ISTA 10 Madrid, Spain

10TH INTERNATIONAL SYMPOSIUM ON TESTATE AMOEBAE

2-6 October 2023 in Madrid, Spain

Abstract booklet

REAL JARDÍN BOTÁNICO

GOBIERNO DE ESPAÑA
MINISTERIO DE CIENCIA E INNOVACIÓN
CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

International Society for Testate Amoeba Research Meeting

Dear Colleagues and Friends,

It is a great pleasure for me to welcome you to the **10th International Symposium on Testate Amoebae ISTA-10**. The meeting is organized by the Royal Botanical Garden-CSIC in Madrid, Spain, **2-6 October 2023**.

Welcome to **Madrid**!

With kindest regards

Enrique Lara

Local Organizing Committee

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This year's ISTA has an X (former Twitter) account in which you can follow the news of the Symposium and its attendant's work: https://twitter.com/ISTAmoebae/

The aim of the account is to present your work on testate amoebas to those that could not come to the ISTA, and to broaden the reach of what we know will be fascinating research to bring together people from different disciplines and promote future collaborations in multidisciplinary projects.

We hope it will be useful for all of us and we will keep open the account for future meetings.

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PROGRAM

	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY
Time	2/10/23	3/10/23	4/10/23	5/10/23	6/10/23
9:00					9:00 Excursion Guadarrama Mountains **Estación de Investigación del Ventorrillo**
10:00-10:15		**Opening Welcome and overview** Enrique Lara (CSIC)	**Welcome**	**Welcome**	
10:15-10:45		**Invited talk 1: Helen Roe** 'Navigating through Murky Waters: Understanding the Role of Testate Amoebae as Nutrient Status Indicators in Lakes'	**Invited talk 2: Valentyna Krashevska** Synthesis of global distribution of testate amoebae diversity, testate amoeba taxonomy and traits database	**Invited talk 3: Kenneth Dumack** Eukaryvorous shell-bearing amoebae – Diversity and feeding mechanisms	
10:45-11:20		**Session 1: Palaeoecology and Palaeoclimatology**	**Session 4: All molecular**	**Session 8: Biogeography & Macroecology**	
10:50		**Kirill Babeshko** Testate amoeba-based palaeoecological reconstructions of mire hydrological regime in permafrost zone (Middle Siberian plateau)	**Jean Claude Ndayishimiye** Diversity and community assembly of testate amoebae and other microbial eukaryotes in diverse biotopes within urban parks: morphological and metabarcoding perspective	**Basil Yakimov** Latitudinal patterns of multi-scale diversity partitioning in soil testate amoebae: A case study in Western Siberia	
11:05		**Martin Souto** Diversity of Testate Amoebae in the Azores archipelago and their use as a paleoenvironmental indicator	**David Singer** Introducing a metabarcoding approach to survey Arcellinida diversity at the species level: fast, cheap and accurate	**Jiahui Su** Climate- and microhabitat-driven patterns of peatland testate amoebae diversity at the continental scale	
11:20		**Robert Booth** The Neotoma Testate Amoeba Database: progress and potential for global change research	**Fernando Useros** Independent colonizations of athalassohaline water bodies by freshwater lobose testate amoeba (Arcellinida, Amoebozoa)	**Yuri Mazei** Relations between testate amoebae, vegetation and environment: patterns and mechanisms at various spatial and temporal scales	
11:35-11:50		Coffee break + Posters	Coffee break	Coffee break	
11:50-12:35		**Session 2: Palaeoecology and Palaeoclimatology**	**Session 5: All molecular**	**Session 9: Biogeography & Macroecology**	
11:50		**Gu Xiuyuan** Sphagnum-dwelling testate amoeba assemblages in mires of Central Siberia and their relationship to water table depth	**Matthew Brown** Developing arcellinid shelled amoebae as genomic models to examine the origin of evolutionary novelty	**Estelle Bruni** Climate niche distribution modelling of Hyalosphenia papilio (Amoebozoa: Arcellinida) conflicts with documented occurrences in the Azores (Portugal) revealing dispersal limitation and the first evidence of island disharmony among microorganisms	
12:05		**Olivia Kuuri-Riutta** Changes in testate amoeba communities after two decades of water table drawdown in a boreal peatland	**Giulia Ribeiro** Unraveling Arsenic Resistance and Adaptive Strategies in Arcellinida: Insights from Transcriptomics and Evolutionary Perspectives.	**Edward Mitchell** Distribution modelling of Apodera vas reveals latitudinal dispersal limitation and range contraction in response to climate warming	
12:20		**Fernanda Charqueño Celis** Response of testate amoebae assemblages to environmental and climatic changes during the last ~700 yrs in Laguna Polo, Patagonia, Argentina	**Alfredo Porfirio de Sousa** From Neoproterozoic and Beyond: Amoebozoan testate amoebae illuminate the diversity of heterotrophs and the origin of complex ecosystems throughout geological time	**Stefan Luketa** Diversity of testate amoebae in the Oriental region	
12:35		**Angela Creevy** Testate amoebae and their role in estimating success of forest-to-bog restoration techniques	**Carmen Soler Zamora** The problem of “shadow species” as illustrated with the taxonomic hotchpotch Cyphoderia ampulla (Rhizaria: Cyphoderiidae)	**Brunella Palacios Ganoza** Changes in testate amoeba community’s functional traits after two decades of water table drawdown in a boreal peatland	
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14:30		**Callum Evans** Response of testate amoeba communities to peatland drain blocking	**Elizabeta Ermolaeva** Effects of taxonomic identification bias on ecological inferences: research community survey on testate amoeba microscopy dataset		
14:45		**Anne Nguyen** Potential effects of sulfur-rich fumigation on testate amoebae assemblages, Smoking Hills area, Northwest Territories, Canada	**Andrey Tsyganov** Testate amoeba unified and standardized shell trait description		
15:00	**Registration**	**Caroline Meyer** Use of moss samples stored in powder form: a solution for large-scale inventories of testate amoebae?	**Clément Duckert** Illustrated morphotype catalogues to improve the consistency of studies based on testate amoebae: example with an elevation gradient in Hawaii.		
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15:30			**Eric Armynot du Châtelet** Morphometry of genera Apodera and Trinema from the Kerguelen archipelago suggests environmentally driven phenotypic plasticity	Coffee break	
15:45			**Julia Török** Morphology, Distribution and Ecological Significance of Sand-dwelling Testate Amoebae in European and North American Freshwater Ecosystems		
16:00			**Anatoly Bobrov** Morphology, Preliminary results of the study of testate amoebae biogeography (based on personal data)	**ISTAR prizes**	
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17:30					
18:00					
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20:00				**Social dinner**	

ABSTRACTS:

INVITED TALKS

'Navigating through Murky Waters: Understanding the Role of Testate Amoebae as Nutrient Status Indicators in Lakes'

Helen M. Roe[1*]

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Testate amoebae (TA) are important nutrient status indicators and show considerable potential for ecological assessment in lakes. In spite of recent advances, the character and specific mechanisms of TA community change in response to nutrient enrichment, and the interactions of testate amoebae with other biological groups in disturbed lakes, still remain poorly understood. This paper will review some of the major developments in understanding TA responses to nutrient loading in lacustrine environments, from seminal early work on the autoecology of key indicator species, to the development of quantitative approaches, including synecological and traits-based approaches. Emphasis will be placed on the insights that can be drawn from other biological groups which typically preserve alongside testate amoebae in lake sediments (e.g., diatoms, plant macrofossils, cladocerans and chironomids). These not only have the potential to elucidate TA responses to specific stressors (e.g., Phosphorus loading), but can aid in understanding wider food-web interactions and major thresholds of ecological change. Four site records will be discussed with high resolution testate amoeba and multi-indicator palaeolimnological data, each with a known history of water quality change. These include lakes with a history of eutrophication linked to land use change, lakes with a complex history of macrophyte development (cf. Prentice et al. 2018), and a lake with a record of historic fish kills. While TA responses can be complex and may partly reflect microhabitat-related controls, the synchronity of the assemblage changes between testate amoebae and other, arguably more well established limnological indicators, clearly underlines the ability of TA to track trophic state. Moreover, traits-based data from a well-studied lake sediment core from SE Scotland with a long history of cultural eutrophication (Loch Leven), further highlights an association between TA morphology (notably test volume and aperture size) in response to nutrient loading; a response which may be mediated by plant macrophyte composition. Similar relationships between test morphology and lake trophic status have been observed in modern lake settings, for example, in County Fermanagh, Northern Ireland. Ongoing lake surface sediment and vegetation sampling are helping to resolve outstanding questions regarding plant community and TA interactions in nutrient-laden lakes. Integrated studies, which combine an understanding of the environmental, molecular ecological and traits-

based responses to this important limnological stressor offer a promising field of future research.

Prentice, S.V., Roe, H.M., Bennion, H., Sayer, C.D., Salgado, J. (2018). Refining the palaeoecology of lacustrine testate amoebae: insights from a plant macrofossil record from a eutrophic Scottish lake. *Journal of Palaeolimnology*, 60: 189-207.

Synthesis of global distribution of testate amoebae diversity, testate amoeba taxonomy and traits database

Krashevska, V. ,[1,2] * Jassey, V., Walcker, R., Mulot, M., Singer, D., Lara, E., Geisen, S., Mitchell, E.A.D, Mazei, Yu., Tsyganov, A. N., Armynot du Châtelet, E., Babeshko, K., Belyakova, O., Beyens, L., Booth, B., Brygadyrenko, V.V., Bynkov, N., Carballeira Coego, R., Charqueño Celis, N.F., Chernyshov, V., Creevy, A., Davidova, R., Duckert, C., Echeverría-Galindo, P.E., Estrada, R., Fernández, L.D., Fournier, B., Gonçalves, V., González Miguéns, R., Hawthorne, D., Herlédan, M., Komlyk, V., Kosakyan, A., Kurina, I., Lahr, D.J.G., Lamentowicz, L., Lamentowicz, M., Loisel, J., Luketa, S., Marcisz, K., Marfina, Parra, A., O., McKeown, M., Pérez Juárez, H., Pontevedra-Pombal, X., Porfírio-Sousa, A.L., Qin, Y., Raposeiro, P.M., Reyes Santos, M., Ribeiro, G.M., Robroek, B., Roland, Th., Shimano, S., Sigala, I, Siemensma, F., Sogame, Y., Souto-Souto, M., Swindles, G., Todorov, M., Tran, H., Väliranta, M., Wanner, M., Whittle, A., Wilmshurs, J., Zhang, H., Wilkinson, D. M.

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A comprehensive understanding of ecological patterns and global environmental challenges relies on global data. Therefore, we undertook an extensive data collection effort of testate amoebae species occurrence at a global scale. Our dataset includes information from 10,889 locations worldwide, encompassing 9,235 terrestrial sites, 1,322 freshwater sites, and 219 marine-interstitial sites. In terrestrial systems alone, we documented the presence of 1,187 species. I will share our latest results on the diversity and endemicity of testate amoebae in different biomes and show the factors responsible for these patterns. The data we have collected serve as the foundation for identifying key ecological factors influencing the diversity and species composition of testate amoebae, providing insights into patterns of microbial diversity.

In order to expand and maintain our collection, the development of a user-friendly, open-access database is essential to facilitate the study of testate amoebae. I will introduce preliminary concepts for the database, including its structure and potential applications. Before proceeding, however, it is crucial to standardize the description of the shell traits to ensure their comparability and integration across different scientific fields. Some news related to trait standardization will also be presented.

