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Author: Richard J. Payne Olga Belyakova Yuri Mazei

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Graphical Abstracts

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Diversity and community ecology of forest epiphyte testate amoebae from European Russia

Richard J. Payne*[a,b], Olga Belyakova[a], Yuri Mazei[a]

[a] Department of Zoology and Ecology, Penza State University, Krasnaya str. 40, 440026, Penza, Russia.

[b] Environment Department, University of York, Heslington, York YO10 5DD, United Kingdom.

* Corresponding author. Tel.: + 447890669343

E-mail address: richard.payne@york.ac.uk (R. Payne)

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Abstract

Testate amoebae are an abundant group of microorganisms which make a significant contribution to the diversity of protist life. Most of the world's potential habitats for testate amoebae have been barely studied and when such places are investigated they frequently reveal novel communities and species. Here we consider the testate amoeba communities associated with boreal forest epiphytes (mosses and lichens); an environment which we argue has been under-researched. We present a dataset of 165 samples from four regions of western Russia and analyse these data in relation to micro-habitat position and selected environmental data. The testate amoebae of epiphytes are abundant but dominated by ubiquitous species. We show that there are trends toward a lower species richness and test concentration with greater elevation on the trunk and in lichens compared to mosses. There are considerable differences in community composition between sampling regions. Of all measured environmental variables only moisture content showed a significant relationship with testate amoeba community structure. Our data highlight how little is known about testate amoeba communities of this habitat and call for greater research efforts, particularly in less-studied regions and biomes.

Keywords: Arcelllinida; Boreal forest; Community Ecology; Biodiversity; Euglyphida.

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Introduction

Testate amoebae are a diverse and abundant group of protists characterised by a hard shell: the test (Meisterfeld, 2002; Smith, Bobrov, and Lara, 2008). Over the last two decades research on testate amoebae (TA) has expanded greatly, promoted in particular by increasing use in palaeoecological studies and the consequent need for modern comparative data (Charman, 1999; Charman, 2001; Mitchell et al, 2008; Payne et al, 2012). However, research is very unevenly distributed with clear biases towards both certain regions (primarily western and central Europe) and particularly towards certain habitats, primarily wetlands (Mitchell et al, 2008). It therefore remains the case that habitats in most of the world have seen little or no testate amoeba research and much fundamental data collection remains to be conducted (Foissner, 1999; Heger et al, 2014).

Research attention on under-studied habitats frequently reveals previously-unknown TA communities and, not infrequently, new species. For instance, interstitial environments on marine and freshwater shorelines might once have been considered unlikely habitats for testate amoebae but a series of studies over the last two decades have shown the presence of relatively diverse communities (Golemanski, 1998; Golemanski, 1998; Golemansky and Todorov, 2004; Golemansky and Todorov, 1999; Nicholls and MacIsaac, 2004) and revealed the presence of new species (Anderson et al, 1996; Nicholls, 2005) and cryptic/pseudo-cryptic diversity (Todorov et al, 2009; Heger et al, 2010). The recent first studies of TA in supra-glacial environments found both that testate amoebae can survive in this seemingly-inhospitable habitat and identified a new species which may be a habitat specialist (Kohshima et al, 2011). It is often assumed that TA are restricted to cooler, moister environments but studies have found several species of TA even in desert soil crusts (Bamforth, 2008). It is therefore clear that when we look in new places we often find new things (Foissner, 1999). To answer fundamental questions about the global diversity of eukaryotic microorganisms basic inventory and taxonomic research is essential, even while this remains unglamorous and unattractive to most research funders (Agnarsson and Kuntner, 2007; Caron et al, 2008; Foissner, 1999; Heger et al, 2014).

A particular motivator for greater quantities of fundamental research has been the increasing recognition of the potential of TA as bioindicators (Payne, 2013). TA have been shown to respond to a long list of anthropogenic factors ranging from air pollution (Meyer et al, 2012), to farming systems (Heger et al, 2012), to chemical weapons disposal (Stoiko et al, 2006). Although most research remains restricted to demonstration studies there is considerable potential for TA to be used routinely in a

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variety of biomonitoring applications (Payne, 2013). This potential provides an incentive to investigate TA response to a broad range of environmental gradients, both to suggest new factors which TA may be able to indicate as well as to give greater confidence that bioindication results are not confounded by non-target variables (Payne et al, 2012).

The focus of this study is the testate amoeba communities of epiphytic vegetation in boreal forests. Approximately 20% of the earth's terrestrial surface is covered with forest (Hansen, Stehman, and Potapov, 2010) and much of the surface of these trees could conceivably harbour testate amoebae in mosses, lichens or on the bark itself. The microbial communities of such habitats are recognised as being under-researched (e.g. Anderson 2014). While there are numerous studies of testate amoebae in forest soils and litter (e.g. Aoki et al., 2007; Krashevska et al, 2007) there are much fewer which consider communities on the trees themselves and most studies which have investigated this habitat have considered a very small number of samples. In Puerto Rico Bamforth (2007) identified 83 taxa in four samples from epiphytic soils; on Ascension island Wilkinson and Smith (2007) identified seven taxa in a single sample of epiphytic moss and in temperate rainforest of western North America Bamforth (2010) identified 70 TA taxa from three epiphytic soil samples. In Thailand Golemansky and Todorov (2000) identified 42 taxa in a sample from tropical forest in Thailand but interestingly two of these taxa were species new to science (*Planhoogenraadia bonneti* and *Centropyxis thailandica*). In Bulgaria (Golemansky et al. (2006) reported the occurrence of 25 testate amoeba taxa from three sites in the Rhopode Mountains and Davidova (2008) identified 34 taxa in epiphytic mosses from south-eastern Bulgaria with the fauna dominated by *Euglypha*, *Centropyxis* and *Trinema* species. On the basis of studies in SW France Bonnet (1973a,b) suggests that communities of this habitat are composed of a mixture of cosmopolitan species resistant to desiccation and species dependent on the surrounding soils. These studies clearly demonstrate that testate amoebae are not only present but abundant and diverse in this habitat.

