Received: 15 June 2022 | Revised: 17 November 2022 | Accepted: 25 November 2022

DOI: 10.1111/jbi.14554

Journal of Biogeography

WILEY

RESEARCH ARTICLE

Ancient vicariance is reinforced by adaptive divergence in the southern beech: Contributions from geogenomics

Mariana Fasanella[1] | Paula Mathiasen[1,2] | Gabriela Juri[1,3] | Dayana G. Díaz[1] | Rodrigo Hasbún[4] | Andrea C. Premoli[1,2]

[1]Instituto de Investigaciones en Biodiversidad y Medioambiente (INIBIOMA-CONICET-COMAHUE), Bariloche, Río Negro, Argentina

[2]Centro Regional Universitario Bariloche, Universidad Nacional del Comahue, San Carlos de Bariloche, Argentina

[3]Instituto de Investigaciones en Recursos Naturales, Agroecología y Desarrollo Rural (IRNAD-CONICET-UNRN). El Bolsón, Río Negro, Argentina

[4]Laboratorio de Epigenética Vegetal, Departamento de Silvicultura, Facultad de Ciencias Forestales, Universidad de Concepción, Concepción, Chile

Correspondence
Andrea C. Premoli, Instituto de Investigaciones en Biodiversidad y Medioambiente (INIBIOMA-CONICET-COMAHUE), Bariloche, Río Negro, Argentina.
Email: andrea.premoli@gmail.com

Funding information
Consejo Nacional de Investigaciones Científicas y Técnicas Argentina, Grant/Award Number: PIP 2021-11220200102500CO; Universidad Nacional del Comahue Argentina, Grant/Award Number: 04/B235; Agencia Nacional de Promoción Científica y Tecnológica Argentina, Grant/Award Number: PICT 2017-0577 and PICT 2019-149; Rufford Foundation, Grant/Award Number: 29211-1; Fondo de Investigación Bosque Nativo CONAF Chile, Grant/Award Number: 068/2012

Handling Editor: Thais Vasconcelos

Abstract

Aim: Geogenomics seeks to understand geological processes linked to lineage divergence. However, the mechanisms that conserve ancient signals in spite of gene flow are still unclear. In the southern beech, the deep lineage divergence produced by vicariant events is associated with ancient marine transgressions. We hereby evaluate the hypothesis that this divergence is maintained by diversifying selection.

Location: Southern Argentina and Chile.

Taxon: *Nothofagus dombeyi*.

Methods: The lineage divergence by means of analysis of molecular variance (AMOVA), principal coordinate analysis, assignment tests and multiple matrix regression analyses were assessed using chloroplast DNA and neutral and outlier single nucleotide polymorphisms (SNPs). Several environmental variables were used to characterize potential within-species niche structuring and genotype–environment associations.

Results: Two deep-rooted latitudinally structured lineages resulted from cpDNA, the northern cluster being more genetically diverse than the southern one. Of the total of 2943 SNPs, 33 identified as outliers and produced two genetic clusters. Neutral SNPs yielded no structure by AMOVA, whereas higher (>75%) F_{ST} values were obtained for cpDNA and outlier SNPs. Precipitation variables were mostly associated with population clusters and suggested two climatic niches, consisting of cold and dry in the south and more variable precipitation, temperature and soil conditions in the north. Associations of genetic distance with environment and geography suggested isolation-by-distance and isolation-by-ecology or isolation-by-environment effects.

Main conclusions: Ancient lineage divergence in *N. dombeyi*, originally driven by vicariance, has been maintained by diversifying selection under distinct environmental conditions that also define distinct within-species niches. Deeply rooted phylogeographical breaks can be conserved in continuously distributed species in the absence of current geographical barriers. Yet, physical gradients exert differential selective pressures, which are maintained in the face of potential gene flow. As a result, selection can lead to geographically localized and differentially adapted groups of populations that can be detected by a combination of traditional phylogeographical and novel genomic methods.

KEYWORDS
cpDNA sequences, cryptic lineages, diversifying selection, genotype-by-sequencing, IBD, IBE, *Nothofagus dombeyi*, single nucleotide polymorphisms

1 | INTRODUCTION

Phylogeographical analyses using the DNA sequences of conserved regions of mitochondria and chloroplasts are used to reconstruct the biogeographical history of taxa. In some of these reconstructions, marked phylogeographical structures indicate potential restrictions to gene flow. Among-lineage differentiation could be driven by tectonic and/or climatic influences that may even occur without noticeable variation in other traits such as morphology (Irwin, 2002), resulting in cryptic divergence (Chenuil et al., 2019; Dufresnes et al., 2020; Shneyer & Kotseruba, 2015; Struck et al., 2018). A great diversity of organisms, including those with cosmopolitan distributions, yield highly divergent genetic lineages. In particular, the geographical discontinuity of intraspecific phylogenies with uniparentally inherited genomes may show genetic 'breaks' sensu Avise et al. (1987) consisting of arrays of related haplotypes that differ from other such groupings by many mutational steps. Intraspecific monophyletic groups distinguished by large genealogical gaps usually arise from long-term extrinsic barriers to gene flow (Avise, 1994; Avise et al., 1987). Similarly, concordant phylogeographical breaks in phylogenetically independent lineages are interpreted as the result of historical biogeographical forces, such as vicariance, that have modelled the genetic architecture of particular regional biotas (Avise, 2009). These divergent phylogroups may even coexist in sympatry as a result of more recent secondary contact between lineages. Thus, geographical breaks typically used to define biogeographical provinces and regions result in associations of taxa that were shaped by both ecological and evolutionary processes. Similarly, vicariant forces may be cryptic and can be detected by the within-species lineage divergence of widespread taxa. Nonetheless, the question still remains as to how these phylogeographical breaks persist over time in spite of potential among-population gene flow. Although differentiation at neutral marker level may be driven primarily by stochastic processes, divergent selection can be strong enough to promote reproductive isolation between populations. Thus, genome-wide differentiation of populations may occur in spite of gene flow (Kirk & Freeland, 2011).

Deep phylogeographical breaks may not coincide with such sudden changes in other traits and can be produced within a continuously distributed species by low individual dispersal distance or small population size (Irwin, 2002). As Neigel and Avise (1993) and Avise (2000) pointed out, phylogeographical discontinuity might arise without a geographical barrier to gene flow. In particular, simulations of uniparentally inherited gene genealogies showed that coalescence time may increase as a result of selection for local adaptation (Irwin, 2012). However, such spatially structured genealogies are not necessarily as predictive of overall patterns of variation in other traits as biparentally inherited genealogies linked to autosomal DNA. Gradual quantitative trait variation is usually found along gradients, as well as in molecular markers such as microsatellites which indicate vast nuclear gene flow (Ribeiro et al., 2011). Nonetheless, gene flow and natural selection along environmental gradients may have opposing effects. While the former may enrich local fitness if new allelic variants are introduced and become available for selection, adaptation may also be arrested if the gene flow rates are so high that the new variants overwhelm locally beneficial genes (Sexton et al., 2014).

Species distribution models (SDMs) use climatic variables to assess the potential ranges of species under several assumptions that should be re-evaluated, such as the belief that species are in equilibrium with their climatic niche, that climate is static over time and is the main distribution driver of each species, and that individual populations respond similarly to variations in climate (Chardon et al., 2020). Thus, intraspecific niche variation and phylogeographical structure have generally been overlooked when modelling species' distributions (Pfenninger et al., 2007), despite the large number of species that inhabit a great variety of climates. Furthermore, populations inhabiting variable geographical conditions can develop genetically based environmental tolerances, resulting in locally adapted populations (Savolainen et al., 2007). Regional environmental dependencies should therefore be modelled in order to capture this geographical variation (Pearman et al., 2010). Uniparentally inherited phylogroups may not only be the result of geographical barriers due to vicariance, but may also be linked to differences in climate and/or contrasting environmental settings; that is, exposed to differential selection regimes, within the range of a species.

The physical heterogeneity of a landscape creates variable settings for adaptation that may result in genetic clines and/or ecotypic variation, depending on how steeply conditions vary along gradients. In addition, genetic patterns may also be the result of genetic exchange, which results in isolation-by-distance (IBD) models such that nearby populations tend to be more genetically alike than distant ones. Likewise, similar environments may result in higher gene flow rates between certain populations (e.g. similar phenology) or in local adaptation due to diversifying selection, which may adjust to isolation-by-environment or isolation-by-ecology (IBE) models (Wang et al., 2013). Thus, spatial heterogeneity in ecological processes significantly contributes to adaptive genetic divergence by means of IBE, in addition to the past and current neutral forces affecting IBD.

