### 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  $150-300\mu$ m 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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### 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.

Unterstützt vom Fonds für die Wissenschaftliche Forschung (FWF).

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

Weisse, T.<sup>1</sup>, Müller, H.<sup>1,3</sup>, Scheffel, U.<sup>1</sup>, Stadler, P.<sup>1</sup>, M. Strüder-Kypke<sup>4</sup> & Foissner, W.<sup>2</sup>

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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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Müller, H., Foissner, W. & Weisse, T. (2005). The role of soil in the ecology of the ciliate *Meseres corlissi*. Annual meeting 2005 of the German Society for Protozoology (DGP) (Abstract)

### 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, lepidosomes, embedded in mucus mainly composed here termed 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, 1.5-2um 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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### 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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Abstract #9

Protist endemicity and dispersal: evidences from flagship species and cryptogams. <u>Wilhelm Foissner</u>, University of Salzburg, Department of Organismal Biology, Hellbrunnerstrasse 34, A-5020 Salzburg. e-mail: <u>wilhelm.foissner@sbg.ac.at</u>

There is a widespread belief that distribution of microorganisms can be successfully studied only by molecular methods. While this is true for bacteria and related organisms, which are extremely small (most < 10  $\mu$ m) and have simple morphologies, it applies only partially to the protists. Many protists have considerable size (200–800  $\mu$ m) and complex morphologies and can thus serve as flagships. Flagship species are so showy, or so novel, that they cannot be overlooked if indeed they were widely distributed. If, for instance, the Australian endemics occurred in Europe and North America then they would have been seen there, long ago. In my lecture, I shall provide slides and short films of such flagship species from limnetic and terrestrial ecosystems, including the tanks of bromeliads and floodplain soils, two almost untouched habitats with high protist diversity. Endemicity requires restricted distribution which is difficult to prove in microorganisms because many disperse in a dormant stage, often called spore or resting cyst. Usually, these are smaller than 100  $\mu$ m and highly resistant to harsh environmental conditions. Thus, they provide evidences for the hypotheses of Fenchel and Finlay that small size and high abundance of microorganisms favour global dispersal and low rates of speciation. However, Fenchel's and Finlay's hypotheses are flawed by macrofungi, mosses and ferns, many of which have a restricted distribution although their spores are usually smaller than 50 µm and are produced in astronomical numbers. For instance, a single mushroom (Agaricus campestris) produces 1.6 x 10<sup>9</sup> spores, while only  $10^6$ – $10^8$  (mainly cystic) protists inhabit a square meter of soil. Thus, I developed the moderate endemicity model which suggests that most of the rare and cryptic protist species have not yet described and that about one third of the protists, described and undescribed, have a restricted distribution. Protist endemicity is caused by geo-historical events (e.g., split of Gondwana and Laurasia and of continents), climatic factors (e.g., wind and water currents, tropical vs. temperate temperature regime), ecological features (e.g., limited cyst viability), and their high age which provided sufficient time to acquire high diversity by colonization of even highly specialized habitats. Supported by the Austrian Science Foundation.

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older growth regions indicating that there might be a succession of phylotypes during the life history of colonies. Diversity of bacteria seemed to be higher in the water column than on corals, while diversity of Archaea was higher on the corals. Data on whole tissue versus surface swabs also indicate that the micro-organisms within the colonies differ from those attached to colonies. The ecological role of these probably non-pathogenic microorganisms remains unknown.