Eukaryvorous shell-bearing amoebae – Diversity and feeding mechanisms

Kenneth Dumack*

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In the last ten years, I conducted a number of research on eukaryvorous amoebae. Many of these previously considered "unculturable" protists, are indeed culturable, but need other eukaryotes as their prey. I will present a summary of the diversity of little-known cercozoan shell-bearing amoebae, especially very minute taxa of the Thecofilosea, whose shell is thin, flexible and organic. While doing so, I wish to convince you that culturing of those taxa is indeed possible and I will try to emphasize the value of culture-based observations. Among these observations, you will find a number of visible proofs of interactions that may have been indicated by correlations in environmental sequencing datasets, but I will also provide you with evidence of the underlying mechanisms. Most interestingly, I will show how the Phryganellina (Amoebozoa) feed on large prey, i.e. filamentous fungi or large algal cells. I will show that *Phryganella* and *Cryptodifflugia* are both capable to manipulate large prey to make their contents accessible. A number of observations support that this process is not supported by chitinases or cellulases, but instead functions mechanically by an actin-mediated, strong push and/or pull movement. Shell morphology already evidences that shell-bearing amoebae are masters of the cytoskeleton - in my presentation you will see that this also applies to how they feed.

ABSTRACTS:

TALKS

Testate amoeba-based palaeoecological reconstructions of mire hydrological regime in permafrost zone (Middle Siberian plateau)

Kirill Babeshko[1,2]*, Gu X. [1,2], Andrey Tsyganov[1,2], Natalia Mazei[1,2], Elena Novenko[1,3], Alexander Komarov[4], Yuri Mazei[1,2],

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We performed palaeohydrological reconstructions of three peat deposits located in permafrost zone of the Central Siberian plateau using testate amoebae. The deepest deposits (8.6 m) were extracted from a palsa peatland (N 67.531603, E 86.634903) which is a perennial frost mounds alternated by hollows. The second core (0.5 m deep) (N 64.16803, E 100.5333) was sampled from an ombrotrophic bog with well-expressed hummock-hollow microtopography. The third peatland is a hollow-ridge complex with 0.5 m thick deposits (N 69.470984 E 91.440841) that slightly inclines towards a lake. The conducted studies have shown that the reconstruction of the mire dynamics and its hydrological regime using testate amoebae in the permafrost zone meets with a number of difficulties. In case of the palsa peatland, the main difficulties are related to the permafrost processes, that results in vertical movements of the permafrost layer due to freezing and thawing. This leads to mixing of peat and disruption of stratigraphy. In other cases, the reconstructed water table levels based on to testate amoebae does not correspond to the indicators of local vegetation, which was reconstructed using plant macrofossils. Plant macrofossils showed more eutrophic conditions. This could be explained by local fires (as shown by charcoal remains), which are known to lead to eutrophication. Thus, the main difficulties for palaeohydrological reconstruction of mires in the permafrost zone are related cryogenic processes in the peat profile and fire-related eutrophication.

The work was supported by the Russian Science Foundation (19-14-00102).

Diversity of Testate Amoebae in the Azores archipelago and their use as a paleoenvironmental indicator

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Keywords: Azores; Environmental reconstruction; Protozoa; Distribution patterns.

Testate Amoebae are a diverse group of microorganisms included mostly in two major phyla: Amoebozoa and Cercozoa. These unicellular ameboid protists with a test are present in a wide variety lacustrine and terrestrial habitat. Within this group some have tests (shells) that are highly resistant to degradation and are preserved in lake sediments. These organisms show great sensitivity to variations in temperature or acidity, to changes in land use, or to alterations in hydrological regime, making them an important class of palaeo-environmental indicators.

Considering that little is known about these organisms in the Azores, we intend with this work to contribute to the development of palaeo-environmental applications and to broaden the knowledge about their diversity. The present work aims to characterize the testate amoebae communities present in several types of habitats of the Azores.

In total 75 taxa were identified, 50 Amoebozoa and 25 Cercozoa, in several habitats distributed over 7 islands of the archipelago. The most common genera for Cercozoa are *Euglypha* and *Trinema* and for Amebozoa, *Nebela* and *Heleopera*. Preliminary analyses reveal a significant separation of the testate amoebae communities between the areas of humid scrubland with abundant moss communities, the peatland areas dominated by mosses of the genus *Sphagnum* and the lakes with submerged and floating vegetation. This knowledge on the ecology of testate amoebae can be applied in future palaeoecological studies and monitoring of present-day ecosystems.

The Neotoma Testate Amoeba Database: progress and potential for global change research.

Robert K. Booth[*], Erica M. Cowper, Jared M. Kodero, Thomas A. O'Rourke, and Alexis R. Stansfield

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Recent studies have highlighted the value of bringing together testate amoeba surface-sample and paleoecological datasets from broad regions to address continental and global-scale research questions. Such efforts can be facilitated by large, well-maintained, publicly available databases, such as the Neotoma paleoecology database (neotomadb.org), which contains site-based records for many types of paleoecological data (e.g., pollen, diatoms, vertebrates). Testate amoeba data are contained within two taxonomically linked databases: 1) stratigraphic records from peatlands and lakes, and 2) surface-samples containing information on modern communities. Over 4500 surface samples and over 100 paleoecological records are now available through Neotoma.

In this study we present a synthesis of ecological and paleoecological studies during the past ~35 years, and discuss how these data are being incorporated into Neotoma and how the research community can get involved in this effort. We report results from two case studies that highlight the potential value of Neotoma including 1) an assessment of species-environment relationships in surface samples from the northern hemisphere and 2) a comparison of subfossil and modern communities to determine the strength of modern-analogues. Our results highlight the central importance of moisture in controlling peatland testate amoeba community composition, and attest to the spatial stability of species-environment relationships. However, subfossil-modern comparisons reveal that poor modern analogues are common, even after removing taxa with weakly idiosomic tests. Research and educational applications of Neotoma are numerous and its structure allows for taxonomic revision and the accommodation of new types of data, giving it flexibility as research approaches develop.

Sphagnum-dwelling testate amoeba assemblages in mires of Central Siberia and their relationship to water table depth

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Testate amoebae have received a considerable attention for their functional significance and application as indicators of environmental conditions, especially in mire ecosystems. The aim of this study is to investigate the species composition of *Sphagnum*-dwelling testate amoebae in mires located in the Central Siberia. In total, 336 samples were collected in 33 mire ecosystems (60.35 - 69.48° N, 86.62°- 102.38° E) with the corresponding measurements of the water table depth (WTD, cm). We identified 173 testate amoebae taxa belonging to 45 genera. The values of WTD ranged from -3 to 87 cm. The most abundant taxa (the relative abundance to the total counts) were *Assulina muscorum* (9.9%), *Hyalosphenia papilio* (8. 5%), *Trinema lineare* (6.4%), *Cryptodifflugia oviformis fusca* (5.6%) and *Archerella flavum* (4.7%). The number of testate amoeba taxa per sample varied from 2 to 38 (mean 17.6 ± 7.22 SD). We calculated the optima and tolerance for testate amoebae taxa observed in six or more samples using the weighted averaging approach. The most xerophilic taxa were *Trigonopyxis minuta* (opt. 50.4, tol. 23.9), *Bullinularia indica* (opt. 33, tol. 18.4), *Trigonopyxis arcula* (opt. 31.7, tol. 18.7) and *Euglypha simplex* (opt. 29.6, tol. 12.8). The hydrophilic taxa were *Arcella gibbosa* (opt. 1, tol. 3.8), *Amphitrema wrightianum* (opt. 1.3, tol. 3.3), *Arcella vulgaris* (opt. 1.5, tol. 5.2) and *Netzelia wailesi* (opt. 2, tol. 4.3). These taxa can be used as indicators of mire surface wetness in the study region. Regional transfer function will be developed on the basis of this dataset.

The work was supported by the Russian Science Foundation (19-14-00102).

Changes in testate amoeba communities after two decades of water table drawdown in a boreal peatland

Olivia Kuuri-Riutta[1*], Brunella Palacios Ganoza[1], Minna Väliranta[2], Edward A. D. Mitchell[3], Eeva-Stiina Tuittila[1]

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Drying and associated changes in vegetation are a suggested consequence of the ongoing climate change for boreal peatlands. This will likely alter the composition of testate amoeba (TA) communities and be further reflected in altered ecosystem functions affected by TA and other soil microorganisms, such as carbon and nutrient cycling. TA are known to respond to water level fluctuations rapidly, and since their tests are well preserved in peat, they are commonly used as proxies to infer past peatland hydrology. However, studies focusing on the impacts of decadal drying and associated secondary changes on TA communities remain scarce. To assess such impacts, we studied TA communities in a controlled water-level drawdown experiment that has been maintained since 2000.

Our experiment is situated in Lakkasuo peatland, Central Finland, and it consists of three experimental water-level drawdown sites (WLD) and corresponding control sites, each pair representing a different peatland type: ombrotrophic bog, poor fen and rich (mesotrophic) fen. We collected 8-10 moss samples from the peatland surface in each of six experimental site-treatment combinations (total number of samples 53) and compared the TA communities. To investigate the transition in TA communities since the beginning of the water lever drawdown, a 30 – 50 cm long peat core was collected from a lawn or low hummock surface from each study site and analyzed in 2-cm intervals.

Overall, the species composition differed both between peatland types and treatments. Based on MANOVA, peatland type was a more important regulator of the TA community than the treatment – only few taxa, namely *Corythion-Trinema* type, *Planocarina marginata*, and *Difflugia lucida* were significantly impacted by water level drawdown, whereas 24 taxa showed significant response to peatland types. NMDS suggests that the change has been the most drastic in the rich fen and the slightest in the bog – a pattern that has been previously observed in the vegetation (Kokkonen 2019). It also indicates that the communities in bog and poor fen WLD sites resemble those of more nutrient-rich conditions compared to the corresponding control sites. All paleoprofiles, including those from control sites, showed a recent change in community composition. Generally, the change reflected adaptation to more nutrient-poor and dryer conditions.

In our presentation, we will present our preliminary results and discuss their potential implications.

Response of testate amoebae assemblages to environmental and climatic changes during the last ~700 yrs in Laguna Polo, Patagonia, Argentina

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Little is known about the paleoecology of testate amoebae in Patagonia, especially in lakes. To fill this knowledge gap, our main goal is to document the paleoecological record of testate amoebae from Laguna Polo (49° 15' 59.4" S, 72° 53' 38.4" W) in Santa Cruz, southern Patagonia. We analyzed a 54-cm long sediment core and conducted paleoecological analyses on testate amoebae in combination with geochemical analyses to characterize the sediment composition (Ca, K, Ti, TOC, TIC, TN, $\delta^{15}N$, $\delta^{13}C$ and C/N). The age model, based on radiocarbon dates and tephrochronology, indicates a basal age of ca. AD 1302. The testate amoebae assemblages are composed of *Centropyxis* with five morphotaxa, *Difflugia* with three, and *Lagenodifflugia*, *Zivkovicia* and Cyclopyxis with one, respectively. According to the redundance analysis (RDA), the assemblages are mainly related to Ca (Pr(>F)=0.007), TOC (Pr(>F)=0.038) and $\delta^{13}C$ (Pr(>F)= 0.099). Then, a Detrended Correspondence Analysis (DCA) was performed using all species and their abundances to summarize environmental changes along the record. DCA axis 1 seems to correlate with the volcanic activity from Volcan Lautaro, whereas DCA axis 2 relates to lake productivity. Finally, the testate amoebae ecological turnover in the last 700 years allows identifying the effect of climate events such as the end of the Medieval Climate Anomaly (MCA, AD 1302-1419), the Little Ice Age (LIA, AD 1419-1722) and the most recent climate change (AD 1959-2018). This work highlights the usefulness of lacustrine testate amoebae for paleoenvironmental reconstructions and the need to understand the sensitiveness of these protists to different environmental drivers.

