Phylogeny and biogeography of an uncultured clade of snow chytrids

C. S. Naff,[†] J. L. Darcy and S. K. Schmidt*[†]
*Department of Ecology and Evolutionary Biology,
University of Colorado, N122 Ramaley Hall, Campus
Box 334, Boulder, CO 80309-0334, USA.*

Summary

**Numerous studies have shown that snow can contain
a diverse array of algae known as 'snow algae'. Some
reports also indicate that parasites of algae (e.g.
chytrids) are also found in snow, but efforts to phy-
logenetically identify 'snow chytrids' have not been
successful. We used culture-independent molecular
approaches to phylogenetically identify chytrids that
are common in long-lived snowpacks of Colorado and
Europe. The most remarkable finding of the present
study was the discovery of a new clade of chytrids
that has representatives in snowpacks of Colorado
and Switzerland and cold sites in Nepal and France,
but no representatives from warmer ecosystems. This
new clade ('Snow Clade 1' or SC1) is as deeply diver-
gent as its sister clade, the Lobulomycetales,
and phylotypes of SC1 show significant (*P* < 0.003)
genetic-isolation by geographic distance patterns,
perhaps indicating a long evolutionary history in the
cryosphere. In addition to SC1, other snow chytrids
were phylogenetically shown to be in the order *Rhizo-
phydiales*, a group with known algal parasites and
saprotrophs. We suggest that these newly discovered
snow chytrids are important components of snow
ecosystems where they contribute to snow food-web
dynamics and the release of nutrients due to their
parasitic and saprotrophic activities.**

Introduction

Approximately 17% of the world's population relies on
snow melt as a source of freshwater for both agriculture
and human consumption (Barnett *et al.*, 2005; Bales
et al., 2006). Atmospheric deposition of nutrients can
alter biogeochemical processes of high-elevation water
systems (Ballantyne *et al.*, 2010), affect water quality from
snowmelt (Brooks and Williams, 1999; Mladenov *et al.*,
2011) and could potentially increase the activity of micro-
organisms within the snowpack. Therefore, identifying the
key members of snow microbial communities of high-
elevation ecosystems is needed in order to understand
nutrient release within the snowpack and its influence
on downstream water quality (Mladenov *et al.*, 2012).
However, studies of the identities and dynamics of non-
algal microbial communities in snow are in their infancy,
especially with regard to their influence on nutrient turno-
ver and food-web dynamics within the snow.

Most previous work on snow microbiology has focused
on bacteria (Segawa *et al.*, 2005; Liu *et al.*, 2009) and
snow algae that are common inhabitants of late melting
snowpacks worldwide (Hoham and Duval, 2001; Remias
et al., 2010); but little attention has been paid to the other
eukaryotic organisms that inhabit snow (Hoham and
Duval, 2001). There are several older reports of the pres-
ence of zoosporic fungi (chytrids) in high-elevation snow-
packs of the Colorado Front Range (Stein and Amundsen,
1967) and Europe (Kol, 1968). Some chytrids are known
parasites of algae and have been shown to affect rates of
turnover and alter food-web dynamics in aquatic ecosys-
tems (Canter and Lund, 1948; Ibelings *et al.*, 2004;
Kagami *et al.*, 2007; Grami *et al.*, 2011; Sime-Ngando
et al., 2011). Therefore, the activity of chytrids in high-
elevation snowpacks could lead to increased nutrient
release from snow, but to date their phylogenetic identity
has not been determined. Therefore, the present study
was undertaken to determine if 'snow chytrids' could
be identified using molecular, culture-independent
approaches. Such methods have been used successfully
to discover major, previously unidentified fungal lineages
in the cryosphere (Schadt *et al.*, 2003; Porter *et al.*, 2008);
groups that were only much later obtained in pure culture
(Rosling *et al.*, 2011). The present study is a first step
in efforts to characterize the diversity and function of
chytrids in high-elevation snowpacks.

Microbial diversity within the snow may also be contrib-
uting to the unexpectedly high diversity of chytrids and
other microbes in alpine soils. Recent work in the Colo-
rado Rockies has shown that soils that lie beneath per-
sistent snow beds ('snow bed' soils) in high-elevation

catchments contain very diverse microbial communities that are capable of rapid cycling of carbon and nitrogen (Ley *et al.*, 2004; Freeman *et al.*, 2009a; King *et al.*, 2010). These snow bed soils are covered with snow for up to 10 months of the year, and are dominated by many groups of previously undescribed chytrids (Freeman *et al.*, 2009b). It is possible that some of the chytrids found in high-elevation soils are actually snow chytrids that only spend part of their life cycle in the soil. These putative snow chytrids may swim up into the snowpack to feed on snow algae (and/or aeolian deposited pollen) and then settle back into the soil during the final stages of snow melt. However, given that no snow chytrids have ever been cultured, almost nothing is known about the their phylogeny or function.

The present study was part of a larger project to describe the diversity and function of the microbial community of high-elevation snowpacks of the Front Range of Colorado and to compare them with snowpack organisms worldwide. Our results revealed that chytrids are among the dominant eukaryotic organisms inhabiting some high-elevation snowpacks and that they constitute several new clades that are currently uncultured and likely restricted to snow and snow-covered environments worldwide.

Results

Long-read sequences (~ 1750 bp) that could be unambiguously identified as chytrids were found on most dates that snow samples were taken in 2011 and 2012 (Table 1). On some dates chytrids dominated the clone libraries. For example, on 15 August 2011, chytrids constituted 75% of surface (0–2 cm) snow sequences and 27% of sequences from deeper depths (Table 1).

Phylogenetic analyses demonstrated that most of the chytrids within high-elevation snowpacks are unrelated to known or cultured chytrids (Figs 1 and 3) and in several cases form large clades that have not been previously recognized. The largest of these groups forms a deeply divergent clade sharing a common ancestor with the order Lobulomycetales (Fig. 1). A phylogenetic comparison with all of the closest relatives (from the SILVA 108 SSU database and GenBank) of these snow sequences revealed that this newly discovered clade contains only sequences from cold environments across the Earth. That is, the only other sequences that fell into this clade were from high-elevation soils in Nepal and Colorado (Freeman *et al.*, 2009b), a mountain lake in France (Lefèvre *et al.*, 2008) and high-elevation snow samples from Switzerland (M. Yuhana, unpublished, GenBank numbers AJ867629 and AJ867630), in addition to snow and ice samples from the present study (Fig. 1). Given the apparent global distribution of this clade in high-elevation ecosystems (Fig. 1) we conducted a phylo-geographic analysis of their distribution using previously described methods (Darcy *et al.*, 2011; Schmidt *et al.*, 2011). These analyses reveal a clear pattern of genetic isolation by geographic distance (Fig. 2, Mantel Test $P < 0.003$).

