ORIGINAL ARTICLE

Phylogenetic perspectives on biome shifts in *Leucocoryne* (Alliaceae) in relation to climatic niche evolution in western South America

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ABSTRACT

Aim Shifts between the western South American sclerophyll and winter-rainfall desert biomes and their relationship to climatic niche evolution and aridity development were investigated in the South American endemic geophytic *Leucocoryne* (Alliaceae) clade.

Location Western South America.

Methods We constructed a molecular phylogeny (internal transcribed spacer, ITS), estimated lineage divergence times, and identified ancestral biomes and biome shifts. The multivariate climatic niche of present-day species was described using occurrence data and bioclimatic variables. Climatic niche similarity was evaluated using Mahalanobis and Fisher distances. Brownian motion (BM) and Ornstein–Uhlenbeck (OU) models of evolution were used to characterize temperature and precipitation niche evolution. Ancestral temperature and precipitation were estimated using the phylogenetic generalized least-squares method.

Results *Leucocoryne* exhibits a low level of phylogenetic biome conservatism. The clade arose in the early Miocene in an ancestral sclerophyll biome and subsequently moved northwards into the arid winter-rainfall biome on two separate occasions, during the late Miocene and Pliocene, respectively, with very recent diversification of species in the winter-rainfall desert. Overall, the multivariate climatic niche showed significant differentiation, and phylogenetic and climatic niche distances were correlated. Temperature and precipitation niche evolution within lineages followed a pattern that is consistent with stabilizing selection (OU model).

Main conclusions The low level of phylogenetic biome conservatism found in *Leucocoryne* is associated with considerable expansion of the precipitation and temperature niche axes. Unidirectional biome shifts from a wetter biome characterized by higher species richness and more continuous vegetation cover, into a drier biome with lower species richness and much sparser vegetation cover, suggest that the availability of and lower biotic resistance within open habitats facilitated biome shifts in *Leucocoryne*. Incursion into the arid winter-rainfall desert and diversification there may have been facilitated by the conservative geophytic life-form of *Leucocoryne*, the generally cool coastal conditions, and the wet/dry climatic cycles occurring since the late Miocene.

Keywords
Ancestral biome, aridity, Chile, climatic niche, *Leucocoryne*, sclerophyll biome, winter-rainfall biome.

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INTRODUCTION

The world's major biomes may be phylogenetically enriched over geological time as a result of colonization by clades originating in other biomes. A number of studies have concluded that clades tend to be characterized by a high level of phylogenetic biome conservatism (Prinzing *et al.*, 2001; Rice *et al.*, 2003; Donoghue, 2008; Crisp *et al.*, 2009), which impedes them from readily crossing over biome lines. Nevertheless, detailed studies in herbaceous plants have shown that related species rarely have identical environmental niches (Smith & Beaulieu, 2009), and significant niche divergence has been detected in clades occurring in arid ecosystems (Ellis *et al.*, 2006; Nakazato *et al.*, 2008, 2010; Evans *et al.*, 2009; Heibl & Renner, 2012). When shifts occur between different biomes, substantial expansion of a clade's climatic niche can be expected (i.e. niche lability, *sensu* Losos *et al.*, 2003), given that individual species of a clade are usually constrained in terms of the climatic conditions under which they occur (Wiens & Donoghue, 2004; Guerrero *et al.*, 2013). Such climatic niche evolution could reflect the accumulation of random variation, or stabilizing selection, whereby lineages diversifying in the colonized biome are able to adapt to novel abiotic and biotic conditions (Butler & King, 2004; Kozak & Wiens, 2010; Wiens *et al.*, 2010; Guerrero *et al.*, 2013). Knowledge of the frequency and direction of biome shifts and of the climatic niche evolution accompanying such shifts is fundamental for our understanding of major biogeographical patterns.

The sclerophyll and desert biomes are represented at mid-latitudes on the western side of the Chilean Andes by the mediterranean shrublands and sclerophyllous forests of central Chile, and the winter-rainfall desert of northern Chile, respectively (Table 1). The winter-rainfall desert extends from 20° S to around 31°–31°30′ S. It is characterized by extremely low (< 150 mm; Table 1) and unpredictable precipitation. It forms part of the greater Atacama Desert, which also comprises areas that receive summer rainfall to the east and as far north as 18° S. The sclerophyll biome lies immediately to the south of the winter-rainfall biome (Fig. 1), extending to around 38° S, where it is replaced by the temperate rain forest. It is also characterized by winter rainfall (di Castri & Hajek, 1976), but receives much higher annual precipitation than the winter-rainfall desert, which can reach over 1300 mm at its southern extreme (Table 1). Sclerophyll elements appeared in central Chile in the early Miocene to become widespread as of the mid-Miocene Climatic Optimum (Hinojosa, 2005; Hinojosa *et al.*, 2006; Armesto *et al.*, 2007). Coastal areas of the Atacama at the latitudes of the winter-rainfall desert were already arid by 20 Ma (early Miocene) (Schlunegger *et al.*, 2010). However, the onset of present-day hyper-arid conditions in the more northerly part of the winter-rainfall desert dates only to the late Miocene (6–8 Ma) (Reich *et al.*, 2009). While the adjacent sclerophyll and winter-rainfall desert biomes show major vegetation differences and many floristic singularities (Guerrero *et al.*, 2011a), a large number of species and genera are distributed in both biomes (Table 1). However, little is known about the timing and direction of biome shifts in such clades, or about the extent and nature of climatic niche evolution (Guerrero *et al.*, 2013).

