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First records of contemporary testate amoeba assemblages from the Kamchatka Peninsula, Russia and potential for palaeoenvironmental reconstruction

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The Kamchatka Peninsula in the far east of Russia is a substantial landmass that is poorly documented in terms of most elements of biodiversity. Here we provide the first study of modern assemblages of testate amoebae, a widespread group of protists that are particularly abundant in soils. We present a data set of 78 widely distributed samples, including forest, fen, scrub and bog habitats. Testate amoebae are abundant and diverse across Kamchatka with 119 taxa identified. The assemblage is primarily composed of widespread taxa, but rarer occurrences such as *Cyclopyxis puteus* hint at important biogeographical differences that will require confirmation with molecular data. Assemblages from mineral soils are significantly different from those of peatlands with the former characterized by small idiosome taxa and the latter by larger taxa, often with secretion tests. Water table depth explained significant variance in the peatland samples, supporting the use of testate amoebae in palaeoecological studies. This study adds to our knowledge of the biodiversity of Kamchatka and the global biogeography of protists, and it paves the way for palaeoecological studies to understand long-term environmental change in this region.

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Testate amoebae are a polyphyletic group of protists characterized by a shell ('test'). Testate amoebae are a particularly diverse group in terms of morphology, varying in length by more than an order of magnitude, and with a wide variety of life history strategies. At least 2000 taxa are documented (Beyens & Meisterfeld 2001; Mitchell *et al.* 2008a), primarily on the basis of the morphology of the test ('morphospecies'). Molecular studies increasingly demonstrate previously unappreciated cryptic and pseudocryptic diversity (Kosakyan *et al.* 2012; Oliverio *et al.* 2014; Singer *et al.* 2018).

Testate amoebae have been identified in a wide range of terrestrial, freshwater and coastal habitats but are most frequently recorded in moist, organic-rich soils (Mitchell *et al.* 2008a). In peatlands, testate amoebae are the dominant protist group, constituting a large proportion of total microbial biomass (Gilbert *et al.* 1998). The functional roles of testate amoebae in microbial foodwebs and biogeochemical pathways are only beginning to be uncovered, but are clearly both important and responsive to environmental change (Wilkinson 2008). Testate amoebae are important consumers of bacteria and fungi, and mixotrophic testate amoebae may also make a non-trivial contribution to primary production (Jassey *et al.* 2012, 2013, 2015; Lara & Gomaa 2016). Since the 1990s, there has been a considerable increase in the volume of research on testate amoebae, from 50 publications in 1990–1995 to 352 publications in 2010–2015 (Mazei *et al.* 2017a), particularly triggered by increasing use in palaeoecological studies (Mazei *et al.* 2017a). The ecology of testate amoebae has been widely investigated in contemporary ecosystems in order to develop species–environment ('transfer function') models to quantify the palaeoecological record, with such studies undertaken in locations from Patagonia to Alaska and Britain to China (Woodland *et al.* 1998; Payne *et al.* 2006; Qin *et al.* 2013; Van Bellen *et al.* 2014, 2017). However, knowledge of testate amoeba diversity and ecology remains geographically biased, and large areas of the global land surface have yet to receive any study at all (Smith *et al.* 2008; Mazei *et al.* 2017a, 2018; Bobrov *et al.* 2019).

Beyond the potential for unrecorded taxa in these regions, such data gaps impair attempts to use testate

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amoebae to understand patterns of protist biogeography and to interpret past assemblages in the palaeoecological record. There are numerous studies on recent testate amoebae in European Russia (Mazei & Tsyganov 2007; Mazei *et al.* 2007; Mazei & Bubnova 2009) and western Siberia (Kurina *et al.* 2010; Mazei & Chernyshov 2011; Mazei *et al.* 2017a), which allowed authors to construct calibration data sets, discuss methodological issues (Kurina & Li 2019) and develop a water table depth transfer function (Tsyganov *et al.* 2017). However, testate amoeba assemblages of the remote Kamchatka Peninsula remain uninvestigated so far. The Kamchatka Peninsula is located in the far northeast of Eurasia, between the Sea of Okhotsk to the west and the North Pacific Ocean to the east (51–60°N, ~160°E). The peninsula is volcanic in origin, with around 300 documented volcanoes in two main ranges formed by the subduction of the Pacific plate: the largely inactive Sredinny Mountains to the west and the Vostochny Mountains to the east (Jones & Solomina 2015). The vegetation of the peninsula includes extensive primary and secondary forests, particularly of *Betula ermannii*, with shrubs replacing trees at higher elevations. Other open habitats include volcanic deserts, alpine habitats and meadows, particularly in areas of former farmland (Jones & Solomina 2015).

The biodiversity of Kamchatka is historically under-researched due to both general difficulty of access, and particularly to Cold War restrictions through much of the 20th century. To our knowledge, there has been no previous research on the modern testate amoeba fauna of the peninsula. The land area of Kamchatka is around 270 000 km^2 so this lack of data represents a substantial gap in global data sets. By comparison, the island of Great Britain is substantially smaller (~210 000 km^2) and has had many dozens of studies of testate amoebae from a wide variety of habitats. Even beyond the Kamchatka Peninsula itself there are very few data on testate amoeba assemblages from far northeast Eurasia, with the only published studies those of Bobrov (2001) from the Sikhote Alin Mountains, further south, and Komarov & Mazei (2010) from southern Chukotka, to the north (the latter only available in Russian).

Although Kamchatka is connected to mainland Eurasia, the peninsula narrows to an isthmus only 100 km wide at its narrowest, and Kamchatka consequently has some of the biogeographical characteristics of an island. At least 16 endemic plant species have been recorded (Jones & Solomina 2015), and endemics are documented in numerous groups, including invertebrates, fish and microorganisms (Whitaker *et al.* 2003; Smetanin 2013; Bolotov *et al.* 2014; Saltykova *et al.* 2015). The peninsula also contains extensive areas of peatland, arguably the habitat where testate amoebae are found with greatest abundance. Kamchatka therefore represents both a substantial data gap in knowledge of global testate amoeba biogeography and a region with particular intrinsic interest in terms of testate amoeba ecology. The potential to use testate amoebae in quantitative reconstructions of past environmental change is another important rationale for conducting ecological study (Payne 2013). Indeed, the only extant data on testate amoebae relate to two palaeoecological studies: Klimaschewski *et al.* (2015) from a site in western Kamchatka and Tilotta (2016) from a site in eastern Kamchatka.

The aims of this study are to (i) reveal key components of the Kamchatka testate amoeba fauna; (ii) find local or regional endemics;(iii) distinguish the key environmental controls on testate amoeba assemblage structure; and (iv) evaluate the importance of testate amoebae as reliable palaeohydrological proxies in Kamchatka peatlands.

