Ecological Distribution of Protosteloid Amoebae in New Zealand

Geoffrey Zahn, Frederick W. Spiegel, Stephen L. Stevenson

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.

PeerJ PrePrints | https://peerj.com/preprints/136v1/ | v1 received: 2 Dec 2013, published: 2 Dec 2013, doi: 10.7287/peerj.preprints.136v1

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

PeerJ PrePrints | https://peerj.com/preprints/136v1/ | v1 received: 2 Dec 2013, published: 2 Dec 2013, doi: 10.7287/peerj.preprints.136v1

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 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.

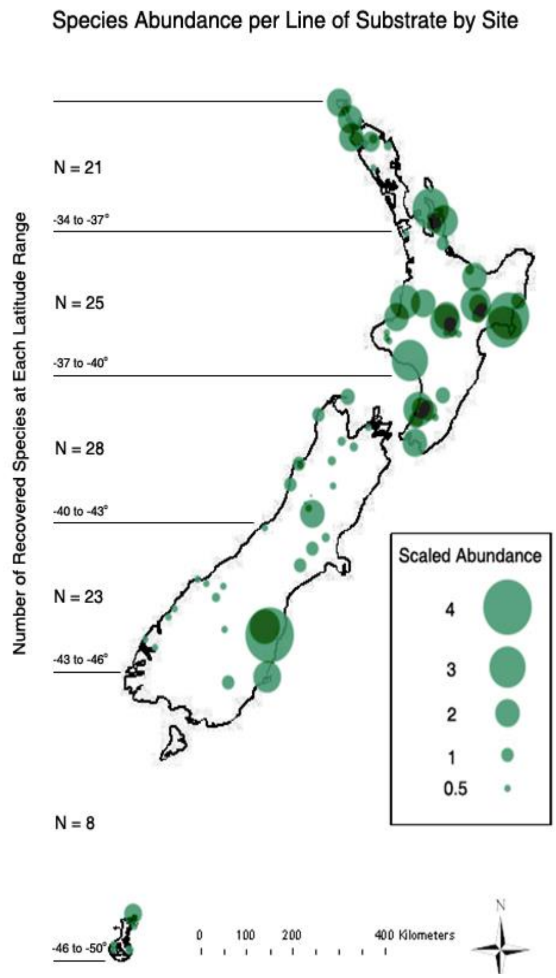

Figure 1 - Sample site markers are scaled to represent the mean number of protosteloid amoebae fruiting bodies encountered for each line of substrate observed from that site. N = species richness observed at each major latitudinal range.

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 objective lens on a compound scope. Species were identified based on sporocarp morphology according

PeerJ PrePrints | https://peerj.com/preprints/136v1/ | v1 received: 2 Dec 2013, published: 2 Dec 2013, doi: 10.7287/peerj.preprints.136v1

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.

PeerJ PrePrints | https://peerj.com/preprints/136v1/ | v1 received: 2 Dec 2013, published: 2 Dec 2013, doi: 10.7287/peerj.preprints.136v1

