14-17 September 2009

Montbéliard (France)

5th International Symposium on Testate Amoebae

14-17 September 2009, Montbéliard, France.

Program and abstracts

Editors : Daniel Gilbert, Université de Franche-Comté and Edward Mitchell, Université de Neuchâtel

5th International Symposium on Testate Amoebae

Welcome to Montbéliard!

We would like to welcome you to Monbéliard for the 5th International Symposium on Testate Amoebae. We are especially pleased by the diversity of people and contributions we will have at this meeting.

Research on testate amoebae has been increasing exponentially in the past decade. This is due largely to the fact that the analysis of testate amoebae is now a common tool in palaeoecological studies. Recent research has also shown that testate amoebae play a very important role in the cycling of elements in terrestrial ecosystems and potentially interact closely with vascular plants in the rhizosphere. Molecular methods are finally becoming available to study the phylogeny and taxonomy of testate amoebae, and help resolve long-standing debates on their diversity and biogeography.

Testate amoebae are also ideal model organisms for research on microbial biodiversity and biogeography, and many other topics. This includes applied aspects: testate amoebae are indeed increasingly used in ecotoxicology and biomonitoring; the potential range of uses of these organisms is continuously expanding and may soon include applications such as forensic science!

The different fields of research related to testate amoebae are all to some degree interconnected and a meeting such as this one is an ideal opportunity for researchers to interact with colleagues specialised on different aspects of these fascinating and beautiful organisms.

The conference is centred around testate amoebae, but the topics are of interest to a very wide range of researchers: protozoologists working on other groups, microbial ecologists, soil ecologists, palaeoecologists, managers, etc.,.

This symposium covers a broad range of topics related to testate amoebae:

- Biology, Physiology & Ecology
  We still know surprisingly little about the basic biology and physiology of testate amoebae. How many of us have been slightly embarrassed not to be able to answer simple questions such as: do testate amoebae have sex? Knowledge on the ecology of testate amoebae is currently strongly biased towards the study of peatlands; much remains to be learned about other ecosystems and most of what is know is based on descriptive studies. Clearly there is much room left for future work!
- Biogeography & Biodiversity
  Testate amoebae are ideal organisms for the study of microbial biogeography and biodiversity. Here too our knowledge is very

fragmentary but several groups are working hard to accumulate better data to clarify the debate. This topic is closely related to the next one.

- Phylogeny & Taxonomy: Morphology vs Molecules
  In the last decade molecular data on testate amoebae has started to accumulate. This, combined with improved biometry based on light and electron microscopy is causing a true revolution in our perception of testate amoeba diversity and taxonomy. The journey has only started and the ride may be rough at times, but we can be certain that the picture will be quite different in 10 years!
- Palaeoecology (peatlands and lakes)
  Palaeoecology has been and still is a leading door through which people working on testate amoebae discover these organisms (this is true for one of us - EM). The value of testate amoebae as indicators of past peatland hydrology in turn calls for improved taxonomy, understanding of dispersal potential, and of environmental controls on community assembly.
- Testate Amoebae and Bioindication
  Sky is the limit! Starting from surface moisture indicators in peatlands and trophy indicators in lakes, testate amoebae are now used or their potential use explored as bioindicators of water, soil and air pollution, cadaver decomposition, soil functioning. It seems that the potential range of applications of these organisms has no limits!

We hope you will have a wonderful time in Montbéliard, that you will find the meeting stimulating, meet new colleagues, exchange views and return home with many new research ideas, which we will be interested to learn about at ISTA-6!

Daniel Gilbert & Edward Mitchell

Acknowledgements

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PROGRAM OF THE CONFERENCES

Please note that smoking inside the building is strictly prohibited

Monday, September 14th 2009

11:00 14:00 Reception of participants, CAPM Building

14:00 14:10 Opening words:
CAPM, University of Franche-Comté, Daniel Gilbert & Edward Mitchell

Biology, Physiology & Ecology (I) Chairman : Edward Mitchell

14:10 14:50 Keynote talk Louis Beyens

Global change and biodiversity: an arctic view

14:50 15:10 contributed talk Andrey Tsyganov

The response of soil testate amoebae assemblages to experimental warming over the whole growing season in high-arctic tundra (Zackenberg, Northeast Greenland)

15:10 15:30 contributed talk Vincent Jassey

Impact of elevated temperature on Testate Amoebae in *Sphagnum fallax* peatland: a microcosm study and perspectives in situ

15:30 15:50 contributed talk Lucasz Lamentowicz

New insights on testate amoebae ecology in minerotrophic peatlands of western Poland

15:50 16:10 contributed talk Irina Kurina

Ecology of testate amoebae (Protozoa: Rhizopoda) in peatlands in the middle taiga of Western Siberia

16:10 16:40 Break

Biology, Physiology & Ecology (II) **Chairman : Daniel Gilbert**

16:40 17:00 contributed talk Martin Vohnik

Testate amoebae vs. fungi: some lessons from damp chambers with pine litter

17:00 17:20 contributed talk Anatoly Bobrov

Phenotypical variability, its reasons and the problem species concept in testate amoebae

17:20 17:40 contributed talk Armynot du Chatelet

Sediment size and mineralogical composition influence on thecamoebian test's construction.

17:40 18:00 contributed talk Humphrey Smith

Environmental influences on variation within natural populations of testate amoebae

18:00 Symposium Photograph!

18:00 19:40 Poster session / icebreaking reception

Tuesday, September 15th 2009

Biogeography & Biodiversity **Chairman : Ralf Meisterfeld**

09:00 09:40 Keynote talk David Wilkinson

Testate amoebae as model organisms for studying the biogeography of free-living microorganisms.

09:40 10:00 contributed talk Francine McCarthy

Some surprising results from a biogeographic study of thecamoebians (testate amoebae) in Alberta, Canada

10:00 10:20 contributed talk Thierry Heger

Molecular data reveal low mitochondrial DNA diversity within Hyalospheniidae morphospecies (Amoebozoa: Arcellinida) from Europe and North America

10:20 10:50 Break

10:50 11:10 contributed talk Enrique Lara

Pseudocryptic speciation and long-range migration in the euglyphid testate amoeba genus *Assulina*

11:10 11:30 contributed talk Yuri Mazei

Changes of testate amoebae community structure along landscape gradient (catena) in forest-steppe region (Russia)

11:30 11:50 contributed talk Milcho Todorov

Diversity, ultrastructure, biometry and ecology of nebelids (Amoebozoa, Arcellinida, Nebelidae) from Bulgaria

11:50 13:50 Lunch break

Phylogeny & taxonomy: morphology vs molecules **Chairman : Louis Beyens**

13:50 14:30 Keynote talk Ralf Meisterfeld

Classification in testate amoebae – practise, problems and perspectives

14:30 14:50 contributed talk Daniel Lahr

Insights into the evolution of the genera *Arcella* and *Cryptodifflugia* from analyses of multiple molecular markers

14:50 15:10 contributed talk Alexander Kudryavtsev

Species diversity and phylogeny of the family Microchlamyiidae Ogden, 1985 (Amoebozoa, Arcellinida)

15:10 15:30 contributed talk Julia Török

Molecular phylogeny of different morphospecies groups of *Arcella* (Amoebozoa, Arcellinida)

15:30 16:00 Break

16:00 18:30 Identification (microscopy session)

Chairmen: Ralf Meisterfeld & Milcho Todorov

18:30 23:00 Visit of Peugeot museum and social dinner

Wednesday, September 16th 2009

Palaeoecology (peatlands and lakes) **Chairman : David Wilkinson**

09:00 09:40 Keynote talk Robert Booth

Testate amoebae as tools for the study of past climate and ecological dynamics: perspectives from peatland studies in North America.

09:40 10:00 contributed talk Jun Yang

Younger Dryas and Holocene succession of testate amoebae assemblages in relation to climatic changes from Cameron Lake, Nova Scotia, Canada

10:00 10:20 contributed talk Yangmin Qin

Testate amoebae as indicators of 20th century environmental change in Lake Zhangdu, China

10:20 10:50 Break

10:50 11:10 contributed talk Helen Roe

Controls on the contemporary distribution of lake testate amoebae within the Greater Toronto Area and their potential as water quality indicators

11:10 11:30 contributed talk Adeline Wall

Response of testate amoeba assemblages to environmental and climatic changes during the Lateglacial-Holocene transition at Lake Lautrey (Jura Mountains, eastern France)

11:30 11:50 contributed talk Richard Payne

The impact of sulphate deposition on testate amoebae in peatlands: Linked experimental and palaeoecological studies in Scotland and Alaska.

11:50 12:10 contributed talk Mariusz Lamentowicz

Last millennium high-resolution palaeoenvironmental study based on testate amoebae from a subalpine peatland: quantitative reconstructions and relations to other proxies

12:10 14:10 Lunch break

Testate Amoebae and bioindication Chairman : Robert Booth

14:10 14:30 contributed talk Dimaris Acosta-Mercado

Can different bryophyte canopy structures stimulate different communities and diversity of testate amoebae? Data from the cloud forest of Sierra de Bahoruco, Dominican Republic.

14:30 14:50 contributed talk Lisa Neville

Using thecamoebians (testate amoebae) as proxies of ecosystem health in Oil Sands impacted regions of Northeastern Alberta

14:50 15:10 contributed talk Caroline Meyer

Effect of Atmospheric Particulate Pollution (PM10) and Nitrogen Dioxide (NO_2) on Testate Amoebae Communities Living in Microsystems "Bryophytes-Microorganisms"

15:10 15:30 contributed talk Daniel Gilbert

Using Bryophyte/associated microorganism microsystems to characterize the impact of contaminants on ecosystems : are testate amoeba the best microbial group for disturbance bioindication ?

15:30 16:00 Concluding words Edward Mitchell & Daniel Gilbert

16:00 18:30 Poster session

17:30 23:00 Free evening

(dinner not included in conference package)

Thursday, September 17th 2009

Field trip to Jura Mountains

CONFERENCE ABSTRACTS

Monday

September 14th 2009

Biology, Physiology & Ecology

KEYNOTE TALK Biology, Physiology & Ecology

Global change and biodiversity: an arctic view

Louis BEYENS

Research Group Polar Ecology, Limnology and Geomorphology, Department of Biology, University of Antwerp, Universtiteitsplein 1, BE-2610 Wilrijk, Belgium

The last decennium, major research efforts are directed to understand how biota and their communities will respond to the changing climate. This question has a time and spatial dimension. As a remarkable side-effect of this question, it was realized by the main biological science community that our knowledge of ecology and geography of many organisms is far from complete, and suddenly biodiversity became again a fashionable research topic. What is living where, and why, and what rules its distribution? From the first records of testate amoebae in the pre-1900 till the present day, regions have been explored for their testate amoebae species richness, communities and ecology. Major compilations deal(t) with it on macro- and on microhabitat scale. Despite all these efforts, and the resulting amount of data, protists in general have long been excluded from ecological textbooks. Indeed, in the paradigm of protists being cosmopolitans it was also thought that their ecology and biogeography should have a different approach than for macro-organisms. Overall, it seemed that the degree of explanation for ecological and related phenomena has a linear relation with the biomass of the individual organism. So it is somewhat easier to explain why for instance a polar bear lives in the Arctic then why some micro-organisms occur there.

I have always believed that eukaryotic unicellullars as testate amoebae should also follow the main biological rules which can be detected in and were derived from the biology of macro-organisms. Already pioneers as Van Oye (1950's) discussed the possibility of testate amoebae as biogeographical indicators. So, when there is for instance an arctic avifauna or arctic vascular plant flora, why should there also not be an arctic testate amoebae fauna? If a crude estimated rate of endemic protists is around 30% (Foissner 2008), there should be one.

Climate induced shifts of biomes are already observed. Will the geographic distribution of testate amoebae communities be influenced by the global warming? Are there testate amoebae species which will behave as invasive plants or animals do? Climate change models indicate that polar regions will experience the most pronounced temperature increase. These regions have a lower biodiversity and also harbour lesser testate amoebae taxa. In which direction will this evolve under increasing warmer conditions, when the treeline will probably rapidly advance in the arctic tundra? The vast majority of morphospecies found in the Arctic are the same as encountered in mid-latitudinal regions, but there seems to be a shift in the importance of some genera, at least in aquatic habitats. Contrary to the tropics for instance, there are few endemic morphospecies in the Arctic. But there are a few taxa which to our knowledge are limited in their distribution to the Arctic, some of them can be considered as arctic flagship taxa. This raises the questions on their origin: when, where, how? A lot of these topics can not be discussed thoroughly without more knowledge on dispersal and colonisation capacities and strategies of testate amoebae in different geographic settings. Warming and transplantations experiments can help in probing the reactions of their communities. Furthermore, since an important part of the year in the polar regions the tundra is covered with snow, we have to know more on their winter ecology.

TALK Biology, Physiology & Ecology

The response of soil testate amoebae assemblages to experimental warming over the whole growing season in high-arctic tundra (Zackenberg, Northeast Greenland)

Andrey N. TSYGANOV [1], Ivan NIJS [2] & Louis BEYENS [1]

[1] Research Group Polar Ecology, Limnology and Geomorphology, Department of Biology, University of Antwerp, Universiteitsplein 1, BE-2610 Wilrijk, Belgium

[2] Research Group Plant and Vegetation Ecology, Department of Biology, University of Antwerp, Universiteitsplein 1, BE-2610 Wilrijk, Belgium

We studied the effect of the experimental warming over the whole growing season on testate amoebae assemblages at different depths in a soil in high-arctic tundra (Zackenberg, Northeast Greenland).

We used Free Air Temperature Increase (FATI) technique in order to increase soil temperature with an average 2.58 ± SD 1.11 and 2.13 ± SD 0.77 ^{o}C at the depth of 2.5 and 7.5 cm, respectively. Testate amoebae assemblages in upper (0 – 3 cm) and lower (3 – 6 cm) soil layers were analyzed separately. Analysis of variance following the logic of BACI (Before-After-Control-Impact) design of the experiment was performed to detect both sustained and short-term effects of the warming on community level indices and difference in them between soil layers. Partial redundancy analysis (RDA) was used to analyze changes in species composition.

The temperature increase resulted in a deeper active layer, while soil moisture was not significantly affected. In terms of univariate community level indices, the significant and sustained effect of the warming was only detected on the Lobose/Filose amoebae index of the thanatocoenosis. The warming prevented accumulation of lobose amoebae shells in both layers, so that the ratio of lobose to filose shells in experimental plots during the 'after' period was situated at the same level as compared to the initial state and was significantly lower than in respective controls. This was mostly caused by the negative relation of lobose species *Centropyxis aerophila* and *Centropyxis aerophila* var. *shpagnicola* to the temperature increase. The positive response to the warming was detected on *Difflugia globulus* species. Nevertheless, the species composition of both active testate amoebae assemblage and thanatocoenosis was not significantly affected by the treatment. Moreover, the warming did not cause any consistent or pulse effect on other univariate community level indices (total number of individuals, total number of species, the Shannon-Wiener diversity index, GINI evenness and the proportion of living individuals) and difference in them between upper and lower layers.

