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Fungal communities at the edge: Ecological lessons from high alpine fungi

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ABSTRACT

One of the least studied ecosystems on Earth is the plant-free zone found between the alpine tundra zone and the zone of permanent ice and snow. This unique ecosystem-type occurs in all of the major mountain ranges on Earth and is especially widespread in the Andes and Himalayas. Here we describe recent molecular-phylogenetic studies of the fungi that inhabit these apparently barren soils. Sites that receive significant amounts of snowfall (in the Himalayas, Rocky and Andes ranges) are dominated by unique clades of zoospore fungi (especially the Spizellomycetales), which likely use the saturated soil conditions during snow melt to complete their life cycles and then remain dormant for most of the year during periods of extreme cold and dryness. In more extreme sites that have very sporadic and shallow snow packs, such as the upper slopes of Llullaillaco Volcano (el. 6 741 m above sea level) on the Chilean-Argentinian border, fungal communities show very little diversity and are dominated by clades of yeasts related to the Antarctic endolithic yeasts in the Filobasidiales group. The other major group found on Llullaillaco form a clade most closely affiliated with the Dothideomycetes. Overall, our phylogenetic approach and spatially explicit sampling scheme allow us to formulate new hypotheses about the ecological functioning of fungi that inhabit critically endangered high elevation ecosystems.

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Introduction

In this paper we focus on the ecology, community structure and phylogenetics of fungi that inhabit apparently barren (plant free) environments of mountainous zones. These areas occur just below the snow line or the “nival zone” (the zone of year-round ice) and above the tundra zone of continuous plant cover. This under-studied area has been referred to as the subnival zone, puna brava, high mountain desert, region of lichens and *Umbilicaria* or aeolian zone among other names (Troll 1973; Swan 1992; King et al. 2008; Humboldt & Bonpland 2009). However, in this paper we refer to this zone as the periglacial zone, following recent precedents (Anderson 2007;

King et al. 2011). The elevational limits of these barren soils vary widely across the globe, being determined mostly by latitude and distance from the nearest large body of water. Fig 1 shows the approximate relationship of the upper and lower boundaries of the periglacial zone with regard to latitude. The highest elevation periglacial soils occur in the permanent high-pressure areas (“horse latitudes” or “sub-tropical ridge”) at approximately 30° north or south latitude – an area that by coincidence contains the highest mountains on Earth: the Andes and Himalayan ranges (Fig 1).

Despite the apparent simplicity of Fig 1, plant-free ecosystems of high elevations are actually quite variable in terms of microclimate and therefore the reasons for the

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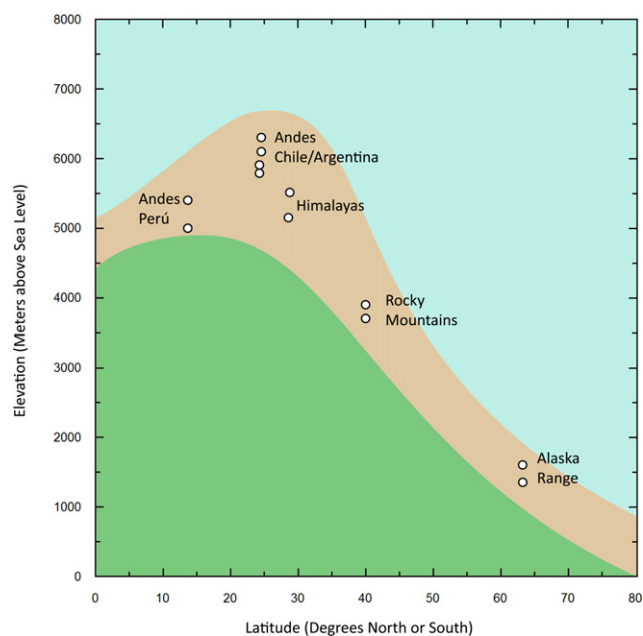


Fig 1 – Latitude (x-axis) and elevation (y-axis) of sites sampled for the present study. The approximate location of the periglacial zone (brown) is shown between the upper limit of continuous plant cover (green) and the snow line. Above the snow line there exists permanent ice cover (blue) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

absence of plants can be quite variable as well. There are three main reasons for the lack of plants: (1) snow pack duration is too long (usually >10 months of the year) to permit plants to complete their life cycles – these systems are called snow-bank soils in the present review; (2) exposed soils that are devoid of snow cover for much of the year and are too cold or dry for plant growth; and (3) early successional soils that have not yet been colonized by plants due to recent deglaciation. It is important to distinguish between these categories because, for example, the types of fungi that are adapted to extremely dry soils are likely to be very different from those found in snow-bank soils that are water saturated for varying periods of time during snow melt (Ley et al. 2004; Freeman et al. 2009). In the present paper we focus on categories 1 and 2 above; fungal communities of recently deglaciated soils are discussed elsewhere (Trappe 1988; Jumpponen 2003).