Site	Latitude/Longitude	Elev. (m)	Habitat	Month/Year Collected	Lines Plated	Site Richness
Tairoa Head Albatross Colony (263)	45°46'30.1000"S, 170°43'41.4998"E	67	Grassland	3/2004	218	10
West of Dunback (264)	45°19'13.3000"S, 170°34'34.2001"E	130	Grassland	3/2004	306	13
West of Morrisons (265)	45°13'16.1000"S, 170°25'24.3001"E	561	Scrub	3/2004	192	11
Boundry Creek Rest Area (266)	44°21'13.5000"S, 169°10'07.7002"E	277	Mixed Dry Forest	3/2004	194	7
Blue Pools (267)	44°09'00.8640"S, 169°16'00.6100"E	277	Beech	3/2004	160	1
Haast Pass (268)	45°06'00.4380"S, 169°21'00.2830"E	716	Beech	3/2004	188	1
South of Haast (269)	44°03'21.1000"S, 168°42'35.3999"E	716	Rainforest	3/2004	320	7
Jacksons Head (270)	43°57'52.6000"S, 168°36'19.4000"E	1	Podocarp/Beech	3/2004	320	11
Road to Hokitika (271)	42°59'00.0790"S, 170°40'00.7961"E	30	Rainforest	3/2004	162	5
Port Elizabeth (272)	42°22'00.5920"S, 171°14'00.3862"E	0	Beach	3/2004	156	18
Punakaiki (273)	42°06'00.9560"S, 171°19'00.7741"E	0	Beach/Nileau	3/2004	336	9
Temple Basin Trail (274)	42°54'44.1000"S, 171°33'32.1001"E	876	Scrub	3/2004	160	7
The Chasin Trail (276)	42°55'09.3000"S, 171°33'30.4999"E	842	Beech	3/2004	162	1
U of Canterbury (277)	43°02'09.0000"S, 171°45'25.9999"E	561	Grassland	3/2004	168	6
Eastern Beech (278)	43°17'28.8000"S, 171°55'01.2000"E	493	Beech	3/2004	158	8
Sharplin Falls (279)	43°37'41.2000"S, 171°25'04.5998"E	463	Beech	3/2004	154	8
Peel Forest (280)	43°53'34.7000"S, 171°15'42.0001"E	289	Podocarp/Beech	3/2004	443	12
Te Anau (281)	45°26'38.0000"S, 167°41'03.0998"E	218	Beech	3/2004	229	3
Mirror Lake (282)	45°01'44.2000"S, 168°00'46.8000"E	350	Beech/Wetland	3/2004	239	2
Lake Gunn (283)	44°53'26.4000"S, 168°05'06.7999"E	485	Beech	3/2004	164	1
Red Tussock Cons. Area (284)	45°33'38.0000"S, 168°02'07.4000"E	480	Native Grassland	3/2004	162	6
Taputaputa Bay (302)	34°26'13.7400"S, 172°42'48.4200"E	5	Teatree	5/2005	40	10
Pine Block Road (303)	34°44'57.7800"S, 173°01'05.8800"E	70	Pine	5/2005	52	12
Ahipara Gum Lands (305)	35°11'40.6800"S, 173°08'06.5400"E	178	Teatree	5/2005	40	9
Herekino Forest Tracks (306)	35°12'35.5200"S, 173°11'27.2400"E	154	Teatree	5/2005	40	10
Mangamuka Forest (304)	35°11'24.2400"S, 173°27'18.7801"E	379	Broadleaf	5/2005	30	10
Puketi Forest (307)	35°16'32.6400"S, 173°41'09.9600"E	16	Podocarp	5/2005	40	13
Harrison Scenic Reserve (308)	35°18'37.2600"S, 174°06'24.7799"E	79	Forest (Coastal)	5/2005	40	9
Trounson Kauri Park (309)	35°43'13.5000"S, 173°39'00.1199"E	234	Podocarp	5/2005	40	1
Mill Bay (310)	36°59'30.7800"S, 174°36'11.2201"E	17	Rainforest	5/2005	44	5
Aratoro Scenic Reserve (359)	38°30'14.7420"S, 175°15'10.8000"E	129	Podocarp	12/2005	40	7
TongariroNP1 (360)	39°14'16.8540"S, 175°33'26.5680"E	1636	Scrub	12/2005	20	1

PeerJ PrePrints | https://peerj.com/preprints/136v1/ | v1 received: 2 Dec 2013, published: 2 Dec 2013, doi: 10.7287/peerj.preprints.136v1

