

This is a repository copy of *First records of testate amoebae from the Novaya Zemlya archipelago (Russian Arctic)*.

White Rose Research Online URL for this paper:
<http://eprints.whiterose.ac.uk/127196/>

Version: Accepted Version

Article:

Mazei, Yuri, Tsyganov, Andrey N, Chernyshov, Viktor et al. (2 more authors) (2018) First records of testate amoebae from the Novaya Zemlya archipelago (Russian Arctic). *Polar Biology*. ISSN 0722-4060

<https://doi.org/10.1007/s00300-018-2273-x>

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

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

2 Yuri A. Mazei^{1,2}, Andrey N. Tsyganov¹, Viktor A. Chernyshov¹, Alexander A. Ivanovsky², Richard J.
3 Payne^{1,3*}

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

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

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

7

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

9

10 **ABSTRACT**

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

25 **KEYWORDS:** Protist; Biogeography; Arctic; Protozoa

26

27 INTRODUCTION

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

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

55 *Study region*

56 Novaya Zemlya is an archipelago in the Russian Arctic (Archangelsk Oblast) between the Barents and
57 Kara Seas (74°N 56°E). The archipelago consists of the two islands of Novaya Zemlya proper
58 separated by the very narrow Matochkin Strait and further small islands to the south (Fig. 1). The

59 two main islands of Novaya Zemlya form a mountainous arc almost 900 km in length, an extension
60 of the Ural Mountain chain separating Europe and Asia (Zeeberg 2002). Approximately 27% of the
61 land area is glaciated, particularly towards the north of the archipelago, and much of the un-
62 glaciated land area is unvegetated with areas of tundra towards the south. South of Novaya Zemlya
63 proper are several smaller islands. Dolgii (also transliterated as Dolgy and Dolgiy) Island is a 38km
64 long island in the Pechora Sea between mainland Russia and Novaya Zemlya. The island has a
65 notably flat topography (maximum elevation 18m) with abundant brackish and freshwater lakes. The
66 vegetation is predominantly tundra and coastal wetlands. Matveev (also transliterated as Matveyev)
67 Island is a similar, smaller island of only 2km diameter, 10km to the north of Dolgii Island. Both
68 islands do not presently have permanent human settlements, although both have been occupied in
69 the past.

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

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

91 MATERIALS AND METHODS

92 *Fieldwork*

93 Fieldwork was conducted on Novaya Zemlya in 2007 and Dolgii and Matveev Islands in 2003. Due to
94 the extreme inaccessibility of these regions the choice of sampling sites was logistically constrained.
95 In Novaya Zemlya sampling was conducted in the region of Blagopoluchiya Bay (Fig. 1) on the
96 eastern shore of the northern (Severny) Island (75.7°N, 63.7°E). This work was conducted as part of a
97 research cruise of the R/V Akademik Mstislav Keldysh, detailed in Flint et al. (2008). This location has
98 very little vegetation. Inland, the Severny Ice Cap lies no more than 10km from the coast with a
99 narrow unglaciated coastal zone with a land surface largely of unconsolidated siltstone shale. We
100 extracted five samples from rare patches of bryophytes and stonecrops (Crassulaceae). From the
101 same region Makkaveev et al. (2013) have presented details of nutrient fluxes and Udalov et al.
102 (2016) and Stepanova and Nedospasov (2017) have described the coastal environment. We believe
103 this sampling site to be amongst the most northerly ever studied for testate amoebae, only
104 exceeded by sites on the archipelagos of Severnaya Zemlya, Franz Josef Land and Svalbard (Andreev
105 et al. 2008; Beyens and Bobrov 2016; Beyens et al. 1986b; Beyens et al. 2000). On Dolgii Island we
106 conducted sampling at a point on the east coast and towards the southern tip (Fig. 1). These
107 sampling regions are representative of the island as a whole with a low-lying topography with
108 extensive small lakes, hummocks of bryophytes and lichens and some areas of shrub vegetation.
109 Eleven samples were extracted of mosses, organic detritus and inorganic sediments (Table 1). On
110 Matveev Island we conducted sampling across this small island (Fig. 1). Four samples were extracted
111 from silt, sand and moss growing in small lakes (Table 1). Sampling on Dolgii and Matveev islands
112 was conducted in parallel with sampling for heterotrophic flagellates, previously described by
113 Tikhonenkov and Mazei (2006). In each sampling site we removed a sample of roughly 10 cm³ which
114 was preserved in an airtight plastic bag and refrigerated immediately (Mazei et al. 2015). Samples
115 were prepared within three months of sampling and preserved with formalin; microscopic analysis
116 was completed within six months of analysis.

