartments. In this study, we isolated eleven Hsp70 cDNA sequences in *Paramecium caudatum*, comprising homologous genes for all major Hsp70 subfamilies, i.e., five with a cytosolic/nuclear localisation, five with an endoplasmic reticulum (ER) localisation and one mitochondrial homolog sequence. Interestingly, the cytosolic as well as the ER-type Hsp70 sequences could be subdivided each into two separate clusters with a closer relationship to corresponding Hsp70 sequences of *Paramecium tetraurelia* than among each other. Furthermore, we investigated the differential gene expression of these five Hsp70 clusters after a heat shock at 34°C using RT-qPCR with specifically designed Taqman® MGBÔ-Probes. Here, we detected that only one cytosolic Hsp70 cluster, including two different sequences, shows a massive upregulation in mRNA levels after heat shock. This finding is comparable to the human Hsp70 family, where also only two genes (HSPA1A and HSPA1B) encode the major heat shock inducible proteins of this gene family.

SSU rDNA and Ultrastructure of Cochliopodiidae, *Thecamoeba* and *Pellita*: Implications for Phylogeny of Discosean Amoebae

Kudryavtsev, Alexander
AG Protozoologie, Institut für Biologie/Zoologie, Freie Universität Berlin,

Phylogenetic analysis of the SSU rRNA gene sequences of Cochliopodiidae, *Thecamoeba verrucosa* and *Pellita* n.sp. (Amoebozoa) shows that all these amoebae belong to Discosea. Cochliopodiidae branch as a sister to Vannellidae; *Th. verrucosa* is robustly the most basal *Thecamoeba*; Thecamoebidae being constantly sister to Acanthamoebidae. *Pellita* n.sp. is either sister to Acanthamoebidae, or one of the basal discoseans. *Cochliopodium* is monophyletic; basal to it is a flattened amoeba with the dorsal, scaleless carbohydrate cell coat (Cochliopodiidae n. gen.) showing that the cochliopodiids were ancestrally scaleless, and the tectum of *Cochliopodium* evolved within this genus. *Th. verrucosa* as well as *Pellita* spp. have a cytoplasmic MTOC near the nucleus and a pronounced microtubular cytoskeleton. In case of *Th. verrucosa* this is a confirmation for the disputed relatedness between Thecamoebidae and Acanthamoebidae. The ultrastructure of *Pellita* is similar to *Gocevia*, however the latter genus seems to be related to *Cochliopodium*, while *Pellita* never groups close to *Cochliopodium* in the molecular trees. The presented data suggest, that a "centrosphere" (cytoplasmic dictyosome-associated MTOC) is one of the ancestral features of Discosea, lost in many branches.

Zellbiologische Charakterisierung von Isolaten der heterokonten Algenklasse Synchromophyceae

Schmidt, Maria
Universität Leipzig

Die Gruppe der heterokonten Algen beinhaltet auf der einen Seite einige der Hauptprimärproduzenten mariner Lebensräume (Diatomeen, Braunalgen) mit hoher Diversität, aber auch Klassen, die nur wenige, bisher kaum näher untersuchte Vertreter beinhalten. Horn et al. beschrieben 2007 eine solche Algenklasse – Synchromophyceae – mit der bisher einzigen Art *Synchroma grande*. Sie ist eine marine, amöboide Alge und hebt sich von allen bekannten Algen mit komplexen Plastiden durch eine spezielle Chloroplastenmorphologie ab. Diese sind in Komplexen organisiert, in denen mehrere Plastiden ihre äußeren zwei Membranen teilen. Es konnten durch mikroskopische Beobachtungen und phylogenetische Untersuchungen an *rbc*L- und 18S rRNA-Gen weitere Algenisolate aus dem Atlantischen Ozean, dem Mittelmeer und dem Karibischen Meer charakterisiert werden, die eine hohe morphologische Ähnlichkeit zu *S. grande* aufweisen und in Stammbäumen eine monophyletische Gruppe der Synchromophyceae erkennen lassen. Diese Algenklasse scheint somit eine Gruppe von Spezies zu umfassen, die in benthischen Lebensräumen weit verbreitet ist und sich tatsächlich durch ihre gemeinsame einzigartige Chloroplastenmorphologie auszeichnet.

Joenia annectens: Ein Termitenflagellat mit einer hohen Diversität an bakteriellen Symbionten

Strassert, Jürgen F. H.[1]; Desai, Mahesh S.[2]; Radek, Renate[1]; Brune, Andreas[2]
[1]Institut für Biologie/Zoologie, FU Berlin; [2]MPI für terrestrische Mikrobiologie

Der Darm holzfressender, niederer Termiten beherbergt eine Vielzahl symbiontischer Mikroorganismen. Während den Flagellaten eine wesentliche Rolle beim Celluloseabbau zukommt, sind Funktion und Identität der Bakterien und Archäen weitgehend ungeklärt. Dies gilt vor allem für die Vielzahl der mit den Flagellaten assoziierten, unkultivierbaren Bakterien. Ultrastrukturelle Untersuchungen an *Joenia annectens* zeigten, dass dieser Termitenflagellat (aus *Kalotermes flavicollis*) von diversen endo- und ektobiotischen Bakterien besiedelt ist. Die phylogenetische Analyse der 16S rRNA-Gensequenzen ermöglichte eine Identifizierung der morphologisch beschriebenen Symbionten. Subzelluläre Lokalisierungen über FISH ergaben, dass die ektobiotischen Bakterien spezifische Entwicklungslinien innerhalb der Spirochaetales und Bacteroidales bilden, während die intrazellulären Bakterien hauptsächlich zu den Endomicrobia gehören. Letztere scheinen in der Flagellaten-Bakterien-Symbiose eine besondere Rolle einzunehmen. So konnte unlängst die Hypothese, dass die Symbionten am Stickstoffstoffwechsel des Flagellaten beteiligt sind, durch die Genomanalyse eines Vertreters der Endomicrobia sowie durch diverse Belege der Co-Speziation zwischen Endomicrobia und *Trichonympha* spp. gefestigt werden.

Kurzvorträge

Characterization of R-Bodies, Inclusion Bodies Produced by *Paramecium* Endosymbionts *caedibacter S*pecies

Schrallhammer, Martina[1,2]; Schweikert, Michael[1]; Altenbuchner, Josef[1]; Petroni, Giulio[2]; Görtz, Hans-Dieter[1]
[1]Universität Stuttgart; [2]Università di Pisa

Caedibacter species are endosymbionts of *Paramecium,* also known as killer particles. They refer the killer trait to their *Paramecium* host. All *Caedibacter* can produce R-bodies, essential components of the killer trait. They function as releasing system for the lethal toxin killing the sensitive *Paramecium*. R-bodies are unusual bacterial inclusion bodies, they differ from normal inclusion bodies by their highly complex and ordered structure. The R-body consists of a protein sheath of well defined dimensions which is tightly coiled inside the *Caedibacter* cell. Triggered by certain stimuli, e.g. a low pH, it unrolls and produces thereby a long hollow cylinder. In the present study, R-bodies were analysed on genetical, molecular and structural level. R-body genes were characterized; complete R-bodies and single Reb proteins have been expressed in *E. coli* and were biochemically and functionally studied after purification. The complete R-body was analysed by different microscopical techniques including electron tomography followed by 3-dimensional reconstructions.

Copemetopus subsalsus, Parablepharisma bacteriophora, and a New *Parablepharisma*-like Organism from Habitats with Oxygen Deficiency – are they Heterotrichids?

Fokin, Sergei[1,2]; Ferrantini, Filippo[1]; Modeo, Letizia[1]; Verni, Franco[1]; Petroni Giulio[1]
[1]Department of Biology, University of Pisa, Italy; [2]Department of Invertebrate Zoology, St. Petersburg State University, Russia

The mentioned three ciliates were found in brackish water habitats with salinity ranging from 5 to 22‰ on the oxic/anoxic border and investigated (reinvestigated) using LM, TEM, SEM and SSU rDNA sequence analysis. Their general morphology reminds that of Heterotrichea, class in which they are traditionally included. In fact, the ciliates have special pattern of the oral ciliature (adoral, paroral, and frontal/fronto-lateral membranelles). The last ones, as well as paroral membrane, are unusually long - from 25 to 100 µm. Special rod-shaped pharyngeal structures were also found. The ciliates are not contractile, with slightly pigmented cortical granules and bacterial endocytobionts; in *Parablepharisma* and *Parablepharisma*-like, also ectocytobiotic sulfur bacteria are present. Molecular data support a close affiliation between *Parablepharisma* and the *Parablepharisma*-like organism. Preliminary phylogenetic analyses reveal that the three species do not branch from within the Heterotrichea but emerge basally within the Intramacronucleata.