TongariroNP2 (361)	39°12'08.9820"S, 175°32'25.8720"E	1134	Beech	12/2005	40	6
DesertRoad (362)	39°18'59.4180"S, 175°43'49.7280"E	1015	Grassland	12/2005	40	2
TongariroNP3 (363)	39°10'10.6140"S, 175°31'26.5440"E	930	Flax/Scrub	12/2005	40	1
AraokiGorge (364)	38°40'16.8240"S, 174°41'40.1028"E	8	Tree Fern/Podocarp	12/2005	40	14
GorgePulloff (365)	38°53'45.9240"S, 174°35'56.4360"E	214	Tree Fern	12/2005	40	11
EgmontNp1 (366)	39°16'45.1560"S, 174°05'05.9280"E	1199	Scrub	12/2005	40	1
EgmontNP2 (367)	39°14'20.6880"S, 174°06'46.1160"E	941	Podocarp/Broadleaved	12/2005	40	2
EgmontNP3 (368)	39°18'28.4760"S, 174°05'50.2800"E	1159	scrub	12/2005	40	1
Wanganui1 (369)	39°49'08.7600"S, 174°50'22.2360"E	120	Mixed Broadleaf	12/2005	60	13
Wanganui2 (370)	39°45'54.2160"S, 175°10'15.1680"E	24	Beech	12/2005	40	10
Manawata (371)	40°20'22.5600"S, 175°49'05.3760"E	76	Broadleaf	12/2005	40	9
Waihini (372)	40°59'46.1760"S, 175°23'22.8120"E	166	Podocarp/Broadleaved	12/2005	40	3
Rimutaka (373)	41°20'56.3280"S, 174°56'15.9000"E	70	Podocarp/Broadleaved	12/2005	40	6
Titahi (374)	41°05'58.8840"S, 174°50'06.5760"E	0	Scrub (Coastal)	12/2005	40	9
QEPark (375)	40°58'19.5600"S, 174°57'36.5400"E	0	Scrub (Coastal)	12/2005	40	15
Otaki (376)	40°51'14.2920"S, 175°14'06.6480"E	128	Secondary Growth	12/2005	40	11
Mahia (377)	39°04'18.0480"S, 177°48'39.4920"E	34	Scrub	12/2005	40	10
Bush (378)	38°52'34.1040"S, 177°51'20.4480"E	543	Secondary Growth	12/2005	40	14
Okita (379)	38°39'53.5320"S, 178°10'49.4040"E	37	Mixed Broadleaf	12/2005	40	10
TeUruwera1 (380)	38°47'56.6880"S, 177°07'22.9440"E	607	Beech/Fern	12/2005	40	8
TeUruwera2 (381)	38°47'02.3280"S, 177°08'04.0200"E	609	Scrub	12/2005	40	14
TeUruwera3 (382)	38°43'43.8240"S, 177°05'11.0760"E	653	Beech/Podocarp	12/2005	40	11
TeUruwera4 (383)	38°39'51.3000"S, 177°02'13.3440"E	661	Beech	12/2005	40	6
HukaFalls (384)	38°38'57.3720"S, 176°05'20.6160"E	580	Broadleaf	12/2005	40	10
LakeTaupo (385)	38°44'41.7840"S, 176°04'07.5000"E	367	Grassland	12/2005	40	7
HinaKapu (386)	38°02'14.6400"S, 176°33'00.0000"E	350	Podocarp	12/2005	40	9
BayPlenty (387)	37°52'15.2400"S, 176°42'32.0400"E	2	Dunes	12/2005	40	4
Hiwy25 (388)	37°18'16.9920"S, 175°53'29.7600"E	65	broadleaf	12/2005	40	9
TwinKauri (389)	36°58'44.6520"S, 175°50'30.9120"E	117	Tree Fern/Kauri	12/2005	40	10
Maungataururu (390)	36°44'54.7440"S, 175°32'15.2520"E	370	Tree Fern/Nikau	12/2005	40	12
SquareKauri (391)	36°59'23.0640"S, 175°34'19.3080"E	306	Kauri/Broadleaved	12/2005	40	9
Hihi (392)	37°06'43.5600"S, 175°38'02.2920"E	59	Nikau/Broadleaved	12/2005	40	11
AUK06-1 (422)	50°50'20.6412"S, 165°55'15.2400"E	9	Forest (Coastal)	3/2006	4	2

PeerJ PrePrints | https://peerj.com/preprints/136v1/ | v1 received: 2 Dec 2013, published: 2 Dec 2013, doi: 10.7287/peerj.preprints.136v1

AUK06-2 (423)	50°50'20.6412"S, 165°55'15.2400"E	9	Forest (Coastal)	3/2006	4	2
AUK06-4 (425)	50°51'11.0412"S, 165°55'12.9000"E	324	Forest (Coastal)	3/2006	4	1
AUK06-9 (430)	50°48'58.6188"S, 166°12'02.5200"E	20	Forest (Coastal)	3/2006	4	2
AUK06-16 (437)	50°32'43.8612"S, 166°12'45.7812"E	11	Forest (Coastal)	3/2006	4	1
AUK06-17 (438)	50°29'34.3788"S, 166°16'51.9600"E	35	Scrub (Coastal)	3/2006	4	3
AUK06-19 (440)	50°31'51.4812"S, 166°18'05.1588"E	6	Scrub (Coastal)	3/2006	4	1
AUK06-20 (441)	50°31'51.4812"S, 166°18'05.1588"E	6	Scrub (Coastal)	3/2006	4	1
Charming Creek (1188)	41°44'24.0000"S, 171°35'42.0000"E	3	Forest (Native)	5/2006	24	1
Truman Track (1187)	42°00'38.8800"S, 171°20'09.6000"E	0	Scrub (Coastal)	5/2006	20	2
Knight's Bush (1281)	45°54'44.1000"S, 169°29'42.5004"E	152	Beech/Broadleaved	5/2007	20	8
Route 6 Nelson (1282)	41°09'47.4984"S, 173°32'55.3992"E	84	Scrub	5/2007	20	1
Kowhai Point (1284)	41°42'44.2008"S, 173°06'46.2996"E	420	Scrub	5/2007	20	5
Lewis Pass (1286)	42°22'26.4000"S, 172°23'46.7988"E	914	Beech	5/2007	16	1
Route 63 (1287)	42°01'52.1004"S, 172°14'35.8008"E	479	Beech	5/2007	16	3
Kahurangi (1288)	41°41'07.5984"S, 172°26'37.1004"E	259	Beech/Broadleaved	5/2007	16	4
Pigeon Saddle (1289)	40°49'57.2988"S, 172°58'08.5008"E	244	Tree Fern/Broadleaved	5/2007	32	6

