High levels of endemism and local differentiation in the fungal and algal symbionts of saxicolous lecideoid lichens along a latitudinal gradient in southern South America

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Abstract
Saxicolous, lecideoid lichenized fungi have a cosmopolitan distribution but, being mostly cold adapted, are especially abundant in polar and high-mountain regions. To date, little is known of their origin or the extent of their trans-equatorial dispersal. Several mycobiont genera and species are thought to be restricted to either the Northern or the Southern Hemisphere, whereas others are thought to be widely distributed and occur in both hemispheres. However, these assumptions often rely on morphological analyses and lack supporting molecular genetic data. Also unknown is the extent of regional differentiation in the southern polar regions. An extensive set of lecideoid lichens (185 samples) was collected along a latitudinal gradient at the southern end of South America. Subantarctic climate conditions were maintained by increasing the elevation of the collecting sites with decreasing latitude. The investigated specimens were placed in a global context by including Antarctic and cosmopolitan sequences from other studies. For each symbiont three markers were used to identify intraspecific variation (mycobiont: ITS, mtSSU, RPB1; photobiont: ITS, psbJ-L, COX2). For the mycobiont, the saxicolous genera Lecidea, Porpidia, Poeltidea and Lecidella were phylogenetically re-evaluated, along with their photobionts Asterochloris and Trebouxia. For several globally distributed species groups, the results show geographically highly differentiated subsclades, classified as operational taxonomical units (OTUs), which were assigned to the different regions of southern South America (sSA). Furthermore, several small endemic and well-supported clades apparently restricted to sSA were detected at the species level for both symbionts.

Key words: glacial refugia, global distribution, pioneer vegetation on rock, subantarctic subregion

Introduction
Saxicolous 'lecideoid' lichens (Hertel 1984) are genera and species usually described originally under the generic name Lecidea sensu Zahlbruckner (1925) and comprise crustose species with apothecia lacking a thalline margin and with non-sepate ascospores. As such they are a heterogeneous, non-mopholetic group and, although some do belong to the genus Lecidea s. str. (Lecideaceae Chevall.), most belong to other genera and/or families, such as Porpidia Körb, Poeltidea Hertel & Hafellner and Cyclohymenia McCune & M. J. Curtis (Lecideaceae), Carbonea (Hertel) Hertel and Lecidella Körb. (Lecanoraceae Körb.). In addition to their morphological similarities, lecideoid lichenized fungi are strongly associated with green microalgal photobionts of the cosmopolitan class Trebouxiophyceae (Hertel 1984, 2007; Buschbom & Mueller 2004; Schmull et al. 2011; Ruprecht et al. 2012a, 2016; Fryday & Hertel 2014; Zhao et al. 2015).

The family Porpidiaceae was erected by Hertel & Hafellner (Hafellner 1984) to accommodate the genus Porpidia along with other genera formerly included in the Lecideaceae that had a Porpidia-type ascus structure. However, Buschbom & Mueller (2004) showed that Lecidea was phylogenetically nested within the Porpidiaceae and so the latter family was included in the synonymy of the Lecideaceae (Lecidea) by Lumbsch & Huhndorf (2010), and this is still the case in the current issue of Outline of Ascomycota: 2017 (Wijayawardene et al. 2018). The genera Lecidea s. str. (Hertel 1984), Porpidia and Poeltidea as well as Cyclohymenia and Farnoldia Hertel are all assigned to this family, although Farnoldia appears to occupy a peripheral position. The other genus investigated here, Lecidella was also originally included in the Lecideaceae but the species of this genus have an ascus structure very similar to the Lecanora-type and so the genus was included in the Lecanoraceae by Hafellner (1984), which is still its current position (Wijayawardene et al. 2018).

The inconspicuous morphology of 'lecideoid' lichens complicates their systematic treatment. Large taxonomic groups are often distinguishable by just a small number of microscopic traits, such as spore size and septation or ascus-type, but species-level identification can be difficult, often relying on subtle characters...
such as excipulum pigmentation and structure or the secondary metabolites produced. The fundamental taxonomic work on leci-deoid lichens (Hafellner 1984; Hertel 1984; Gowen 1989; Knoph & Leuckert 1994; Inoue 1995; Castello 2003; Knoph et al. 2004; Fryday 2005; Fryday & Hertel 2014) has mostly used morphological and chemical characters, but lacks molecular genetic data. Extensive collections, especially from the Southern Hemisphere, are very often older than 50 years which precludes the use of molecular methods because of the common problem of DNA degradation in mycobiont specimens older than 20 years. During the last decade, molecular re-evaluations have helped to redefine the species concepts behind these diverse groups but were mostly focused on the Northern Hemisphere (Buschbom & Mueller 2004; Schmull et al. 2011; Orange 2014; Zhao et al. 2015, 2016; McCune et al. 2017) and Antarctica (Ruprecht et al. 2010, 2012b). However, Hale et al. (2019) recently demonstrated a biogeographical connection between the Lecidea species of western North America and the southern polar regions, helping to provide a better understanding of distribution and speciation patterns in this group. Nevertheless, intermediate latitudes in the Southern Hemisphere remain understudied and recently published results (Ruprecht et al. 2016) have emphasized the extent of the knowledge gap in southern South American leci-deoid lichens, not only from the mycobiont perspective but also from that of the associated green microalga.

The use of DNA sequence data and phylogenetic methods has revealed that cosmopolitan genera often show locally differentiated subgroups or cryptic species, which can be influenced by ecological factors and may be restricted to isolated areas (Walser et al. 2005; Leavitt et al. 2011; Lumbsch & Leavitt 2011; Branco et al. 2015; Kraichak et al. 2015). Lichens, as well as non-lichenized fungi, with an arctic-alpine distribution in the Northern Hemisphere are, however, a notable exception to this pattern, often comprising relatively homogenous genetic entities, mostly at the species level, with widespread distributions. A number of studies, such as those on Porpidia flavicunda (Ach.) Gowen (Buschbom 2007), Flavocetraria cucullata (Bellardi) Kärnefelt & A. Thell and F. nivalis (L.) Kärnefelt & A. Thell (Geml et al. 2010) as well as for several different types of fungi (Geml 2011), indicate continuing intercontinental gene-flow in species that are present in both the Northern and Southern Hemispheres. However, trans-equatorial dispersal is also shown for other, similar lineages, such as the lichenized fungal genus Lichenomphalia Redhead et al. (Geml et al. 2012) or the species Cetraria aculeata (Schreb.) Fr. (Fernández-Mendoza & Printzen 2013). For the algal partners, although the distribution of green-algal photobionts of the genus Trebouxia Pynamy extends across broad intercontinental regions, especially in the Northern Hemisphere (Leavitt et al. 2015), a pattern of trans-equatorial dispersal with low diversification is common (Muggia et al. 2010; Ruprecht et al. 2012a), with one exception in the most extreme areas of continental Antarctica (Ruprecht et al. 2012a). However, for the mycobionts, strong diversification and endemism in the Southern Hemisphere is expected based on morphology, resulting in the development of several distinct species and genera, such as Lecidea aurantia Fryday, L. cambellensis Fryday (Fryday & Hertel 2014) or L. cancriformis C. W. Dodge & G. E. Baker (Castello 2003), Poeltidea (Hertel 1984), Gondwania Soehling et al., Shackletonia Soehling et al. (Arup et al. 2013) and Protounea (Motyka) Krog. (Calvelo et al. 2005).

