



The curse of taxonomic uncertainty in biogeographical studies of free-living terrestrial protists: a case study of testate amoebae from Amsterdam Island

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ABSTRACT

Aim A current debate in microbial biogeography contrasts two views concerning the distribution of free-living microorganisms. The first view assumes a ubiquitous distribution, while the second assumes that at least some species have limited geographical distributions. We tested for limited geographical distributions by identifying testate amoebae morphospecies from an extremely remote oceanic island where the potential for endemism is high.

Location Amsterdam Island, Indian Ocean.

Methods Sixty moss and water samples collected from the top of the volcano to the lowland were investigated for their testate amoeba content. Due to taxonomic uncertainties among the *Argygnia* (*Nebela*) *dentistoma* species complex (including *A. antarctica*), we also performed light and scanning electron microscopy investigations on the shell ultrastructure and biometric analyses on several specimens of this taxon.

Results We identified a total of 43 testate amoeba taxa belonging to 15 genera. Only four testate amoeba taxa had previously been recorded on this island. Testate amoeba communities of Amsterdam Island are dominated by cosmopolitan ubiquitous euglyphid taxa such as *Trinema lineare*, *Assulina muscorum* and *Corythion dubium*. The length and width ranges for *Argygnia dentistoma* on Amsterdam Island overlap with other records of this species and of *A. antarctica*, suggesting that *A. antarctica* is not a distinct taxon.

Main conclusions Although Amsterdam Island is among the most remote islands in the world, an extensive inventory of testate amoeba morphospecies provided no clear evidence for endemism. On the one hand, our detailed morphometric analysis of the *A. dentistoma* complex revealed that *A. antarctica*, a morphospecies previously suggested to display endemism, cannot be confidently distinguished from the cosmopolitan morphospecies *A. dentistoma*. On the other hand, five morphotaxa could not be identified with certainty and might represent new species, potentially with limited distribution. These examples illustrate how taxonomic uncertainties undermine biogeographical studies of testate amoebae. In order to allow better interpretation of morphology-based testate amoeba distribution data, an assessment of genetic diversity among and within morphotaxa in relation to geographical distance for some common testate amoebae should be given high priority.

Keywords

Amsterdam Island, biogeography, cosmopolitan distribution, endemism, Indian Ocean, microorganisms, taxonomy, testate amoebae.

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INTRODUCTION

The current (and long-lasting) debate within microbial biogeography contrasts two opposing views concerning the distribution of microorganisms. The first view (the so-called 'ubiquity theory') assumes that all microorganisms are ubiquitous because their abundance is very large (which sustains global dispersal) (Finlay & Clarke, 1999; Finlay *et al.*, 1999, 2001), while the second view (the so-called 'biogeography theory' or 'moderate endemicity distribution theory') assumes that at least some of them have limited geographical distributions (Foissner, 1997, 1998, 1999, 2008; Stoeck *et al.*, 2007; Vyverman *et al.*, 2007). Testate amoebae provide evidence for both views, and are a good model group for studies of the biogeography of free-living protists. The available data indeed suggest that these protozoa include (1) species small enough to be passively transported over long distances, and (2) species that are large enough to make this rather unlikely (Wilkinson, 1994, 2001; Smith & Wilkinson, 2007).

In the Southern Hemisphere, the testate amoeba diversity of several remote islands located within medium-to-high latitudes have been investigated extensively, mostly over the past few decades [Iles Kerguelen: Bonnet (1981); Iles Crozet: Vincke *et al.* (2004a,b,c); Vincke (2006); Marion Island: Penard (1911); Grospietsch (1971); South Georgia: Smith (1982), Beyens *et al.* (1995); Vincke *et al.* (2006); Ascension Island: Wilkinson & Smith (2006)]. These studies all revealed a large majority of cosmopolitan species. However, a few species showing a more restricted distribution were also recorded. For example, *Apodera vas*, a species reported only from the Southern Hemisphere and the tropics (Smith & Wilkinson, 2007), was observed on several sub-Antarctic islands. Endemism was suggested for a second species, *Argynnia* (*Nebela*) *antarctica*, observed only on Marion Island (Grospietsch, 1971). However, because of the high morphological similarity of this species with *Argynnia dentistoma* Penard, its exact taxonomic status and hence its possible endemic distribution needs to be confirmed using biometric and/or molecular analysis.

