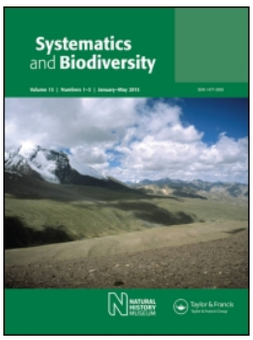

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Research Article

Cryptic species in the Andean hemiparasite *Quinchamalium chilense* (Schoepfiaceae: Santalales)

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The integration of different characters (e.g. morphological, ecological, and molecular) is now recognized as important in species delimitation. In particular, genetic distances between homologous genes have been suggested as one of the main tools to identify species, especially in the case of cryptic species. *Quinchamalium* is morphologically variable and occupies a diverse set of biomes across its distribution in the Southern Andes. Recent work based on morphology has synonymized the entire genus as a single morphospecies, *Quinchamalium chilense*. This widely distributed taxon presents the opportunity to find potential cryptic species. The main objective of this study was to test the existence of cryptic species, based mainly on phylogenetic gene trees, genetic distances, and geographic patterns of haplotypes from molecular markers of the nuclear (ITS) and chloroplast (*trnL-F*) genomes, considering climatic and morphological characteristics. The ITS phylogeny and corresponding haplotype network resulted in three lineages with strong genetic differentiation and distinct geographic patterns. These lineages were informally named *Desert*, *Matorral*, and *Mountain*, based on their geographic distribution in different biomes. The *trnL-F* chloroplast phylogeny did not distinguish *Desert* from *Matorral*, and the haplotype network showed overlap between these last two lineages. Overall, we hypothesize the existence of two cryptic species within *Quinchamalium chilense* (*Mountain* and *Matorral–Desert*) that correspond to genetic, climatic, and morphological differences.

Key words: genetic divergence, molecular lineages, morphology, phylogeny, phylogeography, speciation

Introduction

Specifying criteria or methods to delimit species have been much discussed amongst taxonomists and systematists in the past. In plant systematics, the traditional and most used criterion to identify species has been morphology (Stuessy, 1990), although in many cases it has not been a conclusive criterion (e.g., Lopez Laphitz, Ezcurra, & Vidal-Russell, 2015a; Lopez Laphitz & Semple, 2015). More recently, molecular phylogenetics has decreased the uncertainty when defining species limits (e.g. Acosta, Salariato, & Cialdella, 2016; Piedra-Malagón, Albarrán-lara, Rull, Piñero, & Sosa, 2016; Ruiz-Sanchez, 2011). Nowadays, DNA-based approaches such as barcoding and the identification of species with a threshold measure of divergence between taxa have increased their role in the recognition of diversity, and the genetic distances between homologous genes have been evaluated to identify species (CBOL Plant Working Group, 2009; Fazekas et al., 2009; Yao et al., 2010). In particular, in many taxa where the identification of species is difficult or impossible based on morphological characters alone (Bickford et al., 2007; Lopez Laphitz et al., 2015a; Lopez Laphitz & Semple, 2015), molecular assessments are a powerful tool used for systematics (Gagnon, Hughes, Lewis, & Bruneau, 2015; Ma, Zhao, Wang, Long, & He, 2015; Shepherd, Thiele, Sampson, Coates, & Byrne, 2015).

Authors have used alternative species concepts and different criteria to identify species, and they have been emphasized differently (e.g., Pigliucci, 2003); however, concepts and criteria are not exclusive (de Queiroz, 2007). In this sense, the general lineage concept, introduced by de Queiroz (1998), defines species as separately evolving metapopulation lineages (de Queiroz, 2007; but see Pigliucci, 2003). This concept employs different kinds of evidence such as fixed or

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non-overlapping differences in morphological, behavioural, or ecological characters, molecular divergence thresholds, or geographic isolation, i.e., not only monophyly or reproductive isolation.

Molecular analyses identifying genetically distinct, but morphologically indistinguishable lineages (i.e., cryptic species) have become frequent in some taxonomic groups, i.e., lizards (Raxworthy, Ingram, Rabibisoa, & Pearson, 2007), fungi (Pringle, Baker, Platt, Wares, & Latge, 2005), and lichens (Leavitt, Esslinger, & Divakar, 2012), but have been stated to be surprisingly rare in plants (Bickford et al., 2007; Ma et al., 2015). The investigation of both nuclear and plastid molecular markers and their characteristics, including polymorphisms and genetic distances, has the potential to complement the identification of morphologically similar species, and is the only way in which cryptic species will become visible. The detection of cryptic diversity is important for understanding species distribution ranges, assessing levels of endemism, and predicting species ecology, as well as for determining the conservation status of such cryptic species (Espíndola et al., 2016).

