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Metabarcoding analysis of the fungal biodiversity associated with Castaño Overa Glacier – Mount Tronador, Patagonia, Argentina

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ABSTRACT

Cold environments represent the largest fraction of Earth's biosphere, and are known habitats for cold-adapted microorganisms. The aim of this study was to assess the occurrence and biodiversity of cold-adapted fungi associated with the Castaño Overo glacier, Mount Tronador, Patagonia, Argentina. Samples of naked soil, glacial ice and snow were collected and analyzed using tag-encoded 454 pyrosequencing of the nuclear ribosomal internal transcribed spacer (ITS). A total of 1082 OTUs (operational taxonomic units) and 151,669 sequences were obtained. OTUs obtained from soil samples corresponded mainly to the phylum Ascomycota, whereas for snow and ice samples the phylum Basidiomycota was the most represented group. Metabarcoding analysis showed high biodiversity in glacial environments and allowed the detection of hitherto unknown taxa for Patagonia. To the best of our knowledge, this is the first report of fungal diversity in an extreme glacial environment of Patagonian Argentina using amplicon sequencing.

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1. Introduction

There are multiple habitats on Earth with factors that limit the development of microorganisms, including the ocean floor, hot springs, saltworks, acidic rivers, deserts, glaciers and high altitude areas. An ecosystem is identified as extreme if its physico-chemical conditions are outside the parameters considered optimal for life (from an anthropocentric perspective) of most organisms, such as those that allow the development of model organisms like *Escherichia coli* (Antranikian et al., 2005; Canganella and Wiegel, 2011). Cold environments are one such extreme habitat, with associated factors such as high UV radiation, low water potential, low nutrient availability, high hydrostatic pressure and oxidative stress, among other important factors (Goordial et al., 2013).

Fungi are key microorganisms in cold environments, as symbionts in mycorrhizas or lichens and as saprotrophs that contribute to the cycling of nutrients, and constitute one of the most metabolically active groups of eukaryotes in glaciers (Anesio and Laybourn-Parry, 2012; Hassan et al., 2016). Studies in these environments in Antarctica and the Arctic have shown wide fungal diversity in varied substrates such as bulk soil (Connell et al., 2006; Sterflinger et al., 2012), Antarctic lakes (Gonçalves et al., 2012), cryoconite holes in mountain glaciers (Bergauer et al., 2005; Singh and Singh, 2012), glacier ice and meltwater on Alps and in high mountains in Patagonia, Argentina (de Garcia et al., 2007, 2012, 2014; Turchetti et al., 2008; Libkind et al., 2009; Branda et al., 2010; Brandão et al., 2011). Cold and oligotrophic environments present an ideal location to examine the evolutionary processes of native microorganisms because these habitats facilitate the speciation of endemic organisms (Gostinčar et al., 2009; Cantrell et al., 2011). Additionally, global climate change may increase the rate of desertification and exposed land from glacial retreat in certain parts of the globe (Oerlemans, 2001; Higgens and Vellinga, 2004), allowing the study of communities of soil eukaryotic microorganisms and their adaptation to these habitats (Connell et al., 2008).

The Earth's surface temperatures in the last 2 years have been the warmest since modern record keeping began in 1880 (Cole and McCarthy, 2018). The magnitude of these temperature changes

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impels the study of extreme cold environments, and microorganisms adapted to these environments, because such habitats could be lost in a few years. Paleobotanical studies demonstrate that Arctic soil fungi have responded to climate warming, with the isolation and conservation of extremophile fungi being viewed as a means of avoiding their possible extinction (Zalar and Gunde-Cimerman, 2014).

The environments associated with mountain glaciers of Patagonia are an ideal setting for such studies. The Nahuel Huapi National Park (NHNP) in northwestern ed.Patagonia (Argentina) has a cold to temperate climate and includes vast areas with little or no human influence. Mount Tronador is an old, extinct stratovolcano located in the Patagonian Andes along the Argentina–Chile border in NHNP. Castaño Overa is one of four glaciers located in Argentina (Ruiz et al., 2015). As a result of increases in ultraviolet radiation (UVR) resulting from its proximity to the Antarctic ozone hole, Patagonia is exposed to enhanced levels of solar UV-B radiation for periods during spring (Diaz et al., 2006; Wolfram et al., 2012). Mean annual air temperature in this area is 8.3 °C, with sub-zero temperatures in winter and precipitation, mainly in the form of snow, reaching an annual average of 1186 mm (Hotel Tronador, 41°16′S, 71°39′W, 815 m, period 2013–2017). Studies of yeast biodiversity and novel species with biotechnological value have been conducted in these habitats (de Garcia et al., 2014), but these studies have focused on cultivable yeasts.

The aim of the present study was to evaluate fungal biodiversity associated with Castaño Overa glacier, Mount Tronador (NHNP), in order to begin to elucidate the most abundant groups in this extreme habitat. 454 pyrosequencing was used to study the fungal communities in oligotrophic habitats transitioning from sub-glacial flow through to the development of mesotrophic cold desert habitats to address the following questions: (1) what is the fungal diversity and community composition in snow-ice from Castaño Overa glacier and its associated soil? (2) do diversity and composition of fungal communities vary among the different substrates (soil and snow-ice) and different sites?