The other snow chytrids from the present study fell into the order *Rhizophydiales* (Fig. 3). Unlike 'Snow Clade 1' (SC1) described above, these chytrids were more closely related to known chytrids but still formed several new sub-clades within the *Rhizophydiales*, including 'Snow Clade 2' (Fig. 3).

In order to quantify possible chytrid food sources in the snowpack, both algae and pollen were counted in some of the same samples in which snow chytrids were discovered. These counts revealed very high densities of both snow algae and pollen in the snow (Fig. 4) with significantly higher concentrations of algae and pollen in the top 2 cm of the snowpack ($P < 0.05$).

Discussion

Numerous studies have reported chytrids from cold ecosystems such as high-arctic tundra soils (Booth and

Table 1. Sampling dates, site descriptions and percent of chytrid sequences in eukaryotic clone libraries of the new sequences used in the present study.

| Date	Location	Elevation (m)	Depth (cm)	% Chytrids	Coordinates
26 July 2011	Treeline snow	3384	0–2	25	40°02′56.64″N −105°34′53.30″W
15 Aug. 2011	Talus snow	3742	0–2	75	40°03′27.25″N −105°37′16.76″W
	Talus snow	3742	4–11	26	
12 July 2012	Glacier snow/ice	3792	0–2	30	40°02′59.61″N −105°38′27.91″W
	Glacier snow/ice	3795	0–2	13	
23 July 2012	Glacier I snow/ice	3795	0–2	13	40°02′59.61″N −105°38′27.91″W
	Glacier snow/ice	3795	0–2	33	
22 Oct. 2009	Nepal Snowbed soil	5516	0–4	80	28°38′46.86″N 83°56′′24.10″E
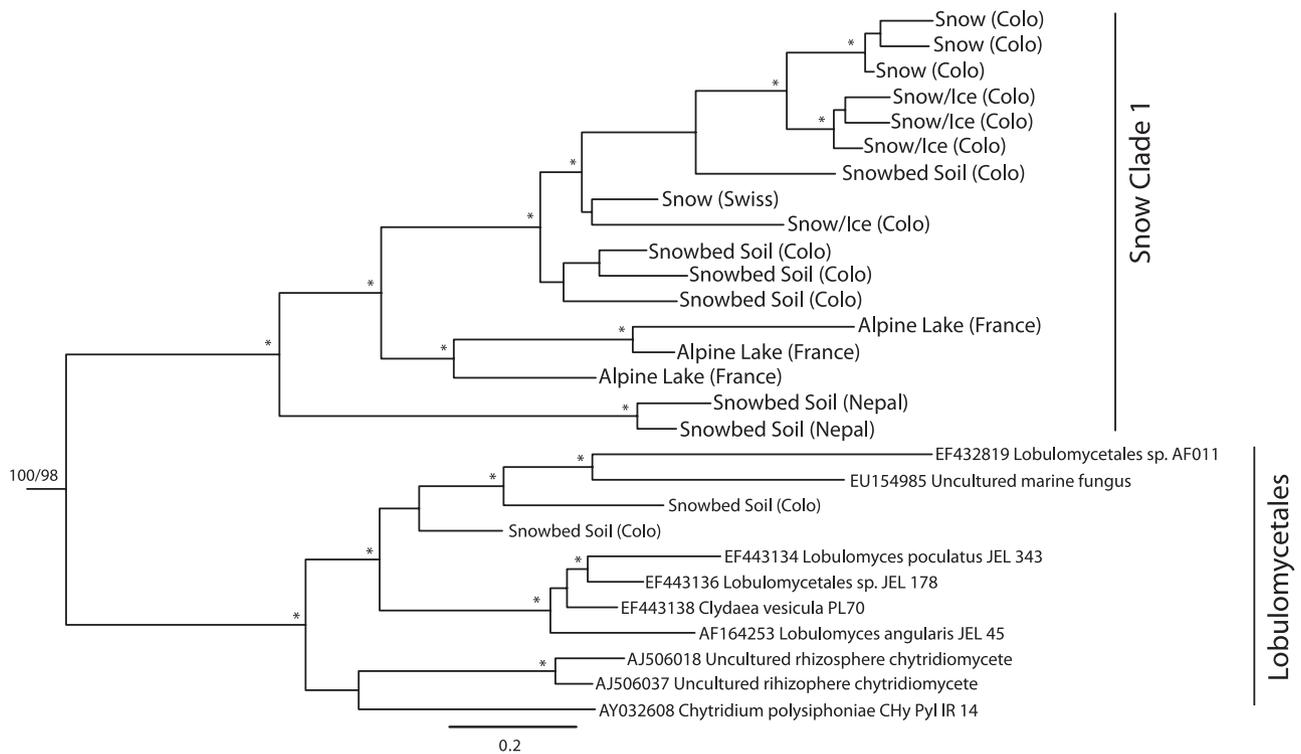
Fig. 1. Phylogenetic tree showing the relationship between 'Snow Clade 1' and the chytrid order Lobulomycetales. The shared node between the two clades has 100% bootstrap support (RaxML, 1000 bootstraps) and posterior probability support of 98% (Mr Bayes, 5 million generations, INVGAMMA model). Asterisks denote nodes with a minimum of 84% bootstrap support and 97% posterior probabilities. 'Snow (Colo)' sequences are from the talus sites studied by Ley and colleagues (2004); 'Snow/Ice (Colo)' are from the Arikaree Glacier site (King *et al.*, 2010); 'Snow (Swiss)' sequences are from high-elevation snow samples from Switzerland (M. Yuhana, unpublished); 'Alpine Lake (France)' are from Lake Pavin in France (Lefèvre *et al.*, 2008); 'Snowbed Soil (Nepal)' sequences are from high-elevation soil samples from the Himalayas (Schmidt *et al.*, 2011; 2012). A more detailed tree (including all GenBank accession numbers) for all of the clades discussed in this paper is available as Fig. S1.

