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A new, colpodid "flagship" (Protozoa, Ciliophora) from soil of a Green River Bed in Botswana (Africa)

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Endemicity is difficult to prove in microscopic organisms because (i) they are not easily recognizable; (ii) many species are dormant (encysted) most of their life; (iii) distinctive morphological features are rare, as compared to higher plants and animals; (iv) the field is distinctly undersearched, and (v) differences may remain unrecognized or misclassified as "site variations" due to the use of holarctic identification literature for species from other biogeographical regions. In this situation, eyecatching "flagships" with conspicuous size and /or morphology are the best distribution indicators. Many such species have been described, but others remain to be discovered, showing our ignorance about even conspicuous taxa. The new flagship was discovered in mud and soil from a green part (flooded mainly during rain season, grassland with a rich mammal fauna in the dry season) of the River Kwai in the Okawango Delta, Botswana, subtropical Africa. "Green River Beds" have never been investigated for protists. Our study shows an extreme diversity with about 100 ciliate species in a single sample. Many of theses species are undescribed, and some are of outstanding size representing biogeographic flagships.

The new flagship belongs to the family Colpodidae, where it represents a new genus and species. It has a size of 150–300µm and occurs in two shape types: the "propeller" form is like the blade of a boat screw and thus very conspicuous when swimming; the second form is conical and shows that the postoral sac occupies the rear end, as in the Marynidae, the sister family of the Colpodidae. However, silver impregnation and scanning electron microscopy show that the new genus belongs to the Colpodidae because the pharyngeal fibres are directed posteriorly. In the lecture, the new flagship is shown in a short film, in silver preparations, and in the scanning electron microscope. There is little doubt that this species is restricted either to Africa or Gondwana.

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The critical nature of protistan test organisms: How to use the synergistic potential of studies on "the same test organisms"?

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As for every biological discipline research in aquatic microbial ecology is often based on classical test organisms, used mostly due to their simple handling and cultivability but irrespective of their real ecological importance and dominance. In principle, several research groups are working on "the same cultured species" and ecologists try to evaluate published and own data to obtain a synergistic picture of the investigated species. In fact, this approach neglects the genotypic, phenotypic and ecophysiological variability of the tested organisms, superficially termed as "the same species". Additionally, the simple extrapolation of data gained in laboratory studies to the situation in the field has to be critically reviewed.
Since nine years, we maintain cultures of one mixotrophic (*Ochromonas* sp. strain DS), two heterotrophic protists (*Bodo* cf. *saltans*, *Cyclidium glaucoma*) and one autotrophic phytoflagellate (*Cryptomonas* cf. *phaseolus*). These isolated strains have been used for a large set of experiments during the last decade, resulting in a detailed characterization of their ecophysiological potential.
In this talk we concentrate on the two bacterivorous protists, namely *Ochromonas* sp. strain DS and *Cyclidium glaucoma*. These organisms were often used to characterize the effects of protistan predators on the structure of bacterial assemblages. Therefore, we compared our own data with available, published data-sets (more than 50 publications). The following topics will be mentioned: i) Many studies include only a poor or even no taxonomic characterization of the tested protists (assuming that we speak about the "same test organism"). ii) We answered many (also rather academic) questions by conducting laboratory studies. In contrast to the adequate knowledge how "the test organisms" behave in lab-experiments, we have only a poor knowledge about the situation in the field. iii) It seems, that some methods work brilliant for laboratory studies but fail to elucidate processes in nature.
For the future we propose a detailed genotypic characterization of the isolated strains (belonging to the "same species") to obtain a detailed picture of the "intra- and inter-culture" variability. Therefore, we plan to collect cultivated strains (identical to the taxa mentioned above) from various research groups and laboratories. Beside the taxonomic affiliation of the cultivated species, a detailed morphological and ecophysiological characterization should be obtained.

Development of species-specific PCR-primers for the sibling species *Stylonychia mytilus* and *Stylonychia lemnae* (Spirotrichea, Ciliophora) based on IDH-sequences

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Based on morphological characters differentiation between the two sibling species *Stylonychia mytilus* and *Stylonychia lemnae* is difficult, especially for non-specialist. They were considered as one species until 1965. At that time cytological and genetic studies showed that the "species" *Stylonychia mytilus* in fact consists of two genetically isolated varieties. Detailed morphological and biochemical analyses confirmed this separation and resulted in a description of a new species, *S. lemnae*. For example, isoenzyme analyses revealed distinct differences in the electrophoretic pattern of isocitrate dehydrogenase (IDH) between both species.

This study was set out to develop a quick method for discrimination between these two species. Therefore, we amplified and sequenced the IDH-gene of several *S. mytilus* and *S. lemnae* clones isolated from different geographic regions.

Our results revealed interspecific as well as intraspecific sequence variation. The species-specific differences allowed the development of species-specific PCR-primers for both *S. mytilus* and as *S. lemnae,* providing a rapid and simple method for the discrimination between these two species even if only one single cell is available.

Furthermore, phylogenetic analyses were carried out using the IDH-sequences. The results show a clear separation between all *S. mytilus* and *S. lemnae* clones supported by high bootstrap values. Further, tendencies indicating geographically structured distribution patterns are denoted.

Distinguishing the sibling species *Stylonychia lemnae* and *Stylonychia mytilus* (Spirotrichea, Ciliophora) by fluorescence in situ hybridization

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Stylonychia lemnae and *Stylonychia mytilus* are members of the *Stylonychia mytilus* complex. Both species are difficult to distinguish by morphological or morphogenetic characters. However, investigations of the isoenzyme pattern of the isocitrate dehydrogenase and the malic dehydrogenase show a distinct differentiation between both species. In the last few years, fluorescence in situ hybridization (FISH) techniques became a suitable and reliable tool for identification and differentiation of closely related species of protozoa, such as ciliates. In the present study, appropriate specific oligonucleotide probes should be developed to distinguish both species.
Therefore, the SSU rDNA of numerous clones of the sibling species *Stylonychia lemnae* and *Stylonychia mytilus* were analysed. Comparing the sequences of both species, a single nucleotide difference was found within the whole gene. Based on this difference a set of two oligonucleotide probes targeting the SSU rRNA of each species was designed. These probes were tested on preserved cells of clones of both species isolated from different geographic regions. All hybridizations performed with the new probes showed intense fluorescence signals in targeted cells only and allow now the unambiguous differentiation of the species *Stylonychia lemnae* and *Stylonychia mytilus*.

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Biodiversity development of terrestrial testate amoebae – is there any succession at all?

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Heterotrophic protists, e.g. testate amoebae, play an important part in primary succession, because these unicellular organisms occur immediately at newly exposed land surfaces in high abundances and biomasses facilitating the establishment of plants and animals. We investigated testate amoebae from soils of different age and stage, which revealed remarkable high abundances and biomasses even at very dry or sandy sites. Emphasis was set on inland dune micro-chronosequences of different plant successional stages (bare sand, *Corynephorus canescens* and *Polytrichum piliferum* as early stages; *Festuca ovina* and *Pinus sylvestris* as late stages). The number of testate amoebae species increased clearly with the successional stage of the vegetation cover, but no consistent replacement or extinction of taxa was observed. The "newcomers" obviously did not reduce the density of the "residents", although the community pattern (abundances, biomasses, dominances) was significantly altered. Organism-free substrate of different quality exposed to the air or adjacent soil was colonised quickly and in high abundance by testate amoebae, but no temporal replacement of species occurred. Cluster analysis of species inventory and abundances of numerous types of soil of different age highlight a classification of amoebal communities towards regional influences rather than local successional stages of vegetation. These data corroborate the fact that belowground communities operate differently than plant ones. Thus, the concept of "community assembly" should be taken into consideration, which is more flexible than "succession". Furthermore, the additive invasion behaviour of testate amoebae points to a lack of interspecific competition, characteristic for a neutral community model.

Phylogenetic and morphological studies on choanoflagellates from the Antarctic and Arctic waters

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As part of the heterotrophic nanoflagellates, which play a very important role in marine Antarctic and Arctic pelagic ecosystems, choanoflagellates form a conspicuous and abundant component. Recent molecular biological studies of marine nanofauna indicated that morphological investigations of these tiny organisms (3-6µm cell size) reveal only limited taxonomic resolution. However, until now only four marine choanoflagellate species of the Acanthoecidae and only seven species of Codonosigidae and Salpingoecidae have been sequenced (18S rDNA). We isloated some choanoflagellates, analyzed them combining morphological and molecular biological methods and summarized information on the polar and global distribution of choanoflagellates. A report will be given on the first results regarding the molecular identity of Arctic and Antarctic isolates of the same morphospecies, on phylogenetic relationships within the choanoflagellates and on the knowledge of the biogeography of choanoflagellates.

Intraspecific genetic variability in *Paramecium* (Ciliophora; Oligohymenophorea)

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We present data on the intraspecific genetic variation in *Paramecium caudatum* and *P. multimicronucleatum*. To test the extent of clonal diversity within these species we sampled at three geographic scales:
A local scale in the vicinity of Leipzig, an intermediate scale throughout Central Europe and a worldwide scale.
We found no intraspecific variability in the 18S RNA/ITS – region, which demonstrates its utility as diagnostic marker to identify species within the genus *Paramecium*. On the other hand, DNA-sequences of the mitochondrial COX I gene revealed extensive intraspecific variation in both species.
Interestingly, the analysis of our data suggests higher sequence divergence between *Paramecium multimicronucleatum* populations.

Diversity of eukaryotes in extremes of anoxicity: The Framvaren Fjord in Norway

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Our research focuses on the diversity of microeukaryotes in different anoxic marine environments using culture-independent as well as culture-dependent approaches. By comparing different sample sites with each other we try to estimate the global dispersal or endemic nature of different organisms and taxonomic groups. Here, we present data from an anoxic environment, the Framvaren Fjord in Norway. With > 1000 clones analyzed thus far and more to come, our Framvaren-study belongs to the most comprehensive molecular diversity inventories in a single environment. Within a wide range of anoxicity levels, Framvaren represents the extremes of anoxicity on Earth: a super-anoxic fjord with sulfide concentrations up to 25 times higher than in the Black Sea. According to current assumptions, species diversity decreases and species abundance increases in extreme environments with only a few specialized species occurring in high numbers. However, in Framvaren we find an unexpectedly high diversity of microeukaryotes. The molecular signatures represent organisms of most major protistan lineages in the eukaryotic tree of life. The level of novelty reaches from novel species, genera and families within well described taxonomic clades, to previously described clades on the highest taxonomic levels known exclusively from their environmental signatures.

Molecular diversity in euglenid flagellates

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Euglenid flagellates are a well defined group of about 1,000 taxa that belong together with kinetoplastids and diplonemids to the supertaxon Euglenozoa. They occupy a variety of fresh water, marine and soil habitats, but mostly prefer water bodies rich with organic contamination, like farm yard mittens, ponds and puddles with decaying plant material. Euglenids are easily identified as such by light microscopical investigation due to a very characteristic set of diagnostic features like cell shape, euglenoid movement, flagella, and ingestion organelles. But only the unique structure of the pellicle serves as an autopomorhic character, because all other characters occur singularly in other organisms as well. Using molecular data to explore euglenid affiliations has been successful so far only within euglenid evolution. Relationships to other protists still remain unclear.
Within euglenid two distinctive lineages can be identified with SSU rDNA data sets: The most known are the phototrophic forms, which evolved after secondary endocytobiosis between a phagotrophic euglenid ancestor and a green alga. As evidence for the secondary endocytobiosis event the established chloroplasts are surrounded by three membranes and an eyespot independent from the chloroplast. The second, less known, clade is built by osmotrophic forms, which exclusively feed on pinocytotic uptake of nutrients via the reservoir membrane. Molecular data show that these two clades do not represent the plesiomorphic nutrition modes in euglenids, but are derived from phagotrophic ancestors: After development of alternative food sources, ingestion apparatuses have been reduced in both lineages. Molecular peculiarities of phagotrophic, phototrophic and osmotrophic euglenids will be discussed.

The species problem and Protist Evolution

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The biological species concept as coined by Ernst Mayr is not applicable to many protists which reproduce by inbreeding or asexually. An extended concept supplementing the biological species concept was suggested by T. M. Sonneborn after intensive studies on differently reproducing species of the *Paramecium aurelia* complex. In his concept based on the hypothesis that inbreeding or asexually reproducing
taxa also evolve as discrete units, he suggested that a species should be recognized as an evolving entity that has undergone a threshold of minimum evolutionary divergence. However, Sonneborn´s idea was poorly received. We examine different morphological and molecular characters discovered and applied in taxonomy since Sonneborn developed his hypothesis. We conclude that there is now an abundance of
objective characters to arrive at sound judgement about the complexity of the genetic differences necessary to delimit species in Sonneborn´s sense when the biological species concept is not applicable. In addition, combined morphological and molecular studies reveal that, although many free-living protests may be globally distributed, geographical patterns and local distribution also occur.

