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# Meeting Report: 5th International Symposium on Testate Amoebae (ISTA-5), Montbéliard, France, 14–17 September 2009 - Present Status of Testate Amoeba Research, Knowledge Gaps and Research Priorities

A group of 55 scientists from 20 countries met for a three days symposium on testate amoebae in the French city of Monbéliard from the 14th to the 17th of September 2009. This meeting follows the previous ISTA meeting held in Antwerp, Belgium in 2006 (Warner and Beyens 2007). The number of attendees, countries represented, talks (30), and posters (28) presented all increased significantly since the previous ISTA, clearly illustrating the present dynamism of research on testate amoebae. The book of abstract is posted on the following website: (http://chrono-environnement.univ-fcomte.fr/spip.php?article631).

The meeting was organised in five sessions: (1) biology, physiology and ecology, (2) biogeography and biodiversity, (3) phylogeny and taxonomy, (4) palaeoecology, and (5) testate amoebae and bioindication. Contributed talks and posters were dominated by descriptive ecological studies on community responses to ecological gradients and species-environment correlation: Over 50% of all contributions dealt with ecology and palaeoecology (Table 1) and 2/3 of these dealt with peatlands and lakes.

## Biology, Physiology and Ecology

The meeting started with the keynote presentation by Louis Beyens (Belgium) who addressed several questions on global change and biodiversity in the Arctic. Both the patterns of diversity (including possible endemic species) and likely effects of global change (climate change, pollution) are less clear for microorganisms, including testate amoebae than macroscopic organisms. More observational (e.g. on species distribution and dispersal potential, eco-physiology) and experimental (e.g. transplants, environmental manipulation) data are clearly therefore required (Beyens et al. 2009).

Humphrey Smith gave a review on existing knowledge on biogeographical records, laboratory experiments and seasonal studies on the effect of environmental factors on testate amoebae. Andrey Tsyganov and Vincent Jassey, presented field and mesocosm experiments on the effect of warming on testate amoebae in the high Arctic tundra and *Sphagnum* mosses respectively. Several studies explored the diversity and ecology of testate amoebae in peatland and forest soil from Russia, Estonia, Poland, China, and Switzerland (by Yuri Mazei, Eve Niinemets, Lucasz and Mariusz Lamentowicz, Irina Kurina, Hongai Li, and co-authors) at different scales (from microecotones to altitudinal gradients) and in some cases over several seasons.

It was especially interesting to see results from some studies on rather unusual topics or generally poorly studied ecosystems and regions. South-America and the Caribbean were well represented with two studies on each neotropical forests (by Valentyna Krashevska and by Dimaris Acosta-Mercado and colleagues) and neotropical rivers (by Fábio Amodêo Lansac-Tôha and by Luiz Felipe Machado Velho and colleagues) (Alves et al. 2008). Marijke Ooms and colleagues reported a clear relationship between soil testate amoebae and an elevation gradient in a freshwater estuarine marsh. Martin Vohnik, Tereza Konvalinkova and colleagues provided two studies on biotic interactions between testate amoebae and fungi, an important topic that follows on previous observations in situ and experiments suggesting a possible tight link between testate amoebae and


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**Table 1.** Topics covered by talks and posters presented at the 5th International Symposium on Testate Amoebae (ISTA-5).

| Dominant theme | *n* | % |
|---|---|---|
| Cell biology | 5 | 9 |
| Genetics (gene evolution, phylogeny, biogeography) | 8 | 14 |
| Taxonomy (morphological) | 5 | 9 |
| Biogeography (morphological data) * | 5 | 9 |
| Ecology * | 18 | 31 |
| Palaeoecology * | 12 | 21 |
| Ecotoxicology and bioindication* | 5 | 9 |
| Total | 58 | 100 |

*Topics requiring sound taxonomy, total of all contributions: 40 or 70%.

soil (including mycorrhizal) fungi (Vohník et al. 2009).