Morphological Taxonomy of Dileptids (Litostomatea, Haptoria): the Quest of Species Characters

Vďačný, Peter[1]; Foissner, Wilhelm[1]
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Dileptids are rapacious ciliates with a conspicuous proboscis belonging to the oral apparatus. In the past century, *Dileptus* species were recognized by body shape and size, the ratio of body and proboscis length, and the nuclear and contractile vacuole pattern. Only in the past decades, further diagnostic features such as extrusome shape, details of the ciliary pattern, shape of the oral bulge opening, and morphometrics were added. In North America, we discovered a new *Dileptus* highly resembling the European *Dileptus anser*, but differing in the contractile vacuole pattern: a dorsal and a ventral row of contractile vacuoles vs. only a dorsal row. One can ask whether this feature is reliable for species discrimination. The contractile vacuole pattern has been successfully used in α-taxonomy of various ciliate genera, e.g., in *Frontonia*. Our studies on several dileptids showed that this feature is as stable, respectively, variable as other characters. When a difference in the contractile vacuole pattern occurred between populations, it was frequently associated with other differences, for instance, the number of the ciliary rows (*Dimacrocaryon amphileptoides* populations), the nuclear apparatus or the extrusome shape (*Dileptus breviproboscis* group). (Supported by the Austrian Science Foundation, FWF project P-19699-B17.)

A Chain Forming Ciliate from Tank Bromeliads

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The leaves of bromeliads are arranged in a way that small ponds, called tanks or cisterns are formed. During the past century, scientists recognized many specific organisms in the tanks, ranging from insects to amphibians, while protists were largely ignored. By chance we recognized many specific tank ciliates, which are now investigated in the course of a project sponsored by the Austrian Science Foundation. One of the most spectacular ciliates is an up to 70 µm long colpodid which makes curious division chains, where the specimens of the second generation are connected by a unique, plug-like structure. This is shown in a film and by scanning electron microscopy. Morphologically, this ciliate represents a distinct family, while the 18S rDNA shows a close relationship with the genus *Colpoda*, suggesting that morphological and molecular evolution are decoupled, as in several other tank ciliates. This species is widespread in bromeliads from Central and South America and grows well in limnetic and terrestrial laboratory cultures. It remains inexplicable why we did not find it in over 100 natural soil and water samples from the same area. (Supported by the Austrian Science Foundation, FWF project P20360-B17 to WF.)

A New, Stunning Peritrich Ciliate from the Rhine River (Germany)

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We discovered a peritrich ciliate with outstanding features during ecological studies in the River Rhine: (i) Micro- and macrozooids form cup-shaped pseudo-colonies on a dish-shaped base, which is attached to a ca. 2 mm long, spirally contracting vorticellid stalk. (ii) The stalk myoneme is only connected to the microzooids. (iii) Each colony contains up to 50 zooids that are not interconnected, but individually merged with the scopula. (iv) The trumpet-shaped, epistylid zooids are ca. 180 µm long and hold an ellipsoidal macronucleus. (v) Myoneme systems of the individually contracting zooids form a tube-like structure in the very narrow posterior cell half. (vi) The silver line pattern is striated. (vii) Pecularities are lacking in the oral ciliature. Especially features (i), (ii), and (iii) suggest establishing a new family and genus for this curious new ciliate. Even if the 18s rDNA sequence places this ciliate close to Vorticella and Pseudovorticella, such phylogenetic position is non-likely according to the current state of knowledge. (These works were supported by DFG project 3545/3-1 to M. Weitere and H. Arndt and FWF project P-19699-B17 to W. Foissner.)

New Testate Amoeba Species from the Plankton of the River Danube

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Six new testate amoeba species has been found in the plankton of the River Danube, three of which are described. *Pseudodifflugia klarae* nov. sp. diff. diagn: *Pseudodifflugia* with 8-14 mm long oval/pyriform rigid, sparsely or densely agglutinated test, undefined aperture, nucleus with bent chromatin rods and single nucleolus. *Bereczkya minuta* nov. gen. nov. sp. diff. diagn: spheric cells (3.5-8 mm) with thin, rigid, sparsely or densely agglutinated test filled entirely by cytoplasm. An ectoplasmic collar is situated in the aperture, from the middle of which a pseudopodial stem with branching filopodia emerges. Slightly wrinkled nucleus with chromatin rods and without nucleolus. *Micramphitrema mülleri* nov. gen. nov. sp. diff. diagn: Tube-like rigid test with mineral particle agglutination on inner organic test layer, tapering to two opposed rigid neck-like apertures. Test circular in cross section, nucleus vesicular, branching filopodia and non-branching tube-like pseudopodia.

A New Clade of Naked Bicosoecids from Different Freshwater Environments

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We found a new group of bicosoecids which does not possess the typical lorica of common freshwater bicosoecids. The groundwater samples originate from North Germany, Cologne and South Africa. Molecular studies revealed a surprising similarity regarding the LSU rDNA for all different isolated strains. The new group forms a branch of bicosoecids with significant distances to all other known bicosoecids such as *Bicosoeca*, *Pseudobodo*, *Cafeteria*, *Siluania* and *Adriamonas*. The new bicosoecids are generally attached to the substrate by their second flagellum and feed on bacteria. Originally only found in groundwater aquifers, recent studies revealed the same species also to be present in the River Rhine. Up to now we found two different species of this new phylogenetic group.

Poster

Phylogeny of *Arcella*: A Comparative Analysis of Different Morphological Species Groups Based on Partial SSU rRNA Gene Sequences

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Arcella EHRENBERG, 1832 has been a favoured research object, due to its relatively large size, unique shell structure and relative ease of culturing. As data increase on lobosean molecular phylogeny, the placing of this well known genus on the phylogenetic tree became of great importance. Furthermore, upon the discovery of phylogenetic relationships among certain *Arcella* species the traditional morphological frames could be validated or abandoned. A short length (ca. 500 bp.) section of the 18S rRNA gene was amplified by pcr using self constructed primers in order to place the genus on the lobosean tree and get a primary insight into the relative position of morphologically distant or similar species. *A. rotundata, A. excavata, A. hemisphaerica, A. gibbosa, A. formosa* and *A. dentata* have been successfully processed. They unambiguously form a distinct group among the testate loboseans, within Tubulina. *A. hemisphaerica* shows the closest similarity with the only published sequence of the species (Tekle & al. 2008). Generic identity of *Arcella artocrea* in Nikolaev & al. 2005 seems to be doubtful, since its consequent grouping with *Centropyxis laevigata*, remarkably distant from the *Arcellas*.

The Monograph of the Hypotricha (Ciliophora, Spirotricha): Three of Six Volumes Already Available

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The Monograph of the Hypotricha is the most detailed revision on this group since Kahl's (1932; Tierwelt Dtl., 25, 399-650) review. The treatise is published in the book series Monographiae Biologicae (MB) edited by Henri J. Dumont and published by Springer (www.springer.com). Three parts of the series are already available, namely, the Oxytrichidae (Berger 1999, MB 78), the Urostyloidea (Berger 2006, MB 85), and the Amphisiellidae and Trachelostylidae (Berger 2008, MB 88). The fourth volume (Kahliellidae, Orthoamphisiellidae, Keronopsidae) is in preparation and will be published in 2009. The species not reviewed in parts 1–4 will be treated in two further volumes. The penultimate part comprises, inter alia, the Uroleptidae, a group previously assigned to the Urostyloidea because of convergencies in the cirral pattern. Part 6 will contain, inter alia, a key to all taxa and addenda to the groups already revised in parts 1–5 so that the series is up-to-date when it is finished. The complete revision will comprise more than 600 species on about 5000 pages and many thousands of figures. The financial support of the Austrian Science Fund (FWF; Project P-20569-B17) is greatly acknowledged.

