Abstract PV 2

The role of *Toxoplasma* rhoptries in invasion and host-pathogen interaction

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Toxoplasma gondii is an obligate intracellular parasite in the phylum Apicomplexa that causes severe central nervous system disorders of immunocompromised (AIDS/transplant/lymphoma) individuals and birth defects to congenitally infected neonates worldwide. In addition to being a globally important pathogen, *Toxoplasma* also serves as a model system for studying Apicomplexan parasites which cause a number of diseases of medical and veterinary importance including *Plasmodium falciparum*, the causative agent of malaria which kills 1-2 million people each year.

One defining feature of these parasites is the presence of rhoptries, unique secretory organelles that are involved in host cell invasion and establishment of the parasitophorous vacuole for intracellular survival. While secretion of the rhoptries has long been implicated in the establishment of infection, only a few rhoptry proteins have previously been reported and little is known about their precise role in this process. We have recently carried out a proteomic analysis of purified rhoptries from *Toxoplasma* and identified many novel proteins in the rhoptry fraction, including a subset of these proteins which localize to the duct-like rhoptry necks. One unexpected finding is that rhoptry neck proteins are released during invasion and localize to the moving junction, a structure that forms the interface between the host and parasite during invasion. The moving junction serves as a “molecular sieve” which allows host lipids but excludes host transmembrane proteins from the forming parasitophorous vacuole. This sieving process likely allows for the successful avoidance of the host endocytic system and host lysosomal destruction.

As a complementary approach to the proteomic analysis, we have also used a monoclonal antibody approach to analyzing the rhoptry fraction. One highlight of this approach has been the identification of a rhoptry-localized protein phosphatase 2C-like protein (TgPP2L) that is targeted to the host nucleus during infection. TgPP2L staining is detected in the host nucleus early following invasion, indicating that its delivery to the host cell is an early event of infection. To determine the precise function of TgPP2L, we have disrupted its gene by homologous recombination. Disruption of the gene results in failure to stain both the rhoptries and infected host nucleus. We have subsequently complemented the disruption using TgPP2L driven by its own promoter which restores both rhoptry and host nuclear staining. Our current goals are to express recombinant TgPP2L to determine if this protein is indeed a phosphatase and use human microarrays to determine host genes that are differentially expressed upon infection with both wild-type and TgPP2L knockout strains of *T. gondii*.

Abstract PV 5

Bakterien in und auf Protozoen: Prokaryotische Symbionten von Termitendarm-Flagellaten

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Die symbiotischen Assoziationen der Termiten mit ihren Darmmikroorganismen sind seit bald einem Jahrhundert Gegenstand des biologischen Interesses. Während sich die frühen Arbeiten zunächst auf die intestinalen Protozoen der niederen Termiten und deren Rolle bei der Verdauung beschäftigten, konzentrierten sich die neueren Untersuchungen auf die prokaryotischen Darmmikroorganismen und ihre metabolischen Aktivitäten. Die Anwendung von Mikrosensortechniken zeigte eine unerwartete Dynamik der physikochemischen Bedingungen im Darm und brachte erste Hinweise auf eine räumliche Strukturierung der mikrobiellen Aktivitäten. Die Vielzahl der verfügbaren Mikrohabitate hat eine entscheidenden Einfluss auf die Zusammensetzung und metabolische Aktivitäten der intestinalen Lebensgemeinschaft.

Die wichtigsten Lebensräume für Prokaryoten im Darm niederer Termiten sind die intestinalen Protozoen, da sie einen Großteil des Enddarms ausfüllen und eine enorme Oberfläche zur mikrobiellen Besiedelung bereitstellen. Obgleich schon lange bekannt ist, dass die meisten Darmflagellaten eng mit prokaryotischen Zellen assoziiert sind, wurde die Bedeutung dieses Phänomens bisher unterbewertet. Schwierigkeiten bei der Kultivierung der Protozoen und das komplette Fehlen von Reinkulturen der prokaryotischen Symbionten erlaubten bis vor Kurzem nur eine morphologische Beschreibung der unterschiedlichen Assoziationen, so dass die Natur der jeweiligen Symbiosen und deren Bedeutung für die beteiligten Partner meist im Dunkeln blieb.

In den letzten Jahren haben die Verfügbarkeit von molekularbiologischen Werkzeugen und die daraus resultierenden experimentellen Möglichkeiten das Interesse an den symbiotischen Verbindungen zwischen Prokaryoten und Termitendarm-Flagellaten erneuert entfacht. Der Vortrag gibt einen Überblick über die Vielfalt der Assoziationen von Protozoen mit prokaryontischen Symbionten, wobei der Schwerpunkt auf den Epibionten und Endobionten auf der Oberfläche und im Zellinnern der Termitendarm-Flagellaten liegt. Die neuesten Erkenntnisse phylogenetischer Studien über die Identität der Symbionten werden zusammengefasst und an konkreten Beispielen vertieft. Soweit es der Stand der Erkenntnisse erlaubt, wird auch auf die funktionelle Bedeutung der Symbionten und ihre mögliche Rolle im Stoffwechsel des Termitendarms eingegangen.

Abstract PV 7

The *Paramecium* genome: a challenge to study the molecular machinery of vesicle transport and fusion along the various trafficking routes

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Trafficking among intracellular membrane compartments, and to and from the cell surface, is largely mediated by vesicular transport. A high degree of specificity and complexity occurs in the regulation of vesicle budding, docking and fusion. Central to the docking and fusion process are SNARE (soluble NSF attachment protein receptors) proteins, a family of well-characterized small transmembrane or membrane-associated proteins that are able to form stable parallel, twisted four-helix bundles, when interacting from opposing membranes. Three of the helices are contributed by Q-SNAREs (syntaxin, SNAP-25) present on one membrane, with the other helix provided by an R-SNARE (synaptobrevin) present on the opposite membrane. Distinct sets of them help maintaining identity of a given intracellular compartment. Although in *Paramecium* several differentiated membrane trafficking pathways co-exist, which are well-described on the LM and EM level, less is known on the molecules involved.
On the basis of the current *Paramecium* genome project we have identified several multigene families encoding protein components relevant for vesicle biogenesis, transport and the docking and membrane fusion machinery, including those acting downstream membrane fusion. For example, multigene families exist for individual subunits of the V-ATPase, actin, synaptobrevin and syntaxin, each of them containing numerous members of which some also contribute to vesicle trafficking. In case of syntaxin *Paramecium* contains up to 26 members that all match the classical build-up of syntaxins, being 'tail-anchored' membrane proteins with an N-terminal cytoplasmic domain and a membrane-bound single C-terminal hydrophobic domain. The membrane anchor is preceded by a conserved SNARE-domain of ~60 amino acids that participates in SNARE complex assembly. In a phylogenetic analysis, most of the *Paramecium* syntaxin genes were found to cluster in groups together with those from other organisms in a pathway-specific manner, allowing an assignment to different compartments in a homology-dependent way. However, some of them seem to have no counterparts in metazoans. Other approaches were to fuse one representative member of each of the syntaxin clusters to GFP and to assess the *in vivo* localization, or to do immuno-localization of individual syntaxins. The combination of all allowed us to identify syntaxins of all important trafficking pathways in *Paramecium*.
Although some of the components are encoded by only a few genes, such as pp63/pf, SNAP-25 or the SNARE-specific chaperone NSF, the existence of multigene families with the unexpected degree of paralog diversification might be a lucky chance to analyze and dissect single protein-protein interactions within these pathways.

Abstract PV 1

The revised higher classification of eukaryotes: from the past to the present

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The International Society of Protistologists recently published a higher classification of eukaryotes as the joint work of 28 scientists. This classification represents our current views on the relationships among eukaryotes, based fundamentally on significant discoveries made in the last quarter of the 20th Century and the early years of this one. By this classification, our Society now formally recognizes the “protists” as pivotal organisms in the diversification of eukaryotes.

Nevertheless, the path to this formal recognition has not been straight. In the late 19th Century, Haeckel recognized the phylogenetic significance of protists, but popular classifications, such as Bütschli’s view of the protozoan protists, segregated the group along academic disciplinary lines. The development of a natural classification of eukaryotes, that is one reflecting true phylogenetic relationships, involved the interplay of scientific concepts and technological advancements in the context of a developing sociological strengthening of protistology as a discipline.

I will trace this development by commenting on concepts, technology, and sociology in four periods. (1) In the early 1900s, professorships were being established and the first journal, the “Archiv” was published in 1902. From 1900-1953 the prime technological approach was light microscopy refined by the transfer of histological stains to the protists, the Society of Protozoologists was founded in 1947, and “The Journal of Protozoology” first published in 1953. Although the focus was on organelles and cell form, the Honigberg et al. classification of 1964 was still very Bütschlian. (2) From 1954-1975, membership in the Society of Protozoologists increased ~3,000%. By the mid-1960s, sections or societies had been established in almost a dozen countries. Nomarski differential interference microscopes were commercialized by Zeiss in 1965 and most major biology departments now had a transmission electron microscope. “Protist” re-emerged as a concept with Whittaker’s five-kingdom classification (1959, 1969), the serial endosymbiosis theory was championed by Margulis (1970) and Taylor (1974), and ultrastructural features in the context of Ehret’s (1960) levels of biological organization were assuming central systematic importance (Lynn, 1976). Nevertheless, the Levine et al. classification (1980) was only a slight modification of the Bütschlian view, with a proliferation of higher “sporozoan” groups. (3) From 1976-1990, gene cloning and sequencing, the polymerase chain reaction, and molecular phylogenetic tools provided new technical approaches to supplement those of microscopy. The International Society of Evolutionary Protistology reached a critical mass at the 1978 meeting in Toronto and was incorporated in 1982, providing a social identity to the “protist” perspective. Conceptual approaches continued those of the previous period with emphasis by Patterson & Brugerolle (1988) on “ultrastructural identities” as synapomorphies of major protist clades, coupled with the pursuit of gene sequences, particularly the small and large subunit rRNA genes by Sogin’s and Adoutte’s groups respectively. While no consensus classification emerged at this time, the “Handbook of Protoctista”, edited by Margulis, Corliss, Melkonian, and Chapman (1990) with 61 authors recognized 35 protist phyla, but not arranged phylogenetically. (4) From 1991-2006, the sociological solidity of the “protist perspective” resulted in the Society of Protozoologists changing its name to the International Society of Protistologists. There has been a continued increase in data and understanding of the genetic diversity of eukaryotes. This leads to a discussion of the six major clades of eukarotes that we now recognize in our revised classification: 1) Amoebozoa; 2) Opisthokonta; 3) Excavata; 4) Rhizaria; 5) Archaeplastida; and 6) Chromalveolata.

Abstract PV 6

Dinoflagellates, some of the oldest and strangest protists: phylogeny, taxonomy, biology seen in a modern context

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The name Berlin occupies a central role in the dawn of dinoflagellate reseach, due to the work of Professor C.G. Ehrenberg. In his 1838 book on the "Infusionsthierchen" , Ehrenberg divided these Thierchen into 12 families, one of which he named Peridinaea. The name was coined after *Peridinium*, a genus Ehrenberg himself created in 1830 in one of his copious contributions filling up the volumes of the Abhandlungen der Königlichen Akademie der Wissenschaften zu Berlin. At the same time, the medical doctor Michaelis discovered that luminescence of the water in Kieler Bay was caused by some of Ehrenberg's *Peridinium* species and related forms.

The name Dinoflagellata goes back to another German, Otto Bütschli in Heidelberg, in the first volume of Dr Bronn's "Klassen und Ordnungen des Thierreichs" (1885). Bütschli classified the Dinoflagellata as one of the four orders of the class Mastigophora.

Dinoflagellates are unique in many respects. Firstly, if we accept that the Acritarchs are primitive dinoflagellates (which is likely), then the group is the oldest known group of eukaryotes, discovered recently in some 1.7-billion-year-old sediments in Northern Territory in Australia.

Considering their great age, it is therefore hardly surprising that the group is so diverse, although it is perhaps more surprising that the group does not appear to have given rise to any truly multicellular forms (or such forms, if they ever existed, have died out).

Present ideas on the phylogenetic relationships of dinoflagellates all agree that their closest relatives are the Sporozoa (Apicomplexa) and the Ciliates. This whole complex, named Alveolata by Tom Cavalier-Smith, is now considered to be phylogenetically related to the heterokont protists, confirming Tyge Christensen's idea of the Chromophyta for all the "brown" algae. This idea has been in discredit for the past 20 years, but the group is now being reborn under the name Chromalveolata.

Dinoflagellates are unique also in containing in their cells several types of chloroplasts: green, blue or brown, indeed in what is now know to comprise a natural groups of dinoflagellates, the cells contain two different chloroplasts, one of which has been transformed to function in light perception only. These chloroplasts are the results of endosymbiotic events, which appear to having taken case independently and successfully on 5-10 occasions. In all other groups of algae, the chloroplasts of each group are monophyletic. Very unexpectedly, recent studies have demonstrated the presence of only approx. 15 genes in each dinoflagellate chloroplast instead of the 100-200 in other chloroplasts, each gene forming a minicircle (only a group of brown species has been examined).

Also, dinoflagellates are unique in producing a series of very diverse toxins, some of which are among the most toxic compounds known, poisoning people and causing economic losses to fishermen and aquaculture owners worldwide.

We have over the past many years concentrated our plankton studies to cover both marine and freshwater dinoflagellates and, when applying modern techniques, we have discovered that present ideas of taxonomy and classification of the dinoflagellates in numerous cases need a fresh start. The old system is very far from being a natural (phylogenetic) one. This applies to both the so-called unarmoured (naked) forms and to many of the armoured forms or, for that matter, to the "poorly armoured" forms whose cells are surrounded by a thin "wall" of plates. Our work on the marine forms goes back to our interest in the toxic species while our more recent interest in the freshwater forms began in the "red lake" Lago di Tovel in the Italian Alps, during a project aiming at understanding why the lake has lost it tomato-red colour.

Abstract PV 3

Die strittige Natur der Hydrogenosomen – Organellen sui generis oder doch stark abgeleitete Mitochondrien?

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Morphologische und insbesondere biochemische Angaben aus den Jahren 1950-1970 zeigten dass in bestimmten, verschiedenen taxonomischen Gruppen zugehörenden, so genannten anaeroben Protisten keine typischen Mitochondrien zu finden sind. Diese Organismen wurden dementsprechend als amitchondrial bezeichnet. Zuerst in Trichomonaden (1973) und nachher auch in einigen Ciliaten, Heterolobosen und Chytridien wurde an Stelle des typischen Mitochondriums ein neues Organell, das Hydrogenosom, entdeckt. Dieses Organell ist durch seinen ungewöhnlichen Metabolismus charakterisiert, besonders durch die Fähigkeit, Wasserstoff als metabolisches Endprodukt zu bilden.

Obwohl ein endosymbiotischer Ursprung dieses Organells bald nach seiner Entdeckung vorgeschlagen wurde, blieb es lange Zeit es eine strittige Frage, ob das Hydrogenosom ein Organell sui generis ist, das unabhängig von Mitochondrien durch einen eigenen endosymbiotischen Prozess enstand, oder ob es seine Existenz demselben Prozess verdankt wie die Mitochondrien. Biochemische Unterschiede zwischen den zwei Organellen, die im Vordergrund von früheren Untersuchungen standen, sprachen eher für die erste Hypothese, die auch durch den in den achtziger-neunziger Jahren allgemein akkzeptierten Stammbaum der Eukaryonten scheinbar unterstützt wurde.

