Using RADseq to understand the circum-Antarctic distribution of a lichenized fungus, *Pseudocyphellaria glabra*

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Abstract

**Aim:** The Southern Ocean landmasses have intrigued biologists for centuries because they share many taxonomic groups. Such disjunct taxa can provide insight into evolutionary processes that connect populations or drive divergence. The lichenized fungus *Pseudocyphellaria glabra*, for example, has a disjunct distribution—separated by the Tasman Sea and the Pacific Ocean—yet whether these locations should be genetically distinct is unclear. The large distances between continents may be expected to prohibit gene flow, but strong and sustained winds in the Southern Hemisphere and the small size of *P. glabra* propagules may facilitate migration. We compared support for these two hypotheses.

**Location:** Southeastern Australia, Tasmania, New Zealand and Southern Chile.

**Taxon:** *Pseudocyphellaria glabra* (Hook. f. & Taylor) C.W. Dodge, 1948 (Ascomycota, Peltigeraceae)

**Methods:** We collected 371 samples across the disjunct range of *P. glabra*. We generated genomic data using restriction site-associated DNA sequencing and reconstructed a Maximum Likelihood phylogeny using 29,098 unlinked SNPs. We then conducted population genomic analyses using 3,756 SNPs including a minimum-spanning network, principal components analysis, discriminate analysis of principal components, and k-means clustering.

**Results:** Maximum likelihood analysis recovered multiple well-supported clades that roughly corresponded to geography. Population genomic analyses identified genetic structuring that generally corresponded with geographic distance; however, some individuals from Chile and Australia were assigned to genetic clusters found in New Zealand, suggesting that recent dispersal events from New Zealand have successfully colonized Chile and Australia.

**Main conclusion:** Populations of *P. glabra* from Australia, Chile and New Zealand are genetically distinct, but frequent long-distance dispersal during the Quaternary probably prevented speciation. This study demonstrates the power of restriction-site associated DNA sequencing for discernment between divergent and connective evolutionary forces that simultaneously influence the population structure of species with disjunct ranges in the Southern Ocean landmasses.

**KEYWORDS**
biogeography, dispersal, evolution, lichen, lichenized fungi, population genetics, subantarctic, systematics
Disjunct distributions of species intrigue scientists for their potential to elucidate ecological and evolutionary processes that drive genetic diversification and speciation. Geographic barriers that create disjunct distributions divide a single randomly mating unit into multiple units, which can then evolve independently through selection and genetic drift, and ultimately undergo speciation. The study of population genomics can potentially identify differing patterns of genetic structure among disjunct populations and infer processes, such as migration, extinction and speciation, that may have contributed to the observed genetic diversity. Population genetic structure is characterized by the number of subpopulations within a species’ range, the frequencies of genetic variants in each subpopulation, and the degree of genetic isolation of the subpopulations (Chakraborty, 1993). Population genetic structure can uncover whether some populations have been isolated for long periods of time and the spatial scale at which populations are differentiated (Werth, 2010). A better understanding of a population’s genetic structure can also have important implications for the conservation of threatened species and the spread of invasive species (Excoffier, 2004).

The floras of major landmasses in the Southern Hemisphere, such as New Zealand, Southern South America and Southeastern Australia, share many taxonomic groups. This pattern is seen in flowering plants (Winkworth, Hennon, Prinzing, & Wagstaff, 2015; Winkworth, Wagstaff, Glenny, & Lockhart, 2002), ferns (Parris, 2001), bryophytes (Miller, 1982) and lichens (Galloway, 1991, 2008a). Almost 100 species of lichens are shared between Australia and New Zealand and over 40 species are shared among Australia, New Zealand and southern South America (Galloway, 1991, 2008a). Two nonexclusive processes may have led to these disjunct distributions of flora in the Southern Hemisphere: (a) vicariance arising from the breakup of Gondwanaland, which separated and isolated ancestral biotas, or (b) long-distance dispersal over large bodies of water.

