The Lichenologist **44**(5): 661–678 (2012)
doi:10.1017/S0024282912000291

Genetic diversity of photobionts in Antarctic lecideoid lichens from an ecological viewpoint

Ulrike RUPRECHT, Georg BRUNAUER and Christian PRINTZEN

Abstract: As part of a comprehensive study on lecideoid lichens in Antarctica, we investigated the photobiont diversity and abundance in 119 specimens of lecideoid lichens from 11 localities in the continental and maritime Antarctic. A phylogeny of these photobiont ITS sequences, including samples from arctic, alpine and temperate lowland regions, reveals the presence of five major *Trebouxia* clades in Antarctic lecideoid lichens. Two clades are formed by members of the *T. jamesii* and *T. impressa* aggregates but for all other clades no close match to any known *Trebouxia* species could be found in sequence databases. One genetically uniform and well-supported *Trebouxia* clade was found only in the climatically unique cold desert regions of the Antarctic (preliminarily called *Trebouxia* sp.URa1), where it is preferentially associated with the highly adapted Antarctic endemic lichen *Lecidea cancriformis*. Levels of genetic photobiont diversity differ slightly, but insignificantly among ecological regions of the Antarctic and do not decrease towards regions with more unfavourable ecological conditions. The genetic diversity of photobionts varies among mycobiont species. Most pairwise comparisons reveal that these differences are insignificant, probably due to the small sample size for most species. The Antarctic lichens studied here are predominantly not specific for a single photobiont species or lineage, except for *Lecidella greenii* and *L. siplei*. These two species are preferably associated with *Trebouxia* sp. URa2, although in the sampling areas of both species, a pool of several other photobionts is available. *Lecidea cancriformis* associates with the highest diversity of photobionts followed by *L. andersonii*.

Key words: climate zones, *Lecidea*, *Trebouxia* clades

Accepted for publication 20 *April* 2012

Introduction

Many extreme terrestrial habitats, especially those in the polar regions, are dominated by lichens. The poikilohydric nature of lichens and the production of UV-screening substances (e.g. quinones, xanthones and melanins) allow these organisms to thrive in areas that are hostile to other life forms. Green-algal lichens are the most successful species under these extreme conditions as they do not depend on the presence of liquid water for reactivation from their dry inactive state, in contrast to cyanobacterial lichens. This is why cyanobacterial lichens are completely absent in the Antarctic cold deserts (Lange *et al*. 1986; Kappen 2000).

'Lecideoid' lichens (Hertel 1984) are species described under the generic name *Lecidea sensu* Zahlbruckner (1925) but not necessarily belonging to the genus in its strict sense. They are mainly characterized by a crustose thallus with green-algal photobionts, apothecia without algae in the exciple and colourless, aseptate ascospores. Most of the species are saxicolous and show a preference for polar and alpine habitats. Consequently, several lecideoid lichen species from the genera *Carbonea, Lecanora, Lecidea* and *Lecidella* appear in continental Antarctica (Hertel 2007; Ruprecht *et al.* 2010, 2012), where

U. Ruprecht and G. Brunauer: University of Salzburg, Organismic Biology, Hellbrunnerstr. 34, 5020 Salzburg, Austria. Email: ulrike.ruprecht@sbg.ac.at
C. Printzen: Senckenberg Forschungsinstitut und Naturmuseum Frankfurt, Abteilung Botanik und Molekulare Evolutionsforschung, Biodiversität und Klima Forschungszentrum (LOEWE BiK-F), Senckenberganlage 25, 60325 Frankfurt, Germany.

they belong to the most abundant species (Hertel 2007; Ruprecht *et al.* 2010, 2012).

The green-algal photobionts of lecideoid lichens have not been studied in detail so far, but the few available studies show that all belong to different species of the genus *Trebouxia* (Hildreth & Ahmadjian 1981; Ettl & Gärtner 1995; Beck 1999). Because of this, and their cosmopolitan distribution, they are ideal objects to study global distribution patterns of cold-adapted lichen photobionts.

Several studies on the mycobiont-photobiont interactions in lichens have shown that both green-algal and cyanobacterial lichens can switch photobionts (Nelsen & Gargas 2009; Otálora *et al.* 2010; Wornik & Grube 2010; Fernández-Mendoza *et al.* 2011), even if the photobiont is transmitted vertically in the form of vegetative propagules (Nelsen & Gargas 2009; Wornik & Grube 2010). Lichens that are specific to certain photobiont species often associate with different genotypes, sometimes even within a single thallus (Piercey-Normore & DePriest 2001; Helms 2003; Blaha *et al.* 2006; Guzow-Krzeminska 2006; Piercey-Normore 2006; Casano *et al.* 2011). This ability to accept different algae as photobionts might be a survival strategy. Blaha *et al.* (2006) suggested that low photobiont specificity might extend the ecological range of lichens. The results of some recent studies suggest that ecological factors, especially climate, may have an impact on photobiont selection (Beck *et al.* 2002; Yahr *et al.* 2006; Fernández-Mendoza *et al.* 2011; Peksa & Škaloud 2011). Helms (2003) demonstrated that the occurrence of certain *Trebouxia* clades correlates with the macroclimate, but the type of substratum (calcareous or siliceous rock, different tree species) seems also to be a major factor in photobiont choice (Helms 2003; Werth & Sork 2010). Since most lecideoid lichens in Antarctica grow on siliceous rock, substratum can probably not explain differences in photobiont occurrence and selection. The most important ecological factor to influence the photobiont selection of mycobionts can therefore be assumed to be the (micro-)climate. Furthermore, photobiont availability could be purely stochastic, in which case photobiont selection could be entirely defined by the specificity and preferences of the mycobiont. In general, Antarctic lichens are quite resistant to cold and arid conditions (Kappen & Valladares 2007; Green 2009). Because the algal partner has to perform net photosynthesis under much lower temperatures than in most other biomes of the world, temperature has often been invoked as a key criterion for photobiont choice in Antarctic lichens (Kappen 1993; Green *et al.* 1999; Pannewitz *et al.* 2006). An influence of temperature on *Trebouxia* photobionts was indeed revealed in several studies (e.g. Tschermak-Woess 1988; Casano *et al.* 2011).

Systematic revisions of lichenized fungi mostly ignore the photobionts. As a result, the identity of the photobionts at species level is known only for a small fraction of all lichens described (Honegger 1996). Lecideoid lichens from Antarctica are no exception, the more so because these lichens are hardly accessible. This contribution is part of a comprehensive study on lecideoid lichens, mainly from continental Antarctica (Ruprecht *et al.* 2010, 2012). Our major goals were: 1) to get a first overview on the genetic diversity of photobionts of Antarctic lecideoid lichens; 2) to identify algal clades with special ecological (mainly climatic) preferences; 3) to identify photobionts that occur exclusively in Antarctica and might be particularly well adapted to the cold and dry climate of the continental Antarctic.

Material and Methods

Collecting sites and material

Saxicolous lecideoid lichens were collected in several regions of the Antarctic continent (Fig. 1, Ruprecht *et al.* 2010, 2012). Terrestrial life is limited to ice-free areas that make up only 0·34% of Antarctica (Peat *et al.* 2007). Water availability is the main factor for lichen growth in the extreme cold deserts. The open sea and/or frequent fog are the main providers of humidity and have a dominant effect on the climatic conditions (Adams *et al.* 2006; Green *et al.* 2007; Ruprecht *et al.* 2012).

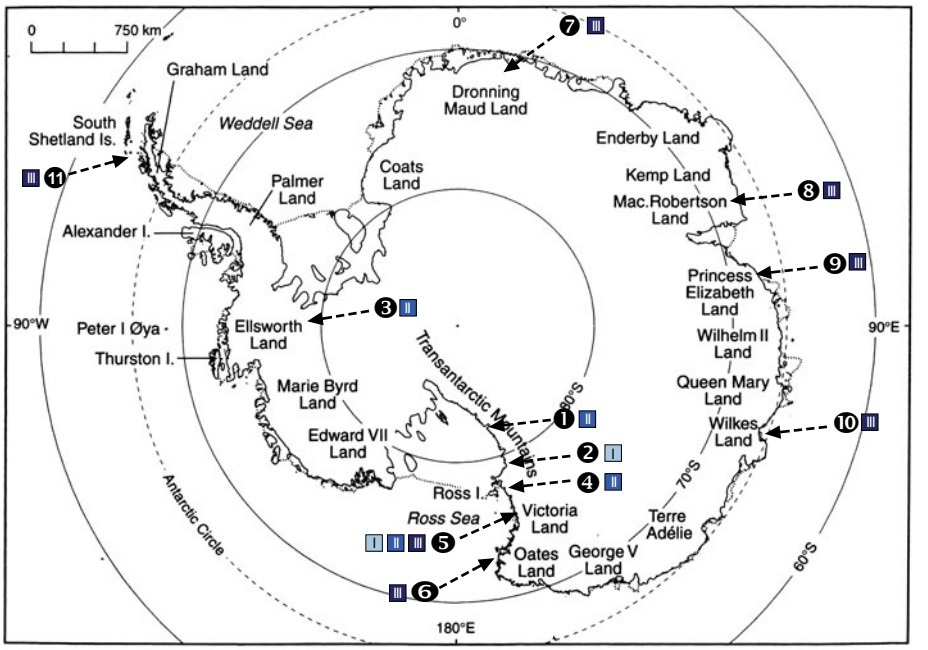


FIG. 1. Location of collecting sites in the maritime and continental Antarctica. Site numbers (ordered by latitude) are indicated together with the signs for the three habitat types (see text). 1) Mt Kyffin, 2) Darwin Area, 3) Ellsworth Land, 4) Taylor Valley, 5) Granite Harbour, Sperm Bluff, Battleship Promontory, 6) Cape Hallett, 7) Dronning Maud Land, 8) Mac. Robertson Land, 9) Princess Elisabeth Land, 10) Wilkes Land, 11) Antarctic Peninsula, South Shetland Islands. General map from Øvstedal & Lewis Smith (2001). In colour online.

