

27. Jahrestagung der Deutschen Gesellschaft für Protozoologie



05.-08.03.2008 in Rostock Warnemünde

Tagungsprogramm

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- 12.10-13.40 Mittagspause
- 13.40-14.20 **Plenarvortrag**
Ramon Massana
In situ diversity of marine heterotrophic flagellates
- 14.20-15.40 **Diversität** Chair: **Martin Schlegel**
- 14.20 Alexandra Stock, Klaus Jürgens, Thorsten Stoeck
Microbial Eukaryotes in the anoxic Gotland Deep
- 14.40 Barbara M. Auinger, Karin Pfandl, Jens Boenigk
Molecular population studies from plankton samples preserved with Lugol's iodine solution - a quantitative approach
- 15.00 Áron Keve Kiss, Keve Tihamér Kiss, Miklós Puky
Unexpected morphological and functional diversity of heterotrophic flagellates in a small floodplain pond
- 15.20 Jürgen F. H. Strassert, Mahesh Desai, Andreas Brune, Renate Radek
*Symbiontische Flagellaten der Gattungen Devescovina und Metadevescovina: Eine Revision ihrer Diversität in der Termite *Incisitermes marginipennis**
- 15.40-16.00 Kaffeepause
- 16.00-18.00 **Posterpräsentation (1-15)** Chair: **Markus Weitere**
- anschließend Mitgliederversammlung der Fördergesellschaft der DGP
- ab 19.00 gemeinsames Abendessen im Hotel

Freitag, 07.03.2008

- 9.00-9.40 **Plenarvortrag**
Alexandra Z. Worden
*The biology and ecology of marine photosynthetic
picoeukaryotes*
- 9.40-10.40 **Phylogenie** Chair: **Jens Boenigk**
- 9.40 Gela Preisfeld, Ingo Busse
 *Invasion und Ausbreitung mobiler Introns in den
 Plastiden euglenider Flagellaten*
- 10.00 Jun Gong, Thorsten Stoeck, Yi Zhenzhen, Miao Miao,
 Zhang Qianqian, David McL. Roberts, Warren Alan,
 Song Weibo
 *SSU rRNA phylogenies support that class Nassophorea
 is non-monophyletic, and class Phyllopharyngea is
 derived from the nassophoreans (phylum Ciliophora)*
- 10.20 Helmut Berger
 *How did the last common ancestor of the hypotrichs
 (Ciliophora, Spirotricha) look like?*
- 10.40 Peter Vd'ačný, Wilhelm Foissner
 *Conjugation in a new Dileptus (Ciliophora, Litostoma-
 tea) and its phylogenetic significance*
- 11.00-11.20 Kaffeepause
- 11.20-12.40 **Protisten in** Chair: **Anja Scherwaß**
 Biofilmgemeinschaften
- 11.20 Annette Schlüssel, Hartmut Arndt
 *Size dependent interaction of flagellates with micro-
 currents around obstacles*
- 11.40 Ute Risse-Buhl, Anja Scherwaß, Hartmut Arndt, Sandra
 Kröwer, Kirsten Küsel
 *Flow velocity at the liquid-solid interface influences
 detachment and creeping behavior of biofilm associa-
 ted ciliates*

- 12.00 Helge Norf, Hartmut Arndt, Markus Weitere
*Konsument und Ressource: Bottom-Up vs. Top-Down
Regulierung periphytischer Ciliatengemeinschaften*
- 12.20 Jennifer Wey, Helge Norf, Hartmut Arndt, Markus
Weitere
*Biofilme als Teil einer Metacommunity: Der Einfluss
von Migration aus dem Plankton auf substratassoziierte
Protozoen-Gemeinschaften*
- 12.40-14.00 Mittagspause
- 14.00-15.00 zur freien Verfügung
- 15.00-15.30 Dieter G. Weiss, Sergei A. Kuznetsov, Willfried Krö-
ger, Manfred Hauser
*Protozoan cell and organelle motility studies at highest
resolution: AVEC-DIC microscopy and motion ana-
lysis*
- 15.30-15.50 Kaffeepause
- 15.50-16.30 **Plenarvortrag**
T. Stoeck
A hidden world of protists in our oceans
- 16.30-17.30 **Posterpräsentation** Chair: **Klaus Hausmann**
(16-23)
- 18.00 Mitgliederversammlung im Tagungssaal
- ab 19.30 geselliger Abend im IOW

Sonnabend, 08.03.2008

- 9.30-10.10 **Plenarvortrag**
Emily Roberts, Emma Wootton, Mikhail Zubkov
The sweet taste of plankton: How the Dinoflagellate, Oxyrrhis marina, recognizes its prey
- 10.10-10.30 Kaffeepause
- 10.30-11.30 **Paramecium** Chair: **Hans-Dieter Görtz**
- 10.30 Helmut Plattner, Thomas Wassmer, Ivonne M. Sehring and Roland Kissmehl
Many reasons to be acid - Paramecium's organelles endowed with a H^+ -pump
- 10.50 Madlen Häntzsch, Thomas U. Berendonk, Martin Schlegel, Detlef Bernhard
Primer development for the detection of the genus Paramecium with special focus on the Paramecium aurelia – complex
- 11.10 Yoshimitsu Nakamura, Manabu Hori, Fujishima Mashiro
The endonuclear symbiotic bacterium Holospora obtusa reversibly changes types of surface antigens expressed in the host Paramecium caudatum
- 11.30-12.30 **Ökologie II** Chair: **Thomas Weisse**
- 11.30 Jan Bechert, Richard Bräucker
Gravitation and light dependent swimming behaviour in Ophryoglena flava. A direction dependent photokinesis?
- 11.50 Wilhelm Foissner
Mimicry in a haptorian ciliate
- 12.10 Janez Mulec, Julia Walochnik
Amoebae in carbonate precipitating microenvironments of karst caves
- 12.30 Verabschiedung

Posterpräsentation am Donnerstag (1-16)

Ökologie

1. Thomas Posch, Judith F. Blom
Ochromonas spp. - A small but powerful all-rounder
2. Michael Moser, Ulrike Scheffel, Peter Stadler, Thomas Weisse
Protists in an acid mining lake - life at the extreme
3. Steffen Jost, Jens Boenigk
Niche separation and coexistence of chrysophytes: The case study 'Loibersbacher Teiche'
4. Mario Prast, Ulrike-Gabriele Berninger
Impact of ciliates on nitrifying bacteria in River Salzach (Austria) sediments
5. Mar Monsonís Nomdedeu, Christine Willen, Lutz Becks, Hartmut Arndt
Bestimmung der Wachstumsrate und der Fressrate von Tetrahymena pyriformis als Basis von Parameterabschätzungen für Modellanalysen
6. Ruth Anderson, Diane McDougald, Staffan Kjelleberg, Klaus Jürgens
Differential resistance to flagellate predation of two Vibrio species subjected to carbon starvation
7. Christine Kern, Rhena Schumann
Heterotrophe Protozoen in einer mikrobiellen Matte im Windwatt "Bock" (Darß-Zingster Boddenkette)
8. Pablo Quintela-Alonso, Ramón Anadón-Álvarez, Soledad Ruiz-Álvarez
Use of ciliates (Protozoa: Ciliophora) as indicators of water quality in two brackish lagoons of the Northwest of Spain

Diversität

9. Anke Oertel, Wilhelm Foissner
Drei neue, haptoride Boden-Ciliaten
10. Jens Boenigk
Niche separation and coexistence of chrysophytes: The Alpine Lake Gradient Analysis (ALGA)
11. Hans-Werner Breiner, Thorsten Stoeck
Improving target-taxa recovery in domain-specific environmental clone libraries
12. Felix Weber, Alexander P. Mylnikov, Wilhelm Foissner, Klaus Jürgens
Distribution and diversity of protists in pelagic redoxclines of the central Baltic Sea
13. Frank Nitsche, Hartmut Arndt, Markus Weitere, Frank Scheckenbach, Klaus Hausmann, Claudia Wylezich
New Choanoflagellate species from deep sea and from brakish waters
14. Wilhelm Foissner, Klaus Jürgens
Some interesting new ciliates from the microaerobic and anaerobic bottom of the Gotlandtief (220 m below NN), Baltic Sea
15. Áron Keve Kiss, Éva Ács, Keve Tihamér Kiss
Rare and likely new heterotrophic flagellate species in plankton of River Danube

Posterpräsentation am Freitag (16-23)

Phylogenie

16. Kristina Hamilton
The Syndinea, a little explored group within the Alveolates
17. Alexander Kudryavtsev, Claudia Wylezich, Martin Schlegel, Julia Walochnik, Rolf Michel
Ultrastructure and molecular phylogeny of the genus Flamella Schaeffer, 1926 (Amoebozoa)
18. Sabine Agatha, Wilhelm Foissner
Conjugation in the spirotrich ciliate Halteria grandinella (Müller, 1773) Dujardin, 1841 (Protozoa, Ciliophora)

Biofilmgemeinschaften

19. Anne Böhme, Ute Risse-Buhl, Kirsten Küsel
Changes in the three-dimensional structure of a multispecies bacterial biofilm due to grazing of Chilodonella uncinata and Spumella sp.
20. Marcel Kathol, Helge Norf, Hartmut Arndt, Markus Weitere
Biofilmassoziierte Ciliaten als wichtige Konsumenten von Plankton

Paramecium

21. Ivonne M. Sehring, Dean Fraga, Roland Kissmehl, Martina Reis, Robert Hinrichsen, Helmut Plattner
Protein phosphatase 2B (PP2B, calcineurin) in Paramecium
22. Christine Klöppel, Jonathan Creutz, Helmut Schmidt, Martin Simon
Functional analysis of Phosphatidyl-Inositol specific Phospholipases (PI-PLCs) in Paramecium tetraurelia
23. Simone Marker, Eva Schloter, Helmut Schmidt, Martin Simon
Antisense transcripts in Paramecium: a global strategy of regulation of gene expression?