The literature provides some indications that TA communities may vary between different habitats on trees. Lackey (1940) found diverse protist communities, including six testate amoeba taxa, in wet holes in *Nyssa aquatica* (Tupelo) trees in Alabama USA. Although no comparative data was collected from other habitats in the same area the presence of larger taxa typical of wetter environments (e.g. *Centropyxis aculeata, Difflugia pyriformis*) suggests that such features may harbour distinctive TA communities.

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The most extensive study of epiphytic testate amoebae to-date has been in Latin America. Krashevska et al. (2010) studied 54 samples from an Ecuadorean rain forest in relation to altitude and elevation on the tree trunk. 113 taxa were identified with species richness declining and community composition changing with height of the sample on the trunk. Diversity was greatest at intermediate altitudes.

From this brief review it will be clear that existing studies are typically of small size, most of these are from the tropics and temperate mid-latitudes and most publications primarily present species lists with no exploration of environmental controls. These are limitations we intend to address in this study. We hypothesised that:

H1 Trees in the taiga zone will support diverse testate amoeba communities.
H2 Tree holes will have communities which are distinct from those of trunks.
H3 Diversity and abundance of testate amoebae will decline with elevation on the trunk.
H4 Epiphytic mosses will support greater density and diversity of testate amoebae than lichens.
H5 Communities of epiphytes will have lower diversity and different composition compared to those of adjacent soils.

Material and Methods

One hundred and sixty five samples were taken between May 2008 and June 2009. Sampling was conducted in four contrasting regions of European Russia, selected to span a range of tree species, climate and air pollution regimes. In each region 3-28 trees were selected with this number largely determined by the tree species richness. Trees were chosen randomly within an area of 1 ha. The regions were: Karelia, north-west Russia in the northern taiga zone (30 samples from *Picea abies;* July 2008; 66.51-66.53 N 32.94-32.98 E), Penza, east-central European Russia in the forest-steppe zone (82 samples from *Quercus robur, Alnus glutinosa, Betula pubescens, Fraxinus excelsior, Populus tremula*; May-June 2008; 52.82-53.87 N 45.06-46.81 E), Mordovia, east-central European Russia in the southern taiga zone (15 samples, tree species not differentiated; June 2009; 54.10 N 46.29 E) and four urban parks in the city of St Petersburg (35 samples from *Tilia cordata, Fraxinus excelsior, Acer platanoides, Picea abies, Populus alba, Quercus robur, Sorbus aucuparia;* September 2008; 59.69-59.72 N 30.38-30.46 E). The Penza and Mordovia regions are adjacent but the data are treated separately due to differences in the forest structure and the fact that samples were extracted in different sampling campaigns in

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different years. Climate of the study regions is warm summer continental to continental sub-Arctic (Köppen classification Dfb and Dfc) with mean annual temperature of 0.4-5.8°C and mean annual precipitation of 471-661mm. Climate statistics for each region are given in Supplementary Table 1.

Our target sampling strategy was to remove all cover of mosses and lichens from an area of 100cm^2 at the base of the tree and 10, 50, 100 and 200cm elevation on the trunk. However, it was not always practically possible to sample at these heights so there is some variability in height sampled, and some samples removed were subsequently misplaced. To delimit the sampling space we used a frame (10x10cm) placed centrally on the trunk and gently scraped the tree surface to remove all epiphytes. We aimed to avoid situations with a mixture of mosses and lichens in order to make comparisons between different substrates. To test whether rotting holes in the tree trunk host distinct communities we took additional samples from this habitat. For 17 sites we took samples of upper 3 cm of litter horizon from adjacent to the base of the tree to compare epiphytic to ground-based faunas in the same immediate area. In the Mordovia region trees were not identified to species level and sample height was not recorded. In all sites we recorded substrate moisture by weighing, drying at 110°C for six hours and re-weighing the samples.

Samples for TA analysis were prepared following the method described in Mazei et al. (2011). 1cm^3 of sample was soaked in water for 24 hours, stirred, filtered at 0.5mm, the suspension left to settle for a further 24 hours, and supernatant decanted off. This method minimises physical damage to tests and avoids the loss of small tests in back-sieving (Avel and Pensa, 2013; Payne, 2009). Samples were examined using a BIOMED-2 microscope and tests identified using taxonomic guides including Mazei and Tsyganov, (2006). We used a high taxonomic resolution with close attention to the differentiation of similar species and sub-species (Bobrov et al, 1999). All tests were counted in the samples, yielding an average count total of 194 (range 0-4124). Live individuals and empty tests were not differentiated.

We analysed the dataset using a variety of univariate and multivariate techniques. Explanatory variables considered were sampling region, substrate type (lichen or moss), moisture content and sample position including elevation and whether the samples were from litter, holes or trunks. For species richness and test concentration we tested for differences between groups using one-way ANOVA. Data were tested for heteroscedasticity using Levene's test and pairwise comparisons tested using Tukey's test. Non-parametric alternatives using Kruskal-Wallis test with comparisons by Bonferroni-corrected Mann-Whitney tests were used where required. Correlations with moisture and elevation were tested using Spearman's rs. To explore differences in community composition between

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samples we used a non-metric multi-dimensional scaling ordination on Bray-Curtis dissimilarity (Beals, 1984; Bray and Curtis, 1957). We tested the explanatory power of environmental variables using redundancy analysis on Hellinger transformed data with P-values produced by permutation tests.

