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Ecological Distribution of Protosteloid Amoebae in New Zealand

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Abstract: During the period of March 2004 to December 2007, samples of aerial litter (dead but still attached plant parts) and ground litter were collected from study sites representing a wide range of latitudes (34° S to 50° S) and a variety of different types of habitats throughout New Zealand (including Stewart Island and the Auckland Islands). The objective was to survey the assemblages of protosteloid amoebae present in this region of the world. Twenty-nine described species of protosteloid amoebae were recorded, along with the heterolobesean acrasid, Acrasis rosea. Of the species recovered, Protostelium mycophaga was by far the most abundant and was found in more than half of all samples. Most species were found in fewer than 10% of the samples collected. Seven abundant or common species were found to display significant preferences for aerial litter or ground litter microhabitats. There was some evidence of a general pattern of a decrease in species richness and diversity with increasing latitude and precipitation and elevation.
Introduction

The term “protosteloid amoebae” refers to a paraphyletic assemblage of unicellular eukaryotes within the supergroup Amoebozoa that exhibit spore dispersal via sporocarpic fruiting. For most of their life cycle, protosteloid amoebae exist as single amoeboid cells that may or may not possess flagella (Shadwick et al. 2009). These organisms are thought to be important consumers of bacteria and other microorganisms (Adl & Gupta, 2006). Although global inventories carried out thus far suggest that protosteloid amoebae occur in every type of terrestrial system (Ndiritu, Stephenson, & Spiegel, 2009), very little is known about their ecology. The results obtained from previous studies (Moore, Stephenson, Laursen, & Woodgate, 2000; F. W. Spiegel & Stephenson, 2000; S. Stephenson et al., 2004) have provided some evidence that ecosystems located at higher latitudes support fewer species and a show a decline in species abundance. Because of its location, size, and isolation, New Zealand provided an excellent opportunity to investigate these patterns.

New Zealand is the most isolated land mass of its size in the world (Cavender, Stephenson, Landolt, & Vadell, 2002) and represents a unique ecosystem with a highly endemic flora (Fleet, 1986). Protosteloid amoebae have been known from New Zealand (Olive & Stoianovitch, 1969), and is the location from which the type specimen of Schizoplasmodium cavostelioides was originally isolated (Olive, 1967). The study sites from which samples were obtained in the present study were located on both the North Island (113,729 km²) and the South Island (151,215 km²) as well as Stewart Island (1,746 km²) and the Auckland Islands (625 km²). Collectively, these islands provide a well-characterized and diverse array of habitats that extend over a wide range of latitudes (34.44° S to 50.85° S). The primary focus of the present study was to exhaustively sample as much of this range as possible in order to characterize the ecological distribution of the protosteloid amoebae present.

Materials and Methods
During the period of March 2004 to December 2007, three separate collecting trips were made to the North Island, South Island and the Auckland Islands (Figure 1 and supplementary table 1). Samples were obtained from Stewart Island in 2006, but yielded no observations. Study sites encompassed a variety of elevations (extending from 0 m to 1636 m), every major vegetation type found in New Zealand, and ranged from 34.44° S to 50.85° S latitude. A total of 247 samples of aerial litter and 234 samples of ground litter were taken collected from 82 different study sites. These samples were placed in small paper bags, air dried, and transported to the laboratory for processing. In order to achieve a broad coverage of many different types of dead plant material, sampling efforts did not include systematic replications of substrate types or habitats, but multiple samples from many habitats were collected.

In the laboratory, samples were cut into small pieces, wetted with sterile water, and plated in lines on minimal nutrient agar (0.002 g malt extract, 0.002 g yeast extract, 0.75 g K₂HPO₄, 15.0 g Difco Bacto Agar, 1.0 L deionized [DI] H₂O) as described by Spiegel et al. (F. Spiegel, Stephenson, Keller, Moore, & Cavender, 2004), yielding 6,533 lines of substrate that were examined in 1,175 plates. Daily observations were made for a minimum of seven days using bright-field microscopy with the 10X
objective lens on a compound scope. Species were identified based on sporocarp morphology according to Olive (1967, 1970) and Spiegel et al. (F. Spiegel, Shadwick, Lindley, Brown, & Nderitu, 2010). Observations of amoeboid and prespore stages were carried out to corroborate sporocarp identifications when necessary.