Abstracts

Plenarvorträge

Heterotrophic nanoflagellates show biogeographies!

Hartmut Arndt, Frank Nitsche, Frank Scheckenbach, Claudia Wylezich

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Protist diversity is still controversially discussed. One group of protistologists assumes a relatively low number of protistan species compared to their low size due to assumed high rates of dispersal and low speciation rates. Another group of protistologists assumes a high protistan diversity similar to other groups of eukaryotes claiming a high number of cryptic species behind each nominal species. We summarise investigations of heterotrophic nanoflagellates regarding their diversity and distribution patterns. We included samples from the largest, though seldom investigated parts of the biosphere, deep sea, groundwater and polar regions. We will show examples clearly indicating biogeographies in nanoflagellates (in addition to those already known for ciliates). According to our recent estimates, protists as the most abundant eukaryotic key players in most ecosystems worldwide, the evolutionary oldest and phylogenetically most diverse eukaryotes should account for a considerable (if not the largest) proportion of Earth's eukaryotic diversity. The knowledge of the number of protist species is not trivial but fundamental for the understanding the Earth's biodiversity and ecosystem functioning.

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In situ diversity of marine heterotrophic flagellates

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Heterotrophic flagellates are ubiquitous protists that play key roles in planktonic marine food webs. They are the main consumers of prokaryotes and participate directly in nutrient remineralization. Both direct measurements and size-fractionation grazing experiments have revealed that these assemblages are numerically dominated by very small cells of 2-3 μm in diameter, which mostly remain unidentified. Recent molecular surveys of marine picoeukaryotes have revealed a huge diversity and the presence of novel lineages. Here I will show that some of these novel lineages, in particular some organisms forming novel clades within the stramenopile radiation (MAST, Marine Stramenopiles), account for a significant part of heterotrophic flagellates assemblages. Phylogenetic probes against different MAST groups have been designed and optimized for FISH to follow the abundance and distribution in the environment of the corresponding organisms. These probes can be further applied in experiments to infer the growth and grazing rates of these still uncultured flagellates. Tools to open the black box of marine heterotrophic flagellates, which are composed of diverse groups with diverse functions, start to be available.

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The biology and ecology of marine photosynthetic pico-eukaryotes

Alexandra Z. Worden

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Protists are important players in marine systems, in part because of their massive contributions to carbon fixation. The picoeukaryotes (protists <2 micrometers in diameter) can contribute a significant portion of the primary production within their size class, rivaling their cyanobacterial counterparts, *Prochlorococcus* and *Synechococcus*. These protists are extraordinarily diverse and photosynthetic species from the ‘supergroups’ Plantae and Chromalveolates appear to be widespread and highly successful in marine systems. We have sequenced the genomes of two cultured representatives of the species *Micromonas pusilla*, a prasinophyte ancient within the green lineage (Plantae). We have also pursued methods to sequence the genomes of uncultured picoeukaryotes, specifically those belonging to the Haptophyta (Chromalveolates). Together with efforts to characterize these populations in the natural environment, such approaches are allowing us to refine hypotheses on niche differentiation and dynamics.

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A hidden world of protists in our oceans

Thorsten Stoeck

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An earlier notion of protistologists was that much of the protistan diversity has been described. Consequently, microbial eukaryotes are often viewed as “...a full deck, or at least as full as we could get, i.e. that we were sampling the vast majority of extant eukaryotes” (Baldauf 2003 in Science 300). However, the phylogenetic analyses of SSU rDNA genes amplified from environmental genomic DNA extracts (rRNA approach) has changed our perception of global microbial biodiversity and unveiled a hidden world of protists. Previously inaccessible environments and organism became more transparent and molecular tools gave more detailed insights into the complex protistan community structures in these systems. I here give a short review of the present state of protistan diversity research in oceanic realms from a molecular perspective. An emphasize will be on protistan life in extreme environments.

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The Sweet Taste of Plankton: How the Dinoflagellate, Oxyrrhis marina, Recognizes it's Prey

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Through selective feeding, protists play a fundamental role in structuring bacterial and phytoplankton communities within the marine environment. Although recent evidence indicates that protozooplankton can select food based on cell surface properties of their prey, the underlying mechanisms are poorly understood.

Previously, using haemagglutination experiments, we identified a Ca²⁺-dependent, mannose-binding lectin (MBL) on the marine dinoflagellate *Oxyrrhis marina*. Feeding experiments, involving live and bead prey, demonstrated the employment of this lectin as a feeding receptor, used for recognizing and selecting prey.

Further characterization of the lectin has revealed it is highly specific in terms of its carbohydrate-binding properties. These results highlight the necessity of purifying protozoan receptors if detailed investigations into biochemical feeding mechanisms are to be undertaken. The purified high specificity receptor is particularly valuable in determining the composition of prey cell surface glycoconjugates, and in establishing which ligands are involved in the predator-prey interaction.

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Vorträge

Does UV radiation influence the depth preference of planktonic freshwater ciliates?

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University of Innsbruck, Austria

Three ciliate species numerically dominate the planktonic ciliate assemblage during the ice-free period in a UV-transparent alpine lake. As each of the three species apparently preferred to stay in a certain depth, we tested whether their sensitivity to solar UV radiation could explain this pattern. Moreover, we assessed their vertical migration behaviour during a diel cycle. Overall, we detected no significant diel vertical migration in the water column but a species-specific differential sensitivity to ambient levels of UV radiation. *Balanion planctonicum* was negatively affected by UV radiation, coinciding with its preference for the deepest water layers. Moreover, the abundance of *B. planctonicum* was significantly correlated to chlorophyll a indicating a dependence on a constant food supply. *Urotricha cf castalia* was found closer to the surface in low abundance but it was tolerant to UV radiation. The *Chlorella*-bearing *A. chlorelligera* was also UV-tolerant which can be attributed to the presence of UV-protecting compounds synthesised by its symbionts. We show that UV radiation is a decisive environmental factor that drives the species-specific distribution of ciliates in a transparent alpine lake.

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Testing the competition-colonization trade-off with benthic ciliates

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Ecological theory postulates a trade-off between competition and colonization ability. This hypothesis was tested with artificial benthic communities in two microcosm experiments. Tiles covered by a bio-film of a benthic diatom, bacteria and heterotrophic flagellates were used as artificial substrates. Six benthic ciliate species served as test organisms. For each species, colonization ability was measured by connecting two microcosms and detecting the time necessary to reach the initially uncolonized microcosm. For measurement of competitive ability, species were added pair-wise to unconnected microcosms. The species with the higher biomass at the end of the experiment was regarded as the better competitor.

Species strongly differed in their colonization ability. Colonization time ranged from 1.7 to 8.7 days. A clear winner and a clear loser were found in the competition experiment, but also some species with similar competitive abilities. The relationship between competitive and colonization ability was positive rather than negative, contrary to the competition-colonization trade-off hypothesis.

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Der Einfluss von Temperatur auf das dynamische Verhalten von Zwei-Beute-Ein-Räuber Systemen - Modellanalysen

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Nicht nur in aquatischen mikrobiellen Nahrungsnetzen ist die Temperatur ein wichtiger abiotischer Faktor zum Beispiel für das Wachstum, die Fress- und die Reproduktionsraten.

Temperaturschwankungen und die Wetterbedingungen im Freiland unterliegen nichtlinearen Schwankungen und sind häufig chaotischer Natur. Mit Hilfe des vereinfachten mathematischen Modells eines Zwei-Beute-ein-Räuber Systems unter Chemostatbedingungen (Takeuchi & Adachi 1983) haben wir die Auswirkungen von konstanten, chaotischen und stufenweise erhöhten Temperaturen untersucht. Trotz dieses idealisierten mathematischen Modells liefern die Ergebnisse Hinweise für die Durchführung der entsprechenden Experimente im Labor. Aufgrund der verschiedenen Temperaturszenarien können Hypothesen für Veränderung des dynamischen Verhaltens eines entsprechenden experimentellen Systems abgeleitet werden.

