Diversity and biogeography of testate amoebae

Humphrey Graham Smith · Anatoly Bobrov · Enrique Lara

Abstract Testate amoebae are amoeboid protists inhabiting a test (shell). They occur globally in soils, wetlands and freshwater, especially peats and mosses. They are of ancient origin, dating from at least the Mesozoic, with possible ancestors as old as the Neoproterozoic. Approximately 2,000 taxa have been described—a number which could easily rise to 4,000 with comprehensive recording. Whilst many protists appear to be cosmopolitan as morphospecies, some of the larger testate species (exceeding 100 μm) have long been considered, controversially, to be geographically restricted. Definitive conclusions have often been confounded by gaps in distributional data and misidentification. Recent increases in recording from previously little known regions, and the rise of molecular taxonomy, have started to resolve outstanding issues—processes still far from complete. Accordingly, biogeographical studies have concentrated on "flagship" species—those which can be identified with certainty and are sufficiently recorded to determine their ecological ranges. *Apodera vas* (Certes) has been proved to be largely restricted to the Gondwanaland continents and sub-Antarctic islands, but absent from the Holartic despite the availability of much suitable habitat. An early analysis postulated a Mesozoic origin of the species and a distribution influenced by continental drift. Recent molecular evidence could imply a later origin. Either way, its current distribution is clearly influenced by the pattern of global wind currents and lack of lowland tropical habitat. By contrast a "Gondwana-tropical" group of species appears to be restricted to latitudes unaffected by glaciation. Instances of local endemism, such as restriction to a single island, are also known, which await molecular evidence for substantiation.

Keywords Biogeography · Cosmopolitanism · Diversity · Ecology · Endemism · Flagship species · Fossil testates · Testate amoebae

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Introduction

Testate amoebae constitute a functional polyphyletic group of those amoeboid protozoa in which a single eukaryotic cell is enclosed within a shell or test (size range 5–300 µm) with an oral aperture, through which filose or lobose pseudopodia protrude during locomotion or feeding. They occur worldwide in a range of terrestrial, wetland and freshwater habitats, but most frequently in moist acid soils and peats, with high organic content and low nutrient turnover, and also in standing waters, lake margins and the biofilms of sewage treatment plants. They are universally associated with mosses, even in more arid conditions, such as moss cushions on tree bark and rocks.

Testate amoebae are phylogenetically divided into those with lobose and those with filose pseudopodia (Cavalier-Smith 2004). Testate lobose amoebae are Amoebozoa (Nikolaev et al. 2005) and include the larger species (>100 µm)—especially those in the families Difflugiidae, Centropyxidae, Arcellidae and Hyalospheniidae. Many species of the difflugiids and centropyxids have agglutinate tests composed of mineral particles gathered from the environment. Many large species of hyalospheniids (e.g. those in genera *Nebela* and *Heleopera*) have tests composed of idiosomes acquired from consumption of euglyphids as prey (Meisterfeld 2002a). Testate filose amoebae by contrast are closely related to the Foraminifera and Cercozoa (Longet et al. 2004) and include the families Euglyphidae and Trinematidae. These contain small species (<100 µm) with siliceous tests composed of idiosomes biosynthesised by the resident amoeba.

Testate amoebae research has contributed importantly to debate about the relevance to protists of Beijerinck's dictum *"Everything is everywhere, the environment selects"*. Apologists for this paradigm have presented evidence for the cosmopolitan dispersion of all microorganisms up to ca 1 mm in size (Finlay 2002; Finlay and Fenchel 2004). Whilst their evidence is based largely on freshwater ciliate records, they have also sought to extend it to soil testates (Finlay et al. 2001; Esteban et al. 2006). However there are numerous examples of testate taxa which appear to be geographically restricted at global, regional and local scales (Foissner 2006), selected examples of which are discussed below. Larger, and thus heavier, testate species disperse less rapidly than others. Natural barriers, such as adverse winds or extended areas without appropriate habitat, slow down their progression. For a single propagule to overcome these obstacles and found a new population becomes a statistically improbable event; therefore it may take thousands or millions of years until it happens. Whilst it is true that many microbial morphospecies, including the smaller testate amoebae, tend to a cosmopolitan distribution, this process is not fixed in time. What we observe nowadays is an instantaneous (and incomplete) picture of the distribution of testate species. It is inevitable that present-day patterns will evolve over succeeding millennia as new species appear, some species spread and others become extinct.