Erforschung der Biogenese der Hydrogenosomen erschlossen ihre weitgehende Übereinstimmung mit der Biogenese typischer Mitochondrien, ein Befund, der für den gemeinsamen Ursprung beider Organellen zeugte. Analyse der phylogenetische Verteilung und auch neuere Angaben über die Biochemie verschiedener Hydrogenosomen unterstützten weitgehend die Schlussfolgerung, dass die typische aerobe Mitochondrien und die anaeroben Hydrogenosomen Derivate eines einzigen, ursprünglichen endosymbiotischen Ereignisses sind und dass ihre Unterschiede nur durch spätere Evolution entstanden sind.

Diese Befunde zeigen, dass die klassische Definition des Mitochondriums erweitert werden muss, um die metabolische Diversität der Organellen zu erfassen, die einen gemeinen Ursprung haben.

Abstract PV 6

Zur Nanofauna der Tiefsee: Protistologische Biodiversitätsforschung im Rahmen verschiedener METEOR-Expeditionen

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Über 60% der Erdoberfläche werden von Wassermassen über 1000 m bedeckt. Damit ist die Tiefsee das größte zusammenhängende Habitat der Erde. Organismen erfahren in der Tiefsee extreme Bedingungen, wie einen hohen hydrostatischen Druck und geringe Nährstoffkonzentrationen. Dazu kommt ständige Dunkelheit sowie eine konstante, meist niedrige Temperatur. Die Summe dieser Faktoren führt zu der Ausbildung einzigartiger Lebensformen unter der Makrofauna mit einer hohen aber bisher noch unzureichend untersuchten Diversität. Die Protozoen der Tiefsee waren mit Ausnahme der Foraminiferen bis vor kurzem kaum untersucht. So wurde auch die im Stoffumsatzgeschehen wichtige Nanofauna (bestehend vor allem aus Flagellaten und Gymnamöben) bisher meist ignoriert. Pionieruntersuchungen der letzten Jahre haben jedoch neue Erkenntnisse über die Struktur der Nanofauna in verschiedenen Tiefseebereichen hervorgebracht, die im Rahmen des Übersichtsvortrages vorgestellt werden.
Überraschendes Ergebnis der ersten taxonomischen Studien zur Nanofauna der Tiefsee war, dass viele Arten gefunden wurden, welche bereits aus Oberflächengewässern bekannt waren. Diese Erkenntnis basierend auf Morphotypen, ließ sich später auch anhand der nachgewiesenen Genotypen bestätigen. Das Auftreten gleicher Arten in Tiefsee und Oberflächengewässern hat die Frage nach möglichen Rekrutierungswegen der Tiefseenanofauna aus dem Oberflächenwasser aufgeworfen. Dabei konnten zwei Mechanismen, und zwar zum einen über partikelassoziierten Transport und zum anderen über Konvektion von Wassermassen, identifiziert werden. Dennoch, aufgrund sowohl morphologischer als auch genetischer Merkmale lassen sich auch neue Formen in der Tiefsee nachweisen, die die Existenz von eigenen Tiefseearten und damit ein Nebeneinander von Ubiquisten und speziell angepassten Arten vermuten lassen.
Bezüglich der räumlichen Verteilung der Nanofauna in der Tiefsee lassen sich klare vertikale Gradienten mit einer starken Abnahme der Quantität mit der Tiefe sowohl für planktische als auch für benthische Protozoen nachweisen. Dieser vertikale Gradient scheint vor allem mit dem abnehmenden Angebot an nutzbaren organischen Substanzen aus der euphotischen Zone zusammenzuhängen. Aber auch innerhalb homogener Tiefen kann es starke Unterschiede in der Struktur der Nanofauna geben. Auf der großräumigen Skala, etwa zwischen verschiedenen Tiefseebecken gleicher Tiefe, lassen sich strukturelle Unterschiede erkennen. Auf der kleinräumigen Skala lassen sich deutliche Abhängigkeiten der Quantität und Diversität der Nanofauna von den Substrateigenschaften nachweisen. So beherbergen Hartsubstrate eine gegenüber den umgebenen Weichsubstraten stark erhöhte Dichte und Diversität an Protozoen. Manche Tiefseegebiete sind extrem arm an solchen Strukturen. Hier kann es zu Konzentrationen der Nanofauna auf künstlichen Hartsubstraten anthropogener Herkunft kommen.

Notizen

P 2

Tintinnopsis cylindrica Daday, 1887: a frequently recorded, but morphologically insufficiently known tintinnid (Ciliophora, Spirotricha)

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Although *Tintinnopsis cylindrica* Daday, 1887 is apparently widely distributed in the plankton of marine and brackish coastal waters, its ciliary pattern remained unknown. Without detailed knowledge of the cell morphology, however, the proposed synonymies cannot be proved. Hence, the cell and lorica features of *T. cylindrica* are redescribed from live and protargol-impregnated specimens collected in mixo-polyhaline basins of the Beltringharder Koog and the Speicherkoog Dithmarschen, two polders at the North Sea coast of northern Germany. The somatic ciliary pattern of *T. cylindrica* is complex, comprising (i) a ventral kinety with ~ 36 mono-kinetids, (ii) a posterior kinety with ~ 26 dikinetids, (iii) a dorsal kinety with ~ 36 dikinetids, (iv) a right ciliary field with ~ 11 kineties, (v) a left ciliary field with ~ 10 kineties, and (vi) lateral ciliary field with ~ 11 kineties. Accordingly, the species differs from its congener *Tintinnopsis cylindrata* Kofoid and Campbell, 1929 that has merely a right and left ciliary field and two ventral organelles. On the other hand, the genera *Codonella* Haeckel, 1873; *Codonellopsis* Jörgensen, 1924; *Cymatocylis* Laackmann, 1910; *Helicostomella* Jörgensen, 1924; *Leprotintinnus* Jörgensen, 1900; and *Stenosemella* Jörgensen, 1924 share this pattern. In *Tintinnopsis cylindrica*, the oral primordium comprises ~ 22 collar membranelles and one buccal membranelle and develops hypoapokinetally posterior to the lateral ciliary field as in *Codonella cratera* (Leidy, 1877) and *Cymatocylis convallaria* Laackmann, 1910.

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P 3

Biodiversity of aloricate Oligotrichea (Ciliophora, Spirotricha)

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Aloricate Oligotrichea are an important component of the marine and freshwater microzooplankton due to their high abundances and growth rates. In the taxonomic revisions of this ciliate group, Maeda & Carey (1985, Bull. Ocean Res. Inst., Univ. Tokyo 19) and Maeda (1986, Bull. Ocean Res. Inst., Univ. Tokyo 21) compiled 14 valid genera and 127 valid species. During the last two decades, circa 22 new genera of aloricate Oligotrichea were established, about 70 species were described, and approximately 40 species were redescribed, necessitating a fresh revision of this ciliate group. After such a taxonomic reassessment for a monograph in preparation, 23 genera are regarded as valid and 1 genus name as unavailable. Another 3 genera are rejected and 11 monotypic genera cannot unequivocally be identified. According to my reconsideration, 159 species are valid and 18 species are subjective synonyms of the valid ones. Furthermore, 15 unidentified or misidentified populations probably represent new species which are, however, not established as the descriptions do not include either live observations or the investigation of silver-impregnated material. On the other hand, 46 further species cannot unequivocally be identified (nomina dubia) and 8 species names are unused since the year 1899 (nomina oblita). Hence, the estimated number of aloricate species within the Class Oligotrichea, excluding the nomina dubia and nomina oblita, is currently close to 175, corresponding to an increase by about 40% (circa 25% without the 15 populations mentioned above) during the last twenty years of research in freshwater and marine neritic habitats. Presumably, several further species await their discovery in the oceanic realm.

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KV 2

Größenunterschiede charakterisieren Arten und Unterarten des Artenkomplexes *Stylonychia mytilus* (Ciliophora, Spirotrichea)

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Der Artenkomplex *Stylonychia mytilus* enthält die Zwillingsarten: *S. mytilus* und *S. lemnae*. Werden die Längen der Zellen von Klonen beider Arten gemessen, zeigt sich:

1. Beide Arten können an ihren Größen unterschieden werden.
2. *S. mytilus* umfasst zwei an ihren Größen unterscheidbare Unterarten, eine große mitteleuropäische und eine kleinere asiatisch-australische Unterart.

Durch Kreuzung der beiden Unterarten von *S. mytilus* sollte festgestellt werden, ob die Zellkerne oder das Cytoplasma die Zellgröße bestimmen.

KV 22

Experimentelle Demonstration von Chaos in einem mikrobiellen Nahrungsgewebe

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Warum Populationen räumlichen und zeitlichen Schwankungen unterlegen sind, stellt eine zentrale Frage in der Ökologie und Evolutionsforschung dar. Die Tatsache, dass schon einfache biologische Systeme das Potenzial haben, verschiedene deterministische Verhaltensweisen – von Gleichgewichtsverhalten, stabilen Grenzzyklen bis hin zum chaotischen Verhalten – zu zeigen, hat zu einem großen Interesse unter Theoretikern geführt. Obwohl Theoretiker für zahlreiche idealisierte mathematische Populationsmodelle zeigen konnten, dass die verschiedenen Dynamiken in Abhängigkeit bestimmter Parameterwerte auftreten, sind die Nachweise in realen Systemen sehr rar und selten eindeutig. Durch genau definierte Versuchsbedingungen konnten wir in einem Zwei-Beute- Ein-Räuber-System zum ersten Mal eindeutig die verschiedenen Dynamiken in Abhängigkeit eines Kontrollparameters experimentell nachweisen. Als Beute fungierten zwei Bakterienarten, Als Räuber der Ciliat *Tetrahymena*. Durch Änderung der Chemostatdurchflussrate konnte chaotisches und periodisches Verhalten, sowie stabile Zustände der drei Populationen erreicht werden.

Extreme genetic divergence of the CO I gene within the genus *Paramecium*

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Generally, high sequence divergence at higher taxonomic levels (family, subfamily) has been observed in ciliates, but high resolution phylogenies at the species level are rare and exclusively based on nuclear markers. Here, we present the first phylogeny at a species level for a free living protozoan, which is based on the mitochondrial cytochrome oxidase I (CO I): the phylogeny within the genus *Paramecium*. The topology of this phylogeny is similar to previously published phylogenies based on 18S sequences, but the resolution is much higher. The analysis of the genetic distances between species reveals that they are the highest ever to be reported for a eukaryotic genus. For example, the genetic distance (measured in aminoacid substitutions) between *P. caudatum* and *P. bursaria* exceeds the genetic distance between humans and corals.

Problems in the systematic classification of some species of hypotrichs (Ciliophora, Spirotrichea)

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For a very long time the higher-level systematics of hypotrichs (= stichotrichs according to Small & Lynn) followed mainly the ventral cirral pattern. Since about three decades, several other morphological and ontogenetic features are successfully used, for example, the dorsal kinety pattern (e.g., kinety fragmentation of oxytrichids) or the rigid body of the stylonychines. Many species can be assigned without any problems to higher taxa like, e.g., the stylonychines, the urostyloids, or the amphisiellids because they show at least one apomorphy of a suprageneric taxon. On the other hand, a considerable number of species is known which cannot be classified in a certain group. Two types can be distinguished, namely (i) those species which lack any higher-level apomorphy (e.g., *Saudithrix terricola* Foissner et al.); and (ii) those which have two or more apomorphies, which, however, assign them to different taxa. Examples for the second type are (i) *Neokeronopsis spectabilis* (Kahl) which has a midventral pattern like the urostyloids and a dorsal kinety fragmentation like the oxytrichids; and (ii) a new, *Uroleptus*-like species with a rigid body which would assign it to the stylonychines. In contrast, the molecular data of this species suggest a close relationship with *Oxytricha*.

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Survival strategies of chrysomonad flagellates: microdiversity

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The molecular diversity of protists and its ecophysiological significance is largely unknown. We analysed the ecophysiological microdiversity of 90 monoclonal strains of the *Spumella* morphotype (Chrysomonadida). We identified different levels of ecophysiologically meaningful phylogenetic resolution. We showed that morphotype as well as 18S rRNA phylotype allow for certain generalisations but neither suffice to resolve observed ecophysiological microdiversity. Overall genotypic variation is neutral with respect to some ecological factors but highly meaningful with respect to other factors and ecophysiological microdiversity is below the resolution of the 18S rRNA gene. The results suggest that the morphospecies concept is inappropriate to reflect ecological differentiation in the investigated protists. Our data further demonstrate that the assumption of similar ecophysiological adaptation of strains belonging to the same population is valid.

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Survival strategies of chrysomonad flagellates: biogeography

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The geographic distribution and the question of endemism in protists is one of the most controversial topics in microbial ecology. The determination of ecophysiological adaptation of protists to different geographic zones provides a means to critically test the basic assumption of the ubiquity theorem, i.e., that geographical barriers do not matter for protist distribution. We investigated the molecular and ecophysiological variation within a chrysomonad morphospecies both, on a local and a global scale. Here we show that adaptation of protists from the geographically isolated Antarctic continent deviates significantly from the global trend of temperature adaptation. Geographic isolation therefore provides a means for local adaptation and consequently speciation in protists.

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Survival strategies of chrysomonad flagellates: temperature adaptation

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We investigated the adaptation to thermal niches of flagellates originating from cold, temperate and tropical sites. Cold adapted flagellates showed low maximum growth rates and survived only maximal temperatures around 20°C whereas strains from temperate and warm regions showed high maximum growth rates and survived temperatures up to 30 – 35 °C. Only at low temperatures, i.e., below 5°C, the cold adapted flagellates showed higher growth rates as compared to strains from temperate to warm regions. Despite a relatively high optimum temperature the cold adpated strains had therefore a competitive advantage only at temperatures below 5°C. Temperature adaptation of different SSU rRNA clades of chrysomonad flagellates differed systematically thus indicating a certain suitability of the SSU rRNA sequence as ecological marker. However, temperature adaptation differed even between closely related strains. The specific ecotypes of flagellates are therefore hardly resolved by SSU rRNA sequence information.

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Survival strategies of chrysomonad flagellates: predation and feeding

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We investigated the survival strategies and the competitive strength of pigmented and colourless bacterivorous flagellates using two model organisms, i.e. the bacterivorous chrysophytes *Poterioochromonas malhamensis* and *Spumella* sp., when fed small ultramicrobacteria (0.044 μm^3) and large bacteria (0.38 μm^3). The numerical responses indicated that small bacteria were not as good a food source as large bacteria even when bacterial cell volume has been taken into account. The heterotrophic *Spumella* had growth rates, which exceeded those of the mixotrophic *Poterioochomonas* at high bacterial concentrations of both sizes of bacteria. At very low prey abundances *Poterioochromonas* grew better than *Spumella*, mainly because maintenance requirements were very high for *Spumella*. At bacterial biovolumes which can be encountered in oligomesotrophic lakes the growth curves of two flagellates intersect. Experiments carried out with mixed flagellate cultures showed that *Poterioochromonas* ingested *Spumella* cells. Applying our data to natural abundances of small and large bacteria as well as pico/nanoplankton we could demonstrate that *Spumella* is an r strategist and relies on mainly large bacteria for growth although small bacteria can contribute significantly to its diet. *Poterioochromonas* is much more a K strategist. Large bacteria also made up for the bulk part of its carbon uptake, but small bacteria, pico-nanoplankton and to a minor extent also photosynthesis contributed to the gross carbon uptake indicating a broader use of different resources.

