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First records of testate amoebae from the Novaya Zemlya archipelago (Russian Arctic)

Yuri A. Mazei[1,2], Andrey N. Tsyganov[1], Viktor A. Chernyshov[1], Alexander A. Ivanovsky[2], Richard J. Payne[1,3*]

1. Penza State University, Krasnaya str., 40, Penza 440026, Russia.

2. Lomonosov Moscow State University, Leninskiye Gory, 1, Moscow 119991, Russia.

3. University of York, Heslington, York YO10 5DD, United Kingdom.

* corresponding author, e-mail: richard.payne@york.ac.uk

ABSTRACT

Testate amoebae have proved a useful group of species to understand the biogeography of larger microorganisms. The Arctic has attracted particular interest in such studies but there are large geographic gaps in current knowledge. Here we present what we believe is the first ever study of testate amoebae from the Novaya Zemlya archipelago in Arctic Russia. We investigated testate amoebae from the northernmost island of Novaya Zemlya proper and two smaller islands to the south: Dolgii Island and Matveev Island. We found that testate amoebae were present and active even in the extreme physical environment of northern Novaya Zemlya. Further south in the tundra zone of Dolgii and Matveev islands, testate amoebae were notably abundant and diverse. There were distinct differences in assemblage between all three islands and particularly between Novaya Zemlya and the two more southerly islands. The assemblage of Novaya Zemlya was distinctive with a surprising abundance of larger taxa. Comparisons to previous data suggest that the testate amoeba assemblages of these islands may show more affinity to those further west in Greenland and Svalbard than those further east in Siberia. Results highlight the limited knowledge of the abundance and diversity of these functionally-significant protists in large areas of the globe.

KEYWORDS: Protist; Biogeography; Arctic; Protozoa

INTRODUCTION

While many of the factors determining the biogeography of larger organisms are well-understood, the biogeography of microorganisms is much less clear and it is uncertain whether the same rules apply (Azovsky and Mazei 2013; Azovsky et al. 2016; Martiny et al. 2006). Few studies have the resources to simultaneously consider all microbial groups so much research on microbial biogeography has focused on distinctive flagship taxa. Testate amoebae (a polyphyletic group of protists) have particular advantages in this respect due to their distinctive and highly variable shells ('tests'), combined with wide distribution, presence in a variety of habitats and a long history of research (Payne 2013; Smith et al. 2008). Studies of testate amoebae have been at the forefront of research questioning the 'everything is everywhere' principle of the Baas-Becking hypothesis (De Wit and Bouvier 2006) for larger microorganisms while examples such as *Nebela ansata* have illustrated the possibility for hyper-local distributions of microbial taxa (Heger et al. 2011; Lara et al. 2015; Smith et al. 2008). As a model for biogeographic studies testate amoebae have the additional advantage that the decay-resistance of their tests means that they can be used to understand microbial community change and environmental forcing on much longer time-scales (centennial to millennial) than other groups which decompose rapidly after death (Mitchell et al. 2008).

The Arctic presents an interesting case for studies of biogeography as many ecosystems are both ecologically uncomplicated and young, providing a simplified model system. Protozoa are one of the least studied groups of terrestrial invertebrates in the Arctic. Over a 100-year history of invertebrate research on the Svalbard archipelago, there are about 1,300 species of terrestrial invertebrates described but only 198 Protozoa (Hodkinson et al. 2013). In their circum-Arctic compilation of data Beyens and Bobrov (2016) identified 378 species of testate amoebae in terrestrial deposits and Beyens et al. (1986a) identified 40 species in aquatic sediments but these totals are very unlikely to represent the full species complement, particularly given the probability of cryptic and pseudocryptic diversity (Kosakyan et al. 2012). There are large spatial gaps in current knowledge of Arctic testate amoeba biogeography with few studies from the Canadian Arctic Archipelago and virtually none from the Russian High Arctic between Svalbard and Severnaya Zemlya. To fill this gap here we present the first data on testate amoebae from Novaya Zemlya.

Study region

Novaya Zemlya is an archipelago in the Russian Arctic (Archangelsk Oblast) between the Barents and Kara Seas (74°N 56°E). The archipelago consists of the two islands of Novaya Zemlya proper separated by the very narrow Matochkin Strait and further small islands to the south (Fig. 1). The two main islands of Novaya Zemlya form a mountainous arc almost 900 km in length, an extension of the Ural Mountain chain separating Europe and Asia (Zeeberg 2002). Approximately 27% of the land area is glaciated, particularly towards the north of the archipelago, and much of the un-glaciated land area is unvegetated with areas of tundra towards the south. South of Novaya Zemlya proper are several smaller islands. Dolgii (also transliterated as Dolgy and Dolgiy) Island is a 38km long island in the Pechora Sea between mainland Russia and Novaya Zemlya. The island has a notably flat topography (maximum elevation 18m) with abundant brackish and freshwater lakes. The vegetation is predominantly tundra and coastal wetlands. Matveev (also transliterated as Matveyev) Island is a similar, smaller island of only 2km diameter, 10km to the north of Dolgii Island. Both islands do not presently have permanent human settlements, although both have been occupied in the past.

Climate data for the period 1961-1990 (station: Malye Karmakuly, south west coast of Novaya Zemlya) show mean annual temperature of -5.5°C with mean July temperature of 6.9°C and mean January temperature of -15.7°C. For the same period mean annual precipitation is 420mm, with monthly totals highest in the autumn (NOAA 2017). The latitudinal span of the archipelago leads to considerable temperature gradients while climate is also affected by marine circulation patterns leading to warmer conditions on the west coast than the east (Coulson et al. 2014).

If Novaya Zemlya is considered as a single island (i.e. ignoring the Matochkin Strait, the >600m wide fjord which bisects the landmass) it ranks as the twentieth largest island in the world. However, the archipelago is recognised as being highly under-researched in terms of biodiversity, partly as a result of its role as a nuclear testing site and consequent access restrictions (Coulson et al. 2014). A substantial proportion of the limited research which has been conducted is only available in inaccessible Russian-language literature (Coulson et al. 2014). To the best of our knowledge the testate amoebae have never been studied. We have not been able to locate any publications and data on testate amoebae from Novaya Zemlya are not included in previous data-compilations of Arctic testate amoeba assemblages and species distributions (Beyens and Bobrov 2016; Beyens and Chardez 1995). This clearly presents a very substantial data gap. This region has particular interest because, in common with other high latitude archipelagos, the islands were most likely entirely ice-covered at the last glacial maximum (Forman et al. 2004; Forman et al. 1999). This means that the current assemblage is likely to have almost entirely developed during the Holocene and provides an interesting example of primary succession across an entire large land-mass over a comparatively short period (Coulson et al. 2014).