Here, we investigate biome shifts and the evolution of the climatic niche in geophytic *Leucocoryne* Lindl. (Alliaceae). This clade is entirely restricted to the sclerophyll and winter-rainfall desert biomes of western South America (Fig. 1), and thus provides an outstanding model for investigating shifts between these two biomes. Water is the limiting factor for plant growth in arid environments (Ehleringer & Mooney, 1983); thus, evolution of the climatic niche is highly relevant for understanding biome colonization in arid and semi-arid areas. We asked the following questions: (1) What is the ancestral biome of *Leucocoryne*? (2) Were biome transitions unidirectional, and when did they take place over the geological history of the clade? (3) Is the climatic niche of the *Leucocoryne* clade conservative or labile? (4) Does evolution of the precipitation and temperature niches of *Leucocoryne* clade fit a gradual drift model (Brownian motion, BM) or stabilizing selection (Ornstein–Uhlenbeck, OU; Butler & King, 2004)? To answer these questions, we undertook phylogenetic reconstruction, determined lineage divergence times, performed ancestral biome reconstruction, identified biome shifts, reconstructed ancestral precipitation and temperature, and characterized changes in the climatic niche.

Table 1 Comparison of the sclerophyll and winter-rainfall biomes of Chile. Plant species richness data follow Arroyo & Cavieres (1997). See also Fig. 1 for the geographical locations of the two biomes. An exclusive taxon is one that is found in one of the two biomes of interest, but not in the other. Exclusive taxa were determined from the original floristic database used in Arroyo & Cavieres (1997). Precipitation is from published meteorological sources (di Castri & Hajek, 1976; http://www.cazalac.org/rp_paises.php?pais_seleccionado=chile).

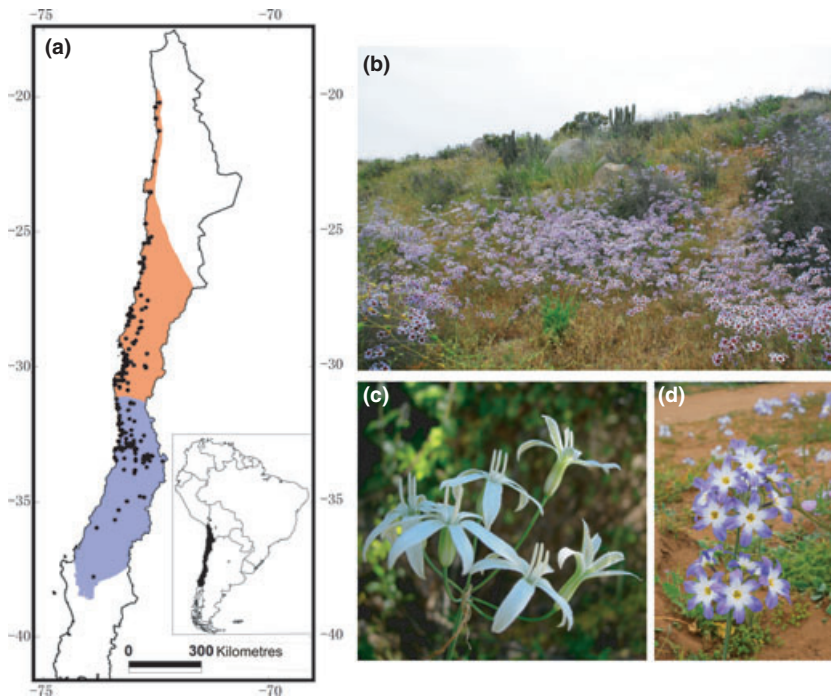| Biome	Land area (10^3 km²)	Annual precipitation (mm)	Vegetation	Total plant families	Exclusive plant families	Total plant genera	Exclusive plant genera	Total plant species	Exclusive plant species
Sclerophyll	155	191–1308	Matorral and sclerophyllous forest	160	36 (22.5%)	620	215 (34.7%)	2537	1536 (60.5%)
Winter rainfall	145	0–134	Desert scrub	127	3 (2.4%)	480	75 (15.6%)	1893	892 (47.1%)

Figure 1 (a) The distribution of *Leucocoryne* in Chile. Black circles are georeferenced occurrences. Blue shading, sclerophyll biome; orange shading, winter-rainfall desert biome. (b) Coastal habitat of *L. purpurea* (Lineage III). (c) Detail of *L. conferta* (Lineage I). (d) *L. coquimbensis* var. *coquimbensis* (Lineage III).

MATERIALS AND METHODS

Leucocoryne is a showy genus of geophytes (Fig. 1) with small tunicate bulbs (1.5 cm diameter). *Leucocoryne* extends over a precipitation range from 1200 mm yr^{-1} in the southern part of the sclerophyll biome at 37° S to ≤ 5 mm yr^{-1} at 20° S in the northern part of the winter-rainfall desert biome. In the sclerophyll biome and the southern part of the winter-rainfall desert, *Leucocoryne* can be found both inland and in coastal locations (Fig. 1). However, with increasing levels of aridity in the winter-rainfall desert, the genus becomes progressively more restricted to the permanent coastal fog belt (Cereceda *et al.*, 2008) (Fig. 1). Species richness peaks in the southern part of the winter-rainfall biome and in the northern extreme of the sclerophyll biome (Jara-Arancio, 2010). Although *Leucocoryne* is capable of abundant seed set, reproduction in many species can be via bulbils (Zöllner, 1972).