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Constructing protistan artificial metagenome libraries from marine eukaryotic assemblages

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Cultivation independent surveys of ribosomal RNA genes amplified from environmental samples have revealed the existence of novel protistan lineages. Many of them are without cultured representatives, as most protists in the ocean are still resistant to our collective cultivation efforts. Ribosomal RNA-based analyses, however, are limited: they do not go beyond the initial discovery of organisms and their phylogenetic affiliations. In prokaryotic diversity studies, analyses of large genome fragments recovered directly from natural microbial communities, represents one successful approach for characterizing uncultivated bacteria and archaea better. Such metagenome libraries represent a major breakthrough in environmental microbiology and may be THE key to uncultured microbes. They identified for example numerous novel genes, proteins, antibiotics, physiological capabilities, and evolutionary events. To our knowledge, no protistan metagenome libraries have been constructed to date. One major reason is that genomic information has to be linked to phylogeny. Else, it is hardly possible to identify the source of this information (pro- or eukaryotic). Here, we present a successful method for the construction of a protistan metagenome library from marine samples and the screening for phylogenetic anchors. Further analyses of these libraries promise significant insights into the genomic structure (G+C content, codon usage, promoter sites, ORFs), genomic potential, evolution and ecological roles of many indigenous protistan species, cultivated or not.

P 31

A preliminary study on reservoir ciliates, Gellingüllü Dam Lake, Yozgat, Turkey

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Gelingüllü Dam Lake is located at south of Yozgat Province, in Central Anatolia Region with 39º36'30N-35º03'20E coordinates and the dam was constructed on Deliceirmak stream which is a tributary of Kizilirmak River. The impoundment period began after November 1993 and the riverine ecosystems started to chance into lotic system.The following study, part of a more complex research, is a preliminary work on ciliates found at the flooded zone of the dam lake. Collected samples were analysed with the non-flooded Petri method which bases on reactivating ciliates from air dried samples. The species were identified by evaluation of morphometric measurements and counts, in vivo and after protargol impregnation technique. Illustration of the specimens were by free-hand sketchs and micrographs. Measurements were performed digitally by IM50 image manager system and Q- win measurement program. Additionally physical and chemical characteristics of samples were determined in order to obtain detailed information of their effect on the distribution of the identified species. Descriptions, micrographs and drawings of some remarkable species are shown and general habitat knowledge is given.

This study is supported by Hacettepe University Scientific Researches Unit.

Problems and prospects for the phylogenetic relationships within the class Colpodea (Ciliophora)

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Previous molecular analyses to test the monophyly of the class Colpodea and the relationships among the orders within the class used limited taxon sampling. Monophyly was only weakly supported, and there were unsuspected groupings that challenge some of the morphological-based ordinal groupings. To classify more accurately these ciliates, we are constructing a molecular phylogeny of the Colpodea using additional taxon sampling and various outgroups. We are analyzing small-subunit (SSU) ribosomal sequences with parsimony, maximum likelihood, and Bayesian methods. Specifically, we have increased sampling within the Cyrtolophosidida and Sorogenida (the potential basal clades), as well as within previously un-sampled orders and families in the rest of the class. Here we present some initial results, emphasizing the ability of SSU to resolve relationships with further taxon sampling. In addition, we report on the presence of two distinct rDNAs in *Bryometopus*, which has a 550 base-pair deletion, and discuss explanations for this unusual sequence.
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A molecular characterization of protozoan communities in aquifers

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There is a remarkable lack of knowledge concerning the role of *Protozoa* in groundwater ecosystems, even though they are known to be important predators of bacteria, influencing microbial abundances and activities in many other ecosystems. Effectively, only little is known about their distribution and diversity, and even less about their functional importance in pristine and contaminated aquifers. This is partially due to a lack of easy accessibility to aquifer sediments, but also due to the requirement of isolation and cultivation of protists for taxonomic classification by means of the classical morphospecies concept. The application of molecular tools may help to solve previous problems in the identification of many (also uncultivable) protozoan species. Nevertheless, suitable 18S rRNA gene primer pairs for protozoa are still rare in the literature, but urgently needed for the investigation of protozoan diversity and their importance for the functionality of an ecosystem.
We evaluated and created eukaryote and group-specific protozoan primers. The primers are currently applied to investigate protozoan diversity variations along a depth profile of a BTEX-contaminated aquifer with extracted nucleic acids from sediment samples. At the highly polluted capillary fringe our analyses have identified different ecotypes of protozoa such as ciliates of the order *Stichotrichida* and flagellates belonging to the *Cercomonadida* to contribute to the indigenous eukaryotic microbiota. Ongoing studies aim to reveal depth-related stratifications and to compare different aquifer samples using both general and group-specific 18S rDNA primer pairs.

P 1

A new "flagship" ciliate from the Niger floodplain breaks flexibility-dogma in the classification of the stichotrichine spirotrichs

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Stichotrichine spirotrichs have a high morphological and ontogenetic diversity, while the 18S rRNA gene sequences are rather similar. While it is often easy to define species and genera, families and higher categories are usually poorly supported. None the less, a main apomorphy, viz., body flexibility separates the urostylid and oxytrichid from the rigid stylonychid stichotrichs, both morphologically and genetically (Foissner et al. 2004, Europ. J. Protistol., 40: 265–281). This dogma is now broken by a new stichotrich from soil of the Niger floodplain in tropical Africa. This ciliate, which is an endemic "flagship" with large body size (average length about 230 µm) and distinct body shape (a "tailed *Stylonychia*"), has a rigid, *Stylonychia* – like body and oral apparatus, while the cirral pattern is urostylid. Accordingly, it would be classified in the family Urostylidae morphologically, assuming that the rigid body is a secondary achievement. However, part of the dorsal bristle rows are dorsomarginal kineties originating from cirral rows; this is a highly characteristic apomorphy of the family Oxytrichidae. Thus, this new ciliate combines "strong" features of urostylid, oxytrichid, and stylonychid stichotrichs. Under these circumstances, we hoped that the 18S rRNA gene sequence would provide deeper insight. Unfortunately, this was not the case because it classified this curious species very near to *Oxytricha granulifera*, type of the genus *Oxytricha*. While an oxytrichid relationship is possible, it is impossible that this species is more closely related to the genus *Oxytricha* than other typical oxytrichid genera, such as *Gonostomum* and *Cyrtohymena*. Genetic misclassification is also indicated by the long branch the species forms on the trees.

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KV 26

New *Holospora* endocytobionts in some common ciliates

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Some of bacterial endocytobionts (BE) of ciliates, mainly *Holospora*-bacteria, may be highly infectious. These EB are widespread in several *Paramecium* spp., but till now have not been found in other ciliates. However, some other EB have similar life cycle with two different stages (I- and R-forms). Our sampling in Italy reviled the EB in some native populations of *Spirostomum minus*, *Frontonia leucas* and yet unclassified *Frontonia sp.* isolated from brackish water pond (12‰). All recorded EB populated the macronucleus (Ma). Both EB from *Frontonia* manifested a strong FISH signal with alpha-proteobacteria-specific and *Holospora*-specific probes. The EB from *S. minus* also belong to the alpha subgroup. The EB from *F. leucas* looks as a typical *Holospora* with dimensions: 1-2.5x0.9-1.0 µm (R- forms) and 5-12x0.7-0.8 µm (I-forms). Aposymbiotic cells of the *Frontonia* spp. can be experimentally infected by homogenate of infected ones and only I-formes can reach and occupy Ma of ciliates. EB in *Frontonia* sp. manifested R-forms with dimensions 2-4x1.3-1.5 µm and I-forms between 10 to 30 µm and had very peculiar spindle-shape form. The third EB from Ma of *S. minus* looks even more deviated from "classical" *Holospora*: 3-6x2-3 µm spindle-shape R-forms and 5-8x4-5 µm oval I-forms with extraordinary cell differentiation with some kind of extrusive device. The rate of infection as well as the number of bacteria in *S. minus* cells were usually rather low. These findings for the first time definitely recorded at least two holosporas out of the *Paramecium* spp.

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Adaptation and acclimation of *Meseres corlissi* (Ciliophora: Oligotrichea) – The role of temperature and soil

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The temperature response of the freshwater ciliate *Meseres corlissi* (Petz & Foissner, 1992) (Ciliophora: Oligotrichea) was investigated over 7.5 - 30.5°C with six clonal cultures isolated from two different habitats in the temperate region of Austria in different seasons. The results were compared to those of a former study with *M. corlissi* from the Dominican Republic (Weisse 2004). Temperature dependent growth rates, cell volumes and production rates of the tropical *M. corlissi* isolate were all significantly different from the six Austrian clones. The latter showed positive population growth at 10°C, while the tropical strain survived only at temperatures >13°C and reached higher growth rates than the Austrian clones at the highest temperatures. The temperature response of the Austrian clones remained stable for up to 600 generations in the laboratory. Cyst formation of the Austrian clones occurred irregularly at all temperatures tested, whereas temperatures <20°C triggered mass encystment of the tropical strain, and cysts occurred only sporadically and at low numbers at temperatures >20°C. Among the Austrian clones several winter isolates differed in their temperature response from a summer strain. In the winter isolates, acclimation to the laboratory conditions became apparent, because the temperature at which growth rates peaked increased with the time of cultivation. As reported earlier, the Austrian isolates of *M. corlissi* require soil extract (SE) for sustained optimal growth (Müller et al., 2006). Our experiments showed that the addition of 5% SE to the culture medium was beneficial at the temperature extremes, in particular.

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Molecular diversity of protozoa in contaminated aquifers

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Protozoan grazing pressure has an important influence on structure and function of bacterial communities. There is some evidence that protozoa may have some impact on contaminant degradation; it is therefore of particular interest to assess the interaction between protozoa and contaminant degrading bacteria. The knowledge about the diversity of protozoa in contaminated groundwater ecosystems is limited. Until now only a few cultivation dependent or independent diversity studies have been performed in these environments.
This study presents data on the diversity of protozoa in contaminated groundwater environments in eastern Germany. The main pollutants are BTEX compounds (benzene, toluene, ethylbenzene, xylene) and MTBE (methyl tertiary-butyl ether). The redox conditions in these systems range from strictly anoxic and sulphate reducing to oxic. Total DNA was extracted and used to construct 18S rRNA gene clone libraries using universal eukaryotic primers. In addition, terminal restriction length polymorphism (T-RFLP) analysis of the 18S rRNA gene pools was applied to compare the eukaryotic community structure between different wells and sites.

KV 25

On the diversity and significance of intracellular bacteria in *Paramecium*

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Few intracellular bacteria have already been observed in the 19th century. In about the middle of the last century, interest arose in the bacterial nature of the killer particles and in the ecological and evolutionary significance of these symbioses. Kappa-particles and many other endosymbionts had been described until about 1975 (review by Preer, Preer and Jurand, 1974). New observations emphasize old views of interactions between killer bacteria and paramecia being extremely complex and it seems hardly acceptable to regard these endocytobioses as examples of mutualism. In recent years, largely based upon rDNA-sequences the phylogenetic relation-ships of some of the symbionts has been studied. When elucidating the phylogenetic position of the old kappa-particles, *Caedibacter taeniospiralis*, it was found that the genus is not monophy-letic (Beier et al. 2002). On the other hand, *Caedibacter* and related bacteria have been detected in protozoa other than ciliates, too, and the host specificity of some of the symbionts has to be investigated anew. In *Paramecium* new killers and non-killers of different bacterial taxa are being found and we have to deduce from the number of new detections that many if not most of the bacterial endocytobionts in *Paramecium* are not yet known, not to consider other ciliates, most of which only little work has been done concerning endosymbioses.

P 6

Molecular phylogeny of the intracellular bacteria in the freshwater dinoflagellate *Peridinium cinctum* (Dinoflagellata)

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An isolate of the freshwater dinoflagellate *Peridinium cinctum*, from Plußsee (Plön, Germany) is known to inhabit two ultrastructurally different bacteria. Molecular phylogeny of the 16S rDNA genes indicates their closest position within the α-proteobacteria and β-proteobacteria, respectively. The closest relative of the α-proteobacterium is determined as *Caedibacter caryophilus*, known as an intranuclear bacterium from the ciliate *Paramecium caudatum*. *Aquamonas fontana* is identified as the closest relative to the β-proteobacterial endocytobiont of *P. cinctum*. In addition, some characteristics of the 23S rDNA sequence of *Caedibacter caryophilus* are shown. These results represent the first molecular identification of endocytobionts in dinoflagellates.

The final proof of the endosymbiont theory

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One hundred years ago, C. Mereschkowsky published a notoriously ignored landmark paper: "Über Natur und Ursprung der Chromatophoren im Pflanzenreiche." (Biologisches Centralblatt, Bd. XXV, No. 18; 15 September 1905). Twenty years after Mereschkowsky's plea for an endosymbiotic origin of plastids, Wallin (1925, 1927) postulated a "bacterial nature of mitochondria". Eventually, the genomics era provided the tools to prove the endosymbiont-hypothesis for the origin of the eukaryotic cell, and the endosymbiont "theory" became an unequivocal historical fact after less than 100 years of biological/biochemical research. The proof for this "singular" event, however, did not deal with the elusive anaerobic relatives of the eukaryotic cell. In particular, the hypothesis that hydrogenosomes, mitosomes, and "mitochondrial" remnants are just variations of mitochondria, was, until recently, based on rather circumstantial evidence. With the discovery of a true missing link, i.e. a hydrogenosome with a genome, this oddity of the endosymbiont "theory" could be settled (Boxma et al. 2005; Martin 2005).

B. Boxma et al. (2005) An anaerobic mitochondrion that produces hydrogen. *Nature* 434, 74-79

W. Martin (2005) The missing link between hydrogenosomes and mitochondria. *Trends Microbiol.* Vol.13, 457-459

Is genetic variability of the alveolate parasite *Hematodinium* host specific?

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Hematodinium, a marine parasite, has been identified in a wide range of crustacean hosts. The species status and host-specificity of this pathogen have not been resolved, as the distinction between morphospecies is ambiguous, and prevalence differs markedly between hosts. Furthermore, not all *in vivo* life-cycle stages have been identified and transmission routes are unknown.

Molecular probes were used to detect *Hematodinium*-like alveolates in the tissues of several crustacean species from the North-East Atlantic. Partial 18S, complete ITS1 and partial 5.8S DNA regions were sequenced, different genotypes identified and their prevalence compared between host species and tissues. Sequence analysis demonstrated *Hematodinium* rRNA variability both between and within host species, and even between tissues of the same individual, thus suggesting both intra- and inter-host genetic variability.

The specificity of *Hematodinium* strains to host species, and it's phylogenetic position within the protists, will be discussed.

P 26

Molecular characterization of ciliate diversity in constructed wetlands

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Constructed wetlands are often used for wastewater treatment due to their high potential to remove bacteria and pathogenic protozoa. It is well known that ciliates are abundant in such constructed wetlands and play an important role in hygienisation processes.
To understand possible correlations between the ciliate community and the effectiveness of water purification, the knowledge about the species assemblage is fundamental. Therefore, we investigated one constructed wetland with focus on ciliate diversity.
Ciliate species were determined by light microscopy and molecular analysis. For this purpose total DNA from water samples was isolated and a fragment of the SSU rDNA gene was amplified with specific primers followed by cloning of the PCR products.
The sequence analysis of several clones resulted in an unambiguous assignment of these clones to previously exactly determined species (sequences were taken from the Genbank). About 20 species could be addressed unambiguously based on their SSU rDNA. In particular the resolution within the species complex of *Paramecium aurelia* is conspicuous.
The application of this molecular method seems to be an effective and reliable tool for the characterization of ciliate community in constructed wetlands.

