

# Ecology of Testate Amoebae from Mires in the Central Rhodope Mountains, Greece and Development of a Transfer Function for Palaeohydrological Reconstruction

Richard J. Payne<sup>a,b,1</sup>, and Edward A.D. Mitchell<sup>c,d</sup>

<sup>a</sup>The Fitch Laboratory, British School at Athens, Odhos Souidhías 52, GR-10676 Athens, Greece

<sup>b</sup>Geography, School of Environment and Development, The University of Manchester, Oxford Road, Manchester M13 9PL, UK

<sup>c</sup>WSL Swiss Federal Research Institute, Ecosystem Boundaries Research Unit, Wetlands Research Group, Station 2, CH-1015 Lausanne, Switzerland

<sup>d</sup>Ecole Polytechnique Fédérale de Lausanne, Laboratory of Ecological Systems, Station 2, CH-1015 Lausanne, Switzerland

**Testate amoebae are useful environmental indicators in ecological and palaeoecological studies from peatlands. Previous quantitative studies have focused on the peatlands of Northern and Central Europe, North America, and New Zealand and have considered a relatively restricted variety of peatland types, mostly ombrotrophic or *Sphagnum*-dominated while more minerotrophic fens have been less studied. Here we present the first quantitative ecological study of testate amoebae from four small mesotrophic fens (pH 5.5–8.1) in the Elatia Forest, northern Macedonia province, Greece. Relationships with the environmental data were investigated using redundancy analysis and mantel tests. Transfer function models were derived using a variety of techniques. Results demonstrate that as for *Sphagnum*-dominated mires hydrology is the most important control on amoebae community composition. Transfer function models should enable water tables to be predicted within 2.5 cm, when data selection is used this is reduced to less than 2 cm. pH is also an important environmental control on testate amoebae communities, a transfer function model enables pH prediction within 0.4 pH units. The hydrological transfer function is the best performing such model yet produced in terms of prediction error. This study provides new data on the ecology of testate amoebae in fens, and the transfer function models should allow quantitative palaeohydrological reconstruction.**

**Key words:** protists; transfer functions; palaeoclimate; peatlands; mire surface wetness.

<sup>1</sup> Corresponding author;  
e-mail richpayne@hotmail.com (R.J. Payne).

**Abbreviations:** Boot, results assessed by bootstrap cross validation e.g. RMSEP<sub>boot</sub> denotes RMSEP assessed by bootstrapping; DWT, depth to water table; Jack, results assessed by jack-knife (also termed ‘leave-one-out’) cross validation e.g. RMSEP<sub>jack</sub> denotes RMSEP assessed by jack-knifing; LOI, loss on ignition; MaxBias; maximum bias; ML, maximum likelihood; PLS, partial least squares; RMSEP, root mean square error of prediction; WA, weighted average; WA-PLS, weighted average partial least squares.

## Introduction

Testate amoebae (Protista) are an abundant group of micro-organisms in many terrestrial and aquatic environments and are particularly numerous in peatlands where they may constitute as much as 30% of the microbial biomass (Gilbert et al. 1998; Mitchell et al. 2003). Testate amoebae species have closely defined preferences for a variety of environmental variables including hydrology, pH and nutrient status and are valuable as bioindicators (Booth 2001; Charman and Warner 1992; Mitchell et al. 1999; Tolonen et al. 1994; Warner 1987). Testate amoebae are characterised by a decay-resistant shell (the test), which may remain preserved for millennia and allow species-level identification long after death. The combination of good preservation and defined ecological niches makes testate amoebae a useful tool in palaeoecological studies (Charman 2001).

Palaeoecological studies using testate amoebae have primarily concentrated on reconstructing hydrological change. In ombrotrophic (precipitation fed) peatlands hydrology is believed to be directly linked to hydroclimate. Therefore by reconstructing the movement of bog water tables it is possible to reconstruct Holocene climatic change. Testate amoebae-climate reconstructions agree well with instrumental climate data and other proxy-climate records (Booth and Jackson 2003; Charman and Hendon 2000; Charman et al. 2001, 2004; Schoning et al. 2005).

Quantitative hydrological reconstruction requires transfer function models that relate community compositions to a measurable environmental variable. Transfer function models for hydrology have now been developed from regions around the world with the greatest concentration in North America and northern and central Europe (Bobrov et al. 1999; Booth 2001, 2002; Charman and Warner 1992, 1997; Lamentowicz and Mitchell 2005; Mitchell et al. 1999; Payne et al. 2006; Tolonen et al. 1994; Warner and Charman 1994; Wilmshurst et al. 2003; Woodland et al. 1998). However, there have been no quantitative studies of testate amoebae in peatlands in southern Europe or more arid regions in general.

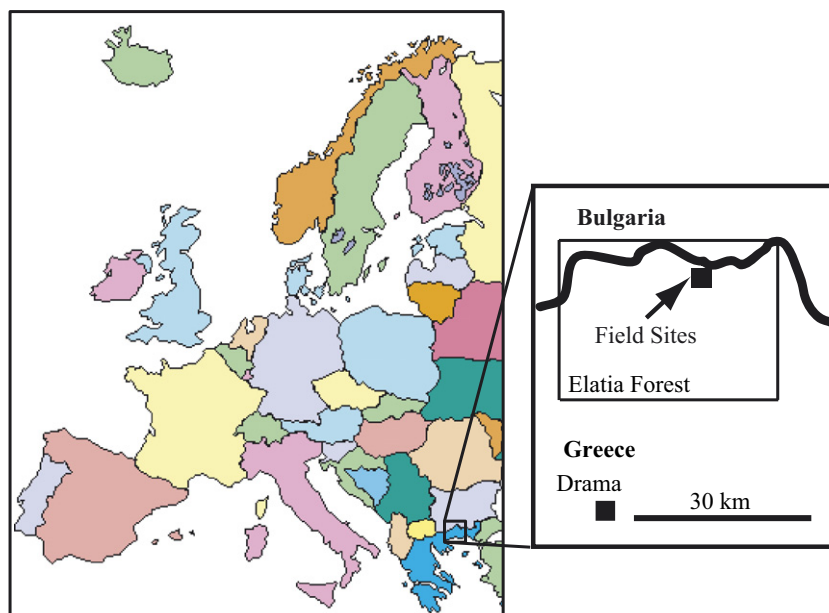
Peatlands are rare in Greece due to the warm/dry climate and prevalence of limestone bedrocks, combined with anthropogenic drainage over several thousand years (Bouzinou et al. 1994, 1997; Christanis 1996; Papazisimou et al. 2002). Peatlands, primarily topogeneous fens exist in several areas, particularly in the north of the country (Botis

