

6th International Symposium on Testate Amoebae

Xiamen, China • 15-18 October 2012



Organizers



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Institute of Urban Environment, CAS



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National Natural Science Foundation of China

6th International Symposium on Testate Amoebae

Xiamen, China 15-18 October 2012

Program & Abstracts

Editors: Jun Yang and Satoshi Shimano







国家自然科学 基金委员会 National Natural Science Foundation of China

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Welcome Letter

Welcome to Xiamen!

Welcome to the 6th International Symposium on Testate Amoebae!

On behalf of the Organizing Committee, we would like to extend our warmest welcome to all the participants in the 6th International Symposium on Testate Amoebae (ISTA6). Previous ISTA symposia have been held in five different cities in four countries in Europe. Building on the series of great successes, ISTA6 is being held for the first time in Asia, at the Institute of Urban Environment, Chinese Academy of Sciences. It is our great honour to host this important event and we are pleased that we have more than 40 delegates from home and abroad to participate this meeting.

Global change, biodiversity conservation and sustainable development are closely related, and have now become a pressing global concern in the 21st century. Testate amoebae are ubiquitous and important components of all aquatic and terrestrial ecosystems. Thus, the study on the widely distributed testate amoebae is of great ecological, environmental and economic significance. Research on testate amoebae, particularly their ecology and applications, has increased rapidly over the past decade.

ISTA6 provides a unique platform to discuss and exchange the latest advances in the different fields of research related to testate amoebae. The three-day symposium will include keynote and general lectures, and poster presentations. The meeting will cover different aspects of testate amoebae, with emphasis on biodiversity and environmental changes, and will be organized in four sessions: 1) Biodiversity & biogeography; 2) Phylogeny & taxonomy; 3) Ecology, biomonitoring & bioassessment; 4) Paleoecology & bioindication. We believe this meeting will promote active exchanges and cooperation and have a positive outcome that will be benefit to all of us for the better of testate amoeba research.

Finally, we hope you will have a wonderful and happy time in Xiamen, enjoy the new research findings on these fascinating and beautiful organisms, and create lifelong professional partnerships and personal friendships.

Jun Yang & Satoshi Shimano



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Acknowledgements

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Support for the organization of this symposium was also kindly provided by:

- ★ Chinese Protozoological Society
- ★ Japan Society of Protozoology
- ★ Japanese Society of Soil Zoology
- ★ Institute of Urban Environment, Chinese Academy of Sciences, China
- ★ Miyagi University of Education, Japan



Organizing Committee

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Environmental Education Center, Miyagi University of Education, Japan

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6th International Symposium on Testate Amoebae

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Scientific Committee

Anatoly Bobrov Moscow State University, Russia

Barry Warner University of Waterloo, Canada

Dan Charman University of Exeter, UK

Daniel Gilbert University of Franche-Comté, France

David Scott Dalhousie University, Canada

David Wilkinson Liverpool John Moores University, UK

Edward Mitchell University of Neuchâtel, Switzerland

Enrique Lara University of Neuchâtel, Switzerland

Francine McCarthy Brock University, Canada

Humphrey Smith Coventry University, UK

Jun Yang Institute of Urban Environment, Chinese Academy of Sciences, China Louis Beyens University of Antwerpen, Belgium

Luiz Felipe Machado Velho Universidade Estadual de Maringa, Brazil

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Robert Booth Lehigh University, USA

Satoshi Shimano Miyagi University of Education, Japan

Tim Patterson Carleton University, Canada

Yongguan Zhu Institute of Urban Environment, Chinese Academy of Sciences, China

Yuhe Yu Institute of Hydrobiology, Chinese Academy of Sciences, China

Yuri Mazei Penza State Pedagogical University, Russia



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General Information

Map of Campus IUE



Registration

8:00-22:00 October 15, 2012: The lobby of the IUE's Hotel.

Venue (Meeting Place)

Room 208, Administration Building, Institute of Urban Environment, Chinese Academy of Sciences, China.

Presentation

All keynote lectures will be of 40 minutes duration (including 5 minutes for discussion). General oral presentations will be allocated 20 minutes (including 5 minutes for discussion). All poster presentations will take place in the area where coffee and tea breaks for delegates will be held.



Accommodation and Service

Every guest room has air-conditioning, free internet access and drinking water in the IUE's hotel.

If your computer cannot connect to the internet automatically, please adjust your settings as follows: Click Start \rightarrow settings \rightarrow network connection \rightarrow click network connection with right mouse key \rightarrow double click Internet protocol (TCP/IP) with left key \rightarrow select gain IP automatically \rightarrow select gain DNS automatically \rightarrow confirm \rightarrow OK!

For ironing or other services, please call Ms Hong Lv at 15985830896.

For local calls, please add 4 before you dial. For room to room calls, please find the appropriate phone number on the list provided in your room and dial the last three digits only.

Please remember to *RETURN* the room card to the registration desk before you leave.

Transportation Information

Taxis are not always available around IUE campus, but directly outside the campus gate there is the BRT (Bus Rapid Transit) Line K1, which runs from the Ferry Terminal to IUE from 6:10 AM to 22:00 PM, provides convenient transport to Xiamen downtown area.

Weather in Xiamen

The weather in Xiamen around October can be extremely pleasant with an average temperature of 20-28 °C. For more information about Xiamen City please visit the websites below:

http://www.chinahighlights.com/xiamen/weather/october.htm http://www.travelchinaguide.com/cityguides/fujian/xiamen/

Brief Agenda

October 15, 2012	Registration
October 16, 2012	Symposium and Xiamen half-day tours
October 17, 2012	Presentation and Discussion
October 18, 2012	Field Trip to Hakka Earth Building, Hua'an, Fujian Province
October 19, 2012	Departure and Optional Trip to Japan



Conference Program

October 15, 2012		
8:00-22:00	<i>Registration</i> (Lobby of the IUE's Hotel) <i>Poster Mounting</i> (2F, near to Room 208, Administration Building)	
7:00-7:50	<i>Breakfast</i> (1 st Floor, Dining Hall)	
12:00-12:30	<i>Lunch</i> (1 st Floor, Dining Hall)	
18:00-19:00	<i>Dinner</i> (2 nd Floor, Dining Hall)	



October 1	6, 2012		
7:30-8:20	<i>Breakfast</i> (2 nd Floor, Dining Hall)		
8:30-8:40	<i>Opening Words</i> (Room 208, Administration Building) (Jun Yang & Satoshi Shimano)		
	Session I: <i>Biodiversity & Biogeography</i> (Chair: Edward Mitchell)		
8:40-9:20	<i>Shouyi Zheng</i> (Keynote Talk) Marine protozoa—Foraminifera: Their scientific and aesthetic applications		
9:20-9:40	Angela Creevy Microbial diversity of a nature reserve: Testate amoebae and diatoms at Mere Sands Wood in North West England		
9:40-10:00	Anatoly Bobrov Species diversity and biogeography of testate amoeba in the Arctic		
10:00-10:30	<i>Group Photo</i> (in front of the Administration Building) & <i>Tea Break</i> (near to Room 208)		
Session II: <i>Phylogeny & Taxonomy</i> (Chair: <i>Yuri Mazei</i>)			
10:30-11:10	<i>Enrique Lara</i> (Keynote Talk) A molecular biology perspective on testate amoebae		
11:10-11:30	<i>Satoshi Shimano</i> Soil testate amoebae in Japan, the first data		
11:30-11:50	<i>Zheng Yu</i> Morphological diversity of <i>Difflugia tuberspinifera</i>		
12:00-13:30	<i>Lunch</i> (2 nd Floor, Dining Hall)		
13:30-19:00	Xiamen Half-Day Tours		
19:00-21:00	Welcome Banquet (Jiali Seafood Restaurant)		



October 1	7, 2012			
7:30-8:30	<i>Breakfast</i> (2 nd Floor, Dining Hall)			
Session III: <i>Ecology, Biomonitoring & Bioassessment</i> (Room 208, Administration Building) (Chair: Daniel Gilbert)				
8:40-9:20	<i>Edward Mitchell</i> (Keynote Talk) Progress and perspectives in testate amoeba ecology			
9:20-9:40	<i>Yuri Mazei</i> Testate amoebae communities in boundaries: Spatial and temporal perspective			
9:40-10:00	<i>Mariusz Lamentowicz</i> Seasonal to decadal temporal patterns of testate amoeba communities in <i>Sphagnum</i> peatlands			
10:00-10:20	<i>Andrey Tsyganov</i> Flourish or flush: Effects of simulated extreme rainfall events on <i>Sphagnum</i> -dwelling testate amoebae in a subarctic bog (Abisko, Sweden)			
10:20-10:50	Tea Break & Poster Session			
Session III: <i>Ecology, Biomonitoring & Bioassessment)</i> (Chair: Satoshi Shimano)				
10:50-11:10	<i>Valentyna Krashevska</i> Response of testate amoebae to nutrient additions along an altitudinal transect of tropical montane rainforests			
11:10-11:30	<i>Vincent Jassey</i> Do testate amoebae occupy the same trophic position in microbial food webs?			
11:30-11:50	<i>Yangmin Qin</i> Ecology of testate amoebae in <i>Sphagnum</i> peatlands of Central China, with particular reference to hydrology and human activities			
12:00-13:30	<i>Lunch</i> (2 nd Floor, Dining Hall)			