117 *Laboratory work*

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

124 *Data analysis*

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

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

140 RESULTS

141 *Overall assemblage composition*

142 We identified 90 taxa (Table 2) but test counts were often low and varied between 2 and 489
143 individuals (mean=116). Several samples contained fewer tests than often considered desirable
144 (Payne and Mitchell 2009). Counts were particularly low from Novaya Zemlya (2-32 tests). The most
145 abundant taxa across all the samples were *Centropyxis aerophila* (18.4% tests), *Trinema enchelys*
146 (10.2% tests), *Euglypha laevis* (8.1% tests) and *Euglypha tuberculata* (4.8% tests). 24 taxa were
147 represented by just a single test. The diversity of the samples was variable with 2-37 taxa per sample
148 (mean=13.4) and a mean Shannon H' of 1.8 (Fig. 2). Individual rarefaction curves strongly suggest
149 that for the majority of samples the count of amoebae was insufficient to identify all taxa (Fig. 3). It
150 was only in a few samples, all from Dolgii Island, where curves showed substantial flattening
151 indicating taxon counts approaching an asymptote. For many samples, particularly those from
152 Novaya Zemlya where counts were low, curves showed a very steep trajectory with no indication of
153 flattening. The overall rarefaction curve similarly did not reach an asymptote (Fig. 4). Rate of
154 increase begins to slow, particularly above ten samples, but the gradient continues to be relatively

155 steep even at the maximum number of samples. It is clear that our results should not be viewed as
156 presenting a comprehensive inventory of the testate amoeba assemblage of these three islands.

157 *Novaya Zemlya assemblages*

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

170 *Matveev Island assemblages*

171 In the Matveev Island samples the most abundant taxa were *E. tuberculata* (18% tests), *E. laevis*
172 (14%) and *Arcella rotundata* (12.9%). Counts were considerably higher than in Novaya Zemlya but
173 still low (mean=50 tests). Taxon richness (mean=10 taxa) and Shannon H' (mean= 1.8) were also
174 higher than Novaya Zemlya. The community composition was very distinctly different from Novaya
175 Zemlya with far more taxa with tests constructed of idiosomes and secretion and many more taxa
176 with filose pseudopodia.

177 *Dolgii Island assemblages*

178 The Dolgii Island assemblage was comparatively similar to the Matveev assemblage but again
179 distinctly different from the Novaya Zemlya assemblage. On this island the testate amoeba counts
180 were the highest (mean= 181 test), as were the taxon richness (mean=19) and Shannon diversity H'
181 (mean=2.2). The most abundant taxa were *T. enchelys* (18.5%), *C. aerophila* (11.2%) and *E. laevis*
182 (9.6%). Although replication of habitats and substrates was insufficient to draw general conclusions
183 it was notable that both species diversity and test count were high in samples from mosses in both
184 lakes and tundra peatlands.

