The use of <63 μm fractions in the separation and identification of testate amoebae in the inter-tidal zone

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ABSTRACT

Accurate and precise reconstructions of past sea levels from saltmarsh sediments depend on a variety of lithostratigraphic and biostratigraphic data. Foraminifera have proved to be good indicators of past sea levels and there is now a large amount of data on their spatial zonation in saltmarshes. Testate amoebae ('thecamoebians') are a closely related group of protozoans which have also been recorded during most of these studies, but only in relatively low numbers and diversity in the >63 μm fraction routinely examined in samples prepared for foraminiferal analyses. This study investigates the potential of the <63 μm fraction to provide improved estimates of testate amoebae species abundance and diversity for the reconstruction of past sea levels. A preparation technique is described for the separation of the <63 μm size fraction and examination using high power light microscopy. Analysis of surface samples on a surveyed transect from the Taf estuary, south Wales, shows that the smaller size fraction contains much more abundant and diverse assemblages than are commonly found in the >63 μm fraction. A total of 36 taxa were recorded with the number of taxa per sample ranging between 3 and 18. The assemblages change over distance and elevation in the middle and upper marsh, suggesting that the sub-63 μm fraction can provide additional data for higher precision estimates of sea level based on sub-fossil assemblages of testate amoebae.

INTRODUCTION

Scott & Medioli (1978) first recognised the potential of foraminifera as accurate indicators of past sea levels and these organisms have since been widely used for this purpose, particularly in eastern North America. The interpretation of sub-fossil foraminiferal assemblages depends on a good understanding of modern distribution patterns in relation to tidal level and as a result there are now a large number of studies demonstrating the zonation of communities in coastal habitats from many areas of the world including eastern North America (e.g., Scott & Medioli, 1980; Scott *et al.*, 1981; 1991; Scott & Martini, 1982; Scott & Leckie, 1990; Gehrels, 1994; de Rijk, 1995a; 1995b), western North America (Patterson, 1990; Jennings & Nelson, 1992; Guilbault *et al.*, 1995; Jennings *et al.*, 1995), South America (Scott *et al.*, 1990), Japan, (Scott *et al.*, 1995), Britain (Horton, 1999), New Zealand (Hayward *et al.*, 1996) and the Pacific Rim (Scott *et al.*, 1996). As part of the process of counting foraminifera in these surveys of modern assemblages, a number of 'thecamoebian[1]' or testate amoebae taxa have also been encountered. These have only been found in relatively low numbers in samples prepared for foraminiferal analysis, but have been recovered from most samples from the upper zones of saltmarshes investigated. They have thus been found to be widespread in saltmarshes, but with assemblages of low diversity, often dominated by one or two taxa only. Testate amoebae have also been studied in freshwater peatlands, with the objective of reconstructing past hydrological conditions and in freshwater lake sediments (Tolonen, 1986). These studies report much higher numbers of individuals in greater diversity both in modern and sub-fossil samples (e.g., Scott & Medioli, 1983; Charman & Warner, 1992; Buttler *et al.* 1996) and the peatland studies use different preparation techniques to those in saltmarsh studies. The difference between taxon abundance and diversity in saltmarsh and freshwater peatlands may thus be explained by the inability of most taxa to tolerate saline conditions or it may be due to the different preparation techniques used. This paper presents preliminary results from testate amoebae analysis of saltmarsh samples from

[1] The term for this group of protozoans varies between authors. Other terms used are 'rhizopods' (e.g., Tolonen, 1986), 'arcellaceans' (Patterson *et al.*, 1985), 'testate amoebae' (Warner, 1990) and testaceans (Tolonen *et al.*, 1992). The term testate amoebae is preferred here as it concurs with the majority of recent taxonomic and ecological literature.

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the Taf estuary, Wales using a new technique modified from that used in freshwater peatlands (Hendon & Charman, 1997). The implications for increasing the amount and usefulness of testate amoebae data in sea-level reconstructions are also discussed.

METHODS

A transect across a saltmarsh on the Taf estuary, south Wales, was established from the mudflat/marsh transition up to the edge of the surrounding upland vegetation (Figure 1). It crosses four main vegetation zones; the *Puccinellia maritima* middle saltmarsh, a broad *Juncus maritimus* zone, and a *Phragmites australis* upper marsh which grades into the woodland-scrub upland vegetation. The transect was surveyed using an electronic total station survey instrument and surface samples of circular cross section 7.5 cm in diameter were taken at locations separated by approximately 5 cm in elevation from each other. The transect was also surveyed to Ordnance Datum using Ordnance Survey benchmarks. Samples were sealed in plastic and sub-sampled and prepared within a week of collection. Subsamples for a series of chemical and sedimentological analyses were required so the top 1 cm of sediment was divided into eight portions, each of 5.52 cm^3. One of these was used for forminiferal and testate amoebae analysis.

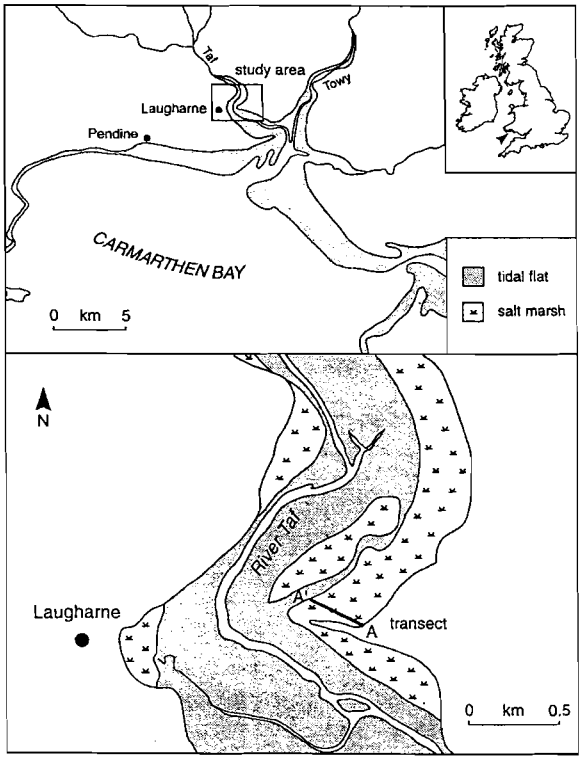

Figure 1. Location of Taf estuary and transect studied.