Testate amoebae and their role in estimating success of forest-to-bog restoration techniques

Angela L. Creevy [1]*, David M. Wilkinson [2], Roxane Andersen [3] and Richard J. Payne (deceased) [4,5]

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Northern peatlands exist in alternative quasi-stable states of unforested open peatlands and forested peatlands (tree-covered). Forested peatlands can be natural or artificial. In the UK, large areas of peatland have been planted for commercial forestry but recent forestry policy recognises the need to remove trees from areas of deep peat and forest-to-bog restoration has significantly increased over the past few decades. This policy shift has brought about substantial interest in the bioindication value of testate amoebae to estimate success of forest-to-bog restoration techniques. Forest-to-bog restoration is somewhat different to other peatland restoration practices because as well as blocking drainage and rewetting, there is also the legacy effect of fertilization and re-profiling, which adds to the complexity of restoration and monitoring. Studies on both raised and blanket bog have shown slow recovery of testate assemblages, especially mixotrophic taxa which had not recovered seventeen years following plantation removal and the establishment of *Sphagnum* mosses in the wetter microforms. This talk discusses the bioindication value of testate amoebae at forest-to-bog restoration sites along with timescales of microbial recovery and suggests restoration techniques that may be helpful for microbial recovery.

Response of testate amoeba communities to peatland drain blocking

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Peatlands represent globally-important habitats and carbon stores. However, human impacts and climate change leave peatlands with a substantial management challenge. Degradation of peatland habitats and their hydrological integrity is increasingly counteracted through the rehabilitation of peatlands including re-wetting and drain blocking. Research into how such management interventions affect peatland microbial communities is limited. Here, we investigate the response of testate amoebae to drain blocking on three small lowland raised bogs in Northern Ireland, UK. We sampled *Sphagnum* adjacent to areas of focused flow near sites of damming in addition to control sites away from dam blocking. These treatments show complex but meaningful results after restoration. We observe several key developments following dam blocking: (i) species diversity increases in both treatments; (ii) unambiguous wet indicator taxa (*Archerella flavum, Centropyxis aculeata* type*, Amphitrema stenostoma,* and *Amphitrema wrightianum*) appear in increasing abundance at dammed treatment sites; (iii) and transfer-function reconstructed water-table depths show wetter conditions in the dammed treatment sites. These findings imply wetter conditions after restoration, where routine monitoring presented no clear trend in water-table depths. We found no statistically significant community-level response to experimental or environmental variables which may be related to antecedent conditions and significant periods of drought during the study period. Thus, caution is advised when implementing testate amoebae for bioindication until their community-level response to restoration is better understood. Nevertheless, this study emphasises the potential of an indicator-taxa based approach to applying testate amoebae as contemporary bioindicators of peatland restoration – particularly on short-term timescales immediately following restoration.

Potential effects of sulfur-rich fumigation on testate amoebae assemblages, Smoking Hills area, Northwest Territories, Canada

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The Smoking Hills (Ingniryuat), in the Horton River area of the western Canadian Arctic is characterized by areas of spontaneously combusting bedrock of the Upper Cretaceous Smoking Hills Formation. Active bocannes expel vapors that contain dark carbonaceous aerosol and trace gases with significant SO_2 and H_2SO_4, which are very similar to anthropogenic industrial pollutants in chemical makeup. While the smoke from the bocannes is a minor contributor to overall Arctic air pollution, their effect at the regional and local scale could be significant. The long duration of the fumigation, with some estimated to have been active for at least 10,000 years, has introduced geochemical anomalies in the nearby soils. This has resulted in elevated acidic conditions and a high relative enrichment of trace metals in soils in the area of fumigating sites.

While there have been only limited studies on the direct impact of sulfur contamination on testate amoebae (TA), studies have shown that TA assemblages are sensitive to sulfate. In these studies, there was a notable significant decrease in small bacterivorous taxa such as *Trinema lineare*, *Corythion dubium*, and *Euglypha rotunda* in comparison to TA species that have less specific feeding preferences. Comparing TA assemblages using multivariate statistical techniques, including partial correspondence analysis, between actively fumigating peatlands vs non-fumigating peatlands in the Smoking Hills area are being assessed to determine if/how fumigation and/or bedrock composition impacts TA assemblage structure.

Use of moss samples stored in powder form: a solution for large-scale inventories of testate amoebae?

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Terrestrial mosses are bioaccumulators of atmospheric pollutants and are used as proxy for air quality. Since 2000, through the BRAMM network (Biosurveillance des Retombées Atmosphériques Métallique par les Mousses), the Museum has been carrying out five-yearly moss sampling campaigns throughout mainland France. Around 3,300 samples with known concentrations of chemical elements are stored in the form of moss powder. These samples were collected under tree cover, far from known sources of contaminant emissions. The study of testate amoebae (TA) in this type of matrix requires prior characterisation of quantitative and/or qualitative losses between fresh samples and grinding material. The extraction method and then the sample processing chain from collection to grinding were tested. The TAs were identified taxonomically using inverted microscopy according to their morphological characteristics. The results show a loss of abundance and diversity as a function of the composition of the tests. Nevertheless, the community structure is similar between powder replicates, although it differs from that of the stem.

Powder samples from 4 sites in the 2021 BRAMM survey were then analysed and the links between diversity, abundance and environmental variables (biogeographical zone, altitude, metal content, etc.) were tested. Our first results show that the structure of TA community in sites sampled in continental zone are similar between them and are different from those taken in the Atlantic zone.

The study of dry moss samples also opens the door to future research on herbarium samples.

Diversity and community assembly of testate amoebae and other microbial eukaryotes in diverse biotopes within urban parks: morphological and metabarcoding perspective

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The urban environment has the potential to host a wide variety of microorganisms. However, gaining a better understanding of microbial community interaction with urban ecosystems requires a deeper understanding of the mechanisms driving the diversity and community assembly of testate amoebae and other microbial eukaryotes in diverse urban biotopes. To address this, we employed high-throughput sequencing of 18S rRNA genes to analyze microbial eukaryotes and microscopy to analyze testate amoebae in five distinct biotopes (tree hole, moss on the tree trunk, land soil, pond water, and pond sediment) within fourteen urban parks located in Moscow (Russia), Xiamen, and Shenzhen (China) during 2020 and 2021 summer seasons. The molecular diversity in 90 samples revealed that Opisthokonta was the most abundant supergroup across all biotopes. Dispersal limitation was identified as the key driving factor in the community assembly process within single or multiple biotopes, with soil recognized as the primary source of dispersal for protozoan amoebae among different biotopes. In comparison, microscopy analysis of tree hole, moss on the tree trunk, land soil, and pond sediment (132 samples) revealed a total of 116 testate amoeba morphospecies (28 genera), with 13–26% of the total species richness shared among all biotopes, indicating high heterogeneity of testate amoeba community in urban parks. The distribution of testate amoebae is largely influenced by the biotope rather than elementary environmental conditions such as pH, moisture content, and the thickness of the leaf litter layer. The work was supported by the Russian Science Foundation (19-14-00102).

Introducing a metabarcoding approach to survey Arcellinida diversity at the species level: fast, cheap and accurate

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Metabarcoding approaches have gained popularity in investigating environmental biodiversity due to their perceived advantages in terms of speed and cost-effectiveness compared to classical morphological studies. However, establishing reliable protocols and data analysis pipelines requires extensive research and development. Additionally, conducting a comprehensive investigation that compares results obtained from morphology and environmental DNA is crucial to evaluate the strengths and weaknesses of each method.

Here, we will introduce a novel workflow for studying species-level diversity of Arcellinida using a metabarcoding approach based on the variable marker COI (mitochondrial cytochrome oxidase). Our protocol has been successfully applied to various environments, including peatlands, soils, estuaries, and sediment from lakes. To accurately assign the obtained environmental OTUs to specific taxa, we developed a custom reference database using sequences obtained from single cells barcoding, ensuring precise taxonomic identification.

Within our environmental dataset, we have successfully identified and detected all infraorders in Glutinoconcha, with the exception of the Hyalospheniformes. The discovery of previously unknown diversity within morphologically homogeneous groups, such as Cylindrothecina, Excentrostoma, and Sphaerothecina, underscores the ecological complexity and challenges traditional morphological identification methods. Further investigations are required to clarify the species boundaries of these groups, as well as to elucidate the ecological niches and interactions of these newly discovered species.

By revolutionizing modern distributional surveys of Arcellinida, our protocol has the potential to establish this order as a model group for a wide range of theoretical and applied studies. It enables rapid and cost-effective analysis of testate amoeba diversity across diverse ecosystems, offering valuable insights into their ecological roles and functions.

Independent colonizations of athalassohaline water bodies by freshwater lobose testate amoeba (Arcellinida, Amoebozoa)

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The salinity barrier is considered as one of the most important frontiers dividing biodiversity and strongly influencing the distribution of organisms. Crossing it opens the possibility for newcomer organisms to colonize new niches and diversify. In turn, these transitions require profound physiological adaptations and are therefore supposed to happen rarely in the evolutionary history of the clades. Most of these transitions have been studied in marine environments, where abiotic and biotic (i.e. competitors and predators) factors difficult transitions.

Here, we explored the biodiversity of Arcellinida testate amoebae in athalassohaline water bodies (i.e. of non-marine origin and with different salt concentration and composition than the sea). These saline lakes experience extreme salinity and temperature fluctuations, which makes them arguably a harsher environment than the sea from the abiotic perspective. Biotic pressures, on the other hand, may be lower due to their island-analogous nature.

We combined microscopical observations, single-cell barcoding and environmental metabarcoding to explore the biodiversity of Arcellinida in different water bodies of Spain and Chile, with salinities ranging from freshwater to near saturation. In general, communities were composed of euryhaline species which were able to tolerate wide salinity ranges, sometimes from freshwater to salinities higher than seawater, in accordance to the high fluctuations present in their original environments. We also found several transitions across the salinity barrier, indicating multiple independent colonizations of the saline environment by Arcellinida from freshwater ecosystems. Since Arcellinida are physiologically able to adapt to salinities higher than those of seawater, salinity alone is not the barrier keeping Arcellinida from transitioning towards the marine environment.