Two New Species of Hypotrichous Ciliates (Ciliophora, Spirotricha) from a Saline Soil in Saudi Arabia

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Two new hypotrichs (stichotrichs) have been discovered in a highly saline soil (12%) from the Al Hasan oasis near the Arabic Gulf in Saudi Arabia. The first species resembles some amphisiellids and is characterised, inter alia, by a distinct gap in the adoral zone of membranelles, a short and a long frontoventral row, a conspicuous postoral row, a second, rather variable left marginal row, and an anteriorly very strongly shortened dorsal kinety 1. The second species belongs to *Cladotricha* and is binucleate, has a buccal row, and one long and two short frontoventral rows. The oral apparatus of *Cladotricha* is gonostomoid, indicating that it forms a monophyletic group (Gonostomatidae Small & Lynn) together with, e.g., *Gonostomum*, *Paragonostomum*, *Wallackia*. The dorsal infraciliature (three bipolar kineties with caudal cirri) was taken over from the ground pattern of the hypotrichs, indicating that the gonostomatids cluster outside the Dorsomarginalia. The financial support by the Center of Excellence in Biodiversity Research, Kind Saud University, is greatly acknowledged.

Exploring a New Ciliate World: Two Peritrichs from Costa Rican Bromeliads

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The water trapped between the leaf axils of bromeliads have long been known to possess a rich, often specific metazoan fauna. Despite this, few studies have focussed on protist diversity. We investigated some bromeliads from Costa Rica and discovered a new *Pseudovorticella* species and *Vorticella gracilis* Dujardin, a still poorly known peritrich. The morphology of *Pseudovorticella* n. sp. was investigated by live observation and silver nitrate impregnation. *Pseudovorticella* n. sp. could be distinguished from its nearest congeners by its two ventral contractile vacuoles, a J-shaped macronucleus, and the average number of silverlines. The second species, *V. gracilis,* was identified using the yellow cytoplasmic colouration, the J-shaped macronucleus, the ventral contractile vacuole, and the average number of silverlines. The yellow colour of *Vorticella gracilis* was very stable between and within populations. Unlike the yellowish variety of *Vorticella convallaria*, i.e. *Vorticella citrina*, *V. gracilis* retained its colour for at least six months, i.e., for the duration of the culture. The oral ciliature and many other features were of the convallaria-type. (Supported by the Austrian Science Foundation, FWF project P20360-B17.)

Four New Species of Haptorid Soil Ciliates from Four Different Biogeographic Regions

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The four new species belong to the Acropisthiina, a group of haptorids where the oral basket is made from nematodesmata originating from the circumoral kinety and the anterior kinetids of the ciliary rows (oralized somatic monokinetids). The new taxa belong to the family Fuscheriidae, which is characterized by having an enchelyodonid general organization and meridional kineties, two of which are differentiated to a dorsal brush anteriorly. A further important genus character is the shape of the extrusomes. Species 1 (from Ayers Rock, Australia) and 2 (meadow puddle from Salzburg, Austria) belong to the genus *Fuscheria* because they have nail-shaped extrusomes; species 3 (soil from the surroundings of Paiku-Tso Lake, Tibet) and 4 (surroundings of Ziway Lake, Ethiopia) represent a new genus each with rod-shaped and acicular extrusomes, respectively. Species 1 and 2, both with about 46 ciliary rows and the oral basket extending two thirds of body length, mainly differ in the number of macronucleus nodules: 12 vs. 1. Species 3 has seven ciliary rows, of which some are curved to the left anteriorly; species 4 has 26 ciliary rows and the oral basket extends in anterior body third. As yet, each species has been found only at its type locality, although about 1500 soil and mud samples have been investigated from sites globally. (Supported by the Austrian Science Foundation, FWF project P-19699-B17.)

Turkish Ciliate (Protozoa, Ciliophora) Fauna

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Although the studies about ciliates have increased worldwide, the data according to the vegetation differences is still limited. Considering the data about turkish ciliate fauna, the purpose of this study is to contribute to the knowledge of ciliates recorded from the inland waters and flooded zones of Turkey. Samples were collected from different sites of wetland and lake areas. All the collections were analysed with the non-flooded petri method which bases on reactivating ciliates from air dried samples and by direct observation after sampling. Morphological characters for all the species were identified by live observation and observation with impregnation methods. The species were defined by the evaluation of morphometric measurements and counts which were performed digitally by IM50 image manager system and Q- win measurement program, few were with optical scale measure. Illustration of the specimens were by free-hand sketchs and micrographs. Until now, totally 175 taxa were recorded from different habitats. Micrographs and drawings of some remarkable species according to the different sampling sites are shown and general habitat knowledge is given.

Uroleptus willii: Morphology, Phylogeny and Ecology of a New Euplanktonic Freshwater Ciliate (Dorsomarginalia, Spirotrichea, Ciliophora)

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Uroleptus willii Sonntag, Strüder-Kypke; Summerer 2008 was discovered in the oligo-mesotrophic Piburgersee in Austria. The morphology, infraciliature and phylogenetic placement (SSrRNA gene sequence) were studied. *Uroleptus willii* is a grass-green fusiform spirotrich of 100 - 150 µm length, bears about 80-100 symbiotic green algae and builds a lorica. The species appears frequently in the summer ciliate assemblage of Piburgersee with about 170 individuals l^{-1} from May through November. The algal symbionts are known to synthesize UV–absorbing compounds. However, morphological features of the genus agree well with those of the Urostyloidea, while the molecular analyses place the genus within the Oxytrichidae. We preliminary assign our new species to the taxon novum 'Dorsomarginalia' proposed by Berger (2006), based on the assumption that the genus *Uroleptus* and the Oxytrichidae are both monophyletic taxa although the monophyly of the latter group has still not been confirmed by molecular data. Supported by the FWF (P21013-B03, P16559-B06).

Plenarvortrag

Consolidating the Foundations of Ecology and Palaeoecology by Studying the Phylogeny, Taxonomy and Biogeography of Testate Amoebae

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Testate amoebae have recently received more attention as ecologists realise that they play important roles in the cycling of C, N, and Si. Testate amoebae differ from most free-living protists by the shell (test) they produce and which makes their study easier. Their shells are preserved in peat and sediments making them useful indicators for palaeoenvironmental reconstruction. However both ecology and palaeoecological applications require a sound taxonomy, which is currently lacking. Recent phylogeny and taxonomy studies combining morphological and molecular methods are revealing complex evolutionary histories, convergent evolution and the existence of cryptic as well as pseudo-cryptic diversity. These findings revive the long-lasting debate about the biogeography of free-living microorganisms. Estimates of testate amoeba global biodiversity are likely to increase dramatically in the future and many regional endemic taxa may be discovered; but before we can be certain about this much more work will need to be done, and we need many more people to study these fascinating organisms!

Exploring the "Rare Protistan Biosphere"

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Recent interrogations of DNA isolated from environmental samples using a tag sequencing strategy have revealed new dimensions in bacterial diversity. Based on these studies, the number of bacterial taxa in a liter of ocean water increases previous estimates at least by one order of magnitude. Such highly diverse and complex bacterial communities are formed by a few dozen abundant taxa plus a large collection of rare taxa (the rare biosphere) that escapes traditional molecular methods like clone library construction and Sanger sequencing. The existence of a rare bacterial biosphere is believed to have enormous consequences for the function(ing) of any ecosystem. We used a tag sequencing strategy to test if the concept of the rare biosphere also applies to microbial eukaryotes. Therefore, we investigates a water sample from a Norwegian fjord, developed a tag sequencing strategy and a pipeline for data processing. Also, we evaluated different target regions of the SSU rDNA as appropriate fragments for tag sequencing and diversity analyses. Here we present our strategy and will reveal if a rare protistan biosphere exists.

The Effect of Geographical Distance and Environmental Conditions on Protistan Diversity in Two Permanently Anoxic Marine Basins

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The tenet that "everything is everywhere, but the environment selects" has influenced microbiology for several decades, and is still debated controversially. However, the proof or disproof of the hypothesis still remains difficult. Scant data from prokaryotic studies suggest that at least over large distances historical separation may overwhelm environmental effects, challenging the above mentioned hypothesis. Though, at smaller spatial scales environmental effects seem to dominate diversity patterns, while distance effects are of secondary importance. We tried to contribute to the ongoing discussion by investigating patterns of protistan diversity from two geographically separated marine anoxic basins (Cariaco Basin, Venezuela, Framvaren Fjord, Norway). To take distance and environmental effects into account, each basin was sampled at two different locations and along an environmental gradient. Biodiversity patterns were analyzed by applying the 454 sequencing technique. Resulting data sets were used to examine overall diversity of samples under study and to compare recorded community patterns based on taxonomic composition and similarity indices. By combining our results with contextual data from both sampling sites we tested for distance effects in relation to contemporary environmental effects.

