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Arcellinida testate amoebae (Amoebozoa: Arcellinida): model of organisms for assessing microbial biogeography

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7.1 Introduction

Although widely recognised as essential participants in ecosystem processes and representing a significant part of the Earth's biodiversity (Clarholm, 1985; Corliss, 2002; Schröter et al., 2003; Falkowski et al., 2004), eukaryotic microorganisms are very poorly understood from evolutionary and biogeographic points of view. Major questions concerning the diversity and the distribution of protists remain completely unresolved. Arcellinida testate amoebae are an excellent group from which to get insights into these questions because they are easy to collect, present in different habitats and they build a shell of characteristic morphology that remains even after the organism's death. In this group, both cosmopolitan and restricted distribution patterns have been documented. Some morphospecies such as *Apodera vas(=Nebela vas)*, *Alocodera cockayni* or the whole genus *Certesella* have been reported as one of the most convincing examples of heterotrophic protists with restricted distributions (Foissner, 2006; Smith and Wilkinson, 2007; Smith et al., 2008). Arcellinida testate amoebae belong to the eukaryotic supergroup Amoebozoa (Nikolaev et al., 2005) and are morphologically characterised by the presence of lobose pseudopodia and a shell (test) composed from proteinaceous, calcareous or siliceous material. It can be either self-secreted or composed of recovered and agglutinated material. The Arcellinida covers a relatively broad range of sizes (mostly between 20 and 250 µm). At least some species have the ability to form a resting stage allowing their persistence under unfavourable conditions and a relatively unlimited dispersal capacity (Corliss and Esser, 1974; Foissner, 1987).

Arcellinida diversity is estimated at about 1500 species, mostly belonging to the genera *Centropyxis*, *Difflugia* and *Nebela* (Meisterfeld, 2002). They are diverse and abundant in virtually all terrestrial and freshwater aquatic habitats on Earth from the tropics to the poles (Meisterfeld, 2002) but they were not reported from truly marine environments. A few species have however successfully colonised brackish water ecosystems such as the marine supralittoral zone (Golemansky, 2007; Todorov and Golemansky, 2007), as well as the saline soils (Bonnet, 1959). The Arcellinida feed on bacteria, plant cells, protists, fungi or small metazoans (Foissner, 1987; Yeates and Foissner, 1995; Gilbert et al., 2000). Moisture conditions and pH are major environmental variables controlling the occurrence of testate amoebae (Charman and Warner, 1992; Charman, 1997) and the response of testate amoebae to different ecological gradients and pollutions make them a useful tool for palaeoecological studies and pollution monitoring (Charman et al., 2004; Nguyen-Viet et al., 2007, 2008; Laggoun-Defarge et al., 2008; Lamentowicz et al., 2008; Mitchell et al., 2008; Kokfelt et al., 2009).

7.2 Biogeography of Arcellinida: historical views

The first investigations on the biogeography of microorganisms date back to the mid nineteenth century when Christian Gottfried Ehrenberg (1795–1876) claimed that mountain ranges separated divergent populations of 'infusoria' (ciliates) (Ehrenberg, 1838, 1850). In his famous voyage on the Beagle, Darwin collected some dust fallen after a storm in the Cape Verde Islands and sent this sample to

Ehrenberg for analysis of the ciliate populations. Surprisingly for them, they found two species observed only in South America and none observed in Africa, in spite of the fact that the wind was blowing westwards and that Africa was closer than America. This apparent contradiction, in addition to the observation that relatively large particles could be transported ('above the thousandth of an inch square'), incited Darwin to think that small organisms have a huge dispersal potential.

The paradigm of the ubiquity of microorganisms came later with the emergence of microbiology/bacteriology as a recognised scientific discipline (O'Malley, 2007). At that time, the study of environmental samples was based on the retrieval and characterisation of pure cultures, and it was observed that identical organisms could be found whenever identical nutritional and physical conditions were provided. Thus, microorganisms had to be ubiquitous, and all environments were provided with a constant input of a 'seed rain' of microbes awaiting the adequate conditions to prosper. This idea was developed mainly by Beijerinck, the founder of the Delft School in Microbiology, who showed that it was possible to predict the composition of a ciliate community knowing the parameters of the environment (Beijerinck, 1913). The famous sentence 'Everything is everywhere, but the environment selects' was later formulated by one of his followers, Baas Becking (Baas Becking, 1934; de Wit and Bouvier, 2006).

