

1 **Vertical stratification of testate amoebae in the Elatia Mires, northern Greece:**  
2 **Palaeoecological evidence for a wetland response to recent climatic change, or**  
3 **autogenic processes?**

4

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14

15 ABSTRACT

16

17 The Elatia Mires of northern Greece are unique ecosystems of high conservation value.  
18 The mires are climatically marginal and may be sensitive to changing hydroclimate,  
19 while northern Greece has experienced a significant increase in aridity since the late 20<sup>th</sup>  
20 century. To investigate the impact of recent climatic change on the hydrology of the  
21 mires, the palaeoecological record was investigated from three near-surface monoliths  
22 extracted from two sites. Testate amoebae were analysed as sensitive indicators of  
23 hydrology. Results were interpreted using transfer function models to provide

1 quantitative reconstructions of changing water table depth and pH. AMS radiocarbon  
2 dates and  $^{210}\text{Pb}$  suggest the peats were deposited within the last c.50 years, but do not  
3 allow a secure chronology to be established. Results from all three profiles show a  
4 distinct shift towards a more xerophilic community particularly noted by increases in  
5 *Euglypha* species. Transfer function results infer a distinct lowering of water tables in this  
6 period. A hydrological response to recent climate change is a tenable hypothesis to  
7 explain this change; however other possible explanations include selective test decay,  
8 vertical zonation of living amoebae, ombrotrophication and local hydrological change. It  
9 is suggested that a peatland response to climatic change is the most probable hypothesis,  
10 showing the sensitivity of marginal peatlands to recent climatic change.

11

12 KEYWORDS: Mires, Peatlands, Climate Change, Testate Amoebae, Palaeohydrology

13

1 INTRODUCTION

2           Climate change may lead to changes in peatland carbon sequestration (Belyea and  
3 Malmer, 2004; Lavoie et al., 2005), gas flux (Chapman and Thurlow, 1998; Regina et al.,  
4 1999; Keller et al., 2004), plant communities (Weltzin et al., 2003; Robroek et al., 2007)  
5 and permafrost melting (Camill, 2005; Turetsky et al., 2007). In many regions of the  
6 world impacts on peatland hydrology have been noted (e.g. Klein et al., 2005). The  
7 peatlands likely to show the greatest impacts are the most marginal sites in regions which  
8 experience the greatest climate change. Such a case may be the Mediterranean region,  
9 where peatlands are rare but are often climatically marginal and are likely to be sensitive  
10 to comparatively minor climatic changes. Mediterranean peatlands are relatively  
11 unimportant in terms of their carbon reserve but are extremely important in terms of  
12 regional biodiversity.

13           Meteorological records for northern Greece show a general trend of reduced  
14 precipitation over the last c.50 years. Climatic impacts on the hydrology of northern  
15 Greece have already been noted and are modelled to increase in severity over coming  
16 decades with consequent impacts for human populations (Mimikou, 1993; Mimikou et  
17 al., 1999; 2000; Bürger, 2002; Baltas and Mimikou, 2005). These changes may have  
18 affected peatlands in the region. Most peatlands in Greece have been heavily impacted by  
19 human activity primarily through drainage for agriculture in the 20<sup>th</sup> Century, and often  
20 have low conservation value (Bouzinou et al., 1994; 1997; Christanis, 1996). An  
21 exception to this is the Elatia Mires of northern Macedonia, where remoteness and Cold  
22 War access restrictions have prevented major impacts. These mires are the most  
23 oligotrophic peatlands in Greece and are probably the only location in the southern

1 Balkans where many wetland endemics typical of more nutrient-poor conditions may be  
2 found. These are the only peatlands in Greece where *Sphagnum* is a permanent presence.  
3 Due to their unique nature the Elatia Mires have been a focus of recent conservation  
4 attention. This study uses a palaeoecological approach based on testate amoebae analysis  
5 to test the hypothesis that the hydrology of the Elatia mires is responding to recent  
6 climatic changes.