### Parasites on Idiosepiidae (Mollusca, Cephalopoda)

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### Abstract

A wide variety of parasites occurs in cephalopods, ranging from viruses, bacteria to isopods. These unsolicited guests are located in every kind of tissue and are most common in muscles, gills, digestive tract and excretory organs. So far only 150 of more than 650 cephalopod species have been examined for parasites. Many detailed studies exist on species of commercial value, but less information is available on infection and parasites in other species. This is the first record of parasites in the cephalopod family Idiosepiidae, a small taxon represented by a single genus (Idiosepius) comprising seven species distributed from Japan over the Indo-Pacific to Moçambique. One conspicuous morphological character of this family is an adhesive organ located on the posterior part of the dorsal mantle side. The animals live in near-shore, shallow waters between sea grass and mangroves. They hide during the day sticking to sea grass leaves or algae for camouflage. In the course of a study of the adhesive organ we detected three groups of parasites (Bacteria, Nem atoda and Ciliophora) within, on and around the adhesive organ. I. Bacteria: Endosymbiotic bacteria account for luminescence or toxin production in some cephalopods. In the present case groups of baculi form-shaped bacteria (2 µm in length, diameter 0.5 µm, lack of cilia) are found in the adhesive organ. The bacteria are enclosed in interstitial cells. Their irregular distribution within the organ indicates an infection rather than a symbiosis. So far, bacteria have been only detected in *Idiosepius pygmaeus* from Thailand. II. Nematoda: Larval nematodes are common in many cephalopod species. From Idiosepius biserialis from Moçambique and Japan unidentified nem atode species are reported. The majority of these worms (10 µm in length, 1.5 µm in diameter) occur in the mantle musculature, the adhesive organ and on the arms. III. Ciliophora: Ciliates are the common ectoparasites of cephalopods. The genus Trichodina has now been found on Idiosepius paradoxus. These protozoa are ectobionts, found as commensals, facultative or obligate parasites on aquatic invertebrates, fish, and amphibians. They have a cylindrical body, rounded distally (diameter 20  $\mu$ m, height 10  $\mu$ m) and three rings of cilia. The body is inverted like a sucker ventrally probably to hold on to the hosts using low pressure. The ciliates infest the mantle epithelia, the inside of the funnel and even the adhesive organ of the squid singly or in groups. Only specimens of Idiosepius paradoxus from Nagoya, Japan (collected with Dr. S. Shigeno and T. Kasugai in April 2005) have been found to be infected; no parasites have been detected on specimens from other locations or other Idiosepius species.

### Soil Biodiversity and Nutrient Turnover in Different Forest Types of Central Europe

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### Introduction

Natural old-growth forests are expected to be high in biodiversity, because the structural richness of the habitat sustains complex relationships between fauna, flora, and microflora. Our hypothesis was that forest type characteristics affect the soil biota and provoke specific nutrient turnover rates. In cooperation between field ecologists, taxonomists, molecular ecologists and biochemists we attempted to link biodiversity and biogeochemistry and gain new insights into the functioning of forest soils (DIANA homepage http://bfw.ac.at/300/2197.html).

### **Material and Methods**

Twelve old-growth forest stands were selected which were situated within the eastern part of Austria, featuring oak-hornbeam, woodruffbeech, acidic beech, spruce-fir-beech, floodplain and Austrian pine forests. All stands were characterized by a natural tree species composition. An overview of the site characteristics and the geographical locations as well as the sampling design is given in Hackl et al. (2000) and Hackl et al. (2004a). The biodiversity of different groups of micro- meso- and macrofauna (Fig. 1) was assessed by the respective specialists after soil sampling or trapping during two vegetation periods. Here we present only data on species richness; abundance data and ecological traits are included in the database.



beeties.

Microbial communities were analyzed by phospholipid fatty acid (PLFA) extraction and analysis, which was done as described by Frostegård et al. (1996). Delta <sup>15</sup>N natural abundance of total soil nitrogen was determined after dry combustion by isotope ratio mass spectrometry (Delta Plus, Finnigan MAT) according to Wanek and Arndt(2002).

### **Results and Discussion**

### Biodiversity

The biodiversity of all taxa was generally large in the investigated natural forest soils (Fig. 2). However, it was not larger as compared to managed forests (Fig. 3). According to the "intermediate disturbance hypothesis" assemblages of late successional stages have less species than those of intermediate stages. We suggest, that the DIANA forests might be considered such late successional stages.

Highest species richness of several taxa, such as protozoa, diptera, coleoptera, bacteria (Fig. 4) and earthworms was found in the nutrient rich floodplain forests (Waitzbauer and Wurth, 2005). The communities of microbes, protozoa, oribatid and gamasid mites as well as collembolans in these forests were distinct from those at the other sites. As compared to the other sites, rank abundance plots for these forests were linear, indicating lower site maturity (Čoja and Bruckner, in preparation). We attribute the high species richness of the floodplain forests to an intermediate disturbance regime. This is brought about by occasional flooding, which may also contribute to the higher abundance of ubiquitous species as compared to forest specialists (Fig. 5). The less fertile beech forests on acidic bedrock showed a large abundance of fungi (Fig. 4) and high biodiversity and abundance of microarthropods, such as gamasid mites and collembola. The pine forests under study were rich in actinomycetes and fungi (Fig. 4), which are more efficient in utilizing recalcitrant pine needles than bacteria. Thirteen new species of protozoa were found in the pine forests (Foissner et al, 2005). Two forest sites which were affected by high nitrogen deposition had a constricted biodiversity of all taxa and contained little microbial biomass (Hackl, 2004a). In addition, these sites were heavily disturbed by digging of wild boars (Fig. 6). Most communities of the soil biota showed clustering according to forest type. There was no direct relationship between the species richness of higher plants and the soil organisms studied.