These data provide additional support for the hypothesis that terrestrial communities are relatively well buffered against short-term temperature increases during a growing season by a vegetation canopy. In spite of this, long-term climate manipulation experiments, involving the winter period, are still needed to be performed.

TALK Biology, Physiology & Ecology

Impact of elevated temperature on Testate Amoebae in *Sphagnum fallax* peatland: a microcosm study and perspectives in situ

Vincent JASSEY[1], Geneviève CHIAPUSIO, Philippe BINET, Marie-Laure TOUSSAINT & Daniel GILBERT

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Sphagnum peatlands currently represent a significant carbon sink but global warming may accelerate organic matter decomposition with a resulting increase of carbon dioxide in the atmosphere. Considering the future importance of temperature increase and the crucial role of microorganisms, little is known about the warming effect on microbial communities in *Sphagnum* peatlands at different depth. Therefore it seems important to understand how *Sphagnum* peatlands will react to higher temperatures. The objective of the project ANR PEATWARM is to use *Sphagnum* peatlands as a model for the analysis of vulnerability in a context of climate change using an experimental system that simulates *in situ* an increase in average temperature. Due to their trophic position at the end of the microbial food webs, Testate amoebae are good integrators of environmental perturbations and may be used as "early warning" of global warming.

In order to identify the relevant biomonitors of elevated temperature, a microcosm study was set up. During two month, 12 peat cores of *Sphagnum fallax* were cultivated at three temperatures (15, 20 and 25°C). The three temperatures were deliberately chosen very different to accentuate differences and to find new avenues of work *in situ*. After two months, *S. fallax* mosses were sampled and cut in two parts: 0-3 and 3-6 cm of the capitulum. Testate amoebae were counted and identified by inverted microscopy at 200 and 400x magnification.

The carbon biomass of Testate amoebae increases with elevated temperature in upper parts (82.3 to 588.2 µgC.cm^{-2}; Fig.1) while in lower parts, the carbon biomass do not change (an average of 150 µgC.cm^{-2}). However, changes in community structure in both parts of *Sphagnum* were observed with notably the dominance of *Hyalosphaenia papilio*.

Figure 1: Carbon biomass of Testate Amoebae (µgC.cm^{-2}) after two growing month at three treatments (15, 20 and 25°C). MANOVA test $P < 0.001$.

Thus this study has identified warming markers among testate amoebae community and has also revealed a reaction of the community between 15 and 20°C. Therefore it is appropriate to monitor these indicators *in situ* in a moderate temperature increase (2-4°C).

TALK Biology, Physiology & Ecology

New insights on testate amoebae ecology in minerotrophic peatlands of western Poland

Łukasz LAMENTOWICZ[1], Maciej GĄBKA [1] & Mariusz LAMENTOWICZ[2,3,4,5]

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[3] Swiss Federal Research Institute WSL, Ecosystem Boundaries Research Unit, Wetlands Research Group, Station 2, CH–1015 Lausanne, Switzerland
[4] Ecole Polytechnique Fédérale de Lausanne (EPFL), Laboratoire des Systèmes Ecologiques, Station 2, CH–1015 Lausanne, Switzerland
[5] Laboratory of Soil Biology, Institute of Biology, University of Neuchâtel, CH–2000 Neuchâtel, Switzerland

There is a growing interest to better understand the ecology of testate amoebae in possibly widest spectrum of environmental conditions. Testate amoebae species ecology and diversity in the *Sphagnum* peatlands is better known than in minerotrophic peatlands. Also taxonomy and diversity of testate amoebae inhabiting fens is more complicated, that makes this work novel and challenging. Furthermore, relationships between aboveground and belowground communities in fens remain still underexploited.

We had an opportunity to investigate those relations using testate amoebae, vegetation and several hydrochemical and physical variables. Minerotrophic, brown moss dominated peatlands (from poor fens to extremely rich fens) in western Poland were sampled. Several samples were also taken from *Sphagnum* to track testate amoebae diversity along poor-rich gradient. We analysed 60 surface samples, where testate amoebae and vegetation were supported by the hydrochemistry. Non Metric Multidimensional Scaling (NMDS) and Multiple Factor Analysis (MFA) was applied to explore relationships between testate amoebae communities, vegetation and environmental parameters i.e. depth of water table (DWT), pH, conductivity, Ca, Mg, temperature, O_2, NH_3, NH_4, Fe, Na, K, SO_4 and DOC.

Results showed that minerotrophic habitats with the brown mosses (especially rich fens) were characterized by a higher number of the testate amoebae species than other microsites. Testate amoebae communities seem to be more related to hydrochemistry than to the vegetation composition.

Such result confirms our former assumptions about importance of water chemistry for testate amoebae in fen's environment that were based on similar type of peatlands but located in Swiss Alps. Next step is to assess a significance of the particular environmental parameters. Scanning Electron Microscope (SEM) pictures were taken to confirm light microscope identification of several more difficult taxa. Obtained data will also be used in further palaeoecological reconstructions from minerotrophic peatlands.

TALK Biology, Physiology & Ecology

Ecology of testate amoebae (Protozoa: Rhizopoda) in peatlands in the middle taiga of Western Siberia

Irina KURINA & Yulia PREIS

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We present new ecological data of relationship between testate amoebae communities and hydrological conditions, trophic level and pH in oligotrophic and meso-eutrophic peatlands in the middle taiga of Western Siberia.

We used the following methods. Testate amoebae were analysed in water suspension with glycerol at magnifications x100 and x400. Hydrological conditions were expressed through the index of watering, which was calculated according to botanical composition of a sample. Trophic level was defined through the ash percentage, pH was measured in water drawn from the substrate.

We obtained the following results. A total of 140 testate amoebae taxa were recorded. Hydrological conditions were shown as the most important factor, which determined the composition and structure of testate amoeba communities (canonical correspondent analysis). Species optimum (weighted average) and tolerance (standard deviation) were calculated for the investigated environmental parameters. All the biotopes were divided into five groups according to distinctions in water-mineral regime: oligotrophic moss hummocks, carpets and bogs, moss and grass fens. These groups differed in composition and structure of testate amoebae communities.

We reach the following conclusions. Our results are consistent with studies of testate amoebae ecology in other regions, they provide additional support for application of these organisms in palaeoecological and biomonitoring research as indicators of water-mineral regime.

TALK Biology, Physiology & Ecology

Testate amoebae vs. fungi: some lessons from damp chambers with pine litter

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In all terrestrial ecosystems, testate amoebae (TA) encounter fungi. There are strong indications that both groups engage in multiple interactions, including mycophagy and decomposition of TA shells, the processes which might be fundamental in nutrient cycling in certain ecosystems. TA often colonize plant litter, the most significant carbon source for plethora of terrestrial heterotrophic organisms, which is primarily decomposed by saprophytic fungi. Here, we present some observations related to TA, obtained during cultivation of saprotrophic fungi colonizing Scots pine (*Pinus sylvestris* L.) litter needles.

The needles were collected from the forest floor in a temperate pine forest, placed in damp chambers (glass Petri dishes with plastic grid overlaid by a moistened filter paper) and cultivated at room temperature and ambient light. In few weeks, a dark septate non-sporulating mycelium started to grow out of some needles, covering the filter paper with a dense mycelial mat. Simultaneously, *Phryganella acropodia* (Hertwig & Lesser) Hopkinson started to reproduce in the chambers with the mycelium.

These observations led us to the following hypotheses: 1) the TA community was more diverse on needles colonized by numerous fungi, than on the filter paper dominantly colonized by the dark mycelium; 2) the dark mycelium determined the distribution of the *P. acropodia* shells; and 3) *P. acropodia* fed on hyphal exudates or bacteria associated with hyphal exudates rather than thick-walled melanised hyphae. To test these hypotheses, we 1) compared the TA spectra on the needles and pieces of the paper colonized by the dark mycelium, randomly dissected along the needles; 2) measured the distances of the *P. acropodia* shells from the dark mycelium and compared them with dots, which were randomly dislocated over the paper using graphic software; and 3) using scanning electron microscopy, we were searching for signs of grazing or digestion on the surface of the dark mycelium in the vicinity of the shells.

In our opinion, all three hypotheses were corroborated: 1) the TA spectrum on the needles (10 genera) was higher than on the filter paper (5 genera); 2) the distances of the *P. acropodia* shells from the dark mycelium were significantly smaller that the distances of the random dots; and 3) there were no signs of grazing/digestion of the hyphae in the vicinity of *P. acropodia* shells attached to the mycelium.

This simple experiment shows that TA might use nutrients from coniferous litter, most likely indirectly through mycelium of saprotrophic fungi; diversity and distribution of fungi colonizing litter might affect diversity and distribution of co-occurring TA; and hyphal exudates or bacteria associating with hyphal exudates might represent an important part of the TA nutrition.

A side lesson: the fungal genus *Pochonia* encompasses well-known nematophagous parasites; conidiophores of *Pochonia bulbillosa* (Gams & Malla) Zare & Gams occurred in our damp chambers mostly in association with *P. acropodia* shells. Do TA have their fungal parasites as well?

TALK Biology, Physiology & Ecology

Phenotypical variability, its reasons and the problem species concept in testate amoebae

Anatoly BOBROV

Faculty of Soil Science, Moscow State University, Vorob'evi Gori, Moscow, Russia, 119991

Exogenous and endogenous factors are identified as the two basic causes of phenotypic variability. Different types of morphological variability of the testate amoebae are discussed. It is noted that observed variability depends on ecological conditions. The concept of ecological (biological) optimum in testate amoebae and the phenotypic completeness of the manifestation of form under different ecological conditions are presented. This raises the problem of validity and completeness of early original species descriptions and the need for the revision of specific and ancestral descriptions. These ideas illustrated by the examples of high morphological variability of testate amoebae in Mesoamerica, one of the centres of high taxonomic diversity. The problem of convergent evolution of phenotypic characteristics in testate amoebae, the problem of species-doubles, dependence of the manifestation of phenotypic variability on the types of their multiplication was discussed. The concept of "logical field of phenotypic variety" for testate amoebae is proposed.

TALK Biology, Physiology & Ecology

Sediment size and mineralogical composition influence on thecamoebian test's construction.

Eric Armynot DU CHATELET[1] & Nicolas TRIBOVILLARD[1]

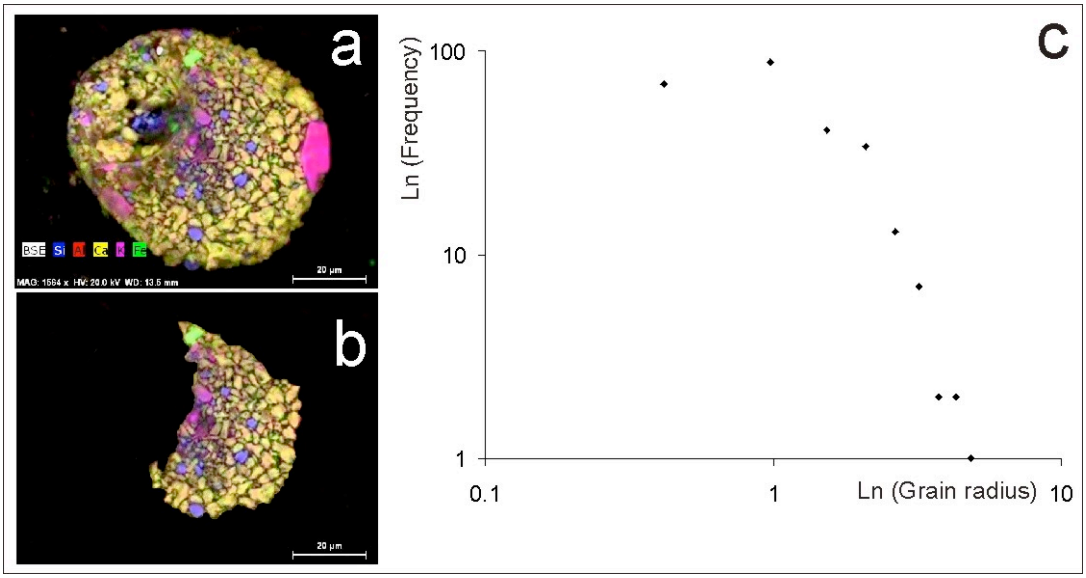
1- Université Lille 1, Laboratoire Géosystèmes (UMR 8157 CNRS), UFR des Sciences de la Terre – bât. SN5, 59655 Villeneuve d'Ascq cedex, France

Thecamoebians are inhabitant of numerous and various continental environment. Hence they are more and more widely used as a tool for past environment reconstructions. Agglutinated thecamoebians use grains from their surrounding environment to construct their tests. In this study we investigate the influence of sediment grain size and mineralogical composition on the test surface structure. This study, with its preliminary results, aims to answer the question: does sedimentary environment have an influence on thecamoebian assemblage that could be a serious bias in past environment reconstruction?

Punctual sampling had been carried out in three lakes in French Alps: Annecy, Anterne, and Gers. These lakes were chosen because of their different bulk mineralogical composition: Annecy is dominated by carbonate, Anterne is dominated by quartz and Gers is dominated by potash feldspar. The cartographical analyses on surface thecamoebians were carried out using an Environmental Scanning Electron Microscope (ESEM) using an Energy Dispersive X-ray Spectroscopy (EDS). The proportion of each mineral was then evaluated by image analysis. The results were compared with similar analysis carried out on surroundings sediments.

The mineralogical analysis show that contrarily to other protists that could have very close way of test building and may chose their own minerals (Agglutinated benthic foraminifera (Armynot Du Chatelet et al., 2008)), mineralogical composition of thecamoebians reflects closely the one of the surroundings, suggesting very little mineralogical discrimination.

By contrast, sediment grain-size seems to be a limiting proxy as no big or very small grains from the surrounding sediment are present in the test. The grain size distribution in the test is log-normal.



Selected specimen from Annecy lake: a) ESEM-EDS analysis of *Centropyxis constricta*. b) Selected zone for grain size and mineralogical composition analysis. c) log-normal grain distribution from this selected area.

The log-normal grain distribution from external area of all analyzed species (*Centropyxis aculeata*, *C. constricta*, *Difflugia oblonga*, *D. corona*) suggests that the agglutinated thecamoebian produce carefully constructed test but are using indiscriminately any mineral from their surroundings. These results suggest that thecamoebians are not influenced by their mineralogical context and could then be used for past environment (climatic!) reconstruction.

Armynot Du Chatelet, E., Recourt, P., Chopin, V., 2008. Mineralogy of agglutinated benthic foraminifera; implications for paleo-environmental reconstructions. Bulletin de la société géologique de France 179, 583-593.

TALK Biology, Physiology & Ecology

Environmental influences on variation within natural populations of testate amoebae

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The influences of environmental factors, other than moisture, upon testate dimensions, distribution and abundance are still poorly known. Elucidation of these is important for our understanding of adaptation to terrestrial environments, to inform the biogeographical debate, and to anticipate the biological effects of climatic change.

