Testate Amoebae in Historical parks of Potsdam, Germany

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Abstract

To explore the potential of urban settings as habitats for testate amoebae, five historical parks in Potsdam (Germany) were sampled at different sites. A total of 32 sampling sites was chosen in proximity to deciduous (Acer, Castanea, Fagus, Tilia, Platanus, Quercus) and coniferous (Fraxinus, Picea, Pinus, Tsuga) trees. Meadows and creeks were also sampled. The overall taxonomic record comprises 76 species and sub-species. High species numbers of >20 per sample were found in meadows and below Fagus, Tilia, and Quercus trees. The species richness per park ranges from 33 to 46 taxa. Most species belong to the eurybiontic ecological group, although litter-inhabiting and hygrophilic and hydrophilic species were also present. Common species found in more than 50% of all samples (superdominants) belong to the genera Centropyxis, Cyclopyxis, Euglypha, and Trinema. Interestingly, the rare Frenopyxis stierlitzi which inhabits tree hollows was found as a recently described species in a new genus Frenopyxis BOBROV & MAZEI 2020 in the Babelsberg Park. The studied testate amoebae are characterized by a high degree of morphological and morphometric plasticity. Therefore, the study of testate amoebae in urban settings will reveal new insights into their ecology and enhance the definition of morphometric variability for single species.

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Introduction

Historical parks play an important role in the preservation of the ancient urban landscapes occupied by old buildings and associated garden areas. Most parks consist of a variety of landscape and relief elements, including flat areas, slopes, groves of trees, meadows, lakes, rivers, and brooks. While the botany of parks is commonly well studied (e.g. Müller 2014), the soil faunae and in particular protists such as testate amoebae are largely unstudied.

Testate amoebae are a group of free-living unicellular organisms enveloped by a discrete shell build of organic material secreted by the organisms themselves, which serves as a matrix or adhesive material for test construction. Shells can be constructed from organic material alone, embedded or covered respectively with additional materials, which are endogenously synthesized (plates and scales from silica or calcium phosphate), or collected from the environment (exogenous, foreign mineral particles) (Fig. 1) (Hedley and Ogden 1974; Meisterfeld 2002).

The shell composition is determined by the role of testate amoebae in the biogeochemical cycles of carbon, silica, calcium, and phosphorus. Testate amoebae are increasingly used in (paleo-) ecological research to characterize and reconstruct e.g. trophic and hydrological conditions (e.g. Kosakyan et al. 2016; Zhang et al. 2022).

Testate amoebae inhabit practically all known habitat types, including lake bottom sediments, soils, and marshes. The Belgian researcher Chardez (1967) identified 12 types of testate amoeba habitats including aquatic, subaerial, aerial, and endogenous environments. Chardez (1967) further divided the population of testate amoebae into three ecological groups: hydrobionts, hydrophiles, and xerophiles. Their geographical distribution covers all continents on Earth, including the Arctic (e.g. Bobrov et al. 2013; Wetterich et al. 2019) and the ice-free regions of the Antarctic (e.g. Smith et al. 2008). Testate amoebae play an important role in soil ecosystems and food chains as predators. In particular, they indirectly stimulate organic matter decomposition processes in the soil carbon and nitrogen turnover cycle (e.g. Schröter et al. 2003).

Because historical parks exhibit landscape heterogeneity, high habitat diversity, and are generally protected areas, they certainly play a role as refugia preserving the species diversity of soil biota. Furthermore, testate amoebae habitats in urban park are probably well-suited environments to test human impacts on protist communities. However, soil protists in historical parks have, as yet, been only minimally studied. Only Balik (1991) considered historical parks when studying the influence of air pollution by traffic on testate amoebae in urban landscapes including parks, while the main biotypes of Europe have been studied to some extent (Beyens 1985; Bunescu et al. 1985; Chardez 1976; Decloître 1979; Foissner 1979; Puppe et al. 2015; Schönborn 1966, 1990; Wanner 1991; Wanner and Dunger 1999).