Southern South America has a complex geological history of crystalline basements that characterize vast areas, such as the Northern and Deseado Massifs towards the north and south of Patagonia, respectively, and the Nahuelbuta Massif along the western Pacific coast (Ramos, 1982, 1989). Overall, these areas can be considered stable terrains that fostered the early evolution of the associated biota, despite significant modifications in sea level and

the impact of marine transgressions, as well as the formation of internal basins that affected the mid-Tertiary landscape features of Patagonia (Bechis et al., 2014; Bertels-Psotka & Cusminsky, 2014). These geologic events resulted in paleoenvironments consisting of fragmented landscapes that affected the ancient lineages evolving at the southern tip of South America. As a result, significant within-species divergence was measured in chloroplast DNA sequences that were similar in sister *Nothofagus* species. Dated phylogenies yielded deep genetic discontinuities (Acosta et al., 2014; Premoli et al., 2012), which were synchronic with marine ingressions that occurred in the Pacific during the Oligocene (Bechis et al., 2014). Detailed phylogeographical analyses yielded shared plastid lineages that reflected long-lasting isolation due to vicariance, but not phylogenetic relationships between taxa. Spatially concordant lineages were also found in many other plant genera, including *Eucalyptus* (McKinnon et al., 2001), *Quercus* (Okaura et al., 2007) and *Populus* (Liu et al., 2017). Similarly, phylogeographical structures shared among sympatric *Nothofagus* provided evidence of cycles of hybridization and introgression, constituting one of the most striking pieces of evidence of widespread chloroplast capture events in plants (Acosta & Premoli, 2010). In addition, the global cooling that took place at the Eocene–Oligocene boundary (Zachos et al., 2008) impacted the gene pool of trees such as *Nothofagus pumilio* (Mathiasen & Premoli, 2010). Thus, the interplay of tectonic and climatic influences produced the spatially heterogeneous and temporally dynamic landscapes in Patagonia that shaped the significant within-species divergence of widely distributed taxa.

Nothofagus dombeyi (Mirb.) Blume, common name 'coihue', is an evergreen species with a broad distribution; it characterizes the low- to mid-elevation temperate forests of southern Argentina and Chile that range from 35° to 43° S latitude. This species can grow 40 m in height and often reaches the uppermost canopy layer; it is one of the largest broadleaf trees of austral forests. It is often found in relatively mesic environments, yet in different forest types and climates. A great deal of genecological variation has been measured in *N. dombeyi* along environmental gradients. Seed weight declines gradually with increased latitude, that is, decreased temperature (Veblen et al., 1996). In addition, common garden and water-stress manipulative experiments yielded genetically based variations in several leaf, whole plant and water-use traits of *N. dombeyi* from contrasting precipitation regimes (Diaz et al., 2020).

The main goal of this study is to test the hypothesis that the genetic structure of populations, originally produced by marine transgressions (vicariance), is maintained by local adaptation and diversifying selection. We analyse populations throughout the distribution range of a widespread species where no barriers to gene flow apparently exist today. The strong environmental/climatic gradients in the region provide an excellent study model, enabling us to disentangle the impact of past and present evolutionary forces. We expect to find a significant association between phylogroups and outlier single nucleotide polymorphisms (SNPs), which, in turn, should be geographically and environmentally concordant, whereas the structure of neutral or non-outlier SNPs will be more similar to a panmictic population.

2 | MATERIALS AND METHODS

2.1 | Sample collection, chloroplast DNA sequencing and SNP genotyping

For cpDNA sequencing, we collected 203 leaf samples from 106 sampling locations across the entire distribution range of *N. dombeyi* (Figure 1a): 44 from Argentina and 62 from Chile. For SNP

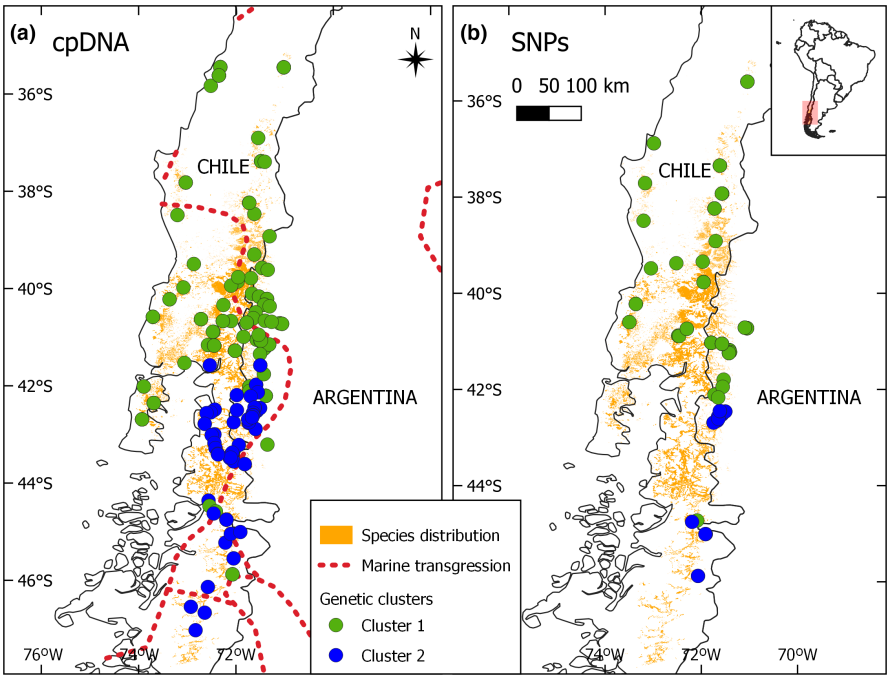

FIGURE 1 Map of southern South America showing the geographical location of the sampling sites of *Nothofagus dombeyi* individuals from Chile and Argentina analysed by (a) chloroplast DNA (cpDNA) and (b) single nucleotide polymorphisms (SNPs). Different colours depict two genetic clusters. References to haplotype numbers and descriptions are shown in Table S4. The red dotted line represents marine transgressions that took place in the Oligocene-Miocene (for details see main text). Distribution of *N. dombeyi* was obtained from the Chilean native Forest cadastre 1:500,000 (CONAF-CONAMA-BIRF, 1999), and native Forest inventory of Argentina (CIEFAP, MAyDS, 2016). Projection EPSG:4326-WGS84.

genotyping, we used 113 individuals from a subset of 36 sampling sites: 17 from Argentina and 19 from Chile (Figure 1; Table S1).

A set of 95 cpDNA sequences was obtained from previous studies (Acosta et al., 2014; Premoli et al., 2012), and the remaining 108 were newly generated for this study (Table S1). Total genomic DNA was extracted from fresh leaves following the ATMAB method described in Dumolin et al. (1995) with the modifications previously used for other *Nothofagus* species (see methods in Mathiasen & Premoli, 2010). DNA was amplified by polymerase chain reaction (PCR) using universal primer pairs for the cpDNA intergenic spacers: *trn*H-*psb*A (HA), *trn*L-*trn*F (LF) and *psb*B-*psb*H (BH) following the PCR amplification conditions previously used for *Nothofagus* in Mathiasen and Premoli (2010), Premoli et al. (2012) and Acosta et al. (2014). PCR products were sent to the sequencing facility in Macrogen Inc. (Seúl, Corea). Novel cpDNA sequences were deposited in GenBank® (accession numbers for LF: OP473928-OP473933, HB: OP473934-OP473939 and HA: OP491426-OP491431). Sequencing data of the three cpDNA regions were aligned with the ClustalW algorithm in MegaX (Kumar et al., 2018) and then concatenated manually into a single combined dataset for posterior analyses. Chloroplast DNA haplotypes were determined from both nucleotide substitutions and indels. Gaps were coded following the simple coding method of Simmons and Ochoterena (2000).

To obtain SNPs, we extracted high-quality total genomic DNA from 100 mg of fresh leaves from 113 individuals, following a modified protocol from Doyle and Doyle (1990); library preparation and high-throughput genotyping by sequencing were performed at the University of Wisconsin Biotechnology Center (DNA Sequencing Facility). DNA concentration and quality were evaluated using picogreen™ (ThermoFisher Scientific) on a Qubit® Fluorometer (Invitrogen). We prepared the GBS genomic library following the protocol detailed by Elshire et al. (2011) with the methylation-sensitive restriction enzyme ApeKI, following Hasbún et al. (2016).