According to temperature and humidity, we roughly divided the snow-free areas into 3 different climatic regions (Table 1):

I. Inland sites with extremely low precipitation and strong, katabatic winds (dry and cold).
II. Inland sites subject to coastal air-masses and/ or frequent fog (intermediate).
III. Coastal sites with open sea during the summer (humid and relatively warm).

In total, we generated ITS sequences of 119 lecideoid lichens from eleven localities in the continental and maritime Antarctic (Fig. 1), and additional samples from the Alps and the Arctic (Ruprecht *et al.* 2010, 2012). In addition, 22 *Trebouxia* sequences from GenBank were included in the dataset (Appendix 1) to infer the phylogenetic position of Antarctic photobionts within the genus *Trebouxia*. In order to identify already described species within our dataset we preferably included GenBank sequences from UTEX strains. *Trebouxia* samples from the Alps, Arctic regions and from South and North America were included in order to get information about intra-specific sequence variation and to see whether the species and haplotypes identified in Antarctic material also occur in other regions of the world. Information on the samples is summarized in Appendix 1.

DNA-amplification, purification, sequencing

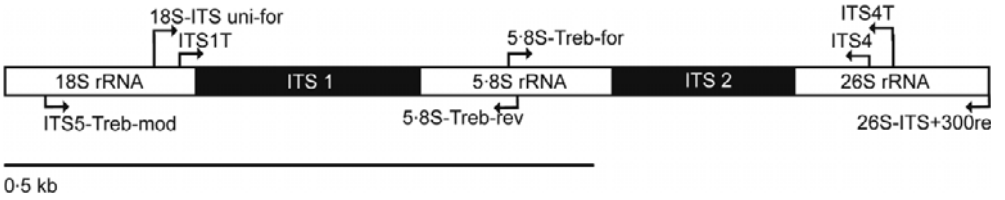
Total DNA was extracted from the thallus or apothecia by using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. The PCR mix contained 1 unit of Kapa HiFi polymerase (PeqLab), 0·2 nM of each of the four dNTPs, 0·3 μM of each primer and *c.* 1 ng genomic DNA. Nested PCRs were performed using GoTaq polymerase (0·5 units). The internal transcribed spacer region (ITS) of the photobionts' nuclear ribosomal DNA was amplified and sequenced with the primers described in Table 2. In cases where the PCR did not produce visible bands in the first reaction, we took 1 μl of this reaction as template for a nested PCR with internal primers. The most successful PCR protocol used the primers 18S-ITS-uni-for and ITS4T for the first PCR, and ITS1T and ITS4 for the nested PCR. PCRs using Kapa Hifi polymerase started with an initial denaturation of 98°C for 2 min, followed by 35 cycles at 98°C for 20 s, 56°C for 20 s and 72°C for 40 s. Cycling conditions for nested PCRs with GoTaq polymerase were as follows: initial denaturation at 95°C for 2 min, followed by 35 cycles at 95°C for 20 s, 55°C for 30 s and 72°C for 50 s. The PCR products were purified using the Qiaquick PCR purification kit (Qiagen) and re-dissolved in sterile distilled water. The purified PCR-products were labelled using the BigDye© Terminator

TABLE 1. *Collecting sites sorted by latitude; the six climate regions are coded in different colours. The grey shaded numbers of the particular regions are consistent with the numbers in Fig. 1. A short habitat description summarizes the most important varieties. In colour online*

Collecting sites					GPS	Colour code	Habitat type	Habitat description	References
Southern hemisphere	Antarctica	1		Mt. Kyffin	S 83° 45'	II	intermediate	inland mountains, surrounded by glaciers, foggy	Green *et al*. (2011)
		2	Darwin Area	Lake Wellman/Smith Valley	S 79° 54'	I	dry and cold	dry inland in between glaciers, strong winds, extremely low precipitation	Ruprecht *et al*. (2010) Simpson & Cooper (2002)
				Diamond Hills	S 79° 51'	I	dry and cold	dry inland in between glaciers, strong winds, low precipitation	
				Brown Hills	S 79° 46'	I	dry and cold	dry inland in between glaciers, strong winds, low precipitation	
				Bartrum Basin	S 79° 45'	I	dry and cold	dry inland in between glaciers, strong winds, low precipitation	
		3		Ellsworth Mountains	S 78° 25'	II	intermediate	intermediate	Convey & McInnes (2005)
		4		Taylor Valley	S 77° 40'	II	intermediate	classical dry valley, low precipitation, foggy	Doran *et al*. (2002)
		5		Sperm Bluff /Mt Suess	S 77° 02'	II	intermediate	inland mountains, surrounded by glaciers	Ruprecht *et al*. (2010)
				Granite Harbour/Botany Bay	S 77° 00'	III	humid and realtively warm	partially open sea during summer	
				Battleship Promontory	S 76° 54'	I	dry and cold	dry inland, low precipitation, sandstone	McKay *et al*. (1993)
		6		Cape Hallett	S 72° 19'	III	humid and realtively warm	open sea during summer	Green *et al*. (2000) Pannewitz *et al*. (2005)
		7		Dronning Maud Land	S 70° 50'	III	humid and realtively warm	open sea during summer	Reijmer & van den Broeke (2001)
		8		Mac. Robertson Land	S 70° 50'	III	humid and realtively warm	open sea during summer	Stickley *et al*. (2005)
		9		Princess Elisabeth Land	S 69° 22'	III	humid and realtively warm	open sea during summer	http://cdiac.esd.ornl.gov/epubs/ndp/ndp032/ndp032.html
		10		Wilkes Land	S 66° 17'	III	humid and realtively warm	open sea during summer	Seppelt *et al*. (1998)
		11		Antarctic Peninsula/ S.Shetland Islands	S 65° 00°	III	humid and realtively warm	Maritime Antarctica, open sea during summer	Monaghan & Bromwich (2008)
				South America	----	IV	----	----	
Northern hemisphere				Europe, USA	----	V	----	mostly high Alpine areas	
				Svalbard, Greenland, Iceland	N 65 -78°00'	VI	----	----	

TABLE 2. *List of primers used to amplify the internal transcribed spacer region rRNA (ITS) and approximate location of priming sites*

Primers	Sequence 5′ to 3′	Temp.	Reference
ITS5-Treb-mod	aggaaggagaagtcgtaacaag	58°C	White *et al.* (1990), modified
18S-ITS-uni-for	gtgaacctgcggaaggatcatt	56°C	This study
ITS1T	ggaaggatcattgaatctatcgt	55°C	Kroken & Taylor (2000)
5.8S-Treb-for	agaacgcagcgaaatgc	57°C	This study
5.8S-Treb-rev	caat5*) tgcgttcaaagat	57°C	This study
ITS4	tcctccgcttattgatatgc	55°C	White *et al.* (1990)
ITS4T	gttcgctcgccgctactcta	56°C	Kroken & Taylor (2000)
26S-ITS+300re	ctatcggtctcccgtcagtat	58°C	This study



* 5 = *inosine*

v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequences were run on an ABI PRISM© 3700 DNA Analyzer (Applied Biosystems) using the PCR primers.

Phylogenetic analysis

Nuclear ITS sequences were assembled and edited using Geneious Pro 5.3.4 (www.geneious.com) and aligned with ClustalW (Thompson *et al.* 1994). The alignment was subsequently refined by using the MUSCLE algorithm implemented in the Geneious program. Poorly aligned positions and divergent regions were eliminated from the alignment using Gblocks 0.91b with default settings (Castresana 2000).

A nucleotide substitution model was chosen using Modeltest 3.7 (Posada & Crandall 1998). The Akaike information criterion selected the tranversional model ($rAC = rCT \neq rAG \neq rAT \neq rCG \neq rGT$, Prosada & Crandall 2001) including a discrete gamma distribution (TVM+G) as the optimal model. A maximum likelihood analysis (ML) was performed using the program Garli 0.96 (http://www.nescent.org/wg_garli/Main_Page) with the estimated TVM (0 1 2 3 1 4) + G model and default settings. A nonparametric bootstrap was used to assess robustness of clades, running 1000 pseudoreplicates.

Maximum parsimony (MP) analyses were performed using PAUP* (Swofford 2003). Heuristic searches with 1000 random taxon addition replicates were conducted with TBR branch swapping and MulTrees option in operation, and equally weighted characters and gaps treated as missing data. Bootstrapping was performed based on 1000 replicates with random sequence additions. Homoplasy levels were assessed by calculating consistency index (CI), retention index (RI), and rescaled consistency (RC) index from each parsimony search. For Bayesian tree inference a Markov Chain Monte Carlo (MCMC) procedure as implemented in the program MrBayes 3.1.2 was used (Huelsenbeck & Ronquist 2001). The analyses were 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). A run with 3·5 million generations starting with a random tree and employing 4 simultaneous chains was executed. Every 100th tree was saved into a file. We plotted the log-likelihood scores of sample points against generation time using TRACER 1.0 (http://evolve.zoo.ox.ac.uk/software.html?id=tracer) to test whether stationarity was achieved by checking if the log-likelihood values of the sample points reached a stable equilibrium value (Huelsenbeck & Ronquist 2001). Subsequently, the first 3500 trees were deleted as the 'burn-in' of the chain. A consensus topology with posterior probabilities for each clade was calculated from the remaining 31 500 trees.

Only clades that received bootstrap support equal or above 70% under parsimony and likelihood and posterior probabilities $\geq 0{\cdot}95$ were considered as strongly supported.