et al. 1993; Bouzinou et al. 1997, 2000; Christanis 1996). Ombrotrophic peatlands are not known in Greece but more oligotrophic mires are present in the uplands of northern Macedonia and Epirus. Although testate amoebae have been studied in Greek soils (e.g. Bonnet 1967, 1986) we are not aware of any previous studies from Greek peatlands. Although peatland testate amoebae are generally believed to be cosmopolitan, the existence of local species (either morphologically distinct or cryptic) or locally adapted populations is possible. It would therefore be inappropriate to assume that studies from elsewhere in Europe are also applicable to Greek mires.

This work investigates testate amoebae ecology from mires in northern Greece. The aims of the study are fourfold: (1) to add to our knowledge of these important micro-organisms and their interaction with their environment, (2) to establish the community composition of these peatland sites and therefore add to our understanding of testate amoebae diversity and biogeography, (3) to produce transfer function models which may allow palaeohydrological and palaeoacidity reconstruction from mires in Greece, and (4) to compare the ecological optima of taxa and performance of the transfer functions with data from other regions.

## Sites

The mires investigated lie in an area of mature coniferous forest in the heart of the Elatia Forest in the Central Rhodope Mountains, approximately 40 km north of Drama (41°29'N, 24°19'E; 1500 masl; Fig. 1). The area has a moist continental climate, annual rainfall is around 1200 mm and mean annual temperature is around 9 °C (Prefectural Local Government of Drama 2000). The study area is underlain by the Elatia-Skaloti granites (Papazisimou et al. 2002) and the combination of these impermeable substrates and the comparatively cool and wet climate has probably allowed the formation of the unusual Elatia mires. Papazisimou et al. (2002) described the general morphology, stratigraphy and vegetation of four small peatland sites, which they classified as transitional mires, and assigned site codes that are also used here (Table 1).



**Figure 1.** Location map of Elatia site.

## Results

### Data Summary

Environmental data show a restricted hydrological gradient compared to other studies (DWT values from  $-1$  to  $15.5$  cm) but significant variance in pH ( $5.5$ – $8.1$ ) and organic content ( $20$ – $92\%$  loss on ignition). Sites KB and DE are notable for drier surface conditions while site BO has less organic sediments than the other sites (Fig. 2). A total of 9386 amoebae of 48 taxa were counted (Table 2); the most abundant were *Euglypha rotunda* type ( $20\%$  of total count), *Centropyxis aerophila* type ( $14.3\%$  of total) and *Trinema lineare* ( $12.6\%$  of total).

### Ordination and Mantel Tests

Principal components analysis results (Fig. 3) show distinct differences in amoebae communities among sites. Sites KB and DE have higher scores on axis one and sites KB and BO have higher scores on axis two. In the redundancy analysis water table depth, pH, organic content and the site variables together explain  $42\%$  of the variance ( $P < 0.001$ ) (Fig. 4). About one-third ( $35\%$ ) of the variance is explained by the site variables and another third ( $33\%$ ) by the environmental variables. Water table depth alone explains  $20\%$  of variance ( $P < 0.002$ ). When variance partitioning

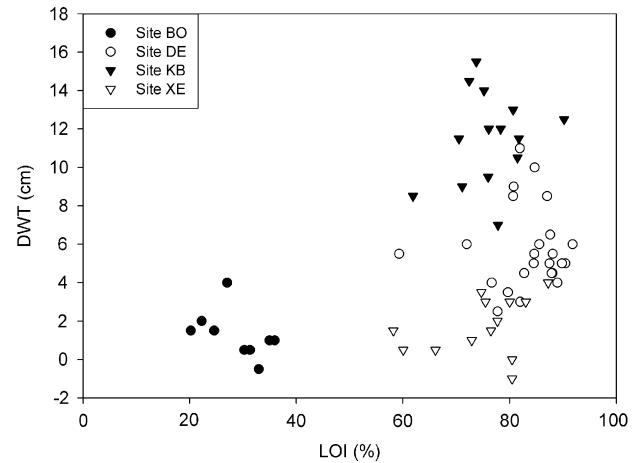
is used site variables independently explain  $7.9\%$  of variance and environmental variables independently explain  $9.8\%$  of variance ( $P < 0.001$ ). With organic content and pH (but not the site variables) partialled out, water table depth explains  $10\%$  of variance ( $P < 0.001$ ).

Principal components analysis of plant samples (Fig. 5) also shows differences among sites and some general similarity with the amoebae data. Sites XE and BO have lower scores on axis one than sites KB and DE. BO samples are tightly clustered on the left of the plot. The redundancy analyses show DWT, pH and LOI explain  $40\%$  of variance ( $P < 0.001$ ). Site variables independently explain  $12.1\%$  of variance and environmental variables independently explain  $8.7\%$  of variance.

The Mantel tests show that all data sets (amoebae, plants, sites and environmental data) are significantly correlated ( $P < 0.001$ ), with the best correlation between the testate amoebae and environmental data ( $r = 0.58$ ). For the amoebae data the correlation with the environmental variables is significantly better ( $r = 0.58$ ) than with the sites ( $r = 0.41$ ), for the plant data these correlations are approximately the same but much lower ( $r = 0.27, 0.26$ ). The amoebae are well correlated with the vegetation ( $r = 0.43$ ). Most of this correlation remains even when the site and environmental variables are partialled out ( $r = 0.32, P < 0.001$ ).