The most probable scenarios for disjunct distributions are that they can be caused 1) by vicariance and mid-distance dispersal, as shown in the genus Chroodiscus (Lücking et al. 2008) through the vicinity and interconnection of continental shelves, or 2) by transition from the Arctic to Patagonia in the Pleistocene, resulting in cryptic specialization, as shown in the bipolar lichen Cetraria aculeata (Fernández-Mendoza & Printzen 2013), or 3) by glacial refugia during the last ice ages at the southern end of South America (Paula & Leonard 2006). A good example of this last scenario is the highly differentiated and endemic lichen species Porpidia navarina U. Rupr. & Türk, which is known only from one of the southernmost islands (Isla Navarino) that was ice free during the Last Glacial Maximum (Douglass et al. 2005; Ruprecht et al. 2016). Additionally, evolutionary processes such as adaptation and subsequent specialization to the harsh climate conditions in Antarctic cold deserts (Ruprecht et al. 2010, 2012b; Schroeter et al. 2011) can also lead to high local differentiation in global species and endemism in the southern polar regions.

Lichens are ideal model-systems to test these hypotheses because several genera and species are globally distributed and form locally differentiated subgroups (Fernández-Mendoza et al. 2011). Additionally, at least double the information is available compared to other organisms because lichens consist of a symbiotic relationship between two or more independently distributed partners. This main symbiotic relationship is formed by a fungus (mycobiont) and green algae and/or cyanobacteria (photobiont). Furthermore, a diverse community of associated bacteria (Grube et al. 2015; Aschenbrenner et al. 2016), algae (Peksa & Skálov 2011; Ruprecht et al. 2014; Moya et al. 2017), endolithic or lichenicolous fungi and basidiomycete yeasts (Lawrey & Diederich 2003; Arnold et al. 2009; Spribille et al. 2016) are part of the lichen thallus.

This study focuses on the geographically isolated, tapering southern end of the South American continent (southern Patagonia, including the islands around Tierra del Fuego and Cape Horn). Due to climatic conditions equivalent to Maritime Antarctica, the southern subpolar region (or subantarctic subregion, which is characterized by an absence of arboreal vegetation and is located between the Antarctic Divergence and Subtropical Convergence: Morrone 2000; Brummitt 2001) is included as part of the Antarctic floral kingdom (Takhatajan & Cronquist 1986). The subantarctic subregion extends northwards through the continent at increasing elevations along the mountain ranges of the southern Andes (Morrone 2000). To the south, Maritime Antarctica is the closest landmass, separated by c. 900 km of ocean and the Antarctic Circumpolar Current (Allison et al. 2010; McCave et al. 2014). These areas are colonized by specialized cold-adapted organisms, which often act as pioneer vegetation (Hertel 1984; Caccianiga & Andreis 2004; Bilovitz et al. 2015). Among other organisms, the globally distributed saxicolous leci-deoid lichens are one of the most frequent components of the biota, forming diverse communities on rocks and boulders, mainly in treeless areas above a temperate rainforest (Fig. 1; Hertel 2007; Goffinet et al. 2012; Ruprecht et al. 2016).

In the current study, we wish to explore the genetic diversity of the two dominant symbionts (myco- and photobiont), along with their distribution and diversification along a latitudinal gradient in southern South America. These were newly estimated with three marker datasets for each symbiont, based on both tree-based and non-tree-based clustering methods, including distance-based clustering, model-based phylogenies and coalescent analyses. To accomplish this, both symbionts of saxicolous lecideid lichen specimens from southern South America were placed in a global context using sequence information from the first author’s project.
framework, from collaborating partners and from international databases (e.g. GenBank). Additionally, newly generated sequences of species that had previously only been described morphologically were included, as well as sequences of uncertain position in the genera *Lecidea* s. str. (Hertel 1984), *Porpidia*, *Poeltidea*, *Lecidella* (mycobiont) and *Asterochloris* Tschermak-Woess and *Trebuoxia* (photobiont).

**Material and Methods**

**Collecting sites and material**

One hundred and eighty-five saxicolous lecideoid lichen samples were collected in southern South America along a latitudinal gradient following the subantarctic climatic subregion by increasing elevation from south (Cerro Bandera, Isla Navarino, Chile, 55°S, 620 m a.s.l.) to north (Cerro Catedral, Bariloche, Argentina, 41°S, 2100 m a.s.l.) and including some areas at a lower elevation in southern Chile.

The specimens were collected from siliceous rock in areas above the tree line that were dominated by subantarctic climatic conditions with an annual mean temperature (BIO1) of 0 to 7.8 °C and an annual precipitation (BIO12) of 320 to 1640 mm (Karger et al. 2017; Fig. 2, Supplementary Material Files S1-1 & S1-2, available online).

All our specimens collected from southern South America (sSA) are deposited in the Herbarium of the University of Salzburg (SZU).

**DNA amplification and sequencing**

Total DNA was extracted from individual thalli using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer’s instructions. The lichen material was scraped off with a sterilized scalpel from the centre of the thallus and included apothecia. The PCR mix contained 0.5 units of GoTaq DNA polymerase, 0.2 nM of each of the four dNTPs, 0.3 μM of each primer and c. 1 ng genomic DNA.

For each symbiont, three markers were amplified and sequenced with the primers presented in Supplementary Material File S1-3, with conditions as described in Ruprecht et al. (2014) and Ruprecht et al. (2016). Unpurified PCR products were sent to Eurofins Genomics/Germany for sequencing.

**Mycobiont.** The internal transcribed spacer region of the nuclear ribosomal DNA (ITS) was amplified for all specimens. Furthermore, the mitochondrial small subunit (mtSSU) and the large subunit of DNA-dependent RNA polymerase 2 (RPB1) were amplified for the *Lecidea/Porpidia/Poeltidea* group (see Supplementary Material File S1-3).
Photobiont. To obtain a first overview of the different taxa at species and/or genus level of the associated green microalgae available to the mycobiont, a broad screening along the internal transcribed spacer region of the nuclear ribosomal DNA (ITS) was performed using the different primer pairs ITS1T and ITS4T (Kroken & Taylor 2000), ITS-sense-A and ITS-antisense-A (Ruprecht et al. 2014) and NS7m and LR1850 (Bhattacharya et al. 1996) as described in Ruprecht et al. (2016). Also, for Trebouxia a chloroplast marker, a variable protein-coding gene including an intergenic spacer region (psbJ-L), and a fragment of the mitochondrial cytochrome oxidase subunit 2 gene (COX2) were chosen (Supplementary Material File S1-3). Additionally, all three markers were sequenced from four Trebouxia cultures (2 × S02/Antarctica, A02/Antarctica and A12/Sweden; Supplementary Material File S1-2d) provided by S. Ott (Düsseldorf), to calibrate the concatenated dataset.

Phylogenetic analyses

Sequences were assembled and edited using Geneious Pro 6.1.8 (www.geneious.com), and aligned with MAFFT v7.017 (Katoh et al. 2002) using pre-set settings (algorithm, auto select; scoring matrix, 200PAM/k = 2; gap open penalty, 1.34–0.123).

Maximum likelihood analyses (ML) were performed using the IQ-TREE web server (Trifinopoulos et al. 2016) with default settings (ultrafast bootstrap analyses, 1000 BT alignments, 1000 max. iterations, min. correlation coefficient: 0.99, SH-aLRT branch test with 1000 replicates) and presented as a consensus tree. The respective evolutionary models were selected with the implemented model finder (Kalyaanamoorthy et al. 2017) of the program IQ-TREE (Supplementary Material File S1-4).

The Bayesian phylogenies were inferred using the Markov chain Monte Carlo (MCMC) procedure as implemented in the program MrBayes 3.2 (Ronquist & Huelsenbeck 2003). The analysis was performed assuming the general time reversible model of nucleotide substitution including estimation of invariant sites and a discrete gamma distribution with six rate categories (GTR + I + Γ; Rodriguez et al. 1990). Two runs with 5 million generations, each starting with a random tree and employing four simultaneous chains, were executed. Every 1000th tree was saved into a file. Subsequently, the first 25% of trees was deleted as the ‘burn-in’ of the chain. A consensus topology with posterior probabilities for each clade was calculated from the remaining 3751 trees. The phylogenies of the mycobiont of the Lecidea/Porpidia/Poeltidea group were rooted with Farnoldia jurana subsp. jurana (Schaer.) Hertel, and for Lecidella with species of the closely related genera Lecanora Ach., Rhizoplaca Zopf and Carbonnea; the algal phylogenies were midpoint rooted. All phylogenies were visualized with the program Figtree v1.4.3 (Rambaut 2014).