Haplotype networks

In order to visualize the haplotype diversity and the occurrence of haplotypes in different regions, we calculated a 95% parsimony probability haplotype network for the cosmopolitan species inferred from the phyloge-

netic tree, using TCS v1.21 (Clement *et al.* 2000) with a fixed connection limit at 15 steps.

Analysis of nucleotide polymorphism

DnaSP v5 (Librado & Rozas 2009) was used for calculating haplotype and nucleotide diversities for the Antarctic photobiont clades, photobionts in different regions of the Antarctic and photobionts found in the eight lichen species that were represented by more than four individuals. Nucleotide diversities were calculated using P-distances, excluding gaps and missing data. We used two-sided *t*-tests to assess whether differences in genetic diversities between different regions or clades were significant.

Results

The final data matrix for the molecular phylogeny comprised 141 OTUs with a length of 640 positions. Of the alignment positions, 226 were parsimony-informative. The MP analyses yielded 6195 equally parsimonious trees 490 steps long (CI = 0·647, RI = 0·953, RC = 0·617).

The ML and Bayesian analyses recovered the same well-supported clades as the MP analysis. The Bayesian consensus tree, with the support values of all three analyses, is shown in Fig. 2. Pie charts show the distribution of the members of each *Trebouxia* clade in different climate zones and habitat types (see also Table 1).

Phylogenetic analysis

The phylogenetic reconstruction revealed five strongly supported, monophyletic groups and one additional weakly supported clade of *Trebouxia* photobionts. The tree is rooted with *T. decolorans, T. arboricola* and two unidentified *Trebouxia* species from Germany and Svalbard. The crown group of the tree is formed by a well-supported clade including sequences of *Trebouxia jamesii* from Genbank and its equally well-supported sister group, here preliminarily called *T.* sp. URa1, which was only found in extremely cold and dry regions in Antarctica. The cosmopolitan and highly variable group of *T. jamesii* includes a well-supported sub-clade that seems to be restricted to dry regions of continental Antarctica, where it occurred in lichen species typical for these extreme habitats (*Carbonea vorticosa, Lecanora fuscobrunnea,* and two undetermined species).

The photobionts of clade URa1 are mainly restricted to *Lecidea cancriformis*, a dominant lichen species of the extremely dry and cold deserts.

The sister group relationship between *T. jamesii* and *T.* sp. URa1 was strongly supported in all analyses. Both clades are sister to a heterogeneous cosmopolitan group which includes Genbank accessions of *T. impressa*. Again, the relationship between these clades is well supported although not as strong as that between *T. jamesii* and *T.* sp. URa1.

The relationships among clades basal to this crown group are poorly resolved and supported, although two large and several smaller clades received high support values. The photobionts of the fourth clade *Trebouxia* sp.URa2 are associated with a wide range of lecideoid lichens (Fig. 2, Table 3). Representatives of this clade are not only found in the Antarctic but also in Europe and the USA. The species was not found in the extremely dry and cold continental Antarctic regions. This cluster is sister to two unnamed Genbank accessions from Greece and Denmark but the relationship lacks support.

The fifth group is also widely distributed and was preliminarily named *Trebouxia* sp. URa3. The voucher specimens were collected in temperate regions, as well as in coastal to dry and even extremely dry regions (Fig. 2).

A small group of well supported clades including *T. asymmetrica* and *Trebouxia* sp. URa4 is sister to *T.* sp. URa3, but the relationships between these clades are poorly resolved.

Haplotype networks

Figure 3 shows the haplotype networks (Clement *et al.* 2000) for *Trebouxia jamesii, T. impressa, T.* sp. URa2 and *T.* sp. URa3. We did not calculate a haplotype network for clade *T.* sp. URa1, because it consisted of only four haplotypes. The high diversity

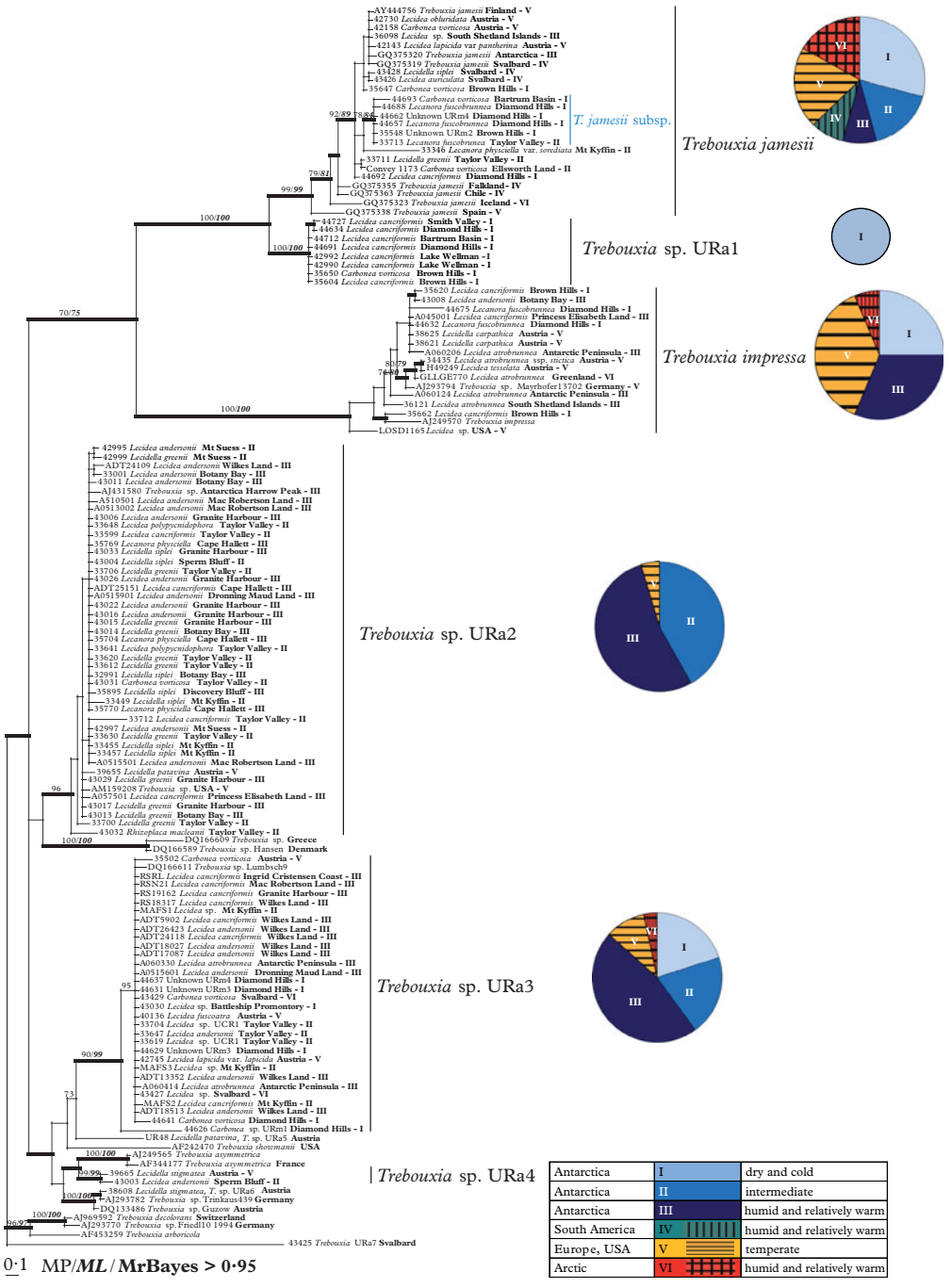


FIG. 2. Phylogeny of Antarctic *Trebouxia* species combined with samples from Austria, USA, the Arctic and downloaded sequences from Genbank. Herbarium numbers are combined with the species names of the mycobiont, the collecting place and the numbers of the habitat types. Antarctic *Trebouxia* species are labelled beside the respective clades. The diagrams beside the particular *Trebouxia* species show the distribution patterns of their occurrence in different climate zones. This Bayesian tree is based on a dataset of ITS sequences with >0·95 support and directly mapped bootstrap values with >70 support MP and ML analyses.

TABLE 3. *Diversity statistics for the major clades of photobionts: number of sequences (N), associated mycobiont species (S) (numbers of GenBank accessions in brackets) and haplotypes (H), and diversities of haplotypes (h) and nucleotides (Pi)*

	T. impressa	*T. jamesii*	*T.* sp. URa1	*T.* sp. URa2	*T.* sp. URa3
N	17	25	8	45	31
S	8 (2)	15 (7)	2	10 (2)	13 (1)
H	12	17	4	18	6
h	0·919	0·943	0·643	0·715	0·301
Pi	0·01807	0·02557	0·00146	0·00442	0·00744
Ecological requirements/ Distribution	not specified/ cosmopolitan	not specified/ cosmopolitan	dry & cold/Antarctic endemic	intermediate/ Antarctic – Alpine	not specified/ cosmopolitan