High elevation, barren soils are arguably the most extreme soil ecosystems on Earth. They share similar attributes (such as extreme temperatures, solar radiation and aridity) with the well-studied soils of the Dry Valleys of Antarctica and the Atacama Desert, but they have the added stressors of low atmospheric pressure and extreme daily temperature cycling across the freezing point. High elevation soils experience the oft-cited cliché of “summer every day and winter every night” (Hedberg & Hedberg 1979; Schmidt et al. 2009a; King et al. 2011). This is especially true of soils in category 2 described above. For example, during the summer at an elevation of 5 576 m on the slopes of the Socompa Volcano on the Chilean-Argentinian border, intense solar radiation during the day heats soils

to temperatures as high as 60 °C, whereas at night soil temperatures drop to below –10 °C (Schmidt, unpublished data). Thus, any organisms living in these soils must be adapted to survive these daily temperature extremes or else remain dormant until the rare occasions when enough snow cover accumulates to shield the soils from such fluctuations.

In the present review we will discuss fungal life in high elevation, barren ecosystems, focusing mostly on recent molecular-based ecological and phylogenetic surveys of sites in the Andes, Himalayas and Rocky Mountains. These sites were sampled during a recent global effort (2008–2009), sponsored by the National Geographic Society, to document microbial life in Earth’s high elevation ecosystems (Freeman et al. 2009; King et al. 2011; Schmidt et al. 2011).

The work reported here builds on recent molecular studies of high elevation barren soils of the Andes, Himalayas and Rocky Mountains (Freeman et al. 2009; King et al. 2010; Schmidt et al. 2011). The most surprising finding from those studies was that chytrids dominated 18S rDNA libraries (across multiple years and primer sets) from soils collected in the Annapurna region of Nepal and in the Colorado Rocky Mountains (Freeman et al. 2009). Chytrids are normally aquatic organisms or at least require an aquatic period to complete their life cycles. Based on year-round measurements of soil water and temperatures we now know that many alpine soils are in fact temporarily aquatic during the snow-melt period. At our chytrid-dominated sites in Colorado there is a 2.5-month period when the deep snow pack melts and the soil under the snow becomes saturated with water (Ley et al. 2004; Freeman et al. 2009). During this period soil temperatures are constant at around 0 °C and fungi dominate microbial activity (Ley & Schmidt 2002). In contrast, the same soils are quite dry during the summer and are subject to intense solar radiation and extreme temperature fluctuations (Ley et al. 2004; King et al. 2011). Therefore our working hypothesis is that chytrids are primarily active under the late-spring snow pack and during the period immediately following snow melt.

The other ecological mystery concerning chytrids in plant-free, high elevation soils is the question of how they obtain carbon and nutrients for growth. This mystery was partially solved for high elevation sites in Colorado when it was shown that large amounts of pine and spruce pollen is blown from lower elevations to these barren sites during late spring and early summer. In addition, gas flux, stable isotope and microscopic observations of high elevation sites in Colorado, Peru and Nepal revealed functioning algal and cyanobacterial communities (Freeman et al. 2009; Schmidt et al. 2009a, 2011) upon which many species of chytrids can feed. Thus, the surprising abundance and diversity of chytrids in high alpine soils can be explained by an abundance of fairly specialized food sources (pollen and algae) and the presence of snow-melt water for at least part of the year.

In the present study we expand on what we have already reported about barren high elevation systems, by broadening the scope of our analyses to include high elevation barren sites that are much drier than previously studied sites in Peru, Colorado and Nepal. These new sites are located at the edges of the Atacama Desert, where the largest volcanoes on Earth rise abruptly out of the desert. In 2009 we sampled barren soils at elevations ranging from 5 200 to 6 300 M above sea level on

the Socompa and Llullaillaco volcanoes that straddle the Chilean-Argentinian border. Our sampling sites were all at least 1 000 m in elevation above the upper limits of the sparse vegetation that occurs in the region (Richter & Schmidt 2002). Sampling of these extreme sites allowed us to determine if chytrids dominate all barren alpine soils or just those that receive high amounts of snowfall.

Materials and methods

Sampling sites and spatial sampling scheme are described in detail elsewhere (Costello *et al.* 2009; Freeman *et al.* 2009; King *et al.* 2010; Schmidt *et al.* 2011). Briefly, in each region we sampled soils (top 4 cm) at scales ranging from 1 m apart to over 40 km apart in a nested sampling scheme (King *et al.* 2008, 2010). In the Himalayas (Nepal) we sampled soils in the Annapurna Conservation Area in two high alpine valleys (Zun Tal, 28°43'19N, 83°54'52E, 5 200 m el., 14 spatially separated samples) and Thorong La (28°47'50N, 83°56'20E, 5 500 m el., six spatially separated samples) approximately 9 km apart during Oct. 2008 (King *et al.* 2011). The Thorong La sites ("Nepal high" in Table 1) are near, but higher than, the sites sampled by Petrovic *et al.* (2000) and the Zun Tal sites ("Nepal low" in Table 1) are a subset of the "low" sites described in Schmidt *et al.* (2011). In the Andes we sampled multiple sites in the Puca valley of Peru at elevations from 5 000 to 5 400 m above sea level (King *et al.* 2008; Schmidt *et al.* 2009a) and sites on Socompa (24°23'46S, 68°14'45W, el. 6 051 m, one site described in Costello *et al.* 2009) and Llullaillaco (24°43'15S, 68°32'09W, el. 6 741 m, six spatially separated samples) volcanoes on the border between Chile and Argentina. These large stratovolcanoes are approximately 47 km apart and were both sampled in Feb., 2009. Sampling sites in Colorado, USA are as described in detail in King *et al.* (2008, 2010) and Freeman *et al.* (2009).