# Fig. 2: Biodiver sity of investiga

Nutrient Turnover

Nitrogen turnover rates were largest in the spruce-fir-beech forest soil and lowest in the acid. beech forest soil (Zechmeister-Boltenstern et al., 2005). In spite of the high turnover rates the nitrogen cycle was almost closed in the spruce-fir-beech stand. Highest loss of N occurred in the floodplain forest as nitrate leaching (Hackl, 2004a). The δ<sup>15</sup>N graph (Fig. 7) shows highest <sup>15</sup>N enrichment in the floodplain forests and <sup>15</sup>N depletion in the acid. beech forest soil. Microbes tend to process the lighter <sup>14</sup>N, which can be leached after conversion to nitrate, leaving 15N enriched material in microbially active soil. Low δ15N, as observed in the acid beech forest, indicates the presence of undecomposed plant material (Högberg, 1997).

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### Conclusions

- Diversity of soil biota in old-growth forests is large, many new species can be found.
- Moderate disturbance (such as flooding and sustainable forest management) may promote species richness but it may change community composition and eliminate rare and specialised forest species. Nitrogen leaching losses may occur.
- Severe disturbance (such as anthropogenic nitrogen inputs and high wild boar density) may negatively affect species richness of the soil biota.
- Nutrient turnover rates are related to physiological groups of the soil community and to forest type. The DIANA database (Fig. 8) has been created to find specific relationships between individual soil taxa and nutrient turnover.

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### References

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FLORIAN WINTER

# Meeting of the Soc.Molec.Biol.Evol. in Aukland, 2005

Reframing the microbial "Everything is Everywhere" debate: Evidence for high gene flow *and* diversity in ciliate morphospecies

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geographic ranges. This high genetic diversity within groups of morphologically-similar organisms the definition of microbial species. important implications for our understanding of global microbial diversity and raises questions about may be related to cycles of isolation in their ephemeral habitats. Such cryptic diversity has gene flow over great distances, as evidenced by the presence of identical haplotypes across broad evidence of high gene flow among geographically separated populations. Our analyses reveal that (1) ciliate morphospecies isolated from ephemeral environments (e.g. freshwater ponds and tide pools) gene flow and low global diversity of microbes, with the 'endemism' hypothesis, which argues for individual morphospecies were comprised of numerous genetic entities and (2) there is considerable redefine this debate. In two different clades of ciliates, we found both high levels of diversity and high global diversity and geographically restricted gene flow. Our analyses of genetic variation in Current debate on microbial diversity contrasts the 'cosmopolitan' hypothesis, which argues for high

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### Diversity in Austrian natural forest soils in relation to nutrient turnover and net greenhouse gas exchange

### S. Zechmeister-Boltenstern for the DIANA Team

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We measured the microbial turnover of carbon (C) and nitrogen (N) in 12 natural forest reserves in Austria, along with estimating potential emission rates of nitrous oxide (N<sub>2</sub>O) and carbon dioxide (CO<sub>2</sub>), and uptake rates of methane (CH<sub>4</sub>). The community composition of soil microorganisms was investigated using PLFA (Phospholipid fatty acid) analysis and molecular tools. The biodiversity of selected taxa of micro-, mesoand macrofauna were studied. The aim was to compare nutrient turnover rates with community composition of the soil biota. Ecophysiological quotients were tested for their ability to make predictions about the carbon dynamics of forest soils. The 12 forests represented the six typical forest types in Central Europe: oak, beech, sprucefir-beech, floodplain, and pine forests.

Forest types had distinct effects on microbial community composition. The nutrient rich floodplain forests sustained a large variety of bacteria, arbuscular mycorrhiza, protozoa and earthworms. Here C and N turnover rates were fastest and leaf litter was quickly decomposed. This was made evident by microbial quotients, xylanase activity, the relative thickness of litter layer and <sup>15</sup>N abundance in the organic soil. The less fertile beech forests on acidic bedrock showed a dominance of fungi and high biodiversity and abundance of microarthropods such as gamasid mites and collembola. Carbon turnover was slowest in the beech forests on acidic bedrock, and slow turnover may lead to the largest net C accumulation.