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Die Bedeutung von Mixotrophie in Gewässern unterschiedlicher abiotischer und biotischer Bedingungen

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Die Zusammensetzung der gesamten planktischen Gemeinschaft als Kontrollmechanismus für das Auftreten mixotropher Protisten wurde in zwei voralpinen Teichen von Herbst 2006 bis Sommer 2007 untersucht. Abiotische Parameter wurden gemessen, Metazooplankton, Ciliaten, Flagellaten und Bakterioplankton quantifiziert und identifiziert, und „Tracer“-Experimente zur Bestimmung der Bakterivorie durch Flagellaten durchgeführt. Der Anteil mixotropher Flagellaten lag in beiden Teichen bei 23 bzw. 29 % der gesamten Flagellatengemeinschaft, wobei die Gattung *Dinobryon* mit bis zu 82 % dominierte. Mixotrophe Ciliaten erreichten Anteile von 20 bzw. 28 % an der gesamten Ciliatengemeinschaft. Ein positiver Zusammenhang zwischen der Ciliaten- und Zooplanktondiversität und dem Anteil oder der Abundanz mixotropher Protisten wurde in keinem der beiden Untersuchungssysteme gefunden. Rotatorien-dominierte Zooplanktongemeinschaften förderten Mixotrophie bei Flagellaten. In einem Teich führte erhöhte Lichtintensität im Frühling und Frühsommer zu einer graduellen Erhöhung im Anteil mixotropher Ciliaten. Systemdynamik, Habitatheterogenität und abiotische Faktoren waren von geringer Bedeutung für die Regulation von Mixotrophie, und ein klares Muster der Regulation von Mixotrophie konnte nicht identifiziert werden.

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The structure of benthic microbial communities of shallow soft sediment habitats

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Our investigation aimed at a better understanding of the dynamics and structure of microbenthic protistan communities. We studied (1) the seasonally changing structure of the organisms forming the “microbial benthic food web” and the prevailing mechanisms controlling their abundances, (2) differences between the structure, biomass and size-distribution of microbial benthic communities in different soft sediments (four marine, nine freshwater sites) from temperate and polar regions (seven and six sites, respectively) and (3) the influence of small macrograzers on the microbial community, employing laboratory experiments. To this end, environmental parameters and the abundances of diatoms, prokaryotes (phototrophic and heterotrophic), phototrophic and heterotrophic nanoflagellates (both differentiated into different size classes) and ciliates from the oxic sediment layer were determined and analysed statistically.

The data and findings suggest that the microbial community of a temperate mudflat goes through a seasonal cycle with a dominance of bottom-up control during winter and spring followed by an increasing influence of top-down effects (such as influence of grazing) from spring to summer. Furthermore, significant differences in the biomass of the microbial components of the benthic community as well as in ciliate species composition and richness were detected between the different sites.

The results of the laboratory experiments suggest that the small macrofauna has a positive stimulating effect on the microbial community by “bioengineering”, rather than a negative effect through grazing. The exclusion of *Arenicola marina* in a long term in situ field experiment demonstrated that other grazers eventually replaced *Arenicola* and filled their ecological niche.

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Anoxicity structures microbial eukaryote communities in the anoxic Gotland Deep

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The anoxic Gotland Deep in the central Baltic Sea is a chemically stratified habitat characterized by gradients of environmental parameters. A major research focus in this deep basin is the investigation of prokaryote microbial communities. Regarding microbial eukaryotes (protists) information is scarce. The aims of our study were: (i) the identification of protists thriving in this oxygen-depleted environment; (ii) their comparison with other marine anoxic environments; and (iii) to reveal if the protistan communities are structured along the stratification gradient. We targeted small subunit ribosomal RNA molecules extracted from environmental sample material and performed phylogenetic analyses and community structure comparisons. The protistan community structure below the chemocline differs significantly from the one immediately above the chemocline. An overlap of taxa between the two communities under comparisons is only minimal, indicating a highly specialized community thriving below the chemocline. We identified (the lack of) oxygen as the dominant factor explaining the structural patterns of the two communities.

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Molecular population studies from plankton samples preserved with Lugol's iodine solution – a quantitative approach

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Linking a specific protist or microalgal SSU rRNA sequence from environmental surveys to a specific morphotype is often problematic. Molecular surveys usually do not provide any information on the morphology of the organism whereas morphological surveys concentrate on preserved samples, which are usually not considered for molecular analyses. One main issue to overcome these problems is to link sequence analysis with morphological investigations from preserved plankton samples on a per cell basis. We present a method for quantitative analysis of planktonic protists and microalgae from field samples preserved with Lugol's iodine solution combining morphological and SSU rRNA gene sequence analysis. We will discuss molecular variation within chrysophyte populations. Our results indicate morphospecies-specific differences: *Ochromonas* sp. and *Dinobryon divergens* were represented by several different genotypes each and for the latter species the dominating genotype differed with habitat. In contrast, *Dinobryon pediforme*, *D. bavaricum* and *Synura sphagnicola* were exclusively represented by a single genotype each and the respective genotype was the same in different samples. In summary, our results highlight the significance of molecular variation within protist morphospecies.

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Unexpected morphological and functional diversity of heterotrophic flagellates in a small floodplain pond

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An *Euglena sanguinea* bloom was observed in a minute pond in the floodplain area of the River Tisza, Hungary. Two 0.5 l volume dipped samples were taken, and left alone for microbial succession. Samples were monitored daily, for one month; living samples were investigated by video microscopy. The heterotrophic flagellate community is extremely rich, we found altogether 54 species. Among these, 12 species are hardly assignable to described ones, many of them are likely new (*Cercomonas*, *Colpodella*, *Heteromita*, *Multicilia*, *Notosolenus*, *Protaspis*, *Salpingoeca* species, and some unknown genera). Adaptive behavioural and feeding traits of flagellate species are investigated. Several different feeding strategies are distinguishable; some species has more than one feeding and food searching type, according to life cycle state. However, a few behavioural elements are also found, which seems to be ineffective for food searching. Between some species, food source partitioning is obvious according to food size and food accessibility, indicating the possibility of coexistence. High morphospecies and functional diversity, and high proportion of undescribed forms, suggests that our knowledge is still limited about the diversity and function of heterotrophic flagellates in freshwater aquatic habitats.

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Symbiontische Flagellaten der Gattungen Devescovina und Metadevescovina: Eine Revision ihrer Diversität in der Termiten *Incisitermes marginipennis*

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Vor über vier Jahrzehnten wurden an Hand lichtmikroskopischer Untersuchungen 19 Flagellatenarten, darunter allein zehn Arten der Devescovinidae, aus der Trockenholztermiten *Incisitermes marginipennis* beschrieben. Die devescoviniden Flagellaten wurden dabei den Gattungen *Devescovina* und *Metadevescovina* zugeordnet. Mit der Absicht die hohe Diversität zu überprüfen, wurden in dieser Studie erneut lichtmikroskopische und erstmals elektronenmikroskopische und molekulare Untersuchungen der Flagellaten durchgeführt. Molekularphylogenetische Analysen durch PCR-Amplifikation und Sequenzierung der 18S rRNA-Gene lieferten nur einen einzigen devescoviniden Genotyp für diese Termiten. Durch die Kombination der morphologischen Merkmale mit den molekularphylogenetischen Befunden konnte gezeigt werden, dass lediglich eine Art der Gattung *Metadevescovina* in *I. marginipennis* aufzufinden ist. Die Gültigkeit der beiden Gattungen *Devescovina* und *Metadevescovina* wird diskutiert.

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Invasion und Ausbreitung mobiler Introns in den Plastiden euglenider Flagellaten

Gela Preisfeld, Ingo Busse

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Die Entstehung der phototrophen Euglenida ist ein beeindruckendes Beispiel für einen evolutionären Quantensprung: In einem einmaligen Endocytobioseereignis wurde eine Grünalge phagozytiert und im weiteren Verlauf der Evolution als zelleigener Chloroplast der Euglenida etabliert. Über die anschließende Evolution des plastidären Genoms ist bis heute nur wenig bekannt. Eine Besonderheit der bereits vollständig sequenzierten plastidären Genome von *Euglena gracilis* und *Euglena longa* ist der einzigartig hohe Anteil an mobiler Gruppe II und III Introns. Wie erste Analysen zeigen, sind Introntranslokation und -verlust als seltene evolutive Ereignisse hervorragende Marker, um die Phylogenese der Plastiden zu rekonstruieren.