Historical development of perceived diversity and biogeography

Whilst it is generally acknowledged that many protozoa have (at least at the morphospecies level) a cosmopolitan distribution, some species of testate amoebae have remained one of the most striking examples of microorganisms that present biogeographical patterns in

their global distribution. The earliest biogeographical assessment of testates was made more than 130 years ago (Bonnet 1983). Ehrenberg concluded that large geographical areas have different faunas of testates but that the European fauna lacks uniqueness. However many protozoologists of the late 19th century were convinced that nonparasitic protozoans are essentially cosmopolitan. Penard (1902) thought that similar habitats in all parts of the world would contain similar faunal assemblages—a view which he later modified (Penard 1938). Heinis (1914) elucidated a circum-austral distribution of some species of *Nebela*. This phenomenon was further investigated by Deflandre (1928, 1936) who also noted a pantropical distribution of some species of *Arcella*. Models were continually revised through the 20th century as more locations were sampled and more species described.

An analysis by Cailleux (1978) of the publications of Decloitre noted the doubling in numbers of described species, sub-species and varieties globally: from 800 in 1952 to 1,600 in 1975. The continental distribution of taxon-richness then known was: Europe 1,031, Africa 648, Australia and Melanesia 428, North and Central America 229, Asia 220, the Arctic 220, Antarctica 89. Decloitre (1985) estimated that 1800 species, sub-species and varieties had been recorded globally. Differences between northern and southern testate faunas were noted repeatedly through the 20th century (Hoogenraad and Groot 1940, 1979; Jung 1942b; Cailleux 1978; Bonnet 1983; Smith and Wilkinson 1987; Wilkinson 2001; Meisterfeld 2002a, b; Foissner 2006), giving rise to models which have related some species' distributions to the palaeogeography of Gondwanaland and Laurasia. The high number of species recorded for Europe must surely be a reflection of the intensity of sampling, rather than true higher species-richness than the other continents. Whilst lower numbers for polar regions may be attributed to hostile environments, when the Americas, Asia and Australasia receive as much attention as Europe, it is reasonable to speculate that taxon-richness could very likely double from ca 2,000 to ca 4,000. The last 30 years have been notable for the increase in records from previously under-sampled regions—the Antarctic and the Far East (Smith 1978; Bonnet 1981; Vincke et al. 2004, 2006a; Yang et al. 2005b), thus providing a more comprehensive data base for biogeographical modelling at the global, regional and local levels.

The fossil record

Testates are certainly ancient taxa. Whilst fossil records are limited and discontinuous, tests exactly resembling those of modern species have been described from 2 and 15 Mya old sediments (Boeuf and Gilbert 1997; Foissner and Schiller 2001). Extant genera are also known from Mesozoic amber at several Holarctic sites dated at ca 100 and 220 Mya old (Poinar et al. 1993; Waggoner 1996; Schönborn et al. 1999; Schmidt et al. 2004). More recently, 'vase-shaped microfossils' with apparent testate affinities have been described from the Neoproterozoic, ca 700–750 Mya old (Corsetti et al. 2003; Porter et al. 2003). These putative testate ancestors were most likely marine, suggesting a switch to terrestrial and freshwater forms during their evolution. Their existence forms part of biologically based arguments that Neoproterozoic conditions may have been less extreme than those claimed for Snowball Earth (Corsetti et al. 2006).

There are considerable records of testates from the Quaternary sediments where they have been used as hydrological and acidity indicators (Charman 1997; Booth 2002; Mitchell et al. 2007), thus providing valuable evidence of Quaternary palaeoenvironmental changes and also of the ecology of extant species.

The whole testate taxa can thus be seen as genetically conservative, with many genera having persisted essentially unchanged (in test morphology at least) for 10's or 100's Mya. At the same time, continuing evolution at the sub-species level may well have occurred in the last few thousands of years (Grospietsch 1971).

Ecological diversity

In biogeographical research it is important to know when the absence of a species from a particular location is truly due to geographical restriction, rather than lack of appropriate habitat. However, whist there is a voluminous literature on the morphological diversity of testate species, the detailed habitat relations of many species are less well known. More is known about relations with moisture than with other environmental variables. An early scheme was proposed by Jung (1936) which classified species into six classes (FI–FVI) according to the moisture content of the habitat. The most extensive recent research on moisture relations has been by Quaternary palaeoecologists who utilize fossil testates as indicators of the moisture content and water tables of peat bogs dating from the Holocene (Charman 1997; Woodland et al. 1998; Booth 2002; Mitchell et al. 2007). This work has enabled lists of species to be determined, ordinated by moisture preference. The most hydrophilic terrestrial species include *Arcella discoides* Ehrenberg, *Apodera vas* (Certes), *Certesella certesi* (Penard) and *Difflugia bacillifera* Penard. Species of drier habitats include *Assulina muscorum* Greeff, *Corythion dubium* Taranek, *Euglypha rotunda* Wailes, *Phryganella acropodia* (Hertwig and Lesser), and *Trigonopyxis arcula* (Leidy) Penard.