### **DIANA** team:

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# 12<sup>th</sup>

# International Congress of Protozoology

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### P4 Biodiversity of Protists: a Different View

### Wilhelm Foissner

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Estimation of diversity and distribution of microorganisms are greatly disturbed by undersampling, the scarcity of experienced taxonomists, and the commonness of misidentifications. Thus, likely more than 50% of the actual diversity is undescribed in many protist groups. Restricted geographic distribution of microorganisms occurs in limnetic, marine, terrestrial, and fossil ecosystems. Similar as in cryptogames and macrofungi, about 30% of the extant suprageneric taxa, described and undescribed, might be morphological and/or genetic and/or molecular endemics. This figure basically matches the early opinion of Fenchel (1993) that "...smaller organisms tend to have wider or even cosmopolitan distribution, a higher efficiency of dispersal, a lower rate of allopatric speciation and lower rates of local and global extinction than do larger organisms". At the present state of knowledge, microorganism endemicity can be proved/disproved mainly by flagship species, distribution data collected by experienced taxonomists on particular species (e.g., the heterotrophic flagellate Hemimastix amphikineta), and excluding sites (e.g., university ponds) prone to be contaminated by foreign invaders. In future, genetic and molecular data will be increasingly helpful. The wide distribution of many microorganisms is usually attributed to their small size and astronomical individual number. However, this is flawed by macrofungi, mosses and ferns, many of which have distinct areals, in spite of their minute and abundant dispersal/means (spores). Thus, I suggest historic events (split of Pangaea etc.), limited cyst viability and, especially, time as further major factors for dispersal and provincialism of microorganisms. Furthermore, the true number of species and their distribution can be hardly estimated by theories and statistics, until reliable investigations of representative ecosystems are available. Generally, the doubts on cosmopolitan distribution of microorganisms can be summarized in a single question: If the world is teeming with cosmopolitan unicellulars, where is everybody? Supported by the Austria Science Foundation (FWF), Project P-15017. Key literature: Fenchel, T. (1993) There are more small than large species? Oikos 80: 220-225; Foissner, W. (2005) Biogeography and dispersal of microorganism: a review emphasizing protists. Endocytobiol. Cell Res. (in press).





# International Congress of Protozoology

July 10-15,2005 Guangzhou · China



### Ciliate Diversity and Significance in Extreme Habitats Wilhelm Foissner

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Organisms in extreme environments live or survive conditions that are outside the range experienced and/or tolerated by the majority of organisms. Extremophiles grow and function in extreme environments whilst cryptobiotes survive exposure to extreme conditions in an ametabolic dormant stage. Many protists can do both, for instance, the anaerobic ciliates which reproduce under such conditions can also generate dormant stages (resting cysts). The following table provides a compilation about ciliate diversity and the number of specific morphotypes in various extreme habitats.

Habitats	Diversity	Specific speci	es Main ecological factors
Antarctic soils	low	few	temperature, spatial isolation
High mountain soils	low	none?	temperature
Saline inland soils	moderate	many	salinity
Hot sand deserts	moderate	many	water, temperature, food
Floodplain soils	high	many	periodic disturbance
Soil from green river bed	ds high	many	water, periodic disturbance
Anoxic soils	low	few	oxygen
Astatic puddles	moderate	many	aperiodic dryness
Bromelian tanks	moderate	many a	periodic dryness, spatial isolation, competition
Leave surfaces	low	few	water, food
Anoxic waters	moderate	many	oxygen, H₂S
Activated slude plants	low	none	oxygen, time
Deep Sea	?	?	pressure
Antarctic shelf ice	moderate	few?	temperature, food, salinity

This compilation indicates that age and variability (niche number) of the habitat play a key role for ciliate speciation and diversity in general. For instance, the old (> 1 million years) sand dunes of the Namib Desert inhabit as many ciliate species as the young (~10,000 years) postglacial soils in Central Europe. In contrast, only few and pollution-tolerant species occur in activated sludge plants, a recent man-made habitat. Moderate biotope variability, as found in floodplain soils, and intense competition, as occurring in Bromelian tanks, accelerate speciation and the origin of endemics. The ecological significance of protists increases with the severity of the habitat because of the decreasing number of multicellular organisms. Supported by the Austria Science Foundation (FWF), Project P-15017