Die Morphospezies: Konzepte und Probleme

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Von den über 30 Artkonzepten sind heute noch folgende sechs in Diskussion: das **Biologische Artkonzept** von Ernst Mayr ("I define biological species as groups of interbreeding natural populations that are reproductively isolated from other such groups".); das **Phylogenetische Artkonzept** sensu Mishler und Theriot ("A species is the least inclusive taxon recognized in a formal phylogenetic classification. As with all hierarchical levels of taxa in such a classification, organisms are grouped into species because of evidence of monophyly. Taxa are ranked as species rather than at some higher level because they are the smallest monophyletic groups deemed worthy of formal recognition, because of the amount of support for their monophyly and/or because of their importance in biological processes operating on the lineage in question".); das **Phylogenetische Artkonzept** sensu Wheeler und Platnik ("We define species as the smallest aggregation of sexual populations or asexual lineages diagnosable by a unique combination of character states".); das **Evolutionäre Artkonzept** von Wiley und Mayden ("An evolutionary species is an entity composed of organisms that maintains its identity from other such entities through time and over space, that has its own independent evolutionary fate and historical tendencies".); das **Hennigsche Artkonzept** ("Species are reproductively isolated natural populations or groups of natural populations. They originate via the dissolution of the stem species in a speciation event and cease to exist either through extinction or speciation".); und das **Ökologische Artkonzept** von van Valen ("a species occupies a certain niche".).

Keines dieser Konzepte erweist sich in der Praxis als leicht handhabbar und kann die Subjektivität der Artabgrenzung nicht wirklich beseitigen. Auch Mayr´s vielgerühmte Definition stößt an Grenzen, wenn man an die asexuellen Organismen und die vielen, die Artgrenzen überschreitenden Hybriden denkt. Daher ist es in der Praxis nach wie vor so, daß eine Art das ist, was der Spezialist als solche einstuft. Das ist sicher subjektiv. Aber man kann eben eine Art nicht "beweisen", weder mit klassischen noch modernen Methoden; man kann sie bestenfalls wahrscheinlich machen. Dennoch: das ist kein Grund aufzugeben! Die molekularbiologischen Daten zeigen fast immer, daß die Morphologen sehr verantwortungsvoll waren, das heißt, weniger Arten errichteten als die Sequenzdaten wahrscheinlich machen.

An Hand einiger Beispiele werde ich das morphologische Artkonzept bei Ciliaten erläutern.

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Molecular typing of protistan species

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What is species? That is the question! If species exist, their evolutionary histories must be different. This separation, whatever its causes, must lead to accumulation of species-specific mutations. It should be possible to use the number of such mutations as a measure of evolutionary separation. It follows that gene divergence could be used as a rather objective species criterion. Is this simple logic supported by empirical observations?
For the sequence divergence to be useful in delineating species, its intra- and interspecific levels should be markedly different. If there is indeed a disparity between the two, a cut off value will emerge distinguishing populations that evolved into different species from those that have not. Using 18S rRNA gene as a marker, we set out to find such a cut off value. Several species of marine benthic ciliates served as test organisms. We detected and contrasted interoperon, intraspecies, and interspecies sequence divergence. While different ciliate species certainly exhibit different levels of interspecific gene sequence variability, the 1% 18S rRNA gene divergence appears to be a typical distance between closely related species. I will discuss the usefulness and limitations of the proposed concept of protistan species defined from molecular prospective.

Taxonomy of the Microsporidia: Morphology vs. Molecular Biology

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Microsporidia are obligate intracellular, eukaryotic parasites with a unique ultrastructure and life cycle. Traditional classifications of microsporidia have been done on the basis of morphologic characters, but the major published classifications differ significantly and the taxonomy of microsporidia remains controversial and is currently under extensive reconstruction. Especially the higher level classification of the Microsporidia has been tenuous because the significance of the homology among the morphological characters that could be identified could not be determined.
Molecular techniques are rapidly becoming an important and integral part of biosystematic studies. Microsporidia are prime candidates for phylogenetic analysis based on DNA sequence data because of the relatively small number of useful morphological characters.
The initial split of the Microsporidia into classes has been based on characters such as whether there is a membrane surrounding the sporoblast (Pansporoblastina versus Apansporoblastina), whether they are diplokaryotic throughout their life cycle or have uninucleate states, and the type of nuclear division (Haplophasea versus Dihaplophasea). There is increasing evidence that, while there are correlations in some cases between these characters and phylogenetic relatedness, these taxonomic schemes have almost no relationship to evolutionary history because these characters are changing relatively rapidly as evolutionary adaptations to host, host environment, and host population parameters. As a result, higher level classifications based on these characters are probably not measures of evolutionary relatedness, and indeed vary greatly among classification systems.
Phylogenies based on molecular sequence data are helping to resolve a large number of questions about the evolution of characters in the Microsporidia. Most of these studies use sequence data from the small subunit rDNA. This is because a large number of sequences have become available for this gene and it has become somewhat of a standard; however, other genes will have to be sequenced to confirm and further clarify microsporidial relationships.

A revised species concept for microalgae

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Since Ehrenberg (1839), species of microalgae have been described based on light microscopic features (the morpho-species concept). Only decades later were clonal cultures of microalgae established on a routine basis (Pringsheim 1921). Until recently, and sometimes even today new species of microalgae have been/are described based on light microscopy of cells from natural samples. Thus, the stability of the diagnostic characters as well as the genetic identity of the cells studied remains largely unknown. Furthermore, because of the lack of cultures, the reproducibility of such studies and hence their conclusions (taxonomic designations) are compromised. In recent years, studies addressing the morphology as well as the genetic identity of microalgal species have questioned the morpho-species concept. It has been shown that representatives of a single algal morphospecies can be genetically as diverse as humans and sea urchins. Many morphological characters used in microalgal classifications are now known to be either plesiomorphic or homoplasic. It is proposed that a revised species concept for microalgae should be based on the biological species concept and linked to non-homoplasious molecular synapomorphies (NHS). Such an approach could in principle also be transferred to asexual taxa or taxa for which sexuality is presently unknown. Some examples from research done in the author's laboratory using this approach will be presented.

Genospecies Versus Morphospecies: Highlights and Pitfalls of Fluorescence In Situ Hybridisation (FISH) with Protists

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During the last few years fluorescence in situ hybridisation techniques were developed to microscopically identify and detect protozoa within complex microbial communities using fluorescently labelled gene-specific nucleotide probes. These techniques can be combined with classical techniques such as silver impregnation methods and electron microscopy preparative techniques. Thus, a single individual organism can be examined and determined by applying different techniques, older morphologically and new genetically based methods. Morphology based identification of protists is standard. The huge number of described species provides lots of information. Direct comparisons can be made easily, if somebody looks carefully at the distinct characters to be compared in between such morphospecies – arguments heard by students from classical taxonomists. Gene specific identification of protists is based on the statement that each morphologic speciation is only the expression of a distinct genomic arrangement, the arrangement of nucleotides, which can be determined exactly. In this review, the highlights and pitfalls of the fluorescence in situ hybridisation (FISH) technique in general is focused. It deals with the technique of probe construction, shows, how a single nucleotide difference can be discriminatory for species identification, discusses the problems of fluorescence signal determination, and how FISH helps bringing together morphospecies and genospecies concepts in protistology.

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Application of morphological and molecular defined groups of organisms (species) in protist ecology

Jens Boenigk, Antonis Chatzinotas, Gianfranco Novarino, Karin Pfandl, Tobias Garstcki

Ecology of specific protistan taxa is largely based on ecophysiological investigations of a few cultivated strains. The increasing awareness of protist microdiversity and intraspecific variation requires to reconsider the current concepts and tools for identifying and grouping protists. Is a morphologically or a molecular defined taxon more suitable for ecological research? To what extend can findings on single strains be generalised for the respective taxon? Molecular data usually provide a higher (phylogenetic) resolution as compared to morphological traits. It is, however, a still unresolved question to what extend the higher resolution of molecular markers increase our understanding of the ecology of protist species. I will present data on more than 60 strains of a common and ubiquitous group of flagellates, i.e. colourless chrysophytes, originating from soil and aquatic habitats. I will discuss the suitability and applicability of morphologically and molecularly defined groups of organisms. Despite a high morphological similarity, the molecular diversity is high. However, even 18S rRNA gene sequences still do hardly allow for a biogeographical restriction of clades. For instance, we found identical genotypes in terms of 18S rRNA gene similarity in samples from Austria, China, New Zealand and Uganda suggesting global distribution. In contrast, ecophysiological experiments indicate consistent trends with respect to the origin of the strains, i.e., to the geographical region. I will discuss the implications of ecophysiological microdiversity for protist ecology and estimates of diversity.

Foreward and reverse genetic methods in analysis of phagosome formation in *Tetrahymena thermophila*

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Phagosomes are formed when particles collected by the oral apparatus, trigger fusion of discoidal vesicles with the membrane of the cytopharyngeal pouch.The growing phagosome incorporates the membranes of these vesicles until it eventually becomes pinched off into the cytoplasm by the action of a local actin-based cytoskeleton.
To dissect genetically the processes involved in phagosome formation we need mutants blocked in phagosome biogenesis. We have obtained by random mutagenesis, a special cross (Uniparental cytogamy) and the use of an efficient enrichment technique a number of different isozygous mutant phenotypes of this pathway: For example, mutants, that are impaired in structure and/or function of their oral apparatus (OA), the solely cellular site where the cell gathers particles and forms phagosomes. Other mutants have functional OA's, collect particles into the pharyngeal pouch, but are unable to form a phagosome. The third type of mutants is able to form a phagosome, but cannot release it into the cytoplasm. These mutants have shown that Tetrahymena can survive and grow unimpaired without phagocytosis, when cultured in suitable media, f. e. chemically defined medium. They also are helpful tools for pharmalogical studies on signalling pathways involved in phagosome biogenesis, but their genes could not be cloned so far.
The availability of an antisense ribosome library (1), in which the antisense vector contains 5' untranslated region inserts derived from a full-length cDNA library corresponding to a large number of protein coding genes, can overcome this shortage. Transformation of conjugating cells with this library by electroporation or biolistic bombardment generates null or hypomorphic phenotypes owing to the presence of antisense ribosomes. We collected transformands blocked in phagosome formation by the same enrichment protocol as described for mutants obtained by random mutagensis. All so far obtained tranformands show structural defects in the oral apparatus or mislocation of the OA. The genes whose silencing caused the mutant phenotype have been cloned by PCR experiments. The availability of the Tetrahymena Genome Database (http://www.ciliate.org.) will speed up identification of genes from further transformands.
To study phagosome formation biochemically we have purified newly formed phagosomes and for comparison also 5 min old phagosomes and separated their proteins by 2-D PAGE. On gels stained with Coomassie-Blue about 150 distinct spots were resolved for each phagosomal stage. From ten spots the proteins were digested, sequenced and used to synthesize their cDNA's by RT-PCR experiments. One of the proteins turned out to be identical to a secreted cysteine protease (Tetrain), two other had high homology to a 25 kD calcium binding protein, previously localized in the cilia only. A fourth protein showed 45% homology to a PtdIns-4P-Kinase. For the other proteins no meaningful homologies were detected by data base searches. The antisense ribosome technique (2) allows specific inhibition of these genes by cloning their reverse orientated 5'-UTR regions into the DN 5318 plasmid. Transformation of conjugating cells with this construct eventually results in selectable mutant phenotypes by this reverse genetic approach.

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Heterogenic distribution of surface-proteins and effects of mRNA abundance: *Paramecium* hesitates to change the serotype

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Proteins with the characteristics of antigens on the surface of *Paramecium* have been first observed by Sonneborn in 1942. The classical methods analyzing the gene-expression were micro- and macronuclear knock-out mutants. Intensive research on the RNAi pathway, not only in *Paramecium*, led to the ability of knock-down experiments by ingestion of dsRNA homologue to the target-gene. We present here the results of silencing serotype 51A of *Paramecium tetraurelia* particularly with regards to the phenotype, which is present in serotype 51D-alpha and also to the serotype shift, which shows interesting characteristics in temporal succession.