Session IV: <i>Paleoecology & Bioindication</i> (Room 208, Administration Building) (Chair: Enrique Lara)		
14:00-14:40	<i>Daniel Gilbert</i> (Keynote Talk) Microecosystem bryophyte-testate amoebae: A good tool of bioindication and biointegration of disturbances	
14:40-15:00	<i>Graeme Swindles</i> Can testate amoebae be used as hydrological indicators in Amazonian peatlands?	
15:00-15:20	<i>Hongkai Li</i> Building testate amoebae-based transfer function from <i>Sphagnum</i> peatland in Xiaoxing'an Mountains, Northeast China	
15:20-15:40	<i>Adeline Wall</i> Testate amoebae distribution in lake sediments	
15:40-16:00	<i>Julie Valentine</i> A multiproxy palaeoecological and palaeoclimatic reconstruction from two raised mires in northwest England	
16:00-16:20	<i>Caroline Meyer</i> Using "moss/soil interface-testate amoeba community" microecosystems as biointegrators of atmospheric deposition of Phenanthrene	
16:20-17:00	Tea Break & Poster Session	
17:00-17:20	Concluding Words (Jun Yang & Satoshi Shimano)	
17:30-18:30	<i>Dinner</i> (2 nd Floor, Dining Hall)	
October 1	8, 2012	
7:30-8:30	<i>Breakfast</i> (2 nd Floor, Dining Hall)	
8:30-17:30	Field Trip to Hakka Earth Buildings	
18:00-19:30	Dinner (2 nd Floor, Dining Hall)	



Dr Humphrey Graham Smith 1945-2012

Dave M Wilkinson

School of Natural Science and Psychology, John Moores University, Byrom Street, Liverpool, L3 3AF, UK

Humphrey Smith, a key figure in the ecology and biogeography of Antarctic Protozoa, died of cancer after a short illness on 3 July 2012. He grew up in Bolton in Northern England and an old school friend remembers a very clever but mischievous child who also had surprisingly adult musical tastes (especially the work of J.S. Bach). Indeed music was to stay important to Humphrey throughout his life as a singer, pianist and organist – in later life he played the organ for several of his local churches. Many people at the 2009 International Testate Amoebae meeting in France will remember him singing in the show caves we visited to try out their acoustics!



Caption for photo: Humphrey Smith (on the left) with Edward Mitchell and Louis Beyens during the last International Testate Amoebae meeting in France in 2009.



His university education was all carried out in Scotland; with a BSc in Ecology from The University of Edinburgh in 1967, a MSc from Aberdeen in 1968 and a PhD on '*Studies of the terrestrial protozoa of the maritime Antarctic*' awarded by Edinburgh in 1973; his thesis was based on work done while employed as a microbiologist by the British Antarctic Survey from 1968 to 1974. As far as I am aware his first scientific paper was a short report on the protozoa of the newly emerged volcanic island of Surtsey published in 1970 but written while he was a Masters student at the University of Aberdeen. However it was Antarctic protozoology that was to dominate his research publications. Humphrey spent a considerable time doing field work in the Antarctic during his PhD studies and returned South again on sabbatical in the southern hemisphere summer of 1983/84 (his students organised a party in a Coventry night club to wish him well on the trip – much beer was consumed, especially by Humphrey!). He also visited the Antarctic once more in retirement – this time as a tourist.

Humphrey's Antarctic work focused on the ecology and biogeography of the terrestrial protozoa. Most of these papers were descriptive studies aimed at identifying the species present and in quantifying their population sizes and many were published in the *British Antarctic Survey Bulletin* – arguably they would have had a wider influence if published in more readily accessible journals. These data later allowed both Humphrey himself, and other scientist, to make use of Antarctic information in global scale analyses of the factors influencing the distribution of protozoa – especially testate amoebae. In collaboration with some of his students he also published several papers on the physiological ecology of Antarctic protozoa based on laboratory work. In 1997 Louis Beyens and Didier Chardez named a new species of testate from Spitsbergen in the Arctic in Humphrey's honour. In their description of *Schoenbornia smithi* they explained that 'this species is named after Dr Humphrey G. Smith (Coventry University, U.K.), the main contributor to our knowledge of testate amoebae in the Antarctic'.

From 1974 he spent the rest of his career at Coventry Polytechnic (which later became Coventry University) continuing his association as an Honorary Research Fellow after his early retirement in 2002. He had a reputation as a charismatic and somewhat eccentric lecturer who always had a lot of time for his students. It was at Coventry that I first met Humph (as he often signed himself in emails) as I was an undergraduate there in the early 1980s. My final year research project took over work that he had started on the biogeography of the genus *Nebela* in the Antarctic and sub-Antarctic zones and which he had been unable to find the time to work on properly himself (in the days before the internet this type of synthetic biogeography could be exceedingly time consuming involving chasing many obscure publications at specialist libraries).



In the 1990's Humphreys research output dropped off as he was very busy with administrative duties, being in charge of student admissions to environmental science and related degrees. However following his early retirement he had more time for research, and the final decade of his life produced a series of papers mainly on aspects of testate biogeography. Of particular note was our joint paper on the distribution of *Nebela (Apodera) vas* which has already become a textbook example of a free-living microbe with a geographically restricted distribution (in the 8th edition of Barry Cox's and Peter Moore's textbook on '*Biogeography*') this textbook recognition rather pleased Humph. He also published a paper in Chinese in collaboration with Jun Yang and myself – although of the three authors only Jun could read the finished article! In addition he further contributed to Chinese protozoology by helping Yangmin Qin and colleagues publish an English language review on the diversity and biogeography of testates in China in 2011.

This brief summary of Humphrey's achievements is in danger of missing out some the most important aspects of his life. Following the death of his mother he used money he inherited in 1998 to buy an area of woodland and set up Mabley Farm which is managed as a working farm and woodland, but also as a site for nature conservation and environmental education. The farm is run by two of his friends who he had met through his involvement with a local nature conservation charity. Woodland management and conservation was another long running interest of Humphrey's. In the many emails about Humph that I saw following his death the words that occurred repeatedly were 'friendly', 'nice' and 'kind'. Many also commented how supportive he had been to other scientists and students. Few of us could hope for a better epitaph!

Note: A more detailed version of this obituary will appear in a future issue of *Acta Protozoologica*.



Abstracts Oral Presentations

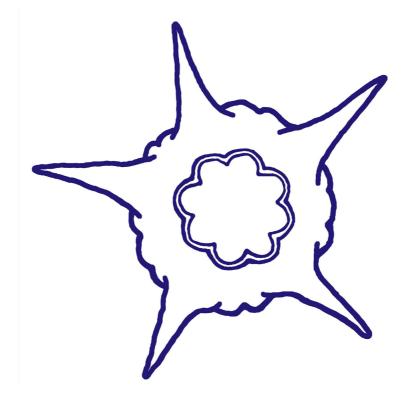


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BIODIVERSITY & BIOGEOGRAPHY



Marine protozoa—Foraminifera: Their scientific and aesthetic applications

Shouyi Zheng

Institute of Oceanology, Chinese Academy of Sciences, Qingdao, 266071 China

Shelled foraminifera averaging about 1 mm in size, are one of the most diversified and abundant group of marine protozoans which are preservable from the Cambrian to the Recent, with about 40,000 fossil and 6000 recent species. As living organisms, they are sensitive to changes in the environment where they inhabit. Their great abundance and diversity of species, ubiquitous distribution of their preservable tests in fossil and recent sediments and the important role they play as excellent bioindicators in oceanography, biostratigraphy, geology, micropaleontology, etc. they have been dubbed "Tiny Giants of the Great Seas". (Bock, 1971)

The aesthetics of foraminifera because of their minute size has been neglected. Microscope revelations of their exquisite morphological beauty show that they are virtually art forms in Nature. According to David Pearson (2001) affinity with the natural world is a universal source of inspiration. As science sees further into the microscopic world of matter and uncovers more of the remarkable structure of living things, nature continues to surprise us and teach us how we might build more cleverly, economically, subtly and ecologically. Engaged in the study of the taxonomy and ecology of foraminifera for more than half a century and knowing their morphology by heart, I embarked on a project of producing proportionately enlarged foram models to let people share the beauty endowed on them by Mother Nature, to inspire their application in design fields such as in garments, lamp, jewelry, pastries, etc., and in organic architecture and to serve as educational tools, popular science exhibits, tourist souvenirs, etc. A new vista of the microscopic foraminifera, a Foraminiferal Sculpture Park consisting of 114 large stone sculptures integrating science, art, and marine culuture, the first of its kind in the world, was officially inaugurated on December 5th, 2009 in Zhongshan City, Guangdong, China. Smithsonian magazine in an article entitled "Beautifully Adapted" wrote "a tribute to foraminifera was long overdue" included the park as one of 10 evolutionary hotspots in the world, placing second.