There are several gaps in our dataset where it was not possible to collect data for a variety of practical reasons. In analysing the data we aimed to use the largest possible dataset to address each of our hypotheses. In practise this required different sub-sets of the complete dataset to be analysed for different hypotheses and so sample size is stated separately for each analysis. A small number of samples failed to yield any tests despite equivalent counting effort. We consider these absences as reflecting a genuine result and therefore include these datapoints in analyses of species richness and abundance, although not community composition. For community composition we consider only the sub-set of data for which all environmental data are available and excluding litter samples .

Results

Overall composition

We identified 67 taxa in the 165 samples (Supplementary Table 2). The testate amoeba community is primarily dominated by relatively ubiquitous taxa (Table 1). The most abundant taxa are *Centropyxis aerophila* and *Corythion dubium*, which each make up around 20% of counted tests. Both of these taxa are frequently encountered in soils and other habitats. There is a particular abundance of taxa with tests formed of idiosomes (13 of the 22 most abundant taxa; Table 1) and of smaller tests (7 of 22 taxa with test length less than ~50µm). Although most tests are of common species the dataset does include some rarer taxa, albeit in small numbers (no new species were identified). Taxa such as *Euglypha simplex, Euglypha capsiosa* and *Corythion asperulum* are quite rarely recorded in studies of testate amoeba ecology.

Species richness

Testate amoeba species richness varied between 0 and 18 taxa. Species richness significantly varied (one-way ANOVA $F_{5,114}$=8.8, P<0.001, n=114) by sampling location defined as: litter samples, holes and the four most abundant elevation categories (Fig. 1a). Pair-wise comparisons show that the only significant difference is that the (relatively small) group of basal trunk (0cm) samples have greater species richness than other samples (P<0.001) although a trend of decreasing species richness with elevation is notable amongst the elevation categories. Considering the larger group of all samples

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associated with sampling height information (n=125) there is a significant negative correlation between sampling height and species richness (Spearman rs=-0.26, P=0.002), however this loses significance when the basal samples are removed (Spearman rs=-0.17, P=0.6). The results therefore show a trend towards lower species richness with elevation; however this is primarily driven by a difference between those samples at ground level and those at greater elevation. There is no significant difference between the species richness of hole and other trunk samples (t-test, P>0.05, n=147). There is a significant difference between the sampling regions (Kruskal-Wallis test K=29.9, P<0.001, n=165) with the Mordovia samples significantly less species rich than all other regions (Bonferroni-corrected Mann-Whitney comparisons P<0.001) and the Penza samples marginally-significantly different from the St Petersburg samples (P=0.03). Species richness is significantly greater in samples from mosses compared to lichens (Mann-Whitney test U=886, P<0.001, n=141; Fig. 1c). This may be related to differences in moisture content with a significant positive correlation between species richness and substrate moisture (Spearman's rs=0.35, P<0.001, n=165; Fig. 2a).

Test concentration

Results for test concentration are in many respects similar to those for species richness. There is a significant difference amongst the six main sampling locations (Kruskal-Wallis K=14.5, P=0.01; Figure 3) but, as for species richness, in pairwise comparisons the only significant difference is that of the basal trunk samples from others (P<0.05). Considering all the samples associated with height information there is a significant negative correlation between elevation and concentration (Spearman's rs=-0.27, P<0.001; Figure 4). However, unlike the species richness data this correlation remains moderately significant when the basal samples are removed (Spearman's rs=-0.22, P=0.01). There is no significant difference between samples from holes and other habitats on the trunk (t-test, P>0.05). There is a significant difference among the sampling regions (Kruskal-Wallis test K=24.4, P<0.001, n=165) and, as for species richness, the Mordovia samples have a significantly lower concentration than all other regions (Bonferroni-corrected Mann-Whitney comparisons P<0.001). The Karelia samples are also marginally-significantly different from the Penza samples (P=0.047). Concentration of tests is significantly greater in samples from mosses compared to lichens (Mann-Whitney test U=1266, P=0.002, n=141) although variability is high (Fig. 3c). As for species richness there is a significant positive correlation between test concentration and substrate moisture content (Spearman's rs=0.40, P<0.001, n=165; Fig. 2b).

Community structure and environmental controls

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The NMDS plots (Fig. 5) highlight clear differences in community composition among study regions. Most apparent is the clustering of samples within the Karelia and Mordovia regions (Fig. 5). Data from the Penza and St Petersburg regions generally show a high degree of overlap with other areas. The NMDS plots do not highlight any difference in community with sample elevation on the trunk (shown here by the size of the points). When tested separately a significant portion of variance is explained by most of the variables in redundancy analyses. The greatest variance is explained by variables associated with location i.e. tree species (21.2%) and sampling region (13.0%; Table 2). This shows clearly that there is a strong element of geographic variability in community, as demonstrated by the NMDS plot, however our sampling design is insufficient to examine the causes of this in greater detail. As the tree species sampled varied between regions it is likely that the variance explained by this variable is also strongly associated with location and both of these variables were therefore included as co-variates in subsequent models. When tested independently, substrate type (i.e. moss or lichen) explains 10.8% and this is the only variable to remain significant when co-variates were introduced, although with much weaker explanatory power (3.1%). Moisture content and sample elevation did not explain significant variance when accounting for sampling region and tree species. There was no significant difference in community between hole and trunk habitats. Our results therefore show strong spatial variability in community but with only comparatively weak links to measured environmental variables. The strongest contrast appears to be between samples from mosses and those from lichens.