Normally surface antigen transformation can be easily induced by temperature shifts. By knocked down gene expression with RNAi we analyzed serotype shifting by indirect immunofluorescence staining of whole cells. The time span in which the “old” and the “new” protein are detectable is much longer than during the shift by temperature. By the comparison of different feeding-clones this phenomenon seems to be associated with the difference in mRNA abundance, a factor which was earlier thought to be regulating serotype expression. Also a heterogenic distribution of surface antigens is obvious: the “old”, silenced protein is located on the cilia-membrane, the new one on the cortex-membrane. No regions with both proteins mixed are detectable.

The expression of serotype 51D-alpha, when 51A is knocked down, correlates with the phenotype of deletions mutants but thought has been given to “cross-reacting” siRNAs silencing other members of the multigene-family. In our case serotypes 51B and 51G were theoretically “cosilenced” and the RNAi experiments obey to the theoretical calculated spectrum of possible phenotypes.

Experience and progress with immuno-localization in *Paramecium* cells

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Immuno-localization on the light and electron microscope (LM, EM) level is a powerful method since it provides insight into potential functions within the three-dimensional context of a cell. Ciliated protozoa, e.g., *Paramecium* cells, are particularly suitable for this type of work due to their regular "design". For instance, cilia and sites for trichocyst exocytosis as well as for coated pit endocytosis are regularly arranged, thus allowing to pinpoint potential molecular players of a complicated game. Another complex aspect is the existence of well defined intracellular trafficking pathways in these cells.

In practice, however, use of antibodies (ABs) available from higher eukaryotic systems in most cases have failed to give any reasonable result on Western blots and in LM and EM immuno-localization studies with ciliates. Frequently this even holds for affinity stains; e.g., a cleavage furrow, though mandatory for cell division, has never been seen in *Paramecium* with fluorescent phalloidin, though it stains other sites containing F-actin.

The solution to this problem is to have recourse to the potential of molecular biology. With *Paramecium*, based on an international genome project, one can increasingly utilize the sequences available for many (now almost all) genes. Immunogenic sites can be prognosticated and the corresponding regions expressed heterologously as peptides for AB production. Alternatively cells can be transfected for overexpression of green fluorescent protein- (GFP-) tagged fusion proteins. This can then also be used, after adequate embedding (-35°C UV polymerization), to perform EM localization studies using anti-GFP ABs.

Among the many examples we have meanwhile analyzed are the 63 kD-phosphoprotein, pp63/parafusin (pf), and the phosphatase/kinase systems using it as a substrate, including phosphatase PP2B (calcineurin) and its activator calmodulin, a cGMP-activated kinase as well as casein kinase type 2 (CK-2). They all turned out to be localized in narrow domains of the cell cortex – in contrast to some other phosphatases and kinases. Other examples are SNAREs (proteins mediating membrane interactions), the SNARE-specific chaperone, NSF, as well as subunits of the H^+-ATPase (proton pump). Another example is actin, with surprising – but also functionally convincing – results. Frequently endogenous antigen copy numbers do not suffice for clear-cut localization, especially in the EM. GFP-labeling then frequently turns out helpful, with anti-GFP ABs as a versatile tool. This approach can frequently be combined with gene silencing. Prerequisite to analyze all this, by cooperation in team having widely different methodologies available, is the "correction" of the aberrant genetic code of *Paramecium* for all genes/gene products to be analyzed.

Low CO_2 inducible carbonic anhydrases in photosynthetic and non-photosynthetic micro organisms

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Cyanobacteria and most eucaryotic algae induce a high affinity CO_2 concentration mechanism (CCM) upon exposure to limiting inorganic carbon concentrations (Ci; CO_2+HCO_3^-). The CCM consists of a close interaction between (i) Rubisco exclusively located to the carboxysomes (cyanobacteria) or pyrenoids (eucaryotic algae), (ii) high affinity uptake systems for Ci species and (iii) differently localised CAs. Some of these CAs are transcriptionally upregulated during acclimation to low Ci but the signal transduction pathway remains unknown. Despite its wide distribution among living organisms the presence of CA in fungi has been controversially discussed. Using a mass spectrometric technique we were able to measure *in vivo* CA activity in *Saccharomyces cerevisiae* which was lacking in a Δ*NCE103*-mutant. Over expressing of the *NCE103* gene product resulted in a highly active protein fraction indicating that it encodes a functional CA. Inactivation of *NCE103* led to a high CO_2 requiring mutant indicating that a functional CA is important for growth under low Ci in *S. cerevisiae* Interestingly, the *in vivo* CA activity was 10-20 times higher in low Ci compared to high Ci cells. Northern blot analysis and *LACZ-NCE103* promoter fusion revealed that expression of *NCE103* is transcriptionally upregulated by low Ci. In addition, we show that CA activities of other heterotrophic micro organisms are also regulated by low Ci. This shows for the first time that low CO_2 inducible CAs are not only restricted to algae but are more widely distributed among living organisms than previously thought.

A ciliate mitochondrion that makes hydrogen

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Since hydrogenosomes are found in various, rather unrelated eukaryotic lineages such as anaerobic flagellates, chytridiomycete fungi, and ciliates, they had been defined operationally as ATP-generating organelles that produce hydrogen (1). These hydrogenosomes exhibit large differences in structure and metabolism, just like mitochondria from various sources (2). However, in contrast to all mitochondria studied so far, hydrogenosomes – with one exception - lack an organelle-associated genome that could establish their evolutionary relationship. Here we show that the hydrogenosomes of the anaerobic ciliate *Nyctotherus ovalis*, from the hindgut of cockroaches, have retained a rudimentary genome.

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Identification and analysis of genes required for DNA elimination in *Paramecium tetraurelia*.

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The *Paramecium tetraurelia* micronuclear genome is estimated to contain approximately 50,000 short (26-882 bp) DNA elements that are efficiently and precisely removed during formation of the somatic macronuclear genome. Our goal is to identify genes that encode proteins required for excision of these internal eliminated sequences (IESs). Differential mRNA display was used to identify genes with increased expression during Paramecium conjugation. A subset of these genes were functionally analyzed by RNAi gene silencing in vegetative and conjugating cells. We found that a Paramecium homologue of UBA2, a SUMO activating enzyme required for modification of proteins in the SUMO pathway, is required for successful macronuclear development. Inhibition of UBA2 expression prevented IES excision but DNA amplification in the anlagen appeared normal. RNAi against UBA2 had no effect on vegetative cell division. Consistent with our data on UBA2, we also found that genes encoding SUMO protein were up-regulated during conjugation. RNAi against SUMO gave the same phenotype as RNAi against UBA2. The data suggest a key role for the SUMO pathway in Paramecium macronuclear development. We believe the SUMO pathway is required to modify key proteins involved in IES excision, perhaps controlling their entry into the anlagen. Interestingly, we found that UBA2 and SUMO are also up-regulated during conjugation in *Tetrahymena thermophila*. We have analyzed another Paramecium gene, called 5A450, which is required for IES excision. RNAi experiments against 5A450 result in the failure of macronuclear development and IES excision, but it has no effect on vegetative cells. A plasmid containing a 5A450-GFP fusion protein transcribed by the native 5A450 promoter shows that the gene product is localized exclusively to the developing macronucleus. No clear homologue of 5A450 has been found in metazoa, but a possible homologue is present in Tetrahymena. The combination of simple RNAi methods and complete macronuclear genome sequence is accelerating the identification of key proteins in macronuclear development.

Ciliate Gene Unscrambling

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Programmed DNA elimination and reorganization frequently take place during cellular differentiation. Macronuclear development in stichotrichous ciliates presents an extreme case of these processes, involving excision of IESs (internal eliminated segments) that interupt protein-coding DNA segments (macronuclear destined segments, MDSs) from micronuclear DNA, as well as removal of transposon-like elements and overall fragmentation, leading to a total genome reduction of 98% in *Stylonychia lemnae*. Approximately 30% of macronuclear precursor sequences are estimated to be scrambled in the germline micronuclear genome, with MDS order permuted and MDSs in either orientation on the micronuclear chromosomes. Thus, massive rearrangements are required to construct functional macronuclear genomes.

The molecular mechanisms of unscrambling to create a functional gene are not yet understood. Recent studies in ciliates indicate that an RNA-based mechanism could direct genomewide DNA rearrangements and serves to disable invading genetic agents (Yao, 2003). A model introduced for *Tetrahymena* proposes that small RNAs might function to specify sequences to be eliminated by a mechanism similar to RNA-mediated gene silencing (Mochizuki, 2002).

Though there is some evidence that RNAi is involved in IES excision, it can not explain the process of gene unscrambling. Instead, template-guided recombination has been proposed recently for unscrambling and IES excision (Prescott, 2003).

In this study we analyze the reorganization of the micronuclear sequence of the actin I gene in *Stylonychia lemnae*. We chose this locus as our model system, because it is the simplest known scrambled gene containing conventionally spliced IESs as well as scrambled MDSs in both orientation. Thus it requires all events of DNA elimination, inversion, and permutation to take place.

Five Nucleotide Carriers in the Symbiont UWE25 are used to exploit Host Metabolites

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Obligate intracellular bacteria live within a highly specialized niche, the eukaryotic cell. Different deep-branching members of the *Rickettsiales* and *Chlamydiales* reside in free living amoebae and paramecia. Interaction between bacterium and host cell can be essential for the reproduction and the survival of the host or strengthen its pathogenesis. On the other hand uptake of primary metabolites provided by the host cell is crucial for many obligate intracellular living organisms.
Recently, the complete genome sequence of the environmental Chlamydia strain UWE25 a symbiont of the protozoan *Acanthamoeba* was identified [1]. Although some reduction of genome size typical for obligate parasites was observed, five (!) genes with sequence similarity to nucleotide transporters (NTT) from plant plastids and other bacteria are present. Most characterized NTTs mediate ATP-transport in counter exchange with ADP (plastids and bacteria). In addition to the ATP/ADP-carrier, a second nucleotide transporter which drives a proton dependent net nucleotide uptake exists in *Chlamydia trachomatis*.
It is tempting to speculate that the five genes of UWE 25 encode nucleotide transporters necessary for the survival of UWE25 by linking symbiont and host metabolism.
We were able to perform a complete biochemical characterization of all five nucleotide transporters of UWE25 after heterologous expression in *E.coli*. NTT1$_{UWE25}$ is utilized for energy parasitism by enabling uptake of host cell ATP in exchange with ADP [2]. The substrate specifity (GTP, CTP, ATP, UTP) of NTT2$_{UWE25}$ closely resembles the characteristics of the second nucleotide carrier of *Chlamydia trachomatis* but in contrast the transport is not proton driven. An unusual substrate spectrum was analyzed in the case of NTT3$_{UWE25}$. It mediates a highly specific uridine-nucleotide transport. Surprisingly, NTT4$_{UWE25}$ is able to transport NAD at high rates. Under physiological conditions it reveals the uptake of amoebal NAD in counter exchange with bacterial ADP [3]. Our results prove that NTT4$_{UWE25}$ is the first known carrier highly specific for NAD. NTT5$_{UWE25}$ catalyzes a novel and unexpected proton dependent net uptake of GTP, GDP and ATP, but barely accepts CTP, UTP and ADP as substrates. With the help of these five nucleotide transporters UWE25 is able to import essential substrates to fuel its own metabolism.

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The unusual resting cyst of *Meseres corlissi* (Ciliophora, Oligotrichea)

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Meseres corlissi Petz and Foissner, 1992 is a planktonic, spirotrich ciliate closely related to the common *Halteria grandinella*. Pure cultures were established with *Cryptomonas* sp. as a main food source. Thus, resting cyst formation and structure could be studied by light and electron microscopy and cytochemical methods. The resting cyst of *M. corlissi* belongs to the kinetosome–resorbing (KR) type and has a conspicuous coat of extracellular organic scales, here termed lepidosomes, embedded in mucus mainly composed of acid mucopolysaccharides, as shown by the strong reaction with alcian blue. The lepidosomes, which likely consist of glycoproteins, are finely faceted, hollow spheres with a diameter of 2–14µm. They are formed underneath the cortex and liberated almost concomitantly to the external surface of the cell before the cyst wall is produced. Resting cyst size is dependent on temperature, the average diameter is 46 µm (without lepidosome coat) at 20° C. The cyst wall, which contains considerable amounts of glycoproteins and a layer of chitin, is smooth, 1.5–2 µm thick, and composed, in the light microscope, of two distinct layers highly resistant to various inorganic and organic solvents. In the transmission electron microscope, the cyst wall consists of five distinct layers (from inside to outside): metacyst, endocyst, mesocyst, ectocyst, and the pericyst, a new term for all materials produced by the encysting cell and deposited upon the ectocyst. Structurally, the five layers of the *Meseres* cyst are similar to those of the stichotrichine (e.g., *Oxytricha*) and phacodinine (*Phacodinium*) spirotrichs, except of the thin ectocyst which is not lamellar but coarsely granular. The lepidosomes are composed of fibres about 20 nm across. The data are compared with respect to the classification of the halteriids. While the general wall structure indicates a stichotrichine relationship, the ectocyst, the lepidosomes, and the chitin layer in the cyst wall suggest the halteriids as a distinct group more closely related to the oligotrichine than stichotrichine spirotrichs.