Cell biology also emerged as an original field at this meeting with studies on endosymbionts of testate amoebae by Julia Török and Daniele Corsaro. Zuzana Burdikova presented examples of possible uses of confocal microscopy to study testate amoeba cell and shell morphology. Eric Armynot du Chatelet used Environmental Scanning Electron Microscopy (ESEM) and Energy Dispersive X-ray Spectroscopy (EDS) to assess if testate amoebae that produce shells by agglutinating mineral particles discriminate among different minerals and the size distribution of particles used for test building.

A clear majority of the contributions presented at the meeting (40 out of 58, or 70%) dealt with topics where reliable taxonomy is essential (e.g. community ecology, palaeoecology, biogeography and ecotoxicology). However, a clear consensus among the participants to the meeting is that progress in taxonomy is urgently needed (see further). But, as for many other groups of protists, the species concept in testate amoebae is far from clear. Anatoly Bobrov discussed the phenotypic variability in relation to some characteristics of species such as ecological amplitude and biogeography.

## Biogeography and Biodiversity

This session started with the keynote presentation by David Wilkinson (U.K.) who gave a broad overview of current research and debates related to testate amoeba biogeography. Descriptive work as well as results from modelling support the idea of (at least partial) limited distribution of testate amoebae and confirm that these protists are indeed good models organisms for studying the biogeography of free-living microorganisms (Smith et al. 2008).

New biogeographical data based on morphological studies were presented from Canada, Russia, Bulgaria, China and Japan. Thierry Heger and Enrique Lara used mitochondrial DNA to assess the genetic variability in relation to geographical distance and size of respectively Nebelids and genus *Assulina*. The results showed complex patterns and also suggest that current biodiversity estimates are under-evaluated. Much more comparable work will be needed to provide firmer bases for the ongoing debate on the biogeography of free-living microorganisms.

## Phylogeny and Taxonomy: Morphology vs. Molecules

An increasing number of groups are now applying molecular methods to study the phylogeny, taxonomy, biogeography and evolution of testate amoebae. In his keynote talk, Ralf Meisterfeld (Germany) gave an overview of the current state of the art in the molecular and morphological taxonomy both the Euglyphid and Arcellinid testate amoebae. As pointed out by H. Smith and A. Bobrov in their respective talks, the question of phenotypic plasticity is clearly a challenge for taxonomists (and ecologists and other users of taxonomy!). Overcoming current limitations in taxonomy will require significant work on basic biology (e.g. live cycles), characterisation of cell characteristics (e.g. number and morphology of nuclei), how environmental conditions affect morphology and growth. A number of

species complexes have been recognised and these can be considered as groups of cryptic or pseudo-cryptic species or as an illustration of phenotypic plasticity. This debate will only be solved by careful combined morphometrical and molecular studies. Basic questions such as the monophyly of Arcellinida are not yet resolved.

Milcho Todorov and Yangmin Qin described two new species in Bulgaria and in China respectively based on morphological characteristics. Four other studies combining molecular and morphological data were presented by Alexandre Kudryavtsev (on Microchlamiidae), Julia Török (on *Arcella*), Thierry Heger (on *Cyphoderia*) (Heger et al. 2010), and Wenjing Zhang (on a new project on genus *Difflugia*). Daniel Lahr presented a multi-gene study of evolution of *Arcella* and *Cryptodifflugia*.

Studies such as these are clearly changing our perception of phylogenetic relationships among testate amoebae, their evolutionary history and will have major implications for their applied uses as bioindicators. These applied uses were one of the justifications for these molecular studies but the results generated are shedding light into hitherto unsuspected complexities in almost every possible aspect: (1) The taxonomy of all groups and therefore biodiversity estimates will need to be revised. (2) This will significantly alter our perception of biogeographical patterns. (3) The evolutionary history and phylogenetic relationships will be reconsidered in the light of molecular data. These are clearly fascinating times for testate amoeba research!

The session ended with a practical session devoted to taxonomy where attendees showed examples of testate amoeba images or "mystery photographs" of unidentified taxa. This was especially useful for young scientists who were able to discuss the taxonomy of problematic taxa with leading taxonomists. This last session clearly illustrated the need for (1) a major effort to make existing knowledge accessible, through compilations of descriptions, translations and the creation of identification key and illustrated guides, and (2) detailed taxonomic studies and revision of many "species complexes".