Illumina high-throughput sequencing was conducted on an Illumina HiSeq 2000 (Illumina) using 100 bp single-end sequencing runs. A total of 26.4 Gb raw sequence data from the 113 individual samples yielded 248,901,409 reads 100 bp long, with no sequences being flagged as poor quality. After trimming low-quality reads ($Q < 30$), we identified 356,177 non-redundant SNPs. We assigned SNP genotypes using the Uneak pipeline (Universal Network Enabled Analysis Kit) contained within the program Tassel v5.0 (Trait Analysis by aSSociation, Evolution, and Linkage; Bradbury et al., 2007). The Uneak pipeline works by first trimming reads to 64 base pairs and collapsing identical reads into tags. Once identified via the Uneak pipeline, the raw SNP data were filtered to include only loci sequenced in 95% or more of the targeted individuals, and the individuals were filtered to include only those genotyped at >70% of the SNP loci. The Uneak pipeline does not depend on a reference genome, so is suitable for *N. dombeyi*. After removing SNPs that did not pass the genotyping quality control criteria, following Hasbún et al. (2016) we kept 2965 SNPs. We tested all these SNPs for pairwise linkage disequilibrium, and in SNP pairs with a coefficient of determination $r^2 > 1$ one of the SNP pairs was removed, generating a new genetic matrix without the correlated markers (2943 SNPs).

2.2 | Climatic niche characterization

To characterize the climatic niche of each genetic cluster, we ran general linear models (GLMs) with 72 climatic variables using Statistica v7 (StatSoft Inc., 2004) to detect which climatic variables differed between the genetic clusters. The 72 variables were as follows: 30 climatic variables generated with the ClimateSA v1.0 software package (available at http://tinyurl.com/ClimateSA), based on the methodology described by Hamann et al. (2013), 19 bioclimatic variables with 30-arcsec spatial resolution from WorldClim 2 (www.worldclim.org; Fick & Hijmans, 2017), 16 environmental variables from the ENVIREM database (Title & Bemmels, 2018) and seven soil quality variables from the Harmonized World Soil Database v1.2 (http://www.fao.org/soils-portal/data-hub/soil-maps-and-databases/harmonized-world-soil-database-v12/en/; Fischer et al., 2008) (Table S2). All variables were standardized by subtracting each value from the sample mean and dividing it by the standard deviation using Statistica (StatSoft Inc., 2004). We tested for multicollinearity of environmental variables through a stepwise procedure which sequentially excludes variables with a high variance inflation factor (VIF), using the 'vifstep' function in the R package 'usdm' with a threshold value of 10 (Naimi, 2015). After solving multicollinearity problems, we carried out a principal component analysis (PCA) with the nine remaining variables (Table S3) in Statistica.

2.3 | Chloroplast DNA sequence data analyses

2.3.1 | Genetic diversity and structuring

We assessed the levels of cpDNA variation by calculating the following molecular diversity parameters: variation in alignment size (in base pairs, bp), number of haplotypes (H), percentage of polymorphic sites (P%), and nucleotide (π) and haplotype (h) diversities; DnaSP v4.10.9 was used for these calculations (Rozas et al., 2003). The relationships between haplotypes were reconstructed by a Median-Joining network with the program Network v4.201 (Bandelt et al., 1999). Bayesian inference analysis was performed using Beast v1.6.2 (Drummond & Rambaut, 2007). The input files were generated using the program Beauti v1.6.2 (provided in the Beast package) with the following settings: the substitution model was GTR + I, following the results obtained under the Akaike information criterion in jmodeltest v2.1.10 (Darriba et al., 2012); a strict clock model and the Yule process were used as the tree prior; and the Monte Carlo Markov chain (MCMC) was run for 5×10^7 generations, sampling every 1000 cycles. A maximum clade credibility (MCC) tree was generated after 25% burn-in of the obtained trees and a 50% posterior probability limit with median node heights using TreeAnnotator v1.6.1 (provided in the Beast package). We

also estimated the divergence time between main genetic clusters using Beast, with a combination of safe but late and early but risky fossil age constraints as the calibration point scenario and the parameter settings described in Acosta et al. (2014). The obtained tree was visualized in FigTree v1.4.4 (Rambaut, 2012), and the kernel density plots for each node were obtained using the divergence dates and credibility intervals by analysing the log files with Tracer v1.7.1 (Rambaut et al., 2018). We examined the cpDNA genetic structure of individuals using the Bayesian clustering approach implemented in the program Baps v6 (Corander et al., 2008) and several different methods implemented in Genalex v6.5 (Peakall & Smouse, 2012). The Baps analysis consisted of a population mixture analysis with spatial clustering of individuals with linked loci varying the number of clusters (K) from K = 2 to K = 10 with 10 replicates each. The optimal number of K was based on the change of log marginal likelihood of the Bayes factor. The degree of genetic differentiation among clusters was then assessed through analysis of molecular variance (AMOVA) and pairwise Φ_{PT} values among the genetic clusters identified by Baps. We evaluated their significance by permutations based on 999 replicates using Genalex. Finally, we implemented a principal coordinates analysis (PCoA) to visualize genetic dissimilarities between individuals in Genalex.

2.4 | Genomic analysis

2.4.1 | F_{ST} outlier tests, population structure, AMOVA and PCA

To detect local adaptation, we used three different approaches: Outflank (Whitlock & Lotterhos, 2015) based on the expected distribution of F_{ST}, Pcadapt (Luu et al., 2016) outlier detection based on PCA and Bayescan (Foll & Gaggiotti, 2008), which controls for the geographical relationships between clusters. For Outflank, we chose 5% as the trim points and a minimum expected heterozygosity of 0.10 to infer the distribution. For Pcadapt, we used k = 2 based on the scree plot which presents the percentage of variance explained by each principal component (PC). The parameters for running Bayescan were 20 pilot runs of 5000 iterations, followed by a sample size of 5000 with a thinning interval of 10 among 50,000 iterations.

For outlier SNPs and neutral SNPs (total SNPs minus outliers), we ran assignment tests in Structure (Pritchard et al., 2000) for population cluster values of K = 1 to 6. We performed 10 independent runs for each K with 10,000 burn-in iterations followed by 100,000 MCMC steps. We assumed the admixture model and included prior information on populations. The number of distinct clusters was determined using Structure Harvester (Earl & Vonholdt, 2012) based on the conservative Evanno's method (Evanno et al., 2005). We re-ran the analysis for each identified population cluster to look for sub-structuring. Cluster assignment was visualized using Distruct (Rosenberg, 2004).

For both groups of SNPs (outliers and neutral), we performed a PCA using the R package 'adegenet v 2.1.3' (Jombart, 2008). Genetic clusters identified by the population structure analysis were used for AMOVA and to compute genetic diversity parameters: private alleles (Np), percentage of polymorphic loci (P%), observed heterozygosity (Ho), expected heterozygosity (He) and inbreeding coefficient (F_{IS}) for both datasets of SNPs using Genalex.

2.4.2 | Genotype–environment association

We used a combination of variables to analyse gene–environment associations (GEAs). All 2943 SNPs were tested for associations with the nine environmental variables that characterize the climatic niche of each genetic cluster. We used three different GEA methods: linear mixed model regressions implemented in the R package 'gapit' (Lipka et al., 2012), latent factor mixed models implemented in 'lfmm' (Frichot et al., 2013) and a Bayesian approach implemented in Bayenv2 (Günther & Coop, 2013). Association analyses were conducted only with SNPs that had a minor allele frequency (MAF) higher than 3%. SNPs with a threshold value of $-\log10(p\text{-value}) > 2$, a Z-score > 4 (following Frichot et al., 2013) and BF > 4 (following De La Torre et al., 2014) were considered candidates for divergent selection, for MLM, LFMM and Bayenv, respectively.

2.4.3 | Geographical and ecological isolation

We explored the effects of IBD and IBE in the distribution of genetic variation for the three datasets (cpDNA, outlier SNPs and neutral SNPs). The response of genetic variation (dependent variable) to changes in explanatory variables (geographical, environmental and geographical + environmental distances) was assessed with multiple matrix regression with randomization analysis (MMRR function in R; Wang, 2013). The regression coefficients of IBE and IBD (β_E and β_D, respectively) were computed and tested for significance with 999 permutations. The genetic distance matrix was calculated as Euclidean distances, and geographical distances were estimated in Km from the decimal degree coordinates of sampling locations, both in Genalex. The environmental matrix was computed as Gower distances estimated from 10 environmental variables, which showed significant differences between lineages (see the GLMs of environmental variables above) for sampling coordinates, using the R package 'fd' (Laliberté & Legendre, 2010).

3 | RESULTS

3.1 | Climatic niche characterization

All environmental variables included in the GLM analysis showed significant differences ($p < 0.0001$) between the genetic clusters (data not shown). Correlation values between environmental variables ranged from −0.006 to 0.895, but after removing the highly collinear variables (i.e. with VIF > 10) only nine remained from the

original set of 72 (Table S3). The results of the PCA with these nine variables suggested the presence of two climatic niches, with 73% of variation explained by the first three axes (Figure S1). The northern cluster presented a wider niche, inhabiting a variety of temperature, precipitation and soil conditions, while the southern cluster was present in colder and drier climates (Figure S2).