This paradigm seemed to rule the viewpoints of the scientific community on the biogeography and distribution of protists well into the twentieth century. Eugène Penard, a famous pioneer on the study of testate amoebae, was convinced that the objects of his studies could be found everywhere when suitable conditions were met (Penard, 1902). In his 1902 monograph, Penard recorded 92% of all Arcellinida and Euglyphida testate amoebae species described to that date in the Lake Geneva area alone. However, with hindsight, he had described many of the species in that monograph or in earlier studies, so this finding may not be very surprising. Indeed, in the following decades, other researchers such as Heinis (1914) and Deflandre (1928, 1936) analysed samples from other biogeographic regions and observed significantly different faunas. Later, Penard himself eventually revised his opinion of the cosmopolitanism of testate amoebae (Penard, 1938).

These pioneering studies had a major influence on later research. By the end of the twentieth century and the beginning of the twenty-first, the existence of limited geographic ranges in testate amoebae was admitted by almost all specialists. Diatom taxonomists also reached a similar conclusion (Kilroy et al., 2003; Van de Vijver et al., 2005; Vanormelingen et al., 2008).

7.3 Endemic Arcellinida morphospecies

Arcellinida testate amoebae comprise some of the most convincing illustrations of non-cosmopolitan heterotrophic protists. The conspicuous testate amoebae

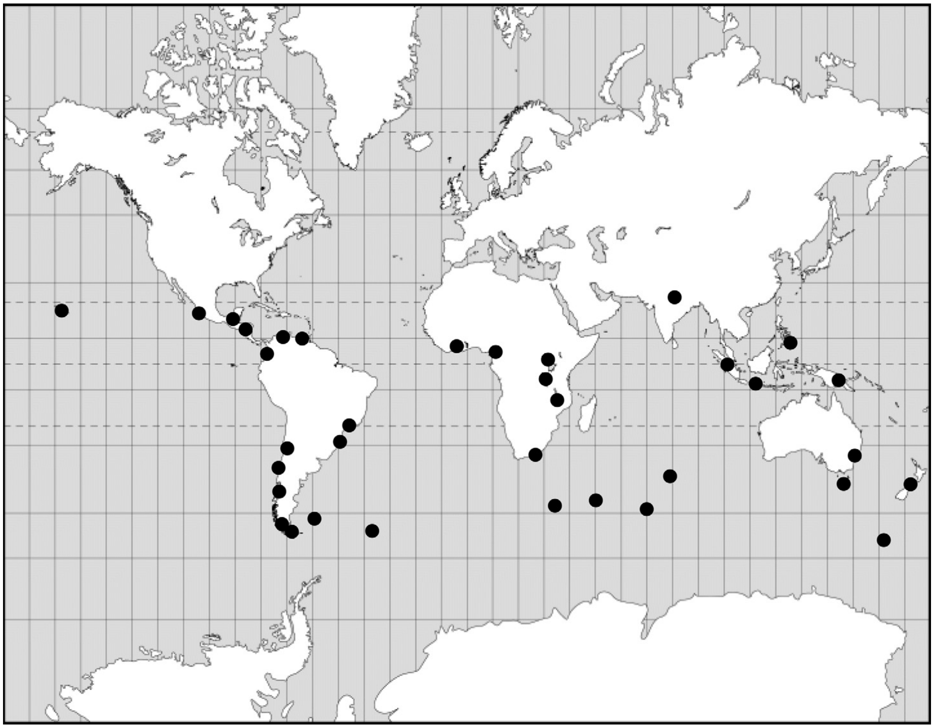

Fig 7.1 Sketch map showing *Apodera vas (=Nebela vas)* records in the southern hemisphere and the tropics (after Smith and Wilkinson, 2007).

Apodera vas and *Certesella* spp., which occur only to the south of the Tropic of Cancer desert belt (Fig 7.1) (Smith and Wilkinson, 2007; Smith et al., 2008), represent classical examples of morphospecies with restricted distributions.

The geographic distributions of these organisms contradict Baas Becking's 'Everything is everywhere, but the environment selects' tenet because in spite of the fact that they were found frequently in the southern hemisphere and low latitudinal zones of the northern hemisphere, they were never encountered in similar Holarctic habitats where most studies on testate amoebae from distinct habitats such as mosses, soils or aquatic environments took place. These genera also stand out by their very distinct morphologies which allow unambiguous identification, making them good examples of 'flagship species' (*sensu* Foissner, 2006). This contrasts with many other taxa of testate amoebae and other free-living protists whose geographic distribution cannot be established with any degree of certainty because of taxonomic uncertainties (Smith et al., 2008; Heger et al., 2009). It is therefore quite likely that besides these flagship species, many more taxa also have limited distributions (see further).