Discussion

Community composition

Our dataset includes several taxa which are rarely-recorded in testate amoeba studies. However, it is difficult to conclude that such taxa are necessarily particularly abundant in this environment or specialists of this habitat. Attempts to investigate the macroecology of testate amoebae (and other protists) are frequently hampered by taxonomic uncertainty with several underlying causes including the long-term decline in taxonomic research, the lack of clear criteria for splitting or grouping similar species and the fact that the majority of testate amoeba counting is necessarily conducted by less-experienced researchers (Heger, Mitchell et al, 2009; Mitchell and Meisterfeld, 2005; Mitchell, Lamentowicz et al, 2014; Payne, Lamentowicz, and Mitchell, 2011). Although molecular methods may ultimately clarify relationships between TA taxa this is still at an early stage and the most important contribution from the molecular research conducted to-date has arguably been to demonstrate how

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little we know. For instance, revealing the surprising taxonomic affinities of well-known species (Gomaa, Mitchell, and Lara, 2013) and the extent of cryptic diversity (Kosakyan, Heger et al, 2012). As none of the rarer taxa we identify in this study are so distinctive as to be considered unmistakable we therefore consider the parsimonious explanation to be that these taxa have simply been recorded elsewhere under other names. We do however note that as well as the presence of these distinguishable rare morphospecies many of the most abundant taxa we locate are small euglyphids (*Trinema* spp., *Corythion* spp., *Euglypha* spp.) and the difficulty of studying such species under conventional light microscopy means that these are taxa highly likely to hide cryptic diversity. Whether epiphytic habitats in this region harbor any true endemic species remains an open question. The overall structure of the community we identify with *Centropyxis aerophila* and small taxa of the genera *Euglypha*, and *Trinema* particularly abundant has similarities to epiphytic samples from previous studies (Davidova, 2008; Bonnet 1973a&b). Adding our larger dataset to the results of previous studies implies that TA communities associated with epiphytes are predominantly composed of species with broad ecological tolerances.

Environmental controls

Our dataset has some important limitations when considering correlations with environmental variables. The dataset includes samples from widely-separated regions which means that it successfully captures considerable variability but also that samples were taken in different regions at different times, complicating comparisons due to seasonal and inter-annual variability in communities (e.g. Warner et al. 2007). Many environmental factors (climate, soils, air quality) and the tree species sampled differ between these regions and there are many, potentially important, environmental factors which are not quantified in our dataset including pH and nutrient status.

While there are clear differences in communities between the sampling regions it is difficult to draw conclusions about the causes of these differences. Epiphytic plants are known to be highly sensitive to air pollution and epiphytes are frequently used in biomonitoring studies (e.g. Geebelen, Hoffmann 2001). Roberts and Zimmer (1990) demonstrated significant positive correlation between cultured protist species richness of epiphytic lichens and distance along urban-rural transects. As our dataset includes both sites in a major city and sites in remote regions these trees are likely to experience a considerable difference in air quality and TA are known to respond to many pollutants (Nguyen-Viet et al. 2004,2007; Payne et al. 2012; Meyer et al. 2012). While it is extremely probable that the St Petersburg sites experience higher levels of Nitrogen, heavy metal and particulate deposition than sites

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in more remote regions our data do not include sufficient replication at the regional scale to allow us to assess what the impacts of this exposure may be. It is notable, for instance, that there is no significant difference in species richness between Karelia and St Petersburg despite the probable difference in air quality. We cannot draw any firm conclusions on the causes of these regional differences because the timing and tree species sampled also varied. The Mordovia region had by far the lowest species richness and concentration. While environmental data were not collected in this region we speculate that this result was driven by very thin and sparse epiphyte cover on these trees. Bonnet (1973) suggests that epiphyte TA faunas may also be affected by the soil type of the surrounding ecosystem, which may be an additional cause of difference among regions.

Our data suggest some environmental controls on testate amoebae in these samples although the relationships between community composition and measured environmental variables were weak. In all datasets there is a significant difference between samples from mosses and lichens. Recently there has been speculation regarding the potential role of protists in the lichen symbiosis. Anderson (2014) highlights the diversity of protists in this habitat and Wilkinson et al. (2014) theorize potential mechanisms involving predation of functionally-important bacteria and roles in Si storage and cycling. Our data demonstrate that epiphytic lichens host diverse testate amoeba communities but these communities are less abundant and diverse than those of bryophytes. Differences between these two substrate types may be driven by greater abundance of living amoebae in bryophytes due to greater retention of moisture and greater plant layer thickness allowing tests to be more easily maintained *in situ*. Alternatively, as our data included both living and dead amoebae, it is possible that decomposition of empty tests might be more rapid in lichens -which might have implications for Wilkinson et al. (2014)'s speculations on a role in the silica cycle.

There is a significant positive correlation between moisture content and test concentration and diversity, although it is not possible to firmly identify any correlation with community composition when accounting for other factors. The importance of moisture to testate amoebae is well-known with some taxa tolerant of desiccation but the group as a whole occurring most abundantly in wet environments. Studies in a wide range of environments have found moisture availability to be a significant environmental control on testate amoeba biology and ecology (Wanner 1999; Charman 2001). It is therefore relatively unsurprising that moisture is important to communities in this habitat, although to our knowledge this is the first study to provide data to show this.

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In the concentration and, less-convincingly, the species richness data there is indication of a relationship with elevation of the sampling point. Relationships with elevation have been previously demonstrated by Kashevska et al. (2010) from an Ecuadorian rain forest. Consistent with our results these authors found a decline in diversity with height but contrary to our results found highest abundance in the highest samples; a result they did not anticipate and were unable to explain. Our result most likely relates to moisture content with higher samples likely to be drier and experience greater variability in moisture availability. The contradiction between our data and those of Krashevska et al. (2010) highlights how little is known of the ecology of TA in these habitats.