Taxonomic uncertainty is one of the principal sources of concern with respect to the ubiquity vs. endemism debate (Mitchell & Meisterfeld, 2005). For example, possible taxonomic confusion with closely related species makes it impossible at the moment to assess if *A. antarctica* should be considered to be an endemic species on Marion Island. Moreover, the 'force-fitting' of European and North American names to specimens from other regions may lead to inaccurate distribution records. Such a bias has been shown to explain at least part of the initial underestimation of diatom endemism in Antarctica (Sabbe *et al.*, 2003). Another problem that may also lead to inaccurate conclusions on biogeographical patterns is the uneven sampling efforts across different regions of the world (Foissner, 2006).

Contrary to the above-mentioned localities, Amsterdam Island (Indian Ocean) has not yet been investigated extensively for testate amoebae, despite its unique and quite interesting

geographical position in the context of microbial biogeography. Amsterdam Island and the neighbouring Saint Paul Island are among the most remote islands in the world. Australia, South Africa and Antarctica are located 3200, 4200 and 3300 km, respectively, away from these islands. To our knowledge there has only been one prior study of testate amoebae from Amsterdam Island. Richters (1908) mentioned the presence of four taxa: *Diffflugia globulosa*, *Euglypha seminulum* (current name: *Assulina seminulum* Ehrenberg 1848), *Nebela collaris* and *Nebela vas* (*Apodera vas* Certes 1891). Given the size and diversity of habitats on the island, these four taxa obviously represent only a small part of the potential overall testate amoeba diversity of Amsterdam Island. In this study, our aim was to assess the testate amoeba biodiversity of Amsterdam Island and, more generally, to test the hypothesis of their limited distribution.

MATERIALS AND METHODS

Study site

Amsterdam Island is located in the Indian Ocean (37°41' S–77°31' E), north of the subtropical convergence (Fig. 1). The volcanic island covers a surface of 55 km². It is entirely volcanic and constituted of a single cone culminating at 881 m (Mont de la Dives). It is geologically very young, and probably formed during the past 700,000 years (Giret, 1987). Steep cliffs surround much of the island. The climate is temperate oceanic (Frenot & Valleix, 1990) with a mean annual temperature of 13.8°C, and mean temperatures of 11.2°C in the coldest month (August) and 17.0°C in the warmest month (February) (Caroll, 2003). Relative humidity is generally high (*c.* 80%) due to the frequency of low cloud ceilings. Precipitation is usually high, with an annual average of 1114 mm distributed over 239 days and falling primarily as rain. The austral summer (January–March) is drier (78 mm month⁻¹) than the other seasons (100 mm month⁻¹). Permanent water bodies are scarce and restricted to the higher plateau in the centre of the island (Plateau des Tourbières) and the west–south-west part of the island. Almost all other areas have neither permanent nor semi-permanent water bodies due to the steepness of the slopes and the permeability of the lava. Due to high relative humidity, the summit is often foggy, and these conditions have allowed extensive peat development in the volcanic caldera.

On the higher central plateau (Fig. 1), the vegetation has a typical sub-Antarctic character and is dominated by a large number of bryophyte species (including *Sphagnum* spp.), grasses and small ferns. On the slopes, the vegetation cover becomes thicker with decreasing altitude, with clear changes in the dominant covering species: larger ferns and grasses replace the moss vegetation and gradually the *Sphagnum* cover also disappears with decreasing altitude (B. Van de Vijver, personal observation). On the lower parts of the island, remnants of the once very extended *Phyllica arborea* Thouars forests can be found. This species is endemic to Amsterdam Island and Tristan da Cunha islands (Richardson *et al.*, 2003). The

