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Diversity of gymnamoebae in grassland soil in southern Scotland

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Summary

As part of a study of the natural history of protozoa at a grassland site at Sourhope, in Scotland, the potential for soil samples to support the growth of naked amoebae was assessed using an indirect dilution/culture method. Populations of up to 73,000 per gram dry weight of soil were detected, and a total of 48 putative species were distinguished using light microscopy. A further six putative species were detected when a different culturing method was employed. The wide range of families and genera represented included *Acanthamoeba*, *Saccamoeba*, *Cochliopodium*, *Vexillifera*, *Korotnevella* and the Vahlkampfiidae, and several new species were isolated. Naked amoebae of lens-like, acanthopodial and dactylpodial morphotypes were detected most frequently, and were most widely distributed across the one-hectare site.

Key words: biodiversity, ecology, soil gymnamoebae, enumeration

Introduction

The principal objective of the soil protozoan diversity project funded by the NERC Soil Biodiversity Thematic Programme was to obtain a comprehensive characterisation of the natural history of the free-living protozoa in the soil of a grassland farm in Scotland. As part of this project, the potential for soil samples to support the growth of naked amoebae has been assessed using an indirect dilution/culture method to reveal the culturable diversity of naked amoebae in the soil (Finlay et al., 2000). Amoeboid organisms are notoriously difficult to identify to the level of species. Instead, the diversity of naked amoebae in the samples was assessed by assigning each population to a morphotype, according to the scheme of Smirnov and Goodkov (1999a).

Material and methods

Naked amoebae were enumerated in 150 soil samples collected over 14 months from 25 plots at the Natural Environment Research Council Soil Biodiversity Thematic Programme experimental site at the Macaulay Land Use Research Institute's Sourhope Research Station, near Kelso in southern Scotland (grid reference NT854 196), as described in Finlay et al. (2000). The enumeration protocol was modified from

the method developed by Anderson and Rogerson (1995) in the following way; 5 g aliquots of air-dried, sieved soil were flooded with filtered rainwater and incubated at 15°C for four weeks prior to dilution and enumeration. The purpose of this modification was to assess the potential of soil to support a community of naked amoebae in the presence of other types of soil microorganisms (Finlay et al., 2000). Consequently, this method estimates the total number of trophozoites and cysts of naked amoebae present in the flooded soil at the end of the incubation period. Following dilution of the flooded soil samples, and aliquoting into multiwell plates, the development of subsequent cultures was monitored over 35 days using an inverted microscope (up to 400× magnification). The number of wells containing naked amoebae was counted and the morphology and lengths of the naked amoebae observed in the wells were recorded. Each population was assigned to a morphotype according to the scheme of Smirnov and Goodkov (1999a). Some strains were established in culture by distributing aliquots from a well containing a clonal population to non-nutrient agar spread with a small quantity of *Escherichia coli* (Page, 1988). The strains were investigated using routine light and electron microscopy techniques.

To recover other amoebae species that did not multiply in the multiwell plates, 0.05-0.1 g of air-dried (sieved) soil from four different plots (collected on 9 Febuary 2000) was placed in duplicate Petri dishes (9 cm diameter) with Prescott's and James's mineral medium (Tompkins et al., 1995), a small volume of Sigma Cereal Leaf-Prescott liquid (op. cit.) and a boiled wheat grain. The Petri dishes were incubated at 15°C in a moist chamber. The development of naked amoeba popu-lations in the Petri dishes was observed over a period of five weeks, at intervals of 2-7 days.

Results and discussion

This investigation revealed the presence in the soil of 48 putative gymnamoebae species belonging to 12 morphotypes (Table 1), this is close to the maximum reported in the literature for a single habitat of any type (Smirnov and Goodkov, 1995; Rodriguez-Zaragoza and Garcia, 1997; Butler and Rogerson, 2000). A range of common isolates which were identified to the level of genus or species is shown in Fig. 1.