Taxon sampling

For taxon sampling, we followed the treatment of Muñoz Schick & Moreira Muñoz (2000), which is based largely on earlier work by Zöllner (1972). We sampled one additional species described by Mansur & Cisternas (2005), bringing the total number of taxa studied to 17 (15 species and two subspecific taxa). Fresh leaf material was collected in the field or from greenhouse plants grown from seeds collected in the field (one species) and dried in silica gel. We used six species of Alliaceae as outgroups: *Miersia chilensis* Lindl., *Nothoscordum bivalve* (L.) Britton, *Nothoscordum inodorum* (Aiton) G. Nicholson, *Tristagma bivalve* (Lindl.) Traub, *Tristagma*

nivale Poepp. and *Zoellnerallium andinum* (Poepp.) Crosa. These taxa were selected because of their recognized morphological and karyological affinities with *Leucocoryne* (Zöllner, 1972; Crosa, 1988; Araneda *et al.*, 2004; Jara-Arancio *et al.*, 2012). We also included *Phycella bicolor* (Ruiz & Pav.) Herb. and *Rhodophiala phycelloides* (Herb.) Hunz. (Amaryllidaceae), given the sister relationship of Amaryllidaceae and Alliaceae (Janssen & Bremer, 2004). Localities and other data are given in Appendix S1 in Supporting Information.

DNA extraction, amplification and sequencing

Genomic DNA was extracted with the DNeasy Plant Kit (Qiagen, Valencia, CA, USA). We amplified the internal transcribed spacer (ITS) using the primers ITS1 (5′-TCCG TGGTGAACCTGCGG-3′) and ITS4-1 (5′-CCTCCGCCTTA TTGATATG-3′) (White *et al.*, 1990). The PCR used a final volume of 30 μL, which contained 8 μL DNA (1 ng μL^{-1}), 10.1 μL distilled water, 3 μL MgCl$_2$ (25 mM), 3 μL 10× buffer, 3 μL of 1 mM dNTP mixture, 1.2 μL of each primer (10 μM) and 0.5 μL Taq polymerase (1 U μL^{-1}). DNA was denatured at 97 °C for 25 s, followed by 26 amplification cycles of 30 s at 96 °C, annealing for 30 s at 53 °C, with a temperature change of one degree per second to the annealing temperature, elongation for 2 min at 72 °C and a final extension of 7 min at 72 °C. Samples were sent to Macrogen (Seoul, South Korea) for purification and sequencing. Sequences were loaded, edited and aligned using CHROMASPRO 2.33 (Technelysium, Brisbane, Australia) and BIOEDIT 7.0 (Hall, 1999), and have been deposited in GenBank (see Appendix S1).

Phylogenetic analysis, divergence times and ancestral biome reconstruction

Parsimony and Bayesian analyses were performed. For the parsimony analysis (PAUP* 4.0; Swofford, 2003), we used a heuristic search with branch-swapping by bisection and tree bisection–reconnection (TBR), and bootstrap analysis (Felsenstein, 1985) with 1000 replicates (Efron *et al.*, 1996). Nodes with bootstrap values $\geq 70\%$ were considered to be supported (Hillis & Bull, 1993). For Bayesian inference (MRBAYES 3.2.1; Ronquist & Huelsenbeck, 2003), the best substitution model (GTR+G) was selected using the Akaike information criterion (MRMODELTEST 2.2; Nylander, 2004). We ran 5.0×10^6 generations, after which the automated diagnostic statistic (average standard deviation of split frequencies) was < 0.01 (Ronquist & Huelsenbeck, 2003). Posterior probabilities of clades were determined from the post-burn-in sample (80%) of 10,000 reconstructed trees. Nodes with credibility ≥ 0.95 were considered to be supported (Ronquist & Huelsenbeck, 2003). We searched the literature and the Paleobiology Database (http://paleodb.org/cgi-bin/bridge.pl) for fossil material of *Leucocoryne* and of Alliaceae, without success. The ITS Bayesian phylogeny was therefore time-calibrated assuming a relaxed molecular clock, using BEAST 1.4.8 (Drummond & Rambaut, 2007). For calibration, we used two substitution rates, each corresponding to annual or perennial herbs, as reported in Richardson *et al.* (2001): 3.5×10^{-9} substitutions per site per year and 3.0×10^{-9} substitutions per site per year.

To infer the ancestral biome of the *Leucocoryne* clade, we optimized the biome of each species of *Leucocoryne* onto the Bayesian ITS tree, using parsimony and maximum likelihood as implemented in MESQUITE 2.74 (Maddison & Maddison, 2008). We used di Castri's (1973) northern latitudinal limit (31°–31°30′ S) for the eumediterranean climate (sclerophyll vegetation) as a guide for assigning species to a biome. Thirteen of the 17 taxa are entirely restricted to one of the two biomes according to this criterion. Among the remaining taxa, one was found predominantly in the sclerophyll biome, extending *c.* 0.5° north of this limit, while three were found predominantly in the winter-rainfall biome extending between a few minutes and *c.* 1° south of di Castri's (1973) limit. As this limit is very approximate, these transgressions did not justify placing the four taxa into more than one biome. Biomes of all outgroups were also included in the reconstruction. Most of the latter occurred principally in the sclerophyll biome and were coded accordingly. Two outgroups (*N. inodorum* and *T. nivale*) are distributed in several biomes in southern South America, but not principally in either the sclerophyll or winter-rainfall biomes. These were placed in a third category.