The goal of this study is to provide a baseline knowledge and a starting point for future protist studies on urban parks by example of historical parks in Potsdam, Germany.

![Figure 1. Scanning electron microscope (SEM) images of some testate amoebae species abundant in the parks of Potsdam: A - *Centropyxis platystoma* (shell closely covered with mineral grains), B - *C. ecornis* (shell closely covered with mineral grains), C - *Arcella rotundata* (completely organic shell), D - *Cyclopyxis eurystoma* var. *parvula* (shell closely covered with mineral grains), E - *Nebela barbata* (shell closely covered with silica plates), F - *N. tincta* (shell closely covered with silica plates). Magnifications are given for Figure 1A to 1D. Magnification of Figure 1E: x1300 and Figure 1F: x1200.](image-url)
Results

A total of 76 species (and subspecies) of testate amoebae were found in 32 soil and bottom samples from five historical parks in Potsdam, Germany (Supplementary Material B). The maximum number of species and subspecies, >20 per sample, was found under tree crowns and near tree trunks of *Fagus sylvatica* (28), *Tilia cordata* (25), *Quercus rubra* (23), as well as on grass meadows (25, 20) while habitats near conifers show species numbers per sample of < 20. The lowest species and subspecies numbers of <5 were associated with *Quercus robur* (4), *Fagus sylvatica* (3), and *Platanus acerifolia* (2) sites, and in creek 1 bottom sediments (3). Blank samples without testate amoebae were not observed in the present study.

High numbers of testate amoebae specimens are only found in association with certain tree species. For example, below *Fagus sylvatica* in different parks, the number of individuals in the surface soil layer ranged from 3 to 283 specimens.

The maximum species richness was found in soil samples near deciduous trees and in meadows, but not near conifers. The analysis of the species frequency of testate amoebae in soil samples from historical parks allowed us to distinguish abundance groups, which are outlined in detail below:

Superdominants (more than 50% of samples or more than 15 samples): *Centropyxis sylvatica*, *Cyclopyxis eurystoma*, *C. eurystoma* var. *parvula*, *Euglypha laevis*, *Trinema complanatum*, *T. lineare*.

Dominants (30 to 50% of samples, 10 to 15 samples): *Centropyxis sylvatica* var. *minor*, *Cyclopyxis kahli*, *Valkanovia elegans*, *Assulina muscorum*, *Corythion dubium*, *C. dubium* var. *orbicularis*, *Trinema lineare* var. *minuscula*.

Subdominants (15 to 30% of samples, 5 to 10 samples): *Arcella arenaria* var. *compressa* (Please note, that *A. arenaria* has been recently transferred to the genus *Galeripora*; González Miguéns et al. 2022), *Centropyxis compressa*, *C. delicatula*, *C. orbicularis*, *Trigonopyxis arcula*, *Plagiopyxis callida*, *Hyalosphenia minuta*, *Phryganella acropodia*, *Euglypha compressa*, *E. compressa* f. *glabra*, *E. cuspidata*, *Tracheleuglypha acolla*, *Corythion dubium* var. *minima*, *Trinema enchelys*.

Trinema complanatum, T. complanatum f. elongata, T. lineare var. terricola, T. penardi, Cryptodifflugia oviformis, C. oviformis f. fusca.


The group of super-dominant species included eurybiontic species. This was an expected result, since the studies were conducted in park zones with different anthropogenic load such as trampling and mowing of grass cover, and, as a rule, with the dominance of deciduous tree species. The group of sub-dominant species with respect to indicator species characterizing microhabitat diversity was the most diverse. These include the litter group comprising Arcella arenaria var. compressa, Euglypha compressa, E. compressa f. glabra, E. cuspidate, E. simplex, and Corythion dubium var. minima. Furthermore, subdominant species characterizing habitats with acidic pH (Trigonopyxis arcula, Hyalosphenia minuta) and slightly acidic to neutral pH (Tracheleuglypha acolla, Geamphorella lucida) were found. Subdominant species that prefer humid and aquatic habitats are all species found of the genus Diffugia, as well as rare species such as Centropyxis compressa, C. delicatula, and Frenopyxis stierlitzi (Fig. 3).

