Preparation and analysis of testate amoebae in peatland palaeoenvironmental studies

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SUMMARY

The use of testate amoebae in studies of peatland hydrology and palaeohydrology has been increasing, and considerable efforts have been made over the past decade to standardise techniques as much as possible. In this article we briefly describe the common procedures used to collect, prepare and analyse testate amoebae in peat-based studies of palaeohydrology and palaeoclimatology. Although specific methods are dependent on the questions and objectives of individual studies, the methods presented here should serve as a good starting point for peatland palaeoenvironmental applications.

KEY WORDS: palaeoclimate, palaeohydrology, transfer function, water table depth.

1. INTRODUCTION

Testate amoebae are routinely used as indicators of past changes in peatland hydrology (Charman 2001, Mitchell *et al*. 2008). These single-celled organisms respond quickly to environmental change, produce decay-resistant and taxonomically distinctive shells (Figure 1), and are generally well preserved and abundant in Holocene peat deposits. In oligotrophic peatlands, testate amoeba community composition is primarily controlled by the moisture content of the surface peat, allowing the development of transfer functions to infer changes in past water table depths. Over the past couple of decades, transfer functions have been developed and validated in many regions of the world (e.g. Charman & Warner 1992, Charman 1997, Charman & Warner 1997, Woodland *et al*. 1998, Bobrov *et al*. 1999, Mitchell *et al*. 1999, Lamentowicz & Mitchell 2005, Schnitchen *et al*. 2006, Payne *et al*. 2006, 2008, Charman *et al*. 2007, Payne & Mitchell 2007, Booth 2008, Lamentowicz *et al*. 2008a,c, Swindles *et al*. 2009, Markel *et al*. 2010). Cross-validation of these transfer functions reveals that water table depths can typically be reconstructed with a mean error of about 6–8 cm (Figure 2). Additional validation for the use of testate amoebae in studies of past climate variability has come from comparative studies of testate amoeba-inferred water table depths and instrumental records of climate for the past few centuries (Charman *et al*. 2004, 2009, Schoning *et al*. 2005, Charman 2007, Booth 2010, Lamentowicz *et al*. 2010), comparison of Holocene water table depth reconstructions from within the same region (e.g. Hendon *et al*. 2001, Booth *et al*. 2006, Charman *et al*. 2006), multi-proxy studies from within the same sediment cores (e.g. Nichols *et al*. 2006, Lamentowicz *et al*. 2008b) and comparison with long-term observed water table measurements (Charman *et al*. 2004).

A range of techniques has been used to collect, isolate, identify and analyse testate amoebae from modern and fossil peats, but recent research has mostly followed the protocols outlined by Hendon & Charman (1997) and Charman *et al*. (2000). However, increasing numbers of researchers are now utilising testate amoebae in palaeo-environmental studies, leading to some modifications of these protocols. In this paper, we briefly describe common field and laboratory procedures that are used to reconstruct peatland palaeohydrology and palaeoclimate using testate amoebae. These general procedures can be modified to address the specific questions and objectives of individual studies.

2. MODERN SAMPLING METHODS

For modern calibration studies, communities of testate amoebae are collected from surface peat. Sampling should be directed toward capturing the range of environmental variability within a peatland (e.g. microtopography, vegetation). At sites where