3.2 | Chloroplast DNA genetic diversity and structuring

The analysis of 203 cpDNA sequences resulted in 24 haplotypes that were geographically structured into two major monophyletic clades, hereafter referred to as genetic clusters (Figure 1a, Figure 2 and Figure S3). The length of the aligned cpDNA sequences varied between 1632 and 1670 bp and presented 21 variable sites, 13 of which corresponded to substitutions, and eight to indels (Table S4).

The median-joining analysis revealed a single network that included the 24 haplotypes with no internal reticulations (Figure 2a). We identified two main latitudinally structured haplotype groups separated by 12 mutational steps: first, haplotypes from the northern distribution range were closely related (1 to 3 mutations of difference); and second, haplotypes from the southern range were also very alike, but very different from the northern ones (Figures 1a and 2a). The MCC tree obtained with cpDNA sequences (Figure S3) showed two main monophyletic clades with high Bayesian posterior probabilities, hereafter clusters 1 and 2, from north to south, which were highly spatially structured (Figure 1a). Haplotype H34, which has a basal position in cluster 1, has a particular DNA sequence that shares characteristics with haplotypes from both genetic clusters (Table S4).

Molecular dating analysis showed deep lineage divergence times between the two *N. dombeyi* haplotype groups of 47.56 Ma (Figure S3). Haplotypes within cluster 1 continued to diverge at ~32.87 and 19.39 Ma as distinct spatially structured subclusters. Within the subclusters, the haplotypes also diverged periodically from ~16.2 Ma to <2 Ma (Figure S3).

The Baps analysis identified five genetic clusters (Figure 2b) with high posterior probability (0.99); however, the KL-divergence matrix (Table S5) showed that four of the clusters were weakly differentiated (KL-distance <0.01) and could be grouped; therefore we decided to use the genetic clustering K = 2 (Figure 2b) for further analyses. The results of the PCoA with 203 cpDNA sequences also suggested the presence of two major genetic clusters (Figure 2c), which were concordant with the monophyletic clades and the results of the Baps analysis. The northern genetic cluster presented the highest values of genetic diversity in terms of the number of haplotypes (20 out

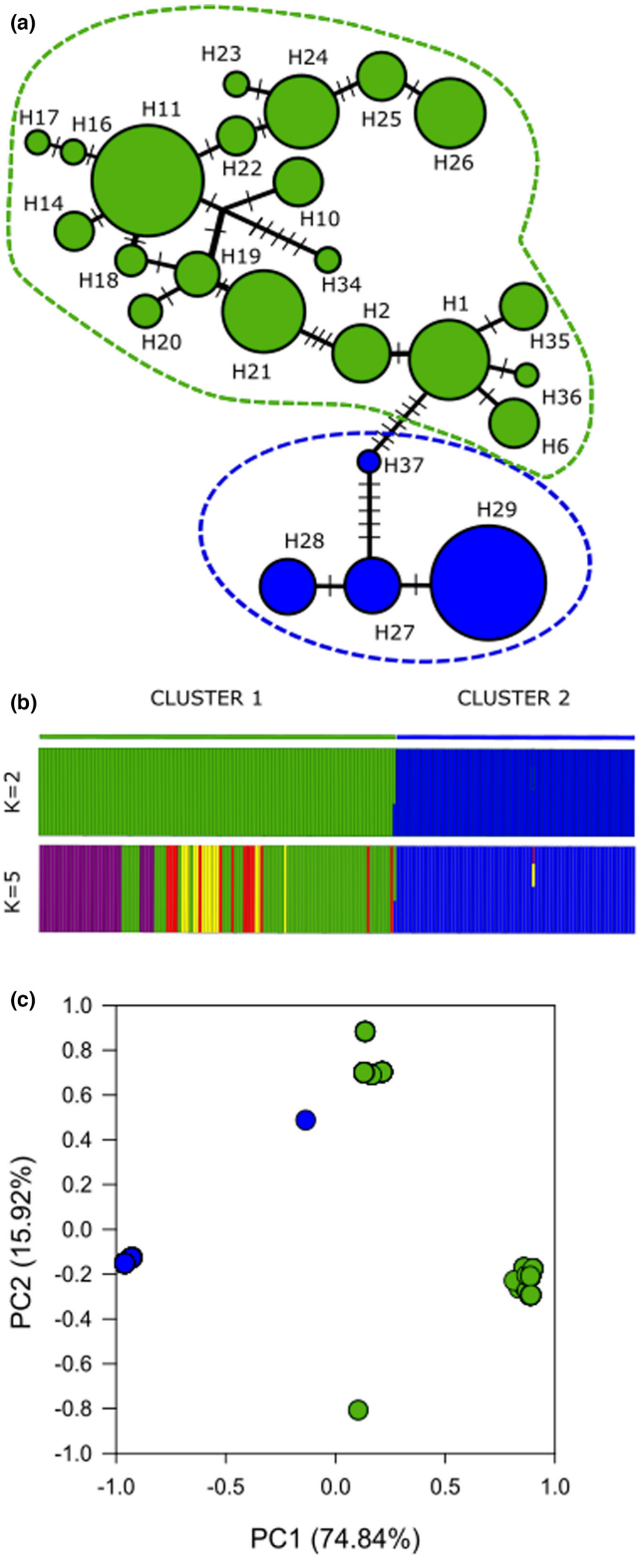

FIGURE 2 Concatenated sequences of three non-coding regions of chloroplast DNA (*trn*L-*trn*F, *trn*H-*psb*A and *psb*B-*psb*H) obtained for *Nothofagus dombeyi* populations from Chile and Argentina evaluated by (a) median-joining network showing the relationship between the 24 haplotypes; circle size is proportional to haplotype frequency, and lines indicate mutational events; (b) Baps analysis depicts spatial clustering of genetic groups for K = 5 and K = 2; and (c) principal coordinate analysis showing the first two coordinates (PC) which explain 90.76% of the total haplotype variation. Colours correspond to genetic clusters shown in Figure 1.

of 24), polymorphism (90%), and nucleotide and haplotype diversity, whereas the southernmost cluster presented the lowest diversity values. Similarly, 20 haplotypes were recorded in the north (cluster 1), whereas only four were found in the south (cluster 2); all the haplotypes were unique to each cluster (Table S6).

3.3 | Outlier tests, population structure and PCA

Local adaptation based on F_{ST} outliers was assessed using 2943 SNPs in 113 individuals. Outflank identified 69 SNPs with moderate to high F_{ST} values (between 0.12 and 0.99), Pcadapt identified 45 SNPs with *p*-values <0.01 and Bayescan identified 25 SNPs with moderate F_{ST} values (between 0.10 and 0.38; q-value = 0). After comparing the results of the three univariate GEA analyses, we found that 33 SNPs were identified as outliers in at least two of the three analyses (Figure 3a and Table S7). From now on, we will name these 33 SNPs as outlier SNPs, and the other 2910 as neutral. The MAF of all 33 outlier SNPs (Table S7) increased with latitude, indicating that rare alleles are most common in cluster 2, that is, the south.

Population structure analyses showed the presence of two major genetic clusters for outlier SNPs (Figure 1b, Figure 3b,c). In contrast, neutral SNPs yielded signals of admixture (Figure 3b) and no evident genetic structure (Figure 3d). PCA for outlier SNPs showed that 73% of the total observed variation was explained by the first two PCs (Figure 3c). The first two components of the PCA can be explained by five outlier SNPs that yielded almost unique homozygous genotypes and alleles for cluster 2 in PC1 (Figure S4). In contrast, the PCA for neutral SNPs explained only 5.4% of the observed total variation (Figure 3d), and the results of the Structure Harvester analysis showed no clear spatial segregation of genotypes (Figure S5).

3.4 | Analyses of molecular variance

The AMOVA with cpDNA sequence data showed high genetic divergence between the two genetic clusters ($\Phi_{PT} = 0.813$, $p < 0.001$; Table S8). The AMOVA at population level for outlier SNPs showed that most of the genetic variation occurred between genetic clusters ($F_{ST} = 0.764$, $p < 0.001$; Table S9). On the other hand, neutral SNPs showed no significant between-cluster genetic differentiation ($F_{ST} = 0.005$, $p = 0.113$; Table S10).

3.5 | Genotype–environment association

Bayenv, MLM and LFMM GEA analyses showed that 7 SNPs had significant associations with climatic variables for at least two of the three analyses (Table S11), three of which were also detected as outlier SNPs based on F_{ST} analysis. The strongest clinal pattern in allele frequencies (from cluster 1 to cluster 2; 0.011 to 1, respectively) was found in SNP18154, associated with mean monthly potential evapotranspiration (PET) of the wettest quarter (PETWeQ). Climatic variables with significant effects were precipitation (bio19), evapotranspiration (PETWeQ) and temperature (Temp10mon and continentality); except for continentality, the other three variables attained higher values in the northern cluster 1 (Figure S6).