The temperature relations of testate amoebae are still poorly known. A broad trend of decreasing species-richness in the southern hemisphere with increasing latitude and with declining mean January temperatures has been noted (Smith and Wilkinson 1987; Smith 1996). Similarly, testate species-richness of ten physiographic regions of Tibet and Yunnan, China, showed a strong positive correlation with the mean temperature of the warmest month (Yang et al. 2005b). However, monthly mean temperatures are an extremely crude indicator of habitat suitability within soils, wetlands and freshwater; there are doubtless many exceptions to the general trend (Vincke et al. 2006a); detailed microclimate data would be necessary to establish such a relationship with precision. In vitro experiments on the effect of temperature on growth rates and respiration of *Corythion dubium* Taranek and *Euglypha rotunda* Wailes demonstrated the ability of these species to tolerate conditions in the Antarctic, where these species are prominent in the testate communities (Smith 1973; Cowling 1983). Laybourn and Whyman (1980) found that increasing temperature promoted a higher reproductive rate in *Arcella vulgaris* Ehrenberg up to 20°C, but no further increase above that temperature. It seems likely that many testate species show some measure of facultative psychrophily, but much more research will be required to establish this as a general phenomenon of the taxon.

The eclectic nature of the feeding strategies of testate amoebae, and their influence upon other ecosystem components, have become better known in recent years. They were previously assumed to be largely bacteriophagous, since they can be routinely cultured successfully with bacteria as the sole food source. However, there is evidence that, in natural ecosystems, their feeding habits are very much more diverse; that many species will consume whatever prey items are most readily available and thus their diet may vary seasonally and with habitat. This topic has been reviewed by Schroeter (2001). His model of testate trophic relations divides testates into the smaller "panphytophagous" species, which graze on bacteria, algae, fungi (hyphae, spores and yeasts) detritus and humus

particles, and the larger "predaceous" difflugid and hyalospheniid species, which additionally feed on small testates, ciliates and micrometazoa (rotifers and nematodes). Subsequent analysis of the food preferences of *Nebela* spp. in *Sphagnum* peatland by Gilbert et al. (2003) has given results consistent with Schroeter's model: some 80% of their diet consisted of micro-algae (especially diatoms) and fungi; during Summer they also consumed ciliates, rotifers and small testates.

Testate amoebae species certainly show variation in their ability as pioneer colonisers of new habitats, and thus in their position along the r-K continuum. Species of the genera *Assulina, Centropyxis, Corythion, Euglypha, Phryganella* and *Trinema* are repeatedly reported as the earliest testate species to appear in the microbial succession occurring in volcanic tephra (Smith 1985), reclaimed mining spoil (Wanner and Dunger 2002) and permeable pavement biofilms (Coupe et al. 2003). These may reasonably be seen as r-strategists in relation to other testates, though all testate species could be judged K-selected in comparison with flagellate and ciliate pioneers. Early testate colonists persist in the succession as microbial communities become more species-rich and complex, often in high numbers. Thus later colonists are in addition to, and not instead of, the pioneers (Wanner and Xylander 2005).

Intra-specific diversity

Many testate species show considerable intra-specific variation in size and shape. Observations on individuals collected in the field sometimes show gradual transition between species that can be dependant on environmental variables such as moisture or availability of test-building material (Cash et al. 1919). Test morphology can also be experimentally influenced in clonal cultures. As long ago as 1916, Jennings showed that many different morphotypes of *Difflugia corona* Wallich could be obtained by artificial selection. More recently, it has been shown that the size of the test and oral aperture in *Cyclopyxis kahli* Deflandre and *Cyclopyxis eurystoma* Deflandre can be influenced by temperature and food supply, and that these induced changes are reversible over a few generations (Wanner and Meisterfeld 1994; Wanner 1999). Interestingly, the range of morphotypic variation that could be induced artificially never exceeded that observed in wild populations. Biometric analysis of 32 natural populations of 24 species by Bobrov and Mazei (2004) revealed that maximum length exceeded minimum length by a factor of x1.2 to x2.2—a significant degree of variability within local populations, which may reasonably be supposed to have a substantial degree homogeneity of both genes and environment. A global review of 130 individuals of the hyalospheniid *Apodera vas* (Certes) by Smith and Wilkinson (2007) likewise revealed a maximum to minimum length ratio of 2.3. Thus, for identification purposes, within-species 'lumping' may be more genetically valid than 'splitting'. It is likely that many, if not all, testate taxa are characterised by an important phenotypic plasticity, that can be seen as conferring a selective advantage and may be a significant factor in enabling their long persistence through geological time.