185 *Differences between islands*

186 There were clear differences between the assemblages of the three islands sampled. The NMDS
187 results (Fig. 5) highlight a close grouping of the five samples from Novaya Zemlya with high scores on
188 axis one and also relatively high scores on axis two. The NMDS also generally separates the Matveev
189 and Dolgii samples. Although these are less distinctly different, the Dolgii samples tend to have
190 higher NMDS1 scores and generally higher NMDS2 scores. Cluster analysis highlights similar
191 compositional relationships between the three islands (Fig. 6). For instance, at the third level of
192 grouping the Novaya Zemlya samples are assigned a distinct group composed of only these five
193 samples. By contrast, the Dolgii and Matveev samples are more mixed, being split amongst the two
194 other groups. Taken overall the results imply distinct differences between Novaya Zemlya and the
195 two more southerly islands and subtle differences between Matveev and Dolgii islands.
196 PERMANOVA showed the difference between all three islands to be highly statistically significant
197 (PERMANOVA $F_{6,7,4,6}=3.9$, $p=0.0001$). In pairwise tests the Novaya Zemlya assemblage is highly
198 significantly different from both the Matveev (Mann-Whitney pairwise $p=0.008$) and Dolgii (Mann-
199 Whitney pairwise $p=0.0004$) assemblages whereas the Dolgii and Matveev assemblages are weakly
200 significantly (Mann-Whitney pairwise $p=0.037$) different from each other.

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

214 DISCUSSION

215 Our research reveals that testate amoebae are present even in the harsh conditions of northern
216 Novaya Zemlya. The presence of live tests confirms that these assemblages are active. Further south
217 in the less extreme climates of Matveev and Dolgii islands testate amoebae are relatively diverse
218 and abundant. The assemblage composition includes many taxa which are widely known in soils,

219 lakes and wetlands across the Arctic and sub-Arctic zones. Some taxa are less-frequently recorded
220 including *Sphenoderia macrolepis*, *Oopyxis cophostoma*, *Diffflugia ovalisina* and *Paulinella*
221 *chromatophora*. It is possible that the environmental conditions of the sampling sites favoured the
222 presence of these rarer taxa but taxonomic uncertainty and limited sampling make it difficult to
223 conclude this with certainty. We did not identify any tests which were not assignable to known
224 species of testate amoebae. Across the Arctic region the most common taxa are known to be
225 *Trinema lineare*, *Assulina muscorum* and *Centropyxis aerophila* (Beyens and Bobrov 2016). While *T.*
226 *lineare* (4.1%) and *C. aerophila* (18.4%) were both abundant in our samples, surprisingly *A.*
227 *muscorum* was not found at all in any of the samples despite the extremely wide distribution of this
228 taxon and known abundance in habitats similar to those sampled here. We do not have any
229 explanation for this surprising absence which deserves future consideration. Our dataset is
230 insufficiently large to identify all taxa and more extensive sampling would be desirable to have
231 confidence in the absence of such taxa.

232 The assemblage composition of the Novaya Zemlya samples is curious for the lack of diversity in
233 testate amoeba genera and test compositions, with a dominance by *C. aerophila* and *C. sylvatica*.
234 While these taxa are both widespread in the Arctic their abundance is somewhat surprising as these
235 are amongst the larger testate amoeba taxa commonly identified. Indeed, the assemblage also
236 included three tests of the very large (mean length c.200µm) taxon *Centropyxis ecornis*. This
237 contrasts with a general finding that taxa with small tests predominate in moisture-limited
238 environments. For instance, studies of testate amoebae in hot desert soil crusts have found a
239 predominance of small taxa (e.g. *Cryptodiffflugia*, *Euglypha*) (Bamforth 2004; Bamforth 2008) and
240 mean biovolume has been considered to correlate positively with moisture supply (Fournier et al.
241 2012; Van Bellen et al. 2017). All of the taxa we identified from unconsolidated and well-drained
242 substrates in very cold conditions are also widely known from wetlands and some are even
243 considered typical of wetter conditions within these wet habitats. *C. aerophila* is known to be an
244 abundant taxon in Arctic lakes and ponds (Beyens et al. 1986a; Trappeniers et al. 1999). It may be
245 that in cold conditions body size confers advantages in moisture-limited environments that it does
246 not in warmer environments. Conceivably the reduced surface area to volume ratio enables
247 amoebae to better resist freezing. The cushion plants sampled may also have provide a microclimate
248 which is buffered from that of the wider environment with less extreme temperature and higher
249 moisture.