Table 1 – Some environmental characteristics of the sampling sites. Data for Nepal, Socompa and Colorado are from King *et al.* (2011), Costello *et al.* (2009) and King *et al.* (2008, 2010), respectively. Values in parentheses are standard errors ($n = 3-18$). More environmental and microbiological data for the sites in Colorado and Nepal can be found in Schmidt *et al.* (2011) and Freeman *et al.* (2009)

Sites	Elevation m.a.s.l. ^a	% water	pH	% carbon
Nepal				
Low	5146	1.4 (0.2)	7.1 (0.04)	0.5 (0.01)
High	5509	1.7 (1.1)	7.2 (0.06)	1.4 (0.3)
Llullaillaco				
Low	6030	0.24 (0.1)	4.2 (0.03)	0.02 (0.006)
High	6330	0.25 (0.2)	4.6 (0.1)	0.01 (0.005)
Socompa	5235	<d.l. ^b	5.2	0.03
Colorado	3660–3800	7.4 (2.5)	4.2	1.9 (0.7)

a m.a.s.l. = meters above sea level.
b <d.l. = below detection limit.

From three spatially separated samples at each site (new sites for this study were Nepal low, Nepal high, Llullaillaco high and Llullaillaco low, Table 1) we extracted DNA and constructed clone libraries as described below. Data from previously constructed clone libraries from Colorado (six libraries) sites ranging in elevation from 3 600 to 3 800 m above sea level (Freeman *et al.* 2009) and Socompa (one library, Costello *et al.* 2009) were also used in the phylogenetic analyses described below. DNA extraction, 18S targeted PCR, cloning and Sanger sequencing techniques for samples from all sites were done as described in detail elsewhere (Schmidt *et al.* 2011). 18S primers, 4Fa-short (5'-ATCCGGTTGATCCTGC-3') and 1492R (5'-GGTTACCTTGTACGACTT-3'), were used because these have been shown in past studies to amplify sequences from all the major fungal groups and do not show PCR bias toward chytrids (Freeman *et al.* 2009). Tests of sequence similarity were performed using the parsimony insertion technique from ARB against the SILVA 104 reference database, and double-checked using BLAST searches of the broader NCBI nucleotide database (Ludwig *et al.* 2004). Final multiple sequence alignments were made using the fast aligner feature of ARB and adjusted by hand as necessary. Ambiguously aligned regions were excluded at this point using a filter that requires at least 40 % identity per residue. Mothur (version 1.19) was used to cluster sequences into 1 % OTUs based on the average neighbor algorithm (Schloss *et al.* 2009). MrBayes 3.1.2 was used to infer the phylogeny of each dataset using the GTR model for nucleic acid evolution with gamma-distributed rate variation across sites, plus a fraction of invariable sites (Huelsenbeck & Ronquist 2001). Generation number, burnin value and the heating parameter were adjusted to ensure the runs had converged onto the stationary distribution while maintaining optimal swapping rates. The phylogenetic structure of fungal communities across sites was compared using UniFrac, which quantifies shared evolutionary history (shared branch length) for each pair of environments (Lozupone & Knight 2005). For this analysis a separate inference of phylogeny for the full (unclustered) dataset was required. Due to the computational limitations of MrBayes for even this modest dataset, we implemented the neighbor joining algorithm and the maximum composite likelihood model from MEGA5 (Tamura *et al.* 2011).

Results and discussion

Fungal communities of all of our sites in the periglacial zone of the Andes, Rockies and Himalayas are shown in Figs 2–4. Compared with clone library determinations of fungal communities of vegetated arctic and alpine systems (Schadt *et al.* 2003; Wallenstein *et al.* 2007; Nemergut *et al.* 2008), these communities have extremely low diversity (except for chytrids) and are dominated by several distinct clades of fungi from across the fungal kingdom. The disparate nature of this limited diversity reflects the dynamic nature of these environments and provides a framework for understanding how different fungal lineages exploit the various niches, which open and close on a seasonal basis. In the following section, we discuss the major groups of fungi found so far in barren high elevation ecosystems and present preliminary hypotheses for their ecological roles in these systems.

