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Can testate amoeba-based palaeohydrology be extended to fens?

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ABSTRACT

Numerous palaeoecological studies have used testate amoeba analysis to reconstruct Holocene hydrological change in peatlands, and thereby past climatic change. Current studies have been almost exclusively restricted to ombrotrophic bogs and the period since the fen-bog transition. Although the critical link between peatland surface wetness and climate is less direct in minerotrophic peatlands such records may still be of value where there are few others, particularly if multiple records can be derived and inter-compared. Expanding the temporal and spatial scope of testate amoeba-based palaeohydrology to minerotrophic peatlands requires studies to establish the primacy of hydrology and the efficacy of transfer functions across a range of sites. This study analyses testate amoeba data from wetlands spanning the trophic gradient in the eastern Mediterranean region. Results demonstrate that different types of wetlands have distinctly different amoeba communities, but hydrology remains the most important environmental control (despite water table depth being measured at different times for different sites). Interestingly, Zn and Fe emerge as significant environmental variables in a sub-set of sites with geochemical data. Testate amoeba-hydrology transfer functions perform well in cross-validation but frequently perform poorly when applied to other sites, particularly with sites of a different nutrient status. It may be valid to use testate amoebae to reconstruct hydrological change from minerotrophic peatlands with an applicable transfer function; however it may not be appropriate to use testate amoebae to reconstruct hydrological change through periods of ecosystem evolution, particularly the fen-bog transition. In practise, the preservation of amoeba shells is likely to be a key problem for palaeoecological reconstruction from fens.

KEYWORDS: Palaeoclimate, Palaeohydrology, Palaeoecology, Protists, Ombrotrophic, Minerotrophic, Bogs

INTRODUCTION

The classic model of peatland development is the hydroseral succession: a lake develops into a bog by successive replacement of aquatic macrophytes by emergent plants by fen communities, ultimately growing beyond the reach of groundwater to reach ombrotrophy (Tansley 1939). While more recent research has demonstrated considerable complexity in the pattern and timing of this progression (Walker 1970, Klinger 1996, Hughes and Barber 2004) most temperate peatlands will still pass through the majority of these stages, each characterised by differing plant communities and environmental conditions. The divisions between many of these stages are indistinct and terminology is not always consistently applied, it may therefore be best to consider this sequence as a 'trophic gradient' leading to ombrotrophy.

As decomposition rates are slow in the saturated, anoxic conditions below the surface of peatlands biological remains are well preserved and palaeoecological studies can be used to reconstruct both the developmental history of a peatland and changes in its wider environment. Over the last two decades increasingly sophisticated methods have been used to derive proxy-records of Holocene climatic change from peats (Blackford 2000, Chambers and Charman 2004). The majority of these palaeoclimatic studies have focussed on ombrotrophic bogs due to the direct link between mire surface wetness and hydroclimate. With a few exceptions (e.g. Booth 2010; Booth et al. 2004; Hendon et al. 2001) the less direct relationship between surface wetness and climate in minerotrophic peatlands has meant that such sites have been avoided for palaeoclimate reconstruction. This has limited the record both spatially and temporally- restricting reconstructions to areas with ombrotrophic peatlands and limiting the length of the record to the period since the fen-bog transition. The former means the methodology cannot be applied to many more arid regions of the world where ombrotrophic peatlands are absent, while the latter means the approach is limited when studying changes in the early Holocene when many peatlands had not yet reached ombrotrophy, such as the 8.2ka BP 'event'. The hydrology of minerotrophic peatlands may be affected by a wide variety of geomorphological, tectonic and anthropogenic process in their catchments so the link between surface wetness may be more complex and less direct than in ombrotrophic peatlands. Palaeohydrological records from fens will always require more cautious interpretation than records from bogs, but may still be valuable in situations where any palaeoclimatic information is scarce, such as the eastern Mediterranean region considered here. The problems of any one record might be overcome using integrated regional networks of multiple sites. Extending the spatial and temporal scope of peatland palaeoclimatology to fens requires untangling competing controls on the organisms used as hydrological proxies in palaeoecology. If palaeohydrological reconstructions are to be extended back through the fen-bog transition studies need to establish the primacy of hydrology in sites which span the trophic gradient.

One of the most widely used palaeoecological techniques in peatlands is testate amoeba analysis. Testate amoebae are a polyphyletic group of protists characterised by a decay-resistant shell (the test) which is well-preserved in peats. In ombrotrophic mires it appears that hydrology is the strongest environmental control on testate amoeba communities validating their application for palaeohydrological (and thereby, palaeoclimatic) reconstruction and providing the basis for the development of transfer functions (Charman 2001; Mitchell et al 2008). These transfer functions, uniquely for peatland palaeoecological records, allow quantified reconstruction of hydrological change in terms of a measurable variable, the depth to water table (DWT). Studies of testate amoeba ecology have been overwhelmingly carried out in ombrotrophic bogs. Although many quantitative studies of testate amoeba ecology have included a few minerotrophic (but generally oligotrophic) samples (e.g. Mitchell et al. 1999; Lamentowicz and Mitchell 2005; Payne et al. 2006; Booth 2002) fewer have been primarily focused on fens (Payne et al. 2007; Opravilova and Hajek 2006). Few studies have investigated testate amoeba ecology along a longer trophic gradient and these have generally been either very localised (Heal 1961, Ruitenburg and Davids 1977) or have only considered a part of this gradient (Tolonen 1992, 1994; Opravilova and Hajek 2006). The purpose of this study is to investigate testate amoeba ecology along the full trophic gradient from eutrophic reedswamps to ombrotrophic bogs. In particular the study aims to address whether the hydrological controls on amoebae are consistent along the trophic gradient. Specific research questions are:

1. How do testate amoeba communities vary along the trophic gradient? What are the dominant environmental controls? (A particular focus is geochemical variables).
2. What testate amoeba taxa are typical of sites at different positions along the trophic gradient?
3. Are testate amoeba hydrological preferences consistent along the trophic gradient?
4. Do transfer functions perform adequately for sites at different positions along the trophic gradient from the training set?
5. Can testate amoebae be used for palaeohydrological reconstruction in eastern Mediterranean peatlands?

SITES and METHODS

Field Sites

The geographical focus of this study is the eastern Mediterranean region, loosely defined to extend to the Black Sea littoral and eastern Levant (Fig. 1). A large dataset was assembled from three previously-published studies (Table 1, Table 2): the data of Payne and Mitchell (2007) from the Elatia Mires of northern Greece, the data of Payne et al. (2008) from a mire in northeast Turkey and the data of Payne et al. (2010) from the Hula wetland in Israel; and new data from a further seven sites throughout the region (Fig. 1). Details of the sites are shown in Table 2. The sites cover a wide geographic range and a wide range of ecoregions spanning the deserts of the Levant to the mountains of the Balkans. Wetlands span the trophic gradient from eutrophic sites (some with anthropogenic nutrient sources) such as the Hula and Ioannina Fen, to oligotrophic raised bogs such as Ispani-2. Due to climatic constraints on peatland development the sites do not form a true chronosequence. Sampling intensity varied from a single sample (site ESA) to 42 samples (Hula Nature Reserve) per site. Clearly a single sample is insufficient to characterise the amoeba community of a site and such sites are included for general interest and biogeographical relevancy. As there is no generally accepted wetland classification system for this region the sites were classified into four broad and relatively unambiguous groups based on vegetation and topography (Table 3).