The breakup of Gondwanaland occurred between 155–80 Ma (Stevens, 1980) and predates the ages of most lineages at the species level, but at the genus and family level, vicariance could explain the many shared groups among southern landmasses (Galloway, 1988; Moncalvo & Buchanan, 2008). However, molecular data suggest that many Southern Hemisphere plant distributions have arisen only within the last 10 Myr (Winkworth et al., 2002). Review articles investigating molecular phylogenies have shown the importance of wind dispersal for seed- and spore-bearing plant species that are distributed across two or more continents (McGlone, 2005; Moreira-Muñoz, 2007; Sammartin & Ronquist, 2004). The West Wind Drift in particular plays an important role in dispersal (Raven, 1973); these cyclonic eastward winds, moving clockwise around Antarctica, are four times as strong as their counterparts in the northern hemisphere. This pattern was initiated during the Miocene when Australia and South America separated from Antarctica, intensified during the Tertiary, and was probably the strongest during the Pleistocene glacial periods (2 Ma–14,000 ya; Lamb, 1959). To this day it is a dominant weather pattern (Winkworth et al., 2002). At least some of the floristic similarities of southern landmasses are direction-dependent and driven by the West Wind Drift pattern (Muñoz, Felicísimo, Cabezas, Burgaz, & Martínez, 2004).

Lichens are symbiotic organisms consisting of a fungus and photosynthetic green algae or cyanobacteria, or both simultaneously. In austral lichen taxa, Galloway (2008b) describes two types of mycobiotas – namely, the paleoaustral lichens, which are Gondwanan groups with poor dispersal abilities, and the neoaustral lichens that dispersed after the breakup of Gondwanaland, through long-distance dispersal of vegetative propagules via birds, ocean currents, or the West Wind Drift. Our focal species, Pseudocyphellaria glabra, has a disjunct range (Figure 1a,b) in Southeastern Australia (incl. Tasmania), New Zealand, Chile, Argentina, Lord Howe Island, Macquarie Island and the subantarctic islands of New Zealand, and is considered a neoaustral lichen species (Galloway, 1991, 2008a; McCarthy, 2001). Indeed, molecular genetic evidence suggest that P. glabra is relatively young (Widhelm et al., 2019).

We recently estimated divergence times in the former family Lobariaceae (Widhelm et al., 2019). The family is now considered the lobarioid clade of Peltigeraceae based on a temporal banding approach that reclassified higher level taxonomic groups in the Lecanoromycetes (Kraichak, Huang, Nelsen, Leavitt, & Lumbsch, 2018). The time-tree estimated a crown age for the lobarioid clade around 70 Ma (Widhelm et al., 2019). This time-tree included one sample each of the closely related species P. glabra, P. homoeophylla and P. freycinetii. The divergence estimates were around 13 Ma for the branching of P. freycinetii and around 3 Ma for P. glabra from P. homoeophylla. If the estimates are accurate, the entire lobarioid clade diversified rapidly at the end and after the breakup of Gondwana (155–80 Ma, Stevens, 1980) and P. glabra, P. homoeophylla and P. freycinetii evolved relatively recently when the continents were very close to their present configuration.

In New Zealand, P. glabra has the widest range and ecological tolerance of all Pseudocyphellaria species (Galloway, 2007). It can be found in all forest types but is most abundant in the wettest of temperate rain forests. It is a large foliose species that is mostly epiphytic but is also found on moss covered rocks (Figure 1c–e). The species reproduces sexually with microscopic ascospores, and vegetatively with phylidia that can be present in copious quantities. Both types of reproductive propagules could be dispersed via West Wind Drift, birds and ocean currents.

The disjunct distribution of P. glabra (Figure 1a,b) makes it an excellent study system for understanding the pattern of genetic differentiation among populations sampled from different landmasses and how much the Pacific Ocean, Tasman Sea, Bass Strait and Cook Strait limit genetic exchange among populations. We test two hypotheses: (1) if large bodies of water isolate populations of P. glabra, then these populations will be genetically distinct, and (2) given the strong wind patterns in the Southern Hemisphere, P. glabra populations on different landmasses will be connected by frequent and ongoing long-distance dispersal (i.e. gene flow). To test these hypotheses, we collected 371 samples from southeast Australia, North and South Islands of New Zealand, and Chile. We used restriction
site-associated DNA sequencing data (RADseq), which is relatively affordable, possible in non-model organisms, and can generate thousands of genetic loci across many individuals (Davey & Blaxter, 2010). RADseq delivers higher resolution population genetic information than traditional methods for phylogeographic and population genetic analyses. Thus, we implemented RADseq to understand the distribution of genetic variation of *P. glabra* across Australia, New Zealand and Chile and to determine whether large bodies of water cause major breaks in gene flow.