Species observations were recorded as presence or absence for each plated line of substrate and this resolution was used for comparisons between sites. All climate data were extracted from the New Zealand National Climate Database (http://cliflo.niwa.co.nz/). Sample-based rarefaction curves were generated using Ecosim 7 (Gotelli & Entsminger, 2009). The effects of latitude, elevation, and precipitation gradients, and microhabitat on species richness and abundance were tested with the General Linear Model ANOVA in Minitab® Statistical Software version 16.

Results

Twenty-nine species of protosteloid amoebae, including the minuscule myxomycete *Echinostelium bisporum*, were recovered in the present study. While not traditionally grouped together with the now defunct “Protostelids” (Shadwick, Spiegel, Shadwick, Brown, & Silberman, 2009), the small fruiting bodies of *E. bisporum* display a protosteloid growth form and are commonly encountered using the current methods, so it has been included in this study. Species were grouped into abundance categories consistent with similar studies (Aguilar, Spiegel, & Lado, 2011; Ndiritu et al., 2009) such that species recovered from: >10% of samples = abundant; 5-10% = common; 1-5% = occasional; <1% = rare.

Seven species were found to be abundant across all study site locations while ten were considered commonly occurring (Table 1). *Protostelium mycophaga* was by far the most commonly encountered species, accounting for twenty-five percent of all fruiting body observations. Eighty-one out of eighty-two sites were positive for fruiting bodies of protosteloid amoebae (99%). The only site that did not yield any observable collections, located on Stewart Island, was left out of subsequent analyses.
The number of collections varied at each site due to local conditions, such as a lack of suitable standing plant material, but of the 481 total collections made, 299 of them yielded identifiable fruiting bodies of protosteloid amoebae (62%). These numbers are consistent with previous studies (Aguilar et al., 2011; Ndiritu et al., 2009; S. L. Stephenson, Landolt, & Moore, 1999).

Microhabitat (aerial vs. ground litter) did not have a significant influence on either the abundance or species richness of fruiting amoebae as a whole (P=0.888, One-way ANOVA; P=0.746; One-way ANOVA, respectively), but several species species displayed significant preferences. Of these, Protostelium mycophaga, Protostelium nocturnum, Protostelium mycophaga var. little, and Soliformovum expulsum were significantly more likely to be found on aerial litter, while Schizoplasmodiopsis pseudoendospora, Nematostelium gracile, and Schizoplasmodiopsis vulgare showed a significant preference for ground litter (Table 2).

Microhabitat also made no difference in correlations between larger environmental factors (i.e. latitude, elevation, etc.) with fruiting amoebae abundance and species richness, except for certain species that displayed significant preferences.

| Species Name | Abbreviation | Total Encounters | Frequency per Sample | Category | Aerial | Ground |
|--------------|--------------|------------------|----------------------|----------|--------|--------|
| Protostelium mycophaga** | Pm | 598 | 2.06 | A | 398 | 200 |
| Schizoplasmodiopsis pseudoendospora* | Sps | 323 | 1.2 | A | 119 | 204 |
| Nematostelium gracile* | Ng | 239 | 1.05 | A | 83 | 156 |
| Soliformovum irregularis | Si | 213 | 1.14 | A | 130 | 83 |
| Schizoplasmodiopsis vulgare*** | Sv | 197 | 0.95 | A | 40 | 157 |
| Protostelium nocturnum*** | Pn | 182 | 0.98 | A | 136 | 46 |
| Schizoplasmodiopsis amoeboida | Sa | 174 | 1.06 | A | 92 | 82 |
| Protostelium arachisporum | Pa | 73 | 0.33 | C | 43 | 30 |
| Protostelium pyriformis | Ppyr | 57 | 0.41 | C | 27 | 30 |
| Schizoplasmodioides cavisteloides | Sc | 51 | 0.28 | C | 38 | 13 |
| Tychosporium acutostipes | Ta | 49 | 0.42 | C | 29 | 20 |
| Cavistelium apophysatum | Ca | 43 | 0.25 | C | 15 | 28 |
| Nematostelium ovatum | No | 41 | 0.31 | C | 14 | 27 |
| Protostelium mycophaga var. little*** | lilPm | 34 | 0.25 | C | 33 | 1 |
| Endostelium zonatum | Ez | 31 | 0.19 | C | 17 | 14 |
| Echinosteliosis oligospora | Eo | 28 | 0.2 | C | 14 | 14 |
| Soliformovum expulsum* | Se | 27 | 0.3 | C | 21 | 6 |
| Echinosteliosis bisporum† | Eb | 16 | 0.16 | O | 7 | 9 |
| Protosteliosis fimicola | Pf | 12 | 0.12 | O | 7 | 5 |
| Microglomus pauxilis | Mp | 9 | 0.07 | O | 1 | 8 |
| Clastostelium recurvatum | Cr | 8 | 0.09 | O | 3 | 5 |
| Protostelium mycophaga var. repeater | Pmrep | 7 | 0.05 | O | 7 | 0 |
| Schizoplasmodiopsis micropunctata | Sm | 5 | 0.05 | O | 5 | 0 |
| Protostelium okumakama | Po | 5 | 0.05 | O | 1 | 4 |
| Schizoplasmodiopsis reticulata | Sr | 4 | 0.01 | R | 2 | 2 |
| Ceratomyxa hemisphaerica | Ch | 2 | 0.01 | R | 0 | 2 |
| Protosporangium articulatum | Partic | 1 | 0.01 | R | 1 | 0 |
| Protosporangium bisporum | Pbisp | 1 | 0.01 | R | 1 | 0 |
| Soliformovum ovatum | So | 1 | 0.01 | R | 0 | 1 |