A brief review is presented of existing knowledge from biogeographical records, laboratory experiments on clonal cultures and seasonally monitored populations. New data are presented on the variation in dimensions of natural populations in terrestrial habitats on South Georgia and the South Orkney Islands, Antarctic zone. An initial hypothesis that temperature might have a direct effect upon test dimensions (as has been shown in *in vitro* cultures) has not been detected. However, there are strong negative correlations between test dimensions and population specific growth rates for *Phryganella acropodia* and *Phryganella paradoxa* – possibly related to these species' obligate agglutinate test construction. Weaker correlations, or none at all, are apparent for *Centropyxis aculeata, Centropyxis aerophila, Assulina muscorum* and *Corythion dubium.*

Tuesday

September 15th 2009

Biogeography & Biodiversity

KEYNOTE TALK Biogeography & Biodiversity

Testate amoebae as model organisms for studying the biogeography of free-living microorganisms.

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Free-living microorganisms are fundamental to most major ecological processes however we still know surprisingly little about their biogeography – and by implication their biodiversity. This is illustrated by latitudinal patterns in diversity. In most groups of macroorganisms there is a well known pattern of higher taxon richness in the tropics - however for the majority of groups of free living microbes (including testates) it is still unclear if there is a tropical peak in taxon richness! Over the last 20 years there has been a significant debate within microbial ecology on the extent to which free-living microbes have cosmopolitan distributions – with some ciliate biologists arguing that effectively all free living microbes are cosmopolitan. The outcome of this debate has large implications for understanding microbial biogeography and biodiversity and hence our understanding of the workings of the Earth System.

Testate amoebae make very useful model organisms for studying such patterns. They are often unusually large by the standards of many microbes, so presumably more likely to show dispersal limitation than smaller microbial taxa. In addition their morpho-species are usually easier to identify than those from many other microbial groups – leading to fewer ambiguities in the literature about taxon identifications so allowing provisional large scale data sets to be assembled from the literature (note: its not that there are no problems with morpho-species identifications in testates, but just that these are more limited than in many other microbial groups!). In addition it is usually easier to assign size data to testates than to many other protist groups – making it easier to ask questions about the potential effect of size on distribution.

I will argue that, while many testate morpho-species are cosmopolitan, the group shows unambiguous examples of taxa that are more limited in their distribution. There is also some evidence that smaller testates are more likely to be cosmopolitan than the larger morpho-species (with non-cosmopolitan distributions becoming increasingly likely over the size range of 20 – 150 μm). This is consistent with recent (as yet unpublished) modelling work showing that probability of widespread distribution by atmospheric circulation declines at microbial sizes or around 20-30 μm.. I also briefly discuss the potential role of humans in the biogeography of free living microbes. Although the role of humans has been discussed extensively in the context of aquatic (marine and fresh water) microbes there is almost nothing in the literature on the potential importance of humans for soil-living microbes. I also briefly speculate on how the growing amount of molecular data may alter my biogeographical conclusions, which are based on a classic morpho-species approach.

TALK Biogeography & Biodiversity

Some surprising results from a biogeographic study of thecamoebians (testate amoebae) in Alberta, Canada

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Thecamoebians (testate amoebae) were analyzed from twelve lakes along a transect through Alberta in five drainage basins and four vegetation zones, and representing a variety of environmental and limnological parameters. Climate, as reflected in the vegetation zones, appears to exert the greatest control on species and strain distributions, although multiple sites sampled within a single lake at different times yielded substantial differences. Lake chemistry (e.g. hardness and concentrations of Na^+, K^+) exerts an important secondary factor. Conductivity, DO at the sediment/water interface, water temperature, and pH had surprisingly little influence on the composition and diversity of thecamoebian assemblages.

The most surprising finding was that natural lakes in Alberta harbour lower diversity assemblages than lakes and wetlands constructed by Oil Sands companies to remediate process-affected water. This, together with the apparently selective incorporation of bitumen into thecamoebian tests, suggests that the by-products of oil sands extraction are a net resource exploited by these protists, at least below threshold levels of naphthenic acids and conductivity. Because relatively little is known regarding the biogeography and ecology of these ubiquitous organisms, other surprises will undoubtedly be uncovered before a clear understanding of the controls on their distribution emerges. One of the difficulties compounding such studies is the lack of standardization of methodology and taxonomy, however.

TALK Biogeography & Biodiversity

Molecular data reveal low mitochondrial DNA diversity within Hyalospheniidae morphospecies (Amoebozoa: Arcellinida) from Europe and North America

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The biogeography of microorganisms is of great importance to the use of testate amoebae as paleobioindicators on a global scale and for diversity estimations. There is a controversial debate whether free-living protists are cosmopolitan or whether at least some species have limited geographical distributions. An often-cited example of the latter case is a terrestrial testate amoeba of the Arcellinida group, *Apodera vas*, which has thus far only been found in the Southern Hemisphere and parts of the northern tropics. In contrast, most other species of the Arcellinida group are thought to have a cosmopolitan distribution. However, the geographical distributions of testate amoeba have only been studied within the context of morphological species descriptions. Such morphological observations must be viewed with caution because of the potential existence of cryptic and pseudo-cryptic species.

Here, we used partial sequences of the mitochondrial Cytochrome *c* Oxidase Subunit 1 gene to investigate the genetic diversity within three well characterized Hyalosphoeniidae morphospecies (*Hyalosphenia papilio, Nebela carinata* and *Nebela marginata*) of distant oligotrophic peat-bogs in Europe and North America. Our results show very little genetic variation within each morphospecies even between geographically very distant sites, with identical sequences occurring at European and American sites.

These data support the interpretation that comparatively large free-living microbes (e.g., *H. papilio* >100 µm*, N. carinata* >140 µm and *N. marginata* >140 µm) can have unexpectedly wide geographical distributions.

TALK Biogeography & Biodiversity

Pseudocryptic speciation and long-range migration in the euglyphid testate amoeba genus *Assulina*

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Testate amoebae from genus *Assulina* are commonly used as bioindicators to infer past water table levels in peat bogs. Researchers generally recognise three morphospecies based on the size of their shell: *A. muscorum* (40-50µm), *A. seminulum* (70-80µm) and *A. scandinavica* (>100µm).

Sequencing of the COI gene, a mitochondrial marker commonly used for genetic barcoding of metazoans, and careful scanning electron microscopic imaging revealed the presence of at least two genetically distant species within *A. muscorum* and three within *A. seminulum*. The size and shape of the scales appears to be the most important criterion for the discrimination of the species. Furthermore, our studies showed the presence of identical COI genotypes within *A. seminulum* in Switzerland, Sweden, Poland and Estonia, thus revealing that this species has the potential to disperse at least at the scale of the western half of the European continent.

TALK Biogeography & Biodiversity

Changes of testate amoebae community structure along landscape gradient (catena) in forest-steppe region (Russia)

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We present new data on species composition and community structure of testate amoebae along landscape gradient (catena) in the forest-steppe native zone (Middle Volga region, Russia).

Studies were conducted during July 2007 in Ostrovtsovskaya forest-steppe (Middle Volga Territory) along the catena that is disposed in the landscape slope (from 198 till 179 meters above-sea level) of south-west exposition. There are 5 plant associations along the catena: steppe herb meadow is situated on a watershed (eluvial landscape), blackthorn is on a gently slope, aspen forest is on a steep slope (transit landscapes), alder forest and osier-bed are on a gully (accumulative landscape). There are typical black earth in the watershed and gently slope, black earth podzolized in the steep slope and sod-gley soil in the gully.

Cyclopyxis kahli and *Centropyxis aerophila sphagnicola* are the most common and abundant species in all positions along the catena. They are the dominant species on eluvial (28.9%) and transit (27.9%) landscapes. Within the accumulative landscape there are set of hydrophilous species (*Difflugia pyriformis* and *Quadrulella symmetrica* in the alder forest) as well as hygrophilous species (*Centropyxis sylvatica* and *Cyclopyxis eurystoma* in the osier-bed) predominate.

Along the catena species richness is increases from 5–7 species in xerophytic steppe meadow and blackthorn to 13–14 species in forests. Abundance is also increases in the same direction: from 80–320 ind. per gram of absolutely dry soil on watershed till 900–1550 in the gully.

The work was supported by the Russian Foundation for Basic Research (grant № 07-04-00185a).

TALK Biogeography & Biodiversity

Diversity, ultrastructure, biometry and ecology of nebelids (Amoebozoa, Arcellinida, Nebelidae) from Bulgaria

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Taxonomical, ultramorphological, biometrical and ecological data for 25 nebelids, found till now in different habitats from Bulgaria are presented. New ultra-morphological and biometrical data for 19 of them are provided. Six of the previously recorded from Bulgaria species have not been found in our study and because of that ultra-morphological and biometrical data for them are not presented. One of the studied species (*Nebela golemanskyi* sp. nov.) showed great peculiarities and was described as a new species.

Some ecological preferences in the distribution of the found species are pointed out. According to their preferable habitats, the next four characteristic ecological groups of nebelids were established:

- Sphagnicolous species frequently occurring and dominating in the *Sphagnum* peatlands (*N. speciosa, N. galeata, N. tubulosa, N. (Argynnia) vitraea, N. (Argynnia) dentistoma, N. bohemica, N. penardiana*);
- Inhabitants of the litter in the beech forests (*N. collaris, N. (Porosia) bigibbosa, N. wailesi* and *N. (Argynnia) dentistoma*);
- Aerophilic species inhabiting drier environments - mostly soil- and epiphytic mosses (*N. tincta, N. parvula* and *N. minor*);
- Characteristic freshwater inhabitants (*N. (Argynnia) bipes*).

Our study shows that nebelids are widely distributed in different habitats from Bulgaria, but there are some peculiarities in their composition, compared to the nebelids distributed in the Central and West European countries. For instance, some species which are usual and frequent inhabitants of the *Sphagnum* peatlands in the Central and West European countries in Bulgaria are entirely absent (*N. (Physochila) tenella, N. (Physochila) griseola*) or they are very rare (*N. flabellulum, N. carinata, N. marginata*). Besides, in the distribution of *N. (Porosia) bigibbosa* is established a clear preference to the litter of beech forests, where this species is frequently occurring. It was occasionally found in the litter of mixed deciduous-coniferous or coniferous forests, but always with a low population density. It is noteworthy that *N. (Porosia) bigibbosa* have never been found in *Sphagnum* environments from Bulgaria.

The morphology and ultrastructure of testate amoebae were studied by scanning electron microscope JEOL JSM-5510. Statistical analyses were performed using the computer program STATISTICA, version 7.0.

Phylogeny & taxonomy: morphology vs molecules

KEYNOTE TALK Phylogeny & taxonomy: morphology vs. molecules

Classification in testate amoebae – practise, problems and perspectives

Ralf MEISTERFELD

RWTH Aachen University, Institute for Biology II, Unit of Cellular Neurobionics

Low as well as high level classification of testate amoebae is still mainly based on characters of the test. Besides several practical advantages of such an approach there are also serious drawbacks.

One problem that is rarely considered is the influence of building materials on the overall shape of agglutinate tests. The biometry of the test is a very useful tool to distinguish between closely related species, but even clones can have significantly different size classes if the number of nuclei is different! In extremes we have discontinuous size distributions!

Other factors that influences test size are food quality and quantity or the water content of the substrate.

The perhaps biggest problems are the so called species complexes. These complexes are often locally common, abundant and they often seem to have a global distribution.

In many cases we do not have clear species boundaries, we have perhaps adaptive peaks and sometimes more intermediate than typical specimens. A consequence of this uncertainty has been the description of many species, subspecies, varieties etc.

The question is whether molecular characters can structure the species complexes and can help to evaluate the usefulness of morphological characters for low level classification.

We have studied different species-complexes of the Euglyphidae, Trinematidae and Arcellinidae with LM, SEM and additionally the SSU genes, internal transcribed spacers (ITS1) and in *Arcella* the cytochrome c oxidase gene (CO1).

Sequence variation in Arcellinida is usually higher than in Euglyphida and can be as high as 4% within morphospecies. Due to many introns ITS1 sequences show a large length variation (up to 100% within genera). Especially in *Trinema* paralogous sequences are common. These are features that make a reliable alignment impossible. More promising is the CO1 gene. It is possible to amplify it from single cells. So far all sequences have an almost similar length and can be aligned easily. Sequence variation within and between species is high enough to analyse difficult species-complexes.

Molecular methods expand the possibility to improve the classification of testate amoeba dramatically. But unfortunately, there is no simple correspondence between morphological and molecular characters. Many of the new clades and sequences cannot be assigned to existing species!

The global as well as the local genetic diversity is higher than expected. Collective morpho-species only **seem** to have a global distribution.

Keeping in mind the ecologists and paleoecologists who are the main users, a low level classification of testate amoeba that is useful requires a **link to test morphology**. **Therefore the question cannot be molecular versus morphological taxonomy but a combination of both**.

TALK Phylogeny & taxonomy: morphology vs. molecules

Insights into the evolution of the genera *Arcella* and *Cryptodifflugia* from analyses of multiple molecular markers

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Multigene analyses are essential for resolving the phylogenetic position of microbial eukaryotes. To this end, we are characterizing SSU-rDNA and multiple protein-coding genes from three species of testate amoebae: *Arcella hemisphaerica*, *Arcella vulgaris* and *Cryptodifflugia operculata*. Analyses of SSU-rDNA with this broader taxon sampling corroborate the monophyly of Arcellinida and indicate that evolutionary relationships are different than previously predicted from test morphology. Further, we characterized multiple divergent paralogs in surveys of actin, one of the most abundant eukaryotic proteins. We find over 30 distinct haplotypes in *Arcella* spp. that vary up to 25% in the nucleotide sequence. However, most haplotypes retain the same amino-acid sequence. Some paralogs were determined to be recombinants of each other based on analyses with recombination detection software. We used maximum likelihood methods to reconstruct the evolution of actin gene families including outgroups such as *Dictyostelium discoideum*. We suggest that this gene family evolves by concerted evolution following a birth-death process, given that we find high conservation in amino-acid sequences and assortment of paralogs between closely related populations. Furthermore, we have detected multiple actin paralogs in *Cryptodifflugia operculata* that group separately from the ones in the genus *Arcella*, and independently from the actin family expansion in *Dictyostelium*. These data suggest that extensive expansion of the actin gene family happened independently within subsets of the Arcellinida.

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TALK Phylogeny & taxonomy: morphology vs. molecules

Species diversity and phylogeny of the family Microchlamyiidae Ogden, 1985 (Amoebozoa, Arcellinida)

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The family Microchlamyiidae is a widely distributed group of testate amoebae with a flexible organic test composed of an alveolate dorsal part and a membranous ventral part. Members of this family have been found in freshwater, some brackish water, and some soil habitats worldwide. Since the description of the first microchlamyiid 150 years ago, the family was considered to be containing only one genus *Microchlamys* with two species described in detail (*Microchlamys patella* and *M. sylvatica*). However, the results of our study during recent years have led to an establishment of the second genus *Spumochlamys* containing now three described species (*Spumochlamys iliensis*, *S. perforata* and *S. bryora*). In addition, we have recently isolated two new freshwater microchlamyiids, showing that the diversity of the family has been largely underestimated.