Microbial diversity of a nature reserve: Testate amoebae and diatoms at Mere Sands Wood in North West England

Angela L Creevy, Jane Fisher, Dave M Wilkinson, Simone Durr

School of Natural Sciences and Psychology, John Moores University, Byrom Street, Liverpool, L3 3AF, UK

The United Nations (UN) has recently launched The Decade of Biodiversity 2011 - 2012. Given that microorganisms comprise much of the biodiversity on earth, and play a fundamental role in the ecology of all the visible members of communities, microbial diversity is poorly researched compared with macroorganisms. Using testate amoebae and diatoms as model groups, the goal of this study is to compare the microbial diversity and distribution of a nature reserve with previously studied macro-organisms.

Field sampling of terrestrial soils and aquatic lake sediment was conducted in September 2011. The morphospecies concept was used to determine species richness and abundance within each microhabitat, and the potential existence of cryptic and pseudo-cryptic species was highlighted for further Scanning Electron Microscopy (SEM) analysis. Organic matter content was determined using loss on ignition and indicative pH measurements were obtained. In addition, the abundance of nematodes was quantified. So far, more than sixty species of testate amoebae and fifty species of Diatoms (work in progress) have been recorded at the reserve. An interesting finding was the testate species composition and abundance of nematodes present in the ornithogenic island soils and litter. It's questionable whether these testates are indicative of the nutrient influx from guano or whether some have arrived as passengers of migratory wetland birds. A decrease in testate diversity was observed from the sediment beneath homogenous patches of aquatic vegetation and invasive macrophyte Crassula helmsii. The existence of potential cryptic species was identified in the Cyphoderiidae family and Achnanthidium minutissimum complex for diatoms. Further research intends to correlate patterns in species richness and composition of testate and diatom communities.

The prevention of extinction is one of the strategic goals of the UN Decade of Biodiversity. In addition to providing species lists, this method is proving useful in obtaining abundance data for eukaryotic microorganisms, comparable to that of macroorganisms. Molecular research would complement this traditional morphology-based estimation of microbial diversity.



Species diversity and biogeography of testate amoeba in the Arctic

Louis Beyens¹, <u>Anatoly Bobrov</u>²

¹Universiteit Antwerpen, Campus Drie Eiken, Dep Biologie, ECOBE, Universiteitsplein 1, 2610 Wilrijk, België

²Faculty of Soil Science, Moscow State University, Leninskie Gori, GSP-2, Moscow 119992, Russia

In this study, around 1800 from 43 different localities (single - and multispot) encompassing the whole Arctic are analysed for their species diversity. In total, 384 taxa (of species and infraspecies level) were found, belonging to 53 genera. The top 10 genera with most taxa are in decreasing order: *Difflugia* (70 taxa), *Centropyxis* (62), *Euglypha* (30), *Arcella* (26), *Nebela* incl *Argynnia* and *Porosia*, *Cyclopyxis* (16), *Trinema* (13), *Cryptodifflugia* (12), *Plagiopyxis* (10), *Corythion* (9).

However, taking into account the know number of taxa per genus worldwide, it is obvious that some genera as e.g. *Assulina*, *Corythion* and *Trinema* are much better represented. Also viewed this way, *Centropyxis* is a far more represented genus than *Difflugia*. This points to the decreasing importance of this genus in the north. Looking at the distribution of the taxa, it is *Trinema lineare* which occurs in most localities with a presence score of 86%, followed by *Assulina muscorum* (84%), *Centropyxis aerophila* (81%), *Corythion dubium* (77%), *Euglypha laevis* (65%), *Euglypha rotunda* (63%), *Trinema enchelys* (60%), *Phryganella acropodia* (58%), *Phryganella acropodia* v. *sphagnicola* (56%), *Diffluglia globulus* (56%), *Euglypha strigosa* f. *glabra* (56%).

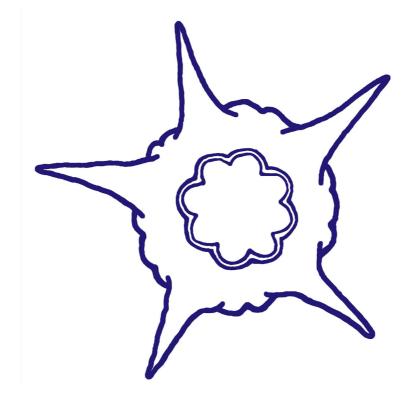
Although the cosmopolitic and also mainly ubiquistic taxa are the best represented in the overall Arctic biome, we suggest, on basis of a cluster analysis, that several major testate amoeba "provinces" can be distinguished in the Arctic. Although not yet satisfactory explained, these "provinces" are probably reflecting and caused by climatic and geographic-historical parameters. It seems too that in general the eastern arctic sites (Siberia and the Russian archipelago's) have a lesser species diversity than the arctic sites from Greenland and the Canadian Arctic (incl Nunavut).

From the rarer taxa, the ones described from the Arctic, and observed in several arctic localities are of special interest. This is a limited group with o.a. *Centropyxis pontigulasiformis* and *C. gasparella*, and some of them can be considered as arctic flagship species. These taxa raise the question when and where they speciated, and how they spread from their site of origin to other arctic regions.

Russian part of this work was supported by Russian Foundation for Basic Research (grants number 11-04-01171-a, and 11-04-91332-NNIO a).



Tuesday Morning 16 October 2012



Phylogeny & Taxonomy



A molecular biology perspective on testate amoebae

Enrique Lara

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Testate amoebae are large and conspicuous protists that have been noticed since the very first attempts of scientists to peer into microbial life. However, the difficulties encountered in keeping them into culture has been a historical burden that prevented testate amoebae for getting the visibility of other protist groups such as ciliates. As a consequence, many fundamental aspects of their biology and even their phylogenetic identity have remained undiscovered until recently. Nowadays, the application of molecular biology tools to testate amoeba research is reshaping our picture of their phylogeny, diversity and biogeography. In addition, it is revealing certain details of their life history that were difficult to observe, such as the existence of sex, or the exact identity of their symbionts. These new approaches are likely not only to increase the value of testate amoebae as tools for bioindication research, but also open exciting new perspectives in research in palaeontology, biodiversity, biogeography, microbial evolution, ecology, and biogeochemistry.



Soil testate amoebae in Japan, the first data

Anatoly Bobrov¹, <u>Satoshi Shimano²</u>

 ¹Faculty of Soil Science, Moscow State University, Leninskie Gori, GSP-2, Moscow 119992, Russia
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For the first time in the vast population of soil material investigated testate amoebae in mountain and lowland forests of Japan. An analysis of 152 samples of forest soil islands of Honshu, Yaku, Kitadaitou-jima, Minamidaitou-jima and Kume-jima. The territory of Japan is of interest to protozoology as an example of a unique island biogeography of large and small islands. It is part of the eastern part of the Holarctic. The island of Honshu comes in two biogeographic provinces - Japanese Evergreen Forest and Oriental Deciduous Forest. The most southern islands make up the local biogeographical unit - Ryukyu Islands.

The samples found about 250 taxa of testate amoebae. We describe two new species - *Deharvegia japonica* and *Assulina discoides*. The bulk of the population belongs to evribiontnoy and cosmopolitan group of testate amoebae. While the findings are not specific to species of the Holarctic, such as *Centropyxis deflandriana*, *C. latideflandriana*, *C. stenodeflandriana*, *C. sacciformis*, *Cyclopyxis lithostoma*, *Distomatopyxis couillardi*, *Hoogenraadia humicola*, *Planhoogenraadia daurica*, *Assulina discoides*, *Deharvengia japonica*, *Quadrulella quadrigera*, *Q. quadrigera* v. *africana*, *Wailesella* sp. can raise the question of the uniqueness of the south-eastern part of the Holarctic.

We believe that such a high frequency of species common to the Indo-Malayan, Australian and Neotropical regions gives reason to raise the question from the standpoint of protozoology on the special status of biogeographic Japan.

Russian part of this work was supported by Russian Foundation for Basic Research (grants number 11-04-01171-a); Japanese part of this work was supported by JSPS (Japan Society for the Promotion of Science) "Evaluation of the effect of global warming on structural change and chain relations of microbe and protist communities in Japan." (grants number no. 20380082).



Morphometric analysis of six natural populations of *Difflugia tuberspinifera*

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The morphometrical variability of six natural populations of *Difflugia tuberspinifera* was investigated using the statistic methods based on 374 samples from Yangtze River and Pearl River valleys. The size frequency distribution analysis indicated that D. tuberspinifera is a size-monomorphic species with normal distribution of shell height, shell diameter and aperture diameter. The size of spine length, collar height, foreside length and number of conical spines are the most variable. The correlation analysis showed that most characters are inter-correlated with P < 0.05. The shell height, shell diameter, aperture diameter, spine length, collar height, rear end length, foreside length, number of aperture tooth-like structures and number of conical spines differed significantly between different populations (P < 0.0001), but principal component analysis (PCA) did not clearly distinguish the six populations based on the morphometric data. However, the cluster analysis separated the six populations into two groups, the Pearl River-Yangtze River group and the Yangtze River group. Further, the populations with different shell characters can coexist within the same river valley. Thus, there is a high morphological variability or diversity within the populations of D. tuberspinifera which is related with local environmental conditions.

This work was supported by the Knowledge Innovation Program of the Chinese Academy of Sciences (KZCX2-YW-QN401) and the National Natural Science Foundation of China (31172114 and 30800097).