Barrett, 1976; Barr, 1980) and high-mountain lakes (Koob, 1966). More recent studies of extreme plant-free soils of Antarctica, Colorado and Nepal revealed that chytrids dominate fungal communities in wetter soils and soils under persistent snowpacks ('snow bed soils') (Bridge and Newsham, 2009; Freeman *et al.*, 2009b; Schmidt *et al.*, 2012). These surprising observations led us to hypothesize that some chytrids from polar and alpine regions may spend at least part of their life cycle in the snow. This hypothesis is supported by microscopic observations of chytrid zoospores and sporangia associated with snow algae communities (Stein and Amundsen, 1967; Kol, 1968; C.S. Naff, unpubl. data, 2011) and the phylogenetic results of the present study. We found several unique clades of chytrids (Figs 1 and 3) that were detected not only in surface layers of the snow (0–2 cm deep) but also deeper layers (4–11 cm) in the same samples that contained high populations of snow algae and an abundance of pollen (Fig. 4). Sequences falling into 'Snow Clade 1' (SC1) were also found in a culture-independent study of high-elevation snow samples from

Switzerland (M. Yuhana, unpubl. data, Fig. 1), further supporting the hypothesis that there exists a unique clade of snow chytrids.

SC1 also encompasses some chytrid sequences that are not exclusively found in snow (Fig. 1) but could have originated from snow. These non-snow sequences were obtained from culture-independent studies of soils that are covered by deep snowpacks for most of the year ('snow bed soils'), and a mountain lake in France that is fed by snow melt (Fig. 1). In addition, the soil sequences encompassed by this clade are from soils (Freeman *et al.*, 2009b) under the Colorado snow banks studied in the present study or from the highest seasonally snow-covered soils (> 5500 m above sea level) studied by Schmidt and colleagues (2011) in Nepal. These high-elevation soil chytrids were previously referred to as the 'Unknown 2' clade by Schmidt and colleagues (2012), and based on their proximity to deep snow banks they could easily be snow chytrids. Likewise, the only other sequences from the SILVA 108 SSU database or GenBank that fall into SC1 (Fig. 1) correspond exactly to
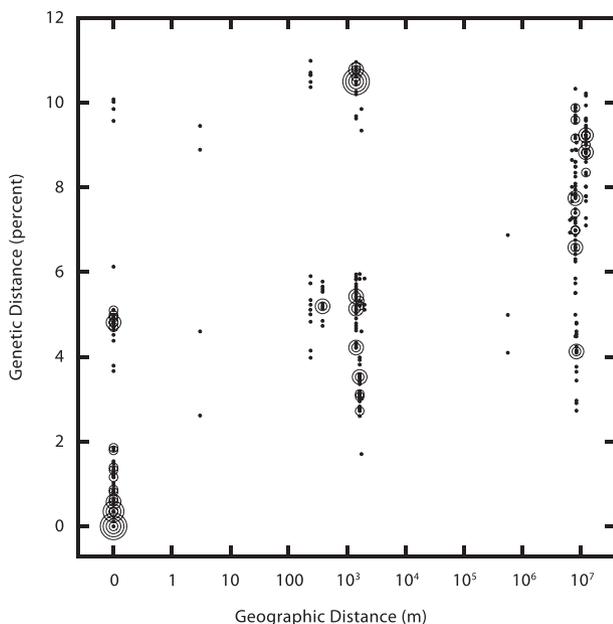
Fig. 2. Isolation by distance plot of genetic difference between phylotypes (y axis) as a function of the geographic distance between sites (x axis). A total of 72 pair-wise points were used to make the figure. Concentric circles are indicative of the number of overlapping pair-wise comparisons, with bin sizes of 1, 2, 3–4, 5, and > 5 for the smallest to largest circles. This pattern of isolation by distance was statistically significant (Mantel test, $P = 0.003$), indicating that members of SC1 are genetically different at each site sampled globally. It is not yet known if this pattern is caused by dispersal limitation or environmental variables.

'Novel Clade 1' from culture-independent studies of a snow-fed mountain lake (Lefèvre *et al.*, 2008; Jobard *et al.*, 2012). Thus all of the sequences in Fig. 1 could have originated in snow, but more work must be done to verify this hypothesis. A phylotype (MPE2-22) whose closest relative is a member of SC1 (GQ995409) was also recently sequenced from a 'moss pillar' in an Antarctic lake (Nakai *et al.*, 2012).

Regardless of the actual ecological niches (snow/ice vs near-snow environments) of the members of SC1, it is still quite remarkable that all of the currently known sequences in this clade are from cold environments. This observation demonstrates that we are only just beginning to scratch the surface of the hidden biodiversity of the terrestrial cryosphere (Costello and Schmidt, 2006; Fell *et al.*, 2006; Dennis *et al.*, 2012; Schmidt *et al.*, 2009; Jumpponen *et al.*, 2012; Lynch *et al.*, 2012). In addition, the presence of this new clade only in cold environments should inform future attempts (cf. Carreiro and Koske, 1992) to isolate chytrids from this group, but to date no organisms from this group have been isolated in pure culture.

All of the other snow chytrids found in the present study fell into the chytrid order *Rhizophydiales*. Phylotypes in the *Rhizophydiales* were found at the highest elevation Snow/Ice site in Colorado (Arikaree Glacier), the lower elevation snow sites at treeline, and in soils from beneath talus snow beds (Fig. 3). Some of these novel phylotypes were previously detected in high-elevation talus soils and were called unknown clades C15 through C19 by Freeman and colleagues (2009b). Phylogenetic relatives of clades C15 and C19 have recently been isolated from high-elevation (> 5000 m) sites in the Andes (Freeman *et al.*, 2009b), but there still are several clades in this group with no cultured relatives (e.g. 'Snow Clade 2', Fig. 3). In addition, the *Rhizophydiales* constituted the second most abundant group of fungi (after the *Spizello-mycetales*) from high-elevation (> 5500 m) soils in the Himalayas (Freeman *et al.*, 2009b; Schmidt *et al.*, 2011), but no work has been done to determine if these Himalayan fungi originated in the snow. Indeed, very little is known about the ecology of the *Rhizophydiales* (Gleason *et al.*, 2010), but some members of this group from warmer areas can utilize pollen (*Rhizophydium sphaerotheca*) and algae (*Rhizophydium fragilaria*) for growth (Powell, 1993; Letcher *et al.*, 2008; Letcher and Powell, 2012) and therefore we are attempting to isolate these unknown clades of *Rhizophydiales* from snow samples by using pollen and snow algae as bait.