Climatic niche evolution

Multivariate and univariate approaches were employed to determine whether the climatic niche of *Leucocoryne* is conservative or labile, and to investigate the mode of climatic niche evolution (random variation versus stabilizing selection), respectively. We compiled georeferenced occurrence data from Chilean herbaria (CONC, Universidad de Concepción; SGO, Museo Nacional de Historia Natural; ULS, Universidad de La Serena). The number of occurrences ranged from one or two for the local endemics (*L.* aff. *vittata*, *L. pauciflora*, *L. talinensis*) to 49 in *L. ixioides* (Appendix S1). To increase the number of occurrences in the three local endemics, four pseudo-occurrences were added for the two taxa with only one true occurrence, and three for the single taxon with only two true occurrences, using GOOGLE EARTH 6.2.2.7373 (Google, Mountain View, CA, USA; http://www.google.com/intl/es/earth/index.html). The four pseudo-occurrences were located 1 km to the east, west, north and south of the true occurrence. Where three pseudo-occurrences were generated, these were located 1 km east and west of one of the true occurrences, and l km to the east of the second true occurrence. Nineteen bioclimatic variables, at a resolution of 1 km², were extracted from WorldClim climate data (Hijmans *et al.*, 2005) using DIVA-GIS 7.5 (Hijmans *et al.*, 2001). The majority of geographical coordinates for collecting localities were given in degrees and minutes and lacked seconds. At a 1-km² resolution, the real locations could therefore have occasionally fallen into one of the adjacent grid cells. This potential source of error was not considered problematic for the purposes of our analysis, as neither precipitation nor temperature will vary significantly over these distances for *Leucocoryne* localities.

Using occurrence data to describe the climatic niche relies on the premise that spatial surfaces of meaningful niche dimensions determine species' distributions (Graham *et al.*, 2004; Soberón & Peterson, 2004). As bioclimatic variables are usually strongly correlated, and in order to reduce collinearity, we choose a subsample of bioclimatic variables from a correlation matrix implemented in XLSTAT 2012.5.01 (Addinsoft, New York, NY, USA), using a threshold value of $r^2 < 0.7$ for pairs of variables. After initial screening, seven bioclimatic variables were retained (BIO1, annual mean temperature; BIO3, isothermality; BIO5, maximum temperature of the warmest month; BIO6, minimum temperature of the coldest month; BIO9, mean temperature of the driest quarter; BIO12, annual precipitation; and BIO17, precipitation of the driest quarter). In order to test whether species varied in their multivariate climatic niches, principal components analysis (PCA) was conducted on the filtered bioclimatic data. Differences in climatic niche space were evaluated using multidimensional comparisons of the first four principal components, which accounted for $> 95\%$ of the variation. We examined whether the four principal lineages of the *Leucocoryne* clade varied in their climatic multidimensional space using Fisher distances. In addition, associations between the Mahalanobis and Fisher distances, and between those distances and the phylogenetic distance matrices, were evaluated with a Mantel test (10,000 randomizations; Mantel, 1967). Phylogenetic distances were computed in R (R Development

Core Team, 2006) using the ADEPHYLO package (Jombart *et al.*, 2010). All multivariate analyses were executed in XLSTAT.

Univariate analyses were performed on the medians for precipitation and temperature across all occurrence localities; medians were preferred over means, as the former are less influenced by extreme values. We analysed the stability of the precipitation and temperature niche within the *Leucocoryne* clade over time with the morphological disparity index (MDI) using the R package GEIGER (Harmon *et al.*, 2008). This compares the observed disparity to that expected under an unconstrained Brownian motion model of evolution (Harmon *et al.*, 2003). Negative values of MDI indicate that disparity tends to be distributed among subclades (due to niche evolution among subclades and conservatism within subclades), whereas positive values of MDI indicate that disparity tends to be distributed within subclades (Evans *et al.*, 2009). Following Kozak & Wiens (2010), we used the OUCH package (Butler & King, 2004) in R to assess the likelihood of observing climatic divergence under a random drift model (Brownian motion, BM) or a stabilizing selection model (Ornstein–Uhlenbeck, OU) of evolution, prior to reconstructing ancestral annual temperature and annual precipitation. We assessed the fit of the model using the sample-size-corrected Akaike information criterion (AIC$_c$). Given the good OU fits, we estimated ancestral values for precipitation and temperature in the chronogram using phylogenetic generalized least squares (PGLS). For reconstruction of the ancestral states, we used COMPARE 4.6b (Martins, 2004).

RESULTS

Phylogenetic analysis, divergence times and ancestral biome reconstruction

The ITS matrix for the 25 taxa comprised 785 aligned positions, of which 294 were parsimony informative. Under maximum parsimony, 516 trees were retained (consistency index = 0.7; homoplasy index = 0.21; retention index = 0.85). The maximum-parsimony consensus and Bayesian inference ITS trees were well supported and congruent (Fig. 2). *Leucocoryne* is monophyletic and comprises three well-resolved lineages in both parsimony and Bayesian inference, and a fourth that is well resolved under parsimony and with a marginally non-significant support value of 0.92 under Bayesian inference. Phylogenetic reconstructions retrieved six pairs of sister species (Fig. 2) among which the pairs *L. alliacea*–*L. conferta* and *L. narcissoides*–*L. dimorphotela* have high node support.