Biogeography

Confounding factors in assembling distributional data

Considerable data have been accumulated, through the 19th and 20th centuries, of species assemblages in sampled materials from a wide range of habitats in most areas of the Earth.

Overwhelmingly, current models of testate taxonomy, phylogeny, ecology and distribution are derived from data on morphospecies descriptions based on test characteristics. Whilst identification of clades by molecular methods is not yet far advanced, there is sufficient evidence to cause wide uncertainty about the value of the criteria traditionally used for taxon discrimination in testate amoeba taxonomy. Molecular phylogenetic analysis is now being used to elucidate the relationships among species and, therefore, to evaluate the validity of the criteria used in species identification. It appears that, within the genus *Euglypha* (possibly the most abundant and widespread of testate genera) valid characters for species separation can only be observed with the electron microscope (Wylezich et al. 2001; Lara et al. 2007a). This makes them unsuitable for large scale biodiversity surveys. Within the family Hyalospheniidae, it has been suggested that the taxonomic weight of criteria used in species or genus definition may not reflect evolution within the group.

Accordingly, the classification of the whole group may have to be re-evaluated, with the result that some species might well be redefined (Lara et al. 2007b).

Even with traditional taxonomy, there are uncertainties caused by under-recording in some parts of the world, by inconsistencies amongst authors as a result of misidentification or use of synonymies, and by differing views of the validity of sub-specific nominations ('splitters' *versus* 'lumpers'). Attempts to circumvent such confounding factors have been a continuing feature of testate biogeographical research.

The "flagship" species

The uncertainties encountered in species identification have long been a pitfall in the evaluation of the geographical distribution of testate species. Lack of clear definition has confounded unequivocal conclusions about biogeography and made it difficult to interpret ancient literature. A way to circumvent these problems is to identify 'flagship' species (Tyler 1996; Foissner 2006). These are species which present a very characteristic morphology that cannot be confused with any others, and which have a restricted distribution globally, even though their habitats are not restricted to that region. To qualify as "flagship" there should not be any intermediate forms known with other species. Since they have a striking and unambiguous morphology, ancient records testifying their presence can be trusted. At present, it makes sense to concentrate attention on the distribution of these flagship species, because they are the ones for which no misidentification can be possible. It is, however, important to note that other species, which are less conspicuous, could very well also show geographically limited distributions. It is probable that these taxa will be better studied using molecular tools, such as sequencing variable genes and/or microsatellite markers.

Cosmopolitanism versus global endemism in the Hyalospheniidae

The family of testate amoebae whose biogeography has been most studied is undoubtedly the Hyalospheniidae sensu Schultze (1877). They are defined by a laterally compressed ovoid or pyrifrom test and acrostome with terminal aperture. An acrostome and pyriform shell are considered to be ancestral traits, and molecular studies have proven that this family is paraphyletic (Nikolaev et al. 2005; Lara et al. 2007b).

Apodera vas (Certes) is certainly the best known example of a testate amoeba with a geographically limited distribution (Smith and Wilkinson 2007). The shape of its test is