Cryopreservation of the Ciliate *Meseres corlissi*

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Few studies exist on the cryopreservation of free-living ciliates, which is reflected by the fact that in protozoan culture collections ciliates are rarely stored in cryopreserved state. Here we present results of a study on the cryopreservation of the spirotrich ciliate *Meseres corlissi* (CCAP 1647/1), which included trophic cells, wet resting cysts and desiccated resting cysts in dry soil. Trophic cells proved to be recalcitrant to all cryopreservation methods tested. Conventional two-step methods, i.e. controlled rate cooling and "Mr. Frosty" cooling employing 5% dimethyl-sulphoxide (DMSO) as a cryoprotectant, were effective in preserving wet and dry cysts. Alternatively, a simple one-step method was tested: rapid cooling over liquid nitrogen or plunge freezing, without the need to employ cryoprotectants and costly refrigeration equipment. This approach was successful with desiccated cysts in dry soil, provided the residual moisture in these samples was < 30%. Cysts cryopreserved with this method gave rise to healthy, thriving cultures after 1 year of storage in liquid nitrogen.

Strain Deposition at the ATCC

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American Type Culture Collection (ATCC) is the oldest, largest and most diverse culture collection world wide. The culture collection includes a wide range of biological materials for research, including microbial strains, cell lines, molecular genomics tools and bioproducts. A frequently asked question from European scientists is how to deposit a strain at ATCC. This presentation will give an overview of the acquisition philosophy, share the correct way to deposit a strain/culture at ATCC and show where to get the necessary information and forms.

Protist Diversity, Distribution and Bacterivory in Baltic Sea Pelagic Redoxclines

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The steep physico-chemical gradients that characterize the transition from oxic to suboxic and sulphidic waters, also termed pelagic redoxcline, in the central Baltic Sea has been extensively studied for bacteria, revealing an important and highly productive bacterial community. However, much remains to be known of the protist community in this environment. The aim of this study was to asses the diversity and distribution of the protist community along the vertical oxygen gradient, using both molecular and classical microscopy techniques; and to estimate their grazing impact on the redoxcline bacterial community. For this we have assembled the data from two field studies conducted on board two cruises in 2007 and 2008, studying Landsort Deep and Gotland Deep. The results reveal the influence of the biochemical gradients in the redoxcline on the composition and distribution of the protist community, with the most marked changes at the sulphidic interface. Additionally they demonstrate an increased importance of ciliates compared to flagellates, and an enhanced grazing impact at the oxic/anoxic interface.

Identification of Potential Activity Markers in Protists by Molecular Techniques

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The aim of our study was to create a cultivation-independent molecular biological screening tool to distinguish active and inactive protistan taxa. This differentiation is necessary if the "realized" diversity (i.e. active cells) present in an ecosystem has to be compared to the overall diversity (active plus inactive cells). Focusing on heterotrophic protists we selected four pure cultures from different supergroups to differentiate two growth phases, i.e. inactive resting cysts and active vegetative cells. Three potential gene markers (18S rRNA, actin, cytochrome c oxidase I) were chosen and their transcription level during these two phases quantified using quantitative RT-PCR. Sequencing of the three gene markers allowed the design of specific PCR primers amplifying a short fragment of about 100 to 150 base pairs. The results of the quantitative PCR showed that all three markers are down regulated after encystation at different degrees: Transcription levels of 18S rRNA genes in cysts varied between different species thus indicating that this gene might not be suitable as an activity marker. In contrast, transcription levels of actin or cytochrome c oxidase I revealed that cultivation-independent detection of active protists should potentially be possible.

Natural Chemical Stress, Multiple Stress Resistance, and Longevity in Aquatic Organisms

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In natural aquatic environments, humic substances (HSs) comprise the majority of organic carbon, exceeding all living organisms by at least one order of magnitude. Freshwater organisms are subject are exposed to HSs. Contrary to outdated, but still recycled paradigms, HSs are taken up, interact with a variety of receptors, provoke oxidative stress, and induce transcriptional responses, such as induction of stress proteins or biotransformation and antioxidant enzymes. In sum, exposure to HSs causes a variety of stress symptoms and should be considered adverse. Yet, we show that animals are actively looking for such stressful environments. What is the reason for this obviously absurd behavior? If the chemical stress is mild, exposed invertebrates as well as vertebrates gain multiple stress resistance and may considerably expand their lifespan.

Exposed unicellular planktonic phototrophs respond by avoidance reactions and reduce their photosynthetic activity with cyanobacteria being more sensitive to HSs-mediated stress than eukaryotes. Thus, this stress structures the planktonic photosynthetic guild. With terrestrial plants, there are first indications that also HSs-exposed phototrophs gain multiple stress resistance.

Functional Analysis of Putative RNA-dependent-RNA-Polymerases (RdRPs) in *Paramecium tetraurelia*: Distinct Pathways of dsRNA- and Transgene-Induced Silencing

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In RNAi pathways, eukaryotic RdRPs are involved in different ways. Across kingdoms different RdRPs have been shown to copy ssRNA into dsRNA and to amplify dsRNA. In nematodes, RdRP has also been identified to participate in the direct synthesis of secondary siRNAs. The *Paramecium* macronuclear genome contains four genes encoding for RdRPs (1-4) with only RdRP1 and RdRP2 showing intact catalytic domains. A transcriptional analysis revealed constitutive expression of all four genes in vegetative cultures with no significant upregulation in cultures undergoing post-transcriptional gene silencing. A comparison concerning the involvement of RdRPs in vegetative silencing either induced by dsRNA or injected transgenes shows, that RdRP1 and RdRP2 are independently necessary for dsRNA silencing, but not for transgene silencing. In the latter case RdRP3 was demonstrated to be an essential component, in spite of a highly mutated catalytic domain. As a consequence of RdRP knock down, the resulting deficit of a silencing phenotype in the respective experiments was consistent with a loss of siRNAs.

Different Classes of Small RNAs in *Paramecium* Involved in RNAi and Regulation of Endogenous Gene Expression

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Differences in PAGE-migration behaviour of transgene- and dsRNA induced siRNAs suggest different pathways of synthesis, which is consistent with the involvement of different RdRPs. These small RNAs are of different origin and the responsible mechanisms are quite different. We can show that a component of the *Paramecium* transgene silencing mechanism, RdRP3, is involved in the regulation of vegetative gene expression as its silencing disrupts the stable expression of surface antigens, leading to a permanent shifting of surface antigens. This is accompanied with transcriptional up-regulation of silent antigen genes. In vegetative cultures we can show antisense RNA of all areas of surface antigen genes, and we are also able to detect small RNA species, giving rise of an homology dependent regulation mechanism regulated by different RNAs. The underlying mechanisms of this kind of vegetative, endogenous silencing remains unknown.

How To Find and Describe Intracellular Bacteria in Ciliates - an Example

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In the last couple of years several intracellular bacteria have been described according to the rules for proper description of microorganisms. Usually, intracellular bacteria depend upon their hosts and culture in vitro seems difficult if not impossible. Characterization of intracellular bacteria thus cannot be based upon data obtained with classical microbiological methods. Though the first steps of microscopical detection and even micrographic demonstrations can be done following simple protocols, these steps may only be the beginning of a long investigation with cloning, amplification and sequencing of appropriate genes and, finally, the verification of having presented a sequence of the bacteria of interest. In this presentation routine methods for detection of intracellular bacteria in ciliates shall be shown and further methods for their characterization shall be discussed on the background of pitfalls and possible problems. Sometimes, as in the case of an intracellular bacterium in *Paramecium sexaurelia*, such projects may take decades to come to a first successful end. It was now possible with a group of authors to describe *Paraholospora nucleivisitans* more than thirty years after the first observation, but a lot of new questions arose.

Intracellular Ca^{2+}-Release Channels in *Paramecium tetraurelia*

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A database search of the *Paramecium* genome reveals 34 genes related to Ca^{2+}-release channels of the IP_3 or ryanodine receptor type (IP_3R, RyR). Phylogenetic analyses show that these Ca^{2+}-release channels (CRCs) can be subdivided into six groups (*Pt*CRC-I to *Pt*CRC-VI), each one with features in part reminiscent of IP_3Rs and RyRs. We have characterized the *Pt*CRC-IV-1 gene family, whose relationship to IP_3Rs and RyRs is restricted to their C-terminal channel domain. CRC-IV-1 channels localize to cortical Ca^{2+} stores and also to the endoplasmic reticulum. This is in contrast to a recently described true IP_3 channel, a group II member (*Pt*IP_3R_N-1), found associated with the contractile vacuole system. Silencing of either one of these CRCs results in reduced exocytosis of dense core vesicles, though for different reasons. Knockdown of *Pt*IP_3R_N affects trichocyst biogenesis, while CRC-IV-1 channels show an impaired release of Ca^{2+} from cortical stores in response to exocytotic stimuli. As CRC-IV-1 channels possess features recalling both, IP_3Rs as well as RyRs, these channels may be representative for a common ancestral precursor from which IP_3R and RyR channels might have arisen during evolution of metazoans.