OTU- and cluster delimitation

For each phylogenetically coherent group (Lecidea/Porpidia/Poeltidea, Lecidella, Asterochloris and Trebouxia), the ITS marker was used to generate operational taxonomical units (OTUs) using automatic barcode gap discovery (ABGD; Puillandre et al. 2012) to additionally define the well-supported intraspecific (cryptic) subgroups with a distance-based method. The default settings

Fig. 2. World map showing the locations of the included accessions obtained from GenBank and from our own database. Pink circles show the collection points of the mycobiont and green circles of the photobiont accessions. The enlarged map (inset) shows the sampling sites from this study in southern South America.
were used, except that ‘X’ (relative gap) = 0.9 and the distance JC69 were chosen. OTUs with a sequence similarity lower than 97.5% were divided into subunits using phylogenetic criteria based on Leavitt et al. (2015) for Trebouxia (Supplementary Material File S1-5).

An alternative cluster delimitation specifically looking for boundaries between population and species-level processes was implemented by a recursive multi-tree application of the general mixed Yule-coalescent model (bGMYC; Pons et al. 2006; Reid & Carstens 2012; Fujisawa & Barraclough 2013) on the ITS phylogeny of Lecidea/Porpidia/Poeltidea to define species-clades and/or species-complexes on a higher scale. For this purpose, substitution model adequacy was estimated using ML reconstructions in IQ-TREE (Minh et al. 2013; Kalyaanamoorthy et al. 2017), resulting in the selection of a TIM2 transition model with empirical frequencies and a Ratefree (Yang 1995; Soubrier et al. 2012) model with 4 relaxed gamma categories. Time explicit phylogenetic reconstructions were carried out in Beast v2.5.1 (Bouckaert et al. 2014) using a strict clock, the suitability of which was previously assessed in MEGA (Tamura et al. 2011), and a constant size coalescent prior, which is the most conservative tree model in terms of including false positives. A maximum clade credibility tree was calculated in TreeAnnotator (Bouckaert et al. 2014) using most recent common ancestor (mrca) dates. The bGMYC analyses were carried out as implemented in the homonymous R package. A single GMYC analysis was iteratively run on a subset of 1000 trees chosen randomly from the posterior distribution, using a chain length of 50 000 sampling steps, a burn-in of 40 000 and a thinning parameter of 100. The results of all GMYC analyses are summarized in a matrix of pairwise co-assignment probabilities for each haplotype. To obtain a consensus partition we processed the co-assignment matrix using an arbitrarily chosen co-assignment threshold of 0.5, and a less arbitrary approach making use of k-medoid clustering (Kaufman & Rousseeuw 1990) and optimum average silhouette width as validation criterion to estimate the optimum number of clusters (Ortiz-Alvarez et al. 2015). For the latter we used the function pamk as implemented in the R package ‘fpc’ (Henning 2014) on the co-assignment matrix converted into its dissimilarity correlate. Clustering validation was carried out using the function cValid from the homonymous R package (Brock et al. 2008); the discrepancy between stability metrics highlights the discrepancy between the number of clusters and the number of sequences per cluster.

For the multi-marker trees, no conflict was found with the single marker trees, thus they are not shown.

OTUs with a sequence similarity lower than 97.5% were divided into subunits using phylogenetic criteria based on Leavitt et al. (2015) for Trebouxia. This concept was used as analogous to an appropriate delimitation method for cryptic (molecular) diversification on a molecular basis to show the biogeographical distribution of the OTUs (Tables 1–3). These parameters were used for all four overall phylogenies based on the ITS marker. The sequence similarity values of the OTUs with at least three accessions are summarized in Supplementary Material File S1-5. The biogeographical distribution of the locally differentiated OTUs was added beside the OTU, voucher and species information in the figures.

Assignment of unknown accessions to mycobiont species was also based on morphological data, but these data are not included here. Extending the analyses to include morphological characters would increase their complexity and result in an unacceptable decrease in clarity, while also resulting in either the molecular or morphological datasets, or more probably both, not receiving the full treatment they deserve. Therefore, the analysis of the morphological data, along with the taxonomic consequences resulting from the analyses of both datasets, will be dealt with in a separate publication.

**Lecidea/Porpidia/Poeltidea-ITS.** (Fig. 3, Supplementary Material Files S2-1a & b). This molecular phylogeny includes all relevant taxonomically identified sequences of the genera Lecidea, Porpidia, Poeltidea and Cyclohymenia provided by the project framework of the first author or downloaded from the NCBI database (GenBank) to place the newly generated sequences of this study into a global context. Redundant sequences in terms of characters and distribution were not included. The final data matrix for the phylogeny comprised 204 single nrITS sequences with a length of 598 characters and includes sequences of specimens of the genera Lecidea (157) Porpidia (42), Poeltidea (3) and Cyclohymenia (2); it was rooted with Farnoldia jurana subsp. jurana as outgroup. Figure 3 displays the collapsed tree at OTU level, the voucher information included in the OTUs is provided in Supplementary Material File S2-1a and the complete tree is shown in S2-1b. The phylogenetically delimited groups revealed were assigned to OTU-, species-, cluster- and genus level.

All three analyses (distance-based, model-based and bGMYC) showed similar topologies, but at different levels. The groups gained with a distance approach (OTUs) were used to show intra-specific and/or cryptic speciation; model-based approaches were used for the assignment at species level; clusters based on bGMYC were used for grouping closely related species or dividing highly heterogeneous species.

Altogether, 121 OTUs (87 Lecidea, 30 Porpidia, 1 Cyclohymenia, 3 Poeltidea) were classified. These OTUs were assigned to species-groups (25 Lecidea, 18 Porpidia, 1 Cyclohymenia, 2 Poeltidea) and clusters (19 Lecidea, 8 Porpidia, 1 Cyclohymenia, 3 Poeltidea).

The backbone of this phylogeny is not supported but at least four main groups can be recognized (Fig. 3). The first group, with low support, is formed solely by species of the genus Lecidea s. str. However, it can be considered a consistent group because it is strongly supported by the three-marker phylogeny (Supplementary Material File S2-2). The Southern Hemisphere lineage (Porpidia navarina) is situated outside the Lecidea

**Results**

**Phylogenetic analyses, OTU and cluster delimitation**

Four overall phylogenies for the saxicolous genera Lecidea/ Porpidia/Poeltidea (Lecidaceae), Lecidella (Lecanoraceae), Asterochloris and Trebouxia (Trebouxiophyceae) were estimated using the ITS marker (Figs 3–6, Supplementary Material Files S2-1a & b, available online). Additionally, two multi-marker trees for Lecidea/Porpidia/Poeltidea (ITS/mtSSU/IRPB1; Supplementary Material File S2-2) and Trebouxia (ITS/pssL/COX2; Supplementary Material File S2-3) were also estimated.