The geographically widespread and morphologically variable genus *Quinchamalium* Molina, endemic to southern South America, consists of yellow-flowered hemiparasitic perennial herbs distributed throughout the Andes from Peru to Argentina, in open habitats within a vast range of altitudes (0–3,800 m a.s.l.). A recently published morphological analysis examining species boundaries within *Quinchamalium* (Lopez Laphitz et al., 2015a) resulted in a reduction from 21 to only one morphospecies, *Q. chilense* Molina. These results cannot be considered conclusive, however, as they did not include phylogenetic information. Until now, the molecular phylogenetic studies that treat this genus were performed exclusively at higher taxonomic levels (genera and families), and the number of individuals of *Quinchamalium* represented in them was low (Der & Nickrent, 2008; Vidal-Russell & Nickrent, 2008a).

Because of its wide geographic distribution that includes different biomes and no clear morphological discontinuities through its entire range, *Quinchamalium* presents an excellent opportunity to interpret molecular lineages in relation to environmental characteristics as potential cryptic species. In the present study, evaluations of morphological and climatic characteristics of individuals of *Q. chilense* sampled from localities across its known distribution were integrated with analyses of chloroplast and nuclear DNA markers to (1) infer phylogenetic relationships within the genus, (2) estimate intra- and inter-lineage divergence, (3) correlate lineages with climatic and morphological variables, and (4) delineate potential cryptic species taking in account monophyly and genetic differentiation as well as the phylogeographic patterns of those lineages.

Materials and methods

Sampling and data collection

Specimens of *Quinchamalium chilense* were obtained from multiple localities throughout the species' geographic range (latitudinal range 7.1° to 50.4°S along the Andes, including Argentina, Bolivia, Chile, and Peru). These specimens also represented the range of morphological diversity found in the species.

Molecular markers selection, DNA extraction, and sequencing

Total genomic DNA was extracted from silica-dried leaf or flower tissue or in some cases herbarium samples from 86 specimens of *Quinchamalium chilense* using Wizard SV Genomic DNA Purification System kit (Promega, Wisconsin). Based on variation in other members of Santalales such as Loranthaceae (Amico, Vidal-Russell, & Nickrent, 2007; Vidal-Russell & Nickrent, 2008b), two molecular markers were selected. The first was the chloroplast encoded *trnL-F* region that consists of the *trnL* 5' exon, the intron, the *trnL* 3' exon, the non-coding spacer, and the *trnF* exon. The second was the nuclear encoded ribosomal DNA internal transcribed spacer consisting of ITS-1, 5.8S rDNA, and ITS-2 (hereafter abbreviated ITS). PCR amplification reactions in a final volume of 25 μL included: 1× buffer (Promega, Wisconsin, USA; 10 mmol/L Tris HCl, 50 mmol/L KCl, pH 8.3), 2 mM de $MgCl_2$, 50 μM dNTPs, 1 unit Taq polymerase, 0.2 μM of each primer, and 1 μL of diluted genomic DNA. For ITS amplifications, 5% dimethylsulfoxide (DMSO, final concentration) was added. The approximately 500 bp ITS region was amplified ($n = 77$) using the primer pair 18S 1830 forward (Nickrent, Schuette, & Starr, 1994) and 26S 40 reverse (Nickrent, Blarer, Qiu, Vidal-Russell, & Anderson, 2004). ITS was amplified using the following PCR profile: 94°C for 5 min; 40 cycles of 94°C for 30 s, 52°C for 30 s, 72°C for 1 min; 72°C for 10 min. The *trnL-F* region ($n = 65$) was amplified using the primer pair c forward and e forward each in combination with f reverse as described in Taberlet, Gielly, Pautou, & Bouvet (1991). For the chloroplast region, a touch down profile was used for amplification consisting of 5 min at 95°C, 5 cycles of 30 s at 94°C, 30 s at 52°C, and 1 min at 72°C, followed by 33 cycles of 30 s at 94°C, 30 s at 48°C, and 1 min at 72°C, with a final extension of 10 min at 72°C. In all reactions negative controls, lacking genomic DNA, were run to check for DNA

contamination. The amplified products were sequenced by Macrogen Inc. (Seoul, South Korea).

Phylogenetic analyses, inter- and intra-lineage diversity

Electropherograms were visually checked in 4Peaks (© 2004–2015 Nucleobytes B.V.), and sequences were aligned with the MUSCLE algorithm implemented in AliView 1.17 (Larsson, 2014) and adjusted manually if needed. Statistics such as nucleotide (π) and haplotype (h) diversity were computed using DnaSP v.5 (Librado & Rozas, 2009). The distance between each pair of sequences was then estimated by Kimura's (1980) two-parameter model using the MEGA program package (Tamura, Dudley, Masatoshi, & Kumar, 2007). Phylogeny reconstruction was carried out with haplotypes or ribotypes (identical sequences were collapsed to one). Each gene was analysed independently and together with Maximum parsimony, Maximum likelihood and Bayesian inference. Maximum parsimony and bootstrap analyses (MPBS, 1000 replicates) were conducted in TNT (Goloboff, Farris, & Nixon, 2000) with heuristic searches and the tree bisection-reconnection algorithm for branch swapping. Gaps were considered homologous if they shared identical boundaries and length. They were coded as a substitution only if they were shared by more than one taxon.