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Mycosporine-like amino acids (MAAs) in *Chlorella*-bearing ciliates from lake plankton.

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To know whether there is another benefit from the mutualistic relationship between *Chlorella* and its specific ciliate host as already known from literature, we assessed the existence of UV-absorbing compounds (mycosporine-like amino acids, MAAs) in different mixotrophic ciliate species, i.e., *Askenasia chlorelligera, Pelagodileptus trachelioides*, *Stokesia vernalis, Uroleptus* sp., and *Vorticella chlorellata*. MAAs are intracellular water-soluble compounds with high molar extinction coefficients having absorption maxima between 309 and 360 nm, which presumably originate in the shikimate pathway known to be present in microalgae but not in ciliates. In summer 2004, we collected living ciliates in two lakes, Piburger See and Gossenköllesee which differ in altitude, incident UV-radiation, underwater UV attenuation and other environmental parameters, e.g., water temperature and maximum depth. Single ciliates were picked out of the samples, cleaned individually, and starved to ensure the absence of remaining algae in food vacuoles. MAAs were identified by HPLC analysis in extracts of 25% (f.c.) aqueous methanol. In the mixotrophic ciliates analysed, we found (i) at least seven different MAAs, e.g., mycosporine-glycine (λ_{max} = 310 nm), and, (ii) one to several MAAs in one ciliate species.

Symbiotic system "Ciliate cell – *Chlorealla-like* algae – Bacteria". Examples for some of green ciliates.

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Not uncommonly the ciliate cell contains several endocytobionts at the same time, in different compartments or, less frequently, in the same one. Different bacteria may be antagonistic, especially when occupying the same compartment. It is known that in the green *Paramecium bursaria* such antagonictic relationships are existed between endocytobiotic *Chlorella*-like algae and any other bacterial infection in the host cytoplasm. Only in white *P. bursaria* the cytoplasm can be frequently (but not always!) populated with different bacteria or other microorganisms. Apparently it is not the cases for other green ciliates: *Climacostomum virens, Stentor amethystinus and Euplotes daidaleos.* Our investigation reveals for these three species presence of trilateral symbiotic system – host cell, *Chlorella*-like algae and bacteria. Numerous bacteria in the cytoplasm of *C. virens,* according to in situ hybridization, belong to *Alphaproteobacteria*. The same was reviled for abundant bacterial population in *S. amethystinus*. The cytoplasm of *E. daidaleos* always populated with *Betaproteobacteria.* In all of the systems relationships between algae and endocytobiotic bacteria are not antagonistic. Moreover, the loss of *Chlorella-like algae* from *E. daidaleos* produced strong unstability for the own bacterial endocytobionts population. Possible diversity of green and bacterial endocytobionts of the systems are discussed in connection with the level of host-endocytobionts adaptations.

UVR-induced photooxidative stress in the mixotrophic ciliate *Paramecium bursaria*

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To investigate whether endosymbiosis affects the degree of oxidative stress, the concentration of different reactive oxygen species (ROS) was assessed under endosymbiotic and aposymbiotic conditions of *Paramecium bursaria.* Ciliate cultures with and without *Chlorella*-symbionts grown either in the log- or in the stationary phase were cultivated in a 14:10 h light:dark cycle (0.16 mE m^{-2} s^{-1} PAR (photosynthetic active radiation) and 0.27 W m^{-2} UVA) at 25°C. The ciliates were screened for ROS (H_2O_2 and $O_2^{\bullet-}$) under normal conditions (basal ROS-content in culture) and after short-term exposure (2h) to UVA and UVB (8.60 and 2.47 W m^{-2}, respectively) by flow cytometry. Further the antioxidative enzymes catalase (CAT), superoxide-dismutase (SOD), and glutathione reductase (GR), which may protect against UVR-induced oxidative damage were investigated spectrophotometrically.
Under basal conditions, ROS production was very similar between endosymbiotic and aposymbiotic ciliates. After UVR-exposure a significant increase in ROS production was found in the stationary phase of aposymbiotic *P. bursaria*. The normal CAT-content was higher in the log-phase than in the stationary phase of symbiotic and aposymbiotic ciliates. No significant difference was found in SOD-content between both stocks under normal conditions. CAT activity displayed a significant decrease under symbiotic and aposymbiotic conditions after short-term UVR-exposure. SOD activity decreased significantly after short-term exposure in the log-phase of both conditions and showed a significant increase in the stationary aposymbiotic *P. bursaria*. No GR activity could be detected. The results indicate that there are significant differences in photooxidative stress under endosymbiotic and aposymbiotic counterparts. The aposymbiotic *P. bursaria* seems to be more stressed by UVR, however, it also shows higher contents of the antioxidant enzyme superoxid-dismutase.

KEY WORDS: Antioxidants; *Chlorella* sp.; Endosymbiosis; Flow cytometry; Oxidative stress

New insights in the molecular relationship between *Paramecium* and its endocytobiont *Caedibacter*

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Endocytobiotic bacteria of the genus *Caedibacter* confer a killer trait upon their host *Paramecium*. Paramecia infected by *Caedibacter* are capable of killing non-infected, so called sensitive, Paramecia, which results in a selective advantage for infected Paramecia. All bacteria that belong to the genus *Caedibacter*, express R-bodies - large protein ribbons coiled into cylindrical structures within the bacteria. These R-bodies are, together with a not yet identified toxin, responsible for the killer trait. The genes of the R-body proteins are located on extrachromosomal elements. In case of *Caedibacter taeniospiralis*, this extrachromosomal element is a plasmid (pKAP), other species of *Caedibacter* carry bacteriophages instead. The toxin-gene is also suspected on these extrachromosomal elements. In order to gain new insights in the molecular relationship between *Paramecium* and its endocytobiont *Caedibacter*, the plasmid pKAP298 from *Caedibacter taeniospiralis* strain 298 was completely sequenced and annotated. The size of the plasmid is about 49 kBp, 63 putative coding ORFs could be identified, as well as 4 transposons and a group II-intron. 23 of the 63 identified ORFs possess on protein-level similarities to already known proteins. There is strong evidence, that the toxin-gene mentioned above is ORF43. A transcriptional analysis of the plasmid pKAP298 was performed and active genes on pKAP298 were identified. Future projects will focus on up- and down-regulated genes in infected Paramecia and on the identification of their function via Gene-Silencing.

Rumen Ciliate Movement and Morphology – Movies and Images of Species from the Rumen Ciliate Culture Collection ERCULE

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Within the rumen fluid ciliates of the orders Trichostomatida and Entodiniomorphida are very abundant, forming dense communities of many beautiful species. Here, movies and images of some species available from the Rumen Ciliate Culture Collection ERCULE are presented and commented upon. It gives an impression about the moving behaviour of the trichostomatids *Dasytricha ruminantium* and *Isotricha prostoma* and of the entodiniomorphids *Epidinium ecaudatum, Entodinium caudatum, Metadinium medium, Eudiplodinium maggii, Polyplastron multivesiculatum*. Three-dimensional images of *Ophryoscolex caudatus, Epidinium caudatum* and several other rumen ciliates were made. Therefore, hybridized (fluorescence in situ hybridisation, FISH) cells with rRNA targeted oligonucleotide probes were analysed and digitally recorded by confocal laser scanning microscopy. The images point up the complexity of the cell shapes of rumen ciliates. These documents show the problems of morphospecies determination quite plainly and may contribute to the discussion of the morphospecies concept.

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Cell Invasion and Intracellular Fate of Microsporidia

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Microsporidia have a unique mechanism to infect host cells. Their spores contain a long coiled polar tube that can be extruded from the spores and that can penetrate the membranes of new host cells. The infective sporoplasm is then injected through the polar tube inside the cell. This method of invading new host cells used by microsporidia is one of the most sophisticated infection mechanisms in biology and ensures that the microsporidian enters the host cell unrecognized and protected from the host defence reactions. Recent studies have shown that, in addition to this mechanism, microsporidia gain access to host cells by phagocytosis. However, after phagocytosis, the unique infection mechanism of the microsporidia is used to escape from the maturing phagosomes and to infect the cytoplasm of the cells. We have visualized this process by using a differential immunofluorescence staining technique in combination with fluorescence microscopy and FACS analysis. In addition the characteristics of the phagosomes harbouring the phagocytosed microsporidien spores were determined by staining with several lysosomal markers. Gaining access to host cells by endocytosis, and escaping destruction by egressing quickly from the phagocytic vacuole to multiply outside the lysosome is a common phenomenon in biology. How this is done by microsporidia is a unique principle by which an obligate intracellular organism has solved this problem utilising its polar tube. The unique extrusion apparatus of the microsporidia has successfully put this phylum on the world's parasitological map, resulting in a group of obligate intracellular organisms, capable of infecting almost any type of host and cell.

Molecular diversity within *Acanthamoeba* morphological group I

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Acanthamoebae are ubiquitously occurring potentially pathogenic protozoa, however, not all representatives of the genus can act as pathogens. In 1977 Pussard and Pons divided the genus into three gross groups on the basis of cyst-morphological features. While most strains isolated from human infections belong to morphological group III and, particularly, group II, group I strains seem to lack virulence. *Acanthamoeba* morphological group I consists of three species which all show cysts of at least 18 µm in diameter. The cysts have a rounded outer wall clearly separated from the inner wall to which it is joined by radiations forming a star shaped structure. However, it has been shown, that the shape of cyst walls can be altered by varying growth conditions and very often polymorphisms even within one clone can be observed. As the validity of the described *Acanthamoeba* species generally has been challenged, a new system dividing the genus into 15 18S rDNA sequence types has been established during the past years. Thereby it has been shown that group II and group III are not only morphologically more alike, but are also genetically closer related than either of them is to group I.

The aim of the current study was to reveal the diversity within *Acanthamoeba* morphological group I and to prove whether it could be justified to split up the genus. The 18S rDNA gene of several *Acanthamoeba* sp. group I strains belonging to different species was sequenced and a cluster analysis was performed including various other acanthamoebae of which sequence data are available. Moreover, the morphological, physiological and immunobiological features of these strains were assessed and compared. It was shown that antigenic patterns of *Acanthamoeba* spp. correlate to the three morphological groups and that also in physiological and immunobiological properties group I seems to be very distinct from the other two groups. Moreover, it was shown that group I does not only show more than two times the 18S rDNA sequence dissimilarity to group II and group III that can be observed between these two groups, but that also within group I sequence dissimilarity is high.

Altogether, this study indicates, that the genus *Acanthamoeba* might need to be reviewed and once again points up the difficulties in the classification of organisms for which the biological species concept is not applicable.

The impact of protists on activity and structure of bacterial community in a rice field soil

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The influence of protists on community structure and function of bacteria in water-saturated soil was studied using microcosms. Sterilized rice field soils were re-inoculated with size-fractionated natural assemblages of protists and bacteria or bacteria alone. Inoculation with protists stimulated microbial activitiesas was demonstrated by depth profiles of O_2 and by CO_2 emission. Increased nitrogen mineralization by inoculation of protists was observed both in oxic surface layer (0-3 mm in depth) and anoxic sub-layer (10-13 mm in depth). The development of the protistan community in the surface layer was revealed by PCR-denaturating gradient gel electrophoresis (DGGE). Sequences retrieved from the DGGE bands showed close relationships with flagellates, ciliates, amoeba, and fungi. Cercozoa were the dominant closest relatives. The amount of extracted DNA in the surface layer without protistan inocula was higher than with them and linearly increased with time, suggesting that protists may control microbial biomass by grazing. Inoculation with protists altered the surface bacterial community revealed by PCR-terminal restriction fragment length polymorphisms (T-RFLP). Gram-positive bacteria such as Bacilli and Clostridia were dominated if protists were present, while betaproteobacteria dominated without them. Our results demonstrate the significant impact of protists on the bacterial community and activity in a water-saturated soil.