**Table 1.** Details of four peatland sites used in this study. See Papapazisimou et al. (2002) for further details of the mires.

Site code	Major plant species	Size	Mean pH	Mean LOI (%)	Mean DWT (cm)	Evidence of external impacts
BO	<i>Carex</i> spp., <i>Mentha spicata</i>	20 × 30 m	7.6	27	1	Animal tracks
XE	<i>Carex</i> spp., <i>Cyperus longus</i> , <i>Juncus effusus</i> , <i>Juncus articulatus</i> , <i>Eriophorum latifolium</i> , <i>Plagiomnium elatum</i> , <i>Mentha spicata</i> , <i>Parnassia palustris</i> , <i>Ranunculus</i> spp., <i>Hypnum lindbergii</i>	50 × 30 m	6.7	75	1.8	Litter around site margins
DE	<i>Juncus</i> spp., <i>Eriophorum latifolium</i> , <i>Sphagnum flexuosum</i> , <i>Plagiomnium elatum</i> , <i>Mentha spicata</i> , <i>Myosotis</i> spp., <i>Aulacomnium palustris</i>	50 × 80 m	6.6	82	5.8	
KB	<i>Juncus effusus</i> , <i>Carex</i> spp., <i>Eriophorum latifolium</i> , <i>Myosotis</i> spp., <i>Geum</i> spp., <i>Ranunculus</i> spp., <i>Mentha spicata</i> , <i>Plagiomnium elatum</i> , <i>Sphagnum flexuosum</i> , <i>Aulacomnium palustris</i>	20 × 50 m	6.4	76	11.5	Animal tracks. Some evidence of historic logging activities

**Figure 2.** Scatter plot of depth to water table (DWT) against Loss on Ignition (LOI) for samples from the four Elatia Mires.

### Species-Environment Modelling

Results of transfer function modelling show the best performing model for hydrology is a two-component WA-PLS model when assessed by  $RMSEP_{jack}$  but a maximum likelihood model when assessed by  $RMSEP_{boot}$  (Table 3). The bootstrapped results, and therefore the maximum likelihood model, are preferred due to the greater number of cycles employed by this technique. For pH a weighted average model performs best by both  $RMSEP_{jack}$  and  $RMSEP_{boot}$ . Model performance is consistent across the hydrological gradient without the under-prediction of water table depths for the driest sites seen in previous studies (Mitchell et al. 1999; Payne et al. 2006; Fig. 6). Removing rare taxa produces a general increase in  $RMSEP_{boot}$  for both pH and water table depth (Fig. 7). The entire data set was therefore used for the subsequent stage of data filtering. Removing samples with high residuals produces an improvement in model performance for pH but a more complex pattern for water table depth. Samples with residuals greater than 3.5 cm were excluded from the water table data and samples with residuals greater than 0.6 from the pH data. These cut-off points represent approximately 20% of the total environmental gradients, a similar proportion to some previous studies (Charman 1997; Payne et al. 2006). Using these filtered data  $RMSEP_{boot}$  is reduced to 1.93 cm for water table depth and 0.35 for pH at the expense of reducing the number of samples to 57 for DWT and 56 for pH (Table 4). The DWT gradient is reduced by 1 cm and the pH gradient by 0.1.

**Table 2.** Taxa encountered in this study, abbreviations (Abbrev) used in Figure 4, number of occurrences (N) and pH and water table (DWT) optima (Opt) and tolerances (Tol) calculated using weighted averaging. It is important to note that weighted averaging does not produce the best-performing model. These data are included to allow comparisons with other studies and are not intended to be used for palaeoecological inference.