7.4 Assessing the distribution of *Apodera vas* and *Certesella* morphospecies in Mesoamerica

Mesoamerica has been the place of contact between South American and North American fauna and flora, an event that took place 3 Mya when the Panamá isthmus was closed allowing macroorganisms to disperse between the two American continents (Webb, 1991). However, in comparison with what is known with macroorganisms the potential impact of the rise of the isthmus on the dispersion of microorganisms between North and South America (and/or vice versa) remains completely unknown. Mesoamerica represents an interesting test case for the ongoing debate over microbial biogeography: if long-distance dispersal is easy for microorganisms then the existence or absence of this isthmus should not affect distribution patterns. However, if microorganisms do not disperse easily over long distances, then the presence of an isthmus will affect distribution patterns by allowing southern taxa to migrate northwards or vice versa.

Because of the possible existence of distinct faunas of free-living protists in South and North America, Mesoamerica is a key region for the study of protistan biogeography. However, although the biogeographic distributions of *Apodera vas* and *Certesella* spp. are reasonably well documented at a global scale, their distributions in Mesoamerica have so far not been investigated systematically (Heinis, 1911; Kufferath, 1929; Bonnet, 1977a; Madrazo-Garibay and López-Ochoterena, 1982; Smith and Wilkinson, 2007; Smith et al., 2008). To our knowledge, only two studies based on a very small number of samples reported the presence of *Apodera vas* in Costa Rica, Guatemala and Mexico (Golemansky, 1967; Laminger, 1973) while *Certesella* spp. were only reported in Guatemala (Laminger, 1973). To this day, the second northernmost reported continental occurrence of *Apodera vas* is Mexico (Laminger, 1973); Nepal is the northernmost occurrence (Bonnet, 1977b). The aim of this study is to estimate the distribution of the 'southern hemisphere and tropical endemic Hyalospheniids' (SHTEH; i.e. *Apodera vas* and members of genus *Certesella*) along a south–north latitudinal Mesoamerican transect extending from Panama to the south of Mexico.

7.5 Methods and sampling area

To assess the biogeographic distributions of *Apodera vas* and *Certesella* morphospecies, over 200 moss and litter samples were collected along a transect from Panama to Mexico. In addition to the latitudinal gradient, this region is characterised by altitudinal gradients, resulting in a broad diversity of biomes from wet lowland rainforest and cloud forest to dry scrublands and desert. In this study, wet moss and wet litter samples were collected in cloud and mesophilous forests between April and May 2007 (Table 7.1) because such moist substrates were described as suitable habitats for *A. vas* and *Certesella* spp. (SHTEH) (Smith and Wilkinson, 2007 and references therein). To extract testate amoebae, moss and litter samples were shaken energetically in water and then sieved and back-sieved using appropriate mesh sizes (250 and 20 µm). The occurrence of *Apodera vas* and *Certesella* spp. was checked using a light microscope under 200× magnification. Cells from *Apodera vas* specimens from Monte Cristo (El Salvador) were isolated and documented by scanning electron microscopy (SEM). The SEM was performed as described in Heger et al. (2009). Up to now, three *Certesella* species have been described (Meisterfeld, 2002). However, given that the taxonomy of genus *Certesella* is still relatively unclear, we adopted a conservative approach and did not distinguish among *Certesella* species.