unmistakable (Fig. 1a) and thus makes a perfect flagship species. First discovered in mosses from Tierra del Fuego by Certes (1891), it has been recorded from many other locations in the Southern hemisphere, including South Africa (Gericke 1932), Australia (Richters 1908; Meisterfeld and Tan 1998), New Zealand (Charman 1997), Kerguelen (Bonnet 1981), Iles Crozet (Vincke et al. 2004, 2006a), Marion Island (Grospietsch 1971) and South Georgia (Smith 1982; Beyens et al. 1995). It has also been reported from certain locations in the Northern hemisphere including Sumatra (Hoogenraad and Groot 1940), Nepal (Bonnet 1977), West Africa (Golemansky 1963; Bonnet 1978) and Central America (Laminger 1973a). However, it has *never* been found north of the tropical desert belt, despite the numerous studies undertaken in the Northern hemisphere (Mitchell and Meisterfeld 2005). This absence is even more remarkable in that *A. vas* is a very frequent species within its geographical range. So numerous are the records that its ecological range can be determined with certainty as occupying moist terrestrial to semi-aquatic habitats (Charman 1997) in the cool temperate to sub-Antarctic zone (Smith and Wilkinson 2007) including high montane habitats at tropical locations up to 4,000 m (Laminger 1973a; Bonnet 1980). However, it can occur in relatively unstable habitats such as forest litter, where the moisture content is variable; it must therefore have a good capacity for encystment (Bonnet 1969). The colonisation capacities of this species are illustrated by its presence on some of the most remote islands on Earth in the Southern Ocean: Kerguelen, Iles Crozet, Marion Island and South Georgia. The chance of the species having persisted on these islands in refugia during the Pleistocene here is remote indeed (Wilkinson 1990), so colonisation must have occurred during the last 10 millennia.

In an attempt to explain the continental distribution of A. *vas*, Smith and Wilkinson (1987) suggested that it appeared on the palaeo-continent of Gondwanaland in the Jurassic period. Its last common ancestor with its closest relatives, the cosmopolitan *Nebela tubulata* Brown, *N. wailesi* Deflandre and *N. lageniformis* Penard lived in Pangea more than 190 Mya ago, and *A. vas* evolved in allotropy after the separation of Gondwanaland. This hypothesis would imply that the species is very old, at least 140 Mya; it is therefore to be expected that its sister species, also found in the north, would be quite distantly related. However, molecular data currently available suggest that *A. vas* may be closely related to the cosmopolitan *N. lageniformis*. The two species present only 3% variation on a sequence of a SSU rRNA gene fragment, which includes the most variable regions of that gene. If this close similarity were confirmed by multigene analysis, it would suggest that the amount of time passed after the divergence of the two taxa might not be so large, and certainly much shorter than the separation between Gondwanaland and Laurasia (Lara et al. 2007b).

The alternative explanation is that *Apodera vas* speciated much more recently in the cool temperate Southern hemisphere (most probably South America) and the cysts were subsequently dispersed by wind to the other continents and also to the remote islands of the Southern Ocean. The strong circum-Antarctica westerly wind has been frequently cited as an effective agent for the dispersal of colonising propagules (McDowall 2005). The spread northwards of cysts as airborne particles through the Capricorn tropic, because the pattern of air currents, is highly unlikely. However, progressive land colonisation through suitable environments created by high mountain ranges, where cold and humid climates prevail (e.g. Andes, African mountains, Himalayas) is much more feasible. In contrast, crossing the Northern desert belt around the Cancer tropic (e.g. Mexican and Sahara deserts) would be much more unlikely owing to absence of suitable habitat and an adverse wind regime. This hypothesis is plausible if *Apodera vas* arose when these barriers were already present.

At least 18 other taxa of nebelid Hyalospheniidae also appear to have Gondwanaland-specific distributions (Smith and Wilkinson 1987) including *Certesella certesi* (Penard),
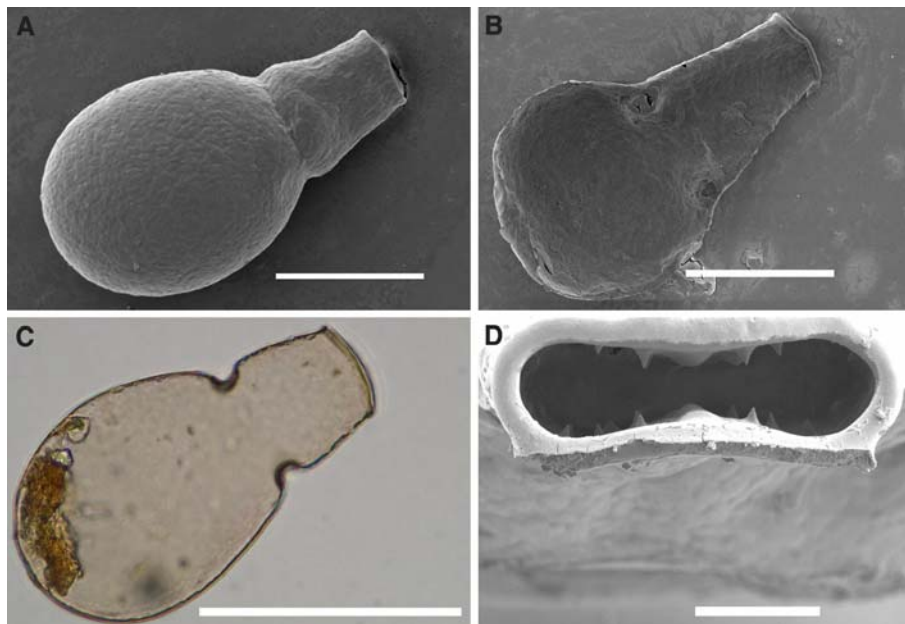
Fig. 1 Illustration of some species of Hyalospheniidae from the southern hemisphere. **A** = *Apodera vas* from Marion Island; **B** = *Certesella* sp. from Marion Island; **C** = *Alocodera cockayni* from Argentine Patagonia; **D** = A detail from the pseudostome of *Certesella murrayi* shows the typical punctuation inside the neck of the shell. Scale bars represent 50 μm (**A**, **B**, **C**) and 10 μm (**D**)