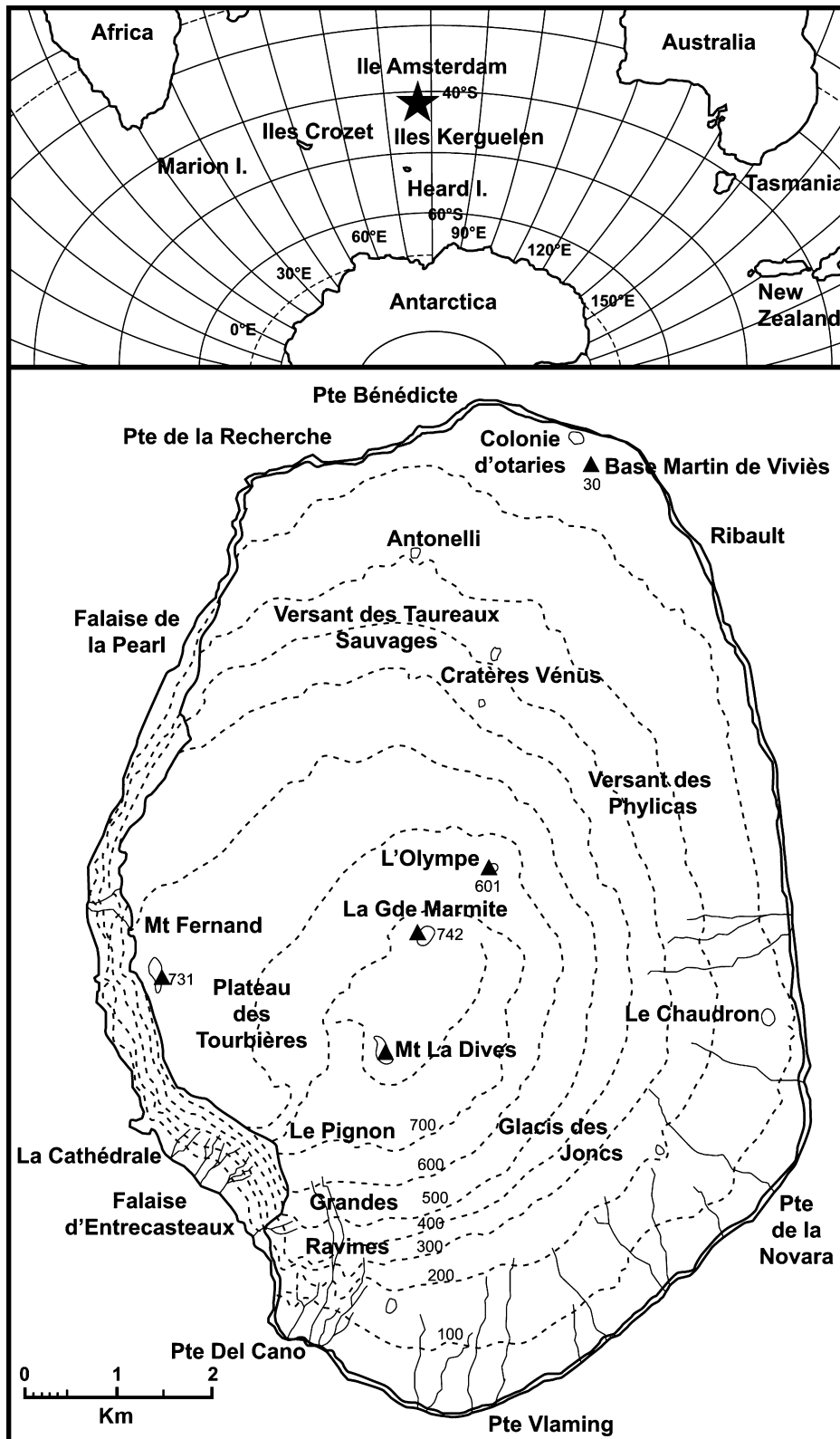


Figure 1 Geographical position of Amsterdam Island in the Southern Indian Ocean and detailed map of the island.

endemic fauna is rather poor and comprises a few marine bird species such as the rare Amsterdam albatross (*Diomedea amsterdamensis* Roux *et al.*, 1983; Jouventin & Roux, 1983)

and several larger fur seal colonies (*Arctocephalus tropicalis* Gray). Fires and the introduction of cattle (*Bos taurus* Linnaeus) on the island have caused major ecological changes.

The cattle population is now controlled, and parts of the island are recovering. A small, permanently occupied base (Martin de Viviers) is situated on the northern shore.

Sampling

During two short visits to Amsterdam Island in 1998 and 1999, 83 samples were collected from all over the island, 60 of which were analysed in this study. In order to obtain a broad overview of the diversity and distribution of the testate amoeba communities on the island, samples were taken from different habitat types: freshwater ponds, rivers and mosses (ranging from aquatic to dry terrestrial). Additional sampling was performed during the austral summer of 1999–2000. Samples were fixed in the field with 3% formaldehyde.

