The *Sphagnum* ponds of Simmelried in Germany: A biodiversity hot-spot for microscopic organisms

FOISSNER W.[1] & KREUTZ M.[2]

[1]*Universität Salzburg, FB Organismische Biologie, Hellbrunnerstrasse 34, A-5020 Salzburg.*
e-mail: wilhelm.foissner@sbg.ac.at
[2] *Private Laboratory, Magdeburger Strasse 2, D-78467 Konstanz. e-mail: makreu@gmx.de*

The Simmelried is a three hectare-sized moorland near to the town of Constance, southern Germany. It formed after the last (Würm) ice-age, that is, about 15,000 years ago, when a regressing glacier tongue produced a terrain with water-filled depressions between streamlined boulder depositions. We documented by colour micrographs and scanning electron microscopy 656 species of bacteria, autotrophic and heterotrophic protists, and micro-metazoa (mainly rotifers). Sixty of these were naked and testate amoebae which could be identified to species level; 40 could not, and most of these are likely undescribed. Some of the 60 species identified are rare and never have been documented by micrographs, for instance, *Dinamoeba mirabilis*, *Polychaos fasciculatum*, *Mastigella* sp., *Lecythium hyalina*, *Diaphoropodon mobile*, *Plagiophrys parvipunctata*, *Apogromia mucicola*, and *Rhogostoma schleusseri*. The 656 species documented represent only two thirds of the taxa actually seen. We estimate that further analyses will increase this figure to 1000–1200 species. Thus, a considerable diversity accumulated over 15,000 years, emphasizing the great distribution capacity of micro-organisms. On the other hand, some common species are lacking (e.g., the ciliate *Colpidium colpoda*, the euglenid *Phacus pleuronectes*, and rotifers of the genera *Proales* and *Floscularia*) and many undescribed species were discovered. While a mass of undescribed species is comprehensible in amoebas, flagellates and ciliates, which are poorly researched, this is surprising in well-known groups, such as euglenids and chrysophytes. Thus, we must face the possibility that some of the undescribed species are regional or local endemics. The high species richness, including many undescribed species, suggests the Simmelried as a regional biodiversity centre worth to be protected by law.

Pathology Associated With *Coleps* Spp. In Tilapia

[1]Lester H. Khoo, [2]Wilhelm Foissner and [3]Alistair D.M. Dove

[1]University of Pennsylvania, School of Veterinary Medicine, New Bolton Center, 382 West Street Road, Kennett Square, PA 19348; [2]FB Organismische Biologie, Universität Salzburg Hellbrunnerstr. 34A-5020 Salzburg, Austria; [3]Marine Sciences Research Center, Stony Brook University, Stony Brook NY 11794

Tilapia (*Oreochromis niloticus*) were submitted to the Pennsylvania Animal Diagnostic Laboratory System from a commercial producer who was raising the fish in a recirculating system. The major complaint was that production in the various tanks of the system was not up to expected standards. No major mortalities were noted at this time. Fish were submitted live and were subjected to a full diagnostic work up which included gill biopsies, skin scrapes, necropsy, and microbiological culture of the kidney and histopathological examination of samples of all the various tissues collected in 10% neutral buffered formalin. Microscopic examination of the skin scrapes and gill clips revealed a large number (approximately 15 to 20 per high power field) of a small (approximately 75um X 30um), barrel shaped holociliate that was later identified as *Coleps* sp. The gills also appeared swollen, congested and slightly blunted. Although *Vibrio angullarum* was isolated from one fish (out of five), there were no corresponding histopathological findings that would indicate a bacterial infection. Significant histopathological findings were renal tubular mineralization as well as lamellar edema and epithelial erosion of the gills. Occasionally, sections of organisms consistent with *Coleps* sp. were also present in between the gill lamella. It was thought that branchial lesions might be a result of a combination of factors including water quality (the total ammonia nitrogen was reported to be high), formalin treatments and as well as possible irritation by the protozoa. Similar appearing organisms were also rarely seen in the affected renal tissue. *Coleps* sp. are protozoans that have distinctive protective calcerous plates. They are mainly regarded as free living scavengers that feed on dead and dying tissue and microorganisms. However, they have been reported to kill zebra fish (4-9 day old) larvae in production systems as well tropical fish and rainbow trout.

KV 5

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

DUNTHORN, Micah[1], Wilhelm FOISSNER[2] and Laura A. KATZ[1]

[1]University of Massachusetts, Amherst, MA 01003, USA, [2]Department of Organismal Biology, University of Salzburg, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria

Previous molecular analyses to test the monophyly of the class Colpodea and the relationships among the orders within the class used limited taxon sampling. Monophyly was only weakly supported, and there were unsuspected groupings that challenge some of the morphological-based ordinal groupings. To classify more accurately these ciliates, we are constructing a molecular phylogeny of the Colpodea using additional taxon sampling and various outgroups. We are analyzing small-subunit (SSU) ribosomal sequences with parsimony, maximum likelihood, and Bayesian methods. Specifically, we have increased sampling within the Cyrtolophosidida and Sorogenida (the potential basal clades), as well as within previously un-sampled orders and families in the rest of the class. Here we present some initial results, emphasizing the ability of SSU to resolve relationships with further taxon sampling. In addition, we report on the presence of two distinct rDNAs in *Bryometopus*, which has a 550 base-pair deletion, and discuss explanations for this unusual sequence.
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559

KV 12

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

KATZ, Laura A.[1], George B. McMANUS[2], Oona L.O. SNOEYENBOS-WEST[1], Autumn GRIFFIN[1], Katarzyna PIROG[1], Barbara COSTAS[2] and Wilhelm FOISSNER[3]

[1]University of Massachusetts, Amherst, Massachusetts, USA, [2]University of Connecticut, Groton, Connecticut, USA, [3]Department of Organismal Biology, University of Salzburg, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria

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

560

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

FOISSNER, Wilhelm[1], and Thorsten STOECK[2]

Department of Organismal Biology, University of Salzburg, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria, [2]School of Biology, Department of Ecology, TU Kaiserslautern, Erwin-Schrödinger-Strasse 14, D-67663 Kaiserslautern, Germany

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

561

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P 32

Local ciliate endemism in an anoxic alpine lake?

KOLODZIEJ, Karolina[1], Wilhelm FOISSNER[2] and Thorsten STOECK[1]

School of Biology, Department of Ecology, TU Kaiserslautern, Erwin-Schrödinger-Strasse 14, D-67663 Kaiserslautern, Germany, [2]Department of Organismal Biology, University of Salzburg, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria

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

562

KV 1

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

BERGER, Helmut[1, 2], Khaled A.S. AL-RASHEID[3] and Wilhelm FOISSNER[2]

[1]Technisches Büro für Ökologie, Radetzkystrasse 10, A-5020 Salzburg, Austria, [2]Department of Organismal Biology, University of Salzburg, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria, [3]King Saud University, Zoology Department, P.O. Box 2455, Riyadh 11451, Saudi Arabia

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

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561