MATERIALS AND METHODS

Fieldwork

Fieldwork was conducted on Novaya Zemlya in 2007 and Dolgii and Matveev Islands in 2003. Due to the extreme inaccessibility of these regions the choice of sampling sites was logistically constrained. In Novaya Zemlya sampling was conducted in the region of Blagopoluchiya Bay (Fig. 1) on the eastern shore of the northern (Severny) Island (75.7°N, 63.7°E). This work was conducted as part of a research cruise of the R/V Akademik Mstislav Keldysh, detailed in Flint et al. (2008). This location has very little vegetation. Inland, the Severny Ice Cap lies no more than 10km from the coast with a narrow unglaciated coastal zone with a land surface largely of unconsolidated siltstone shale. We extracted five samples from rare patches of bryophytes and stonecrops (Crassulaceae). From the same region Makkaveev et al. (2013) have presented details of nutrient fluxes and Udalov et al. (2016) and Stepanova and Nedospasov (2017) have described the coastal environment. We believe this sampling site to be amongst the most northerly ever studied for testate amoebae, only exceeded by sites on the archipelagos of Severnaya Zemlya, Franz Josef Land and Svalbard (Andreev et al. 2008; Beyens and Bobrov 2016; Beyens et al. 1986b; Beyens et al. 2000). On Dolgii Island we conducted sampling at a point on the east coast and towards the southern tip (Fig. 1). These sampling regions are representative of the island as a whole with a low-lying topography with extensive small lakes, hummocks of bryophytes and lichens and some areas of shrub vegetation. Eleven samples were extracted of mosses, organic detritus and inorganic sediments (Table 1). On Matveev Island we conducted sampling across this small island (Fig. 1). Four samples were extracted from silt, sand and moss growing in small lakes (Table 1). Sampling on Dolgii and Matveev islands was conducted in parallel with sampling for heterotrophic flagellates, previously described by Tikhonenkov and Mazei (2006). In each sampling site we removed a sample of roughly 10 cm^3 which was preserved in an airtight plastic bag and refrigerated immediately (Mazei et al. 2015). Samples were prepared within three months of sampling and preserved with formalin; microscopic analysis was completed within six months of analysis.

Laboratory work

Samples were prepared for microscopy using the method of Mazei and Chernyshov (2011) involving suspension in deionised water followed by shaking and suspension in a petri dish. Testate amoebae were identified using light microscopy (Biomed, Russia) at a magnification of 160× and tests identified following Mazei and Tsyganov (2006). The full volume of each sample was counted and all tests recorded. Live individuals were differentiated in the Novaya Zemlya samples but not the Dolgii and Matveev Island samples.

Data analysis

We first considered the overall structure of the data using ordination. We used a non-metric multi-dimensional scaling (NMDS) ordination based on Bray-Curtis dissimilarity of relative abundance data to assess differences between samples (Bray and Curtis 1957). To quantify the relationships between individual samples we conducted a cluster analysis based on Ward's method. To statistically test for differences between the three islands we used a one-way permutational analysis of variance (PERMANOVA) with 9999 permutations based on a Bray-Curtis dissimilarity matrix of relative abundance data.

We considered two indices of the diversity of the identified assemblages: total taxon richness and Shannon's H' diversity index. We tested for differences in diversity between the three islands using Kruskal-Wallis tests with Mann-Whitney pairwise comparisons as the data did not meet the assumptions of ANOVA. To consider the adequacy of our sampling we first used individual rarefaction to test whether our counts for individual samples were sufficient to reach an asymptote. We then used sample rarefaction of the entire dataset to assess how taxon richness changed with number of samples considered (Colwell et al. 2004). All data analyses were carried out using PAST vers.3.04 (Hammer et al. 2001).

RESULTS

Overall assemblage composition

We identified 90 taxa (Table 2) but test counts were often low and varied between 2 and 489 individuals (mean=116). Several samples contained fewer tests than often considered desirable (Payne and Mitchell 2009). Counts were particularly low from Novaya Zemlya (2-32 tests). The most abundant taxa across all the samples were *Centropyxis aerophila* (18.4% tests), *Trinema enchelys* (10.2% tests), *Euglypha laevis* (8.1% tests) and *Euglypha tuberculata* (4.8% tests). 24 taxa were represented by just a single test. The diversity of the samples was variable with 2-37 taxa per sample (mean=13.4) and a mean Shannon H' of 1.8 (Fig. 2). Individual rarefaction curves strongly suggest that for the majority of samples the count of amoebae was insufficient to identify all taxa (Fig. 3). It was only in a few samples, all from Dolgii Island, where curves showed substantial flattening indicating taxon counts approaching an asymptote. For many samples, particularly those from Novaya Zemlya where counts were low, curves showed a very steep trajectory with no indication of flattening. The overall rarefaction curve similarly did not reach an asymptote (Fig. 4). Rate of increase begins to slow, particularly above ten samples, but the gradient continues to be relatively steep even at the maximum number of samples. It is clear that our results should not be viewed as presenting a comprehensive inventory of the testate amoeba assemblage of these three islands.

Novaya Zemlya assemblages

In the Novaya Zemlya samples counts of testate amoebae were low but a substantial proportion of these tests (22%) were occupied by living amoebae, with live individuals present in all but one sample. This suggests that an active community is present even in this very harsh physical environment. Nine species and subspecies were identified with the most abundant taxa being *C. aerophila* and *Centropyxis sylvatica*. None of the taxa identified can be considered uncommon; most are very widespread in soils and wetlands worldwide. Two tests of *Plagiopyxis intermedia* are notable as this is a relatively infrequently recorded taxon. All taxa were of lobose testate amoebae and all but a single individual of *Arcella arenaria* was of a taxon with a xenosome test. Most individuals were of the similar genera *Centropyxis, Cyclopyxis* and *Plagiopyxis* and many frequently-abundant genera in soils and lakes were not present at all (e.g. *Euglypha, Nebela, Difflugia, Trinema*). Taxon richness was low, varying from two to seven taxa and samples had a correspondingly low diversity with Shannon H’ varying from 0.56 to 1.53 (Fig. 2).

Matveev Island assemblages

In the Matveev Island samples the most abundant taxa were *E. tuberculata* (18% tests), *E. laevis* (14%) and *Arcella rotundata* (12.9%). Counts were considerably higher than in Novaya Zemlya but still low (mean=50 tests). Taxon richness (mean=10 taxa) and Shannon H’ (mean= 1.8) were also higher than Novaya Zemlya. The community composition was very distinctly different from Novaya Zemlya with far more taxa with tests constructed of idiosomes and secretion and many more taxa with filose pseudopodia.

Dolgii Island assemblages

The Dolgii Island assemblage was comparatively similar to the Matveev assemblage but again distinctly different from the Novaya Zemlya assemblage. On this island the testate amoeba counts were the highest (mean= 181 test), as were the taxon richness (mean=19) and Shannon diversity H’ (mean=2.2). The most abundant taxa were *T. enchelys* (18.5%), *C. aerophila* (11.2%) and *E. laevis* (9.6%). Although replication of habitats and substrates was insufficient to draw general conclusions it was notable that both species diversity and test count were high in samples from mosses in both lakes and tundra peatlands.