Preparation techniques used for saltmarsh samples have predominantly been based on wet-sieving of 10 cm^3 samples from the upper 1 cm of sediment and retaining the 63-500 μm fraction. This size fraction is considered to include all identifiable adult foraminiferal tests, but to exclude larger detritus and smaller sand and silt size residue which would obscure tests and hamper counting. Counts are carried out on a dissecting microscope at relatively low magnifications (x20-x60) to totals of 3-500 on entire or split samples. However, although this is an appropriate technique for extraction of foraminifera, it is probably less than ideal for testate amoebae, since many taxa are known to be much smaller than 63 μm in length. Thus, preparation techniques for peatland samples often involve no lower sieve size and all fragments smaller than 300-750 μm are retained (e.g., Tolonen, 1986; Warner, 1990), or a very fine 15 μm sieve is used to exclude only small detritus in samples which are more humified (Hendon & Charman, 1997). Sample size is 1-2 cm^3 and this yields very large numbers of tests. Counting is carried out at high magnification (x400 and x1000 for examination of critical features) on the samples which are mounted in an aqueous medium on a microscope slide with a coverslip. Volumetric splitting of samples is not necessary since to calculate total concentrations an exotic marker of *Lycopodium* spores is added to the initial preparation in a similar way to that used in pollen analysis (Stockmarr, 1971). However, saltmarsh sediments contain large amounts of debris of all size ranges and the entire 15-300 μm fraction on a slide is impossible to count because most or all of the tests are obscured. Instead, a combined approach is used here, with standard preparation of foraminifera samples for the >63 μm fraction, examined under a dissecting microscope. The 15-63 μm fraction is then mounted and examined separately for testate amoebae at x400.

Each 5.52 cm^3 sub-sample was stained with Rose Bengal and sieved through 500 and 63 μm sieves. The >63 μm fraction was counted for forams and testate amoebae at x20-x60 under a dissecting microscope until all the tests in the sample or a split had been counted. The <63 μm fraction was sieved through a 15 μm mesh and the >15 μm fraction retained. Four *Lycopodium* tablets containing approximately 12,500 spores each were dissolved in HCl, rinsed in distilled water and added to this fraction. This was mixed well with glycerol, mounted under a 22x50 mm coverslip sealed with nail varnish, and scanned at x400 for testate amoebae and juvenile foraminifera. Counting continued until the whole slide had been examined or until over 100 testate amoebae tests were counted. Normally 150 testate amoebae would be counted (Woodland *et al.*, 1998) but despite the small size range mounted, the saltmarsh samples yielded very dirty slides which were time-consuming to count. Thus until the potential utility of the analyses had been determined, 100 tests was considered an adequate compromise between the desirability of

higher counts and the time constraints in the study. Test concentrations were calculated from the number of *Lycopodium* spores counted using standard formulae (Stockmarr, 1971).

SYSTEMATICS AND TAXONOMY

The terms 'thecamoebians' and 'testate amoebae' are informal classifications often used to describe the group of test forming protozoans which primarily inhabit freshwater environments. Existing work on saltmarsh assemblages (e.g., Medioli *et al.*, 1981) often uses the older classification of Loeblich & Tappan (1964) in which they are placed within two separate classes, Rhizopodea von Siebold 1845, subclass Lobosia Carpenter 1861, Order Arcellinida Kent 1880 and Reticularea Lankester 1855, Subclass Filosia Leidy 1879. This system does not clearly differentiate between testate amoebae and foraminifera which also fall within the Reticularea. An alternative classification system used by most workers on freshwater peatlands (e.g., Warner, 1990), is based on The Committee on Systematics and Evolution of the Society of Protozoologists (1980), and elevates Subclass Filosia Leidy 1879 to Class Filosea Leidy 1879, thus separating the testate amoebae more clearly from the foraminifera which occur within the Class Granuloreticulosea de Saedeleer 1934. Both the foraminifera and testate amoebae are within the Subphylum Sarcodina Schmarda 1871, Superclass Rhizopoda von Siebold 1845. Since the Classes Lobosea and Filosea contain many other organisms, for all practical purposes testate amoebae are considered to be those species of the Lobosea and Filosea which form an outer test.

The taxonomy and identification of testate amoebae is somewhat confused and there are major disagreements about what constitute different species. Although sexual reproduction does occur (Mignot & Raikov, 1992), it is probably rare and most reproduction is asexual which presents difficulties with biological species definitions (Medioli & Scott, 1983). Consequently, a morphological species concept is more suitable, as applied in the ciliates, for example (Finlay *et al.*, 1996). This still leaves room for considerable disagreement and previously divided species have sometimes been grouped into very large single species complexes (Medioli & Scott, 1983). It can be shown in laboratory studies using clonal cultures, that many apparently morphologically distinct species found in nature are actually the same species (Medioli *et al.*, 1987) and the logical conclusion of this is that for species definition, these larger groups should be adopted and that the previously separated taxa should be regarded as ecophenotypes. However, if the purpose of a study is to use the organisms as ecological indicators, then the separation of 'real' species from ecophenotypes is largely irrelevant. If a relationship can be shown between species/ecophenotype occurrence and the environmental parameter(s) of interest, then this can be used to infer conditions from other assemblages where the conditions are unknown, such as in fossil samples. In such a process, grouping of ecophenotypes is likely to lead to a loss of information. In this study we have elected to separate taxa at the lowest level which is workable and repeatable between observers, despite the fact that some of these taxa are likely to be ecophenotypes (Medioli *et al.*, 1987). We have still grouped some species (see Appendix 1) as we feel they are inseparable for practical purposes, especially when the tests are without active pseudopodia, as they all are once they have been prepared for counting. Identification of the taxa was achieved using a variety of texts including Cash *et al.* (1905-1919), Penard (1902), Grospietsch (1958), Corbet (1973), Ogden & Hedley (1980), and Ellison & Ogden (1987).