Developing arcellinid shelled amoebae as genomic models to examine the origin of evolutionary novelty

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The enduring nature of arcellinid shells combined with their species-specific morphology has allowed for arcellinid shelled amoebae to serve as important microorganisms for understanding early evolution of eukaryotic life on earth and serve as important bioindicators for environmental monitoring and ecology studies. This conspicuous phenotypic feature also makes them an obvious group to investigate how evolutionary novelties arise, and how genetic information and the environment are responsible for the diversity of shell phenotypes seen in this species rich group of microbial eukaryotes. Despite the shell being the most recognizable character of Arcellinida, serving as the most important identifying taxonomic characteristic, we currently lack a molecular understanding of shell formation process, impairing evolutionary interpretation of this key feature of arcellinids, which in turns complicates matters for ecological inference. To begin addressing these bottlenecks, we will establish transient and stable (CRISPR/Cas9 mediated) genetic manipulation tools in the arcellinid *Arcella intermedia*, with the validation of previous successes that demonstrate *Arcella* being an amenable organism to different experimental manipulation. Additionally, we will generate high-quality reference and draft genomes from arcellinid species that represent both the known biodiversity and known shell formation strategies seen in the group. This project's outcomes will enable both *in silico* and *in situ* investigations into this evolutionary novelty's origin. Simultaneously genomics will illuminate both the critical issue of taxonomic identity (linking morphology with genetics) thus leading to more accurate interpretations in studies of based solely on shell morphologies.

Unraveling Arsenic Resistance and Adaptive Strategies in Arcellinida: Insights from Transcriptomics and Evolutionary Perspectives.

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Arsenic is a toxic metalloid with carcinogenic potential, and its resistance is crucial for organism survival. However, limited knowledge exists regarding eukaryotes, particularly Arcellinida, a lineage of testate amoebae. Transcriptomics provides insights into the molecular mechanisms underlying an organism's response to environmental stressors, particularly in less studied groups such as testate amoebae. This study presents the origin and evolution of arsenic resistance mechanisms in eukaryotes, with a particular emphasis on Arcellinids. The study identified homologs of arsenic resistance genes and found pieces of their evolutionary history through gene duplication and horizontal gene transfer events. We hypothesized that some arsenic resistance mechanisms likely pre-date the last common ancestor of eukaryotes. The study found that *A. uspiensis* exhibited low-moderate tolerances to arsenic, with survival and cell division observed under low concentrations (10 ppm) and survival without cell division under moderate concentrations (50 ppm). Arsenic exposure led to upregulation of cellular processes, including DNA repair, metabolism, immune response, and cell cycle regulation. *A. uspiensis* also upregulated gluconeogenesis pathways related to the constitution of reserves for the organism. Specific arsenic resistance enzymes were constitutively expressed, and *A. uspiensis* employed specific tolerance enzymes, antioxidant enzymes, and anti-apoptotic signaling to mitigate the harmful effects of arsenic. *A. uspiensis* enters a low-activity state with limited resistance mechanisms in the presence of arsenic, likely waiting for improved conditions to regain normal activity. This research contributes to understanding eukaryotic arsenic resistance and provides insights into the adaptive strategies employed by organisms facing arsenic contamination.

From Neoproterozoic and Beyond: Amoebozoan testate amoebae illuminate the diversity of heterotrophs and the origin of complex ecosystems throughout geological time

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Heterotrophic microbial eukaryotes play a pivotal role in marine and terrestrial ecosystems, contributing to carbon and nutrient cycles. These microorganisms, capable of phagocytosis, act as predators of bacterial communities and other microeukaryotes, occupying a significant position in complex food webs. The origin and diversification of these heterotrophic microeukaryotes remain unclear. Fossil evidence and molecular data suggest that the emergence of predatory microeukaryotes and the transition to a eukaryote-dominant marine environment occurred around 800 million years ago. Vase-shaped microfossils (VSMs) represent the oldest known evidence of heterotrophic microeukaryotes in marine environments and terrestrial habitats. In this study, we investigate the early divergence and diversification of Arcellinida and related amoebozoan taxa using a relaxed molecular clock approach. Our phylogenomic analysis reveals a well-resolved tree of amoebozoan testate amoebae, including a monophyletic Arcellinida with three suborders and five infraorders. Through calibration using fossils and rigorous clock models, we estimate the timing of diversification of Arcellinida during the early Neoproterozoic, shedding light on the expansion of life during this period. Our results suggest a well-established complexity in shallow marine ecosystems, involving both phototrophic and heterotrophic microeukaryotes during the Neoproterozoic, followed by an invasion of freshwater systems and subsequent diversification of Arcellinida in the Phanerozoic. The findings indicate the need for some revision of the classification of amoebozoan testate amoebae, with taxa reassigned to different orders. Overall, this study provides valuable insights into heterotrophic microeukaryotes' evolutionary history and ecological significance in Earth's ecosystems.

The problem of “shadow species” as illustrated with the taxonomic hotchpotch *Cyphoderia ampulla* (Rhizaria: Cyphoderiidae)

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Accurate species delimitation based on an integrative approach is indispensable for a proper estimation of biodiversity. However, although delimitation methods often point to the existence of new taxa, these are never described, or their description is delayed for decades due to time constraints or lack of training in new molecular technologies. As a consequence, invalid species names are carried over the years, with negative repercussions for ecology, biogeography and conservation. To give visibility to this problem, we coined the term "shadow species" to describe these taxonomic units with an informal status that, although sufficient data exist to characterise their independence, their description is never formalised. It is therefore crucial to resolve cases of shadow species as soon as possible or, better still, to avoid their existence by describing the taxa as soon as the necessary data are available. As an example, here we resolve the case of the shadow species *Cyphoderia ampulla* (Rhizaria; Cercozoa; Euglyphida; Cyphoderiidae).

SESSION 6: DIVERSITY

Effects of taxonomic identification bias on ecological inferences: research community survey on testate amoeba microscopy dataset

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Keywords

Taxonomic Bias; Morphological Identification; Testate Amoeba; Comparative Analysis; Expert Judgement; Theory-Practice Gap; Methodology; Learning; Ecological Assessment; Bioindication

Abstract

Differences in perception and judgment and their influences on knowledge are being evaluated in many domains of natural sciences and beyond. Among them, taxonomic uncertainty creates a known, but mostly ignored, identification bias in ecological studies. This bias undermines reproducibility, which is especially critical in comparisons among studies and meta-analyses.

Testate amoebae (TA) are commonly used as proxies for palaeoenvironmental reconstructions and as bioindicators in peatlands and lakes. Such work is always based on morphological identification, which for many taxa is challenging due to the lack of clear morphological criteria and identification keys.

We conducted a survey within the TA research community, using microscopy images of TA observed in surface *Sphagnum* and peat samples. These images were shared with all research groups working with TA. Observers with varying degrees of expertise participated, and broad descriptive data about each participant was documented. Additionally, we recorded self-evaluated level of confidence for each identification. We hypothesized that TA taxonomic identification bias would affect estimates of diversity and ecological interpretation including applications such as bioindication.

This is the first attempt to simultaneously include all the abovementioned metrics in one analysis. Notably, no expert judgement from our side or from any previously made research was used. Our results create a fundament for work on methodological guidelines and exploration of approaches allowing us to overcome identification bias. This will help make TA-based ecological studies more robust.

Testate amoeba unified and standardized shell trait description

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Studying the diversity of testate amoebae offers valuable insights into ecological, paleoecological, and evolutionary questions. However, a major challenge arises from the varied terminology used to describe their morphological traits across different publications and languages. This lack of standardization hampers precise taxonomic descriptions and hinders ecological studies that rely on functional characteristics of these organisms. Moreover, selective pressures exert on test traits, which provides them with a special relevance in evolutionary studies. To address this issue, it is essential to unify and standardize the terminology used to describe the morphological traits of testate amoebae. This will greatly facilitate future research and enhance the integration of these organisms into various scientific disciplines. In our work, we have undertaken the task of revising, organizing, and visually representing the most important morphological traits of testate amoeba shells. Additionally, we have compiled a comprehensive glossary containing all relevant terminology. By providing a unified and accessible resource, our efforts aim to support both aspiring microbiologists and experienced researchers in this fascinating field of study. This standardized framework will enable easier communication, presentation, and discussion of research findings, ultimately advancing our understanding of testate amoebae and their ecological significance.

Illustrated morphotype catalogues to improve the consistency of studies based on testate amoebae: example with an elevation gradient in Hawaii.

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Due to the state of the taxonomy and the general difficulty to identify them, testate amoebae are rarely identified to the species level in routine work and are instead treated into broad morphotypes. We present here the usage of an illustrated morphotype catalogue, collections of specimen pictures grouped into morphological Operational Taxonomic Units (mOTUs), to serve as a basis for identification within or between studies. These catalogue should be done beforehand by experts based on samples from the region that will be the focus of the study and can then be handled to analysts for the routine work, and only at the end of study are mOTUs assigned taxonomic names (if possible). Morphotype catalogues have the advantage of being transparent records of the study and have been shown to significantly reduce analysts bias and to increase comparability between studies. Also, this approach prevents force-fitting ambiguous specimens to a name, which in turn greatly limits the pollution of databases with unreliable observations. We here present an example of a morphotype catalogue to assess the diversity patterns of testate amoebae along an elevation gradient on the islands of Hawaii and Maui.

Morphometry of genera *Apodera* and *Trinema* from the Kerguelen archipelago suggests environmentally driven phenotypic plasticity

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Apodera vas is an emblematic species with a broad distribution from the southern hemisphere up to Tropic of Cancer region. Although infrequent, this species has been intensively studied. Documented morphological and morphometrical variability suggest the existence of species complex, but the relationships between this variability and environmental constraints is unknown. In contrast, species of the genus *Trinema* are present in most analyzed samples globally, with several species described based on morphological characteristics.

The Kerguelen archipelago is located in the subantarctic part of the southern Indian Ocean. With 7200km², the essentially magmatic territory presents a wide range of soil types, hydrological conditions, and vegetation, all showing strong gradients. We analyzed 166 samples from four sampling campaigns (1972, 2018, 2019 & 2021), in which 180 *Apodera vas* individuals and 921 *Trinema* individuals (*T. enchelys, T. lineare, T. complanatum*) were measured. It appears that the genus *Trinema* is present throughout the archipelago, while *Apodera* is present mostly in the west where land surface temperature variations are the strongest but rare or absent in the east with more stable conditions. We observed a strong morphological variability; the largest individuals being found in non-degraded and hydromorphic mosses and the smallest individuals in saxicolous mosses and environments degraded by erosion. These contrasts may either indicate the presence of two or more species within the genus *Apodera* or alternatively phenotypic plasticity. Changes in environmental conditions may cause the migration or disappearance of sensitive species such as *Apodera vas*.

Morphology, Distribution and Ecological Significance of Sand-dwelling Testate Amoebae in European and North American Freshwater Ecosystems

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Microhabitats such as the interstitial spaces between sand grains in mineral sediments have long been considered unsuitable for freshwater testate amoebae. However, these habitats provide a home for a rare assemblage of testate amoebae species in the Danube River and some other European aquatic habitats. Morphometric analyses have provided comprehensive insights into the morphology of certain *Corythionella*, *Cyphoderia*, and *Psammonobiotus* species. The current distribution of the rare *Cyphoderia* species, *C. calceolus* and *C. myosurus*, reflects the Pleistocene hydrographic patterns observed in Central Europe. *Corythionella* species in freshwater exhibit a distinct distributional pattern, influenced by various factors, including anthropogenic impacts. Both *Psammonobiotus* and *Corythionella* species have also been recorded in North America. In the Danube River, *Psammonobiotus* and *Corythionella* species co-occur, occasionally alongside rare species such as *Cyphoderia calceolus*, *C. myosurus* and *Paramphitrema* species. Beyond the Danube, these rare filose testate amoebae species occur in nutrient-poor deep lakes, recently established reservoirs on rivers, wetland areas formed on glacial sand deposits, or in riparian zones of rivers with sand and gravel. The presence of these species serves as a reliable indicator of low organic matter content and can be used as valuable proxies for ecological characterization of Holarctic freshwater ecosystems.