The data sets were assessed for the best-fitting model of nucleotide substitution using the Akaike Information Criterion (AIC) as implemented in JModeltest 0.1.1 (Darriba, Taboada, Doallo, & Posada, 2012). The selected model was GTR+G for all regions (ITS, *trnL-F*). Maximum likelihood analyses were conducted in PAUP* (Swofford, 2003). Tree heuristic searches were performed with a Neighbour Joining (NJ) starting tree, using Tree Bisection Reconnection (TBR) branch swapping algorithm. Bootstrap nodal support (MLBS) was determined by analysing 100 replicates with same settings as the original search. Bayesian phylogenetic analyses were performed using the parallel Metropolis-coupled Markov chain Monte Carlo algorithm in MrBayes version 3.1.2 (Altekar, Dwarkadas, Huelsenbeck, & Ronquist, 2004; Ronquist & Huelsenbeck, 2003). Two independent Bayesian analyses with four chains each were performed for five million generations. Trees and parameters were saved every 100 generations, producing 50,000 trees each run. Model parameters were estimated as part of the analysis; uniform prior probabilities were assigned to all parameters and a Dirichlet prior distribution was assigned to the state frequencies. The burn-in was determined by identifying stationary distribution using the –ln likelihood score. The variance between runs in all cases was below 0.001, thus runs were combined thereby increasing the number of trees in the posterior probability (PP) distribution. Bayesian inference (BI) posterior probabilities (PP) of clades (i.e. clade credibility values) and tree probabilities were calculated after 25% of generations were discarded as burn-in.

Haplotype networks

Data sets of both markers (ITS and *trnL-F*) were subject to a phylogenetic network analysis (minimum spanning network) with PopART (Leigh & Bryant, 2015) under default settings, with all sequences incorporated.

Climatic and morphological characters compared with genetic lineages

Previous multivariate evaluation (principal component and discriminant analyses) of 17 morphological and 19 climatic variables emphasized the importance of some variables to characterize the variation within *Q. chilense* (Lopez Laphitz et al., 2015a). The mean, standard deviation, and range values for these variables were calculated for each lineage. The morphological variables were leaf shape (LS, i.e. length/width ratio), floral tube length (FTL), and floral morph (FM, i.e. tube-length/style-length ratio as estimator of short-styled thrum flowers). The climatic variables were annual precipitation (AP) and annual temperature (AT). Significant differences in these variables amongst phylogenetic lineages were analysed with the non-parametric Kruskal–Wallis test associated with a chi-square approximation. Finally, non-parametric multiple comparisons were calculated to identify the lineages that differed in morphological or/and climatic characteristics.

Results

Phylogenetic analyses

Alignment of the concatenated data set (ITS + *trnL-F*) resulted in a matrix of 1221 sites of which 89 were parsimony informative. Individual gene alignment lengths were 580 bp for ITS and 639 bp for *trnL-F*. The percentage of parsimony-informative sites was 8.4% for ITS while for *trnL-F* it was 5.4%. The number of sequences for ITS was 74 from which there were 37 ribotypes identified, and 63 individuals for *trnL-F* were represented by 32 haplotypes. Upon analysis, the two data sets both showed two main lineages, when analysed separately and with all criteria (Fig. 1; 1 for ITS and 2 for *trnL-F*). Overall, the phylogenetic relationships, based on ITS, were congruent with the different criteria (MP, ML, and BI), resolving three main lineages that were named according to their geographic distribution in different biomes (Fig. 2) as *Desert*, *Matorral*, and *Mountain*. The MP analysis of the