2. Materials and methods

2.1. Study area and sampling

Soil, ice, and snow samples were aseptically collected from Castaño Overa glacier, Mount Tronador (41°09′S, 71°53′W) in January 2015. GPS positions were recorded *in situ* (Supplementary Table 1). Five soil samples were collected from two sites, Soil Site 1 (SS1) and Soil Site 2 (SS2), in moraines surrounding the Castaño Overa glacier. Five plots of 1 × 1 m were delimited, with five subsamples of soil 100 g collected from each plot and placed into sterile plastic bags (Connell et al., 2006). Three ice samples were collected from three Castaño Overa fissures (each one with three replicates) and five snow samples were collected from the surface of the glacier (Fig. 1). All samples were stored in sterile plastic bags. Ice and snow samples were melted aseptically at room temperature and then filtered through Millipore® membrane filters (pore size 0.45 mm, diameter 47 mm) (de Garcia et al., 2012).

2.2. Soil chemistry

Subsamples of soil from each site were pooled to obtain a composite aliquot to be used for physical and chemical analysis. Soil pH was measured in a 1:2.5 (w/v) soil-to-water ratio, soil moisture was determined gravimetrically (Barrett et al., 2002), organic carbon was assessed by wet-oxidation (Okalebo et al., 2002), total N was estimated by the micro-Kjeldahl method and phosphorus concentration was assayed by mineralization of the sample and dry digestion (Gregorich and Carter, 2007). All of the analyses were carried out by the Soil Group from the Centro Regional Universitario Bariloche, Universidad Nacional del Comahue, Río Negro, Argentina. Only soil samples were chemically analyzed, because snow-ice samples were not suitable for the corresponding analyses.

2.3. Direct DNA extraction from snow, ice and soil

DNA was extracted from soil and snow-ice using MOBIO PowerMax® Soil and MOBIO PowerWater® kits, respectively, as per the manufacturer's instructions. 454 Pyrosequencing (Roche) amplification of ribosomal ITS regions was performed using the primer ITS1F (5-TCCGTAGGTGAACCTGCGG-3) and ITS4 (5-TCCTCCGCTTATTGATATGC-3) (Zhang et al., 2015). A single-step PCR profile using HotStarTaq Plus Master Mix Kit (QIAGEN, Valencia, CA) was used under the following conditions: 94 °C for 3 min, followed by 28 cycles of 94 °C for 30 s, 53 °C for 40 s and 72 °C for 1 min, and then a final elongation step at 72 °C for 5 min. Following PCR, all amplicon products from different samples were mixed in equal concentrations and purified using Agencourt AMPure beads (Agencourt Bioscience Corporation, MA, USA). Samples were sequenced utilizing Roche 454 FLX titanium instruments and reagents, following the manufacturer's guidelines.

2.4. Amplicon data processing

The sequence data was processed using an analysis pipeline (www.mrdnalab.com, MR DNA, Shallowater, TX). Sequences were depleted of barcodes and primers, and then short sequences (<200 bp), sequences with ambiguous base calls, and sequences with homopolymer runs exceeding 6 bp were removed. Sequences were then denoised and operational taxonomic units (OTUs) were defined by clustering at 3% divergence (97% similarity), followed by the removal of singleton sequences and chimeras (Dowd et al., 2008a, 2008b; Edgar, 2010; Capone et al., 2011; Eren et al., 2011;

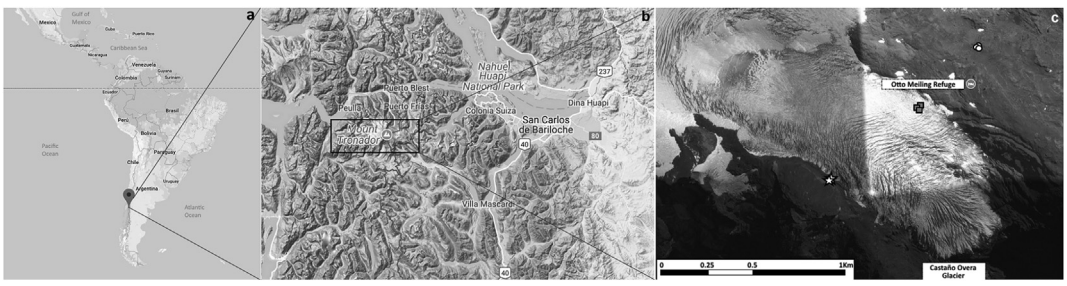


Fig. 1. Locations of Castaño Overa Glacier and sampling sites. Circles represent soil samples S1–S3 collected from site 1 (SS1), white stars represent soil samples S4–S5 from site 2 (SS2) and gray squares correspond to ice-snow samples G6–G10 from glacier sites SG1–SG3.

Swanson et al., 2011). Final OTUs were taxonomically classified using BLASTn against a curated database derived from UNITE and NCBI. Taxonomy followed Kirk et al. (2008), Liu et al. (2015), McLaughlin and Spatafora (2014, 2015), Wang et al. (2015) and the MycoBank database (http://www.mycobank.org).

2.5. Data analysis

The sequences of selected OTUs were analyzed with related sequences from GenBank. The phylogenetic relationships were estimated using MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets (Kumar et al., 2016). Phylogenetic trees were constructed using the neighbour-joining algorithm, and bootstrap values were calculated from 1000 replicate runs. The Kimura two-parameter model was used to estimate evolutionary distance.