The species diversity as reflected by the Simpson’s Diversity Index is equally high with values of about 0.9 for Babelsberg Park, Glienicke Park, Albert Einstein Science Park (Telegrafenberg) and Sanssouci Park, but distinctly lower with 0.5 in the New Garden (Table 1).

In the six soil samples from Babelsberg Park, the highest species richness of testate amoebae was found with 46 taxa (Table 1). Of these, 28 taxa were identified in samples from below Fagus sylvatica, 25 taxa from below Tilia cordata, and 20 taxa in meadow samples. The eurybiontic species Centropyxis sylvatica var. minor, Cyclopyxis eurystoma, Corythion dubium, and Trinema complanatum dominated in the Babelsberg dataset where additional 20 taxa were found sporadically. Because the species list here is the richest, the diversity of ecological groups is also more extensive. Along with litter species from the genera Valkanovia, Assulina, Euglypha, Corythion, Trinema, and Cryptodifflugia, we found species not typical for meadow park landscapes such as Centropyxis delicatula, Trigonopyxis arcula, and Geamphorella lucida, three species from genera Heleopera and Hyalosphenia, and two species from obligate hydrobiontic Diffugia species. Of particular interest is the presence of Frenopyxis stierlitzi (Bobrov and Mazei 2020) from the recently described genus Frenopyxis that typically inhabits tree hollows (Fig. 4).

In New Garden, 40 species were found (Table 1) in six samples including 19 taxa from below Picea abies, 13 from below Fraxinus americana, 25 from grass meadow, and 14 from below Fagus sylvatica. The dominant eurybiontic species were Cyclopyxis eurystoma, C. eurystoma var. parvula, and Euglypha laevis. Meanwhile Centropyxis delicatula and hygrophilic species of the genera Heleopera, Hyalosphenia, and Euglypha were rare.

In Glienicke Park, 39 species were found in six samples (Table 1). We found 13 species in samples of grass meadow near the ruins of the old building, 21 below Fagus sylvatica, 16 below Platanus hispanica, and 15 below Acer pseudoplatanus. Only one eurybiontic species, Centropyxis sylvatica, was dominant. But a group of hygrophilic species from the genera Heleopera, Hyalosphenia, and Euglypha was also found. It should also be noted that the rare species Centropyxis delicatula was identified as a moisture-prefering species.

We found 37 species taxa in the Albert Einstein Science Park (Telegrafenberg) (Table 1). The highest species richness of 23 taxa was found below Quercus rubra. Fifteen taxa were found below Fagus sylvatica. The eurybiontic species Centropyxis sylvatica, C. sylvatica var. minor, Cyclopyxis

**Figure 3.** Light microscope images of rare testate amoebae species in samples from the Parks of Potsdam: A - Centropyxis delicatula; B - Frenopyxis stierlitzi.
were present, among which the rare species *E. simplex* should be highlighted, as well as species from the genera *Trinema* and *Cryptodifflugia*. *Cyclopyxis eurystoma* v. *parvula*, *Phryganella acropodia*, and *Trinema complanatum* dominate the testate amoeba communities of Sanssouci Park.

**Discussion**

An interesting specific feature of the park population is the presence of species from hygro-hydrophilic groups, as well as litter species, i.e., a complex of species more characteristic of forest ecosystems. Although samples were taken under the crowns of different tree species, a herbaceous layer typical of meadow communities existed under the crowns.

To assess the degree of similarity of the testate amoeba population in the studied samples from the five historical parks of Potsdam, statistical analyses of neighbor joining clustering and PCA were carried out (Figs 5, 6). The species diversity of the testate amoeba communities was also evaluated using various indices (Table 1).

