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Testate amoebae as non-pollen palynomorphs in pollen slides: Usefulness and application in palaeoenvironmental reconstruction

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Abstract.

Testate amoebae are a frequently used palaeoecological proxy for reconstructing changes in palaeohydrological conditions, particularly in studies of *Sphagnum*-dominated peatlands. Their use in palaeoecological studies has increased following the development of transfer functions, allowing for the quantitative reconstruction of water-table depth changes through time. Increasingly, they are included in non-pollen palynomorph (NPP) studies alongside a wide range of other proxies, representing a valuable tool, particularly in multi-proxy studies.

Testate amoebae have been used for qualitative assessment of palaeohydrology in NPP studies and may aid the verification of environmental interpretations of conditions inferred from curves of NPP with unknown ecology and taxonomy. Their usefulness in such studies is limited by the destruction of tests owing to harsh chemical treatments used in pollen preparation methods. This makes community distribution data of testate amoebae derived by these methods largely unsuitable for quantitative assessment of water-table depth. Furthermore, many palynological studies combine testate amoebae as one single curve, losing further ecological detail. Patterns of change of surviving species, most commonly of *Assulina*, *Archerella*, *Arcella*, *Hyalosphenia* and *Archerella flavum*, remain relatively unaffected and therefore can still be useful for interpreting qualitative changes in hydrological conditions through time, particularly when coupled with other proxies.

The authors would like to dedicate this paper to the memory of the late Dr Richard John Payne.

1. Testate amoebae.

Testate amoebae are a polyphyletic group of eukaryotic micro-organisms, belonging to a minimum of three different taxonomic phyla (Mitchell *et al.*, 2008; Ruggiero *et al.*, 2015). The term 'testate amoebae' itself has no specific taxonomic meaning, but instead refers collectively to the wide variety of eukaryotic micro-organisms that build shells (tests).

Insert Figure 1 here.

Conservative estimates suggest there are around 2500 species of testate amoebae globally (Mitchell *et al.*, 2008). The small size of most species allows for their dispersal across great distances, limited only by the narrow ecological tolerances exhibited by some species (Charman *et al.*, 2000). Testate amoebae are highly abundant in moist habitats such as lakes, wetlands and soils (10^3 - 10^4 individuals per g^{-1} dry weight peat) (Mitchell *et al.*, 2008). Each taxonomic group develops their own tests differently, with some tests composed of endogenous plates (idiosomes) or secretions, with or without agglutinated environmental particles (xenosomes) (Charman *et al.*, 2000). The chemical composition of these tests also varies greatly, being either siliceous, calcareous or organic (Charman *et al.*, 2000). Species exhibit a large amount of morphological variability, ranging in size between 4-400 μ m and exhibiting a diverse range of body-plans and surface ornamentations (Payne *et al.*, 2012) (Figure 1 and 2). Testates are easily identified to species level, mostly cosmopolitan, are often abundant in sediments, have specific environmental preferences and are often well preserved in Quaternary sediments for thousands of years (Charman *et al.*, 2000). These features make testate amoebae useful proxies in palaeoecological studies.

2. Testate amoebae in palaeoecology.

Scientific interest in testate amoebae has increased in the last 20 years, increasing from an annual average of 5 papers involving testate amoebae during the 1990s to over 50 in 2015 (Kosakyan *et al.*, 2016). Testate amoebae have also been increasingly adopted as research tools by a range of disciplines, with studies in over 150 scientific journals between 1996-2015, an increase from 40 journals between 1976-1986 (Kosakyan *et al.*, 2016). In palaeoecology, testate amoebae have most commonly been applied in studies of *Sphagnum*-dominated peatlands in the northern hemisphere (Hendon and Charman, 1997; Mitchell *et al.*, 2008). The waterlogged, acidic conditions of peat allow for the preservation of archives of environmental change through time, spanning the time since their initiation after the beginning of the Holocene to the present day (Blackford and Chambers, 1991; Chambers and Charman, 2004).