Alpha-diversity indices (i.e., Chao1, Good's coverage estimator and the Shannon Index) and beta-diversity metrics were calculated. Rarefaction curves were produced. To estimate community similarity among samples, a hierarchical cluster analysis was applied on the basis of the abundance of OTUs in the communities using Bray–Curtis dis similarity and a dendrogram inferred with the average linkage method. These analyses were performed in R, using OTUs for community procedures in the *Vegan* package (Oksanen et al., 2011). A principal components analysis (PCA) was carried out using IBM SPSS Statistics software v. 23 (IBM Corp.), to determine which soil physicochemical characteristics best explained the variance among samples.

3. Results

3.1. Sequence data

The raw data from 10 samples (five of soil and five of snow-ice) consisted of 219,068 sequences. After removing short sequences, chimeric sequences and OTUs of non-fungal organisms, 151,669 sequences passed the quality control, resulting in a total of 1082 OTUs at 97% similarity. Soil samples had more variable numbers of OTUs, ranging from 168 to 365, while the numbers in snow-ice samples ranged from 171 to 255. The length of sequences ranged from 225 to 553 bp.

3.2. Diversity and structure of fungal community

The 1082 OTUs spanned eight phyla, 24 classes, 44 orders and 163 families, with 4% (43 OTUs) being classified as unknown taxa. There were few (6.6%) OTUs shared between soil and snow-ice, with the majority being specific to each of the two substrates (Supplementary Table 2 and Supplementary Figure 1). Both rarefaction curves and Good's coverage estimator suggested that 454 pyrosequencing captured the dominant phylotypes in all samples (Table 1). Furthermore, curves revealed that for a common sampling effort (9296 reads), soil samples show higher richness (number of OTUs) than snow-ice samples (Fig. 2).

Shannon-Weaver Index values, in both soil and snow-ice, ranged between 3.56 and 4.44, but there were no significant differences in index values between sites or between substrates ($P > 0.05$). Nevertheless, soil sample S2 showed the most diverse profile, with a value of 4.44. The highest number of sequences was recovered from snow-ice. Dikarya related sequences dominated soil and snow-ice substrates, both for the number of sequences (87.14%) and in the number of OTUs (918 of 1082) (Fig. 3).

3.3. Differences in community structure between soil and ice-snow samples

The relative abundance of phylogenetic groups from soil and snow-ice, based on ITS sequences, was obtained. A comparison of the fungal components in the two substrates showed that the phylum Ascomycota dominated the soil samples, and that Basidiomycota were most frequent in snow-ice samples.

For soil samples, 23 classes were identified, with the Leotiomycetes (Ascomycota) being the most common taxon (relative abundances of 11–42%), followed by the Sordariomycetes, Dothideomycetes and Eurotiomycetes. Members of the Geoglossomycetes were only present in sample S5 (at a relative abundance of 11%), and sample S1 showed the highest proportion of Chytridiomycetes, with 45% of OTUs being related to this class (Fig. 4).

Fifteen classes/unknown classes were shared between soil samples SS1 and SS2, representing 60% of total classes (Supplementary Figure 2a). Classes unique to SS1 were the Saccharomycetes (2.07%), Archaeorhizomycetes (1.73%), Pucciniomycetes (0.55%), Orbiliomycetes (0.15%), Cystobasidiomycetes (0.1%), Monoblepharidomycetes (0.06%), Entorrhizomycetes (0.03%) and Neocallimastigomycetes (0.03%), with SS2 consisting exclusively of Geoglossomycetes (5.47%) and unknown Basidiomycota (0.01%) (all values are percentages per site).

For snow-ice samples, 13 classes were identified, and two unknown-classes were obtained, with the Microbotryomycetes (Basidiomycota), which was present in all samples, being the most abundant group (63–94% per snow-ice sample), followed by the Monoblepharidomycetes (0.5–26% per sample), and sample G10 showing the highest proportion of this class.

In snow-ice samples, nine classes were identified at each site (Supplementary Figure 2b). The Malasseziomycetes was unique to site one (SG1, 0.01%) and the Arthoniomycetes was only present at site two (SG2, 0.02%). At site three, only the Glomeromycetes were present (0.12%). Shared classes between SG1 and SG2 were the Tremellomycetes (0.6% and 0.02%, respectively) and unknown Basidiomycota (0.02 and 0.003%, respectively). Finally, an unknown group of Ascomycota was shared between SG2 and SG3 (0.03 and 0.01%, respectively). Fourteen classes and unknown classes were shared between soil and ice-snow substrates in total.

The most abundant ascomycete orders in soil and snow-ice were the Helotiales and Capnodiales, respectively, while the Sebacinales and Leucosporidiales were the most abundant basidiomycete orders in soil and snow-ice, respectively. Members of the Chytridiomycetes and Monoblepharidomycetes were also found, with a high proportion of Spizellomycetales in soil samples and Monoblepharidales in snow-ice samples (Supplementary Table 3).

Similarity of the community among sites was assessed, comparing the relative abundance and distribution of OTUs by sample. Cluster analysis, using Bray-Curtis dissimilarity, separated the communities into three main groups (Fig. 5). One group contained all communities from snow-ice, whereas soil communities were further separated into two clusters, one in site 1 (S1–S3) and the other in site 2 (S4–S5). OTUs identified to species level were less abundant compared with OTUs from higher levels of taxonomic identification, with 202 OTUs at the species level over 1112 OTUs with <97% of identification, for both soil and snow-ice samples. Over this total, 183 were related to sequences of isolated species, while the remainders were related to environmental amplicons.