Taxa	Abbrev	N	DWT Opt (cm)	DWT Tol (cm)	pH Opt	pH Tol
<i>Arcella arenaria</i> Greeff 1866	AAREN	11	5.9	3.9	6.6	0.3
<i>Arcella catinus</i> Penard 1890	ACAT	1	3.5	—	6.9	—
<i>Arcella discoides</i> Ehrenberg 1843	ADISC	24	3.7	3.5	6.9	0.6
<i>Arcella hemisphaerica</i> Perty 1852	AHEMI	8	1.2	0.8	7.0	0.7
<i>Arcella vulgaris</i> Ehrenberg 1830	AVULG	25	6.7	3.5	6.6	0.4
<i>Assulina muscorum</i> Greeff 1888 type	AMUSC	11	7.1	3.3	6.4	0.6
<i>Centropyxis aerophila</i> Deflandre 1929 type	CAERO	60	3.6	3.4	7.0	0.6
<i>Centropyxis aculeata</i> Ehrenberg 1830 type	CACU	34	1.0	2.0	7.2	0.6
<i>Corythion dubium</i> Taranek 1881	CDUB	24	7.6	3.0	6.2	0.5
<i>Cryphoderia ampulla</i> Ehrenberg 1840	CAMP	39	2.5	2.5	7.0	0.6
<i>Diffugia avellana</i> Penard 1890	DAVEL	15	4.2	3.6	6.6	0.5
<i>Diffugia gassowskii</i> Ogden 1983	DGASS	14	5.0	3.1	6.6	0.4
<i>Diffugia lucida</i> Penard 1890	DLUCI	31	6.4	3.2	6.5	0.3
<i>Diffugia microstoma</i> Thomas 1954	DMIC	1	8.5	—	6.5	—
<i>Diffugia oblonga</i> Ehrenberg 1838	DOBLO	27	3.7	3.2	6.7	0.6
<i>Diffugia penardi</i> Hopkinson 1909	DPEN	19	2.7	3.3	7.4	0.6
<i>Diffugia pristis</i> Penard 1902 type	DPRIS	21	2.7	2.1	7.0	0.6
<i>Diffugia pulex</i> Penard 1902 type	DPUL	40	2.8	2.6	6.8	0.5
<i>Diffugia rubescens</i> Penard 1891	DRUB	10	4.7	1.6	6.5	0.5
<i>Diffugia</i> 'species 2'	DSP2	4	5.9	5.5	6.7	0.2
<i>Euglypha acanthophora</i> Ehrenberg 1841	EACAN	2	6.0	3.5	6.6	0.2
<i>Euglypha ciliata</i> Ehrenberg 1848 type	ECIL	32	7.9	3.1	6.3	0.5
<i>Euglypha ciliata</i> Ehrenberg 1848 var. <i>glabra</i> type	ECILG	36	6.3	3.2	6.5	0.4
<i>Euglypha cristata</i> Leidy 1874	ECRIS	6	9.6	2.4	6.1	0.6
<i>Euglypha rotunda</i> Wailes 1911 type	EROT	60	7.6	4.2	6.6	0.4
<i>Euglypha strigosa</i> Ehrenberg 1872	ESTRI	4	7.8	3.6	6.2	0.4
<i>Euglypha tuberculata</i> Dujardin 1841	ETUB	55	4.9	3.9	6.7	0.6
<i>Heleopera petricola</i> Leidy 1879	HPET	1	9.0	—	6.6	—
<i>Heleopera rosea</i> Penard 1890	HROS	47	6.4	3.4	6.5	0.4
<i>Heleopera sphagni</i> Leidy 1874	HSPHA	4	9.4	3.2	6.8	0.4
<i>Lesquereusia spiralis</i> Ehrenberg 1840	LSPIR	3	4.6	0.8	6.4	0.6
<i>Lesquereusia modesta</i> Rhumbler 1895	LMOD	1	5.0	—	6.9	—
<i>Nebela dentistoma</i> Penard 1890	NDENT	39	4.0	3.9	6.9	0.7
<i>Nebela lageniformis</i> Penard 1890	NLANG	21	7.2	2.9	6.5	0.5
<i>Nebela militaris</i> Penard 1890	NMILI	3	9.7	2.2	6.7	0.2
<i>Nebela penardiana</i> Deflandre 1936	NPEN	31	7.6	4.3	6.4	0.4
<i>Nebela tubulosa</i> Penard 1890	NTUB	18	3.7	2.6	6.8	0.3
<i>Paraquadrula irregularis</i> Deflandre 1932	PIRE	4	1.2	0.5	7.9	0.1
<i>Phryganella acropodia</i> Hertwig & Lesser 1874 type	PACRO	29	2.7	3.4	7.1	0.7
<i>Pontigulasia elisa</i> Penard 1893	PELI	1	0.0	—	6.7	—
<i>Quadrullella symmetrica</i> Wallich 1863	QSYM	55	6.2	3.9	6.6	0.4
<i>Sphenoderia fissirostris</i> Penard 1890	SFIS	21	5.6	2.9	6.7	0.3
<i>Sphenoderia lenta</i> Schlumberger 1845	SLEN	11	5.4	3.2	6.6	0.3
<i>Tracheleuglypha dentata</i> Moniez 1888	TDENT	59	5.4	3.9	6.7	0.5
<i>Trigonopyxis arcula</i> Leidy 1879	TARC	16	9.4	4.0	6.6	0.3
<i>Trinema complanatum</i> Penard 1890	TCOMP	19	11.2	3.0	6.7	0.3
<i>Trinema enchelys</i> Ehrenberg 1838	TENCH	41	3.0	2.6	7.0	0.6
<i>Trinema lineare</i> Penard 1890	TLIN	59	5.9	5.1	6.9	0.6

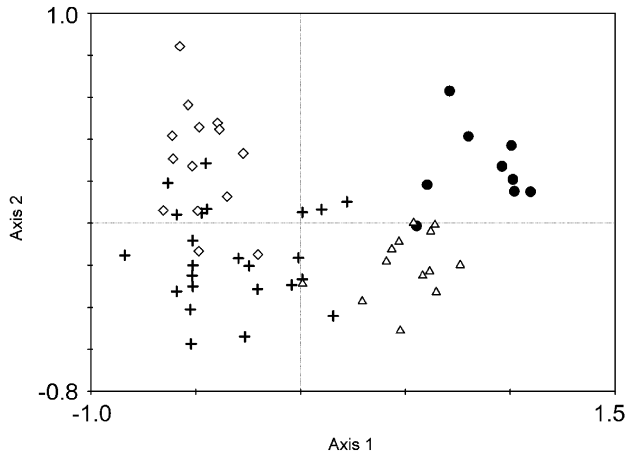
*Trinema lineare* apparently has a bimodal distribution on the water table gradient (Fig. 8). Removing this taxon improves performance of the model for the full data set from a  $RMSEP_{jack}$  of 2.4 to 2.2 cm although this substantially reduces the total number of tests included.

**Discussion**

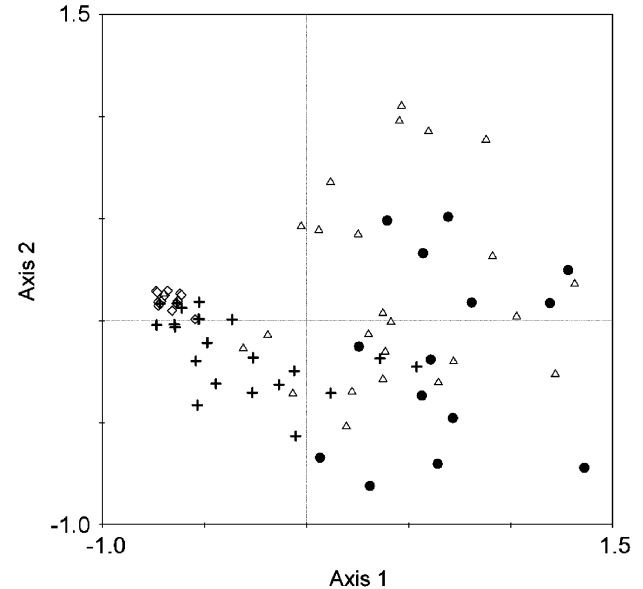
**Testate Amoebae Ecology**

The ordination results show significant variance in the amoebae data is explained by hydrology, pH