Due to the very short character of the stay during ships' stopovers on the island, only a very limited number of abiotic parameters could be measured. Water pH was measured using a WTW Multimater (Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany). Moss moisture values were estimated using the moisture scale by Jung (1936), ranging from FI = submerged mosses to FVIII = dry – no water. Although this method may seem rather subjective, the moisture classes can be related to real moisture equivalents in percentages (Meisterfeld, 1977).

Testate amoeba analysis

To extract testate amoebae, samples were shaken energetically in distilled water and passed through a sieve with a mesh diameter of 250 μm . Testate amoebae were then concentrated by centrifugation (5 min at $1125 \times g$). Rose Bengal was added to differentiate the test without or with protoplasm (corresponding to dead or living individuals at the moment of sampling). In each sample, 150 testate amoebae were identified at $\times 400$ magnification. After the count of 150 individuals was reached, a short check for potential supplementary species was performed on additional slides. The following taxonomic references were used for identification: Deflandre (1928, 1929, 1936); Decloitre (1962, 1978, 1979, 1981); Grospietsch (1964, 1971); Ogden & Hedley (1980); Ogden (1983); Vincke (2006).

Because of taxonomic uncertainties in the *A. dentistoma* complex, we performed a light microscope morphometric analysis (length and width) on 38 specimens of *A. dentistoma* (*sensu lato*) from 12 different samples. Investigations on the shell ultrastructure of *A. dentistoma* were performed by scanning electron microscopy (SEM) on six different specimens from two different samples (numbers 27 and 46). Specimens were isolated using a glass micropipette, positioned on an aluminium stub, then air-dried in a desiccator for 1 week. The samples were coated with gold in a Bal-Tec SCD005 sputter (Bal-Tec AG, Balzers, Liechtenstein). Specimens were observed with a Philips XL30 FEG microscope at a tension of 3 kV (Philips Electron Optics, Eindhoven, the Netherlands).

RESULTS

Diversity and species composition

Forty-three taxa (including species, infra-specific taxa and morphotypes of uncertain taxonomic affiliation) belonging to 15 genera were observed in the 60 moss and water samples analysed (Appendices S1 and S2 in Supporting Information). The three most abundant taxa, *Trinema lineare*, *Assulina muscorum* and *Corythion dubium*, together made up 64% of the total count (Appendix S2), while the relative abundance of each of the 40 other taxa was lower than 5%. The genera *Euglypha* and *Diffflugia* showed the highest diversity, with 10 and six taxa, respectively, while the genus *Nebela* was represented only by *Nebela lageniformis*. *Heleopera sphagni*, *Diffflugia angulostoma* and *Arcella arenaria* were each observed in only a single sample during the screening of samples after the total count of 150 individuals had been reached. Of the 43 taxa recorded, five taxa could not be assigned a precise name based on their morphology: *Assulina* sp. 1, *Cyphoderia* sp. 1, *Diffflugia* sp. 3, *Diffflugia* sp. 7 and *Euglypha* cf. *cuspidatum*.

Morphological comparisons among the *Argygnia dentistoma* species complex

Based on light and SEM observations, the structure and the composition of the *Argygnia dentistoma* shells from Amsterdam Island samples correspond to the original description of *A. dentistoma* by Penard (1890). The shells were composed mainly of circular agglutinated scales around the pseudostome and with circular or elongated scales on the rest of the shell (Figs 2a & 3a–i). The shell ultrastructure differs considerably among individuals due to the high morphological variability of the scales used to build the shell (Fig. 3a–i). The shell of some individuals is even composed of a few large, elongated particles that may consist of agglutinated diatoms (Fig. 3b).

The mean length (*L*) and width (*W*) of the specimens observed were 79.2 ± 1.5 and 60.1 ± 1.4 μm , respectively. As shown in Fig. 4, the range of biometric values is large and overlaps with the corresponding values of *A. dentistoma* from the Czech Republic (Fig. 2b) ($L = 96 \pm 1$; $W = 64 \pm 1$; $n = 10$) (Lara *et al.*, 2008), *A. dentistoma* from Marion Island (Fig. 2c) ($L = 91.0 \pm 1.0$, $W = 78.1 \pm 0.9$ μm ; $n = 22$) (Grospietsch, 1971) and *A. antarctica* from Marion Island (Fig. 2d) ($L = 78.7 \pm 0.9$, $W = 55.9 \pm 0.8$ μm ; $n = 38$) (Grospietsch, 1971).