The cluster analysis showed a relative similarity of Sanssouci Park and New Garden testate amoeba populations, and a closer similarity of Babelsberg Park and Albert Einstein Science Park (Telegrafenberg) populations (Fig. 5). The analysis also highlighted a similar testate amoeba fauna in samples from Glienicke Park. The conclusions of the cluster analysis are also confirmed by the results of the PCA scatter diagram (Fig. 6). Close similarity is due to the high numbers of eurybiontic species such as *Centropyxis sylvatica*, *Cyclopyxis eurystoma*, *C. eurystoma* v. *parvula*, *Euglypha laevis*, *Trinema complanatum*, and *T. lineare*.
The indices of species diversity of a community characterize its ecological state. As conditions become more favorable the number of species increases and the dominant group may include several taxa, i.e., the community is polydominant. Conversely, under extreme or unfavorable conditions, both the total number of taxa and the number of dominants decrease. The number of taxa for park meadow sites is rather high (Table 1), especially in samples from Babelsberg Park which amount to 46 taxa. In addition, the dominant group in Babelsberg Park includes five taxa, in Glienicke Park four, and two to three taxa each in the rest.

The dominance index is highest in the New Garden testate amoebae community, and lowest in samples from Sanssouci Park and Babelsberg Park (Table 1). The explanation is that in the New Garden population Cyclopyxis eurystoma var. parvula is the absolute dominant, and its absolute value is more than 30%, which is a very high amount. Therefore, the most favorable ecological conditions for this group of protozoa at the time of sampling were found in Sanssouci Park and Babelsberg Park. This is also confirmed by the values of the Simpson’s, Menhinick’s, and, to a greater extent, Shannon indices (Table 1).

In the entire dataset, we found in total 17 species represented by one or more polymorphic forms and variants that sum up to 38 taxa, while 35 taxa represent monomorphic species and three taxa have been identified only to genus level (Supplementary Material B). The testate amoebae of Potsdam parks are characterized by a high degree of morphological and morphometric plasticity most likely as a result of the adaptation to park conditions with characteristic open spaces and anthropogenic pressure as likewise observed in other environments (e.g. Bobrov and Mazei 2004; Wanner 1999). This conclusion is based on the peculiarity of the intraspecific structure of various subspecies and variants. The polymorphism in testate amoebae is defined as simultaneous presence of several phenotypes in the population or interrupted diversity of forms (Schönborn 1992; Bobrov et al. 2002). Polymorphic species can be characterized by different size forms (e.g. major, minor, microstoma) (Bobrov et al. 2004), unless they do not represent distinct although physically very similar (sibling) species.

The ecological plasticity of a certain species is determined by the size of its ecological niche. The wider is the ecological optimum of a species, the less likely is the occurrence of morphological and

Figure 4. The newly described species Frenopyxis stierlitzi (Bobrov and Mazei 2020) as seen (A) in an SEM image and (B) in a light microscope image.

Figure 5. Neighbor-joining clustering of testate amoebae populations in the historical parks of Potsdam. Euclidian distance.

Figure 6. PCA scatter diagram of testate amoebae populations in the historical parks of Potsdam. Euclidian distance.
morphometric modifications that aim at survival in a certain unfavorable ecological situation. Therefore, the morphological and morphometrical plasticity might to some degree reflect the heterogeneity of ecological conditions at the level of habitats and microhabitats (e.g. Mitchell et al. 2008). The ratio of the number of polymorphic forms and variants to the total number of species to per park site (Table 1) might therefore provide insights of the habitat diversity captured by the sampling sites in the different parks. Following this assumption higher habitat diversity is seen in the record of Babelsberg Park with the highest share of polymorphs in the total number of taxa (0.39) if compared to the record of Park Sanssouci with a respective share of 0.16. The latter probably reflecting less diverse ecological habitat conditions seen in the sampled sites.