The genera *Microchlamys* and *Spumochlamys* differ from each other in the relationships between the cell body and the test. In the former genus the cell body is separated from the test with the thin flexible membrane forming also the ventral part of the test and an aperture, while in the latter, the cell body is directly attached to the dorsal part of the test that directly continues into the membranous ventral part. Different microchlamyiid species are often hardly distinguishable with the light microscopy, however they clearly differ from each other in the relief of the dorsal test surface revealed by scanning electron microscopy, and this can be a useful tool for species identification within the family.

Molecular phylogenetic analysis based on small-subunit ribosomal RNA gene sequences of four microchlamyiid species (*S. bryora*, *S. perforata* and two new species) places the Microchlamyiidae within the Arcellinida, but their relationships with other testate amoebae are poorly resolved due to the very high divergence of the microchlamyiid sequences, and the lack of close relatives in the currently available arcellinid sequence set. Based on morphology and gene sequence analysis, one of the studied new species is a good candidate for the inclusion into a new genus. In the phylogenetic trees it branches as a sister group to the *Spumochlamys* spp., however the support for this position is weak, and the distance between the sequences is large. At the microscopical level, this species differs from the others in having a peripheraly located granular nucleus, while others have a vesicular nucleus.

The presented data clearly indicate, that the Microchlamyiidae may still contain a lot of yet undescribed species awaiting discovery. Being easy to isolate and grow in a pure clonal culture, these amoebae may be an excellent model for studying the biology of arcellinids, biogeographical distribution and the species problem using both, morphological and molecular approaches.

This study was partially supported with the DAAD fellowships A/05/00103 and A/07/09265 to AK, and the SCOPES cooperative grant from the Swiss National Science Foundation, project IB73A0-111064 to JP.

TALK Phylogeny & taxonomy: morphology vs. molecules

Molecular phylogeny of different morphospecies groups of *Arcella* (Amoebozoa, Arcellinida)

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Arcella EHRENBERG, 1832 has been a favoured research object, due to its relatively large size, unique shell structure and relative ease of culturing. As data increase on lobosean molecular phylogeny, the placing of this well known genus on the phylogenetic tree became of great importance. Furthermore, upon the discovery of phylogenetic relationships among certain *Arcella* species the traditional morphological frames could be validated or abandoned.

Using cultured and environmental samples, partial 18S rRNA gene sequences of various length were amplified by PCR using universal and self constructed primers in order to place the genus on the lobosean tree and get a primary insight into the relative position of morphologically distant or similar species of the genus.

A. rotundata, A. excavata, A. hemisphaerica, A. gibbosa, A. formosa, A. discoides and *A. dentata* have been successfully processed. They unambiguously form a distinct group among the testate loboseans, within the Tubulina. *A. hemisphaerica* shows the closest similarity with the only published sequence of the species (Tekle & al. 2008). Generic identity of *Arcella artocrea* in Nikolaev & al. 2005 seems to be doubtful, since its consequent grouping with *Centropyxis laevigata*, remarkably distant from the *Arcellas*.

Wednesday

September 16th 2009

Palaeoecology (peatlands and lakes)

KEYNOTE TALK Paleoecology

Testate amoebae as tools for the study of past climate and ecological dynamics: perspectives from peatland studies in North America.

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Testate amoebae have been increasingly used as tools in paleoenvironmental research, with recent studies providing detailed reconstructions of past climate and ecological variability. Relationships between environmental conditions and community composition form the basis of these studies, and species-environment relationships have been examined and modeled within a variety of depositional environments. For example, in oligotrophic peatlands the composition of testate amoeba communities is related to surface-moisture conditions, allowing the reconstruction of past changes in water-table depth from subfossil assemblages. Significant progress has been made in recent decades in quantitatively reconstructing past moisture variability using testate amoebae, including the development of transfer functions from many previously understudied regions and the ability to infer changes in mean annual water-table depth.

In this presentation, I describe recent research on the use of testate amoebae as paleohydrological indicators, drawing primarily from ongoing efforts in North American peatlands. These efforts include the development of a large modern calibration dataset ($n>1000$) for paleohydrological inference, studies focused on the role of seasonal moisture variability in structuring testate amoeba communities, investigations into the potential impact of taphonomy and threshold effects on water-table depth reconstructions, and the development of a network of testate amoeba-based records of late Holocene moisture variability. Through the use of these and other examples, my primary objectives will be to 1) detail new developments and remaining challenges in the use of testate amoebae in paleohydrological inference, 2) highlight recent applications of testate amoebae to studies of multidecadal-scale drought and ecological variability at regional-to-continental scales, and 3) outline research priorities and opportunities for international collaboration aimed at furthering our understanding of this valuable group of environmental proxies.

TALK Paleoecology

Younger Dryas and Holocene succession of testate amoebae assemblages in relation to climatic changes from Cameron Lake, Nova Scotia, Canada

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As single-celled organisms with a short regeneration time, testate amoebae respond more rapidly in terms of community structure and density to environmental changes than larger animals and plants. In this study, detailed testate amoebae records from a sediment core were obtained from Cameron Lake, Nova Scotia, Canada, to examine long-term variability in the community structure during the Younger Dryas (YD) and Holocene.

A total of 104 species and subspecies representing 26 genera were identified and the highest species number and abundance were found among the genera *Difflugia* and *Centropyxis*. Decreases in species richness and diversity were highly correlated to the YD, North Atlantic Preboreal Oscillation (PBO) and 8.2 cal kyr cooling events. Further, the most dramatic changes in species composition occurred at the YD/Holocene transition (10.0 ^{14}C kyr. B.P.) when the dominant genus abruptly shifted from *Centropyxis* to *Difflugia* mainly due to both climatic amelioration and rapid lake acidification. The middle Holocene warming and eutrophication were characterized by (1) an abrupt increase in species abundance (2280 to 6290 ind./ml), (2) an increase in the eutrophication indicator, *Difflugia limnetica* (also known as *Cucurbitella tricuspis*), and (3) a decrease in relative abundance of the oligotrophic indicator, *Centropyxis aerophila*. Interestingly, the YD reversal and 8.2 cal kyr cooling events were clearly recorded by testate amoebae, although they did not cause marked changes in the terrestrial vegetation.

These results provide evidence that testate amoebae have wider potential application as climate proxy, in addition to indicators of local paleolimnological changes.

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TALK Paleoecology

Testate amoebae as indicators of 20th century environmental change in Lake Zhangdu, China

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We investigated the response of lacustrine testate amoeba communities to 20th century environmental changes in Lake Zhangdu, China. The lake and surrounding region have undergone dramatic changes in the past century, including the expansion of agricultural and industrial activities, and associated hydrological modifications that led to the isolation of the lake from Yangtze River.

We assessed the potential effects of these activities on testate amoeba communities of the lake by examining subfossil assemblages in contiguous samples along a 35-cm long, ^{210}Pb dated sediment core. A total of 25 testate amoeba taxa belonging to 4 genera (*Difflugia*, *Centropyxis, Neztelia*, and *Pentagonia*) were encountered in the core. The largest change in the composition of testate amoeba communities occurred in the 1960s, coincident with the expansion of human activities in the watershed. Testate amoeba communities shifted from species characteristic of relatively oligotrophic lakes (e.g. *Difflugia biwae, D. tuberspinifera* and *D. pristis*) to species more common to mesotrophic and eutrophic systems (e.g. *Difflugia oblonga*, *D. corona, D. smilion* and *D. lanceolata*).

Our results provide valuable baseline data on testate amoebae and water conditions before and after major 20th century human impact on the lake ecosystem. Lake Zhangdu was recently reconnected with the Yangtze River as part of ongoing conservation efforts, and future monitoring of testate amoebae communities could be used to inform management and assess restoration success.

TALK Paleoecology

Controls on the contemporary distribution of lake testate amoebae within the Greater Toronto Area and their potential as water quality indicators

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Testate amoebae were examined from 71 surface sediment samples collected from 21 lakes and ponds in the Greater Toronto Area to i) elucidate the controls on faunal distribution in modern lake environments; and ii) to consider the utility of testate amoebae in quantitative studies of water quality change. This area was chosen because it includes a high density of kettle and other lakes which are threatened by urban development and where water quality has deteriorated locally as a result of contaminant inputs, particularly nutrients. Sampled lakes included a large urban pond in downtown Toronto, four kettle lakes in suburban areas and seven in more rural or 'green space' areas within urbanising regions. Samples were also collected from six Stormwater Management (SWM) ponds to provide an evaluation of thecamoebian response to metal and other common urban runoff contaminants.

Fifty eight samples yielded statistically significant testate amoebae populations. The most diverse faunas were recorded in lakes beyond the limits of urban development, although the faunas of all lakes showed signs of sub-optimal or stressed conditions. The faunas from the SWM ponds were sparse or barren. The assemblages were divided into five clusters using Q-mode cluster analysis, supported by Detrended Correspondence Analysis. Canonical Correspondence Analysis (CCA) was used to examine species-environment relationships and to examine the observed clusterings. Twenty four measured environmental variables were considered, including water property attributes (e.g. pH, conductivity, dissolved oxygen), substrate characterisitcs, sediment-based phosphorus and 11 environmentally available metals.

The thecamoebian assemblages showed a strong association with phosphorus, reflecting the eutrophic status of many of the lakes, and locally to elevated conductivity measurements which appear to reflect road salt inputs associated with winter de-icing operations. Substrate characteristics, total organic carbon and metal contaminants (particularly Cu and Mg) also influenced the faunas of some samples. A series of partial CCAs show that of the measured variables, phosphorus has the strongest influence on assemblage distribution, explaining 6.98% of the total variance. Monte-Carlo permutation tests show that this result is highly significant ($p<0.002$). The sensitivity of lake thecamoebians to eutrophication corroborates previous work based on fossil assemblages.

TALK Palaeoecology

Response of testate amoeba assemblages to environmental and climatic changes during the Lateglacial-Holocene transition at Lake Lautrey (Jura Mountains, eastern France)

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High resolution multi-proxy studies on a sediment sequence from Lake Lautrey (Jura Mountains, eastern France) gave a palaeoenvironmental data base for the present study. Thus, this sequence offered the opportunity to test the response of lacustrine testate amoebae to climate changes for the Lateglacial-Holocene transition.

Testate amoeba assemblages showed variations to successive climatic events at Lake Lautrey. On one hand, changes in the composition of testate amoeba assemblages (dominating species and assemblage structure), as well as in the accumulation rate (tests/cm²/year) corresponded to major climatic phases (i.e. Oldest Dryas, Bølling/Allerød Interstadial, Younger Dryas, Preboreal). On the other hand, decreases in the accumulation rate preferentially marked the minor short-lived cooling events, such as Older Dryas event or Gerzensee oscillation. However, the Preboreal oscillation, well registered by other proxies at Lake Lautrey, cannot be recognised in the testate amoeba record. In addition, the influence of environmental factors such as organic matter inputs is highlighted in this study.

This work supports the interest of lacustrine testate amoebae for palaeoclimatic reconstructions. Nevertheless, a better understanding of the relation between climate and lacustrine testate amoebae requires (1) further high-resolution studies based on multi-proxy approaches, and (2) the development of modern analogues (present-day ecology of species).

TALK Palaeoecology

The impact of sulphate deposition on testate amoebae in peatlands: Linked experimental and palaeoecological studies in Scotland and Alaska.

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Peatlands are exposed to sulfate deposition from both anthropogenic sources, primarily fossil fuel burning, and natural sources, primarily volcanoes. Peatlands exposed to sulphate deposition exhibit marked changes in methane flux, indicative of changes in prokaryote communities. Here we investigate whether sulphate deposition may also affect testate amoeba communities. Experimental studies were conducted on two peatlands in Scotland. In the first experiment the testate amoebae communities of plots treated with sodium sulphate almost ten years ago were compared with untreated control plots. In the second experiment a single plot treated with sulphuric acid was compared with control plots seven years after treatment. Although there is some variability in response between sites and sampling areas all replicates showed statistically significant differences particularly noted by reduced abundance of small bacterivorous taxa (*Euglypha rotunda, Trinema lineare, Corythion dubium*).

As the impacts of sulphate deposition appeared relatively distinct in experimental studies we subsequently carried out palaeoecological studies to see if testate amoebae might record a palaeoecological response to sulphate deposition. Volcanic eruptions are known to deposit considerable quantities of sulphate on peatlands in Alaska and volcanic ash (tephra) layers allow a volcanic event to be precisely positioned in the palaeoecological sequence. We investigated the palaeoecological record across two tephra layers replicated in five near-surface peat monoliths from across a southern Alaskan peatland. Although the response is somewhat variable a reduced abundance of small bacterivorous taxa coincident with the tephra layers is present in most profiles. Further replication of both experimental and palaeoecological studies is desirable, however results to date do strongly suggest that a reduced abundance of these species may be a general response to sulphate deposition. These changes may relate to a shift from methanogenic archaea to sulphate reducing bacteria, suggesting the interesting possibility that testate amoebae analysis may allow detection of past episodes of sulphate-suppressed methanogenesis.

TALK Palaeoecology

Last millennium high-resolution palaeoenvironmental study based on testate amoebae from a subalpine peatland: quantitative reconstructions and relations to other proxies

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Subfossil testate amoebae (Protists) and stable oxygen isotopes supported by pollen were used to reconstruct the hydrological history of the last 1000 years from Mauntschas mire in the south-eastern Swiss Alps (Upper Engadin valley; 1818 m a.s.l.). This peatland, located at the bottom of an Alpine valley, recorded local hydrological changes that can be related to precipitation/temperature changes since AD 1000. Using the testate-amoeba training set developed from samples collected in 2007 in peatlands in the same valley we reconstructed depth to the water table in Mauntschas mire. In the former calibration study (Lamentowicz et al., 2009) concentrating on the instrumental period (starting AD 1864) we showed that decreasing water tables were correlated with increasing temperatures as measured nearby. However, further analyses also showed correlation between winter precipitation and the mire wetness. Despite the apparently complex causes for the water table fluctuations, in the wider time frame we observed a clear hydrological signal that might be related to climate changes in the Alps as follows. Since AD 1000 the presence of *Archerella flavum* indicated wet conditions. The habitat became yet moister towards AD 1300 when the wettest conditions of the last millennial history of the peatland were reconstructed. After AD 1300 testate amoebae indicated a gradual decrease of the water table. During AD 1580–1630 *A. flavum* indicated increased moisture increased; this period may be related to a cold anomaly recorded in the Alps. Between AD 1670 and 1715 the water table decreased again. The second and most pronounced wet phase was recorded AD 1715–1850 with the maximum water table ca. AD 1780. During the instrumental period (1864–2003) the water table decreased gradually, following the trend of increasing temperatures. In the pollen diagram the maximum wetness of the Little Ice Age (ca AD 1800) is reflected by the Cyperaceae and *Selaginella* maxima. When the climate became warmer and drier after AD 1850 (end of the Little Ice Age) *Sphagnum* spores became more abundant. There is also a striking similarity between reconstructed water depth and the *Pinus* non-*cembra* curve, suggesting temperatures as the common driving factor. The $\delta^{18}O$ chronology from *Sphagnum* stems shares similarities with the water table reconstruction during the instrumental period as well as before, with an anti-correlated phase at the end of the 19th century. $\delta^{18}O$ chronologies from *Sphagnum* and *Polytrichum* are highly correlated and lead to the conclusion that $\delta^{18}O$ fractionation in both species and in different parts of the plant occurs in a similar way. A multiproxy analysis of $\delta^{18}O$ and testate amoebae might reveal underlying hydrology processes in Mauntschas mire.