In all cases the ML analyses (IQ-TREE web server; Trifinopoulos et al. 2016) of the six phylogenies recovered the same tree topology as the MrBayes analysis (Ronquist & Huelsenbeck 2003). Therefore, we present here only the Bayesian tree with support values of the ML analyses.
Fig. 3.collapsed phylogeny on OTU-level including all available relevant taxonomically identified sequences of the genera Lecidea, Porpidia, Poeltidea and Cyclophymenia using the marker ITS. Voucher information included in the OTUs is provided in Supplementary Material File S2-1a and the complete tree is shown in S2-1b (available online). Further information is also available in Supplementary Material Files S1-2a & S1-2c. Alphanumeric codes represent OTUs and numbers in brackets indicate the number of sequences comprising that OTU (see also Supplementary Material File S1-5). The biogeographic distribution (NH, Northern Hemisphere; C, cosmopolitan; sSA, southern South America; Ant, Antarctica) has been added beside the OTUs. New sequences of specimens from sSA are in bold italics and those from other parts of the world are in bold. The vertical bars beside the phylogeny show the affiliation to clusters and genera. The bootstrap values with ≥ 95 support of ML analyses were directly mapped on the Bayesian tree with ≥ 0.90 (grey) and ≥ 0.95 (black) support posterior probability values (branches in bold). In colour online.
group and next to the second group, which is an intermixed group of Lecidea and Porpidia species including two well-supported accessions of the genus Cyclohymenia. The third group is formed solely by species of the genus Porpidia and the fourth by the Southern Hemisphere genus Poeltidea.

The Lecidea group forms 17 clusters. The well-supported, heterogeneous and cosmopolitan species cluster L01 is formed by the L. atrobrunnea (Ramond ex Lam. DC.) Schaer. clade with one southern polar OTU (Lcd05) and a well-supported sister group containing Lecidea sp. 1 (Lcd09a, b; endemic to SsA), L. promiscens Nyl. (cosmopolitan) including three SsA OTUs (Lcd10-12), and L. swartzioidea Nyl. (Northern Hemisphere). Cluster L02 includes L. confluens (Weber) Ach. and an accession from GenBank (Porpidia speirea (Ach.) Kremp et al. 2011). The placement of this latter accession in the Lecidea s. str. group could be caused by an incorrect species assignment.

Fig. 4. Phylogeny of the genus Lecidella with accessions from SsA (see Supplementary Material File S1-2a, available online) integrated in the species concept and most of the published accessions (Zhao et al. 2015; see also Supplementary Material File S1-2c) using the marker ITS. OTU numbers precede voucher numbers and the published species names from GenBank, as well as the biogeographic distribution information, for each OTU is included (NH, Northern Hemisphere; C, cosmopolitan; SsA, southern South America; Ant, Antarctica). New sequences of specimens from SsA are in bold italics and those from other parts of the world are in bold. The vertical bars beside the phylogeny show the affiliation to the clades. The bootstrap values with ≥ 95 support of ML analyses were directly mapped on the Bayesian tree with ≥ 0.90 (grey) and ≥ 0.95 (black) support posterior probability values (branches in bold). In colour online.
analyses were directly mapped on the Bayesian tree with this species as closely related to (mens from sSA are marked in bold italics. The vertical bars beside the phylogeny show the affiliation to the genera. The bootstrap values with Files S1-2a & S1-2c, available online). OTU numbers precede voucher numbers and the published species names from GenBank as well as the biogeographic distribution legend:

**NH** Northern Hemisphere  
**C** Cosmopolitan  
**SSA** southern South America  
**Ant** Antarctica

Fig. 5. Phylogeny of the genus Asterochloris including all available relevant taxonomically identified sequences using the marker ITS (see Supplementary Material Files S1-2a & S1-2c, available online). OTU numbers precede voucher numbers and the published species names from GenBank as well as the biogeographic distribution for each OTU is included (NH, Northern Hemisphere; C, cosmopolitan; SSA, southern South America; Ant, Antarctica). New sequences of specimens from SSA are marked in bold italics. The vertical bars beside the phylogeny show the affiliation to the genera. The bootstrap values with ≥ 95 support of ML analyses were directly mapped on the Bayesian tree with ≥ 0.90 (grey) and ≥ 0.95 (black) support posterior probability values (branches in bold). In colour online.

(Lecidea confluens and P. speirea are morphologically quite similar and mainly distinguished by the asci-type). This assumption is supported by two accessions of P. speirea from China that are included in the phylogeny and are part of cluster P03. This agrees with the findings of Buschbom & Mueller (2004), who described this species as closely related to P. tuberculosa (Sm.) Hertel & Knoph, which was confirmed in this phylogeny.

Cluster L03 (Lecidea sp. 2) forms a highly supported and very homogenous clade, comprising specimens from Antarctica, the Arctic and sSA, and is formed by a single cosmopolitan OTU (Lcd18).

Cluster L04 is formed by the cosmopolitan L. andersonii Filson complex and Lecidea sp. 3 clade related to L. laboriosa Müll. Arg. (Northern Hemisphere), a Californian specimen of Lecidea violascens H. Magn. and an undetermined specimen from the Austrian Alps (Lecidea sp. UR00280, Lcd34). The first group contains distinct OTUs of Antarctic, North American and Chinese species of L. polyypicnidophora U. Rupr. & Türk, the cosmopolitan L. andersonii, the Antarctic Lecidea sp. UCR1 and two not assignable accessions from North America. The second group (Lecidea sp. 3 clade) is formed solely of species from southern South and North America, including OTUs (Lcd27-32) containing specimens from both areas.

Several well-distinguished species form strongly supported clusters, such as the variable accessions from the Northern Hemisphere and maritime Antarctica of the L. silacea Ach. clade together with an unidentified specimen from sSA (Lecidea sp. UR00130, Lcd 40, cluster L05) and L. fuscoatrina Hertel & Leuckert (cluster L06), followed by L. plana (J. Lahm) Nyl. (Northern Hemisphere; cluster L07), L. lithophila (Ach.) Ach. (Northern Hemisphere, maritime Antarctica; cluster L08), and by the Northern Hemisphere species L.
Fig. 6. Phylogeny of the genus Trebouxia with accessions from sSA integrated into the species concept and most of the published accessions of Leavitt et al. (2015) using the marker ITS (see Supplementary Material Files S1-2b & S1-2d, available online). OTU numbers precede voucher numbers and the published species names from GenBank as well as the biogeographic distribution information for each OTU is included (NH, Northern Hemisphere; C, cosmopolitan; sSA, southern South America; Ant, Antarctica). New sequences of specimens from sSA are in bold italics and those from other parts of the world are in bold. The vertical bars beside the phylogeny show the affiliation to the clades. The bootstrap values with $\geq 95$ support of ML analyses were directly mapped on the Bayesian tree with $\geq 0.90$ (grey) and $\geq 0.95$ (black) support posterior probability values (branches in bold). In colour online.
leucothallina Arnold, L. obluridata Nyl. and an unidentified accession (MK990108, Lcd46) from North America (cluster L09).

Two recently described species, Lecidea aptrootii M. Khan et al. (Khan et al. 2018) and L. uniformis McCune (McCune et al. 2017), are molecularly very closely related to the accession of L. fuscoatra (L.) Ach. var. grisella (Flörke) Nyl. (Zhao et al. 2016), a taxon which was recognized by Aptroot & Van Herk (2007) at the species level as L. grisella. Together with L. fuscoatra, they form the strongly supported cluster L10. This is sister to cluster L11, which includes the Antarctic (OTU Lcd58) and, so far, endemic species L. cancriformis and closely related, but heterogeneous, accessions solely from sSA (Lcd52-60). Another new and endemic species from sSA (Lecidea sp. 4, Lcd61-64) forms cluster L12, and an unidentified specimen (UR00096, Lcd65) forms the highly supported cluster L13.