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## A New Family of Haptorid Ciliates (Protozoa, Ciliophora)

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We studied the morphology of three rare haptorid ciliates, using live observation and silver impregnation: Apertospathula sp. from saline grassland soil of Venezuela; a new genus and species from field soil of Venezuela; and Spathidium porculus (Penard, 1922) from Sphagnum mud in Germany. Simple ethanol fixation (50-70%, v/v) is recommended to reveal the ciliary pattern of "difficult" ciliates, such as S. porculus, by protargol impregnation. The three genera investigated have a distinct feature in common, viz., a lasso-shaped oral bulge and circumoral kinety, where the right half is slightly to distinctly longer than the left and the circumoral kinety is open ventrally. Thus, they are classified into a new spathidiid family, which probably evolved from a Bryophyllum-like ancestor by partial reduction of the oral bulge and circumoral kinety. Apertospathula sp. has a wart-like process, the palpus dorsalis at the anterior end of the dorsal brush. The right branch of the circumoral kinety is only slightly longer than the left one. The new genus and species from field soil of Venezuela has a straight oral bulge and circumoral kinety the right branch of which extends to posterior body end, while the left ends in anterior third. Spathidium porculus, a curious ciliate with a snout-like dorsal elongation of the oral bulge, the palpus oralis, has a highly characteristic ciliary pattern and thus represents also a new genus: the oral pattern is as in the new genus from Venezuela, but the oral bulge and the circumoral kinety extend spirally to posterior body end, while the somatic ciliary rows course meridionally. This is achieved by inserting some shortened ciliary rows in the curves of the oral bulge. A paper containing all new taxa is in press.





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### Flagships as Ultimate Proof of Protistan Endemicity: Evidences from Spathidiids (Protozoa, Ciliophora) Kuidong Xu<sup>1, 2</sup> and Wilhelm Foissner<sup>2</sup>

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Estimation of diversity and distribution of protists is greatly disturbed by undersampling, the scarcity of experienced taxonomists, and the frequency of misidentifications. Notwithstanding, the postulated cosmopolitism and ubiquity of protists are flawed by the fact that many flagship species have been found only in one or two biogeographic regions. Flagships with conspicuous morphology and/or size are the elephants of the microscopic world, and it is unlikely that such species would be overlooked if indeed they were widely distributed. We show five spathidiid flagships and provide evidences of endemicity, based on a thorough revision of the group and the investigation of over 2,000 freshwater and soil samples collected worldwide. The spathidiids are a group of free-living, rapacious haptorid ciliates with an oblique anterior end (oral bulge) studded with toxicysts. Among them, Myriokaryon lieberkuehnii is a size-flagship measuring 900-2000 × 70-120 µm. Likely, it is a limnetic cosmopolitan and thus has been described already in 1889 by the German protistologist Otto Bütschli. In contrast, a related genus and species, Cephalospatula brasiliensis Foissner, 2003 might be endemic to South America, where it has been found at three sites. Like M. lieberkuehnii, C. brasiliensis is a large, vermiform species (350 × 35 µm) with a unique, golfclub-shaped oral bulge. Arcuospathidium cultriforme, a knife-shaped species with an average size of 240  $\times$  40  $\mu$ m, is likely cosmopolitan and has been described already in 1922 by the Swiss protistologist Eugene Penard. In contrast, A. lorjeae Foissner, Agatha & Berger, 2002, which differs from A. cultriforme by the 10 µm long brush bristles, has been discovered only recently in Namibia (Africa) and Brazil (South America), suggesting that it is a Gondwanan flagship. A not yet described genus and species has been found only in Central and South America. Although it is only a middle-sized species  $(135-160 \times 55-65 \mu m)$ , it is highly conspicuous due to its gigantic mouth. Last but not least, we shall show Protospathidium namibiense and Protospathidium n. sp., two middle-sized species (about 210 × 20 μm, 250 × 27 μm) that are very similar at first glance, but are distinct species with restricted geographic distribution on more detailed investigation. Our data show that many ciliate flagship species remain to be discovered, emphasizing our ignorance of the protist world. Supported by the FWF, Project P-15017

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