Certesella murrayi (Wailes) (Fig. 1b, d) and *Alocodera cockayni* (Wailes) (Fig. 1c), which also have characteristic, unmistakable shapes. Of these, *C. certesi* is the next most frequently recorded of the Gondwana-specific species after *A. vas* and most likely has a comparable biogeographical history. It shows an ecological difference in that its range extends to the Antarctic zone. *Certesella murrayi* and *A. cockayni are* rarer species with more disjunct distributions. *Alocodera cockayni* has been recorded several times from New Zealand as well as South America (Charman 1997), whereas *C. murrayi* may be endemic to South America (Vucetich 1978).

The existence of Northern-specific species is much more difficult to elucidate because the tropics and Southern hemisphere have been so much less sampled than Northern regions. Therefore, evidence that a species is truly absent from the South is much less likely to be conclusive. Furthermore, difficulties of access to literature can lead authors to misinterpretations. Hoogenraad and Groot (1979) cited the species *Nebela carinata* (Archer) Leidy, *N. marginata* Penard, *N. tubulosa* Penard, and *Hyalosphenia papilio* Leidy as Northern endemics, although they had been found previously in Congo by Gauthier-Lièvre (1954) and Decloitre (1965). Tentatively, the large *Nebela speciosa* Deflandre appears a good candidate for Northern endemism; its very large size (>200 μm, and even 278 μm) makes it a good flagship species and it has only been reported from North America, Germany and England (Deflandre 1936; Grospietsch 1958; Laminger 1973b; Ogden 1984). From its morphology, it appears that its closest relative might be *Nebela tubulosa* Penard (Ogden 1984).

Regional endemism

Several mid-20th century attempts were made to define regional differences in testate fauna. Jung (1942a) argued for the existence of regional faunas as a result of limited dispersal abilities of some species—particularly the forest dwelling species which have decreased ability to form cysts (Bonnet 1983). Jung (1942b) described eight new nebelid species which, like *Certesella murrayi*, appear to be restricted to South America—discoveries which are consistent with the general paradigm that the South American continent represents a biodiversity "hotspot" globally.

Decloitre (1953) sought to distinguish between the faunas of both temperate zones and the tropics, whilst Oye (1960) distinguished the faunas of the Palaearctic, the Nearctic and the Southern hemisphere. Schönborn (1966) argued that species which differ on the different Southern hemisphere continents must have evolved after the breakup of Gondwanaland. Stout (1969) compared the testate faunas of New Zealand, Australia and Europe using the family taxonomy then in favour (% figures approximate):

New Zealand: Euglyphidae 40%, Nebelidae 33%, Centropyxidae 9%, Difflugiidae 7%, Arcellidae less than in Australia and Europe.

Australia: Euglyphidae 40%, Nebelidae 25%, Centropyxidae 22%, Difflugiidae 7%, Arcellidae more than in New Zealand.

Europe: Euglyphidae 40%, Nebelidae 14%, Centropyxidae 22%, Difflugiidae 7%, Arcellidae as in Australia.

The constant figure for the Euglyphidae is notable. This family contains species almost all less than 100 µm long. These, more than species of other families, can be expected to have cosmopolitan distributions at the morphospecies level.