Differences between islands

There were clear differences between the assemblages of the three islands sampled. The NMDS results (Fig. 5) highlight a close grouping of the five samples from Novaya Zemlya with high scores on axis one and also relatively high scores on axis two. The NMDS also generally separates the Matveev and Dolgii samples. Although these are less distinctly different, the Dolgii samples tend to have higher NMDS1 scores and generally higher NMDS2 scores. Cluster analysis highlights similar compositional relationships between the three islands (Fig. 6). For instance, at the third level of grouping the Novaya Zemlya samples are assigned a distinct group composed of only these five samples. By contrast, the Dolgii and Matveev samples are more mixed, being split amongst the two other groups. Taken overall the results imply distinct differences between Novaya Zemlya and the two more southerly islands and subtle differences between Matveev and Dolgii islands. PERMANOVA showed the difference between all three islands to be highly statistically significant (PERMANOVA $F_{6.7,4.6}$=3.9, p=0.0001). In pairwise tests the Novaya Zemlya assemblage is highly significantly different from both the Matveev (Mann-Whitney pairwise p=0.008) and Dolgii (Mann-Whitney pairwise p=0.0004) assemblages whereas the Dolgii and Matveev assemblages are weakly significantly (Mann-Whitney pairwise p=0.037) different from each other.

In terms of diversity there were similar differences in assemblage between the three islands (Fig. 2). The samples from Novaya Zemlya were unambiguously the least diverse and the Dolgii samples the most diverse. In terms of taxon richness there were some clear differences between the islands and these were highly significantly different (Kruskal-Wallis H=11.6, p=0.002). In pairwise comparisons the only significant difference was between the Dolgii and Novaya Zemlya samples (Mann-Whitney pairwise p=0.003). This difference in taxon richness is relatively unsurprising given the low total counts in the Novaya Zemlya dataset. In terms of Shannon H’ which accounts for sample size and considers evenness as well as richness there was also a difference in diversity between the islands (Kruskal-Wallis H=11.4, p=0.003). In this case there were significant pairwise differences between the Novaya Zemlya assemblage and both the Dolgii (Mann-Whitney pairwise p=0.003) and the Matveev (Mann-Whitney pairwise p=0.037) assemblages. Overall the results clearly suggest that the Novaya Zemlya samples tend to have lower diversity than the other two islands which are more similar albeit with a trend to greater diversity in Dolgii than Matveev Island.

DISCUSSION

Our research reveals that testate amoebae are present even in the harsh conditions of northern Novaya Zemlya. The presence of live tests confirms that these assemblages are active. Further south in the less extreme climates of Matveev and Dolgii islands testate amoebae are relatively diverse and abundant. The assemblage composition includes many taxa which are widely known in soils, lakes and wetlands across the Arctic and sub-Arctic zones. Some taxa are less-frequently recorded including *Sphenoderia macrolepis, Oopyxis cophostoma, Difflugia ovalisina* and *Paulinella chromatophora*. It is possible that the environmental conditions of the sampling sites favoured the presence of these rarer taxa but taxonomic uncertainty and limited sampling make it difficult to conclude this with certainty. We did not identify any tests which were not assignable to known species of testate amoebae. Across the Arctic region the most common taxa are known to be *Trinema lineare, Assulina muscorum* and *Centropyxis aerophila* (Beyens and Bobrov 2016). While *T. lineare* (4.1%) and *C. aerophila* (18.4%) were both abundant in our samples, surprisingly *A. muscorum* was not found at all in any of the samples despite the extremely wide distribution of this taxon and known abundance in habitats similar to those sampled here. We do not have any explanation for this surprising absence which deserves future consideration. Our dataset is insufficiently large to identify all taxa and more extensive sampling would be desirable to have confidence in the absence of such taxa.

The assemblage composition of the Novaya Zemlya samples is curious for the lack of diversity in testate amoeba genera and test compositions, with a dominance by *C. aerophila* and *C. sylvatica.* While these taxa are both widespread in the Arctic their abundance is somewhat surprising as these are amongst the larger testate amoeba taxa commonly identified. Indeed, the assemblage also included three tests of the very large (mean length c.200µm) taxon *Centropyxis ecornis*. This contrasts with a general finding that taxa with small tests predominate in moisture-limited environments. For instance, studies of testate amoebae in hot desert soil crusts have found a predominance of small taxa (e.g. *Cryptodifflugia, Euglypha*) (Bamforth 2004; Bamforth 2008) and mean biovolume has been considered to correlate positively with moisture supply (Fournier et al. 2012; Van Bellen et al. 2017). All of the taxa we identified from unconsolidated and well-drained substrates in very cold conditions are also widely known from wetlands and some are even considered typical of wetter conditions within these wet habitats. *C. aerophila* is known to be an abundant taxon in Arctic lakes and ponds (Beyens et al. 1986a; Trappeniers et al. 1999). It may be that in cold conditions body size confers advantages in moisture-limited environments that it does not in warmer environments. Conceivably the reduced surface area to volume ratio enables amoebae to better resist freezing. The cushion plants sampled may also have provide a microclimate which is buffered from that of the wider environment with less extreme temperature and higher moisture.

The differences between the three islands are likely to partially reflect climate and consequent ecological differences. It is relatively unsurprising that the sites in Novaya Zemlya with very sparse vegetation and extensive exposed moraine host different testate amoeba assemblages from the wet tundra environment sites sampled on Dolgii and Matveev islands. Both temperature and moisture availability will differ considerably and these are known to be important drivers of testate amoeba assemblages. It is conceivable that glacial history of the sites may also be a factor. The Novaya Zemlya sampling locations are within a few kilometres of the current glacial margin and may well have been ice-covered in the relatively recent past whereas Dolgii and Matveev islands were most likely ice-free by at least the early Holocene (Forman et al. 1999). The more southern islands have therefore had considerably more time for amoeba communities to become established and develop. A further factor may be their geographic position; the Dolgii and Matveev island sites are far closer to the mainland and therefore the biogeographic barrier provided by the sea is likely to be much more permeable.

The more subtle differences between Matveev and Dolgii islands are more surprising. In this case the differences may relate to both the physical and human environment. The soils of Matveev Island are typically thinner than on Dolgii island and the waterbodies less extensive. Matveev Island was occupied by humans in the Soviet period with a lighthouse and radio station established whereas sampling sites on Dolgii island were further from previous habitation sites. Given the small size of Matveev island this means that most of the island’s habitats have been disturbed by human activity to a greater or lesser extent. Greater relative abundance of taxa such as *Arcella rotundata* could, for instance, represent a legacy of aquatic pollution (Jiang 2006).

Our results for testate amoebae parallel some of the findings of Tikhonenkov and Mazei (2006) for heterotrophic flagellates in many of the same locations on Dolgii and Matveev islands. Our data show that, similarly to the flagellates, most testate amoebae are of taxa with widespread distributions and that the true diversity of assemblages may be greater than that detected. Our results also similarly imply greater numbers and diversity of testate amoebae in mosses than in other substrates in these lakes and wetlands. For testate amoebae this is unsurprising as the high abundance of testate amoebae in moss-dominated habitats is well-established.

Based on a synthesis of published Arctic testate amoeba data Beyens and Bobrov (2016) suggest that there are distinct testate amoeba assemblages associated with different Arctic regions. Novaya Zemlya falls in a zone which is not adequately delimited, between a suggested Svalbard/Greenland fauna to the west and a Siberian fauna to the east. The Svalbard/Greenland group is typified by a lesser number of *Difflugia* and *Arcella* taxa. If this separation is robust our results suggest that the Novaya Zemlya region can probably be considered to show more commonality with the Svalbard/Greenland region than with Siberian sites further east. However the presence of such ecoregions for testate amoebae requires further research, ideally using molecular methods as well as morphology.