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SSU rRNA phylogenies support that class Nassophorea is non-monophyletic, and class Phyllopharyngea is derived from the nassophoreans (phylum Ciliophora)

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The hypostome ciliates have been generally classified into two classes, Phyllopharyngea and Nassophorea. The status of Nassophorea and its relationship with Phyllopharyngea is one of the most controversial issues in ciliate systematics. In this study we focus on the phylogenetic interrelationships of Nassophorea and Phyllopharyngea based on small subunit ribosomal RNA gene sequences. The three nassophorean subgroups, synhymeniids, microthoracids and nassulids, are found to be monophyletic, with synhymeniids being placed as a sister group of Phyllopharyngea, and microthoracids as a sister of synhymeniids + Phyllopharyngea clade in all phylogenies; while the exact placement of nassulids is still uncertain. Constraint topologies for Nassophorea monophyly are rejected using approximately unbiased tests at confidence of 94.4% or using multi-scale bootstrap tests at probability of 99.2%. These new relationships among these taxa are well supported by morphological, ontogenetic and ultrastructural data, which, in turn, corroborate the nassophorean origin of phyllopharyngean subgroups, the cyrtophorids, chonotrichians, suctorians, and rhynchodians. The evolution of phenotypic characters of main lineages in phyllopharyngeans and nassophoreans are hypothesized, and a new higher classification is proposed.

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How did the last common ancestor of the hypotrichs (Ciliophora, Spirotricha) look like?

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The Hypotricha Stein (= Stichotrichia Small & Lynn) are a major group of the spirotrichs. The knowledge of the ground-pattern (= ground-plan) of an evolutionary unit is of fundamental importance for the establishment of a phylogenetic system. This pattern is the combination of all features (e.g., morphological, ontogenetic, ecological, molecular) of the last common ancestor (stem-species) from which a monophylum evolved, that is, it is summary of apomorphies and more or less young plesiomorphies. Some supposed apomorphies of the Hypotricha are: (1) 18 frontal-ventral-transverse cirri; (2) three dorsal kineties; (3) parental somatic ciliature completely replaced during cell division. Some plesiomorphies of the hypotrichs are: (1) cirri present; (2) body dorsoventrally flattened; (3) body flexible; (4) frontal-ventral-transverse cirri originate from six anlagen; (5) caudal cirri present; (6) cortical granules present; (7) lack of kinetodesmal fibres in interphasic specimens; (8) benthic; (9) telomeric repeat sequence TTTTGGGG. For details, see Monographs of hypotrichs by Berger (1999; *Monographiae Biol.* 78:1–1080; oxytrichids), Berger (2006; *Monographiae Biol.* 85: 1–1303; urostyloids), and Berger (2008; *Monographiae Biol.*, in press; amphisiellids). The financial support of the Austrian Science Fund (FWF; Project P-20569-B17) is greatly acknowledged.

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Conjugation in a new Dileptus (Ciliophora, Litostomatea) and its phylogenetic significance

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Details on sexual processes in *Dileptus* have been reported for only two species, i.e., *D. anser* and *D. gigas*. However, the knowledge on body and ciliary changes is scant because these studies did not use silver impregnation, where both processes can be followed concomitantly. Thus, we studied conjugation in a new haptorid ciliate, *Dileptus* sp. Conjugation is similar to that in congeners, that is, it is temporary, heteropolar, and the partners unite bulge-to-bulge with the proboscis. Some peculiarities occur in the nuclear processes, i.e., there are two synkaryon divisions producing four synkaryon derivatives, of which two become macronucleus anlagen, one becomes the micronucleus, and one degenerates. Unlike spathidiids, *Dileptus* shows massive changes in body shape and ciliary pattern before, during, and after conjugation, that is, early and late conjugants as well as early exconjugants resemble *Spathidium*, while mid-conjugants resemble *Enchelyodon*. These data give support to the hypothesis that spathidiids evolved from a *Dileptus*-like ancestor by reduction of the proboscis. *Dileptus* exconjugants differ from vegetative cells by the smaller size, the stouter body, the shorter proboscis, and the number of ciliary rows, suggesting one or several postconjugation divisions.

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Size dependent interaction of flagellates with microcurrents around obstacles

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Boundary layers serve as an environment for heterotrophic nanoflagellates in running waters. Although the influence of surface topography on hydrodynamics for macrofauna was studied several times, investigations in the layer between 2 and 5 μm are rare. Former studies showed that even low flow velocities can cause the detachment of nanoflagellates. The present study analysed, if the detachment of morphologically similar cercomonads in currents behind micro-topographies (*Ancylus fluviatilis* shells) varies with their cell sizes. Three different cercomonads with sizes from 5 to 27 μm and one bodonid of 5 μm size were analysed. At a flow velocity of 0.3 m s^{-1} small flagellates were not affected, but large cercomonads showed high detachment rates. These data indicated a size dependent effect of microcurrents on nanofauna. In addition, the influence of a complex surface topography like an artificially modelled biofilm was studied for the current-sensible flagellate *Cercomonas crassicauda*. The results conveyed that only flagellates in sheltered regions stayed attached. Individuals at exposed areas were washed away by the current and were not capable of actively moving into the sheltered areas.

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Flow velocity at the liquid-solid interface influences detachment and creeping behavior of biofilm associated ciliates

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Since water flow in the laminar boundary layer at liquid-solid interfaces is sufficient to detach heterotrophic flagellates, we investigated if morphological features and feeding modes of ciliates are advantageous for a delayed detachment at increasing flow velocities. Sessile ciliates showed the highest resistance to fast flow velocities. More contractions and longer extension times of a sessile ciliate indicated a reduced filtration activity at flow velocities $>300 \mu\text{m s}^{-1}$. Only the vagile flat gulper feeder *Chilodonella uncinata* withstood flow velocities $\geq 2600 \mu\text{m s}^{-1}$. Vagile flat filter feeder which extended more than $60 \mu\text{m}$ into the water and vagile round filter feeder showed lower resistance to fast flow velocities. Vagile species that withstood detachment between 300 and $1100 \mu\text{m s}^{-1}$ had a higher displacement rate and showed a positive rheotactic response allowing species to colonize more favorite habitats and balancing drift caused by the unidirectional flow of water.

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Konsument und Ressource: Bottom-up vs. top-down Regulierung periphytischer Ciliatengemeinschaften

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Ciliatengemeinschaften werden in zeitlich und räumlich unterschiedlichen Intensitäten sowohl über Ressourcen („bottom-up“) als auch über Räuber („top-down“) gesteuert. Während diese Prozesse im Plankton gut untersucht sind, wissen wir bis heute wenig über die Steuerung oberflächenassoziiierter Ciliatengemeinschaften, obwohl Ciliaten in solchen Biofilmen in hohen Dichten vorkommen können.

In Fliesszellenexperimenten mit Rheinwasserdurchfluß wurde sowohl durch Zugabe planktischer Bakterien als auch durch Stimulation benthischer Bakterien die Bedeutung erhöhter Ressourcenverfügbarkeit für die Struktur periphytischer Ciliatengemeinschaften untersucht. Es zeigte sich, dass neben der direkten Ausnutzung erhöhter planktischer Bakteriendichte durch suspensionsfressende Ciliaten auch ein indirekter Weg der Ressourcennutzung auftreten kann. Die Stimulation von Biofilmbakterien führte zu deutlich geringeren Effekten, was sich teilweise durch die Bildung fraßresistenter Formen erklären lässt. Ferner zeigten sich starke saisonale Abhängigkeiten in der Stärke der Antwort auf den Ressourcenstimulus – nicht zuletzt dadurch bedingt, ob potentielle Grazer periphytischer Ciliaten anwesend waren.

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Biofilme als Teil einer Metacommunity: Der Einfluss von Migration aus dem Plankton auf substratassoziierte Protozoen-Gemeinschaften

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Das Konzept der Metacommunity geht davon aus, dass Migration eine entscheidende Rolle zur Aufrechterhaltung der Diversität und Abundanzen von lokalen Organismengemeinschaften spielt. Biofilme, die mikrobiellen Gemeinschaften auf aquatischen Grenzflächen, stehen potentiell im ständigen Austausch mit dem Plankton, dessen Bedeutung für die lokalen Gemeinschaften wurde bisher jedoch kaum untersucht. Auf der ökologischen Rheinstation der Universität zu Köln wurde in Fließzellenversuchen die Migration von Protozoen aus dem natürlichen Fluss-Plankton in Biofilme durch Größenfraktionierung manipuliert. In einer Kreuzmanipulation konnte gezeigt werden, dass die frühe Biofilmbesiedlung durch heterotrophe Flagellaten (HF) besonders durch Immigration aus dem Plankton und nicht durch lokale Ressourcenverfügbarkeit gesteuert wird. In Versuchen mit vorgewachsenen (1-2 Wochen alten) Biofilmen zeigte sich im Gegensatz hierzu ein überraschend geringer Einfluss der Immigration, und zwar sowohl auf HF- als auch auf Ciliatengemeinschaften.