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Testate Amoebae and bioindication

TALK Testate Amoebae and bioindication

Can different bryophyte canopy structures stimulate different communities and diversity of testate amoebae? Data from the cloud forest of Sierra de Bahoruco, Dominican Republic.

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The diversity of soil testate amoebae is primarily influenced by water availability and chemistry, vegetation type- bryophytes in particular, and to a lesser extent, to pH. These results may be biased since most studies were made in *Sphagnum* spp. peatlands, which are highly acidic and ombrotrophic systems. In *Sphagnum*-dominated vegetation the potentially limited length of the environmental gradients determines which alternate factor remains most important to explain testate amoeba diversity.

However, in bryophytes, water availability may be correlated to morphology as evidenced by the presence of evolved structures, such as the hyalocysts of *Sphagnum* that enhance water accumulation or limit water loss. Therefore, to get a finer resolution of the underlying mechanism that regulates, in bryophytes, testate amoeba diversity and distribution of functional groups, we studied the relationship between bryophyte canopy structure and testate amoeba species diversity in a subtropical cloud forest in the Dominican Republic. Since rough bryophyte canopies with an open, loose or spaced branching architecture tend to have higher water loses due to higher turbulence and mass transfer than dense cushions or smooth canopies, we hypothesized that different bryophyte canopy structures in terms of roughness will have different levels of testate amoeba diversity.

After assessing the canopy structure of 19 replicated bryophyte species with LED MOSS SCAN, and extracting the testate amoebae with the EQPS method, we found 86 species of testate amoebae. *Herberthus* sp showed the highest testate amoeba mean species richness and *Leucoloma schwaneackeanum* the lowest, 40 ± 6.6 (SD), and 24 ± 22.2 (SD), respectively. For density, *Bazzania* had the highest mean 321 ± 246.8 ind g d.wt. $^{-1}$, and *Leucoloma* with the lowest 133 ± 190.8 ind g d.wt. $^{-1}$. The distribution of testate amoeba feeding groups and biomass was similar among the different bryophyte canopy structures with herbivores being the dominant feeding strategy followed by bacterivores. Although there were significant differences in canopy roughness (maximum for *Thuidium urceolatum* and minimum for *Octoblepharum erectifolium*), there were no significant differences in testate amoeba richness, abundance or distribution of feeding groups.

The data suggest that bryophyte canopy structure and presumably, water availability, does not have an immediate effect on the diversity and community composition of testate amoebae in tropical cloud forests of the Dominican Republic. A possible reason for this lack of correlation is the overall high relative moisture throughout the year.

TALK Testate Amoebae and bioindication

Using thecamoebians (testate amoebae) as proxies of ecosystem health in Oil Sands impacted regions of Northeastern Alberta

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Thecamoebians (testate amoebae) have been identified in the benthic zones in a series of oil sands reclamation wetlands in northeastern Alberta. The size and relative distribution of the thecamoebian community appears to respond to a variety of different chemical parameters in aquatic ecosystems impacted by oil sands operations. Ecosystems more impacted by OSPM (oil sands process material) contain sparse, low diversity populations dominated by *Centropyxis aculeata, Centropyxis constricta* and *Arcella vulgaris.* These taxa appear to be more resilient to high concentrations of naphthenic acids (up to 69 ppm) and sodium chloride (conductivities > 2000), the major byproducts of oil sands extraction.

More abundant and diverse thecamoebian populations rich in difflugiid species, particularly *Difflugia oblonga* and *Difflugia urceolata,* appear to be restricted to environments with concentrations of naphthenic acids < 11ppm and relatively low conductivities. As the degree of OSPM impact shifts toward a more natural ecosystem the difflugiid/ centropyxid population responds within a year. Samples were also collected during the spring, summer, and fall months to establish seasonal variation of the thecamoebian population and its relation to the reclamation study. Thecamoebians can thus be used as bio-indicators during remediation efforts in oil sands impacted ecosystems. In addition, comparing thecamoebian assemblages in a variety of engineered aquatic ecosystems in which the chemical changes have been monitored for close to 20 years, allows us to better understand the ecology of the individual taxa.

As a control the species diversity of the aquatic environments impacted by oil sands activity was compared to a variety of natural environments across Alberta.

TALK Testate Amoebae and bioindication

Effect of Atmospheric Particulate Pollution (PM10) and Nitrogen Dioxide (NO_2) on Testate Amoebae Communities Living in Microsystems "Bryophytes-Microorganisms"

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We studied the relationships of nitrogen dioxide atmospheric pollution and metal trace elements contained in atmospheric particles which were accumulated in Bryophytes to microbial communities of Bryophytes at 3 differently polluted sites in France (rural, urban and industrial) over an 8-month period.

We used the following methods. Metal trace elements in mosses were analyzed by instrumental neutron activation (INAA) and by inductively coupled plasma mass spectrometry (ICP-MS) by the CEA. The temperature, relative humidity, but also wind speeds and the levels of NO_2 were measured. The microbial communities were extracted from the terrestrial mosses *(Scleropodium purum*) and the biomass of the testate amoebae was determined.

We obtained the following results. The biomass of testate amoebae was significantly higher at the rural site than at the urban and industrial sites ($p=0.0008$) at the end of this study. After 8 months of exposure, *Assulina muscorum* and *Bullinularia indica* had disappeared from the industrial site. At the urban site, the testate *Difflugia* genus had disappeared. The Redundancy Analysis (RDA) showed that the physico-chemical variables ([NO_2], relative humidity and temperature) and the trace elements which were accumulated in Bryophytes ([Cu], [Sr]) explained 51.3% of the variance in the microbial community data.

We reach the following conclusions. The results of this study suggest that testate amoebae could be a good bioindicator of atmospheric pollution (NO_2 and PM10) and could be used for ecological biomonitoring. However, other variables such as other atmospheric pollutants (PAH, VOC,…) also influence the structure of this testate amoebae community.

Using Bryophyte/associated microorganism microsystems to characterize the impact of contaminants on ecosystems: are testate amoebae the best microbial group for disturbance bioindication ?

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Previous studies showed that microbial communities living in terrestrial or aquatic mosses have a good potentiality to be early indicators of the presence of contaminants (or of some nutrients) in ecosystems. Indeed, the comparison between abundance and biomass of different microbial groups such as primary producers / predators / decomposers can give valuable information about the nature of a disturbance. These studies also showed that some microbial groups, and particularly the unicellular eukaryotic predators, seem to respond significantly to many kinds of contaminants, probably because they are affected both directly (through toxic effect) and indirectly (e.g. through trophic interactions) by the disturbance.

Testate amoebae stand out among the different groups of potential microorganism bioindicators. Indeed, the presence of a shell constitutes an important advantage for future applications. It allows to collect and to preserve samples without fixative, and to store them easily. Furthermore identification of morpho-species by trained technicians is possible. Testate amoebae may therefore be used as atmospheric or water contamination indicators in the near future. However, more studies are needed in different ecosystems and under different contaminations and ultimately to standardize their use and define a “testate amoeba bioindication index”.

Thursday

September 17th 2009

Field trip to Jura Mountains: ecosystems, wine, cheese & culture

POSTER ABSTRACTS

POSTER Biology, Physiology & Ecology

Confocal Laser Scanning Microscopy and Two Photon Excitation Microscopy as a Tool to Study Testate Amoebae

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We applied Confocal Laser Scanning Microscopy (CLSM) and Two-Photon Excitation (TPE) microscopy techniques to visualize testate amoebae (TA), their test, morphology and physiology. TA are usually examined using Scanning Electron Microscopy (SEM) and Environmental SEM (E-SEM). SEM allows much higher resolution than CLSM, but is limited to viewing surface of the specimen. It is not possible to acquire images of living TA and physiology of the amoeba inside the test by SEM or E-SEM. The goal of the study was to examine the potential of CLSM and TPE for imaging of TA.

We tried 17 fluorescent dyes to label different structures of TA. We succeeded in an efficient application of fluorescent dyes such as acid fuchsine, DIOC $_3$(3) and propidium iodide to study TA. We detected autofluorescence of test and fluorescence of the chlorophyll A from symbiotic algae in mixotrophic species. CLSM enabled us to acquire images of TA from depths up to 40 μm, whereas TPE was able to penetrate to 60 μm. Stereological methods were employed to estimate the volume of the biomass of TA visualized by CLSM.

Ecologists often face challenges with the identification of TA or to estimate the volume of cells. The possibility of obtaining 3D reconstructions from the CLSM data can be very helpful. The amount and quality of data about the morphology, biometry, distribution, and ecological preferences varies among TA species. Especially, the cytoplasm, the types of pseudopodia and nuclei for the majority of these species have not been observed and this causes difficulties in their systematic identification. CLSM and TPE may contribute to improving knowledge on TA morphological characteristics with implications for taxonomical and ecophysiological research.

As far as we know, this is the first time the CLSM, TPE, 3D reconstruction and stereological methods have been used for studying inner organization of TA.

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POSTER Biology, Physiology & Ecology

Monitoring the effects of Oil Sands Process-Affected Water (OSPW) on thecamoebian assemblages: an experimental approach

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Thecamoebian (testate amoeba) assemblages have been shown to respond over short time periods to environmental conditions in aquatic reclamation options under development at oil sands operations in northeastern Alberta. This research has shown that testate amoebae are a useful bio-monitoring tool for reclamation in the Oil Sands region. Thecamoebian responses to Oil Sands Process Water (OSPW) have been monitored in the field at lacustrine and wetland test sites established by Syncrude Canada Ltd. and Suncor Energy Ltd.

In order to better establish testate amoeba as a permanent fixture in the oil sands reclamation project a controlled laboratory experiment is presently underway, in which thecamoebians from a natural aquatic system on site at Syncrude's Mildred Lake Operation are being cultured and monitored in order to understand their response to different concentrations of OSPW. Survival and changes in relative community composition (difflugiids vs. centropyxids) will be used to establish the dilution of OSPW in which thecameobians can survive and examine how a natural assemblage changes over time in response to increased concentrations of OSPW.

POSTER Biology, Physiology & Ecology

Interactions of testate amoebae and microfungi on spruce litter needles

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The goal of our study is to examine the association of testate amoebae (TA) and saprophytic fungi colonizing coniferous litter. The specific questions are: 1) what is the spectrum of fungi colonizing the litter? 2) what is the spectrum of TA colonizing the litter? 3) is there any relationship between the fungal and TA spectra? 4) what is the feeding mode of the TA associated with the needles colonized by the saprotrophic fungi (fungivory, bacterivory…)? Answering these questions might improve our understanding of processes influencing the distribution of TA and their possible roles in nutrient cycles involved in litter decomposition.

The main impulse for this study were regular observations of TA associated with spruce (*Picea abies*) litter needles in damp chambers (Petri dishes with moistened filter paper), which were originally designed to isolate the litter-colonizing fungi. To examine this possible association, we collected spruce litter needles colonized by fungi and evaluated the TA spectrum using a scanning electron microscope (SEM). TA species, their frequency and association with fungal mycelia growing on the surface of the needles were recorded.

Cultures of *Arcella discoides* were established on submerged agar plates. Several microfungi colonizing the litter were successfully isolated and were cultivated in damp chambers with *A. discoides*. This cultures are evaluated for more than 2 months (number of TA individuals + their association with the fungal mycelium).

To evaluate potential degradation of fungal cell wall and/or utilization of shell of *A. discoides* we observed activity of chitinase in the Confocal Laser Scanning Microscopy. We cultivated the fungus in slide cultures and placed *A. discoides* on fungal mycelia and supplied them with fluorogenic substrate 4-methylumbelliferyl-β-D-N-acetyl-glucosaminide. We compare activity of chitinase between live and death mycelium with TA.

We suppose active role of both partners (microfungi and TA) in their interaction.

This study was supported by projects GAČR, No. 206/09/P295 and LC 06063

POSTER Biology, Physiology & Ecology

Testate amoebae contribution to the zooplankton species richness and abundance in distinct environments of the Paraná River floodplain, Brazil: a long-term study.

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In the present study we investigated the contribution of testate amoebae to the species richness and abundance of zooplankton community in the Paraná River floodplain. Samples were performed in distinct environments (connected and unconnected lakes, channels and rivers) during eight year, between 2000 and 2008, twice a year, in potamophase and limnophase periods.

We identified 96 infrageneric taxa of testate amoebae belonging to eight families. Arcellidae, Difflugidae and Centropyxidae were the most specious and abundant families in the different environments. Regarding to species richness, testate amoebae were the second most important group in the plankton samples, in all environments, hydrological periods and years studied, being less specious only than rotifers.

Although higher values of testate amoebae abundance have been recorded in lentic environments, the results evidenced, in relation to rotifers and microcrustaceans, a higher relative contribution of testate amoebae in the lotic environments.

Considering the different families, distinct abundance patterns were observed between lentic and lotic environments. In this way, Difflugiidae predominated in lentic environments while Centropyxidae was the most abundant in lotic ones.

Temporally, we observed a remarkable variation in testate amoebae contribution to the zooplankton diversity and abundance, among the studied years. These variations are discussed in relation to the intensity and amplitude of the flood pulse.

Supported by: CNPq/PELD

POSTER Biology, Physiology & Ecology

Distribution of testate amoebae in different bog microecotypes along the gradient: hummock-lawn-hollow in Selisoo Bog, NE-Estonia

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We present data on the distribution of testate amoebae assemblages in NE-Estonian peat bog (Selisoo bog) in different microecotypes along the gradient from hummock to closed pool in upper 30 cm of peat layers.

Five 30-cm-long peat cores were taken along the 10-m long transect from the centre of hummock to hollow. Samples were analyzed in 2-cm interval and both percentages and concentrations of testate amoebae and pollen were determined. Humification analyses were used to assess the decomposition degree of peat. To describe patterns in testate amoebae species composition along environmental gradients, we used a nonmetric multidimensional scaling. To find relationship between the concentrations of testate amoebae species and environmental variables, Pearson's correlation coefficients were computed.

Our results show, that almost every sample point from hummock to hollow is characterized by different testate amoebae assemblages. There exist species-indicators, which are specific to certain bog microecotype (e.g. *Nebela militaris* in hummock, *Difflugia oblonga* in floating peat carpet), but there also occur species, which are specific to other environment variables (depth, decomposition degree, distance to water table level) in peat column (e.g. *Trinema lineare*, *Cryptodifflugia oviformis*) or are related to local vegetation (e.g. *Amphitrema flavum*). Statistical analysis showed that distinct microecotypes are the best described by concentration data of testae amoebae species in peat. Distribution of species with dry environment preference is closely related to occurrence of tree and heather (*Calluna vulgaris*) pollen and high decomposition degree of peat, all indicating dry environment or hummock microecotype. Despite different testate amoebae species communities, the vertical distribution of testate amoebae in almost all studied samples from different microecotypes shows the trend of water level drop during last decades in Selisoo, being the most prominently observable in hummock and distinct lawn microecotypes. High concentrations of *C. oviformis* occurring frequently in a dividing stage deeper than 16 cm below peat surface, indicate the species active life at that depth.