Preliminary results of the study of testate amoebae biogeography

(based on personal data)

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The composition of communities of testate amoebae inhabiting different types of habitats in different geographic regions of the Earth, from benthic and marsh to soil, has been studied. More than a thousand samples were analyzed. Databases of testate amoeba communities were compiled according to our own research from different geographical regions - 1) testate amoebae of the East European Plain from the White to the Black Sea, 2) testate amoebae of the Arctic and Subarctic from Greenland to Alaska, 3) testate amoebae of the eastern transect from Kamchatka to New Zealand, 4) testate amoeba of the American transect from Canada (British Columbia) to Argentina (Terra del Fuego).

The characteristics of Late Pleistocene and Holocene paleocommunities of testate amoebae in the Arctic and Subarctic are considered. In total, more than 600 species and subspecies taxa of testate amoebae have been found. Differences and similarities in the composition of testate amoeba communities from different geographical regions are shown.

We also analyzed the composition of ninety-eight testate amoebae communities inhabiting different *Sphagnum* species, both monodominant communities and rarer polydominant ones, such as *Sphagnum cusdatum+S.majus*. The average sample was more than 300 testate amoebae for each *Sphagnum* community. Two hundred three species and subspecies of testate amoebae have been found, complexes of dominant and rare species of bog habitats have been identified. The results of the research can be used in works on the indication of the ecological conditions of sphagnum habitats and their geographical distribution.

Latitudinal patterns of multi-scale diversity partitioning in soil testate amoebae: A case study in Western Siberia

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The relationship between species diversity and spatial scale is a central topic in spatial community ecology but it remains poorly studied for testate amoebae especially for the case of turnover and nestedness components of β-diversity. We adopted a hierarchical sampling design (at climatic sub-zone, ecosystem type, biotope and replicate sample levels) to perform multi-scale partitioning of β-diversity of testate amoeba assemblages along a latitudinal gradient in the Western Siberian Plain. We found a prominent latitudinal gradient in among-ecosystem component by comparing β-diversity profiles among zones. β-diversity at all levels of hierarchy is primarily determined by rare species, especially in the northern regions, where rare taxa almost exclusively accounted for the diversity at the ecosystem level. In addition, β-diversity is generally dominated by the turnover component at all hierarchical levels in lower latitudes, whereas nestedness prevailed at among-ecosystem scale in higher latitudes. These findings indicate that microbial assemblages in northern latitudes are spatially homogeneous and constrained by historical drivers at larger scales whereas in southern regions it is dominated by the turnover component both at the micro-habitat and ecosystem levels and therefore determined by recent vegetation and environmental heterogeneity.

The work was supported by the Russian Science Foundation (19-14-00102).

Climate- and microhabitat-driven patterns of peatland testate amoebae diversity at the continental scale

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The role of environmental factors that shape large-scale distribution of protists remains understudied. We aimed to disentangle the effects of latitudinal and longitudinal gradients on the distribution of *Sphagnum*-dwelling testate amoebae and to understand the effects of environmental variables across different microhabitats. We analyzed abundance data for 843 samples collected from 76 peatlands across the most of Eurasia (50.8 – 69.5 °N, 29.0 – 159.6 °E). Linear mixed-effects models were applied to determine environmental drivers of testate amoebae α- and β-diversity. We found the prominent reversed latitudinal gradient for α-diversity which is mostly driven by temperature: the lower the temperature, the higher the α-diversity. This gradient depends on microhabitat and it is present only for lawn and hummock microhabitats but not for hollow microhabitat. We also found effects of temperature seasonality and annual precipitation on α-diversity. β-diversity positively depends on precipitation seasonality but this relation is significant only for hummock microhabitats. Our results indicate that various climatic factors determine α- and β-diversity of testate amoebae in a continental scale and those relations are microhabitat specific.

The work was supported by the Russian Science Foundation (19-14-00102).

Relations between testate amoebae, vegetation and environment: patterns and mechanisms at various spatial and temporal scales

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The relationships among testate amoeba assemblages (TAA), vegetation, micro- and macro-ecological characteristics, and ecosystem processes (CO_2 and CH_4 emission) were investigated in a range of terrestrial ecosystems using observational and experimental approaches in modern and palaeoecological settings. Our results indicate that species structure of *Sphagnum*-dwelling TAA in mires across of the Northern Caucasus Mountains are greatly controlled by local vegetation, especially bryophytes, whereas the effects of the micro-environmental characteristics was weaker. Among the micro-environmental data, acidity was the main factor affecting the species composition of TAA followed by water table depth and temperature. A study on the effects of hierarchical scales (climatic zones, ecosystem types, biotopes and sample replicates) in the Arctic tundra showed the greatest contribution of the large-scale component on the species structure of TAA, whereas the contribution of local environmental heterogeneity was much lower. Long-term (ten thousand years) dynamics of TAA were affected by large-scale climatic events (glaciation), medium-term (several thousand years) dynamics was related to the hydrological regime and vegetation changes and short-term dynamics was controlled by microenvironment and colonization rates. The short-term (two years) effects of warming, precipitation decrease, and shadowing on microbial communities were investigated in a field experiment (East European Plain). The results revealed changes in both diversity and species structure of TAA. Shadowing strongly affected molecular diversity of protists that resulted in increasing abundance of Fungi, Cercozoa and Lobosa. The species diversity and community heterogeneity of TAA was positively correlated with CO_2 emission, which was positively related with water table depth and negatively with CH_4 fluxes. These findings indicate the potential for development of a testate amoeba-based transfer function for reconstruction of greenhouse gas emission.

The work was supported by the Russian Science Foundation (19-14-00102).

Climate niche distribution modelling of *Hyalosphenia papilio* (Amoebozoa: Arcellinida) conflicts with documented occurrences in the Azores (Portugal) revealing dispersal limitation and the first evidence of island disharmony among microorganisms

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Keywords

Microbial biogeography; Species distribution modelling (SDM); Island biogeography; Island disharmony; Soil biodiversity; Free-living protists; Testate amoebae; *Hyalosphenia papilio*

Abstract

Assessing the biogeography of most microbial eukaryotes is challenging due to taxonomic and distributional data shortfalls. However, testate amoebae stand out as exceptions due to their high diversity and abundance in soils, and their relative facility of morphological identification. *Hyalosphenia papilio* (Leidy) is a large, conspicuous circum-boreal testate amoeba species that is widely distributed in *Sphagnum* peatlands of the Holarctic realm. Previous studies highlighted complex biogeographical patterns of the species, its phylogenetic diversity being much higher in North America than in Eurasia. The Azores (Portugal) being isolated in the North Atlantic Ocean and hosting extensive *Sphagnum* peatlands (i.e., potentially favourable habitats), we hypothesized that the archipelago could have been a steppingstone between North America and Europe where *H. papilio* populations could have established, possibly resulting in specific phylotypes. However, despite the presence of companion testate amoebae species of similar size, *H. papilio* seems to be absent from the *Sphagnum* peatlands in the Azores, suggesting that soil microbial

eukaryotes also display island disharmony. Here, using high resolution species distribution models at various scales (global and regional), we confirm that suitable habitat for *H. papilio* exist in the Azores. This study confirms that the potential distribution of microorganisms can strongly vary in response to climate, but that dispersal limitation exists, similar to what is well known for macroscopic organisms. Moreover, our models highlight the pertinence of including microorganisms in species distribution modelling to decipher microbial biogeography patterns and prioritize areas for field surveys.

Distribution modelling of *Apodera vas* reveals latitudinal dispersal limitation and range contraction in response to climate warming

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Keywords

Climate change; Conservation biogeography; Cosmopolitanism; Endemism; Gondwana; Microbial biogeography; Palaeogeography; Soil biodiversity; Species distribution modelling (SDM); *Apodera vas*

Abstract

The diversity and distribution of soil microorganisms and their potential for long-distance dispersal (LDD)are poorly documented, making the threats posed by climate change difficult to assess. If microorganisms do not disperse globally, regional endemism may develop and extinction may occur due to environmental changes. Here, we addressed this question

We documented the global distribution of *Apodera vas* and built a climatic niche model to predict its past (LGM), present, and future (2071-2100) potential distributions in responses to climate, assess whether its distribution could be explained by dispersal (matching climatic niche) or vicariance (palaeogeography), the likelihood of cryptic diversity, and potential threat from climate and land-use changes

Climatic conditions for *A. vas* currently exist in the British Isles, demonstrating a lack of inter-hemispheric LDD, congruent with the vicariance hypothesis. Longitudinal LDD is however confirmed in isolated and geologically young peri-Antarctic islands. Models show range contraction from LGM to current and from current to future with favourable conditions developing on the Antarctic Peninsula.

This study illustrates the value of climate niche models for research on microbial diversity and biogeography, along with exploring the role played by historical factors and dispersal limitation in shaping microbial biogeography. We assess the discrepancy between latitudinal and longitudinal LDD for *A. vas*, which is possibly due to contrast in wind patterns and/or likelihood of transport by birds. Our models also suggest that climate change may lead to regional extinction of terrestrial microscopic organisms, thus illustrating the pertinence of including microorganisms in biodiversity conservation research and actions.

Diversity of testate amoebae in the Oriental region

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Keywords

Biodiversity, biogeography, endemism, faunistics, taxonomy

Abstract

The first boom in testate amoeba biogeography occurred during World War II and the period that followed (1940-1970). This period is characterized by intensive research on tropical and subtropical testate amoebae based on light microscopic observations. After the period of intensive research in the holotropical kingdom, emphasis has been placed on the study of polar and subpolar regions. In these regions, researchers discovered mainly small, cosmopolitan testate amoebae, so the description of new species was very rare. Considering that this research direction did not have a great impact on biogeography, we can say that the second boom of this discipline started around 2010 with new research on holotropical testate amoebae. The second boom of the testate amoeba biogeography was achieved through extensive research in the Neotropical and Ethiopian regions using the methods of faunistics, molecular and descriptive taxonomy. However, research on testate amoebae in the Oriental region has recently been sporadic. The focus of our recent research has been on the Oriental region and on the boundaries of this region with the Palaearctic and Australian regions. The basic geographic features of the Oriental region and its relationships to other biogeographic regions are presented here. The history of research on the diversity of testate amoebae in this region is analyzed along with biographies of important researchers. A list of all registered testate amoeba taxa in the Oriental region has been compiled. The taxonomic status and geographic distribution of the characteristic taxa for this region were analyzed.

Changes in testate amoeba community's functional traits after two decades of water table drawdown in a boreal peatland

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Testate amoebae (TA) participate in photosynthesis and nutrient cycle in boreal peatlands. The functional role of TA can be investigated e.g., via functional traits – characteristics that reflect the life strategies of the individual. Due to their sensitivity to hydrological conditions and long preservation in peat, they are also a much-used proxy for peatland hydrology.

Peatland drying and associated changes in vegetation are a possible consequence of the ongoing climate change. While TA are known to respond to water level fluctuations rapidly, studies focusing on the effects of long-term drying remain scarce, even though it likely affects TA functional traits and thus the ecosystem processes they participate in. To assess the impact of long-term drying on peatland TA, we will study their functional traits in a controlled water-level drawdown experiment that has been maintained since 2002. This allows us to address not only the direct impact of drying but also that of associated changes in e.g., vegetation.