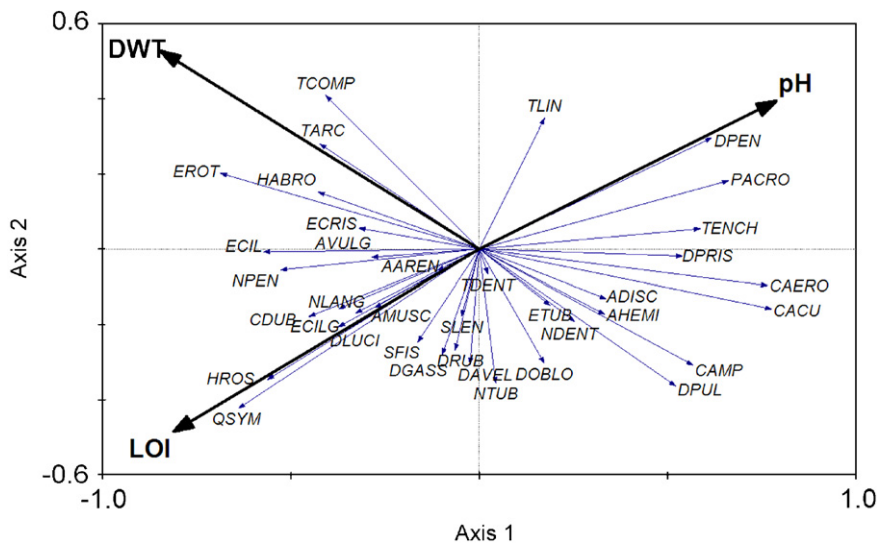
and organic content of the sediment. Amoebae communities are distinctly different among sites and are related to vegetation, independently of either the environmental data or site-specific factors. This suggests a strong link, likely a trophic one through the microbial food webs, between amoebae and vegetation (Ledeganck et al. 2003). In the redundancy analyses, 10% of the species



**Figure 3.** PCA ordination plot of testate amoebae samples (log-transformed data). Diamonds show BO samples, crosses show XE samples, triangles show DE samples and circles show KB samples.



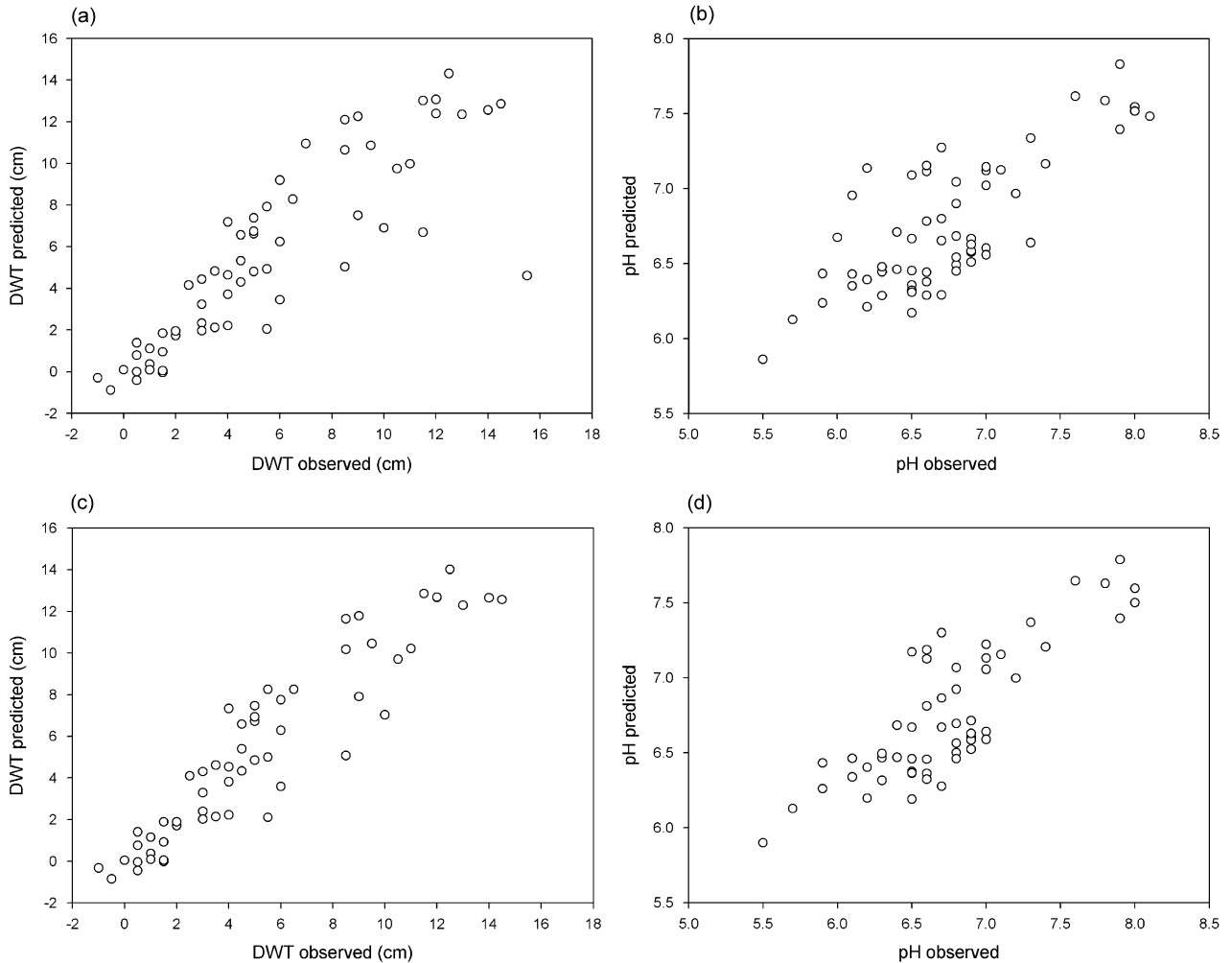
**Figure 5.** PCA ordination plot of plant samples. Data converted to percentage scale (see text for details) and square-root transformed. Site symbols are as for Figure 3.