We reach the following conclusions. Firstly, testate amoebae assemblages in different microecotypes even as near as 10 m from each other, are distinctly different. So, palaeohydrological changes in bog must be reconstructed with attention paid on the variation of bog microecotypes in time. Secondly, if possible, concentrations of testate amoebe in sediment should be calculated and presented, as these describe microecotypes better than percentages and data of different studies and localities were easier to compare. Thirdly, when collecting samples for modern training sets, some species living below surface must be kept in mind and samples from lower portion of peat must also be studied.

POSTER Biology, Physiology & Ecology

Modern testate amoebae communities of a tidal fresh water marsh (Notelaar, Belgium): First step to predict water level changes of the Scheldt estuary (Belgium).

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We investigated present testate amoebae communities on an inland tidal fresh water marsh (Notelaar) of the Scheldt estuary with the aim of making a transfer function for reconstructing former water levels. These former inland water level fluctuations will be related to a series of land loss and – reclamation events that took place at the mouth of the Scheldt during the period of 1550-1800. The relations found will be used as model to estimate the effect of nowadays wetland restorations on the water level of the Scheldt.

Randomly on the marsh we took at every sample site two soil samples (2 cm deep = +/-sedimentation of 1 year) along an altitudinal gradient. The first soil sample was used for testate amoebae analysis. The second soil sample was taken with fixed volume for sediment analysis (particle size, bulk density, loss on ignition) Furthermore at each sample site notes were made on the vegetation type (vegetation cover of *Salix sp*., *Phragmites sp*., or other vascular plants) and height was measured using DGPS or Total Station (vertical error: +/- 1cm). The sample sites are spread over an altitudinal gradient of 1.23 m (5.34 m TAW- 6.57 m TAW). Soil samples for testate amoebae analysis were prepared using the method of Charman 1998. The counted samples exposed a very rich testate amoebae fauna of more than 80 species, mostly consisting of fresh water species and ubiquitous (most common: *Trinema lineare, Trinema enchelys* and *Tracheuglypha dentata*) and also containing one marine interstitial species *Cyphoderia littoralis*, probably transported upstream by the river.

In total 37 testate amoebae species (>2% relative abundance for at least one sample) were used in multivariate analysis. The RDA revealed a clear relationship between testate amoebae and height, presenting *Trinema complanatum, Arcella arenaria, Difflugia globulus, Difflugia globulosa* and *Cyclopyxis kahli* as highly positive correlated species.

POSTER Biology, Physiology & Ecology

Characterization of intracellular bacteria from clonal cultures of Arcella spp. (Amoebozoa, Arcellinida)

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In order to address the phylogeny of *Arcella* clonal cultures were established using several species of the genus. Most of them did not survive longer than one-two years. However, one *Arcella rotundata* and one *A. intermedia* clone keep on growing for 4 and 3 years, respectively. Our former study has already proved the presence of certain prokaryotic endocytobionts in *Arcella* spp. (Török et al. 2008).

Applying the same culture method and FISH, repeated molecular characterization of bacterial isolates originating from the *A. rotundata* clone recovered a totally different association (including members of the Alpha-, Gammaproteobacteria and Firmicutes) when compared to the previous results. *A. intermedia* also hosted Alpha- and Gammaproteobacteria. Unequivocally environmental taxa without any symbiotic or pathogenic affinities were not among the characterized isolates.

A remarkable phenomenon was the occurrence of *Rhizobium*, an obligate symbiont in three strains isolated from *A. rotundata*. Two further strains were classified into the hazardous *Bacillus cereus* group. The only gammaproteobacterium (probably *Pseudoxanthomonas*) has been reported from animal tissues. In the *A. intermedia* host two strains of the Alphaproteobacteria were characterized as probably *Brevundimonas* which contains a rare opportunistic pathogene species. The recovered gammaproteobacterium was a member of the Pseudomonadales, Moraxellaceae, comprising either *Moraxella* or *Enhydrobacter* with great probability. Both genera contain pathogenic species besides environmental ones. The food organism, *Enterobacter aerogenes* has never been detected among the sequences which we consider as a proof for the adequacy of the applied culture method.

The characterized taxa suggest that *Arcella* species provide suitable ecological conditions supporting the growth of a wide variety of bacteria. Our future task is to elucidate the factors that account for the presence and dynamism of these associations.

Török, J.K., Pollák B., Heéger Zs., Csikós Gy., Márialigeti K. (2008): First evidence on bacterial endocytobionts in the testate amoeba genus Arcella (Amoebozoa, Arcellinida). Protistology. An International Journal. 5(4):303-312.

POSTER Biology, Physiology & Ecology

Testate amoebae composition in different habitats of a Paraná River floodplain lake, Brazil

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Although testate amoebae, in aquatic environment, are organisms essentially benthonic or associated to littoral vegetation, studies have recorded a high taxa richness of these protozoans in plankton samples, especially in shallow and small water bodies of floodplain systems, as well as in plankton of running waters.

This study aimed to carry out a survey on testate amoebae composition in different habitat types (plankton, sediments and aquatic macrophytes associated fauna) of a shallow lake of the Paraná River floodplain. Sampling was performed monthly, from April 2007 to March 2008.

We recorded 89 infrageneric taxa belonging to 9 families, among them Difflugiidae, Centropyxidae and Arcellidae were the most representative. In regard to the species distribution, 82 species were recorded in the sediment, 71 associated to aquatic macrophytes, whereas 53 species were registered in plankton samples.

An analysis of Non-Metric Multidimensional Scaling (NMDS) evidenced clear patterns of testate amoebae species composition to the different habitats, suggesting distinct community structure for each compartment.

In summary, although the flood pulse has been considered the main function force, driving the distinct aquatic communities in floodplain systems, our results evidenced that testate amoebae assemblages composition are more distinct spatially, among habitats, than temporally, along an seasonal cycle.

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POSTER Biology, Physiology & Ecology

Seasonal and altitudal patterns of testate amoeba communities in *Sphagnum*-dominated peatlands of the Swiss Alps and Jura mountains

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Testate amoebae play a central role in the microbial loop of *Sphagnum* peatlands as important grazers of bacteria, fungi and microscopic eukaryotes. They are very sensitive to the moisture (or water table depth) gradient and their belowground communities change over different temporal scales from seasons to millennia in response to changes in micro-environmental conditions. However it is less clear if they also respond to temperature gradients. To address this question, we studied the temporal patterns or testate amoeba communities along a 1300 m elevation gradient in Switzerland from June 2008 to April 2009. We sampled four *Sphagnum*-dominated peatlands ranging from about 600 to 1900 m a.s.l. We hypothesize that the density, diversity and community structure of testate amoebae changes a) along the altitudinal gradient, b) seasonally.

The non-metric multidimensional scaling ordination showed a clear altitudal pattern from the lowest (driest with longest growing seasons; Loormoss) to the highest (wettest with shortest growing season; Hochrajen) site.

The average total testate amoeba concentration was highest in August ($80x10^3$ ind./g) and May ($61x10^3$ ind./g), then decreased during the vegetation season in October ($49x10^3$ ind/g). Highest concentration among living individuals was recorded in August ($23x10^3$ ind. g^{-1}) and April ($26x10^3$ ind. g^{-1}), the lowest in October ($10x10^3$ ind. g^{-1}) and March ($14x10^3$ ind. g^{-1}). The next step is to relate changes in concentration to microbial biomass and other measured parameters.

POSTER Biology, Physiology & Ecology

Searching for endosymbionts in free-living amoebae

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Endosymbiotic organisms are widely present in protists, including either mutualistic or parasitic forms. Most investigations focused on the interactions between naked amoebae, mainly amphizoic species (e.g. *Acanthamoeba* and *Naegleria*), and pathogenic bacteria like *Legionella*. Testate amoebae are generally not investigated, and, as a consequence, no data is available about the presence in their populations of such intracellular bacterial symbionts.

In previous studies, we analysed the occurrence of potentially pathogenic bacteria in naked amoebae. We included into this investigation the *Chlamydiae*, a peculiar group of intracellular bacteria infecting all vertebrates, some invertebrates, and amoebae. We proceeded through : i) screening of amoebae isolated onto agar; and ii) recovery of endosymbionts into amoeba coculture. However, culture-independent surveys indicated a high biodioversity within the *Chlamydiae*, suggesting more complex ecologic roles.Various species-level taxa of chlamydiae were recovered from the environment, several of which being unable to infect *Acanthamoeba*, suggesting for a larger host range in natural habitats.

We are currently investigating the occurrence of *Chlamydiae* and other endosymbionts among testate amoebae. Preliminary results obtained with *Nebela tincta* (n = 15) (Amoebozoa, Arcellinidae) tend to exclude the presence of chlamydiae in this species. We will enlarge our investigations to other populations and species of testate amoebae. Our hypothesis of the presence of chlamydiae in testate amoebae is indirectly supported by the results of Török et al, which demonstrated recently the presence of various bacterial endosymbionts in other arcellinids (*Arcella* spp.).

Corsaro D & D Venditti (2009) Detection of *Chlamydiae* from freshwater environments by PCR, amoeba coculture and mixed coculture. Res. Microbiol. 160:547-552.
Török JK, B Pollák, Z Heéger, G Csikós & K Márialigeti (2008) First evidence of bacterial endocytobionts in the lobose testate amoeba Arcella (Amoebozoa, Arcellinida). Protistology 5: 303-312.

POSTER Biogeography & Biodiversity

The fauna of testate amoeba in Japan. – Catalog and literature database –

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In this paper, we present a catalog of Japanese testate amoeba, and taxonomical problems in Japan. Recently, Asian testate amoeba faunae ware studied by some young scientists (e. g. Yang, 2005). They described some new species and also mentioned Asian endemic species. Previously, some Japanese scientists worked on the taxonomy of testate amoeba. However, some of the taxonomical descriptions were not adequate.

We collected more than 1300 literatures on the ecology and taxonomy of Japanese protists. We made a database on these literatures. Dr. Meisterfeld provided us with species name list from his database. We checked them and added some species names from our database.

The following results were obtained. In Japan, 341 species were recorded based on ecological studies (187 papers) and taxonomical studies (5 papers). Our database includes 2615 records. And the species recorded from Japan is included subspecies and forma, but not “sp. as only genus name”.

In some cases, the taxonomic description of species was needed additional studying based on Japanese specimens, topotype (e.g. some of Dr. Hada’s papers). Now we (Aoki, Shimano, Meisterfeld) are currently preparing a catalog of Japanese testate amoeba and clarifying the taxonomical problem. We hope our infrastructure-building of the testate amoebaean taxonomy will ensure the monitoring of diversity reduction caused by climate change.

Acknowledgements: We thanks to Dr. Meisterfeld provided us species list from his database. This study was supported by JSPS grant, no. 21310024, Kiban (B) “infrastructure-building of the protist taxonomy for the monitoring of diversity reduction caused by the climate change” and “The biodiversity of Lake Biwa and its drainage basin: Taxonomy, Morphology and Systematics using molecular biology techniques.” Lake Biwa Museum General Research 2006-2010.

POSTER Biogeography & Biodiversity

Biodiversity of testate amoebae from mosses and lichens on rocks (Karelia, Russia)

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We present new data on species composition and community structure of testate amoebae in the different microbiotopes on rocks within northern taiga native zone (Karelia, Russia).

We collected samples in July 2008 from mosses and lichens which are growing on rocks and large stones (height 2.0–2.5 m) in three points. First one was a small island in the White Sea, second – in a sea bank, third – in a continental part (on watershed). The following microbiotopes at the heights from 0.5 to 2.5 m above the ground were investigated: 1) small surfaces covered by foliose lichen *Hypogymnia physodes*, 2) large surfaces covered by the same lichen species, 3) surfaces covered by fruticose lichen *Cladonia* sp., 4) large moss cushions formed by *Dicranum* sp., 5) small moss cushions. For comparison testate amoebae communities from teresstrial biotopes were investigated. They are surfaces of soils covered by mosses *Hylocomium splendens, Polytrichum commune, Pleurozium schreberi*. Altogether 40 microbiotopes were analyzed.

45 species and forms of testate amoebae were identified, including 42 – in soil mosses and 43 – in biotopes on rocks. Four species were found in more than half samples: *Corythion dubium* (97%), *Assulina seminulum* (85%), *Trinema enchelys* (72%), *Euglypha simplex* (52%). The most abundant are C. *dubium* (31.4% on the average), *T. enchelys* (12.4%), *Trinema lineare* (5.5%), *Nebela tincta* (5.2%), *Corythion orbicularis* (4.9%).

As far from the sea species diversity and abundance are increase. For instance, abundance of shells on rocks grows from 1900 ind. per gram of absolutely dry substrate in the island, throw 8400 ind./g on the sea bank till 30700 ind./g in continental part. The same pattern reveals in terrestrial biotopes: 14500 – 16500 – 29800. Moreover in the island and sea bank testate amoebae abundance on rocks 2–7 times less than in terrestrial biotopes, whereas in continental part differences are disappeared. In terms of species richness differences between biotopes remained the same in all regions: in the island there are 6.3 species (per sample on the average) in rocks and 8.0 species in terrestrial mosses, in the sea bank – 7.5 and 11.3, in continental part – 10.8 and 16.0.

Specificity of community structure is connected not with specificity of microbiotope but with remoteness from the sea. On the island there are following dominant composition: *Corythion dubium – Centropyxis orbicularis – Euglypha ciliata glabra – Trinema enchelys*, on the sea bank – *Corythion dubium – Trinrema enchelys – Nebela parvula*, on the continental part – *Trinema enchelys – Nebela tincta – Trinema lineare – Corythion dubium – Nebela militaris*.

The lowest abundance (1900–4500 ind./g) and species richness (7.4–7.7 species per sample) are characteristic for most dry conditions (moisture is 37–48%) of rock lichens *Hypogymnia physodes*, *Cladonia* sp. The more complex communities (with abundance 24200–38000 ind./g and species richness 11.5–13.5) are formed in the moist (moisture 79–92%) lichens *Hypogymnia physodes*, moss cushions and terrestrial mosses.