OTUs such as *Penicillium lividum*, *Venturia hystrioides*, *Gibberella* sp. and *Phialocephala helvetica* dominated in soil, while in snow-ice, *Phenoliferia psychrophenolica*, *Epicoccum nigrum*, *Preussia* sp. and *Phaffia rhodozyma* showed the highest abundances. *Gibberella* sp.

Table 1
Fungal diversity parameters, number of OTUs and sequences in each sample.

	Soil					Snow-Ice				
Sample	S1	S2	S3	S4	S5	G6	G7	G8	G9	G10
No. reads*	12769	9002	9367	14409	13664	15655	16950	16410	25112	18331
No. OTUs	345	271	263	165	263	194	197	243	254	169
Frequency of most abundant OTU (%)	25.73	7.26	18.57	15.19	15.71	15.61	14.04	15.45	12.18	15.01
Diversity indices										
Good's coverage	0.96	0.97	0.97	0.98	0.98	0.98	0.98	0.98	0.98	0.98
Shannon (H)	3.56	4.44	3.93	3.69	3.6	3.74	3.81	3.45	3.66	3.64
Chao1	410.3	316.1	327.2	173.5	290.6	202.1	213.1	295.1	272.2	178

*Final number of reads obtained after removing primers, short (<200 bp) and low-quality sequences and chimeras.

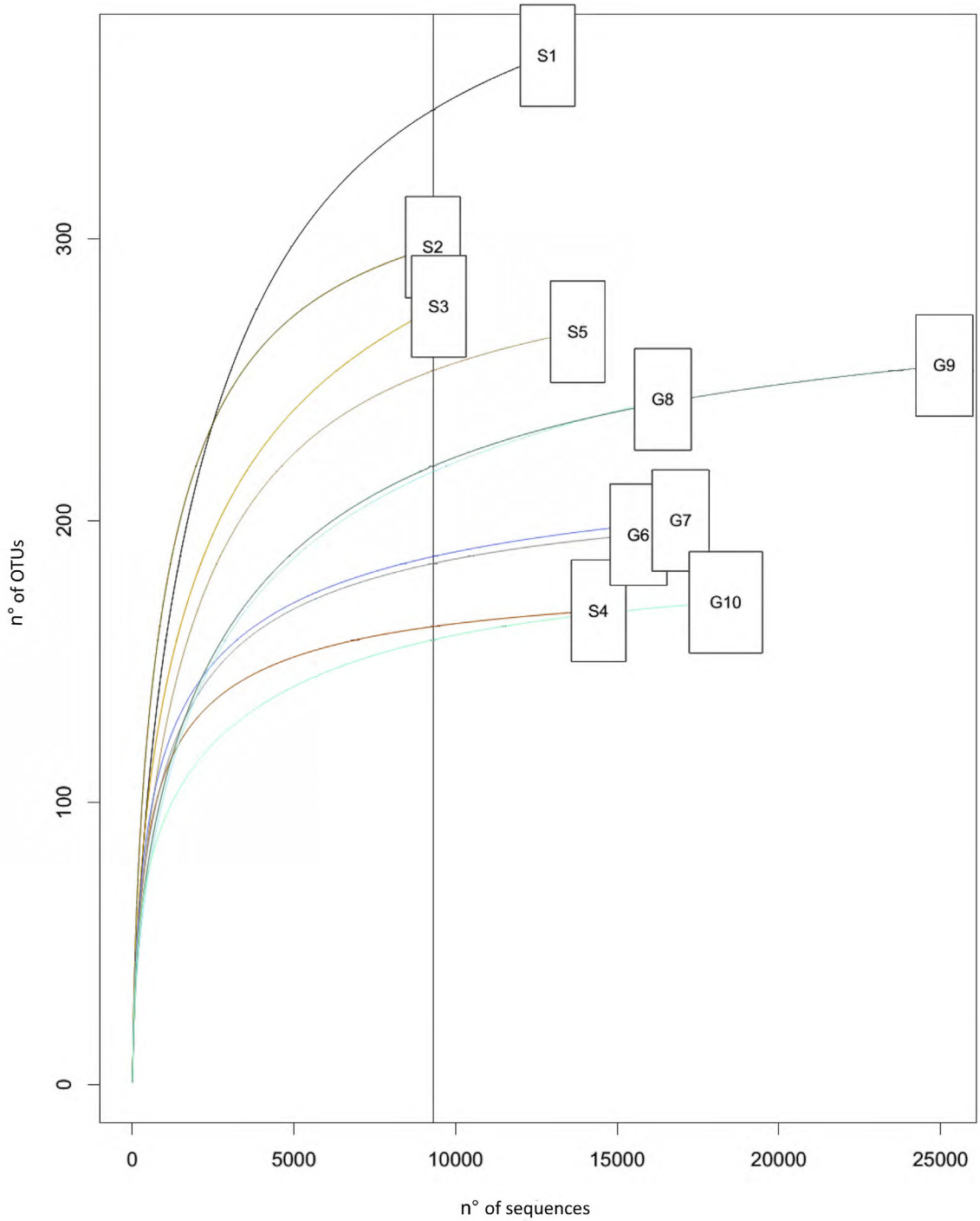


Fig. 2. Rarefaction curves of estimated OTU richness in soil (S1–S5) and glacier snow-ice (G6–G10).