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Protozoan cell and organelle motility studies at highest resolution: AVEC-DIC microscopy and motion analysis

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One of the most important achievements in light microscopy during the last decades was the combination of finest optical techniques with analogue and digital real-time image processors that resulted in a new quality of insight into motility mechanisms. Among these techniques we found most suitable video-enhanced contrast differential interference contrast (VEC-DIC) microscopy developed by Bob Allen with a visualization limit of 20 nm objects. We show video sequences of *Paramecium*, *Heliophrya*, *Thalassomyxa* and others demonstrating actin- and microtubule-based organelle movements and phagocytosis at high resolution. Tracking of the paths of individual cells and organelles allows the quantitative analysis of their motility parameters.

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Many reasons to be acid– Paramecium’s organelles endowed with a H⁺-pump

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The vacuolar H⁺-ATPase (V-ATPase) is a multisubunit ATP-driven proton pump. Its main function is to establish an electrochemical proton potential across membranes using ATP hydrolysis and this is essential for numerous cellular processes in all eukaryotes. The holoenzyme is formed by two subcomplexes, the transmembraneous V₀- and the cytoplasmic V₁-complexes. Sequencing of the whole genome of the ciliate *Paramecium tetraurelia* enabled us to identify virtually all the genes encoding V-ATPase subunits in this organism and to study the localization (GFP fusion proteins, immuno- fluorescence, immuno-gold/EM) of the enzyme and its manifold roles (post-transcriptional gene silencing effects). The number of V-ATPase genes in this free living protozoan is strikingly higher than in any other species previously studied. Especially abundant are V₀-a-subunits with as many as 17 encoding genes. This abundance creates the possibility of forming a large number of different V-ATPase holoenzymes by combination and has functional consequences by differential targeting to various organelles, by specific effects in membrane trafficking, biogenesis of dense core secretory vesicles (trichocysts), osmoregulation and regulation of Ca²⁺ homeostasis after exocytosis stimulation.

Refs.: Wassmer et al. 2005 J. Cell Sci. 118:2813; dito 2006 Mol. Biol. Cell 17:917; dito 2008 Eur. J. Physiol. (invited review, in press).

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Primer development for the detection of the genus Paramecium with special focus on the Paramecium aurelia – complex

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The genus *Paramecium* is one of the best-known and intensively studied taxon within the Ciliophora. Due to their cosmopolitan distribution, their importance for water purification and their easy cultivation, *Paramecium* species have become important model organisms. Although the members of the genus *Paramecium* possess unique morphological characteristics, molecular methods are an additional opportunity for species determination, especially for non-specialists. Therefore, we present a set of primers which enable the unambiguous and easy assignment of ciliates belonging to the genus *Paramecium*. In addition, we present results on the development of hierarchic multiplex primers, which will allow the simultaneous detection of representatives belonging to the genus *Paramecium* as well as to the *Paramecium aurelia* –complex. Furthermore, the *P. aurelia* – complex was investigated in more detail. We sequenced about 2200bp including the whole SSU rDNA and partial ITS- regions of 13 species within the *P. aurelia* - complex. Based on these sequences, further species-specific primers will be developed to discriminate these morphological undistinguishable species.

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The endonuclear symbiotic bacterium Holospora obtusa reversibly changes types of surface antigens expressed in the host Paramecium caudatum

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The Gram-negative bacterium *Holospora obtusa* is an endonuclear symbiont of the ciliate *Paramecium caudatum*. The surface antigen (SAg) with a molecular mass of about 266 kDa was extracted only from the aposymbiotic cells by salt/ethanol treatment, but not from the symbiotic cells. Instead, two SAGs of 188 kDa and 149 kDa were extracted from the symbiotic cells. Indirect immunofluorescence microscopy and immunoblot with a monoclonal antibody raised for the 266-kDa protein showed that the antigen was present only on the surface of the aposymbiotic cell but not on the symbiotic cell. When *H. obtusa* were removed from the cells by treatment with penicillin-G-potassium, resulting aposymbiotic cells recovered the 266-kDa SAg but lost both, the 188- and 149-kDa SAGs from the cell surface. These results show that *P. caudatum* changes the surface antigen depending on the presence or the absence of *H. obtusa* in the macronucleus. Although *Paramecia* change the types of surface antigen by various stresses such as temperature-shifts and starvation, 188 kDa and 149 kDa SAGs were induced only by the infection of *H. obtusa*.

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Gravitation and light dependent swimming behaviour in Ophryoglena flava. A direction dependent photokinesis?

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Ciliates show a distinct coupling between membrane potential and ciliary movement. Hence, it is possible to determine the state of excitation of the cell by analyzing the swimming behaviour.

Ophryoglena flava, a holotrich sarcophagous ciliate, shows an active reaction to the gravity vector, compensating about 55% of the cell's sedimentation rate.

A kinesis is commonly defined as a change in locomotion velocity dependent on the value of the stimulus and not on stimulus direction. Gravikinesis, as exception, depends as well on the value as on the direction of acceleration. This has been demonstrated by means of centrifugal experiments and in parabolic flights.

Besides from graviresponse, *Ophryoglena flava* also shows a reaction to unidirectional light, which may be caused by the organelle of Lieberkühn. We present new data of wavelength and light direction dependent photoreactions in *Ophryoglena*. The data imply that photoreaction in this species is composed of photoorientation and a light direction dependent kinesis.

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Mimicry in a haptorian Ciliate

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Typically, haptorids have toxicysts for killing other ciliates, their preferred prey. Further, they have so-called cortical granules which are usually less than 2 μm in size and colourless. Possibly, these granules are mucocyst-like extrusomes with a defensive function. In African and North American soils, we discovered a red haptorian ciliate, possibly belonging to the genus *Enchelyodon*. The colour is due to cortical granules which have a similar absorption spectrum as those of the red heterotrich ciliate *Blepharisma*, whose pigment granules have a defensive function against predators. The new *Enchelyodon*, an about 200 μm long, cylindroidal ciliate, is the first coloured haptorid that ever has been found. Among 30 food items offered (various ciliates, flagellates, micrometazoans ...), *Enchelyodon* fed only on *Blepharisma* spp. When this prey is lacking, it makes red resting cysts. However, some become smaller and colourless, indicating that the colour depends on the specific prey. Based on some preliminary experiments, I suggest that *Enchelyodon* mimics the toxic *Blepharisma* to escape predators, for instance, the large *Bursaria* and *Dileptus*. (Supported by FWF, project P-19699-B17.)

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Amoebae in carbonate precipitating microenvironments of karst caves

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In karst caves microorganisms are involved in rock-building and rock-weathering processes. The aim of the current study was to reveal and to compare the occurrence of free-living amoebae in these habitats.

Altogether, 11 samples from 4 Slovenian karst caves and 9 different habitats were investigated for amoebae by the plate culture method. Isolated amoebae were identified by their morphologic characters and genotyped by DNA sequencing.

Five samples were positive for amoebae, including *Acanthamoeba castellanii* genotype T4, *Echinamoeba silvestris*, *Hartmannella vermiformis*, and a vahlkampfiid amoeba. The abundance of acanthamoebae and hartmannellae in caves is interesting not only because the former are potential pathogens, but also because both can serve as vehicles for many different bacteria and may thus play a significant role for the perpetuation of the microfauna in caves. From stromatolitic stalagmites, a typical biogenic speleothem, a vahlkampfiid amoeba was isolated that might represent a new species, or even genus in the vicinity of *Neovahlkampfia*. The amoeba cannot grow at temperatures $>30^{\circ}\text{C}$, has prominent uroid filaments, but does not transform to a flagellate in the enflagellation test.

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Poster

Ochromonas spp. - A small but powerful all-rounder

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Up to date there are more than 600 publications about *Ochromonas* spp. (incl. *Poteroiochromonas*), regarding several scientific aspects, e.g., cell biology, toxicology, biodegradation, ecology, biophysics, biochemistry, molecular biology, and genetics. In the 60ies / 70ies most articles concerned biochemical and cellular aspects (ultrastructure) of *Ochromonas* and its requirements for vitamins and glucose. More recently research focussed on the aut- and synecology of this chrysophyte. The most cited work (188 times, Ford 1953) demonstrates the requirement of vitamin B₁₂ by *Ochromonas*. In contrast, more than 150 publications in peer-reviewed journals (mainly from the 50ies / 60ies) have never been cited, thus exiting knowledge run the risk of being ignored or forgotten. In our review we highlight that this genus is a physiological all-rounder, which blurs the boundaries between animals and plants. Depending on the cultivation conditions, *Ochromonas* behaves like an autotrophic, heterotrophic, osmotrophic or even cannibalistic organism, able to combine these nutrition modes. We summarize the impressive spectrum of substances which are released, used, or degraded by *Ochromonas*, present data on its worldwide distribution, and evaluate the impact on food webs.