7.6 Results and discussion

This survey confirmed the widespread occurrence of SHTEH in Mesoamerica. For the first time, *Apodera vas* was recorded in Panama, Salvador and Nicaragua while *Certesella* morphospecies were recorded for the first time in Panama, Costa Rica, Nicaragua, Salvador and Mexico (Table 7.1, Figs 7.2 and 7.3). We found *Certesella* spp. and *Apodera vas* specimens in most samples collected from favourable forest habitats (i.e. permanently wet moss and litter samples). The exact northernmost distribution limit of SHTEH in Mexico remains unclear. The region located between Mexico City and the USA was indeed poorly or not investigated. All samples with *Apodera vas* or *Certesella* spp. were collected at elevations between about 1000 and 3100 m a.s.l. *Apodera vas* and *Certesella* spp. co-occurred in seven of the 35 samples (Table 7.1), confirming that these species have relatively similar ecological tolerances (Charman, 1997). Our data also confirm that as far as suitable microhabitats occur, these SHTEH can live in relatively distinct ecosystems. For instance, we found *Apodera vas* and *Certesella* spp. in the Monteverde cloud forest where mean annual precipitation and temperature were 2500 mm and 18.8 °C (Clark et al., 2000) as well as in the Volcan Poás forest where precipitation exceeded 3400 mm and mean annual temperature was lower than 13 °C (Rowe et al., 1992; Martinez et al., 2000). In the literature, these species with restricted distributions were also reported in South American peatlands (Zapata et al., 2008) and New Zealand peatlands (Charman, 1997). Interestingly, some moss species harbouring SHTEH in the south of the Tropic of Cancer desert belt are also present in the Holarctic habitats where SHTEH were never reported. For instance, *Apodera vas*

Table 7.1 Locations and characteristics of all *Apodera vas* and *Certesella* spp. records in Mesoamerica.

Apodera vas	*Certesella* sp.	Sampling location	Country	Coordinates	Sampling date	Substrate	Ecosystem	Altitude (m)	Reference
	*	Parque La Amistad	Panama	08°53′58.5″N 82°37′11″W	30.3.2007	moss	Cloud forest	~2455	new record
*		Parque La Amistad	Panama	08°54′04.9″N 82°37′13″W	30.3.2007	moss	Cloud forest	~2450	new record
	*	Parque La Amistad	Panama	08°54′05″N 82°37′05″W	30.3.2007	moss	Cloud forest	~2380	new record
*		Parque Nacional Chirripo	Costa Rica	09°27′N 83°34′W	11.4.2007	moss	Cloud forest	~2350	new record
*		Parque Nacional Chirripo	Costa Rica	09°27′53″N 83°34′03″W	11.4.2007	moss	Cloud forest	2066	new record
*		Parque Nacional Chirripo	Costa Rica	09°27′15.6″N 83°32′55.1″W	11.4.2007	moss	Cloud forest	~2400	new record
*		N.A	Costa Rica	c. 9–10°N 82–84°W	1966	N.A	N.A	2000–4000	Laminger (1973)
*	*	Volcan Barva	Costa Rica	10°08′N 84°06′W	15.4.2007	moss	Cloud forest	~2830	new record
*		Volcan Barva	Costa Rica	10°08′N 84°06′W	15.4.2007	moss	Cloud forest	~2830	new record
*	*	Volcan Poás	Costa Rica	10°11′27″N 84°13′58.6″W	13.4.2007	moss	Cloud forest	~2575	new record

Table 7.1 (*cont.*)

Apodera vas	***Certesella* sp.**	**Sampling location**	**Country**	**Coordinates**	**Sampling date**	**Substrate**	**Ecosystem**	**Altitude (m)**	**Reference**
*	*	Volcan Poás	Costa Rica	10°11′27″N 84°13′58.6″W	13.4.2007	moss	Cloud forest	~2575	new record
*	*	Volcan Poás	Costa Rica	10°11′27″N 84°13′58.6″W	13.4.2007	moss	Cloud forest	~2575	new record
	*	Volcan Poás	Costa Rica	10°11′27″N 84°13′58.6″W	13.4.2007	moss	Cloud forest	~2575	new record
*		Monteverde reserva	Costa Rica	10°18′N 84°47′W	18.4.2007	moss	Cloud forest	~1570	new record
*		Monteverde reserva	Costa Rica	10°18′N 84°47′W	18.4.2007	moss	Cloud forest	~1570	new record
*		Monteverde reserva	Costa Rica	10°18′N 84°47′W	18.4.2007	moss	Cloud forest	~1570	new record
*		Monteverde reserva	Costa Rica	10°18′N 84°47′W	18.4.2007	moss	Cloud forest	~1570	new record
*		Santa Elena reserva	Costa Rica	10°20′N 84°47′W	20.04.2007	moss	Cloud forest	~1550	new record
*		Volcan Mombacho	Nicaragua	11°50′07″N 85°58′46″W	20.04.2007	moss	Cloud forest	1123	new record
*		Volcan Mombacho	Nicaragua	11°50′03″N 85°58′48″W	20.04.2007	moss	Cloud forest	1143	new record