Our working hypothesis is that snow chytrids have a complex life cycle that includes an active phase when they feed on abundant algae and/or pollen in the snow-pack and a quiescent phase where they lie dormant in the soil awaiting sufficient snow accumulation to become active. This hypothesis is consistent with the fact that phylotypes of some of these chytrids are detected in both snow and soil samples from high elevations and with the fact that the wettest time of year in the high alpine of many mountain ranges is during spring snow melt (Ley *et al.*, 2004; Schmidt *et al.*, 2011). It is also a possibility that these chytrids are not even native to high-elevation sites but rather are being dispersed over long distances (through the atmosphere) to polar and alpine environments as has been shown recently for snow-dwelling bacteria in the genus *Polaromonas* (Darcy *et al.*, 2011). *Polaromonas* spp. are ubiquitous in high-elevation snow and ice but show remarkably little genetic diversity even when comparing very geographically distant sites. To determine if this signal of global dispersal is also evident in our genetic data for snow chytrids, we carried out isolation by distance analyses (Darcy *et al.*, 2011; Schmidt *et al.*, 2011). In contrast to results for globally dispersed *Polaromonas* spp., SC1 shows very strong genetic divergence across all geographic distances (Fig. 2). A similar pattern of extreme genetic isolation has recently been demonstrated for other polar and alpine micro-eukaryotes such as rotifers (Robeson *et al.*, 2011) and algae (De Wever *et al.*, 2009; Schmidt *et al.*, 2011).
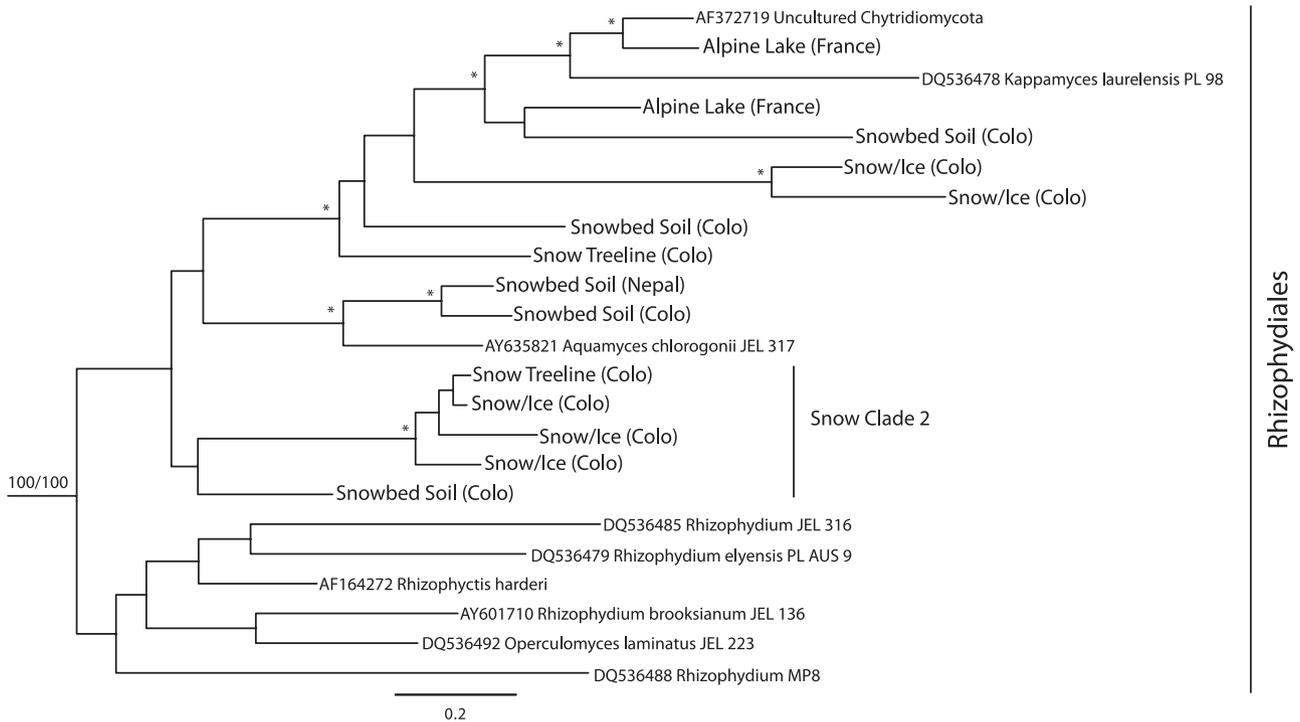
Fig. 3. Phylogenetic tree showing the relationship of snow chytrid phylotypes (in the *Rhizophydiales*) to their closest relatives from GenBank. Asterisks denote nodes with a minimum of 75% bootstrap support and 65% posterior probabilities. 'Snow Treeline (Colo)' sequences are from snow at the forest tundra ecotone as described by Brooks and colleagues (1996); 'Snow/Ice (Colo)' are from the Arikaree Glacier site (King *et al.*, 2010); 'Alpine Lake (France)' are from Lake Pavin in France (Lefèvre *et al.*, 2008); 'Snowbed Soil (Nepal)' sequences are from high-elevation soil samples from the Himalayas (Schmidt *et al.*, 2011; 2012).