Field and Laboratory Methods

Samples of surface sediment and any leaf litter (varying from 3-5cm deep) were collected from the sites between 2005 and 2007. Throughout this paper the term 'sediment' is used *sensu lato* to include both truly autochthonous peats and mixed autochthonous-allochthonous deposits present in swamps and fens. A hole was made in the peat surface and depth to water table (DWT) measured after leaving for a period of between one and 12 hours for water table to equilibrate. The peat or moss surface was taken as the zero level; negative values represent a submerged peat surface. In the laboratory a sub-sample of sediment was suspended in deionised water and pH measured using a ratio of 1cm^3 peat to 25 or 30ml water. A further sub-sample was dried overnight at 110°C and then burned at 550°C for at least five hours with weights pre- and post-drying and pre- and post-incineration used to calculate percent moisture and loss on ignition (LOI). Due to a variety of logistical constraints a complete set of environmental data was not obtainable for all sites. For a few sites in the Levant (Hula Nature Reserve, Hula (Agmon), Aamiq, Fassouri and ESA) additional sediment samples were acid-digested and geochemically analysed using ICP-AES (elements included in analyses here are: Al, B, Ba, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Pb, Sr and Zn; see Payne et al. 2010 for details). Sample preparation for testate amoebae was based on a modified version of the water-based method of Hendon and Charman (1997). Sub-samples of 1-2 cm^3 were placed in a beaker and either boiled for ten minutes or immersed in boiling water for 30 minutes. Samples were filtered and the fraction either 15>300 μm or 15>250μm (in the case of Elatia samples) retained. Slides were prepared by mixing with glycerol. In most cases at least 150 tests were counted, however in the Aamiq site (Lebanon), concentrations were very low and only 50 tests could be counted, this may be insufficient to adequately characterise the amoeba community (Payne & Mitchell 2009). A conservative taxonomic scheme was adopted based on the widely-used approach of Charman et al. (2000). To ensure taxonomic consistency a variety of minor changes were made to the taxonomic schemes presented in the original papers. Full details of taxonomy are presented in Appendix 1; particularly notable are the broad *Centropyxis* *aerophila* and *Phryganella acropodia* types, both of which incorporate several species. Samples selected as outliers in data filtering for transfer function performance in the three component studies were also excluded here.

Data analysis

Ordination was used to explore the data structure and identify possible environmental controls on amoeba communities. Species data was Hellinger transformed to allow the use of ordination techniques based on a linear response (Rao 1995, Legendre and Gallagher 2001). Principal components analysis (PCA) was used to investigate the data structure, followed by a series of (partial) redundancy analyses (RDAs) to test the strength of correlations with environmental variables. Significance testing used Monte Carlo permutation tests (999 permutations). For the geochemical data a forward-selection procedure was used to identify a minimal suite of variables. All ordination analyses were carried out using Canoco vers. 4.53 (ter Braak and Šmilauer 1997-2004). *K*-means partitioning was used to identify groupings of samples based on untransformed data using the program K-MEANS vers 2.0 (Legendre 2001). The Calinski-Harabasz (1974) pseudo-*F*-statistic was used to select an optimum number of groups (Legendre and Legendre 1998). To identify diagnostic species of sites at different positions along the trophic gradient the Dufrêne-Legendre Indicator Value method was implemented (IndVal: Dufrêne and Legendre 1997) using INDVAL vers. 2.0 with significance tested using 999 permutations. This approach aims to identify species which have both high specificity and high fidelity to a single group; a taxon with a maximal indicator value of 100% would be found in all samples of a group and only in that group. Unlike the widely used two-way indicator species (TWINSPAN) method IndVal allows flexibility in classification and does not need require the awkward concept of pseudospecies (Dufrêne and Legendre 1997; McGeoch and Chown 1998).

Testate amoeba-hydrology transfer functions have previously been developed from three areas: the Hula Nature Reserve (Payne et al. 2010); Sürmene Ağaçbaşı Yaylası peatland (Payne et al. 2008) and the Elatia mires (Payne and Mitchell, 2007). These areas occupy contrasting positions along the trophic gradient; Sürmene Ağaçbaşı Yaylası is an ombrotrophic bog, the Elatia mires are mesotrophic fens and the Hula is a Papyrus-dominated marsh with sediments that are not sufficiently organic to be strictly termed peat. To investigate how the hydrological controls on amoeba communities vary along this gradient these transfer functions were applied to each other and to data from other sites.

RESULTS and DISCUSSION

Testate amoeba communities in wetlands along the trophic gradient

The total dataset consists of 211 samples from 15 sites; with the taxonomic modifications 63 taxa remained in the dataset of which the most abundant were *Trinema lineare* and *Centropyxis aerophila* type. A plot of mean pH against mean loss on ignition shows a rather consistent overall relationship with the exception of the very acidic Asi Gonia site (Fig. 2). Site positions in this plot do not entirely agree with the *a priori* site classification in Table 3; this is partly due to variability in the loss on ignition measurements with values elevated in sites with considerable surface leaf litter (e.g. many samples of the Hula Nature Reserve) and reduced in a few sites where surface sediments were less organic than deeper layers (e.g. Elatia-BO and ESA). The LOI values suggest surficial sediments in several sites are less than 65% organic matter and are therefore insufficiently organic to be termed peat *sensu stricto*.

In the PCA the samples clearly tend to cluster within sites (Fig. 3). The first axis of the PCA divides samples along the trophic gradient. Samples from the ombrotrophic sites (Sürmene Ağaçbaşı Yaylası and Ispani-2) are closely grouped on the right of the plot. On the left are samples from reedswamps, a eutrophic fen and graminoid-dominated mesotrophic fens. Samples from bryophyte-dominated mesotrophic fens have slightly higher scores on PCA1 with some overlap between the samples from Asi Gonia and Sürmene Ağaçbaşı Yaylası. PCA2 divides the non-ombrotrophic sites with the Elatia sites having notably low scores and the Hula samples a considerable range along this axis. PCA3 divides samples from the two ombrotrophic sites- Sürmene Ağaçbaşı Yaylası and Ispani-2 with the latter having higher scores and less variability. PCA3 also separates Aamiq from similar reed-dominated wetlands (Hula, Ioannina etc). A comparison of groupings from *K*-means partitioning with the *a priori* classification shows some similarity (Table 4). The optimum value of *K* was determined to be 5; *K*-means groups 3-5 are essentially restricted to ombrotrophic bogs, including only a single sample from a minerotrophic site. Samples from bryophyte-dominated fens are largely restricted to group 2 while samples from other fens and reedswamps are divided between groups 1 and 2.