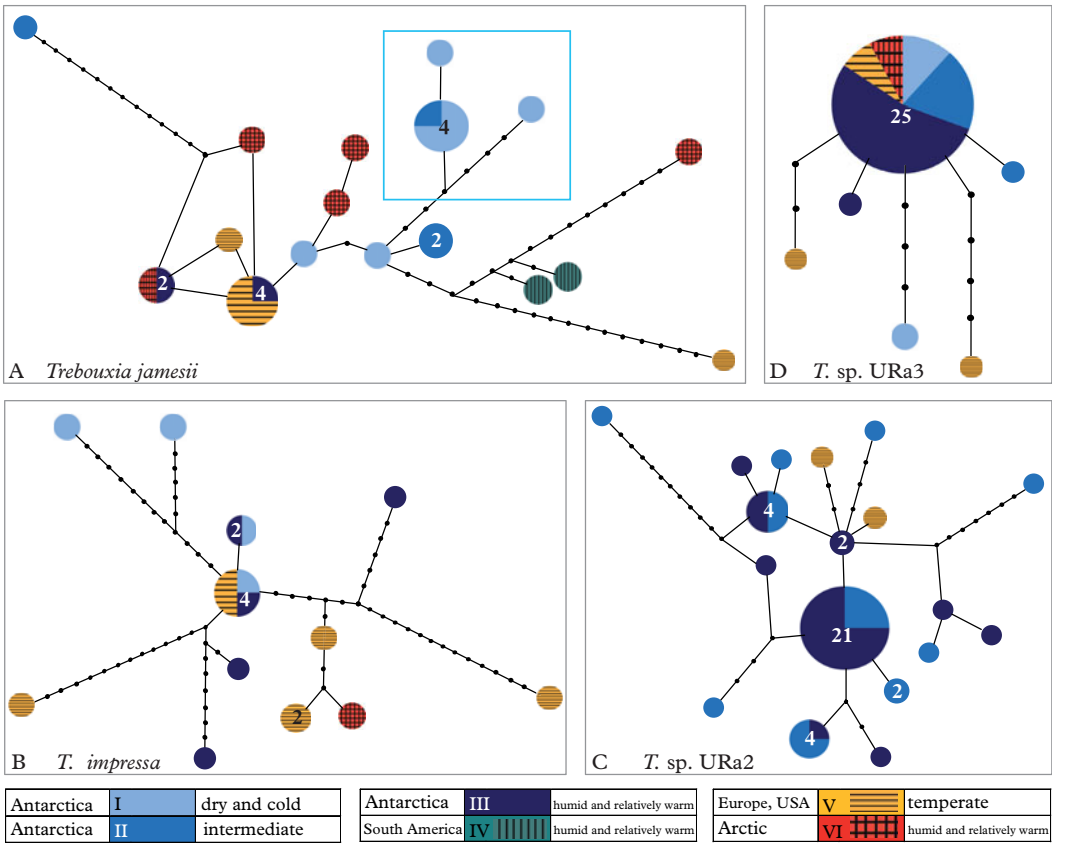


FIG. 3. Haplotype networks. A, *Trebouxia jamesii* with highlighted endemic sub-clade; B, *T. impressa*; C, *Trebouxia* sp. URa2; D, *Trebouxia* sp. URa3. Numbers of haplotypes ≥2 are indicated within the dots.

of *T. jamesii* (Fig. 3A) is indicated by a high number of haplotypes that are separated by many mutational steps. One of the haplotypes shows a bipolar distribution, while another was found in the maritime Antarctic and in alpine regions of the Northern Hemisphere. Six individuals belonging to three haplotypes form a clade that was only found in the cold and dry Antarctic deserts. The other haplotypes are scattered throughout the habitat types and geographic regions.

Trebouxia impressa (Fig. 3B) shows twelve haplotypes, again separated by several mutational steps. The central haplotype with four accessions occurs in humid and extremely dry Antarctic habitats, as well as in Alpine areas.

In contrast to these networks, *T.* sp. URa2 (Fig. 3C) consists of 13 relatively closely related haplotypes that were restricted to more humid Antarctic regions and also include two haplotypes from Austria and the USA. Twenty-five out of 31 individuals of *T.* sp. URa3 (Fig. 3D) belong to a single haplotype that was found in both polar regions and Alpine regions in Austria. Five haplotypes were each only represented by a single individual. One photobiont sequence of *Carbonea* sp. URm1 (44626, *T.* sp. URa3, see Fig. 2) was not included in the haplotype network, because it was separated from the other haplotypes by too many mutational steps.

Analysis of nucleotide polymorphism

Table 3 shows diversity statistics for the major clades of photobionts. *Trebouxia jamesii* is the most diverse of the five photobiont species recognized here. In our dataset, this photobiont also associates with the highest number of lecideoid lichen species (15). *Trebouxia* sp. URa2 and URa3 associate with 10 and 13 different lecideoid lichen species respectively, although they display a considerably lower haplotype and nucleotide diversity. *Trebouxia impressa*, although almost as diverse as *T. jamesii*, was found in only eight lichen species. Most pairwise comparisons between the clades are non-significant, but *Trebouxia* URa1 shows significantly lower diversity than *T. impressa, T. jamesii* and *T.* sp. URa2, and *T.* sp. URa2 is significantly less diverse than *T. impressa* (two-sided *t*-tests, data not shown).

Neither species composition nor genetic diversity and haplotype richness differ significantly between the three Antarctic climate zones (data not shown). Every habitat contains four photobiont species in different frequencies. The highest number of haplotypes was found in the coastal habitats (19), followed by the extremely dry habitats (15) and the foggy inland sites (14). The extremely dry habitats show the highest haplotype and nucleotide diversities (Table 4). Table 4 also shows that mycobiont species richness is considerably lower in dry interior sites than in intermediate and coastal sites.

Eight lichen species were represented by more than four individuals in this phylogenetic study. Diversity statistics focusing on these eight investigated species of lecideoid lichens differ with regard to the number of associated photobiont species and also their haplotype diversity (Table 5). However, the number of photobiont species that were found to be associated with a lichen species only once is apparently correlated with the number of individuals investigated. With one notable exception, the haplotype diversities of photobionts do not differ significantly between mycobiont species. Regarding the photobiont diversity, *Lecidea cancriformis*, however, is significantly more diverse than *L. andersonii* ($0{\cdot}02 > P > 0{\cdot}01$), *Lecidella siplei* ($0{\cdot}02 > P > 0{\cdot}01$) and *Lecidella greenii* ($0{\cdot}005 > P > 0{\cdot}001$).

Discussion

In spite of decades of research on Antarctic lichens (see Øvstedal & Lewis Smith 2001), the lichen flora of this continent is still insufficiently known. While the physiological properties of Antarctic lichens and their photobionts have been studied intensely (Kappen 1993, 2000; Green *et al.* 1999; Pannewitz *et al.* 2006; Barták *et al.* 2007), very little is known about the diversity and distribution of Antarctic lichen photobionts. In this study we therefore attempted

TABLE 4. *Ecological characterization and diversity statistics of the three climate zones in Antarctica, calculated with dnaSP. Occurrence of lichen species (mycobiont) combined with the photobionts, Numbers of sequences (N), photobiont species (S) and haplotypes (H), and diversities of haplotypes (h) and nucleotides (Pi). In colour online*

Habitat type		I	dry and cold			II	intermediate						III	humid and relatively warm					
		3	4	10	8	3	3	2	2	7	4	7	3	18	5	10	5	3	3
Mycobiont		*Carbonea vorticosa*	*Lecanora fuscobrunnea*	*Lecidea cancriformis*	others	*Lecidea andersonii*	*Lecidea cancriformis*	*Lecidea polypycnidophora*	*Lecidea* sp. UCR1	*Lecidella greenii*	*Lecidella siplei*	others	*Lecanora physciella*	*Lecidea andersonii*	*Lecidea atrobrunnea*	*Lecidea cancriformis*	*Lecidella greenii*	*Lecidella siplei*	others
Photobiont	*T. jamesii*	2	2	1	2	-	-	-	-	1	-	3	-	-	-	-	-	-	2
	T. URa1	1	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	T. impressa	-	2	1	-	-	-	-	-	-	-	-	-	1	3	1	-	-	-
	T. URa2	-	-	-	-	2	2	2	-	6	4	2	3	11	-	2	5	3	1
	T. URa3	-	-	-	6	1	1	-	2	-	-	2	-	6	2	6	-	-	-
	N	25				28							47						
	S	4				4							4						
	H	15				14							18						
	h	0·920				0·889							0·814						
	Pi	0·10369				0·05737							0·06241						

TABLE 5. *Associations of mycobionts and photobionts: numbers of sequences (N), photobiont species (S) and haplotypes (H), and diversities of haplotypes (h) and nucleotides (Pi)*

		Mycobiont							
		Carbonea vorticosa	*Lecanora fuscobrunnea*	*Lecanora physciella*	*Lecidea andersonii*	*Lecidea atrobrunnea*	*Lecidea cancriformis*	*Lecidella greenii*	*Lecidella siplei*
	N	6	5	4	23	7	22	12	8
	S	3	2	2	4	2	5	2	2
	H	6	4	2	12	7	12	6	6
	h	1·000	0·900	0·500	0·874	1·000	0·887	0·803	0·893
	Pi	0·10010	0·10195	0·07508	0·05358	0·08482	0·09780	0·02505	0·03769
Photobiont	*T. jamesii*	3	3	1	–	–	1	–	1
	T. sp. URa1	1	–	–	–	–	7	–	–
	T. impressa	–	2	–	1	5	3	1	–
	T. sp. URa2	–	–	3	15	–	4	11	7
	T. sp. URa3	2	–	–	7	2	7	–	–

to characterize the green-algal photobionts of a group of widely distributed crustose lichens, relating their occurrence with geographic origin and ecological differences of Antarctic ecosystems. Green algae of the genus *Trebouxia* are among the most common photobionts of lichens (Ettl & Gärtner 1995; Rambold *et al*. 1998). *Trebouxia impressa*, *T. jamesii* and a few unidentified genetic lineages have previously been identified as photobionts of various Antarctic lichen species (Aoki *et al.* 1998; Romeike *et al.* 2002; Fernández-Mendoza *et al.* 2011). In our analysis of lecideoid lichens, we could also identify these two photobionts in different lichen species (Fig. 2). In addition, we found three major clades (URa1, URa2, and URa3) that, based on ITS sequences, cannot be assigned at present to any described species of *Trebouxia*. Another clade (URa4) of *Trebouxia* present in Antarctica was detected in only two specimens, one *Lecidella stigmatea* from Austria and one *Lecidea andersonii* from the coastal continental Antarctic, and will not be discussed here.