The potential of testate amoebae as palaeoecological indicators was first recognised during the early to the middle of the 20th century (e.g, Harnisch, 1926; 1927; Grospietsch, 1953; Aaby 1976) . The abundance of certain taxonomic groups was known to correspond with specific environmental gradients, particularly moisture and water-table depths (Tolonen, 1986). This knowledge allowed for the subjective evaluation of changes in peatland water table levels, based upon changes in testate amoebae community structure. Recent developments have moved to make testate amoebae-derived reconstructions of palaeohydrology quantitative, using advanced statistical techniques examining the relationships between community structure and specific environmental variables. The

development of large ecological datasets relating testate amoebae community structure to ecological gradients has allowed for the development of statistical transfer functions (Birks, 1995; Amesbury *et al.*, 2016). These statistical models describe the relationships between species and environmental variables of interest. In testate amoebae studies, water-table depth is the most significant factor affecting testate amoebae community structure, although pH and the availability of micro-nutrients are also important (Tolonen *et al.*, 1992). Once the present-day ecological preferences of testate communities are known, these can then be applied to fossil assemblages, allowing quantitative estimates of changes in ecological gradients through time to be derived (Warner and Charman, 1994). Since this development, the use of testate amoebae in palaeoenvironmental studies has increased exponentially (Charman *et al.*, 2000; Payne *et al.*, 2012). Transfer functions have been developed from various sites around the world (e.g. Payne and Mitchell, 2007; Lamentowicz and Mitchell, 2005; Amesbury *et al.*, 2016) and have been applied in a large number of studies (e.g. Warner and Charman, 1994; Booth and Jackson, 2003; Swindles *et al.*, 2007). Testate amoebae are frequently used to augment other proxies for hydrological variability, such as plant macrofossil and peat humification analysis (Blackford and Chambers, 1991; Payne *et al.*, 2012).

Insert figure 2 here

3. Testate amoebae as NPPs in palynological slides

The purpose of this article is to discuss the potential usefulness of testate amoebae as indicators of palaeohydrological change in palynological studies. Several methods have been used for the extraction of testate amoebae from sediments (Tolonen, 1986; Hendon and Charman, 1997; Booth *et al.*, 2010). These techniques tend to fall into two categories: Preparations for the specific removal of testate amoebae analysis generally based upon a combination of dispersal in boiling water and sieving (Hendon and Charman, 1997) and preparations based upon pollen preparation methods. The latter undergo a sequence of chemical treatments for the removal of unwanted mineral and organic detritus (Faegri and Iversen., 1989). In such studies, testate amoebae are typically categorised along with other non-pollen remains that survive the harsh chemical pre-treatments used to create pollen slides. These sub-fossils are collectively termed Non-Pollen Palynomorphs (NPPs) and include a vast range of micro-organism remains including those of fungi, cyanobacteria, rotifers and algae (van Geel, 2002). These remains of aquatic animals, plants and fungi tend to be autochthonous, and are therefore likely to be more sensitive to local ecological changes than pollen (Payne *et al.*, 2012). Their inclusion in palynological studies can therefore enhance the richness of interpretations derived

from palaeoecological studies with limited additional investment of effort, expense, or time (Mighall *et al.*, 2006; van Geel, 2002).