**Conclusions**

In the present study of the testate amoebae, the data on the particularities of their population in five historical parks of Potsdam, Germany were obtained for the first time. It fills to some extent a gap in protozoology and also expands our knowledge of the protist populations of urban historic parks. This work accomplished a comparative analysis of the population of testate amoebae in urban parks hosting diverse assemblages. The recently described species *Frenopyxis stierlitzi* inhabiting the hollows of linden trees previously known from sampling sites in the Natural City Park of Moscow (Russia) was re-found in Babelsberg Park (Potsdam, Germany).

We hope that our work will attract the attention of zoologists studying other groups of unicellular and multicellular animals and will allow us to understand more deeply the peculiarities of how animal complexes form in urban historic parks and the role of those parks in the picture of biodiversity of different geographical regions.

**Methods**

In 2016, surface soil samples were taken near the trunks of different tree species in the historical parks of Potsdam, Germany (Fig. 2). The sampling sites include locations in Sanssouci Park (founded in 1744), New Garden (created in 1787), Glénicke Park (created in 1816), Babelsberg Park (created in 1833), and Albert Einstein Science Park (Telegrafenberg created in 1874). Two samples were taken from the bottom sediments of two creeks near the main entrance to Sanssouci Park. Sampling site descriptions are given in Supplementary Material A.

The sampling sites are specified as follows:


Sampling sites included prominent tree trunks, tree under-crown space, the litter as well as the uppermost (top 0–3 cm) soil. In places the sampling sites were covered by mosses. In addition, the bottom of two small creeks was sampled. A total of 32 samples was taken, weighing an average of 50 grams, including eight samples from Sanssouci Park and six samples each from New Garden, Glénicke Park, Babelsberg Park, and Albert Einstein Science Park (Telegrafenberg).

Testate amoebae were isolated from semi-decomposed litter that had been deposited in the bottom of tree hollows, or from a mixture of decomposed wood and leaves in the shallow hollow of a tree-trunk base (taking the uppermost 1 cm). Samples were kept in a refrigerator before analysis (Mazei et al. 2015). The samples were suspended in water and passed through a 500 lm mesh filter to remove large particles. Testate amoebae shells were concentrated with a centrifuge. A drop of suspension was placed on a slide, and glycerol was added. Normally, five subsamples from each sample were examined. The samples were studied using BA300 (Motic, USA) and Axioplan 2 (Carl Zeiss, Germany) light microscopes under x400 magnification and a Jeol 6060 SEM (SEMTech Solutions, USA) at a voltage of 15 kV under x800 to x1,500 magnification. The number of identified tests per sample depended on the density of the testate amoebae and ranged from 2 to 28 specimens.
per sample. Species identification is based on classical identification keys such as Gel'tser et al. (1995) and Charman et al. (2000).

To assess the degree of similarity of the testate amoeba population between the parks, statistical analyses of neighbor joining clustering and Principal Coordinates Analysis (PCA) were carried out. The species diversity was also evaluated using various indices like the Dominance Index, Simpson’s Index, Simpson’s Index of Diversity, the Shannon (-Wiener) Diversity Index, and Menhinick’s Diversity Index.

Dominance indices are weighted toward the abundance of the commonest species. A widely used dominance index is Simpson’s Diversity Index. It considers both richness and evenness. Simpson’s Index (D) measures the probability that any two individuals drawn at random from an infinitely large community will belong to the same species. Simpson’s Index of Diversity 1-D represents the probability that two individuals randomly selected from a community will belong to different species. The value of this index ranges between 0 and 1. The Shannon (-Wiener) Diversity Index considers the number of species living in a habitat and their relative abundance. Menhinick’s Index is based on the ratio of the number of species and the square root of the total number of individuals.

**Conflict of Interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**CRediT authorship contribution statement**

Anatoly Bobrov: Conceptualization, Investigation, Resources, Writing – original draft, Visualization, Funding acquisition, Supervision. Sebastian Wetterich: Writing – original draft, Writing – review & editing, Visualization. Lutz Schirrmeister: Writing – original draft, Writing – review & editing, Visualization.

**Data availability**

Data will be made available on request.

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**Appendix A. Supplementary Material**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.protis.2022.125911.

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