The accessions of *T. jamesii* form a genetically diverse clade with several sub-clades (Figs 2 & 3A, Table 3). Lichens associated with *T. jamesii* are found in all Antarctic habitats investigated by us. The comparison with sequences from other parts of the world revealed that one ITS haplotype from the maritime Antarctic (including the South Shetland Islands) also occurs in Austria and Finland. This result is in line with those of Fernández-Mendoza *et al.* (2011), who found one ITS and one GPD-haplotype of *T. jamesii* with a bipolar distribution. The wide geographic distribution of *T. jamesii* is related to its large genetic diversity, its occurrence as a photobiont of many lichens (see e.g. Kroken & Taylor 2000; Blaha *et al.* 2006; Piercey-Normore 2006; Hauck *et al.* 2007; Fernández Mendoza *et al.* 2011) and its wide ecological amplitude. In the Antarctic, *T. jamesii* is accepted by a whole range of different species of *Carbonea*, *Lecidea*, *Lecidella* and *Lecanora* (Fig. 2, Table 5) and occurs in cold deserts as well as in humid maritime regions. The delimitation of *T. jamesii* and *T. simplex* (synonymized by Friedl 1989) is still far from clear and it is likely that more than one phylogenetic species is hidden behind these names. Hauck *et al.* (2007), for example, found an apparently undescribed sister clade to *T. simplex* and *T. jamesii* f. *angustilobata*, which they preliminarily named *T. hypogymniae.* A closer look at the phylogenetic tree in Fig. 2 shows one sub-clade that was exclusively found in the extreme cold Antarctic deserts, and that is firmly embedded in the *T. jamesii* clade. All members of this sub-clade were collected in habitat types I and II (Table 1, Figs 2 & 3) and therefore seem to be well adapted to the extreme Antarctic climate. Extended sampling and additional molecular markers may eventually reveal that this is a cryptic species endemic to Antarctica.

The presence of undescribed lineages of *Trebouxia* in Antarctica is further supported by clade *T. sp*. URa1, a lineage that is preferentially associated with *Lecidea cancriformis.* None of the identified *Trebouxia* accessions in GenBank matched closely with our sequences in this clade. As in the case of the *T. jamesii* sub-clade, the fact that this clade appears restricted to the extremely dry and cold deserts suggests that it represents a new species that may be adapted to this hostile environment. Its preferred mycobiont, *L. cancriformis*, is in turn quite unselective and accepts almost all *Trebouxia* species detected in our study. Low photobiont selectivity has previously been reported from Antarctic lichens (Wirtz *et al.* 2003) and explained by selection against photobiont-specific mycobionts and harsh environmental conditions. The fact that the unspecific *L. cancriformis* is one of the most dominant species in the extreme cold deserts and occurs in the entire continental Antarctic (Ruprecht *et al.* 2010, 2012) further supports this interpretation.

A wide distribution of *T. impressa* was previously reported in the literature (Ettl & Gärtner 1995; Aoki *et al*. 1998; Romeike *et al*. 2002). However, the other two unnamed lineages of *Trebouxia* in our dataset are more widely distributed. *Trebouxia* URa3 is almost as widely distributed as *T. jamesii*. *T.* sp. URa2 was also found in temperate areas (Austria and USA, Fig. 3C) but does not occur in cold desert regions. Specimens with this algal clade were all collected in

coastal or coastal-influenced Antarctic habitats (Table 1, Figs 2 & 3). *Trebouxia impressa* and members of both undescribed clades form associations with almost all of the lichens sampled, although *T.* sp. URa2 was not found with *Carbonea vorticosa* and *Lecanora fuscobrunnea*, which prefer extremely dry climates (Table 5).

Perhaps the most surprising result of our study concerns the diversity of photobionts in different climatic regions of the Antarctic. Haplotype and nucleotide diversity of the photobionts show no significant difference among the three Antarctic climate zones (Table 4). Species composition differs slightly but there is no 'typical' species pattern in any of the three climatic zones. This result is counterintuitive because the harsh environmental conditions have often been postulated to exert strong selective pressure on Antarctic organisms (Kappen 1993; Broady & Weinstein 1998; Domaschke *et al.* 2012), a force expected to limit genetic diversity. In the case of the lichen photobionts studied here, there is no evidence of reduced levels of genetic diversity due to selective pressure in more extreme cold desert regions. Figure 3C even indicates that haplotypes of *T. sp.* URa2 found in the humid, coastal regions are genetically more similar than haplotypes from the dryer regions, although the same overall number of haplotypes was found in both regions. *T.* sp. URa3 is genetically much more uniform and preferably found in the more humid Antarctic areas, but at least haplotype numbers do not differ among the three climatic regions. Domaschke *et al.* (2012) reported significantly lower levels of genetic diversity and haplotype richness in Antarctic populations of *Cetraria aculeata* and offered two different explanations: demographic bottlenecks through colonization of the Antarctic or selection for a few well-adapted haplotypes. The data presented here indicates that demographic factors may play the more important role in shaping the genetic diversity of Antarctic lichens and their photobionts.

None of the mycobionts of 12 different lecideoid and 2 sterile Antarctic lichens studied here are specific to a single photobiont species or lineage, although *Lecidella greenii* and *L. siplei* are preferably associated with *Trebouxia* sp. URa2. The sampling areas of both species contain a pool of several other photobionts (Fig. 2; Appendix I). This supports the findings of other researchers that true selectivity for a particular photobiont species is found in only a few lichens (Otálora *et al.* 2010). The major factor shaping photobiont-mycobiont associations seems to be the local availability of acceptable photobionts (Beck *et al.* 1998; Kroken & Taylor 2000; Piercey-Normore & DePriest 2001; Fernández-Mendoza *et al.* 2011). Our finding that *Lecidea cancriformis* associates with the highest number of photobionts (Table 5) can thus be explained in two different ways. Firstly, *L. cancriformis* is the most widespread lichen in our sample and may therefore simply have a better chance of encountering different photobionts. This may, for example, explain the contrast to *L. andersonii* which is mainly restricted to coastal influenced – dry habitats and shows slightly lower photobiont diversity than *L. cancriformis*. *Carbonea vorticosa,* a lichen with a preference for cold and dry habitats, also associates with fewer *Trebouxia* clades than *L. cancriformis*, but this may be a sampling artefact. Secondly, the harsh environmental conditions in dry parts of the continental Antarctic may enforce lower photobiont specificity, by allowing more unspecific lichens such as *L. cancriformis* to colonize more different habitats (Wirtz *et al.* 2003). Our dataset does not allow testing of these hypotheses. It is interesting to note, however, that *Lecidella greenii* and *L. siplei*, which are largely restricted to the coastal humid to dry climate zones (II & III), were with a single exception only associated with *Trebouxia* URa2, although there is a pool of several *Trebouxia* species available in the area. If a larger sample supports this observation, it would indicate that photobiont specificity causes restricted occurrence and not vice versa.

We are grateful to H. T. Lumbsch (Chicago) and R. Türk (Salzburg) for advice and support. Selina Becker (Frankfurt) is thanked for technical assistance and Mikhail Andreev (St. Petersburg) and Rod Seppelt (Tasmania) for providing additional samples from other regions of continental Antarctica.

This study was financially supported by the research funding programme 'LOEWE – Landes-Offensive zur Entwicklung Wissenschaftlich-ökonomischer Exzellenz' of Hesse's Ministry of Higher Education, Research, and the Arts. Antarctica New Zealand is thanked for logistic support and Shulamit Gordon for coordinating LGP, to which this research contributed. Waikato University is thanked for financial support. This manuscript is an output of the FRST-funded IPY Research Programme *Understanding, valuing and protecting Antarctica's unique terrestrial ecosystems: Predicting biocomplexity in Dry Valley ecosystems* located at Biological Sciences and ICTAR (International Centre for Terrestrial Antarctic research) at Waikato University.

REFERENCES

Adams, B. J., Bardgett, R. D., Ayres, E., Wall, D. H., Aislabie, J., Bamforth, S., Bargagli, R., Cary, C., Cavacini, P., Connell, L. *et al.* (2006) Diversity and distribution of Victoria Land Biota. *Soil Biology & Biochemistry* **38:** 3003–3018.

Aoki, M., Nakano, T., Kanda, H. & Deguchi, H. (1998) Photobionts isolated from Antarctic lichens. *Journal of Marine Biotechnology* **6:** 39–43.

Barták, M., Váczi, P., Hájek, J. & Smykla, J. (2007) Low-temperature limitation of primary photosynthetic processes in Antarctic lichens *Umbilicaria Antarctica* and *Xanthoria elegans*. *Polar Biology* **31:** 47–51.

Beck, A. (1999) Photobiont inventory of a lichen community growing on heavy metal-rich rock. *Lichenologist* **31:** 501–510.

Beck, A., Friedl, T. & Rambold, G. (1998) Selectivity of photobiont choice in a defined lichen community: inferences from cultural and molecular studies. *New Phytologist* **139:** 709–720.

Beck, A., Kasalicky, T. & Rambold, G. (2002) Mycophotobiontal selection in a Mediterranean cryptogam community with *Fulgensia fulgida*. *New Phytologist* **153:** 317–326.

Blaha, J., Baloch, E. & Grube, M. (2006) High photobiont diversity in symbioses of the euryoecious lichen *Lecanora rupicola* (Lecanoraceae, Ascomycota). *Biological Journal of the Linnean Society* **88:** 283–293.