2 | MATERIALS AND METHODS

2.1 | Sampling

Fresh population samples of *P. glabra* were collected in the Australian states of Tasmania and Victoria (TAS and VIC), throughout the North and South Islands of New Zealand (NZN and NZS, including two samples from Auckland and Enderby Islands), and in Chile (CHI) during three field trips from 2015 to 2017 (see Figure 2b for collection sites from all major land masses are shown on the maps (b) and represented by colours on the tip labels...
locality maps). A total of 371 specimens were sampled from 41 sites. At each site, if possible, at least 20 individual thalli were collected on separate trees. We also sampled P. freycinetii in Chile and used this specimen as an outgroup. All specimens are vouchered in F (Table S1).

2.2 | DNA isolation and quality assessment

Genomic DNA was extracted from a roughly 1 cm² portion of vegetative tissue and a mortar and pestle and the DNAs were isolated using ZR Fungal/Bacterial DNA MiniPrep™ kit (Zymo Research) adhering to the manufacturer’s directions except that macerated tissues were incubated at 65°C for 1 hr in lysis buffer before proceeding to the isolation steps. DNA concentration was determined using the AccuBlue (Biotium, Inc.) kit and the Tecan plate reader (Tecan Genomics). DNA isolation and quality assessment

2.3 | Metagenomic DNA sequencing and mycobiont reference genome assembly

Metagenomic DNA from sample VIC 2287A (DNA# 14816) was sequenced at the University of Illinois at Chicago Sequencing Core on an Illumina (Illumina Inc.) NextSeq500 platform. Trimmomatic (Bolger, Lohse, & Usadel, 2014) was used to remove poor quality reads and adapter sequences. Bases were trimmed when the average quality of 4-base sliding windows was below 15 and bases at the start and end of reads had a quality below 10. All trimmed reads shorter than 25 bp were filtered out (LEADING:10 TRAILING:10 SLIDING-WINDOW:4:15 MINLEN:25). After quality filtering, trimmed paired-end reads were assembled using SPAdes 3.5.0 (Bankevich et al., 2012) with default parameter settings.

The assembled metagenome was used as an input for MetaWATT Binner 3.5.3 (Strous, Kraft, Bisdorf, & Tegetmeyer, 2012), which was used to cluster contigs that originated from the mycobiont population of DNA data. The MetaWATT binning procedure uses multivariate statistics of tetrancleotidic frequencies combined with the use of interpolated Markov models. Because MetaWATT was originally designed only for prokaryotic organisms, we produced a custom database of nine axencyclic cured lichen-forming fungal nuclear genomes (see Table S2 for Genbank accession numbers) to identify contigs of Lecanoromycetes origin.

To create the custom database, we performed gene prediction using AUGUSTUS 2.5.5 (Stanke & Morgenstern, 2005) on all 10 genomes. Amino acid sequences of the generated gene models were used by MetaWATT for the identification of bins. Only reads identified as Lecanoromycetes were selected from the metagenomic contigs. Finally, we created a Bowtie2 (Langmead & Salzberg, 2012) database from the selected scaffolds for the mapping approach to filter for mycobiont RAD loci.

2.4 | RADseq library prep and Illumina sequencing

Restriction site associated DNA libraries were prepared and sequenced from whole DNA isolations at the University of Wisconsin-Madison Biotechnology Center. DNA concentration was verified using the Quant-it™ PicoGreen® dsDNA kit (Life Technologies). Libraries were prepared as in Elshire et al. (2011) with minimal modification; in short, 50 ng of DNA was digested using the 5-bp cutter ApeKI (New England Biolabs) after which barcoded adapters amenable to Illumina sequencing were added by ligation with T4 ligase (New England Biolabs). Libraries were prepared in batches of 192 samples and adapter-ligated samples were pooled and amplified to provide library quantities amenable for sequencing, and adapter dimers were removed by SPRI bead purification. Quality and quantity of the finished libraries were assessed using the Agilent Bioanalyser High Sensitivity Chip (Agilent Technologies, Inc.) and Qubit® dsDNA HS Assay Kit (Life Technologies), respectively. Libraries were standardized to 2 nM. Cluster generation was performed using HiSeq SR Cluster Kit v3 cBot kits (Illumina Inc.) Flowcells were sequenced using single read, 100 bp sequencing and HiSeq SBS Kit v4 (50 Cycle) (Illumina Inc.) on a HiSeq2500 sequencer. Images were analyzed using the standard ILLUMINA PIPELINE, 1.8.2.