Material and methods

Fieldwork and laboratory analysis

Two campaigns of field sampling were conducted. In summer 2005, 18 sites were sampled from across Kamchatka, including locations in the southern (Petropavlovsk vicinity), central (Esso vicinity) and northern Peninsula (Ossora vicinity), and adjacent Karaginskii Island in the Bering Sea. Samples were extracted (typically one per site) from a range of vegetation types including forests, meadows and wetlands from both peat and mineral soils (Table S1). Mineral soil samples represented 1–2 cm of litter or grass tussocks and 1 cm of humus horizon; peat soils represented the upper 3–5 cm of *Sphagnum* stems. In summer 2016, 37 samples were extracted from 16 individual peatlands in the West Kamchatka Lowland and throughout the central depression. One to four samples were extracted per site, in order to span the microtopographic gradient (Table S1, Fig. 1). In both these sampling campaigns, site selection aimed to span the range of testate amoeba habitats; however, given the remoteness of the region, the selection was also necessarily opportunistic and constrained by logistical considerations. In summer 2016, an intensive sampling campaign was also undertaken at one blanket bog site in West Kamchatka: Kiumshichek (Fig. 1). Twenty samples were extracted along N–S and E–W transects across the peatland.

In each of the modern sampling locations, a sample of surface soil or sediment was extracted, including any mosses and plant litter (Mazei *et al.* 2015). Where *Sphagnum* mosses were selected, sample size exceeded 25 capitula (Mazei *et al.* 2017b) and samples were stored refrigerated until analysis, which was conducted within 3 months (Mazei *et al.* 2015). In 16 samples from Kiumshichek and 22 samples from the rest of Kamchatka, water table depth was measured by making a hole in the peat surface and leaving the water to equilibrate for periods from 5 min to 24 h.

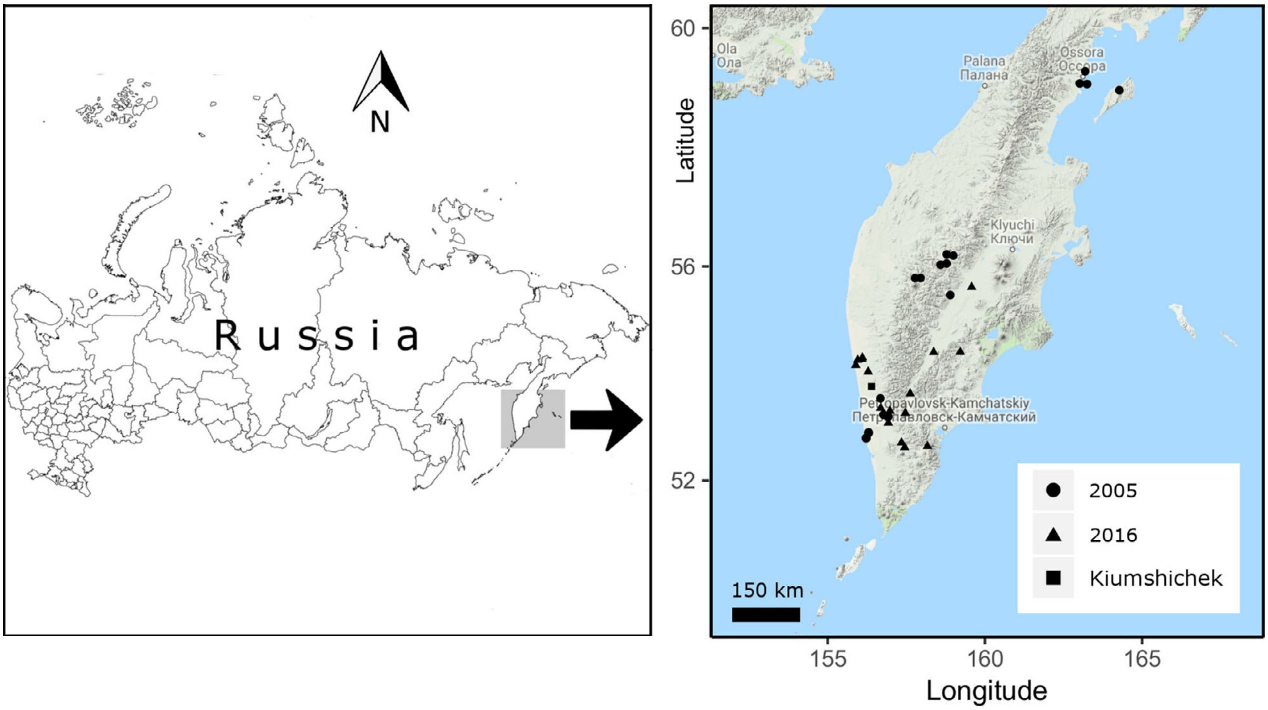

Fig. 1. Location map of Kamchatka sampling sites.

In the laboratory, samples were prepared for analysis of testate amoebae using a method based on suspension in water, physical agitation and settling (Mazei & Chernyshov 2011). Samples were mounted in glycerol and analysed at 400× magnification using taxonomic guides including Corbet (1973), Mazei & Tsyganov (2006) and Todorov & Bankov (2019). 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, which led to count totals that varied from six to 2466 tests (mean = 268), with all but 13 samples having counts greater than the 'standard minimum' of 50 tests (Payne & Mitchell 2009). Count data were collated and recorded as relative abundance.

Ecological data analysis

Our analysis of contemporary testate amoeba communities focused on assemblage variability, environmental controls and differences between peat and mineral soils. We first used non-metric multidimensional scaling (NMDS) on a Bray–Curtis (Bray & Curtis 1957) dissimilarity matrix to visualize similarities and differences between the amoeba assemblages of all four sets of samples. We then compared the 11 modern samples from mineral soils to the samples from peat soils. We tested for differences in assemblages using permutational multivariate analysis of variance (PERMANOVA, 999 permutations) based on Bray–Curtis distance using the function 'adonis' in the 'vegan' package in R (Oksanen *et al.* 2019; R Core Team 2020). In order to compare the species richness of testate amoebae in data sets with unequal counts we performed a rarefaction analysis with the function 'rarefy' in the 'vegan' package (Oksanen *et al.* 2019). In our subsequent data analysis, we focused on the larger pool of samples from peatlands. To assess the environmental determinants of testate amoeba assemblage composition, we used direct ordination to test relationships between assemblages and potentially important environmental factors. We considered four variables: water table depth (WTD), altitude, mean annual temperature and mean annual precipitation. Altitude data were obtained from local mapping or, when this was not available (seven sites), from the global Shuttle Radar Tomography Mission (SRTM) data set (Farr *et al.* 2007). Climate data were extracted from the WorldClim database of interpolated meteorological data (Hijmans *et al.* 2005). We focused on the 37 samples for which data on all four of these variables were available. Species data were Hellinger transformed (Rao 1995) and redundancy analysis (RDA) was used to test the explanatory power of each variable. Data analysis was conducted in R using packages including 'vegan' and 'rioja' (Oksanen *et al.* 2019; Juggins 2009).