Cluster L14 is formed by the very common, heterogeneous and cosmopolitan species group L. lapicida (Ach.) Ach. It includes the cosmopolitan L. lapicida var. pantherina (Ach.) Ach. and is sister to the Northern Hemisphere L. lapicida var. lapicida (Ach.) Ach. These subspecies show no morphological differentiation and are separated only by their different chemotypes but, according to Hertel (1995), they have different ecological requirements. However, cluster L14 contains the two most common OTUs restricted to sSA (Lcd66 and Lcd70), as well as OTUs restricted to Antarctica (Lcd70), the Northern Hemisphere or with an alpine-Antarctic distribution (Lcd67). The now cosmopolitan species L. medusula (C. W. Dodge) Hertel, previously reported

| Species - distribution | n | OTU - distribution |
|------------------------|---|-------------------|
| Lecidea atrobrunnea - clade* | globally | 2 | LP_Lcd05 southern polar |
| Lecidea auriculata - clade | globally | 14 | LP_Lcd81b, 83 sSA |
| Lecidea lapicida - clade | globally | 48 | LP_Lcd66, 67, 70, 75 sSA |
| Lecidea promiscens | globally | 9 | LP_Lcd10 - 12 sSA |
| Lecidella stigmataea | globally | 13 | LL_ST07, 08, 09, 15-20 sSA |

Table 1. Geographical distributions of selected mycobiont species and OTUs, arranged in groups according to shared distributions at different levels of clustering. Subtotals of numbers of species, specimens (n) and OTUs according to shared distributions are presented, with the total given below. Species and OTUs with different distributions (globally/sSA) are marked in bold. Accessions occurring in Antarctica and sSA are summarized as southern polar. * = species that were found in two different categories and were included only once in the total sum. See Supplementary Material File S1-2a (available online) for further information on the listed specimens.
only for the Southern Hemisphere (Fryday & Hertel 2014), forms cluster L15 with specimens from sSA, the Arctic and the Austrian Alps at the base of this clade. Finally, an Arctic specimen of L. protobacina Nyl. and an unidentified specimen from continental Antarctica (T48883b, Lcd80) form the clusters L16 and L17. The species included in this group are all part of Lecidea s. str. (Hertel 1984).

The second group, which contains species of the genera Lecidea s. str. and Porpidia, is highly supported, with the Southern Hemisphere L. kalbii Hertel (cluster L19) and the newly described species/genus Cyclophymenia epithillica McCune & M. J. Curtis (cluster C01) basal to the group, although with weak support. The cosmopolitan clade L. auriculata Th. Fr. is divided at OTU level into Northern and Southern Hemisphere accessions (Lcd81a,b-84) and L. tessellata Flörke is formed by a single cosmopolitan OTU (Lcd85, cluster L18). The well-supported, Northern Hemisphere group of Porpidia species includes P. albocaeruleascens (Wullen) Hertel & Knoph, P. degelii (H. Magn.) Lendemer, P. rugosa (Taylor) Coppins & Fryday (cluster P01), P. hydrophila (Fr.) Hertel & A. J. Schwab (cluster P02) and P. flavicunda, P. melinodes (Korb.) Gowan & Ahit, P. speirea and P. tuberculosa (cluster P03). However, species of the genus Porpidia are clearly distinguishable morphologically from species of Lecidea, by their ascus type and additionally by the associated green microalgal genus of the family Asterochloris (Wirth et al. 2013; Ruprecht et al. 2016).

The third group is formed by four clusters of Porpidia species and is divided into two well-supported main subgroups. One is formed by cluster P05, which includes the heterogeneous cosmopolitan clade inc. sed. Porpidia sp. 1 together with P. cinereoastra (Ach.) Hertel & Knoph, P. contraivonemosa (Arnold) Knoph & Hertel, P. musiva (Korb.) Hertel & Knoph, P. irrigua A. Orange and Porpidia sp. (UR00248, Porp17), with P. striata Fryday and P. islandica Fryday et al. forming the highly supported clusters P06 and P07. The other strongly supported subgroup is formed by the heterogeneous and cosmopolitan cluster P08, including P. flavocruenta Fryday & Buschbom, several heterogeneous accessions of P. macrocarpa (DC.) Hertel & A. J. Schwab, and the quite differing accessions from Turkey and China that were identified as P. crustulata (Ach.) Hertel & Knoph.

The genus Poeltidea, which occurs only in the Southern Hemisphere, forms the fourth group and consists of three clusters, POE01 and POE02 (Poeltidea perista (Nyl.) Hertel & Hafellner clade), and POE03 (Poeltidea sp. 1).

Lecidea/Porpidia/Poeltidea-ITS/mtSSU/RPB1. (Supplementary Material File S2-2). The final data matrix of this phylogeny contains 204 concatenated sequences of the markers ITS, mtSSU and RPB1 with a length of 2115 characters; it includes sequences of specimens of the genera Lecidea, Porpidia and Poeltidea and was rooted with Farnoldia jurana subsp. jurana as outgroup. Specimens from the project framework of the first author (Antarctica, Arctic, Austria) with the same three markers were added.

All available sequences from this study were included to show the abundance of the different specimens in the studied areas of sSA. The phylogenetically delimited groups revealed were assigned to OTU-, species- and genus level.

This multi-marker phylogeny is not fully comparable to the overall single marker (ITS) phylogeny of Lecidea/Porpidia/ Poeltidea because of the limited availability of sequences of the chosen markers in GenBank. The topology is, in most cases, similar and it forms the same four groups, but in many cases they show greater support. The Lecidea group, at least, is strongly supported in this phylogeny. Due to the limited availability of Porpidia sequences in GenBank, the topology of this group is slightly different, with P. navarina clustering together with P. cinereoastra and P. macrocarpa, but with low support. Finally, the genus Poeltidea with two species is still at the base of this phylogeny.

The OTUs obtained from the ITS sequences are still very well supported. Many clades clearly show a local differentiation at OTU level and are endemic to sSA. The two largest clades (OTUs Lcd66 and Lcd70) are part of the cosmopolitan L. lapicida cluster and are the most abundant accessions. These are followed by Porpidia navarina and Lecidea sp. 1 (both species endemic to sSA), L. promiscens (cosmopolitan species but with sSA OTUs) and other smaller groups.

Lecidella-ITS. (Fig. 4). To place the new accessions of Lecidella from sSA into a global context, the species concept and most of the published accessions of Zhao et al. (2015) were used. Additionally, several sequences from GenBank and from the project framework of the first author were added to this dataset.
Redundant sequences in terms of characters and distribution were not included. The final data matrix of this phylogeny contains 76 sequences of the marker ITS with a length of 538 characters, and was rooted with species of the genera *Carbonea*, *Leconora*, and *Rhizoplaca* to obtain well-defined units in the genus *Lecidella*.

The backbone of the phylogeny is unresolved but four strongly supported main clades are formed; three (*L. enteroleucella* (Nyl.) Hertel, *L. stigmatella* (Ach.) Hertel & Leuckert and *L. elaeochroma* (Ach.) Choisy) are the same as those identified by Zhao et al. (2015), with the fourth clade (*Lecidella* spp. nov.) containing two species occurring only in Antarctica and sSA. All available sequences for this group were included to show the abundance of the different accessions. The phylogenetically delimited groups revealed were assigned to OTU-, clade- and genus level.

*Lecidella enteroleucella* is still the only member of the first clade as described by Zhao et al. (2015).

The second clade (*L. stigmatella*) is completely unresolved. *Lecidella patavina* (A. Massal.) Knoph & Leuckert and *L. stigmatella* are intermixed and not assignable. These two species differ most noticeably in that the hymenium of *L. patavina* is inspersed with oil droplets, whereas that of *L. stigmatella* is not. That the accessions of these two species are intermixed perhaps indicates that either only one species is involved or that the defining character has been interpreted inconsistently. *Lecidella greenii* U. Rupr. & Türk and *L. sipleti* (C. W. Dodge & G. E. Baker) May. Inoue form well-supported lineages and the 12 sequences for this group are included to show the abundance of the different accessions. The phylogenetically delimited groups revealed were assigned to OTU-, clade- and genus level. The backbone of this clade is unresolved. The phylogenetically delimited groups revealed were assigned to OTU-, clade- and genus level. The topology of the species from GenBank shows a similar pattern to the phylogeny already described in Ruprecht et al. (2014).

