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An Initial Account of the Terrestrial Protozoa of Ascension Island

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Summary. An initial account of the terrestrial protozoan fauna of Ascension Island (7° 57´s, 14°22´w), based on nine samples from across the range of the island's habitats, describes 52 taxa: 11 flagellates, 8 gymnamoebae, 1 helizoa, 11 ciliates and 21 testate amoebae. These tend to be cosmopolitan species with wide habitat tolerances (e.g. many are also found in polar regions). The testate amoebae are missing the larger (>150 µm) taxa, suggesting these may show dispersal limitation. There is evidence that compared to an area of continental land mass the protozoan fauna may be relatively species poor, however unlike the case with the macroscopic organisms, there is no evidence for endemic species on this island. These results are discussed in the context of microbial biogeography, especially in the context of debates over the claimed cosmopolitan nature of microbial distributions.

Key words: Ascension Island, biogeography, ciliates, flagellates, gymnamoebae, soil protozoa, testate amoebae, testate rhizopods, thecamoebians.

INTRODUCTION

From our perspective at the start of the XXI-st century, the XVIII and XIX centuries appear to have been a wonderfully romantic time to be a naturalist. The adventurous researcher could travel to scientifically unknown regions, describing not only new species but studying entire floras and faunas for the first time. While it is now very difficult to find regions where the macroscopic organisms have been completely unstudied, many such locations still exist for exploration by microbiologists. Ascension Island, in the tropical South Atlantic, is one such place. As far as we are aware there have been no studies of the terrestrial protozoa, despite the fact that the island's remote location makes it potentially interesting in the context of microbial biogeography. For example, data from the island would be highly relevant to ongoing debates about the extent of cosmopolitan distributions in microbial biogeography (e.g. Foissner 1999, Wilkinson 2001, Finlay 2002, Dolan 2006, McArthur 2006). In this paper we present data on protozoa isolated from soil and bryophyte samples from nine sites on Ascension Island; ranging across the diversity of the islands' microclimates from the very wet to the extremely arid and discuss the relevance of these data to important ideas in microbial biogeography.

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Ascension Island is a recent volcanic island approximately one million years old, covering 97 km^2 in the South Atlantic Ocean (7°57'S, 14°22'W) (Ashmole and Ashmole 2000, Gray *et al.* 2005). Prior to its discovery by humans at the start of the sixteenth century, it appears to have had a limited flora, comprising 25-30 vascular plant species, around 10 of which were endemic (Cronk 1980, Ashmole and Ashmole 2000). Recent data on the conservation status of the surviving native plants are provided by Gray *et al.* (2005). Much of the ecological work on the island has concentrated on either the sea birds (reviewed by Bourne and Simmons 1998, Ashmole and Ashmole 2000) or the Green Turtles *Chelonia mydas* (e.g. Broderick *et al.* 2006). The invertebrates have been shown to include a number of endemic species, including at least five endemic pseudoscorpiones (Duffey 1964, Ashmole and Ashmole 1997). From the XIX century onwards there was a programme of plant and animal introductions with the aim of making the environment more pleasant for humans (Duffey1964, Wilkinson 2004). Before these introductions even high on the islands' mountain, where water is plentiful, the largest plants were ferns and a single species of endemic shrub *Oldenlandia adscensionis*, which was probably always rare (Cronk 1980). These introductions are of particular relevance for our study of the terrestrial protozoa as they created the cloud forest system on Green Mountain (Fig. 1a) which was included in our sampling sites and because some of these plants were imported to the island with soil (Hart-Davis 1972) which is virtually certain to have contained protozoan taxa. Most of the island is very arid with limited vegetation cover (Fig. 1b).

METHODS

Field sites

Samples were collected from nine sites (7 soil and 2 bryophyte samples) covering a range of potential habitats for soil microbes. Soil samples were collected from the soil surface after any litter layer had been removed. All samples were collected during July 2003. Details of the sites are given in Table 1 and some examples are shown in Fig. 1. After collection all samples were dried for storage (either air or oven dried, depending on the air humidity at the time of collection), it is likely that all samples were x-rayed during their passage to our laboratories in Britain.

Laboratory methods

Samples were analysed for protozoan genera and species by culturing for all taxa, and by direct observation for testate amoebae. Bacterial food for the cultures was provided in the form of *Klebsiella aerogenes* (NCIB strain 418) grown on rich nutrient agar: Beef extract 10 g, bacteriological peptone 6 g, yeast extract 2 g, NaCl 5 g, Agar 15 g, distilled water 1l. After 3 days' incubation at 25°C, *Klebsiella* was harvested and transferred to non-nutrient agar plates. Five replicate plates were used for each soil sample. Approximately 2g of soil were inoculated onto each plate seeded with *Klebsiella* and flooded with Buschnell-Haas solution: $CaCl_2.2H_2O$ 0.02 g, $FeCl_3.6H_2O$ 0.05 g, K_2HPO_4 1.0 g, KH_2PO_4 1.0 g, $MgSO_4.7H_2O$ 0.2 g, NH_4NO_3 1.0 g, distilled water 1l. Cultures were incubated at 20°C and examined by phase-contrast microscopy after 3, 6, 10, 25, 43 and 67 days' incubation. A final examination was made one year later.