Given the evidence for moisture content as an environmental control on TA communities in this habitat and others we might expect to find different communities in tree holes compared to tree trunks. It might be expected that such features would catch and retain water and therefore host distinct communities adapted to these wetter conditions (*cf*. Lackey 1940). However, our data provide no support for this conjecture; we find no evidence for such differences in community composition, species richness or concentration. Our data, counter-intuitively, show higher concentration and species richness in samples from the base of tree trunks compared to samples of adjacent litter. This might relate to stemflow and therefore greater moisture availability at the base of the tree or conceivably to interactions with mycorrhizas. However we note that the number of samples from tree bases is very small and therefore this result should not be given strong weight.

Conclusions

Our data show that epiphytes from European Russia host abundant TA, although these are primarily species with broad ecological preferences. In future studies molecular data will be desirable to provide a less-ambiguous assessment of the true diversity of this habitat and any (pseudo-)cryptic diversity. Our results provide first steps towards understanding the ecology of TA in this habitat. Our data show that there is considerable difference between communities associated with lichens and bryophytes and TA occur more abundantly and with greater diversity in locations with greater moisture availability. However there is also considerable variability amongst our study regions which suggests that other, currently unknown, factors are important in structuring these communities.

We believe that epiphytes on trees are likely to be a significant habitat for testate amoebae. Studies around the world have demonstrated the presence of diverse communities, including some new

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species, despite the fact that research conducted to date has been limited. Global forest area is estimated at 32,688,000 km^2 ((Hansen et al, 2010) data for the year 2000). If, as a crude first approximation, we assume a constant tree stem density of 100,000 km^{-2} and average stem diameter of 30cm based on the intensively-studied plots of Maltamo et al, (2004) then, if we (very conservatively) consider that testate amoebae are restricted to the first 3m of the trunk, and only to the trunk this habitat could provide in the order of $9x10^6$ km^2 'potential living surface'. This is roughly 7% of the global terrestrial land surface. Even if we account for the fact that the actual volume that testate amoebae may be able to exist in on tree surfaces is quite thin (depth perhaps 3mm) compared to that in soils (depth perhaps 5cm) then the actual volume of 'living space' in trees may still approach 0.5% of the total, which we would argue is non-trivial.

An additional argument for the importance of this habitat is that much of our evidence for the geological history of testate amoebae comes from fossils preserved in amber (e.g. Girard 2012; Schmidt et al. 2010). Epiphytic testate amoebae are likely to be disproportionately represented in these samples. As new taxa are frequently described from such fossil samples it is important to have sufficient contemporary data to be confident that these taxa are not extant.

Epiphytic environments in forests are probably a comparatively widespread habitat for TA and are perhaps deserving of greater research attention than has thus-far been the case.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at...

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FIGURE AND TABLE CAPTIONS

Figure 1. Species richness of epiphytic testate amoebae communities from western Russia by a) sample types, b) regions, and c) substrate type. Error bars show +/- one standard deviation. Bars with different letters denote significant differences (see text for full results). Note that the epiphytic categories presented exclude some samples taken at other heights or with height not recorded but these are included in the overall epiphyte averages shown in separate bar on the right.

Figure 2. Correlation between substrate moisture content (%) and species richness (a) and test concentration (b).

Figure 3. Test concentration of epiphytic testate amoebae communities from western Russia by a) sample types, b) regions, and c) substrate type. Error bars show +/- one standard deviation. Bars with different letters denote significant differences (see text for full results).

Figure 4. Correlation between test concentration and elevation of sample.

Figure 5. NMDS ordination of epiphytic testate amoeba data based on Bray-Curtis dissimilarity of a) percentage, and b) concentration data. Point size is proportional to elevation of sampling point with the exception of Mordovia samples for which this information is not available and a constant diameter is used.

Table 1. Abundance of major epiphytic testate amoeba taxa of western Russia (>0.5% of all tests). List is based on epiphytic samples only (i.e. excluding ground litter). For full list of species identified see Supplementary Table 2.

Table 2. Results of redundancy analyses of Hellinger transformed testate amoeba data. Significance tested by permutation test. The analyses are based solely on samples from trunks with a full set of environmental data, (with the exception of tests for difference between communities of trunks and rot-holes).

Supplementary Table 1. Climatic conditions of the studied regions (from www.pogodaiklimat.ru).

Supplementary Table 2. Full species list.