The two substitution rates provided very similar divergence times (Fig. 2). Divergence of *Leucocoryne* within the family Alliaceae, as indicated by the stem node age, occurred in the early Miocene (Fig. 2). The stems of Lineage I diverged in the Miocene (Fig. 2). Lineages II, III and IV diverged considerably later, in the Pliocene (Fig. 2). Considerable diversification in Lineage III occurred in the Pliocene

(Fig. 2), while diversification within Lineage IV took place relatively recently, in the Pleistocene. According to maximum parsimony, the inferred ancestral biome of *Leucocoryne* is the sclerophyll biome (Fig. 3). Results for maximum likelihood were concordant, although there was some ambiguity. The ancestor of the *Leucocoryne* clade corresponds to the sclerophyll biome. Lineages I and IV are restricted to the sclerophyll biome. Lineage II and most of Lineage III are distributed in the winter-rainfall desert biome to as far north as 23° S and 20° S, respectively (Fig. 2). Lineage IV is found predominantly in the northern part of the sclerophyll biome, with one species extending to the southern extreme of the sclerophyll biome (*L. ixioides*). These results indicate that biome shifts in *Leucocoryne* have occurred on two occasions and always undirectionally, out of the sclerophyll biome and into the winter-rainfall biome.

Climatic niche evolution

Extensive variation was revealed in the multivariate climatic niche space occupied by *Leucocoryne* species. Fisher distances indicated that the climatic niches of the four principal *Leucocoryne* lineages were different (Appendix S2). Considerable divergence of the climatic niche over the evolutionary history of *Leucocoryne* is supported by positive correlations between Mahalanobis and Fisher distances and phylogenetic distances (Mantel test: Mahalanobis, $r = 0.272$, $P = 0.001$; Fisher, $r = 0.319$, $P < 0.001$), respectively. Mahalanobis and Fisher distances were also significantly correlated ($r = 0.763$, $P < 0.001$).

Reconstruction of ancestral states showed the common ancestor of the *Leucocoryne* clade to be associated with an estimated 340 mm annual precipitation and 12 °C annual temperature (Fig. 4). This combination of precipitation and temperature in western South America is associated with sclerophyll vegetation, both now and in the past (Armesto *et al.*, 2007). The ancestral states for precipitation and temperature thus agree well with our results for biome reconstruction, where the sclerophyll biome was also found to be ancestral (Fig. 3). Overall, among lineages, annual temperature varied from 12 to 17 °C across the clade, while annual precipitation varied over two orders of magnitude (3–535 mm) (Fig. 4). The highest reconstructed annual precipitation is found in the sclerophyllous Lineage IV, and the lowest is found in the winter-rainfall desert Lineage III (Fig. 4).