Our experiment is situated in Lakkasuo peatland, Central Finland, and it consists of three experimental water-level drawdown sites (WLD) and corresponding control sites that represent three peatland types: ombrotrophic bog, poor fen, and mesotrophic fen. We collected 8-10 TA samples from peatland surface from each site (total 53) and compared the community weighted means of selected functional traits in different sites. To verify that the differences are caused by our experiment, and to investigate the transition in TA communities more carefully, a 30 – 50 cm long peat core was collected from a lawn surface from each study site and analyzed in 2-cm intervals.

In this presentation, we will present our preliminary results and discuss their potential implications.

ABSTRACTS:

POSTERS

Poster: Contribution to the taxonomy of the *Padaungiella lageniformis* complex (Arcellinida: Hyalospheniformes): Is Emei Mount (Sichuan Province, China) home of a new semicryptic species?

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Testate amoebae are eukaryotic microorganisms that have an amoeboid unicellular body situated in a shell of different composition, shape and size. The shell in the vast majority of species has a constant shape and contains different structures that can be used as taxonomic characters. Given that these organisms have been studied since the beginning of the 19th century, a large number of species, varieties and forms have been described, and there are different opinions about their taxonomic statuses. One of the most common and widespread moss-dwelling testate amoeba is *Padaungiella lageniformis*, which is morphologically very variable and exactly represents a complex of species with unclear boundaries. There are different understandings about the taxonomic status of a few species from this complex. The semicryptic species may be concealed in cases where there is considerable morphological variation but without clear boundaries supporting species delimitation, such as in the *Padaungiella lageniformis* complex. We found that the arithmetic mean of the values for four ratio characters (shell width/shell length, neck length/shell length, neck length/shell width and neck width/neck length ratios) are key taxonomic characters for separating semicryptic species within this complex. In this case, semicryptic species can be distinguished only on the basis of average morphometric values for populations, not for individuals. This species concept was applied for the first time in this study to define testate amoeba species, and thanks to it we were able to define and formally describe new *Padaungiella* species from Emei Mount, Sichuan Province, China.

Keywords: morphometrics, semicryptic species, species concept, taxonomy, testate amoebae

Poster: The genus *Heleopera* (Arcellinida: Volnustoma) in subtropical China with the first record of *H. baetica* after its description from Spain

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The genus *Heleopera* includes seven species and a few infraspecific taxa. Their shell is always laterally compressed, usually colorless or yellowish, but also with reddish or purple species. The shell is composed of collected euglyphid idiosomes and sand grains or diatoms. These materials are often coated and reinforced with siliceous material. The aperture is terminal, lenticular or slit-shaped, with a thin but distinct organic rim. We recorded the presence of four moss-dwelling taxa from the genus *Heleopera* in subtropical China: *H. baetica*, *H. petricola*, *H. petricola* var. *amethystea*, and *H. sylvatica*. In 2021, Soler-Zamora with co-authors described *H. baetica* from the cyanobacterial mats on cave walls in southern Spain. They treated this species as endemic to the caves in the Iberian Peninsula and a bioindicator of sub-aquatic habitats. Our finding of this species from the Guilin Mountains in the Sichuan Province of China indicates that it is not endemic to Spain, but that it also occurs at the opposite end of Eurasia. This species is not specialized for living in cyanobacterial mats on cave walls, but also lives in green mosses in the subtropical forests. We have concluded that this species inhabits the karst areas in the subtropical climate zone (in the wider sense).

Keywords: biogeography, endemicity, Heleoperidae, morphometrics, taxonomy, testate amoebae

Poster: Morphological variability of several species of the family Centropyxidae (Arcellinida: Excentrostoma) from Maluan Mountain, Guangdong Province, China

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The family Centropyxidae comprises many species adapted to live in soils, especially forest litter. The ventral position of the aperture has been considered an adaptation to the dry environment. We performed a morphometric analysis of moss-dwelling species of the family Centropyxidae from Maluan Mountain, Guangdong Province, China. We recognized several morphotypes within the *Centropyxis aerophila* complex and discussed their taxonomic statuses. The morphometric analysis of *Centropyxis spinosa* showed that the population from Maluan Mount is characterized by significantly larger shells compared to previously registered populations of this species, i.e. there is very little overlap. Future detailed morphometric investigations of typical populations of *C. spinosa* are necessary in order to determine the conspecificity of all populations of this rare but widespread species. Also, the taxonomic position of this species within the family Centropyxidae was discussed. We did a detailed morphometric analysis of *Centropyxis sacciformis*, a rare species with a limited distribution. This species has not previously been compared with species from the *Centropyxis deflandriana* complex, although they are very similar - morphologically, morphometrically, and biogeographically. We considered in detail the taxonomic history of these species, and created a key to the identification of taxa within this group of species.

Keywords: Centropyxidae, morphometrics, pseudocryptic species, taxonomy, testate amoebae

Poster: Morphological variability of some *Centropyxis* species (Arcellinida: Excentrostoma) from Emei Mount, Sichuan Province, China

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The altitudinal zonation of forest formation on East Asian mountains on a latitudinal gradient from 20° to 30°N shows a transition between tropical and temperate zonation types. The natural forests that remain are only found in some isolated stands and in preserved areas or steeply sloping sites. They harbor a high diversity of species and support the coexistence of three tree life forms, evergreen broadleaved, deciduous broadleaved, and coniferous trees. The fauna of the testate amoebae that inhabit these subtropical forests in the Palearctic-Oriental transition zone is almost completely unexplored. The eastern slope of Emei Mt in the Sichuan Province contains a remnant of the native primary forests that represent most of the natural vegetation patterns of the subtropical forests of China. We analyzed the morphological variability of moss-dwelling *Centropyxis* species (Excentrostoma: Centropyxidae) from Emei Mount. The genus *Centropyxis* is one of the largest testate amoeba genera that includes more than 130 nominal names (species and varieties), but many descriptions are not adequate. We recorded the presence of four widespread and common species: *C, aerophila*, *C. elongata*, *C. plagiostoma*, and *C. sylvatica*. Also, we registered the presence of an unidentified morphotype that is characterized by a characteristic triangular aperture. Morphometric analysis was done on several hundred individuals, and methods of univariate, bivariate, and multivariate statistics were applied.

Keywords: *Centropyxis*, Centropyxidae, morphometrics, taxonomy, testate amoebae

Poster: Testate amoebae from the heart of the Oriental region: First records from the Malay Peninsula

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The Malay Peninsula forms the southernmost extension of the great peninsular region of Indochina, with which it is connected by the Isthmus of Kra. South of this isthmus, the Malay Peninsula extends for about 1000 km, first to the south, then to the southeast almost parallel to Sumatra, ending at Cape Piai, the southernmost point of the Asian continent. The central mountain range is in line with the continental axis and forms a distinct watershed between the streams flowing east and west into the surrounding seas. To date, the diversity of testate amoebae in the Malay Peninsula has not been explored, despite it being one of the world's biodiversity hotspots. We conducted a taxonomic study of testate amoebae inhabiting green mosses near the forest stream in Phang Nga, one of the southern provinces of Thailand in the central part of the Malay Peninsula. We recorded about 30 species of testate amoebae, most of which have a cosmopolitan distribution. The population of *Centropyxis laevigata* differs from the typical populations of this species by slightly smaller dimensions of the shell. We have morphologically and morphometrically characterized a new morphotype that resembles *Nebela barbata*. Future research on testate amoebae from the Malay Peninsula is needed to fill a gap in our knowledge of the biogeography of the Oriental region.

Keywords: Arcellinida, biogeography, Euglyphida, morphometrics, Oriental region, taxonomy

Poster: Description of some *Quadrulella* species (Amoebozoa: Arcellinida: Hyalospheniformes) from subtropical China

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The Oriental region can be considered as a unit that hosts fauna different from the adjacent Palaearctic fauna in the north and Australasian fauna on its southern border. The border between the Oriental and the Palaearctic region in China has always been a point of discussion. Sometimes the 30° northern latitude is chosen as an arbitrary northern limit for the Oriental fauna in China. In southern China, the transition from the temperate Palaearctic to the tropical Oriental region occurs through an even gradation that is the result of the long-standing progressive mixing of the Oriental and Palaearctic elements of biodiversity. The genus *Quadrulella* includes a large number of species characteristic of the holotropical kingdom, although the diversification of this genus in the Oriental region has not been specifically studied. We conducted a taxonomic study of moss-dwelling *Quadrulella* species from the subtropical part of China which belongs to the Oriental region and take samples at two localities in the Guangxi and Guangdong provinces. We registered and morphologically characterized several morphotypes differing in shell shape and size, as well as in the size of the shell plates. The registered morphotypes were compared with previously described taxa of the genus *Quadrulella*, and some of them were described as new species for science. All registered taxa were compared with each other using multivariate statistical methods.

Keywords: biogeography, morphological variability, morphometrics, taxonomy, testate amoebae

Poster: Taxonomic identity of three rare *Gibbocarina* morphotypes (Amoebozoa: Arcellinida: Hyalospheniformes) from the Oriental region

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The genus *Gibbocarina* includes only three species (*G. galeata*, *G. gracilis*, and *G. penardiana*) and several rare infrasubspecific taxa usually recorded from tropical and subtropical climate zones. These testate amoebae are characterized by elongated-pyriform shells and differ from similar genera by the presence of a hollow keel. In the literature from the mid-20th century, there are descriptions of the populations from the Oriental region whose taxonomic status is unresolved. Namely, researchers have recorded a series of populations that make a morphological and morphometric continuum with populations from Africa and Europe. On the Emei Mount (Sichuan Province, China) we recorded a population characterized by morphometric values corresponding to the typical populations of *Nebela penardiana* var. *minor*. Significantly smaller individuals of this taxon we registered on the Maluan Mount in Guangdong Province (China) and the Malay Peninsula (Thailand). In sympatry with a small morphotype of *N. penardiana* var. *minor* in the moist mosses near the stream in a tropical rainforest, we recorded the presence of *G. gracilis*. Based on morphometric data for about a thousand individuals from the mentioned populations, multivariate morphometric analysis was conducted and the results obtained are compared to the data available in the literature.

Keywords: biogeography, morphological variability, morphometrics, taxonomy, testate amoebae

Poster: Morphological variability of several testate amoebae of the family Arcellidae (Amoebozoa: Arcellinida: Sphaerothecina) from Shangchuan Island, South China Sea

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The genus *Arcella* consists of about 110 described species and infraspecific taxa. Their shells are composed entirely of proteinaceous organic material arranged in hexagonal units. The shape of the shell is more or less campanulate, with a central and circular aperture and radial symmetry. The genus *Arcella* differs from the recently established genus *Galeripora* by the absence of pores around the aperture and the absence of an organic layer covering the outer surface of the shell. Different morphotypes may represent different species or instead reflect intraspecific variability, so morphological variability in testate amoebae can lead to taxonomic complications due to environmental influences. We recorded 10 species from the family Arcellidae in a wetland on Shangchuan Island in the South China Sea. Among the recorded species is a morphotype resembling *Arcella guadarramensis* González-Miguéns & Lara, recently described from Spain. This morphospecies occurs in Spain in two clades that are clearly separated morphometrically, but have the same morphology. Namely, clade L includes the individuals with shell diameters between 114 and 126 µm (based on six individuals), while clade M includes the individuals with shell diameters between 141 and 146 µm (based on four individuals). The individuals included in our study are significantly larger and are characterized by a slightly different morphology. We presented the multivariate morphometric analysis of all registered taxa based on about 1000 individuals.