Beyens and Bobrov (2016) suggest that some testate amoeba species can be considered Arctic region endemics and propose two examples of distinctive potential ‘flagship’ species: *Centropyxis pontigulasiformis* and *Centropyxis gasparella*. Interestingly, neither were located in these samples despite the fact that *C. pontigulasiformis* is known from Svalbard to the west (Beyens et al. 1986a) and *C. gasparella* is known from the north Siberian coast to the east. Beyens and Bobrov (2016) theorise that such taxa may have survived the last glacial in Arctic refugia, therefore conceivably their absence in our samples might relate to the absence of nearby refugia. However it should be cautioned that our analyses suggest that sampling is unlikely to have captured all testate amoeba taxa present in this region so these taxa may have been present in our sites but not located in our sampling.

CONCLUSIONS

Testate amoebae are a group of protists which are both functionally-significant in many ecosystems and have considerable potential as model organisms for study of global microbial biogeography. However, use of testate amoebae to understand protist biogeography is compromised by limited understanding of abundance and diversity in many regions of the world. Basic inventory work from little-known parts of the planet is essential to allow the potential to be realised. Here we contribute to filling a large data gap for the Russian Arctic, showing the remarkable ability of testate amoebae to survive even in harsh polar environments. Our results are an important step towards better understanding the biogeography of protists in the Arctic. Future work needs to focus on expanding coverage and combining existing morphospecies datasets to draw broader-scale conclusions and on supplementing morphological identification with molecular analyses.

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Compliance with Ethical Standards

The authors declare no conflict of interest.

References

Andreev A, Lubinski D, Bobrov A, Ingólfsson Ó, Forman S, Tarasov P, Möller P (2008) Early Holocene environments on October Revolution Island, Severnaya Zemlya, Arctic Russia. Palaeogeogr, Palaeoclimatol, Palaeoecol 267:21-30

Azovsky A, Mazei Y (2013) Do microbes have macroecology? Large-scale patterns in the diversity and distribution of marine benthic ciliates. Global Ecol Biogeogr 22:163-172

Azovsky AI, Tikhonenkov DV, Mazei YA (2016) An estimation of the global diversity and distribution of the smallest eukaryotes: Biogeography of marine benthic heterotrophic flagellates. Protist 167:411-424

Bamforth SS (2004) Water film fauna of microbiotic crusts of a warm desert. J Arid Environ 56:413-423

Bamforth SS (2008) Protozoa of biological soil crusts of a cool desert in Utah. J Arid Environ 72:722-729

Beyens L, Bobrov A (2016) Evidence supporting the concept of a regionalized distribution of testate amoebae in the Arctic. Acta Protozool 55:197-209

Beyens L, Chardez D (1995) An annotated list of testate amoebae observed in the Arctic between the longitudes 27E and 168W. Arch Protistenk 146:219-233

Beyens L, Chardez D, De Landtsheer R, De Baere D (1986a) Testate amoebae communities from aquatic habitats in the Arctic. Polar Biol 6:197-205 doi:10.1007/bf00443396

Beyens L, Chardez D, De Landtsheer R, De Bock P, Jacques E (1986b) Testate amoebae populations from moss and lichen habitats in the Arctic. Polar Biol 5:165-173

Beyens L, Chardez D, Van de Vijver B (2000) A contribution to the protist-diversity in the polar regions: testate amoebae data from the Russian Arctic. In: Ceulemans R, Bogaert J, Deckmyn G, Nijs I (eds) Topics in Ecology: structure and function in plants and ecosystems. Universitaire Instelling Antwerpen, Antwerp, pp 101-110

Bray JR, Curtis JT (1957) An ordination of the upland forest communities of southern Wisconsin. Ecol Monogr 27:325-349

Colwell RK, Mao CX, Chang J (2004) Interpolating, extrapolating, and comparing incidence-based species accumulation curves. Ecology 85:2717-2727

Coulson S et al. (2014) The terrestrial and freshwater invertebrate biodiversity of the archipelagoes of the Barents Sea; Svalbard, Franz Josef Land and Novaya Zemlya. Soil Biol Biochem 68:440-470

De Wit R, Bouvier T (2006) ‘Everything is everywhere, but, the environment selects’; what did Baas Becking and Beijerinck really say? Environm Microbiol 8:755-758

Flint M, Zatsepin A, Kucheruk N, Poyarkov S, Rimskii-Korsakov N (2008) Multidisciplinary studies of the ecosystem of the Kara Sea: Cruise 54 of R/V Akademik Mstislav Keldysh. Oceanology 48:883-887

Forman S, Lubinski D, Ingólfsson Ó, Zeeberg J, Snyder J, Siegert M, Matishov G (2004) A review of postglacial emergence on Svalbard, Franz Josef Land and Novaya Zemlya, northern Eurasia. Quat Sci Rev 23:1391-1434

Forman SL, Lubinski DJ, Zeeberg JJ, Polyak L, Miller GH, Matishov G, Tarasov G (1999) Postglacial emergence and late Quaternary glaciation on northern Novaya Zemlya, Arctic Russia. Boreas 28:133-145

Fournier B, Malysheva E, Mazei Y, Moretti M, Mitchell EAD (2012) Toward the use of testate amoeba functional traits as indicator of floodplain restoration success. Eur J Soil Biol 49:85-91

Hammer Ø, Harper D, Ryan P (2001) PAST-palaeontological statistics, ver. 1.89. Palaeontologia Electronica 4

Heger T et al. (2011) Rediscovery of *Nebela ansata* (Amoebozoa: Arcellinida) in eastern North America: biogeographical implications. J Biogeogr 38:1897–1906

Hodkinson ID et al. (2013) Terrestrial and Freshwater Invertebrates. In: Meltoft, H (ed) Arctic Biodiversity Assessment. Status and Trends in Arctic Biodiversity. CAFF, pp 195-233

Jiang J-G (2006) Development of a new biotic index to assess freshwater pollution. Environm Poll 139:306-317

Kosakyan A, Heger TJ, Leander BS, Todorov M, Mitchell EAD, Lara E (2012) COI barcoding of nebelid testate amoebae (Amoebozoa: Arcellinida): extensive cryptic diversity and redefinition of the Hyalospheniidae schultze. Protist 163:415-434

Lara E, Roussel-Delif L, Fournier B, Wilkinson DM, Mitchell EA (2015) Soil microorganisms behave like macroscopic organisms: patterns in the global distribution of soil euglyphid testate amoebae. J Biogeogr 43:520–532

Makkaveev P, Polukhin A, Khlebopashev P (2013) The surface runoff of nutrients from the coasts of Blagopoluchiya bay of the Novaya Zemlya Archipelago. Oceanology 53:539-546

Martiny JBH et al. (2006) Microbial biogeography: putting microorganisms on the map. Nature Rev Microbiol 4:102-112

Mazei Y, Chernyshov V, Tsyganov AN, Payne RJ (2015) Testing the effect of refrigerated storage on testate amoeba samples. Microb Ecol 70:861-864

Mazei Y, Tsyganov AN (2006) Freshwater testate amoebae. KMK: Moscow

Mazei YA, Chernyshov V (2011) Testate amoebae communities in the southern tundra and forest-tundra of Western Siberia. Biol Bull 38:789-796