2.5 | RADseq data assembly

The raw reads of P. glabra samples from the HiSeq2500 sequencing were processed and assembled as described earlier for metagenomic datasets of lichens (Grewe, Huang, Leavitt, & Lumbsch, 2017). This process used ipyRAD (Eaton & Overcast, 2016) to demultiplex raw reads and pyRAD (Eaton & Ree, 2013) for the remaining steps. An additional mapping step was conducted after the generation of consensus sequences (pyRAD step 5) that used Bowtie2 to filter for mycobiont loci with a reference sequence. Raw Illumina RAD sequences are referred to as read and the clustered reads per individual sample as loci; the final matrices are alignments of homologous loci from multiple samples with nucleotide substitutions referred to as SNP. In pyRAD, we set the datatype to genotype-by-sequencing, ploidy to haploid (1), a similarity threshold for the clustering of reads within and between individuals to 90% (0.90), and a minimum coverage of four samples per locus (4). For the reference-based filtering of RAD loci, we used Bowtie2 with adjusted parameters to allow one permitted mismatch (~N 1), a seed length of 20 (~L 20), up to 20 seed extension attempts (~D 20) and a maximum ‘re-seeding’ of 3 (~R 3). Following an initial round with all sequenced samples, we re-ran step 7 of pyRAD and excluded 70 samples with fewer than 1,000 recovered loci, resulting in a final dataset with 301 samples that had an average of 7,045 (SD = 4,719) loci per sample. We used the filtered pyRAD output files from these datasets, such as unlinked_snps, alleles, and vcf, for further phylogenetic and population genomic analyses as described earlier for lichen RADseq datasets (Grewe, Lagostina, Wu, Printzen, & Thorsten Lumbsch, 2018).
2.6 | Phylogenomic analysis

A maximum likelihood (ML) phylogenetic analysis was conducted with iq-TREE 1.6.12 (Nguyen, Schmidt, von Haeseler, & Minh, 2015) using the concatenated unlinked SNPs file generated by pyRAD that contained all *P. glabra* samples with at least 1,000 loci and the one outgroup *P. freycinetii* (302 samples total). ModelFinder (Kalyaanamoorthy, Minh, Wong, von Haeseler, & Jermiin, 2017) identified GTR+F+ASC+R5 model as the best fit using the Bayesian information criterion (BIC) and that model was used for the ML tree search with 1,000 bootstrap replicates using the rapid bootstrapping algorithm. The resulting unrooted phylogenetic tree was drawn to scale with the Interactive Tree of Life (https://itol.embl.de/).

2.7 | Estimation of population subdivision

The differences in the population structure of *P. glabra* samples between different landmass populations were calculated by creating a reduced dataset that included all sites with a minor allele frequency (MAF) larger than 0.05 and greater than 50% coverage using VCFtools 0.1.15 (Danecek et al., 2011). We converted the reduced file to a genlight object using the adegenet 2.0.2 R package. The genlight object was further modified to account for haploid genomes, and the population membership for samples based on landmass were assigned. This modified genlight object was used for population genetic analyses in R.

Population structure of the 301 *P. glabra* samples was visualized with a minimum spanning network (MSN). The MSN analysis clusters multilocus genotypes (nodes) and draws edges that represent the genetic distances between nodes. The genlight object was used to reconstruct the MSN along with a distance matrix that was calculated using bitwise.dist() function from poppr (Kamvar, Tabima, & Grünwald, 2014), which calculated allelic differences between all samples. The function plot_poppr_msn() was used to visualize the MSN.

A principal component analysis (PCA) was conducted to summarize genetic variation between samples using the genlight object. We used the gIPCA function in adegenet (Jombart et al., 2010) retaining the first four principal components. To visualize the PCA, we used ggplot2 (Wickham, 2016), where colour represents landmass populations and ellipses enclose 95% of the data for each population.

We used the Discriminant Analysis of Principal Components (DAPC) to estimate the genetic structure of 301 *P. glabra* samples from the five landmass populations (CHI, NZN, NZS, TAS and VIC). The DAPC is a multivariate statistical approach that combines a PCA with a discriminant analysis (DA) for a separation of genomes based on their variance between groups rather than the total variance of the sample. The DAPC was implemented in the adegenet 2.1.1 R package (Jombart et al., 2010). Initially, the genlight data were transformed using a PCA and then components of PCA were submitted to a DA. The results of the analyses were visualized in two ways: one showing genetic variation in genomic space and the other in a STRUCTURE-like plot that shows the predicted group membership.