Keywords: endemism, morphometrics, new species, taxonomy, wetlands

Poster: New insight into the taxonomic status of the rare enigmatic testate amoeba *Difflugia australis* var. *minor* (Amoebozoa: Arcellinida): Does the euglyphid amoeba hide within an arcellinid-like shell?

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Testate amoebae are a polyphyletic group characterized by the presence of a shell around the amoeboid body. The order Arcellinida includes species with a very diverse shell structure, but the presence of lobopodia is characteristic of all of them. Testate amoebae with filopodia and shells covered with self-synthesized plates belong to the order Euglyphida. Often the difference between these two phylogenetically distant and morphologically well-defined groups is clear, so there is no taxonomic confusion about the membership of species in either of these groups. However, in a wetland on Shangchuan Island (South China Sea), we recorded testate amoebae originally thought to belong to the arcellinid genus *Difflugia* and identified them as *D. australis* var. *minor* Gauthier-Lièvre & Thomas, 1958. This species was originally described from a stream in the Sambailo region, Guinea, Africa. The authors did not provide any information on the characteristics of the cytoplasm, so it can be assumed that the description is based only on the shells. In 1971, Godeanu described *D. australis* var. *minor* from the Danube and its tributaries in Romania. Based on morphological and morphometric comparisons, we conclude that the record of *D. australis* var. *minor* from Romania is the population of a euglyphid *Pareuglypha reticulata* Penard, 1902. The results of our original research on the morphological variability of both taxa indicate that *D. australis* var. *minor* is a synonym of *P. reticulata*. Future research is needed to determine whether *P. reticulata* is a phenotypically highly variable species or a species complex.

Keywords: convergent evolution, morphometrics, species synonym, taxonomy, testate amoebae

Poster: Shangchuan Island (China) is a home of Asian relatives of *Difflugia szczepanskii* (Amoebozoa: Arcellinida), an endemic testate amoeba to Europe

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The genus *Difflugia* is the oldest and the largest genus of testate amoebae with about 300 nominal species and infraspecific taxa. This abundance of species is, at least in part, due to a combination of inadequate descriptions and the lack of good diagnostic features. Namely, it has often been the case that even slight deviations in shell shape have resulted in the establishment of new forms, varieties or species, regardless of the range of variability that individual *Difflugia* taxa may exhibit. In 1965, Schönborn described *Difflugia szczepanskii* from the oligotrophic Hancza Lake in Poland, characterized by oval and laterally compressed shells with irregularly round aperture. According to the original description of this species, the shell is hyaline, covered with a meager amount of particles, with the following measurements: shell length 60–80 µm and shell width 30–35 µm. In 2012, Davidova rediscovered this species based on 25 individuals from Bulgaria. Her specimens are in good agreement with the original description concerning the form and structure of the shell, but established measurements are smaller: shell length 50–60 µm and shell width 24–30 µm. In the summer of 2022, we took water samples from a wetland on Shangchuan Island (South China Sea, China). We measured 40 individuals similar to *Difflugia szczepanskii* and registered two clearly separated size-classes: small (shell length 50–56 µm, shell width 27–31 µm) and large (shell length 63–81 µm, shell width 28–38 µm). Similarities and differences between our morphotypes and populations previously described in Europe were discussed.

Keywords: biogeography, endemism, morphometrics, taxonomy, testate amoebae

Increased phototrophy in *Archerella flavum* associated with recent warming on Alaska's North Slope.

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Testate amoebae are vital to peatland microbial food webs and are well-suited for paleoecological studies exploring microbial responses to recent and ongoing arctic climate change. Mixotrophic testate amoebae acquire energy via algal symbionts (zoochlorellae) in addition to heterotrophic food sources, and recent work indicates that carbon uptake by mixotrophs may be significant at the ecosystem level. Previous studies suggest $\delta^{13}C$ of mixotrophic tests reflects the relative carbon contributions from heterotrophic and phototrophic sources. In this study, we measured $\delta^{13}C$ of testate amoebae in living *Sphagnum* to confirm this previous work, then measured changes in the $\delta^{13}C$ of *Sphagnum* and *Archerella flavum*, a mixotrophic species, along a ^{14}C-dated peat core from the North Slope of Alaska. A spooling-wire interfaced with an isotope ratio mass spectrometer enabled measurements from small sample sizes (10-20 individuals). Our objective was to assess the hypothesis that a longer snow-free season over the last several decades led to greater relative use of algal symbionts by this mixotrophic species. Our sub-decadally resolved record reveals that *Archerella flavum* shifted toward greater relative use of algal symbionts in the late 1960s and 1970s, when warming became pronounced in the region. Furthermore, comparison with instrumental records collected since 2007 confirm the sensitivity of phototrophy to changes in the length of the snow-free season. More research is needed to better understand how these changes may impact broader microbial communities and carbon cycling processes in high-latitude peatlands.

TaxRef: the French taxonomic referential, a help or a curse?

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On behalf of the French administration, I am working on updating the national taxonomic reference system (TaxRef) for the testate amoebae (TA) of France and its overseas territories. In relation to global reference systems (GBIF, WORMS, etc.), TaxRef lists: 1) the reference names of taxa, 2) homotypic and heterotypic synonyms and 3) status of presence. Each information is associated with a bibliographic reference. Other information can be entered, such as life traits, habitat or relationships between species (predation, interactions).

Updating TaxRef for TA has led me to consult a rich bibliography. Unfortunaly, whether the articles are old or new, the taxa are mentioned according to two codes: the botany code (variety and form authorised) and the zoology code (lowest taxonomic level is the subspecies). Some species are even described using quadri-names (e.g. *Trinema complanatum var gigantea f. punctata* Decloître, 1982). The choice of a common code becomes necessary. I propose to use the zoology code because TA are closer to animals. We should publish a legal article in conjunction with the International Commission on Zoological Nomenclature.

The next step in using zoology code is to determine what happens to species whose names follow another code. How long does it take to review the types of species described according to the botanical code? In the meantime, is the status quo maintained or are species systematically transformed? I propose to use TaxRef as a database to store information on types and to associate their scans with it, but I need your help.

Is the Latitudinal Diversity Gradient applicable to unicellular eukaryotes? A Comparative Study of Arcellinida (Amoebozoa) in Brazilian Amazon and Temperate Mosses

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The paradigm for microbial diversity is 'Everything is everywhere, but the environment selects'. Moreover, the latitudinal diversity gradient (LDG) is a well established pattern of global species distribution. However, most microbial morphotypes (along with their ecology) have not been well characterized, and microbial diversity in the tropics is essentially unknown. In this work in progress, we aim to compare the Arcellinida (Amoebozoa) diversity of a moss sample from the pristine Amazon (Serra do Imeri) with the diversity found in mosses from the temperate regions. Alpha diversity, relative abundance and shell morphology of Arcellinida (Amoebozoa) will be analyzed for comparison. We identified taxa through the use of optical microscopy and scanning electron microscopy (SEM). Alpha diversity and relative abundance has been calculated with species curves. We compared the morphotypes found with previous taxonomic descriptions and SEM images. We found up to 3 times more Arcellinida taxons than in recent registers from mosses of temperate regions. More than 130 SEM pictures from different individuals were taken. Some shells of characteristic rare species (e.g. *Apodera vas* and *Certesella martiali*) showed phenotypical novelties. Based on our preliminary findings, we found evidence supporting the applicability of the LDG in terms of species richness, however, some registers of rare morphotypes with novel phenotypes reinforces the perspective that molecular information from neotropical populations needs to be accessed to discuss if the lineages found in the tropics are the same as the registered in temperate zones.

Peatland Initiation, Development and Restoration in an Urban Area during Mid- and Late Holocene: reconstruction based on testate amoebae, pollen and plant macrofossils remains

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Peatlands play an important role in the global carbon cycle, but have been exploited over many centuries that reduces their carbon storage capacity. To investigate peatland development during the Late Holocene and their restoration after peat extraction we applied a multi-proxy paleoecological (testate amoebae, pollen, plant macrofossils, loss on ignition, peat humification, etc.) approach to undisturbed and floating vegetation mat deposits of the Gorenki peatland (Meshchera Lowlands, East European Plain). Peatland development started around 2550 BCE as a water-logged eutrophic birch forest (terrestrial paludification) surrounded by a broadleaf forest. Around 2400 BCE, the peatland turned into an open mire with *Sphagnum* mosses, sedges, and willows. During 900–800 BCE, the mire transformed into a wet mesotrophic peatland surrounded by a spruce forest. First human settlements and deforestation around 300–400 CE coincided with oligotrophization of the mire. The growth of Slavic population in the region in 14th century CE caused transformation of indigenous spruce-broadleaf forests into the croplands; mire became drier and forested. Since peat extraction was abandoned in the beginning of 20th century CE, the mire underwent self-restoration started from a formation of the *Sphagnum cuspidatum/obtusum* quagmire on the floated peat remains. The *Sphagnum* mat stabilized during 1960–2000 CE. During the last twenty years, agricultural activity decreased and pine forests was restored in the adjacent area; the floating mat became drier and more oligotrophic that can lead to the formation of a bog in the absence of considerable anthropogenic impact.

The work was supported by the Russian science Foundation (19-14-00102).

Poster: The species concepts in the taxonomy of testate amoebae

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Speciation in protists is often decoupled from morphological divergence, creating complexes of cryptic, semicryptic, and pseudocryptic species. For these organisms, discontinuity in the variation of morphological traits is more difficult to identify than for multicellular organisms with complex body organization. The species are cryptic if it is impossible to identify individuals consistently and accurately from morphology alone. We cannot formally name these entities, so they stay "hidden" under the Latin name of morphologically defined species (morphospecies). In part the controversy around the taxonomic inflation that may accommodate the phylogenetic hypotheses resides in the practicality of diagnosing the cryptic species, a problem particularly acute in morphologically austere groups, such as testate amoebae. After studying populations in more detail, many cryptic species become less cryptic and they are recognized as pseudocryptic and semicryptic species. The species are pseudocryptic if individuals can be identified from morphology providing sufficient care is taken, but are so similar that there is a high probability of misidentification, even by a competent taxonomist. The species are semicryptic if individuals can be identified consistently and accurately from morphology if and only if the provenance is known, either in terms of geographical origin or population characteristics. Populations of different species may have different mean values for one or more morphological traits but if the ranges of values overlap, individuals cannot be identified and the species are semicryptic.