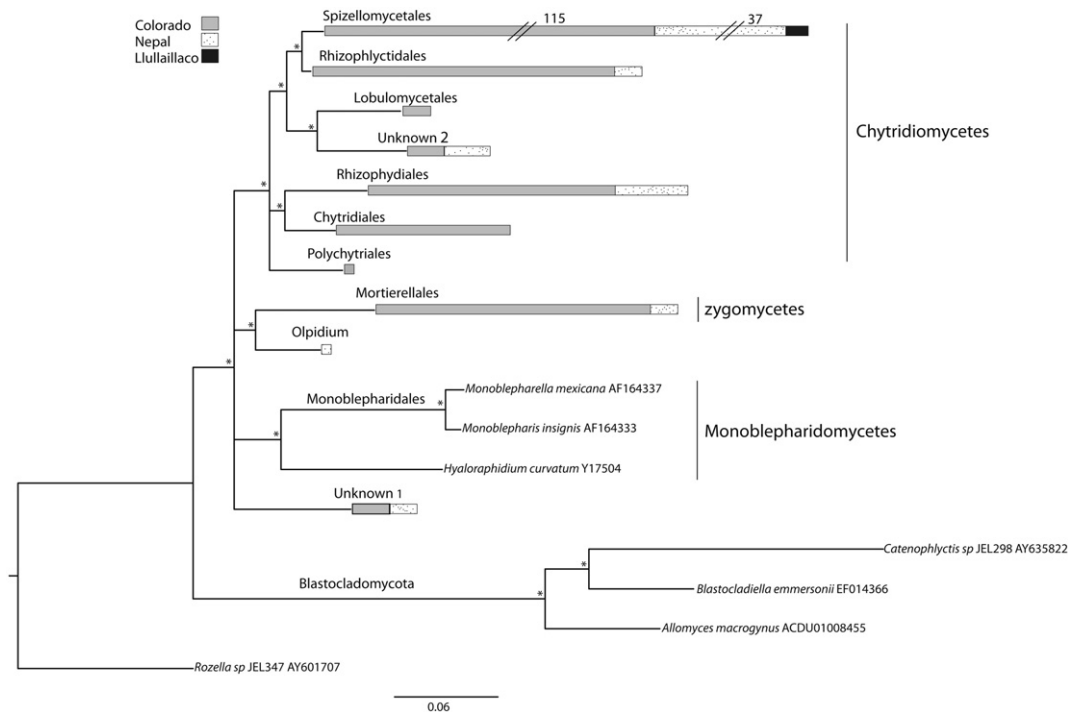


Fig 2 – Bayesian consensus tree showing the zoosporic fungi and zygomycetes of barren high elevation soils of Rocky Mountains (Colorado), Himalayas (Nepal) and the Dry Andes (Llullaillaco Volcano). The size of the rectangle is proportional to the abundance of sequences identified in each region (the smallest rectangle equals one sequence). The tree was rooted with *Rozella* sp. JEL347 (AY601707). Asterisks identify nodes with > 70 % posterior probabilities. The full tree on which this simplified tree is based, and a list of guide sequences used, is presented as **Supplementary Fig 1**.

Chytridomycota and Zygomycota

As discussed previously, chytrids dominated libraries of plant-free high elevation sites in Nepal and Colorado, making up 75 and 70 % of all fungal sequences, respectively (Freeman et al. 2009) and were easily isolated from high elevation periglacial soils of Peru, Bolivia and New Zealand (Freeman et al. 2009; Simmons et al. 2009). In contrast, no chytrids were detected in a preliminary sampling of Socompa Volcano (Costello et al. 2009) and chytrids made up less than 3 % of all fungal sequences from our extensive sampling effort on Llullaillaco Volcano. Fig 2 shows the phylogenetic relationships of the major groups of chytrids and related groups (e.g. zygomycetes) from clone libraries of our Nepal, Colorado and Llullaillaco sites. Seven distinct clades affiliated with the phylum Chytridiomycota were found in high elevation soils including the orders Spizellomycetales, Rhizophlyctidales, Lobulomycetales, Rhizophydiales, Chytridiales, Polychytriales and Olpidium. Multiple sequences fell into the order Mortierellales belonging to the zygosporic subphylum Mortierellomycotina (Hoffmann et al. 2011). The remaining sequences formed two distinct undescribed clades within the Chytridiomycota (Fig 2).

The clade with the most sequences (48 % of total library) corresponded to the order Spizellomycetales, with representatives being found in Nepal, Colorado and on Llullaillaco (Figs 2 and 3). In addition, all of the chytrid-related sequences from Llullaillaco Volcano formed a unique subclade within the Spizellomycetales, with an average of 10 % difference from any other known sequences (Fig 3). No other chytrids or zygomycetes

were detected on Llullaillaco or the nearby Socompa Volcano. It is likely that the Llullaillaco clade represents a yet undescribed, xerotolerant clade of chytrids. Previous research indicates that the spizellomycetes are found in harsh soil environments such as arid grasslands, sand dunes, glacial till and Arctic clay (Wakefield et al. 2009). The fact that spizellomycetes have the ability to decompose substrates such as pollen or can be parasitic may play a role in their geographical distribution. Lab experiments have shown that some species can tolerate temperatures up to 90 °C as well as desiccation (Gleason et al. 2004). Gleason et al. (2010a) showed that some spizellomycetes can grow at a pH as low as 2.9 while others can tolerate pH higher than 11 as well as surviving changes in osmotic potentials. Additionally, six Spizellomycetales isolates were able to survive temperatures of –15 °C for 7 d (Gleason et al. 2010b).