Only one accession from sSA clusters together with the cosmopolitan species *A. woessiae* Škaloud & Peksa. This highly supported and homogeneous clade is formed by 19 specimens (OTU Ast24) occurring only on Isla Navarino and one accession from the other side of the Beagle Channel (Tierra del Fuego) in the southernmost part of sSA. Four other accessions (OTUs Ast20–23) are placed in the main group with low support.

*Trebouxia*-ITS. (Fig. 6). To place the new accessions of *Trebouxia* from sSA into a global context, the species/OTU concept and the dataset reduced to one accession of each OTU of Leavitt et al. (2015) was used. Additionally, several new sequences from GenBank and from the project framework of the first author were added to this dataset. Redundant sequences in terms of characters and distribution were not included. The final data matrix of this phylogeny contains 157 ITS sequences with a length of 805 characters and was midpoint-rooted.

The phylogenetically delimited groups revealed were assigned to OTU- and clade level, as described in Leavitt et al. (2015). The backbone of the phylogeny is unresolved but four strongly supported main clades were formed (A, I, S and G) that correspond to those of Leavitt et al. (2015). Altogether, clade A includes 39 OTUs with the specimens of this study forming part of two cosmopolitan (A02 and A04) and four locally differentiated (A36–A39) OTUs. A04 has a sequence similarity of 96.7% and was subdivided into two subunits (A04a and A04b).
Three different groups were formed by accessions assigned to Clade I. Due to a sequence similarity below the threshold of 97.5%, the subOTU I01i of Leavitt et al. (2015) was divided into two subunits (I01i and I01j) with a cosmopolitan and southern polar distribution, plus an independent OTU I17 occurring solely in sSA. The addition of the diverse accessions from sSA to the existing OTU I01, which already has 10 subOTUs (I01a–i) described by Leavitt et al. (2015), resulted in most of them being transferred into distinct OTUs. However, these have not been renamed here since this is an open system and a regrouping with new accessions is expected in the future.

Fifty percent of the sSA accessions of this marker are contained in the cosmopolitan OTUs S02 and S07 of clade S. S02 was subdivided into four subunits (S02, S02a by Leavitt et al. (2015) and S02b, S02c in this study), with the accessions from sSA being assigned only to S02 together with Northern Hemisphere specimens. S02b is formed solely by a specimen from continental Antarctica; a similar finding was described in Ruprecht et al. (2012a; T. jameisi ssp. is equivalent to S02b in this study). Another strongly supported OTU (S16) consists only of accessions from sSA. The cosmopolitan OTU S02 contains three accessions from the southernmost areas of sSA and is part of a strongly supported clade dominated by specimens from Iceland.

No accession from this study is part of clade G.

**Trebouxia-ITS/psbJ-L/COX2.** (Supplementary Material File S2-3). The data matrix of this phylogeny contains 217 concatenated sequences of the markers ITS, psbJ-L and COX2 with a length of 1693 characters. Only sSA specimens are included to demonstrate the instaspecific differentiation. This dataset was calibrated with four cultured Trebouxia specimens (see Supplementary Material File S1-3d; 2 × S02/Antarctica, A02/Antarctica and A12/Sweden). Interestingly, the psbJ-L sequences of A02 of the cultured specimen from Antarctica are different from the North American specimen from Leavitt et al. (2015).

The dataset still shows the same number of OTUs as the ITS phylogeny (Fig. 6) and the grouping is the same. Clade A shows nine well-supported groups at species level (A02, A12, A38, A39, A04a, A04b, A36, A37), with A36 and A04b being closely related. The subOTUs A04a and A04b are more separated than in the ITS phylogeny due to the marker psbJ-L.

Clade I is divided into two subOTUs (I01i and I01j) and one newly developed distinct OTU (I17) because of its heterogeneous structure. No COX2 sequences were included because they were not assignable.

More than half of the sequences are included in the homogenous and cosmopolitan OTUs S02 and (a smaller part) in S07. These groups are closely related and share similar COX2 sequences. OTU S16, which occurs only in sSA, is clearly separated at species level from S02 and S07, and S20 is situated at the base of clade S.

**Distribution of species and OTUs: globally distributed vs. restricted to sSA and/or southern polar regions**

Altogether, 185 mycobiont specimens forming 54 OTUs assigned to 24 species of the genera Lecidea, Porpidia, Poeltidea and Lecidella were identified. Four species of the genus Lecidea (Lecidea sp. 2, Lecidea sp. 3, L. medusula, L. tessellata) and Porpidia macrocarpa are globally distributed at species and OTU level.

By far the most abundant accessions are formed by locally differentiated OTUs (Lcd66, Lcd70; 48 accessions) and belong to the cosmopolitan species-clade Lecidea lapicida (cluster L14), with the next most frequent groups being Lecidella stigmatae (13) and Lecidea promiscens (9). Finally, a total of 12 species or single sequences are so far described and/or known only from sSA or the southern polar regions (Table 1, Figs 3 & 4, Supplementary Material Files S2-1a, b & S2-2).

The photobionts comprise 202 accessions that are assigned to 20 OTUs. Most of the algal specimens (128) belong to globally distributed taxa, especially Tr_S02 with 84 accessions, followed by Tr_S07, Tr_A02, Tr_A12, Tr_A04a, Tr_S20 and a single accession of Asterochloris woessiae from a lower elevation area close to Esquel, Argentina. The very heterogeneous OTU Tr_I01i (Leavitt et al. 2015) was divided into two subOTUs: I01i with a global distribution and I01j that occurs in Antarctica and sSA. OTU I17 occurs solely in sSA. A surprisingly high number of 74 accessions (Asterochloris and Trebouxia) form 13 clearly separated OTUs and have, so far, been found only in sSA (Table 2, Figs 5 & 6, Supplementary Material File S2-3).

In summary, for the mycobiont the percentage distribution of accessions at OTU level shows a high rate of local differentiation and endemism for the sSA specimens versus those that are globally distributed (87:13). In particular, Parque Nacional Torres del Paine and Morro Chico, with 100% each, and the southernmost sampling point, Isla Navarino, with 90% have the highest amount of specialized accessions. In contrast, the photobiont OTUs show a higher rate of globally distributed accessions (32:68). However, both symbionts show no significant specialization along the latitudinal gradient in southern South America (Table 3).

**Discussion**

For the four re-evaluated groups Lecidea/Porpidia/Poeltidea, Lecidella (mycobiont), Asterochloris and Trebouxia (photobiont), the geographically isolated southern end of the South American continent supports a high degree of locally differentiated subclades (OTUs) in globally distributed species, as well as lineages currently known only from sSA at the species, coalescent-defined cluster and genus level. This was, to some extent, unexpected for these mostly globally distributed genera and can partially be explained by the lack of sequence information for most of the Southern Hemisphere lecideoid mycobiont species (Knoph & Leuckert 1994; Fryday & Hertel 2014). This also applies to the photobionts because of the limited availability of molecular studies for the southern polar regions (e.g. Muggia et al. 2010; Fernández-Mendoza et al. 2011; Ruprecht et al. 2012a).