Similar evolutionary patterns were detected for temperature and precipitation. Both variables were associated with positive MDI values (1.87 and 0.26, respectively), suggesting that the temperature and precipitation disparities tend to be distributed mostly within lineages. The sample-size-corrected Akaike information criterion (AIC$_c$) revealed temperature and precipitation to fit the OU model best (temperature: BM = 658, OU = 77; precipitation: BM = 2142581, OU = 2074), indicating the evolution of temperature and precipitation niches to be consistent with stabilizing selection, rather than accumulation of random variation over time.
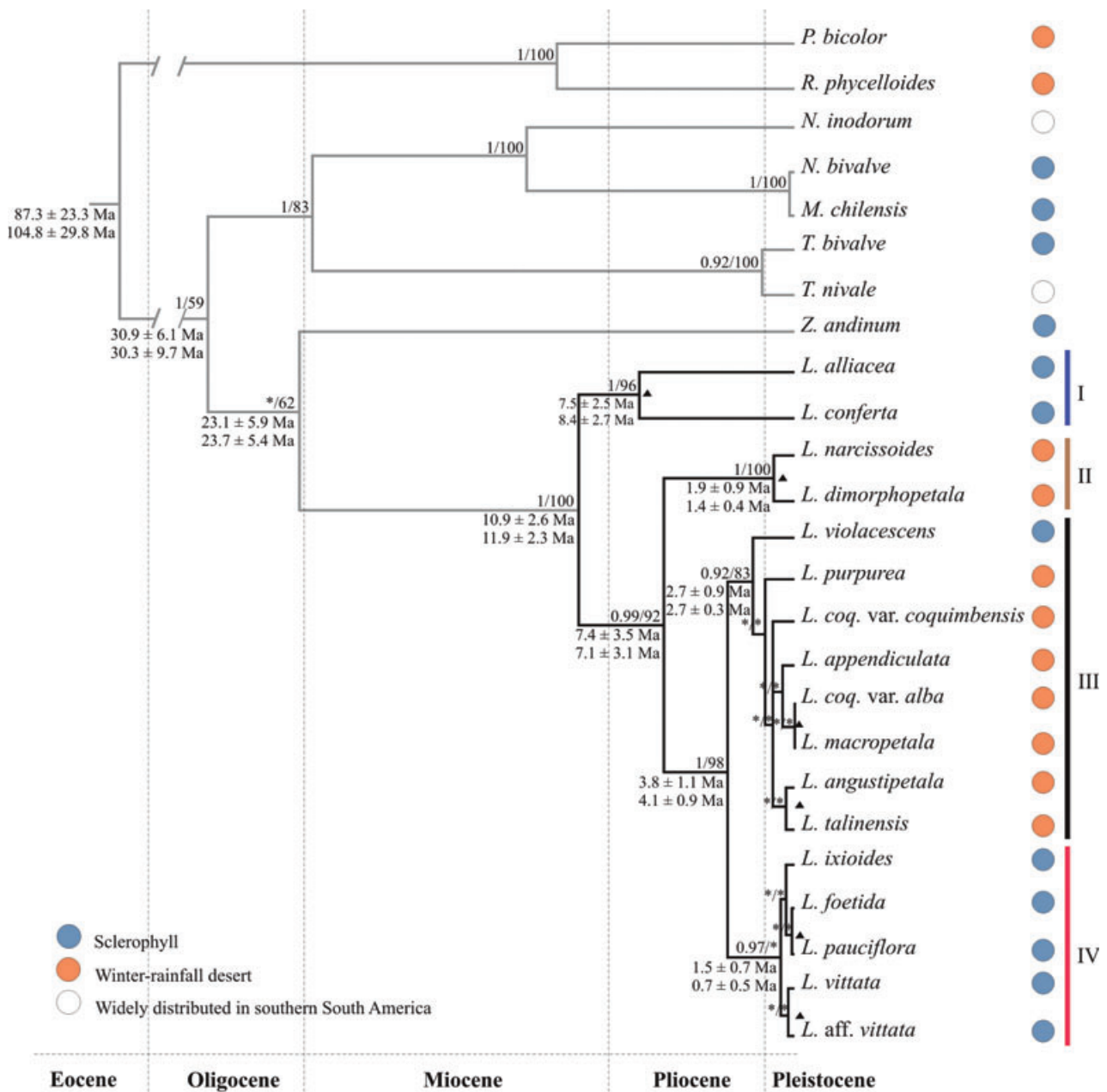
Figure 2 Bayesian inference phylogenetic tree for ITS sequences for 17 taxa of the genus *Leucocoryne* in Chile. Branch lengths with slashes at the base of the tree have been shortened by 75%. Credibility/parsimony bootstrap values are found above the branches. Asterisks indicate nodes without support. Triangles indicate supported pairs of sister species. Estimation of divergence times (millions of years ago, Ma) with Bayesian statistics (mean and standard deviation) based on 3.5×10^{-9} (upper) and 3.0×10^{-9} (lower) substitutions per site per year are found below the branches. Biomes are shown by circles: blue, sclerophyll; orange, winter-rainfall desert; white, widely distributed in southern South America, including in the sclerophyll biome, and marginally in the winter-rainfall biome. The principal lineages are indicated by roman numerals (I–IV). Black lines indicate the external group and grey lines the internal group. The outgroup species are *Miersia chilensis*, *Nothoscordum bivalve*, *N. inodorum*, *Tristagma bivalve*, *T. nivale* and *Zoellnerallium andinum* (Alliaceae) and *Phycella bicolor* and *Rhodophiala phycelloides* (Amaryllidaceae).

DISCUSSION

The *Leucocoryne* clade made two independent incursions from a total of four lineages, out of its ancestral sclerophyll biome into the arid winter-rainfall desert in the late Miocene and Pliocene, respectively. Thus, it may be concluded that