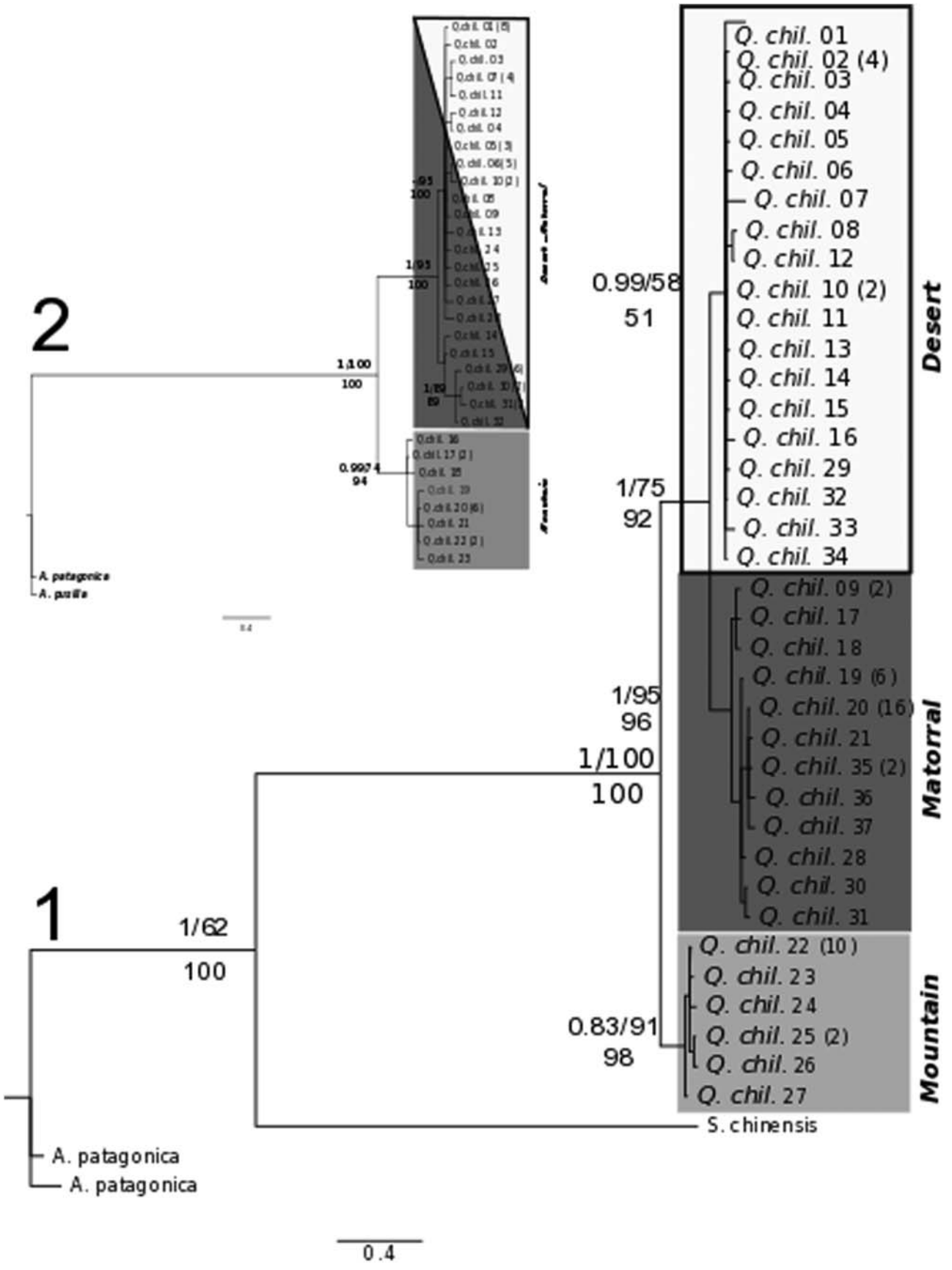

Fig. 1. Phylogeny of *Quinchamalium chilense* based on the analysis of 1: ITS, 2: *trnL-F*. The tree numbers above each node represent BI Posterior Probability/ ML Bootstrap Support, and numbers below each node represent MP Bootstrap Support, (-) represents nodes with no support. Numbers next to each tip represent the different haplotypes (numbers between parentheses are the frequency of that haplotype).