Palaeoecological potential

A secondary aim of the study was to investigate the potential for testate amoebae to be used in palaeoecological studies in Kamchatka. We assessed: (i) whether there are systematic differences between modern and fossil assemblages; and (ii) how test abundance varies with depth.

We considered the testate amoeba assemblages of a core extracted from the centre of the Kiumshichek site. The core had a total length of 50 cm and was extracted using a Russian corer (Aaby & Digerfeldt 1986) with dimensions of 50 cm length and 5 cm diameter. Subsamples were removed in 0.5-cm contiguous slices through the top 20 cm and 1-cm contiguous slices throughout the rest of the core. The samples were analysed for testate amoebae as above (Mazei & Chernyshov 2011) to a depth of 45 cm to cover the target period. The radiocarbon age (^{14}C AMS) of the peat deposits at the depth of 41.5 cm was 1418±59 a BP (calibrated age at 95% confidence interval: 1187–1203 (1.4%), 1243–1246 (0.2%), 1256–1415 (91.4%), 1464–1478 (1%), and 1502–1515 cal. a BP (0.9%); calibration curve IntCal13 (Reimer *et al.* 2013), package 'clam' (Blaauw 2020)). These palaeoecological data will be discussed in full elsewhere but here are used to address modern/fossil testate amoeba assemblage comparability and variability in test preservation with depth. NMDS on a Bray–Curtis dissimilarity matrix was used to compare the test assemblages of the fossil and modern communities, differences in assemblage composition were tested using PERMANOVA (999 permutations, Bray–Curtis dissimilarity) and the variability in test counts with depth was enumerated using the function 'adonis' in the 'vegan' package in R (Oksanen *et al.* 2019).

Results

Across all samples analysed we identified 119 taxa (species and sub-species). The most abundant overall were *Cryptodifflugia oviformis* (8.9% of the total counts), *Hyalosphenia papilio* (8.9%), *Assulina muscorum* (7.8%), *Hyalosphenia elegans* (6.6%) and *Archerella jollyi* (5.9%). The data set includes a number of infrequently recorded taxa including *Arcella costata*, *Cyclopyxis puteus*, *Nebela barbata*, *Nebela barbata psilonata*, *Planocarina maxima* and *Pseudodifflugia jungi* (Table 1, Fig. 2). An ordination of the overall data set (Fig. 3) highlights differences between the component data sets, most strongly typified by lower axis 1 scores for the 2005 samples (which include mineral soils as well as peat soils) and higher scores for the palaeoecological samples. The reasons for these differences are explored further below.

Our analysis shows considerable differences between the samples from mineral soils and from peat soils. The samples are clearly separated in ordination space with mineral soil samples having lower scores on axis 1 (Fig. 4). Testing with PERMANOVA shows the differences between assemblages from mineral and peat soils to be highly significant (F = 5.11, p = 0.001). Our test is unbalanced with many more peat soils than mineral soil samples; this can affect the performance of all commonly used tests of multivariate difference with ecological data, including PERMANOVA (Anderson & Walsh 2013). However, in this case, the difference is very clear even without statistical testing. Taxa such as *Phryganella hemisphaerica*, *Trinema lineare* and *Euglypha laevis* were more abundant in mineral soils (Fig. 5). Taxa such as *Hyalosphenia papilio*, *Archerella flavum* and *Hyalosphenia elegans* were more abundant in peat soils. The total number of species in mineral soils (56 taxa) was lower than in peat soils (109 taxa basing to raw counts and 72.6 taxa if rarefied to the total counts of testate amoebae in mineral soils).

To establish the environmental controls on peatland amoeba assemblages we tested the explanatory power of four environmental variables with each of the variables as sole predictor. Mean annual temperature, mean annual precipitation and altitude did not explain significant variance in these tests ($p > 0.05$). However, water table depth was significant, explaining 5.5% of species variance with moderate significance ($p = 0.02$). Taxa including *Hyalosphenia elegans* and *Nebela tincta* were positively associated with WTD, *Cryptodifflugia oviformis* and *Trinema lineare* were negatively associated while *Archerella jollyi* and *Assulina muscorum* were orthogonal (Fig. 6).

To establish the potential for using testate amoebae for palaeoecological reconstruction in Kamchatka, we compared modern and fossil assemblages. Initial palaeoecological research at Kiumshichek shows significant differences between palaeoecological and modern assemblages. In an NMDS, palaeoecological samples had higher axis 1 scores (Fig. 7) and significantly different from modern assemblages (PERMANOVA F = 9.0, p = 0.001). The samples from the greater sampling depths are more different from the modern assemblages and located further on the right side of the ordination diagram. The total number of species in palaeo-samples (47 taxa) was lower than in surface peat (82.3 taxa if rarefied to the total counts of testate amoebae in palaeodata set). These differences may be partially associated with test decomposition. There is a strong decline in countable test numbers with depth and only samples in the top 16 cm had achievable count totals over 50 tests (Fig. 8).

Discussion

Biogeography of testate amoebae

As a group, testate amoebae have attracted particular interest because of the insights that they can provide into microbial biogeography. Testate amoebae provide one of the best demonstrations of regional endemism in protists (Smith & Wilkinson 2007; Heger *et al.* 2011), and genetic data are increasingly allowing the factors structuring distributions in space and time to be understood (Heger

Table 1. Full taxa inventory (alphabetical) identified in this study.