Many palynological studies incorporate testate amoebae into their NPP counts. NPP data are typically used in conjunction with other palaeoecological proxies (e.g. Pollen, peat humification, plant macrofossils). Specific examples include Lacourse and Davies, (2015), who inferred changes in relative water-table depth by comparing the testate amoebae assemblages within the pollen slides with fungal remains, identifying a hydrological shift coincident with the transition from forested to bog conditions. Huntley *et al.*, (2013) compared declines of *Arcella artocrea* with concurrent declines in copepod spermatophores, interpreting this change as reflecting a hydrological shift from standing water to the development of a *Sphagnum* moss lawn, triggered by drainage prior to modern road construction. van der Linden *et al.*, (2006) in a multi-proxy study of anthropogenic and climate changes from a Swedish bog, counted testate amoebae as NPPs in both pollen and plant macrofossil samples. However, despite including these in the resultant diagrams, these curves were not used in the interpretations, as specific analysis of testate amoebae using water-based methods was also undertaken in this study. Most studies of this type are from peat or similar organic soils, with a smaller number from lacustrine sediments and few from archaeological contexts (Payne *et al.*, 2012). Testate amoebae offer an obvious interpretive benefit in non-pollen palynomorph studies, as unlike many NPPs for which little known taxonomic or ecological knowledge exists; these are reasonably well established for testate amoebae (van Geel, 2002). Trends in community distributions of testate amoebae may be used to independently verify if changes in water table predicted by other NPPs, most notably fungal spores, reflect hydrological changes in peatland studies (Yeloff *et al.*, 2007).

4. Limitations.

4.1. Differential preservation due to chemical preparation methods.

It is well documented that certain species of testate amoebae are vulnerable to the harsh chemical treatments associated with pollen preparation (e.g. acetolysis) due to their fragility in comparison with more resistant pollen grains (Aaby, 1976; Tolonen, 1986; Payne *et al.*, 2012). A range of different methods are used to prepare palynological slides, however it is currently unknown whether certain methods are more-or-less destructive towards tests (Payne *et al.*, 2012). A study by van der Molen and Hoekstra (1988) subjected samples from the same cores to both palynological and water-based methods, to compare differences in the types and number of tests preserved by each method. Their results showed that whilst 11 species were identified from the samples prepared by water-based methods, only two species were identified in slides prepared using pollen type preparations.

One of the common preparatory steps in pollen analysis requires sieving the sample through a 100-125 μm mesh sieve, which may have the effect of separating the larger tests from the pollen sample (van der Linden *et al.*, 2006). These factors may account for the differential preservation and severely reduced concentration of tests in palynological slides compared with water-based methods (Hendon and Charman, 1997; Payne *et al.*, 2012)

Species whose tests are composed of organic secretions (e.g. *Archerella*, *Arcella*, *Hyalosphenia* sp.) or organic coated idiosomes (e.g. *Assulina* sp.) are more common in pollen slides, owing to their resistance to the strong acid treatments associated with pollen studies (Payne *et al.*, 2012).

Archerella flavum are the most frequently encountered species, whereas species with agglutinated tests are the least resistant to destruction (Hendon and Charman, 1997; Payne *et al.*, 2012).

However, some species with xenosomic-type tests, including *Centropyxis* and *Phryganella*, are reasonably well represented in pollen slides (Payne *et al.*, 2012).

A review of the available literature of palynological studies identifying testate amoebae showed that only 20 species were represented, with the most represented genus being the *Arcella*, with eight species (Payne *et al.*, 2012). Most of the taxa identified within pollen preparations were relatively cosmopolitan, although some relatively rare taxa, such as *Arcella stellaris* and *Phryganella nidulus* were preserved (Payne *et al.*, 2012). Despite the loss of subtle changes in abundance due to the artificial absence of rare species, changes in species composition within palynological slides may still reflect ecologically meaningful changes that more-or-less mirror the results of water-based methods. However, the loss of these species gives artificial weighting to those that survive; therefore, these assemblages are not accurate representations of the fossil record (Payne *et al.*, 2012). Despite this, patterns of change remain relatively intact between samples from different preparations, such that the ecological information of these taxa may be retained (Payne *et al.*, 2012). The effects of low-count totals may be addressed in NPP studies by simply increasing the number of individuals counted (e.g. Muller *et al.*, 2003).