DISCUSSION

Testate amoeba diversity

Of the 43 testate amoeba morphotaxa observed in the moss and water samples, three ubiquitous taxa, *Trinema lineare*, *Assulina muscorum* and *Corythion dubium*, dominated the communities. These species, and in particular *T. lineare*, are frequently reported in the literature as being dominant taxa in

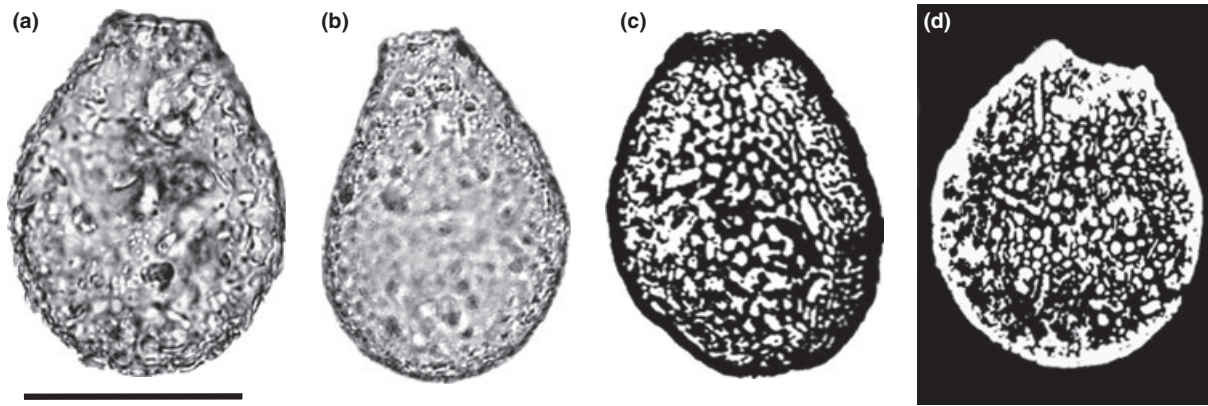


Figure 2 Light microscopy pictures illustrating four different specimens of the *Argynnia* complex: (a) *A. dentistoma*, Amsterdam Island, present study; (b) *A. dentistoma*, Czech Republic (Lara *et al.*, 2008); (c) *A. dentistoma*, Marion Island (Grospietsch, 1971); (d) *A. antarctica*, Marion Island (Grospietsch, 1971). Scale bar = 50 μm .

different environments (Wanner, 1991; Ledeganck *et al.*, 2003; Mattheussen *et al.*, 2005; Vincke *et al.*, 2006). This ubiquity probably reflects the high reproduction rate and the wide ecological tolerance of this species.

Among the recorded taxa, only two (*Assulina seminulum* and *Diffflugia globulosa*) had previously been recorded on this island (Richters, 1908), therefore the 41 other taxa represent new records. While our study substantially increases the known diversity of testate amoebae on Amsterdam Island, it is clear that the analysis of 60 samples is not sufficient to find all the taxa living there.

We did not find two of the four species that were previously reported by Richters in 1908: *Nebela collaris* and *Apodera vas*. This is surprising, as the environmental preferences of these species correspond well with the nature of the samples (i.e. wet mosses) that were investigated in the present study. Moreover, these taxa are both quite large, so they are unlikely to be overlooked while scanning through the samples after the count of 150 individuals was reached. It is of course possible that both records are the result of a misidentification. Indeed, some misidentifications of *N. collaris* have been reported in the literature. For instance, Penard (1902) mentioned the presence of a probable specimen of *Argynnia dentistoma* among the *N. collaris* drawings published by Leidy (1879). In contrast, misidentification of *Apodera vas* seems unlikely because of the unmistakable morphology of this species (Mitchell & Meisterfeld, 2005). It is also possible, but less likely, that samples became mixed up or contaminated during the study of Richters (1908). Alternatively, *N. collaris* and *A. vas* might have disappeared as a result of ecosystem disturbances resulting in habitat loss. Indeed, Amsterdam Island has been strongly affected by overgrazing and fire during the past centuries (Micol & Jouventin, 1995; Frenot *et al.*, 2001). However, the suitable habitat for *A. vas* (wet mosses; Smith & Wilkinson, 2007) is still present on the top of the island today and certainly never disappeared. Unfortunately, Richters (1908) does not give precise indications on the

sampling location, so the former distribution of the two species on the island is unknown. A palaeoecological study based on peat cores from the peatland located on the top of the island would be useful to obtain more insight into the impact of human disturbances on Amsterdam Island and the possible changes in testate amoeba diversity through time.