2.8 | Clustering analysis

We identified the number of genetic clusters (*k*) in the reduced genlight object using the function find.clusters from the R adegenet package (Jombart et al., 2010). We ran successive K-means from 1–10 values of *k*, retaining all principal components (PCs). For each model, BIC goodness of fit was computed to identify the optimal *k*. For each value of *k*, this process was repeated 10 times to explore the variability for each value. We explored multiple values of *k* by visualizing with a DAPC barplot of the posterior probabilities of group assignment for each sample.

3 | RESULTS

3.1 | Reference genome assembly and RADseq results

We assembled a draft reference genome of *P. glabra* to filter mycobiont RAD loci. The Illumina NextSeq sequencing of the whole *P. glabra* (2287A VIC) thallus resulted in 42,803,904 metagenomic (lichenized fungal, algal, cyanobacterial and microbial) paired-end reads. First, we trimmed these raw data, which reduced the paired-end reads to 40,247,418 (94% of raw data). These were then assembled into 1,038,157 scaffolds (N50 = 189,136 bp; L50 count of 513 kbp) with a total size of 494 Mbp (including 1,866 scaffolds of sizes larger than 10 kbp). Further metagenomic binning identified 30.65 Mbp of the assembly as mycobiont derived, from which we selected 1,142 scaffolds (N50 = 401 bp; L50 count of 17,123 kbp) with sizes larger than 10 kbp. The sorted draft genome of *P. glabra* had a total size of 19.36 Mbp.

3.2 | Phylogenomic analysis

The ML tree (Figure 2a) was estimated from the 302 sample RADseq matrix with 29,098 distinct alignment patterns and 11,997 parsimony informative sites. The tree has five well-supported, major clades. Clade A (100% bootstrap support) contained samples from CHI, NZN and NZS. Clade B (100% bootstrap support) had one sample from NZN and 10 samples from VIC, while Clade C (100% bootstrap support) had the rest of the Australian samples from VIC and TAS. Clade D (100% bootstrap support) contained the majority of samples from CHI. Clade E (97% bootstrap support) had samples from NZN and NZS. The one sample from Enderby Island (17205_NZS) forms a sister-group to Clades D and E.
3.3 Population genomic analyses

For population genomic analyses, we included only SNPs with a MAF >0.05 and more than 50% coverage. This filtering approach reduced the RADseq dataset to a total of 3,717 SNPs used to differentiate the genomes by their variation with MSN, PCA and in a non-parametric approach with a DAPC. The outgroup sample of *P. freycinetii* is not included in this dataset.

The MSN analysis shows that populations are not fully differentiated geographically (Figure 3). Clusters of samples in the network correspond to major clades in the ML tree. Individuals from Chile tend to be associated with samples from New Zealand.

With the PCA we observed that PC1 distinguished samples from Australia (TAS and VIC: Figure 4, far left), New Zealand (NZN and NZS: Figure 4, middle) and Chile (CHI: Figure 4, far right). The Australian populations from TAS and VIC were undifferentiated as were the two New Zealand populations (NZN and NZS). Most of the CHI samples were genetically distinct, but some could be found within the New Zealand 95% ellipses.

The results of the DAPC scatterplot (Figure S1) are like the PCA with Australian (TAS and VIC) samples occupying the same area. The same is true for the New Zealand samples with NZN and NZS overlapping in the plot. The samples from Chile form their own cluster in the top left. The barplot detected high levels of admixture (Figure 5). Samples from New Zealand have high levels of admixture, as most individuals are assigned to both populations NZN and NZS. A similar result is seen among TAS and VIC samples. Some individuals from Chile and Australia are either completely assigned to a New Zealand population or have lower levels of admixture.