The second largest clade represented in our libraries was the order Rhizophydiales with distinct sub-clades within this group being found in both Colorado and Nepal. Some aquatic species of this order are parasites of algae, but soil isolates are usually saprotrophs on pollen, keratin or chitin (Letcher et al. 2006). The Rhizophlyctidales order was the third largest clade, being heavily represented in Colorado soils and rare in high elevation soils of Nepal. This order contains extremo-tolerant members that are resistant to extremes of temperature, pH and desiccation (Gleason et al. 2010a, b). The best-studied member of this order is *Rhizophlyctis rosea*, a known cellulose decomposer (Letcher et al. 2008).

The newly described order Lobulomycetales was represented only in the Colorado clone libraries, but the original

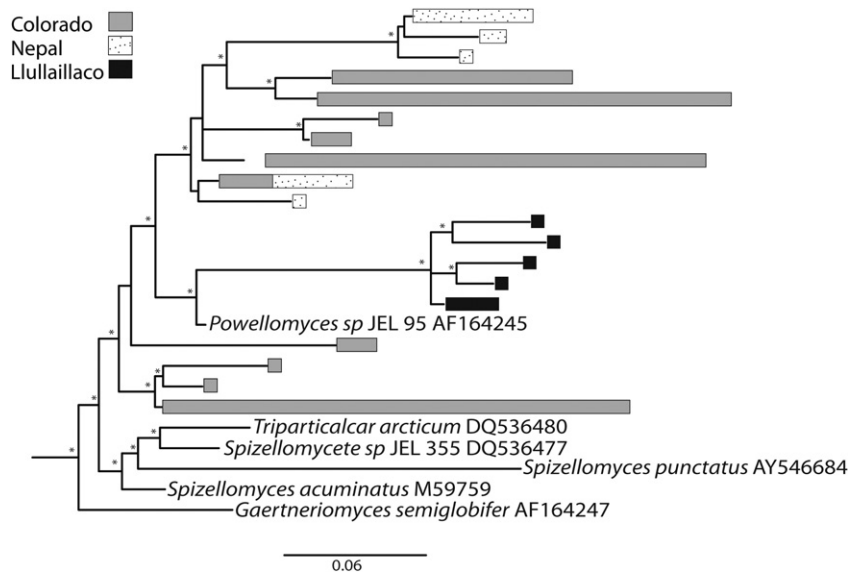


Fig 3 – Expanded tree of the Spizellomyces clade from Fig 2 showing geographic distribution of sub-clades from Llullaillaco, Colorado and Nepal. The lengths of the rectangles are proportional to the abundance of sequences within each OTU. Each OTU was formed at the 1 % level using the average neighbor algorithm. The smallest rectangle equals one sequence. Asterisks indicate node support of > 70 % posterior probability.

type species was isolated from recently uncovered subglacial sediments in the high Andes of Peru (Freeman et al. 2009; Simmons et al. 2009). The Peru type sequence (*Chytridiales* sp. AFO11) was also recently identified as the closest relative of several previously unclassified sequences collected at Mars Oasis in Antarctica (Bridge & Newsham 2009). Other species in this order are found in terrestrial, marine and acidic freshwater systems (Simmons et al. 2009).

Several large unidentified groups of putative chytrids were also identified in the present study (Fig 2). The first unknown clade shares a common ancestor with the order Monoblepharidales. Since the Monoblepharidales belong to a different class than the orders discussed above, this may suggest a new order of the class Monoblepharidomycetes. The second unknown clade shares a common ancestor with the Lobulomycetales. However, since little is known about the ecology of the Lobulomycetes, little can be inferred about the ecological role of these new fungi. Even though the Lobulomycetes were originally placed within the order Chytridiales and the genus *Chytrium* (Simmons et al. 2009), which was based on morphological features, there is no phylogenetic evidence that this unknown clade is closely related to the Chytridiales.

Finally, representatives of the zygomycete subphylum Mortierellomycotina (Hoffmann et al. 2011) were present in samples from both Colorado and Nepal. The ecological role of the Mortierellales in high elevation systems is not known, however some of their closest relatives are rapidly growing “snow moulds” of vegetated alpine and subalpine ecosystems (Schmidt et al. 2008, 2009b).