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Analysis of the flow field induced by the sessile ciliate *Opercularia asymmetrica* (Oligohymenophorea, Peritrichia)

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The cilia beat of *Opercularia asymmetrica* generates a flow field that convectively transports suspended particles and dissolved substances to the oral cavity of the organism. By use of optical micro-flow measurement and theoretical methods the flow environment of two neighbouring cells is studied. Both, yeast cells (*Saccharomyces cerevisiae*) and artificial flow tracers are used for the visualisation of the flow field. The tracers are rejected by the protozoa and deviate from the fluid path lines, while yeast cells follow the flow almost perfectly. This is shown through a dimensional analysis of the involved hydrodynamic forces on the tracers. The measured flow field exhibits maximum velocities of 25 µm/s at around 20 µm distance ahead of an individual ciliate and extends 200 µm from it. A nicking motion of the ciliate is observed and found not to obey any periodic law. Multiples of protozoa exhibit an alternating cilia beat regime generating a non-stationary flow field. It can be shown through theoretical methods that fluid exchange is enhanced in this alternating regime compared to a flow field generated by a single ciliate. Fluid exchange depends on the distance of the ciliates from each other and on the alteration frequency of the cilia beat. The comparison of an analytical Stokes' flow solution with the observed fluid flow serves to determine the force required to maintain the flow field against viscous dissipation. The force magnitude is ten to hundred picoNewton.

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Zoophagie-Nachweis bei *Pleurozia purpurea* in Experiment und Natur

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Ungefähr 600 Arten fleischfressender Pflanzen sind seit dem ersten wissenschaftlichen Beweis für die Existenz von Carnivorie im Pflanzenreich beschrieben worden. Gemeinsame Merkmale der zahlreichen Gattungen sind die Zugehörigkeit zu den höheren Pflanzen und die Spezialisierung auf Insekten als Beutetiere. Schon lange war bekannt, dass einige niedere Pflanzenarten Organe bilden, deren Morphologie stark an die Fallen höherer Pflanzen erinnert. Dieses Gebiet der Botanik wurde lange Zeit unbehandelt gelassen und die sog. Wassersäcke der foliosen Lebermoose wurden bis vor wenigen Jahren hauptsächlich als Wasserspei-cherungsorgane interpretiert. Erstmalig ist durch zahlreiche Präparationen die komplexe Wassersackmorphologie der Lebermoosart *Pleurozia purpurea* geklärt und dokumentiert worden. Umgewandelte Blattteile bilden einen sackartigen Hohlraum mit einer reusenartigen Öffnung in das Innere. Diese ist mit einem hyalinen Häutchen verschlossen, welches sich nur nach innen öffnen lässt. So stellt ein solcher Wassersack eine scheinbar perfekte Tierfalle im Miniaturformat dar. Angeregt von diesen Beobachtungen wurde das *Blepharisma americanum* als Testbeute in zahlreichen Versuchsreihen mit *P. purpurea* zusammengebracht. Die Ergebnisse beweisen eindeutig, dass die Wassersäcke der niederen Pflanze effektive Fallen für Ciliaten darstellen. Durch das als Klappe fungierende Häutchen ist es für gefangene Tiere unmöglich, aus den Fallen zu entkommen. Wie Untersuchungen am Naturstandort in Glencoe, Schottland, bewiesen haben, fängt *P. purpurea* in der Natur ein breites Spektrum von Protozoen und kleinen Metazoen. Die Tiere werden von dem zoophagen Moos zwar weder getötet noch gibt es Indizien für eine Verdauung seitens der Pflanze, jedoch ist anzunehmen, dass *P. purpurea* von dem Tierfang und der damit verbundenen Quelle lebensnotwendiger Elemente in ihrem oligotrophen Habitat, den Regen gespeisten Hochmooren, profitiert.

P 24

Ciliates in the elimination of suspended bacteria from sewage plants: experiments under continuous conditions using 30L fermenters

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Pathogenic bacteria that gain resistance e.g. during antibiotic treatment of patients are shed with human excretions into the waste water and transported to sewage treatment plants where they are enriched and may exchange resistance genes by horizontal gene transfer. Most of the classical pathogenic bacteria do not sediment with the sludge but remain suspended and therefore are finally discharged in relatively high numbers (e.g. up to 10^3 antibiotic resistant coliforms per ml) into the environment where they are a major threat for human health. In their natural habitats free swimming ciliates are able to filter-feed on suspended bacteria even at low cell densities. Therefore their aptitude for reducing the bacterial cell number in the efflux of sewage purification plants was investigated.
Our results show a reduction of different suspended bacteria by free swimming ciliates in a scale of 200 ml. After up-scaling to 25 l we showed that *Colpidium campylum* and *Tetrahymena pyriformis* decreased bacteria population up to 20% per h (10000 ciliates/ml) in batch-fermentation. Continuous fermentations of Ciliates and *Escherichia coli* were performed for 100 h. In order to maintain a stable bacteria ammound, 10 or 20% of the original bacteria concentration was added every our.
From these promising results, we conclude that *T. pyriformis* can be used as a novel and economical organism for waste water processing.

KV 30

The evolutionary history of dinoflagellates

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I will summarize the current understanding of the overall phylogeny of dinoflagellates. Molecular phylogenetic analyses using ribosomal and multiple protein genes consistently place dinoflagellates within the Alveolata and indicate that their closest relatives are *Oxyrrhis*, perkinsids, and a larger clade consisting of colpodellids and apicomplexans. Although the deepest phylogenetic relationships within the dinoflagellates are currently unresolved, the relationships within certain dinoflagellate subclades are robustly supported with molecular sequence data. Molecular phylogenetic frameworks will be compared with established hypotheses of dinoflagellate evolution based on morphological data. In this context, I will discuss preliminary results from several projects currently underway: (1) identifying the nearest relatives to 'core' dinoflagellates, (2) reconstructing the initial dinoflagellate radiation with a broad phylogenetic dataset of heat shock protein 90 sequences, (3) inferring the phylogenetic relationships within the orders Noctilucales and Dinophysiales and (4) addressing the molecular phylogeny and ultrastructural character evolution within polykrikoids and warnowiids.

P 4

Ebriid phylogeny and the expansion of the 'Cercozoa'

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Ebria tripartita is a phagotrophic flagellate present in marine coastal plankton communities worldwide. This is one of two extant species in the Ebridea, an enigmatic group of eukaryotes with an unclear phylogenetic position. Ebriids have a peculiar combination of ultrastructural characters including a large nucleus with permanently condensed chromosomes and an internal skeleton composed of siliceous rods. The taxonomic history of the group has been tumultuous and has included a variety of affiliations, such as silicoflagellates, dinoflagellates, 'radiolarians' and 'neomonads'. Today, ebriids are treated as a eukaryotic taxon *incertae sedis*. We conducted phylogenetic analyses of small subunit rDNA sequences from two multi-specimen isolations of *E. tripartita*. The newly recognized ebriid clade was most closely related to sequences from described species of *Cryothecomonas* and *Protaspis*. These molecular phylogenetic relationships were consistent with current ultrastructural data from all three genera, leading to a robust placement of ebriids within the 'Cercozoa'.

P 5

Dinoflagellate, euglenid or cercomonad? The ultrastructure and molecular phylogeny of *Protaspis grandis* sp. nov.

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Protaspis is an enigmatic genus of marine phagotrophic biflagellates that have been tentatively classified by different protozoologists with several different groups of eukaryotes, including dinoflagellates, euglenids and cercomonads. This uncertainty led us to investigate the phylogenetic position of *Protaspis grandis* sp. nov. with ultrastructural and small subunit (SSU) rDNA sequence data. Our results demonstrated that two heterodynamic flagella emerge through funnels, that they are positioned subapically within a depression and separated by a distinctive protrusion. A complex multi-layered wall surrounds the cell. Like dinoflagellates and euglenids, the nucleus contains permanently condensed chromosomes and a large nucleolus throughout the cell cycle. Pseudopodia, containing numerous mitochondria, emerge from a ventral furrow through a longitudinal slit, which is positioned posteriorly from the protrusion and flagellar apparatus. Mitochondria have tubular cristae, and batteries of extrusomes are present and ejected through pores in the cell wall. The SSU rDNA sequence demonstrated a very close relationship between the benthic *Protaspis grandis* and the planktonic *Cryothecomonas longipes*. These are first ultrastructural and molecular phylogenetic data for the genus *Protaspis* and they indicate that the current taxonomy of *Protaspis* and *Cryothecomonas* is in need of reevaluation.

P 21

Grazing-Raten von Biofilm assoziierten Ciliatengemeinschaften in Abhängigkeit von der Temperatur

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Ciliaten können in enorm hohen Dichten auf Biofilmen in natürlichen Gewässern vorkommen. Viele dieser Ciliaten ernähren sich von planktischer Nahrung, womit sie neben der Makrofauna (z.B. Muscheln) eine wichtige Rolle in der bentho-pelagischen Kopplung in flachen Seen und Fließgewässern spielen können. Hier stellen wir ein gewässerangebundenes Fließzellensystem vor, mit dem sich die Grazingraten von seminatürlichen Ciliatengemeinschaften auf natürliches Plankton quantifizieren lassen. Diese Gemeinschaften lassen sich dazu *in situ* analysieren. Speziell wurde hier vor dem Hintergrund der globalen Klimaerwärmung der Einfluss der Temperaturerhöhung auf die Grazingraten von Ciliatengemeinschaften untersucht. Dabei konnte gezeigt werden, dass sich das Grazingverhalten der Ciliatengemeinschaften gegenüber dem Grazingverhalten der Makrofauna deutlich unterscheidet. So zeigten die Ciliaten einen linearen und keinen exponentiellen Anstieg der Grazingraten mit der Temperatur. Bei hohen Temperaturen (>25°C) nahm die Grazingrate von Muscheln ab, während die der Ciliatengemeinschaft weiter stieg. Hinzu kamen Unterschiede in der Größenpräferenz von Nahrungspartikeln. Während Biofilme sowohl Bakterien als auch Nanoplankton konsumierten, konsumierten Muscheln kaum Bakterien. Insgesamt legen die Ergebnisse nahe, dass sich der Effekt von Temperaturerhöhung auf die bentho-pelagische Kopplung in biofilmdominierten Systemen (z.B. Gewässer mit viel Hartsubstrat) deutlich von dem in makrozoobenthosdominierten Systemen unterscheidet.

KV 12

Reframing the 'everything is everywhere' debate: evidence for high gene flow and diversity in ciliate morphospecies

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Current debate on microbial diversity contrasts the 'cosmopolitan' hypothesis, which argues for high gene flow and low diversity, with the 'endemism' hypothesis, which argues for high diversity and geographically restricted gene flow. Our analyses of genetic variation in ciliate morpho-species isolated from ephemeral environments (freshwater ponds and tide pools) redefine this debate. In 2 different clades of oligotrich ciliates (in the genera *Halteria/Meseres* and *Strombidium*), we found both high levels of diversity and evidence of high gene flow as indicated by the presence of identical haplotypes across broad geographic ranges. Five recognizable morphospecies of *Halteria/Meseres* were found to be composed of 7 different clades, differing by as much as 7.6% sequence divergence at the ITS locus (ITS1, ITS2 and 5.8S rDNA). Two recognizable morphospecies of *Strombidium* (*S. oculatum* and *S. stylifer*) resolved into 10 distinct clades, differing by as much as 15.7% at the same locus. For both groups of ciliates, the genetic divergence underlying these morphospecies may be related to cycles of isolation in their ephemeral habitats. By comparison, there is both low diversity and high gene flow in published data on ciliates from open coastal water, a more stable environment over evolutionary time-scales. Our analyses indicate that models of microbial diversity must test for ecologically driven patterns in the interactions of gene flow and species richness to account for observed patterns of high dispersal and high gene flow. Supported by NSF, FWF, the Tomlinson Funds and a Fulbright Fellowship.

KV 4

Untersuchungen zur Phylogenie der Reticulomyxidae

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Im Spätsommer 2005 konnte in einem kleinen See südlich Berlins das Massenvorkommen eines unbeschriebenen plasmodialen Organismus mit Bidirektionalströmung in Zellkörper und Pseudopodien beobachtet und analysiert werden. Diese Süßwasserforaminifere mit mehreren Merkmalen der Arten *Reticulomyxa filosa* und *Wobo gigas* sowie mit eigenen morphologischen, physiologischen und molekulargenetischen Besonderheiten wird hier erstmals vorgestellt. Die Entwicklungsstadien dieser neuen Art umfassen frei schwebende Propagationseinheiten (Protoplasma-Tropfen - blebs), gestielte solitäre Cysten und agreggierte ungestielte Cysten, *Lieberkühnia*-artige runde Zellen, wurmförmige kleine und netzartige große Plasmodien sowie Migrationsstadien, die bis zu 4 cm lang werden können. Die größeren Plasmodien bedecken mitsamt den Granuloreticulopodien Areale von bis zu 4–6 cm^2. Die Organismen sind stationär und von einer Hülle umgeben, die beweglichen Stadien sind nackt. Zugleich wird – als Vertreter der Außengruppe – eine nahe verwandte unbeschriebene Brackwasser-Foraminifere vorgestellt, deren Fortpflanzungs- und Ausbreitungsstrategien Einblicke in die Phylogenie der Reticulomyxiden erlauben.
Die kennzeichnenden Lebensstadien werden in kurzen Videosequenzen vorgestellt.

Quantitative RT-PCR: single cell screening vs. culture analysis

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Since quantitative RT-PCR has become a standard technique analysing transcript abundance, the method revealed great advantages in specifity and sensitivity compared to northern blotting. Working with unicellular organisms the analysis of single cells has become possible and it seemed questionable if the data of single cells varies in great scale from the values determined from RNA isolates from whole cultures, displaying only a global average of single cell values.
We analysed the transcriptional level of GAPDH, a catalytic enzyme involved in glycolysis, in single cells and cultures and found it constantly expressed in *Paramecium tetraurelia*. We used it therefore for reference in all studies analysing transcript abundances of surface antigens. Surface antigen mRNA abundances were found to vary in great scale between single cells of serotype pure cultures in spite of the level of GAPDH. The comparison of single cells to whole cultures revealed that single cell analysis becomes essential if (i) cells of the culture do not react simultaneously to trigger-initiators, e.g. RNAi treatment or if (ii) the determination of transcript-ratios is wanted for the description of regulatory mechanisms. Therefore molecular biologists have to decide whether the analysis of cultures describes their phenomenon enough in detail or the analysis of single cells would make sense in spite of being expensive and labor-intensive.