We explored different numbers of k using the find.clusters function in the adegenet R package. For this analysis, we used BIC goodness-of-fit statistics for 1–10 clusters (10 replicates each k) and found that four, five and six clusters had the lowest but similar BIC scores (Figure 6). We generated barplots for k values from three to six (Figure S2). In the barplots (Figure 6) Group 1, which contains most of the individuals from Chile, is always well-differentiated from other samples. Group 1 assignment corresponded with Clade D in the ML phylogeny. Groups 2 and 3 encompass all the individuals from New Zealand, Australia, and some samples from Chile. In the k = 4 plot, Group 4 encompassed most samples from TAS and VIC and corresponded to Clade C in the ML phylogeny, and five samples from VIC were assigned to Group 3. In the k = 5 plot, many of the Group 3 samples were assigned to Group 5, and when k = 6 some samples from VIC were assigned to group 6. Groups 5 and 6 corresponded to subclades of the major clades found in the ML tree.

4 DISCUSSION

The biogeography of the Southern Hemisphere has intrigued scientists for hundreds of years. When Joseph D. Hooker visited these areas during an Antarctic Expedition between 1839 and 1843 he was impelled to write Charles Darwin to explain the biological similarities of Australia, New Zealand and Southern South America (Skottsberg & Pantin, 1960). Hooker became convinced that these affinities were only partly explained by dispersal and more so by a fragmented
great Southern Continent (Galloway, 1988). This idea was supported later by Wegener’s theory of continental drift (Wegener, 1966) and subsequently by plate tectonics (Hughes, 2001). The breakup of Gondwana did indeed isolate many groups of organisms, including lichens, and these ideas dominated explanations of disjunct distributions of species by biogeographers for many years (Brundin, 1966; Raven & Axelrod, 1972; Swenson, Hill, & McLoughlin, 2001). Today, however, dispersal is seen as the primary mechanism for the disjunct distribution at the species level (McGlone, 2005; Moreira-Muñoz, 2007; Sanmartín & Ronquist, 2004). Long-distance dispersal was first recognized as an important force in the Southern Hemisphere when palynological data revealed temporal differences in the first appearance of shared taxa (i.e. Australia first, New Zealand second, etc.) The pollen fossil record suggests that transoceanic dispersal occurred throughout the Tertiary (Macphail, 1997; Mildenhall, 1980; Pole, 1994, 2001). Molecular phylogenetic studies further corroborated patterns of eastward dispersal of austral plants (table 1 in Winkworth et al., 2002). Similarly, our results showed that dispersal, rather than vicariance, better explains the genetic pattern of currently disjunct distributed *P. glabra*.

Our results suggest that the vast water bodies separating continents of the Southern Hemisphere do not form an absolute barrier to gene flow among populations of the lichenized fungus *P. glabra*, but the Tasman Sea and Pacific Ocean are still large enough
to isolate genetically distinct populations. However, geographically distant individuals may be more genetically similar than geographically proximate ones (some CHI and VIC individuals assigned to New Zealand genetic clusters in the DAPC and clustering analyses). The study finding corroborated the biology of the species, where lichenized fungi, which have reproductive propagules with a small size and come in large quantities, tend to disperse well and the disjunct distribution of *P. glabra* can be explained by recent dispersal events (Galloway, 1991, 2008a, 2008b).

Our ML results (Figure 2a) showed that samples of *P. glabra* formed multiple distinct lineages and did not show clearly delimited geographic structure. Individuals of *P. glabra* from all major landmasses were polyphyletic, but most samples from Chile and Australia formed well-supported clades (Figure 2a, Clades C, B, and D). New Zealand samples are polyphyletic, suggesting a greater genetic variability in this region, which is also observed in the DAPC and clustering analyses. One sample from Enderby Island formed a distinct lineage sister to Clades D and E. Perhaps more dense sampling of New Zealand’s subantarctic islands would reveal other genetically isolated clusters.

Population genomic clustering results from the RADseq dataset confirm that *P. glabra* populations are isolated by large bodies of water, such as the Tasman Sea and Pacific, because they are genetically distinguishable from one another. Relatively smaller bodies of water, such as the Bass Strait (separating TAS and VIC) and Cook Strait (separating NZN and NZS), are not large enough to isolate populations and dispersal is homogenizing the populations. Alternatively, if the Bass and Cook Straits were land bridges during the last glacial maximum (Lambeck & Chappell, 2001; Proctor & Carter, 1989), it might be that not enough time has passed for populations on each side to become genetically differentiated. Admixture between New Zealand and Chile was detected in the STRUCTURE-like plot of the DAPC analysis (Figure 5). Seven samples from Chile were assigned to the New Zealand landmass based on genetic data; this could be caused by relatively recent long-distance dispersal. This result agrees with the ML phylogeny, where some CHI samples form a small sub-clade in Clade A, clustering with samples from NZN and NZS and are not associated with most CHI samples in Clade D. There was slightly less admixture between New Zealand and Australia samples. Only six Australian samples from VIC were assigned to New Zealand genetic clusters. This could be due to occasional east-to-west dispersal across the Tasman Sea. Although the prevailing wind pattern is west-to-east at high altitudes, some cyclonic weather systems at lower altitudes provide opportunities for east-to-west dispersal, and birds are also capable of carrying spores against the dominant wind patterns (Wardle, 1978).