Ascomycota and Basidiomycota

Ascomycota and Basidiomycota were relatively rare in all samples from Colorado and Nepal (12 % and 14 %, respectively), but made up a majority of the clones in the

much drier soils on Llullaillaco and Socompa volcanoes (Fig 4). The major groups of basidiomycetes from these barren high elevation soils have phylogenetic affinities with fungal lineages that were first discovered in Antarctica (Vishniac 1985; Vishniac 2006a). Dominating both of our highest elevation sites were putatively novel relatives of the Filobasidiales, which includes the Antarctic endolith, *Cryptococcus friedmannii* (Takashima & Takashi 1999) also found in Icelandic soils (Vishniac 2006b; Fonseca et al. 2011; Kurtzman et al. 2011). The original *C. friedmannii* isolate is rare in Antarctica compared to other yeasts, but is the only documented endolith, and has a T_{max} of <25 °C in culture (Vishniac 1985). A recent large-scale ecological study of these yeasts revealed complex distribution patterns around the globe that were explained by a combination of both deterministic habitat-filtering, as well as stochastic dispersal factors (Vishniac 2006b). In particular, the *albidus* clade of the Filobasidiales was found to dominate arid desert and tundra sites, a capacity perhaps attributable to their conspicuous polysaccharide capsule. More focused work using other marker genes (e.g. the D1/D2 region; Peterson & Kurtzman 1991; Kurtzman et al. 2011) is needed to better define the relationship of our yeast clades to the *albidus* clade.

The other major group of Basidiomycota found at our sites (mostly Colorado) are members of the Microbotryomycetes (Fig 4) that includes *Leucosporidium antarcticum*, an ostensibly aquatic yeast, with a T_{max} of <19 °C in culture. However, other reports of its presence in northern European and Asian peat bogs and willow catkins suggest a broad capacity for both aquatic and transitional environments (Fell et al. 1969; Kockova-Kratochvilova et al. 1972; Summerbell 1983; Vishniac 2006a). Compared to other Antarctic psychrophilic and psychrotrophic yeasts, *L. antarcticum* produces the most trehalose in culture at 15 °C (~7 % w/w dry cells), and has a highly unique profile of intrinsic and inducible heat shock