No.	Taxa
1	*Alabasta militaris* (Penard, 1890) Duckert *et al.*, 2018
2	*Arcella arenaria compressa* Chardez, 1974
3	*Arcella arenaria* Greeff, 1866
4	*Arcella artocrea* Leidy, 1879
5	*Arcella catinus* Penard, 1890
6	*Arcella costata* Ehrenberg, 1847
7	*Arcella discoides* Ehrenberg, 1843
8	*Arcella gibbosa* Penard, 1890
9	*Arcella rotundata* Playfair, 1918
10	*Arcella vulgaris* Ehrenberg, 1830
11	*Archerella flavum* Archer, 1877
12	*Archerella jollyi* van Oye, 1956
13	*Argynnia dentistoma* Penard, 1890
14	*Argynnia retorta* (Leidy, 1879) Štěpánek, 1953
15	*Argynnia vitraea* Penard, 1899
16	*Assulina muscorum* Greef, 1888
17	*Assulina scandinavica* Penard, 1890
18	*Assulina seminulum* (Ehrenberg, 1848) Leidy, 1879
19	*Bullinularia indica* (Penard, 1911) Deflandre, 1953
20	*Centropyxis aculeata* (Ehrenberg, 1838) Stein, 1857
21	*Centropyxis aerophila* Deflandre, 1929
22	*Centropyxis aerophila sphagnicola* Deflandre, 1929
23	*Centropyxis constricta* (Ehrenberg, 1841) Deflandre, 1929
24	*Centropyxis discoides* (Penard, 1890) Deflandre, 1929
25	*Centropyxis ecornis* (Ehrenberg, 1841) Leidy, 1879
26	*Centropyxis elongata* (Penard, 1890) Thomas, 1959
27	*Centropyxis gibba* Deflandre, 1929
28	*Centropyxis orbicularis* Deflandre, 1929
29	*Centropyxis plagiostoma* Bonnet et Thomas, 1955
30	*Centropyxis sylvatica* (Deflandre, 1929) Bonnet et Thomas, 1955
31	*Centropyxis sylvatica minor* Bonnet et Thomas, 1955
32	*Corythion dubium* Taránek, 1881
33	*Corythion orbicularis* (Penard, 1910) Iudina, 1996
34	*Cryptodifflugia oviformis* Penard, 1890
35	*Cryptodifflugia pusilla* Playfair, 1917
36	*Cryptodifflugia sacculus* Penard, 1902
37	*Cyclopyxis aplanata microstoma* Schönborn, 1966
38	*Cyclopyxis arcelloides* (Penard, 1902) Deflandre, 1929
39	*Cyclopyxis eurystoma* Deflandre, 1929
40	*Cyclopyxis kahli* Deflandre, 1929
41	*Cyclopyxis puteus* Thomas, 1960
42	*Cyphoderia ampulla* (Ehrenberg, 1840) Leidy, 1879
43	*Difflugia bacillariarum* Perty, 1849
44	*Difflugia bacillifera* Penard, 1890
45	*Difflugia globulosa* Dujardin, 1837
46	*Difflugia humilis* Chardez, 1991
47	*Difflugia lucida* Penard, 1890
48	*Difflugia oblonga* Ehrenberg, 1838
49	*Difflugia pristis* Penard, 1902
50	*Euglypha anadonta* Bonnet, 1960
51	*Euglypha bryophila* Brown, 1911
52	*Euglypha ciliata* (Ehrenberg, 1848) Leidy, 1878
53	*Euglypha ciliata glabra* Wailes, 1915
54	*Euglypha compressa* Carter, 1864
55	*Euglypha compressa glabra* Wailes, 1915
56	*Euglypha cristata decora* Jung 1942
57	*Euglypha cristata* Leidy, 1879
58	*Euglypha cuspidata* Bonnet, 1959
59	*Euglypha filifera* Penard, 1890
60	*Euglypha laevis* (Ehrenberg, 1832) Perty, 1849
61	*Euglypha simplex* Decloitre, 1965
62	*Euglypha strigosa* (Ehrenberg, 1871) Leidy, 1878
63	*Euglypha tuberculata* Dujardin, 1841
64	*Euglypha rotunda* Wailes, 1915
65	*Gibbocarina galeata* (Penard, 1902) Kosakyan *et al.*, 2016
66	*Gibbocarina gracilis* (Penard, 1910) Kosakyan *et al.*, 2016
67	*Heleopera petricola amethystea* Penard, 1902
68	*Heleopera petricola* Leidy, 1879
69	*Heleopera rosea* Penard, 1890
70	*Heleopera sphagni* Leidy, 1874
71	*Heleopera sylvatica* Penard, 1890
72	*Hyalosphenia elegans* Leidy, 1879
73	*Hyalosphenia papilio* Leidy, 1879
74	*Hyalosphenia subflava* Cash, 1909
75	*Lagenodifflugia bryophila* (Penard, 1902) Ogden, 1987
76	*Lagenodifflugia va*s (Leidy, 1874) Medioli et Scott, 1983
77	*Longinebela tubulosa* (Penard, 1902) Kosakyan *et al.*, 2016
78	*Nebela barbata* Leidy, 1874
79	*Nebela barbata psilonata* Jung 1942
80	*Nebela collaris* (Ehrenberg, 1848) Leidy, 1879
81	*Nebela longicollis* Penard, 1890
82	*Nebela tincta* (Leidy, 1879) Awerintzew, 1906
83	*Padaungiella lageniformis* (Penard, 1902) Lara et Todorov, 2012
84	*Padaungiella tubulata* (Brown, 1911) Lara et Todorov, 2012
85	*Padaungiella wailesi* (Deflandre, 1936) Lara et Todorov, 2012
86	*Phryganella acropodia* (Hertwig et Lesser, 1874) Hopkinson, 1909
87	*Phryganella hemisphaerica* Penard, 1902
88	*Physochilla cratera* Wailes, 1912
89	*Physochilla griseola* Penard, 1911
90	*Placocista glabr*a Penard, 1905
91	*Placocista jurassica* Penard, 1905
92	*Placocista lens* Penard, 1899
93	*Placocista spinosa* (Carter, 1865) Leidy, 1879
94	*Plagiopyxis callida* Penard, 1910
95	*Plagiopyxis callida grandis* Thomas, 1958
96	*Plagiopyxis declivis* Thomas, 1958
97	*Plagiopyxis penardi* Thomas, 1958
98	*Planocarina carinata* (Archer, 1867) Kosakyan *et al.*, 2016
99	*Planocarina marginata* (Penard, 1902) Kosakyan *et al.*, 2016
100	*Planocarina maxima* (Awerintzew, 1907) Kosakyan *et al.*, 2016
101	*Pseudodifflugia gracilis terricola* Schlumberger, 1845
102	*Pseudodifflugia jungi* van Oye, 1944
103	*Quadrulella symmetrica* (Wallich, 1863) Schulze, 1875
104	*Schoenbornia humicola* Schönborn, 1964
105	*Sphenoderia fissirostris* Penard, 1890
106	*Sphenoderia lenta* Schlumberger, 1845
107	*Tracheleuglypha dentata* Deflandre, 1938
108	*Trachelocorythion pulchellum* (Penard, 1890) Bonnet, 1979
109	*Trigonopyxis arcula* (Leidy, 1879) Penard, 1912
110	*Trigonopyxis minuta* Schönborn et Peschke, 1988
111	*Trinema complanatum* Penard, 1890
112	*Trinema enchelys* (Ehrenberg, 1838) Leidy, 1878
113	*Trinema leidyi* Chardez, 1981
114	*Trinema lineare* Penard, 1890
115	*Trinema lineare truncatum* Chardez, 1964
116	*Trinema penardi* Thomas et Chardez, 1958
117	*Valkanovia delicatula* (Valkanov, 1962) Tappan, 1966
118	*Valkanovia elegans* Schönborn, 1964
119	*Wailesella eboracensis* Wailes, 1911