The evidence for dispersal in the RADseq data is bolstered by divergence time estimates of Widhelm et al. (2019) that showed *P. glabra* originated long after the breakup of Gondwana. We also conducted a molecular clock analysis of ITS sequences from 34 *P. glabra* individuals from all major landmasses and *P. freycinetii* as outgroup and found similar divergence times (Figure S3; Table S3) using a previously reported molecular evolution rate of 3.41 substitution/site/year × 10^-9 (Leavitt, Esslinger, Divakar, & Lumbsch, 2012). Furthermore, the presence of *P. glabra* on Lord Howe Island, Macquarie Island and the subantarctic islands of New Zealand suggest a highly dispersive capacity of the species (McCarthy, 2001). The dominant west-to-east wind pattern (i.e. West Wind Drift) is probably facilitating the migrations of wind dispersing organisms (Galloway, 1991; Muñoz et al., 2004). Similarly, birds such as the migratory albatross and shearwaters could carry propagules and spores in both directions (Gillespie et al., 2012).

Our results suggesting long-distance dispersal are in line with what has been reported in other studies on lichens where intercontinental dispersal has formed genetically distinct populations, but evidence for recent dispersal suggests that these are still connected by gene flow. Buschbom (2007) investigated the population genetics of *Porpidia flavicunda*, which has a circumarctic distribution, using three nuclear loci. There were no fixed nucleotide polymorphisms, and identical haplotypes were widely shared. Migration rate analysis suggested that *P. flavicunda* dispersal was sufficiently high to prevent major population differentiation by genetic drift (Buschbom, 2007). *Cetraria aculeata* occurs in the Arctic, the Antarctic, and at high altitudes in the Americas and Eurasia. Population genetic and phylogenetic analyses of *C. aculeata* populations suggested that the species originated in the Northern Hemisphere and had a stepwise dispersal via the Andes through the Pleistocene (Fernández-Mendoza & Printzen, 2013). In another bipolar taxon, *Mastodia tesselata*, phylogenetic and population genetic analyses suggested that *M. tesselata* actually consists of two species that diverged during the Miocene in the Southern Hemisphere. One species has a range in Antarctica and the other in Southern South America. The South American species became bipolar during a single trans-tropical dispersal event to Alaska (Garrido-Benavent, de los Ríos, Fernández-Mendoza, & Pérez-Ortega, 2018). Another example is in the genus *Sticta*, where at least four independent long-distance dispersal events from continental sources to Hawaii were inferred using a five-gene phylogeny (Widhelm et al., 2018).

Circumantarctic and bipolar species ranges are also found in other spore-bearing organisms. The moss *Polytrichum juniperinum* has a bipolar and circumantarctic disjunct range and reportedly recently dispersed from South America to Australia/New Zealand and was reported using phylogenetic methods (Biersma et al., 2017). The moss *Pyrrhobryum mnioides* has likely undergone both ancient cryptic speciation reflecting vicariance between South America and Australia/New Zealand (80 Ma), and recent dispersal between Australia and New Zealand is likely (McDaniel & Shaw, 2003). Patterns of vicariance and long-distance dispersal were both invoked to explain the current distribution of species of the tree ferns of the family Cyatheaceae, including long-distance dispersal was reported from Australasia to South America in Cyathea (Noben et al., 2017).