We propose two hypotheses that may explain the dispersal pattern of the snow chytrids; First, snow chytrids may be readily dispersed and their survival depends on the ability to adapt to different environmental pressures. Under this hypothesis, the spatial separation of clades within the Snow Chytrids is not due to dispersal limitation, but instead subclades are adapted to specific environmental conditions. Factors such as food sources (type and abundance), microclimate and snowpack depth and duration may be sufficiently different between these sites to permit or bar the presence of specific members of SC1. Further investigation of the microbial communities present at the various sites mentioned in this paper is needed in order to elucidate the variables and to determine if they may be limiting factors for colonization. An alternative hypothesis is that Snow Chytrids are structured by dispersal limitation suggesting that they have been evolving in isolation in each environment. It is possible that members of SC1 are psychrophilic, and are geographically restricted to cold 'islands' in the cryosphere. Dispersal between islands would be difficult for such organisms, since passing through more temperate regions may prove fatal for propagules. Because members of SC1 have yet
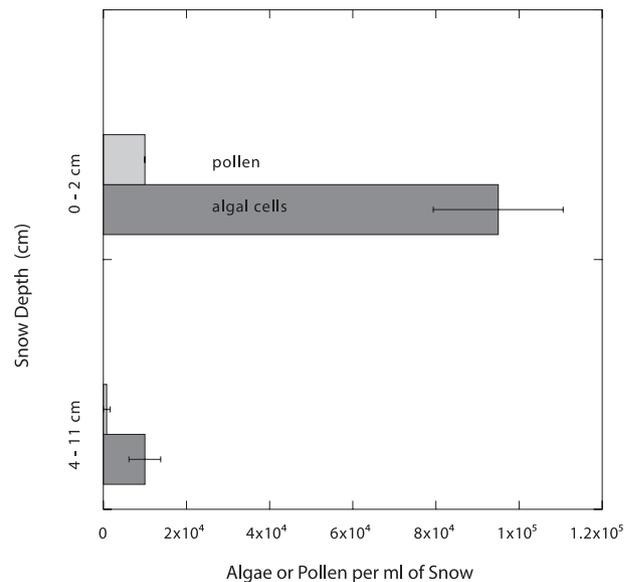
Fig. 4. The concentration of snow algae and pollen in surface (0–2 cm) and below surface (4–11 cm) snow samples collected on 15 August 2011. There were significantly higher concentrations of algae and pollen within the top 2 cm of snow compared with the concentrations found between 4 and 11 cm ($P < 0.05$).

to be isolated in culture, it remains to be seen whether they employ the same quiescence strategies as some other chytrids (Gleason *et al.*, 2004; 2010). Dormancy mechanisms are strong enablers of dispersal capacity (Lennon and Jones, 2011), and the ability to form a persistent state may be expected of organisms living in environments with high exposure to ultraviolet radiation, such as snowfields. Common algae living in the same snowfields (e.g. *Chlamydomonas nivalis*) contain photoprotective pigments (astaxanthin) and encyst to form persistent states (Hoham and Duval, 2001). Since members of the SC1 have been found in the same locations, it is likely that they too have the capability to encyst or otherwise form hardy states.

Finally, it must be noted that all of the clades containing putative snow chytrids show deep phylogenetic divergence, rivaling that of order-level groups such as the Lobulomycetales (Simmons *et al.*, 2009). This divergence is readily apparent in Figs 1–3 despite the fact that no snow chytrids are presently in culture. Culture-independent studies are valid for demonstrating deep-levels of phylogenetic structure and in fact have led to important discoveries in fungal phylogenetics (e.g. Porter *et al.*, 2008; Nilsson *et al.*, 2011). The deep level of divergence observed for snow chytrids likely indicates a long history of evolution in the cryosphere, perhaps even during periods of extreme greenhouse events, when chytrids and other micro-eukaryotes may have persisted in high-mountain and high latitude refugia (Stoeck *et al.*, 2007; De Wever *et al.*, 2009). However, much more data are needed from more snow-covered environments to further address questions about the evolution and biogeography of SC1 and other novel groups discovered in the present study.

Conclusions

Overall our results indicate that there are several deeply divergent, novel clades of chytrids present in late-season snowpacks of the Colorado Front Range. From an ecological perspective, these findings are not surprising because it has been known for a long time that microbial life can thrive in any environment where free water is even minimally available. More work is needed to understand the ecological interactions within the snowpack, but just the presence of chytrids in the snow indicates that turnover of algal and other substrates (e.g. pollen) is likely taking place within the snowpack – resulting in the release of nutrients.

From an evolutionary perspective, our results indicate that novel phylotypes of chytrids exist at each of the geographically isolated sites studied. Whether this is a result of genetic drift, selective pressures or a combination of both, remains to be further elucidated. At present it

appears that snow chytrids dispersed globally, probably during a colder epoch, and are now continuing to evolve in isolated pockets of the cryosphere. It remains to be seen if these denizens of the cold will survive future increases in global temperatures.

Experimental procedures

Sample collection

Snow samples were collected at sites on Niwot Ridge and in the Green Lakes Valley of the Front Range of Colorado where intensive biogeochemical and microbiological work has been done in the past (Ley *et al.*, 2004; Freeman *et al.*, 2009a; b; King *et al.*, 2010; Mladenov *et al.*, 2012). Most of the present study was done in the vicinity of the talus sites described by Ley and colleagues (2004; 40°03′ 34″N; 105°37′ 0″ W; 3739 m) and the high-elevation sites on and next to Arikaree Glacier (King *et al.*, 2010; 40°02′ 57″N; 105°38′ 25″ W; 3810 m). These sites are located above the tundra zone (West *et al.*, 1999) and are mostly devoid of vegetation. One sample (26 July 2011) was taken below the tundra near the tree-line site described by Brooks and colleagues (1996). All samples were collected from red and green snow patches during the summer of 2011 and 2012 (on 26 July, 4 and 15 August, and 19 September 2011; and 12 and 23 July 2012). Samples were taken earlier in 2012 than in 2011 because of the unusually early melt out of the snowpack in 2012. Snow samples were taken aseptically from the top 2 cm of snowpack and from 4 to 11 cm below the surface of the snowpack. Snow samples were collected in 50 ml sterile Falcon tubes (BD Biosciences, Bedford, MA, USA), placed on ice in a cooler and returned to the lab where they were frozen at −70° until utilized.