Local differentiation (cryptic speciation) appears quite common in lichen-forming fungi (Leavitt et al. 2011; Lumbsch & Leavitt 2011; Kraichak et al. 2015; Dal Grande et al. 2017), but has rarely been described for the most common and widespread lichen photobiont taxa Trebouxia (Fernández-Mendoza et al. 2011; Ruprecht et al. 2012a; Leavitt et al. 2015) and Asterochloris (Škaloud et al. 2015). In particular, the lichen cluster (L14, Fig. 3) Lecidea lapicida, which occurs in polar and high mountainous regions worldwide (Hertel 1984; Hertel & Andreev 2003; Hafellner & Türk 2016), shows locally differentiated accessions at the OTU level that are currently known only from sSA. Surprisingly, Austrian and Antarctic (Lcd67) accessions are closely related, but they are clearly distinct from the two main OTUs (Lcd66 and Lcd70).
that comprise 48 of the 185 sSA specimens. Another cosmopolitan (Northern Hemisphere and sSA) and common lichen species is *L. auriculata*, which includes a quite homogenous but widely distributed OTU (Lcd81b, 13 specimens) restricted to sSA. The heterogeneous clades of *Porpidia macrocarpa*, which include specimens from Antarctica and the Austrian Alps, show a similar pattern. *Lecidea atrobrunnea* is the only exception, forming two southern polar OTUs with accessions from both Antarctica and sSA (Fig. 3). A similar pattern is also known for *Usnea aurantia-coatra* (Jacq.) Bory (Laguna Defior 2016) and *Cetraria aculeata* (Fernández-Mendoza et al. 2011). A different example is *Lecidea cancriformis*, which has, so far, been described as endemic to Antarctica and is one of the dominant crustose lichens in the most extreme areas of the continent (Castello 2003; Hertel 2007; Ruprecht et al. 2010). The Antarctic accessions belong to a single, well-supported OTU (Lcd58; Fig. 3, Supplementary Material Files S2-1a, b & S2-2, available online) but there are seven closely related OTUs occurring in sampling areas north of Tierra del Fuego. However, the whole cluster L11, including *L. cancriformis*, remains confined to the southern polar regions. Several other species, as well as the genus *Poeltidea*, occur solely in the southern polar regions. *Lecidea medusula*, which was previously only investigated morphologically and thought to be endemic to the Southern Hemisphere (Hertel 2009), is shown to be a cosmopolitan species. However, in total, the cosmopolitan species/OTUs (*Lecidea* sp.2, *Lecidea* sp.3, *L. medusula*, *L. tessellata*, *Porpidia macrocarpa*) are outnumbered by those that are currently known only from sSA (Table 1).

The *Lecidea* phylogeny, based on the data of Zhao et al. (2015), reveals a new southern polar species-level clade (*Lecidella spp. nov.*) with accessions from sSA and continental Antarctica. The specimens are not morphologically assignable to the available species descriptions (e.g. Knoph & Leuckert 1994; Ruprecht et al. 2012b; Wirth et al. 2013). All the other sequences added to the phylogeny of Zhao et al. (2015) form well-supported and distinguished OTUs endemic to sSA.

Interestingly, several cosmopolitan and abundant mycobiont species occurring in continental and maritime Antarctica, for example *Lecidea andersonii*, *L. polyphyllum* Hertel 2007; Ruprecht et al. 2010, 2016; Hale et al. 2019) and *Lecidella siplei* (Ruprecht et al. 2012b), were not found in the sSA regions.

In contrast to the mycobiont, the photobiont shows the opposite pattern. In particular, the genus *Trebuoxia* is known as widely distributed globally, often with low diversification (Muggia et al. 2010; Ruprecht et al. 2012a). These findings are supported in this study with more than half of the accessions assigned to two cosmopolitan OTUs of the genus *Trebuoxia* (S02 and S07) plus some smaller groups (A02, A04a, A12; Leavitt et al. 2015; Fig. 6, Table 2, Supplementary Material File S2-3). The remaining *Trebuoxia* and *Asterochloris* accessions form highly diverse and locally differentiated and/or endemic groups, which was unexpected. The contrasting distribution behaviour of the cosmopolitan photobionts could be caused mainly by their wide choice of mycobiont partners (Kroken & Taylor 2000), allowing them access to the different distribution strategies of the various lichens.

The most southern sampling area at Isla Navarino shows, for the mycobiont, not only locally restricted OTUs, but also strongly supported endemic species (e.g. *Porpidia navarina*; Ruprecht et al. 2016), which is also the case for the photobiont *Asterochloris* (OTU Ast24). As this area was ice-free during the Last Glacial Maximum (Douglass et al. 2005), the likely reason can be explained by the concept of glacial refugia, where the cold and glacial phases were the drivers of population divergences and (cryptic) speciation after transition from the Northern to the Southern Hemisphere (Paula & Leonardo 2006; Stewart et al. 2010; Fernández-Mendoza & Printzen 2013). Furthermore, the two most southern areas of the South American continent sampled (Parque Nacional Torres del Paine and Morro Chico), which both have 100% locally differentiated OTUs and endemic species for the mycobiont (Table 3), are influenced by the violent westerly gales caused by the split of the Humboldt Current to the north and the Antarctic Circumpolar Current (ACC) to the south (Silva et al. 2009). This further leads to the assumption that mycobiont dispersal is limited through this asymmetrical wind system, which is caused by the undertow of the ACC, driven by westerly winds over the circumpolar streamlines (Allison et al. 2010). Moreover the flow speed of the ACC between the Last Glacial Maximum and the Holocene has remained almost unchanged (McCave et al. 2014). Nonetheless, several other strongly supported species groups that occur only in other areas of sSA, such as *Lecidella* sp. 1 at 2000 m.a.s.l. close to Esquel (42.8°S) or endemic lineages in *Trebuoxia* (S16, A38, A39), hint at further, and so far unknown, separation events at the remote and climatically extreme southern end of the American continent.

**Taxonomy**

It is well known that the mycobiont genera *Lecidea* and *Porpidia* (Fig. 3) are not clearly separated and our phylogeny confirms that the species currently included in *Porpidia* do not form a monophyletic group (i.e. Buschbom & Mueller 2004; Schmull et al. 2011; Ruprecht et al. 2016; Fig. 3, Supplementary Material Files S2-1 & S2-2b). Additionally, the newly described species/genus *Cycloymenia epithithca* with peritheliod apothecia and an apparently *Porpidia*-type ascus (McCune et al. 2017) is situated among these two genera. In general, species of *Lecidea* and *Porpidia* are morphologically differentiated by ascus-type (*Lecidea* or *Porpidia*), larger ascospores with the presence of a perispore in *Porpidia* and different genera of associated green microalgae as photobionts (*Trebuoxia* sp. in *Lecidea* sp., *Asterochloris* sp. and *Trebuoxia* sp. in *Porpidia* sp.; Ruprecht et al. 2016). *Chlorella* sp. as described by Li et al. (2013) was not found in the sSA *Porpidia* species. Although several new sequences were added for species that were previously only described morphologically (e.g. *L. kalbii*, *L. promiscens*, *L. swartzioidea*, *L. lithophila*, *Poeltidea perusta*), and sequences for other *Porpidia* species obtained from GenBank, the re-evaluated phylogeny could not be resolved. However, three species morphologically assigned to *Lecidea* s. str. (L. auriculata, L. tessellata, L. kalbii; Hertel 1984; Wirth et al. 2013; Fryday & Hertel 2014) form, together with several *Porpidia* species and the genus *Cycloymenia*, a highly supported but intermixed group. Although clearly defined groups for *Porpidia* are easily recognized and a name at the genus level is available for at least one of them (*Haplocarpon*), it would be premature to formally recognize these groups as genera because of the uncertain systematic position of the rare type species, *Porpidia trullisata* (Kremp.) Körb, for which molecular data are not yet available. However, the three other groups in our phylogeny are formed solely by species of *Lecidea* s. str., *Porpidia* and *Poeltidea*, respectively.