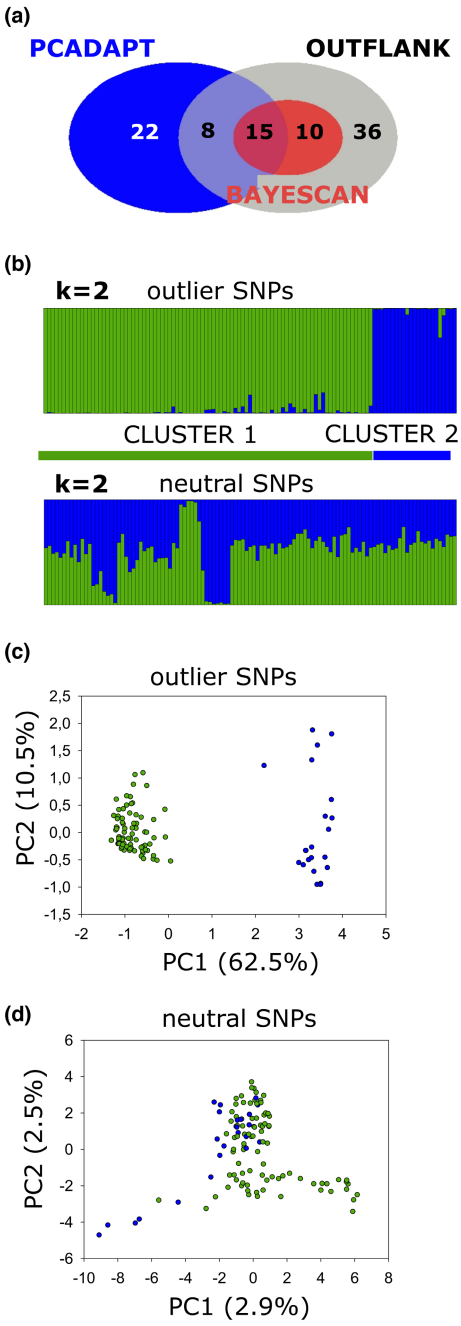

FIGURE 3 Genomic analyses based on single nucleotide polymorphisms (SNPs) of *Nothofagus dombeyi* populations. (a) Venn diagram of distinct methods applied to detect outlier loci; (b) Structure analysis showing spatial clustering for K = 2 for outliers and neutral SNPs, respectively; (c) principal component analysis (PC1 vs PC2) by means of outlier and (d) neutral SNPs.

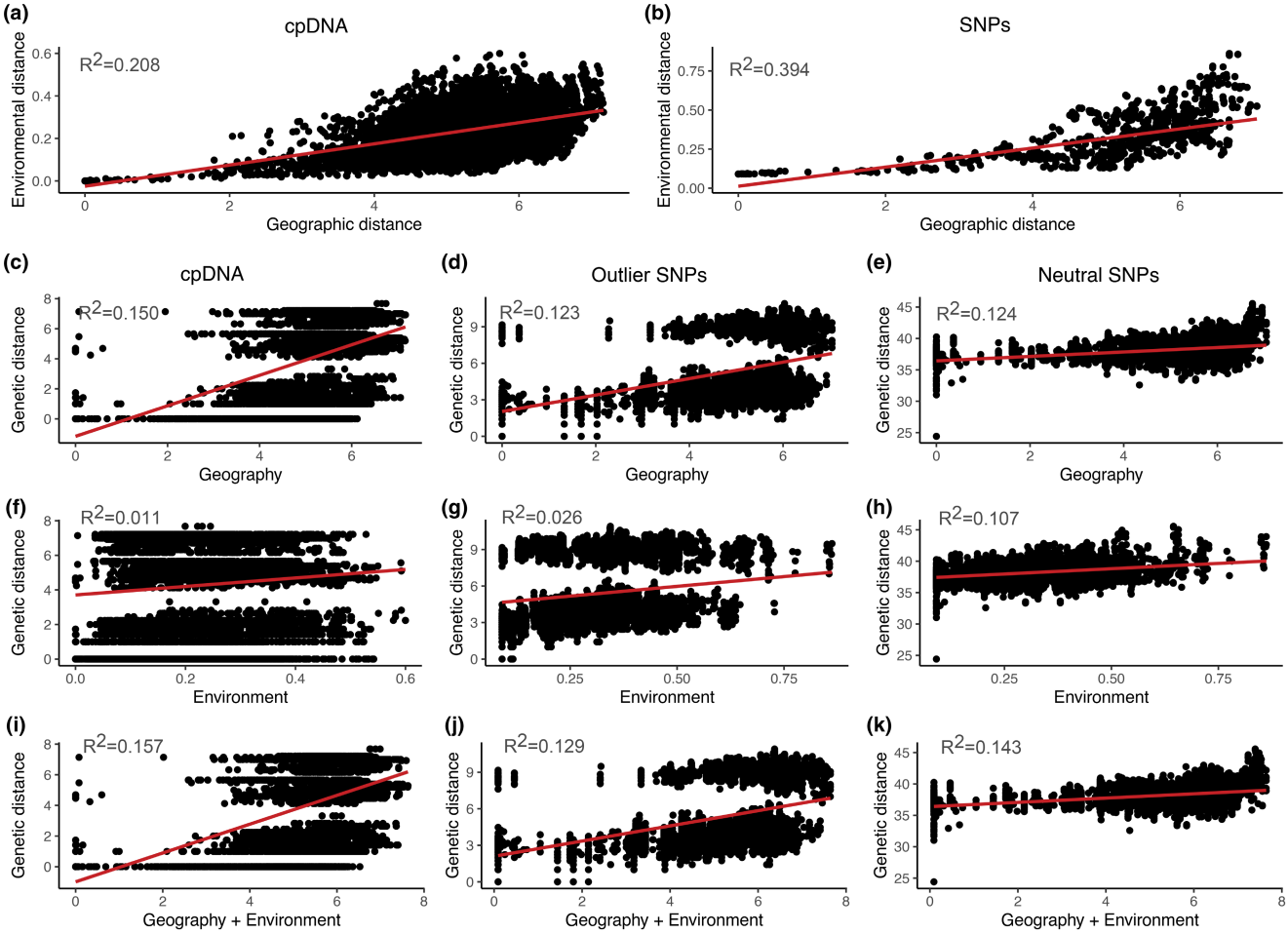

FIGURE 4 Relationship between geographical, environmental and geographical + environmental distances of *Nothofagus dombeyi* from Chile and Argentina with chloroplast DNA (cpDNA) and single nucleotide polymorphisms (SNPs) by multiple matrix regression analyses. The scatterplots show the relationship between (a–b) geographical and environmental distances and the effects of (c–e) geography; (f–h) environmental and (i–k) the combination of geographical and environmental influences on genetic distances. Regression lines and significant ($p < 0.01$) R^2 values are shown on each graph.

3.6 | Geographical and ecological isolation

The MMRR analysis revealed a strong relationship between geographical location and environmental distance matrices for both cpDNA ($R^2 = 0.208$, $p = 0.001$) and SNP data ($R^2 = 0.394$, $p = 0.001$) (Figure 4a,b). Also, IBD played a significant role in the genetic differentiation of cpDNA lineages, as did IBE to a lesser extent, while the combined effects of geography and the environment contributed significantly to explain genetic differentiation (Table S12; Figure 4). Outlier and neutral SNPs also showed significant IBD and IBE patterns (Table S12; Figure 4). When considering both environment and geography, the results showed weak but significant patterns of IBD and IBE (Figure 4) based on both neutral and outlier SNPs ($R^2 = 0.143$, $p < 0.01$; $R^2 = 0.129$, $p < 0.01$, respectively) (Table S12). In addition, IBD and IBE analyses for cpDNA and outlier SNPs showed clear differentiation of two geographically local and environmentally different discrete clusters, as evidenced by the dot patterns in the scatterplot, while no distinctive genetic groups were detected for neutral SNPs (Figure 4).