DNA extraction, PCR amplification and 18S gene clone library construction

A MO BIO Power Water kit with filters (MO BIO Laboratories, Carlsbad, CA, USA) was used to extract DNA from the snow following the manufacturer's protocol. PCR was carried out using the Amplitaq Gold 360 Master Mix (Applied Biosystems, Carlsbad, CA, USA) and the 18S primer set 4Fa-short (5′-ATCCGGTTGATCCTGC-3′) and 1492R (5′-GGTTAC CTTGTTACGACTT-3′) to amplify small subunit ribosomal DNA. These primers were used by Freeman and colleagues (2009b) who showed that they were not biased towards chytrids compared with other common primers for eukaryotes. Thermal cycling reactions were run for 34 cycles with an annealing temperature of 49°C. DNA amplification was confirmed using gel electrophoresis. Isolated bands of DNA were excised and purified using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA) and were then ligated into pCR®4-TOPO vectors (TOPO TA Cloning kit, Invitrogen, Carlsbad, CA, USA) and transformed into TOP10 competent *Escherichia coli* cells following the manufactures protocol. Successfully transformed cells were grown on Luria Broth agar (MO BIO) with 50 μg ml⁻¹ of ampicillin, and were inoculated into 96-well plates containing 1.5 ml of Luria Broth (MO BIO). They were incubated at 37°C while shaken at

200 r.p.m. for 16 h and then centrifuged. The pellets were sent to Functional Biosciences (Madison, WI, USA) where the plasmids were bidirectionally sequenced using the primer sets T7 and M13R.

Phylogenetic analyses

The software program Sequencher 4.6 (Gene Codes, Ann Arbor, MI, USA) was used to trim vector from bidirectional sequences and assemble them into contiguous reads. BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to identify the sequences, which were then aligned using SINA (http://www.arb-silva.de/aligner/), and imported into ARB (v. 9.6) via a parsimony insertion. Additional related sequences (from both cultured and uncultured chytrids) used in the phylogenetic analysis were selected from the ARB database (v 9.6) and GenBank. The sequence dataset was hand-curated, and a lane mask function was used to filter out alignment columns with less than 40% shared identity. The program Mothur (Schloss *et al.*, 2009) was used to cluster phylotypes sharing 99% sequence identity into operational taxonomic units (OTUs) using the average neighbour algorithm. Representative sequences of approximately 1750 bp were picked from each OTU for use in phylogenetic analyses. First, a rooted tree was created using the maximum likelihood program RaxML (Stamatakis, 2006) with the inverse gamma distribution (INVGAMMA) model, with 1000 bootstraps to provide node support values. Then, confidence levels for each node, in the form of posterior probabilities, were determined using the Baysian inference of the maximum likelihood method. MrBayes (v. 3.1) was run with the following parameters: INVGAMMA model, burn-in of 1250, temperature setting of 0.08, and run to convergence with five million generations (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003).

To test whether the novel clade of chytrids shows significant biogeographic structure, we constructed genetic isolation by geographic distance matrices as previously described (Darcy *et al.*, 2011; Schmidt *et al.*, 2011). Briefly, a matrix of geographic distances between sample locations was computed in R (R Development Core Team, 2012) using the Fields package (Furrer *et al.*, 2012; http://CRAN.R-project.org/package=fields) and a genetic distance matrix for all sequences was created using Mothur (Schloss *et al.*, 2009). To test for a correlation between these matrices, a Mantel test was performed in R using 1000 randomized permutations.

Algal and pollen counts

To quantify possible chytrid food sources in the snow samples, pollen and algae were counted using phase contrast and fluorescent (for chlorophyll autofluorescence) microscopy (400× magnification) and a hemocytometer with a volume of 0.1 μl (Bright Line Counting Chamber, Hausser Scientific, Horsham, PA, USA). Counts were done on surface samples (top 2 cm) and below surface samples (4–11 cm) from the 15 August time point. Statistical analyses comparing surface and subsurface pollen and algal counts were done in R (R Development Core Team, 2012) using a *t*-Test.

Nucleotide sequence accession numbers

The SSU rRNA sequences generated in this study were submitted to the GenBank database under Accession Numbers KC561936–KC561975.

References

Bales, R.C., Molotch, N.P., Painter, T.H., Dettinger, M.D., Rice, R., and Dozier, J. (2006) Mountain hydrology of the western United States. *Water Resour Res* **42:** WO4832. doi:10.1029/2005WR004387.

Ballantyne, A.P., Brahney, J., Fernandez, D., Lawrence, C.L., Saros, J., and Neff, J.C. (2010) Biogeochemical response of alpine lakes to recent changes in dust deposition. *Biogeosciences* **7:** 8723–8761.

Barnett, T.P., Adam, J.C., and Lettenmaier, D.P. (2005) Potential impacts of a warming climate on water availability in snow-dominated regions. *Nature* **438:** 303–309.

Barr, D.J.S. (1980) An outline for the reclassification of the Chytridiales, and for a new order, the *Spizellomycetales*. *Can J Bot* **58:** 2380–2394.

Booth, T., and Barrett, P. (1976) Taxonomic and ecological observations of zoosporic fungi in soils of a high-arctic ecosystem. *Can J Bot* **54:** 533–538.

Bridge, P.D., and Newsham, K.K. (2009) Soil fungal community composition at Mars oasis, a southern maritime Antarctic site, assessed by PCR amplification and cloning. *Fungal Ecol* **2:** 66–74.

Brooks, P.D., and Williams, M.W. (1999) Snowpack controls on nitrogen cycling and export in seasonally snow-covered catchments. *Hydrol Processes* **13:** 2177–2190.

Brooks, P.D., Williams, M.W., and Schmidt, S.K. (1996) Microbial activity under alpine snowpacks. *Biogeochem* **32:** 93–113.

Canter, H.M., and Lund, J.W.G. (1948) Studies on plankton parasites. I. Fluctuations in the numbers of *Asterionella formosa* Hass. in relation to fungal epidemics. *New Phytol* **47:** 238–261.

Carreiro, M.M., and Koske, R.E. (1992) Room temperature isolations can bias against selection of low temperature microfungi in temperate forest soils. *Mycologia* **84:** 886–900.

Costello, E.K., and Schmidt, S.K. (2006) Microbial diversity in alpine tundra wet meadow soil: novel Chloroflexi from a cold, water-saturated environment. *Environ Microbiol* **8:** 1471–1486.

Darcy, J.L., Lynch, R.C., King, A.J., Robeson, M.S., and Schmidt, S.K. (2011) Global distribution of *Polaromonas* phylotypes – evidence for a highly successful dispersal

capacity. *PLoS ONE* **6:** e23742. doi:10.1371/journal. pone.0023742.