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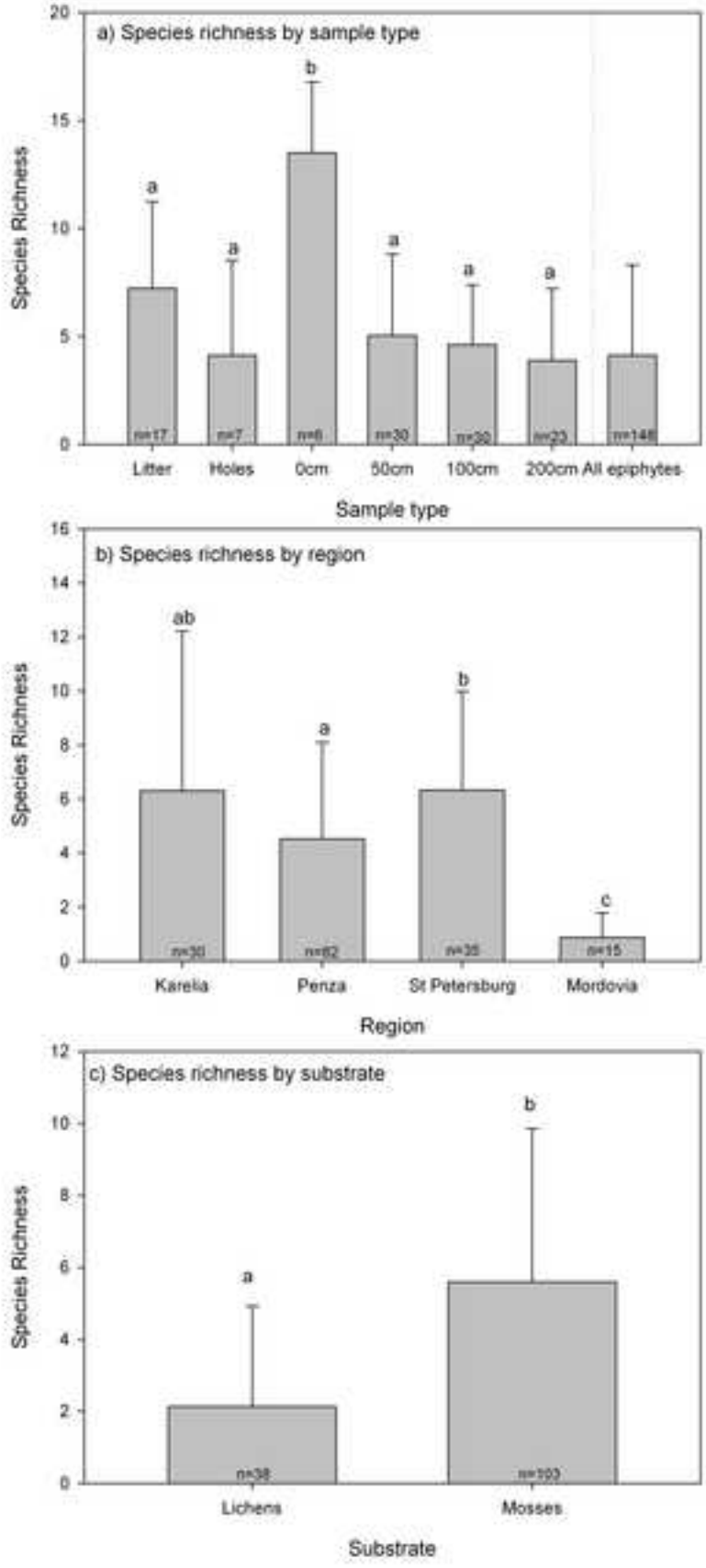
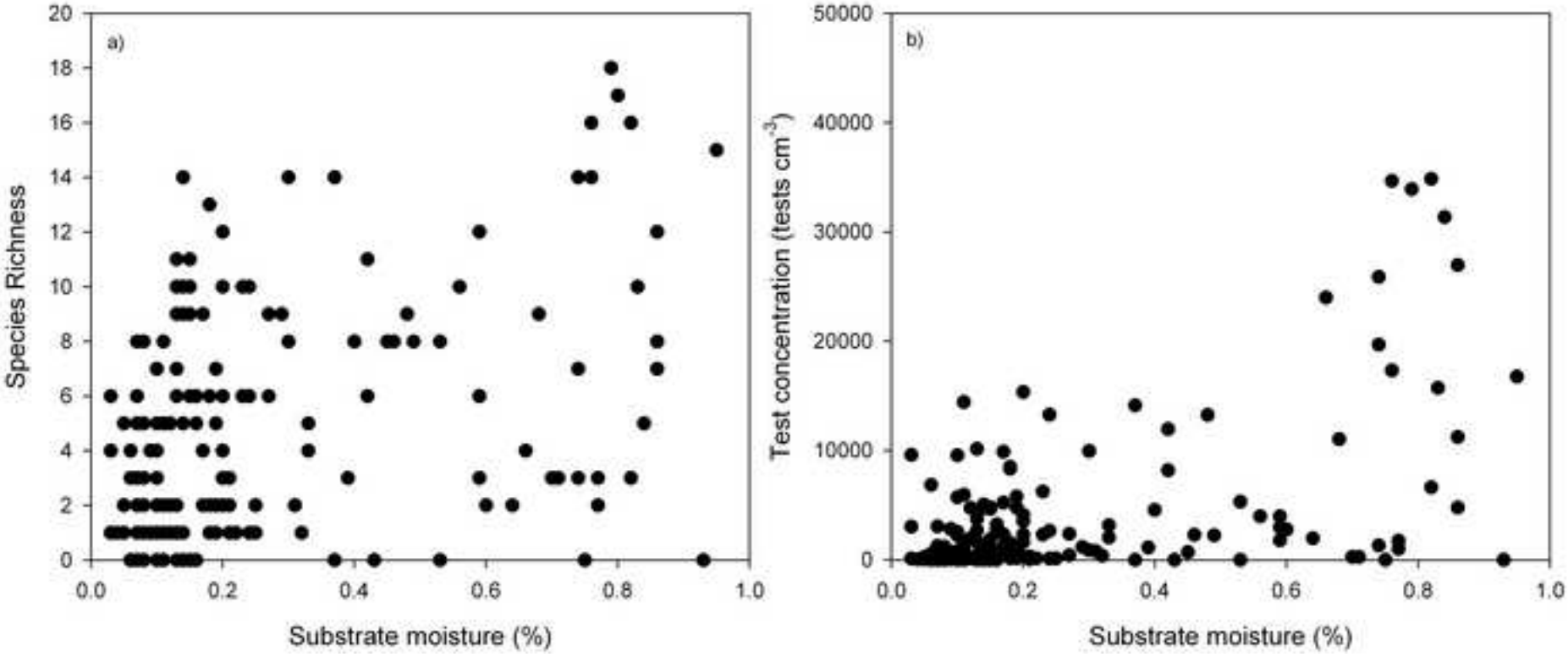