4 | DISCUSSION

We used an empirical approach to show that phylogeographical breaks prompted by vicariant events can be maintained by selection despite gene flow. Combined phylogeologic analyses of *N. dombeyi*, employing cpDNA and geogenomic methods, yielded latitudinally structured genetic clusters that are geographically concordant with adaptive SNP variants associated with distinct climatic niches. The original latitudinal structure of *N. dombeyi* is the product of ancient vicariant events that started as early as the Oligocene, which coincides with fossil and tectonic evidence (Bechis et al., 2014; Bertels-Psotka & Cusminsky, 2014). Although the age and tectonic setting of the associated marine deposits are still a matter of debate, recent studies based principally on U–Pb geochronology and Sr isotope stratigraphy reinforced the early hypothesis (Ramos, 1982) that marine transgressions flooded a vast part of southern South America, including the present Patagonian Andes (Encinas et al., 2018). The structure of *N. dombeyi* chloroplast DNA lineages was similar to those found in *Nothofagus pumilio* (Mathiasen & Premoli, 2010). In

addition, a combined analysis of all the *Nothofagus* species inhabiting southern Patagonia, using cpDNA sequences, yielded concordant phylogeographical patterns. These patterns were used to reconstruct the effects of isolation that promoted divergence in the presence of ancient basins (Acosta et al., 2014; Premoli et al., 2012). Whereas it is widely accepted that genealogical gaps usually arise from long-term extrinsic barriers to gene flow (Avise, 1994; Avise et al., 1987), Irwin (2002) suggested that phylogeographical breaks might develop in continuously distributed species in the absence of geographical barriers. This occurs if the average individual dispersal distances or population size of the species is low. Although *N. dombeyi* is predominantly outcrossed, local seed dispersal occurs (Veblen et al., 1996), which particularly in closed forests results in significant fine-scale spatial genetic structures (Premoli & Kitzberger, 2005). Therefore, the original cpDNA genetic signature prompted by a vicariant event was probably maintained over time by limited dispersal, thus maintaining the phylogeographical break.

Although no clear genetic structure was detected for neutral SNPs through PCA, significant F_{ST} values, indicating the degree of between-cluster divergence by AMOVA, were two orders of magnitude less than those between cpDNA phylogroups and outlier SNP clusters. Thus, the high homogeneity yielded by neutral SNPs suggests no restriction to gene flow among *N. dombeyi* populations, as previously recorded for different provenances using biparentally inherited microsatellites (Diaz et al., 2022). Nonetheless, adaptive divergence may limit among-population gene flow (Ye et al., 2017). Genomic analyses of *N. dombeyi* inhabiting precipitation extremes yielded significant yet lower between-site divergence for neutral than for outlier SNPs (F_{ST} = 0.039 and 0.483, respectively) (Diaz et al., 2020). The divergence of the outlier SNPs was similar to the degree of differentiation in quantitative characters (Q_{ST} = 0.42) at leaf and whole plant levels under common garden conditions, thus being indicative of genetically based variation driven by selection. In addition, adaptive variation with latitude was reported in *N. dombeyi* for seed weight and percent germination (Donoso, 1987), showing that climate gradients and geography exert differential selective pressures on trait variation, which can be maintained in the face of potential gene flow. As a result, selection can lead to geographically localized and differentially adapted groups (Irwin, 2012).

Outlier SNPs showed significant climatic associations, particularly with precipitation-related variables, indicating that water accessibility is very important for *N. dombeyi*, whose successful establishment depends on favourable wet conditions for growth (Suarez & Kitzberger, 2010). Our results suggest that climate adaptation in *N. dombeyi* might have occurred due to many changes in allele frequencies as a result of selection pressures related to climate. In particular, outlier SNP groups were associated with two climatic niches, which suggests that widespread taxa inhabiting heterogeneous environmental and climatic envelopes may adjust accordingly.

Discrete genetic clusters produced by cpDNA sequences (Figures 2a, 4c and Figure S3) prove that the two groups are highly differentiated. Similar to cpDNA, outlier SNPs yielded two genetic groups. The SNP groups, however, had a relatively stronger association with environmental variables (20% of total variance compared to 7% for cpDNA), as they are adapted to different climates as a result of diversifying selection. Nonetheless, the similar genetic structure of both cpDNA and outlier SNPs, driven mainly by geography, indicates that the nuclear genome still contains signals of past vicariant events like those shown in the black spruce (Prunier et al., 2012), which were erased in neutral SNPs by high gene flow rates in *N. dombeyi*. While geography and climate are highly correlated in *N. dombeyi*, the former seems to better explain the strong phylogeographical structure and deep coalescence times found in cpDNA as a result of ancient historical processes. Past hybridization-introgression cycles resulted in sympatric *Nothofagus* sharing cpDNA haplogroups (Acosta & Premoli, 2010), which may have reinforced local divergence from a common gene pool by vicariance.

Allele frequencies for outlier SNPs changed considerably in the southern cluster, many SNP loci being dominated by a single allele which was different to that found in the northern one (Figure S4); this indicates marked differences in the relative abundance of genotypes throughout *N. dombeyi* range. However, the almost fixed frequencies of alternative homozygous genotypes for some outlier SNP loci suggests extranuclear inheritance, and thus distinct haplotypes for such markers, while the low-frequency heterozygotes may imply some degree of cpDNA heteroplasmy. Ongoing studies that include sequencing of entire chloroplasts of *Nothofagus* species will contribute to clarify the potential adaptive value of cytoplasmic genomes.

The significant association found between environmental variables with different cpDNA lineages and outlier SNPs is a clear indication of intraspecific variation in the ecological niche (Van Valen, 1965) as a result of populations inhabiting distinct climatic envelopes. Within-species niche variation has been interpreted as niche specialization, which is considered widespread in a great diversity of taxa (Carlson et al., 2021). Thus, the evidence presented here highlights the relevance of within-species niche variation in SDMs, which by design do not usually account for intraspecific-level variation. Overlooking this spatial heterogeneity may result in poor performance of SDMs in research into global change, reconstruction of past distributions, and management of future populations. In sum, the two distinct clusters of *N. dombeyi* populations, which have been deeply rooted since the mid-Tertiary, have been maintained by natural selection, and should be considered distinct conservation units.

5 | CONCLUSION

In this work, we show that a vicariance signal yielded by conserved sequences of the chloroplast, and associated with geography, may be reinforced by adaptation to distinct climatic niches, as portrayed by outlier SNPs. Local divergence can be maintained over time despite gene flow, as depicted by neutral SNPs. Our results indicate that although no natural barriers to gene flow currently exist among *N. dombeyi* populations, the phylogeographical differences between lineages, produced by ancient vicariance, are still evident and are maintained by diversifying selection in different environments.

ACKNOWLEDGEMENTS

This research was supported by funds from the Agencia Nacional de Promoción Científica y Tecnológica Argentina PICT 2017-0577 to MF, the Rufford Foundation Small Grant 29211-1 to GJ and projects PICT 2019-149, Consejo Nacional de Investigaciones Científicas y Técnicas Argentina (CONICET) PIP 2021-11220200102500CO and Universidad Nacional del Comahue Argentina 04/B235 to ACP. The authors thank the DNA Sequencing Facility at the University of Wisconsin Biotechnology Center for providing genotype-by-sequencing and support services. We thank APN (Administración de Parques Nacionales of Argentina) and CONAF (Corporación Nacional Forestal of Chile) for permission to sample plant material from protected areas. We are thankful to Audrey Shaw for English editing services. M.F., P.M, G.J, D.D. and A.C.P. are members of CONICET (National Research Council of Argentina), and R.H. is an associate professor of Universidad de Concepción, Chile.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Newly generated chloroplast DNA sequences are deposited in GenBank, Accession numbers OP473928-OP473939 and OP491426-OP491431. Data matrices containing genomic information that supports the findings of this study are available from Universidad Nacional del Comahue Institutional Digital Repository RDI Unco URI: http://rdi.uncoma.edu.ar/handle/uncomaid/16887 and Dryad, Dataset, https://doi.org/10.5061/dryad.280gb5mt3.

ORCID

Mariana Fasanella https://orcid.org/0000-0002-1607-3832
Paula Mathiasen https://orcid.org/0000-0003-4970-5776
Rodrigo Hasbún https://orcid.org/0000-0002-4852-6154
Andrea C. Premoli https://orcid.org/0000-0002-4632-1497

REFERENCES

Acosta, M. C., Mathiasen, P., & Premoli, A. C. (2014). Retracing the evolutionary history of *Nothofagus* in its geo-climatic context: New developments in the emerging field of phylogeology. *Geobiology*, *12*(6), 497–510.

Acosta, M. C., & Premoli, A. C. (2010). Evidence of chloroplast capture in south American *Nothofagus* (subgenus *Nothofagus*, *Nothofagaceae*). *Molecular Phylogenetics and Evolution*, *54*, 235–242. https://doi.org/10.1016/j.ympev.2009.08.008

Avise, J. C. (1994). *Molecular markers, natural history and evolution*. Chapman and Hall.

Avise, J. C. (2000). *Phylogeography: The history and formation of species*. Harvard Univ. Press.

Avise, J. C. (2009). Phylogeography: Retrospect and prospect. *Journal of Biogeography*, *36*, 3–15.