RESULTS

Test numbers

Data on testate amoebae numbers are presented as concentrations in Table 1. The figures are all given in numbers per cm^3 and should be multiplied by a factor of ten for comparison with most previously published data. Total concentrations vary between 1726 and 65566 tests/cm^{-3} which is high by comparison with other studies based on >63 μm size fractions, where numbers may reach about 1000 per 10 cm^3 or less (Scott & Martini, 1982; Scott *et al.*, 1995), or exceptionally up to 9000 per 10 cm^3 (Scott *et al.*, 1991). They are also much higher than the numbers recorded in the >63 μm size fraction of the same subsamples which have a maximum of 116 per cm^3 (Table 1).

The numbers of stained specimens recorded are very low by comparison with the unstained tests. Staining was always clear where it occurred and was always associated with cellular contents so stained individuals can be considered live or encysted. The low numbers of living tests may be due to the early sampling time (April) when productivity may not have reached its peak. Another possibility is that the large numbers of empty tests are primarily allochthonous in origin and that relatively few tests live *in situ*. This seems unlikely since preservation of empty tests was very good and there was no difference in the appearance of live and dead tests. In addition the strong zonation in relation to distance and elevation (see below) suggests that post-mortem transport of tests is minimal. Relatively low numbers of live foraminifera were also found which supports this idea and may indicate that sedimentation rates are rather low with large residual populations present. This is an area where more work needs to be done on seasonal variations in living and dead populations in the surface sediments.

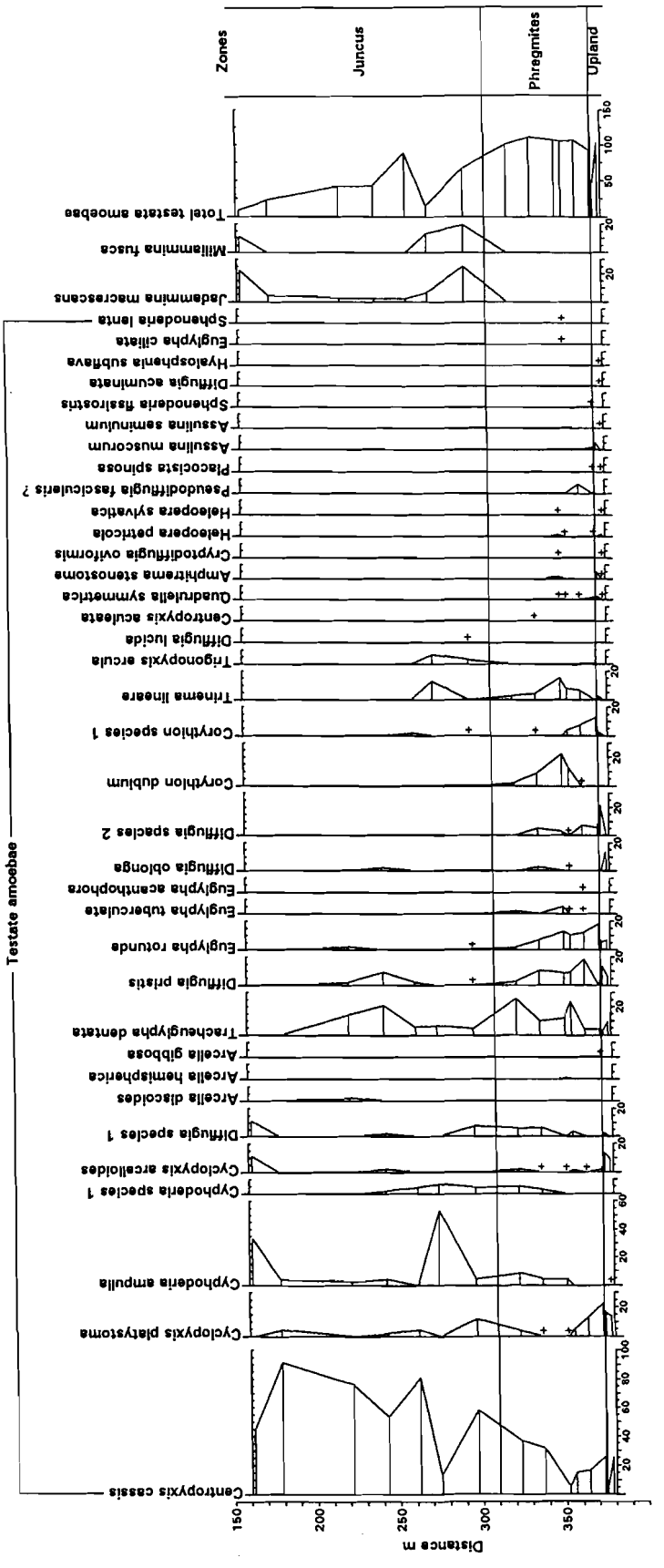

Figure 2. Percentage abundance changes in major testate amoebae taxa against distance in metres. Plant zones are marked on the diagram for reference with the upper marsh at the bottom of the y axis. All taxa (including foraminifera) plotted as a percentage of the total testate amoebae count. Total testate amoebae is expressed as the number of individuals counted in each sample. Taxa occurring at <5% in <3 samples are excluded. See Table 1 for minor taxa.

Diversity

Although there are several taxa which have not been identified clearly yet (see Appendix), assuming these are accepted as different taxa the diversity of the assemblages varies between 3 and 18 taxa per sample. These are also much higher than previously reported figures from the >63 μm fraction from saltmarsh samples, where taxon numbers are often limited to two to three dominant taxa with perhaps another three or four additional taxa in smaller numbers. The total number of taxa encountered is 36, although a number of these occur in only one or two samples in relatively low numbers. This is despite the relatively small number of samples examined from a single transect. It seems likely that this taxon number would be increased by counting of further samples from different transects on this and other marshes. The diversity in the >63 μm fraction is limited to two taxa with a single taxon *Centropyxis cassis* being the only one present in almost all the samples. A number of the taxa encountered are illustrated in Plates 1 and 2.