Figure2


a)
Species Richness
Substrate moisture (%)
b)
Test concentration (tests cm-3)
Substrate moisture (%)

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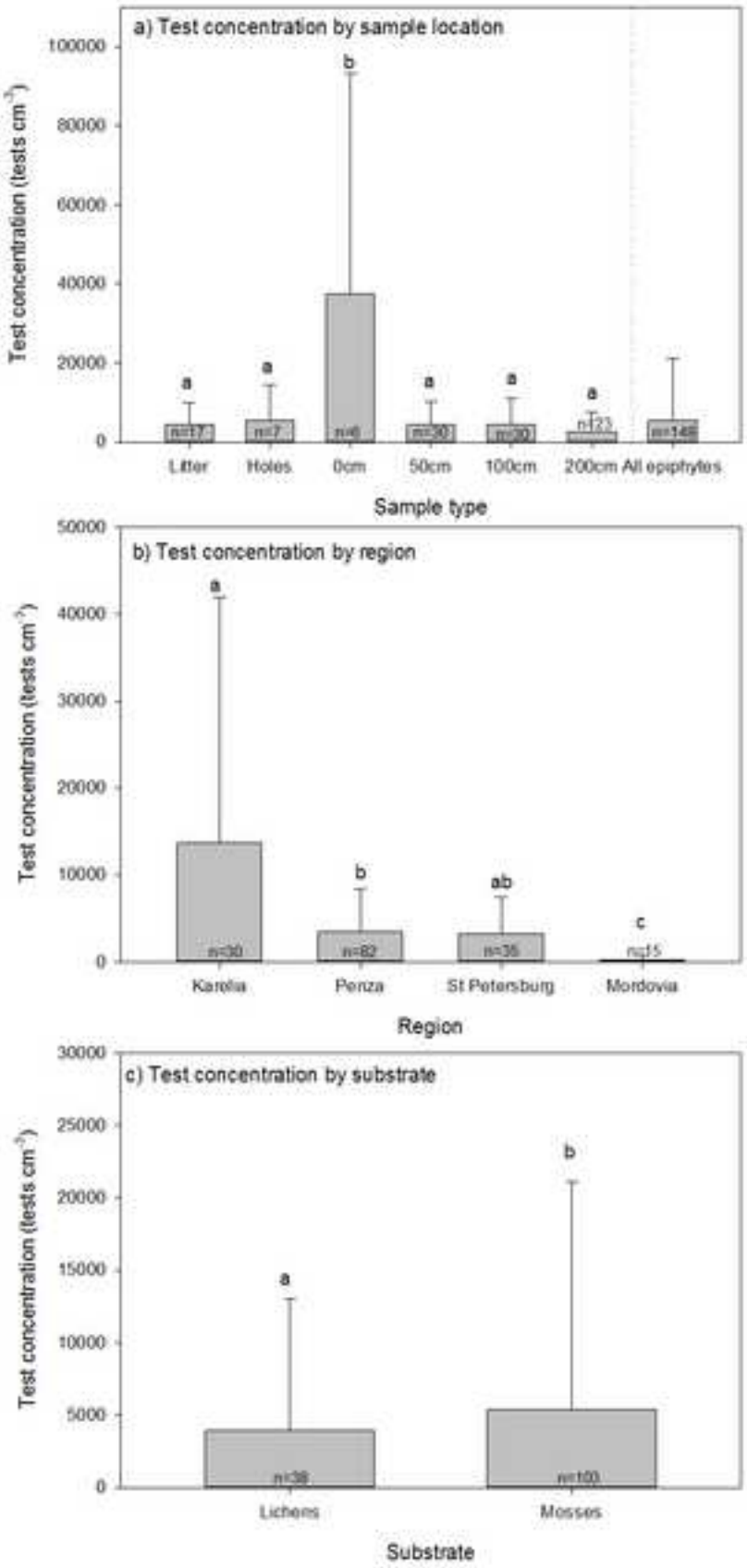

Figure4

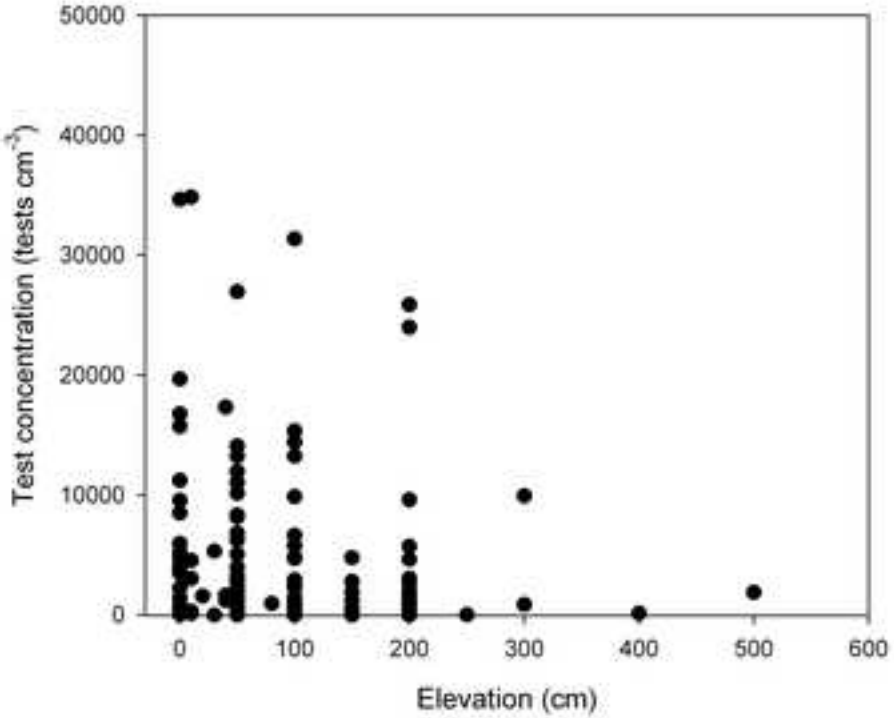
50000
40000
30000
20000
10000
0
Test concentration (tests cm-3)
0
100
200
300
400
500
600
Elevation (cm)