De Wever, A., Leliaert, F., Verleyen, E., Vanormelingen, P., Van der Gucht, K., Hodgson, D.A., *et al.* (2009) Hidden levels of phylodiversity in Antarctic green algae: further evidence for the existence of glacial refugia. *Proc R Soc B* **276:** 3591–3599.

Dennis, P.G., Rushton, S.P., Newsham, K.K., Lauducina, V.A., Ord, V.J., Daniell, T.J., *et al.* (2012) Soil fungal community composition does not alter along a latitudinal gradient through the maritime and sub-Antarctic. *Fungal Ecol* **5:** 403–408.

Fell, J.W., Scorzetti, G., Connell, L., and Craig, S. (2006) Biodiversity of micro-eukaryotes in Antarctic Dry Valley soils with < 5% soil moisture. *Soil Biol Biochem* **38:** 3107–3119.

Freeman, K.R., Pescador, M.Y., Reed, S.C., Costello, E.K., Robeson, M.S., and Schmidt, S.K. (2009a) Soil CO_2 flux and photoautotrophic community composition in high-elevation, 'barren' soils. *Environ Microbiol* **11:** 674–686.

Freeman, K.R., Martin, A.P., Karki, D., Lynch, R.C., Mitter, M.S., Meyer, A.F., *et al.* (2009b) Evidence that chytrids dominate fungal communities in high-elevation soils. *Proc Natl Acad Sci USA* **106:** 18315–18320.

Furrer, R., Nychka, D., and Sain, S. (2012) *Fields: Tools for Spatial Data, Version 6.6.3*. Boulder, CO, USA: National Center for Atmospheric Research. [WWW document]. URL http://CRAN.R-project.org/package=fields.

Gleason, F., Lectcher, P., and McGee, P. (2004) Some Chytridiomycota in soil recover from drying and high temperatures. *Mycol Res* **108:** 583–589.

Gleason, F.H., Schmidt, S.K., and Marano, A.V. (2010) Can zoosporic true fungi grow or survive in extreme or stressful environments? *Extremophiles* **14:** 417–425.

Grami, B., Rasconi, S., Niquil, N., Jobard, M., Saint-Béat, B., and Sime-Ngando, T. (2011) Functional effects of parasites on food web properties during the Spring diatom bloom in Lake Pavin: a linear inverse modeling analysis. *PloS ONE* **6:** e23273.

Hoham, R.W., and Duval, B. (2001) Microbial ecology of snow and fresh-water ice with emphasis on snow algae. In *Snow Ecology*. Jones, H.G., Pomeroy, J.W., Walker, D.A., and Hoham, R. (eds). New York, USA: Cambridge University Press, pp. 168–215.

Huelsenbeck, J.P., and Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17:** 754–755.

Ibelings, B.W., De Bruin, A., Kagami, M., Rijkeboer, M., Brehm, M., and Van Donk, E. (2004) Host parasite interactions between freshwater phytoplankton and chytrid Fungi (Chytridiomycota). *J Phycol* **40:** 437–453.

Jobard, M., Rasconi, S., Solinhac, L., Cauchie, H.-M., and Sime-Ngando, T. (2012) Molecular and morphological diversity of fungi and the associated functions in three European nearby lakes. *Environ Microbiol* **14:** 2480–2494.

Jumpponen, A., Brown, S.P., Trappe, J.M., Cázares, E., and Strömmer, R. (2012) Twenty years of research on fungal–plant interactions on Lyman Glacier forefront – lessons learned and questions yet unanswered. *Fungal Ecol* **5:** 430–442.

Kagami, M., von Alert, E., Ibelings, B.W., de Bruin, A., and Van Donk, E. (2007) The parasitic chytrid, *Zygorhizidium*, facilitates the growth of the cladoceran zooplankter, *Daphnia*, in cultures of the inedible alga, *Asterionella*. *Proc R Soc B* **274:** 1561–1566.

King, A.J., Freeman, K.R., McCormick, K.F., Lynch, R.C., Lozupone, C.A., Knight, R., and Schmidt, S.K. (2010) Biogeography and habitat modelling of high-alpine bacteria. *Nat Commun* **1:** 53. doi: 10.1038/ncomms1055.

Kol, E. (1968) *Kryobiologie, Biologie und Limnologie des Schnees und Eises 1. Kryovegetation*. Stuttgart, Germany: E. Schweizerbart'sche Verlagsbuchhandlung.

Koob, D.D. (1966) Parasitism of *Asterionella formosa* Hass by a chytrid in two lakes of Rawah wild area of Colorado. *J Phycol* **2:** 41–45.

Lefèvre, E., Roussel, B., Amblard, C., and Sime-Ngando, T. (2008) The molecular diversity of freshwater picoeukaryotes reveals high occurrence of putative parasitoids in the plankton. *PLoS ONE* **3:** e2324. Doi:10.1371/journal. pone.0002324.

Lennon, J., and Jones, J.P. (2011) Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nat Rev Microbiol* **9:** 119–130.

Letcher, P.M., and Powell, M.J. (2012) *A Taxonomic Summary and Revision of Rhizophydium (Rhizophydiales, Chytridiomycota)*. Tuscaloosa, AL, USA: University Printing, The University of Alabama.

Letcher, P.M., Vélez, C.G., Barrantes, M.E., Powell, M.J., Churchill, P.F., and Wakefield, W.S. (2008) Ultrastructural and molecular analyses of Rhizophydiales (Chytridiomycota) isolates from North America and Argentina. *Mycol Res* **112:** 759–782.

Ley, R.E., Williams, M.W., and Schmidt, S.K. (2004) Microbial population dynamics in an extreme environment: controlling factors in talus soils at 3750 m in the Colorado Rocky Mountains. *Biogeochemistry* **68:** 313–335.

Liu, Y., Yao, T., Jiao, N., Kang, S., Xu, B., Zeng, Y., *et al.* (2009) Bacterial diversity in the snow over Tibetan Plateau Glaciers. *Extremophiles* **13:** 411–423.

Lynch, R.C., King, A.J., Farías, M.E., Sowell, P., Vitry, C., and Schmidt, S.K. (2012) The potential for microbial life in the highest elevation (>6000 m.a.s.l.) mineral soils of the Atacama region. *J Geophys Res* **117:** G02028. doi:10.1029/2012JG001961.