250 The differences between the three islands are likely to partially reflect climate and consequent
251 ecological differences. It is relatively unsurprising that the sites in Novaya Zemlya with very sparse

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

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

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

278 Based on a synthesis of published Arctic testate amoeba data Beyens and Bobrov (2016) suggest that
279 there are distinct testate amoeba assemblages associated with different Arctic regions. Novaya
280 Zemlya falls in a zone which is not adequately delimited, between a suggested Svalbard/Greenland
281 fauna to the west and a Siberian fauna to the east. The Svalbard/Greenland group is typified by a
282 lesser number of *Diffflugia* and *Arcella* taxa. If this separation is robust our results suggest that the
283 Novaya Zemlya region can probably be considered to show more commonality with the
284 Svalbard/Greenland region than with Siberian sites further east. However the presence of such

285 ecoregions for testate amoebae requires further research, ideally using molecular methods as well
286 as morphology.

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

297 CONCLUSIONS

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

308

309 ACKNOWLEDGEMENTS

310 Analysis and publication of data was supported by the Russian Science Foundation, grant 14-14-
311 00891 to YuM and Russian Foundation for Basic Research, grant 16-04-00803 to AT. UK-Russia
312 research cooperation was supported by a Royal Society International Exchange grant to RJP
313 (IE150173).

314 Compliance with Ethical Standards

315 The authors declare no conflict of interest.

317 **References**

318

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

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

324 Azovsky AI, Tikhonenkov DV, Mazei YA (2016) An estimation of the global diversity and distribution
325 of the smallest eukaryotes: Biogeography of marine benthic heterotrophic flagellates. *Protist*
326 167:411-424

327 Bamforth SS (2004) Water film fauna of microbiotic crusts of a warm desert. *J Arid Environ* 56:413-
328 423

329 Bamforth SS (2008) Protozoa of biological soil crusts of a cool desert in Utah. *J Arid Environ* 72:722-
330 729

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

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

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

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

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

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

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

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

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

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

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

356 Forman SL, Lubinski DJ, Zeeberg JJ, Polyak L, Miller GH, Matishov G, Tarasov G (1999) Postglacial
357 emergence and late Quaternary glaciation on northern Novaya Zemlya, Arctic Russia. *Boreas* 28:133-
358 145

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

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

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

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

367 Jiang J-G (2006) Development of a new biotic index to assess freshwater pollution. *Environm Poll*
368 139:306-317

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

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

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

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

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

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

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

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

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

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

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

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

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

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

397 Trappeniers K, van Kerckvoorde A, Chardez D, Nijs I, Beyens L (1999) Ecology of testate amoebae
398 communities from aquatic habitats in the Zackenberg area (Northeast Greenland). *Polar Biol* 22:271-
399 278