RNA/DNA ratios in protists as a measure of growth rate and activity

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The analysis of the gene of the small subunit of ribosomal RNA (SSU rRNA) amplified from environmental samples is the gold standard to study the molecular diversity of protistan species in nature. But this rRNA approach provides only little information beyond the fact of their existence, distribution and molecular phylogeny. For example, rDNA-derived clone libraries are not able to distinguish between inactive, encysted or even dead organisms and active members of the microbial community under study. However, this is especially important in environments with steep physico-chemical gradients (e.g. anoxic systems), within which microbial community structure is changing on a small temporal and spatial scale. Recently, we applied a novel method in eukaryote microbiology to identify the active members of extant protistan communities. This technique targets the rRNA molecule directly rather than its gene. This is based upon the theory that significant concentrations of ribosomal RNA are only present in active and growing protists as was shown for prokaryotes and multicellular eukaryotes. However, to our knowledge, this theory has not yet been confirmed for protistan species. Here, we present the first experimental data, which display a significantly higher RNA/DNA ratio with increasing growth rates and activity in protistan cultures.

P 32

Local ciliate endemism in an anoxic alpine lake?

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The global distribution of protist species has been subject to intense and controversial discussion. The view that microbial eukaryotes are dispersed ubiquitously, is contrasting the view that protist species have biogeography. We isolated and cultivated a ciliate from a remote anoxic alpine lake (Alatsee, Allgäu), which supports the latter hypothesis. This organism is morphologically and morphometrically similar to the oligohymenophorean ciliate *Urocentrum turbo* except for one character: it lacks trichocysts, a characteristic feature of *U. turbo*, easily visible with light microscopy. The novel isolate only displays cytoplasmic trichocyst-"Anlagen", visible after protargol staining. Trichocysts are a common structure in oligohymenophorean ciliates, being used for defense. Thus, these structures can be considered as a plaesiomorph character in these organisms. We argue, that the novel isolate, which forms a stable population in lake Alatsee, is on the verge of forming a new species, descending from *U. turbo*. Such an organism has not been described from any place else. Thus, we assume that it is characteristic of the anoxic lake Alatsee. Interestingly, the sequences of the 18S rDNA, ITS1, 5.8S rDNA and ITS2 genes are nearly identical. We conclude, that these phylogenetic markers are not able to resolve an early speciation process in these, and most likely many other, organism. Thus, we argue that protist diversity is even higher than suggested by molecular surveys. Isolated anoxic systems are discussed as predestined sites of allopatric speciation.

P 9

Diversity of testate amoebae (Protozoa, Testacea) in different litter types of a mountain rain forest in Southern Ecuador

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We investigated the species composition of testate amoebae in two different types of tree litter (Purdiaea nutans, Graffenrieda emarginata) exposed for one year at two altitudes (1850 m and 2270 m) of a mountain rain forest in southern Ecuador.
At 1850 m Purdiaea litter, a mixture of both litter types and Graffenrieda litter contained 40, 68 and 70 species of Testacea, respectively. At 2270 m species diversity was strongly reduced, litterbags with Purdiaea, mixed and Graffenrieda litter contained only 6, 17 and 19 species, respectively.
In conclusion, Purdiaea and Graffenrieda litter supported very different communities of Testacea. Only common species with cosmopolitan distribution occurred in Purdiaea litter, in contrast litter of Graffenrieda supported a high diversity of Testacea including new species with potentially restricted tropical distribution. Our results indicate that the species distribution of Testacea in the mountain rain forest is strongly influenced by plant litter type.

KV 18

Einfluss der Hochwasserdynamik auf benthische Ciliaten der Mittelelbe

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Ciliaten in der Stromsohle großer Flüsse sind durch morphologische Besonderheiten, die der Drift entgegenwirken, an das Leben auf Oberflächen im Sediment angepasst, sodass bei einer mittleren Strömungsgeschwindigkeit (Elbe 1,1 m s^{-1}) im Hauptstrom, trotz der hohen Resuspen-sionsrate, hohe Ciliatenabundanzen im Sediment zu finden sind. Buhnenfelder zeichnen sich durch ihre geringe Strömungsgeschwindigkeit als Sedimentatitons- und Akkumulationszentren auch für Ciliaten aus. Ziel der Untersuchung war es, den Einfluss eines Hochwassers auf die Ciliatengemeinschaften der Hauptstrom- und Buhnenfeldsohle, zu untersuchen. Die benthischen Ciliaten wurden in 4 Längsbereisungen der Mittelelbe bei mittlerem Abfluss und bei erhöhtem Abfluss mit Hilfe eines Stechrohres aus dem Sediment entnommen. Die Gesamt-abundanz der Ciliaten in der Hauptstromsohle variierte bei Stkm 232,5 zwischen 260 Ind. cm^{-3} bei mittlerem Abfluss und 16 Ind. cm^{-3} bei Hochwasser. Sessile Peritrichia (v. a. *Vorticella infusionum*) wurden bei Hochwasser in der Hauptstromsohle fast gar nicht gefunden. Dafür stieg die Abundanz räuberisch lebender Spezies, wie *Monodinium balbianii balbianii* an. In der Buhnenfeldsohle bei o. g. Stkm war die Abundanz der Ciliaten mit 400 Ind. cm^{-3} doppelt so hoch als bei mittlerem Abfluss. Planktische Arten, wie *Urotricha farcta* traten bei Hochwasser im Buhnenfeld vermehrt auf. Diese Ergebnisse zeigen, daß ein Hochwasser in beiden Beprobungsarealen zu einer Veränderung der Ciliatenzönose führte. Es verursachte jedoch im Hauptstrom durch starke hydrodynamische Kräfte eine fast komplette Auswaschung einzelner Arten aus dem Sediment, während es im strömungsärmeren Buhnenfeld durch Eintrag von planktischen Organismen aus der fließenden Welle zum temporären Abundanzanstieg kam.

KV 15

Morphological diversity of the genus *Cochliopodium* (Himatismenida): how to put it in order?

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The genus *Cochliopodium* is currently among the most diverse amoebozoan genera, comprising 16 species more or less fully characterized morphologically, among which 12 were first isolated during the recent decades. Currently used characters for species identification within the genus are tectum scales ultrastructure and the morphology of locomotive form. According to the scale structure 4 species groups can be outlined within *Cochliopodium* – "*actinophorum*", "*spiniferum*", "*bilimbosum*", and "*gulosum*". Two species, *C. gallicum* and *C. larifeili*, cannot be assigned to any of these groups. Other morphological features are either shared by all the groups (like nuclear structure or dictyosomes), or, like shape of the locomotive form, variable within the groups, and the same alternative states may occur in different groups. The groups can be better distinguished by their habitats. The "a*ctinophorum*" group is almost exclusively freshwater and soil, while "*gulosum*" is marine. "*Spiniferum*" and "*bilimbosum*" occur in both marine and freshwater habitats. The analysis of these data allows a careful suggestion, that at least "*actinophorum*" and "*gulosum*" may constitute separate phylogenetic lineages. However, this can be altered with the application of molecular characters in the analysis, as it happened recently with vannellids. Anyway, the degree of diversification within *Cochliopodium* favors subdivision of this genus into several ones, raising its status to a family.

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Locomotion and F-actin distribution in *Cochliopodium* sp. (Amoebozoa, Himatismenida)

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We observed and recorded locomotion of *Cochliopodium* sp. (a new species), and revealed the distribution of F-actin in the moving cells by TRITC-phalloidin staining. During locomotion amoebae produce a broad peripheral sheet of hyaloplasm, whose leading edge extends over the substratum, attaches to it and contracts together with formation of a new leading edge. Lateral parts of hyaloplasm contract in the uroidal direction. Posterior edge of the cell is usually raised over the substratum, producing trailing filaments from the ventral surface, and the granuloplasm is dragged in the direction of movement. TRITC-phalloidin staining reveals an actin network in the leading hyaloplasm, and pronounced bundles of actin ca. 0.3-1.2 µm thick, oriented transversally or obliquely to the direction of movement. These bundles appear to originate from centers anchored in the posterior or lateral plasma membrane and sometimes extend into frontal actin network. We suggest, that the formation and contraction of actin network at the leading edge of the cell may propel the advancement of amoeba, while microfilament bundles provide the retraction of lateral and posterior parts of the cell to drag them forward together with granuloplasm. This spatial organisation of F-actin in *Cochliopodium* is obviously different from that of *proteus*-type amoebae, and more similar to that of flattened cells of *Protostelium*, *Dictyostelium* or fibroblasts.

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In vivo and *in vitro* horizontal gene transfer in ciliates

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Horizontal Gene Transfer (HGT) means a non-reciprocal exchange of genes between species that, *per definition,* are otherwize isolated genetically. A preliminary phylogenetic analysis of a macronuclear gene library from *Nyctotherus ovalis* from the hindgut of cockroaches revealed a number of potential HGT's, but the frequency of the transfers and the spectrum of transferred genes seems to be substantially different from that observed in rumen ciliates. Therefore, we studied the possibility of gene transfer *in vitro* using cultures of the ciliates *Tetrahymena thermophila* and *Euplotes crassus*. The eukaryotic plasmid pEGFP, which encodes the EGFPand Neomycin phosphotransferase, was used as a source of exogenous DNA. Potential gene transfer was monitored by screening the ciliates for EGFP fluorescence, which, however, did not provide evidence for any HGT of complete EGFP genes. Also, Reverse Transcriptase (RT) PCR could not provide any evidence for HGT. Since both detection methods rely on the proper expression of the potentially transferred DNA, we are now screening for the presence of fragments of plasmid DNA, which potentially were integrated into the macronuclear genome of *Euplotes crassus*.

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Testate amoebae under molecular light; family hyalosphaenidae revisited

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Members of the lobose testate amoebae (order Arcellinida Kent, 1880) are a widespread group of unicellular eukaryotes encountered in many terrestrial, freshwater and even marine environments. Because of their often very narrow ecological tolerance, they are valuable bioindicators in ecology and paleoecology. However, the phylogeny and taxonomy are still mostly based on morphological characters and are far from being clear. This is particularly the case with the family Hyalosphaenidae (sensu Ogden and Hedley, 1980), which taxonomy has changed several times but is still not fully convincing. Here we present a phylogeny of this group based on SSU sequences, in which we demonstrate the paraphyly of genera *Heleopera* and *Hyalosphaenia*, and we resolve the phylogenetic relationships within genus *Nebela* s.l.

Spontaneous recomplementation of RNA induced macronuclear deletions in *Paramecium tetraurelia*

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Double-stranded RNA (dsRNA) processed into small ssRNA molecules triggers RNA interference but was also reported to be involved in epigenetic regulation, such as the development of the ciliate macronucleus. In *Paramecium*, epigenetic mechanisms were studied intensively in the X-irradiation mutant d48, lacking the gene for surface antigen A in its macronucleus but not in its micronucleus. In this study we introduced dsRNA homologues to surface antigen A into *Paramecium* cells prior to induction of autogamy, producing a deletion mutant with a noA-phenotype, similar to d48. Single cell real-time PCR analysis revealed a significant reduction of the A-gene copy number in comparison to wild type macronuclei, varying between cell lines originating from different autogamous cells. Interestingly, after a certain time of cultivation cells underwent a spontaneous gradual recomplementation process, proceeding with vegetative fissions, but independently from further macronuclear rearrangements. The current copy number of a cell line was determined by the initial degree of deletion after dsRNA treatment, indicating that remaining gene copies promote the amplification process of the gene itself, similar to the postautogamous development of the new macronucleus. The cell lines regained wild type level in respect of the A-gene copy number but, nevertheless, serotype A was never expressed again. The phenomenon observed in these dsRNA deletion mutants arouses a new discussion of the characteristics of the d48 mutation, its A-gene deletion persisting over innumerable vegetative fissions and sexual processes of the macronucleus.

KV 9

Übertragungswege bei *Histomonas meleagridis* – Was ist dran am Althergebrachten?

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Histomonas meleagridis ist ein kosmopolitischer Einzeller, der bei vielen Hühnervögeln auftritt und insbesondere bei Puten schwere Schäden anrichtet (sog. Blackhead Disease). Trotz dieser großen wirtschaftlichen Bedeutung sind die Kenntnisse zur Morphologie, zu den Übertragungswegen und auch zur Chemotherapie äußerst limitiert. Es existieren sehr kontroverse Berichte. So wurden begeißelte Stadien im Blinddarmlumen beschrieben, dazu amöboide, gewebsinvadierende Formen in der Darmwand und Leber sowie angebliche Dauerstadien in den Eiern des Nemtoden *Heterakis gallinaceum.* Die für Trichomonadida als wirksam beschriebenen Medikamente haben keine Wirkung auf *Histomonas.* Unsere elektronenmikroskopischen Untersuchungen der Stadien in den Wänden des Darmes und der Leber zeigten Zysten, die denen der Gattung *Blastocrithidia* sowohl in Form als auch Größe sehr ähnelten. Diese mögliche Zugehörigkeit der von *Histomonas meleagridis* zu den Blastocrithidien (Ordnung: Kinetoplastida) würde das Wirkversagen von Metronidazol bei Histomoniasis erklären.

KV 3

Molecular phylogeny of lobose testate amoebae (Arcellinida)

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The Arcellinida are the largest group of the testate amoebae. Three suborders - Arcellina, Difflugina and Phryganellina are distinguished. We have sequenced the 18s rRNA genes of *Microchlamys patella*, *Pyxidicula operculata* (Arcellina), *Difflugia acuminate*, *Netzelia tuberculata*, *N. wailesi* (Difflugina) and *Cryptodifflugia oviformis* (3 isolates), *C. horrida* (Phryganellina). The 9 new sequences are longer than average and highly variable. *Pyxidicula opercultata* has the shortest (1838 bp) and *Cryptodifflugia horrida* the longest sequence (3010 bp). These differences are the result of several inserts in the variable regions of the gene. The exceptional length of the 18s of *C. horrida* is caused by two additional inserts of 320 and 401 bp. The sequences of the three morphologically identical isolates of *Cryptodifflugia oviformis* are significantly different! This indicates the existence of cryptic species in lobose testate amoebae. The hypothesis that the order Arcellinida is a monophylum is not supported! This is independent of the methods used for tree reconstruction. The position of the three suborders in the trees is unstable and depends largely on the methods used. *Heleopera sphagni* is always the most basal taxon of the Arcellinida and never groups with the rest of the Difflugina. One reason for this behaviour could be that the analyses suffers from a long branch-artefact. The Arcellina are only weakly supported in the distance tree and appear polyphyletic in the Bayesian analysis and the Maximum Likelihood tree. *Pyxidicula operculata* is either a sister group to the Phryganellina or to the Phryganellina + Difflugina (– *Heleopera sphagni)*.

KV 20

Bedeutung von Temperaturerhöhung in der frühen Entwicklung biofilmassoziierter Ciliatengemeinschaften

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Die Formulierung ökologischer Folgen globaler Klimaveränderung, darunter ungewöhnliche Temperaturvariationen, stellt eine zentrale Herausforderung aktueller ökologischer Forschung dar. Protozoen eignen sich dabei besonders gut für die experimentelle Untersuchung der Folgen von Temperaturerhöhungen auf Lebensgemeinschaften da (i) durch ihre hohen Wachstumsraten viele Generationen in kurzer Zeit untersucht werden können und (ii) sie von enormer Wichtigkeit für das Stoffumsatzgeschehen in verschiedenen Ökosystemen sind. Hier stellen wir ein Fließzellensystem vor, dass sich zur Nutzung als Gewässer-Bypass-System eignet und sich insbesondere durch die Möglichkeit zur experimentellen Manipulation und der *in-situ*-Beobachtung von biofilmassoziierten Protisten auszeichnet. Durch die offene Anbindung an das Gewässer und die damit verbundene Möglichkeit zur Migration weisen die Biofilme ein hohes Maß an Natürlichkeit auf. In unseren Versuchen haben wir Biofilme bei verschiedenen Temperaturen von 0 - 6°C über der Freilandtemperatur kultiviert. Ein überraschendes Ergebnis war, dass sich durch die reine Temperaturmanipulation keine strukturellen Unterschiede in biofilmassoziierten Ciliatengemeinschaften zeigten. Die experimentelle Erhöhung von Nahrungsressourcen (DOC, planktische Algen) führte hingegen zu einer deutlichen temperaturbedingten Stimulation der Ciliatengemeinschaft. Unsere Ergebnisse weisen auf einen limitierten Effekt von Temperaturerhöhungen in nährstoffärmeren und eine deutliche Reaktion in nährstoffreicheren Systemen hin.