Assemblage composition

Figure 2 shows the relative abundance of the testate amoebae plotted against distance on the transect. There is a clear zonation from the highest levels of the marsh down to a point some 220m from the marsh edge where numbers became too low for reasonable counts to be made. The uppermost sample was at 5.07m OD and the lowest sample where testate counts were made was at 4.28m OD. The *Puccinellia* plant zone does not contain significant numbers of testate amoebae. Counts of foraminifera from the same samples show that the highest sample in which foraminifera occur is at 4.80m OD where only *Jadammina macrescens* is found. The lowest point where testates were counted is dominated by *J. macrescens, Miliammina fusca* and *Trochammina inflata. Haplophragmoides* is also found in some quantity between these elevations. Below 4.27m OD, calcareous foraminifera characteristic of low marsh and mudflat environments begin to occur.

A few testate amoebae taxa occur only in the two uppermost samples which are from the edge of the upland, but all other taxa are found on the main marsh expanse. There are particularly diverse assemblages in the next six samples which mainly occur within the *Phragmites australis* dominated zone of the marsh, although the last two samples in this group straddle the lower boundary of this zone. There is some zonation of testate amoebae within the *Phragmites* zone, with *Cyphoderia* species increasing in abundance towards the lower edge and *Difflugia pristis, Euglypha rotunda* and *Corythion dubium* decreasing. *Centropyxis cassis* is dominant over the *Juncus maritimus* marsh but *Tracheleuglypha dentata* is also relatively common and there are sporadic occurrences of other taxa. A number of foraminifera were also encountered in the samples from the *J. maritimus* marsh, either of *Miliammina fusca* or *Trochammina* type individuals which could not be identified to species level. While many of these exceeded 63 μm in maximum dimensions, they were clearly small enough to go through the sieve mesh. This suggests that the entire foraminiferal population is not being described by examination of the larger size fraction alone, although this may be a relatively small proportion of the total.

Most of the taxa of testate amoebae found are known to be always smaller than 63 μm in maximum dimensions (e.g., *Euglypha rotunda*) or to have size ranges which cross this threshold (e.g., *Centropyxis cassis*). It is, therefore, not surprising that the former were not recorded in the larger size fraction but it is interesting to note that even the commonest taxa in that fraction (*Centropyxis cassis*) was still only recorded in relatively low numbers.

CONCLUSION

The results demonstrate clearly that sub-63 μm fractions can be used to provide additional data on testate amoebae distribution on saltmarshes. Both the number of individuals and the diversity of the described assemblages are greatly increased compared to assessments based on the >63 μm fraction. This is not surprising given that many taxa of testate amoebae are restricted to the smaller size range, but the magnitude of the difference in abundance is surprising and suggests that previous studies have seriously underestimated the abundance of these organisms on saltmarshes. Furthermore, the additional taxa found yield an informative zonation in relation to elevation along the transect, particularly for the upper marsh and supra-tidal zone where the foraminifera data are rather poor at discriminating smaller differences in elevation at this particular site. These areas of marshes are of particular interest in sea-level studies, because sedimentation rates are generally low. As a result, a shift in environment, signified by a change in microfossil assemblage, is more likely to be caused by a change of sea level rather than a change in sedimentation rate. In the lower parts of the marsh, sedimentation often obscures any environmental change that might be attributed to a change in sea level (Allen, 1990). Further work on a greater number of sites and transects is clearly required before definitive statements are made, but this pilot study suggests that testate amoebae may help provide higher precision estimates of past sea levels if sub-fossil assemblages can be recovered from saltmarsh sediments. Although the smaller size fraction is more difficult to work with than the larger material, it does provide a significant additional amount of data. Future work will concentrate on comparing the costs and benefits of testate amoebae analyses with diatom and foraminifera analyses and on combining the techniques to provide an

efficient multi-proxy technique for higher precision sea level reconstruction.

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REFERENCES

Allen, J.R.L. 1990. Constraints on measurements of sea-level movements from salt-marsh accretion rates. *Journal of the Geological Society*, **147**, 5-7.

Buttler, A., Warner, B.G., Grosvernier, P. & Matthey, Y. 1996. Vertical patterns of testate ameba (Protozoa: Rhizopoda) and peat-forming vegetation on cutover bogs in the Jura, Switzerland. *New Phytologist*, **134**, 371-382.

Cash, J., & Hopkinson, J. 1905. *The Freshwater Rhizopoda and Helioza.* Vol 1 Rhizopoda part 1, 150 pp. The Ray Society, London.

Cash, J., & Hopkinson, J. 1909. *The British Freshwater Rhizopoda and Helioza.* Vol. II Rhizopoda part 2, 166 pp. The Ray Society, London.

Cash, J., Wailes, G.H., & Hopkinson, J. 1915. *The British Freshwater Rhizopoda and Helioza.* Vol 3 Rhizopoda part 3, 156 pp. The Ray Society, London.

Cash, J., Wailes, G.H., & Hopkinson, J. 1919. *The British Freshwater Rhizopoda and Helioza.* Vol. IV Supplement to the Rhizopoda, 130 pp. The Ray Society, London.

Charman, D.J. & Warner, B.G. 1992. Relationship between testate amoebae (Protozoa: Rhizopoda) and microenvironmental parameters on a forested peatland in northeastern Ontario. *Canadian Journal of Zoology*, **70**, 2474-2482.

Committee on systematics and evolution of the Society of Protozoologists 1980. A newly revised classification of the Protozoa. *Journal of Protozoology*, **27**, 37-58.

Corbet, S.A. 1973. An illustrated introduction to the testate Rhizopods in *Sphagnum* with special reference to the area around Malham Tarn, Yorkshire. *Field Studies*, **3**, 801 - 838.

De Rijk, S. 1995a. *Agglutinated foraminifera as indicators of salt marsh development in relation to Late Holocene sea level rise.* PhD thesis, University of Amsterdam, 188 pp.

De Rijk, S. 1995b. Salinity control on the distribution of salt marsh foraminifera (Great Marshes, Massachusetts). *Journal of Foraminiferal Research*, **25**, 156-166.

Ellison, R.L. & Ogden, C.G. 1987. A guide to the study and identification of fossil testate amoebae in Quaternary lake sediments. *International Review of Hydrobiology*, **72**, 639-652.

Finlay, B.J., Coliss, J.O., Esteban, G. & Fenchel, T. 1996. Biodiversity at the microbial level: the number of free-living ciliates in the biosphere. *The Quarterly Review of Biology*, **71**, 221-237.