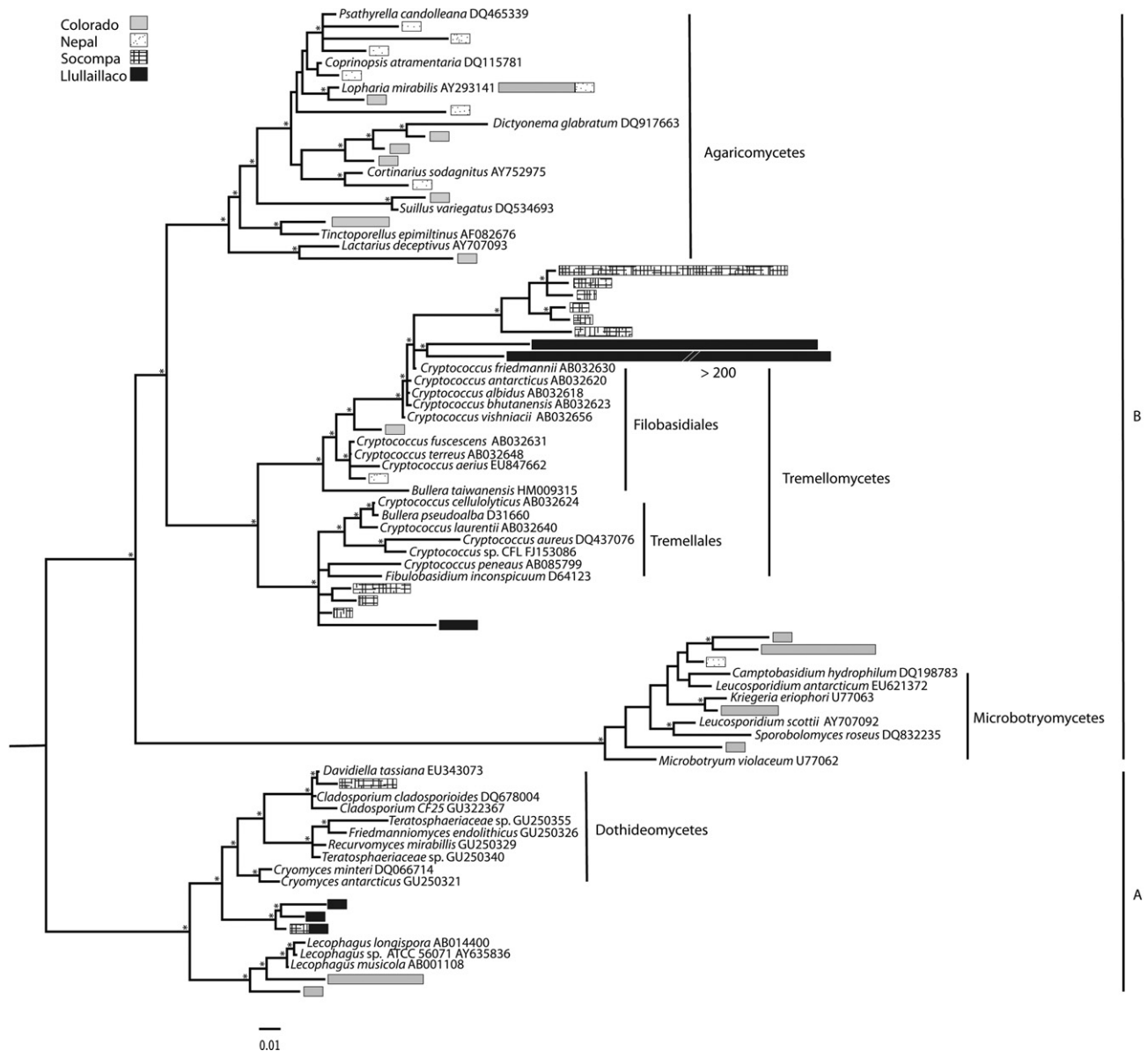


Fig 4 – Bayesian consensus tree of all periglacial Ascomycota (A) and Basidiomycota (B) and their closest cultured relatives. The length of the rectangles is relative to the abundance of sequences within each OTU, with the smallest rectangle equaling one sequence. Asterisks indicate node support of > 70 % posterior probability. Clade designations for guide sequences were assigned using systematics which is consistent for analyses using both 18S and the D1/D2 regions (Takashima & Takashi 1999; Schoch et al. 2006; Boekhout et al. 2011).

proteins (Deegenars & Watson 1998). The occurrence of the *L. antarcticum*-like fungi in our wettest sites, and the absence of common Antarctic yeasts *Candida*, *Dioszegia* and *Mrakia*, provide an intriguing starting point for understanding the physiological adaptations needed for high mountain soil life.

Although a few Agaricomycetes sequences were recovered from the Colorado Rocky Mountains and the Himalayas, most are typically associated with plant-dominated soils. Given the proximity of these two periglacial systems to forest ecosystems, we suggest windborne deposition of spores as the most plausible explanation for the presence of Agaricomycetes. One possible exception however, is the small cluster of

sequences around *Dictyonema glabratum* (Fig 4). This lichen has been documented growing in the high plains of the Andes up to 4 300 m.a.s.l. on exposed rock and soil surfaces, although it had not previously been documented in the periglacial zone (Larcher & Vareschi 1988).

Ascomycetes were relatively rare in our clone libraries, making up 0 %, 2 % and 3 % of clones in Nepal, Colorado and the dry Andes, respectively. All of the ascomycetes detected in the Colorado soils were related to the genus *Lecophagus*, which are known predators of rotifers and tardigrades. These fungi use adhesive pegs to immobilize rotifers and tardigrades, before penetrating the body cavity with digestive hyphae

(Tanabe *et al.* 1999; McInnes 2003). *Lecophagus* has previously been isolated from damp terrestrial environments, as well as from cold Antarctic soils and freshwater lake margins (McInnes 2003). The discovery of these fungi in high elevation soils corroborates the recent finding of an unexpectedly high diversity of bdelloid rotifers and tardigrades in the same Colorado soils studied here (Robeson *et al.* 2009, 2011).

In contrast to the relatively wet sites sampled in Colorado, all of the ascomycete sequences from the dry Socompa and Llullaillaco volcanoes were relatives of the Dothideomycetes, which includes xerotolerant species of the genus *Cladosporium* (Fig 4). These fungi are sometimes called the black rock fungi and have been found on or within barren rock faces. Many Dothideomycetes form deeply melanized meristematic colonies, and produce extracellular polysaccharides to ameliorate UV and osmotic stresses (Selbmann *et al.* 2005). Using their unique colony morphology that helps insulate them from unfavourable conditions by decreasing their surface to volume ratio, these fungi appear to have optimized a slow but persistent growth strategy for their extreme rocky habitats (Ruibal *et al.* 2009; Lynch & Schmidt, unpublished data).

Ecological breadth of high elevation fungi

Based on where we have detected each fungal clade and the relative dryness of each site (duration of snow cover), we can begin to form hypotheses concerning the ecological breadth of fungi discussed in this paper. Fig 5 shows our initial, qualitative estimations of where some of the clades discussed above are found with respect to the estimated duration of snow cover in high elevation ecosystems. We present Fig 5 not as an absolute statement about where individual species might be found, but as summary of the likely ecological range of broad taxonomic groups. The groups with the broadest ecological ranges are the Filobasidiales and the spizellomycetes, both of which have radiated widely in barren high elevation soils, as

shown in Figs 3 and 4. The Filobasidiales have been especially successful in the drier sites that we studied (Socompa and Llullaillaco) and in dry areas of Antarctica (Vishniac 2006a). The spizellomycetes have a similar broad range, but have seemingly been more successful in wetter high elevation systems; in the present study they were found at only one of six sites on Llullaillaco volcano and were not detected on Socompa, whereas different phylotypes of this clade were detected in all samples from our wetter sites (Colorado and Nepal). The broad ecological tolerances of the spizellomycetes are also supported by the work of Gleason *et al.* (2010a, b), who demonstrated a wide range of ecological tolerances of different spizellomycete species.