P 29

Heterotrophe Nanofauna in der Luft

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Bisher gab es wenige detaillierte Untersuchungen zur Verdriftung heterotropher Protozoen mit dem Wind. Es konnten erstaunlich viele verschiedene Protozoentaxa (102) in luftexponierten Fallen nachgewiesen werden. Protozoen waren sowohl in künstlichem Süßwassermedium als auch in künstlichem Seewasser zu finden. Außerdem konnten erstmalig Zystenbildungen einiger Protozoenarten nachgewiesen werden. Die meisten registrierten Arten sind als Bodenbewohner und Zystenbilder prädestiniert, weltweit verfrachtet zu werden. Erstaunlicherweise konnten jedoch einige Taxa, die in der Literatur als Kosmopoliten beschrieben werden, im Laufe der Bearbeitungszeit nicht nachgewiesen werden. Damit bleibt die Aussage Finlay's (2002) zur weltweiten Verbreitung von Mikroorganismen weiter spekulativ. Die bisherigen Arbeiten stellen allerdings nur einen ersten Einstieg in die Problematik der weltweiten Verbreitung dar, die durch molekularbiologische Untersuchungen künftig ergänzt werden sollen.

P 16

Survival strategies of chrysomonad flagellates: suspended sediments

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The effect of suspended fine sediments on a flagellate community originating from oligomesotrophic Lake Mondsee was investigated using different clay minerals and artificial silicate beads. On the community level suspended clays did not affect flagellate abundance. This was different for specific taxa, i.e., the abundances of *Spumella*-like flagellates and *Ochromonas-Chromulina* decreased significantly. In order to understand this taxon-specific response, the influence of different clay characteristics, specifically of particle concentration and particle size, was investigated for *Spumella*-like flagellates in laboratory studies. These experiments confirmed, that at bacterial abundances in oligotrophic and mesotrophic lakes, i.e., $1 – 4 \times 10^6$ bacteria ml^{-1}, the flagellates are severely food limited. The presence of suspended sediments generally decreased the growth at any food concentration tested. This decrease was reflected in shifts of the parameters of the numerical response. While the half saturation coefficient and the threshold food concentration for positive growth increased linearly with increasing suspended particle concentration the maximal growth rate decreased exponentially. The clearance rates strongly decreased when small and large particles were present but were only slightly affected for intermediate particles of 3µm in diameter. We assume that small particles in the size range of ingestible bacteria interfere with the feeding process and cause lower clearance rates, while intermediate particles may serve as substrate for the flagellates and enables the flagellates to optimise their clearance rates. Probably due to wall effects, clearance rates again decreased for larger suspended particles.

P 17

Survival strategies of chrysomonad flagellates: habitat specifity

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We investigated the flagellate community of a stream and its exchange with the flagellate community of the surrounding soils. Besides the direct exchange of species between the compartments, losses due to grazing and to turbulence in running waters were also part of the investigation. Samples were taken at 12 sampling points along the Fuschler Ache and from surrounding soil sites. Abiotic parameters were directly measured in the field. We investigated the flagellate community in terms of abundance and basic species composition in the water gradient. Further, we isolated 40 chrysomonad flagellates from representative aquatic and terrestric sites. The flagellates were identified based morphology as well as on SSU rRNA sequence data. Both, change of species composition and a drastic reduction in flagellate abundance during the first few km of the Fuschler Ache was observed. Based on our data soil borne flagellates were introduced in the Fuschler Ache and made up for a significant part of the stream community.

Mapping components along roads and crossroads of vesicle trafficking in *Paramecium* cells

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We have cloned genes encoding various components contributing to vesicle trafficking in *P. tetraurelia*. Such components may serve organelle biogenesis (H^+-ATPase), vesicle movement (actin), docking and eventual fusion (SNAREs, i.e., synaptobrevin, syntaxin and SNAP25, as well as the SNARE-specific chaperone, NSF).
With most components we find a multiplicity of paralogs that can be assigned to subfamilies. Representative members have been analyzed in more detail. This included the following techniques. (i) Producing antibodies against characteristic immunogenic peptides, followed by conventional or confocal immuno-fluorescence or immuno-gold EM-analysis. (ii) Overexpression as GFP-fusion proteins, eventually also followed by immuno-EM. (iii) Gene-silencing and functional studies.
With the exception of NSF and SNAP25, we can specifically localize isoforms of different components at distinct sites of vesicle trafficking pathways. This seems to be one aspect explaining the unexpected degree of paralog diversification found in *Paramecium*. Even, along one trafficking road, components may be exchanged, thus mediating specificity of individual steps or crossroads.
Part of the work presented has been done in collaboration with the lab of Jean Cohen (Gif-sur-Yvette), i.e., NSF and H^+-ATPase. - Supported by Deutsche Forschungsgemeinschaft.

Nitrification in aquatic sediments – Do ciliates have an impact?

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Nitrification, the oxidation of NH_4^+ to NO_3^- via NO_2^- in an oxic environment, is catalyzed by nitrifying bacteria. Bacterivorous interstitial ciliates might affect nitrification because grazing pressure is known to change bacterial activity and community structure. Selective grazing, e.g. ciliates discriminating for or against nitrifying bacteria, can be excluded as a recent study has shown (Neubacher et al. in prep.).
Sandy sediments from the Rivers Salzach (Austria) and the Baltic Sea (Germany) were transferred into laboratory flumes and manipulations such as freezing (to remove macrofauna), autoclaving and addition of ciliates from cultures and combinations of these were conducted. Nitrification rates were determined using slurry-assays, extensive supplementary data such as O_2, NO_3^-, NO_2^- and NH_4^+ microsensor profiles and various biotic and abiotic parameters were collected. Analysis of data is not yet completed, but first results show a tendency that increased numbers of ciliates lead to higher nitrification rates. Furthermore the impact of the polychaete *Nereis diversicolor* on nitrification was studied.

KV 31

Evolution of phagotrophic euglenids

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Understanding the evolution of ingestion devices is essential for a comprehension of euglenid phylogeny, especially of phagotrophic evolution. For informal classification four different types of ingestion apparatuses representing an increase in structural complexity have been compiled. Unfortunately, this scheme does not reflect any phylogenetic scenario resulting from molecular analyses. Type I apparatuses comprise the MTR/pocket type found in euglenids like *Petalomonas* and *Calycimonas*. They are also found in bodonids and thought to portray the plesiomorphic condition. Type II can be found in *Ploeotia* and is composed of plicate vanes and supporting rods with few peripheral microtubules. A type III ingestion apparatus is built of curved vanes and two supporting rods each comprising a central microtubule bundle and can be found in common forms like *Peranema* and *Anisonema.* The highly complex and protrusible siphon-like organ of *Entosiphon sulcatum* represents type IV. Comparison of standard molecular approach data and morphological data reflects the difficulties to put together a hypothesis about how the ingestion apparatus evolved in this group.

P 18

The impact of the composition of the plankton community on the presence or absence of mixotrophic protists in two freshwater ponds

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The existence of mixotrophe protists has long been known. However, their role in the microbial food web is still not fully understood. By their simultaneous functioning on different trophic levels, mixotrophs create a separate niche, allowing them to coexist within a diverse microbial community.
Based on this background, our study investigates two different lakes – one inhabited by planktivorous fish and one without– in Salzburg. Possible differences in the structure of the microbial food web are likely caused by this difference. In this context, the effect on the abundance of mixotrophic protists was investigated.
Abundances of central components of the planktonic community (bacteria, flagellates, ciliates and zooplankton) were estimated and the contribution of mixotrophic flagellates to total bacterivory was quantified using established protocols for grazing experiments. Our results show a significant difference in the abundance of mixotrophs in the two types of lakes. Reasons and consequences are discussed.

Einfluss der Fließgeschwindigkeit auf Biofilm assoziierte Ciliaten

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In kleinen Fließgewässern mit hohen Fließgeschwindigkeiten leben Ciliaten meist in Assoziation mit einer stationären Oberfläche in dünnen und kompakten Biofilmen. Bei geringen Fließge-schwindigkeiten entwickelt sich ein eher dicker, hoch poröser Biofilm. Ciliaten haben mannig-faltige Anpassungen an hohe Fließgeschwindigkeiten entwickelt, wie z.B. die direkte Anheftung mittels Stiels (Peritrichia) oder die dorso-ventrale Abflachung der Zelle und Reduktion der Cilien (Phyllopharyngia, Haptoria). In dieser Studie soll der Einfluss der Fließgeschwindigkeit auf die Struktur und Dynamik der Ciliatengemeinschaft in Fließgewässerbiofilmen untersucht werden. Als Substrat für den Biofilm wurden Glasobjektträger verwendet, die in der Ilm, einem thüringischen Fluss dritter Ordnung, ein bis 14 Tage exponiert wurden. Das Substrat wurde oberhalb (0,1 m s^{-1}) und unterhalb (0,4 m s^{-1}) eines anthropogen errichteten Wehres und an einer schnellfließenden Referenzstelle (0,4 m s^{-1}) exponiert. An der Referenzstelle stieg die Gesamtabundanz der Ciliaten kontinuierlich von 2 auf 294 Individuen cm^{-2}. Hier dominierten Arten der Gruppen Haptoria (*Litonotus* spp.) und Phyllopharyngia (*Chlamydonella alpestris*) die eine abgeflachte Körperform besitzen. Im langsam fließenden Reservoir wurden schon nach eintägiger Exposition höhere Abundanzen (bis zu 60 Individuen cm^{-2}) als an den Stellen hoher Fließgeschwindigkeit gefunden, mit dem Abundanzmaximum nach 5 bis 7 Tagen. Im Reservoir, mit sehr hohen Bakteriendichten, dominierte unter anderem der bakterivore Ciliat *Uronema nigricans* (Hymenostomatia) mit einer rundlichen Körperform. Die Fließgeschwindigkeit beeinflusste neben den morphologischen Typen, der taxonomischen und funktionellen Zusammensetzung der Ciliatengemeinschaft auch die Besiedlungsdynamik.

Three dimensional structure of *Acinetobacter* biofilms in flow chambers in presence and absence of a protozoan predator (*Tetrahymena pyriformis*)

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The presence of protozoans in biofilms has shown in first studies in some studies strong influence on the biomass of bacterial biofilms (see e.g. Haws, 2005). In this presentation the results of the analysis of the three-dimensional structure in flow chamber experiments will be shown. We investigated the influence of *Tetrahymena pyriformis* on biofilms built by *Acinetobacter spec*. under flow cell conditions (see e.g. Christensen et al., 1999). For this purpose, we used different flow rates in the flow cells. For the reason of quantification of biofilm parameters the programm „3D for LSM" has been used.
First results suggest that *Acinetobacter* biofilms are more vulnurable under comparable low flow than under ‚normal' flow conditions (1,6 and 4 ml/min respectively). Furthermore, in some cases we could find a slightly different three-dimensional structure of the bacterial biofilms under ‚normal' flow conditions in the presence of the ciliates. Possible reasons for these results will be discussed in the poster presentation.

KV 33

The R-SNAREs (synaptobrevins) of *Paramecium*

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Membrane interactions are mediated by soluble N-ethylmaleimide sensitive factor attachment protein receptors (SNAREs) which are characterised by the possession of a heptad repeat coiled-coil SNARE motif. During membrane fusion, these SNARE domains form a stable, SDS-resistant SNARE-complex that is thought to be sufficient to accomplish membrane fusion. Because of the presence of a conserved arginine or glutamine residue in the central layer of the SNARE domain, SNAREs are also classed into R-SNAREs and Q-SNAREs.. We discovered a set of R-SNAREs (synaptobrevins) in the ciliate *Paramecium tetraurelia* consisting of nine families that are encoded by 15 genes and are expressed simultaneously. This high number of genes underlines the complexity of the endomembrane system in *Paramecium*. Most *P. tetraurelia* synaptobrevins (*Pt*Sybs) possess a SNARE domain and show homology to the amino terminal longin domain of the longin family of R-SNAREs such as Ykt6, Sec22 and VAMP7. Interestingly, some of the *Paramecium* R-SNAREs do not exhibit the otherwise highly conserved arginine at the zero layer of the SNARE domain. Structural modelling revealed nearly identical structures for all SNARE domains, but more deviant structure predictions were made for amino terminal longin domains of the *Pt*Sybs. We localised members of several *Pt*Syb families with GFP-constructs and with specific antibodies at the light and electron microscopic level and examined the function of synaptobrevins in *Paramecium* exemplary for a R-SNARE of the osmoregulatory system, *Pt*Syb2.

P 37

Purification and characterization of an acid-stable extracellular lipase from *Tetrahymena thermophila*

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The ciliate *Tetrahymena thermophila* is known to secrete a myriad of enzymes of biotechnological interest. An extracellular lipase was purified and further characterized. The corresponding gene was identified in the genome of *Tetrahymena thermophila.* In order to improve lipase production, the mutant strain *T. thermophila* pPT_Lipase was generated. By induction with cadmium the lipase production of this strain could be increased 20-fold. The enzyme was purified 34-fold from supernatant of *T. thermophila* pPT_Lipase and the wildtype strain 1868.4 by ammonium sulfate precipitation, hydrophobic interaction chromatography, anion exchange chromatography, and gel filtration. The enzyme was homogenous as judged by SDS-polyacrylamide gel electrophoresis. Enzyme activity was measured using trioleine as substrate. The extracellular lipase showed optimal activity at pH 4,25 and remaining activity at alkaline pH. It was acid stable down to a pH of 2 when incubation was performed at room-temperature. After incubation at pH 4 and 65°C for 30 min the enzyme retained 40 % of it´s activity. The presence of a mixture of conjugated and unconjugated bile-salts seemed to slightly inhibit enzyme activity. The amino acid sequence of the lipolytic enzyme showed high homology with other already characterized extracellular lipases. It contains the highly conserved lipase motiv Gly/X/Ser/X/Gly and the amino acids of the catalytic triad.

P 7

Divergence between North American and Eurasian clones of *Stylonychia lemnae* inferred from sequence analyses

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The species *Stylonychia lemnae* (Ciliophora, Spirotrichea), a member of the *Stylonychia mytilus* complex, shows a global distribution and occurs in many freshwater habitats in temperate climate zones. Different morphological and molecular analyses as well as countless mating experiments resulted in clear discrimination of *S. lemnae* from the sibling species *S. mytilus*. Recently we showed that the SSU rDNA of these both sibling species consistently differs in one single nucleotide, which can be used for species discrimination based on fluorescence in situ hybridization.
In 1989, Ammermann and colleagues described some peculiarities (e.g. the occurrence of naturally amicronuclear strains) for the North American clones of *S. lemnae*. Sequence analyses of several North American clones of *S. lemnae* revealed the typical "*lemnae*"-nucleotide pattern within their SSU rDNA gene. Surprisingly, these analyses yielded an additional single nucleotide difference, which separates all North American clones of *S. lemnae* from numerous clones of the same species isolated from different regions in Europe and Asia.

