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Taxonomic Confusion Blurs the Debate on Cosmopolitanism versus Local Endemism of Free-Living Protists

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The Debate over Cosmopolitanism

There is currently a hot debate whether free-living protists are cosmopolitan, (Finlay 2002; Finlay and Clarke 1999; Finlay and Fenchel 1999; Finlay et al. 1999, 2001) or whether some, and perhaps indeed most, of them have limited geographical distributions (Foissner 1997, 1998, 1999). This question has important implications for the estimates of biodiversity: if free-living protists species have a global distribution, then their global diversity is low (Finlay and Clarke 1999; Finlay et al. 1999) but if some protists at least have a restricted geographical distribution, and if this pattern is not simply a consequence of their narrow ecological requirement, then their global diversity might be very high (Foissner 1999).

Like ciliates, testate amoebae provide evidence for both views. Indeed, the available data suggests that they include both species small enough to be transported passively over long distances and species large enough for this to be unlikely (Foissner 1999; Smith 1996; Wilkinson 1994, 2001).

In this paper, we follow the taxonomic nomenclature of Arcellinida used in the second edition of the illustrated guide to the protozoa (Meisterfeld 2002).

The Case of *Apodera (Nebela) vas* (Certes)

Among the testate amoebae, *Apodera (Nebela) vas* (Certes) (Fig. 1) has frequently been cited as an example of a species with a limited geographical distribution, occurring only in the Southern Hemisphere or even in former Gondwana (Deflandre 1936; Van Oye 1944). Indeed, during a discussion over the cosmopolitanism of microorganisms, at the 4th European Congress of Protistology, and 10th European Conference on Ciliate Biology in 2003 in Italy, Prof. Foissner challenged Prof. Finlay and other proponents of the ubiquitous distribution of microbial species to find *Apodera vas* in Europe.

The argument can be summarized as follows: Given the relatively large size of this species (130—210 μm), its distinct morphology, and the larger number of studies on testate amoebae carried out in the Northern Hemisphere, it is highly unlikely that it would have been overlooked. Its true distribution is, of course, as for all testate amoebae, not well characterized. It is quite obvious that it is neither restricted to former Gondwana nor to the Southern Hemisphere, having for example been reported in sub-Saharan Africa north of the equator, Hawaii, Venezuela and Central America. In fact we report here the finding of this species in a *Sphagnum* moss collected in a swamp in the Eastern Arc Mountains, Tanzania (Coordinates: S7°49.5691′E35°55.5678′) (Fig. 1). Nevertheless, the vast majority of studies on testate amoebae have been done on samples taken in Europe and North America, and this species has never been reported in these regions.

Finlay et al. (2004) argue that under-sampling and rarity may explain why some species have never been reported in some places. While it is obvious that under-sampling is indeed a problem, in the case of *Apodera vas* the sampling bias is extremely in favour of Europe where it **has not** been found and not of the southern locations where it **has** been found repeatedly. For example, the specimen illustrated in Figure 1 was found in the only *Sphagnum* sample analysed by the first author from Tanzania. The species was there, but we did not find it in hundreds of *Sphagnum*

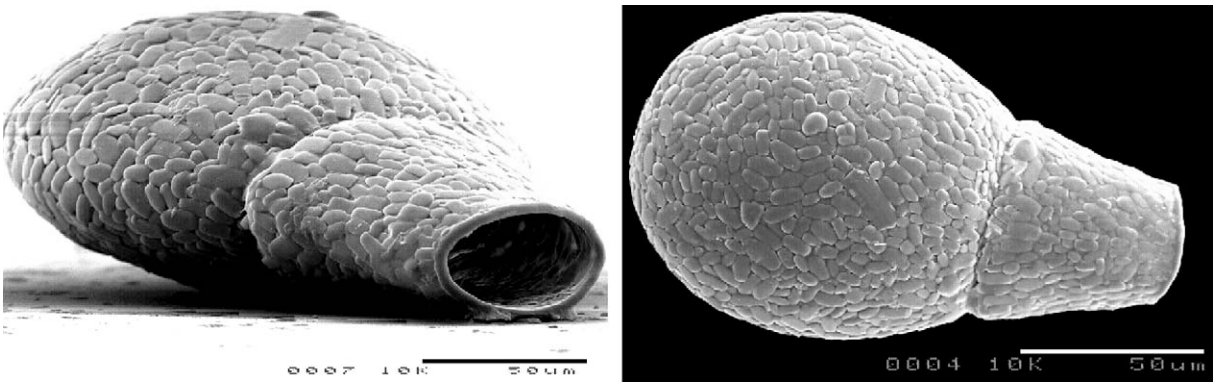

Figure 1. *Apodera vas* (Certes), a testate amoeba found in South and Central America and Sub-Sahara Africa but lacking from North America and Eurasia. This individual is from a *Sphagnum* moss collected in Tanzania. SEM taken at the University of Alaska Anchorage by K. Kishaba, J. Kudenov and E. Mitchell.

samples (of many species taken across the whole range of habitats in which these mosses can be found) or other habitats from Europe and North America, and neither did other European and North American testate amoeba specialists. The odds for this to be due to random chance are extremely small.