Mitchell EAD, Charman DJ, Warner BG (2008) Testate amoebae analysis in ecological and paleoecological studies of wetlands: past, present and future. Biodivers Conserv 17:2115-2137

NOAA (2017) Malye Karmakuly climate data. ftp://ftp.atdd.noaa.gov/pub/GCOS/WMO-Normals/TABLES/REG_II/RA/20744.TXT. NOAA. Accessed September 2017

Payne RJ (2013) Seven reasons why protists make useful bioindicators. Acta Protozool 52:105-113

Payne RJ, Mitchell EAD (2009) How many is enough? Determining optimal count totals for ecological and palaeoecological studies of testate amoebae. J Paleolimnol 42:483-495

Smith HG, Bobrov A, Lara E (2008) Diversity and biogeography of testate amoebae. Biodivers Conserv 17:329-343

Stepanova S, Nedospasov A (2017) Features of hydrophysical and hydrochemical conditions in Blagopoluchiya Bay (Novaya Zemlya Archipelago). Oceanology 57:65-74

Tikhonenkov DV, Mazei YA (2006) Heterotrophic flagellates from freshwater biotopes of Matveev and Dolgii Islands (the Pechora Sea). Protistology 4:327-337

Trappeniers K, van Kerckvoorde A, Chardez D, Nijs I, Beyens L (1999) Ecology of testate amoebae communities from aquatic habitats in the Zackenberg area (Northeast Greenland). Polar Biol 22:271-278

Udalov A, Vedenin A, Simakov M (2016) Benthic fauna of Blagopoluchiya Bay (Novaya Zemlya Archipelago, Kara Sea). Oceanology 56:655-665

van Bellen S et al. (2017) An alternative approach to transfer functions? Testing the performance of a functional trait-based model for testate amoebae. Palaeogeogr Palaeoclimatol Palaeoecol 468:173-183

Zeeberg J (2002) Climate and glacial history of the Novaya Zemlya Archipelago, Russian Arctic: with notes on the Region's history of exploration. Rozenberg, Amsterdam

FIGURE CAPTIONS

Fig. 1 Location map of sites sampled for testate amoebae in this study.

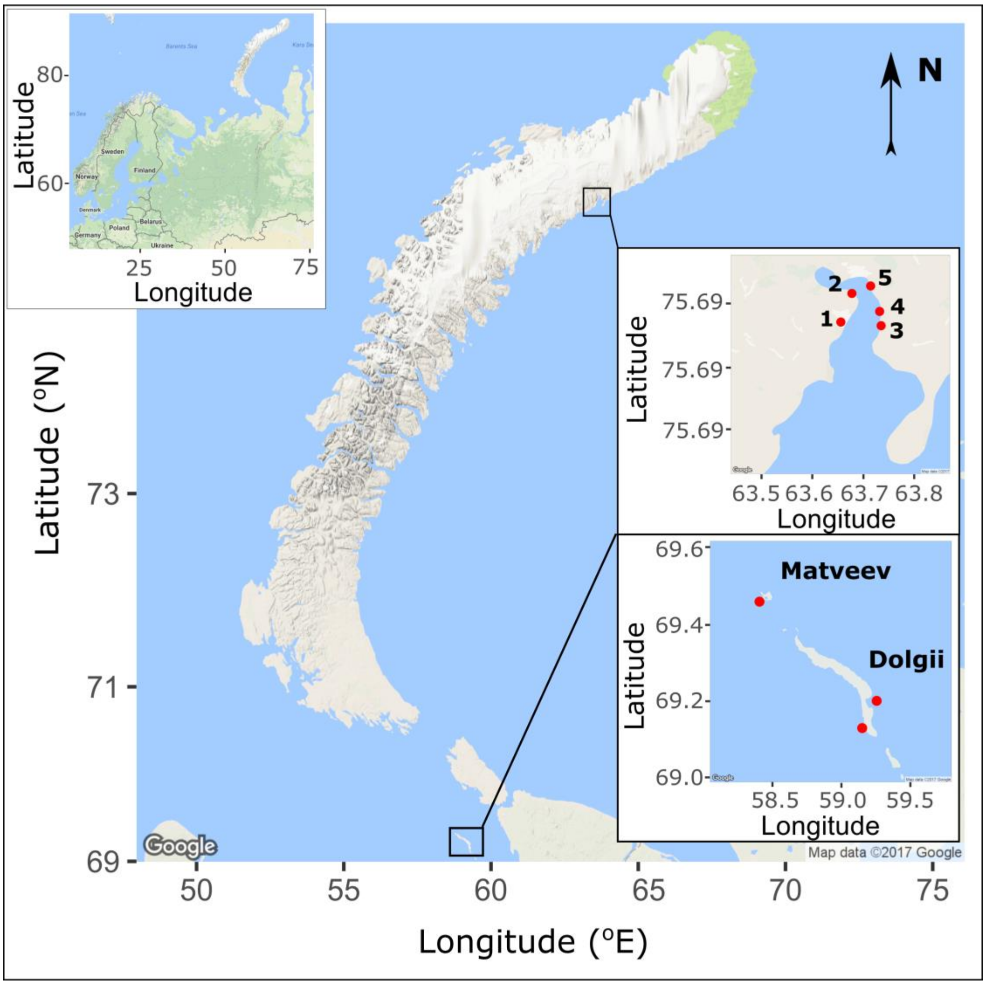

Fig. 2. Boxplots showing diversity of testate amoeba assemblages from the three islands: A) shows absolute taxon richness and B) shows Shannon H' diversity index. Different letters above bars denote significant differences in Mann-Whitney pairwise comparisons.

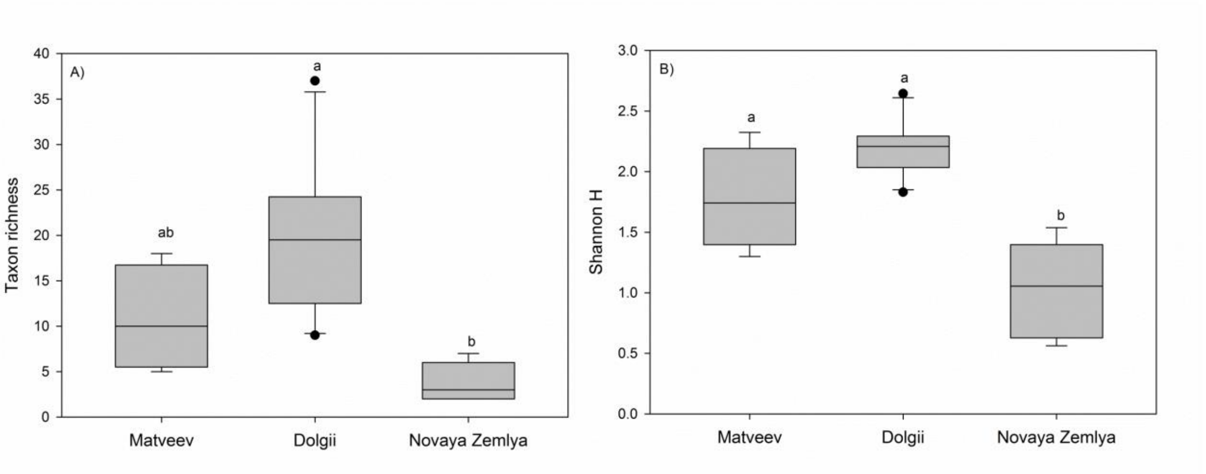

Fig. 3. Individual sample rarefaction curves of testate amoeba composition.