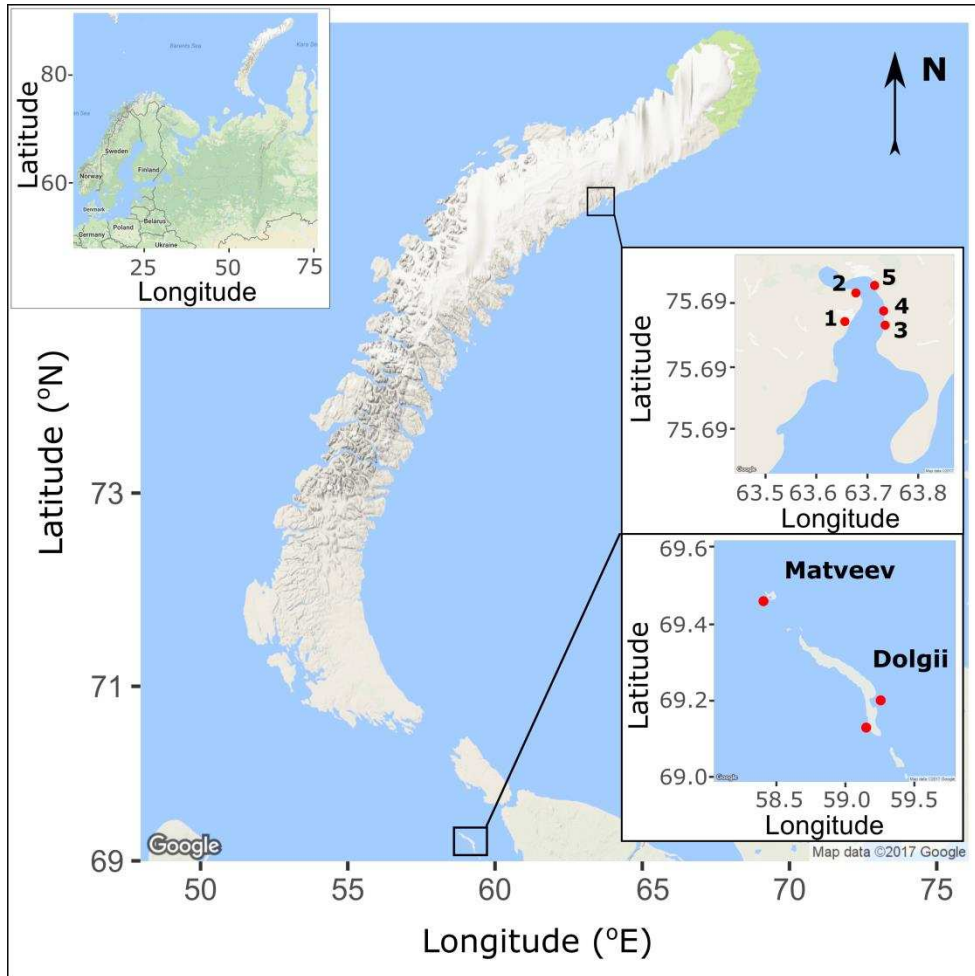
400 Udalov A, Vedenin A, Simakov M (2016) Benthic fauna of Blagopoluchiya Bay (Novaya Zemlya
401 Archipelago, Kara Sea). *Oceanology* 56:655-665

402 van Bellen S et al. (2017) An alternative approach to transfer functions? Testing the performance of
403 a functional trait-based model for testate amoebae. *Palaeogeogr Palaeoclimatol Palaeoecol*
404 468:173-183