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Protists in an acid mining lake – life at the extreme

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Acid mining lakes (AML) are extreme habitats with pH ranging from 2 to 5. Due to the oxidation of pyrite, associated with brown and black coal deposits, these lakes have been acidified and exhibit very high iron concentrations. Biodiversity is reduced to a few protist and rotifer species. Protists play a major role in these lakes, and the mixotrophs *Chlamydomonas acidophila* and *Ochromonas sp.* may contribute most to primary production and bacterivory; ciliates are less important in the food web. In a new research project funded by the Austrian Science Fund (FWF), we compare the protist communities in two AML located in Langau, Lower Austria, and Lusatia, Eastern Germany. We investigate the pH tolerance and the pH adaptation of the major players to test the hypothesis that acidotolerant species benefit from competitive release under acidic stress. First experimental results with *Chlamydomonas acidophila* demonstrate that these organisms are well adapted to low pH. Highest growth rates were achieved at pH ranging from 2.5 to 3.5, whereas no growth was obtained at neutral pH.

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***Niche separation and coexistence of chrysophytes:
The case study 'Loibersbacher Teiche'***

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The Loibersbacher Teiche are slightly acidic ponds within the Alpine Lake Gradient investigated in our group. We investigated the seasonal plankton dynamics, the effect of zooplankton predation on the plankton community and the pH adaptation of the dominant chrysomonad taxa, i.e. *Ochromonas* sp., *Dinobryon pediforme* and *Synura sphagnicola*. Despite a similar community composition and largely similar abiotic parameters in the two investigated ponds, the plankton community develops differently in both ponds. We will discuss potential key factors involved in the differential plankton community development.

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Impact of ciliates on nitrifying bacteria in River Salzach (Austria) sediments

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Rivers are of great biogeochemical importance for the transport and processing of matter. Within the river, sediments play an essential role as location of biogeochemical processes, due to the steep gradients at the water-sediment-interface, their high bacterial population and their connection to the groundwater and the riparian zone. While nitrification is well studied in terms of autecological studies on the relevant nitrifying bacteria, the catalyzing organisms have been rarely regarded as part of a food web. This study focussed on the interaction between nitrifying bacteria and ciliates. Ciliates are important bacterivores in sediments. We designed an experimental study with natural sediments transferred to laboratory flumes, where the ciliate abundance was manipulated. The sediments were either untreated, autoclaved or frozen to achieve different compositions of inhabiting fauna and nutrient levels. Total bacteria, nitrifying bacteria, flagellates and ciliates were counted. Nitrification potentials and *in situ* concentrations of ammonium and nitrate were measured with slurry assays and microsensors, respectively. The results showed that the abundance of ammonium-oxidizing bacteria increased in treatments with high ciliate abundance. The contribution of nitrifying bacteria to the total bacterial abundance and their activity, i. e. the nitrification potential of the sediments, were very low in our fluvial sediments when compared to other systems such as marine sediments.

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Bestimmung der Wachstumsrate und der Fressrate von Tetrahymena pyriformis als Basis von Parameterabschätzungen für Modellanalysen

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Modellierung ist ein wichtiges Instrument in der Ökologie. Insbesondere mikrobiologische Modelle sind dafür gut geeignet. Wir untersuchen unterschiedliches Verhalten in der Populationsdynamik eines Chemostat-Systems, das aus zwei Bakterien (*Pedobacter* sp. und *Brevundimonas* sp.) und einem Ciliaten (*Tetrahymena pyriformis*) besteht. Für dieses Modell müssen Parameter wie die Fressrate und die Wachstumsrate bestimmt werden.

Die Wachstumsrate wurde bei verschiedenen Temperaturen bestimmt, um das Modell unter wechselnden Temperaturbedingungen zu untersuchen. Zur Bestimmung der Bakterien-Fressrate von *Tetrahymena* wurde der Inhalt der Vakuolen untersucht.

Da die Fresspräferenz von *Tetrahymena* ebenfalls ein essenzieller Parameter für die Modellierung ist, wurde auch sie bestimmt.

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Differential resistance to flagellate predation of two Vibrio species subjected to carbon starvation

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Non-differentiating bacteria when subjected to carbon starvation induce a series of physiological and morphological changes that entail an increased resistance to a number of stress factors. Among these is a reduction in size, known to confer a higher resistance to ingestion by phagotrophs. However, we hypothesize that this is not the only defence mechanism exhibited by these bacteria against protist predation. To test this, we subjected two model bacteria for carbon starvation studies, *Vibrio angustum* and *Vibrio vulnificus*, to predation by the flagellate *Cafeteria roenbergensis*, using different initial bacterial concentrations and physiological states (carbon starved and non-starved). Our results show a marked difference in the resistance of the two bacteria to predation. While non-starved *V. vulnificus* seemed to constitute an adequate food source for *Cafeteria*, in it's starved it did not allow protist growth except at the highest concentration. On the contrary, starved *V. angustum* did not appear to exhibit significant resistance to predation by *Cafeteria*. Since both bacteria include a similar size reduction in their response to starvation, these results point to an additional resistance mechanism to predation in *V. vulnificus*.

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Heterotrophe Protozoen in einer mikrobiellen Matte im Windwatt „Bock“ (Mecklenburg-Vorpommern)

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In einer mikrobiellen Matte im Windwatt „Bock“ an der südlichen Ostseeküste wurde erstmals die Gemeinschaft heterotropher Protozoen untersucht. Dabei spielte auch ein möglicher Fraßdruck auf die Mattenstrukturbildner, vor allem die Cyanobakterien *Microcoleus chthonoplastes* und *Lyngbya aestuarii*, und die Bakterienfauna eine Rolle. Bisher wurde postuliert, dass in mikrobiellen Matten der Fraßdruck und die Bioturbation durch Pro- und Metazoen nur in gering sind. Dies galt als eine wesentliche Voraussetzung zur Etablierung der Matte. Diese wirkt durch ihre Struktur Sediment verfestigend und funktioniert daher in gewissem Maß als natürlicher Küstenschutz.

Von September 2007 bis März 2008 wurden monatlich Proben der mikrobiellen Matte und vergleichend dazu von einem mattenfreien Standort entnommen. Die Abundanzen und Zusammensetzung der Protozoen im Sediment sowie der mikrobiellen Matte wurde lichtmikroskopisch erfasst.

In der mikrobiellen Matte war die Protozoenabundanz gegenüber dem mattenfreien Standort im Windwatt erhöht. Insbesondere heterotrophe Nanoflagellaten, aber auch zahlreiche andere vorwiegend als bakterivor beschriebene Arten traten besonders häufig in der Cyanobakterienmatte auf. Die isolierten Ciliaten ingestierten einzellige Grünalgen, Diatomeen und Cyanobakterien, konnten aber die fädigen Mattenbildner selbst nicht nutzen. Auch *in situ* wurde kein Grazing dieser großen Cyanobakterien beobachtet. Die in der mikrobiellen Matte auftretenden Protozoen beschleunigen wohl hauptsächlich durch Bakterivorie, fördern so die Primärproduktion und damit die Mattenstabilität sowie deren Wachstum.

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Use of ciliates (Protozoa: Ciliophora) as indicators of water quality in two brackish lagoons of the Northwest of Spain

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The Saprobic System was used to test the saprobity variation on two brackish lagoons of the Galician coast (northwest of Spain). Presence or absence of certain species is consequence of the environmental water conditions, related with the decomposition of the organic material. After consulting the last studies related with the saprobic and indicator value of different species of ciliated protozoan with saprobic character, we have included each lagoons in one of the five saprobic degrees or classes described by Zelinka and Marvan's method. Prevalence of β -mesosaprobic and α -mesosaprobic species in both lagoons indicates a moderate-high level of saprobity, which was also quantified by the method of Pantle and Buck (1955). Index "S" values were inside the α -mesosaprobic area, with a water quality of class III during most of the months, according to the classification of Friedrich (1990).