Payne *et al.*, (2012) tested the suitability of testate community structures derived from palynological methods for generating transfer function-based reconstructions of water table depth. Testate data derived from water-based manipulations were manipulated (loss of taxa not recorded in NPP studies, lower test count totals, loss of rarer taxa). The resultant transfer function derived estimates of water table depth showed that the combined manipulations effectively 'destroyed' the palaeoecological signal apparent from the original, water-based reconstructions. Whilst the effects of low-count totals could be addressed, the loss of individual tests cannot be accounted for (Payne *et al.*, 2012). It is therefore unlikely that transfer functions applied to palaeoecological data can be

used to derive accurate reconstructions. However, Amesbury *et al.*, (2016) argue that such reconstructions should be considered with caution in any case, recommending that reconstructions be considered as directional rather than an accurate reconstruction of water table depths. This argument was corroborated by the comparison of testate-amoebae based water-table depths from modern samples with instrumental measurements from the same location by Swindles *et al.*, (2015). Their results indicated that whilst reflecting directional changes, testate amoebae-based water-table depths gave inaccurate estimations of either the mean or median recorded depths. It could therefore be argued that whilst accuracy and ecological detail is lost, the overall direction of the curves remains similar and may offer some benefits in aiding ecological qualitative interpretations. Payne *et al.*, (2012) also recommend plotting ordination scores in the same manner as Rull *et al.*, (2008) to integrate this information.

4.2. Lack of taxonomic detail.

In addition to the above caveats, palynological studies often lack taxonomic detail (Payne *et al.*, 2012). Identification in palaeoecological studies remains largely based upon shell morphologies, which at lower taxonomical levels is often unresolved (Mitchell *et al.*, 2008). Payne *et al.*, (2012) note that some of the highest number of taxa identified in palynological studies were analysed by trained testate amoebae analysts (e.g. Wheeler *et al.*, 2010). Palynologists in studies using NPPs are infrequently specialists in testate amoeba analysis, therefore the degree of taxonomic resolution in NPP studies can be expected to be lower than in testate-amoeba specific studies. Uncertainties at the higher taxonomic levels have minimal impact upon the usefulness of testate amoebae in palaeoecological studies (Mitchell *et al.*, 2008). However, common species which survive palynological preparation are often easily identified with confidence using the number of available guides, keys and monographs (e.g. Leidy, 1879, Ogden and Hedley, 1980, Mazei and Tsyganov, 2006; Charman *et al.*, 2000; Clarke, 2003; Mazei and Warren, 2012; 2014; Meisterfeld, 2002).

Despite most tests being readily identifiable to at least the genus level, in some palynological studies testates are merely represented as curves of their total counts (e.g. Wood and Wilmshurst, 2012). Such approaches severely limit meaningful interpretation of the testate amoebae assemblages. For example, Miehe *et al.*, (2009) counted total testate amoebae, claiming that their increased presence was indicative of nutrient enrichment from anthropogenic activities, despite the well-known negative impact of experimental nutrient addition upon the abundance of testate amoebae (Gilbert *et al.*, 1998). Wood and Wilmshurst (2012) similarly represented testate amoebae as a single curve, interpreted as indicators of 'wetter' conditions, although this interpretation was derived in conjunction with data from other proxies.

Some studies identify testate amoebae to the genus level. For example, since *Assulina* sp. are both easily identified and well represented in pollen preparations (Charman, 2001), percentage values of this genus are frequently represented as single curves in NPP studies. However, this taxon is represented by at least three distinct species (*Assulina muscorum*, *A. seminulum* and *A. scandinavica*), each with their own specific ecological preferences. Similarly, Borrromei *et al.*, (2010) interpreted samples with increased percentage concentrations of undifferentiated *Arcella* species as representing shallow, meso-eutrophic or mesotrophic freshwater pools. This interpretation is contestable because *Arcella* is a large genus, containing species occupying a range of environmental situations, and is therefore likely to be too general (Payne *et al.*, 2012).

