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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.

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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 km^2) and the South Island (151,215 km^2) as well as Stewart Island (1,746 km^2) and the Auckland Islands (625 km^2). 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

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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.

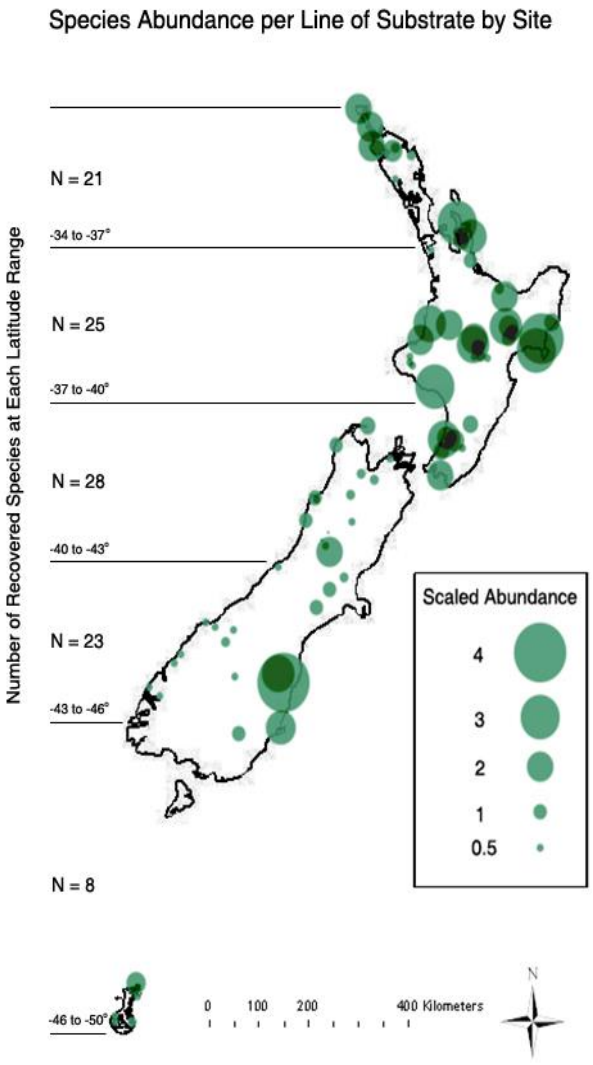

Figure 1 - Sample site markers are scaled to represent the mean number of protosteloid amoebae fruiting bodies encountered for each line of substrate observed from that site. N = species richness observed at each major latitudinal range.

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 K_2HPO_4, 15.0 g Difco Bacto Agar, 1.0 L deionized [DI] H_2O) 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

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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.

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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,

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 amoeboidea	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
Schizoplasmodium cavostelioides	Sc	51	0.28	C	38	13
Tychosporium acutostipes	Ta	49	0.42	C	29	20
Cavostelium 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
Echinosteliopsis oligospora	Eo	28	0.2	C	14	14
*Soliformovum expulsum**	Se	27	0.3	C	21	6
Echinostelium bisporum†	Eb	16	0.16	O	7	9
Protosteliopsis fimicola	Pf	12	0.12	O	7	5
Microglomus paxillus	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 okumukumu	Po	5	0.05	O	1	4
Schizoplasmodiopsis reticulata	Sr	4	0.01	R	2	2
Ceratiomyxa 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
Schizoplasmodium obovatum	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)*

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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