Avise, J. C., Arnold, J., Ball, R. M., Jr., Bermingham, E., Lamb, T., Neigel, J. E., Reeb, C. A., & Saunders, N. C. (1987). Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics*, *18*, 489–522.

Bandelt, H. J., Forster, P., & Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, *16*, 37–48.

Bechis, F., Encinas, A., Concheyro, A., Litvak, V. D., Aguirre-Urreta, B., & Ramos, V. A. (2014). New age constraints for the Cenozoic marine transgressions of northwestern Patagonia, Argentina (41°–43°S): Paleogeographic andtectonic implications. *Journal of South American Earth Sciences*, *52*, 72–93.

Bertels-Psotka, A., & Cusminsky, G. C. (2014). Nuevas especies de ostrácodos de la Formación Ñirihuau (Oligoceno) en su área tipo (alrededores de San Carlos de Bariloche), provincia de Río Negro, República Argentina, *Ameghiniana*, *36*(1), 71–81. Retrieved from https://www.ameghiniana.org.ar/index.php/ameghiniana/article/view/2476

Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., & Buckler, E. S. (2007). TASSEL: Software for association mapping of complex traits in diverse samples. *Bioinformatics*, *23*(19), 2633–2635. https://doi.org/10.1093/bioinformatics/btm308

Carlson, B. S., Rotics, S., Nathan, R., Wikelski, M., & Jetz, W. (2021). Individual environmental niches in mobile organisms. *Nature Communications*, *12*, 4572. https://doi.org/10.1038/s41467-021-24826-x

Chardon, N. I., Pironon, S., Peterson, M. L., & Doak, D. F. (2020). Incorporating intraspecific variation into species distribution models improves distribution predictions, but cannot predict species traits for a wide-spread plant species. *Ecography*, *43*, 60–74. https://doi.org/10.1111/ecog.04630

Chenuil, A., Cahill, A. E., Délémontey, N., Du Salliant, E., & Fanton, H. (2019). Problems and questions posed by cryptic species. A framework to guide future studies. In E. Casetta, J. Marques da Silva, & D. Vecchi (Eds.), *From assessing to conserving biodiversity. History, philosophy and theory of the life sciences* (Vol. *24*, pp. 77–106). Springer.

CIEFAP, MAyDS. (2016). Actualización de la Clasificación de Tipos Forestales y Cobertura del Suelo de la Región Bosque Andino Patagónico. Informe Final CIEFAP. https://drive.google.com/open?id=0BxfNQUtfxxeaUHNCQm9lYmk5RnM

CONAF-CONAMA-BIRF. (1999). *Catastro y Evaluación de Recursos Vegetacionales Nativos de Chile* (p. 89). Proyecto CONAF-CONAMA-BIRF.

Corander, J., Marttinen, P., Sirén, J., & Tang, J. (2008). Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. *BMC Bioinformatics*, *9*, 539.

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods*, *9*(8), 772.

De La Torre, A. R., Roberts, D. R., & Aitken, S. N. (2014). Genome-wide admixture and ecological niche modelling reveal the maintenance of species boundaries despite long history of interspecific gene flow. *Molecular Ecology*, *23*(8), 2046–2059.

Diaz, D. G., Ignazi, G., Mathiasen, P., & Premoli, A. C. (2022). Climate-driven adaptive responses to drought of dominant tree species from Patagonia. *New Forests*, *53*, 57–80. https://doi.org/10.1007/s11056-021-09843-4

Diaz, D. G., Mathiasen, P., & Premoli, A. C. (2020). Subtle precipitation differences yield adaptive adjustments in the Mesic *Nothofagus dombeyi*. *Forest Ecology and Management*, *461*, 117931. https://doi.org/10.1016/j.foreco.2020.117931

Donoso, C. (1987). Variación Natural en Especies de Nothofagus en Chile. *Bosque*, *8*(2), 85–97. https://doi.org/10.4206/bosque.1987.v8n2-03

Doyle, J. J., & Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus*, *12*, 13–15.

Drummond, A. J., & Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, *7*, 214.

Dufresnes, C., Nicieza, A. G., Litvinchuck, S. N., Rodrigues, N., Jeffries, D. L., Vences, M., Perrin, N., & Martínez-Solano, I. (2020). Are glacial refugia hotspots of speciation and cytonuclear discordances?

Molecular Ecology, *29*(5), 986–1000. https://doi.org/10.1111/mec.15368

Dumolin, S., Demesure, B., & Petit, R. J. (1995). Inheritance of chloroplast and mitochondrial genomes in pedunculate oak investigated with an efficient PCR method. *Theoretical and Applied Genetics*, *91*, 1253–1256.

Earl, D. A., & Vonholdt, B. M. (2012). Structure harvester: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*, 359–361.

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One*, *6*(5), e19379.

Encinas, A., Folguera, A., Bechis, F., Finger, K. L., Zambrano, P., Pérez, F., Bernabé, P., Tapia, F., Riffo, R., Buatois, L., Orts, D., Nielsen, S. N., Valencia, V. V., Cuitiño, J., Oliveros, V., Del Mauro, D. G., & Ramos, V. A. (2018). The late oligocene–early miocene marine transgression of patagonia. In A. Folguera, et al. (Eds.), *The evolution of the Chilean-Argentinean Andes*. Springer Earth System Sciences.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, *14*(8), 2611–2620.

Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: New 1km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, *37*(12), 4302–4315.

Fischer, G., Nachtergaele, F., Prieler, S., van Velthuizen, H. T., Verelst, L., & Wiberg, D. (2008). *Global Agro-ecological zones assessment for agriculture (GAEZ 2008)*. IIASA.

Foll, M., & Gaggiotti, O. (2008). A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. *Genetics*, *180*(2), 977–993.

Frichot, E., Schoville, S. D., Bouchard, G., & Francois, O. (2013). Testing for associations between loci and environmental gradients using latent factor mixed models. *Molecular Biology and Evolution*, *30*(7), 1687–1699.

Günther, T., & Coop, G. (2013). Robust identification of local adaptation from allele frequencies. *Genetics*, *195*(1), 205–220.

Hamann, A., Wang, T., Spittlehouse, D. L., & Murdock, T. Q. (2013). A comprehensive, high-resolution database of historical and projected climate surfaces for western North America. *Bulletin of the American Meteorological Society*, *94*, 1307–1309.

Hasbún, R., González, J., Iturra, C., Fuentes, G., Alarcón, D., & Ruiz, E. (2016). Using genome-wide SNP discovery and genotyping to reveal the main source of population differentiation in *Nothofagus dombeyi* (Mirb.) Oerst. in Chile. *International Journal of Genomics*, 3654093. https://doi.org/10.1155/2016/3654093

Irwin, D. E. (2002). Phylogeographic breaks without geographic barriers to gene flow. *Evolution*, *56*, 2383–2394. https://doi.org/10.1111/j.0014-3820.2002.tb00164.x

Irwin, D. E. (2012). Local adaptation along smooth ecological gradients causes phylogeographic breaks and phenotypic clustering. *The American Naturalist*, *180*(1), 35–49.

Jombart, T. (2008). Adegenet: An R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*(11), 1403–1405.

Kirk, H., & Freeland, J. R. (2011). Applications and implications of neutral versus non-neutral markers in molecular ecology. *International Journal of Molecular Sciences*, *12*, 3966–3988.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGAX: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*, 1547–1549.

Laliberté, E., & Legendre, P. (2010). A distance-based framework for measuring functional diversity from multiple traits. *Ecology*, *91*, 299–305.

Lipka, A. E., Tian, F., Wang, Q., Peiffer, J., Li, M., Bradbury, P. J., Gore, M. A., Buckler, E. S., & Zhang, Z. (2012). GAPIT: Genome association and prediction integrated tool. *Bioinformatics*, *28*(18), 2397–2399.

Liu, X., Wang, Z., Shao, W., Ye, Z., & Zhang, J. (2017). Phylogenetic and taxonomic status analyses of the Abaso section from multiple nuclear genes and plastid fragments reveal new insights into the North America origin of Populus (Salicaceae). *Frontiers in Plant Science*, *7*, 2022. https://doi.org/10.3389/fpls.2016.02022

Luu, K., Bazin, E., & Blum, M. G. B. (2016). Pcadapt: An R package to perform genome scans for selection based on principal component analysis. *Molecular Ecology Resources*, *17*(1), 67–77.

Mathiasen, P., & Premoli, A. C. (2010). Out in the cold: Genetic variation of *Nothofagus pumilio* (Nothofagaceae) provides evidence for latitudinally distinct evolutionary histories in austral South America. *Molecular Ecology*, *19*, 371–385.