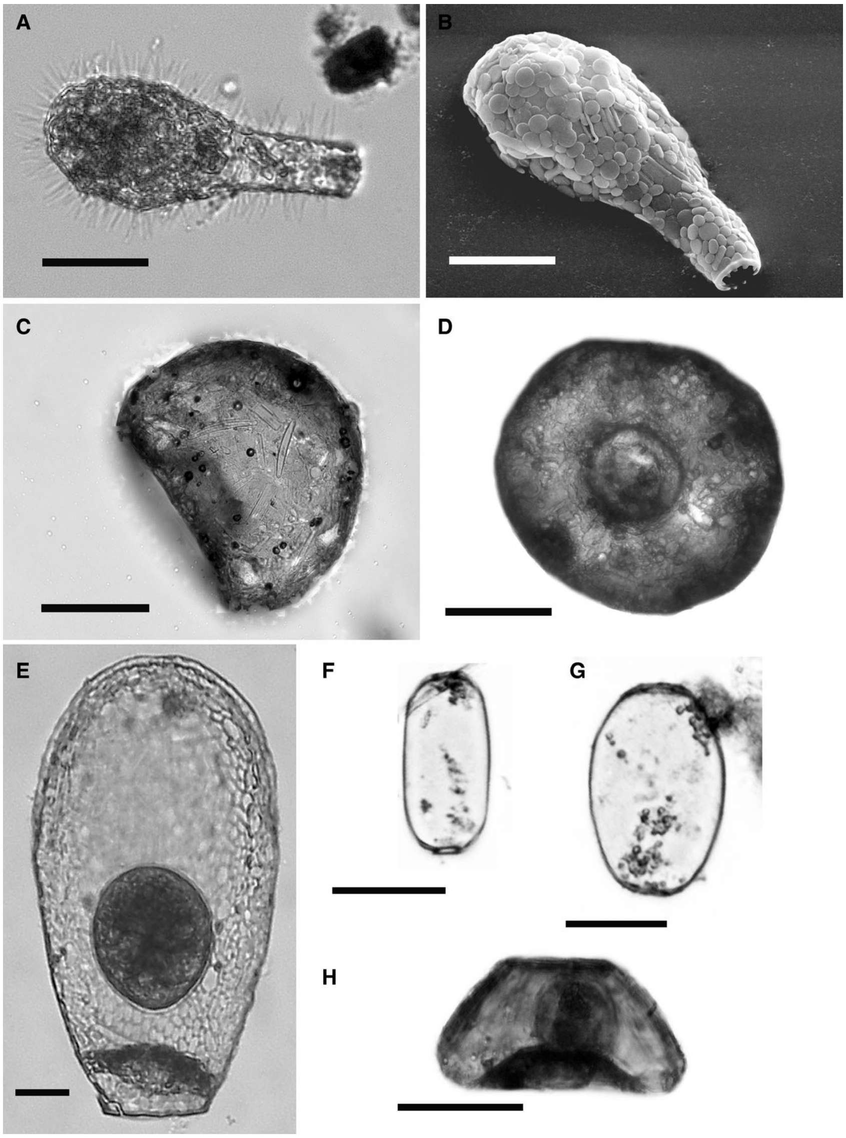

Fig. 2. Micrographs of some of the notable testate amoebae identified in this study. A. *Nebela barbata*. B. *Nebela barbata psilonata*. C, D. *Cyclopyxis puteus*. E. *Planocarina maxima*. F. *Archerella flavum*. G. *Archerella jollyi*. H. *Arcella costata*. Scale bars 40 μm. A–C, E–H – lateral view, D – apertural view. A, C–H – light microscope micrographs, B – scanning electron micrograph.

et al. 2013; Singer *et al.* 2018). No previously unknown morphospecies were located in our samples, and the vast majority of the taxa we identify are extremely common across the Holarctic realm (Amesbury *et al.* 2016, 2018; Beyens & Bobrov 2016). The most abundant taxa here are both frequently recorded, and frequently dominant in testate amoeba studies. However, some infrequently recorded taxa are also present. As always with studies on morphology-based taxonomy alone, caution is required when drawing contrasts with other studies produced by different analysts (Payne *et al.* 2011), but some findings are clear.

In the Kiumshichek peatland site we identified five individuals of *Arcella costata* (Fig. 2). This is a relatively infrequently recorded taxon and an unusual find in terms of habitat, as most records are associated with open water (Nogueira 2001; Bini *et al.* 2007). The observation is additionally notable as records of this taxon have come disproportionately from the tropics (van Oye 1926), particularly the Americas (Bovee 1965; Green 1975; Nogueira 2001). Our record of this taxon in Kamchatka's peatland is a rare outlier in the boreal realm, although the taxon has also recently been noted in Korea (Jung 2016) and in arctic Yakutia and European Russia (Bobrov & Wetterich 2012; Tsyganov *et al.* 2017). Taxonomic confusion (particularly with *A. conica*) is plausible with this species, but we believe our identification is secure.

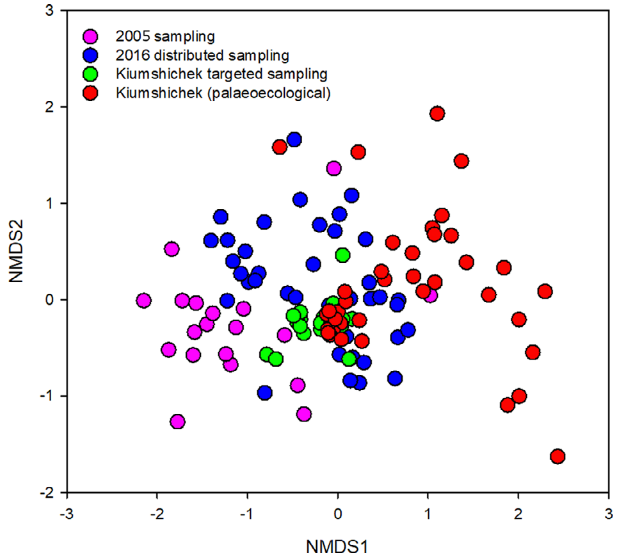

Fig. 3. NMDS ordination of testate amoeba assemblages (Bray–Curtis dissimilarity) in all data sets. 2016 distributed sampling – surface samples taken in 2016 except those from Kiumshichek; Kiumshichek target samples – surface samples taken in 2016 from Kiumshichek.

We also recorded three individuals of *Cyclopyxis puteus*, a distinctive taxon with a strongly invaginated tube-like pseudostome where misidentification is unlikely (Fig. 2). This taxon has been recorded globally, including in Europe (Lousier 1982) and arctic North America (Beyens *et al.* 1990) but is notably present in several previous studies in the Russian Far East, despite the relative scarcity of such studies, suggesting disproportionate abundance (Bobrov 2001; Bobrov *et al.* 2003). Our record appears to be the first from peatland, with mineral soils being more frequent habitats.