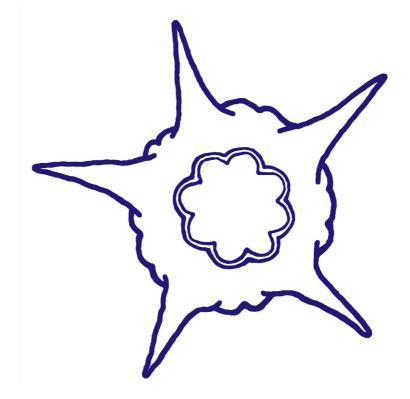
Tuesday Afternoon 16 October 2012



Xiamen Half-Day Tours



Wednesday Morning 17 October 2012



ECOLOGY, BIOMONITORING & BIOASSESSMENT



Progress and perspectives in testate amoeba ecology

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Testate amoebae are an ideal model group of organisms to study the ecology of free-living soil microorganisms. Thanks to their morphological diversity and presence of a test (shell) they are arguably the easiest group of microorganisms to study using classical microscopy methods. As a result, numerous studies have explored their community ecology, potential as bioindicators, and to a lesser extent as new models in ecotoxicology. In addition, several studies conducted over the last decade have revealed that they are also functionally very important as key players in biogeochemical cycling. Clearly testate amoebae are not simply a curiosity topic! Poor taxonomy remains one of the major limitations to the more general study of testate amoebae. As a consequence, a relatively crude taxonomic resolution (morpho-taxa) is generally used in ecological as well as palaeoecological studies. While this approach gives satisfactory results, improved taxonomy is badly needed to make this research more robust. Ongoing, albeit slow (!) progress in taxonomy is already causing the revision of some ideas, especially in biogeography and global biodiversity estimates. The next step will be to assess to which degree cryptic and pseudo-cryptic species differ in their ecology and functional roles in ecosystems. However simple functionally relevant morphological traits such as shell and aperture size have recently been shown to correlate to environmental gradients and to be good predictors of the trophic position of species. Such a functional trait approach is generally limited to macroscopic organisms; applying it to testate amoebae allows to using these organisms to address novel ecological questions thereby further demonstrating the interest of testate amoebae as model organisms in ecology. The last decade has seen an impressive increase in the number of active researchers working on testate amoebae - and a corresponding number of publications. Once a rather obscure research topic, the study of testate amoebae is quickly becoming a dynamic and highly motivating field of research with many young scientists and recently appointed academics in different regions of the world. These are great times to be working on these beautiful and fascinating organisms!

The overview presented in this keynote talk is the result of multiple research project conducted in collaboration with a number of colleagues. I would especially like to thank the senior researchers working on testate amoebae for their enthusiasm and willingness to share their knowledge with the younger generations. A perfect example of this was the late Humphrey Smith whom many colleagues were hoping to see in Xiamen and to the memory of whom I would like to dedicate this keynote talk.



Testate amoebae communities in boundaries: Spatial and temporal perspective

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In order to examine how testate amoebae communities reflect ecosystem boundary effects we studied local assemblages in course of spatial and temporal (seasonal) environmental heterogeneity of different nature. Firstly, we revealed the patterns of changes in community structure of testate amoebae communities along the six types of "water land" boundaries in Bear River (Middle Volga territory): the new frontier, terrigenous and reogeneous border, reogeneous and terrigenous hemiecotones, and full ecotone. For all types of boundaries testate amoebae communities are divided into terrestrial and aquatic variants. Aquatic types of the communities are dominated by the hydrophilic species of the genera Arcella, Difflugia, Cyphoderia, Pseudodifflugia. For terrestrial communities pedobiont and eurybiont groups from the genera Centropyxis, Euglypha, Plagiopyxis, Trinema are charactersitic. The decreasing of the abundance and species richness of testate amoebae in the boundary zones was detected. The clear-cut division of community types was observed in the new frontier and the borders. In hemiecotones and full ecotone the continuous changes of community composition structure from water to land was observed. Secondly, we investigated changes of community structure along a floodplain transect in alluvial soils of the islands in the Ilych River (North Urals territory). Along the floodplain transect (willow meadow - deciduous forest - coniferous forest), the testate amoebae community changed directly. There are spatially homogeneous (low beta-diversity) testacean communities but species rich on the local level (high alpha-diversity) within forests. Within willows and meadows, communities are characterized by low alpha-diversity and high heterogeneity that leads to high gamma-diversity. Thirdly, we studied temporal changes of testate amoebae communities in the swampy forest, located near Leonidovka village in Penza region (Middel Volga territory). Abundance and species composition in different local communities are affected by moisture content in habitat. The greatest abundance of testate amoebae was observed in April, the minimum occurred in May and October. Community of testate amoebae in the early spring was dominated by Nebela bohemica, N. militaris, Trigonopyxis minuta, Euglypha ciliata glabra, Arcella arenaria. Spring-summer complex is represented by species that tolerate to dryness Corythion dubium, Phryganella hemisphaerica, E. laevis, E. ciliata, Centropyxis aerophila, C. minuta. In late summer and autumn the community is dominated by Ph. hemisphaerica, C. dubium, C. minuta.

This work was supported by the Russian Foundation for Basic Research (grant 10-04-00496-a) and grant of the President of Russian Federation (grant MD-4426.2012.4).



Seasonal to decadal temporal patterns of testate amoeba communities in *Sphagnum* peatlands

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The presentation will show various approaches to investigate testate amoeba community change through time, from seasonal to centennial time scales and in relation to an elevation gradient. We conducted a study in four Sphagnum-dominated peatlands along a 1300 m elevation gradient in the Jura Mountains and the Alps of Switzerland over one full year. In this study we explored the abundance and diversity of testate amoebae in relation to the elevation gradient, seasons, and micro-environmental variables (water table depth and hydrochemistry). Testate amoeba density varied seasonally with highest values recorded in spring and summer, but species richness did not vary significantly over the year. The structure of communities varied both seasonally and along the altitudinal gradient. Seasonal patterns in density and community structure suggest a response to micro-climatic and hydrochemical changes (e.g. spring flush of nutrients associated to snow melt). We next used a palaeoecological approach to assess to which climatic variable (from instrumental meteorological data) decadal changes in testate amoeba communities were most strongly correlated over the last 140 year. Strongest correlations were observed with relative humidity, especially in the spring (April-May). While some of these results are in line with previous observations or hypothesized patterns and correlation, others are more intriguing and call for more descriptive (using both neo- and palaeo-ecological approaches) and for experimental studies aiming at better understanding the factors that control testate amoeba population dynamics and changes in community structure. This research is important because a good understanding of seasonal patterns is crucial for sound interpretation of modern (e.g. biomonitoring) and long-term (palaeoecology) data.



Flourish or flush: Effects of simulated extreme rainfall events on *Sphagnum*-dwelling testate amoebae in a subarctic bog (Abisko, Sweden)

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Extreme precipitation events are recognised as important drivers of ecosystem responses to climate change and can considerably affect high-latitude ombrotrophic bogs. Therefore, understanding the relationships between increased rainfall and the biotic components of these ecosystems is necessary for an estimation of climate change impacts. We studied overall effects of increased magnitude, intensity and frequency of rainfall on assemblages of Sphagnum-dwelling testate amoebae in a field climate manipulation experiment located in a relatively dry subarctic bog (Abisko, Sweden). The effects of the treatment were estimated using abundance, species diversity and species structure of living and empty shell assemblages of testate amoebae in living and decaying layers of Sphagnum. Our results show that increased rainfall reduced the mean abundance and species richness of living testate amoebae. Besides, the treatment affected species structure of both living and empty shell assemblages reducing proportions of hydrophilous species. The effects are counterintuitive as increased precipitation-related substrate moisture was expected to have opposite effects on testate amoeba assemblages in relatively dry biotopes. Therefore, we conclude that other rainfall-related factors such as increased infiltration rates and frequency of environmental disturbances can also affect testate amoeba assemblages in Sphagnum and that hydrophilous species are particularly sensitive to variation in these environmental variables.

The fieldwork of the first author at the Abisko Research Station was financially supported by grants of the Royal Swedish Academy of Science (ANS Scholarship 2008). Financial support to Frida Keuper was offered by the Darwin Centre for Biogeosciences (grant 142.161.042) and ANS Scholarship 2008.



Response of testate amoebae to nutrient additions along an altitudinal transect of tropical montane rainforests

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We investigated the response of testate amoebae to moderate additions of nitrogen (N) and phosphorus (P) to montane tropical rainforests along an altitudinal transect in southern Ecuador. Addition of P detrimentally affected testate amoebae with the density of live cells being reduced by 28% (P<0.00002). In contrast, addition of N increased the density of testate amoebae on average by 21% (P=0.002). Notably, the combined addition of P and N negated the positive effect of the addition of N only. Generally, the density of testate amoebae increased in the order P<N+P<Control<N. However, the effects of nutrient additions varied with altitude (P=0.01). The combined addition of N and P only reduced the density of live cells at 1000 and 2000 m, whereas at 3000 m the density in the N+P treatment exceeded that in the control. Generally, altitude explained most of the variance in the density of live cells which increased in the order 1000<3000≤2000 m (P<0.0001).