At Sourhope, naked amoebae of acanthopodial, monotactic and eruptive morphotypes appear to be the most diverse (Table 1), which is consistent with the fact that these morphotypes comprise the highest numbers of described species. When compared with the known diversity of amoebae within the phylum Rhizopoda (sensu F.C. Page), most families of free-living, aerobic amoebae are represented at Sourhope by a wide diversity of morphologies and sizes. Notable exceptions are the Amoebidae and the Thecamoebidae. The apparent absence of representatives of these families is remarkable as the Amoebidae includes one of the few real "soil" genera of gymnamoebae – the genus *Deuteramoeba*, and rugose species of *Thecamoeba* are also considered to be typical "soil" species. It is possible that the way the soil was treated (air-dried) destroyed these types of amoebae, or that they were present in low numbers (below the detection range of the enumeration method; 1,000-50,000 per gram dry weight of soil) or that the enrichment methods used did not allow them to grow in culture. The latter possibilities are supported by experimental data; when aliquots of a single sample of soil from plot 4C were cultured in duplicate Petri dishes, a total of 20 putative species could be differentiated, of which eight had not been detected in any of the six samples from this plot enumerated using the multiwell plate technique, and four of these eight had not been observed in any of the multiwell plate cultures generated from the 150 soil samples collected from across the site. A total of six putative species (including the largest observed, a *Rhizamoeba* sp.) multiplied in Petri dishes but were not detected in multiwell plate cultures, so even more species may have been detected if additional culture techniques had been employed.

A further problem is that the number of species differentiated may be limited not only by the extent of the recovered diversity but also by the limit to the number of notably different "sketches" of amoebae that an experienced investigator can keep in mind (about 50). It is particularly difficult to differentiate similar amoebae species within monotactic and eruptive morphotypes, especially in mixed cultures, which may result in the underestimation of the number of different species.

As, historically, naked amoebae have been under-sampled on a global scale, it is unremarkable to discover new species (Smirnov and Goodkov 1995, 1999b). During this investigation, five new species were clearly distinguished; *Vannella persistens* (Smirnov and Brown, 2000), one *Vexillifera* species and two *Saccamoeba* species (using light and electron microscopy), and a new species of *Paravahlkampfia* (differentiated using ribo-somal DNA comparisons; J.F. De Jonckheere, pers. comm.). In addition, a *Cochliopodium* strain was found to be almost identical to the incomplete description of '*Cochliopodium* sp. 2' published by Bark (1973), en-abling the description to be completed and the species named as *Cochliopodium barki* (Kudryavtsev, Brown and Smirnov, in press). To date, five strains, which could be confidently assigned to species based on light and electron microscopy observations, have been deposited with the Culture Collection of Algae and Protozoa (CCAP), UK: *Rosculus ithacus* (CCAP No. 1571/3);