**Figure 4.** Redundancy analysis of log-transformed testate amoebae data showing major taxa and environmental variables.

**Table 3.** Model performance for depth to water table (DWT; cm) and pH assessed by four measures. Results shown are for the best performing model variant, i.e. classical Weighted Average (WA) with inverse deshinking, two-component weighted average partial least squares (WA-PLS) model, two-component partial least squares (PLS) model for pH and three-component PLS model for DWT. For pH determination, additional components in WA-PLS did not outperform WA.

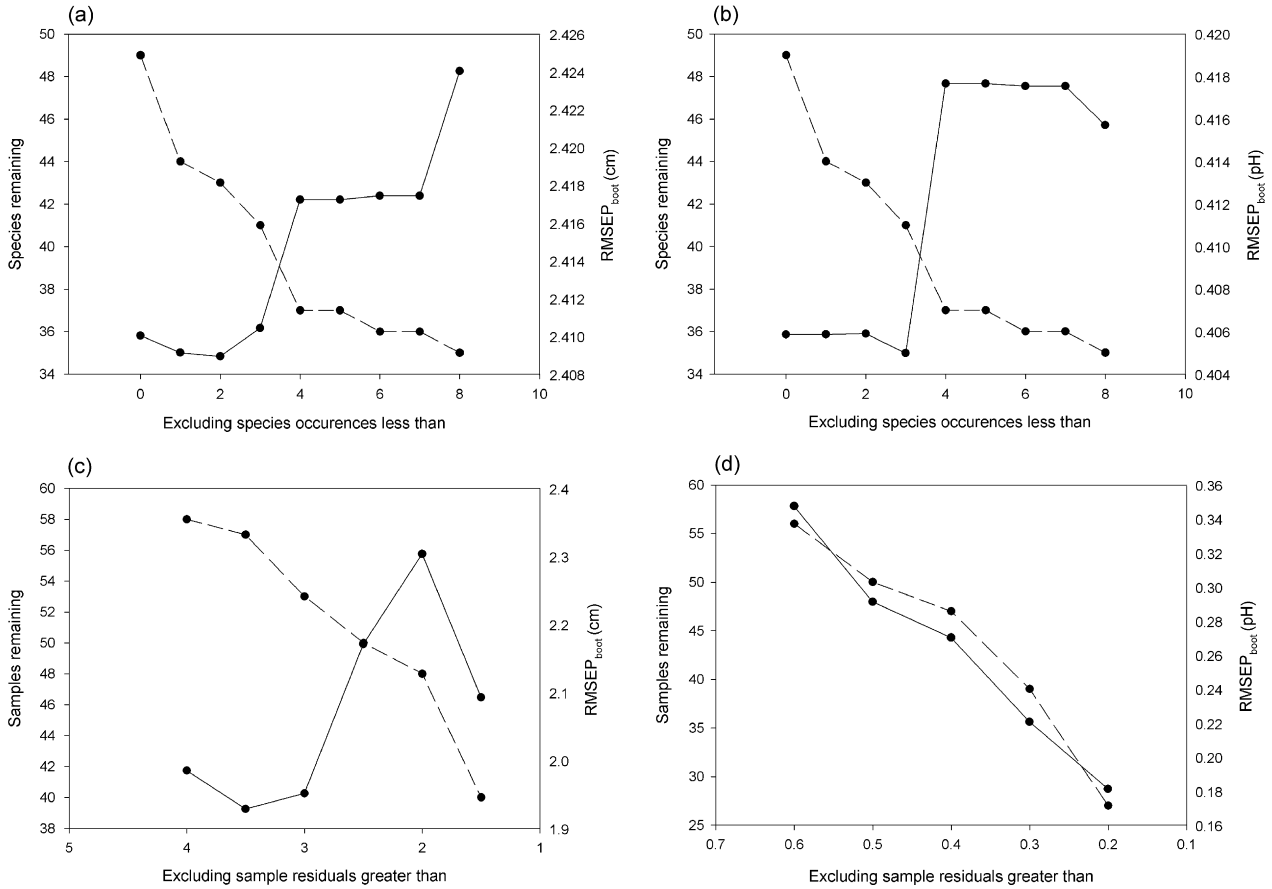
	RMSEP <sub>jack</sub>	Max Bias <sub>jack</sub>	$R^2_{jack}$	RMSEP <sub>boot</sub>	Max Bias <sub>boot</sub>	$R^2_{boot}$
<i>DWT</i>						
WA	2.54	5.80	0.64	2.60	5.88	0.65
WA-PLS	2.35	4.11	0.69	2.47	4.34	0.69
ML	2.48	5.30	0.68	2.41	4.77	0.74
PLS	2.52	4.60	0.65	2.75	4.68	0.65
<i>PH</i>						
WA	0.40	0.61	0.50	0.41	0.62	0.51
WA-PLS	—	—	—	—	—	—
ML	0.46	0.47	0.52	0.46	0.56	0.52
PLS	0.42	0.69	0.46	0.44	0.71	0.46



**Figure 6.** Observed against model predicted DWT (a and c) and pH (b and d) values pre- (a and b) and post-filtering (c and d) using best performing ML model for DWT and WA model for pH.

variance is explained by water table data. This compares well to other studies, which have found values of 9.1% (Charman 1997), 9.7% (Booth 2001), and 11.7% (Booth 2002). Sites variables are more important to the vegetation than amoebae data. A similar result from a study on a