One of the most interesting features of the ordination plots is that they show relatively clear differences between the ombrotrophic and non-ombrotrophic sites, but very little difference between minerotrophic sites with peat and those with sediments which are less organic (e.g. Hula, Fassouri). *K*-means partitioning identifies multiple distinguishable communities in ombrotrophic bogs but struggles to separate the amoeba communities of even very dissimilar non-ombrotrophic sites such as the sub-tropical lowland Fassouri reedswamp and the montane fen of Elatia-XE. It seems that the change in environmental conditions during the fen-bog transition is a critical one for testate amoeba communities, but the differences among minerotrophic peatland types and even between minerotrophic peatlands and wetlands with less organic sediments are relatively unimportant. Unsurprisingly, the testate amoeba communities of more oligotrophic fens with bryophytes show greatest similarity to those of ombrotrophic peatlands. There is limited overlap between the Asi Gonia and some of the Sürmene Ağaçbaşı Yaylası samples. Interestingly the Sürmene Ağaçbaşı Yaylası samples which plot closest to the Asi Gonia samples are from the southern sector of the site which has been heavily damaged by peat-cutting and (to a lesser degree) grazing (Byfield and Ozhatay 1997, Payne et al 2007, 2008). This overlap may suggest that the damage to this area, either through hydrological modification or nutrient input, has led to the reintroduction of more minerotrophic conditions. The findings that three of the five *K*-means groups are restricted to ombrotrophic sites and samples from Sürmene Ağaçbaşı Yaylası show much the greatest scatter in ordination space of any site may suggest that the transition to ombrotrophy provides a greater variety of niches for amoebae. Different micro-topographic areas on bogs have distinctly different amoeba communities (Mitchell et al. 1999); it may be that the development of hummock-hollow topography allows a broader variety of amoeba communities to co-exist in bogs than in the more homogeneous surface environment of other wetlands.

A recent topic of considerable interest has been the biogeography of testate amoebae. Certain distinctive taxa appear to be restricted to limited geographical areas and continental drift has been postulated as a possible cause of this pattern (Smith and Wilkinson 2007; Smith et al. 2008). The sites of this study span Europe and Asia (and lie relatively close to Africa) so it is interesting to note that there are quite limited differences between sites in different continents- for instance there is considerable overlap in PCA space between Aghios Phloros, Rezina and Ioannina in SE. Europe and Hula and Aamiq in W. Asia (Fig. 3). It should however be borne in mind that the taxonomic scheme used here groups some species, and there may be further cryptic species which are difficult to distinguish at all using light microscopy (Todorov et al. 2009; Heger et al. 2010)

While this dataset does not allow modelling of the relationship between amoeba communities and trophic status it is possible to identify taxa typical of different stages of the trophic gradient. IndVal results (Table 5) show the best indicators of ombrotrophic conditions are *Assulina* species, which are largely restricted to these sites. *Quadrulella symmetrica* and *Euglypha ciliata* type are indicative of minerotrophic sites with bryophytes while *Difflugia pulex* type, *Cyphoderia ampulla* and *Centropyxis aerophila* are indicators of sedge- and rush-dominated minerotrophic sites. Indicators of reed-dominated sites include *Phryganella acropodia* type and *Difflugia minutissima* type (see Appendix 1 for details of these groupings). These indicators may allow the semi-quantitative use of testate amoeba to track peatland development in palaeoecological records. However, it should be remembered that these values may reflect the peculiarities of this data and should not be used uncritically. Differences may be found between regions and many of these taxa are widely present in a range of ecosystem types, so for instance the minor presence of *Centropyxis aerophila* in a palaeoecological sequence should not be taken as evidence that the ecosystem at the time was a sedge- or rush-dominated fen.

Environmental controls on testate amoebae

Redundancy analysis shows that all environmental variables explain more than a third of the overall variance; however when site variables are partialled out this is reduced greatly to barely 5% (Table 6). The majority of the variance can be explained by site variables, further illustrating the differences shown by the PCA (Fig. 3). Results suggest the strongest individual environmental control is wetness (depth to water table) with pH explaining a smaller proportion of the variance. Both % moisture and loss on ignition explain significant variance independently of other environmental variables, but not of site variables. The simple 'trophic index' (Table 3) explains a surprisingly large proportion of variance independent of environmental variables-7.7%, but this is probably at least partly related to the large inter-site differences in amoeba communities.

It is an almost (but not entirely) ubiquitous finding of studies of testate amoeba ecology in wetlands that hydrology explains the large proportion of variance in community structure, and therefore unsurprising that this control proves so strong here (e.g. Booth 2002, 2007; Lamentowicz and Mitchell 2005; Payne et al. 2006). Testate amoeba taxa have been shown to respond to increased moisture by increasing abundance, decreasing encystment and increasing test size (Heal 1961; 1963; Laminger 1978; Louisier 1974a&b). Even the diet of amoebae may be sensitive to moisture; Laminger (1978) suggests that during wetter periods *Trinema enchelys* shifts its diet from organic detritus to bacteria and smaller testate amoebae. Such changes are likely to be related to the thickness of water films and motility of amoebae (Bonnet 1964, Lousier 1975). That DWT is still the strongest environmental variable, despite samples being extracted at different points in different years illustrates the strength of the hydrological control and may be related to the ability of amoeba communities to adapt relatively rapidly to changing moisture regime (Lousier 1974a&b; Warner et al. 2007). The positive correlation of *Assulina muscorum*, *Assulina seminulum* and *Corythion dubium* with DWT, and negative correlation of *Centropyxis aerophila* type agrees with the known hydrological preferences of these taxa (Fig. 4). It is interesting that there is little correlation between % moisture and DWT, and while DWT is correlated with taxa known to be more xerophilic (probably no testate amoeba taxa are strictly xerophilic) % moisture is not correlated with taxa believed to be hydrophilic. This result shows the complexity of the hydrological control on amoebae. Although amoebae clearly do not respond to depth to water table *per se*, this measurement appears to consistently provide the most robust proxy for the hydrological parameters which do affect amoebae (*cf.* Mitchell et al. 1999).

As would be expected, loss on ignition is negatively correlated with both the trophic index and pH. Axis one clearly represents the trophic gradient with species typical of oligotrophic peatlands (*Heleopera rosea, Assulina* spp.) having higher scores and species typical of eutrophic peatlands having lower scores (e.g. *Tracheleuglypha dentata, Centropyxis aerophila*). The position of *Trinema lineare* in this plot is interesting; this taxon is often taken to be xerophilous (which probably at least partly reflects its frequent conflation with *Corythion dubium*) but is extremely abundant in some samples from fens and is identified as an indicator of reed-dominated fens and swamps by IndVal. In northern Greece the relative abundance of this species along the hydrological gradient was shown to have a remarkable U-shaped distribution with the taxon frequent at the extremes but rare in the middle (Payne and Mitchell 2007). Such a pattern might be explained by the greater abundance of other taxa, predation, or competition in the middle of the gradient or by the presence of cryptic species. The RDA shows this taxon is most abundant in wetter niches, but is most strongly associated with the trophic gradient, being more abundant in eutrophic sites, a finding supported by smaller-scale previous studies (e.g. Ruitenburg and Davids 1977). This emphasises the importance of differentiating trophic from hydrological control on species distributions. It is notable that of the taxa negatively correlated with LOI (and positively correlated with the trophic index) many have xenosome tests (*P. acropodia* type*, C. aculeata* type*, C. aerophila* type etc). This may suggest that the relative absence of inorganic particles in ombrotrophic sites is a limiting factor for these taxa (Heal 1961).