In answer to Foissner's challenge, Finlay, Esteban, and Fenchel published an article in which, rather than either providing evidence for the presence of *Apodera vas* in Europe, or acknowledging that there may be some exceptions to the cosmopolitan distribution of free-living microbes, they use a taxonomic argument against the lack of global distribution of *Apodera vas*, namely that there has been confusion between *Apodera vas*, which has not been found in Europe and North America (Fig. 1), and *Pontigulasia*, which has been found in Europe and North America (Fig. 2) (Finlay et al. 2004). However, we disagree with this taxonomic argumentation. While we can only agree with the questionable validity of many testate amoebae taxa, it is very hard to believe that such confusion has taken place and a taxonomic clarification is therefore needed.

It should first be noted that the species depicted by Finlay et al. as *Pontigulasia* has now been moved to a new genus, *Lagenodifflugia* Medioli & Scott, 1983 (Medioli and Scott 1983; Ogden 1987). The old genus *Pontigulasia* Rhumbler, 1986, characterized by a pyriform shell, often with a constriction of the neck, is now separated into *Pontigulasia* Rhumbler, 1986 (with a centrally located bridge joining the two lateral walls of the shell in the neck region), *Lagenodifflugia* (with a diaphragm in the neck region with a single, central, circular opening), and *Zivkovicia* Ogden, 1987 (with a diaphragm in the neck region with two openings).

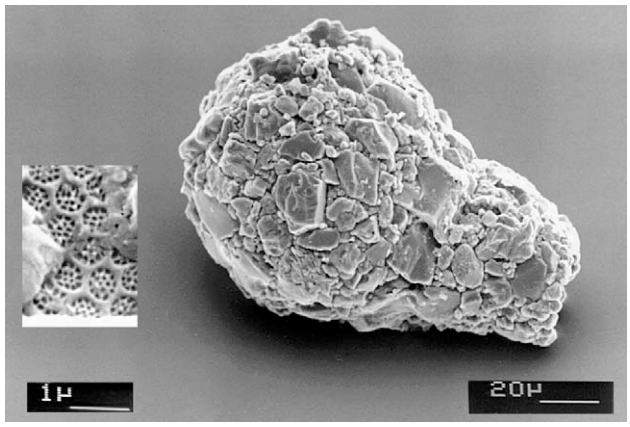

Figure 2. *Lagenodifflugia vas* (Leidy, 1874), collected in Grietherbusch (Germany). The inset shows the structured organic cement typical for *Lagenodifflugia*, *Pontigulasia* and *Zivcovicia*. Reproduced from "The Illustrated Guide to the Protozoa" with permission from the Society of Protozoologists (now the International Society of Protistology).

More importantly, for this debate, the genera *Apodera* and *Pontigulasia*/*Lagenodifflugia*/*Zivkovicia* clearly differ by at least two morphological characters: (1) One of the defining characteristics of the genera *Pontigulasia, Lagenodifflugia*, and *Zivkovicia* is the presence of a distinct internal dividing wall at the base of the neck. This characteristic clearly separates *Pontigulasia, Lagenodifflugia*, and *Zivkovicia* from *Difflugia*, and also from *Nebela* and *Apodera* (Ogden 1987; Rhumbler 1986) and is visible under the microscope unless hidden by an apertural plug or other debris. This wall is not straight in the case of *Lagenodifflugia*. (2) The two taxa also differ with respect to the cement used for test construction: *Pontigulasia, Lagenodifflugia*, and *Zivkovicia*

produce a structured cement (Fig. 2), while *Apodera* produces a sheet-like cement (Meisterfeld 2002).

Finlay and co-authors interpret the constriction between the main part of the test and the neck as a homology when it is more likely only an analogy. Indeed, such a constriction can also be found in other taxa such as *Cucurbitella* and *Lesquereusia*, which are very unlikely to be closely related to *Nebela*/*Apodera*. We therefore do not believe that the two taxa belong to a same "continuum", as suggested by Finlay et al. (2004). However it must be noted that, for the discussed taxa, the stability or lack thereof of distinguishing morphological characters has not yet been assessed under controlled conditions and no molecular data is currently available. Therefore one could argue that the phylogenetic significance of these morphological characters is poorly known.