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Drei neue, haptoride Boden-Ciliaten

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Die Ordnung Haptorida umfasst holotriche Ciliaten, die eine sogenannte Dorsalbürste besitzen. Sie führen eine räuberische Lebensweise und besitzen in der Regel Extrusome vom Toxicysten-Typ, um ihre Beute zu überwältigen. Es wurden die Morphologie und Infra-ciliatur dreier neuer Arten aus Moosen und den oberen Bodenschichten feuchter Standorte Venezuelas und Australiens mit Standardmethoden untersucht. Die erste Art gehört zur Gattung *Enchelys*, die hauptsächlich limnische und nur wenige bodenbewohnende Arten umfasst. Sie wurde in einer Algenkruste auf einem Granitfelsen, einem sogenannten "Laja", in Venezuela gefunden und unterscheidet sich von ihren Verwandten durch den flaschenförmigen Körper und den langen, geschwungenen Makronukleus. Die beiden anderen Arten besitzen keine lichtmikroskopisch erkennbaren Extrusome und stellen daher eine Besonderheit innerhalb der Haptoriden dar. Bisher wurden nur bei *Coriplites terricola*, einer Art mit zweireihiger Dorsalbürste, keine Extrusome gefunden. Eine der neuen Arten konnte der Gattung *Coriplites* zugeordnet werden, während für die Zweite aufgrund ihrer dreireihigen Dorsalbürste eine neue Gattung aufgestellt wird. (Gefördert vom FWF, Projekt P 19699 – B17.)

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Niche separation and coexistence of chrysophytes: The Alpine Lake Gradient Analysis (ALGA)

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We currently investigate microeukaryotes with a focus on chryso-
monad flagellates in an alpine gradient comprising 40 lakes. The analysis
comprises morphological surveys based on light and electron micro-
scopy as well as molecular surveys. We further isolated more than
100 chrysophyte strains from these lakes. For these strains we investi-
gate molecular signatures as well as the ecophysiological adaptation.
Resting stages, i.e. cysts, are analysed from material captured in sedi-
ment traps and linked to the vegetative cells by means of molecular
methods. Both, chryso-
monad cysts as well as vegetative cells are ana-
lysed on a seasonal basis for selected lakes. Aspects of the micro-
diversity and population dynamics are covered by cultivation-inde-
pendent molecular methods. Our main goal is to gain insights in the
coexistence, niche differentiation and distribution pattern of the target
organisms by a holistic approach combining molecular, morphological,
ecophysiological and palaeolimnological data. We will present an
overview on the current activities and first results.

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Improving target-taxa recovery in domain-specific environmental clone libraries

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The sequence analysis of environmental SSU rDNA clone libraries is the gold standard to evaluate microbial diversity in a specific habitat. However, besides microbial target taxa such clone libraries often times include large numbers of non-target taxa. For example, when analyzing microbial eukaryotes, large proportions of the clone library often times include molecular signatures of metazoa or higher plants. Considering the limits (time and supplies) of most laboratories the number of randomly chosen clones for sequence analysis is restricted. Thus, it is a major aim to have a high number of target-clones while keeping the number of non-targets as small as possible. In order to increase the efficiency of target-taxa recovery we developed a technique that identifies non-target taxa prior to choosing clones for sequencing. This method is based on ^{33}P -labeled oligonucleotide hybridization to membrane-bound vector-transformed bacterial colonies. We applied this technique to planktonic samples from the hypersaline and anoxic L'Atalante deep-sea basin in the eastern Mediterranean, which included a high number of crustacean clones (>50% of all clones). Our approach makes the screening of taxon-specific gene signatures in environmental clone libraries no longer a question of luck and coincidence.

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Distribution and diversity of protists in pelagic redoxclines of the central Baltic Sea

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In the central Baltic Sea (e.g., Gotland Deep) the pelagic redoxcline, comprising the transition from suboxic to anoxic and sulphidic water layers, is characterized by steep physico-chemical gradients (oxygen, N- and S- compounds, manganese, iron etc.) and high chemoautotrophic bacterial activities, fueled by different redox reactions. Much less than on prokaryotes is known on the functional role and diversity of protists within redoxclines. Our goal was to quantify the vertical distribution of different functional groups of protists (e.g., nanoflagellates, ciliates), to assess their diversity and taxonomic identity by microscopical and molecular techniques (e.g., RNA/DNA fingerprints, sequencing) and to estimate their importance as bacterial consumers (size-fractionation, FLB disappearance). The results reveal that, similar as for the prokaryotes, the biogeochemical gradients in the redoxcline determine the composition, distribution and probably also ecological function of the protist communities, with strongest shifts at the sulphidic interface.

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New choanoflagellate species from Deep Sea and from brackish waters

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Despite their high abundance and their high importance for the oceanic matter flux, heterotrophic nanoflagellates are only poorly studied in the deep-sea regions. Studies on the choanoflagellate distribution during two deep-sea expeditions, to the South Atlantic (5038 m) and Antarctica (Weddell Sea, 2551 m), revealed the deepest records of choanoflagellates so far. A new species, (*Lagenoeca antarctica*) with a conspicuous spike structure on the theca is described from deep Antarctic waters. A second species, *Salpingoeca abyssalis* sp. n., was isolated from the abyssal plain of the South Atlantic (5038 m depth). Floating and attached forms were observed. Phylogenetic analyses based on a fragment of SSU rDNA revealed *Salpingoeca abyssalis* to cluster together with a marine isolate of *Salpingoeca infusionum* while *Lagenoeca antarctica* clusters separately from the other codosigid and salpingoecid taxa. *Salpingoeca abyssalis* and an undetermined *Monosiga* species seems to be the first choanoflagellate species recorded from the abyssal plain.

Additionally, a new species of acanthoecid choanoflagellate isolated from brackish waters of the Danshui estuary in North Taiwan is described. Due to the similar morphology shared with *Diplothea costata* the new species has been placed in the same genus and named *Diplothea elongata*. A phylogenetic analysis of partial SSU rDNA sequences from *Diplothea costata* and *D. elongata* supports this taxonomic affiliation. This is a large and distinctive choanoflagellate which has not been reported in any previous study, suggesting that it may be an endemic species of restricted distribution.

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Some interesting new ciliates from the microaerobic and anaerobic bottom of the Gotlandtief (220 m below NN), Baltic Sea

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Few data are available on ciliates from deep marine environments. Thus, we investigated the so-called Gotland depth in the Baltic Sea. Samples were taken from microaerobic and anaerobic sites close above the bottom at a depth of about 220 m. Ciliates were studied in vivo and after silver impregnation. We found about 20 species, most belonging to the haptorids, prostomatids, and scuticociliatids, some of which have an outstanding morphology and likely represent new genera and species. Obviously, deep marine environments are a further, almost untouched diversity pool, such as floodplain soils and tanks of bromeliads. Studying ciliates from deep marine environments poses two major problems: their abundance is usually very low and they cannot be cultivated with ordinary laboratory conditions. The poster shows three likely undescribed species, viz. a *Metacystis* (Haptoria ?) with several caudal cilia, a *Plagiocampa*-like prostomatid with large cortical alveoli, and a *Holophrya*-like prostomatid with a specific brush pattern. (Supported by FWF, P-19699-B17.)

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Rare and likely new heterotrophic flagellate species in plankton of River Danube

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Samples were collected on 07. Nov. 2007 and 22. Nov 2007 from the plankton of River Danube, (Hungary). 10 l water was filtered through a 10 µm mesh size plankton net to 100 ml aliquot and left alone in laboratory. Microbial succession occurred on aggregates. Heterotrophic flagellates were investigated daily, for two weeks, in live samples by video microscopy. Altogether 55 species were found in two samples. Some species are rarely found, and important supplements can be given to their descriptions (e.g. *Phyllomonas contorta*, with anterior flagellum, now as *Ancyromonas contorta*; *Thaumatomastix setifera* sensu Lauterborn without long spines and scales but likely with stereocilia). 8 species, found by several specimens, cannot be assigned to described ones, thus are likely new species (*Cercomonas*, *Heteromita*, *Notosolenus*, *Protaspis*, *Rhizaspis* species). 11 species found by only few specimens are likely new species or genera, and have to be investigated in further studies. This unexpected heterotrophic flagellate diversity, and the high proportion of undescribed morphospecies claim more attention to the investigation of large rivers, which, as a melting-pot of species maintain huge protistan diversity.

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The Syndinea, a little explored group within the Alveolates

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The Syndinea, also known as the Alveolate Group II, comprise of parasitic genera (*Amoebophrya*, *Hematodinium* and *Syndinium*) and picoplankton from the aphotic zone. The Syndinea are regarded as a key group in understanding protist evolution as some of their morphology and gene sequences are highly similar to that of dinokaryotes, perkinsids and even apicomplexans. Depending on the gene and method used for phylogenetic analyses the Syndinea are placed ancestral, or as a sister clade, to the dinokaryotes or the perkinsids. Further, tree topology within the Syndinea suggests that this group may be as genetically diverse as apicomplexans and ciliates, with the genus *Amoebophrya* being distantly related to *Hematodinium* and *Syndinium*. To date, ultrastructural studies of this enigmatic group of alveolates have been restricted to the three parasitic genera, with most observations reported from the crustacean parasite *Hematodinium*. This poster provides an insight into our knowledge of the Syndinea by comparing structural characteristics and phylogenetic analyses.