Broady, P. & Weinstein, R. N. (1998) Algae, lichens and fungi in La Gorce Mountains, Antarctica. *Antarctic Science* **10:** 376–385.

Casano, L. M., del Campo, E. M., García-Breijo, F. J., Reig-Armiñana, J., Gasulla, F., del Hoyo, A., Guéra A. & Barreno E. (2011) Two *Trebouxia* algae with different physiological performances are ever-present in lichen thalli of *Ramalina farinacea*. Coexistence versus competition? *Environmental Microbiology* **13:** 806–818.

Castresana, J. (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* **17:** 540–552.

Clement, M., Posada, D. & Crandall, K. A. (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9:** 1657–1660.

Convey, P. & McInnes, S. J. (2005) Exceptional Tardigrade-dominated ecosystem in Ellsworth Land, Antarctica. *Ecology* **86:** 519–527.

Domaschke, S., Fernández Mendoza, F., García, M. A., Martín, M. P. & Printzen, C. (2012) Low genetic diversity in Antarctic populations of the lichen-forming ascomycete *Cetraria aculeata* and its photobiont. *Polar Research* **31:** 17353.

Doran, P. T., Priscu, J. C., Lyons, W. B., Walsch, J. E., Fountain, A. G., McKnight, D. M., Moorhead, D. L., Virginia, R. A., Wall, D. H., Clow, G. D., *et al.* (2002) Antarctic climate cooling and terrestrial ecosystem response. *Nature* **415:** 517–520.

Ettl, H. & Gärtner, G. (1995) *Syllabus der Boden-, Luft- und Flechtenalgen.* Stuttgart: Gustav Fischer.

Fernández-Mendoza, F., Domaschke, S., García, M. A., Jordan, P., Martín, M. P. & Printzen, C. (2011) Population structure of mycobionts and photobionts of the widespread lichen *Cetraria aculeata*. *Molecular Ecology* **20:** 1208–1232.

Friedl T. (1989) *Systematik und Biologie von* Trebouxia (*Microthamniales, Chlorophyta*) *als Phycobiont der* Parmeliaceae (*Lichenisierte Ascomyceten*). Ph.D. thesis, University of Bayreuth.

Green, T. G. A. (2009) Lichens in arctic, antarctic and alpine ecosystems. *Rundgespräche der Kommission für Ökologie, Ökologische Rolle der Flechten.* **36:** 45–65.

Green, T. G. A., Schroeter, B. & Sancho, L. G. (1999) Plant life in Antarctica. In *Handbook of Functional Plant Ecology* (F. I. Pugnaire & F. Valladares, eds): 495–543. New York, Basel: Marcel Dekker Inc.

Green, T. G. A., Schroeter, B. & Sancho, L. (2007) Plant life in Antarctica. In *Functional Plant Ecology*, 2nd Edn. (F. I. Pugnaire & F. Valladares, eds): 389–433. Boca Raton, Florida: CRC Press.

Green, T. G. A., Sancho, L. G., Türk, R., Seppelt, R. D. & Hogg, I. D. (2011) High diversity of lichens at 84°S, Queen Maud Mountains, suggests preglacial survival of species in the Ross Sea region, Antarctica. *Polar Biology* **34:** 1211–1220.

Guzow-Krzeminska, B. (2006) Photobiont flexibility in the lichen *Protoparmeliopsis muralis* as revealed by ITS rDNA analyses. *Lichenologist* **38:** 469–476.

Hauck, M., Helms, G. & Friedl, T. (2007) Photobiont selectivity in the lichens *Hypogymnia physodes* and *Lecanora conizeaoides*. *Lichenologist* **39:** 195–204.

Helms, G. W. F. (2003) *Taxonomy and symbiosis in associations of* Physciaceae *and* Trebouxia. Ph.D. thesis, Georg-August Universität, Göttingen.

Hertel, H. (1984) Über saxicole, lecideoide Flechten der Subantarktis. *Beih. Nova Hedwigia* **79:** 399–499.

Hertel, H. (2007) Notes on and records of Southern Hemisphere lecideoid lichens. *Bibliotheca Lichenologica* **95:** 267–296.

Hildreth, K. C. & Ahmadjian, V. (1981) A study of *Trebouxia* and *Pseudotrebouxia* isolates from different lichens. *Lichenologist* **13:** 65–86.

Honegger, R. (1996) Morphogenesis. In *Lichen Biology* (T. H. Nash III, ed.): 65–87. Cambridge: Cambridge University Press.

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

Kappen, L. (1993) Plant activity under snow and ice, with particular reference to lichens. *Arctic* **46:** 297–302.

Kappen, L. (2000) Some aspects of the great success of lichens in Antarctica. *Antarctic Science* **12:** 314–324.

Kappen, L. & Valladares, F. (2007) Opportunistic growth and desiccation tolerance: the ecological success of poikilohydrous autothrophs. In *Functional Plant Ecology* (F. I. Pugnaire & F. Valladares, eds): 7–66. Boca Raton, Florida: CRC Press.

Kroken, S. & Taylor, J. W. (2000) Phylogenetic species, reproductive mode, and specificity of the green alga *Trebouxia* forming lichens with the fungal genus *Letharia*. *Bryologist* **103:** 645–660.

Lange, O. L., Kilian, E. & Ziegler, H. (1986) Water vapor uptake and photosynthesis of lichens: performance differences in species with green and blue-green algae as phycobiont. *Oecologia* **71:** 104–110.

Librado, P. & Rozas, J. (2009) DnaSP v.5 A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **15:** 1451–1452.

McKay, C. P., Nienow, J. A., Meyer, M. A. & Friedmann, E. I. (1993) Continuous nanoclimate data (1985–1988) from the Ross Desert (McMurdo Dry Valleys) cryptoendolithic microbial ecosystem. In *Antarctic Research Series, Vol. 61: Antarctic Meteorology and Climatology: Studies Based on Automatic Weather Stations* (D. H. Bromwich and C. R. Stearns, eds): 201–207. Washington, DC: American Geophysical Union.

Monaghan, A. J. & Bromwich, D. H. (2008) Advances in describing recent Antarctic climate variability. *Bulletin of the American Meteorological Society* **9:** 1295–1306.

Nelsen, M. P. & Gargas, A. (2009) Symbiont flexibility in *Thamnolia vermicularis* (Pertusariales: Icmadophilaceae). *Bryologist* **112:** 404–417.

Otálora, M. A. G., Martínez, I., O'Brien, H., Molina, M. C., Aragón, G. & Lutzoni, F. (2010) Multiple origins of high reciprocal symbiotic specificity at an intercontinental spatial scale among gelatinous lichens (Collemataceae, Lecanoromycetes). *Molecular Phylogenetics and Evolution* **56:** 1089–1095.

Øvstedal, D. O. & Lewis Smith, R. I. (2001) *Lichens of Antarctica and South Georgia: A Guide to Their Identification and Ecology.* Cambridge: Cambridge University Press.

Pannewitz, S., Green, T. G. A., Maysek, K., Schlensog, M., Seppelt, R., Sancho, L. G., Türk, R. & Schröter, B. (2005) Photosynthetic responses of three common mosses from continental Antarctica. *Antarctic Science* **17:** 341–352.

Pannewitz, S., Green, T. G. A., Schlensog, M., Seppelt, R., Sancho, L. & Schroeter, B. (2006) Photosynthetic performance of *Xanthoria mawsonii* C. W. Dodge in coastal habitats, Ross Sea region, continental Antarctica. *Lichenologist* **38:** 67–81.

Peat, H. J., Clarke, A. & Convey, P. (2007) Diversity and biogeography of the Antarctic flora. *Journal of Biogeography* **34:** 132–146.

Peksa, O. & Škaloud, P. (2011) Do photobionts influence the ecology of lichens? A case study of environmental preferences in symbiotic green alga *Asterochloris* (Trebouxiophyceae). *Molecular Ecology* **20:** 3936–3948.

Piercey-Normore, M. D. (2006) The lichen-forming ascomycete *Evernia mesomorpha* associates with multiple genotypes of *Trebouxia jamesii*. *New Phytologist* **169:** 331–344.

Piercey-Normore, M. D. & DePriest, P. T. (2001) Algal switching among lichen symbioses. *American Journal of Botany* **88:** 1490–1498.

Posada, D. & Crandall, K. A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* **14:** 817–818.

Posada, D. & Crandall, K. A. (2001) Selecting models of nucleotide substitution: an application to Human Immunodeficiency Virus 1 (HIV-1). *Molecular Biology and Evolution* **18:** 897–906.

Rambold, G., Friedl, T. & Beck, A. (1998) Photobionts in lichens: possible indicators of phylogenetic relationships? *Bryologist* **101:** 392–397.

Reijmer, C. H. & van den Broeke, M. R. (2001) Moisture source of precipitation in Western Dronning Maud Land, Antarctica. *Antarctic Science* **13:** 210–220.

Rodriguez, F., Oliver, J. L., Marin, A. & Medina, J. R. (1990) The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology* **142:** 485–501.

Romeike, J., Friedl, T., Helms, G. & Ott, S. (2002) Genetic diversity of algal and fungal partners in four species of *Umbilicaria* (lichenized Ascomycetes) along a transect of the Antarctic Peninsula. *Molecular Biology and Evolution* **19:** 1209–1217.

Ruprecht, U., Lumbsch, H. T., Brunauer, G., Green, T. G. A. & Türk, R. (2010) Diversity of *Lecidea* (Lecideaceae, Ascomycota) species revealed by molecular data and morphological characters. *Antarctic Science* **22:** 721–726.

Ruprecht, U., Lumbsch, H. T., Brunauer, G., Green, T. G. A. & Türk, R. (2012) Insights into the diversity of *Lecanoraceae* (Lecanorales, Ascomyceta) in continental Antarctica (Ross Sea region). *Nova Hedwigia* **94:** 287–306.