Gene Silencing Discloses Multiple, but Widely Specific Functional Roles of the Actin4 Subfamily in *Paramecium tetraurelia* Cells

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Paramecium tetraurelia contains an actin multigene family with 30 members. The actin4 subfamily consists of two paralogs which show less that 50% identity to the other isoforms. Actin4 has been localized to several structures in *P. tetraurelia* cells, i.e. the cleavage furrow (which could never be visualized before), nascent food vacuoles, as well as the oral apparatus, cilia and the surface pattern. Gene silencing experiments showed that only *Paramecium* cells silenced in actin4 could not divide anymore and subsequently died. We found a remarkable coherence between actin4 expression and the capacity of vegetative nuclear division of both, micro- and macronucleus, paralleled by reduced cell division. Over longer silencing periods, reduced actin4 expression entails reduced phagocytic activity paralleled by accumulation of "acidosomes" near the cytopharynx where they normally fuse with phagosomes. Additional (ultra-)structural changes observed were extensively elaborated misshaped early endosomes ("terminal cisternae") and increased autophagy. In sum, the structural/functional alterations we see mainly concern subcellular components to which *Pt*Actin4 has been localized, with the additional observation of an effect on karyokinesis.

Pharmacology of Ciliated Protozoa - Drug (In)Sensitivity and Experimental Drug (Ab)Use

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Most data on the effects of drugs as inhibitors, modulators or stimulators have been collected with higher eukaryotic, mainly mammalian cells. Although in cell biological experiments with lower eukaryotes, including ciliates, the same drugs have frequently been applied, many results remained questionable for several reasons. Most drugs had to be used in unusually high concentrations. Moreover, drug effects have rarely been verified at a biochemical or molecular level. Data steadily emerging from genomics of ciliates, mainly Paramecium tetraurelia and Tetrahymena thermophila, show that drug binding sites have only occasionally been conserved during evolution. They may vary or be totally absent in ciliate orthologs or specifically in certain paralogs. We here try to evaluate data available so far on the pharmacology of ciliates. In the future, domain analysis and drug screenings may detect compounds specifically effective in specific ciliated protozoa, including pathogenic forms, and, thus, yield an important basis not only for cell biology but also for ecotoxicology.

Reference: Plattner, H., I.M. Sehring, C. Schilde and E.-M. Ladenburger. Pharmacology of Ciliated Protozoa - Drug (In)Sensitivity and Experimental Drug (Ab)Use. Int. Rev. Mol. Cell Biol. (in press)

Increase of Tyrosinase Transcription After UV-B Irradiation in the Cellular Slime Mould *Dictyostelium discoideum*

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To examine the defense system of *Dictyostelium discoideum* to pathogens a tool has to be established, which can measure the level of Tyrosinase transcription after contact with lethal impacts. We chose UV-B radiation because it is known that UV-B is a powerful DNA damage factor. Studies on the unicellular fungus *Cryptococcus neoformans* revealed that melanin is embedded into the cell wall to protect the cells against injury and especially pathogens. The pigment Melanin is synthesized by the oxidation of phenols catalyzed by Tyrosinase. To test the hypothesis that UV-B radiation also increases the Tyrosinase transcription level in *Dictyostelium*, slime moulds were irradiated with UV-B light (312 nm) and transcription level of Tyrosinase was then examined using real-time PCR. Our results show a surprising UV-resistance of these protists because the median lethal irradiation rate (LD50) was reached first after 52 min of irradiation. Further, our results show that the level of the Tyrosinase transcript increased 13-fold during the irradiation. These results suggest that *Dictyostelium* may utilize a melanin based system to protect itself effectively against lethal radiation and perhaps pathogens.

A Secreted, Cytosolic Phopshatidyl-Specific Phospholipase C Affecting Special GPI-Anchors on the Surface of *Paramecium*

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Surface proteins anchored by a GPI- (glycosylphosphatidylinositol) in the outer membrane are widely distributed on eukaryotic cells. The GPI-anchor is cleavable by a phospholipase C (PLC) leading to a release of the surface proteins, and this process was postulated to be essential in several systems. This seems surprising as also the infectious prions causing fatal neurodegenerative diseases in human and cattle are GPI anchored and prion release is linked to its pathogenicity. We report the characterization of six PLCs in Paramecium, which all show PLC-characteristics of higher eukaryotes regarding catalytic domains and -architecture. As all of these endogenous PLCs are shown to release GPI-anchored surface proteins in vitro, we identified two enzymes (PtPLC2 and 6), that RNAi phenotypes show strong defects in release of GPI-anchored surface proteins in vivo. Moreover, these RNAi lines also show abnormal surface protein distribution and trafficking, implicating that these PLCs have more functions than GPI cleavage. As we find GFP-fusion proteins either in the cytosol and in surface protein extractions, these PLCs are obviously secreted. This is the first description of an endogenous PLC affecting GPI-anchors in vitro and in vivo.

Molecular Characterisation of the Four RdRP Genes in *Paramecium tetraurelia*

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RNA-dependent RNA polymerases (RdRPs) are widely distributed enzymes in eukaryotes, holding functions e.g. in several RNAi-related mechanisms. These mechanisms are involved in posttranscriptional as well as transcriptional regulation of endogenous gene expression and control of viruses and transposable elements. We characterised four genes encoding for putative RdRPs in *P.tetraurelia*, RdRP1, RdRP2, RdRP3 and RdRP4 in respect of sequence similarity, expression, as well as number, sequence and splice efficiency of introns. Each of the genes was found to have an intact ORF and to be expressed at a low level, suggesting to be subject to selective pressure. As a pecularity, two of the RdRP4 introns were found to be inefficiently spliced. Moreover, a comparison of the amino acid sequences revealed RdRP1 and RdRP2 to hold catalytic domains which are highly homologous to those of other eukaryotes, such as *C.elegans* or *A.thaliana*, but not to viral RdRPs. In contrast, RdRP3 as well as RdRP4 are lacking some of the most conserved amino acids of the RdRP domain, including one aspartate which was proved to be essential for catalysis. This study represents a basis for the functional analysis of RdRP-involving RNAi-related mechanisms and rises discussion of the *Paramecium* gene regulation.

Enrichment of Micronuclei and Detection of Microsatellites in *Paramecium caudatum*

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All *Paramecium* species possess one "somatic" macronucleus and different numbers of transcriptional inactive micronuclei. In *P. caudatum* the micronuclei can be found in an indentation of the macronucleus, which hinders an easy separation. Here we present an easy, low cost method to enrich micronuclei of *Paramecium caudatum*. This method yields micronucleus DNA, which can easily be used for subsequent DNA analyses.

Furthermore, we tried to detect microsatellites in the micronucleus DNA of Paramecium. These simple sequence repeats (SSRs) are tandemly repeated motifs of 1 – 6 bp found in prokaryotic and eukaryotic genomes and can be used as powerful genetic markers. Until now no microsatellites have been described for any *Paramecium* species. We speculate that microsatellites occur in the micronuclear genome. We present the current state of microsatellite detection in micronuclear DNA of *P. caudatum*.


Ökologie

Plenarvortrag

Microbial Battlegrounds - The Multiple Facets of Protozoa-Bacteria Interactions in Aquatic Communities

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Protozoa and bacteria coexist in a wide range of communities of natural, technical, and medical importance. Generally, this interaction is characterized by the extensive grazing activity of protozoa on bacterial prey populations. The long coevolutionary history of protists and bacteria suggest that a series of (co-)adaptations have evolved governing fitness and population dynamics of both players. We argue that selective grazing has given rise to diverse routes of bacterial defense and has promoted major transitions in bacterial evolution, such as multicellularity, chemical defense and pathogenesis. To test this hypothesis, we have been carrying out comparative studies on cellular and molecular antagonisms in planktonic and sessile model communities. Our studies assess (i) the mechanisms employed by protozoa to efficiently feed on bacteria, (ii) the role of cooperative and chemical defence, and (iii) the cellular targets used by bacteria to suppress trophic regulation and exploit the 'eukaryotic niche' presented by protists. Our findings reveal biofilm formation, chemical communication and targeted effector production as key elements in these interactions and suggest that these molecular adaptations may shape diversity and community dynamics at the bacterial-eukaryotic interface more than we currently appreciate.