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POSTER Biogeography & Biodiversity

Micro- and macroscale changes in density and diversity of testate amoebae of tropical montane rain forests of southern Ecuador

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We investigated changes in diversity and density of testate amoebae in epiphytes of trees in tropical montane rain forests in the Andes of southern Ecuador. Local – microscale [tree height of 0 (base of tree trunk), 1 and 2 m; TH I, TH II and TH III, respectively] and regional – macroscale (forests at 1000, 2000 and 3000 m) changes were investigated. At the macroscale diversity and density of testate amoebae peaked at intermediate altitude (2000 m) with an average richness of 53 species and average diversity of 15,165 ind. g^{-1}, exceeding that in litter and soil. At the microscale diversity of testate amoebae reached a maximum at TH I, whereas density of testate amoebae reached a maximum at TH III. The percentage of empty shells at the macroscale was at a maximum at 2000 m and at the microscale at TH I, whereas the percentage of live forms was at a maximum at 3000 m and at TH III. The overall diversity of testate amoebae in epiphytes was high (114 species). However, only two to nine species were dominant representing 54-85% of total living testate amoebae. These consisted predominantly of cosmopolitan species occurring in humid habitats of low pH, such as *Assulina muscorum* and *Euglypha strigosa*.

Results of the present study suggest significant variations in density and diversity of testate amoebae at both the micro- and macroscale. However, for testate amoebae density the macroscale appears most important whereas changes in diversity of testate amoebae are more pronounced at the microscale.

POSTER Biogeography & Biodiversity

Changes of testate amoebae community structure along two moss-soil microecotones in spruce-forest and birch wood in northern taiga (Karelia, Russia)

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We present new data on community structure changes along two moss-soil microecotones in the northern taiga native zone (Karelia, Russia).

Samples were collected in July 2006 in spruce forest and birch wood in the north Karelia within northern taiga native zone. Firs transect was chosen in flood-plain birch forest. It was started in moss plot covered by *Sphagnum angustifolium* and finished within fen-podzolic soil with well developed carpet of leaves and grass felt. Second transect was placed in spruce forest and started in moss plot covered by *Sphagnum flexuosum* and finished in peaty-podzolic soil with well developed carpet of coniferous waste. The extension of each transect was 35–40 cm. Within each transect 5 zones were chosen (2 in moss part, 1 in transition, 2 in soil part) on the distance 7–8 cm from each other.

Within first transect abundance was 230–250 thousands ind. per gram of absolutely dry substrate in moss, 230–290 – in carpet of leaves, whereas significantly decreases (130 thsd. ind. / g) in the border between moss and soil. Species richness in moss is 28 species (on average between replicates), 26 – in soil, 25 – in the border. Set of dominant species includes 12 species. There are three community variants: i) in mosses – *Trinema lineare, Cryptodifflugia oviformis*, *Centropyxis orbicularis*; ii) in soil – *Nebela bohemica*, *Assulina seminulum, Centropyxis orbicularis, Trinema lineare, Heleopera sylvatica*; iii) in the border – species from the neighboring groups (*C. orbicularis, A. seminulum, C. oviformis*), as well as specific forms *Euglypha tuberculata*, *Tracheleuglypha dentata*, *Nebela wailesi*. It is interesting that *Trinema lineare* that was very abundant in soil and moss biotopes in the border is not abundant that resulted in decreasing of total abundance here.

Within spruce forest abundance changes from 150–230 thsd. ind./g in moss, 140–200 – in soil, 100–110 – in the border. Species richness is 20 species in the moss, 25 species in the soil, and 23 species in the border. Set of dominant species includes 13 species. There are three community variants: i) in mosses – *Trinema enchelys, Trinema lineare, Trinema complanatum, Centropyxis orbicularis, Cyclopyxis arcelloides*, *Nebela parvula*, *Arcella catinus*, *Centropyxis aculeata*, *Corythion dubium*, *Centropyxis orbicularis*; ii) in soil there are the same species – *T. enchelys, T. lineare, T. complanatum*, *C. orbicularis*, as well as specific *Cyclopyxis eurystoma, Trigonopyxis arcula*, *Trigonopyxis minuta*, *Assulina seminulum, Sphenoderia fissirostris*; iii) in the border there are no specific species, whereas dominants are presented by eurytopic species *T. enchelys, T. lineare, T. complanatum*,

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POSTER Biogeography & Biodiversity

Biodiversity of testate amoebae from Russian Arctic (Tazovsky peninsula, Matveev and Dolgii Islands)

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We present new data on species composition and community structure of testate amoebae from Russian Arctic.

Material was collected in two regions. In small islands Dolgij and Matveev samples from lake sediments, sphagnum quagmires, terrestrial mosses were taken in September, 2003. In the Tazovsky peninsula samples from fens, soils, terrestrial mosses, lichens were collected in July, 2008. Altogether 84 samples were quantitatively analyzed using standard methods for soil testate amoebae (Geltzer et al., 1985).

In the biotopes of Tazovsky peninsula 98 testate amoebae species and forms were identified. Most abundant species were *Trinema enchelys, Corythion dubium, Trinema lineare, Assulina seminulum, Euglypha laevis*. The abundance varied from 6000–7000 ind. per gram of absolutely dry substrate in the most dry microbiotopes (moss "cushion" in yernik, lichens in birch elfin woodland) up to 250000–450000 ind./g in sphagnum on the bank of the glacial lake and in fens.

Testate amoebae community formed two variants in accordance with moisture of biotopes. In moist conditions (fen, bank of the lake) there are *T. enchelys, T. lineare. E. laevis, Euglypha tuberculata, Trinema complanatum elongata* predominating. In dry conditions *Phryganella acropodia, Ph. hemisphaeica, Corythion dubium* are most abundant. Other characteristic species are *Hyalosphenia papilio, Archerella flavum, Placocista spinosa* in moss biotopes, *Assulina muscorum, A. seminulum, Nebela collaris, N. bohemica, Trigonopyxis arcula* in lichens, *Trigonopyxis minuta* in tundra felt.

In the biotopes of Matveev and Dolgij islands 89 species and forms were identified. The most diverse genera are *Centropyxis* (include 13 species and forms), *Difflugia* (12), *Nebela* (12), *Euglypha* (11), *Arcella* (10).

In terms of structure testate amoebae community is divided into two variants: i) from moss biotopes in lakes and terrestrial ecosystems; ii) bottom sediments of lakes. In mosses *Trinema enhelys, T. lineare, Euglypha laevis, E. tuberculata, Cyphoderia perlucidus* are predominated, whereas species from the genus *Difflugia, Centropyxis, Arcella*, as well as *Phryganella acropodia* and *Archerella flavum* are most abundant.

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POSTER Biogeography & Biodiversity

Pentagonia zhangduensis nov. spec., (Lobosea, Arcellinida), a new freshwater species from China

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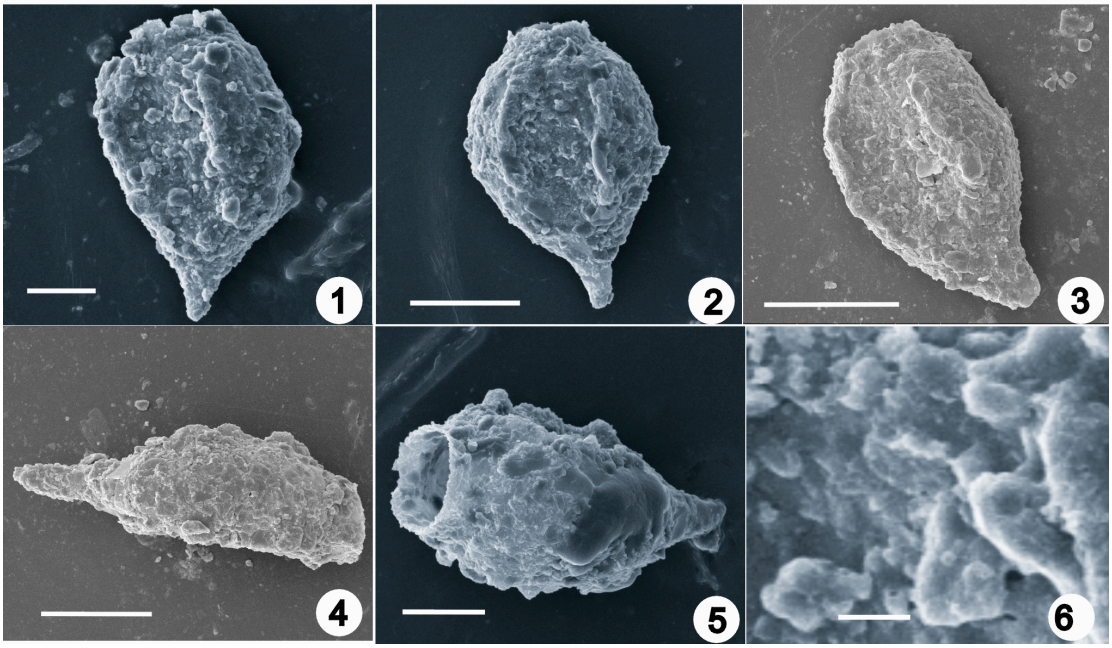
The morphology of a new testate amoeba *Pentagonia zhangduensis* nov. spec. was investigated using light and scanning electron microscopy. The new species was discovered in the sediments of Lake Zhangdu, Hubei Province, China. The low coefficients of variation and normal size frequency distribution suggest that *P. zhangduensis* is a size-monomorphic species. *P. zhangduensis* differs from the one other species in this genus (*P. maroccana*), by its larger size and its quadrangular cross section with two parallel longitudinal ridges on each of the two flattened sides of the test.



SEM photographs of *Pentagonia zhangduensis*. Figs 1,2: front view showing the parallel longitudinal ridges. Fig. 3: back view showing the longitudinally parallel ridges. Fig. 4: lateral view showing the compressed shell. Fig. 5: apertural view showing a narrowed collar and circular aperture. Fig. 6: detail of the test surface, note the flattish pieces of quartz. Scale bars are 100 µm (1-4); 50 µm (5); 10µm (6).

POSTER Biogeography & Biodiversity

A taxonomical study of *Difflugia biwae* Kawamura, 1918 as a local endemic species of Ancient Lake Biwa

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The environment is currently threatened due to global warming, by such things as glacial collapse, thawing of permafrost and rising sea level. The ancient fresh water Lake Biwa is experiencing a reduction in turn over (also called "Deep breath"). This phenomenon involves colder, denser, surface water sinking to the bottom of the lake, partly due to colder surface temperatures and melting mountain snow flowing downhill. Turn over provides dissolved oxygen to the deepest parts of a body of water. Ordinarily, turn over occurs from autumn to spring. But nowadays, global warming makes the winter season warmer. The reduction in turn over means that less oxygen and hydrogen sulfate reaches the bottom of the four million years old lake where many endemic species live. This greatly limits the populations of benthic organisms and fish. In recent years, the lake has been just cold enough to promote turn over, but next year it may not be cold enough based on temperature patterns. This might lead to the extinction of species. Already, many species of big lake all over world are approaching extinction because of temperature changes thus far.

For instance, Lake Biwa Environmental Research Institute has been investigating the planktonic fauna/ flora every month for more than 40 years. *Difflugia biwae* Kawamura, 1918, formerly considered as a local endemic species of Lake Biwa, was also discovered by Yang (2005) in Lake Mulan in China. In Lake Biwa 40 years ago, it had been reported as the dominant species in the investigation. In 2007, about 20 individuals were rediscovered, but they are not living cells, only tests. *D. biwae*, has became first endangered protozoan species in Japan to our knowledge. Kawamura (1918) described this species in Japanese, so we present the detail of this species.

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POSTER Biogeography & Biodiversity

Morphology, ultrastructure and biometry of *Nebela golemanskyi* sp. nov. (Amoebozoa, Arcellinida, Nebelidae) from Bulgaria

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A new sphagnicolous species of the genus *Nebela* isolated from wet *Sphagnum* mosses gathered at Vitosha Mountain (Bulgaria) is described.

Its morphology and ultrastructure was studied by scanning electron microscope JEOL JSM-5510. Statistical analyses were performed using the computer program STATISTICA, version 7.0.

At first glade, in small magnification on light microscope the new species might be identified as *N. speciosa*. However, scanning electron microscopy clearly shows the differences between the two species, which are expressed in the following:

1.) The shell of *N. golemanskyi* is composed of very characteristic voluminous, globular or rarely elongated, rounded and hollow plates, usually with large parts between them interspersed with small beads of organic cement; in *N. speciosa* the shell plates are smaller, more compressed and are put close to each other;

2.) The shell of *N. golemanskyi* is almost uncompressed and has no lateral margins, while in *N. speciosa* the shell is always compressed laterally and has small lateral margins;

3.) The shell of *N. golemanskyi* is comparatively shorter than and not as elongated as in *N. speciosa*, and the ratio width/length in the new described species is higher than that in *N. speciosa* (0.6 and 0.4, respectively);

4.) The aperture of *N. golemanskyi* is roughly circular to oval, not convex and bordered by a thin collar of organic cement; in *N. speciosa* it is regular oval, slightly convex in broad view and concave in lateral view, and surrounded by a thick collar of organic cement.

The new described species is comparatively rare and inhabits wet *Sphagnum* peatlands (1800 – 2000 m a.s.l.) in the Vitosha Mountain (Bulgaria). It was found in associations with the next dominant species: *Hyalosphenia papilio, N. galeata, N. speciosa, N. penardiana, N. (Argynnia) dentistoma, N. (Argynnia) vitraea, Quadrulella symmetrica* and *Cyphoderia ampulla.*

POSTER Phylogeny & taxonomy

Comparing SSU and COI markers for assessing phylogenetic relationships among Cyphoderiidae (Cercozoa: Euglyphida)

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The current taxonomy of euglyphid testate amoebae is essentially based on morphological characteristics of the shell. However, the segregation between morphospecies can be very difficult due to the lack of diagnostic characters. Cryptic species were recently reported within different morphospecies. There is therefore a need for developing molecular tools for establishing the relationships between different euglyphid species.

Here we assessed and compared the effectiveness of the mitochondrial Cytochrome *c* Oxidase Subunit 1 (COI) and SSU rDNA genes for determining the phylogenetic relationships among *Cyphoderia* species. Sequences were obtained from eleven populations belonging to six *Cyphoderia* morphospecies. Although the COI fragments (500 bp) used in these analyses were much shorter than the SSU rDNA fragments (1700 bp), COI and SSU rDNA tree topologies were very similar. Mean inter-population COI sequence divergences were in average 2.7 greater than the SSU rDNA sequence divergences while mean COI and SSU rDNA intra-population sequence divergences were always very small (<0.05%).

Altogether, these results demonstrate the feasibility of using the mitochondrial COI as a taxonomic "barcoding" marker for Cyphoderiidae or other euglyphid protists.

POSTER Phylogeny & Taxonomy

Phylogeny and genetic diversity of the genus *Difflugia* (Amoebozoa: Arcellinida)

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Our understanding of the phylogenetic evolution, global biodiversity, and biogeography of unicellular eukaryotes is still fragmentary, and one of the major reasons is the lack of primary molecular data on protozoa. The genus *Difflugia* Leclerc, 1815 is the earliest described taxon and the most diverse group of testate amoebae. It is very common in freshwater habitats and is a key ecological player in fluxes of energy and matter, but its phylogenetic position among unicellular eukaryotes and the phylogenetic relationships among its species remain unclear. Thus the establishment of solid phylogenetic relationships among "species" is necessary for determining the taxonomic significance of diagnostic morphological characters used to identify species. Furthermore, the biodiversity of *Difflugia* is poorly understood because the biological species concept is not applicable to these organisms, which reproduce by inbreeding or asexually.