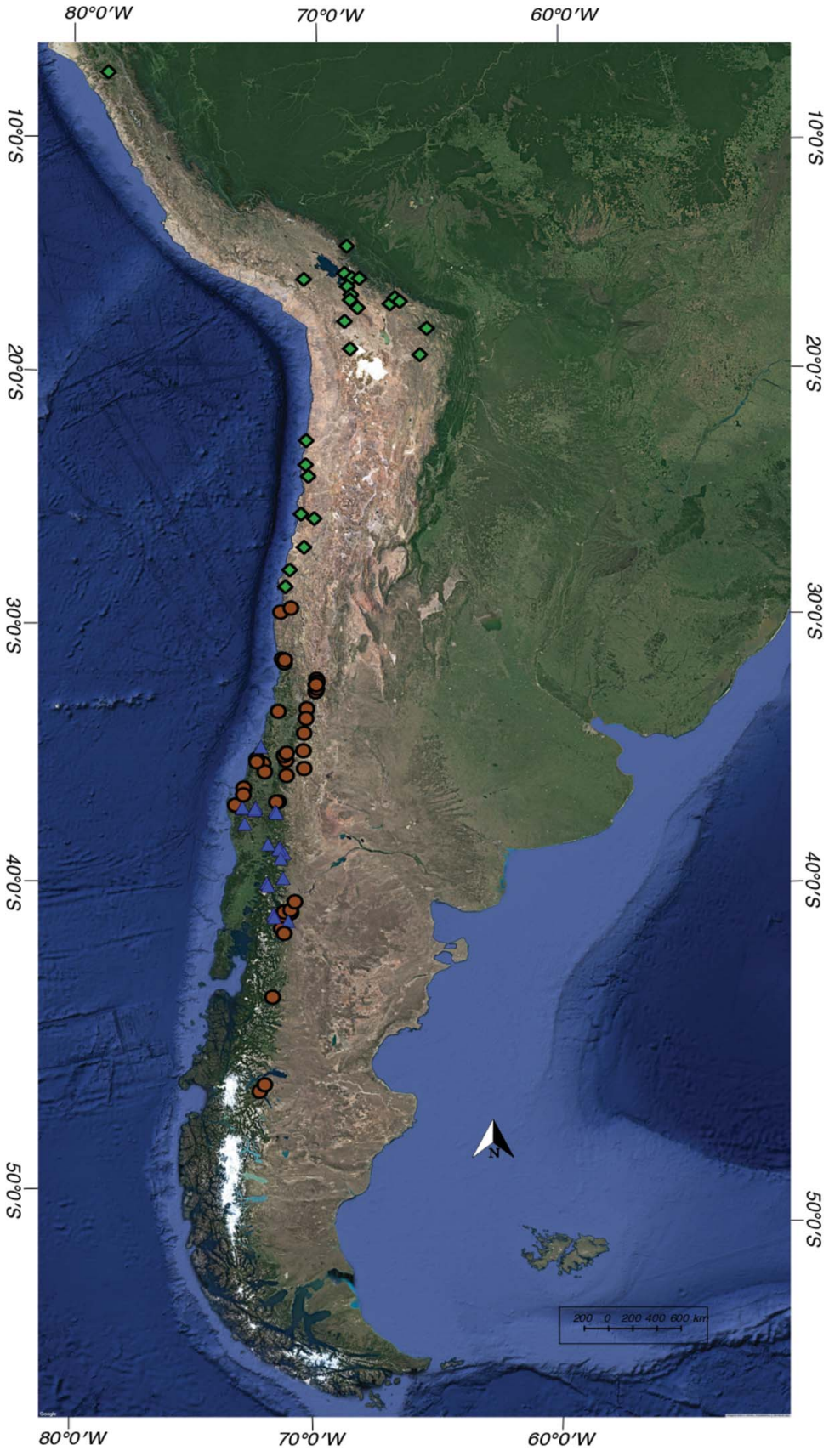

Fig. 2. Distribution map of the genus *Quinchamalium*. Symbols represent different phylogenetic lineages: Mountain, blue triangle; Desert, green diamond; Matorral, brown-circle.

ITS data set recovered 72 equally parsimonious trees 470 steps long. These trees had a consistency index (CI) of 0.91 and a retention index (RI) of 0.94. The 50% majority rule consensus phylogram from the BI analysis and the ML topology were entirely congruent with the MP strict consensus tree; they differed only in resolution and clade support values. The genus was therefore resolved into three well-supported lineages (Fig. 1.1): Desert (0.99 PP/58 MLBS/51 MPBS), Matorral (1.00 PP/95 MLBS and 96 MPBS), and Mountain (0.83 PP/60 MLBS/98 MPBS). Each of the three lineages had a different geographic distribution, with areas of sympatry in the centre (between clades Matorral and Mountain; Fig. 2). The Desert lineage is found in the Desert biome of southern Peru, western Bolivia, and northern Chile, from 15° to 30°S (Fig. 2). The Matorral lineage is distributed mostly in the sclerophyllous Matorral of central Chile but also in shrublands in the ecotone between the Patagonian Steppe and the Southern Temperate Forest, between 30° and 47°S (Fig. 2). The Mountain lineage is distributed at high elevations (>1,500 m a. s. l.) in the mountains of southern Chile and Argentina from 35.5° to 41.5°S (Fig. 2).

The analyses of the chloroplast *trnL-F* region did not resolve the same three lineages as was found with ITS. All topologies estimated with the different criteria (MP, ML, and BI) using the *trnL-F* region were congruent, resolving two main lineages. Following the previous nomenclature, the first one was named Desert–Matorral and the second Mountain (Fig. 1.2). The MP analysis of the *trnL-F* region recovered 49 equally parsimonious trees 279 steps long. These trees had a consistency index (CI) of 0.92 and a retention index (RI) of 0.96. The 50% majority rule consensus phylogram from the BI analysis and the ML topology were entirely congruent with the MP strict consensus tree; they differed only in resolution and clade support values. Therefore in the *trnL-F* analyses the genus was resolved into two well-supported lineages (Fig. 1.2): Desert–Matorral (1.00 PP/95 MLBS and 100 MPBS), and Mountain (0.99 PP/74 MLBS/94 MPBS). In contrast to the ITS phylogeny, the Desert and Matorral lineages were not resolved.

Table 1. Genetic distances (Kimura 2 parameter model: K2P) calculated for chloroplast and nuclear DNA regions (*trnL-trnF* region above the diagonal and ITS below the diagonal) between the three phylogenetic clades labelled: Desert, Matorral, and Mountain (Fig. 1).