Table 1 - A=abundant, C=common, O=occasional, R=rare *p<0.05; **p<0.01; ***p<0.001 (All tests: significant difference between Aerial and Ground litter abundance; one-way ANOVA test)
and annual precipitation) and community richness or abundance.

The strongest indicators of community richness and abundance were elevation and precipitation, while latitude also played a significant role. Increases in all three factors led to predictable declines in protosteloid amoebae community measures (Figure 2). The most abundant and diverse communities were found in drier, more northerly locations close to sea level. This trend has been observed in other work (Spiegel, unpublished data) though potential mechanisms for the observations have not been explored.

Discussion

The main focus of this study was to provide a comprehensive survey of the protosteloid amoebae of New Zealand and to investigate the distribution of these species along gradients of climate, elevation, and latitude. A sample-based rarefaction curve (Figure 3) suggests that sampling effort was

![Figure 2 - Regressions of all observations of fruiting bodies' richness and abundance against latitude, elevation, and annual rainfall. Latitude is in degrees below the equator, Elev. is meters above sea level, Rainfall is annual precipitation received during the year collected.](image_url)
sufficient to recover the bulk of the known and
described species diversity present. This study
also provided an excellent opportunity to
observe the distribution of an easily
observable group of microbes across a large
latitudinal transect. Broadly, we were able
demonstrate that latitude, elevation, and
precipitation had an influence on the abundance and richness of protosteloid amoebae in New Zealand.

The sampling method varied somewhat between collecting trips. The first collections were
physically separated by substrate type (i.e. a separate bag for each type of litter collected), whereas the
subsequent collections were pooled together (i.e. all aerial litter in one bag and all ground litter in
another bag). This change was made for convenience, since many study sites had limited amounts of
litter present and it was difficult to find substrate species that yielded both aerial and ground litter in the
same general area. Cursory analysis of the two sampling methods suggested that species observations
were not affected by initial pooling of samples and thus sampling methods were treated as equal for all
subsequent analyses. The sampling protocol did not allow for rigorous testing of this assumption, but
this is beyond the scope of the present study. Additionally, the number of plated lines of substrate per
study location varied from 4 to 486 as shown in supplementary table 1. For most sites (68%), at least
forty lines of substrate were plated for observation.

These heavily observed sites may display a bias toward an increase in the observations of rare
species when compared with sampling locations such as the Auckland Island sites, in which only four lines
of substrate were observed. Of the five rare species identified, two (*Ceratiomyxa hemisphaerica* and
*Protosporangium bisporum*) were only found at sample locations from which 486 lines were plated and
none were found at any locations from which less than 32 lines were plated. These rare species account
for only nine distinct observations, and excluding them from further analyses had no impact on the significance of results, so they have been left in. The most common species, Protostelium mycophaga, was found at only one sample location from which 486 lines were plated.

The effectiveness of various levels of observational effort for the detection of protosteloid amoebae was quantified by Aguilar et al. (2011) and it was found that four lines of substrate per sample was enough to detect 80% of species present, while eight lines per sample was able to yield 90% of the species present. Substantial increases in observational effort yielded only one or two additional rare species. In the present study, site richness was not significantly correlated with the number of plated lines per study location ($R^2=0.033$, $P=0.103$). Interestingly, six of the nine observations of rare species occurred at sites in which forty lines of substrate were plated, further suggesting that sampling efforts greater than that did little to increase the effectiveness of ecological surveys for rare species of protosteloid amoebae. It is apparent that comparisons between abundant, common, and occasional species may be safely made using the current study’s sampling and observation protocol.

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