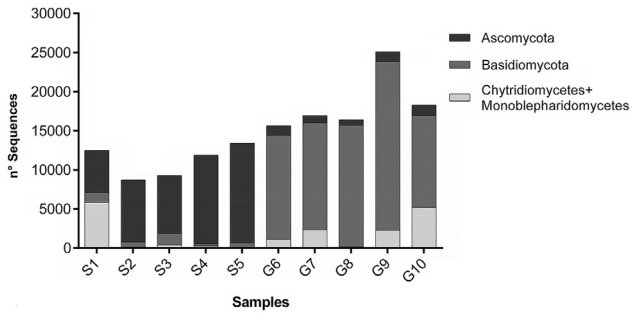


Fig. 3. Absolute abundance of sequences by sample.

had the highest number of sequences shared between the two substrates (1% of 151,669 sequences). The most abundant fungi in all samples (3.9–5.5%) were OTUs 10, 17 and 45, which were related to members of the Microbotryomycetes.

Due to the large number of unidentified OTUs within the Microbotryomycetes, a selection of the most abundant Microbotryomycetes-like OTU sequences is shown in Supplementary Table 4. A phylogenetic tree was constructed showing the placement of OTUs in the Microbotryomycetes (Fig. 6). OTU 72 was related to *P. psychrophenolica*, a cold adapted species belonging to a psychrophile ecoclade (Gadanho and Sampaio, 2009). OTUs 17, 10, 769, 27 and 23 were grouped together as a sister group to psychrophilic yeasts isolated from glacial ice from Castaño Overa glacier and marine ice from Antarctica (strains CO1.5, CO2.3, CRUB 1741, 10.2 and, 10.6). OTUs 28 and 14 were grouped together. OTUs 17, 10, 769, 27 and 23 differed by 32 (SD: 3) nucleotide substitutions (9.8%) from Antarctic yeast (strain 10.2), while OTU 72 differed by 34 nucleotide substitutions (9.04%) from *P. psychrophenolica* AG21.

3.4. Soil chemistry

The moisture content of soil samples ranged from 9.9% (S5) to 27% (S1). Both carbon, nitrogen and phosphorus concentrations were highest at site 1 (S1, S2 and S3). Soil samples were slightly acidic with pH values ranging from 4.8 to 5.4 (Supplementary Table 5). The PCA showed that the first two principal components explained 85.3% of total variance (Fig. 7a). PC1 represented a gradient in phosphorus, moisture, carbon and nitrogen concentration, and PC2 represented a gradient in pH value. The samples from two sites were clearly separated by the variables comprising component 1, with the differences between samples corresponding largely to pH value (Fig. 7b).

4. Discussion

4.1. Diversity of fungal communities

To the best of our knowledge, this work represents the first analysis of fungal communities within oligotrophic soil and snow-ice from a glacial environment of Patagonia (Argentina), using a high throughput NGS method. Studies carried out in soil and glaciers in different part of the world have shown a total number of OTUs ranging between 900 and 2500 (Tedersoo et al., 2014), and 298 to 1611 (Gutiérrez et al., 2015; Rime et al., 2016), respectively. In our study we obtained 1112 total OTUs in soil and snow-ice substrates, close to the upper limit of richness in similar extreme environments.

Soil and snow-ice samples showed differences based on the most representative OTUs. Only a few OTUs were present in both substrates, suggesting a limited fungal community interchange at the terminal moraine. Rime et al. (2016) found a similar pattern in recently deglaciated soils and endogenous supra- and subglacial habitats, suggesting a lower dispersal capability of fungi than bacteria in the same habitat.

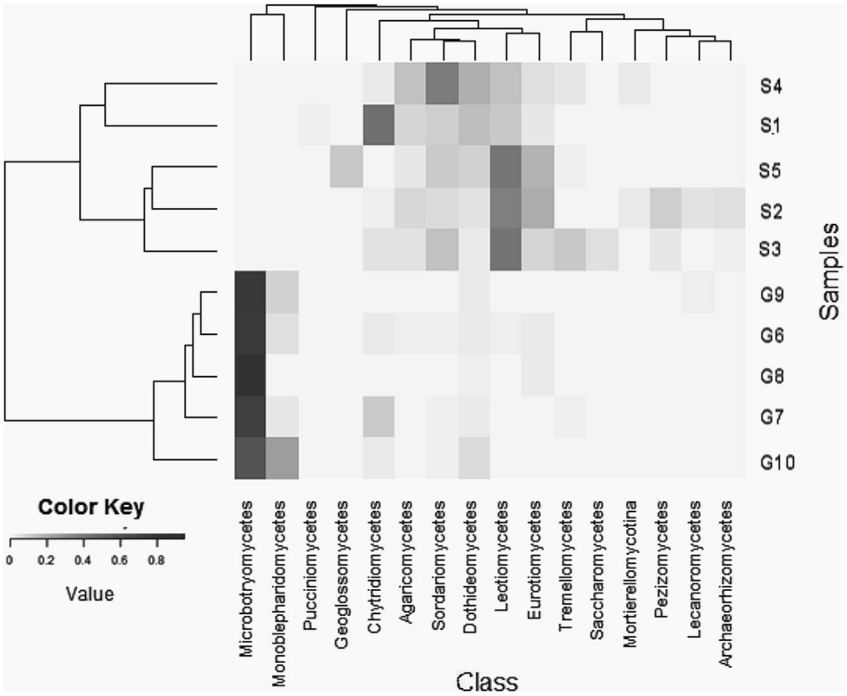
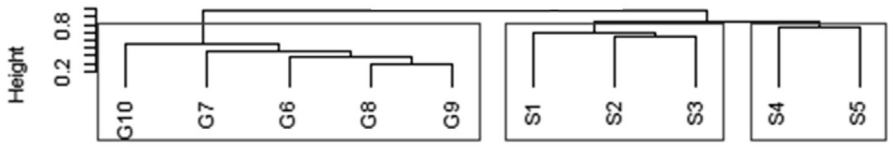