Seasonal changes of a benthic microbial community in an intertidal fine sediment

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Protists play an important role in the nutrient cycle of pelagic microbial food web as a link between microbial and "classic" food web. Detailed information about the function of protists in benthic microbial food web is rare, while it is assumed that in benthic habitats protists play an important role. This study aims at investigating the seasonal composition of the microbial community and the forcing factors in an intertidal mudflat.
Samples of the upper 3 mm of the sediment from an intertidal mudflat were collected over a period of one year and bacteria as well as protists were enumerated by epifluorescence microscopy. The results of this study indicates that the benthic microbial systems of fine grained sediments follow a seasonal cycle with a dominace of bottom up control in winter and spring followed by increasing influence of grazing from spring to summer. Especially diatoms seem to be heavily grazed by large protists (HNF >10 µm) and an increasing number of ciliates and meiofauna during summer. Simultaneously, an enhanced activity of deposit feeders and thus an intensified positive feed-back to primary production can be assumed, whereas cyanobacteria and bacteria appear to be controlled by temperature and organic carbon input. Significant correlations between diatoms, bacteria and heterotrophic nanoflagellates led to the assumption that small HNF (1-5 µm) feed mainly on macromolecules, HNF 5-10 µm on bacteria and smaller nanoflagellates (HNF and PNF 1-5 µm).
Nevertheless, we suggest that for benthic intertidal microbial food webs the model of Fretwell (1977) holds true. It predicts that top-down forces are dominating trophic dynamics, but the structure of the food web is determined by the fundamental bottom-up attributes such as primary production.

Prädation von Ciliaten auf Biofilme: Einfluss auf die dreidimensionale Struktur

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Ciliaten können in Biofilmen in hohen Abundanzen, vor allem aber mit einer hohen Biomasse auftreten. Da sie zudem hohe Freßraten auf Bakterien ausüben können, ist anzunehmen, dass sie durch ihre Prädation besonders auf Bakterien in Biofilmen einen signifikanten Einfluss ausüben. In Laborversuchen wurde in einem Zwei-Art-System die Prädation des Ciliaten *Tetrahymena pyriformis* auf *Acinetobacter spec*. untersucht. Dabei wurden die Biofilme in Fließkammern mit permanentem Durchfluss an frischem Medium angezüchtet und das Wachstum mit und ohne Einfluss der Ciliaten verfolgt. Durch die Markierung mit *gfp* (grünfluoereszierendem Protein) konnte das Wachstum der Bakterien und damit die Entwicklung der 3D-Struktur der Biofilme während des gesamten Versuchszeitraumes *in situ* am Laser-Scanning-Mikroskop beobachtet werden. Die Ergebnisse dieser Untersuchungen werden im Vortrag dargestellt und mit Resultaten der Anfärbung der dreidimensionalen Struktur von Biofilmen im Freiland verglichen und diskutiert.

Effects of flow velocity on the attachment of heterotrophic flagellates

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Many heterotrophic flagellates live on surfaces in rivers, streams and lakes. Some of them adhere permanently and others crawl over the surface with more temporary attachments. The flow velocity presents for the organisms with both problems and benefits. The movement of the surrounding water enhances the mass transfer, but it may increase the difficulty of adherence on the surfaces.
At different flow velocities, the attachment to surfaces was investigated for five species of heterotrophic flagellates differing in their morphology and type of attachment: Two species are gliding closely to the surface. One species is free-swimming or attached by a protoplasmic thread, and two species form colonies clustered at the end of a stalk. The attachment was investigated by an *in-situ*-monitoring on an inverse microscope.
The species attached by a stalk have a high resistance against high flow velocity (up to 1.2 m s^{-1}) followed by the gliding forms. Flagellates with a protoplasmic thread are tearing off at a flow velocity higher than 0.6 m s^{-1}. In addition the difference rate of surface structures on the attachment characteristics was investigated. The laboratory investigations were compared with observations on flagellate distribution in the field.

Reduction of primary oral ciliature in Trimyema, a member of the "riboclass" Plagiopylea

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A "riboclass" (Lynn (2003) Europ. J. Protistol. 39, 356-364) is a vernacular taxon comprising organisms with dissimilar morphology but with ribosomal RNA similarity. Among ciliates, the best-known example for a conflict between morphology and molecular systematics is Halteria grandinella, a look-like oligotrich but a molecular stichotrich, found in close neighbourhood to Oxytricha granulifera. Likewise disturbing at the first glance is the assignment of the Trimyema to the Plagiopylea, which is definitively different in morphology from Plagiopyla, but constantly goes with it in RNA trees, with very high bootstrap values. Since only one kind of tree, either the morphological one or the molecular one can be correct, a scenario has to be developed which removes the supposed inconsistency. For Trimyema is it the gradual reduction of the primary oral ciliature (POC), culminating in the complete loss of the POC in
typical plagiopylids. The gradual loss of the POC is illustrated in the Prostomatea (Coleps et al.), which is the sistergroup to the Plagiopylea, both together forming the sistergroup to the Oligohymenophorea. Reduction and loss of the POC, which is often preceded by an apicalization of the POC, seems to have occurred repeatedly and independently in ciliates. Examples for this convergent evolutionary trend seem to be the karyorelictine ciliate Kentrophoros, the Litostomatea and Prostomatea, the astomes and the apostomes among the Oligohymenophorea and perhaps all Phyllopharyngea, which, to my understanding, have developed a secondary oral ciliature after complete loss of the primary one; they are the deuterostomes among the ciliates.

The role of soil in the ecology of the ciliate *Meseres corlissi*

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The type population of the oligotrich ciliate *Meseres corlissi* is adapted to life in a small astatic meadow pond, with alternating phases of population growth of active ciliates and dormancy of resting cysts. In mud samples from the natural site, which were dried and stored at room temperature, those cysts survived for >2 years (Müller, unpubl.). In the present study, the ecology of this population was investigated using two clonal strains, which were isolated from soil samples collected at the type location in November 2002 (strain E4) and in December 2003 (strain M10) and grown in the laboratory on a diet of cryptomonads with the addition of Eau de Volvic. We observed that growth, encystment/excystment and long-term cyst survival depended strongly on the presence/absence of soil or soil-extract in the culture medium. Relevant results were: 1) Growth was enhanced greatly by addition of soil extract (SE) or sterilized garden soil (SGS) to the culture medium. 2) Encystment could be induced by dilution of SE in the culture medium. In contrast, dilution of ciliates or food algae, at a constant SE level, had no significant effect, and there was no indication for a relationship between cyst formation and temperature. 3) Excystment could be triggered by adding SE or SGS to the culture medium. 4) Cysts of our laboratory strains, stored in dry SGS, remained viable for > 4 months. The nature of the 'soil factor' responsible for these effects remains at present unknown. The results reported in this study contrast those of an earlier investigation with another isolate of the same species, originating from a very different habitat. Weisse (2004) studied a *Meseres corlissi* strain isolated from a water sample collected from the reservoir of a tree bromelia in a fog rain forest in the Dominican republic. Different from the Salzburg clones, the strain from the Dominican republic could grow at high rates in SE-free medium, and the main factor controlling encystment/excystment was temperature. The findings from these two studies, therefore, point to the importance of local adaptation of geographically separated populations of this cosmopolitan, but rare ciliate species. Two additional studies on the ecology of *M. corlissi* (Gächter & Weisse 2005, Weisse et al. 2005, this meeting) support this interpretation.

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Variable response to temperature among five clones of *Meseres corlissi*

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The temperature response of the freshwater ciliate *Meseres corlissi* (Ciliophora: Oligotrichea) was investigated with four clonal cultures isolated from the type location, an astatic meadow pond in Salzburg (Petz & Foissner 1992), and compared to an earlier study with another isolate from the Dominican Republic (Weisse 2004). The Salzburg clones were isolated in November 2002 (D2 and E4) and in December 2003 (M3 and M10). The American *Meseres corlissi* (DR) originated from a different habitat, the water reservoir of a tree bromelia. The species identity of all isolates was verified by morphological (Foissner 2005, Foissner et al. 2005) and genetic investigations (Strüder-Kypke et al., in prep.). All strains were kept in modified Woods Hole Medium with the addition of Eau de Volvic and *Crytomonas* sp. as food. The experiments were performed at temperatures ranging from 7,5 to 30,5°C under 14:10 light:dark rhythm and lasted for 36-72 h. The overall temperature reponse was significantly different between the DR isolate and the Salzburg clones. Population growth rates of the latter were positive at 10°C, at which temperature the DR strain did not grow, but lower than that of the DR isolate at higher temperatures (25-30°C). Among the Salzburg clones significantly different growth rates were observed between the clones that were isolated at different times. The response of cell volume and cellular production to temperature was also significantly different between the DR and the Salzburg strains. In contrast to the former, volume of the four Salzburg clones decreased linearly with increasing temperature up to 25°C. We conclude that the observed differences in growth rates, cell volume and production rates between the DR and the four Salzburg clones reflect their adaptation to the temperature range in their respective natural habitat.

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Monograph of the Hypotrichs (Ciliophora). Part 3. - A biodiversity study

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For non-taxonomists, revisions and monographs are often the main source of information about a group of organisms. Monographs are also appreciated by specialists because time-consuming searches about, for example, nomenclature, taxonomy, or ecology can be avoided. Further, the diversity of a higher taxon is thoroughly documented. Part 3 of the monograph of hypotrichs contains all genera which do not belong to the oxytrichids (Berger 1999), urostylids (Berger 2005), or the euplotine spirotrichs. According to Berger (2001; updated version, see http://protozoology.com) about 77 genera comprising circa 240 species are concerned. They belong to various higher taxa, for example, the Amphisiellidae, the Kahliellidae, the Strongylidae. On the assumption that 30–50% of the species are synonyms, species indeterminata, or species belonging to other higher taxa, about 120–170 valid species are known at present. The last detailed revision of hypotrichous ciliates (stichotrichs according to a new terminology) was published by Kahl (1932). A short review was provided by Borror (1972). The present project is planned over a three year period and comprises, inter alia, a critical inventory and monographic treatment of the available data since 1758 (the data about the genera treated are distributed in more than 1500 papers), morphological investigation of some species using live observation and silver staining, and investigation of cell division of some key species because morphogenetic data are often very useful for the analysis of phylogenetic relationships of hypotrichs (Berger & Foissner 1997). The financial support of the project by **APART** (Austrian Programme for Advanced Research and Technology; Austrian Academy of Science; Project 10940) is greatly acknowledged.

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Description of a new vahlkampfiid amoeba, based on morphological, ultrastructural and molecular characteristics

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A new species of vahlkampfiid amoeba, *Vahlkampfia signyensis* n. sp., was isolated from two soil sites at Signy Island, South Orkney Islands, Maritime Antarctic. Trophozoites of the species had a typical vahlkampfiid morphology, showed eruptive movement and did not form flagellates. However, *V. signyensis* differs from other described species of the genus in a range of morphological and ultrastructural characters, as well as its 5.8S rDNA sequence. According to its 5.8S rDNA sequence, the new species is most closely related to *V. avara*. An isolate of the new species had a temperature growth optimum of only 10 ºC, and did not grow at either 30 ºC or 37 ºC. The low optimal growth temperature is adaptively significant for life in the Maritime Antarctic, and hence an example of a non-morphological but ecologically relevant protist species character.