Geochemical controls on testate amoebae

Analysis of the samples with ICP-AES data shows the strongest geochemical correlate is Zn while Fe is also significant (Table 6). Sr, which was shown to be a significant environmental variable in the Hula data (probably acting as a proxy for a range of other geochemical variables), is not significant in this dataset once site variables are partialled out (both Zn and Fe were significant variables in some formulations of the Hula data (Payne et al. 2010)). The reasons why these variables emerge so strongly are not immediately apparent. Previous studies have shown associations between testate amoebae and a variety of geochemical variables including Ca, K and Mg (Mitchell et al. 2000, Lamentowicz et al. 2008, Opravilova and Hajek 2006) but such relationships are not apparent in this data. Zn and Fe are essential nutrients required in particular for enzyme production, but can also be toxic in higher concentrations. Fe is a component of the tests of some taxa with possible functions including structural rigidity, extra-cellular storage, or even a detoxification mechanism keeping metals away from the cell membrane (Deflandre 1929; Hedley et al. 1976; Ogden 1987; 1988). In urban mosses Nguyen-Viet et al. (2007) found negative relationships between two taxa (*Trinema lineare* and *Euglypha ciliata* var.

glabra) and Zn, but no significant relationship with overall amoeba community or species richness, and no relationships between Fe and amoebae. The species responses provide few clues to the mechanisms driving the apparent associations with Zn and Fe here. Tests of genera which have been suggested to accumulate iron (Centropyxidae and possibly Arcellidae) are not positively correlated with Fe and of the two taxa which Nguyen-Viet et al. (2007) suggest may be deleteriously affected by Zn, only one (*E. ciliata* (var. glabra not differentiated)) shows such a relationship here. The concentrations of many metals are highly correlated so the apparent significance of these elements in our analyses does not necessarily mean that it is Zn and Fe which are themselves controls on amoeba communities, it is perhaps more likely that these simply represent broader gradients. It is also possible that any relationship is indirect through controls on plant communities (impacting the amoeba's physical environment and biotic interactions in the rhizosphere) or other microbial groups.

Hydrological reconstruction along the trophic gradient

To test the efficacy of transfer functions when applied to sites at different positions along the trophic gradient the three transfer functions were applied to each of the other two training sets and to additional data sets. Additional data sets with measured DWT values, more than five samples and a reasonable range of DWT values were included, narrowing the selection to three: Asi Gonia, Ispani-2 and Ioannina. Optimal transfer function models (Table 1) were used with standard errors estimated by bootstrapping (1000 cycles). Figure 5 shows model predicted against measured DWT values and proportion of tests included in the model. A 1:1 relationship is not to be expected as the water table depths were measured at different times in different years, however we can hypothesise a linear relationship. R^2 is used as a simple index of this relationship.

For most datasets, with most transfer functions, the correlation between measured and predicted DWT values is weak (Fig. 5). In one case there is no correlation at all and in two cases there is a negative correlation. For the Asi Gonia data there is very little correlation between measured and predicted DWT values using any of the transfer functions. The situation is little better for the Ispani-2 data. The best correlations (excluding cross-validations) are achieved when analysing the Hula data with the Elatia transfer function (R^2=0.51) and the Elatia data with the Hula transfer function (R^2=0.56). These values compare relatively well against R^2≈0.8 in boot-strap and jack-knife cross-validation (Table 1). Some correlation is apparent with the Ioannina data analysed using the the Sürmene Ağaçbaşı Yaylası transfer function (R^2=0.36) and the Sürmene Ağaçbaşı Yaylası data analysed with the Elatia transfer function (R^2=0.24). However in both these cases the correlation is helped by one data point from the wetter end of the hydrological gradient and if this is excluded the correlation becomes much weaker. In palaeoecological reconstruction it would probably only be valid to use the Elatia transfer function for the Hula data and vice versa.

An obvious explanation for poor model performance with independent test sets would be that the test set contains many taxa which are not included in the training set. On the basis of Fig. 5 a relationship between the strength of correlation and proportion of tests included in the model is not clear. Although reasonable correlations are only produced when a relatively high proportion of tests are included in the model, a high proportion of tests does not necessarily lead to a good correlation between measured and model-predicted DWT. The overwhelming majority of tests from the Ioannina dataset are of taxa included in the Hula and Elatia models and yet there is little correlation between measured and model-predicted values. Similarly a high proportion of tests from the Asi Gonia dataset are included in the Elatia and Sürmene Ağaçbaşı Yaylası models and yet there is also very little correlation with measured values. To directly test the relationship between proportion of tests included and transfer function performance (assessed by R^2) samples with low proportions of tests included in the model were successively removed. Data were filtered by percent of tests of taxa included in the transfer function using cut-off points increasing in increments of 10% from 10% to 90%. Only the data sets from the Elatia Mires, the Hula and Sürmene Ağaçbaşı Yaylası peatland were used for these analyses. Results (Fig. 6) show that there is no simple relationship: only using those samples where a high proportion of tests have their taxa included by the transfer function does not give a higher R^2 value. In fact in many cases excluding samples with poorer fit reduces the strength of correlation between measured and model-predicted values. This result therefore shows that the poor performance of the transfer function models (illustrated by Fig. 5) is not simply caused by a low proportion of tests being included in the transfer function model and therefore valuable hydrological indicator taxa not being included. The transfer function models also perform poorly even if almost all the taxa, and therefore almost all the individuals can be included in the model. Three possible causes of this problem can be suggested- either the hydrological preferences of some amoeba taxa are poorly modelled by the transfer function (possibly due to rarity), there are cryptic species with differing ecological niches, or there are regional differences in the hydrological preferences of amoeba taxa. All of these possibilities are worrying. The problems are particularly acute if applying a transfer function to sites in a different geographical region or to a different peatland type, however this is not the sole problem. The Sürmene Ağaçbaşı Yaylası transfer function is derived from a *Sphagnum*-dominated ombrotrophic mire complex in northeast Turkey, however it performs very poorly with data from the Ispani-2 mire, another *Sphagnum*-dominated ombrotrophic site only 200Km distant.

As a simple representation of the similarity of species optima between the three transfer functions weighted averaged optima are plotted by their relative positions along the hydrological gradient, in order to minimise the importance of the differences in the measured hydrological gradient due to the timing of the studies (cf. Booth 2001). Nine taxa were found in all studies (Fig. 7). While there is some general relationship in optima between transfer functions this is not the case for all taxa. For some taxa there is very wide disparity between optima, particularly notable in *Centropyxis aerophila* type, *Difflugia pulex* type and *Trigonopyxis arcula*. For *Trigonopyxis arcula* this difference is probably explained by a very low abundance in the Hula site (and some uncertainty over identification in this site: Payne et al. submitted), both the other two taxa are groups which may include species with differing hydrological preferences. It should be remembered that this approach ignores the possibility for real wetness differences between the sites, which is quite likely in these data. An increasing emphasis in studies of testate amoebae for environmental reconstruction is combining transfer functions to expand their geographic potential and reduce the possibility of no-analogue problems (e.g. Booth 2007). If the three datasets used here are uncritically combined it is possible to produce a transfer function with an $\text{RMSEP}_{\text{boot}}$ of only 10.1cm, not much poorer than some published results (Fig. 8). However it is important to note that this result is largely because dry samples are restricted to the Turkish site.

Palaeoecological application of testate amoebae in eastern Mediterranean peatlands.