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

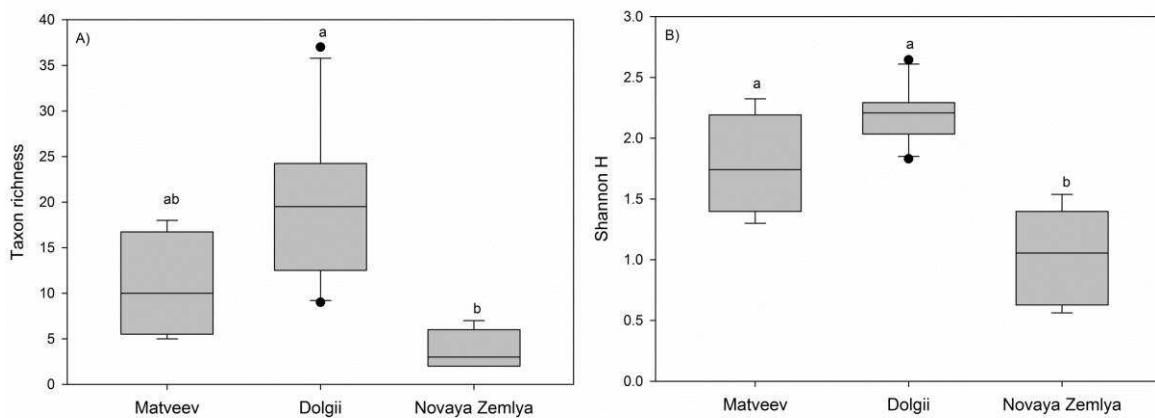
407

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



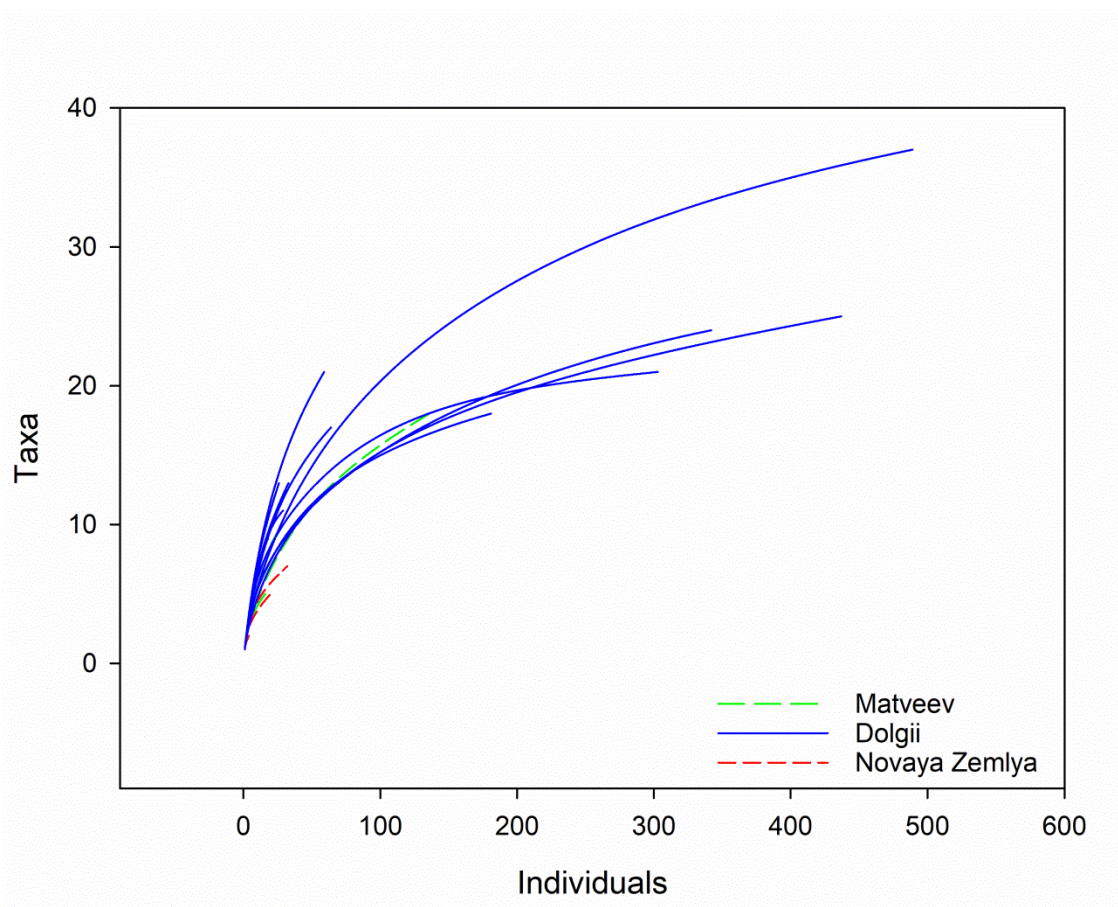
410

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



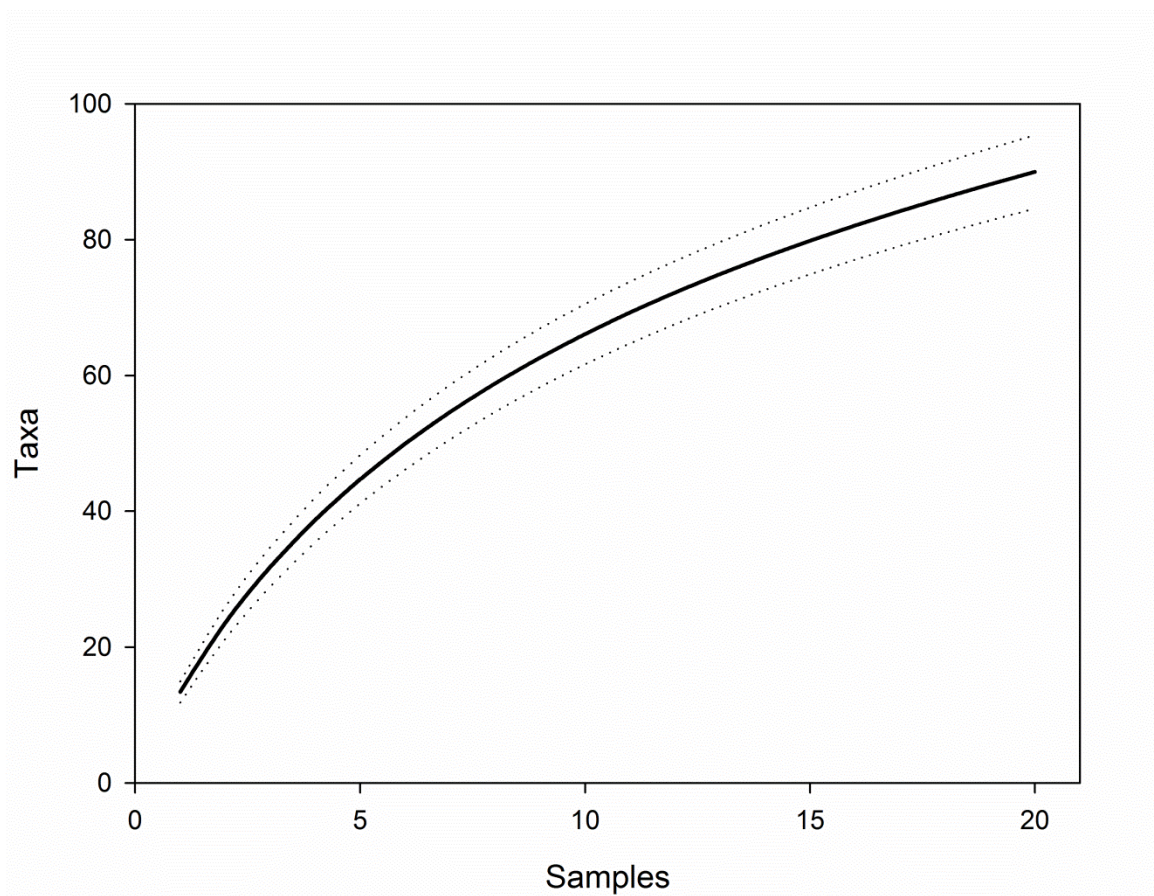
414

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



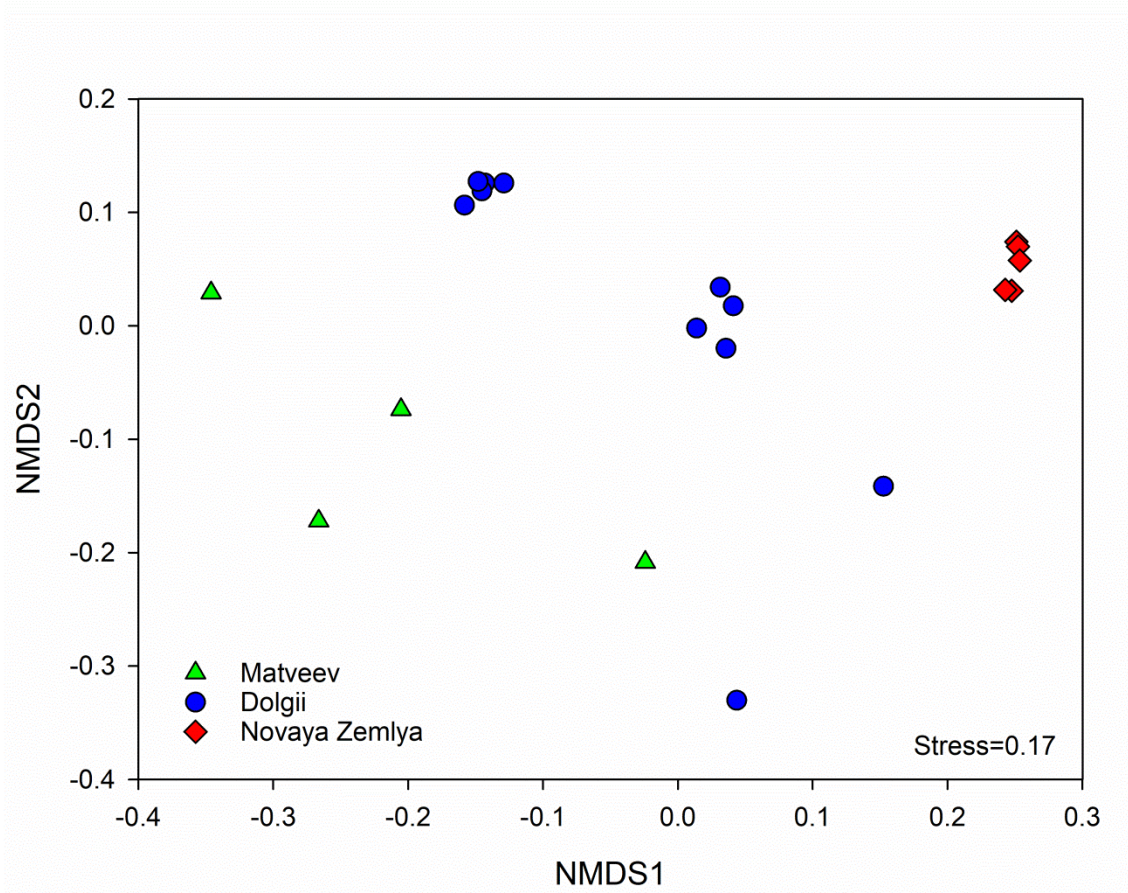