Bonnet (1980) proposed a "Gondwana-tropical" group of species and included in it some species of *Hoogenraadia, Planhoogenraadia, and Lamptopyxis*, whose ranges cover the tropical areas of Africa, South and Central America, Southeast Asia, New Guinea and Philippines, but lie north of circum-Austral zone. Subsequently (1983) he broadened the group by including some species of *Centropyxis, Cyclopyxis, Deharvengia, Ellipsopyxis, Plagiopyxis*, and *Protoplagiopyxis.* Korganova (1994) reported that most of the testate species found on 13 Pacific islands of Tonga and Western Samoa were cosmopolitan, but 7 species belonged to the Gondwana-tropical group. Species such as *Hoogenraadia humicola* Bonnet, *Planhoogenraadia media* Bonnet, *and Distomatopyxis couillardii* Bonnet and Gomez-Sanchez (Bobrov 2001) occupy a particular place among the faunas of the southeastern Palaearctic (Fig. 2), the ranges of *Hoogenraadia* and *Planhoogenraadia* occupying mainly the Holarctic (Bonnet and Gomez-Sanchez 1994; Todorov and Golemansky 1999; Beyens and Meisterfeld 2002). The northern boundary of these taxa's geographical ranges seems to correlate with the maximum extent of Pleistocene glaciation.

Until recently, the testate amoebae of the Far East were unknown to western scientists. Evidence is now emerging of the existence of a distinctive testate fauna with the description of four species, specific to the Far East: *Collaripyxidia dongtongiensis* (Balik and Song 2000), *Difflugia tuberspinifera* (Yang et al. 2004), *Difflugia biwae* (Yang and Shen 2005), and *Difflugia mulanensis* (Yang et al. 2005a).

Evidence of Nearctic endemism comes from the discovery of several new species from Canada. *Paraquadrula ogdeni* and *Netzelia labeosa* have been described from the arctic North West Territory (Beyens and Chardez 1997), whilst *Corythionella golemanski*, *Cyclopyxis acmodonta* and *Arcella formosa* have been described from wetland and freshwater in Ontario (Nicholls 2003, 2005).
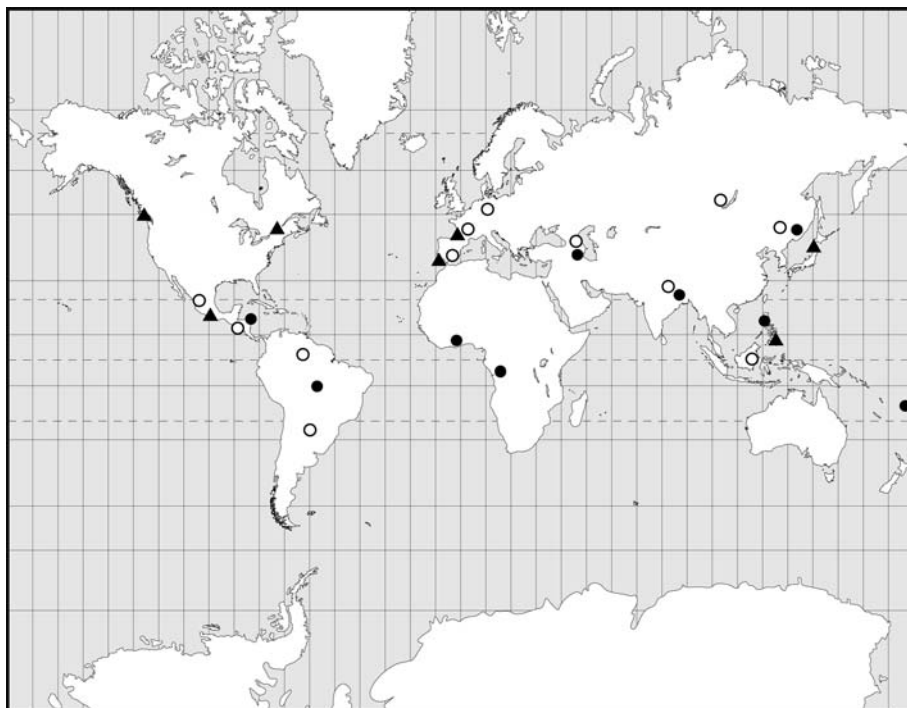
Fig. 2 The recorded global distribution of three genera of the Gondwana-tropical group of testate amoebae. ●, Genus *Hoogenraadia;* ○, Genus *Planhoogenraadia;* ▲, Genus *Distomatopyxis*

A possibly unique soil environment existed in north-eastern Siberia during the late Pleistocene as shown by the studies of Bobrov et al. (2003, 2004) on Quaternary perma-frost deposits on the Bykovsky Peninsula of the Laptev Sea (71° N)—an area which is now Arctic tundra, but possibly warmer during Pleistocene interglacial periods. An *Argynnia* species was found in samples dated to 45,300 + 1,200/–1050; 44,280 + 1,320/–1,120; and >41,830 years BP. Nowadays, only two species of this 15-species genus, *A. dentistoma* (Penard) and *A. vitraea* (Penard), are known to have widespread cosmopolitan distributions (Ogden and Hedley 1980). Other species have disjunct recorded distributions in Canada, Eurasia, Australia and the Antarctic (Deflandre 1936; Meisterfeld and Tan 1998). No species of this genus, except *A. dentistoma*, has been found in the present day Arctic (Beyens and Chardez 1995).