Fig. 4. Heatmap showing the total abundance of each class in each sample of soil (S1–S5) and glacier snow-ice (G6–G10) from Castaño Overa glacier. The branch lengths of the cluster dendrogram show the similarity level.Clustering among samples was based on a distance matrix computed with Bray–Curtis dissimilarity.



Substrate	Species	G10	G7	G6	G8	G9	S1	S2	S3	S4	S5	Ab (%)
Both substrates	*Hyaloraphidium curvatum*	0	12.76	0	0.49	6.61	3.01	8.90	5.62	50.16	12.45	1.46
	Gibberella sp.	1.41	0	0	0	0	0.47	1.20	0.80	91.83	4.28	0.98
	Penicillium sp. I	32.74	0	0	2.54	0.38	18.91	0.38	25.38	7.87	11.80	0.52
	Preussia sp. I	0	53.48	0	1.88	26.18	1.69	0.94	1.88	12.62	1.32	0.35
	Gyoerffyella sp.	0	0	0	0.21	1.68	5.05	8.63	22.32	8.21	53.89	0.31
	Articulospora tetracladia	0	0	0	0.30	0	3.61	5.72	70.78	18.37	1.20	0.22
	Sphaerulina polyspora	5.25	0	49.69	40.43	0	1.23	0.93	2.47	0	0	0.21
Snow-Ice	*Epicoccum nigrum*	58.59	0	0	0.80	40.61	0	0	0	0	0	0.41
	Phenoliferia psychrophenolica	100	0	0	0	0	0	0	0	0	0	0.35
	Phaffia rhodozyma	0	95.45	4.55	0	0	0	0	0	0	0	0.13
	Placopsis santessonii	0	0	0	5.29	94.71	0	0	0	0	0	0.12
	Hormonema sp.	0	91.67	0	0	8.33	0	0	0	0	0	0.09
	Leucosporidium sp.	0	0	35.29	1.47	63.24	0	0	0	0	0	0.04
	Rhodotorula ingeniosa	5.88	94.12	0	0	0	0	0	0	0	0	0.03
Soil	*Penicillium lividum*	0	0	0	0	0	0.29	0	0.29	1.83	97.59	1.36
	Venturia hystrioides	0	0	0	0	0	70.53	0.09	1.02	12.52	15.84	0.77
	Phialocephala helvetica	0	0	0	0	0	24.95	63.41	11.64	0	0	0.63
	Trichocladium opacum	0	0	0	0	0	12.27	61.61	13.19	8.71	4.22	0.50
	Phoma sp.	0	0	0	0	0	0	0	0	99.53	0.47	0.28
	Mortierella elongata	0	0	0	0	0	8.06	36.02	2.13	50.95	2.84	0.28
	Truncatella angustata	0	0	0	0	0	6.91	1.32	42.76	22.70	26.32	0.20

Fig. 5. Dendrogram representing the similarity between the composition of fungal OTUs in soil samples (S1–S5) and glacier snow-ice (G6–G10) from Castaño Overa glacier. The branch lengths of the cluster dendrogram show the similarity level. Clustering is based on a distance matrix computed with Bray–Curtis dissimilarity.

The rarefaction curves obtained for all samples showed different richness patterns depending on the substrate. Soil samples showed greater values when compared with snow-ice, and the estimated coverage indicated that at least 96.7% of the OTUs richness was detected in this sampling. The greater diversity in soil samples can be explained in part by fungal groups that generally are associated with plants or lichens in these soils (Sterflinger et al., 2012; Rime et al., 2016).

The results obtained through NGS technology allowed us to acquire information on diversity and on taxonomic fungal groups that have not been isolated from the studied environment. In addition, by using NGS methods, higher levels of diversity were detected when comparing the taxonomic groups identified in this work with previous research of cultivable yeasts and/or filamentous fungi of Patagonia and Antarctica (Cecilia Mestre et al., 2011; de Garcia et al., 2012; Godinho et al., 2012).