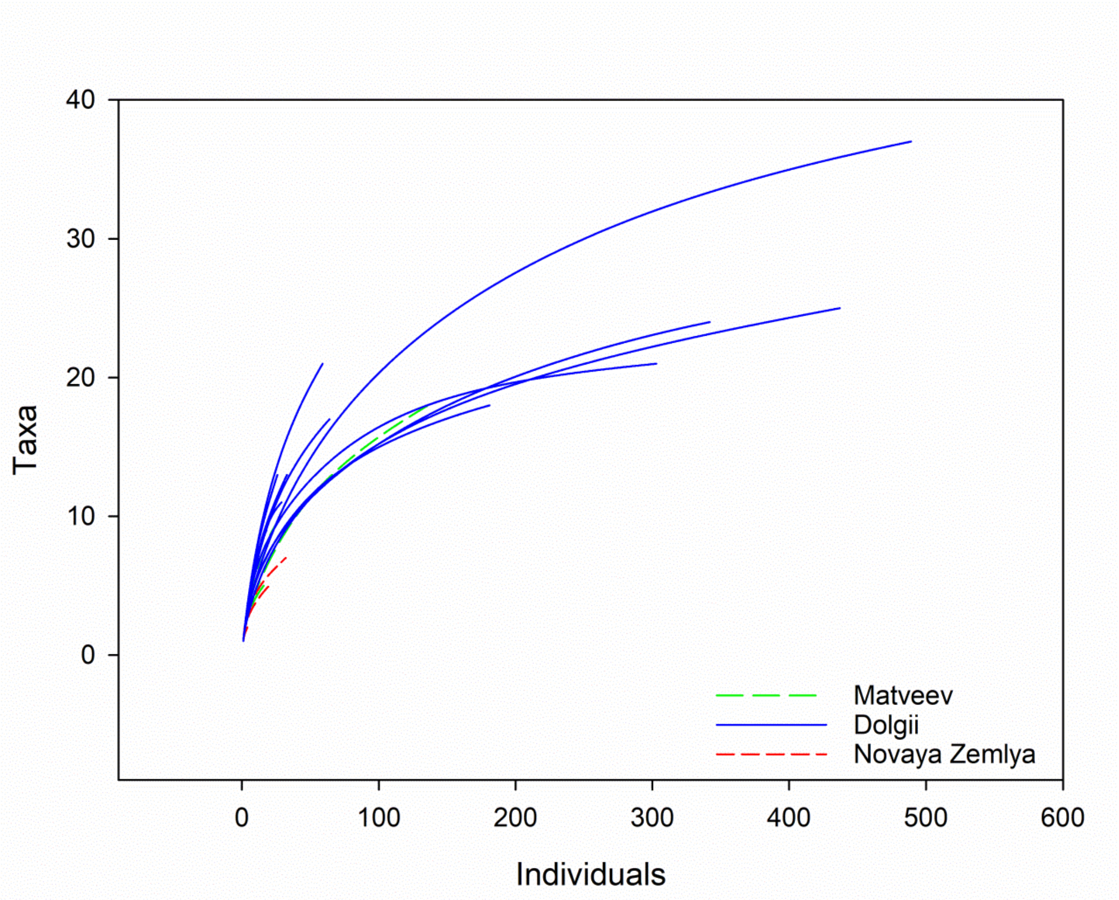

Fig. 4. Overall sample rarefaction curve for entire dataset based on Mao’s Tau showing standard errors (dotted lines).

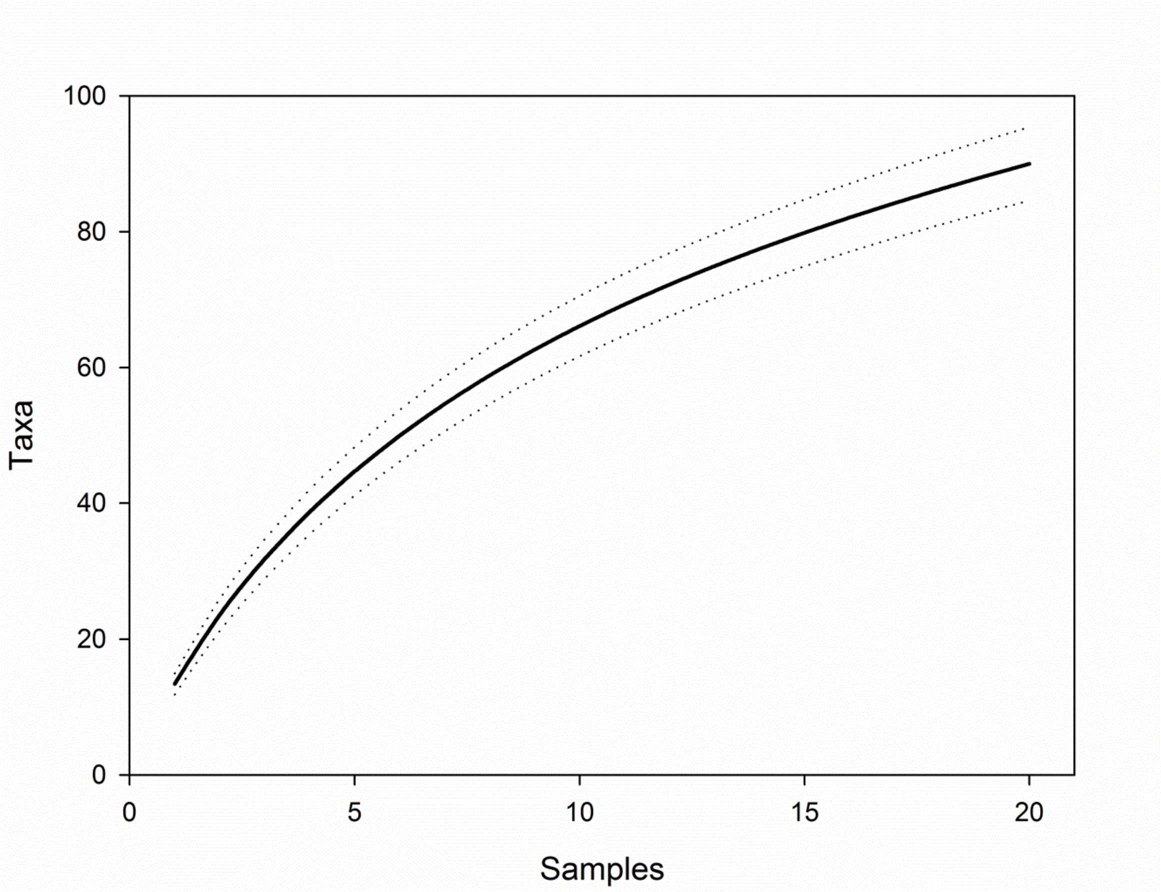

Fig. 5. Non-metric multi-dimensional scaling ordination on Bray-Curtis dissimilarity of testate amoeba relative abundance data from all three islands.