Leucocoryne is characterized by a low level of phylogenetic biome conservatism. On a broader geographical scale, Crisp *et al.* (2009) showed biome stasis at speciation to outweigh biome shifts by more than 25:1. Nevertheless, these authors found biome shifts between the sclerophyll and desert biomes to be easier than among any other pair of biomes. Our
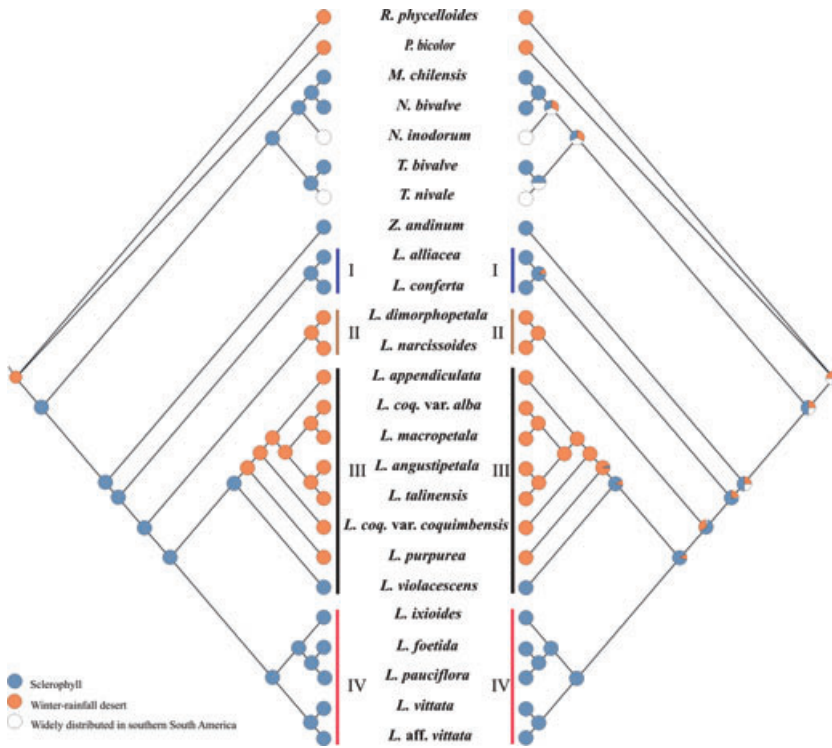
Figure 3 Ancestral biome reconstruction for Chilean *Leucocoryne* on the Bayesian tree using parsimony (left) and maximum likelihood (right). Biomes are shown in the circles; blue, sclerophyll; orange, winter-rainfall desert; white, widely distributed in southern South America, including in the sclerophyll biome, and marginally in the winter-rainfall biome. The principal lineages are indicated by roman numerals (I–IV). The outgroup species are *Miersia chilensis*, *Nothoscordum bivalve*, *N. inodorum*, *Tristagma bivalve*, *T. nivale* and *Zoellnerallium andinum* (Alliaceae) and *Phycella bicolor* and *Rhodophiala phycelloides* (Amaryllidaceae).
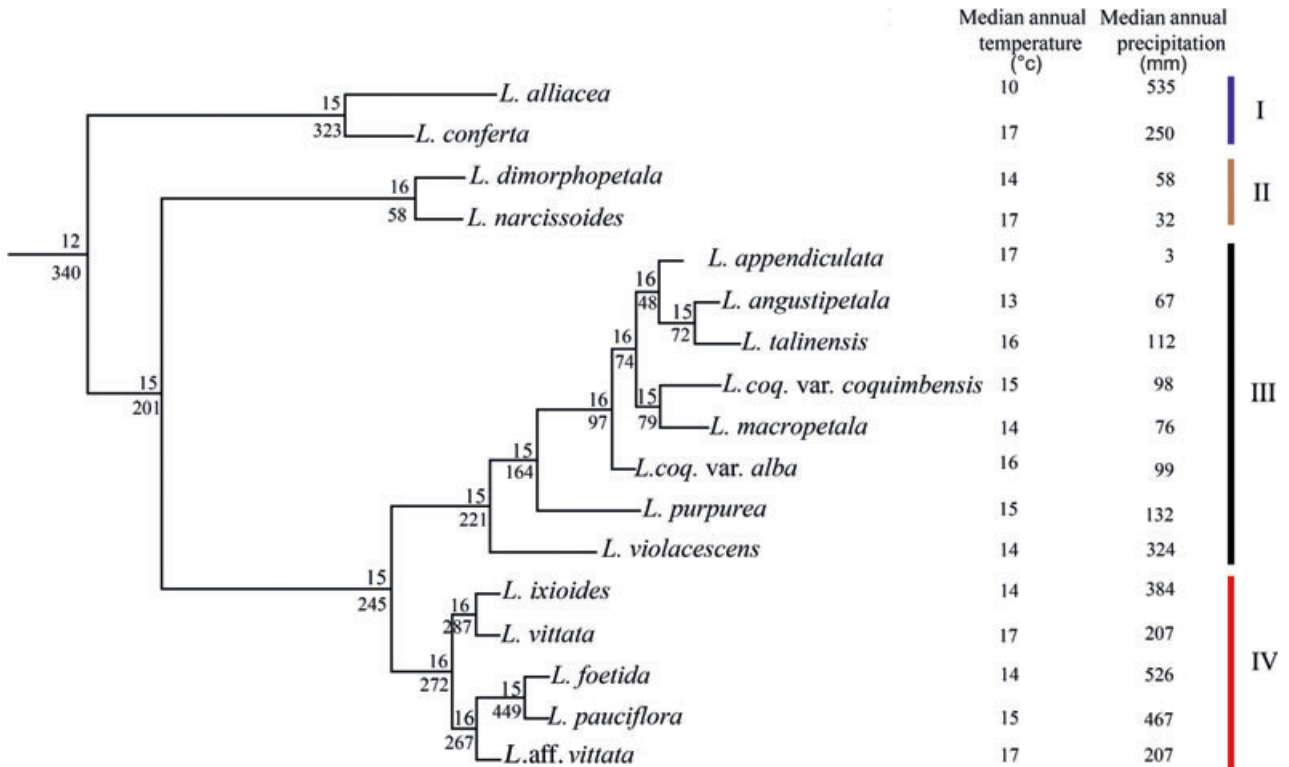

Figure 4 Reconstruction of ancestral states of median annual temperature (°C) (above branches) and annual precipitation (mm) (below branches) for *Leucocoryne* in Chile. The principal lineages of *Leucocoryne* are indicated by roman numerals (I–IV).

results for *Leucocoryne* confirm that biome shifts can occur between sclerophyll and desert vegetation, including on a somewhat finer geographical scale.

Interestingly, the direction of biome shifts in *Leucocoryne* has been from the wetter to the drier biome, with the sclerophyll biome of central Chile acting as a biogeographical

source and the winter-rainfall biome as a sink. This raises the interesting question as to why biome shifts have occurred from a more physically benign biome into a physically harsher biome. Given that the initial shift from the sclerophyll biome into the winter-rainfall desert occurred relatively early – in the late Miocene (Fig. 3) – the unidirectional biome shifts in *Leucocoryne* are unlikely to reflect insufficient evolutionary time for the development of new sclerophyll clades from winter-rainfall desert ancestors. Species richness (Table 1) and especially vegetation cover (USGS Land Cover Institute, 2012) are lower in the winter-rainfall desert than in the sclerophyll biome. The preferred direction of shifts from the sclerophyll biome to winter-rainfall desert thus might be due to less biotic resistance to invasion in the winter-rainfall biome, in parallel with known occurrences in contemporary plant invasions (Levine *et al.*, 2004).