7

## 8 SITES and METHODS

9

10 The Elatia Mires lie in the Elatia Forest, approximately 70 km north of the city of  
11 Drama and 5 km south of the Bulgarian frontier at around 1500m asl. (41°29'N, 24°19'E;  
12 Fig. 1). Four small peatlands are situated in clearings within a natural coniferous forest  
13 dominated by *Picea abies*, the only such forest in Greece (Papazisimou et al., 2002). A  
14 comparatively cool and moist climate combined with impermeable granite bedrocks have  
15 allowed peat formation. Peat deposits are shallow, probably not exceeding 1 m of  
16 continuous peat, although deeper, buried, peat deposits are also found in one site. The  
17 mires have been termed the sites 'transitional' due to their mix of features typical of fens  
18 and bogs (Papazisimou et al 2002). Samples were extracted from the two most  
19 oligotrophic sites: Dexameni mire (site DE; mean pH 6.5) and Krya Vrissi mire (site KB;  
20 mean pH 6.4). Vegetation of the mires includes *Juncus effusus*, *Carex* spp., *Eriophorum*  
21 *latifolium*, *Myosotis* spp., *Geum* spp., *Ranunculus* spp., *Mentha spicata*, *Plagiomnium*  
22 *elatum*, *Sphagnum flexuosum*, *Aulacomnium palustris* and *Climacium dendroides*  
23 (Papazisimou et al 2002).

1 Peat monoliths between 16 and 26 cm in length were extracted by cutting down  
2 from the peat surface. Two monoliths were removed from central areas of the Krya Vrissi  
3 mire and one from Dexameni; denoted KB1, KB2 and DE respectively. Peat stratigraphy  
4 was noted and humification recorded on the Von Post scale (Von Post, 1924).

5 Testate amoebae analysis was used to reconstruct changes in hydrology through  
6 the length of the monoliths. Testate amoebae are shell-forming unicellular  
7 microorganisms that are abundant in peatlands and sensitive to peatland hydrology. By  
8 analysing the changing community composition down the length of a peat core and  
9 interpreting the results with a transfer function model it is possible to reconstruct how  
10 mire wetness has varied over time (Charman, 2001; Mitchell et al., 2008). Such  
11 reconstructions have been validated by comparison with instrumental data and  
12 independent proxy-climatic records (Charman and Hendon, 2000; Charman et al., 2004;  
13 Schoning et al., 2005).

14 Peat sub-samples for testate amoebae analysis were boiled in deionised water,  
15 filtered at 250  $\mu\text{m}$  and then back-filtered at 15  $\mu\text{m}$  with the 15 $>$ 250  $\mu\text{m}$  fraction retained  
16 (Hendon and Charman, 1997). Slides were made up with glycerol and amoebae identified  
17 following the taxonomic scheme described in Payne and Mitchell (2007); a count of 150  
18 tests was aimed for. Amoebae diagrams were constructed using C<sup>2</sup> ver. 1.4 (Juggins,  
19 2003) and zoned using optimal sum of squares partitioning (Birks and Gordon, 1985) in  
20 ZONE ver. 1.2 (Juggins, 1992). Quantitative environmental reconstruction was carried  
21 out using the transfer function model developed by Payne and Mitchell (2007). Depth to  
22 water table (DWT) was reconstructed using a maximum likelihood model (RMSEP<sub>boot</sub>  
23 1.9cm) and pH using a weighted average model (RMSEP<sub>boot</sub> 0.4). The reconstructed

1 values are termed testate amoebae inferred depth to water table (TI-DWT) and testate  
2 amoebae inferred pH (TI-pH). Bootstrapped error estimation with 1000 cycles was used  
3 to provide sample-specific error estimates.

4

## 5 Chronology

6 Establishing reliable chronologies for recent peat deposits has been a persistent  
7 challenge for peatland palaeoecologists (Turetsky et al., 2004) and is particularly  
8 problematic for these sites as they are non-ombrotrophic and include unusual plant  
9 communities. A search for cryptotephra (following the method of Pilcher and Hall,  
10 1992) failed to identify any shards and a search for spheroidal carbonaceous particles  
11 (following the method of Rose et al, 1995) failed to find adequate concentrations. Two  
12 radiometric methods,  $^{210}\text{Pb}$  and  $^{14}\text{C}$  analysis were applied to the peat profiles.  $^{210}\text{Pb}$  was  
13 analysed by assuming equilibrium with its grand-daughter  $^{210}\text{Po}$ . Peat samples were  
14 dissolved in strong acids with a  $^{209}\text{Po}$  yield tracer, plated onto copper disks and activity  
15 measured by  $\alpha$ -spectrometry. Four samples from towards the base of the monoliths were  
16 AMS radiocarbon dated (Goodsite et al, 2001; Goslar et al, 2005). Bulk samples were  
17 used due to the absence of *Sphagnum* (Nilsson et al. 2001). Samples were carefully  
18 prepared to minimise risk of external contamination and rootlets were picked out.