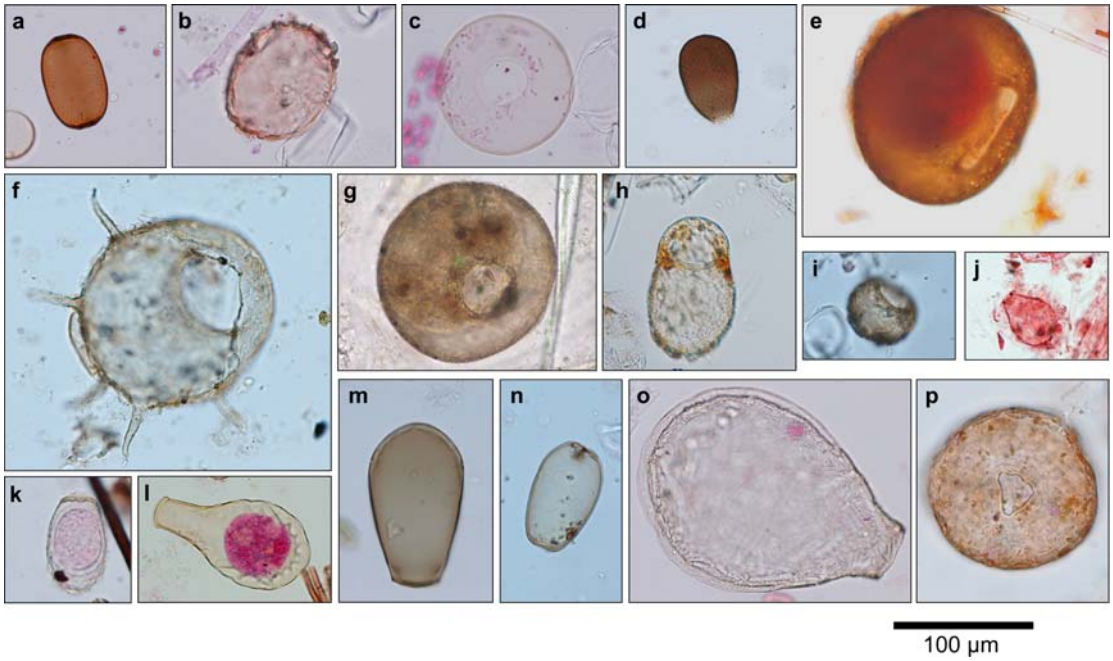

Figure 1. Photomicrographs of some testate amoeba taxa commonly encountered in peatland studies: a) *Archerella* (*Amphitrema) flavum*, b) *Amphitrema wrightianum*, c) *Arcella discoides* type, d) *Assulina muscorum*, e) *Bullinularia indica*, f) *Centropyxis aculeata* type, g) *Centropyxis ecornis* type, h) *Centropyxis platystoma* type, i) *Cyclopyxis arcelloides* type, j) *Difflugia pulex* type, k) *Heleopera sylvatica*, l) *Hyalosphenia elegans*, m) *Hyalosphenia papilio*, n) *Hyalosphenia subflava*, o) *Nebela carinata*, p) *Trigonopyxis arcula*.

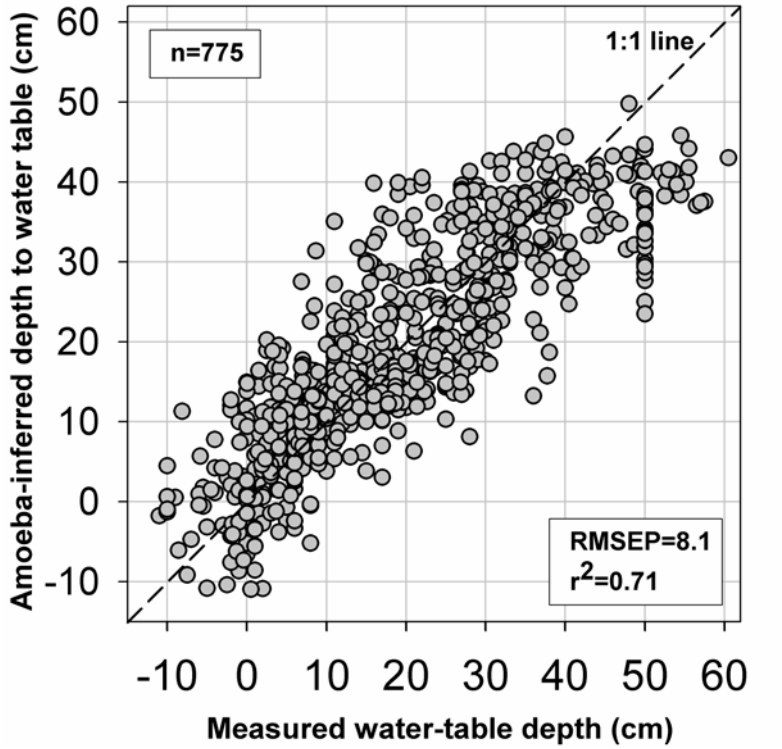

Figure 2. Cross-validation (leave-one-out) of a transfer function developed from North American peatlands, including sites in mid-continental and eastern North America (Booth 2008), the Rocky Mountains (Booth & Zygmunt 2005) and Alaska (Markel *et al*. 2010), using a simple weighted average model.