With high levels of dispersal, inferring the ancestral range of *P. glabra* is not straightforward. The higher levels of genetic diversity in New Zealand suggest that *P. glabra* originated in New Zealand. Furthermore, the sister species *P. homoeophylla* is only found in New Zealand and its genetic variability is also found in New Zealand.
 Zealand (Galloway, 2007). If *P. glabra* originated in New Zealand after divergence from *P. homeoeophylla*, with our estimates of recent divergence times (within the last 5 Ma), dispersal would have been the only way the species colonized and populated Australia and Chile. When dispersal was successful, most likely a ‘founder takes all’ scenario played out, where only one genotype became established, leading to high-density blocking that inhibited the establishment of the new genotype (Waters, Fraser, & Hewitt, 2013). The populations in Australia and Chile have lower genetic diversity than New Zealand, which is in line with the ‘founder takes all’ hypothesis. The greater genetic diversity of *P. glabra* in New Zealand could also have been influenced by the diversity of New Zealand’s geological processes (sinking, uplift, tilting, sea level change, erosion, volcanism, glaciation). For example, during the Pliocene (5.333–2.58 Ma), when *P. glabra* populations were diversifying, the Southern Alps experienced rapid uplift (Wallis & Trewick, 2009). The orogeny of mountain ranges is known to promote genetic diversity (Badgley et al., 2017; Hoorn et al., 2010). In lichens, the uplift of the Andes was shown to increase genetic diversity in *Sticta* (Widholm et al., 2018) and *Peltigera* (Magain, Mialdikowska, Goffinet, Sérusiaux, & Lutzoni, 2017).

However, the Chilean endemic *P. freycinetii* is sister to *P. glabra/P. homeoeophylla* clade (Widholm et al., 2019), so *P. glabra* might have diverged from *P. freycinetii* in Chile. Divergence could have been ecological-sympatric, with the two taxa being derived from a common ancestor in Chile. In Chile, *P. freycinetii* is found in subalpine and steppe habitats between 600–1,200 m altitude and grows on soil or mosses, while *P. glabra*, in Chile and throughout its entire range, has a wider ecological amplitude, being found at sea level and in lowland forests, scrub, moorlands, grasslands, and subalpine and high alpine scrub (Quilhot, Cuellar, Díaz, Riquelme, & Rubio, 2012). After divergence in Chile, *P. glabra*, with wider ecological amplitude and sexual and asexual modes of dispersal, was possibly better at dispersing to other landmasses. After dispersal to New Zealand, another lineage became isolated and lost the ability to produce vegetative propagules, leading to the speciation event that formed *P. homeoeophylla*. In Tasmania, another one of these speciation events could have generated the endemic *P. soredioglabra*, which is hypothesized to be a sorediate sister species to the phyllidiate *P. glabra*, but this has not been tested with molecular data. Both species have a yellowish thallus (usnic acid in upper cortex); broad, spreading, adnate lobes; a dark brown tomentum on the lower cortex; a white medulla; yellowish thallus (usnic acid in upper cortex); broad, spreading, adnate lobes; a dark brown tomentum on the lower cortex; a white medulla; white pseudocyphellae; green photobiont; and an identical chemical composition (Kantvilas & Elix, 1999). To further clarify the original range of *P. glabra*, the focus of future population genomic analyses should focus on more sampling of *P. glabra*, *P. homeoeophylla* and *P. soredioglabra*.

5 | CONCLUSION

The populations of *P. glabra* occurring in Australia, Chile and New Zealand are genetically structured, but long-distance dispersal, via dominant wind patterns and possibly by migratory birds, has established populations on landmasses between −20S and −60S degrees latitude in the Southern Ocean. The relatively wide ecological tolerance and ability to thrive in a variety of habitats and substrates (Galloway, 2007) may allow *P. glabra* to be more successful at long-distance dispersal than closely related *Pseudocyphellaria* species, such as *P. soredioglabra*, *P. homeoeophylla* and *P. freycinetii*, which are only found in New Zealand and Chile, respectively. The results suggest that dispersal keeps populations connected, even though they are divided by the large barriers of the Pacific Ocean and the Tasman Sea.

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DATA AVAILABILITY STATEMENT

Raw RAD sequences: GenBank BioProject PRJNA614916. Final datasets used for phylogenetic and population genetic analyses and input data and scripts for DAPC are at GitHub https://github.com/twidholm/P-glabra-RADseq.

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REFERENCES


BIOSKETCH

Todd J. Widhelm is the Collection Manager of Fungi at the Field Museum in Chicago, Illinois, USA. This paper was part of his dissertation, supervised by Roberta Mason-Gamer and Thorsten Lumbsch, which focused on the phylogenetics and population genetics of lichenized fungi.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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