*		Parque Montecristo	Salvador	14°25′N 89°21′W	24.4.2007	moss	Cloud forest	2140	new record
*		Parque Montecristo	Salvador	14°25′N 89°21′W	24.4.2007	moss	Cloud forest	~2000	new record
*		Parque Montecristo	Salvador	14°25′N 89°21′W	24.4.2007	moss	Cloud forest	~2000	new record
*		Parque Montecristo	Salvador	14°25′N 89°21′W	24.4.2007	moss	Cloud forest	~2000	new record
*		Parque Montecristo	Salvador	14°25′N 89°21′W	24.4.2007	moss	Cloud forest	~2000	new record
*	*	N.A	Guatemala	c. 14–15°N, 89–92°W	1966/1970	N.A	N.A	2000–4000	Laminger (1973)
*	*	Biotopo del Quetzal	Guatemala	15°12′N 90°13′ W	27.04.2007	moss	Cloud forest	~1650	new record
	*	Biotopo del Quetzal	Guatemala	15°12′N 90°13′W	27.04.2007	moss	Cloud forest	~1650	new record
	*	Biotopo del Quetzal	Guatemala	15°12′N 90°13′W	27.04.2007	moss	Cloud forest	~1650	new record
	*	Biotopo del Quetzal	Guatemala	15°12′N 90°13′W	27.04.2007	moss	Cloud forest	~1650	new record
	*	Biotopo del Quetzal	Guatemala	15°12′N 90°13′W	27.04.2007	moss	Cloud forest	~1650	new record
*		Biotopo del Quetzal	Guatemala	15°12′N 90°13′W	27.04.2007	moss	Cloud forest	1750–1900	new record

Table 7.1 (*cont.*)

Apodera vas	***Certesella* sp.**	**Sampling location**	**Country**	**Coordinates**	**Sampling date**	**Substrate**	**Ecosystem**	**Altitude (m)**	**Reference**
	*	Biotopo del Quetzal	Guatemala	15°12′N 90°13′W	27.04.2007	moss	Cloud forest	1750–1900	new record
*		Ixtlán	Mexico	17°17′45″N 96°23′ 53″W	5.5.2007	moss	Cloud forest	2350	new record
*	*	Ixtlán de Juárez	Mexico	17°22′49″N 96°26′ 51″W	6.5.2007	moss	Mesophilous forest	3050	new record
*		Tequila	Mexico	18°43′32″N 97°04′ 37′ ′W	8.5.2007	litter	Forest	1770	new record
*		Tequila	Mexico	18°43′32″N 97°04′ 37″W	8.5.2007	litter	Forest	1770	new record
*		N.A	Mexico	c. 19°N, 97°W	1966	N.A	N.A	2000ñ4000	Laminger (1973)
*		Between Desierto de los Leones and Cruz Blanca	Mexico	19°16′N, 99°19′W	1967	moss	Temperate forest	3100–3700	Golemansky (1967)

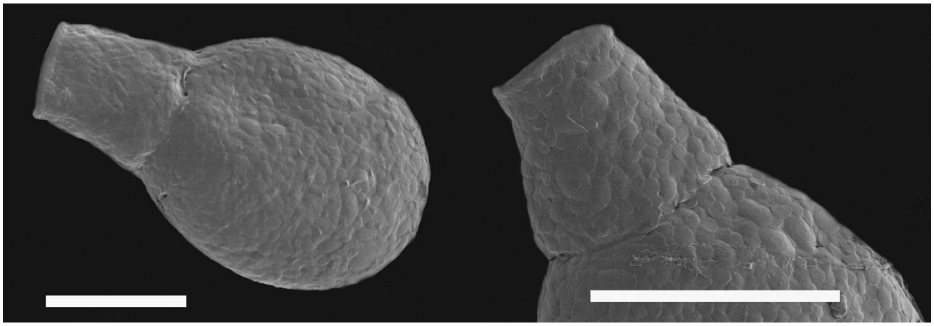

Fig 7.2 Scanning electron pictures illustrating *Apodera vas* from Salvador. Scale bars 50 µm.