Table 1 - Collection site locations and characteristics. Number of lines plated refers to the number of lines of substrate observed from each site.

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

PeerJ PrePrints | https://peerj.com/preprints/136v1/ | v1 received: 2 Dec 2013, published: 2 Dec 2013, doi: 10.7287/peerj.preprints.136v1

between larger environmental factors (i.e. latitude, elevation, 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.

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 2 - 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)*

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 sufficient to recover the bulk of the known and described species diversity present. This study also

PeerJ PrePrints | https://peerj.com/preprints/136v1/ | v1 received: 2 Dec 2013, published: 2 Dec 2013, doi: 10.7287/peerj.preprints.136v1

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.

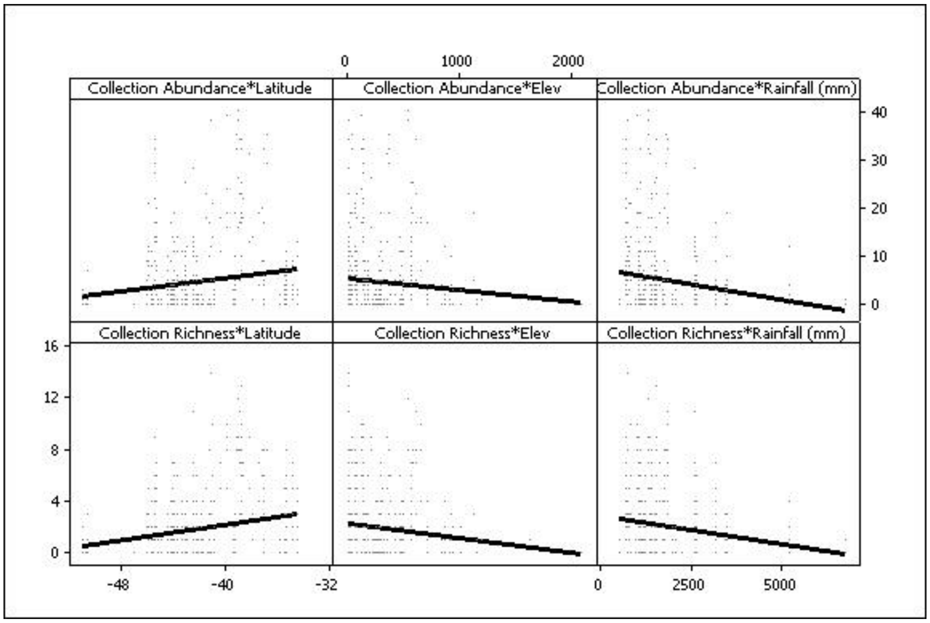

Figure 2 - Regressions of all observations of fruiting bodies' richness and abundance against latitude, elevation, and annual rainfall. Latitude in in degrees below the equator, Elev. is meters above sea level, Rainfall is annual precipitation received during the year collected.

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 Table 1. For most sites (68%), at least forty lines of substrate were plated for observation.

PeerJ PrePrints | https://peerj.com/preprints/136v1/ | v1 received: 2 Dec 2013, published: 2 Dec 2013, doi: 10.7287/peerj.preprints.136v1

These heavily observed sites may display a bias toward an increase in the observations of rare species (Table 1) 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 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.

Acknowledgements

Special thanks to John Shadwick and David Orlovich for their help gathering and processing samples.