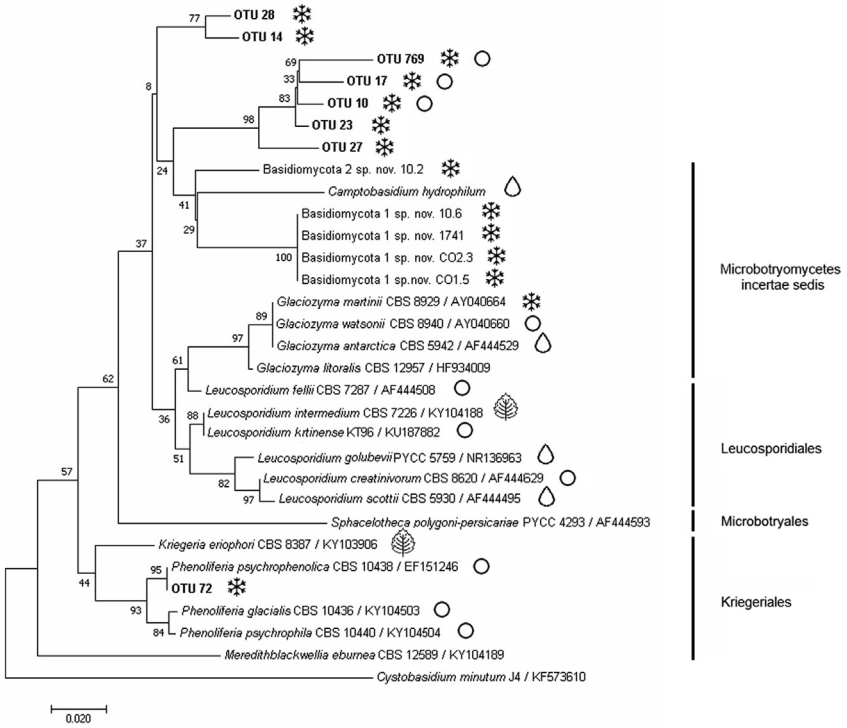


Fig. 6. Phylogenetic placement of Microbotryomycetes-like OTUs obtained by neighbour-joining (distance K2P method) of the ITS region. The figures represent the substrate from which the sequences or isolations were obtained: leaf: phylloplane; circle: soil or sediments; snowflake: glacier ice and water drop: water. Bar, number of substitutions accumulated per 100 nucleotides. Boostrap values were calculated from 1000 replicates.

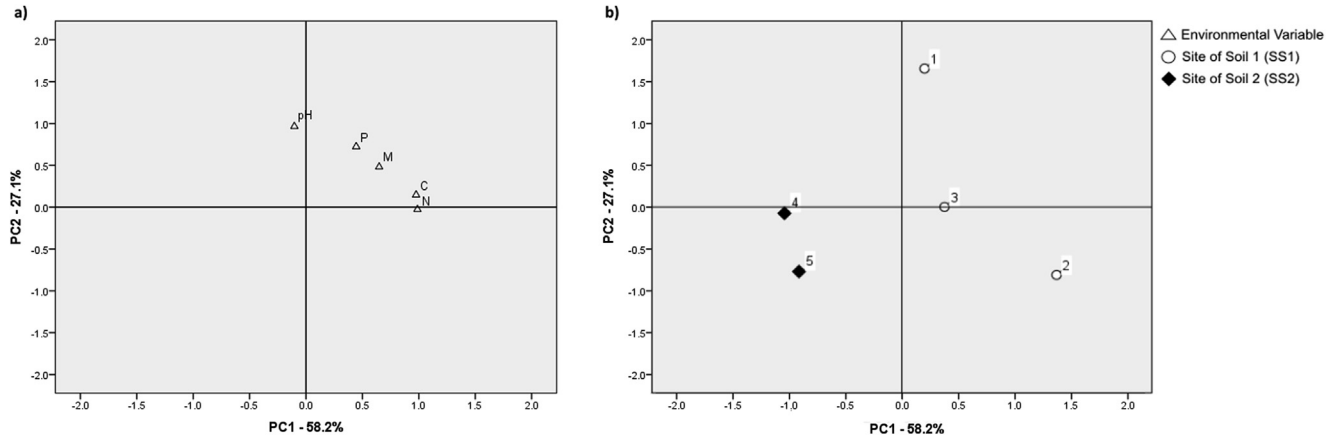


Fig. 7. PCA of the different physicochemical characteristics determined for the soils samples. (M: moisture; P, C and N: concentrations of phosphorus, carbon and nitrogen, respectively).

4.2. Differences in community structure between soil and ice-snow samples

We found that ascomycetes dominated in the soils from the Mount Tronador glacier. This is similar to observations from Antarctic soils, which are also dominated by ascomycetes (Connell et al., 2006, 2008; Arenz et al., 2014; Cox et al., 2016). The most abundant OTUs were members of the genera *Claussenomyces* sp., *Rhizoscyphus* sp. and *Phialocephala* sp., which are associated with decaying wood or live plants (ericoid mycorrhiza, dark septate endophytes). Similar results have been obtained for soils close to huts in the Antarctic (Arenz et al., 2014). For the class Leotiomycetes, studies show that these fungi tend to increase in diversity towards the poles, with it being one of the most abundant ascomycete classes in Arctic tundra, and on Antarctic islands (Tedersoo et al., 2014; Zhang et al., 2015; Cox et al., 2016), with the results obtained here supporting these observations.

In our research, chytrids were present in both soil and snow-ice samples. These zoosporic fungi are rarely isolated from extreme environments, but in recent years the analysis of environmental DNA has allowed further insights into the ecology of these basal fungi. For example, Chytridiomycota have been found in Antarctic soils (Bridge and Newsham, 2009) and a new clade of snow chytrids have been described in snowpacks of Colorado and Switzerland (Naff et al., 2013). Moreover, the presence of chytrids has recently been discovered in glacier meltwater and sediments in a fjord in Chilean Patagonia (Gutiérrez et al., 2015). The orders Spizellomycetales and Rhizophydiales are the most common chytrids in soil. Some species in these orders have been shown to tolerate desiccation and freezing in laboratory experiments (Gleason et al., 2004, 2010). We have observed the presence of this group in sample S1 with the highest abundance of Chytridiomycota. This soil sample had the highest pH value, moisture and P concentrations, suggesting that the influence of glacial meltwater could create favorable conditions for the dispersal and development of chytrids in soil, similar to other high elevation environments such as Colorado and Nepal, where the chytrid-like sequences dominate (Freeman et al., 2009).