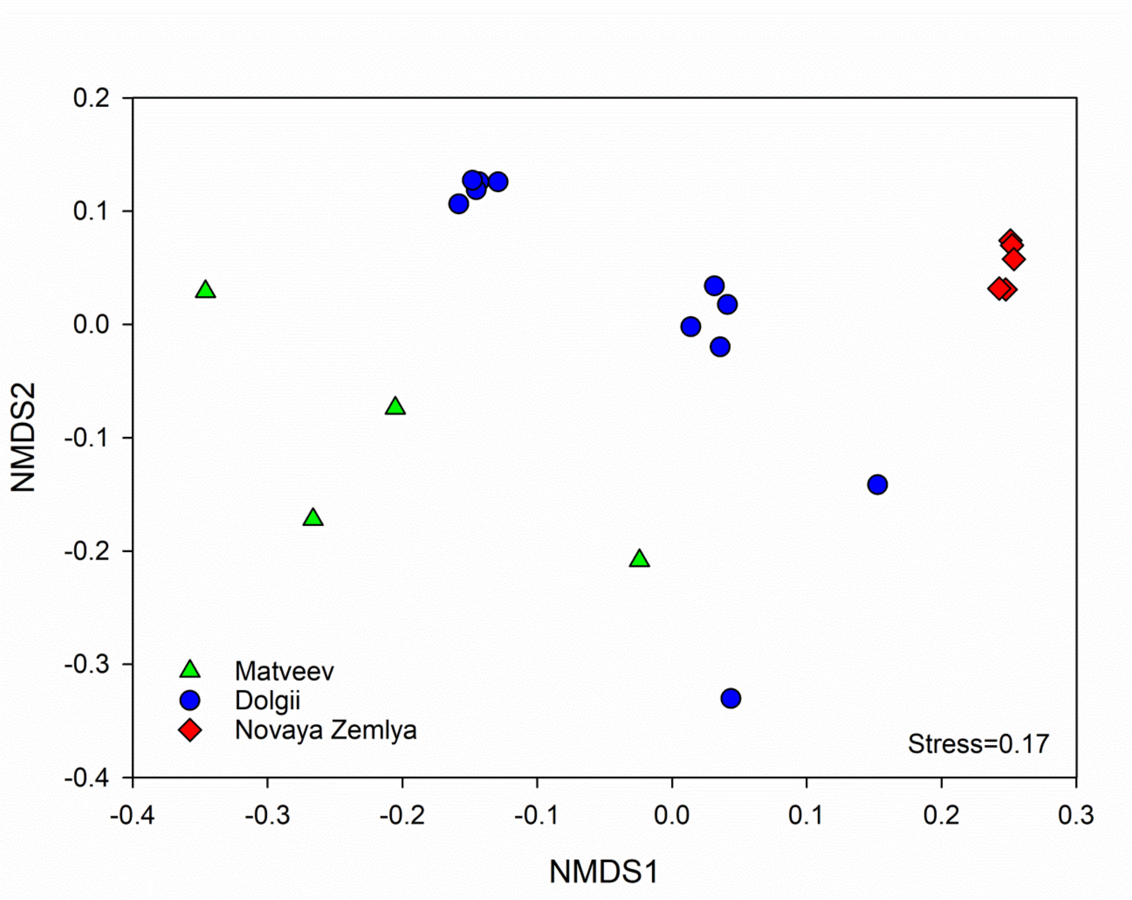

Fig. 6. Cluster analysis dendrogram of testate amoeba relative abundance data for all samples. Analysis based on Ward's method clustering.

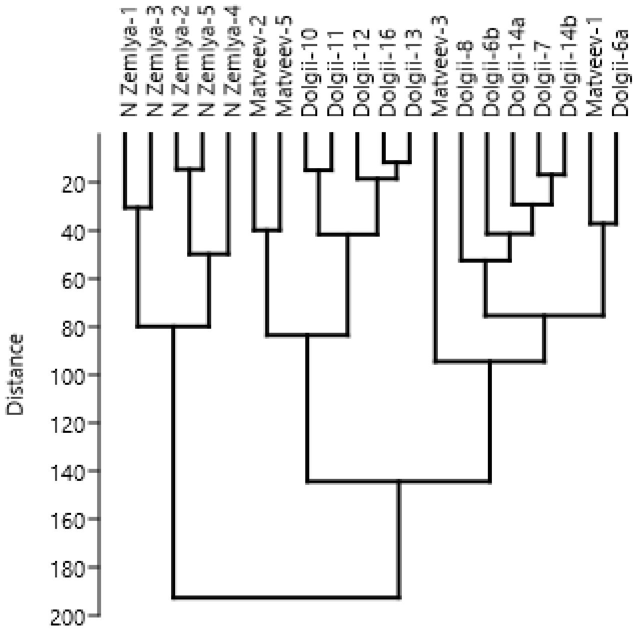

Table 1. Details of samples analysed for testate amoebae in this study.

Island	**Sampling date**	**Habitat**	**Substrate**
Novaya Zemlya 1	15/09/2007	Bare rock and gravel	Cushion stonecrops (Crassulaceae)
Novaya Zemlya 2	15/09/2007	Bare rock and gravel	Cushion bryophytes
Novaya Zemlya 3	15/09/2007	Bare rock and gravel	Cushion stonecrops (Crassulaceae)
Novaya Zemlya 4	15/09/2007	Bare rock and gravel	Cushion stonecrops (Crassulaceae)
Novaya Zemlya 5	15/09/2007	Bare rock and gravel	Cushion bryophytes
Matveev 1	12/09/2003	Lake	Sandy sediments
Matveev 2	12/09/2003	Small lake in tundra	Moss
Matveev 3	12/09/2003	Lake	Silty sediment
Matveev 5	12/09/2003	Lake	Moss
Dolgii 6a	15/09/2003	Lake	Organic detritus
Dolgii 6b	15/09/2003	Lake	Organic detritus
Dolgii 7	15/09/2003	Lake	Moss
Dolgii 8	15/09/2003	Lake	Sandy sediments
Dolgii 10	15/09/2003	Tundra	Moss
Dolgii 16	15/09/2003	Tundra	Moss
Dolgii 11	18/09/2003	Tundra	Moss
Dolgii 12	18/09/2003	Lake	Moss
Dolgii 13	18/09/2003	Lake	Moss
Dolgii 14a	18/09/2003	Lake	Organic detritus
Dolgii 14b	18/09/2003	Lake	Organic detritus

Table 2. Testate amoeba taxa identified and their relative abundance across all samples from each studied island in the archipelago (rounded to one decimal place).