McKinnon, G. E., Vaillancourt, R. E., Jackson, H. D., & Potts, B. M. (2001). Chloroplast sharing in the Tasmanian eucalypts. *Evolution*, *55*, 703–711. https://doi.org/10.1111/j.0014-3820.2001.tb00806.x

Naimi, B. (2015) Usdm: Uncertainty analysis for species distribution models. R Package Version 1.1-15. http://CRAN.R-project.org/package=usdm

Neigel, J., & Avise, J. C. (1993). Application of a random walk model to geographic distributions of animal mitochondrial DNA variation. *Genetics*, *135*(4), 1209–1220.

Okaura, T., Quang, N. D., Ubukata, M., & Harada, K. (2007). Phylogeographic structure and late quaternary population history of the Japanese oak *Quercus mongolica* var. crispula and related species revealed by chloroplast DNA variation. *Genes & Genetic System*, *82*(6), 465–477. https://doi.org/10.1266/ggs.82.465

Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: Genetic analysis in excel. Population genetic software for teaching and research – An update. *Bioinformatics*, *28*, 2537–2539.

Pearman, P. B., D'Amen, M., Graham, C. H., Thuiller, W., & Zimmermann, N. E. (2010). Within-taxon niche structure: Niche conservatism, divergence and predicted effects of climate change. *Ecography*, *33*, 990–1003. https://doi.org/10.1111/j.1600-0587.2010.06443.x

Pfenninger, M., Nowak, C., & Magnin, F. (2007). Intraspecific range dynamics and niche evolution in *Candidula* land snail species. *Biological Journal of the Linnean Society*, *90*(2), 303–317. https://doi.org/10.1111/j.1095-8312.2007.00724.x

Premoli, A. C., & Kitzberger, T. (2005). Regeneration mode affects spatial genetic structure of *Nothofagus dombeyi* forests. *Molecular Ecology*, *14*, 2319–2329.

Premoli, A. C., Mathiasen, P., Acosta, M. C., & Ramos, V. A. (2012). Phylogeographically concordant chloroplast DNA divergence in sympatric *Nothofagus* s.s. how deep can it be? *New Phytologist*, *193*, 261–275.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*, 945–959.

Prunier, J., Gérardi, S., Laroche, J., Beaulieu, J., & Bousquet, J. (2012). Parallel and lineage-specific molecular adaptation to climate in boreal black spruce. *Molecular Ecology*, *21*(17), 4270–4286.

Rambaut, A. (2012). *FigTree v1. 4. Molecular evolution, phylogenetics and epidemiology*. University of Edinburgh, Institute of Evolutionary Biology.

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarisation in Bayesian phylogenetics using tracer 1.7. *Systematic Biology*, *67*(5), 901–904. https://doi.org/10.1093/sysbio/syy032

Ramos, V. A. (1982). Las ingresiones pacíficas del Terciario en el Norte de la Patagonia (Argentina). *III Congreso Geológico Chileno (Concepción) Actas I*, 262–288.

Ramos, V. A. (1989). The birth of southern South America. *American Scientist*, *77*(5), 444–450.

Ribeiro, A. M., Lloyd, P., & Bowie, R. C. K. (2011). A tight balance between natural selection and gene flow in a southern African arid-zone endemic bird. *Evolution*, *65*, 3499–3514.

Rosenberg, N. A. (2004). DISTRUCT: A program for the graphical display of population structure. *Molecular Ecology Notes*, *4*(1), 137–138.
Rozas, J., Sánchez-DelBarrio, J. C., Messeguer, X., & Rozas, R. (2003). DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, *19*, 2496–2497. https://doi.org/10.1093/bioinformatics/btg359
Savolainen, O., Pyhäjärvi, T., & Knürr, T. (2007). Gene flow and local adaptation in trees. *Annual Review of Ecology, Evolution, and Systematics*, *38*, 595–619. https://doi.org/10.1146/annurev.ecolsys.38.091206.095646
Sexton, J. P., Hangartner, S. B., & Hoffmann, A. A. (2014). Genetic isolation by environment or distance: Which pattern of gene flow is most common? *Evolution*, *68*, 1–15.
Shneyer, V. S., & Kotseruba, V. V. (2015). Cryptic species in plants and their detection by genetic differentiation between populations. *Russian Journal of Genetics: Applied Research*, *5*(5), 528–541. https://doi.org/10.1134/s2079059715050111
Simmons, M. P., & Ochoterena, H. (2000). Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology*, *49*(2), 369–381.
StatSoft, Inc. (2004) STATISTICA (data analysis software system), version 7. www.statsoft.com.
Struck, T. H., Feder, J. L., Bendiksby, M., Birkeland, S., Cerca, J., Gusarov, V. I., Kistenich, S., Larsson, K. H., Liow, L. H., Nowak, M. D., Stedje, B., Bachmann, L., & Dimitrov, D. (2018). Finding evolutionary processes hidden in cryptic species. *Trends in Ecology & Evolution*, *33*(3), 153–163. https://doi.org/10.1016/j.tree.2017.11.007
Suarez, M. L., & Kitzberger, T. (2010). Differential effects of climate variability on forest dynamics along a precipitation gradient in northern Patagonia. *Journal of Ecology*, *98*, 1023–1034.
Title, P. O., & Bemmels, J. B. (2018). ENVIREM: An expanded set of bioclimatic and topographic variables increases flexibility and improves performance of ecological niche modeling. *Ecography*, *41*, 291–307.
Van Valen, L. (1965). Morphological variation and width of ecological niche. *The American Naturalist*, *99*, 377–390.
Veblen, T., Donoso, C., Kitzberger, T., & Rebertus, A. J. (1996). Ecology of southern Chilean and Argentinean *Nothofagus* forests. In T. Veblen, R. S. Hill, & J. Read (Eds.), *The ecology and biogeography of Nothofagus forests* (p. 403). Yale University Press.
Wang, I. J. (2013). Examining the full effects of landscape heterogeneity on spatial genetic variation: A multiple matrix regression approach for quantifying geographic and ecological isolation. *Evolution*, *67*, 3403–3411.
Wang, I. J., Glor, R. E., & Losos, J. B. (2013). Quantifying the roles of ecology and geography in spatial genetic divergence. *Ecology Letters*, *16*, 175–182.
Whitlock, M. C., & Lotterhos, K. E. (2015). Reliable detection of loci responsible for local adaptation: Inference of a null model through trimming the distribution of F(ST). *The American Naturalist*, *186*(S1), S24–S36.
Ye, J.-W., Zhang, Y., & Wang, X.-J. (2017). Phylogeographic breaks and the mechanisms of their formation in the Sino-Japanese floristic region. *Chinese Journal of Plant Ecology*, *41*, 1003–1019.
Zachos, J. C., Dickens, G. R., & Zeebe, R. E. (2008). An early Cenozoic perspective on greenhouse warming and carbon-cycle dynamics. *Nature*, *451*, 279–283.

BIOSKETCH

The research team is interested in the ecological and evolutionary divergence and diversity of different lineages of tree species of Patagonia.

Mariana Fasanella is a researcher at CONICET and works on the genetic adaptation of Patagonian trees using genomic markers and environmental variables.
Paula Mathiasen is a population geneticist and phylogeographer. Her research is focused in population genetics, phylogeographic and paleogenetic studies related to ecological, evolutionary and biogeographic processes of native trees from South American temperate forests.
Gabriela Juri is a doctorate candidate that studies the establishment and evolution of reproductive barriers among sympatric Nothofagus species' pairs of subgenus Nothofagus as a model group.
Dayana Diaz is a doctorate student at Universidad Nacional del Comahue. Her research focuses in population genetics, phylogenetic and geographical variation related to ecological and evolutionary processes and to the conservation of woody species native to Patagonia.
Rodrigo Hasbun Zaror have a PhD in Biotechnology and currently is Associated Professor at the Departamento de Silvicultura, Universidad de Concepción. His research focuses on biodiversity conservation and breeding using biotechnological tools. His current interest is to understand and develop strategies of plant adaptation to climate change based on genetic and epigenetic variation.
Andrea Premoli main interests are population genetics and genomics applied to the study of the factors that affect the evolution of plant species and the conservation of natural resources, particularly woody species of the southern and subtropical montane forests of South America.
Author contributions: MF, PM and ACP conceived the ideas, analysed the data, interpreted the results and wrote the manuscript. MF, PM, GJ, DD, RH and ACP collected the data and performed the laboratory work. GJ, DD and RH contributed to revision of the final version of the manuscript.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Fasanella, M., Mathiasen, P., Juri, G., Díaz, D. G., Hasbún, R., & Premoli, A. C. (2023). Ancient vicariance is reinforced by adaptive divergence in the southern beech: Contributions from geogenomics. *Journal of Biogeography*, *50*, 564–575. https://doi.org/10.1111/jbi.14554