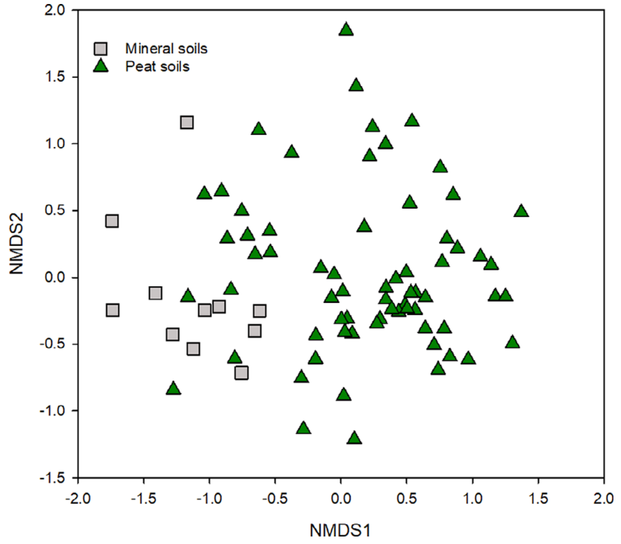

Fig. 4. NMDS ordination of testate amoeba assemblages (Bray–Curtis dissimilarity) for modern samples from peat soils and mineral soils in all data sets.

In one sample from the Kiumshichek site we identified five individuals of *Planocarina maxima* (Fig. 2). This is another relatively rarely recorded taxon, although it has been located in recent studies in Russia (Babeshko *et al.* 2015). The taxon is notable by virtue of being one of the largest species in the abundant family Hyalospheniidae. The recorded distribution appears to be limited and the taxonomic status is unclear, so we are hesitant about drawing inferences from our records. We also identified three *Cryptodifflugia* taxa including *C. pusilla*. In this case the relatively infrequent recording in previous studies is almost certainly because multiple *Cryptodifflugia* species have been grouped in many studies (particularly those focused on palaeoecology), often being referred to as *C. oviformis* or *C. oviformis*-type (Bobrov & Mazei 2017).

Another notable taxon we recorded is *Nebela barbata* and its glabrous variety *Nebela barbata psilonata* (Fig. 2). The former is characterized by numerous spines covering the shell (a feature that is not typical for Hyalosphenidae), and both have apertural denticles or lobes resembling those of the flagship species *Pseudonebela africana* (Lahr & Gomez e Souza 2011). The latter differs from the two *Nebela* taxa by a circular cross-section, shorter neck and shell, and the absence of the apertural lip. Despite these distinct characteristics, there are still some issues in identification of these taxa. The absence of spines has been observed for *N. barbata* in previous studies (Cash & Hopkinson 1909; Hoogenraad & Groot 1940); however, these individuals were not assigned to *N. barbata psilonata*. In addition, some of the individuals of *N. barbata psilonata* observed in this study (Fig. 2) had a circular cross-section so they potentially could belong to an unknown variety that requires further investigation. *Nebela barbata* has a widespread geographical distribution (Eurasia, America, Indonesia) and generally inhabits wet *Sphagnum* mosses, but it is a rather rare component in these assemblages (Hoogenraad & Groot 1940). It is difficult to estimate the geographical distribution of *N. barbata psilonata* due to these identification issues, but it was originally described by Jung (1942) from south Chile and has been recorded there more recently (Fernández *et al.* 2015).

Our samples included abundant *Archerella flavum* and the closely related *A. jollyi* (Fig. 2). *A. flavum* is one of the most distinctive and well-known testate amoebae and one of the most abundant taxa in European, North American, and Siberian peatlands (Kurina *et al.* 2010; Amesbury *et al.* 2016, 2018; Mazei *et al.* 2017a). However, *A. flavum* appears to be less abundant in testate amoeba data sets from China, and previous authors have speculated on the causes of this difference (Qin *et al.* 2013; Song *et al.* 2018). Recently Li *et al.* (2015) have identified *A. flavum* in the Lesser Khingan Mountains of northeast China, not far from the Russian border, and our data confirm abundant presence further northeast again in Kamchatka. It therefore seems probable that the

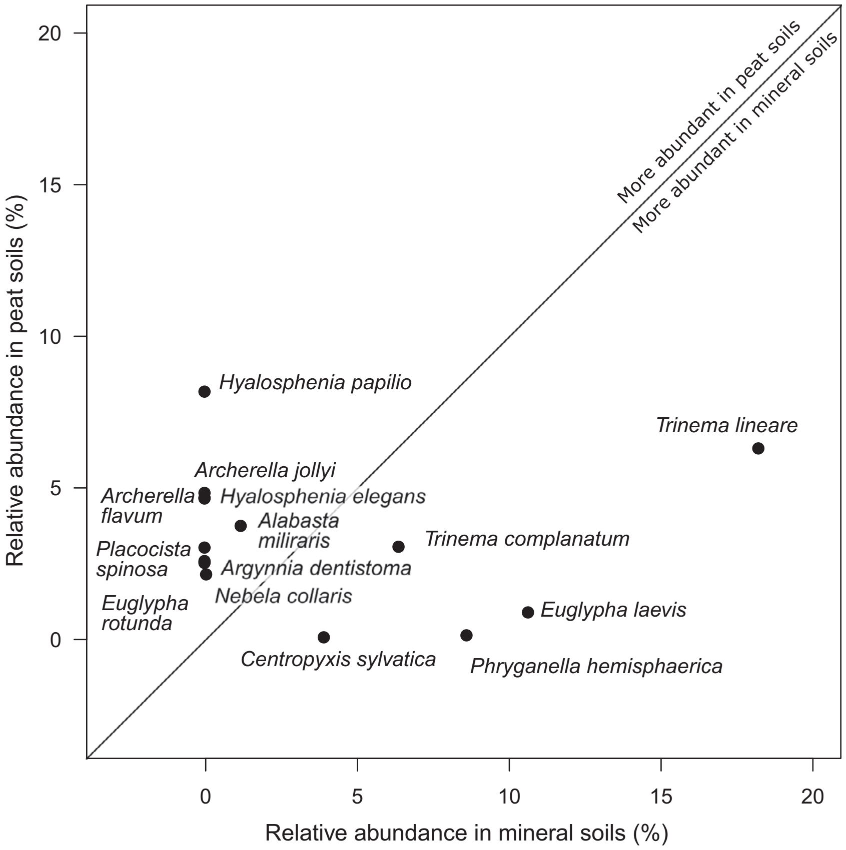

Fig. 5. Difference in species relative abundance between peat soils and mineral soils. Showing mean relative abundance across samples for the 15 taxa with greatest absolute difference between the two data sets.

lack of this taxon in the rest of China may reflect climatic or habitat differences (perhaps associated with relative lack of *Sphagnum*) rather than restricted dispersal.