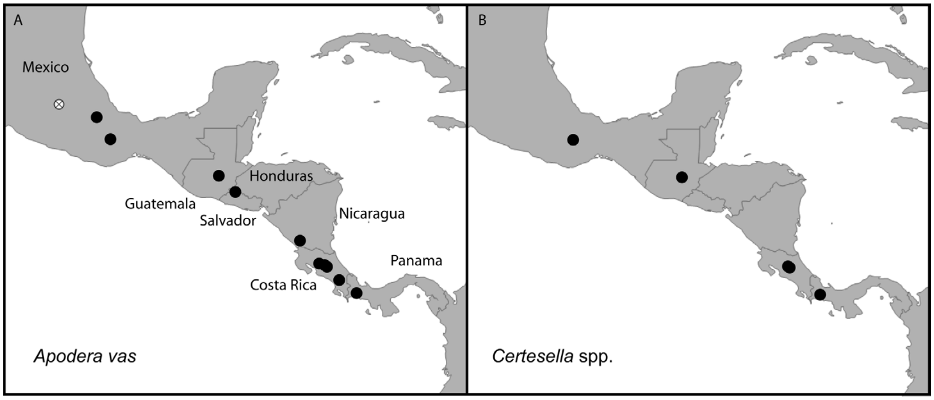

Fig 7.3 Sketch maps showing (A) the occurrences of the testate amoebae *Apodera vas* and (B) *Certesella* spp. along a Mesoamerican transect between Panama and the south of Mexico. Black dots indicate new records while the white dot represents the *Apodera vas* record published by Golemansky (1967). The records published by Laminger (1973) were not reported on this map because of the lack of accurate coordinates. Details of sites are given in Table 7.1.

were found in a *Sphagnum magellanicum* sample collected in Laguna Esmeralda (near Ushuaia, Tierra del Fuego, Argentina; E. Lara, unpublished data) but were never reported from holarctic *Sphagnum magellanicum* samples. Altogether, these data support the interpretation that the absence of these species in Holarctic habitats is not explained by the lack of a specific habitat in the northern hemisphere. Abundant favourable moist soil and moist litter habitats are indeed present in Europe and North America from the Boreal tundra to temperate rain forests. Thus, the SHTEH example is clearly inconsistent with the 'Everything is everywhere, but the environment selects' tenet.

7.7 Evaluating the consequences of potential cryptic species within endemic Arcellinida morphospecies

Cryptic diversity has been commonly reported in several free-living protist morphospecies (de Vargas et al., 1999; Slapeta et al., 2005; Heger et al., 2010). Within the endemic *Certesella* and *Apodera vas* morphospecies, the presence of a hidden diversity is therefore also possible. In the case of *Apodera vas*, several indices such as its polymorphic shell (Smith and Wilkinson, 2007; Zapata and Fernández, 2008) and its relatively wide environmental tolerances indeed suggest the presence of cryptic species (species which cannot be discriminated by morphology alone) or pseudocryptic species (species with subtle morphological dissimilarities, possibly visible only by scanning electron microscopy) within this morphospecies. Each of these potential hidden species can have either a distribution corresponding to the actual *Apodera vas*/*Certesella* morphospecies distributions or a more restricted distribution. In order to evaluate the genetic diversity within these endemic morphospecies, molecular-based studies are needed.

7.8 Potential factors governing the distribution of Arcellinida

The debate over cosmopolitan vs. limited endemism in free-living microorganisms has mostly focused on the question of taxonomy: defenders of cosmopolitanism are usually ‘lumpers’ while defenders of endemism are usually ‘splitters’ (see Finlay et al., 2004 and answer by Mitchell and Meisterfeld, 2005 for an example). Recent developments in taxonomy and especially in molecular taxonomy have provided several examples showing that the splitters may be right and that we have even underestimated the true diversity of testate amoebae (Lara et al., 2008). However, this debate is only one part of the whole story. Several other important factors influence the distribution of free-living microorganisms and all of these suffer from a clear lack of data or conceptual framework:

1. Dispersal by wind. Free-living microorganisms are assumed to be small enough to be easily transported by wind (Finlay, 2002). However, although several studies have revealed the presence of microorganisms (mostly bacteria and fungi) in the atmosphere and highlight the importance of atmospheric transport for microbial large scale dispersion (Darwin, 1846; Griffin et al., 2002; Kellogg and Griffin, 2006; Gorbushina et al., 2007; Hervas et al., 2009; Pearce et al., 2009), no data are available for testate amoebae. Atmospheric circulation models could be used to model potential long-distance

dispersal of microorganisms under different scenarios (e.g. size of the organisms) (Wilkinson et al., In prep).