Mladenov, N., Sommaruga, R., Morales-Baquero, R., Camarero, L., Laurion, I., Diéguez, M.C., *et al.* (2011) Dust inputs and bacteria influence dissolved organic matter in clear alpine lakes. *Nat Commun* **2:** 405. doi:10.1038/ ncomms1411.

Mladenov, N., Williams, M.W., Schmidt, S.K., and Cawley, K. (2012) Atmospheric deposition as a source of carbon and nutrients to an alpine catchment of the Colorado Rocky Mountains. *Biogeosciences* **9:** 3337–3355.

Nakai, R., Abe, T., Baba, T., Imura, S., Kagoshima, H., Kanda, H., *et al.* (2012) Eukaryotic phylotypes in aquatic moss pillars inhabiting a freshwater lake in East Antarctica, based on 18S rRNA gene analysis. *Polar Biol* **35:** 1495–1504.

Nilsson, R.H., Ryberg, M., Sjökvist, E., and Abarenkov, K. (2011) Rethinking taxon sampling in the light of environmental sequencing. *Cladistics* **27:** 197–203.

Porter, T.M., Schadt, C.W., Rizvi, L., Martin, A.P., Schmidt,

S.K., Scott-Denton, L., *et al.* (2008) Widespread occurrence and phylogenetic placement of a soil clone group adds a prominent new branch to the fungal tree of life. *Mol Phylogenet Evol* **46:** 635–644.

Powell, M.J. (1993) Looking at mycology with a Janus Face: a glimpse at chytridiomycetes active in the environment. *Mycologia* **85:** 1–20.

R Development Core Team (2012) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. ISBN 3-900051-07-0, [WWW document]. URL http://www.R-project.org/.

Remias, D., Karsten, U., Lütz, C., and Leya, T. (2010) Physiological and morphological processes in the Alpine snow alga *Chloromonas nivalis* (Chlorophyceae) during cyst formation. *Protoplasma* **243:** 73–86.

Robeson, M.S., King, A.J., Freeman, K.R., Birky, C.W., Martin, A.P., and Schmidt, S.K. (2011) Soil Rotifer communities are extremely diverse globally but spatially autocorrelated locally. *Proc Natl Acad Sci USA* **108:** 4406–4410.

Ronquist, F., and Huelsenbeck, J.P. (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19:** 1572–1574.

Rosling, A., Cox, F., Cruz-Martinez, K., Ihrmark, K., Grelet, G.-A., Lindahl, B.D., *et al.* (2011) Archaeorhizomycetes: unearthing an ancient class of ubiquitous soil fungi. *Science* **333:** 876–879.

Schadt, C.W., Martin, A.P., Lipson, D.A., and Schmidt, S.K. (2003) Seasonal dynamics of previously unknown fungal lineages in tundra soils. *Science* **301:** 1359–1361.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., *et al.* (2009) Introducing Mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* **75:** 7537–7541.

Schmidt, S.K., Nemergut, D.R., Miller, A.E., Freeman, K.R., King, A.J., and Seimon, A. (2009) Microbial activity and diversity during extreme freeze-thaw cycles in periglacial soils, 5400 m elevation, Cordillera Vilcanota, Perú. *Extremophiles* **13:** 807–816.

Schmidt, S.K., Lynch, R.C., King, A.J., Karki, D., Robeson, M.S., Nagy, L., *et al.* (2011) Phylogeography of microbial phototrophs in the dry valleys of the high Himalayas and Antarctica. *Proc R Soc B* **278:** 702–708.

Schmidt, S.K., Naff, C.S., and Lynch, R.C. (2012) Fungal communities at the edge: ecological lessons from high alpine fungi. *Fungal Ecol* **5:** 443–452.

Segawa, T., Miyamoto, K., Ushida, K., Agata, K., Okada, N., Kohshima, S., *et al.* (2005) Seasonal change in bacterial flora and biomass in mountain snow from the Tateyama Mountains, Japan, analyzed by 16S rRNA gene sequencing and real-time PCR. *Appl Environ Microbiol* **71:** 123–130.

Sime-Ngando, T., Lefèvre, E., and Gleason, F.H. (2011) Hidden diversity among aquatic heterotrophic flagellates: ecological potentials of zoosporic fungi. *Hydrobiologia* **659:** 5–22.

Simmons, D.R., James, T.Y., Meyer, A.F., and Longcore, J.E. (2009) Lobulomycetales, a new order in the Chytridiomycota. *Mycol Res* **113:** 450–460.

Stamatakis, A. (2006) RaxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22:** 2688–2690.

Stein, J.R., and Amundsen, C.C. (1967) Studies on snow algae and fungi from the Front Range of Colorado. *Can J Bot* **45:** 2033–2045.

Stoeck, T., Kasper, J., Bunge, J., Leslin, C., Ilyin, V., and Epstein, S. (2007) Protistan diversity in the Arctic: a case of paleoclimate shaping modern biodiversity. *PLoS ONE* **2:** e728. doi:10.1371/journal.pone.0000728.

West, A.E., Brooks, P.D., Fisk, M.C., Holland, E.A., Kai, R., and Schmidt, S.K. (1999) Landscape patterns of CH_4 fluxes in an alpine tundra ecosystem. *Biogeochemistry* **45:** 243–264.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Full phylogenetic tree showing the relationship between the three clades discussed in this paper (Snow Clade 1, order Lobulomycetales, and order Rhizophydiales) and the out-group (order Chytridiales). *Asterisks denote nodes with minimum of 90% bootstrapping support (RaxML, 1000 bootstraps) and 100% posterior probabilities (Mr Bayes, 5 million generations, INVGAMMA model). #The pound sign denotes nodes with 100% posterior probability support but where bootstrapping support was not available. [1]Tree tips labelled with a '1' are from this study: 'Snow' covering the talus sites described in Freeman and colleagues (2009b), 'Snow/Ice' on top of Arikaree Glacier (King *et al.*, 2010), and 'Snowbed Soils' from the Himalayan sites described in Schmidt and colleagues (2011; 2012). [2]Tree tips labelled with a '2' are sequences from 'Snowbed soils' in Colorado (Freeman *et al.*, 2009b). [3]Tree tips labelled with a '3' correspond to sequences obtained from prior studies: Swiss Snow (M. Yuhana, unpublished) and Alpine Lake, France (Lefèvre *et al.*, 2008).