	K2P distance		
ITS \trnL-F	*Matorral*	*Desert*	*Mountain*
Matorral	0	0.006	0.057
Desert	0.035	0	0.057
Mountain	0.064	0.072	0

Intra- and inter-lineage genetic divergence

The pairwise genetic distances (p-distances) computed within *Quinchamalium chilense* resulted in mean values of 0.031 for ITS and 0.025 for *trnL-F*. As a complementary analysis, the Kimura model (Kimura, 1980) was used to measure inter-lineage divergence (Table 1) and within-lineage divergence. Coincidentally with the phylogenetic and haplotype network outcomes, distances between the Desert and Matorral lineages are less than those between Mountain and Desert or Mountain and Matorral (Table 1). Hence Desert and Matorral taxa are genetically more similar to each other than either is to Mountain. It is also notable that between the lineages with no clear differentiation (i.e., Desert and Matorral), the distance calculated for the chloroplast marker (0.006) is nearly 10 times less than that obtained for ITS (0.035).

The ITS within-lineage divergences were 0.006, 0.005, and 0.002 for Desert, Matorral, and Mountain respectively. In the same way, the *trnL-F* within-lineage divergences were 0.003, 0.008, and 0.004 for Desert, Matorral, and Mountain respectively. Therefore, each within-lineage genetic divergence resulted in an order of magnitude lower than between lineages (Table 1) for both markers.

Haplotype networks

Haplotype networks are depicted on Fig. 3.1 based on nuclear ITS (37 ribotypes from 74 individuals) and Fig. 3.2 based on chloroplast *trnL-F* (32 haplotypes from 63 individuals). The difference between these two networks reflects the differences between the phylogenies inferred with each marker mainly owing to the incomplete resolution of the Desert and Matorral lineages. It is clear that ITS recovers a strong geographic pattern between the three lineages (Fig. 3.1), and that there exists sharing of chloroplast haplotypes between Matorral and Desert (Fig. 3.2). These networks also reflect the high number of mutations between the Mountain haplotypes and the other two; in fact this pattern is repeated for both markers.

Climatic and morphological characters compared with genetic lineages

Statistically significant climatic and morphological differences exist amongst the three lineages (Table 2). Comparisons of means of annual precipitation resulted in three different groups (Desert, Matorral, and Mountain, from lowest to highest precipitation; Table 2). However, annual mean temperature discriminated only two groups (Desert and Matorral-Mountain, from highest to lowest temperatures; Table 2). Comparisons of two of the three morphological variables (leaf shape and floral morph) resulted in two groups (Desert-Matorral and Mountain). Floral tube length showed differences only between Mountain and Desert;