Gehrels, W.R. 1994. Determining relative sea-level change from salt-marsh foraminifera and plant zones on the coast of Maine, U.S.A. *Journal of Coastal Research*, **10**, 990-1009.

Grospeitsch, Th. 1958. *Wechseltierchen (Rhizopodon). Einfurhrung in die Kleinlebewelt.* Kosmos. Stuttgart.

Guilbault, J-P., Clague, J.J. & Lapointe, M. 1995. Amount of subsidence during a late Holocene earthquake - evidence from fossil tidal marsh foraminifera at Vancouver Island, west coast of Canada. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **118**, 49-71.

Hayward, B.W., Grenfell, H., Cairns, G. & Smith, A. 1996. Environmental controls on benthic foraminiferal and thecamoebian associations in a New Zealand tidal inlet. *Journal of Foraminiferal Research*, **26**, 150-171.

Hendon, D. & Charman, D.J. 1997. The preparation of testate amoebae (Protozoa:Rhizopoda) samples from peat. *The Holocene*, **7**, 199-205.

Horton, B.P. 1999. The distribution of intertidal foraminifera at Cowpen Marsh, Tees Estuary, UK: implications for studies of Holocene sea-level changes. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **149**, 127-149.

Jennings, A.E. & Nelson, A.R. 1992. Foraminiferal assemblage zones in Oregon tidal marshes - Relation to marsh floral zones and sea level. *Journal of Foraminiferal Research*, **22**, 13-29.

Jennings, A.E., Nelson, A.R., Scott, D.B. & Aravena, J.C. 1995. Marsh foraminiferal assemblages in the Valdivia Estuary, south-central Chile, relative to vascular plants and sea level. *Journal of Coastal Research*, **11**, 107-123.

Loeblich, A.R. & Tappan, H. 1964. Sarcodina, chiefly 'Thecamoebians' and Foraminiferida. *In*: Moore, R.C. (ed.) *Treatise of invertebrate palaeontology, Part C, Protista II.* University of Kansas Press. 900 pp.

Medioli, F.S. & Scott, D.B. 1983. *Holocene Arcellacea (Thecamoebians) from eastern Canada.* Cushman Foundation Special Publication no. 21, Washington D.C. 23 pp.

Medioli, F.S., Scott, D.B. & Abbott, B.H. 1987. A case study of protozoan intraclonal variability: taxonomic implications. *Journal of Foraminiferal Research*, **17**, 28-47.

Medioli, F.S., Scott, D.B., Collins, E.S. & McCarthy, M.G. 1990. Fossil thecamoebians: present status and prospects for the future. *In*: Hemleben, C., Kaminski, C., Kuhnt, W. & Scott, D.B. (eds) *Paleoecology, biostratigraphy and taxonomy of agglutinated foraminifera.* NATO ASI Series C-327, 813-839. Kluwer.

Mignot, J.P. & Raikov, I.B. 1992. Evidence for meiosis in the testate amoeba *Arcella*. *Journal of Protozoology*, **39**, 287-289.

Ogden, C.G. & Hedley, R.H. 1980. *An atlas of freshwater testate amoebae.* British Museum (Natural History), Oxford University Press, 222 pp.

Patterson, R.T. 1990. Intertidal benthic foraminiferal biofacies on the Fraser River Delta, British Columbia: Modern distribution and paleoecological importance. *Micropaleontology*, **36**, 229-244.

Patterson, R.T., Mackinnon, K.D., Scott, D.B. & Medioli, F.S. 1985. Arcellaceans ('thecamoebians') in small lakes of New Brunswick and Nova Scotia: modern distribution and Holocene stratigraphic changes. *Journal of Foraminiferal Research*, **15**, 114-137.

Penard, E. 1902. *Faune Rhizopodique du Bassin du Léman.* Henry Kündig, Genève. 714 pp.

Scott, D.B., Collins, E.S., Duggan, J., Asioli, A., Saito, T. & Hasegawa, S. 1996. Pacific Rim marsh foraminiferal distributions - implications for sea-level studies. *Journal of Coastal Research*, **12**, 850-861.

Scott, D.B. & Martini, I.P. 1982. Marsh foraminifera zonations in western James and Hudson Bays. *Naturaliste Canadienne*, **109**, 399-414.

Scott, D.B. & Medioli, F.S. 1978. Vertical zonation of marsh foraminifera as accurate indicators of former sea-levels. *Nature*, **272**, 538-541.

Scott, D.B. & Medioli, F.S. 1983. Agglutinated rhizopods in Lake Erie: modern distribution and stratigraphic implications. *Journal of Paleontology*, **57**, 809-820.

Scott, D.B., Hasegawa, S., Saito, T., Ito, K. & Collins, E. 1995. Marsh foraminiferal and vegetation distributions in Nemuro Bay wetland areas, eastern Hokkaido. *Transactions and Proceedings of the Palaeontological Society of Japan*, **180**, 282-295.

Scott, D.B., Schnack, E.J., Ferrero, L., Espinosa, M. & Barbosa, C.F. 1990. Recent marsh foraminifera from the east coast of South America: comparison to the northern hemisphere. *In*: Hemleben, C., Kaminski, C., Kuhnt, W. & Scott, D.B. (eds) *Paleoecology, biostratigraphy and taxonomy of agglutinated foraminifera.* NATO ASI Series C-327, 717-737. Kluwer.

Scott, D.B., Suter, J.R. & Kosters, E. 1991. Marsh foraminifera and arcellaceans of the lower Mississippi Delta: controls on spatial distributions. *Micropaleontology*, **37**, 373-392.