To relate changes in the structure of testate amoeba communities to changes in the availability of potential prey, we also analyzed the response of soil microorganisms to nutrient additions. Further, to trace mechanisms responsible for nutrient-mediated changes in the density of microorganisms and testate amoebae, we analyzed abiotic factors such as litter C-to-N ratio, pH and water content which likely change with altitude and fertilisation. The results suggest that even moderate and short-term addition of nutrients to tropical rain forests alters the density, community structure and activity of microbial consumers and their prey.

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Do testate amoebae occupy the same trophic position in microbial food webs?

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A major impediment in determining microbial food web structure probably stems from the difficulty in identifying interspecific links and in collecting the required quantitative dietary data. Microbial communities are composed of numerous species and potentially highly complex food webs with numerous feeding links. Discovering the structure of these food webs is an important goal in ecology because they determine major population to ecosystem processes. Here we present an analysis of microbial food web structure in Sphagnum-peatlands by exploring trophic links of testate amoeba consumers. We analyzed (1) the density and biomass of microbial communities, (2) stable isotope (δ^{13} C and δ^{15} N) signatures of microorganisms (i.e. microalgae, testate amoebae, nematods, rotifers), and (3) the feeding habits of main testate amoeba species. We showed that different testate amoeba species such as Archerella flavum, Hvalosphenia papilio and Nebela tincta cover several trophic levels and that their feeding type (bacterivory, algivory, or omnivory) translated both into their isotopic values and trophic position. Our study further confirmed the energetic benefits of endosymbiotic algae for mixotrophic species. The presence of algal endosymbionts modifies the trophic position of *H. papilio* when the density of its preferential prey is low. A sound description of microbial food webs is essential for building food web models and describing microbial food web structure and function, especially in species-rich systems where food web structure and ecosystem function are strongly influenced by human activities.

This research is a contribution of μ POL-AIR project (Use of *Sphagnum*-peatlands to quantify the deposition of long-range air pollutants and to evaluate their impact on environment). μ POL-AIR is supported by PRIMEQUAL program (support of French Ministry of Environment and ADEME) (2010-Q.3-Chorus2100082984).



Ecology of testate amoebae in *Sphagnum* peatlands of central China, with particular reference to hydrology and human activities

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Testate amoebae are a diverse and abundant group of soil protozoa, which constitute a large proportion of microbial biomass in many ecosystems and probably fill important roles in ecosystem functioning. These microorganisms have attracted the interest of palaeoecologists as the preserved shells of amoebae and the known hydrological preferences of many taxa allow the reconstruction of past hydrological change. In *Sphagnum*-dominated peatlands surface wetness reflects hydroclimate, so testate amoebae play an increasingly important role in the reconstruction of Holocene climate change.

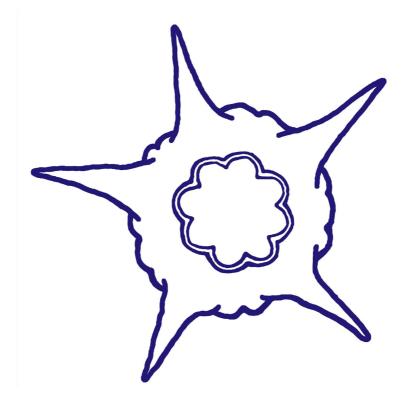
Here we study the ecology of testate amoebae in peatlands from central China in relation to hydrology, pH and metals. We demonstrate that the testate amoeba community structure is correlated with depth to water table (DWT), and that the hydrological preferences of species generally match those of previous studies. We developed a weighted average DWT transfer function that allows the prediction of water table depth with a cross-validated mean error of less than 5 cm. Our results demonstrate the potential for testate amoebae to be used for palaeohydrological reconstruction in China. Such studies could contribute to our understanding of the Holocene climatic changes in China, particularly regarding past Asian monsoon activity.

In addition, our results also suggested that testate amoebae communities have been changed between different sites, which reflected the disturbance of human activities.



Wednesday Afternoon

17 October 2012



PALEOECOLOGY & BIOINDICATION



Microecosystem bryophyte-testate amoebae: A good tool of bioindication and biointegration of disturbances

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Microecosystems constituted of bryophytes and their associated microbial communities constitute whole ecosystems integrating above- and belowground linkages that can be used in the study of environmental disturbances, such as pollution or global warming, on biodiversity and ecosystem functioning. For many years, we highlighted the potential of these microecosystems to assist in the study of the response of (i) an organism, (ii) a population, and (iii) the microbial communities' structure to environmental changes, showing the utility of such systems in bioindication and biointegration studies.

Bioindication studies showed that several testate amoeba species (TA) were sensitive to atmospheric pollution. For example, Assulina muscorum and Bullinularia indica are sensitive to industrial particulate pollution while Paraquadrulla irregularis is sensitive to atmospheric NO₂ pollution. Arcella sp. and Centropyxis sp. are sensitive to particulate and phenanthrene atmospheric pollution. We further showed that the use of simple morphological traits of TA could highlight modifications of the microbial communities' structure during environmental perturbations (biointegration studies). For example, the trophic level of TA can be inferred from the measure of their pseudostome size, i.e. species with a large pseudostome are considered as having a high trophic level. The study of the warming impact on TA communities showed a decrease of 70% of the biomass of TA with a large pseudostome size, suggesting a decrease of the pressure of predation on lower trophic levels, and therefore a modification of the structure of microbial communities. Similarly, we found that particulate pollution modified the relative importance of TA community in microecosystems. Thus, the use of such microecosystems reveals central and relevant information on the anthropogenic disturbances of ecosystems. Nevertheless further studies are required to develop this tool and standardise it for potential general use.

This research is a contribution of µPolair project (Use of *Sphagnum*-peatlands to quantify the deposition of long-range air pollutants and to evaluate their impact on environment), the city of Besançon and the Pays de Montbéliard Agglomeration (PMA, France). µPolair project is supported by the PRIMEQUAL program.



Can testate amoebae be used as hydrological indicators in Amazonian peatlands?

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There has been much recent interest in terms of the diversity, extent and carbon stock of Amazonian peatlands. However, we know very little about their formation and ecohydrological development over time. There is also uncertainty over the future of Amazonian peatlands, as this region has encountered severe droughts over the last decade which may become more frequent with future climatic change. The aim of this research project is to investigate the use of testate amoebae as hydrological indicators in domed peatlands in Peruvian Amazonia. Such domed peatlands operate under an ombrotrophic regime for most of the time, but are affected occasionally by high-magnitude floods.

One hundred contemporary litter samples, a comprehensive suite of environmental data, and peat cores were collected from Aucayacu peatland in 2011. Testate amoebae analysis is currently being carried out on these samples. Initial results suggest that dry litter ridges in the peatland are dominated by the taxon *Hyalosphenia subflava* whereas wet hollows and pools are dominated by *Centropyxis aculeata*. Testate amoebae are preserved in 1-m peat cores from Aucayacu and San Jorge (another domed site in the same region), albeit in low concentrations. Initial ¹⁴C dating suggests that these cores span approximately the last 1,000 years. Multivariate statistical analyses will determine the most important environmental controls on the distribution of testate amoebae in Aucayacu and transfer functions will be developed and rigorously tested before application to subfossil material. This research has important implications for understanding long-term ecohydrological changes in tropical peatlands and the data will be used in conjunction with specially-adapted peatland development models to examine the responses of tropical peatlands to climate change.

This work was funded by a Royal Society (UK) research grant to G.T. Swindles.



Building testate amoebae-based transfer function from *Sphagnum* peatland in Xiaoxing'an Mountains, Northeast China

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Testate amoebae have been routinely used in peat based paleoenvironment research. In this study, we aimed to explore the control factors on testate amoebae composition in peatlands in Xiaoxing'an Mountains, one of main peatland distribution areas in Northeast China, and build transfer functions for quantitively reconstructing paleoenvironment. 91 samples were collected from *Sphagnum* dominated pealands, covering the different microhabitats as more as possible. Six environmental variables, namely depth to water table (DWT), moisture content, pH, conductivity, bulk density and ash content were measured.

All samples were identified with light microscope by 200 times magnification. 55 taxa, 38936 shells were observed. Redundancy analysis (RDA) model with hellinger transformation of species data was used to explore the relationship between testate amoebae composition and environmental variables. The results RDA show that six variables explain 23.15% total variance. All of six variables are significant, while DWT and moisture have greater marginal effect (DWT: 11.85%; moisture: 8.38%). These two variables were chosen as the target variable to build transfer function with WA model and WA-PLS model. Leave-one-out cross validation was used to assess the relative performance of these models. When full data are included the best model for DWT is WA.cal of which RMSE_{jack} is 11.95 cm and r_{jack}^2 is 0.57, while WA-PLS component 2 is better for moisture gives RMSE_{jack} of 2.09% and r^2_{jack} of 0.53. When samples with residual higher than 20% of environmental gradient were excluded from training set, the performances of transfer function models were clearly improved. For DWT, WA.inv outperform the other models, RMSE_{iack} reduce to 7.07 cm and r^2_{iack} to 0.74. WAPLS Component 3 is the best-performance model for moisture, which produces RMSE_{jack} of 1.57%, and the r_{jac}^2 of 0.64. The DWT and moisture of peat can be reconstructed with the mean error of 7.07 cm and 1.57% respectively if the profiles samples have the similar species composition with this study.