416

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



419

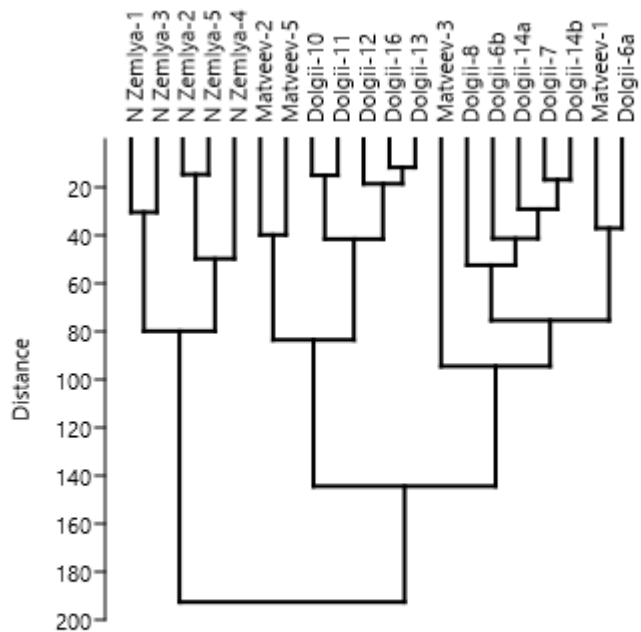
420 Fig. 5. Non-metric multi-dimensional scaling ordination on Bray-Curtis dissimilarity of testate
421 amoeba relative abundance data from all three islands.



422

423 Fig. 6. Cluster analysis dendrogram of testate amoeba relative abundance data for all samples.

424 Analysis based on Ward's method clustering.



425

426

427 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

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

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

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

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

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