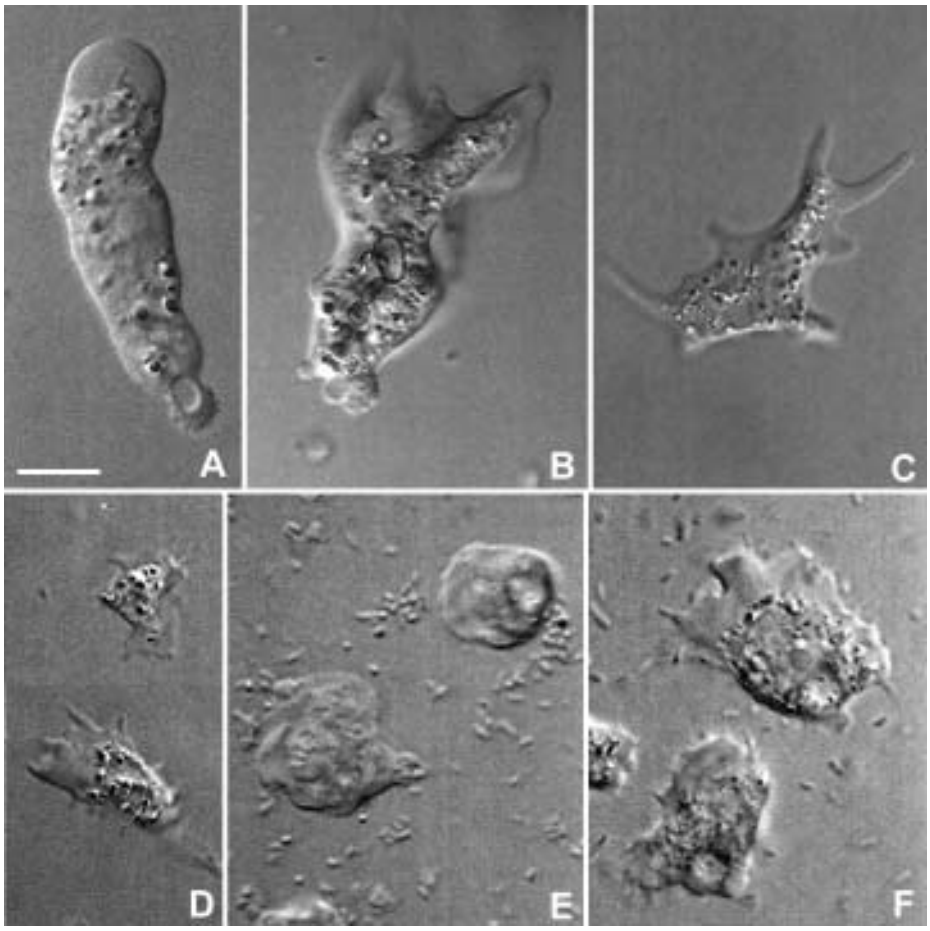

Fig. 1. Naked amoebae isolated from Sourhope soil (selection). a - *Saccamoeba* sp., b - *Mayorella vespertilioides;* c - *Korotnevella stella;* d - *Vexillifera bacillipedes;* e - *Platyamoeba placida;* f - *Acanthamoeba* sp. Scale bar: 10 μm.

Platyamoeba placida (CCAP No. 1565/14); *Cochliopodium barki* (CCAP No. 1537/4); *C. minus* (CCAP No. 1537/5), and *Vannella persistens* (CCAP No. 1589/13).

The relative frequency of detection of the different morphotypes of naked amoebae across the site is shown in Table 1. Acanthopodial and dactylopodial morphotypes were found most frequently. A wide variation was found in the frequency of detection of the 45 putative naked amoebae species. Four genera – *Cochliopodium* spp. (lens-like), *Rosculus ithacus* (flabellate), *Korotnevella* spp. (dactylopodial) and *Acanthamoeba* spp. (acanthopodial) were significantly more widespread across the site, and more frequently detected than the others. There was a positive correlation between frequency of detection of a morphotype and its distribution across the 25 plots (data not shown), which is consistent with random dispersal of naked amoebae across the site.

On the question of the ubiquity of naked amoeba species. Some of the species identified in the soil from Sourhope (*Mayorella vespertilioides, Korotnevella stella, K. bulla, Saccamoeba limax, Platyamoeba stenopodia*, for example) have been found in other regions of the world (Page, 1976; Smirnov and Goodkov, 1995; Smirnov, unpubl. data), and may be ubiquitous. If each putative species observed during this investigation actually represents a distinct species, then the diversity of naked amoebae cultured from the soil of this one-hectare grassland site represents approximately one fifth of the estimated diversity of naked amoebae in non-marine environments; 220 species (Finlay, 2001). This high ratio of local to global species richness is consistent with the theory that protozoan species have a global distribution (Finlay et al., 2001; Finlay, 2002). In contrast, the newly-described species *Vannella persistens* (Smirnov and Brown, 2000), and several other unidentified species, appear to be unique to Sourhope, and certain types of naked amoebae were apparently absent, which could be interpreted as meaning that some naked amoebae species may be restricted in distribution by unknown ecological factors. However, additional sampling has, in the past, revealed the presence of "endemic" species in geographically distant

Table 1. Naked amoebae morphotypes detected at Sourhope.