Scott, D.B., Williamson, M.A. & Duffett, T.E. 1981. Marsh foraminifera of Prince Edward Island: their recent distri-

bution and application for former sea level studies. *Maritime Sediments and Atlantic Geology*, **17**, 98-129.
Scott, D.K. & Leckie, R.M. 1990. Foraminiferal zonation of Great Sippewissett salt marsh (Falmouth, Massachusetts). *Journal of Foraminiferal Research*, **20**, 248-266.
Stockmarr, J. 1971. Tablets with spores used in absolute pollen analysis. *Pollen et Spores*, **13**, 615-621.
Tolonen, K. 1986. Rhizopod analysis. *In*: Berglund, B.E. (ed.), *Handbook of Holocene palaeoecology and palaeohydrology*, 645-666. John Wiley.
Tolonen, K., Warner, B. G. & Vasander, H. 1992. Ecology of testaceans (Protozoa: Rhizopoda) in mires in southern Finland: I. Autecology. *Archiv fiir Protistenkunde*, **142**, 119-138.
Warner, B. G. 1990. Testate amoebae (Protozoa). Methods in Quaternary ecology no. 5. *Geoscience Canada*, **5**, 65-74.
Woodland, W.A., Charman, D.J. & Sims, P.C. 1998. Quantitative estimates of water tables and soil moisture in Holocene peatlands from testate amoebae. *The Holocene*, **8**, 261-273.

APPENDIX - TAXONOMY

The mounting and examination of samples under coverslips introduces some problems into the identification of testate amoebae, mainly arising from the limited movement of the tests which means that only unidirectional views of some large, flat taxa can be observed. The following list and notes are a brief summary of the main authorities used here. Also noted are major synonyms and lists of species which may be included within our taxa due to the difficulty of seeing differentiating features in tests in particular orientations or where pseudopodia are required for identification. A full discussion of the rationale for testate amoebae identification will be presented in a future paper. Several taxa were identified to genus level only and are numbered on Figure 2.

Amphitrema stenostoma Nüsslin 1884

Arcella discoides Ehrenberg 1872

Includes:
Arcella rotundata Playfair 1917
Arcella rotundata var.aplanata Deflandre 1928
Arcella polypora Penard 1890
Arcella megastoma Penard in Wailes 1913

Arcella gibbosa Penard 1890

Includes:
Arcella bathystoma Deflandre 1928
Arcella crenata Playfair 1917

Arcella hemispherica Perty 1852

Assulina muscorum Greeff 1888

Assulina seminulum (Ehrenberg 1848) Leidy 1879

Difflugia seminulum Ehrenberg 1848
Assulina seminulum var.*Scandinavica* Penard 1890

Centropyxis aculeata (Ehrenberg 1830, 1832) Stein 1859

Arcella aculeata Ehrenberg 1830, 1832
Centropyxis discoides Penard 1890 and Deflandre 1929

Centropyxis cassis (Wallich 1864) Deflandre 1929

Difflugia cassis Wallich 1864
Includes:
C.aerophila Deflandre 1929
C.orbicularis Deflandre 1929
C.aerophila var. *sphagnicola* Deflandre 1929
C.aerophila var. *sylvatica* Deflandre 1929

Centropyxis platystoma (Penard 1890) Deflandre 1929

Difflugia platystoma Penard 1890
Centropyxis constricta (Ehrenberg 1843, Leidy 1879) Deflandre 1929

Corythion dubium Taranek 1881

Includes:
Trinema enchelys Leidy 1878
T.complanatum Penard 1890
T.penardi Thomas and Chardez 1958

Cryptodifflugia oviformis Penard 1890

Cyclopyxis arcelloides (Penard 1902) Deflandre 1929

Centropyxis eurystoma Deflandre 1929
Centropyxis minuta Deflandre 1929
Centropyxis arcelloides Penard 1902
Centropyxis laevigata Penard 1890
Centropyxis aplanata Deflandre 1929
Centropyxis penardi Deflandre 1929
Centropyxis kahli Deflandre 1929
Includes:
Phryganella hemisphaerica Penard 1902
Phryganella acropodia (Hertwig and Lesser 1874) Cash and Hopkinson 1909
Phryganella nidulus Penard 1902
Difflugia globulus (Ehrenberg 1848) Cash and Hopkinson 1909

Cyphoderia ampulla (Ehrenberg 1840) Leidy 1878

Difflugia acuminata Ehrenberg 1838

Difflugia lucida Penard 1890

Difflugia oblonga Ehrenberg 1830, 1832

Difflugia pristis Penard 1902

Euglypha ciliata (Ehrenberg 1848) Leidy 1878

Euglypha rotunda Wailes and Penard 1911

Includes:
Euglypha laevis (Ehrenberg 1845) Perty 1849

Euglypha tuberculata Dujardin 1841

Includes:
E.scutigera Penard in Wailes and Penard 1911.

Heleopera petricola Leidy 1879

H.petricola var. *amethystea* Penard 1899

Heleopera sylvatica Penard 1890

Hyalosphenia subflava Cash and Hopkinson 1909

Placocista spinosa (Carter 1865) Leidy 1879

Euglypha spinosa Carter 1865
Includes:
Placocista jurassica Penard 1905

Pseudodifflugia fascicularis Penard 1902

Quadrulella symmetrica (Wallich 1863) Schulze 1875

Difflugia proteiformis var. *symmetrica* Wallich 1863
Quadrula symmetrica Schulze 1875
Nebela (Quadrulella) symmetrica Cockerell 1911

Sphenoderia fissirostris Penard 1890

Sphenoderia lenta Schlumberger 1845

Tracheleuglypha dentata Penard 1890

Trigonopyxis arcula (Leidy 1879) Penard 1912
Difflugia arcula Leidy 1879
Includes:
Trigonopyxis minuta Schönborn and Peschke 1988
Trigonopyxis microstoma Hoogenraad and de Groot 1948

Trinema lineare Penard 1890

Table 1. Estimated total (stained and unstained) testate amoebae and small foraminifera concentrations from the <63 μm fraction of the Taf samples. Summary data for the >63 μm size fraction also shown. Figures are all in numbers per cm^3 except for the numbers of taxa and numbers of tests counted. nc indicates >63 μm fraction not counted. Concentrations of stained individuals given in parentheses.