19

## 20 RESULTS

### 21 Testate amoebae

22 Testate amoebae were found through the length of the monoliths but apparent  
23 concentrations were low (counting time approximately 6-8 hours per sample). The three

1 testate amoebae diagrams show similar trends (Fig. 2). At the base of the profiles (zones  
2 DE-1, KB1-1 and the lower portion of KB2-1) the community composition is noted by  
3 abundant *Diffflugia* spp., particularly *Diffflugia pulex* type. Above this section there is a  
4 decline in these taxa leading to a more diverse community noted by *Centropyxis*  
5 *aerophila* (zones DE-2, KB1-2 and the upper portion of KB2-1). The next significant  
6 change is a marked increase in *Euglypha rotunda*, and in KB1, *Euglypha ciliata* type. The  
7 uppermost samples are different from those directly below, recognized as a separate zone  
8 in KB2 (zone KB2-3) and DE (zone DE-3).

9         Overlap between the palaeoecological data and the modern training set is very  
10 good. Over 99% of all amoebae counted are included in the training set; the total for  
11 individual samples does not fall below 97%. Water table reconstructions show a similar  
12 pattern between sites (Fig. 3). From the base of the sequences to c.6 cm depth there is low  
13 amplitude variability with no obvious similarity between profiles. At 6 cm there is a rapid  
14 increase in values that significantly exceeds bootstrapped error estimates, representing a  
15 significant lowering of water table in all sites. At the top of the sequence there is a slight  
16 decline in TI-DWT values and then a resumed increase in sites KB1 and DE, although  
17 there is a continued decline in site KB2. pH reconstructions show gradual lowering of pH  
18 values, increasing in rate above c.6 cm. The changes in pH are less pronounced than  
19 those in DWT and (particularly in KB2) only marginally exceed the error estimates.

20         The main amoebae community change is a shift in the most abundant taxon to  
21 *Euglypha rotunda* from *Centropyxis aerophila* type and *Diffflugia pulex* type. *C.*  
22 *aerophila* is generally regarded as typical of moderately wet conditions, while *E. rotunda*  
23 is probably most typical of intermediate conditions (Charman et al., 2000). The indicator

1 value of *D. pulex* type is uncertain. The majority of transfer functions to encounter both  
2 taxa show *E. rotunda* to have a higher DWT optimum than *C. aerophila* type (or  
3 equivalent *C. cassis* type) (Payne et al., 2006; 2007; in press; Woodland et al., 1998;  
4 Charman and Warner, 1997; Charman, 1997; Warner and Charman, 1994; Lamentowicz  
5 and Mitchell, 2005). The transfer function results therefore agree with the known  
6 autecology of the taxa in interpreting this change as a shift to drier conditions.

7

### 8 Chronology

9         The  $^{210}\text{Pb}$  results show no decline in activity with depth and a sequence of peaks  
10 and troughs (Fig. 4). It was not possible to determine the “unsupported” component of the  
11  $^{210}\text{Pb}$ ; insufficient material was available for direct  $^{226}\text{Ra}$  analysis and the base of the  
12 unsupported layer was not reached. The lack of a monotonic decrease in activity with  
13 depth undermines the attempt to use the method for dating. There are two possible causes  
14 of these results; very rapid peat accumulation, as suggested by the radiocarbon dates, and  
15 movement of lead within the peat profile given the minerotrophic nature of the sites  
16 (MacKenzie et al., 1998).