Taxonomy and biogeography

Our analyses of shell size variation among *Argynnia* specimens did not support the morphological distinction of the two species *A. antarctica* and *A. dentistoma*, suggested by Grospietsch (1971). Indeed, the size variability of the *Argynnia* morphotypes collected on Amsterdam Island was continuous, and did not comprise any clusters of individuals with similar sizes (Fig. 4). Furthermore, the length and width of *Argynnia* specimens from Amsterdam Island bridge the size gaps between *A. antarctica* and *A. dentistoma*: the size of the smallest individuals we observed is similar to that of *A. antarctica* from Marion Island (Grospietsch, 1971) and the larger ones are similar in size to *A. dentistoma*, described by Grospietsch (1971) (Fig. 3). Our size variability reported for *A. dentistoma* (52–92 μm) is also similar to the variability reported in the original species description (80–130 μm ; Penard, 1890). High variability in shell size is frequently reported for testate amoeba species. For instance, the shell length of *Apodera vas* ranges from 90 to 210 μm (Smith & Wilkinson, 2007). Similarly, Lahr & Lopes (2007) reported a $3.4 \times (100\text{--}340 \mu\text{m})$ range in test height for *Netzelia walesii*. This level of morphological variability generally suggests the existence of more than one taxon. Unfortunately, there are no molecular data to assess if testate amoeba taxa with such high morphological variability are composed of one or more taxa.

In support of the possible variability of testate amoeba morphology, Chardez (1989) and Wanner (1999) showed, using clonal cultures, that abiotic and biotic environmental factors, such as food source, temperature and insecticides, affected the test morphology of several testate amoeba species.