The extent to which it will be possible to obtain palaeoecological data from Eastern Mediterranean peatlands is still somewhat uncertain. In bogs, initial study of a core from the Sürmene Ağaçbaşı Yaylası peatland has shown countable test concentrations thoughout (Payne et al. 2008); further work aims to complete a full palaeoecological study from this site. Palaeoecological study of a core from the Imnati mire in Georgia has also been successfully undertaken (Payne 2009) and initial analysis suggests it would probably also be practicable to apply testate amoeba-based palaeoecology to the Ispani-2 mire. However the situation in fens is more uncertain. Studies of near-surface monoliths from the Elatia Mires have managed to count sufficient tests to obtain palaeoecological records from two sites, but these records only cover the very recent past (Payne & Pates 2009). It was not possible to obtain any palaeoenvironmental data from other sites. Only a few poorly preserved tests were encountered in a core with intermittent peat from Skafidi, Kythera (Greece) and tests were only present in the uppermost levels of a core from site ESA in Jordan (the single sample included in the dataset here). Tests were absent from even surface samples in the Azraq wetland (Jordan). Results so far therefore suggest that palaeoecological results will be obtainable from ombrotrophic sites but successful study of minerotrophic sites may not always be possible.

CONCLUSIONS

This study clearly demonstrates that different types of organic wetlands have differing testate amoeba communities. Ombrotrophic peatlands appear to have amoeba communities which are quite different from those of other wetlands, while there is relatively little difference between the amoeba communities of more nutrient rich fens and those of non-peat wetlands. Species indicative of differing positions on this gradient are identified and may assist changes to be tracked in palaeoecological records. Across the trophic gradient the dominant controls appear to be hydrology and pH; major nutrients were not directly measured in this study but are likely to be important in determining the differences between the amoeba communities of different wetland types. Surprisingly, Zn and Fe emerge as important geochemical variables, the reasons for which are currently unclear.

Although hydrology is a strong control on amoeba communities and models perform well in cross-validation, transfer functions perform poorly when applied to other sites. Partly this is inevitable due to the use of one-off water table measurements, but problems appear more fundamental. There are apparent differences in the relative position of hydrological optima of some taxa between studies suggesting either that optima are poorly characterised or problems with cryptic species of differing optima. Caution needs to be used when applying transfer functions beyond the area from which they were derived, and particularly to sites of differing trophic status. It may be possible to successfully use testate amoebae to reconstruct hydrological change in fens (recent research in North America has produced very encouraging results: Booth 2010) but may not be possible to use testate amoebae to reconstruct hydrological change through the fen-bog transition and other major ecosystem changes.

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FIGURES

Figure 1. Location of sampling sites in this study. Further detail of surface sampling sites is given in Table 2.

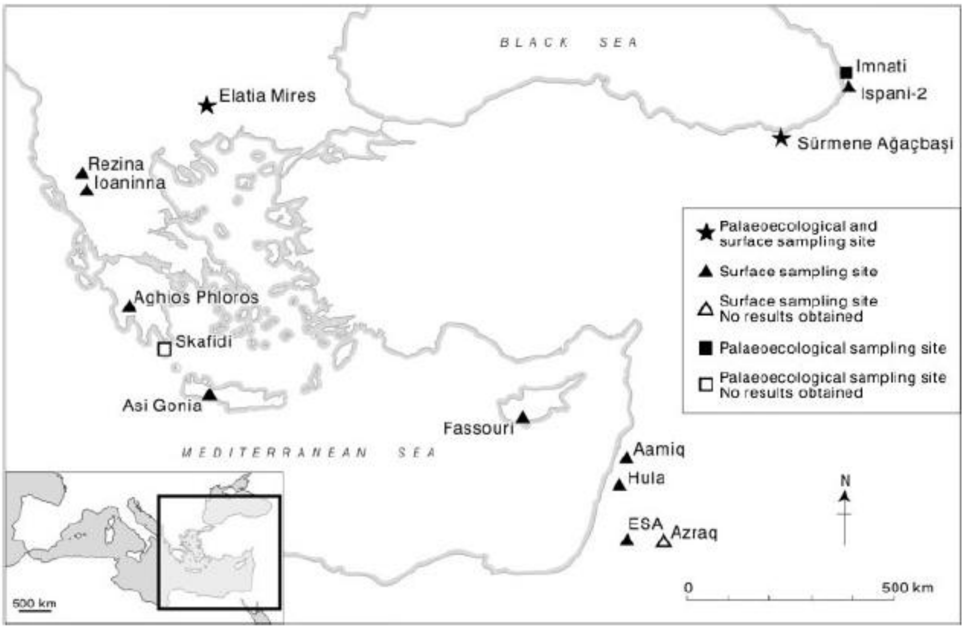


Figure 2. Scatter plot of mean loss on ignition against mean pH by site.

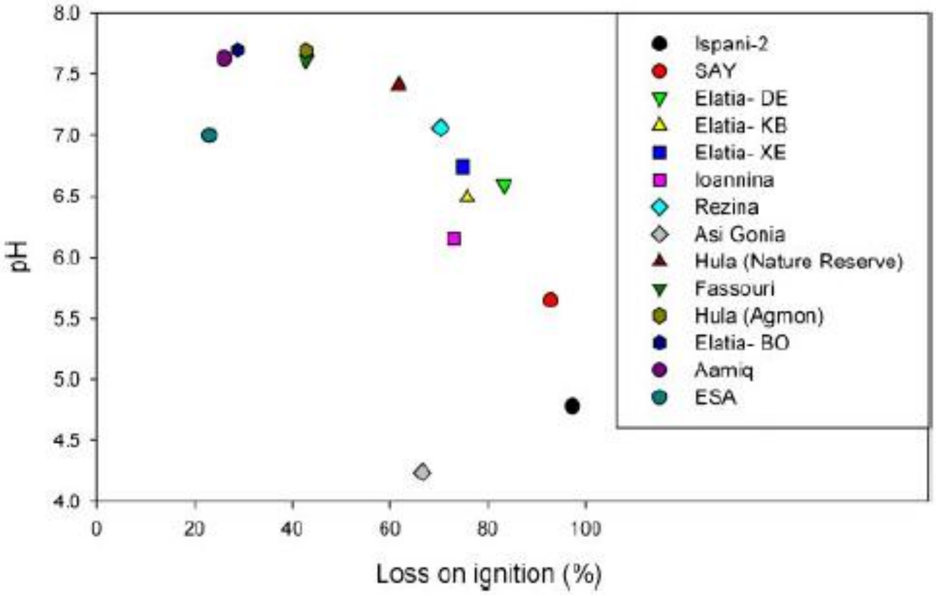


Figure 3. Principal components analysis (PCA) of Hellinger-transformed testate amoeba data. Showing site groupings identified in Table 3.