Taxon	**Relative abundance (%)**		
	Novaya Zemlya	Matveev	Dolgii
Arcella arenaria Greeff, 1866	0.6	0.9	0.3
Arcella arenaria compressa Chardez, 1974	0.0	0.0	0.4
Arcella arenaria sphagnicolla Deflandre, 1928	0.0	0.0	1.0
Arcella catinus Penard, 1890	0.0	0.0	1.5
Arcella conica (Playfair, 1918) Deflandre, 1928	0.0	0.0	0.0
Arcella discoides Ehrenberg, 1843	0.0	0.0	0.1
Arcella discoides scutelliformis Playfair, 1918	0.0	0.0	0.1
Arcella hemisphaerica Playfair, 1918	0.0	7.0	0.3
Arcella rotundata Playfair, 1918	0.0	12.9	0.5
Arcella rotundata alta Playfair, 1918	0.0	0.0	0.3
Archerella flavum Archer, 1877	0.0	1.7	2.6
Argynnia dentistoma Penard, 1890	0.0	0.2	0.4
Argynnia vitraea Penard, 1899	0.0	0.0	0.1
Assulina collaris Kufferath, 1932	0.0	0.0	0.2
Assulina seminulum Greeff, 1888	0.0	0.2	0.0
Centropyxis aculeata (Ehrenberg, 1838) Stein, 1857	0.0	1.7	0.1
Centropyxis aculeata oblonga Deflandre, 1929	0.0	0.0	1.8
Centropyxis aerophila Deflandre, 1929	48.3	0.9	11.2
Centropyxis aerophila sphagnicola Deflandre, 1929	13.0	0.0	0.5
Centropyxis cassis (Wallich, 1864) Deflandre, 1929	0.0	0.0	0.6
Centropyxis ecornis(Ehrenberg, 1838) Leidy, 1879	2.6	0.0	3.0
Centropyxis elongata (Penard, 1890) Thomas, 1959	0.0	0.0	2.1
Centropyxis gibba Deflandre, 1929	0.0	0.2	0.0
Centropyxis oblonga Deflandre, 1929	0.0	0.0	0.1
Centropyxis orbicularis Deflandre, 1929	0.0	0.0	0.7
Centropyxis platystoma (Penard, 1890) Deflandre, 1929	0.0	2.6	2.6
Centropyxis platystoma armata Deflandre, 1929	0.0	0.0	0.2

Centropyxis sylvatica (Deflandre, 1929) Bonnet et Thomas, 1955	14.5	0.0	0.0
Corythion dubium Taránek, 1881	0.0	0.0	0.1
Corythion orbicularis (Penard, 1910) Iudina, 1996	0.0	0.0	0.0
Cryptodifflugia crenulata globosa Playfair, 1917	0.0	0.0	0.2
Cyclopyxis arcelloides (Penard, 1902) Deflandre, 1929	0.0	0.0	0.3
Cyclopyxis eurystoma Deflandre, 1929	4.0	0.0	0.0
Cyclopyxis kahli Deflandre, 1929	12.9	0.0	0.0
Cyphoderia ampulla (Ehrenberg, 1840) Leidy, 1879	0.0	1.4	0.0
Cyphoderia perlucidus Beyens et Chardez, 1986	0.0	0.0	2.9
Cyphoderia trochus Penard, 1899	0.0	0.0	0.3
Cyphoderia ventricosa Chardez, 1991	0.0	0.0	0.4
Difflugia amphoralis Cash et Hopkinson, 1909	0.0	0.9	0.7
Difflugia ampululla Palyfair, 1918	0.0	0.0	1.2
Difflugia bacillifera Penard, 1890	0.0	0.9	0.4
Difflugia brevicolla Cash et Hopkinson, 1909	0.0	0.0	1.4
Difflugia geoshpaerica Ogden, 1991	0.0	6.1	3.0
Difflugia globulosa Dujardin, 1837	0.0	3.6	2.1
Difflugia lucida Penard, 1890	0.0	0.0	0.1
Difflugia minuta Rampi, 1950	0.0	1.7	1.5
Difflugia oblonga Ehrenberg, 1838	0.0	0.0	0.1
Difflugia ovalisina Beyens et Chardez, 1957	0.0	1.4	2.4
Difflugia penardi Hopkison, 1909	0.0	0.0	0.1
Difflugia petricola Cash, 1909	0.0	0.0	0.1
Euglypha acanthophora Bonnet, 1960	0.0	0.0	0.0
Euglypha capsiosa Coûteax, 1978	0.0	0.2	0.1
Euglypha ciliata (Ehrenberg, 1848) Leidy, 1878	0.0	0.0	0.1
Euglypha compressa Carter, 1864	0.0	0.0	0.2
Euglypha cristata Leidy, 1879	0.0	0.0	0.1
Euglypha cristata decora Jung, 1942	0.0	0.0	0.7
Euglypha denticulata Brown, 1912	0.0	0.0	0.1
Euglypha laevis (Ehrenberg, 1832) Perty, 1849	0.0	14.0	9.6

Euglypha scutigera Penard, 1911	0.0	0.0	0.2
Euglypha simplex Decloitre, 1965	0.0	0.0	0.9
Euglypha strigosa (Ehrenberg, 1871) Leidy, 1878	0.0	0.0	0.3
Euglypha strigosa glabra Wailes, 1898	0.0	0.0	0.3
Euglypha tuberculata Dujardin, 1841	0.0	18.0	2.2
Heleopera sphagni Leidy, 1874	0.0	0.0	0.3
Lesguereusia epistomium Penard, 1893	0.0	0.0	0.2
Lesguereusia spiralis (Ehrenberg, 1840) Bütschli, 1888	0.0	0.0	0.1
Nebela bohemica Taránek, 1882	0.0	0.0	0.3
Nebela collaris (Ehrenberg, 1848) Leidy, 1879	0.0	0.0	0.5
Nebela galeata Penard, 1902	0.0	0.0	0.4
Nebela galeata orbicularis Deflandre, 1936	0.0	0.0	0.0
Nebela gracilis Penard, 1910	0.0	0.0	0.1
Nebela militaris Penard, 1890	0.0	0.0	0.1
Nebela tincta (Leidy, 1879) Awerintzev, 1906	0.0	1.4	0.3
Oopyxis cophostoma Jung, 1942	0.0	0.0	0.5
Padaungiella lageniformis (Penard, 1902) Lara et Todorov, 2012	0.0	0.0	0.1
Padaungiella wailesi (Deflandre, 1936) Lara et Todorov, 2012	0.0	0.0	0.5
Paragudrula irregularis (Archer, 1877) Deflandre, 1932	0.0	7.3	0.0
Paulinella chromatophora Lauterborn, 1895	0.0	0.0	1.2
Phryganella acropodia (Hertwig et Lesser, 1874) Hopkinson, 1909	2.5	0.0	0.6
Phryganella hemisphaerica Penard, 1902	0.0	9.5	4.0
Physochilla tenella Penard, 1893	0.0	0.2	0.0
Plagiopyxis intermedia Bonnet, 1959	1.6	0.0	0.0
Sphenoderia fissirostris Penard, 1980	0.0	0.2	0.0
Sphenoderia macrolepis Leidy, 1879	0.0	0.0	0.2
Tracheleuglypha dentata Deflandre, 1928	0.0	0.6	0.2
Trinema complanatum Penard, 1890	0.0	0.0	1.6

Trinema enchelys (Ehrenberg, 1838) Leidy, 1878	0.0	0.4	18.5
Trinema grandis (Chardez, 1960) Golemansky, 1963	0.0	4.2	0.1
Trinema lineare Penard, 1890	0.0	0.0	7.4
Trinema penardi Thomas et Chardez, 1958	0.0	0.0	0.1