P 43

The hydrogenosomal metabolism of the anaerobe ciliate *Nyctotherus ovalis*

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Hydrogenosomes are membrane-bounded organelles of certain anaerobic eukaryotes hat produce hydrogen and ATP (Muller 1993). In 2005, Boxma et al. showed that the hydrogenosomes of *N. ovalis* contain a "mitochondrial" genome, and, potentially, more than 50 "mitochondrial" proteins encoded by nuclear genes of mitochondrial origin. Since it could be shown that these hydrogenosomes possess a functional mitochondrial complex I and II, and, in addition, perform a "fumarate respiration" like certain anaerobic mitochondria (Tielens et al. 2002), we searched for additonal "mitochondrial" genes that are potentially involved in the hydrogenosomal metabolism. Here we show that is possible to identify metabolic key-genes, which provide evidence for the major metabolic routes in the hydrogenosomes of *N. ovalis*.
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Characterization of R-body genes in *Caedibacter caryophilus*

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Certain species of *Paramecium* can be infected by *Caedibacter*, a polyphyletic genus of bacterial endosymbionts which produce refractile inclusion bodies (R-bodies). These R-bodies are proteinaceous ribbons typically coiled within the bacterial cell. They are presumption for *Caedibacter* cells to confer the killer trait to their host. Killer paramecia release toxic particles (*Caedibacter* cells carrying R-bodies) and their ingestion ultimately kills sensitive paramecia. Genetic determinants of R-bodies, the *reb* genes, have been characterized (Heruth *et al.*, 1994) in *C. taeniospiralis* (*Gammaproteobacteria*). In the present study consensus-degenerate hybrid oligonucleotide primers and RL-PCR (Random and Limited) were used to characterize the *reb* genes of *C. caryophilus* (*Alphaproteobacteria*). Data mining showed the presence of so-called *reb*-like genes in different free-living *Alpha*-, *Beta*- and *Gammaproteobacteria*, which have not been reported to produce R-bodies. Sequences of *reb* and *reb*-like genes were used for phylogenetic analysis and the results suggest that two concurring phenomena likely occurred in the spreading and evolution of *reb* genes: horizontal gene transfer and gene duplication.

P 10

Euplotes trisulcatus KAHL 1932 – A benthic microaerophile or a planktonic anaerobe?

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We observed an abundant euplotid ciliate in the anoxic water column of the super-sulfidic Framvaren Fjord in southwest Norway. The organisms was isolated and cultured immediately after sampling. Silver nitrate staining (Chatton-Lwoff technique) identified the organism as *Euplotes trisulcatus* KAHL 1932. This organism has been characterized as a typical marine benthic interstitial ciliate. This was supported by the facts that (i) until now it was recovered exclusively from marine sediments and (ii) that the organism's morphology is well adapted for life in between and on top of sediment grains. Typically, the prevailing view is, that such protozoa are microaerophiles, and that their vertical zonation reflects the distribution of oxic microhabitats within the sediment. Here, we present data challenging this hypothesis: The source of isolation as well as ecophysiological experiments question, if *E. trisulcatus* is obligat interstitial and aerobic. Based on this and other observations, we argue, that the case of *E. trisulcatus* is not unique and that we might have to rethink the definition and ecology of so-called "interstitial" ciliates.

Actuarioloa framvarensis Stoeck et al., 2005: the type species of a novel genus within the Kinetoplastea (Euglenozoa)

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Kinetoplastea are ubiquitously distributed flagellated protists of high ecological and medical importance. We isolated an organism from the oxic–anoxic interface of the anoxic Framvaren Fjord (Norway), which phylogenetically branches within an unidentified kinetoplastean sequence clade (SSU rDNA). Ultrastructural studies revealed a typical cellular organization that characterized the isolate as a member of the order *Neobodonida* VICKERMAN 2004. This order contains five genera, four of which are morphologically different from the novel organism (*Dimastigella*, *Cruzella*, *Rhynchobodo* and *Rhynchomonas*). The arrangement of the microtubular rod that supports the apical cytostome and the cytopharynx differed from the diagnosis of the fifth described genus (*Neobodo* VICKERMAN 2004) within the order Neobodonida. On the basis of both molecular and microscopical data, a novel genus within the order Neobodonida, *Actuariola* gen. nov., is proposed. We phylogenetically, morphologically and ecologically characterize its type species, *Actuariola framvarensis* STOECK et al 2005 and provide an *in situ* tool to access the organism ecology in nature.

A probabilistic model of cell size reduction in *Pseudo-nitzschia delicatissima* (Bacillariophyta)

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The pennate planktonic diatom *Pseudo-nitzschia delicatissima* is very common in temperate marine waters and often responsible for blooms. Recently, extensive samples of *Pseudo-nitzschia* have been taken from coastal waters. Mating and cell size reduction experiments were carried out and served as a data basis for a probabilistic model of cell size reduction which is here presented and discussed. We use a non-stationary continuous-time Markov Chain to model the successive loss in cell diameter as a stochastic process from an initial cell size of about 80 µm over six cell states to an absorbing state, equivalent to cell death. We derived a Maximum Likelihood estimator for the population age, given a cell size distribution of a population. The estimator was validated using a test data set. Ongoing work will incorporate other features like spore stages and the possibility to enter a cycle of sexual reproduction to compensate for the loss in cell size. As the Markov Chain now models typical cell size changes, we are therefore able to predict and detect deviations from a normal population development without the need to collect large data samples.

P 40

Introns in macronuclear genes of *Nyctotherus ovalis*

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The anaerobic heterotrichous ciliate *Nyctotherus ovalis* lives in the hindgut of cockroaches. The macronucleus of this ciliate possesses highly processed genes on gene-sized minichromosomes. These “mini chromosomes” consist out of a single open reading frame flanked by short leader- and trailer sequences, and telomeres. Some of the open reading frames are interrupted by introns, that will be removed from the primary RNA transcript. The majority of the introns is small, and ranges between 18 and 27 nucleotides. All introns have a typical 5’ and 3’ flanking region, allowing the identification of candidate genes by bioinformatic analysis. This information might be of importance to get information about the potential timescale for the acquisition of “foreign” genes by lateral gene transfer. Here we show that sequencing of cDNA's for candidate genes can provide the necessary information.

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KV 34

Post-transcriptional control enables mutual exclusive expression of surface antigens in *Paramecium*

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One of the most striking features of surface antigen expression in any protist is the exclusive presence of a single antigen species on the cell surface. As this behaviour of gene expression is essential for antigen function it still remains unknown which mechanisms of regulation occur in *Paramecium* to avoid expression of more than one member of the multigene family. Knocking down the expression of serotype 51A by RNAi we analysed the development of transcript abundances during the induced serotype-shift from 51A to 51D. Therefore surface antigen related transcripts were analysed using quantitative RT-PCR; parallel the presence of proteins on the cell surface was determined by indirect immunofluorescence staining to enable the comparison of transcript- and protein-status of the cultures.
However, quantitative PCR is a very sensitive method and therefore we were able to find not expressed 51D transcripts in wild type 51A cultures. An un-exclusive transcription in such a way was not reported before. Further progress of the shift was characterized by enforced transcription of the 51D gene. We found that in spite of still high abundant 51A transcripts only 51D transcripts were processed into proteins and delivered to the cell surface. Therefore we conclude that post-transcriptional control is a major component regulating mutual exclusive expression of surface antigens. On the other hand our experiments revealed that transcriptional control should also be involved in regulation as the transcriptional level of 51D still raised when already pure 51D protein was synthesised speaking for the existence of a feedback mechanism. We therefore demonstrate that regulation of surface antigen expression occurs on several levels and that the mechanism behind is more complicated than expected.

Chlorella-bearing ciliates in sunlit waters of Piburger See (Tyrol, Austria)

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We analysed the ciliate assemblage composition over one annual cycle at monthly or biweekly intervals in the sunlit water layers (0-9 m depth) of Piburger See (PIB). In particular, we assessed the contribution of *Chlorella*-bearing ciliates with relation to changes in levels of incident natural solar radiation. Ciliates were collected at the deepest site of the lake and analysed qualitatively by live observations (10 µm plankton net samples), as well as quantitatively by the protargol-staining (QPS) method. In addition, the existence of UV-absorbing compounds (mycosporine-like amino acids, MAAs) in the ciliates was assessed by HPLC. Finally, other biotic and abiotic parameters (Chl-*a*, Temp, O_2) of the lake were measured. We found that throughout the year *Chlorella*-bearing ciliates were mainly distributed in the upper 6 m of the water column in summer/autumn with a mean abundance of 2.6 cells ml^{-1} (max. 13.0 ml^{-1}). At certain times and depths in summer, >70% of the total ciliate abundance was represented by green *Halteria bifurcata*, *Pelagohalteria viridis,* and *Askenasia chlorelligera*. In several ciliate species, we detected MAAs, such as mycosporine-glycine (λ_{max} = 310 nm) and palythine (λ_{max} = 310 nm). In addition, changes in MAAs composition over the year, e.g., in *Pelagodileptus trachelioides* were observed. Our results suggest that in summer *Chlorella*-bearing ciliates in PIB are more competitive than heterotrophic ones.

Is *Spirostomum teres* a light-sensitive heterotrich ciliate?

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Based on observations of the vertical distribution of *Spirostomum teres* (*ca*. 500 x 50 µm) in oligo-mesotrophic Piburger See (PIB), Neidl (1989) hypothesized that the presence of the pigment flavin in the citreous cortical granules makes this species prone to be photosensitive, when exposed to natural solar radiation and elevated O_2-concentrations, as found in the epiliminion. In fact, this species has never been detected in the upper 13 m of the water column of PIB. However, there is little information about how its distribution changes over time and how it relates to different environmental conditions. Thus, we investigated (i) the spatial distribution of hypolimnetic ciliates including *S. teres*, in samples from 12-24 m depth over a one year cycle by qualitative and quantitative analyses (live & QPS), in relation to incident natural solar radiation and abiotic parameters (Temp, O_2, etc.), and (ii) we identified the nature of the pigment, as well as tested the light-sensitivity of the *S. teres* under laboratory conditions. Here we show the first results of those analyses.

KV 13

Protistan species richness: predicting the unpredictable

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The prominent part of eukaryotic diversity is represented by unicellular organisms, the protists. Their species number in nature may be in the hundreds of thousands, but most have never been observed or otherwise detected; the existence of these species is predicted. Available estimates are vague and problematic, and do not provide baseline information on either local or global species richness. The quality of protistan richness predictions however is an important issue as they serve as a basis for all of the paradigms of biodiversity, its role, function and meaning. Local species richness cannot be measured directly, for the same reason that the global diversity has yet to be quantified: its' simply too large for the methodology available. One of the best tools for protistan detection is the rRNA approach, but even large SSU rDNA clone libraries seem to capture only a small fraction of the original richness. Thus, the number of protistan species in all but the simplest communities can only be estimated statistically, typically on the basis of a small subset of species (or their molecular signatures) observed directly. Uncertainties in species richness prediction are due in large parts to the way statistical tools are used, if not indeed misused. We developed a powerful synthetic statistical approach to quantify protistan diversity. It provides statistically sound estimates at any level of taxonomic hierarchy. We apply this approach to a large original 18S rDNA dataset and show that protistan species richness in anoxic arctic sediments is spectacularly high. We argue that our methodology provides estimates of species richness that are general, have biologically meaningful SEs, and meet other fundamental statistical standards. This approach can be an essential tool in biodiversity research.

KV 7

Phylogeny of trichostome ciliates (Ciliophora, Litostomatea) endosymbiotic in the Yakut horse

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Ciliates of the Subclass Trichostomatia inhabit the fermentative regions of the digestive tract of herbivores. Most small subunit ribosomal RNA (SSrRNA) gene sequences that are available are from species isolated from the rumen of cattle or sheep and from marsupials. No ciliate species endosymbiotic in horses have yet been analyzed. We have sequenced the SSrRNA genes of five ciliate species, isolated from the caecum and colon of four Yakut horses: *Cycloposthium edentatum, Cycloposthium ishikawai, Tripalmaria dogieli, Cochliatoxum periachtum*, and *Paraisotricha colpoidea*.
Based on their morphology, *Cycloposthium*, *Tripalmaria* and *Cochliatoxum* are classified as Entodiniomorphida, while *Paraisotricha* is considered a member of the Vestibulifera. Preliminary phylogenetic analyses using Bayesian inference, distance, and parsimony methods, confirm these placements. The two *Cycloposthium* species cluster together with the published *Cycloposthium* species isolated from a wallaby in Australia. *Tripalmaria* and *Cochliatoxum* branch as the sister group to the Entodiniomorphida or basal to it. The Vestibulifera remain paraphyletic with *Paraisotricha* branching basally to all other trichostome species, but not closely related to *Isotricha, Dasytricha* or *Balantidium*.

KV 28

The potential role of symbiotic *Chlorella* in UV-protection of freshwater ciliates

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Algae-bearing ciliates have been observed to be in advantage over other species at times of low food supply or under micro-aerobic conditions near the oxycline, but their occurrence also in sunlit waters of eutrophic environments, suggests the existence of other benefits in this mutualistic association. We investigated the role played by symbiotic *Chlorella* to minimize the damaging effects of UV radiation in *Paramecium bursaria*, *Uroleptus* sp., and *Stokesia vernalis*. An ultrastructural characterisation of the ciliates and symbiotic algae, with special emphasis on (1) cell wall and shape of zoochlorellae and (2) their abundance and distribution within the ciliate, revealed clear differences among the investigated species. Results from UV-experiments and HPLC-analyses of sunscreen-compounds in combination with the morphological analyses suggest at least two different UV-protection strategies of the ciliate-*Chlorella* symbiosis: production of algal-derived colourless sunscreen compounds known as mycosporine-like amino acids (MAAs), in *Uroleptus* sp. and *Stokesia vernalis* and internal self-shading in the case of *Paramecium bursaria*.

P 30

Running water environment – Unexpected wealth of rare testate amoeba species (Protozoa: Testacealobosia, Filosea)

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The testate amoeba communities of running waters (2 large rivers: Danube and Tisza and a Transdanubian lowland channel) were investigated in Hungary during 1996-2005 to reveal the taxonomic composition and abundance. Besides the usual overhelming majority of *Difflugia*, *Centropyxis* and other genera possessing mainly xenosomous test, some very rare and peculiar genera and species - mainly filoseans - have been detected. These include *Hyalosphenia punctata, Clypeolina marginata, Nadinella tenella, Centropyxiella sp.* (*arenaria*?) *Cyphoderia calceolus*, *Schaudinnula arcelloides*, *Corythionella golemanskyi, Placocysta lens*. The coexistence of some of the species was conspicuous in more samples.
Hyalosphenia punctata has been found in three independent watersheds in Hungary. Its lobose character is now demonstrated by the extended lobopodia of the living specimen.
Centropyxiella spp. are considered as obligate marine species, however, some specimens of *C. arenaria* occurred in the samples. Salinity probably does not limit its distribution, since it has a wide tolerance according to the literature. *Corythionella* was also considered to be marine until the description of the freshwater *C. golemanskyi* which since has been found in the Danube River as well.
One common character of the majority of the above species is the very thin, fragile test, made either of siliceous plates or thin organic one covered in thin external plates, similar to the marine psammobionts, suggesting a lesser demarcation between freshwater and marine testacean faunas.