We also find it hard to believe that taxonomists might have fallen victim to the following circular reasoning: because *Apodera vas* is believed to occur only in the Southern Hemisphere, its discovery in other regions is reported under a different name, e.g. *Pontigulasia*. In fact, although the genus *Nebela* s.l. contains some problematic species that require revision (e.g. the *Nebela tincta* complex), it also contains mainly, and perhaps mostly, very well defined species (e.g. *N. militaris, N. carinata, Porosia bigibbosa*). We therefore cannot agree with the statement of Finlay et al. (2004) that the taxonomy of *Nebela* is "labyrinthine" and that the challenge of finding *Apodera vas* in Europe is a "nebulous" one.

Despite the fact that Finlay and co-workers do not claim to have any "specialist knowledge on the taxonomy of these organisms", in an attempt to prove their point they nevertheless develop their case about *Apodera vas* using the argument of an apparent taxonomic confusion which we believe is wrong and which thus required clarification. The fact that some protozoologist might have misidentified or poorly described *Apodera vas* or other taxa in the early 20th century should not be used as an argument for the lack of clear taxonomic identities in a genus as a whole (*Nebela*), or testate amoebae in general.

Is there more than Size?

Reducing the cosmopolitan versus local distribution question only to body size may not allow finding a satisfying answer for all groups of organisms. While this approach may work for many groups, perhaps most of them, it is more than likely that exceptions exist. Finlay and co-workers's data sets are, for example biased towards aquatic habitats, which most likely allow an easier dispersal of microorganisms than soils. The degree of cosmopolitanism of microorganisms is perhaps not only a matter of size, but may also depend on their habitat: aquatic and wetland species and those living in soil litter and mosses are more likely to be transported over long distances than species living in less accessible habitats such as deeper soil horizons. It follows that the likelihood of finding a local endemic free-living protozoa species should depend on the type of habitat required for its development. For example, marine, and especially planktonic microorganisms apparently have a very good potential for long-distance dispersal and it would therefore appear unlikely to find examples of restricted geographical distribution in such organisms. However, cryptic species of both benthic and planktonic foraminifera have been discovered, some of which have cosmopolitan distribution while others have not (Darling et al. 2000, 2004; Hayward et al. 2004). It would thus be interesting to compare at which size the threshold between cosmopolitanism and local distribution is detected for different habitat types such as closed basin lakes, peatlands, mineral soils, estuarine habitats, etc. Doing this exercise may reveal differences that can have a significant impact on global biodiversity estimates.

Is Taxonomy the Limiting Factor?

The controversy over *Nebela vas* illustrates very well how taxonomic uncertainties add to the confusion over the cosmopolitanism versus provincialism of testate amoebae and other microorganisms.

On the one hand, excessive splitting of taxa, which certainly exists in some cases, may give the impression of a higher rate of endemism than actually exists (Finlay et al. 2004). Given the known variability of shell morphology in some testate amoebae taxa (Schönborn 1992; Schönborn and Peschke 1988; Wanner 1999; Wanner and Meisterfeld 1994; Wanner et al. 1994a,b), until their taxonomic validity is clarified, many of the described species and even more of the subspecies and varieties should not be used as evidence for limited distribution ranges as many of them were not confirmed or observed by anyone else than the person who first described

them. In that respect we agree with the reasoning of Finlay et al. (2004).

On the other hand, it is clearly extreme, and most probably wrong, to consider that all species of a genus such as *Nebela* are nothing more than intergrades of a continuum. Furthermore, the existing literature on testate amoebae biogeography is mostly based on the observation of morphotypes and the possible existence of cryptic species that may have limited geographical distributions, or very restricted ecological requirements, has not yet been addressed much.

Finally, it may not be possible to give a definitive answer to the cosmopolitanism versus endemism question because the first thing on which an agreement should be reached is the definition of the species, which is challenging for organisms, such as testate amoebae, that reproduce mainly asexually. Therefore, the debate over the global versus local distribution of micro-organisms may in great part simply parallel the one opposing "lumpers" and "splitters". The cosmopolitanism debate clearly illustrates the need for a major taxonomic effort. For individual groups such as the Arcellinida or the euglyphid testate amoebae, it should be possible to find a consensus over a definition of the species. A sound taxonomy is the base on which other fields of biology can build. The debate over cosmopolitanism is just one illustration of this fact. Some funding agencies have recognized this (e.g. the US NSF with its "Systematic Biology and Biodiversity Inventories Cluster" within the Division of Environmental Biology), but for others taxonomy does not yet seem to have been recognized as a priority.

It is clearly not possible to understand the subtleties in the taxonomy, biogeography and ecology of all groups of living organisms. Perhaps as a result of this complexity, the debate over the cosmopolitan distribution of free-living protists has now become quite emotional. Relying on ancient data of uncertain value is not the way to solve the problem. To make progress, we now need to (1) improve the taxonomy of free-living protists by combining morphological and molecular characters, (2) intensify the sampling effort in regions under-represented in the existing data sets, and (3) take into account the habitat specificity of the species.

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