5. Conclusions.

Testate amoebae are useful indicators of palaeohydrology and are being increasingly included in NPP studies along with a wide range of other microfossils and proxies, particularly in studies of *Sphagnum*-dominated peatlands. They represent a valuable tool in palaeoecological studies, particularly in multi-proxy studies.

Testate amoebae have been used effectively in several NPP studies. They are useful for the qualitative assessment of palaeohydrological change, particularly when used in conjunction with other NPPs. In addition, testate amoebae have an advantage over most NPPs in that they form a group of NPPs where the taxonomy and ecology of species frequently identified are relatively well known, and therefore may aid in the verification of environmental interpretations of trends exhibited by NPPs for which their ecology and taxonomy is currently unknown.

However, the usefulness of testate amoebae as a proxy for palaeohydrological conditions is hindered by the loss of tests in both number and disproportionately by species. This limitation is likely to result from the loss or destruction of the tests during pollen preparation methods. This means that community structures of testate amoebae from palynological studies do not accurately represent the fossil record. However, patterns of change remain relatively unchanged, due to the survival of many cosmopolitan species and the disproportionate loss of rarer species. In spite of this, the surviving fossil communities cannot be expected to provide accurate reconstructions of water table depth when applied to transfer functions, although it is argued in this article that the directional changes that survive in the reconstructions may be used as qualitative guides to aid interpretation. A further caveat in many palynological studies involving testate amoebae is that they often lack taxonomic detail, unnecessarily grouping testates in one single curve for the total sum of tests counted. This curve is less ecologically meaningful than deriving an individual curve for each species identified.

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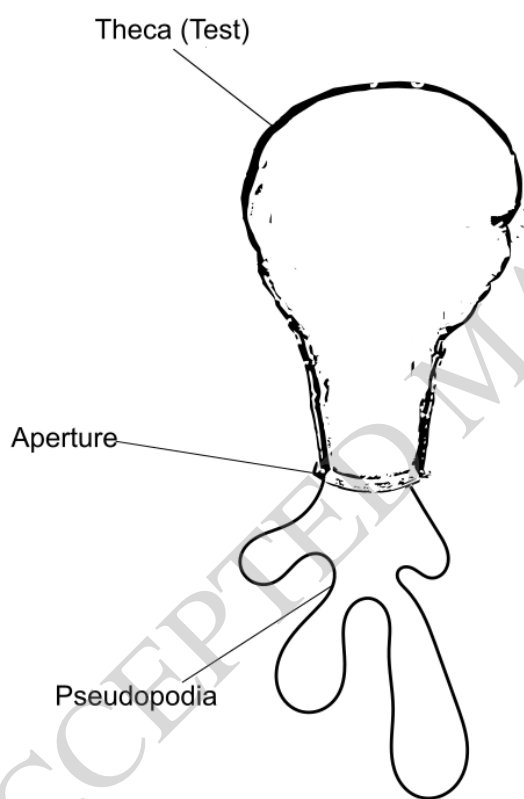
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Figure captions:

Figure 1. Testate amoebae schematic showing the various features which can be used to identify a test to the highest possible taxonomic resolution.

Figure 2: A selection of tests demonstrating the range of morphologies, test features and surface ornamentations possessed by testate amoebae. A: *Longinebela tubulosa* Kosakyan et al., 2016; B: *Arcella artocrea* Leidy, 1876; C: *Hyalosphenia papilio* Leidy, 1874; D: *Bullinularia indica* Penard, 1907; E: *Placocista spinosa* F: *Nebela flabellulum* Leidy, 1874; G: *Centropyxis aculeata* type Ehrenberg, 1832; Penard, 1899; H: *Amphitrema wrightianum* Archer, 1869; I: *Euglypha cristata* Leidy, 1974. Photographs taken by author (L.A.).

Testate amoebae schematic



Test morphology

-Size/shape/compression

-Microsculpturing

-Pore number/position

-Aperture size/shape/position

Test composition

-Agglutinated/Xenosomes/Idiosomes

-Siliceous, calcareous, proteinaceous