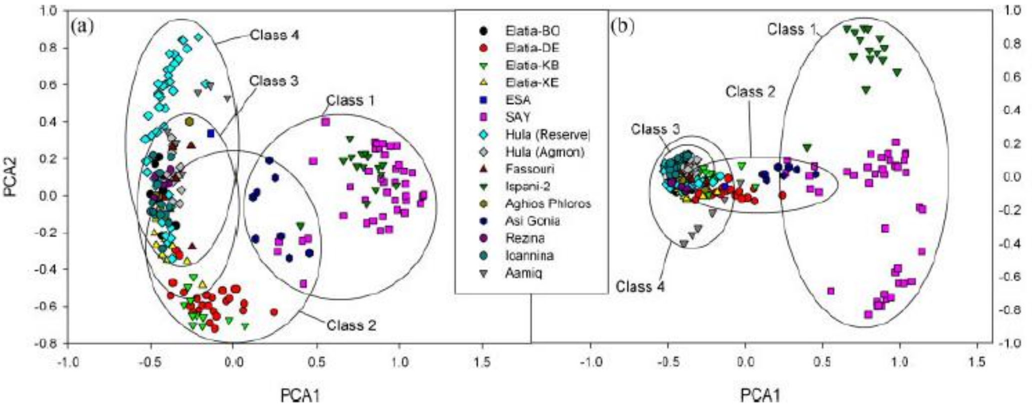


Figure 4. Redundancy analysis (RDA) of Hellinger-transformed testate amoeba data, also showing selected taxa and environmental variables. Plot a) is based on all data and plot b) only on samples with geochemical data.

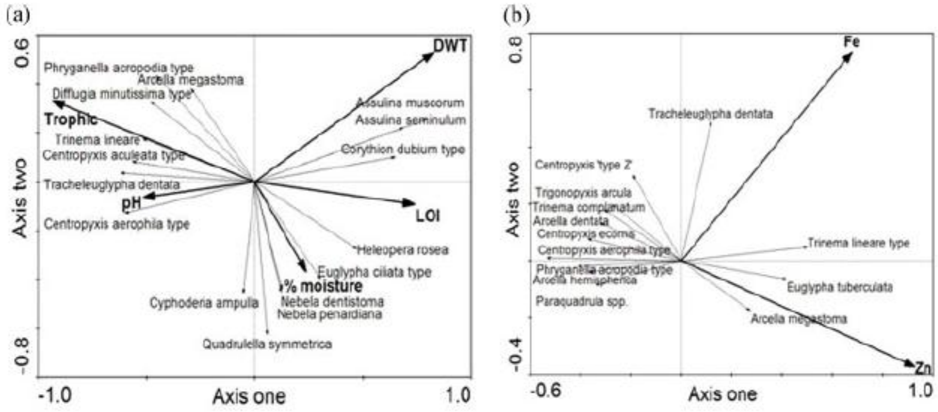


Figure 5. Model predicted against measured depth to water table (DWT) for six datasets using three transfer functions. Main plots show model prediction with boot-strapped standard error estimate (1000 permutations), against measured values. Also showing (on the right of the plots) box and whisker plot showing percent of total tests included in the model by sample. Cross-validation results for transfer function datasets are shown for reference. R^2 values are shown for each comparison; R^2 values are given in parentheses where relationship is negative and R^2_{boot} values are shown for cross-validations. For brevity the Sürmene Ağaçbaşı Yaylası peatland dataset is referred to as 'Turkey data'.

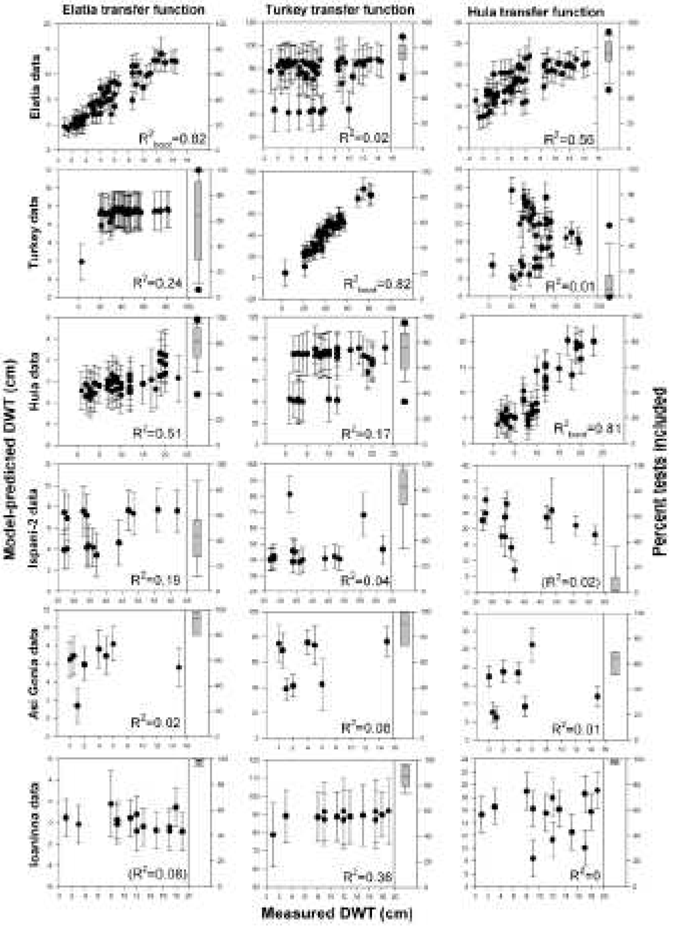


Figure 6. Impact of filtering data for percent of tests included in transfer function model. Showing, R^2 between measured and predicted values (circles) and number of samples remaining (crosses). R^2 values shown by white circles represent negative correlations.

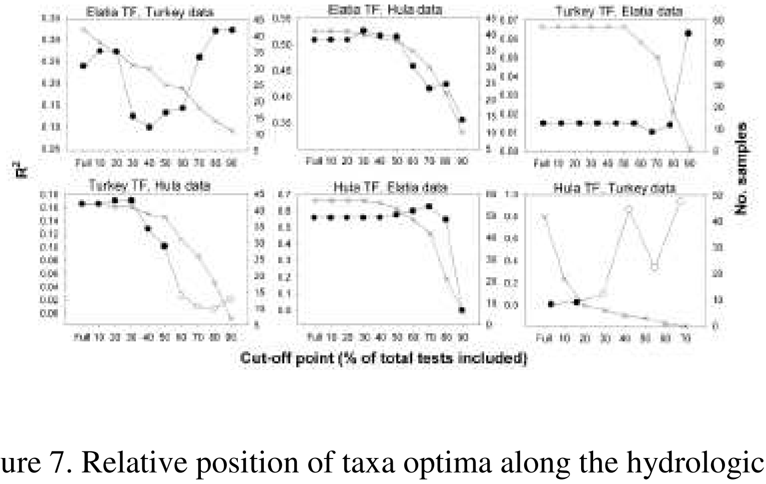


Figure 7. Relative position of taxa optima along the hydrological gradient estimated by weighted averaging.

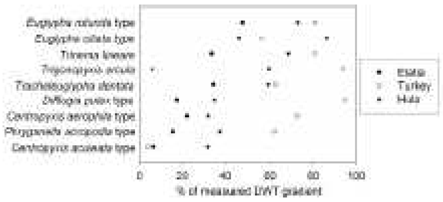
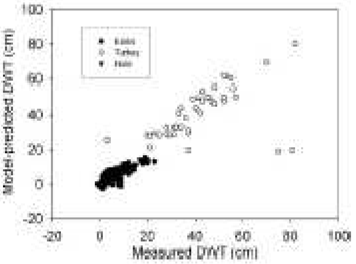


Figure 8. Observed against transfer function predicted depth to water table using a combined model.



TABLES

Table 1. Transfer function model performance. These values differ slightly from those in the original studies due to the taxonomic harmonisation carried out (Appendix 1).