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Ultrastructure and molecular phylogeny of the genus Flamella Schaeffer, 1926 (Amoebozoa)

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Previous morphological studies failed to infer the position of the genus *Flamella* Schaeffer, 1926 among Amoebozoa, resulting in several conflicting hypotheses. We studied morphology and ultrastructure of three new *Flamella* spp., and inferred the phylogenetic relations of the genus using SSU rRNA gene sequences of five species. Trophic amoebae of *Flamella* spp. demonstrate mitochondria with tubular cristae, cytoplasmic microtubules, and no typical dictyosomes. Instead, their cytoplasm contains small flattened vesicles, especially in the perinuclear region, that might correspond to the Golgi complex. Cysts are double-walled in all species, with several ostioles covered by operculae. These features are identical to those of *Comandonia operculata* Pernin et Pussard, 1979, suggesting a synonymy of *Flamella* and *Comandonia*. In the SSU rRNA phylogenetic trees *Flamella* is monophyletic and robustly forms a single lineage with *Filamoeba* spp. We include the genus *Flamella* in the family Filamoebidae Cavalier-Smith et al., 2004. This family constantly groups with the Conosa (i.e. Archamoebae+Mycetozoa) branch of Amoebozoa. Partially supported by the DAAD fellowship A/05/00103 to AK, grants Schl 229/7-1,2 from DFG and 06-04-49387 from RFBR.

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Conjugation in the Spirotrich Ciliate Halteria grandinella (Müller, 1773) Dujardin, 1841 (Protozoa, Ciliophora)

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The isogamontic conjugants fuse partially with their ventral sides to a homopolar pair. The first maturation division embraces dramatic transformations: (i) the partners obtain an intimate interlocking arrangement; (ii) the number of bristle kineties is reduced from 7 to 4 in each partner; and (iii) the right conjugant loses its buccal membranelles, the left the whole adoral zone. The remaining collar membranelles arrange around the pair's anterior end and are shared by both partners; finally, the couple resembles a vegetative specimen in size and outline. The vegetative macronucleus fragments before pycnosis. The micronucleus performs three maturation divisions, but only one derivative each performs the second and third division. The synkaryon divides twice, producing the future micronucleus, a macronuclear anlage, and two disintegrating derivatives. Scattered somatic kinetids occur, but disappear without reorganization. An incomplete oral primordium originates on the ventral side of both partners. The conjugation of *Halteria* resembles in several respects that of spirotrich hypotrichs; however, the majority of morphological, ontogenetical, and ultrastructural features still indicates an affiliation with the oligotrich and choreotrich spirotrichs. Accordingly, the cladistic analysis still contradicts the genealogies based on the sequence of the small subunit rRNA genes. Supported by the Austrian Science Foundation (Projects P17752-B06 and P19699-B17).

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Changes in the three-dimensional structure of a multispecies bacterial biofilm due to grazing of *Chilodonella uncinata* and *Spumella* sp.

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Protozoa seem to play a major role in structuring biofilm morphology due to grazing activity and motility. Vagile filter feeding protozoa decrease the biofilm thickness and stimulate the formation of microcolonies, which are known as bacterial defense strategies against grazing. In the present study the impact of two biofilm associated species on the three-dimensional structure of a multispecies bacterial biofilm was investigated in a continuous laminar flow system. The gulper feeder *Chilodonella uncinata* (Phyllopharyngia, Ciliophora) growing with the filter feeder *Spumella* sp. (Crysophyceae, Chrysophyta) caused an increase of biofilm porosity of 197 %, a biofilm volume loss of 68 % and a biofilm surface area loss of 63 %. In contrast, protozoa increased the ratio of biofilm surface area to volume (bsa/v) by about 26 %. The impacts were even higher with the flagellate alone. The higher bsa/v and porosity might improve exchange of nutrients and gases between the biofilm and its surrounding fluid. Additionally, a three-fold higher formation of bacterial microcolonies was observed in the presence of the vagile *Chilodonella uncinata* and the sessile *Spumella* sp., but not with the sessile *Spumella* sp. alone. We suggest that microcolony formation was caused by the vagility of *Chilodonella uncinata*.

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Biofilmassoziierte Ciliaten als wichtige Konsumenten von Plankton

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Große Fließgewässer führen eine komplexe planktische Gemeinschaft, deren Biomasse von Einzellern dominiert wird. Die Umsatzprozesse dieser Mikroorganismen sind von entscheidender Bedeutung für das Stoffumsatzgeschehen der Gewässer. Jüngere Untersuchungen haben gezeigt, dass dieses Potamoplankton oft durch benthische Konsumenten kontrolliert wird. Jedoch wurden in diesem Zusammenhang meist benthische Makroorganismen betrachtet, während die benthische Mikrofauna (vor allem biofilmassoziierte Ciliaten) in diesem Zusammenhang ignoriert wurde. Hier stellen wir erste Messungen von Verlustraten natürlicher Planktongemeinschaften aus dem Rhein an biofilmassoziierte Konsumenten vor. Die Quantifizierung der Gemeinschaftsgrazingraten erfolgte in Fliesszellensystemen, die im Bypasssystem zum Rhein stehen. Diese Methode ermöglicht es, den direkten Einfluss semi-natürlicher Biofilme auf planktische Organismen zu quantifizieren. Die Ergebnisse zeigen, dass biofilmassoziierten Konsumenten einen hohen Fraßdruck auf das Picoplankton und auf das Nanoplankton ausüben können. Die Verlustraten sind gekoppelt an die jahreszeitlich unterschiedliche Gesamtabundanz der Konsumenten und abhängig von der taxonomischen Struktur der Biofilme. Biofilmassoziierte Konsumenten sollten somit neben der benthischen Makrofauna als wichtiges Bindeglied zwischen dem planktischen und benthischen Nahrungsnetz betrachtet werden.

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Protein phosphatase 2B (PP2B, calcineurin) in Paramecium

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Protein phosphatase 2B (PP2B) or calcineurin (CaN) is a Ser/Thr phosphatase consisting of two subunits, the catalytic, calmodulin- (CaM-) binding CaN-A subunit and the regulatory Ca²⁺-binding CaN-B subunit. Based on the *Paramecium* genome project, we could identify 7 subfamilies of the CaN-A subunit and one subfamily of the CaN-B subunit, each subfamily with two members of considerable identity on the amino acid level (≥ 55 between, and $\geq 94\%$ within subfamilies). Within CaN-A subfamily members, the catalytic domain and the CaN-B binding region are maximally preserved and their molecular modeling resulted in a 3D structure almost identical to a human ortholog. Overall similarities of CaN-A and CaN-B to orthologs from other organisms are between 41 and 54 %. Heterologous expression in *E. coli* and Western blot analysis resulted in binding of established, specific antibodies against the mammalian subunits. Silencing of the CaN-A1 gene resulted in a reduction of stimulated trichocyst exocytosis just as with the “non-discharge” mutant nd7. Although silencing also affects Ca²⁺ dynamics upon exocytosis stimulation, the underlying mechanism and its effect on trichocyst release remains to be established.

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Functional analysis of Phosphatidyl-Inositol specific Phospholipases (PI-PLCs) in Paramecium tetraurelia

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PI-PLCs may have different functions in a cell: as they may accept either PI (Phosphatidylinositol) or GPI (Glycosyl-Phosphatidylinositol) their involvement concerns quite different metabolic pathways. We show here the functional splitting of six different PI-PLCs between extracellular hydrolysis of GPI-anchors but also in intracellular modes of operation.

GPI-cleavage during antigenic variation but also the artificial cleavage of these anchors during salt/ethanol extraction was shown the role for one group of PLCs.

Moreover, T-maze assays show the involvement of two PI-PLCs in the signal transduction pathway in response to extracellular folate and interestingly other PI-PLCs are shown to release IP₃ (inositol-3-phosphate) in the control mechanism of osmoregulation. Sequence analysis and comparison of the catalytic domains suggests that this kind of functional splitting is achieved by different intra- (and extra-) cellular locations of the specific enzymes.

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Antisense transcripts in Paramecium: a global strategy of regulation of gene expression?

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RNA species operate on multiple levels of gene regulation, including transcriptional and posttranscriptional mechanisms. Antisense transcripts of coding and non-coding regions are known to have a broad range of functions, such as repression of translation, RNAi-mediated mRNA targeting and also transcriptional repression.

We analysed vegetative cells for presence of antisense RNA of coding regions of single-copy, paralogous genes and genes of a multi-gene family, the variable surface antigens. An RT-PCR-based method revealed that antisense transcripts are omnipresent RNA species, mostly covering the whole open reading frame. Moreover, presence of intron-less and intron-containing antisense transcripts suggest different origins and independent mechanisms of synthesis.

Since antisense transcripts hold the potential of homology-based gene regulation, they are candidates for the regulation of paralogue and multigene expression. Mutual exclusion of variable surface antigen expression e.g. might be realised by RNAi-related mechanisms including antisense RNA.

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