There are also some notable taxa not recorded in our samples. Despite the abundance of wet fen environments in the sampling, we did not identify any individuals of the genus *Quadrulella*, a highly visible and distinctive taxon and very typical of this habitat across the Holarctic. In the Sikhote Alin Mountains (>1500 km southwest of Kamchatka), Bobrov (2001) identified several unexpected taxa of the genera *Planhoogenraadia* and *Hoogenraadia*. The latter is particularly significant because available evidence suggests this genus is primarily located in tropical regions, being first described in Africa and with almost all subsequent records in tropical and subtropical regions of Africa, Asia and the Americas (Bobrov *et al.* 2015). The finding from Sikhote Alin appears to be the northernmost recorded location, and it is worthy of comment that neither *Planhoogenraadia* nor *Hoogenraadia* species were identified in this study, suggesting that the distribution does not extend further northeast to Kamchatka. There may be several factors affecting this, including the cooler climate, differences in vegetation (the Sikhote Alin findings were associated with *Quercus mongolica*, which is rare or absent in Kamchatka) and the geographical barrier of the Sea of Okhotsk.

Our total inventory of 119 taxa (Table 1) is high relative to most testate amoeba studies of a similar spatial scale. This might imply that the Kamchatka assemblage is particularly diverse. This is plausible given the strong gradients in many significant environmental variables. However, comparisons of this nature are confounded by the varying level of taxonomic precision adopted by analysts, preventing any robust conclusions being drawn. Taken overall, our results imply considerable commonality between the testate amoeba assemblages of Kamchatka and the rest of the boreal biome but with some hints of biogeographical differences. Given the emerging extent of cryptic diversity in testate amoebae, molecular data would be required to fully assess microbial biogeography.

Ecology of testate amoebae

Our data also cast light on the ecology of testate amoebae in Kamchatka. The first key finding is the scale of the difference between assemblages from peat soils and mineral soil. Our results show strong differences in assemblages with small euglyphid taxa particularly

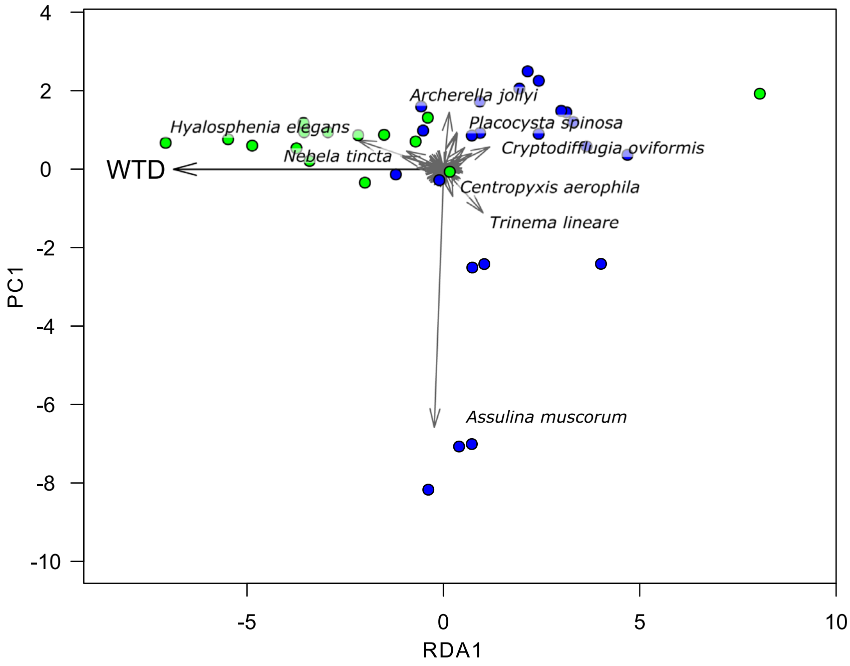

Fig. 6. Redundancy analysis of testate amoeba samples from peatlands with water table depth (WTD). Site scores and WTD vector divided by three for clarity, only selected species labelled. WTD explains 5.5% of species variance. Axis 2 is unconstrained (PCA scores, the axis explains 22.4% of the total variance). Green points show Kiumshichek samples, blue points show other sites.

associated with mineral soils and larger taxa, often with tests constructed of organic secretions (e.g. *Archerella*, *Hyalosphenia*), particularly abundant in the peat samples. Quantifying these differences has implications for palaeoecological reconstruction in peatlands, for instance understanding the formation and dynamics of 'forest-to-bog' ecotones (Ratcliffe *et al.* 2017).

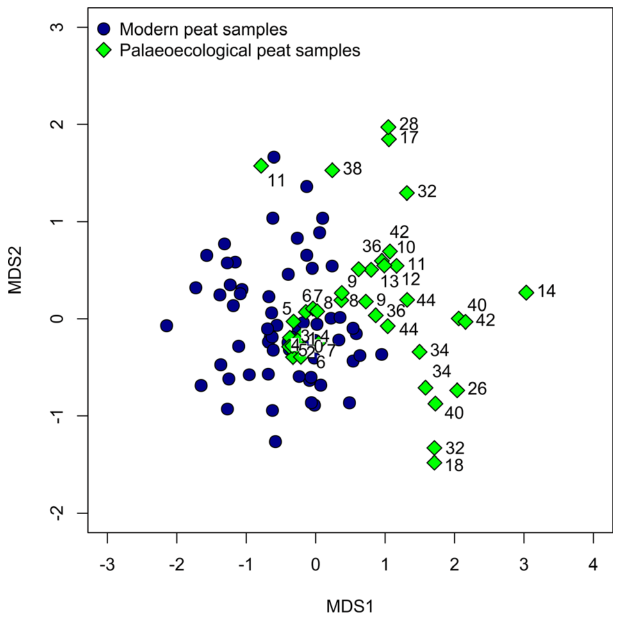

Fig. 7. NMDS ordination of testate amoeba assemblages (Bray–Curtis dissimilarity) from modern and palaeoecological peat samples (STRESS = 0.18). Numbers are sampling depth (cm) for palaeoecological samples (the replicate numbers were removed from the centre of the diagram to make it less cluttered).

Our results also show a significant relationship between testate amoebae and WTD. This finding is unsurprising as this relationship has been demonstrated in numerous previous studies in peatlands globally (Tolonen *et al.* 1992, 1994; Payne *et al.* 2012; Amesbury *et al.* 2016, 2018). The proportion of variance explained in Kamchatka peatlands is at the lower end of the range for similar studies, but is still significant. Although multiple environmental variables affect species composition of testate amoeba assemblages, variables related to peat surface wetness, especially WTD, generally explain most of the variance. The direct comparison of this estimate might be complicated due to different environmental settings and/or statistical procedures, but in many studies on modern testate amoeba ecology, WTD alone explains less than 20% of the total variance in assemblages: 13.5% (Amesbury *et al.* 2013); 7.0% (Charman *et al.* 2007); 7.6% (Lamarre *et al.* 2013); 7.1% (Tsyganov *et al.* 2017); see Payne *et al.* (2012: fig. 1) for an overview. Occasionally, the proportion of the variance explained by WTD reaches >20% (23.8%; Qin *et al.* 2013). Despite this, WTD still represents the most important 'measurable' environmental variable. This low explanatory power is not uncommon in ecology and should normally still allow a quantification of past water tables from fossil assemblages (Van Bellen *et al.* 2016).