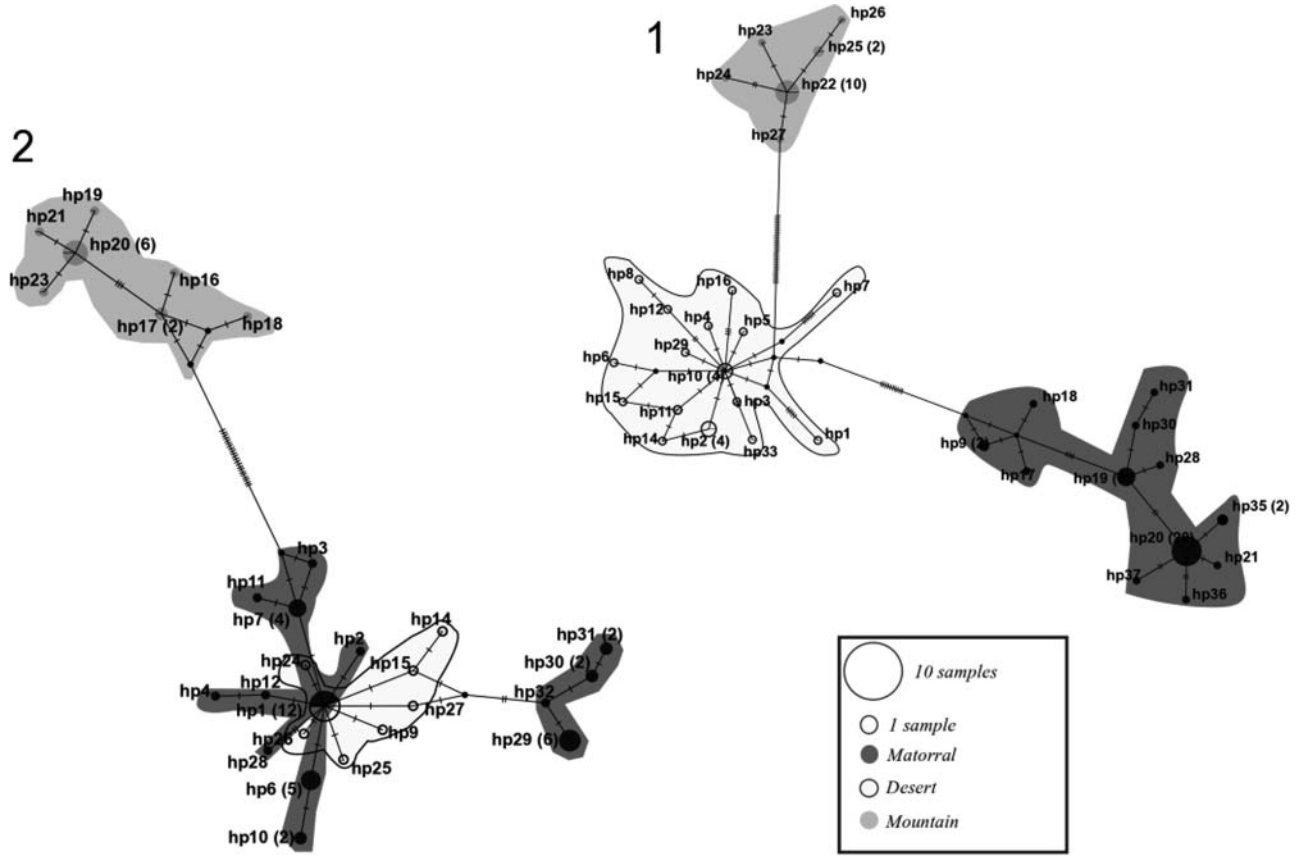

Fig. 3. 3.1. ITS Median Joining Network haplotype shows the relationship between the three phylogenetic lineages. 3.2. *trnL-F* Median Joining Network haplotype shows the relationship between the three phylogenetic lineages (Mountain, Desert, and Matorral).

Table 2. Kruskal–Wallis P-values comparing climatic and morphological characteristics between the three phylogenetic lineages (Desert, Matorral, and Mountain). Mean values, standard deviations, and range values (Min-Max between parentheses) are indicated for each variable. Superscript letter indicates homogeneous groups based on the non-parametric test of means comparisons. Significant differences indicated with *. FM values between 30–49 are considered *a priori* thrum flowers, and values higher than 50 are considered *a priori* pin flowers.

Var. description	Desert	Matorral	Mountain	P-value
Climatic				
Annual mean temperature (°C)	12.1±1.6[a] (2.4−20.2)	7.0±0.8[b] (-4.5-16.7)	7.1±1.1[b] (-2-13.5)	0.0082*
Annual mean precipitation (mm)	271±103[a] (6−1284)	805±46[b] (435−1008)	1245±114[c] (753−1117)	<0.0001*
Morphological				
Leaf shape (length/width)	18.6±7.8[a] (8.4−31.9)	22.5±11.2[a] (9−63)	13.8±6.5[b] (6.0−28.4)	<0.0001*
Total length of floral tube (mm)	0.88±0.1[a] (0.6−1.2)	1.0±0.3[ab] (0.5−1.8)	1.0±0.2[b] (0.7−1.4)	0.0168*
Floral morph (style length/floral tube length*100)	80.3±15.7[a] (50−111)	80.3±11.4[a] (55−103)	57.7±15.6[b] (42−92)	<0.0001*

Matorral did not differ from the other two lineages in floral tube length. The Mountain lineage is found in areas with relatively higher precipitation and it has somewhat broader leaves and lower values for the floral morph ratio (i.e., higher proportions of thrum compared with pin flowers).

Discussion

Phylogenetic analyses, lineage divergence, and haplotype networks

The phylogenetic tree and haplotype network from the nuclear ITS marker yielded three well-supported lineages (Desert, Matorral, and Mountain; Fig. 1.1). The mean pairwise genetic distances were smaller within the whole *Quinchamalium chilense* group than the distances amongst the three well-supported ITS lineages.

Amongst the molecular tools available, DNA barcoding is powerful for the identification and delimitation of cryptic species (e.g., Liu, Moller, Gao, Zhang, & Li, 2010). For plants, barcode species delimitation considers around 2–10% divergence between two lineages to represent separate species (Fazekas et al., 2009, Fig. 2b; Yao et al., 2010). As the highest frequency of interspecific divergence in plants falls between 0.005 and 0.03 (Fazekas et al., 2009, Fig. 2b), we consider that if two lineages have more than 3% divergence but no discontinuities in morphological multivariate space, then we can talk about cryptic species. There are controversies in using a 'molecular yardstick' to define species. However, for conservation effort it is also useful and desirable to have genetic divergence data in order to conserve genetic diversity within communities (Shapcott et al., 2015).