Figure5

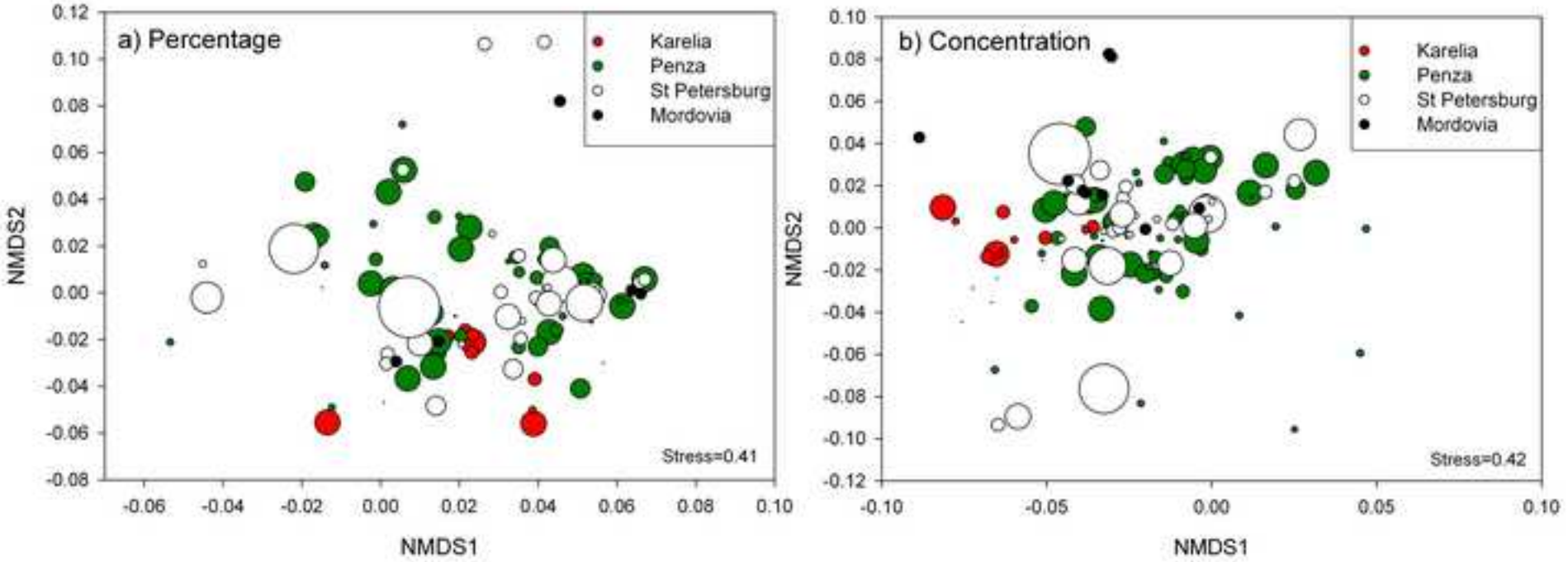

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Table 1. Abundance of major epiphytic testate amoeba taxa of western Russia (>0.5% of all tests). List is based on epiphytic samples only (i.e. excluding ground litter). For full list of species identified see Supplementary Table 1.

Taxon	**Overall %**
Arcella arenaria compressa	2.58
Assulina muscorum	2.09
Assulina seminulum	3.60
Centropyxis aerophila	20.57
Centropyxis aerophila sphagnicola	4.55
Corythion dubium	19.12
Corythion orbicularis	4.59
Difflugia lucida	1.24
Euglypha ciliata glabra	1.83
Euglypha laevis	0.62
Euglypha rotunda	1.21
Euglypha simplex	0.80
Euglypha strigosa	0.52
Euglypha strigosa glabra	0.59
Nebela tincta s.l.	1.35
Phryganella acropodia	4.37
Phryganella hemisphaerica	15.77
Tracheleuglypha dentata	0.68
Trinema complanatum	2.50
Trinema enchelys	3.86
Trinema lineare	1.32
Trinema penardi	1.42

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Table 2. Results of redundancy analyses of Hellinger transformed testate amoeba data. Significance tested by permutation test. The analyses are based solely on samples from trunks with a full set of environmental data, (with the exception of tests for difference between communities of trunks and rot-holes).

Explanatory variables	**Co-variates**	**% variance explained**	**P-value**
Region	-	13.0	0.001
Tree species	-	21.2	0.001
Moisture content	-	4.0	0.001
Elevation	-	1.3*	ns
Substrate	-	10.8	0.001
Holes vs Trunk	-	1.5	ns
Moisture content	Tree species Region	1.1	ns
Elevation	Tree species Region	1.1	ns
Substrate	Tree species Region	3.1	0.001
Holes vs Trunk	Tree species Region	0.7	ns

*This analysis gives a non-significant result, however when controlling by sampled trees a significant result (P=0.001) is found.

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