Table 2. Site characteristics of wetlands in this study.

Table 3. Classification of sites into groups based on vegetation and topography.

Table 4. Comparison of groupings identified by *K*-means partitioning and *a priori* classification (Table 3).

Table 5. Indicator species of four groups of wetlands in this study (Table 3) showing taxa with IndVal ≥30 significant at P=0.001. See Dufrêne and Legendre (1997) for details of the IndVal approach.

Table 6. Results of redundancy analysis of testate amoeba data showing percent variance explained by various combinations of variables and co-variables and P-values determined by Monte Carlo permutation tests (999 permutations).

APPENDIX 1. Full species list and details of taxonomic scheme used in this study.

Table 1. Transfer function model performance. These values differ slightly from those in the original studies due to the taxonomic harmonisation carried out.

Dataset	**Model structure**	**Samples**	**Taxa**	**$RMSEP_{jack}$ (cm)**	**$RMSEP_{boot}$ (cm)**	**Max $Bias_{jack}$ (cm)**	**Max $Bias_{boot}$ (cm)**	**R^2_{jack}**	**R^2_{boot}**
Elatia	Maximum Likelihood	57	46	1.8	2.0	1.4	2.8	0.80	0.82
Sürmene Ağaçbaşı Yaylası	Maximum Likelihood	42	34	7.1	8.1	21.4	20.4	0.82	0.82
Hula	Weighted average (inverse deshrinking)	41	23	2.7	2.8	3.5	3.7	0.81	0.81

Table 2. Site characteristics of wetlands in this study.

Site	Location	Site type	Dominant plant species of sampling area	Human impacts	N	Further site details and previous research
Sürmene Ağaçbaşı Yaylası [SAY]	NE. Turkey	Ombrotrophic (blanket?) bog	*Sphagnum palustre, Sphagnum fuscum*, *Carex* spp.	Peat cutting and grazing.	53	Payne et al. (2007, 2008), Byfield & Őzhatay (1997), Aytuğ et al. (1975)
Ispani-2	W. Georgia	Percolation bog	*Sphagnum papillosum, Sphagnum rubellum, Molinia litoralis*	Minor peripheral drainage	15	Joosten et al. (2003), Connor et al. (2007), De Klerk et al. (2009)
Elatia-BO	NE. Greece	Mesotrophic fen	*Carex* spp., *Mentha spicta*	Little.	9	Payne and Mitchell (2007), Papazisimou et al. (2002)
Elatia-DE	NE. Greece	Mesotrophic fen	*Plagiomnium elatum, Juncus effusus, Sphagnum flexuosum*	Little.	24	Payne and Mitchell (2007), Papazisimou et al. (2002)
Elatia-KB	NE. Greece	Mesotrophic fen	*Plagiomnium elatum, Juncus effusus, Sphagnum flexuosum*	Little.	14	Payne and Mitchell (2007), Papazisimou et al. (2002)
Elatia-XE	NE. Greece	Mesotrophic fen	*Carex* spp., *Mentha spicta*	Little.	13	Payne and Mitchell (2007), Papazisimou et al. (2002)
Rezina	W. Greece	Mesotrophic fen	*Carex rostrata*	Little obvious impact. Some grazing.	5	Willis (1992)
Asi Gonia	Crete (S. Greece)	Mesotrophic fen	*Scirpoides holoschoenus, Juncus effusus*, *Sphagnum* spp.	Grazing and burning.	8	Atherden & Hall (1999)
Ioaninna	W. Greece	Eutrophic fen	*Phragmites australis*	Nutrient enrichment of adjacent lake.	14	Christanis (1996)
ESA	Jordan	Rheotrophic fen(?)	Unclear	None known.	1	-
Aghios Phloros	SW. Greece	Drained fen (Samples from peripheral spring)	*Phragmites australis*	Drained peatland.	5	Papazisimou et al. (2005)
Hula* (Nature Reserve)	N. Israel	Reedswamp	*Cyperus papyrus, Phragmites australis*	Retained fragment of drained wetland. Nutrient enrichment.	43	Hambright and Zohary (1998, 1999), Payne et al. (in press)
Hula* (Agmon)	N. Israel	Reedswamp	*Phragmites australis*	Restored area of drained peatland.	11	Hambright and Zohary (1998, 1999), Payne et al. (in press)
Aamiq*	Lebanon	Reedswamp	*Phragmites australis*	Hydrological modification	6	-
Fassouri*	Cyprus[1]	Reedswamp	*Phragmites australis*	Hydrological modification	5	-

* Spelling varies by transliteration system.

[1] While situated on the island of Cyprus this site is technically part of the United Kingdom as it lies within the Akrotiri ‘Sovereign Base Area’.

Table 3. Classification of sites into groups based on vegetation and topography.

Class	Vegetation type	Typical species	Sites
1	*Sphagnum*- dominated (ombrotrophic bog)	*Sphagnum* spp.	Sürmene Ağaçbaşı Yaylası Ispani-2
2	Bryophyte-dominated, mesotrophic and oligotrophic fens	Brown mosses, *Sphagnum* spp.	Elatia-DE Elatia-KB Asi Gonia[†]
3	Rush- and sedge-dominated mesotrophic fens	*Juncus* spp., *Carex* spp.	Rezina Elatia-XE Elatia-BO[‡] ESA[∞]
4	Reed-dominated fens and swamps	*Phragmites australis, Cyperus papyrus*	Ioannina Hula Nature Reserve Hula-Agmon Aghios Phloros Fassouri Aamiq

[†] The vegetation of this site contains large quantities of rushes but is included in this category as the presence of *Sphagnum* suggests comparatively nutrient-poor conditions.

[‡] Although surficial sediments are inorganic peat sediments are found a short distance below the surface so this classification is considered most appropriate.

[∞] There is a degree of uncertainty in this classification.

Table 4. Comparison of groupings identified by *K*-means partitioning and *a priori* classification (Table 3).

Trophic class*	Site type	K-means groups				
		1	**2**	**3**	**4**	**5**
1	*Sphagnum*- dominated ombrotrophic bog		5	13	14	25
2	Bryophyte-dominated mesotrophic and oligotrophic fens	1	34			
3	Rush- and sedge-dominated mesotrophic fens	10	25			1
4	Reed-dominated fens and swamps	35	48			

* see Table 3.

Table 5. Indicator species of four groups of wetlands in this study (Table 3) showing taxa with IndVal ≥30 significant at P=0.001. See Dufrêne and Legendre (1997) for details of the IndVal approach.

Class 1	Class 2	Class 3	Class 4
***Sphagnum*- dominated ombrotrophic bogs**	**Bryophyte-dominated, mesotrophic and oligotrophic fens**	**Rush- and sedge-dominated mesotrophic fens**	**Reed-dominated fens and swamps**
Assulina muscorum (93.3)	*Euglypha ciliata* type (73.8)	*Difflugia pulex* type (51.8)	*Phryganella acropodia* type (65.2)
Assulina seminulum (86.0)	*Quadrulella symmetrica* (73.6)	*Cyphoderia ampulla* (51.1)	*Difflugia minutissima* type (54.6)
Corythion dubium (43.7)	*Nebela penardiana* (69.8)	*Centropyxis aerophila* type (50.9)	*Trinema lineare* (42.5)
Hyalosphenia papilio (38.6)	*Difflugia lucida* (57.8)	*Difflugia pristis* type (47.2)	*Arcella megastoma* (41.8)
	Euglypha rotunda type (56.4)	*Centropyxis aculeata* type (47.1)	*Tracheleuglypha dentata* (35.5)
	Arcella vulgaris (52.0)	*Trinema enchelys* (42.8)	
	Heleopera rosea (45.8)	*Euglypha tuberculata* (38.9)	
	Nebela lageniformis (40.4)	*Arcella discoides* (34.2)	
	Trinema complanatum (36.3)	*Difflugia penardi* (31.1)	

Table 6. Results of redundancy analysis of testate amoeba data showing percent variance explained by various combinations of variables and co-variables and P-values determined by Monte Carlo permutation tests (999 permutations).