In contrast to the spizellomycetes and Filobasidiales, some groups of fungi studied herein showed very restricted ecological ranges. Perhaps the most extreme example is the clade affiliated with the Dothideomycetes (Figs 4 and 5). This group was found only in the soils of the Socompa and Llullaillaco volcanoes, although they have been found on high mountain rock surfaces elsewhere (Ruibal *et al.* 2009). *Cladosporium* spp. in this group are well known for their tolerance of desiccation and resistance to UV radiation (Selbmann *et al.* 2005) and may be one of the only fungal lineages in the present study to have evolved strategies to grow during periods of extreme drought. It is likely that almost all of the other fungal lineages discussed above are organisms that can survive as spores during periods of extreme drought, but still require at least short periods of higher water availability to complete their life cycles.

Community structure of high elevation ecosystems

Recent advances in phylogenetic analysis of microbial communities allow us to compare community structure from a statistical perspective (Martin 2002; Schadt *et al.* 2003).

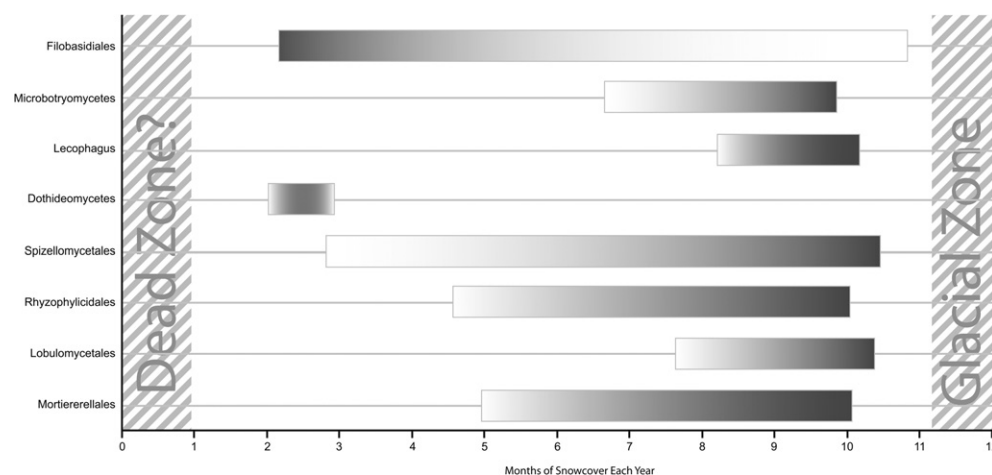


Fig 5 – Estimated ecological ranges for fungal groups with reference to duration of snow cover. Duration of snow cover was estimated for each group based on which sites they were found in (Figs 2–4) and field observations of the duration and depth of snow cover, as reported elsewhere (King *et al.* 2010). Duration of snow cover influences fungal growth both in terms of water availability and the insulating properties of snow (e.g. Schadt *et al.* 2003) which protects fungi from the extreme temperature fluctuations at each of the sites studied here.

We used the UniFrac approach, which tests whether there is less shared evolutionary history for each pair of environments than would be expected by chance (Lozupone & Knight 2005). These analyses revealed that all pair-wise comparisons for the chytrid and zygomycete communities (Fig 2) were highly significantly different (P values <0.0001). Fig 2 reveals this significance is driven by the profusion of zoosporic lineages that are endemic to each mountain range. Despite the paucity of biological knowledge regarding these basal fungi, our culture-independent efforts have resulted in a compelling case for either long-term geographic isolation and/or evolutionary specialization for local environmental conditions. Community analysis of the higher fungi (ascomycetes and basidiomycetes) also produced significant differences ($P < 0.05$) when comparing the dry Andes versus the Rockies, but the two dry Andean mountains, Llullaillaco and Socompa, were not significantly different from each other in community structure ($P = 0.53$) despite being 47 km apart. Unfortunately comparisons with Himalayan soils were not possible due to limited sample sizes for the higher fungi. Overall, the dominance of novel endemic basal fungal lineages in wetter high mountain soil systems suggests that improving our understanding of these basal fungi should yield important ecological insights, as well as strengthen our understanding of the forces that have shaped fungal evolution.

Conclusion and future directions

The major new conclusion of the current study is that chytrids do not dominate all plant-free high alpine soils. Our earlier work showed that chytrids dominated clone libraries of high elevation soils of Nepal, Colorado and culturing efforts in Peru (Freeman et al. 2009), all of which are in arid regions yet receive much more snowfall than the extreme dry soils of Socompa and Llullaillaco volcanoes. These high volcanoes are in one of the driest areas on Earth, allowing us to examine extremely dry soils at the highest elevations yet studied in a systematic way. These soils harbored some of the simplest fungal communities yet described (only three fungal lineages, compared to at least 15 major lineages in the Dry Valleys of Antarctica; Fell et al. 2006; Schmidt et al. 2011). Future ecophysiological and genomic studies of the fungi in these soils should yield new insights into fungal adaptations to stressors such as diurnal temperature fluctuations across the freezing point and low atmospheric pressures.

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Supplementary material

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.funeco.2011.10.005.

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