Detection of ingested nitrifying bacteria in food vacuoles of ciliates using FISH (fluorescence in situ hybridization)

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The role of bacteria in important biogeochemical cycles such as the nitrogen cycle has been well studied in the past, but little attention has been paid to the factors controlling their abundances and community structure. Ciliate grazing is likely to have a major impact on the bacterial community and thus might also have an influence on the nitrogen cycle. As a case study we examined ciliate grazing on the nitrite-oxidizing bacteria of the genus *Nitrospira*.
FISH allows detection of bacteria inside the food vacuoles of ciliates. Thus, it should be possible to identify nitrifying bacteria within the food vacuoles of ciliates. The relative selection for or against *Nitrospira* and the qualitative and quantitative detection of the ingested nitrifying bacteria are the aims of this study. All experiments in this study were designed as *in vitro* experiments with cultures of different bacteria and ciliate species, in particular the bacteria *Nitrospira moscoviensis*, *Pseudomonas fluorescens*, *Polynucleobacter* spec. and the ciliates *Paramecium aurelia* and *Tetrahymena pyriformis*.
FISH uses fluorescetly (CY3) labelled 16S rRNA-targeted oligonucleotide probes to detect the ingested bacteria. Starved ciliates were fed a range of different food concentration, also using different incubation periods. The experiment was stopped by fixation with paraformaldehyde. For the enumeration of ciliates and bacteria, samples were concentrated on filters with the pore size of 1.2µm and 0.2 µm, respectively. By combined staining with FISH and DAPI the free and ingested *Nitrospira* are marked and can be detected by epifluorescence microscopy. They can be distinguished from other bacteria used as ciliate food in the cultures. The first results suggest a selection against *Nitrospira moscoviensis.*

Updated Key to the Genera of the Order Oligotrichida (Ciliophora, Spirotricha)

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Oligotrichid ciliates (Ciliophora, Oligotrichida) are an important component in the marine and limnetic microzooplankton. The somatic ciliature of the oligotrichids is highly reduced, typically consisting of only two ciliary rows. Nevertheless, the diversity of patterns created by these two rows is considerable. Recently, the investigation of their evolution led to the establishment of three new families and four new genera and a revised classification. The order Oligotrichida now comprises the families Cyrtostrombidiidae Agatha, 2004; Pelagostrombidiidae Agatha, 2004; Strombidiidae Fauré-Fremiet, 1970; and Tontoniidae Agatha, 2004 with the sufficiently known genera *Cyrtostrombidium* Lynn & Gilron, 1993; *Limnostrombidium* Krainer, 1995; *Pelagostrombidium* Krainer, 1991; *Laboea* Lohmann, 1908; *Omegastrombidium* Agatha, 2004; *Strombidium* Claparède & Lachmann, 1859; *Spirostrombidium* Jankowski, 1978; *Parallelostrombidium* Agatha, 2004; *Tontonia* Fauré-Fremiet, 1914; *Paratontonia* Jankowski, 1978; *Pseudotontonia* Agatha, 2004; and *Spirotontonia* Agatha, 2004. These families and genera are keyed dichotomously, using simple characters usually recognizable in live and preserved specimens, such as a contractile tail, a neoformation organelle (permanent tube in which stomatogenesis takes place), and cyrtos-like pharyngeal fibres combined with the lack of ventral membranelles. The upper limit of the extrusome girdle and the distended cell surface usually indicate the position and curvature of the girdle kinety, a further important taxonomic feature. This key is designed for users not specifically trained in the identification of ciliates.
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Diversity and evolution of gut ciliates from mammals

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The foreguts and hindguts of many herbivorous mammals host a community of very diverse microorganisms. Ciliates form the major part of the eukaryotic population. Phylogenetic analysis of the ciliate 18S rDNA sequences from ruminants and Australian marsupials suggest that the ciliates from these animals are monophyletic. However, the evolutionary history of gut ciliates from various mammals is still a matter of discussion. Although detailed knowledge about the diversity of the ciliates is the key to address this question, the conventional approach based on morphology has limitations when one tries to assess the ciliate diversity. The limited number of morphological characters, a general problem in the current protist taxonomy, and in particular, difficulties to isolate the ciliates from gut samples for a detailed characterization are the major problems for the classical analysis.

In order to investigate the ciliate population of the hindgut of mammals using a molecular approach, we extracted DNA from rumen fluid from cattle, sheep, a goat, and the European red deer *Cervus elaphus*, and from feces of hindgut fermenters (horse, elephant, zebra). 18S rDNA clone libraries from these DNAs were constructed, and randomly chosen clones were partially sequenced. Phylogenetic analysis shows that all the gut ciliates of mammals (plus marsupials) fall within a monophyletic lineage. Clones obtained from these hindgut fermenters cluster separately from the clones found from the ruminants. In contrast to the rumen ciliates from the domestic animals, where many ciliates are shared by different hosts, our study suggest that at least a significant fraction of the ciliate populations of the various hindgut fermenters might be host specific.

Eukaryotic diversity in Dutch soil

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During the last decade, remarkable advances have been made studying prokaryotic diversity using culture-independent, molecular approaches. Also, the biodiversity of microbial eukaryotes has been studied in aquatic ecosystems on the basis of nuclear small subunit ribosomal RNA (SSU rRNA) genes, revealing an unexpected eukaryotic diversity. Surprisingly, molecular approaches to study the phylogenetic diversity of microbial eukaryotes in terrestrial environments are rare. Here we describe an analysis of the diversity of microbial eukaryotes in historical soil samples, derived from an archive of air-dried soil samples documenting the land reclamation by drainage of a former sea bottom (Wieringermeer polder, The Netherlands) over the years 1942-1975. These samples had been analysed previously with respect to the diversity of prokaryotes, in particular of the *Bacillus*-group, which exhibits significant changes in the composition of this group during the drainage process (Tzeneva et al. 2004). Denaturing gradient gel electrophoresis (DGGE) of amplified SSU rDNA revealed a high diversity of eukaryotes, which obviously underwent substantial changes over the years. To explore the diversity of microbial eukaryotes in more detail, a nuclear SSU rRNA gene library was generated from a DNA sample of the year 1975. Our study revealed diverse, previously undescribed eukaryotic sequences that could be assigned to fungi and a variety of aerobic and anaerobic protist groups: cercozoans, ciliates, xanthophytes (stramenopiles), heteroloboseans, and amoebozoans, pointing out that the molecular diversity of many protist groups can be assessed even in air-dried, historical soil samples.

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The Testate Lobose Amoebae (Order Arcellinida Kent, 1880) Finally Find their Home within Amoebozoa

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Testate lobose amoebae (order Arcellinida Kent, 1880) are common in all aquatic and terrestrial habitats, yet they are one of the last higher taxa of unicellular eukaryotes that has not found its place in the tree of life. The morphological approach did not allow to ascertain the evolutionary origin of the group or to prove its monophyly. To solve these challenging problems we analysed partial small-subunit ribosomal RNA (SSU rRNA) genes of seven testate lobose amoebae from two out of the three suborders and seven out of the thirteen families belonging to the Arcellinida. Our data support the monophyly of the order and clearly establish its position among Amoebozoa, as a sister-group to the clade comprising families Amoebidae and Hartmannellidae. Complete SSU rRNA gene sequences from two species and a partial actin sequence from one species confirm this position. Our phylogenetic analyses including representatives of all sequenced lineages of lobose amoebae suggest that a rigid test appeared only once during the evolution of the Amoebozoa, and allow reinterpretation of some morphological characters used in the systematics of Arcellinida.

Molecular Diversity and Phylogeny of Rumen Ciliates

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Rumen ciliates were discovered by Gruby and Delafont (1843), and in 1927 a detailed taxonomy written by Dogiel became available. Since that time a wealth of morphological studies has led to the identification of more than 250 species of ciliates in the various ruminants, but it became evident that morphological methods alone were not sufficient to access the true diversity of rumen ciliates. Only recently, molecular genetic techniques allowed a more detailed survey of the diversity and phylogeny of the rumen protozoa[1,2,3]. However, the analysis of the extremely complex communities of rumen ciliates has remained fragmentary until now.
Here we describe a molecular analysis to unravel the diversity and phylogeny of the rumen protozoa from Cervids. We extracted DNA from the total rumen contents of ten samples, which were derived from four different hosts (moose, roe deer, fallow deer and red deer). 18S rRNA genes were amplified by PCR, and cloned. Libraries were created, and about 500 clones (approx. 50 from each library) were sequenced partially. These sequences were subjected to a phylogenetic analysis - including sequences from the EMBL database and from the type strains isolated in the course of the ERCULE project (QLRI-CT-2000-01455). Our data revealed a gigantic diversity of rumen ciliates in European Cervids. In particular, we found evidence that the diversity of rumen protozoa in the roe deer had been underestimated substantially.

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The mitochndrial genome of *Euplotes*

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Until now, only 3 mitochondrial genomes of ciliates, i.e. from *Tetrahymena thermophila, Tetrahymena pyriformis,* and *Paramecium aurelia* have been sequenced and analyzed in more detail (1-3). These mitochondrial genomes are rather large if compared with animal mitochondria, and – in contrast to most other mitochondria – linear and not circular. Here we describe the molecular cloning of parts of the mitochondrial genome of *Euplotes crassus* and the first results of our sequencing effords. The mitochondrial genome of *Euplotes crassus* is of particular interest because it is (i) only distantly related to the mitochondrial genomes mentioned above, and (ii), potentially, closely related to the hydrogenosomal genome of the anaerobic, hypotrichous ciliate *Nyctotherus ovalis,* which possesses a macronuclear genome structure similar to *E. crassus.* So far we have identified the genes encoding the mitochondrial ribosomal RNAs, several components of the mitochondrial electron transport chain, and a number of mitochondrial ribosomal proteins. Phylogenetic analysis confirms that the mitochondrial genes of *E. crassus* are much more related to their homologues from *N. ovalis* than to the above-mentioned organelle genomes of other ciliates.

Hydrogenosomes are not the same!

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Hydrogenosomes are organelles, approximately 1-2 micrometer in size, that compartmentalize the terminal reactions of the anaerobic cellular energy metabolism. They were first described in the parabasilid flagellate, *Tritrichomonas foetus*, in a seminal publication by Lindmark and Müller (1973) as subcellular compartments that produce hydrogen and ATP. Since this time hydrogenosomes or variations of them have been described in quite a number of rather different unicellular eukaryotes adapted to microaerobic or anoxic environments (Roger 1999; Yarlett 2004). Several researchers considered these organelles as variations of mitochondria adapted to anaerobic environments (Embley et al. 2003), but it is still a matter of debate whether or not these ancestral "mitochondria" had an aerobic metabolism using oxygen as a terminal electron acceptor - or whether these organelles functioned anaerobically, and, consequently, produced hydrogen like present-day hydrogenosomes (Tjaden et al. 2004). Here we will review the various types of hydrogenosomes and related organelles. We will show that these anaerobic organelles evolved by evolutionary tinkering – repeatedly and independently from the same ancestral organelle.

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Rumen ciliates possess macronuclear “midi-chromosomes”

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The rumen of cow, sheep and goat is populated by a complex community of anaerobic microorganisms. Rumen ciliates account for a substantial fraction of the biodiversity and up to 50% of the biomass. Interestingly, these ciliates are monophyletic, and so far nothing was known about the genetic organisation of their genomes. Here we show that rumen ciliates host “midi-chromosomes” in their macronuclei. Their size, between 40 and 120 kb, is substantially larger than the size of the well-known mini-chromosomes of certain hypotrichous and heterotrichous ciliates, but much smaller than that of the macronuclear chromosomes of *Tetrahymena* and *Paramecium.* The genetic structure of their micronuclei remains to be unravelled.

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Ultrastructural comparison of two strains of the marine HNF *Caecitellus* cf. *parvulus* isolated from deep-sea sediment and surface water of the South Atlantic

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Whereas the deep sea as an extreme habitat (high pressure, absence of light, poor nutrients concentration, low temperature) covers more than 90% of the world ocean bottom, the knowledge on deep sea protists is still very limited. Numerous protists found in the deep-sea sediments are known from surface waters. However, molecular studies revealed quite different genotypes within morphospecies of heterotrophic nanoflagellates (HNFs), identified by light microscopy. Therefore the studied strains of *Caecitellus parvulus* (stramenopiles) which we collected during an expedition with RV METEOR (cruise 48/1, DIVA I, year 2000) from deep-sea sediments and from the surface water of the oligotrophic South Atlantic, Angola Basin, are designated as *C.* cf. *parvulus*. We found light and electron microscopical differences between a deep-sea strain and a surface water strain of this flagellate. We also found differences between both investigated strains of *C.* cf. *parvulus* from the South Atlantic compared to the ultrastructure of two *C. parvulus* strains described by others from the North Atlantic and Pacific: There are significant differences in the length of the posterior flagellum, in the appearance of the surface coat and in the number of microtubules in flagellar root 3, which surrounds the oral region and forms the cytoskeleton of the feeding basket. The highest number of microtubules within the feeding basket shows the deep-sea strain of *C.* cf. *parvulus* with 11 microtubules more than in *C. parvulus*. It is discussed whether the resulting larger feeding basket is indicative for a different ecological niche of *C.* cf. *parvulus*.

Identification of a serotype H analogue in *Paramecium primaurelia* as an RNAi-phenotype

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Two species of the *Paramecium aurelia* complex are well known in the case of serotypes: *Paramecium prim-* and *tetraurelia*, containing multigene-families encoding for these proteins. Scientific work of many decades showed the stable expression of eleven serotypes in *Paramecium tetraurelia* in contrast to three serotypes in *Paramecium primaurelia*. The genome-project obtained that the multigene family of serotypes contains many more members than previously known and expected. We compared the serotype-system of *Paramecium prim- and tetraurelia* and identified in the *P.primaurelia* genome several unknown sequences of serotypes, which can be categorized in two classes: genes with identical copies in the *P.tetraurelia* genome and genes with similarity to *P.tetraurelia* genes:
Fragments of two genes were sequenced, showing identity (or nearly identity) to serotypes of the *P.tetraurelia* genome (51I and 51D-alpha), whereas these genes seem not to be expressed in *P.primaurelia* in contrast to their copy in *P.tetraurelia*. A further fragment similar to 156S (*P.primaurelia*) was isolated which expression we also cannot prove.
Knocking down the expression of serotype 156G of *P.primaurelia* by RNAi-experiments, we induced the expression of an alternative serotype. The unknown serotype, whose nucleic acid sequence is very similar to serotype 51H of *P.tetraurelia*, extends the spectrum of expressed serotypes of *P.primaurelia* to a number of four.