Our multivariate analysis suggests that there was expansion of the climatic niche during the diversification of the *Leucocoryne* clade. Lineage III diversified as of the late Pliocene; species of this lineage are today found in enclaves that punctuate coastal areas of the hyper-arid desert (but see Dillon *et al.*, 2009; Guerrero *et al.*, 2011b), suggesting fragmentation of a previously more continuous ancestral distribution during wetter periods. From the Miocene (Placzek *et al.*, 2009) onwards, and into the Pleistocene (Díaz *et al.*, 2012), wet/dry cycles have occurred repeatedly in the arid and hyper-arid areas of northern Chile. In accordance with such repeated wet/dry cycles, precipitation became strongly reduced as of the late Pliocene (Amundson *et al.*, 2012), at the time when Lineage III was diversifying. During wet cycles, *Leucocoryne* populations may have undergone expansion, whereas contraction and local divergence may have occurred during dry cycles, leading to an overall expansion of the clade's climatic niche and speciation. Supporting the latter, we found the precipitation and temperature niches in *Leucocoryne* to resemble the pattern expected under stabilizing selection (Butler & King, 2004; Wiens *et al.*, 2010; Burbrink *et al.*, 2012). Moreover, precipitation and temperature showed positive disparities, which are associated with an increase in niche breadth within subclades (Evans *et al.*, 2009). Similar range expansions and contractions probably also occurred within Lineage IV, also with mostly allopatrically distributed species. This particular lineage is found mostly in the northern and driest part of the sclerophyll biome, where significant fluctuations in precipitation have been reported in the Quaternary (Maldonado & Villagrán, 2006).

The expansion of the climatic niche of the *Leucocoryne* clade, allowing survival of certain species under very arid conditions, begs the question as to whether biome shifts were accompanied by the gradual evolution of new adaptations to an arid climate as opposed to dependence on pre-existing genotypic variation. Some major radiations in arid climates have been accompanied by *in situ* evolution of key morphological and anatomical innovations (Klak *et al.*, 2004). However, pre-existing phenotypic characters, in the form of tubers, are known to have facilitated adaptation to seasonal

winter-wet and summer-dry climates in the cosmopolitan carnivorous plant genus *Drosera* (Droseraceae) (Yesson & Culham, 2006), and expansion into seasonally arid or xeric environments from more mesic environments in South American *Oxalis* (Heibl & Renner, 2012).

The geophytic habit of the *Leucocoryne* clade is well suited to survival under the high interannual variation in precipitation found in the winter-rainfall desert. *Leucocoryne* bulbils can remain dormant below ground for many years until adequate precipitation is received. On the other hand, as a result of the cooling effect of the coastal fog bank (Cereceda *et al.*, 2008), temperatures in coastal areas of the most arid part of the winter-rainfall desert are relatively low, allowing the delicate *Leucocoryne* bulbs to resist desiccation. El Niño years (Holmgren *et al.*, 2006) and cool temperatures are associated with extensive populations of *Leucocoryne*, covering many kilometres (compare Fig. 1). Coupled with the above, bulbil production by *Leucocoryne* species allows underground nutrient storage and vegetative reproduction, signifying less dependence on the annual appearance of leaves and flowers, and the often unreliable pollination service in the coastal winter-rainfall desert (González & Pérez, 2010).

In conclusion, *Leucocoryne* shows a low level of phylogenetic biome conservatism. The existence of specific pre-existing life-history characteristics as seen in *Leucocoryne* and other clades discussed above, might turn out to be critical in paving the way for climatic niche evolution when shifts between biomes with very different climates are involved. To a degree, the large-scale unidirectional biome shifts in *Leucocoryne* resemble biological invasions, whereby invading species establish more easily in open areas characterized by lower biotic resistance (Levine *et al.*, 2004). Further phylogenetic studies are needed to determine whether the patterns found in this study are typical of other clades centred in the sclerophyll and winter-rainfall desert biomes of Chile.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Voucher information and GenBank accession numbers.
Appendix S2 Fisher distances between the climatic niches of four *Leucocoryne* lineages.

BIOSKETCH

Paola Jara-Arancio undertook her doctoral work at the University of Chile, Santiago, Chile, working on phylogenetic reconstruction of *Leucocoryne*. She is currently a postdoctoral fellow at the Institute of Ecology and Biodiversity (IEB) in Chile (http://www.ieb-chile.cl/), where she is extending her phylogenetic work to other clades. Her main research interests are in the evolution of the desert flora and the high Andean flora of Chile.

The research interests of the team authors are diverse, covering systematics, evolution, biogeography, Tertiary palaeobotany, and the conservation of southern South American plant and animal groups, especially in mediterranean, desert and high-elevation ecosystems.

Author contributions: P.J.A., P.C.G. and M.T.K. conceived the ideas and study design; P.J.A., P.C.G. and G.A. collected material and contributed data; P.J.A., P.C.G. and M.A.M. performed the phylogenetic reconstructions and analysed the data; M.T.K., P.J.A., P.C.G., L.F.H. and M.A.M. undertook the writing.

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