Direct examination for testate amoebae was made by a modification of Couteaux' (1967) filtration method. For this 0.5 g soil was suspended in 250 ml distilled water and agitated for 1 h, 20 ml aliquots of suspension were then filtered through 0.45 grade Millipore membrane filters. Filters, bearing residue, were air-dried at room temperature. Filter segments were cleared in xylene and mounted in Canada balsam, with ten replicates for each sample. Species were identified from test characteristics, with the aid a wide range of publications and web-sites. Important sources used in the identification of testates and other protozoa included those by Sandon (1927), Curds (1969), Ogden and Hedley (1980), Page (1988), Patterson and Headley (1992), Charman *et al*. (2000), Clarke (2003), and Mitchell (2003).

Sample pH's were measured on a saturated paste (left 20 min to equilibrate) using a Pye model 292 pH-meter. Organic matter content by loss on ignition was determined through the ignition of dried samples for 4 h at 550°C (Berglund 1986). Because of limited material, loss on ignitions could only be performed on 4 of the 7 soil samples.

RESULTS

Descriptions of the sites along with the pH of the substrate and organic matter content, where available, are given in Table 1; photographs showing some of the sample sites and illustrating the range of variation in terrestrial habitats on Ascension Island are shown in Fig. 1. Occurrence of non-testate amoebae species are shown in Table 2. These comprise the flagellates, gymnaboebae, ciliates and heliozoa; all of which were identified from cultured samples. Occurrence of testate amoebae are shown in Table 3; these were the results of direct counts from samples and/or culturing of samples as indicated in this table. Although considered to be taxa most usually characteristic of damp and organic rich soils (e.g. Smith and Coupe 2002), testates were found in all our sites even very arid ones with extremely low organic matter (although at site 6 they were only represented by a single empty test). In most cases the testates were found in direct counts, suggesting that they were ecologically active in these soils and bryophytes; four

Figs 1a, b. Ascension Island habitats and sampling sites (for details of sampling sites see Table 1). **A** - *Bamboo* sp. cloud forest at the summit of Green Mountain. All the plants which can be seen in this photograph are introduced species. Prior to these introductions the dominant plants on the mountain were a number of fern species which are now either rare or extinct. Sample 1 was taken from soil at this site while sample 2 was taken from epiphytic moss growing on the Bamboos. **B** - Away from Green Mountain most of Ascension is very arid. Sample 7 was collected from just below the summit of Sisters Peak (the highest point in the photograph), while sample 8 was taken from lower down on the peak (on the opposite side of the Peak from the one shown in the photograph) in an area near the road with some invasive Mexican Thorn *Prosopis juliflora* plants.

Table 1. Sampling locations and soils data (pH and organic mater content by loss on ignition) for the protozoan sampling sites. Grid References are for the British Ordnance Survey's map of the Island (series G 892, edition 4-GSGS).

Site (Grid reference)	Location	Altitude (m)	pH	Loss on ignition (%)	Comments
1 (725 209)	Summit Green Mountain	850	4.9	36.4	Soil
2 (724 209)	Summit Green Mountain	850	4.3	no data - but pure plant material.	Epiphytic Moss
3 (725 207)	Elliot's Path, Green Mountain	750	5.2	29.2	Soil from fern rich site
4 (725 207)	Elliot's Path, Green Mountain	750	5.6	no data	Soil from under grasses, within 10 m of site 3.
5 (716 213)	Cronk's Path, Green Mountain.	660	6.3	no data	Soil from wooded habitat.
6 (743 248)	Hummock point	sea level	5.7	0.8	Soil; very arid, habitat of endemic Spurge *Euphorbia origanoides*
7 694 238)	Sisters Peak, just below the summit.	420	6.4	no data	Soil; arid, limited plant cover e.g. *Ipomoea pescaprae*
8 (699 232)	Base of Sisters Peak, near the road.	240	6.1	2.2	Soil with some limited vegetation of Pine *Pinus* and Thorn *Prosopis juliflora*
9 (737 207)	Near Devils Ash Pit	520	5.8	no data - but primarily plant material.	Moss and lichen "crust."

taxa were only found after culturing. This study identified 52 taxa of protozoa from the soils and bryophyte habitats of Ascension Island. Prior to this study we could find no records of any of these groups for the island in the literature. Total species richness of protozoan taxa for each site are given at the base of Table 3.