Sphagnum moss dominates, about 10cm^3 of the upper photosynthetic part of the moss is usually collected for analysis. This typically represents the uppermost *ca*. 5cm of the moss, although more or less may be collected depending on the density of the *Sphagnum*. The upper 1–2 cm (i.e. capitulum) is often removed prior to analysis because vertical variation in testate amoebae occurs along the stem, and samples collected from the lower portion of the stem exhibit higher taxonomic diversity (Mitchell & Gilbert 2004) and are generally thought to be more similar to the death assemblage that is incorporated into the peat record. At sites lacking *Sphagnum*, samples are generally taken from brown moss carpets and vascular plant remains.

In association with each modern testate amoeba sample, water table depth is measured, often in conjunction with other environmental variables (e.g. pH, conductivity, N, P, Ca, Mg, DOC). Water table depth measurements that reflect the average experienced during the growing season are best for comparison with testate amoeba communities, and these can be obtained through repeat measurements (e.g. Woodland *et al*. 1998) or other integrative estimates such as polyvinyl chloride (PVC) tape discolouration (Belyea 1999, Booth *et al*. 2005). However, PVC tape discolouration has had mixed success in recent studies (Payne *et al.* 2006, Schnitchen *et al*. 2006, Booth 2008, Markel *et al*. 2010), and when integrative estimates of water table depth are not possible, instantaneous measurements (i.e. measured on the day of sampling) are still useful, so long as extremely dry conditions are avoided (Charman *et al*. 2000, Booth 2008).

3. FOSSIL SAMPLING METHODS

Subsamples of 1–2 cm^3 are collected from along a peat core, each typically spanning 0.5–1 cm of peat. Given the rapid response time of testate amoebae to environmental change and the likely increasing sensitivity of peatland hydrology to autogenic change over longer timescales (Charman *et al*. 2006), analysis of contiguous or nearly contiguous subsamples is recommended for studies of past hydroclimatic variability.

4. ISOLATION OF TESTS FROM PEAT

Testate amoebae are usually isolated from modern and fossil peat using a sieving procedure without any chemical reagents (Hendon & Charman 1997, Charman *et al*. 2000). A modified version of this procedure is presented here.

1. Each peat sample is placed in beaker (100–250 ml) with distilled water (~50–100 ml) and a clean stirring rod. One or two tablets of *Lycopodium* spores can be added as an exotic marker to permit the calculation of test concentrations (tests cm^{-3}) and accumulation or influx rates (tests cm^{-2} year^{-1}). The number of tablets is dependent on the peat volume used, with one tablet per cm^3 of peat typically adequate.

2. Samples are boiled in distilled water for approximately ten minutes, stirring occasionally to disaggregate peat and disperse the *Lycopodium* spores. Alternatively, some analysts recommend soaking the samples overnight in distilled water. The boiling step may be omitted if living and dead individuals are to be differentiated in modern samples, as boiling may remove some cells from the tests. However, for transfer function development, both living and dead tests are usually tallied together because the objective is to characterise the assemblage that becomes incorporated into the fossil record.

3. Distilled water is added to cool off the samples, and the material is typically washed through 300 µm and 15 µm sieves. The 300 µm sieve removes coarse particulate matter from the samples, and the 15 µm sieve filters some of the smaller particulates and tends to make analysis easier and more efficient. A source of vibration can be held against the 15 µm sieve to speed the fine-sieving process (a dremel tool works quite well). Some analysts recommend using a 10 µm sieve (Beyens & Meisterfeld 2001) or no microsieve at all (Payne 2009), to avoid the loss of particularly small taxa (Wall *et al*. 2009). However, most palaeoclimate work requires the examination of numerous samples (often continuous analysis along sediment cores), and microsieving makes analysis more efficient. The choice of whether or not to use fine sieving depends on the objectives of the study, but for quantitative environmental reconstructions the calibration and fossil data should ideally be obtained using the same procedure (Payne 2009). The material retained in the 15 µm sieve is washed into 50 ml centrifuge tubes and centrifuged at 3,000 rpm for five minutes.