Analysis	***n***	**Explanatory variables**	**Co-variables**	**% variance**	***P***
1	193 [a]	DWT, pH, LOI, %moisture, Trophic index	-	35.2	0.001
2	193 [a]	DWT, pH, LOI, %moisture	Sites	5.3	0.001
3	193 [a]	DWT	pH, LOI, % moisture	8.7	0.001
4	193 [a]	DWT	pH, LOI, % moisture, Sites	3.0	0.001
5	193 [a]	pH	DWT, LOI, % moisture	1.6	0.001
6	193 [a]	pH	DWT, LOI, % moisture, Sites	0.4	0.019
7	193 [a]	LOI	DWT, pH, % moisture	2.6	0.001
8	193 [a]	LOI	DWT, pH, % moisture, Sites	0.3	ns
9	193 [a]	% moisture	DWT, pH, LOI	1.2	0.001
10	193 [a]	% moisture	DWT, pH, LOI, Sites	0.2	ns
11	193 [a]	Trophic index	DWT, pH, LOI, % moisture	7.7	0.001
12	64 [b]	Zn, Fe*	Sites	12.6	0.001
13	64 [b]	Zn	Fe, Sites	7.9	0.001
14	64 [b]	Fe	Zn, Sites	2.4	0.02

* Minimal suite selected using forward selection from all non-hydrological environmental variables based on a cut-off at P=0.05.

[a] All samples with full suite of environmental data.

[b] All samples with geochemical data.

Appendix 1. Full species list and details of taxonomic scheme used in this study.

Taxon	**Definition of groups and taxonomic notes**	**Taxa differentiated in component studies combined here.**
Amphitrema stenostoma Nüsslin 1884		
Arcella arenaria Greef 1866 type	Synonymous with *Arcella catinus* type of Charman et al. (2000).	*Arcella arenaria* type, *Arcella catinus.*
Arcella dentata Ehrenberg 1830		
Arcella discoides Ehrenberg 1872 type	Follows Charman et al. (2000).	
Arcella hemispherica Perty 1852		
Arcella megastoma Penard 1902		
Arcella vulgaris Ehrenberg 1830		
Archerella flavum (Archer 1877)	Formerly *Amphitrema flavum.*	
Assulina muscorum Greef 1888		
Assulina seminulum Ehrenberg 1848		
Bullinularia indica Penard 1907		
Centropyxis aculeata (Ehrenberg 1830) type	Follows Charman et al. (2000), includes all spined forms.	
Centropyxis aerophila Deflandre 1929 type	Includes all sub-rounded centropyxidae with ovoid sub-terminal aperture. Includes *Centropyxis platystoma* and *Centropyxis cassis* types of Charman et al. (2000).	*Centropyxis aerophila* type, *Centropyxis platystoma* type.
Centropyxis ecornis (Ehrenberg 1841) type	Includes *Centropyxis ecornis* and *Centropyxis laevigata.*	*Centropyxis ecornis, Centropyxis laevigata.*
Corythion dubium Taranek 1881		
Cyphoderia ampulla Ehrenberg 1840		
Cyphoderia trochus Penard 1899		
Cryptodifflugia oviformis Penard 1890		
Cyclopyxis 'type Z'	Unidentified taxon, see Payne et al. (submitted).	
Difflugia avellana Penard 1890		
Difflugia gassowskii Ogden 1983		
Difflugia lucida Penard 1890		
Difflugia minutissima Penard 1904 type	Very small ovoid *Difflugia* tests (<20µm length).	
Difflugia oblonga Ehrenberg 1838 type	Follows Charman et al. (2000).	
Difflugia penardi Hopkinson 1909		
Difflugia pristis Penard 1902 type	Follows Charman et al. (2000).	
Difflugia pulex Penard 1902 type	Small ovoid *Difflugia* tests (approx 20-40µm length).	
Difflugia rubescens Penard 1891		
Difflugia spp.	Other *Difflugia* taxa including unidentified or very rare taxa	*Difflugia* 'type X', *Difflugia* 'species 2', *Difflugia microstoma, Difflugia* cf. *lacustris, Difflugia* cf. *glans, Difflugia elegans.*
Euglypha acanthophora Ehrenberg 1841		
Euglypha ciliata (Ehrenberg 1848) type	Includes *Euglypha ciliata* and *Euglypha compressa.*	*Euglypha ciliata* var. glabra, *Euglypha ciliata* type, *Euglypha compressa.*
Euglypha cristata Leidy 1874		
Euglypha filifera Penard 1902		
Euglypha rotunda Wailes 1911 type	Follows Charman et al. (2000).	
Euglypha strigosa (Ehrenberg 1872)		
Euglypha tuberculata Dujardin 1841		
Heleopera petricola Leidy 1879		
Heleopera rosea Penard 1890		
Heleopera sphagni Leidy 1874		
Heleopera sylvatica Penard 1890		
Hyalosphenia papilio Leidy 1875		
Lesquereusia epistomium Penard 1902		
Lesquereusia modesta Rhumber 1895		
Lesquereusia spiralis (Ehrenberg 1840)		
Nebela dentistoma Penard 1890		
Nebela lageniformis Penard 1890		
Nebela militaris Penard 1890		
Nebela penardiana Deflandre 1936		
Nebela tincta (Leidy 1879) type	Not distinguished from similar taxa without pores.	
Nebela *tubulata* Brown 1911		
Nebela tubulosa Penard 1890		

Phryganella acropodia (Hertwig & Lesser 1874) type	All ‘bowl-shaped’ tests. Synonymous with *Cyclopyxis arcelloides* type of Charman et al. (2000).	*Phryganella acropodia* type, *Cyclopyxis eurystoma, Cyclopyxis arcelloides* type
Plagiopyxis spp.	All *Plagiopyxis* species.	*Plagiopyxis* spp., *Plagiopyxis* cf. *callida.*
Paraqudarula spp.	All *Paraquadrula* species.	*Paraquadrula* spp., *Paraquadrula irregularis.*
Pontigulasia elisa Penard 1893		
Quadrulella symmetrica Wallich 1863		
Sphenoderia fissirostris Penard 1890		
Sphenoderia lenta Schlumberger 1845		
Tracheleuglypha dentata Moniez 1888		
Trinema complanatum Penard 1890		
Trinema enchelys Ehrenberg 1838		
Trinema lineare Penard 1890		
Trigonopyxis arcula (Leidy 1879)		
Habrotrocha angusticollis Murray 1905[1]		

[1] This test-forming bdelloid rotifer is commonly found among testate amoebae communities and has been included in the analyses here.