In any case the mutligene families of serotypes contain many more members than previously obtained. The four new genes described were not expressed under standard cultivation-conditions. Expression of three of them was never obtained and the third one only in knock down experiments of stable serotypes. This leads to the assumption that most of the regular expressed serotypes are discovered, but multigene families contain more unexpressed members.

Silencing gene expression of *Paramecium* by feeding: knock down efficiency and its relation to transcriptional activity in *E.coli*

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Within the last few years RNAi has become a powerful tool for studying gene expression. Feeding technology is possible in several organisms, meaning that dsRNA is delivered into the organism by dsRNA producing bacteria. Also in *Paramecium* this technique gets more and more attention, due to the advantage of its low costs.
The reason for this study is the obvious phenomenon that some fragments are able to produce phenotypes whereas others don't. Possible explanations are discussed namely dsRNA length, sequence and the position of the fragment in the target-gene.
We silenced expression of serotypes of *Paramecium* with different fragments of each gene and counted the cross-working siRNAs to other co-silenced members of the multigene family. Here we compare the phenotypes produced by each fragment with the relative amount of bacterially expressed dsRNA in synchronized cultures and describe a correlation of silencing efficiency in *Paramecium* and transcriptional activity in *E.coli*. On the other hand we found fragments producing dsRNA in *E.coli* but do not show phenotypes in *Paramecium*, so another discriminating-mechanism should exist.

Generation of Functional Heterokaryons of *Tetrahymena thermophila*

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Functional heterokaryons are cell lines of *T. thermophila* bearing a dominant marker conferring drug resistance in the micronucleus and the allele for drug sensitivity in the macronucleus. Such lines are very useful for positive selection of cells that have successfully completed conjugation. To construct strains of *T. thermophila* with enhanced secretion of acid hydrolases by mutagenesis and genetic crosses we need new lines of the available heterokaryons and new ones with different drug resistance markers.

Therefore, a functional heterokaryon was regenerated by crossing the existing functional heterokaryon Cu 438.1 (*Pmr-1 /Pmr-1* (pm-s, IV)) to wild type cells. From drug resistant progeny (*Pmr-1/Pmr-1*$^{+}$ (pm-r, x)) assorters sensitive for the drug were obtained and their mating types were determined. To establish strains conferring drug resistance homozygously in the micronucleus pairs from the first round of genomic exclusion were isolated and the non-star exconjugants were characterized by further genetic crosses. The growth kinetics and secretion kinetics of such a clone were measured.

Furthermore, the reaction of *T. thermophila* to various drugs of prokaryotic and eukaryotic origin were tested in order to generate functional heterokaryons bearing novel drug resistances. The drugs tested so far had adverse effects on growth, however did not completely kill the cells within 3-4 days at concentrations up to maximal 1 mg/ml. We presently test other drugs that can fulfil these demands.

Generation and characterisation of mutants from *Tetrahymena thermophila* deficient in secretion of proteases

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To use *Tetrahymena thermophila* for heterologous expression and secretion of foreign genes, mutant clones are needed, that must not secrete protease into the nutrient medium.
Methods to create mutants of *T. thermophila* by random mutagenesis and UPC (Uniparental Cytogamy) are well established. So far there is no procedure available to detect mutants that lost their ability to secrete proteases to the nutrient media. Because there is no possibility known to enrich these mutants, it is necessary to test a high number of clones. Furthermore cell-lysis must not occur during the screening process, because intracellular proteases might lead to incorrect results.
After the random mutagenesis and UPC (Uniparental Cytogamy), the resulting whole genome homozygous progeny was cloned into 96-well culture plates. To detect mutant clones deficient for protease secretion, we developed two different screening procedures. These screening procedures allow testing of a high number of clones and are based on general protease substrates such as casein-derivates and gelatine.
These screening methods allowed us to identify a first mutant that was further described regarding the characteristics of secretion. Batch-fermentation of the mutant and the reference strains were carried out and the kinetics of enzyme secretion of the proteases and two other enzymes were investigated.
The results show that the mutant releases significant fewer amounts of active protease to the nutrient media as the reference strains. Similar results were obtained for the β-hexosaminidase secretion, while the activity of the acid phophatase was not reduced in the mutant strains.
Further control experiments are necessary to decide, whether the mutant is deficient in secretion in general or has a deficiency specific for protease secretion.

First report of the peculiar parasitic green alga *Helicosporidium* in a bark beetle

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In the body of the bark beetle *Dentroctonus micans* (Coleoptera, Scolytidae) from a wood near Trabzon in Turkey masses of small (6 µm) spores were found. In top view they appeared rounded to oval and depicted maximally three small cells in the centre. A peripheral ring-like structure appeared to be a helix with four windings in side views. It corresponds to a long slender cell as could be seen in ruptured spores. Ultrathin sections corroborated the light microscopic results. The spores contain three internal cells that are enclosed by windings of a peripheral, helical slender cell. In mature spores, cell organelles are hardly seen. The internal cells (sporoplasms) have a thick hyaline outer layer and a dark, finely granular inner cytoplasm containing rounded electron lucent (probably lipid) and electron dark (probably glycogen) inclusions. The filamentous cell is much thinner. Its cytoplasm bears rounded, electron lucent inclusions.

These curious spores belong to the Helicosporidia. In the first report by Keilin (1921) a helicosporidium from a dipteran larva was described. The filamentous peripheral cell is the main characteristic feature for diagnosis. To date, only one species, *Helicosporidium parasiticum*, is described but in fact the isolates from different hosts (insects, mites, crustaceans, trematodes) may represent several, morphological similar species.

The discovery of *Helicosporidium* in the dangerous pest, *Dentroctonus micans*, offers a chance to find pathogenic protists for use in biological control of bark beetles. The ability to evoke pathogenic effects on insects could already been demonstrated for *Helicosporidium* infections.

A morphological disturbance of *Paramecium bursaria* after an infection with the yeast, *Yarrowia lipolytica*.

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Chlorella-free *P. bursaria* can be infected with various species of yeasts and bacteria, which are however kicked-out from the host cell when the host cell was further re-infected with original symbiotic *Chlorella* cells. (Görtz, 1982, Abendschein and Görtz, 1994 unpublished). A budding yeast *Yarrowia lipolytica* is one of such infective microorganisms. We recently found that *P. bursaria* cells infected with the yeast fall in incomplete cell divisions, resulting in chained cells and, in some cases, clumps of cells hard to describe. The cytoplasm of such morphologically disturbed cells was filled with chained and branched yeast cells, which seemed to hinder cell divisions of the host cell. On the other hand, *Chlorella*-bearing *P. bursaria* cells repelled the infection with the yeast.

In our poster we show results of infection experiments with the yeast and re-infection experiments of yeast-bearing *P. bursaria* with *Chlorella* cells.

First molecular data on *"Caedibacter macronucleorum"* from *Paramecium duboscqui*

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A number of ciliate endocytobionts, mostly cytoplasmic ones, confer on the host killer-traits – the ability to kill the cells of "sensitive" clones, lacking these bacteria. The genus *Caedibacter* comprises 5 validly described species all conferring the killing trait to the host. These organisms were originally described as members of the same genus mainly because they all present an obligatory symbiotic lifestyle and the presence of an easy recognizable morphological trait, the R-body, a structure that appears to play an important role in the killing effect. Recently, 16S rRNA data evidenced that *Caedibacter* is a polyphyletic assemblage with *C. taeniospiralis* belonging to *Gammaproteobacteria* and *C. caryophilus* to *Alphaproteobacteria* (Beier et al., 2002). Another working group recorded the presence of the same species, *C. caryophilus*, in different hosts and within different cell compartmentalization, the macronucleus in *Paramecium caudatum*, and the cytoplasm in *P. novaurelia* (Kusch et al., 2000). These data indicated the limit in taxonomy and systematic of the previously used characters. We molecularly characterized *"C. macronucleorum"*, described from the macronucleus of the euryhaline ciliate *Paramecium duboscqui* (Fokin and Görtz, 1993). 16S rRNA molecular data support a close affiliation of *"C. macronucleorum"* to *C. caryophilus* within the *Alphaproteobacteria*. Although 16S rRNA similarity between these species does not support alone the establishing of *"C. macronucleorum"* as a separate species, interesting differences are observed in the 16S rRNA gene internal excised element (IEE) typical of this group of organisms (Springer et al.,1993). 16S rRNA IEE is not subjected to the strict functional evolutionary constrain of 16S rRNA and, for this reason, accumulates mutation more rapidly. Whereas *C. caryophilus* strains isolated from *P. caudatum* and *P. novaurelia* share identical IEE supporting the hypothesis of symbiont exchange among different host, the differences observed in *"C. macronucleorum"* IEE support an evolutionary separation of this lineage. Further studies involving DNA-DNA hybridization will be necessary to better evaluate the evolutionary distance between *C. caryophilus* and *"C. macronucleorum"*.

Ciliate-ciliate parasitism in lake plankton between a suctorian and a hypotrich ciliate.

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In summer 2004 we observed the infection of a planktonic hypotrich, i.e., *Uroleptus* sp. by a suctorian, most likely of the genus *Podophrya* (Suctorea: Podophryidae). The genus *Podophrya* includes planktonic and parasitic suctorians which are able to form a stalk and resting cysts. Hypotrich ciliates are sometimes infected by suctorians and this "relationship" might be host-specific, e.g., *Podophrya grelli* DIECKMANN, 1985 infects only *Stylonychia lemnae* and *Sphaerophrya parurolepti* FOISSNER, 1980 only *Paruroleptus caudatus*. Although more than 30 other ciliate species were present within the same water sample from Piburger See we could not observe any of them infected. Even at low magnification (X 40-100) the parasites attached to the host were clearly visible. Heavily infected hypotrichs were seriously deformed and very sensitive to pipette transfer. We followed the infection cycle in the living ciliates and after a quantitative protargol staining procedure (QPS): (i) attachment of the ciliated suctorian with tentacles at the hypotrich, (ii) loss of the cilia of the suctorian, (iii) inducement of a pellicular invagination in the hypotrich cell which remained open to the environment, (iv) cell division of the suctorian inside the hypotrich, (v) release of swarmers/ resting cysts into the water and presumably death of the host cell.

Control of benthic ciliate communities and meiofauna by grazers in marine, brackish and freshwater sediments: a cross system comparison

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Three laboratory and one *in situ* experiments were conducted to investigate potential top-down control of ciliate communities and meiofauna in marine, brackish and freshwater sediments. The laboratory experiments were established by adding some macrometazoans: small snails, mud shrimps, polychaetes, and chironomid larvae. The field experiment manipulated the presence/absence of the polychaete, *Arenicola marina*, from mid and low intertidal mudflats, respectively. The laboratory experiments show that dominant ciliate species and individual meiofaunal groups had different responses to different macrograzers, though the grazing effects on total ciliates and meiofauna were nonsignificant. The field experiment revealed that ciliate abundance, biomass, species richness, and diversity were not significantly affected by the exclusion of *Arenicola*. However, the species composition was distinctly changed, and there was a general trend towards higher biomass and functional shifts between carnivorous dominance and omnivorous/herbivorous dominance due to the shifts of dominant species. In contrast, meiofauna abundance was significantly enhanced or decreased, depending on sampling dates and sites. Remarkably, with the increase of the dominant, carnivorous ciliate biomass, there were tendencies of the decrease of meiofauna abundance, and *vice versa*. As the predacious ciliates were frequently dominant in sediments, they might play an important role in the microbial food web and thus obscure the effects of macrograzer predation on meiofauna and ciliates. Furthermore, both ciliates and meiofauna were more influenced by the sampling location (different tidal levels) than by *Arenicola* exclusion; they were also influenced by physical disturbance, suggesting the importance of the potential nontrophic interactions in the benthic microbial food web.