Cyclopyxis puteus Thomas (a rare and infrequent species today) was also recorded from a sample dated *ca.* 45,000 BP. This species has predominantly a Holarctic distribution in temperate and boreal forest (Bobrov 2001; Todorov 2001); however it has been recorded from Arctic tundra in Canada and Spitsbergen (Beyens and Chardez 1995). There is also a single record of a solitary specimen from the sub-Antarctic Iles Crozet (Vincke et al. 2006b). Given the large size of this species (195 μm diameter), the possibility of misi-dentification is remote; however this latter record appears anomalous and emphasizes the difficulty of defining the distribution patterns of rare species with certainty. It is also interesting to note the records of the *Nebela bigibbosa* Penard from the late Holocene samples, ^{14}C dated to 1,360 ± 35; 1,240 ± 60 and 1,080 ± 35 years BP (Bobrov et al.

2003, 2004). This species typically inhabits forest litter (Todorov 2002). The only previous record of it from the high Arctic is that of Penard (1903) from Spitsbergen.

A bi-polar comparison of recorded testate fauna has been made by Wilkinson (2001) which revealed that the largest testates (up to 245 μm) tended to occur in either the Arctic or the Antarctic, but not both, whilst the bi-polar species had a maximum size of 135 μm. This analysis is currently being repeated, incorporating more recent data and extending it to include the "Third Pole"—the high Himalayan mountains of Tibet and northwestern Yunnan, China, utilizing the work of Yang et al. (2005b) who has assembled a comprehensive list of 207 species across 10 physiographical regions. This tri-polar comparison is expected to yield valuable insights.

The characteristic fauna of various regions appears to be determined by an interaction of factors of a general biogeographical nature—the availability of habitats in each climatic zone, the cyst-forming and dispersal abilities of different species, and the existence of geographical barriers. It seems certain that regional endemism is a real phenomenon amongst testate species, but our understanding is so far incomplete, pending the acquisition of more comprehensive data.

Local endemism

Many cases of endemism to smaller localities, such as a single island, have been documented. Again, southern hemisphere nebelids are prominent. An often cited example is *Argynnia antarctica*, a species apparently endemic to the sub-Antarctic Marion Island, described by Grospietsch (1971), with supporting biometrical evidence. Although no molecular data are available, it appears to be recently evolved since it is likely that the whole testate fauna of Marion Island (like South Georgia and other sub-Antarctic islands) colonised the island since the end of the Pleistocene (Wilkinson 1990). It can be argued from its morphology that it evolved from the cosmopolitan *Argynnia dentistoma* (Penard), or from the Southern hemisphere endemic *A. teres* Jung, and that it has not yet had time to reach southern Africa. Other candidate species for insular endemism in the southern hemisphere include *Nebela similes* and *Nebela australis* on Tierra del Fuego (Vucetich 1972) and *Nebela subspherica* in New Zealand (van Oye 1956). An example from the Arctic is the description of *Schoenbornia smithi* from Spitsbergen by Beyens and Chardez (1997).

A less plausible example is the description by Decloitre (1964) of a new variety of *Nebela bohemica (N. bohemica* Taranek var. *adeliae)* as endemic to Terre Adélie, Antarctica. It is considerably larger than previously described specimens of *N. bohemica* (Deflandre 1936). However, in the absence of other evidence, it is valid to consider this as a phenotypic "variety" only. As in all cases of claimed endemism, it is possible to find morphologically closely related forms which have a wider distribution.

Recently reported testate species from the remote Ascension Island in the tropical mid-Atlantic (Wilkinson and Smith 2006) consist of cosmopolitan species only. This observation is consistent with the fact that this arid island was largely devoid of suitable habitats for testates until the 19th century, when imported plants with accompanying soil were artificially established there, inducing microclimatic changes (Ashmole and Ashmole 2000). It may be inferred that most of the testate fauna of Ascension Island is the result of recent arrival (since 150 years ago) and that endemic sub-speciation has not yet had time to occur.

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