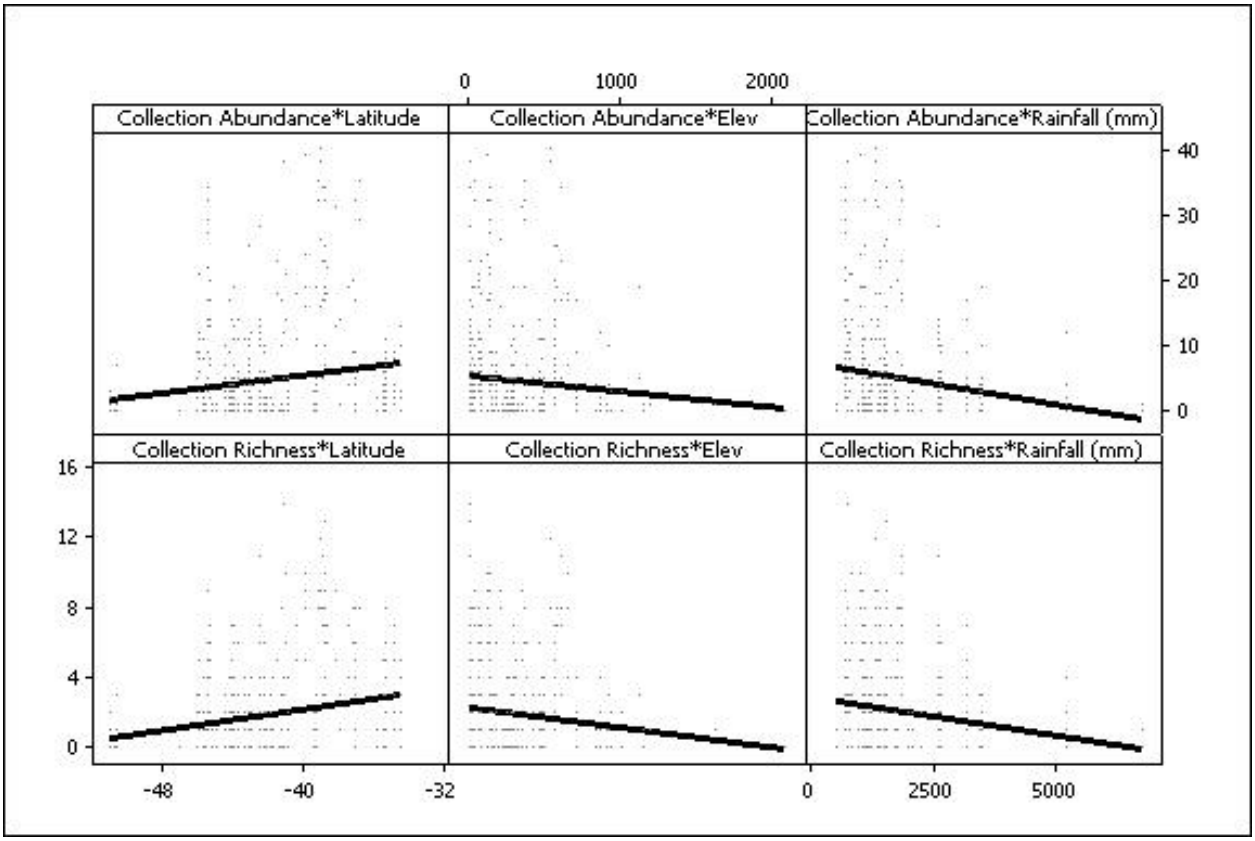

Figure 2 - Regressions of all observations of fruiting bodies' richness and abundance against latitude, elevation, and annual rainfall. Latitude in in degrees below the equator, Elev. is meters above sea level, Rainfall is annual precipitation received during the year collected.

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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.

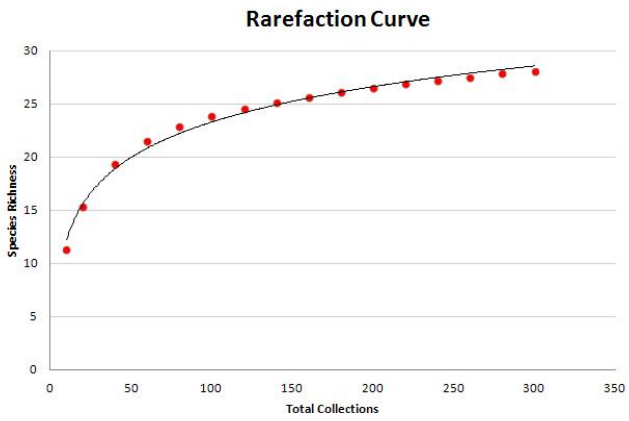

Figure 3 - Rarefaction curve showing sampling effort.

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 Aukland 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

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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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