Horizontal distribution of ciliates in Piburger See (Tyrol, Austria)

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The ciliate community of the nine meter layer of Piburger See (PIB), in relation to several biotic and abiotic parameters was investigated by a horizontal sampling strategy during one day in April 2002. The horizontal spatial distribution of ciliates, in respect to taxonomic composition and abundance was monitored in order to evaluate the representativeness of the standard sampling point (M) situated at the deepest site of PIB. Ciliate samples of the nine meter depth layer were observed alive and stained by a Quantitative Protargol Stain (QPS). Bacterial abundances were counted by flow cytometry. 2D-graphs of the nine meter layer were performed by inverse distance weighting calculations with Arc View GIS 3.2. The nine meter layer of PIB was homogeneous regarding physical and chemical parameters. Ciliate and bacterial abundances measured at M reflected the average abundances (7.3 cells ml^{-1}; 1.3 x 10^6 cells ml^{-1}) of the respective water depth. However we found gradients for ciliates and bacteria with variations in numbers by a factor of 2. Our results indicated that: (i) samples from the standard sampling point (M) were representative for the situation in the complete nine meter layer; (ii) horizontal currents in PIB were supposedly responsible for the gradients of ciliates and bacterial numbers and, therefore the distribution patterns were mainly due to passive drift-processes; (iii) horizontal patterns of single species, as e.g. *Pelagostrombidium fallax* indicated the availability of some species to move actively towards regions with more favourable conditions.

The effect of metazooplankton on protozoans during clear-water phase assessed by *in situ* size-fractionation experiments

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During the lake clear-water phase high metazooplankton abundances play a major role in planktonic food chain. This period is characterised by low algal biomass and this implicates an increase in predation on protozoans. The aim of this study during the clear water phase was multiple: Assessment of the effect of metazooplankton on protozoans and analysis of food-chain interactions between ciliates and flagellates. Moreover, the reliability of such *in situ* experiments was evaluated. An *in situ* size-fractionation design was installed within the epilimnion of two drinking water reservoirs during five days in order to investigate predator-prey relationships. A first experiment was conducted in the meso-eutrophic reservoir of Esch-sur-Sûre (Grand- Duchy of Luxembourg) in May 2003 and a second in the oligo-mesotrophic reservoir "Oleftalsperre" (Germany) in May 2004. Flagellates were counted by the epifluorescence microscopy. Ciliates composition, abundance and biovolume were determined in Utermöhl settling chambers by inverted microscopy. A clear top-down effect on the protozoan abundance was observed when metazooplankton was removed. A strong decrease in autotrophic and heterotrophic nanoflagellates occurred during the course of the experiment in presence of predators. Inversely, in absence of predators, diversity of autotrophic nanoflagellates increased. Ciliates increased in abundance and their taxonomical composition changed during the experiment in predator-free treatment. Although the used size fractionation-design requires mechanical skills it is nonetheless a good instrument for *in situ* food chain studies in lakes.

Unexpected Effects of Prey Dimensions and Morphologies on the Size Selective Feeding by Two Bacterivorous Flagellates (*Ochromonas* sp. and *Spumella* sp.)

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Current models on protistan size selective feeding assume that contact probability is the factor that largely explains observed food preferences. Contact probability is generally expected to be positively correlated to prey size and therefore to explain observed food selection for larger prey items. We critically tested these basic assumptions on size selective feeding using the interception-feeding chrysomonad nanoflagellates *Ochromonas* sp. and *Spumella* sp. Mechanisms of differential feeding were studied during distinct stages of the selection process, i.e., contact probability, capture efficiency, ingestion efficiency and differential digestion, by means of high resolution video microscopy. Food selection was investigated using a mixture of microspheres ranging from 0.3 to 2.2 µm in diameter, as well as a mixed bacterial community. In contrast to current model assumptions the contact probability was highest for microspheres of intermediate size (0.9 to 1.2 µm), but was not generally positively correlated with prey size over the whole prey size range. Capture and ingestion also proved to be involved in size selection. The pattern of size selective feeding was the same, independent of the food concentration. Our results indicate that contact probability is not generally positively correlated with prey size, but shows a maximum for intermediate-sized prey regarding the offered prey size spectrum of 0.3 to 2.2 µm, and selection steps other than contact probability are crucial for size selection and should be integrated in models on size selection.

Recurrent bloom of filamentous bacteria induced by bacterivorous flagellates in an oligo- mesotrophic lake

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Pelagic bacteria from an alpine lake (Piburger See, Austria) were studied during phytoplankton spring bloom with regard to their seasonal and vertical variations in the assemblage structure. The role of protists, especially heterotrophic nanoflagellates (HNF), and the impact of nutrients, in particular of phytoplankton exudates, on the development of bacterioplankton was investigated. Bacterivorous nanoflagellates played a major role in this lake by regulating the phenotypic structure of bacterioplankton. Shortly after bacterial abundances (small single- celled bacteria) declined HNF numbers increased and reached their peak value (18000 individuals ml^{-1}) contemporaneously with the maximum abundance recorded for filamentous bacteria (7.9×10^4 cells ml^{-1}). Filament biomass contributed up to 47 % of total bacterial biomass and filaments reached cell lengths up to 123 µm. Due to this topical phenomena of filaments in natural systems, we developed a schematic model to explain the formation of filaments with respect to the impact of grazing and nutrients. In addition, we analyzed the assemblage composition via fluorescence in situ hybridization together with catalyzed reporter deposition (CARD- FISH). Using this method we determined the effect of flagellate grazing on the taxonomic structure of the bacterioplankton. Our findings point out that seasonal variations in bacterial phenotypic community structure are recurrent in this oligo- mesotrophic lake and are likely induced by heavy grazing pressure through HNF.

Free-swimming and aggregate-associated flagellates and ciliates in the River Spree

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It is generally assumed that protozoa are important in the microbial food web of aquatic systems, but limited data are available on protozoan abundance and distribution in rivers. We investigated flagellate and ciliate abundances, community structure and spatial distribution of a 6th order lowland river (River Spree, Germany) in relation to environmental abiotic gradients and bacteria. Free-swimming and aggregate-associated flagellates and ciliates were quantified and identified using live observation; for further enumeration and determination samples were also fixed. Bodonids, colorless euglenids and bacterivorous ciliates dominated the protistan community. The abundance of the protists showed a strong seasonality in the investigated period. Protozoa grew mainly in slowly flushed lakes and were then washed out into the adjacent flowing reaches of the River Spree. In these areas, the abundance of protozoa and aggregates decreased in flowing direction. The protistan distribution and growth seem to be structered by an interplay of factors, such as flow velocity, bacteria, nutrients, aggregate composition and particle size. The concentration of aggregates ranged between 500 and 2500 particles/ml. The majority of the aggregates in the river section Krumme Spree were smaller than 500 µm. Therefore, amongst other factors, the colonization of riverine particles by flagellates and ciliates differed from that of marine and lake snow. Dissimilarities between the colonization of aggregates from different aquatic habitats were also caused by the interplay of sedimentation and resuspension in running waters.

Wastewater Treatment − Contribution of Ciliates to the Development and Structure of Aerobic Activated Sludge Granules

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Activated sludge granules are spherical clusters of dense microbial communities. Granular sludge settles impressively faster than activated sludge flocks. It can thus be used to increase the process efficiency in wastewater treatment. The communities of such granules are mainly composed of bacteria, fungi and ciliates. The latter seemed to play a key function in the development of the granules, as mainly ciliates of the subclass Peritrichia are observed in colonizing the surfaces of these granules building there large colonies.
Here, a study is presented, which highlights the role of ciliates in the development of aerobic activated sludge granules. After an initial inoculation with activated sludge from a municipal wastewater treatment plant a lab-scale sequencing batch reactor (SBR) was fed with wastewater of a dairy company. The succeeding development of granules was microscopically observed over a time period of about one month, repeatedly. The process of granule development could be differentiated in three phases: During the initial phase, several ciliate species and bacteria are randomly distributed, not forming flocks or large colonies. Whilst the intermediate phase, activated sludge flocks are built by an enhanced growth of peritrichous ciliates and by the attachment of bacteria and fungi to the stalks of these ciliate colonies. Furthermore, free swimming protists, such as ciliates of the genus *Tetrahymena*, colonise the cavities of the sludge flocks. The final phase is characterised by the condensation of activated sludge flocks forming spherical communities of bacteria and fungi colonised by peritrichous ciliates on its surfaces and by the presence of numerous free swimming protists in the surrounding fluid. Thus, ciliates of the order Peritrichia can be regarded as responsible for the formation of aerobic activated sludge granules as they provide the initial matrix of the granules.

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Impact of flagellate grazing and nutrient availability on the taxonomic structure and activity of planktonic bacteria

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Grazing by bacterivore protists and nutrient availability are known to be the mayor top-down and bottom-up factors bacterial assemblage in freshwater habitats are facing. In a size-fractionation and phosphorus (P)-enrichment experiment we simultaneously studied the impact of these factors on bacterial assemblage composition and activity in mesotrophic Piburger See, Austria. Two size fractions were generated by filtration of raw water samples over 5 µm respectively 0.8 µm pore sized filters to obtain an approach with enhanced flagellate grazing due to elimination of higher protistan predators (< 5 µm), and a predator-free variant (< 0.8 µm). These fractions were filled into bottles, bottles which were enriched with threefold P concentration (15 µg P l^{-1}), and dialysis tubes which allowed free nutrient exchange with surrounding water. All variants were incubated in the lake for four days. In daily sampling, bacterial and flagellate abundance and biomass, bacterial activity, bacterial assemblage composition, protistan grazing rate and P-content were evaluated. Grazing by flagellates yielded in a pronounced reduction of active bacteria, which was visible not only in lower uptake rates of radiolabeled substrates, but also in a decline of high DNA bacteria and a lower detectability with the universal CARD-FISH-probe EUB338. Moreover, a shift in bacterial assemblage composition was observed, with *ß-Proteobacteria* being heavily grazed and *α-Proteobacteria* seeming to be more grazing-resistant. A shift in bacterial assemblage composition was also visible in the approach with P enrichment and even more pronounced in the dialysis tubes, where *ß-Proteobacteria* became dominant and seemed to be more competitive in taking up nutrients.

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Variable response to pH among three clones of *Meseres corlissi*

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In spite of its tremendous importance in relation to natural and anthropogenic acidification, surprisingly little is known on the ecophysiological impact of pH on aquatic protist. The effect of pH on growth rates, cell size and cellular production of the freshwater ciliate *Meseres corlissi* (Ciliophora: Oligotrichea) was investigated with three clonal cultures from two different locations. The clones were isolated from soil samples collected at the type location, an astatic meadow pond in Salzburg, in November 2002 (strain E4) and in December 2003 (strain M10) and from a similar habitat at Kefermarkt, Mühlkreis (Upper Austria; strain KM-P1). The species identity was verified by morphological (Foissner 2005, Foissner et al. 2005) and genetic investigations (Strüder-Kypke et al., in prep.). All strains were kept in the laboratory on a diet of the small cryptophyte *Cryptomonas* sp. in modified Woods Hole Medium (MWH) with the addition of Eau de Volvic. Experiments were performed at 22.5 °C under 14:10 light:dark rhythm for 24-48 h. Since we had observed earlier that growth was significantly enhanced by the presence of soil (SE) or peat extract (PE) in the culture medium (see Müller et al. 2005, this meeting), we added 5-10% of SE or PE to the algae/MHW cocktail at the beginning of each experiment. Positive population growth rates of *M. corlissi* were measured in the pH range from 5.0-8.5. The tolerance to low pH values (5.0-5.5) of all three clones was enhanced by the presence of PE. Results showed minor, but significant differences among the two Salzburg clones isolated from the same location at differerent times. Cell size of strain M10 was, e.g., more sensitive to pH changes than that of E4. Larger differences were measured between the Salzburg and the Kefermarkt clones. In the experiments with SE, the latter clone was more tolerant to moderately low pH values (pH 5.5-6), and its pH optimum was shifted towards the slightly acidic range. The pH optimum was best defined in terms of cellular production rates. Taken together, the results from this study further point to the significance of local ecophysiological adaptation among geographically distant populations of *Meseres corlissi* (Gächter & Weisse 2005, Müller et al. 2005).

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Do Ciliates have an impact on nitrogen transformation in a sandy riverine sediment?

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The nitrogen cycle is one of the most important biogeochemical cycles whose understanding is essential for aquatic ecosystems. Sediments play a special role in the N-cycle because many of the bacteria-catalyzed transformations take place here. Sandy sediments make up approx. 30% of riverine sediments and are inhabited by a large variety of organisms. Among these are ciliates, which as consumers of bacteria may play an important role. There are several direct and indirect possible mechanisms how ciliates can influence nitrogen transformations. Direct influences comprise the uptake and production of nitrogen compounds through consumption and excretion as well as the use of nitrate as an electron acceptor. Selective consumption of nitrate-transforming bacteria or structuring the bacterial community by grazing pressure are possible mechanisms for indirect influences. For our study sediment was taken from the River Salzach and placed undisturbed into flumes. Experimental treatments were the addition of bacterivorous ciliates and excluding macrofauna. Bacterial counts and microsensor profiles suggest that the direct effects of ciliates and macrofauna are at least as large as ciliate grazing impact on bacteria.