17         Radiocarbon dates were all returned as post-bomb and calibrated using CaliBomb  
18 (<http://calib.qub.ac.uk/CALIBomb/frameset.html>). All dates give multimodal probability  
19 distributions ranging from the mid-1950s to post-1995, indicating rapid peat  
20 accumulation (Table 1). As the dates were based on bulk samples it is possible that they  
21 have been contaminated by modern carbon, perhaps through penetration by sedge roots.  
22 However, the samples were prepared carefully to avoid contamination and obvious roots  
23 were removed. Systematic differences between dates on bulk samples and selected



1 macrofossils have not been proven (Blaauw et al. 2004). The dates are internally  
2 consistent in showing the peats to be late 20<sup>th</sup> century in age and are not contradicted by a  
3 date of  $100 \pm 40$  <sup>14</sup>C yrs BP at 31-36 cm from a neighbouring site (Papazisimou et al.,  
4 2002). For two samples from the KB2 monolith, the deeper sample (GdA-1178: 21-  
5 24cm) has a highest probability peak more recent than the upper sample (GdA-1016: 13-  
6 15cm). However this date also has a subsidiary probability peak at 1957-1958 (10.3%).  
7 As there is no stratigraphic reason to suspect a reversal it seems more likely that this  
8 older peak is the correct one.

9         Neither the <sup>210</sup>Pb results nor the radiocarbon dates allow us to establish a secure  
10 chronology for these profiles. However both sets of results can be taken to suggest that  
11 the sediments are very recent. Most probably these peats have accumulated within the last  
12 few decades but it is not possible to be more precise. Nevertheless, there is no reason to  
13 suspect disturbance of the stratigraphy and it is still probable that these profiles do  
14 preserve a continuous record of testate amoebae changes and may reveal recent  
15 hydrological changes in the mires.

16

## 17 DISCUSSION

18

19         The testate amoebae results show a single large change in amoebae community at  
20 around 6 cm depth which can be inferred as a shift to a drier mire surface. This is entirely  
21 consistent with the hypothesis of a lowered water table due to the general reduction in  
22 precipitation in northern Greece. However simple attribution of the changes to a climatic

1 impact is not possible due to the presence of other factors which could also be responsible  
2 for these changes.

3

#### 4 1. Non-climatic hydrological change

5 It seems probable that the Elatia Mires have undergone hydrological change;  
6 however as the sites are not ombrotrophic it is possible that this is unrelated to climate. It  
7 is possible that processes such as forestry, tectonic or geomorphological change in the  
8 wider area could have lead to a change in water input into the mires. Although there is no  
9 particular evidence to suggest that this may be the case the hypothesis cannot be  
10 discounted on the basis of the data presented here.

11

#### 12 2. Vertical zonation of living amoebae

13 Interpretation of the uppermost testate amoebae assemblages may be complicated  
14 if the amoebae are still alive below the surface. Testate amoebae have been noted to  
15 exhibit vertical zonation forced by gradients in light, moisture and mineral material for  
16 test construction (Heal, 1962; Booth 2002; Mitchell and Gilbert 2004; Mazei et al. 2007).  
17 It is not clear that vertical zonation could explain the species changes observed. Taxa  
18 with xenosome tests such as *Diffflugia* spp. and *C. aerophila* are commonly observed in  
19 lower horizons, probably due to availability of material for test construction. However the  
20 typical position of *Euglypha* species varies between studies (Chacharonis 1956; Booth  
21 2002; Mitchell and Gilbert 2004; Mazei and Bobnova 2007). The only study in  
22 transitional mires (Mazei and Bobnova 2007) found vertical zonation to be much weaker  
23 than in bogs. In these sites alive or encysted amoebae were only noted in the top 2-3 cm.

1 It is probable that vertical zonation in this region could explain the unusual communities  
2 in the uppermost samples, but unlikely that vertical zonation is the cause of the major  
3 change at 6cm.

4

### 5 3. Test preservation

6 The taxa which are primarily responsible for the increase in TI-DWT in the upper  
7 portions of the sequence (*E.rotunda*, *E.ciliata*, *C.dubium*) all have tests constructed of  
8 idiosomes. Such tests may be particularly prone to decomposition in the fossil record  
9 (Lousier and Parkinson, 1981; Swindles and Roe, 2007; Payne 2007). Selective test loss  
10 could have led to inaccurate palaeoenvironmental reconstruction in these sites (Mitchell  
11 et al. 2008). A number of strands of evidence suggest this is unlikely to be the major  
12 cause of the changes: 1) during microscopy no apparent reduction in overall test  
13 concentrations was noted with depth (although concentrations were not enumerated), this  
14 might be expected given the high proportion of idiosome tests; 2) no increase in degraded  
15 tests was noted with depth; 3) the taxa concerned decline sharply at around 6 cm,  
16 however they continue through the rest of the profiles in lower concentrations; 4) other  
17 closely related taxa reach their highest concentrations lower in the profiles (e.g.  
18 *E.tuberculata* in KB1); 5) the timescale under consideration is very short so differential  
19 preservation is perhaps less likely than in longer-term studies; 6) when Euglypha is  
20 removed from the reconstructions an increase in TI-DWT at 6cm remains (Fig. 3),  
21 although this is less marked and there are other changes such as a large drop in TI-DWT  
22 at the top of KB1.