Seppelt, R. D., Nimis, P. L. & Castello, M. (1998) The genus *Sarcogyne* (*Acarosporaceae*) in Antarctica. *Lichenologist* **30:** 249–258.

Simpson, A. L. & Cooper, A. F. (2002) Geochemistry of the Darwin Glacier region granitoids, southern Victoria Land. *Antarctic Science* **14:** 425–426.

Stickley, C. E., Pike, J., Leventer, A., Dunbar, R., Domack, E. W., Brachfeld, S., Manley, P. & McClennan, C. (2005) Deglacial ocean and climate seasonality in laminated diatom sediments, Mac.Robertson Shelf, Antarctica. *Palaeogeography, Palaeoclimatology, Palaeoecology* **227:** 290–310.

Swofford, D. L. (2003) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Sunderland, Massachusetts: Sinauer Associates.

Tschermak-Woess, E. (1988) The algal partner. In *CRC Handbook of Lichenology*, Vol. I. (M. Galun, ed.): 39–92. Boca Raton, Florida: CRC Press.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994) CLUSTAL W: improving the sensitivity of progressive sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22:** 4673–4680.

Werth, S. & Sork, V. L. (2010) Identity and genetic structure of the photobiont of the epiphytic lichen *Ramalina menziesii* on three oak species in southern California. *American Journal of Botany* **95:** 821–830.

White, T. J., Bruns, T. D., Lee, S. B. & Taylor, J. W. (1990) Amplification and direct sequencing of fungal ribosomal genes for phylogenies. In *PCR Protocols: A Guide to Methods and Applications* (M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White, eds): 315–322. San Diego: Academic Press.

Wirtz, N., Lumbsch, H. T., Green, T. G. A., Türk, R., Pintado, A., Sancho, L. & Schroeter, B. (2003) Lichen fungi have low cyanobionts selectivity in maritime Antarcica. *New Phytologist* **160:** 177–183.

Wornik, S. & Grube, M. (2010) Joint dispersal does not imply maintenance of partnerships in lichen symbioses. *Microbial Ecology* **59:** 150–157.

Yahr, R., Vilgalys, R. & DePriest, P. T. (2006) Geographic variation in algal partners of *Cladonia subtenuis* (Cladoniaceae) highlights the dynamic nature of a lichen symbiosis. *New Phytologist* **171:** 847–860.

Zahlbruckner, A. (1925) *Catalogus Lichenum Universalis*. Vol III. Leipzig: Gebrüder Borntraeger.

Appendix 1. *Trebouxia* samples used in this study, with information on collecting localities, climate zones, voucher specimens, mycobionts, and Genbank accession numbers

Photobiont	Herbarium Sample ID	Habitat	Climate zone	Mycobiont	Accession number
Trebouxia aboricola	–	–	V	*Chaenotheca phaeocephala*	AF453259
T. asymmetrica	–	France	V	*Toninia sedifolia*	AF344177
T. asymmetrica	–	–	V	*Diploschistes diacapsis*	AJ249565
T. decolorans	–	Switzerland	V	*Xanthoria parietina*	AJ969592
T. impressa	R. Türk 33711*	Antarctica, Taylor Valley	II	*Lecidella greenii*	JN204743
T. impressa	R. Türk 34435	Austria, Kärnten, Hohe Tauern	V	*Lecidea atrobr.*ssp. *stictica*	JN204746
T. impressa	R. Türk 35620	Antarctica, DA, Brown Hills	I	*L. cancriformis*	JN204750
T. impressa	R. Türk 35662	Antarctica, DA, Brown Hills	I	*L. cancriformis*	JN204753
T. impressa	R. Türk 36121	Antarctica, S. Shetland Is.	III	*L. atrobrunnea*	JN204759
T. impressa	R. Türk 38621	Austria, Kärnten, Karnische A.	V	*Lecidella carpathica.*	JN204761
T. impressa	R. Türk 38625	Austria, Kärnten, Karnische A.	V	*L. carpathica*	JN204762
T. impressa	R. Türk 43008	Antarctica, Botany Bay	III	*Lecidea andersonii*	JN204778
T. impressa	R. Türk 44632	Antarctica, DA, Diamond Hills	I	*Lecanora fuscobrunnea*	JN204800
T. impressa	R. Türk 44675	Antarctica, DA, Diamond Hills	I	*L. fuscobrunnea*	JN204806
T. impressa	LE A045001	Antarctica, Princ.s Elisabeth L.	III	*Lecidea cancriformis*	JN204813
T. impressa	LE A060124	Antarctica, Antarctic Peninsula	III	*L. atrobrunnea*	JN204820
T. impressa	LE A060206	Antarctica, Antarctic Peninsula	III	*L. atrobrunnea*	JN204821
T. impressa	GLLGE770	Greenland	VI	*L. atrobrunnea*	JN204834
T. impressa	LOSD1165	USA, South Dakota	V	*Lecidea* sp.	JN204836
T. impressa	–	–	V	*Parmelina carporrhizans*	AJ249570
T. jamesii	R. Türk 33713	Antarctica, Taylor Valley	II	*Lecanora fuscobrunnea*	JN204745
T. jamesii	R. Türk 35548	Antarctica, DA, Brown Hills	I	Unknown URm2	JN204748
T. jamesii	R. Türk 44662	Antarctica, DA, Diamond	I	Unknown URm4	JN204805
T. jamesii	R. Türk 35647	Antarctica, DA, Brown Hills	I	*Carbonea vorticosa*	JN204751
T. jamesii	R. Türk 36098	Antarctica, S. Shetland Is.	III	*Lecidea* sp.	JN204758
T. jamesii	R. Türk 42143	Austria, Tirol, Ötztal	V	*L. lapicida* var.*pantherina*	JN204766
T. jamesii	R. Türk 42158	Austria	V	*Carbonea vorticosa*	JN204767
T. jamesii	R. Türk 42730	Austria, Osttirol, Hohe Tauern	V	*Lecidea obluridata*	JN204768
T. jamesii	R. Türk 43426	Norway, Svalbard	VI	*L. auriculata*	JN204793
T. jamesii	R. Türk 43428	Norway, Svalbard	VI	*Lecidella siplei*	JN204795
T. jamesii	R. Türk 44657	Antarctica, DA, Diamond Hills	I	*Lecanora fuscobrunnea*	JN204804
T. jamesii	R. Türk 44688	Antarctica, DA, Diamond Hills	I	*L. fuscobrunnea*	JN204807
T. jamesii	R. Türk 44692	Antarctica, DA, Diamond Hills	I	*Lecidea cancriformis*	JN204809
T. jamesii	R. Türk 44693	Antarctica, DA, Bartrum Basin	I	*Carbonea vorticosa*	JN204810
T. jamesii	Convey 1173	Antarctica, Ellsworth Mts.	II	*C. vorticosa*	JN204833
T. jamesii	–	Finland	V	*Flavocetraria nivalis*	AY444756
T. jamesii	–	Norway, Svalbard	VI	*Cetraria aculeata*	GQ375319
T. jamesii	–	Antarctica	III	*C. aculeata*	GQ375320
T. jamesii	–	Iceland	VI	*C. aculeata*	GQ375323
T. jamesii	–	Spain	V	*C. aculeata*	GQ375338
T. jamesii	–	Falkland	IV	*C. aculeata*	GQ375355
T. jamesii	–	Chile	IV	*C. aculeata*	GQ375363
T. showmanii	–	USA	V	–	AF242470
Trebouxia sp.	–	USA	V	–	AM159208
T. sp.	–	Greece	V	*Lecanora rupicola*	DQ166609
T. sp. AHP	–	Antarctica	III	*Umbilicaria antarctica*	AJ431580
T. sp. Friedl10	–	Germany	V	*Anaptychia ciliaris*	AJ293770
T. sp. Guzow	–	Austria	V	*Protoparmeliopsis muralis*	DQ133486
T. sp. Hansen	–	Denmark	V	–	DQ166589
T. sp. M.13702	–	Germany	V	*Rinodina milvina*	AJ293794
T. sp. Tr.439	–	Germany	V	*Buellia elegans*	AJ293782
T. sp. URa1	R. Türk 35604	Antarctica, DA, Brown Hills	I	*Lecidea cancriformis*	JN204749
T. sp. URa1	R. Türk 35650	Antarctica, DA, Brown Hills	I	*Carbonea vorticosa*	JN204752
T. sp. URa1	R. Türk 42990	Antarctica, DA, Lake Wellman	I	*Lecidea cancriformis*	JN204770
T. sp. URa1	R. Türk 42992	Antarctica, DA, Lake Wellman	I	*L. cancriformis*	JN204771