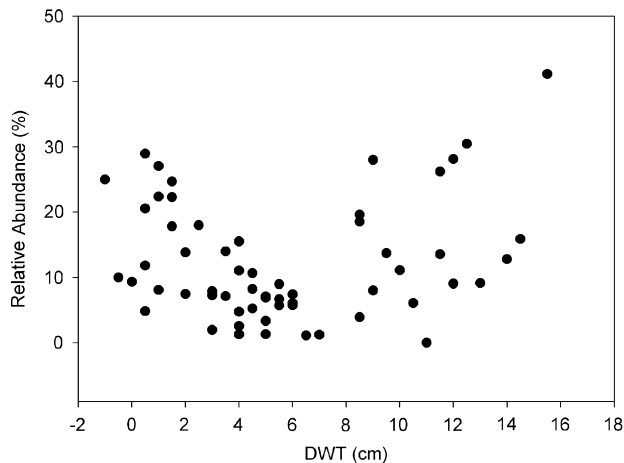
broader scale had been interpreted as evidence that the vegetation is controlled by both the local environment and broader biogeographic patterns, while the generally cosmopolitan testate amoebae responded only to the micro-environmental conditions (Mitchell et al. 2000). Given the proximity of



**Figure 7.** Impact of removing taxa occurring in few samples (a and b) and samples with high residuals (c and d) on model performance assessed by  $RMSEP_{boot}$  (solid lines) using optimal model, and number of taxa or samples remaining in the data set (dashed lines).

**Table 4.** Model performance pre- and post-filtering. Results are shown using the selected maximum likelihood (ML) model for depth to water table (DWT) and weighted average (WA) model for pH.

	$RMSEP_{jack}$	Max Bias <sub>jack</sub>	$R^2_{jack}$	$RMSEP_{boot}$	Max Bias <sub>boot</sub>	$R^2_{boot}$
<i>DWT</i>						
Full data	2.48	5.30	0.68	2.41	4.77	0.74
Filtered data	1.78	2.32	0.82	1.93	2.84	0.82
<i>pH</i>						
Full data	0.40	0.61	0.50	0.41	0.62	0.51
Filtered data	0.34	0.53	0.60	0.35	0.55	0.60



**Figure 8.** Relative abundance of *Trinema lineare* along the hydrological gradient.

the sites in this study the result here is more probably due to non-measured environmental variables that differ between sites and are important controls on vegetation.

*Trinema lineare* apparently has a bimodal distribution on the hydrological gradient (Fig. 8). Possible explanations for this include a greater abundance of other taxa in the middle of the range proportionally decreasing *T. lineare* relative abundance, the inclusion of other similar or cryptic species along with *T. lineare* or simply a greater abundance of *T. lineare* at the extremes due to resource competition or predation in the centre of the gradient.

The transfer function modeling shows that using the full modern data and given good agreement with fossil data it may be possible to predict water table depth with a model-specific error of less than 2.5 cm, or less than 2 cm when data selection is used. Counter-intuitively removing rare taxa actually reduces model performance to a small extent. This suggests the need to adopt a systematic approach when using data-filtering to improve model performance. In absolute terms this is the best performing testate amoebae hydrological transfer function yet produced. The eight previous studies, which have presented RMSEP<sub>jack</sub> results, show optimum values between 3.9 and 9.7 cm (Payne et al. 2006; Woodland et al. 1998). However, there is a general correlation between model performance and total environmental gradient (Payne et al. 2006). The RMSEP<sub>jack</sub> of the optimum ML model in this study represents 15% of the total DWT range for the full data set and 11% for the filtered data set, broadly

similar to previous studies. The good performance of this model is probably largely due to the limited hydrological gradient and lack of very dry sites for which models often perform poorly (Payne et al. 2006).

Comparison of taxa optima with other regions of the world is complicated due to the limited overlap in community composition between this study and previous work combined with the general problem of one-off DWT measurements making quantitative comparisons of taxa optima impossible. However, there is approximate agreement in the relative position of taxa on the gradient. Taxa indicative of wet conditions in other studies such as *Centropyxis aculeata* and *Arcella hemispherica* have low water table depth optima here. Taxa indicative of drier conditions such as *Arcella arenaria* (equivalent to *A. catinus* type), *Assulina muscorum*, and *Trigonopyxis arcuata* have greater water table optima here. Exceptions do exist for some taxa such as *Quadrullella symmetrica* (indicating moderately dry conditions here) and *Phryganella acropodia* type (indicating comparatively wet conditions here). An interesting taxon is *Diffflugia pulex*, which is rare in modern studies and has been considered to represent dry conditions due to its affinities in palaeoecological data (Caseldine and Gearey 2005; Charman et al. 2000; Hendon 1998; Schnitchen et al. 2003). In this study, the taxon has a weighted averaged optimum water table depth of 2.8 cm suggesting it may be more typical of wet conditions. However, this may be complicated by taxonomic confusion or the possibility of cryptic species.

This study provides information on the hydrological preferences of some taxa rarely encountered in other transfer function studies from European mires such as *Cryphoderia ampulla* and *Sphenoderia fissirostris* (both indicative of wet conditions). An improved knowledge of the hydrological preferences of species typical of more nutrient-rich conditions may aid interpretation of the early Holocene palaeoecological record when many mires had not undergone the transition to ombrotrophy (Opravilova and Hajek 2006).

## Classification of the Sites

The Elatia mires were provisionally classified as transitional between fens and bogs (Papazisimou et al. 2002). The data in this study suggests the mires lie on the fen side of the bog-fen divide. The surface pH of these sites ranged from 5.5 to 8.1, significantly greater than bogs (usual pH

around 4). Bogs usually have loss on ignition values over 95%; these sites have values from 20% to 92% (mean 71%). The low organic contents of sediments in some samples and thin peat layers in others means that significant areas of these sites cannot be termed peatland by many definitions. Many of the most abundant testate amoebae taxa in these sites may be typical of nutrient-enriched conditions (*Euglypha rotunda*, *Quadrullella symmetrica*, *Euglypha tuberculata*, *Cyphoderia ampulla*: Corbet 1973; Charman et al. 2000). Taken overall, it is clear that the mires are not ombrotrophic and the existing studies do not demonstrate that they are transitional towards ombrotrophy. The sites are however clearly more oligotrophic than mires elsewhere in Greece. A better classification may be as mesotrophic fens. There are significant differences between the sites representing a continuum from the most eutrophic (BO) to the most oligotrophic (KB and DE).