Population Structure and Perennation Strategies of Chrysomonad Flagellates

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We investigated cyst formation and the ecological trigger of cyst formation in several pigmented and unpigmented taxa. We further analyzed the extent of ecophysiological and molecular homogeneity, or heterogeneity, of distinct protist populations and tested for correlations between molecular distance and ecophysiological adaptation. We investigated the molecular microdiversity and the ecophysiological tolerances for a total of 13 strains originating from two freshwater samples. These local population studies were further compared to the molecular and ecophysiological diversity of 28 strains originating from remote sampling sites. None of the investigated populations are homogenous, but are rather heterogeneously composed of different ecotypes and genotypes, possibly corresponding to cryptic species. This population heterogeneity may partly explain the deviations between studies on single strains and populations in both laboratory and field studies. The molecular distance between the strains was correlated with the salinity and temperature adaptation of the respective strains, contradicting the assumption that SSU rRNA variation reflects accumulated neutral mutations.

The Local Diversity of Heterotrophic Nanoeukaryotes has been Underestimated: A Case Study in the River Danube with Emphasis on Heterotrophic Flagellates

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A single 48 l plankton net sample from the River Danube has been investigated using a simple culture method, and an intensive light microscopic observation with video recording. Altogether 183 small sized (<30 mm) heterotrophic eukaryote morphospecies has been detected, including 130 heterotrophic flagellates and other pikoeukaryotes, 28 naked amoebae, 11 testaceans and 14 heliozoans. This local species richness highly exceeds present morphological and molecular investigations. 44% of heterotrophic flagellates (55 species) are undescribed, and likely new for science. The probable reason for this high species number is the successful reduction of under-reporting (a species present in a sample but not observed/noticed) with our method. Although generalisations would be premature from one sample, we suggest that: 1. under-reporting seriously limits the exploration of local species richness; 2. local microbial eukaryotic diversity may exceed our present knowledge; 3. the total number of nanoeukaryotic morphospecies has been underestimated, many undescribed morphospecies still exist in freshwater habitats.

Flagellates at Extremely Low pH – Specialist vs. Generalist Life Strategies

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Acid mining lakes (AML) are extreme aquatic habitats with strongly reduced biodiversity. They may harbor primarily specialists adapted to the harsh conditions or acidotolerant generalists that benefit from competitive release under acidic stress. To test for these alternative life strategies, we isolated two dominant flagellate species, *Chlamydomonas acidophila* and *Ochromonas* sp., from each of two similar AML (pH ~2.6) located in Lusatia, Germany, and Langau, Austria. The strain *Ochromonas* sp. DS originating from Lake Constance served as reference for a closely related neutrophil species. To account for a potential synergistic stress effect of low pH and high temperature, we measured flagellate growth rates in the laboratory over a combination of 4 pH (2.5, 3.5, 5.0, 7.0) und 3 temperatures (10, 17.5 and 25 °C). Our experimental results demonstrate that *Chlamydomonas acidophila* is specifically adapted to the harsh environmental conditions, while growth rates of *Ochromonas* sp. were positively correlated with increasing pH. In addition to the opposing life strategies, we recorded significant intraspecific differences within the closely related strains from the two AML.

Analyses of Ciliate Diversity Using Single Strand Conformation Polymorphism Analyses (SSCP Analyses)

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In the past years several molecular techniques have been established and applied to analyze ciliate diversity in different habitats. A special method is the single strand conformation polymorphism analysis (SSCP analysis) which is often applied for clinical diagnostics, especially for the detection of diseases caused by mutations. This technique utilizes that different secondary structures of single-stranded nucleic acids result in modified migration mobility of fragments through polymers. Until now SSCP analyses are still underrepresented in investigations of microbial communities. Therefore this study should test the practicability for the analysis of ciliate diversity in constructed wetlands. At first, we amplified 144 clones with newly developed and labeled primers resulting in a 300bp large gene fragment of the 18S rRNA. These PCR products were used for capillary array electrophoresis (CAE-SSCP). The analyses of the peak pattern after separation showed clear differences between the clones. For a detailed assessment sequence analyses were performed afterwards, supporting the results of the SSCP analyses. Additionally, this method can be used for a convenient and sensitive determination of ciliate diversity. Furthermore, an application of the CAE-SSCP for the detection of genera, classes etc. is even more promising.

Kurzvorträge

Significant Habitat Effects Influence Protist Fitness in Acid Mining Lakes

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It is currently controversially discussed if the same freshwater microorganisms occur worldwide wherever their required habitats are realized. We performed transfer experiments with flagellates and ciliates from three acid mining lakes (AML, pH ~2.7) to investigate if similar habitats may affect similar organisms differently. We measured population growth rates of the dominant flagellates (*Chlamydomonas acidophila*, *Ochromonas* sp.) and ciliates (*Oxytricha* spp.) as a proxy of their fitness in a crossfactorial design under standardized laboratory conditions. Each isolate was exposed to the original water from its home habitat and from two similar AML with standard food organisms. Results revealed significant effects of strain, lake (=habitat), and strain x habitat interaction. In conclusion, our study demonstrates that the same habitat may affect strains of the same species differently and that similar habitats may harbour different strains of the same or even different species.

Effekte mechanischer und biologischer Störungen auf biofilm-assoziierte Ciliatengemeinschaften

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Im Freiland können biofilmassoziierte Lebensgemeinschaften wechselnden mechanischen und biologischen Störungen (z.B. Geschiebetrieb, Grazing) ausgesetzt sein. Nach theoretischen Überlegungen, z.B. der „Intermediate Disturbance Hypothesis" (IDH), sollte dabei eine mittlere Störungsintensität einen positiven Effekt auf die Diversität der Gemeinschaft haben, während die Dichte mit steigender Störung kontinuierlich abnimmt. Diese Hypothese wurde für Biofilme des Rheins, die auf Objektträgern unter semi-natürlichen Bedingungen kultiviert wurden, getestet. Dabei wurden sowohl der Einfluss biologischer Störung (Beweidung durch eine Schnecke) auf Bakterien, heterotrophe Flagellaten (HF) und Ciliaten untersucht, als auch die Effekte unterschiedlicher mechanischer Störungsintensitäten zu verschiedenen Jahreszeiten und unterschiedlichen Temperaturen auf die Ciliatengemeinschaft getestet. Bezüglich der Biofilm-bewohnenden Ciliaten zeigten sich (1) abnehmende Abundanzen mit zunehmenden Störungsintensitäten, (2) nur geringe Auswirkungen der Störung auf die taxonomische Struktur, und (3) eine Abnahme der Störungseffekte mit Erwärmung. Des Weiteren waren die Effektstärken der mechanischen Störung für alle untersuchten Gruppen (Bakterien, Algen, HF) ähnlich, während für die biologische Störung deutliche Unterschiede nachgewiesen wurden.

Investigation on the Diversity of Prokaryotes in Tree Canopies of a Temperate Forest

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Microbes are known to play an important role in benthic and soil habitats as well as in planktonic communities. However, the role of prokaryotes and their biodiversity on leave surfaces and in degrading wood of canopies is not thoroughly investigated. Until recently, inhabitants of leaf surfaces, known as a common habitat for terrestrial microorganisms, were regarded as potential phytopathogens only. However there is evidence that they positively influence the plant e.g. by protecting the leaf from freezing or by improving the N2 fixation. We investigated the diversity and the species composition of prokaryotes in the phyllosphere and the dead wood community using culture independent methods. Samples of common oak (*Quercus robur*) and small-leaved lime (*Tilia codata*) in the canopy of the temperate forest Auewald in Leipzig were investigated using molecular methods such as T-RFLP and gen-library construction. First results indicate significant differences of the ribotype richness of Actinobacteria between tree species, whereas no significant differences could be detected between dead wood species. A clone library was constructed. So far 70 clones were screened and the results suggest a high diversity of prokaryotes in dead wood.