Appendix 1. *Continued*

Photobiont	Herbarium Sample ID	Habitat	Climate zone	Mycobiont	Accession number
T. sp. URa1	R. Türk 44634	Antarctica, DA, Diamond Hills	I	*L. cancriformis*	JN204801
T. sp. URa1	R. Türk 44691	Antarctica, DA, Diamond Hills	I	*L. cancriformis*	JN204808
T. sp. URa1	R. Türk 44712	Antarctica, DA, Bartrum Basin	I	*L. cancriformis*	JN204811
T. sp. URa1	R. Türk 44727	Antarctica, DA, Smith Valley	I	*L. cancriformis*	JN204812
T. sp. URa2	R. Türk 32991	Antarctica, Botany Bay	III	*Lecidella siplei*	JN204726
T. sp. URa2	R. Türk 33001	Antarctica, Botany Bay	III	*Lecidea andersonii*	JN204727
T. sp. URa2	R. Türk 33449	Antarctica, Mt Kyffin	II	*Lecidella siplei*	JN204729
T. sp. URa2	R. Türk 33455	Antarctica, Mt Kyffin	II	*L. siplei*	JN204730
T. sp. URa2	R. Türk 33457	Antarctica, Mt Kyffin	II	*L. siplei*	JN204731
T. sp. URa2	R. Türk 33599	Antarctica, Taylor Valley	II	*Lecidea cancriformis*	JN204732
T. sp. URa2	R. Türk 33612	Antarctica, Taylor Valley	II	*Lecidella greenii*	JN204733
T. sp. URa2	R. Türk 33620	Antarctica, Taylor Valley	II	*L. greenii*	JN204735
T. sp. URa2	R. Türk 33630	Antarctica, Taylor Valley	II	*L. greenii*	JN204736
T. sp. URa2	R. Türk 33641	Antarctica, Taylor Valley	II	*Lecidea polypycnidophora*	JN204737
T. sp. URa2	R. Türk 33648	Antarctica, Taylor Valley	II	*L. polypycnidophora*	JN204739
T. sp. URa2	R. Türk 33700	Antarctica, Taylor Valley	II	*Lecidella greenii*	JN204740
T. sp. URa2	R. Türk 33706	Antarctica, Taylor Valley	II	*L. greenii*	JN204742
T. sp. URa2	R. Türk 33712	Antarctica, Taylor Valley	II	*Lecidea cancriformis*	JN204744
T. sp. URa2	R. Türk 35704	Antarctica, Cape Hallett	III	*Lecanora physciella*	JN204754
T. sp. URa2	R. Türk 35769	Antarctica, Cape Hallett	III	*L. physciella*	JN204755
T. sp. URa2	R. Türk 35770	Antarctica, Cape Hallett	III	*Lecanora physciella*	JN204756
T. sp. URa2	R. Türk 35895	Antarctica, Granite Harbour	III	*Lecidella siplei*	JN204757
T. sp. URa2	R. Türk 39655	Austria, Salzburg, Hochkönig	V	*L. patavina*	JN204763
T. sp. URa2	R. Türk 42995	Antarctica, Mt Suess	II	*Lecidea andersonii*	JN204772
T. sp. URa2	R. Türk 42997	Antarctica, Mt Suess	II	*L. andersonii*	JN204773
T. sp. URa2	R. Türk 42999	Antarctica, Mt Suess	II	*Lecidella greenii*	JN204774
T. sp. URa2	R. Türk 43004	Antarctica, Sperm Bluff	II	*L. siplei*	JN204776
T. sp. URa2	R. Türk 43006	Antarctica, Granite Harbour	III	*Lecidea andersonii*	JN204777
T. sp. URa2	R. Türk 43011	Antarctica, Botany Bay	III	*L. andersonii*	JN204779
T. sp. URa2	R. Türk 43013	Antarctica, Botany Bay	III	*Lecidella greenii*	JN204780
T. sp. URa2	R. Türk 43014	Antarctica, Botany Bay	III	*L. greenii*	JN204781
T. sp. URa2	R. Türk 43015	Antarctica, Granite Harbour	III	*L. greenii*	JN204782
T. sp. URa2	R. Türk 43016	Antarctica, Granite Harbour	III	*Lecidea andersonii*	JN204783
T. sp. URa2	R. Türk 43017	Antarctica, Granite Harbour	III	*Lecidella greenii*	JN204784
T. sp. URa2	R. Türk 43022	Antarctica, Granite Harbour	III	*Lecidea andersonii*	JN204785
T. sp. URa2	R. Türk 43026	Antarctica, Granite Harbour	III	*L. andersonii*	JN204786
T. sp. URa2	R. Türk 43029	Antarctica, Granite Harbour	III	*Lecidella greenii*	JN204787
T. sp. URa2	R. Türk 43030	Antarctica, Battleship Prom.	I	*Lecidea* sp.	JN204788
T. sp. URa2	R. Türk 43031	Antarctica, Taylor Valley	II	*Carbonea vorticosa*	JN204789
T. sp. URa2	R. Türk 43032	Antarctica, Taylor Valley	II	*Rhizoplaca macleanii*	JN204790
T. sp. URa2	R. Türk 43033	Antarctica, Granite Harbour	III	*Lecidella siplei*	JN204791
T. sp. URa2	LE A0510501	Antarctica, Mac. Robertson Land	III	*Lecidea andersonii*	JN204814
T. sp. URa2	LE A0513002	Antarctica, Mac. Robertson Land	III	*L. polypycnidophora*	JN204815
T. sp. URa2	LE A0515501	Antarctica, Mac. Robertson Land	III	*L. andersonii*	JN204816
T. sp. URa2	LE A0515901	Antarctica, Dronning Maud Land	III	*L. andersonii*	JN204818
T. sp. URa2	LE A057501	Antarctica, Princ. Elisabeth Land	III	*L. cancriformis*	JN204819
T. sp. URa2	ADT24109	Antarctica, Wilkes Land	III	*L. andersonii*	JN204828
T. sp. URa2	ADT25151	Antarctica, Cape Hallett	III	*L. cancriformis*	JN204830
T. sp. URa2	GZU H49249	Austria, Steierm., Eisenerzer A.	V	*L. tesselata*	JN204835
T. sp. URa3	R. Türk 33346	Antarctica, Mt Kyffin	II	*Lecanora physciella*	JN204728
T. sp. URa3	R. Türk 33619	Antarctica, Taylor Valley	II	*Lecidea* sp. UCR1	JN204734
T. sp. URa3	R. Türk 33647	Antarctica, Taylor Valley	II	*L. andersonii*	JN204738
T. sp. URa3	R. Türk 33704	Antarctica, Taylor Valley	II	*L.* sp. UCR1	JN204741
T. sp. URa3	R. Türk 35502	Austria	V	*Carbonea vorticosa*	JN204747
T. sp. URa3	R. Türk 40136	Austria, Oberösterr., Eferding	V	*Lecidea fuscoatra*	JN204765
T. sp. URa3	R. Türk 42745	Austria, Osttirol, Hohe Tauern	V	*L. lap.*var. *lapicida*	JN204769
T. sp. URa3	R. Türk 43427	Norway, Svalbard	VI	*L.* sp.	JN204794

Appendix 1. *Continued*

Photobiont	Herbarium Sample ID	Habitat	Climate zone	Mycobiont	Accession number
T. sp. URa3	R. Türk 43429	Norway, Svalbard	VI	*Carbonea vorticosa*	JN204796
T. sp. URa3	R. Türk 44626	Antarctica, DA, Diamond Hills	I	*C.* sp. URm1	JN204797
T. sp. URa3	R. Türk 44629	Antarctica, DA, Diamond Hills	I	Unknown URm3	JN204798
T. sp. URa3	R. Türk 44631	Antarctica, DA, Diamond Hills	I	Unknown URm3	JN204799
T. sp. URa3	R. Türk 44637	Antarctica, DA, Diamond Hills	I	Unknown URm4	JN204802
T. sp. URa3	R. Türk 44641	Antarctica, DA, Diamond Hills	I	*Carbonea vorticosa*	JN204803
T. sp. URa3	LE A0515601	Antarctica, Dronning Maud Land	III	*Lecidea andersonii*	JN204817
T. sp. URa3	LE A060330	Antarctica, Antarctic Peninsula	III	*L. atrobrunnea*	JN204822
T. sp. URa3	LE A060414	Antarctica, Antarctic Peninsula	III	*L. atrobrunnea*	JN204823
T. sp. URa3	ADT13352	Antarctica, Wilkes Land	III	*L. andersonii*	JN204824
T. sp. URa3	ADT17087	Antarctica, Wilkes Land	III	*L. andersonii*	JN204825
T. sp. URa3	ADT18027	Antarctica, Wilkes Land	III	*L. andersonii*	JN204826
T. sp. URa3	ADT18513	Antarctica, Wilkes Land	III	*L. andersonii*	JN204827
T. sp. URa3	ADT24118	Antarctica, Wilkes Land	III	*L. cancriformis*	JN204829
T. sp. URa3	ADT26423	Antarctica, Wilkes Land	III	*L. andersonii*	JN204831
T. sp. URa3	ADT5902	Antarctica, Wilkes Land	III	*L. cancriformis*	JN204832
T. sp. URa3	MAF-Sancho1	Antarctica, Mt Kyffin	III	*L.* sp.	JN204837
T. sp. URa3	MAF-Sancho2	Antarctica, Mt Kyffin	III	*L. cancriformis*	JN204838
T. sp. URa3	MAF-Sancho3	Antarctica, Mt Kyffin	III	*L.* sp.	JN204839
T. sp. URa3	RS18317	Antarctica, Wilkes Land	III	*L. cancriformis*	JN204840
T. sp. URa3	RS19162	Antarctica, Granite Harbour	III	*L. cancriformis*	JN204841
T. sp. URa3	RSN2.1	Antarctica, Mac Robertson L.	III	*L. cancriformis*	JN204842
T. sp. URa3	RSRL	Antarctica, Wilkes Land	III	*L. cancriformis*	JN204843
T. sp. T. L. 9	–	–	V	*Lecanora rupicola*	DQ166611
T. sp. URa4	R. Türk 39665	Austria, Salzburg, Hochkönig	V	*Lecidella stigmatea*	JN204764
T. sp. URa4	R. Türk 43003	Antarctica, Sperm Bluff	II	*Lecidea andersonii*	JN204775
T. sp. URa5	UR48	Austria, Salzburg, Hochkönig	V	*Lecidella patavina*	JN204844
T. sp. URa6	R. Türk 36808	Austria, Kärnten, Gailtaler A.	V	*L. stigmatea*	JN204760
T. sp. URa7	R. Türk 43425	Norway, Svalbard	VI	*Lecidea andersonii*	JN204792

* hb.Türk (SZU)