DISCUSSION

This study provides the first data on soil protozoa from Ascension Island. Because of its remote location these data are potentially valuable in understanding the biogeography of free living microbes. Clearly our work

Table 2. Results of culturing of samples for non-testates for the 9 sites (details of sites are given in Table 1).

Taxa	1	2	3	4	5	6	7	8	9
Flagellates									
Bobo saltans Ehrenberg			x	x	x	x			
Cercomonas crassicauda Alexeieff				x					
Heteromita globosa Stein	x	x		x	x		x	x	x
Mastigamoeba limax Moroff	x		x		x				
Menoidium sp. Perty			x						
Monosiga sp. Kent									x
Pseudophyllomitus apiculata (Skuja) Lee						x	x		
Rhynchomonas nasuta Klebs	x								
Spiromonas angusta Dujardin							x		
Spongomonas sp. Stein									x
Spumella elongata Belcher *et* Swale		x		x	x	x	x		x
Gymnamoebae									
Acanthamoeba astronyxis Ray *et* Hayes		x						x	x
Mayorella sp. Schaeffer	x			x					x
Naegleria gruberi Schardinger		x							
Platyamoeba sp. Page							x		
Thecamoeba striata Penard									x
Valkampfia ustiana Page	x				x			x	
Vanella platypodia Gläser			x						
Vexillifera bacillipedes Page		x							x
Helizoa									
Ciliophrys azurina Mikrjukov *et* Patterson								x	x
Ciliates									
Colpoda cucullus Muller			x					x	x
Colpoda steini Maupas		x	x	x	x	x	x	x	x
Colpoda sp. Ehrenberg				x					
Gonostomum affine Stein							x		
Homalozoon sp. Stokes				x					
Leptopharynx sphagnetorum (Levander) Mermod							x	x	x
Litonotus sp. Wrzesniowski	x								
Tachysoma pellionella (Müller-Stein)								x	
Pseudoglaucoma muscorum Kahl									x
Uroleptus sp. (Ehrenberg) Stein							x		x
Uronema nigricans Florentin				x	x				
Total non-testate taxa	6	6	5	10	7	4	9	8	15

is an initial investigation based on the analysis of only nine small samples; however our study is comparable to the information available from several other South Atlantic islands which have been the subject of similar sized investigations (Table 4). Larger sample sizes would undoubtedly produce more extensive species lists. For example early work on Ile de la Possession, Iles Crozet (47°S), based on a similar level of sapling to our study, gave testate lists of eight (Richters 1907) and nine (Smith 1975) species. However substantially more detailed work on this island has recently raised this total to 58 taxa (Vincke *et al.* 2004).

Although based on limited samples our study provides interesting perspectives on several aspects of microbial ecology and biogeography. The testate amoebae data are particularly relevant to biogeographical debates because testate cysts are formed inside the organisms test and so it is relatively easy to assign a size to testate taxa, when discussing its dispersal. This is not the case for many other protozoan groups, for example the cysts of the ciliate *Colpoda cucllus* (which appears in our Ascension species list: Table 2) are 35 µm in diameter while its trophzoites are 40-110 µm long (Sandon 1927). All the testate taxa identified from Ascension are widely distributed around the globe; this is consistent with the 'everything is everywhere' idea which suggests, in its strongest version, that all free living microbes have cosmopolitan distributions and are found wherever the

Table 3. Testate data for the 9 sites. D - signifies found only in direct counts; C - only found in cultures and, B - found both by direct counts and culturing.

Testate amoebae	1	2	3	4	5	6	7	8	9
Arcella hemispherica Perty									D
Arcella arenaria Greef			D						D
Arcella discoides Ehrenberg					D				
Arcella vulgaris Ehrenberg									D
Assulina muscorum Greef		D							
Assulina seminulum Ehrenberg			C						
Centropyxis aerophila Deflandre		D		D			D	D	B
Centropyxis cassis Wallich	D								
Centropyxis constricta Ehrenberg									D
Corythion dubium Taranek		B							
Cyclopyxis eurystoma Deflandre							D	D	D
Cyclopyxis kahli Deflandre				D					D
Difflugia globulosa Dujardin				D	D				D
Difflugia longicollis Deflandre						D			
Euglypha rotunda Wailes									C
Euglypha strigosa Ehrenberg	C								
Lesquereusia spiralis Ehrenberg			D						
Lesquereusia modesta Rhumbler			D		D				
Phryganella acropodia Herwig *et* Lesser	D	D	D				D	D	D
Trinema enchelys Leidy		B	C						
Trinema lineare Penard		C			C				C
Total testate taxa	2	7	6	3	4	1	3	3	11
Total protozoa (testates + other groups)	8	13	11	13	11	5	12	11	26

Table 4. Comparison of our Ascension data with other initial reconnaissance type surveys of soil protozoa on mid-Atlantic islands. Data for the islands other than Ascension from the "Quest" Expedition (Sandon and Cutler 1924) which collected a similar number of samples to our on work.