This work was supported by the National Natural Science Foundation of China (41001121).



Testate amoebae distribution in lake sediments

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This study consisted in documenting the ecology of testate amoebae and their spatial distribution at the water-sediment interface in different lakes. Four lakes were chosen with different trophic status: in France: (1) Lake Pavin (Puy-de-Dôme, a Crater Lake) = oligo-mesotrophic, (2) Lake Clairvaux (Jura) = mesotrophic, (3) Lake Bonlieu (Jura) = eutrophic; and in Japan: (4) Lake Biwa (Shiga) = a mesotrophic north basin and a eutrophic south basin. Cores were collected at different depths using a sediment corer (1.8 to 2.1 cm of diameter) and the first-cm of sediment was cut and fixed in glutaraldehyde at 2% of final concentration, before counting and identification. The results showed well diversified communities that varied in composition, abundance and diversity in each lake. The presence of common species to all lakes is noticed.

The abundance of testate amoebae is best explained by the amount of organic matter depositions and the concentration of dissolved oxygen. Organic matter inputs as well as physico-chemical parameters of lakes (linked to the trophic status and the variation with the depth) are important factors that influence testate amoeba communities. An annual survey at one station in the Lake Biwa suggests that variations in physico-chemical water conditions and other microbial community compositions during the different seasons have an impact on testate amoeba assemblages. That survey also gives evidences on the importance of sediment composition on testate amoeba assemblages in particular the size of the particles (sand *vs* mud) and the organic matter content that seems to be a limited factor.

This work was supported by the Japanese Society for the Promotion of Science (JSPS), the French CNRS (National Centre for Science Research), and the Region of Franche-Comté.



A multiproxy palaeoecological and palaeoclimatic reconstruction from two raised mires in northwest England

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Past climate has been subject to naturally driven changes and in order to understand present/future climate change it is necessary to have an understanding of past climatic anomalies and their causes. It has long been known that palaeoclimatic data can be obtained from peat bogs, however until recently these were entirely qualitative, however the last few decades have seen attempts to obtain more quantitative climatic data from peat bogs.

Testate amoebae are organisms where hydrology is a primary control on species composition, especially in oceanic regions. As they are often well preserved in peats, the current study aims to provide a climate record spanning the mid-late Holocene using testate amoebae, *Sphagnum* leaf counts and colorimetric analysis of humic acids from peat cores obtained from two ombrotrophic bogs in the northwest of England (South Lancashire and Cheshire) – these have a climatically interesting location on the western edge of Europe. Data from testate amoebae at the south Lancashire site are relatively limited, however results obtained from the Cheshire site are promising, and several shifts in climate have been identified suggested by both testate amoebae and colorimetric analysis which suggest very similar changes in bog hydrology. This site is also currently being managed by the conservation body 'Natural England' in an attempt to restore the partly drained bog surface. Results from this study demonstrate that the restoration demonstrate that this is being at least partly successful.

Pollen and plant macrofossils are also being used to reconstruct the past ecology of the region.



Using "moss/soil interface-testate amoeba community" microecosystems as biointegrators of atmospheric deposition of Phenanthrene

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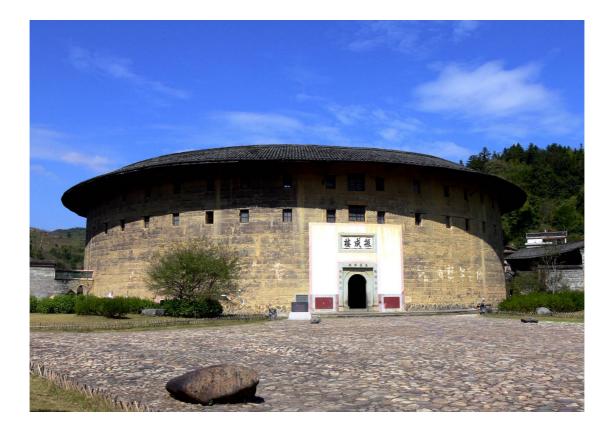
Among ecotoxicological tests on the effects of atmospheric pollutants, microecosystems models like "moss/soil interface- Testate amoebae (TA) community" microecosystems are a good option to understand their impacts on ecosystems. The TA community is sensitive to changes in its environment (climate change, metal or gas pollution) and the response of TA community reflects a response of other microbial groups in microecosystems owing to their trophic position on the top of the microbial food web. Here we studied -under controlled conditions- the effects of atmospheric Phenanthrene (PHE) deposition on the microecosystem "moss/soil interface- TA community" over a 1-month period. PHE is a potentially gaseous PAH generally considered as a model of PAH because of its ubiquity and its preponderance in the atmosphere. We analyzed (1) concentration of PHE in the interface "moss- top of soil", (2) abundance of TA community and (3) the impact of PHE on TA according to their trophic level which is characterized by the pseudostome size. We showed that PHE was accumulated by the moss/soil interface ($45\pm18 \mu g.kgDW^{-1}$) and was significantly negatively correlated $(0.4 < r^2 < 0.7)$ with the total TA abundance and the abundance of 5 species (Arcella sp., Centropyxis sp., Nebela lageniformis, Nebela tincta and *Phryganella* sp.). Among sensitive species, species with a superior trophic level were more sensitive than the other TA species. This result suggests that links between microbial groups in the microecosystems are disrupted by PHE and that this pollutant had a direct (ingestion of the pollutant or direct contact with cell) and / or indirect (decrease of prey) effect on the TA community. The TA community offers a potential integrative tool to understand mechanisms and processes by which the atmospheric PHE deposition affects the links between microbial communities and ecosystems.

This research is a contribution of µPolair project (Use of *Sphagnum*-peatlands to quantify the deposition of long-range air pollutants and to evaluate their impact on environment), the city of Besançon and the Pays de Montbéliard Agglomeration (PMA, France). µPolair project is supported by the French Ministry of the Environment.



Thursday

18 October 2012



Field Trip to Hakka Earth Buildings



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The large scale ecology and biogeography of testate amoebae

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On a global scale testate amoebae are found in terrestrial and freshwater habitats, from tropical forests to the continental Antarctic. They are particularly common in soils with low pH and high organic matter content where they can make up a large proportion of the microbial biomass. In estimating their significance in ecological processes, such as nutrient cycling, it is not just their numbers (or biomass) that are important but also their turn-over – as many generations can be produced during a single year. This, for example, has lead to estimates that they may be as important as plants in the soil silicon cycle (and so potentially important to for silicon entering fresh water and oceans).

Testate amoebae have also been important model organisms in studying the biogeography and diversity of free-living microorganisms and this is the aspect of their biology that my talk will concentrate on. For example two key cases of free-living microbes which clearly do not have a cosmopolitan distribution are the testates Apodera (Nebela) vas and Nebela ansata. Indeed A. vas has now become a textbook example of a microbe with a non-cosmopolitan distribution. An important aspect of my testate related research over the last decade has been to investigate the possible role of size in predicting the likelihood of cosmopolitan distributions. I have taken two approaches to this problem, namely the statistical analysis of polar testate faunas (faunas with similar habitats but a maximum distance apart) and – more recently – by modelling the airborne dispersal of microbes in computer models of the global atmospheric circulation. These studies provide some evidence that cosmopolitan distributions become increasingly more likely in testates between 150 and 20 microns. It is likely that molecular methods will become increasingly important in studies of global testate distribution and diversity over the next decade – for example in identifying potential cryptic species hiding within apparently cosmopolitan taxa.



Digitalisation of the Pernard slide collection of Geneva: Making historical collections accessible to all

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We present an on-going project, which aims to digitalise the collection of Eugène Penard of the Natural History Museum of Geneva, Switzerland. This collection contains 854 slides or over 150 species described at the turn of the end of the 19th century and early 20th century. Each slide contains one or more testate amoeba specimens. This project is funded by Wikimedia.CH (http://www.wikimedia.ch/). Images of each slide will be made freely available to the community on Wikimedia. The information corresponding to each slide will also be entered in the Global Biodiversity Information Facility (GBif) database (http://www.gbif.org/).

Eugène Penard (1855-1954) was a pioneer of testate amoeba taxonomy. His careful observations and detailed descriptions of many testate amoeba taxa still constitute a major reference to this date. The quality of his permanent slides is truly impressive. However despite their excellent state of preservation, microscopy slides are not permanent. Furthermore it is difficult or impossibly for many researchers to travel to individual museums to study them. By making them freely accessible on the internet we hope to help the research community and also to give an opportunity to anyone to discover the incredible morphological diversity (and beauty!) of testate amoebae. This digitalised collection will hopefully also be used as a teaching resource.

This project is funded by Wikimedia CH http://www.wikimedia.ch/



Pacific transect: Testate amoebae of the Commander Islands to Papua New Guinea

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Analysis of soil samples was made Holarctic, Indo-Malayan and Australian parts of terrestrial Pacific - Commander Islands (9 samples), Kamchatka Peninsula (19), the northern coast (Bay of Seal) of the Sea of Okhotsk (13) and southern coast (Uda Bay) of the Sea of Okhotsk (17), Sakhalin Island (39), Japan (152). Far East (70), South Korea (4), China (61), Vietnam (43), the Sunda Islands (28), Papua New Guinea (9), Australia (9). Analysis of data on geographical distribution of testate amoebae showed similar patterns with modern concepts of biogeographic regionalization of terrestrial ecosystems of this part of the earth's land mass.