Sample identification	TAF 17	TAF 19	TAF 22	TAF 23	TAF 25	TAF 26	TAF 27	TAF 28	TAF 29	TAF 30.5	TAF 31	TAF 32	TAF 33	TAF 33.5	TAF 34
Distance along transect m	162	179	222	243	262	275	297	323	337	352	356	364	374	376	378
Total testate amoebae counted (<63 μm)	9	23	42	43	89	15	67	101	111	107	108	108	93	37	103
Number of taxa (<63 μm)	4	3	6	9	6	6	11	12	15	17	17	14	13	13	18
Total testate amoebae concentration cm^{-3} (<63 μm)	1818	2750	5376	10856	21286	1726	6410	65566	43861	30389	16092	23940	24149	56045	23403
Number of taxa (>63 μm)	1	0	1	2	1	0	1	1	1	1		1	nc	nc	nc
Total testate amoebae concentration cm^{-3} (>63 μm)	3	0	15	22	3	0	12	2	17	116		93	nc	nc	nc
Testate amoebae															
Amphitrema stenostoma										568				3029	227
Arcella discoides			128												
Arcella gibbosa													260		
Arcella hemispherica										284					
Assulina muscorum													519	3029	
Assulina seminulum															227
Centropyxis aculeata									395						
Centropyxis cassis	808	2511	4096 (256)	5806	17220 (717)	230	3731 (191)	24019 (649)	13830	1704	2384	3990 (222)	6232 (260)	3029	5907 (227)
Centropyxis platystoma		120		252	957 (239)		765 (96)	2597	395	284	745	3103	5453 (520)	9088	3181 (227)
Corythion dubium								1298	3951 (790)	6816	2086	443			
Corythion species 1					478		96		790		596	1773	3116	1515	
Cryptodifflugia oviformis										284					227
Cyclopyxis arcelloides	202			252				1298	790	284	298	443	519	7574	2272
Cyphoderia ampulla				252	957	115	287 (191)	3246	1185						
Cyphoderia species 1	606	120	128	505 (252)		920	287	5843	1976	1420					227
Difflugia acuminata															227
Difflugia lucida							96								
Difflugia oblonga				252					1185		149				3181 (227)
Difflugia pristis			128	1010	478		96	1948	4742	2556	1490	4433		7574	1590
Difflugia species 1	202			252			478	3895	2766		596			1515	
Difflugia species 2									2371	852	298	1773	1298 (260)	12118	909
Euglypha acanthophora												222			
Euglypha ciliata											149				
Euglypha rotunda			128				96	1298	3161 (395)	3976 (284)	1639	2882 (222)	4414	3029	1590
Euglypha tuberculata								1298		1704	149	443			
Heleopera petricola										568 (284)			260		454
Heleopera sylvatica										284					227
Hyalosphenia subflava															227
Placocista spinosa													260		227
Pseudodifflugia fascicularis												1552			
Quadrulella symmetrica										284	298	222	519	1515	227 (227)
Sphenoderia fissirostris													260		
Sphenoderia lenta											149				
Tracheleuglypha dentata			768 (128)	2272 (252)	1196 (717)	115	287	16878	4347 (395)	3692	3725	1108	1039	1515	2272 (227)
Trigonopyxis arcula						115	191								
Trinema lineare						230		1948	1976	4828 (284)	1192	1552		1515	
Foraminifera															
Trochamminids	404 (202)	120 (120)	128 (128)	252 (252)	478	115	1626 (672)								
Miliammina fusca	202					230	1244 (191)								

