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 20th

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}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

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 20th Century, and often

20   have low conservation value (Bouzinos 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 spicta, Plagiomnium*

22   *elatum, Sphagnum flexuosum, Aulacomnium palustris* and *Climacium dendroides*

23   (Papazisimou et al 2002).

4

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 μm and then back-filtered at 15 μm with the 15>250 μm 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^2 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 ($RMSEP_{boot}$

23   1.9cm) and pH using a weighted average model ($RMSEP_{boot}$ 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 cryptotephras (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}Pb and ^{14}C analysis were applied to the peat profiles. ^{210}Pb was

13 analysed by assuming equilibrium with its grand-daughter ^{210}Po. Peat samples were

14 dissolved in strong acids with a ^{209}Po yield tracer, plated onto copper disks and activity

15 measured by α-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

testate amoebae diagrams show similar trends (Fig. 2). At the base of the profiles (zones

DE-1, KB1-1 and the lower portion of KB2-1) the community composition is noted by

abundant *Difflugia* spp., particularly *Difflugia pulex* type. Above this section there is a

decline in these taxa leading to a more diverse community noted by *Centropyxis*

aerophila (zones DE-2, KB1-2 and the upper portion of KB2-1). The next significant

change is a marked increase in *Euglypha rotunda,* and in KB1, *Euglypha ciliata* type. The

uppermost samples are different from those directly below, recognized as a separate zone

in KB2 (zone KB2-3) and DE (zone DE-3).

Overlap between the palaeoecological data and the modern training set is very

good. Over 99% of all amoebae counted are included in the training set; the total for

individual samples does not fall below 97%. Water table reconstructions show a similar

pattern between sites (Fig. 3). From the base of the sequences to c.6 cm depth there is low

amplitude variability with no obvious similarity between profiles. At 6 cm there is a rapid

increase in values that significantly exceeds bootstrapped error estimates, representing a

significant lowering of water table in all sites. At the top of the sequence there is a slight

decline in TI-DWT values and then a resumed increase in sites KB1 and DE, although

there is a continued decline in site KB2. pH reconstructions show gradual lowering of pH

values, increasing in rate above c.6 cm. The changes in pH are less pronounced than

those in DWT and (particularly in KB2) only marginally exceed the error estimates.

The main amoebae community change is a shift in the most abundant taxon to

Euglypha rotunda from *Centropyxis aerophila* type and *Difflugia pulex* type. *C.*

aerophila is generally regarded as typical of moderately wet conditions, while *E. rotunda*

is probably most typical of intermediate conditions (Charman et al., 2000). The indicator

7

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}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}Pb; insufficient material was available for direct ^{226}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

8

1   macrofossils have not been proven (Blaauw et al. 2004). The dates are internally

2   consistent in showing the peats to be late 20th century in age and are not contradicted by a

3   date of 100±40 ^{14}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 ^{210}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 *Difflugia* 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

11

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 synchroneity 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

4 **Acknowledgements**

5

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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}Pb profile for monolith KB2.

21

22

23

1   Table 1. Radiocarbon dates from the peat monoliths

2

Monolith	Depth (cm)	Date code	Percent modern carbon (pMc)	Calibrated age range (95% probability) (cal. years AD)
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