The Hidden Life in Treetops – A Molecular Survey of Ciliates in the Canopy of a Temperate Floodplain Forest

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Numerous studies have focused on the analyses of ciliate diversity in different habitats resulting in a huge number of detected and described species. A special environment is the canopy of trees which is still hardly investigated due to the reachability. There are only few studies focussing on ciliates on trees, especially in tree-holes or bark. Therefore, we started an analysis of the ciliate assemblage within the canopy of a temperate floodplain forest near Leipzig using molecular methods. The samples (leaves, deadwood and living wood) were taken from two tree species (*Quercus robur* and *Tilia cordata*) at a tree height of 30m. The samples were investigated directly after sampling, as well as 3 and 7 days later using the "non-flooded Petri dish method". After DNA extraction total DNA was amplified using ciliate specific primers. PCR products were used for cloning and the clones were determined by restriction enzyme analyses and sequencing. 50 clones could be assigned to 15 species after BLAST search and the most sequences belong to order Colpodida which are known as typical soil inhabitants. The results show clear differences in the ciliate communities isolated from basswood and common oak as well as from different sources.

Factors Structuring Spatial and Temporal Patterns of Protistan Communities in an Anoxic Water Column

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Multiple studies based either on morphological investigation or on the application of molecular tools revealed that the composition of protistan communities in anoxic waters is dynamic over spatial and seasonal scales. Variations in local community patterns are assumed to reflect changes in environmental conditions, even though the identification of responsible key parameters is difficult. By investigating the composition of three distinct protistan communities along a vertical O_2/H_2S gradient in the Framvaren Fjord (Norway) we tried to make out abiotic and/or biotic factors shaping community composition. Sampling and collection of contextual data was conducted at different sampling sites within the fjord as well as in different seasons. Community patterns were determined by T-RFLP and tested for correlation with e.g. oxygen or hydrogen sulphide concentrations, or composition of prokaryotic communities. While protistan communities displayed no clear correlation to any of the considered abiotic parameters, the composition of prokaryotic communities reflected the redox regime. We hypothesize that variation within protistan communities is at least in parts due to biotic factors and mirrors changes in prokaryotic communities, as a result of prey-predator relationships.

Grazing Activity of Ciliates and Flagellates with Different Feeding Modes Influence Bacterial Biofilm Morphology

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Grazing activity of protists contributes to morphological changes of biofilms. Since the lifestyle and food uptake of gulper feeders is strongly associated to surfaces, gulper feeders may influence biofilm morphology more than filter feeders. We investigated the impact of *Dexiostoma* (filter feeder, Hymenostomatia, Ciliophora), *Chilodonella* (gulper feeder, Phyllopharyngia, Ciliophora), and *Spumella* sp. (interception feeder, Chrysophyceae, Chrysophyta) on the morphology of multispecies bacterial biofilms in small flow cells. The vagile gulper and filter feeders stimulated microcolony formation about 3.5 - 4 times. Microcolony abundance was not altered by the sessile interception feeder. Thus, the stimulation of microcolony formation seemed to be influenced by the mobility of grazing protists. Biofilm volume was not altered in the presence of the filter feeder but was 2.5 - 6.3 times lower in the presence of the gulper and interception feeders compared to ungrazed biofilms. Gulper and interception feeders caused a 1.2 - 1.8 times higher biofilm surface area to biofilm volume ratio (BSA / BV), which might improve exchange of nutrients and gases between the biofilm and its surrounding fluid, hence accelerating microbial growth.

Determination and Influences of Variable Extrinsic and Intrinsic Parameters on Population Dynamics of the Ciliate *Tetrahymena pyriformis*. Model Analyses and Preliminary Results.

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The knowledge regarding intrinsically driven population dynamics is very limited. We use a defined microbial system to study the interactions between extrinsic and intrinsic factors. Changes in the population dynamics of a chemostat system that consists of two prey bacteria (*Pedobacter sp.* and *Brevundimonas sp.*) and a ciliate as predator (*Tetrahymena pyriformis*) are investigated as a model system. In addition, we used a simplified mathematical model after Takeuchi and Adachi (1983) for a two-prey-one-predator system to check for different model outcomes at varying parameter sets.Laboratory experiments of growth rates of each species were a prerequisite to understand the effect of temperature changes on the dynamic behaviour. Experiments with a defined temperature change (of 5 degrees up) resulted in an observable response of *Brevundimonas*, leading to a shift in the associated attractor model (abundance). This was confirmed by mathematical modelling.

Analysing Protist Population Structures by Multiplex Single-Cell PCR from Preserved Plankton Samples

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We extended a previously presented method for quantitative analysis of planktonic protists and microalgae from field samples preserved with Lugol's iodine solution to a multiplex PCR approach covering nucleus encoded as well as mitochondrial genes. We focused on genes with a different phylogenetic resolution and specifically also on genes which are used for barcoding. We will present the methodological advances as well as first results on chrysophyte population studies.

Poster

First Description of *Euplotes raikovi* as Natural Reservoir of *Francisella philomiragia*

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The genus *Francisella* comprehends 2 species, *F. tularensis* and *F. philomiragia;* both are short rod-shaped or coccoid facultative intracellular bacteria. *F. tularensis* is the etiological agent of tularaemia, *F. philomiragia* is often closely linked to water-borne transmission and may cause severe disease with pneumonia and/or septicaemia. Most cases occur in immune-compromised individuals or near-drowning patients. The present study reports the detection of intracellular bacteria in the marine ciliate *Euplotes raikovi*. These bacteria have been characterized by the full-cycle rRNA approach. Phylogenetic analyses positioned them closely to *F. philomiragia*. Fluores-cence in situ Hybridization with a *Francisella*-specific probe confirmed the presence of *F. philomiragia* within the *Euplotes* cells. Hitherto *Francisellaceae* have been found only as endosymbionts in arthropods or as intracellular pathogens in rodents, but never in protists. This finding raises the question if ciliates serve as a natural reservoir for infective bacteria in a much larger extent than previously expected.

High Resolution Electron Microscopy of R-Bodies in *Caedibacter* Species, Endosymbionts in Ciliates of the Genus *Paramecium*, Revealed by Electron Tomography

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Electron tomography has become an important tool to reveal high resolution data of transmission electron microscopical samples after reconstructing the volume from a tilt series, subsequent segmentation and visualization. The possibility to display results as three dimensional data and the generation of virtual sections of few microns thickness give new details in subcellular structures. Preleminaryy results of the investigation of R-bodies from *Caedibacter caryophilus* and *C. taeniospiralis*, endosymbionts of the ciliate genus *Paramecium* are given.

Intriguing Parasite-Host Interactions of the Apicomplexan *Eimeria bovis*

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Eimeria bovis is an obligate intracellular parasite of cattle causing severe haemorrhagic typhlocolitis in calves and high economic losses worldwide. *Eimeria bovis* sporozoites need to invade the endothelial cells of the central lymph capillaries of the villi of the ileum, in order to develop. Once established they develop into first generation macromeronts (300 µm) within a period of 14-18 days. *Eimeria bovis* must rely on several regulatory processes to ensure the sustained development within the host endothelial cell. We have shown that *E. bovis* modulates the host cell cytoskeleton, host cell apoptosis and the gene transcription of immunomodulatory molecules in infected endothelial cells. To characterise early innate interactions between *E. bovis*-infected endothelial cells and PMN we used an *in vitro* system with bovine umbilical vein endothelial cells (BUVEC) as host cells. Adhesion of PMN to *E. bovis*-infected BUVEC was determined by using the parallel plate flow chamber assay which simulates physiological flow conditions. We further analysed the transcription of adhesion molecule genes in infected host cells. PMN adhesion was enhanced in *E. bovis*-infected BUVEC layers as early as 8h p. i. PMN adhered to both infected and non-infected cells within BUVEC, suggesting paracrine cell activation. *Eimeria bovis* infection up-regulated the transcription of genes encoding for P-selectin, E-selectin, VCAM-1 and ICAM-1. *Eimeria bovis* had suppressive effects on TNF-α-mediated up-regulation of adhesion molecule gene transcription. Furthermore, *E. bovis* sporozoites failed to induce CXC chemokine genes in BUVEC (e. g. GRO-α, IL-8, IP-10) and COX-2 and only caused moderate transcription of MCP-1, RANTES, GM-CSF and iNOS genes when compared to *T. gondii* infections. As *E. bovis* sporozoites have to cross the mucosal layer of the ileum to infect lymphatic endothelial cells they might be exposed to interstitial fluid, lymph and leukocytes. To mimic this situation *in vitro*, we exposed *E. bovis* sporozoites to PMN and found enhanced elimination of the parasites. Addition of immune serum clearly increased these reactions, whereas neonatal calf serum had no effect, thus suggesting a PMN-derived antibody-dependent cytotoxicity. Scanning and transmission electron microscopy showed PMN engulfing sporozoites or extending filopodia towards them and occasionally incorporating the parasites. PMN reacted by showing enhanced transcription of the IL-6, MCP-1, GRO-α, TNF-α and iNOS genes after the exposure to sporozoites, while stimulation with merozoite I antigen, in addition, up-regulated IL-8, IP-10 and IL-12 gene transcription. Enhanced *in vitro* oxidative burst and phagocytic activities were observed after the contact of PMN with viable sporozoites. To verify *in vivo* the potential role of PMN, we analysed the general phagocytic and oxidative burst activities of PMN obtained *ex vivo* from *E. bovis* experimentally infected calves. Enhanced levels of both activities were found early p. i. (1-5 days) and towards the end of the first merogony (days 13-22 p. i.) thus supporting the *in vitro* data. Phagocytosis, oxidative burst, production of proinflammatory molecules and neutrophil extracellular trap (NET)-formation were identified as the main PMN-derived effector mechanisms against *E. bovis*. In addition, here, we provide for the first time an indication of *E. bovis*-induced NET-formation, probably facilitating the killing of large-sized pathogens, a hypothesis which is still currently undergoing more detailed investigation.