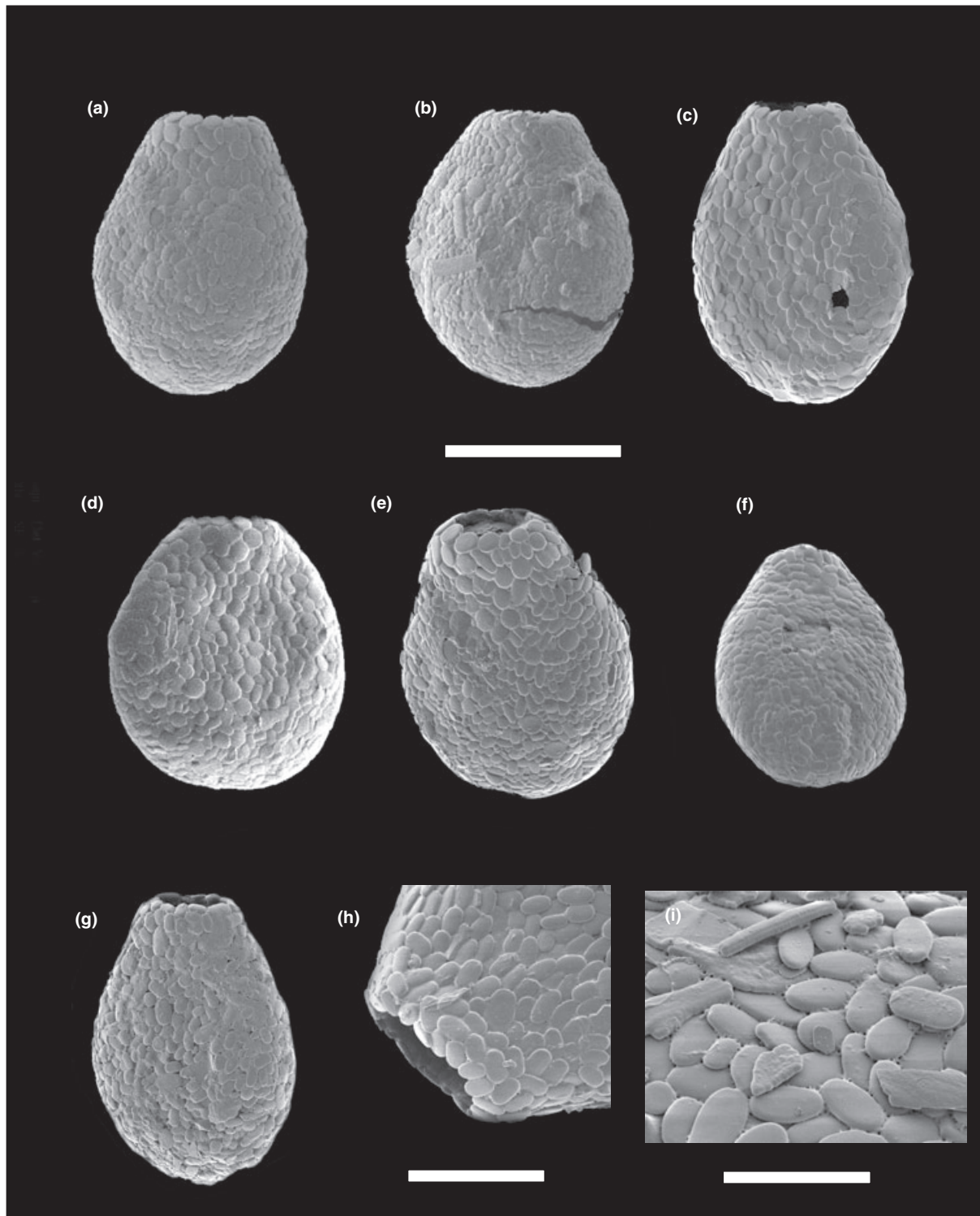


Figure 3 Scanning electron microscopy pictures illustrating different specimens of *Argynnia dentistoma* from Amsterdam Island (a–g) with detailed pictures of the pseudostome (h) and the shell ultrastructure (i). Scale bars of a–g, h, i = 50, 20 and 10 μm , respectively.

These studies suggest that some taxa, at least, may also show considerable morphological diversity in the environment.

Our morphometric data contrast with recent evidence for good correspondence between genetic and morphological

diversity in Nebelid testate amoebae. Indeed, significant genetic differences in the nuclear 18 S ribosomal RNA gene were found in two cases between a given taxon and another one that had been described as a variety: (1) *Nebela penardiana*

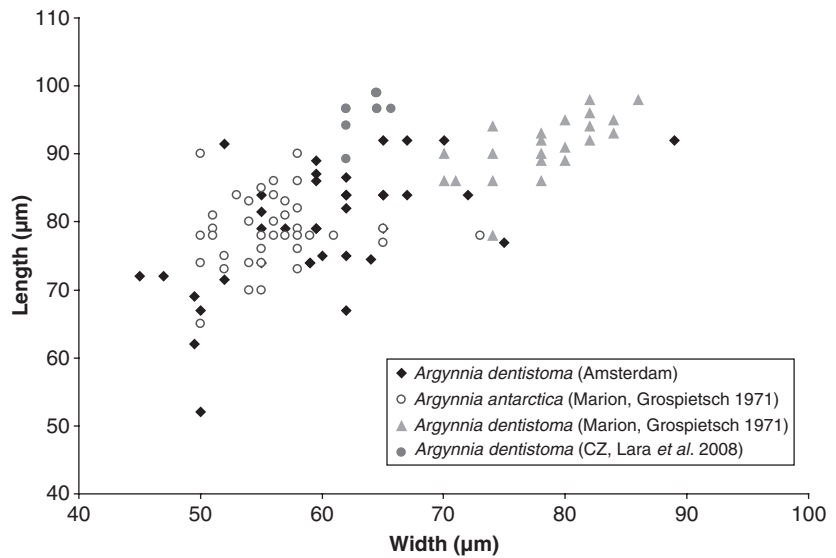


Figure 4 Shell morphometric measurements of four different morphospecies of the *Argynnia dentistoma* complex: diamonds, *A. dentistoma*, Amsterdam Island, present study ($n = 38$); triangles, *A. dentistoma*, Marion Island (Grospietsch, 1971) ($n = 22$); open circles, *A. antarctica*, Marion Island (Grospietsch, 1971) ($n = 38$); closed circles, *A. dentistoma* (Lara *et al.*, 2008) ($n = 10$).

var. *minor* and *N. penardiana*, and (2) *Nebela tincta* var. *major* and *N. tincta* (Lara *et al.*, 2008). In both cases, the morphological differences between the two varieties are comparable with the range of sizes and shapes described within the *A. dentistoma* complex. If the same correspondence exists between morphological and molecular differences within the *A. dentistoma* complex, a molecular study of this complex could reveal more than one valid taxon, which may have restricted distribution.