The core objective of this study is to create a robust phylogenetic hypothesis for *Difflugia* through combined analyses of DNA sequences and morphological data. Our project covers three different levels of diversity: 1) the phylogenetic relationships among "species" (different morphotypes), 2) relationships within closely related "species" (species complexes), and 3) the genetic diversity from different populations of a given morphological species with microsatellite DNA molecular markers. This project will fill a gap in the knowledge of a very common group of organisms while also contributing to research on protist diversity, evolution and biogeography. This study will have direct implication for the use of testate amoebae in paleolimnology: understanding the relationship between morphological and genetic diversity will clarify the true diversity and thus quantity of information available to ecologists and paleoecologists.

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POSTER Palaeoecology

Palaeohydrological record based on testate amoebae from a Baltic bog in N Poland: towards a multi-proxy palaeoclimatic reconstruction

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Baltic bogs of Northern Poland are interesting objects for palaeoclimatic studies because their hydrology conditions developed under the influences of both oceanic and continental climates. The dominance of one or the other type of climate might have changed over time.

We present continuous 6000-years record from an ombrotrophic peatland – Stążki, located 35 km south from the Baltic Sea coast. In this study we used testate amoebae to: 1) reconstruct quantitatively water table changes, 2) identify the major wet and dry shifts that could been caused by changing climate, 3) compare results with the data from Poland and the other regions of Europe. Changes in the bog surface wetness were inferred using the existing and still updated Polish transfer function including 230 surface samples. We identified sixteen wet and fifteen dry shifts. Main periods of high bog surface wetness occurred at 5120-4880 BC, 4720-4670, 4560-4350 BC and 4160-3910 BC. Since 3730 BC frequency of wet periods was decreasing. Longer wet shifts are visible between 140-570 AD and 770 – 950 AD. We determined three main phases in the hydrological history of the bog. The first phase (5300-3250 BC) represents mostly wet conditions indicated by *Amphitrema flavum* and *Hyalosphenia papilio*. Second phase (3250 – 130 BC) was characterized by dry conditions (indicated by *Assulina muscorum* and *A. seminulum*). The last third phase (130 BC – 970 AD) is a period of increased water table and peat accumulation with the dominance of *Hyalosphenia papilio and H. elegans*.

Our results correlate relatively well with other Polish and European data based on various proxy. This study illustrates an important value of testate amoebae analysis in the reconstruction of past hydrological conditions on the border of the continental and oceanic-type climate. Furthermore, our result is an introduction to the further detailed multi-proxy study consisting pollen, plant macrofossils and geochemical data.

POSTER Palaeoecology

Evaluating the effects of Holocene hydroclimatic changes on carbon dynamics in a permafrost peatland, Kuujjuarapik, North-eastern Canada

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In order to improve the comprehension of the impacts of permafrost aggradation and degradation on carbon dynamics, multi-proxy analyses are realized to reconstruct Holocene paleohydrological conditions. Peat cores were extracted from three different environments (modern palsa, collapsed palsa and forested peatland margin) in a permafrost peatland located near Kuujjuarapik, in the southern limit of the discontinuous permafrost zone in subarctic Quebec (55°13'N, 77°41'W).

Testate amoebae and plant macrofossil analyses are used to assess past hydrological conditions since the beginning of peat accumulation in the region. A northern Quebec water-table depth transfer function using testate amoebae assemblages will be developed to infer past water-table depth by analysing modern assemblages from twelve sites following a nordic transect from boreal to subarctic Quebec (Charman et *al*., 2007). This transfer functions will allow the reconstruction of paloeohydrological conditions that influenced peat accumulation through the Holocene in north-eastern Canada. Loss-on-ignition (LOI) has allowed the determination of sediments bulk density as well as organic and related carbon contents through the cores. LORCA and RERCA supported by ^{14}C will be done to reconstruct the peat accumulation dynamics linked with hydroclimatic variability and permafrost development. ^{14}C values indicate that peat began to accumulate 5000 years BP. Testate amoebae analysis shows several important changes that could be associated to permafrost aggradation and changes in hydrology and peat accumulation. LOI and testate amoebae results from collapsed palsa and forested peatland margin show several important differences probably due to differences of microforms dynamics. However, some correlations can be done between testate amoebae and LOI results of the two cores and this might reflect the influence of hydroclimatic fluctuations on carbon dynamics in permafrost peatlands systems.

Charman, D. J., Blundell, A. and ACCROTELM members, 2007, A new European testate amoebae transfer function for paleohydrological reconstruction on ombrotrophic peatlands.

POSTER Palaeoecology

Testate amoebae reveal palaeohydrology and abrupt shift in trophy during the Holocene in small subalpine mire in French Alps

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One of the most important goals in palaeoecology is to distinguish the palaeoclimatic signal recorded in the sediment. Very often this signal co-occurs with human impact e.g. deforestation that makes the palaeoclimatic interpretation difficult. However, testate amoebae analysis provide independent data on the past hydrology (surface wetness) and trophy that complete multi-proxy approach. Testate amoebae (Protista) living in peatlands are important palaeoecological indicators.

In this study we aim to: a) reconstruct the hydrology of a subalpine mire during the last 6000 years using testate amoebae, b) infer possible palaeoclimatic signal, c) identify and explain trophic shifts during the mire history. The study site is located in the Maurienne Valley (Savoie, French Alps), which is one of driest valleys of the Alps. The depth to water table inferred from transfer function reveals a specific hydrology of the bog. Terrestrialization of the lake took place ca 6630 yr cal. BP. From 5620 to 4100 yr cal. BP, water table gradually decreased, the pollen analysis showing local Cyperaceae domination. However, *Sphagnum* expanded during this period, which is indicated by *Amphitrema flavum* between 5620-4100 yr cal. BP. Ca 1690 yr cal. BP, a very wet minerotrophic fen appeared, probably as an effect of deforestation with a profound change of the vegetation structure by land-use, resulting in a better supply of minerotrophic run-off waters.

In the next stage, our data will be compared with major changes in the vegetation throughout the Holocene (pollen and plant macrofossils analyses).

POSTER Palaeoecology

Testate amoeba analysis of lake sediments: impact of filter size and total count on estimates of density, species richness and assemblage structure

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Testate amoebae are informative about palaeoecological conditions, but the methods generally used for their analyses in lake sediments differ from those used for their analyses in peats, making comparisons difficult.

This study examines how filter mesh size and total number of individuals counted affect species richness, Shannon diversity, equitability, density and assemblage structure. We analysed the complete testate amoeba contents of six sediment samples from Lake Lautrey, France.

The abundance of testate amoebae was high (1,403-10,870 shells.cm^{-3}), and species smaller than 63 µm in both length and width represented up to 89% of total abundance and 43% of species richness. A simulation showed that using 47 µm or 63 µm mesh-size filters reduced inter-sample differences and changed the patterns of abundance, species richness and assemblage structure, causing loss of information and leading to potential erroneous palaeoecological interpretation.

Rarefaction analyses suggest that although 170 shells are sufficient to assess the general structure of assemblages, such small sample sizes can underestimate species richness by overlooking taxa with relative abundances <4%. Total counts of 400 shells yield better estimates of assemblage structure and recover at least 50% of total species richness, although species with absolute frequencies below 2% may still be missed. Higher counts are required to obtain reliable estimates of species richness and assemblage structure in samples that have high testate amoeba densities but are dominated by a few small taxa.

Further studies should determine the bioindicator value and functional roles of small and/or rare species in lakes and thus to what extent overlooking them affects palaeoecological interpretations.

POSTER Palaeoecology

A useful index based on testate amoebae for paleolimnological research

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In the freshwater lake deposits, the genera *Difflugia* and *Centropyxis* are the two most important components of testate amoebae communities in terms of their species abundance and richness. Stratigraphic analyses of the postglacial sediments of two Nova Scotia lakes were used to compare the relative abundance and the species richness of *Difflugia* to *Centropysis*. Relatively few *Difflugia* were recorded during Younger Dryas cooling period in the lakes' histories, when *Centropyxis* were relatively abundant and diverse. The increase of relative abundance and species richness of *Difflugia* was in accord with the climate warming events in early Holocene and middle Holocene. Interestingly, both abundance- and species-based indices with natural logarithm transformation are significantly correlated with the GISP2 ice core temperature, and especially the North Atlantic Preboreal Oscillation (PBO) cooling event was also recorded by the indices.

From these data it is suggested that the ratio (with natural logarithm transformation) of *Difflugia* to *Centropysis* may provide a useful paleolimnological index of postglacial climate status. In addition, the advantages of the abundance-based index include its simplicity for non-specialists, the fact that the abundance can be enumerated from the same microscope slide and that the ratio is independent of sedimentation rate.

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POSTER Palaeoecology

First record of testate amoebae on glaciers and a description of *Puytoracia jenswendti* nov. sp. (Rhizopoda, Filosea)

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We report for the first time the presence of testate amoebae inhabiting on glaciers. Three shallow firn/ice cores of 10 m depth were obtained from the Mocho-Choshuenco and Osorno volcanoes, Andean Cordillera, Chile, in October and November, 2005. Testate amoebae were detected in the cores after performing biological analyses. The composition of testate amoebae and their abundance in this first glacier record is presented, in addition to describing a new species corresponding to the genus *Puytoracia.* These glacier testate amoebae species have been previously described in cold ice-free regions in the Arctic and Sub-Antarctic Islands where they have been regarded as primary settlers.

The presence of Testate amoebae was quantified by means of an optical microscope (Olympus BX50WI) using a magnification of 600x. The individuals were acquired with a digital camera Photometrics Cool Snap cf and measured digitally using Image J software to obtain morphometric taxonomic characters. For a better identification of the surplus species a Scanning Electron Microscope Leo Electron Model 420 was used.

The microscopic analysis of 150 samples reveals a total of 454 individuals belonging to 4 taxa that correspond to *Trinema lineare*, *Trinema enchelys*, *Puytoracia bergeri* and to the new taxon *Puytoracia jenswendti* nov. sp. 22 % of the total individuals could not be identified due to overlap and ambiguity of certain taxa, with the exception of individuals belonging to the new species which were well characterized. The abundance of testacea amoebae specimens found in the firn/ice cores has clear seasonal variations, with a maximum in the summer season.

After an extensive review of taxonomic characters we agree with other authors who indicate that the identification of the genus *Trinema* is problematical and ambiguous. In this regard we support the need of genetic and molecular methods in future studies.

This record shows that Tecamebas can provide a new and novel proxy as paleoindicators for firn/ice core dating and estimation of a mass balance record for glaciers.

POSTER Palaeoecology

Developing testate amoeba-based transfer functions of environmental variable in peatlands in the Changbai Moutains, of Northeast China

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During July and August, 2008, 81 *Sphagnum* samples were collected from four peatlands in the Changbai Mountains (Hani, Jinchan, Chichi and Yuanchi) along the hydrological gradient from hollow to hummock. Seven environmental variables were measured in the field (depth to water table - DWT, pH, conductivity, latitude, longitude and altitude) and in the lab (peat moisture). A total of 33 testate amoeba taxa were recorded, all of which occur in other regions in the world.

Detrended correspondence analysis (DCA), redundancy analysis (RDA), and canonical correspondence analysis (CCA) were used to explore the relationships between testate amoeba assemblages and environmental variables. The DCA shows that the four peatlands have similar testate amoeba communities. In the RDA, seven environmental variables explained 31.8% of variation in species data. DWT, pH and moisture of peat individually explained significantly 10%, 9% and 8% respectively (Monte Carlo significance tests p□0.001). Both RDA and CCA show that DWT, pH and moisture of peat are principal factors, which determine the assemblage of testate amoebae in peatlands in the Changbai Mountains, and that testate amoeba-based quantitative paleoenvironmental reconstruction can be developed for these variables.

Different models including PLS, WA, WA-PLS, ML, MAT, and LWWA were used to develop transfer functions. Two cross-validation methods, jack-knifing and bootstrapping using 1000 cycles were employed to assess the relative performance of these models. When the full set of 75 samples is used in training set, the prediction performances are poor. The best models were LWWA_inv for DWT ($RMSEP_{jack}$=10.6cm, r^2_{jack}=0.60), WATOL_inv for pH ($RMSEP_{jack}$=0.28 and r^2_{jack}=0.51), WA_inv for moisture ($RMSEP_{jack}$=3.40%, r^2_{jack}=0.34). Filtering outliers samples representing the top 20% of the range of residuals improved model performance, but setting a threshold at 15% produced even better results: with this threshold the best models were WA-PLS for DWT ($RMSEP_{jack}$=5.5cm, r^2_{jack} =0.81), WA_inv for both pH ($RMSEP_{jack}$=0.16, r^2_{jack}=0.79) and moisture ($RMSEP_{jack}$=1.38%, r^2_{jack} =0.75). This means that DWT, pH and moisture of peat can be reconstructed with the mean error of ±5.5cm, ±0.16 pH units, ±1.4% if the testate amoebae assemblage in peat profile are similar with modern training set.

Compared with ecological studies conducted in other regions of the world, testate amoebae from Changbai Mountains show similar ecological preferences for DWT, surface moisture, and pH. If the ecological preferences of testate amoebae are similar in different parts of the world this suggests that these preferences have also been stable over time. Testate amoebae provide an extra method in quantitative paleoenvironmental reconstruction based on peat sediments. Together with other proxies such as pollen, plant macrofossil, humification degree, and isotope, we can recover a more reliable picture of past environmental changes.

POSTER Bioindication

Forensic science goes underground: Testate amoebae as bioindicators of cadaver decomposition

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Forensic science is a broad discipline that covers a variety of research topics that are relevant to the legal system. One applicable tool for criminal investigations is the use of forensic entomology to estimate the post mortem interval (PMI) by analysing the insects that develop on the cadaver. However, a decomposing cadaver also changes the environment beneath it, but this is a topic that has received very little attention so far.

We investigate the effect of a decomposing cadaver on the underlying soil and whether these changes can be used as a possible tool in forensic science. As testate amoebae have been shown to respond to a wide range of environmental gradients and perturbations we hypothesise that they will also strongly respond to the presence of a decaying body.

We therefore investigate density and community structure of testate amoebae underneath decomposing cadavers. After filtering and fixation the testate amoebae are identified and counted under the microscope. Living and dead individuals are tallied separately.

We collected samples starting in August 2009 from litter and soil underneath a decomposing pig, a fake pig (= bags filled with soil) and a control from three sites in a beech forest in Neuchâtel, Switzerland. The pig as showed in various studies is most acceptable as a model for the decomposition of a human corpse. The sampling will continue until complete resilience of the soil testate amoeba communities.

The main questions in our study are: 1) Do testate amoebae respond to the decaying body? 2) If yes can they be used for forensic investigations - are they an applicable tool? 3) How long does it take until the soil community goes back to normal? 3) Which variables have the strongest influence on the soil community?

This is still a preliminary study. In the next stage we aim at analyzing physical, chemical and biological variables in the litter and soil samples.

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