P 19

The impact of an oxygen gradient on the microeukaryotes in the Framvaren Fjord (Norway)

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The diversity of microeukaryotes from extreme marine environments, such as the Framvaren Fjord in Southern Norway (with sulfide levels in the bottom water 25 times greater than those in the Black Sea), is largely unknown. The aim of our study was to identify the quantitatively most important flagellates in this system and to investigate their ecological role (here: grazing impact on bacteria). To find our whether the grazing-behaviour of microeukaryotes changes with changing oxygen conditions, water samples from the oxic (9 metres), the oxic/anoxic boundary (20 m) and from the anoxic area (22 m) were collected. Flagellates and bacteria were enumerated in these water samples and differences in their vertical distribution, possibly related to the chemical environment were recorded. In addition, bacterivory experiments were conducted. The results of these experiments allow conclusions on the microbial food web in the Framvaren Fjord and the role of certain taxa of heterotrophic flagellates as bacterivores.

P 39

Analysis of copy numbers of genes in *Nyctotherus ovalis* using Q-PCR

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Nyctotherus ovalis is an anaerobic hypotrichous ciliate that thrives in the hindgut of cockroaches. This ciliate possesses one micronucleus and one macronucleus. The macronucleus of *N. ovalis* contains gene sized pieces[1]. These gene sized pieces consist out of a single open reading frame flanked by a leader- and a trailer sequence and telomers. Some leader sequences are very short and do not contain clear signatures indicative of regulatory sequences. It has been speculated also whether gene- expression in *N. ovalis* is controlled by the copy number of gene sized pieces in the macronucleus. Information about copy numbers of these gene sized species is only available by hybridisation data of *Euplotes* and *Oxytricha*. [2,3] Quantative PCR however is a more reliable technique to gain information about copy numbers of genes in the macronucleus.

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KV 11

Diversity of free-living amoebae (FLA) in thermal waters in Hungary

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Free-living amoebae (FLA) are a phylogenetically highly diverse group of eukaryotic, heterotrophic organisms feeding on bacteria, algae and yeasts. Several representatives of the FLA, including the genera *Naegleria, Acanthamoeba*, *Balamuthia* and *Sappinia*, are involved into human disease and the ability to cause an infection in humans has among other criteria been correlated to the ability to grow at ≥37°C. Particularly *Naegleria fowleri*, the causative agent of the primary amoebic meningoencephalitis, exhibits a pronounced thermophily and occurs predominantly in warm waters.
The aim of this study was to reveal the diversity of free-living amoebae in thermal waters in Hungary. Water and swap samples were taken from several different spas and cooling waters in different regions of Hungary. Amoebae were isolated by the plate culture method at 37°C and 42°C, respectively. All isolates were cloned and identified by their morphologic characters. The relationships of the isolates to one another and to other FLA were assessed by sequencing of the ribosomal small subunit RNA gene and subsequent cluster analysis. Interestingly, almost all samples were positive for FLA. The isolated strains were identified as belonging to the genera *Acanthamoeba, Echinamoeba, Naegleria, Platyamoeba* and *Vannella*. Several isolates might represent new species as they exhibit less than 95% sequence identity to any described taxon.

KV 16

Soil testate amoebae – Succession and diversity and consequences for ecosystem processes

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Temporal and spatial distribution of different trophic groups of the soil fauna (testate amoebae, nematodes, enchytraeids, earthworms, and spiders) and land use practices were investigated. Successional stages of red oak and indigenous oak forest stands were differentiated using a false time series (chronosequence) in the post-mining landscape and compared to undisturbed reference sites. Furthermore, these forests were confronted to open habitats of the post-mining landscape (natural primary succession). Communities of testate amoebae were highly differentiated according to history and vegetation type of the investigation sites. Amoebae assemblages in non-forest habitats differed strongly from forest sites, which could be further separated into old-growth and younger areas. Preliminary litterbag-experiments (colonisation of organism-free cellulose) revealed pronounced differences between the sampling sites. Further experiments are planned to quantify soil processes and develop the impact of different land use options on the soil function in the context of a sustainable land use in post-mining landscapes Supported by BMBF, Fkz 01LC0018A.

KV 24

A new protocol to analyse protozoa and bacteria in aerobic activated sludge granules by using FISH

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The ecological function of protozoa in wastewater systems is still rather unexplored. It is known that protozoa are important for purification and clarification of sewage water. In aerobic sequencing batch reactors (SBRs) ciliates of the subclass Peritrichia often colonize granules in large numbers. Aerobic activated sludge granules are dense, spherical biofilms consisting of protozoa, bacteria, extracellular polymeric substances, and sometimes fungi. These granules can dramatically improve purification efficiency and sludge settleability in wastewater treatment. Recent studies showed that mostly peritrichous ciliates provide the structural matrix of such microbial aggregates. This study tries to enlighten the interactions of protozoa, bacteria and fungi during the development and establishment of the granules by a biomolecular approach. To identify the taxonomic entities in the microbial communities the fluorescence in situ hybridisation (FISH) technique is applied. As granules can reach a diameter of several millimeters it was neccessary to develop a protocol which ensures that even microbial cells in the core of a granule can be detected and identified by FISH. Based on this new protocol granules are subjected to a fixation procedure with paraformaldehyde or Bouin`s solution, frozen (-196°C) and cut into slices of 35-40 µm using a cryotome. The slices are placed on gelatine coated microscope slides and hybridized with different fluorescently labeled oligonucleotide probes targeting the 16S rRNA (Bacteria) and 18S rRNA (Protozoa). The long-term aim is to investigate granule structure development multidimensionally, i.e. in space and time, by using that protocol.

P 23

Microscopic investigations on the structure and composition of aerobic activated sludge granules

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Aerobic activated sludge granules are spherical clusters of dense microbial biofilms used to improve sludge settleability and water purification in wastewater treatment processes. This study lays focus on the development and composition of granules from sequencing batch reactors (SBRs) loaded with various types of wastewater. Granules mainly consist of protozoa, bacteria, extracellular polymeric substances, and in some cases of fungi. Mostly ciliates of the subclass Peritrichia and sometimes several representatives of fungi seem to be the key organisms providing the structural matrix of such granules. The ciliates settle on other organisms or particles, thus building flocs. Mainly the stalks and often subsequently the zoids of tree-like colony forming ciliates are colonized by bacteria, the main food of ciliates. After a while most protozoal cells are completely overgrown by bacteria and die. The cellular remains serve as a new substrate for bacteria and swarming ciliates and thereby act like a skeletal structure element within the granules. By this process a granule of up to several millimeters in diameter can develop. Granule size depends on the wastewater type and the operational set-up of the reactor. Density and compactness of granules in the same reactor can be variable. However, it turned out that a completely formed granule always comprises a core and a fringe zone. In this study the development and composition of different types of granules are observed by light microscopy and scanning electron microscopy.

Impact of pH on growth and survival of freshwater ciliates

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In spite of its potential significance as environmental parameter in many inland waters, the pH impact on aquatic protists has received only little attention. The effect of pH on growth, cell volume, and production of four prostome and one oligotrich freshwater ciliate species was investigated in laboratory cultures with *Cryptomonas* sp. as food. Overall, pH had a significant, species-specific impact on all parameters investigated. The food alga, *Cryptomonas* sp., showed a wide pH tolerance, with positive growth rates between pH 4.4 and 9.65. Over this pH range, algal cell volume remained constant. In the ciliates, the pH impact on cell volume was positively correlated to the pH effect on their growth rates in some, but not in every of the strains investigated. Among three species of the genus *Urotricha*, *U. farcta* was the pH most tolerant species, reaching positive growth rates from pH 4.1-9.5; *U. castalia* was the pH most sensitive species (pH range 6.5-8.2). The pH optimum was derived from cellular production rates. The pH optima of the three *Urotricha* species were shifted; their production rates peaked at pH 4.4-5.3 (*U. farcta*), pH 5.9-7.3 (*U. furcata*), and at pH 6.8-7.9 (*U. castalia*). Another prostome species, *Coleps spetai*, and the oligotrich *Meseres corlissi* were also classified as neutrophile species. The pH effect on growth and survival of the ciliates was minor at circumneutral and moderately alkaline pH values, relative to the impact of temperature and food measured in earlier experiments. The width of the pH reaction norm of the ciliates appeared to be related positively to the width of their temperature niche and their natural distribution.

Capacity of ciliates in the elimination of suspended bacteria from sewage plant water

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One of the sources of antibiotic-resistant bacteria in the environment, which are a major threat to human health, are sewage treatment plants where the bacteria are enriched in the biological purification steps and may gain resistance genes by horizontal gene transfer. From here the suspended bacteria are finally released in relatively high numbers into the environment. Free-swimming ciliates are able to filter-feed on suspended bacteria even at low densities – therefore their ability for reducing the bacterial cell number was investigated. The experiments were designed to resemble the conditions of water leaving the sewage plant. In a batch experiment six different ciliate species (starting density 2×10^4 cells/ml) were cultivated together with *Escherichia coli* (starting density 1×10^7 cells/ml) for eight hours, the cell density of both was measured every hour. The two ciliate species (*Tetrahymena pyriformis*, *Colpidium campylum*) showing the highest clearance rate of bacteria were selected and used for further investigation. Additional batch experiments were carried out at five different temperatures (10°, 15°, 20°, 25°, 30° C), where the ciliates were co-cultivated with three different bacteria species (*E. coli, Bacillus subtilis, Pseudomonas putida*) either alone or in combination. The concentration of bacteria and ciliates was measured every two hours. The results showed that the load of suspended bacteria can be reduced five- to fifty-fold, depending on temperature, ciliate species and bacterial species. Further experiments under continuous conditions and larger scale are needed to confirm these results.

KV 23

Genetische Identifizierung von Protozoen im Grundwasser – Erstellung einer 28S rRNA Sequenzdatenbank

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Protozoen sind eine wesentliche Komponente des Stofftransfers in aquatischen Ökosystemen und haben als Bakterivore großen Einfluss auf das mikrobielle Nahrungsnetz. Der Lebensraum Grundwasser ist bezüglich der Nanofauna bislang nur sehr spärlich untersucht und eine zuverlässige taxonomische Einordnung seiner Diversität ist nicht möglich. Zudem basieren die bisher genutzten Methoden zur Untersuchung der Nanofauna auf Kultivierungstechniken und unterliegen daher einer starken Selektivität. Im Projekt wird die Qualität der gesamten (nicht nur der kultivierbaren) Nanofauna sowie deren Quantität mittels ribosomaler Signatursequenzen (Oligonukleotide) untersucht. Mit den entwickelten Oligonukleotidsonden sollen Methoden etabliert werden, die die schnelle und zuverlässige Analyse der Nanofauna-Diversität zulassen.

Zunächst wurde ein Datensatz partieller 28S rRNA Sequenzen (D1-D5 Region) für die Nanofauna erstellt. Hierzu werden Sequenzen aus Grundwasser-Kulturen (LAM) sowie durch Amplifikation aus einzelnen Zellen und Grundwasseraliquoten gewonnen. Auf Basis des Sequenzdatensatzes ist es möglich, gruppen- und artspezifische Oligonukleotidsonden zu entwickeln, um anhand dieser funktionelle Gruppen und einzelne Arten zu detektieren. Im Vortrag werden erste Ergebnisse zur taxonomischen Zusammensetzung der Grundwasser-Nanofauna anhand der 28S rRNA Daten vorgestellt.

P 8

Molecular biological and morphological studies on the reisolated binucleate amoeba *Sappinia diploidea (Thecamoebidae)*

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Strains of the binucleate naked amoeba *Sappinia diploidea* (Hartmann and Nägler,1908, Ges. naturschforsch. Fr. Berlin, 5:112) possessing a complex life cycle were reisolated from the barks of trees and a freshwater pond, identified and re-investigated. In addition to the previous morphologic description (Goodfellow, Belcher and Page, 1974, Protistologica, 2:207) we observed throughout differentiations of the cell surface coat forming a distinct glycocalyx. The phylogenetic analysis based on SSU rRNA gene sequences shows close relatedness of this species to the members of the genus *Thecamoeba* and confirmed the existence of the monophyletic family Thecamoebidae. Until now there was no laboratory culture of this interesting species. As a result of the present finding a strain of *Sappinia diploidea* is deposited with CCAP (UK) and is available for further studies; a neotype is established for this species.

KV 10

Two microsporidian parasites of *Chaetocnema tibialis* (Coleoptera: Chrysomelidae) in Turkey

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Microsporidian spores show typical ultrastructural characteristics such as a polar filament, an anchoring disc and a polaroplast; mitochondria are missing. The number of polar coils provides a useful taxonomic criterion for differentiating species. The first record of a microsporidian from *Chaetocnema tibialis* (Coleoptera: Chrysomelidae) is *Nosema chaetocnemae* Yaman and Radek 2003. The spores are oval, having a size of 3.52 ± 0.41 µm x 2.09 ± 0.26 µm (n = 50). They are diplokaryotic and their polar filaments have 13 coils. Infections are found in the gut, tracheae, muscles and Malpighian tubules. Recently, a new microsporidian parasite of *Chaetocnema tibialis* was recorded in Turkey. The fresh spores are cylindrical and measure 4.12 ± 0.5 µm x 1.4 ± 0.4 µm (n = 50). They are diplokaryotic. The number of polar coils is 8-10. This new microsporidian has a prominent posterior spore vacuole and exclusively infects the Malpighian tubules of the insect. Thus, it differs in several typical features from *N. chaetocnemae*, such as spore size and shape, number of polar coils, and type of infected tissue. As the life cycle of the parasite is not completely known, it could not be identified at the species level. However, our studies confirm two discrete microsporidian infections in *C. tibialis* in Turkey.

KV 13

Linking molecular diversity and functionality in protistan communities

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Analysis of ribosomal DNA (rDNA)-derived clone libraries has proven to be uniquely suited for the initial discovery of organisms providing information on their distribution and molecular phylogeny. Recently, several methods were suggested to bridge the gap between descriptive community analysis and ecological significance of rDNA-derived diversity. One approach uses the construction of RNA-derived clone libraries to reflect the diversity of the metabolically active members of a microbial community. However, this technique has not yet been applied for eukaryotes. Here, we describe and apply a technique for simultaneous extraction of eukaryotic RNA and genomic DNA using a planktonic sample collected below the chemocline of a Danish anoxic fjord, suitable for enzymatic downstream applications. A comparison of the RNA- with the DNA-derived library revealed good correspondence of the general community composition in terms of higher taxonomic ranks. We retrieved nearly 600 protistan target clones, which grouped into 84 different phylotypes (98% sequence similarity). Of these phylotypes, 27% occurred in both libraries, 25% exclusively in the rRNA- and 48% exclusively in the rDNA-library. Most of the library-unique phylotypes were represented by a single (or pair) of cloned sequence type(s). However, main differences between the libraries were the lack of several dinoflagellate and 'uncultured alveolates group I' clones from the rRNA library and of several prasinophyte clones from the rDNA library. These discrepancies between SSU rDNA and SSU rRNA libraries demonstrated the value of generating DNA- and RNA-derived libraries when investigating the diversity and functionality in microeukaryotic communities *in situ*.

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