### Implications for Palaeoenvironmental Reconstruction

Results of this study show that testate amoebae in Greek mires are responsive to hydrology and the transfer function should allow accurate prediction of water tables. Testate amoebae are preserved in the sediments of the Elatia mires and community composition shows good overlap with the modern training set (Richard Payne, unpublished data). It should therefore be possible to use testate amoebae to reconstruct past hydrological change. The Elatia mires are minerotrophic so a direct link between surface wetness and climate cannot be assumed, although recent studies in minerotrophic mires have shown surprisingly good agreement with records from ombrotrophic mires (Booth et al. 2004; Hendon et al. 2001). Comparison to other climate records and replication between sites would be required to establish the climatic sensitivity of the Elatia mires. Records of local hydrological change would also be of value to investigate how these unusual mires have responded to past climatic change and therefore how they may change in a period of increasing aridity (Mauquoy and Yeloff, in press).

### Methods

**Field and laboratory methods:** Samples were collected along transects across the mires. At each sampling point vegetation was surveyed and

recorded on the DOMIN scale (Rodwell 1991), depth to water table (DWT) measured and a surface sample (approximately 5 × 5 × 5 cm) collected. In the laboratory the sample was divided in two. One sub-sample was dried at 110 °C and incinerated at 550 °C to calculate moisture content and loss on ignition (LOI). A second sub-sample of 2 cm<sup>3</sup> was disaggregated in ultra-pure water and pH measured. Testate amoebae were extracted from a sub-set of 61 samples. The peat suspension was boiled for 10 min, filtered at 250 μm and then back-filtered at 15 μm with the 15 > 250 μm fraction retained. Slides were prepared by mixing the material with glycerol (Hendon and Charman 1997). A count of 150 amoebae per sample was aimed for (Min. = 144, Max. = 178).

**Taxonomy:** Taxonomy is a persistent problem in testate amoebae ecology and palaeoecology due to issues with the non-preservation of some diagnostic features, confusion over the criteria for differentiating morphospecies and a degree of intraspecific variability (Charman 1999). A conservative taxonomic approach is adopted here. The following groupings were used: *Centropyxis aerophila* type includes *Centropyxis cassis* and *Centropyxis orbicularis*. *Phryganella acropodia* type includes *Cyclopyxis arcelloides*, *Cyclopyxis eurystoma* and *Pseudodifflugia gracilis* and is synonymous with the *C. arcelloides* type of Charman et al. (2000). *Centropyxis aculeata* type includes *Centropyxis hirsuta*. *Euglypha rotunda* type may include *Euglypha laevis*. *Euglypha ciliata* type includes *Euglypha compressa*. Other types follow Charman et al. (2000). Amoebae were identified with the aid of several taxonomic guides (Charman et al. 2000; Clarke 2003; Corbet 1973; Deflandre 1929, 1936; Ellison and Ogden 1987; Ogden 1983; Ogden and Hedley 1980). The test-forming bdelloid rotifer *Habrotrocha angusticollis* was also counted and included in percentage calculations (Warner 1987).

**Numerical analyses:** Ordination methods were used to (a) examine the general structure of the amoebae data, (b) test the link between the amoebae and environmental data, (c) examine the structure of the vegetation data and (d) test the link between this and the environmental data. Principal components analyses (PCA) were applied to both vegetation and amoebae data. Amoebae data were log-transformed and taxa with less than five occurrences and one sample without DWT measurement excluded. Vegetation data were square-root transformed and DOMIN classes converted to percentage values using the mid-point of each size-class from 4 to 10 with

values of 1%, 2% and 3% for classes 1–3. Links to the environmental data were tested using a series of redundancy analyses (RDA) with significance testing by Monte Carlo permutation tests (999 permutations) in CANOCO ver.4.53 (Ter Braak and Šmilauer 1997–2004). Six environmental variables were included in the analysis: depth to water table (DWT), percent moisture, organic content (loss on ignition: LOI), pH, distance from the site margin and sites dummy variables. Mantel tests were used to investigate the general relationship between the amoebae, plant, sites and environmental data. For the vegetation and testate amoebae data similarity matrices were constructed using the Steinhaus similarity coefficient transformed to distance using  $D = 1 - S$ . The environmental and sites data were standardised (centred-reduced) and a Euclidian distance calculated. Mantel tests were performed between pairs of similarity matrices using the R-package (Casgrain and Legendre 2004).

Transfer function models were developed using weighted average (WA), weighted average-partial least squares (WA-PLS), partial least squares (PLS) and maximum likelihood (ML) techniques (Birks 1995) in  $C^2$  version 1.3 (Juggins 2003). Jack-knifing and bootstrapping were used to provide estimates of root-mean-square error of prediction (RMSEP),  $R^2$  and Maximum Bias. Model performance was improved by selective data exclusion. Initially taxa with low occurrences were excluded; subsequently samples with high residuals were removed (Payne et al. 2006).

## Acknowledgements

This work was primarily funded by a BSA MacMillan–Rodewald fellowship to RJP. Thanks to Kimon Christanis and Stavros Kalaitzidis (University of Patras) for useful discussion of peatlands in Greece and the logistics of fieldwork. This work was carried out by permission of the Greek Institute of Geology and Mineral Exploration (IGME) and the Forest Service of the Prefecture of Drama. This paper is an indirect contribution of EU RECIPE project (reconciling commercial exploitation of peat with biodiversity in peatland ecosystems) through support for E. Mitchell. RECIPE was partly supported by the European Commission, Directorate I, under the programme “Energy, Environment and Sustainable Development” (No. EVK2-2002-00269) and partly, for the Swiss partners, by the OFES (Swiss federal office for education and science), Switzerland. We thank

Ralf Meisterfeld for useful correspondence. Comments by two anonymous reviewers helped improve the manuscript.

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