Morphotype	Number of species	Frequency of detection across the study site (%)
Acanthopodial	9	84
Monotactic	9	29
Eruptive	7	52
Fan-shaped	6	59
Lens-like	4	91
Flabellate	3	57
Dactylopodial	3	69
Lingulate	2	7
Mayorellian	2	20
Flamellian	1	5
Filose	1	4
Lanceolate	1	3

locations (Esteban et al., 2001; Smirnov, 2003) and it is accepted that naked amoebae are a relatively understudied group. Therefore, much further work studying a wide range of local faunas (and accurately identifying the species detected) is required before making any conclusive statement about naked amoebae species distribution on a global scale.

Only small and medium-sized amoebae with size ranges from 3.5-15 μm (*Vannella* sp.) to 56-115 μm (*Rhizamoeba* sp.) were detected in the Sourhope soil (Fig. 2). Almost half of the putative species detected had an average cell length of 18-32 μm, and naked amoebae with this size range were also calculated to be the most abundant in the soil samples (Finlay and Fenchel, 2001). Only one species with an average length below 10 μm was noted, perhaps because we overlooked small cells (which is improbable due to the very detailed, repetitive examination of samples), or because they do not grow under the culture conditions used, or because they really are not very abundant.

In the majority of the re-wetted 150 soil samples treated as described above, naked amoebae became less abundant than flagellated protozoa, but more abundant than testate amoebae and ciliates, with maximum and mean abundance of 73,000 and 17,300 cells per gram dry weight, respectively (Finlay et al., 2000). No seasonal variation was detected in the potential of the soil to support protozoan populations (op. cit.). Throughout the sampling programme, *Cochliopodium* spp. and *Rosculus ithacus* were the most numerous naked amoebae in re-wetted soil samples following a four week incubation. According to the four morphotypes classification system of Anderson and Rogerson (1995), these species would be assigned to morphotype 4 (which includes fan-shaped or discoidal species). Several other recent studies have also found that morphotype 4 amoebae are significantly more common and numerous than other types in a range of soils (Bischoff and Anderson, 1998; Anderson, 2000; Bass and Bischoff, 2001). It has been hypothesized that the morphology and relatively small size of morphotype 4 amoebae may be particularly successful adaptations to soil microhabitats (Bischoff and Anderson, 1998) but we lack understanding of the significance of different morphological characte-ristics in determining the relative success of different naked amoeba species in soil.

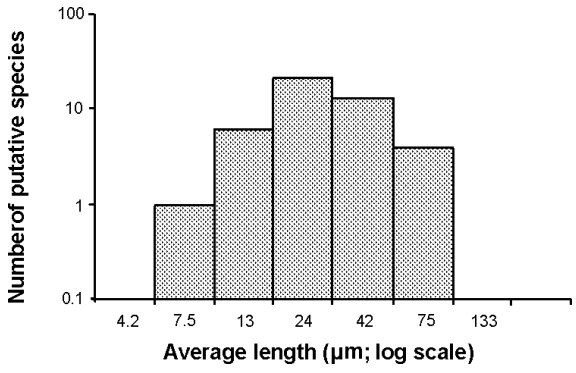

Fig. 2. Frequency size distribution of naked amoebae from Sourhope soil.

Cyst formation is an important ability in a predominantly dry soil environment. A cyst cannot be attributed to any trophozoite observed in mixed culture, but cyst formation was observed in monocultures of eleven species. A new species detected at Sourhope, *Vannella persistens*, is the first *Vannella* species recorded with the ability to form cysts (Smirnov and Brown, 2000). Perhaps other species are capable of forming cysts but culture conditions were unsuitable. Remark-ably, some morphotypes detected in the air-dried soil can be assigned to well-studied genera for which no cysts have been reported (e.g. *Mayorella*, *Korotnevella*), which indicates that cyst formation is not an obligatory adaptation in soil amoebae. However, as with *Vannella*, other species and genera may be confirmed as cyst-forming in the future.

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