## Palaeoecology

Palaeoecology alone accounted for over 20% of all contributions to this meeting, and thus continues to represent a major application for testate amoeba research (Table 1). This session started with the keynote presentation by Robert Booth (USA) who illustrated some important open questions. (1) Past assemblages often do not have any modern analogues. (2) Some important factors such as potential differential preservation of testate amoeba shells in peat and sediments may affect the quality of palaeoenvironmental reconstruction. (3) The strength of different environmental factors in affecting community composition may change over time in relation ecosystem development. (4) Little is still known on seasonal or inter-annual variability. (5) Most existing models use either single measurements of environmental variables or average values but the importance of ecological fluctuations on testate amoebae have not yet been studied and we do not understand how different factors affect testate amoebae. (6) We do not know much about the ecology and potential uses in palaeoecology of testate amoebae from peatland types other than *Sphagnum*-dominated ones.

Historically, palaeoecological studies using testate amoebae on peat deposits are more numerous than studies of lake sediments. However, in this meeting the two topics were well balanced with new projects assessing the use of testate amoebae as palaeoclimatic indicators in lakes of Nova Scotia (Canada), by Jun Yang, the French Jura Mountains, by Adeline Wall, China, by Yangmin Qin, and as indicators of eutrophication in the Canadian Big Lakes area, by Helen Roe.

A general limitation of palaeoecogical studies of peat deposits is the relatively low temporal resolution, as compared to annually resolved records such as tree rings. However, some recent high resolution studies such as presented by Mariusz Lamentowicz and colleagues from the Eastern Alps of Switzerland now reach near annual resolution with sampling intervals of 0.2 mm (Lamentowicz et al. 2010). Other palaeoecological studies of peatlands were presented from France, Poland, and Northern Quebec.

Finally Pamela Santibañez presented an unusual study on testate amoebae from an ice core including the description of a new species. Will testate amoebae now become a new tool in ice cores studies? Beyond this interesting discovery, it would be interesting to know more about cold tolerance of testate amoebae and to which extent they can be active in winter, a subject on which very little is known.

## Testate Amoebae and Bioindication

The use of testate amoebae as bioindicators is clearly one of the leading research topic overall if

palaeoecology is included. However, in recent years there research has also focused on other applied uses of testate amoebae in bioindication, especially for the monitoring of pollution such as heavy metals and ecosystem management or restoration. In parallel ecotoxicological studies are undertaken to assess under controlled conditions how testate amoebae respond to specific pollutants (Nguyen-Viet et al. 2008). The talks presented at the meeting included effects of $NO_2$, and others that had not yet been studied with respect to their effect on testate amoebae: crude oil (Lisa Neville and Francine McCarthy), particulate pollution (PM10) (Caroline Meyer), and sulphur deposition (Richard Payne). In bioindication, testate amoebae may either be used alone or in combination with other micro-organisms as shown by Daniel Gilbert.

A new development that is likely to attract much attention in the future is the use of testate amoebae in forensic science. There are at least two published papers on the topic where soil testate amoebae were used to match a crime scene with soil attached to the shoes of a suspect (Chardez 1990; Swindles and Ruffell 2009). In a different approach, Ildiko Szelecz and colleagues presented new experimental project aiming at assessing how soil organisms including testate amoebae respond to a decaying body. Will the analysis of soil testate amoebae soon become a new standard tool in forensic science?

## Final Points and Decisions

In the final plenary discussion, meeting, participants decided that meetings should be held every three years. The next meeting will take place in China. Meeting participants also agreed that the time was right for establishing a new society of testate amoebae scientists. This society has not yet been formally established but will hopefully become a reality before the next ISTA in 2012 in China (organizers: Jun Yang, China and Satoshi Shimano, Japan). Anyone interested in becoming a member of this new society or contributing to its development should contract EM.

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