2. Dispersal by animals. The main potential factor for natural dispersal of free-living microorganisms other than wind is migratory animals, mainly birds. The feathers of birds constitute ideal sampling and transportation devices for microorganisms. This will be most efficient for birds living in wetlands and ground-nesting and/or feeding taxa. Although several studies have shown that birds can transport high numbers of microorganisms (Wuthrich and Matthey, 1980; Bisson et al., 2009), we are far from having a clear image of the local and global potential dispersal of free-living microorganisms by birds. A potential limitation of birds as vectors of free-living microorganisms is that the nesting habitat of many long-distance migratory birds is very different from their wintering habitats. For example, many shorebirds nest in freshwater wetlands and bogs in the boreal and arctic regions but winter in brackish water wetlands.
3. Dispersal by humans. Wilkinson (2010) discussed the potential role of humans in dispersing terrestrial free-living microorganisms. Given the exponential increase in travel across continents, this is currently a very significant mechanism for long-distance dispersal. Humans may have contributed to the dispersal of microorganisms for millennia through their own migrations, the spread of agriculture and the development of long-distance ocean travel.
4. Survival during transportation. Long-distance transport, especially on birds' feathers or by wind exposes microorganisms to unfavourable conditions such as drought, freezing and UV radiation. Therefore it may be that, although many microorganisms can be transported over long distances, they do not survive and cannot colonise a new habitat. It would therefore be useful to test experimentally the resistance of various microorganisms to the range of conditions they are likely to experience during transport in order to determine if survival plays a role in shaping biogeographic patterns. Many free-living microorganisms are able to encyst and when encysted would presumably survive extended periods of time in the air. However, the encystment and excystment capacities of most microorganisms are not well documented (Corliss and Esser, 1974; Foissner, 1987).
5. Establishment in an existing community. The potential for viable microorganisms falling on soil or into an aquatic environment to establish new populations will depend on several factors such as the ecological conditions of the new environment (do microclimatic and physico-chemical conditions match its own requirements?) and biotic interactions (are potential prey organisms present in the case of a predator, could local predators wipe out the newly

developed population?). The importance of competition in a community assembly of testate amoebae is currently unclear. For example Wanner and Xylander (2005) found no evidence for species turnover in a primary colonisation sequence of sand dunes. Experiments could quite easily be done to test the potential for alien species to become established in existing communities but such experiments have to our knowledge not been performed with Arcellinida.

Taken together these factors show that the dispersal potential of free-living microorganisms is clearly not only a matter of size.

7.9 Perspectives

Palaeoecology could potentially provide useful information on the dispersal potential of Arcellinida. For example, peat deposits and lake sediments could be studied to determine how Arcellinids (re-)colonised those habitats after the last glacial retreat or if their diversity on isolated islands increased with the arrival of humans. However, a potential confounding factor of this latter possible study is that humans usually strongly modify the vegetation of such islands, either directly by clearing forests for agriculture or indirectly by introducing animals such as goats and cows that strongly alter the vegetation. Such changes in turn may affect the ecology of wetlands and lakes, thus causing shifts in communities of microorganisms that are not necessarily related to the establishment of new taxa on the island but simply the colonisation of habitats that became favourable to taxa that already lived on the island but in other locations.

An international network of suction traps has been established since the nineteenth century for the study of plant pests such as aphids (Klueken et al., 2009). These traps could easily be used to sample microorganisms from the air and obtain a quantitative estimate of the colonisation rate and diversity of air-transported microbes.

In order to better understand the Arcellinida gene flow among regions and continents, it would be also highly relevant to assess the phylogeography of selected Arcellinida morphospecies. Indeed, a purely morphological approach does not provide any information on the genetic structure of the populations and the level of gene flow among populations. If a highly variable gene was studied and little or no genetic differentiation among populations of a species was observed, this would provide evidence for a high level of dispersal, consistent with the ‘ubiquity theory’. In contrast, a high degree of genetic differentiation among populations would indicate that limited geographic distributions exist in protists, providing evidence against the ‘ubiquity theory’. In addition, such a study would also contribute to resolving taxonomic uncertainties.

Finally, process-based modelling and sensitivity tests of all model parameters would be useful to assess which factors may play the biggest role in determining the dispersal potential of free-living microorganisms. Models cannot be expected to provide definitive answers, at least not with the current lack of data and understanding on critical parameters that will need to be included in the model, but they would be useful in clarifying which of the many open questions matters most.

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