Species of the genera *Planhoogenraadia, Hoogenraadia, Ellipsopyxis* are to Indo-Malayan and Australian biogeographic regions of the characteristic component of the population of testate amoebae. Representatives *Deharvengia, Distomatopyxis* and *Apolimia* occur less frequently. Important also belongs testate amoebae of the species complex *Centropyxis deflandiana* and *Cyclopyxis lithostoma*, which in this part of terrestrial ecosystems, the Pacific Ocean found just outside the Holarctic or on its southern border.

Testaceans of circum-Australian group - genera *Certesella, Apodera* were not found in any of the samples. The main reason is the small number of samples expected. Findings *Certesella certesi* noted previously in Australia in moist-to-wet Sphagnum (Meisterfeld, Tan, 1998). Northern border of the circum-Australian group testate amoebae is of great interest from the view of biogeography.

This work was supported by Russian Foundation for Basic Research (grant number 11-04-01171-a).



Biogeography of genus *Hoogenraadia* Gauthier-Lievre & Thomas 1958

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Genus *Hoogenraadia* was firstly observed in Africa by Gauthier-Lievre & Thomas in 1958. The characteristic of this genus is the smooth shell with a anterior cap or visor, which makes the aperture a slit opening. Some species of genus *Hoogenraadia* were believed endemic distributions, which support evidence against the cosmopolitan.

The report provides the first description of six species of genus *Hoogenraadia - Hoogenraadia africana* Gauthier-Lievre & Thomas 1958, *Hoogenraadia asiatica* Wang & Min 1987, *Hoogenraadia cryptostoma* Gauthier-Lievre & Thomas 1958, *Hoogenraadia humicola* Bonnet 1975, *Hoogenraadia ovata* Bonnet 1975, *Hoogenraadia sylvatica* Vucetich 1973. According to its own literature and enhanced understanding of the geography of this species, and its ecology. World map shows the distribution of the genus *Hoogenraadia*. The morphological and morphometric characteristics of the species of this genus were described. The results suggested that the morphology of plagiostoma by testate amoebae have not always adaptive significance.

Our work supported morphological data on the six species of genus *Hoogenraadia* and summarized a map on the distribution and biogeography of these species. However, more work is clearly needed to investigate their morphology, biometry and phylogeny before draw the conclusion on either endemic or cosmopolitan distribution.

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Testate amoebae from the Galapagos and mainland Ecuador

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The diversity of testate amoebae has been far less investigated in tropical regions than in temperate regions. This uneven sampling strongly undermines any discussion about the biogeography of testate amoebae. It is therefore critical to explore more intensively the regions of potential interest for biogeographical studies and for which few data are available. Strangely, one of these is the Galapagos archipelago. Although this region has been of central interest for the study of evolution and biogeography, since the time of Charles Darwin only two very incomplete reports currently exist. Ehrenberg (1853) analysed samples collected by Darwin, and Decloître (1973) analysed eight samples from which he described 14 new taxa.

In order to investigate this diverse tropical region more thoroughly we collected soil and moss samples from the Galapagos Islands (94 samples covering all habitats on Santa-Cruz Island from the lowlands to mountain *Sphagnum* peatlands) and mainland Ecuador (36 samples from three sampling locations at 1000, 2000, and 3000 m elevation) and analysed the testate amoeba communities using light microscopy. We compared the diversity of testate amoebae found in these two sets of samples. A total of 225 morphotaxa were identified altogether, of which 98 were observed only in the Galapagos, 75 only in mainland Ecuador, and 52 in both sets of samples. There are potentially 30 new species from the Galapagos and 5 from mainland Ecuador in the material. However, these numbers may have to be increased if detailed morphometrical and molecular analyses can be done on this material.

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Phylogeny of East Asian endemic *Difflugia* (Amoebozoa: Arcellinida) inferred from SSU rRNA gene sequences

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The species of the genus *Difflugia* are abundant free-living testate amoebae and important component of aquatic ecosystems, yet their phylogenetic position within amoebozoa is still poorly known. In this study, we amplified and sequenced the small-subunit ribosomal RNA (SSU rRNA) genes of two morphotypes of *Difflugia tuberspinifera* and one isolate of *D. mulanensis*. These new sequences were compared with published sequences to investigate the molecular phylogeny and the evolutionary relationships of *Difflugia* within the Arcellinida. Both Bayesian and maximum likelihood trees indicated that *Difflugia* is not monophyletic because the all *Difflugia* species are divided into two clades and mixed with the genus *Centropyxis*, and genera *Arcella* and *Netzelia*, respectively. One clade is characterized by a large-sized and pyriform shell without any collar or necklace, the other is characterized by a small or middle-sized and globose shell with a distinct apertural collar or necklace. The results contradict the traditional taxonomy based on shell composition, and corroborate the importance of general shell shape and apertural collar or necklace in the systematics of the genera *Difflugia* and *Netzelia*.

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Recent diversification in the Asian endemic *Difflugia* morphospecies (Amoebozoa: Arcellinida)

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Difflugia is the most species diverse genus of Arcellinid testate amoebae but there are only few molecular data on this group and the taxonomic validity of morphological characters is unclear. We investigated the phylogenetic position within the Arcellinida tree of three endemic Asian *Difflugia* taxa: 1) *D. mulanensis*, 2) a spiny morph of *D. tuberspinifera* and 3) a spineless morph of *D. tuberspinifera* by amplifying the SSU rRNA gene sequences from three independent extractions each.

Our phylogenetic analysis shows that the three taxa are closely related to *D. achlora* and *Netzelia oviformis*. The SSUr RNA gene sequences of the spiny and the spineless morphs of *D. tuberspinifera* differed by only two nucleotides polymorphism and shared identical introns and insertions, suggesting that both morphospecies are evolutionary very closely related to each other, and diverged only recently in evolutionary terms. However, the mechanisms that prompted this evolutionary diversification are still unknown and require further research.

The project was supported by the Knowledge Innovation Program of the Chinese Academy of Sciences (KZCX2-YW-QN401), the National Natural Science Foundation of China (30800097, 31172114 and 41276133), the Xiamen Project of Science and Technology for Distinguished Young Scholars (3502Z20116006), the Science and Technology Cooperation Program Switzerland - Russia grant IZLR Z3_128338 (to E. Lara and E. Mitchell), and the Swiss NSF grant n° PZ00P2_122042 (Ambizione fellowship to E. Lara).



The response of peatland testate amoebae communities (Protists) to in-situ water table changes

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Testate amoebae (TA) living in peatlands are commonly used for palaeoecological reconstructions studies of peatlands and lakes, however, the response of testate amoebae to microenvironmental changes has never been tested experimentally. The aim of the study was to experimentally test the response of TA to changes in water table depth in a field experiment. The study site, Le Russey, is a cutover bog located in the French Jura Mountains. For the experiment, three trenches were dug out to create replicated hydrological gradients. Intact Sphagnum carpets (15 cm diameter) of different origin (hummock, lawn, pool) were collected from the mire and placed at three different water table positions (dry, moist, wet) in September 2008. A second series of samples was seeded with TA extract from the three sampling situations to provide a complete set of species. Moss samples were collected from each plot at three time points (August 2008, May 2009 and August 2009) over a two year period to analyse the changes in TA communities. Results show that density and community structure of TA changed over time. The lowest density of living communities was observed in May 2009 (71,6 ind/mg) and highest concentrations in August (712,1 ind/mg) (Kruskal Wallis test, p<0.01). Furthermore, we recorded significant (Kruskal Wallis test, p < 0.01) drop in species richness and Shannon H. DWT changes had an impact on TA communities. However this effect comes together with an impact of the origin of *Sphagnum* samples and of the added amoebae extract. On that bases we can assume that even though TA are a good and accurate tool used for palaeoecological reconstructions, a great caution should be paid to the setting investigated when interpreting results.



Vegetation and environment determine testate amoebae communities in fens

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Testate amoebae are single celled organisms that are very sensitive to hydrology, pH and minerals content of in the water. Ecology of these organisms in Sphagnum peatlands is actually better understood than those inhabiting minerotrophic fens. In this study we wanted to answer several questions: (i) does vegetation have an important influence on testate amoeba communities? (ii) are particular amoebae species connected with habitats created by specialized mosses and vascular plants? In order to explain those issues we analysed vegetation and testate amoeba communities from 9 fens in Wielkopolska region. Percentage cover of plants was determined at each peatland in one-metre sample plot. Multiple factor analysis (MFA) and moving window analysis were used to explore relationship between testate amoebae, vegetation and hydrochemical variables. In total 141 testate amoebae, 39 moss and 82 vascular plants species were recorded. MFA has shown that hydrochemistry, vascular plants and mosses have similar significance for testate amoebae. However correlation coefficient was the highest for the amoebae and mosses. Shannon diversity and species abundance of testate amoebae, mosses and vascular plant along the poor-rich gradient was the highest in moderately rich and rich fen habitats and decreases in extremely rich fen. Small, idiosomic and symbiotic amoebae were recorded in poor fens while rich fens were important for xenosomic amoebae which use organic and inorganic particles to construct the test. The moving-window analysis shows dependence of the testate amoeba communities to vascular plants and environmental parameters and tends to decline along the poor-rich gradient, while stable response for mosses is evidenced along moderately rich and extremely rich fens.