Serological Detection of *Toxoplasma* Infection in Turkeys

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Since turkey meat consumption increased over the last years, it is important that this meat and the products thereof are free of harmful infectious agents. *Toxoplasma gondii* is a ubiquitous parasite infecting many animal species as well as humans. In order to evaluate the risk posed by turkey meat it is a necessity to have a handy tool for serodiagnostic surveys. Therefore, we developed a kinetic ELISA for the detection of antibodies directed against *Toxoplasma gondii* in the sera of turkeys. Out of nine antigens tested, comprising ROP1, MAG1, SAG1, GRA1, GRA2, GRA6, GRA7, GRA8, and GRA9, only GRA7 and GRA8 and the combination of both were proven to be most suitable for this purpose. Furthermore, different dilutions of serum and detection antibody have been tested. Best results were achieved when a serum dilution of 1:500 and a detection antibody dilution of 1:1000 have been used.

Porcine Coccidiosis – Immune Response to *Isospora suis* in Piglets

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Isospora suis is the causative agent of neonatal porcine coccidiosis, a frequent disease in suckling piglets. Despite its economic and veterinary importance, interactions between the parasite and the immune system of the host are still poorly understood. To address these interactions, piglets were infected with *I. suis* on the third day of life and re-infected after six month. Thereafter lymphocytes were isolated from blood (PBMC), spleen and mesenteric lymph nodes (MLN) and analysed for their antigen-specific reactivity *in vitro* in IFN-γ ELISPOTs and proliferation assays. *Isospora*-specific production of IFN-γ was detected in PBMC and splenocytes. After MACS-depletion of distinct T-lymphocyte subpopulations $CD4^+$ T-helper cells and TcR-γδ-T cells were identified as antigen-specific responders. In contrast, antigen-specific $CD8^+$ cytolytic T lymphocytes seemed to represent the reactive T-cell subset in the MLN.

Kurzvorträge

Isolierung von *Thecamoeba quadrilineata* mit pilzartigen intranukleären Parasiten und Beschreibung ihrer Entwicklung und des Wirtsspektums

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In einer aus Moosproben isolierten *Thecamoeba quadrilineata*-Population wurden einzelne Trophozoiten beobachtet, die intrazelluläre Aggregate von rund-ovalen Endozytobionten (KTq-2) aufwiesen. Elektronenmikroskopische Untersuchungen ließen Sporen mit einem echten Zellkern erkennen, was die Vermutung nahe legte, dass es sich um Pilze handeln könnte, was noch durch die positve Calcofluor White-Färbung unterstützt wurde. Die in Subkulturen der infizierten Amöben beobachtete Entwicklung der Endozytobionten beginnt mit der Phagozytose der Sporen durch den Wirt. Über das Zytoplasma gelangen die Sporen in den Kern, wo erst die eigentliche Entwicklung im oder am Endosom beginnt. Nach dem Heranwachsen differenzieren sich die Parasiten noch immer innerhalb der Kernmembran erneut zu zahlreichen Sporen, die nach dem Zerfall des Wirtes frei werden und von anderen Thekamöben ingestiert werden können. Durch Kokultivierungsversuche wurde die Empfänglichkeit für KTq-2 bei T. striata, T. terricolae und mit Einschränkungen auch bei Sappinia spp. nachgewiesen. Die Ergebnisse werden mit den frühen Beschreibungen von Nucleophaga sp.(Dangeard, 1886; Doflein und Reichenow, 1929) verglichen.

Isolierung von *Acanthamoeba* Genotyp T5 aus dem ZNS

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Akanthamöben kommen ubiquitär vor und können beim Menschen zwei grundsätzlich verschiedene Krankheitsbilder hervorrufen, die Acanthamoeba-Keratitis und die Granulomatöse Amöbenenzephalitis (GAE). Bei der GAE handelt es sich um eine meist letale Entzündung des Gehirns, die allerdings fast ausschließlich bei Immunsupprimierten vorkommt. Derzeit sind innerhalb der Gattung Acanthamoeba über 20 Arten und insgesamt 15 Genotypen (T1-T15) beschrieben. Die weitaus meisten klinischen Isolate zeigen Genotyp T4, jedoch sind durchaus nicht alle Vertreter dieses Genotyps pathogen. Ein 17-jähriger immunkompetenter Patient wurde mit hohem Fieber und Bewusstseinstrübung ins Krankenhaus eingeliefert. Bereits im Vorfeld war vom niedergelassenen HNO-Arzt eine Nasennebenhöhlenentzündung festgestellt worden. Eine Untersuchung des Liquors ergab sowohl in der Kultur als auch in der PCR einen positiven Acanthamoeba-Nachweis. Die Amöben wurden anschließend nach ihrer Zystenmorphologie als Acanthamoeba lenticulata bestimmt und mittels Sequenzierung als Genotyp T5 identifiziert. T5 ist zwar als potentiell virulent eingestuft und zeigt hohe Zytopathogenität in der Zellkultur, ist aber bisher noch nie aus dem ZNS isoliert worden.

Stage-Specific Expression of Six Calcium-Dependent Protein Kinases in *Cryptosporidium parvum* in Vitro

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Cryptosporidium spp. are apicomplexan pathogens with zoonotical impact causing intestinal or even respiratory infections in a wide range of hosts. *Cryptosporidium parvum* possesses seven calcium-dependent protein kinases (CDPKs) and four calmodulin domain related protein kinases (CRKs). Such kinases have previously been described in plants, algae and other apicomplexa. Based on phylogenetical and structural analysis, six kinases with classical domain structure were chosen to be analysed for stage-specific expression. The sequences annotated as cgd3_920, cgd5_820, cgd4_3330, cgd2_1300, cgd2_1060 and cgd7_1840 were recovered from CryptoDB.org and were detected by means of nested 3prime RACE-PCR. A specific antibody was generated for cgd3_920 by immunisation of rabbits. Human ileocecal adenocarcinoma cells (HCT-8) were infected with sporozoites from 4 x 10^5 freshly excysted oocysts of *C. parvum* in 6 well plates. The detection of the transcripts was carried out on 3 h, 21h, 27 h, 43 h and 51 h post infection. Only cgd4_3330 was detected at all points in time, while the transcripts of cgd2_1300, cgd2_1060 and cgd7_1840 were detected at 21 h, 27 h, 43 h and 51 h post infection. Transcripts of cgd3_920 were found at 21 h, 43 h and 51 h post infection.

In the immunoblot the anti cgd3_920 specific antibody showed strong reaction with an antigen at 56 kDa in protein extracts of excysted oocysts, which corresponds to the calculated molecular weight of cgd3_920 of 55.72 kDa, but presented no reaction with antigen extracts of infected HCT-8 cells.

CDPKs coded by cgd3_920, cgd5_820, cgd4_3330, cgd2_1300, cgd2_1060 and cgd7_1840 of *C. parvum* are transcribed in infected HCT-8 cells and CDPK coded by cgd3_920 is translated in sporozoites but not in infected HCT-8 cells at 48 h.