PeerJ PrePrints | https://peerj.com/preprints/136v1/ | v1 received: 2 Dec 2013, published: 2 Dec 2013, doi: 10.7287/peerj.preprints.136v1

References

Adl, M. S., & Gupta, V. S. (2006). Protists in soil ecology and forest nutrient cycling. *Canadian Journal of Forest Research*, *36*(7), 1805–1817. doi:10.1139/x06-056

Aguilar, M., Spiegel, F. W., & Lado, C. (2011). Microhabitat and Climatic Preferences of Protosteloid Amoebae in a Region with a Mediterranean Climate. *Microbial Ecology*, *62*(2), 361–373. doi:10.1007/s00248-011-9843-6

Cavender, J. C., Stephenson, S. L., Landolt, J. C., & Vadell, E. M. (2002). Dictyostelid cellular slime moulds in the forests of New Zealand. *New Zealand Journal of Botany*, *40*(2), 235–264. doi:10.1080/0028825X.2002.9512786

Fleet, H. (1986). *The Concise Natural History of New Zealand*. Aukland, NZ: Heinemann.

Gotelli, N., & Entsminger, G. (2009). *EcoSim: Null models software for ecology.* Jericho, VT: Acquired Intelligence Inc. & Kesey-Bear.

Moore, D. L., Stephenson, S. L., Laursen, G. A., & Woodgate, W. A. (2000). Protostelids from Boreal Forest and Tundra Ecosystems in Alaska. *Mycologia*, *92*(3), 390. doi:10.2307/3761495

Ndiritu, G. G., Stephenson, S. L., & Spiegel, F. W. (2009). First Records and Microhabitat Assessment of Protostelids in the Aberdare Region, Central Kenya. *Journal of Eukaryotic Microbiology*, *56*(2), 148–158. doi:10.1111/j.1550-7408.2008.00382.x

Olive, L. S. (1967). The Protostelida: A New Order of the Mycetozoa. *Mycologia*, *59*(1), 1. doi:10.2307/3756938

Olive, L. S. (1970). The Mycetozoa: A revised classification. *The Botanical Review*, *36*(1), 59–89. doi:10.1007/BF02859155

Olive, L. S., & Stoianovitch, C. (1969). Monograph of the Genus Protostelium. *American Journal of Botany*, *56*(9), 979. doi:10.2307/2440919

Shadwick, L. L., Spiegel, F. W., Shadwick, J. D. L., Brown, M. W., & Silberman, J. D. (2009). Eumycetozoa = Amoebozoa?: SSUrDNA Phylogeny of Protosteloid Slime Molds and Its

Significance for the Amoebozoan Supergroup. *PLoS ONE*, *4*(8), e6754. doi:10.1371/journal.pone.0006754

Spiegel, F., Shadwick, J., Lindley, L., Brown, M., & Nderitu, G. (2010). A Beginner's Guide to Identifying the Protostelids. Retrieved from ftp://slimemold.ddns.uark.edu/slimemold/pdfs/Handbook1_3rd.pdf

Spiegel, F., Stephenson, S., Keller, H., Moore, D., & Cavender, J. (2004). Spiegel, F. W., S. L. Stephenson, H. W. Keller, D. L. Moore, and J. C. Cavender. "Sampling the biodiversity of mycetozoans." Biodiversity of fungi: inventory and monitoring methods. Edited by GM Mueller, G. Bills and MS Foster. Elsevier Academic Press, Burlington, Mass (2004): 547-576. In *Biodiversity of Fungi: Inventory and Monitoring Methods* (pp. 5747–576). Elsevier Academic Press.

Spiegel, F. W., & Stephenson, S. L. (2000). Protostelids of Macquarie Island. *Mycologia*, *92*(5), 849. doi:10.2307/3761580

Stephenson, S. L., Landolt, J. C., & Moore, D. L. (1999). Protostelids, dictyostelids, and myxomycetes in the litter microhabitat of the Luquillo Experimental Forest, Puerto Rico. *Mycological Research*, *103*(2), 209–214. doi:10.1017/S0953756298006996

Stephenson, S., Schnittler, M., Lado, C., Estrada-Torres, A., Wrigley de Basanta, D., Landolt, J., … Spiegel, F. (2004). Studies of neotropical mycetozoans. *Systematics and Geography of Plants*, *74*, 87–108.

PeerJ PrePrints | https://peerj.com/preprints/136v1/ | v1 received: 2 Dec 2013, published: 2 Dec 2013, doi: 10.7287/peerj.preprints.136v1