Clearly, the morphological data do not allow an unambiguous determination of the biogeographical status of *A. dentistoma* on Amsterdam Island. Given the observed high morphological variability, it remains possible that a significant genotypic diversity exists within the *A. dentistoma* complex. A more detailed taxonomic study of the *A. dentistoma* species complex, based on both morphological and molecular approaches, is needed to clarify the potential validity and endemic status of *A. antarctica*. Until this is done, we consider *A. antarctica* to be an invalid taxon and *A. dentistoma* to be cosmopolitan. On the other hand, five morphotaxa could not be identified with certainty and might represent new species, potentially with limited distribution.

Several endemic higher plant species have been described on Amsterdam Island, such as *Plantago stauntonii* (Ronsted *et al.*, 2002). The Amsterdam albatross, *Diomedea amsterdamensis*, breeds only on a small part of the island and is considered one of the rarest birds in the world (Micol & Jouventin, 1995). Moreover, at least one endemic insect species exists (Bameul *et al.*, 1990).

The only microbial group investigated in depth on Amsterdam Island to date is that of the non-marine diatoms (Bacillariophyta). Although the non-marine diatom flora was at first considered to be cosmopolitan (Van de Vijver & Beyens, 1999), the recent description of several species now suggests that an endemic diatom flora is present on the island

(Van de Vijver *et al.*, 2008; M. Cantonati, B. Van de Vijver and H. Lange-Bertalot, unpublished data).

The occurrence of these endemic organisms demonstrates that despite being geologically young (0.69 Ma, according to Giret, 1987), Amsterdam Island is sufficiently old and isolated for speciation to occur within both macroscopic and microscopic organisms. The presence of several biogeographical barriers in the ocean, such as the sub-Antarctic divergence between Amsterdam Island and the islands of the cold-temperate sub-Antarctic region (Stonehouse, 1982), increase the possibility of the development of an endemic (micro-) fauna and flora.

CONCLUSIONS

In contrast to other taxonomic groups, our data do not provide any conclusive evidence for the existence of endemism among testate amoebae, therefore they would rather bring support to the idea that microorganisms are (at least mostly) cosmopolitan. More than 95% of the morphospecies (41 of 43) recorded on Amsterdam Island have already been mentioned by Vincke *et al.* (2004a,b,c) and Vincke (2006) on Îles Crozet and South Georgia, despite the considerable geographical distance between these three sites (Fig. 1). This suggests that the probability of the existence of local endemic species is indeed small. Although Amsterdam Island is very remote, the morphology-based testate amoeba records suggest a lack of speciation. If true, this would mean that biogeographical patterns differ between diatoms and testate amoebae. This would be surprising because there is *a priori* no major difference in the dispersal potential of these two groups of protists.

Our data do not rule out completely the possible existence of endemic testate amoebae. Indeed, as the taxonomic status of several taxa recorded by Vincke *et al.* (2004a,b,c) and Vincke (2006) on Îles Crozet and South Georgia, and in this study, is

not yet established, no definitive conclusion can be drawn regarding their biogeography. The case of the *A. dentistoma* complex discussed here illustrates this problem. Finally, cryptic diversity has recently been discovered in several free-living protist morphospecies (de Vargas *et al.*, 1999; Slapeta *et al.*, 2005; Finlay *et al.*, 2006). Each of these hidden species can have either restricted or cosmopolitan distributions. An assessment of genetic diversity among and within morphotaxa in relation to geographical distance for some common testate amoebae should be given high priority in order to allow better interpretation of morphology-based testate amoeba distribution data. Only when such data become available will we be able to reach firmer conclusions about the geographical distribution of this very common, but still relatively understudied, group of organisms.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Locations and characteristics of all samples of testate amoebae analysed.

Appendix S2 List of all testate amoebae observed.

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