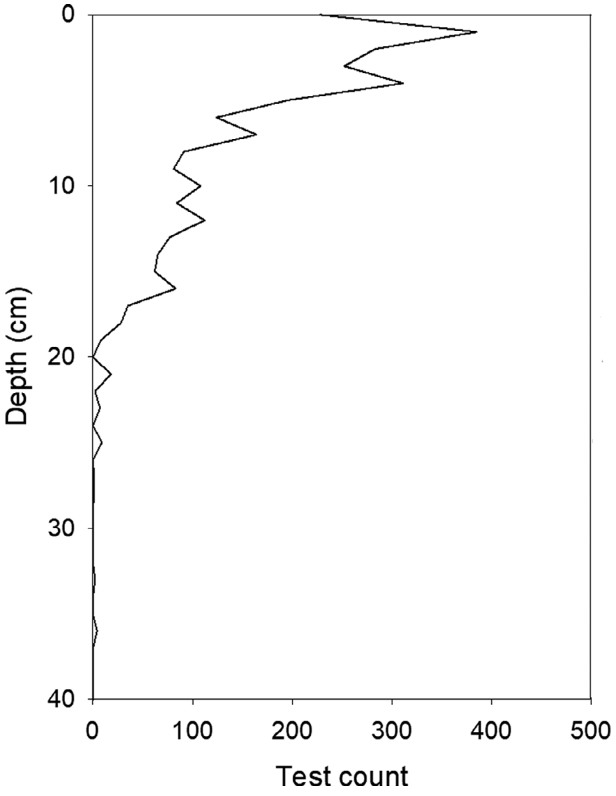

Fig. 8. Decline in test numbers with depth in the Kiumshichek site.

However, the ordination plot suggests that the species' hydrological preferences do not always agree with those identified in other regions. For instance, *Archerella jollyi* (usually grouped with *A. flavum* elsewhere) and *Assulina muscorum* are both here orthogonal to the WTD vector in the ordination (Fig. 6), whereas previous research has demonstrated strong hydrological preferences (*A. jollyi* indicating wetter conditions and *A. muscorum* indicating drier conditions). Similarly, *Placocista spinosa* here is negatively correlated with the WTD vector, despite being an unambiguous indicator of wetter conditions in most studies (Payne *et al.* 2012). These results may point to real differences in hydrological preferences in Kamchatkan peatlands, to cryptic species, or, more prosaically, to the limited size of the data set here or problems with WTD measurements.

Our results showed no significant relationship with other environmental variables tested, i.e. altitude, mean annual temperature and mean annual precipitation. This is surprising, considering that a number of previous studies demonstrated a clear effect of air temperature (Tsyganov *et al.* 2012; Jassey *et al.* 2013, 2015), precipitation (Tsyganov *et al.* 2013a, b) and altitude (Mazei *et al.* 2012; Tsyganov *et al.* 2013a, b; Heger *et al.* 2016) on testate amoeba assemblages. This can be explained by the high sensitivity of testate amoebae as unicellular organisms to local environmental conditions. The latter are not always directly linked to large-scale climatic or geomorphological characteristics, so that well-designed studies with strong control on confounding factors are required to detect the response of testate amoebae. Therefore, while testate amoebae clearly are sensitive to climate to some degree, this appears to be strongly mediated by location-specific factors.

Palaeoecological reconstruction

Our results have potential for the application of testate amoebae for palaeoecological reconstruction in Kamchatka. As discussed above, it is notable that although WTD explains significant variance, the proportion is lower than some studies and the position of several taxa in the ordination (Fig. 6) differs from established knowledge. The development of a robust regional transfer function model would clearly be desirable to permit the quantification of future palaeoecological studies. However, our limited data set is not big enough to develop a well-performed regional transfer function for Kamchatka. Our comparisons of palaeoecological and modern assemblages imply that assemblages present in the past are different to those sampled today, again supporting the need for future research in the region.

Initial palaeoecological work at the Kiumshichek site implies that adequate preservation may also be an issue in the application of testate amoebae in Kamchatka palaeoecology. While declines in test abundance with depth are common in peatlands, the abruptness of this test loss is relatively unusual in an ombrotrophic site (Mitchell *et al.* 2008b). Similar issues have been noted in blanket bogs elsewhere in the world (Charman *et al.* 2001), perhaps related to the relatively dense and humified peat often encountered in such sites. Two previous studies, Tilotta (2016) and Klimaschewski *et al.* (2015), have found adequate preservation over much deeper depths and longer time-periods, so the limited available evidence suggests that preservation is variable between sites. In the case of the Utka peatland site studied by Klimaschewski *et al.* (2015) the contrast is surprising, as the two sites are similar in many ways, both being blanket bogs on the West Kamchatka Plain with similar vegetation.

Conclusions

Given the limited knowledge of Holocene environmental change in Kamchatka and the extensive occurrence of relatively undisturbed peatlands, the application of testate amoeba analysis to understand peatland palaeohydrology and palaeoclimatology is an important research direction. Testate amoebae form diverse assemblages across Kamchatka (in total 119 taxa were identified), which are mainly composed of widespread taxa, but rarer occurrences such as *Cyclopyxis puteus* hint at important biogeographical differences. Our results demonstrate that the local mire ecosystems are characterized by specific assemblages of testate amoebae in comparison to mineral soils. The local environmental variables such as water table depth have a greater impact on the species composition of testate amoebae, as compared with regional climatic characteristics and altitude. This might represent considerable obstacles for palaeoecological reconstructions and particularly point to the need for larger studies to quantify relationships between modern testate amoeba assemblages and regional climate.

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Author contributions. – YAM, EYN and RJP secured funding; AAA, ANT, RJP, KVB, MK, TJS, DAK and NVS conducted fieldwork; KVB and AAB conducted microscopy; RJP and ANT conducted data analysis; RJP wrote the first draft of the manuscript to which all authors contributed with text, comments and ideas. Lead author RJP was tragically killed by an avalanche while trying to reach the peak of Nanda Devi (Indian Himalayas) on 26th May 2019.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at http://www.boreas.dk.

Table S1. Details of the distributed sites sampled for this study with locations (samples from the intensively studied Kiumshichek site are not listed).