The unresolved and intermixed topology of several species in the two main clades of the *Lecidella* phylogeny (*L. stigmatet and L. elaeochromia*; Fig. 4) could not be improved with the additional specimens from sSA. Only an extended species sampling
can help to unravel the inconsistent relationships in both these phylogenies (Lecidea/PorpidiaceaePoeltidea, Fig. 3; Lecidella, Fig. 4).

Conclusions

The species-rich group of leциdeoic lichens found extensively in alpine and polar regions in southern South America comprises highly divergent OTUs of cosmopolitan species, as well as several endemic species. Three factors may contribute to the observed differentiation and endemism: a) the geographical isolation of this southernmost landmass north of Antarctica, b) limited dispersal caused by the Antarctic Circumpolar Current system, and c) the presence of regional glacial refugia.

The diverging patterns of dispersal in the cosmopolitan leциdeoic lichen group are still under-researched. Acquiring larger datasets along the assumed distribution routes of the highest mountain ranges (Garrido-Benavent & Pérez-Ortega 2017; Hale et al. 2019), and a consequent sampling for better global coverage, will help to understand colonization events and specialization in this, so far, quite overlooked group of crustose lichens.

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Supplementary Material. Supplementary Material is found in two files. Supplementary Material File S1, includes Tables S1-1, S1-2a and S1-3–5. Supplementary Material File S2, includes Table S2-1a and Figs S2-1b, S2-2 and S2-3. To view supplementary material for this article, please visit https://doi.org/10.1017/S0024282920000225.

References

Allison LC, Johnson HL, Marshall DP and Munday DR (2010) Where do winds drive the Antarctic Circumpolar Current? Geophysical Research Letters 37, L12605.

Aptroot A and Van Herk CMK (2007) Lecidea grisella sympatric with Lecidea fuscaatra, differing in its rimose instead of areolate thallus. Lichenologist 39, 293–296.

Arnold AE, Miadlikowska J, Higgins KL, Sarvate SD, Gugger P, Way A, Hofstetter V, Kauf F and Lutzoni F (2009) A phylogenetic estimation of trophic transition networks for ascomycetous fungi: are lichens cradles of symbiotrophic fungal diversification? Systematic Biology 58, 283–297.

Arup U, Sochting U and Frödén P (2013) A new taxonomy of the family Teloschistaceae. Nordic Journal of Botany 31, 16–83.

Aschenbrenner IA, Cernava T, Berg G and Grube M (2016) Understanding microbial multi-species symbioses. Frontiers in Microbiology 7, 180.

Bhatthayara D, Friedl T and Damberger S (1996) Nuclear-encoded rDNA group I introns: origin and phylogenetic relationships of insertion site lineages in the green algae. Molecular Biology and Evolution 13, 978–989.

Bilowitz PO, Nascimbene J and Mayrhofer H (2015) Terricolous lichens in the glacier forefield of the Morteratsch Glacier (Eastern Alps, Graubunden, Switzerland). Phyton-Annales Rei Botanicae 55, 193–199.

Bouckaert R, Heled J, Kuhntert D, Vaughan T, Wu CH, Xie D, Suchard MA, Rambaut A and Drummond AJ (2014) BEAST 2: a software platform for Bayesian evolutionary analysis. PLoS Computational Biology 10, e1003557.

Branco S, Glaudeux P, Ellison CE, Kuo A, LaButti K, Lipzen A, Grigoriev IV, Liao HL, Vilgalys R, Peay KG, et al. (2015) Genetic isolation between two recently diverged populations of a symbiotic fungus. Molecular Ecology 24, 2747–2758.

Brock G, Datta S, Pihur V and Datta S (2008) clValid, an R package for cluster validation. Journal of Statistical Software 25, 1–22.

Brommitt RK (2001) World Geographical Scheme for Recording Plant Distributions (Edition 2). Pittsburgh, Pennsylvania: Carnegie Mellon University, Hunt Institute for Botanical Documentation.

Buschbom J (2007) Migration between continents: geographical structure and long-distance gene flow in Porpidia flavicunda (lichen-forming Ascomycota). Molecular Ecology 16, 1835–1846.

Buschbom J and Mueller G (2004) Resolving evolutionary relationships in the lichen-forming genus Porpidia and related allies (Porpidiaceae, Ascomycota). Molecular Phylogenetics and Evolution 32, 66–82.

Cacciani M and Andreis C (2004) Pioneer herbaceous vegetation on glacier forelands in the Italian Alps. Phytocoenologia 34, 55–89.

Calvelo S, Stocker-Wörgötter E, Liberator S and Elias JA (2005) Proteus mucosus (Parmeliaceae, Ascomycota), a genus endemic to southern South America. Bryologist 108, 1–15.

Castello M (2003) Lichens of Terra Nova Bay area, northern Dronning Maud Land (Continental Antarctica). Studia Geobotanica 22, 3–54.

Dal Grande F, Sharma R, Meiser A, Rolshausen G, Budel B, Mishra B, Thines M, Otto J, Piennninger M and Schmitt I (2017) Adaptive differentiation coincides with local bioclimatic conditions along an elevational cline in populations of a lichen-forming fungus. BMC Evolutionary Biology 17, 93.

Douglas DC, Singer BS, Kaplan MR, Ackert RP, Mickelson DM and Caffee MW (2005) Evidence of early Holocene glacial advances in southern South America from cosmogenic surface-exposure dating. Geology 33, 237–240.

Fernández-Mendoza F and Printzen C (2013) Pleistocene expansion of the bipolar lichen Cetraria aculeata into the Southern Hemisphere. Molecular Ecology 22, 1961–1983.

Fernández-Mendoza F, Domaschke S, García MA, Jordan P, Martin MP and Printzen C (2011) Population structure of mycobionts and photobionts of the widespread lichen Cetraria aculeata. Molecular Ecology 20, 1208–1232.

Fryday AM (2005) The genus Porpidia in northern and western Europe, with special emphasis on collections from the British Isles. Lichenologist 37, 1–35.

Fryday AM and Hertel H (2014) A contribution to the family Lecideaceae s. lat. (Lecanoromycetidae inc. sed., lichenized Ascomycota) in the southern subpolar region; including eight new species and some revised generic circumscriptions. Lichenologist 46, 389–412.

Fujisawa T and Barraclough TG (2013) Delimiting species using single-locus data and the Generalized Mixed Yule Coalescent approach: a revised method and evaluation on simulated data sets. Systematic Biology 62, 707–724.

Garrido-Benavent I and Pérez-Ortega S (2017) Past, present, and future research in bipolar lichen-forming fungi and their photobionts. American Journal of Botany 104, 1660–1674.

Geml J (2011) Coalescent analyses reveal contrasting patterns of intercontinental gene flow in arctic-alpine and boreal-temperate fungi. In Fontaneto D (ed.), Biogeography of Microscopic Organisms: is Everything Small Everywhere? Cambridge: Cambridge University Press, pp. 175–190.

Geml J, Kauff F, Brochmann C and Taylor DL (2010) Surviving climate changes: high genetic diversity and transoceanic gene flow in two arctic-alpine lichens, Flavocetraria cucullata and F. nivalis (Parmeliaceae, Ascomycota). Journal of Biogeography 37, 1529–1542.

Geml J, Kauff F, Brochmann C, Lutzoni F, Laursen GA, Redhead SA and Taylor DL (2012) Frequent circumbartctic and rare transequatorial dispersals in the lichenised agaric genus Lichenomphalia (Hygrophoraceae, Basidiomycota). Fungal Biology 116, 388–400.

Goffinet B, Rozzi R, Lewis L, Buck W and Massardo F (2012) Miniature Forests of Cape Horn: Ecotourism with a Hand Lens [Los Bosques en Miniatura del Cabo de Hornos: Ecoturismo con Lupa]. Denton: University of North Texas Press.

Gowan SP (1989) The lichen genus Porpidia (Porpidiaceae) in North America. Bryologist 92, 25–59.
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