	Tristan da Cunha (37°S)	Gough Island (40°S)	St. Helena (16°S)	Ascension (8°S)
Gymnamoebae	5	6	5	8
Testates	7	14	3	21
Ciliates	11	9	8	11
Flagellates	22	16	15	11

correct habitat occurs (e.g. Finlay 2002). However, it is of interest that the Ascension testate fauna is apparently missing the larger taxa. Using the same data set of testate sizes as used by Wilkinson (2001), most of the Ascension taxa have median test sizes (either length or diameter, whichever is largest) below 100 µm and only one (*Centropyxis constricta*) has a median size of 150 µm. The analysis in Wilkinson (2001) suggested that, for testates, limited geographical range becomes commoner above sizes of 100-150 µm. The absence of the larger testate species from our Ascension samples is consistent with this idea. Certainly, using classical morphological approaches, we found no evidence for endemic taxa amongst any of the microbial groups we studied; this result is different from that found in the multicellular plants and animals of Ascension where several endemics have been described (Ashmole and Ashmole 2000).

As well as the tendency of testates to be cosmopolitan in their distribution another striking feature, which runs counter to the commonly observed patterns in macroscopic species, is the lack of specialist tropical species. Strikingly all our testate species, with two exceptions (*Arcella vulgaris* and *Difflugia longicollis*), are also recorded in the Arctic data set of Beyens and Chardez (1995). Indeed *A. vulgaris* has been recorded in the high arctic by other authors (Balik 1994, Hodkinson *et al.* 2004) while *D. longicollis* was found in the sub-Antarctic (Smith and Headland 1983), so all the testate taxa described from the tropical Ascension Island are also found in polar habitats!

Although, as described above, our species lists are far from complete they are consistent with the hypothesis that Ascension has a relatively impoverished soil protozoan fauna; presumably because of a combination of its recent geological origin, remote location and predominance of arid habitats. This is suggested by a comparison of our testate data with that described by Meisterfeld and Tan (1998) in their preliminary study of the testates of the previously unstudied Mount Buffalo National Park in Australia. They sampled a similar number of sites to our Ascension study (8 compared with our 9) but found 50 taxa of testates (compared to our 21).

The ciliates show a similar pattern to the testates, with most of the species identified from Ascension exhibiting a cosmopolitan distribution (Foissner 1998). An apparent exception is *Psedoglaucoma muscorum* which has previously only been recorded from the Holarctic (Foissner 1998). This could suggest that this taxon is more widely distributed than previously thought; as would be suggested by the 'everything is everywhere' ideas of Finlay and colleagues (e.g. Finlay and Clarke 1999, Finlay and Fenchel 1999, Finlay 2002). This interpretation is made more plausible by the taxons small size (<30 µm) and the possibility of confusion with other similar looking taxa such as *Uronema nigricans* and *Cyclidium glaucoma*. However it could have been introduced to the island by humans, as it is known that not only plants but also soil was introduced to the island from the northern hemisphere during the XIX-th century (Hart-Davis 1972). The other groups are also comprised of widespread species, often with broad habitat requirements. For example the flagellate *Rhyncomonas nasuta* has been recorded from fresh and sea water as well as soils (Cowling 1994), while several of our flagellate and gymnamoebae have been recorded in Antarctic dry valleys (Bamford *et al.* 2005).

The common pattern for macroscopic species is that islands are species poor for their size but rich in endemic species (Whittaker 1998). Although our species lists are clearly not comprehensive they do strongly suggest that only part of this pattern is seen in soil protozoa; while our data are consistent with the soils of Ascension being relatively species poor we found no evidence for endemism based on our classical morphological approach to protozoan identification. Clearly there is the possibility that molecular methods may discover 'cryptic' species, which although genetically distinct are not recognisable by morphological methods (Dolan 2006). However, so far, molecular studies on widespread ciliate taxa (e.g. *Colpoda spp* and *Gonostomum affine*) have failed to find such cryptic species (Bowers *et al.* 1998, Foissner *et al.* 2001). A striking feature of the species identified from Ascension is that they are not specialist tropical species and in many cases have also been recorded from polar regions - again this result is counterintuitive to biologists familiar with larger organisms. The testate amoebae data are particularly interesting, being consistent with the idea that the larger testates show limited distributions and for these taxa 'everything is not everywhere'. The soil protozoa of remote islands, such as Ascension, would clearly repay more detailed study.

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