4. Water is decanted and the residues may be stained with Safranine to help highlight the tests during analysis, although this depends on the preference of the analyst. In modern samples, empty and living tests can be distinguished by

staining with Rose Bengal, which tends to preferentially stain cytoplasm.

5. After staining, an additional water wash followed by centrifuging is typically performed to remove residual stain.

6. Residues are then transferred into stoppered vials where they can be stored in water, glycerol, or some other storage medium. Water is recommended if scanning electron microscope work may be undertaken. Slides can be made using water, glycerol, or another mounting medium. Although glycerol performs adequately for routine analysis, the optical properties of water are better.

5. TAXONOMY AND QUANTIFICATION

The large amount of morphological variability amongst the testate amoebae has given rise to a diverse and confusing literature. For peatland research, some good resources for test identification include: Leidy (1879), Penard (1902), Cash & Hopkinson (1905, 1909), Cash *et al*. (1915), Deflandre (1936), Grospietsch (1958), Corbet (1973), Ogden & Hedley (1980), Meisterfeld (2001a,b) and Charman *et al*. (2000). There are also numerous monographs and descriptions of individual taxa in the literature, many of which are referred to by the sources listed here. For routine peatland palaeohydrological applications, a recommended starting point for test identification is Charman *et al*. (2000), as this taxonomic approach has been followed for the majority of modern peatland palaeohydrological studies. Useful modifications to the identification scheme of Charman *et al*. (2000) are presented elsewhere (Booth 2002, 2008). Most common taxa are relatively easily identified, but there are a number of taxon groups that can be difficult to separate from one another and these may be grouped or split at different levels. Because the purpose of many studies is to apply transfer functions, which are often derived by different analysts, taxonomic harmonisation of datasets is important. In general, it is recommended that a high-level taxonomy such as that used by Charman *et al*. (2000) is applied. Taxa may be split to lower levels in counting but may need to be grouped to ensure reliable application of the transfer function. There is evidence that relatively minor differences in morphology such as size variants (e.g. Bobrov *et al*. 1999) and number of pores (Booth & Meyers 2010) can add power to environmental inferences, so it may be worth recording these attributes during counting.

As in other types of microfossil analysis, slides are usually scanned and the abundance of the different testate amoeba taxa is tallied. Slides are typically scanned at 400x magnification, although 1000x is useful for the identification of some taxa. Traditionally, a total of 150 tests has been tallied, but for transfer function applications a total count of 100 individuals is likely to be sufficient for most samples (Payne & Mitchell 2009). Higher counts are needed to identify all taxa and to estimate the relative abundance of rare taxa accurately (Wall *et al*. 2009). Relative abundances of taxa are typically expressed as percentages of the total counted, although concentration and influx can also be calculated.

6. TRANSFER FUNCTION DEVELOPMENT AND APPLICATION

Transfer functions are widely used in palaeoecology and palaeoclimate research to provide quantitative reconstructions of environmental or climatic variables based on a modern training set (assemblage data and associated environmental variables) and a fossil assemblage dataset. Inferred values of the variable of interest are calculated by applying a regression model developed from the modern dataset to the fossil data (Birks 1998). Standard software is now available for developing, testing and applying a range of models (Juggins 2007). A typical procedure would be to test a range of potential models against a modern dataset. There are many possible models including those based on Gaussian responses of taxa to the environmental variable (mostly forms of 'weighted averaging'), those based on linear responses, and those based on matching total assemblages ('modern analogue techniques'). Most studies suggest that weighted averaging produces easily understood, accurate and precise estimates of water table position from testate amoeba assemblages. Once the best-performing model has been identified, it can be applied to the fossil data to produce inferred water table values. Sample-specific error estimates for reconstructed water tables are estimated by using a bootstrapping procedure to assist detection of significant changes.

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