Testate amoebae biodiversity and biomass in the Linje mire in relation to climate change

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The goal of the research is to assess response to the ongoing climate change of key biological components of the peatland functioning - the microbial communities, with a strong focus on testate amoebae. We would like to experimentally test the sensitivity of a bog surface to change in water table depth in combination with temperature increase. Our study site - Linje mire, located in oceanic-continental climatic transition is expected to be a link between the other experimental sites in Europe and Asia [PEATWARM project, research site: Frasne mire French Jura Mountains; Siberia - CliMireSiber, research site: Mukhrino Field Station - Western Siberia (Russia)]. Hypotheses: Response of the biomass and biodiversity will vary among key- functional groups of microorganisms. We will observe significant changes in testate amoeba (as a key functional group) communities in response to manipulated heat waves. Drought will also induce shifts in microbial communities and biochemical processes. Methods: We will use Open Top Chambers (OTC) warming system for the temperature manipulation. Furthermore, we will change the depth of the ground water table to imitate wet and dry shifts in the peatland hydrology. Expected results: Observed changes in microbial communities will lead to labeling of most sensitive climate change indicators in peatlands.

This work is supported by Polish-Swiss Research Programme (PSPB-013/2010).



Nature and origin of mineral grains agglutinated on testate amoebae

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Increasing knowledge on nature and structure of testate amoeba shells is necessary to improve paleoecological (decomposition of the test) and ecological (role of the test in mineral nutrient cycling, bioindication) studies. In this work we tried to identify the mineralogical nature of some test found in *Sphagnum* leaves in comparison to the nature of minerals present in the watershed of peatlands.

Seven ombrotrophic and minerotrophic peatlands were sampled in central and eastern France for inspecting environment diversity. The chemical determination of the minerals agglutinated within the test walls of testate amoebae was carried out by means of elemental chemical mapping using an Environmental Scanning Electron Microscope (ESEM) equipped with an EDS device. Testate amoebae tests were imaged for elemental mapping (Si, Al, Na, Mg, K, Ca, Ti, Fe and sometimes Cl). The maps were performed on C-coated specimens by means of an Environmental Scanning Electron Microscope (ESEM) equipped with an Energy Dispersive Spectroscopy (EDS) device. Pointed chemical measurements of forty minerals grains were analyzed using an Electron probe micro analyzer (EPMA) (CAMECA SX100) equipped with four wavelength dispersive spectrometers (WDS). X-ray profiles and quantification were carried out at 15kV and 20nA. The mineralogical composition of surrounding rocks was evaluated using geological maps. Main results showed that the composition of test is closely linked to the composition of near terrains suggesting that short-distance atmospheric transport of dry particles is the principal process explaining the nature of test composition.

This work was supported by the PRIMEQUAL program (French Ministry of the Environment, ADEME) and its μ POLAIR project.



Can testate amoebae associated to GDGTs be used to reconstruct past temperatures from peat deposits?

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Peatland dwelling testate amoebae are good indicators of changing environmental conditions. They are thus used in both ecological and paleoecological studies, especially for reconstructing surface moisture (generally as water table depth). More recently they have started to be used in applied ecological research on human impacted peatlands and the history of land-use changes. Within the PEATWARM project, our aim is to determine to what extent these microorganisms could be used as indicators of climate change (moisture and temperature) in temperate regions. Here we propose an identification and calibration of most relevant warming proxies. A high-resolution multi-proxy study of a 4 m peat core collected in the French Jura Mountains using subfossil testate amoebae and branched glycerol diakyl glycerol tetraethers (GDGTs) was used to reconstruct temperature changes during the last 6000 years. GDGTs proxies were shown to overestimate temperature with higher temperatures (ca. 10 °C) than measured annual air temperature (ca. 6 °C) at the surface and were more consistent with spring and summer temperature of the peatland, suggesting that branched GDGT-producing bacteria might be more active during the warmest months of the year. These data suggest that a combined transfer function using both subfossil testate amoebae and GDGT proxies may yield the most accurate palaeotemperature reconstruction from peat deposits.

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Thecamoebians as indicators of seasonally induced hydrological changes in ponds of Lucknow district in the Ganga-Yamuna plains of north India

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The sediment-water interface samples collected from five ponds of Lucknow district were studied to understand thecamoebian response to winter (November) and summer (May) temperature, rainfall and water level in these ponds. The ponds are quite shallow and small, and lie in the heart of the great Ganga-Yamuna plains of North India. This region has dry and hot summer months (April to Early June) followed by rainy monsoon months (mid-June to mid-September). December through February are winter months during which atmospheric temperature becomes significantly low and may also get occasional rains.

Eleven thecamoebian species from these lakes were grouped into two informal assemblages, tentatively named as Assemblage-I and Assemblage-II. Assemblage-I is characterized by presence of *Centropyxis aculeata, C. arcelloides, Arcella vulgaris, A. discoides, Difflugia oblonga*, and *Difflugia gramen*. This assemblage typifies winter months (November) when post-monsoon water level is high in these ponds; have high dissolved oxygen; normal pH; low salinity and exponential growth of aquatic weed *Lemna*. Assemblage-II comprises *Difflugia protaeiformis, Trigonopyxis arcula, Amphitrema flavum, A. stenostoma and Trinema* spp. and characterizes summer months (May) when the day time atmospheric temperature increases to about 46 °C; pH and salinity also increase and dissolved oxygen of water decreases to as low as 5 mg/l; the growth of *Lemna* ceases during this period and the weed detritus settles at the bottom of almost dried ponds. This trend is significant as similar results were obtained from Lake Sadatal about one hundred km away.



Occurrence of testate amoebae in palynological preparations: potential source of new information

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Generally palynological preparations are not used for thecamoebian studies. A simpler method using water is usually preferred so that delicate microfossils or sub-fossils are not damaged or totally destroyed. However, acid resistant thecamoebians have been reported from the palynological slides of Holocene sediments, and Cretaceous and Permian rocks. Thecamoebian studies using standard micropaleontological methods from highly polluted environments often fail to provide meaningful information because most specimens remain dark due to clay and organic particles sticking on the tests. As an alternative to simple use of water a modified palynological method could be used to isolate clean thecamoebians. In this method sediment samples are processed by boiling in 50 ml of 5 % KOH in distilled water for 10 min, and sieved through 150 mesh size (~105 um) to remove larger macroscopic matter. It is followed by acetolysis treatment thus opaque thecamoebian specimens become transparent thus easier to study under transmitted light. The obvious limitations of this method are loss of some information. Determination of the proportion of living thecamoebians (with protoplasm) versus dead ones cannot be done and since only autogenous proteinaceous tests are acid resistant, most other types will not survive harsh chemical and mechanical treatment.

The earliest geological record of thecamoebians is from the Neoproterozoic (~ 742 Ma) Chuar Group, Grand Canyon in Arizona, USA and they are known to occur globally in Neoproterozoic rocks. Despite their long geological history their pre-Holocene occurrences are rare and patchy. Palynological slides of Phanerozoic sedimentary rocks might provide additional data as proven by recent studies on Cretaceous and Permian rocks. It is suggested that careful observation and search for thecamoebians in palynological slides could potentially lead to new information about these microfossils from the entire sedimentary record of the Phanerozoic.



A prelimunary report on thecamoebians from environmentally stressed Himalayan Lake Naintal, India

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Nainital (29°38' N; 79°45' E) is a mountainous (average elevation 2,084 m) city situated in a valley in the lesser Himalaya Mountains. It has temperate summers (maximum temperature 27°C; minimum temperature 7°C); cold winters (maximum temperature 15°C; minimum temperature -3° C) and usually receives snowfall between December and February. The annual rainfall averages around 1294.5 mm. In the center of the city is a crescent shaped Lake Nainital (29°24' N; 79°28' E) which is ~1432 m long and ~42 m wide; its maximum depth is 27.3 m. The lake receives water carrying the household refuge, garbage, silt and huge amounts of sewage through 26 open drains, out of which 3 are perennial and the rest become active only during rainy season. The lake is used as a source of drinking water; fishing and is a major tourist attraction.

This lake is highly polluted primarily due to sewage it receives from human settlement in its catchment areas. Deforestation in the valley especially along the lake shore accelerates transportation of eroded sediments from the slopes thus filling this lake rapidly. The lake water is alkaline (pH 8.4–9.3) and receives toxic substances like heavy metals (manganese, copper, nickel, lead, chromium, cadmium and zinc) that get adsorbed onto the suspended sediments and settle at the bottom of the lake. The association of these metals with exchangeable fraction cause toxic impacts on aquatic life. A low diversity thecamoebian fauna dominated by various Arcellids and Centropyxids inhabits the lake bottom. This assemblage is clearly indicative of environmentally stressed lake.



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