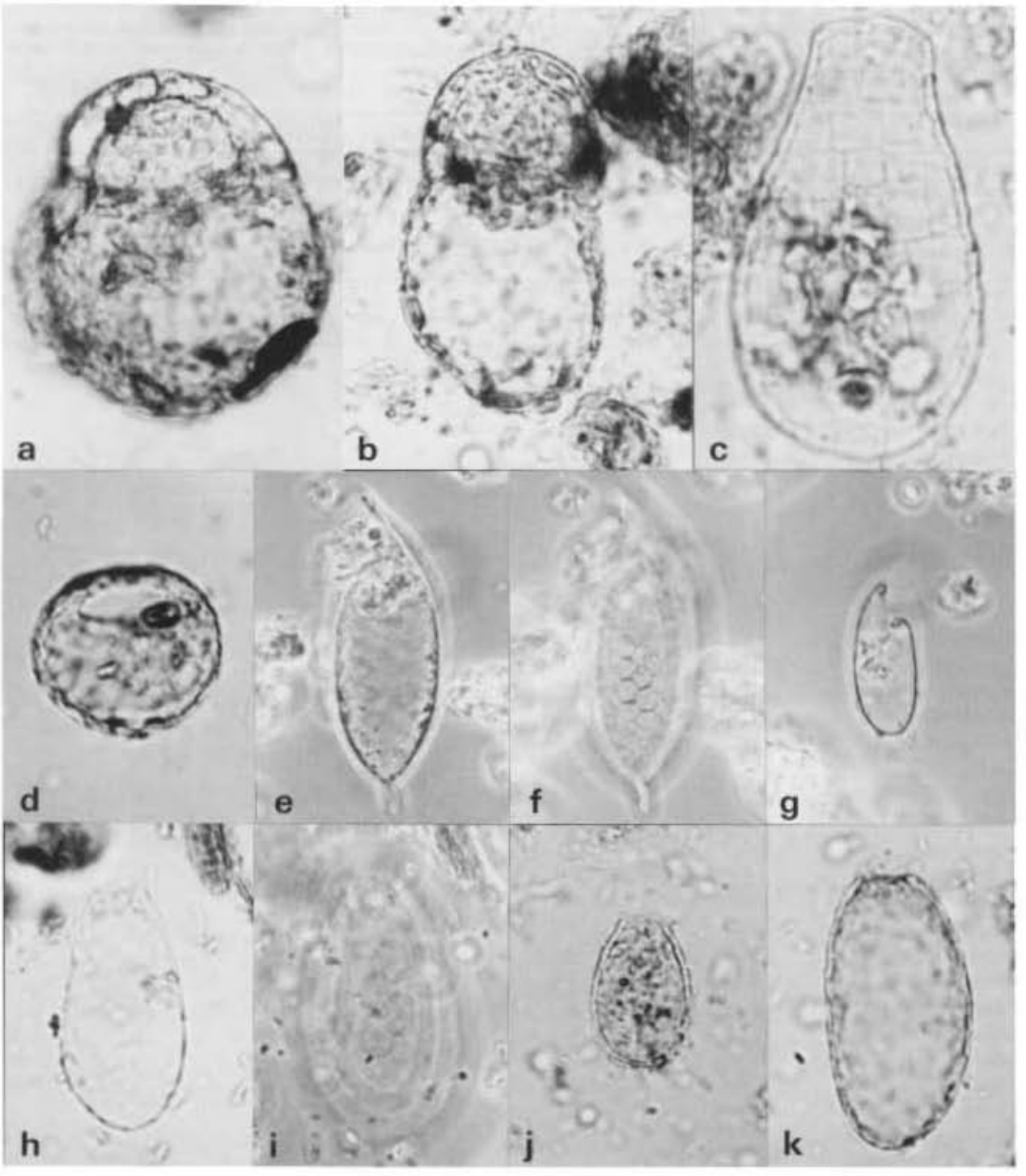

Plate 1. Photomicrographs of selected testate amoebae recovered from the Taf Estuary surface samples. All are plain transmitted light unless specified as phase contrast. All photographs taken at x1000 oil immersion, with x100 Nikon Plan Apochromatic objective lens. a. *Centropyxis cassis*, 64 μm long. b. *Centropyxis platystoma*, 66 μm long. c. *Quadrulella symmetrica*, 80 μm long. d. *Cyclopyxis arcelloides*, 30 μm diameter. e. *Cyphoderia* species 1, 58 μm including aboral horn. f. *Cyphoderia* species 1, phase contrast showing plates (4 μm maximum diameter). g. *Trinema lineare*, phase contrast, 28 μm. h. *Tracheleuglypha dentata*, 44 μm long. i. *Tracheleuglypha dentata*, phase contrast, plates 13 μm maximum diameter. j. *Difflugia* species 1, 38 μm long. k. *Difflugia* species 2, 71 μm long.

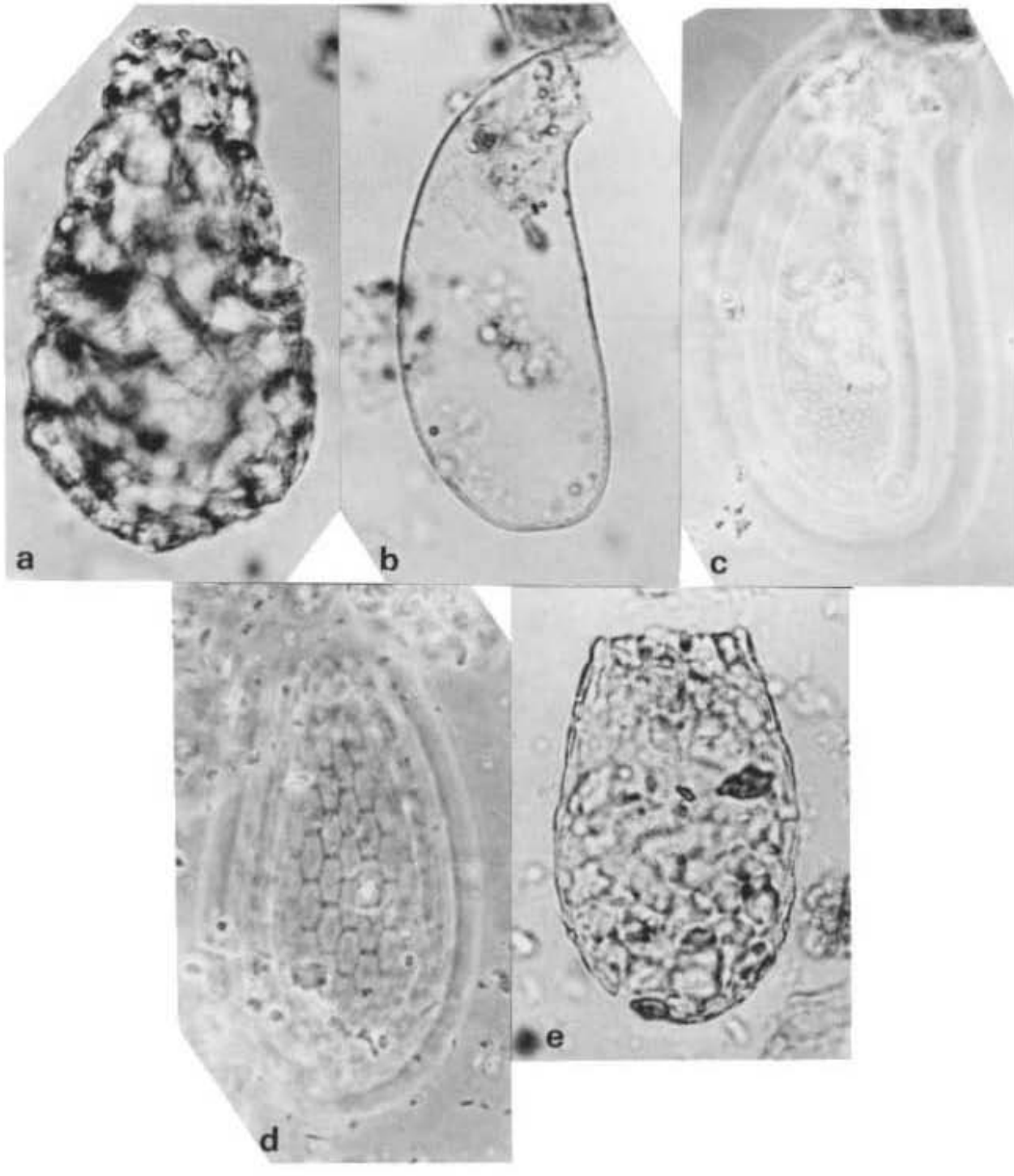

Plate 2. Photomicrographs of selected testate amoebae recovered from the Taf Estuary surface samples. All are plain transmitted light unless specified as phase contrast. All photographs taken at x1000 oil immersion, with x100 Nikon Plan Apochromatic objective lens. a. *Difflugia oblonga*, 93 μm long. b. *Cyphoderia ampulla*, 85 μm long. c. *Cyphoderia ampulla*, phase contrast, showing plate structure (each plate 1.5 μm maximum diameter). d. *Euglypha tuberculata*, phase contrast, showing plate structure. e. *Difflugia lucida*, 71 μm long.