The Monoblepharidales was the most represented order of chytrids in ice-snow samples. This order is generally accepted as being sister to the Chytridiomycetes in the phylum Chytridiomycota (Hibbett et al., 2007; Dee et al., 2015). However, some authors place the taxon as a separate phylum, the Monoblepharidomycota, containing the Monoblepharidomycetes and the Hyaloraphidiomycetes (Powell and Letcher, 2014). In this study, we consider them to be a group within the Chytridiomycota. Monoblepharidales have been isolated from freshwater, soil, brackish and marine environments, with other chytrid orders (Longcore and Simmons, 2001; Gleason et al., 2008), and have been found in water samples of Lake Ontario, Canada (Zhang et al., 2015). This order is considered mostly saprotrophic, with a few known parasites (Powell and Letcher, 2014; Karpov et al., 2017). To date, there is a paucity of information about this taxon in extreme environments. Our study reports its existence in Patagonia, contributing to our knowledge of its geographic distribution and ecology, and setting the basis for future studies on its role in extreme environments.

Basidiomycota dominated in snow-ice samples of Castaño Overa glacier, Patagonia, Argentina, in agreement with previous studies of culturable yeasts from ice and water of Argentine Patagonian glaciers and other cold environments (Margesin et al., 2007; Branda et al., 2010; Thomas-Hall et al., 2010; Uetake et al., 2011; Vaz et al., 2011; de Garcia et al., 2012; Brown et al., 2015; Martinez et al., 2016). Snow-ice substrate harbored sequences related to psychrophilic and psychrotolerant species, such as *P.psychrophenolica*, a basidiomycetous yeast isolated from alpine glaciers (Margesin et al., 2007; Branda et al., 2010) and *P. rhodozyma*, a basidiomycetous yeast associated with temperate forests in the northern and southern hemispheres (Libkind et al., 2007; David-Palma et al., 2014). The detection of *Ph. rhodozyma* in snow-ice samples is in agreement with previous culture-dependent studies describing its distribution in Andean Patagonia (Libkind et al., 2007, 2009, 2011).

For both substrates studied here, the OTUs that we were able to identify to genus/species level were mostly related to ascomycetes. Most of these species are described as soil organic matter decomposers, plant pathogen and root or lichen symbionts, such as *Gibberella* sp., *Sphaerulina polyspora*, *P. helvetica* and *Psoroma buchananii* (McLaughlin and Spatafora, 2015). Nevertheless, unknown microbotryomycetous sequences were dominant in snow-ice samples. The Microbotryomycetes is the second largest class in the Pucciniomycotina, with more than 200 described species that include mostly dimorphic species and yeasts (Boekhout et al., 2011). This taxonomic group encompasses a wide variety of psychrophilic fungi, such as *Phenoliphfia* spp. in the Sporidiobolales (Margesin et al., 2007), *Leucosporidium* spp. in the Leucosporidiales (Sampaio et al., 2003; de García et al., 2015), *Glaciozyma* spp. in the Kriegeriales (Turchetti et al., 2011) and *Camptobasidium hydrophilum* in the Camptobasidiaceae (Marvanová and Suberkropp, 1990). Studies of culturable yeasts of cold environments showed that species in this class are predominant along with species in the Tremellomycetes (de Garcia et al., 2007, 2012; Turchetti et al., 2011,

2013; Edwards et al., 2013; Singh et al., 2013; Zalar and Gunde-Cimerman, 2014).

The OTUs obtained here consisted of few sequences that do match with known species, most of which are in the class Microbotryomycetes. One of the unidentified species was related to *P. psychrophenolica* (OTU 72), which is a cold adapted species that belongs to a psychrophile ecoclade (Gadanho and Sampaio, 2009). The remaining OTUs were close to the *Glaciozyma* clade and were related to a new taxonomic group in which all isolates have been recovered from glacial ice from montane and polar habitats. These isolates possess psychrophilic growth profiles and represent a new yeast genus that will be formally described in a following report (de Garcia, personal communication). This mountain glacial/soil environment possesses a broad range of fungi adapted to low temperatures, freeze-thawing cycles and oligotrophic conditions, which is most probably of considerable ecological and functional significance.

5. Conclusions

The diversity of fungi in cold environments harbors microbial life much more diverse than previously thought. The patterns of distribution of the different classes were common to other studies of fungi inhabiting diverse cold environments. Chytridiomycota occurred at high relative abundance in the glacier. Additional studies are needed to understand the ecological role of chytrids within these and other extreme environments. For glacial environments in particular, the predominant group consisted of previously undescribed species related to psychrophilic yeasts. Future efforts will focus on developing new isolation strategies for their recovery and subsequent genetic and physiological characterization in order to better establish their taxonomy, level of specialization to extreme conditions, and ecological and biotechnological importance.

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Supplementary data

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