23

1 4. Autogenic mire development processes

2 The *Elatia* mires have been termed ‘transitional’ between fens and bogs; it is  
3 possible that the changes in the palaeoecological record are due to ombrotrophication.  
4 Ombrotrophication can be expected to lead to major amoebae community changes as fens  
5 have distinctly different testate amoebae communities from bogs and nutrient status is an  
6 important secondary gradient (Opravilova and Hajek, 2006).

7 Conventionally it has been assumed that an autogenic mechanism drives  
8 ombrotrophication; peat gradually accumulates above the water table until it is no longer  
9 reliant on groundwater and becomes acidified by leaching and the establishment of  
10 *Sphagnum* (Hughes, 2000). This would sit well with the reconstructed increase in TI-  
11 DWT and slight decrease in TI-pH. However, it seems unlikely that this autogenic model  
12 could explain the suddenness of the amoebae change, the 2cm+ offset between  
13 stratigraphic and amoebae community changes and the apparent synchronicity in change  
14 between two sites. More recent studies have suggested allogenic forcing of  
15 ombrotrophication with a lowered water table leading to the peat surface being separated  
16 from groundwater (Hughes, 2000; Hughes and Dumayne-Peaty, 2002; Hughes and  
17 Barber, 2003; Hughes et al., 2000). It is therefore also possible that ombrotrophication is  
18 occurring, but is driven by a real allogenic change.

19

20 The results presented here are consistent with the hypothesis of a climate change-  
21 induced hydrological change; however other explanations cannot be excluded. It is  
22 possible that multiple processes have lead to the observed patterns. If the changes are due  
23 to climate then this study provides the first evidence for the impacts of recent climate

1 change on Mediterranean peatlands. Climate change is likely to be a key challenge to  
2 regional peatland conservation.

3

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5

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7

1

2 FIGURES and TABLE

3

4 Figure 1. Location of the Elatia Mires.

5

6 Figure 2. Testate amoebae profiles from three monoliths. Showing peat stratigraphy,  
7 major testate amoebae species (% of total), testate amoebae total count, humification  
8 expressed on the Von Post scale, and testate amoebae zones. Stratigraphic columns show  
9 moss-dominated peat (solid wavy lines), peat of mixed composition with mosses most  
10 abundant (interrupted wavy lines), peat of mixed composition with macrofossils most  
11 abundant (interrupted vertical lines) and macrofossil-dominated peat (solid vertical lines).

12

13 Figure 3. Testate amoebae inferred depth to water table (TI-DWT) and testate amoebae  
14 inferred pH (TI-pH) from the three peat monoliths with boot-strapped error estimates.  
15 The transfer function model was based on one-off DWT and pH measurements so the  
16 units of reconstruction are depth to water table (cm) and pH based on an October 2005  
17 datum. Also showing TI-DWT and TI-pH reconstructions excluding *Euglypha* species,  
18 which might be lost from the palaeoecological record.

19

20 Figure 4. Radiocarbon dates and  $^{210}\text{Pb}$  profile for monolith KB2.

21

22

23



1 Table 1. Radiocarbon dates from the peat monoliths

2

<b>Monolith</b>	<b>Depth (cm)</b>	<b>Date code</b>	<b>Percent modern carbon (pMc)</b>	<b>Calibrated age range (95% probability) (cal. years AD)</b>
DE	15-16	GdA-1177	120.55 ± 0.37	1958-1961 (9.3%) 1985-1988 (90.5%)
KB1	21-24	GdA-1015	121.45 ± 0.36	1958-1961 (52.7%) 1984-1986 (42.3%)
KB2	13-15	GdA-1016	116.17 ± 0.33	1956-1958 (0.2%) 1989-1991 (94.8%)
KB2	21-24	GdA-1178	110.88 ± 0.53	1957-1958 (10.3%) 1995- (85.1%)

3