Keywords: cryptic species, pseudocryptic species, semicryptic species, species complex, taxonomy

Poster: Taxonomic revision of testate amoebae with agglutinated dorsoventrally flattened shells and close relatives (Arcellinida: Excentrostoma and Sphaerothecina) based on comparative morphology

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The infraorder Excentrostoma comprises 150 valid species within 15 valid genera characterized by bilaterally symmetrical shells adapted to live in soils. Here we accept the two-family concept of the infraorder Excentrostoma based on comparative morphology: Plagiopyxidae (shell with an eccentric cryptostome aperture, usually slit-like) and Centropyxidae (shell with an eccentric oval or circular aperture). The genus *Oopyxis* is most often treated as *incertae sedis*, but we have included them in the family Plagiopyxidae due to aperture position and morphology close to other members of this family. In the family Centropyxidae we have included the genus *Conicocassis*, which is morphologically very similar to the genus *Centropyxis*, but usually treated as *incertae sedis* because molecular data are absent. The infraorder Sphaerothecina includes 174 valid species within 14 valid genera with a more or less spherical or semi-spherical shell and a centrally placed aperture. Their shells are areolar or agglutinated (xenosomes embedded in organic cement). We recognized the structure of the shell as an important character for the separation of the two phylogenetic groups. Within the group with areolate shells, we recognized a family Arcellidae, while within the phylogenetic line with agglutinated shells, we included three families, one of which is erected here based on morphological characters. We singled out the family Trigonopyxidae (comprising the genus *Trigonopyxis* with six valid species) as Glutinoconcha *incertae sedis* because at the moment there are conflicting data about its position in the classification system of the suborder Glutinoconcha.

Keywords: Amoebozoa, high-rank systematics, phylogeny, taxonomic analysis, taxonomy

Poster: An atlas of the wetland-dwelling testate amoebae from Shangchuan Island, South China Sea

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From our point of view in the first half of the 21st century, the 18th and 19th centuries seem to have been a wonderfully romantic time to be a naturalist. The adventurous explorer could travel to scientifically unknown regions and not only describe new species, but also study entire floras and faunas for the first time. While it is very difficult today to find regions where macroscopic organisms have not yet been explored, there are still many such places that can be explored by protistologists. Shangchuan Island, in the subtropical part of the South China Sea, is one such place. We investigated the faunal composition of testate amoebae from a wetland on Shangchuan Island. We recorded more than 30 species, some of which are new to science or rarely found. We provided a morphological description and morphometrics of all registered species and infraspecific taxa, as well as microscopic images illustrating morphological variability within all taxa. Also, identification key was also created for taxa found in a wetland on Shangchuan Island. Since wetlands on small subtropical islands are usually endangered ecosystems, the possibilities for the conservation of the studied locality were considered from the point of view of testate amoeba diversity. For a long time, natural disasters were the main risk factors for ecosystems on Shangchuan Island, but in recent years, human exploitation activities such as tourism, recreation, and agriculture have become the main risk factors.

Keywords: biodiversity, biogeography, faunistics, identification key, taxonomy

Poster: Taxonomic and biogeographical review of the testate amoeba genus *Quadrulella* (Amoebozoa: Arcellinida: Hyalospheniformes)

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Typical representatives of the genus *Quadrulella* are easily recognized by their pear-shaped to ovoid shells, which are characterized by the presence of square plates. Their shells consist of a thick protein layer with square siliceous plates embedded on the outer surface. Square plates are rare in testate amoebae and this feature was used in the late 19th century to classify these amoebae into their own genus and even family. These amoebae did not attract the attention of researchers until the mid-20th century (the first boom in testate amoeba biogeography) when several papers were published describing the fauna of testate amoebae from tropical and subtropical regions. On this occasion, many new species were described that we consider endemic to exotic areas since they do not occur in the temperate climate zone. Thanks to the renewed interest in the Holotropical kingdom in recent years, as well as molecular and population approaches to taxonomy, new papers have been published that shed new light on the diversity of the genus *Quadrulella*. Based on an extensive literature review, we have established five species groups within the genus *Quadrulella*: *Q. symmetrica* group (ovoid to pyriform shells), *Q. longicollis* group (long shells), *Q. tubulata* group (distinct neck), *Q. elegans* group (presence of lateral pores), and *Q. cordobensis* group (irregular aperture). The key to taxa identification is given for each species group, as well as biogeographic analysis.

Keywords: biodiversity, biogeography, Hyalospheniidae, identification key, taxonomy

Poster: Taxonomic and biogeographical review of the *Padaungiella lageniformis* complex (Amoebozoa: Arcellinida: Hyalospheniformes)

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The genus *Padaungiella* was established in 2012 by splitting the species of the genus *Nebela* characterized by a distinct long neck into a new genus. Each species has a controversial taxonomic status whose boundaries with some other entities within this genus are unclear. During the 20th century, many researchers noticed the presence of more or less easily distinguishable varieties within the type species *P. lageniformis*. The researchers identified five of the most variable characters: (a) the fundus can be broadly elliptical or pyriform; (b) in most cases, the shell is very strongly compressed and the neck and fundus have almost the same thickness, but in other cases, the fundus is strongly arched and the neck in the lateral view was more clearly separated from the fundus than usual; (c) the neck can be sharply defined or gradually merges into the fundus; (d) the edge of the aperture sometimes forms a curved lip, while in other cases, a lip cannot be detected; (e) the majority specimens have the surface covered by kleptoidiosomes, but sometimes building units could not be observed very clearly and the proportion of the organic matrix was predominant. All the characters mentioned in the previous studies are qualitative and do not show clear boundaries between varieties. Based on the quantitative characters defined here, we have provided a taxonomic and biogeographical analysis of all taxa that were included at the variety level in *Nebela lageniformis* during its taxonomic history.

Keywords: biogeography, Hyalospheniidae, identification key, pseudocryptic species, taxonomy

Microspatial distribution of soil inhabiting testate amoebae in urban parks according to under-tree crown and hillside position heterogeneity

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We examined species composition, diversity, and structure of testate amoeba assemblages in soils beneath tree crowns (microscale) at three slope positions (mesoscale) in subtropical urban parks in Shenzhen, China. Forty-two species of testate amoebae belonging to 18 genera were identified in 81 samples. Testate amoeba assemblages were the most diverse and abundant in the center of the tree crown comparing with other under-tree habitats. Foot of the hills harbored higher testate amoeba abundance and diversity comparing with upper locations along the hillslopes. The distribution of testate amoebae was mostly driven by under-crown and hillside positions but not by elementary environmental conditions such as pH, moisture content and thickness of leaf litter layer. None of later factors were sufficient in shaping testate amoeba assemblage composition. The findings of our study suggest that in regional studies of soil microbial eukaryotes both micro- (i.e., under-crown) and mesoscale (i.e., the slope position) heterogeneity should be considered.

The work was supported by the Russian Science Foundation (19-14-00102).

Pseudopod Projection and Modulation: Exploring Variability Across the Tree of Amoebozoan Testate Amoebae (Arcellinida: Amoebozoa)

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Testate amoebae are unicellular eukaryotes provided with an outer covering (shell). These organisms protrude pseudopods through the single shell aperture and use them for locomotion, feeding, and other complex behaviors. The pseudopod is considered a key morphological character, and it is used to describe, distinguish, and classify amoeba groups. Despite the central role of pseudopods, the intra-specific morphological variability of this structure and the relationship between this variability and other features of the organisms has been poorly explored, including the correlation between this variability and different substrate types. In this study, we sampled representative species from two diverse infraorders of Arcellinida (Amoebozoa) that inhabit different natural environments and substrates. Using light microscopy, we observed their behavior on three different substrate types: agar plates, plastic bottles, and glass plates with liquid media. The primary objectives were to investigate the range of morphological variability in relation to organismal performance (i.e., locomotion speed), examine whether shell attributes and substrate types correlate with performance, and describe the nature of these correlations and their interplay with the variables. To achieve these objectives, we measured various features including shell diameter and area, pseudopod number per individual, and pseudopod length and width of multiple individuals. We then used these measurements and locomotion speed as variables in generalized linear models (GLMs) for further analysis, constructing separate models for each substrate type. The present study sheds ligth on the variability of pseudopod projection and modulation across the tree of amoebozoan testate amoebae (Arcellinida). Investigating pseudopod variability within Arcellinida leads to a better understanding of their diversity and differential capability to inhabit diverse habitats.

Key-words: cellular motility, microeukaryotic plasticity, variability

Arcella pompholys, a new bubble like species from the High Andes

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Arcellidae is one of the most diverse and ecologically successful families of Arcellinida, occurring almost ubiquitously in terrestrial and freshwater environments, including hypersaline endorrheic basins. They have in common a discoid shape of the test as well as the presence of more than one nucleus at least at some stage of their life cycles. Nevertheless, the morphological evolution of the group is particularly complex, including instances of cryptic speciation and evolutionary convergences. Knowing better the patterns their evolutionary patterns will increase our understanding of diversification in protists. Here, we isolated and described a new species from the Northern Chilean High Andes, with a particularly thin and flexible test that makes the organism resemble a bubble. This particularity of the test remains constant even after all the different subculturing steps. We placed the organism phylogenetically and confirmed its position next to *Arcella intermedia* and *A. vulgaris* with a nuclear (SSU rRNA) and mitochondrial (COI) markers. Tolerance studies show that the new species *A. pompholys* is highly sensitive to salinity and dies at 0.5 g/l salt, which contrasts with the presence of numerous saline water bodies in the surroundings. This example shows the importance of local conditions when estimating the diversity of testate amoebae at a regional scale.

Novel Diversity Next Door: The Discovery of *Cylindrifflugia luciferina* in the Pond of the Royal Botanical Garden

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Molecular approaches are revealing a considerable diversity of testate amoebae, both within Cercozoa and Arcellinida. However, a great deal of this diversity is composed of species that can only be distinguished by examining fine details in the scaling patters, or based on morphometric analysis. Many others are totally cryptic, and phenotypic plasticity hides possible traits that could be used to discriminate species. Still, and despite over 200 years of testate research, new morphotypes that differ significantly from known forms can still be found. Many ecosystems and regions of the world are still widely underexplored, and can potentially reveal new forms. In this case, we present a morph that has been, to our knowledge, never described: *Cylindrifflugia luciferina*, named after its two curved spines that remaining horns. We characterized the species both morphologically and molecularly, based on SSU rRNA sequences. This new species falls within the newly described infraorder Cylindrothecina, close related to *C. bacillifera.* Interestingly, this species does not come from faraway places but lives in the pond of the Royal Botanical Garden. Such an example supports the idea that, even in environments that are easy to sample, testate amoeba diversity is immense and awaits to be explored.

Composition, distribution and ecological characteristics of *Sphagnum*-dwelling testate amoebae in Bulgaria

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We present a summarized data about the composition, distribution and ecology of *Sphagnum*-dwelling testate amoebae from Bulgaria. During the period July 2016 – September 2018, we collected material from spatially separate regions of five mountains, namely Vitosha, Stara Planina, Rila, Pirin and Rhodopes. A total of 266 samples (one qualitative and one quantitative) from 133 locations of 17 *Sphagnum* species were examined. As a result of the present study, we established 148 species from 41 genera and 17 families, which constitutes 84.1% of the 176 sphagnophilic species registered in Bulgaria so far. There is a high degree of faunistic similarity between the different mountains (82.88% - 88.70%), as the number of species varies within narrow range (between 110 in Stara Planina and 116 species in Pirin). The majority of species are widespread and are recorded in all mountains (87 species in total, 58.8% of all records). Most of them are typical *Sphagnum*-dwelling species, belonging to the genera *Gibbocarina*, *Hyalosphenia*, *Longinebela*, *Nebela*, *Playfairina*, *Quadrulella* and *Sphenoderia*. When comparing different regions, the results clearly show that relatively distant areas from different mountains form well-separated clusters. A decisive role for the development of similar fauna has the presence of similar ecological conditions. The relationship between testate amoebae communities and environmental factors has been studied through ordinal analysis. Environmental factors with highest impact on the distribution and community structure are the type of substrate (*Sphagnum* moss), groundwater level (WTD), oxygen content and pH.