Within *Quinchamalium*, lineage-pair comparisons resulted in less genetic distances between the Desert and Matorral lineages than between them and the Mountain lineage (Table 1). For ITS marker the divergence of the Mountain lineage ranged from 6.4% to 7.2% while the divergence value between the Desert and Matorral lineages was almost half this: 3.5%. The *trnL-F* marker showed the same pattern but to a lesser degree: the divergence of the Mountain lineage from both Desert and Matorral was 5.7%, whereas the genetic distance between Desert and Matorral was one order of magnitude less: 0.6%. Furthermore, the haplotype phylogenetic tree of *trnL-F* did not distinguish a Desert lineage from a Matorral one (Fig. 1.2); however, in both phylogenies the Mountain lineage formed a well-supported clade. The within-lineage genetic distances were much lower than the between-lineage distances, indicating homogeneity of the three lineages, especially for the Mountain lineage for ITS.

Finally, the genetic structure is clearly associated with geography, as haplotypes of the Mountain lineage do not overlap geographically with those of the Matorral–Desert lineage in any of the gene networks. However, in the case of the chloroplast network, the geographic areas Matorral and Desert share *trnL-F* haplotypes, whereas this does not happen in the ITS network (Fig. 3).

Considering the amount of genetic divergence and the lack of discontinuities in morphological features along the geographic range of *Quinchamalium* (Lopez Laphitz et al., 2015a), we could hypothesize the existence of two or three cryptic species within the *Quinchamalium* genus, with the Mountain lineage being the most divergent. The *trnL-F* topology shows all of the Matorral individuals in the same clade with Desert individuals (Fig. 1.2), while the ITS topology shows them as reciprocally monophyletic. Under the hypothesis of cryptic species, the incongruence between these two gene phylogenies could be due to hybridization events between the Desert and Matorral lineages or to incomplete lineage sorting. The sharing of chloroplast haplotypes across species boundaries is a well-documented phenomenon (French, Brown, & Bayly, 2016; Hollingsworth, Graham, & Little, 2011; Hollingsworth, Li, van der Bank, & Twyford, 2016; Nevill, Després, Bayly, Bossinger, & Ades, 2014). Incomplete lineage sorting of ancestral polymorphisms (Ma et al., 2015; Michalski & Durka, 2015) is a process where reciprocal monophyly has not been reached (Olave, Sola, & Knowles, 2014). Although we cannot exclude any of these two processes, the ITS phylogeographic pattern is partial evidence that the chloroplast undifferentiated lineages (Desert and Matorral) could be incipiently divergent and potentially cryptic species. Also, if hybridization with gene flow is on-going, we would not expect to see a clear distinction between Desert and Matorral in the ITS data. On the other hand, identifying three instead of two cryptic species would result in a conflict between the chloroplast marker's phylogeny and current species-level taxonomy. However, this conflict and especially the sharing of chloroplast haplotypes across species boundaries has been documented several times before (e.g., Barrett et al., 2014; Hollingsworth et al., 2011, 2016; Nevill et al., 2014). More evidence such as assessing genome-wide genetic marker discovery and genotyping using next-generation sequencing (e.g., SNPs) will be necessary to accept the three ITS lineages as clearly different cryptic species.

Overall, integrating phylogenies, genetic distances and haplotype networks, we identified at least two cryptic species within *Quinchamalium*: Desert–Matorral and Mountain. Previous work attempting to delimit species based upon quantitative morphological analyses in *Quinchamalium* was not conclusive (Lopez Laphitz et al., 2015a) and for this reason only one morphospecies was identified and described (*Q. chilense* Molina; Lopez Laphitz, Ezcurra, &

Vidal-Russell, 2015b). Therefore, here we re-evaluated groups that are slightly differentiated morphologically and congruent with genetic lineages, using an integrative perspective to study cryptic diversity. In conclusion, the presence of at least two main genetic lineages, Mountain and Desert–Matorral, in *Quinchamalium* could be considered different cryptic species.

Climatic and morphological characters compared with genetic lineages

Including the geographic perspective in cryptic species delimitation is particularly important because *Quinchamalium* experiences a wide variety of environmental conditions in its extensive north-south distribution, from warm desert through matorral, to cold high-mountain environments surrounded by temperate forests. For this reason, showing DNA haplotypes with a strong phylogeographic pattern supports the differentiation of two cryptic species, one present mostly in the northern Chilean desert and in the Chilean matorral, that extends south to open areas in the east of the temperate forest of South America, and the other one inhabiting high elevation areas (mountain peaks) within the temperate forest. Although exhaustive morphological analyses were not successful in finding morphological discontinuities that correlated with separate species within *Quinchamalium* (Lopez Laphitz et al., 2015a), it is noteworthy that statistical analyses using as a factor the ITS lineages (with three levels: Desert, Matorral and Mountain) resulted in significant differences between Mountain and the other groups for precipitation, leaf shape, and floral morph. Although these differences in morphology are not clear-cut and can go almost unnoticed, they corroborate and/or complement the detection of two cryptic species. For instance, cryptic species Mountain, which inhabits an area with higher precipitation than Desert–Matorral, is characterized by relatively broader leaves and a distinctive floral morph (i.e. plants with thrum as well as pin flowers). Narrower leaves in the Desert–Matorral plants can be expected in its area with less precipitation (Ezcurra, Ruggiero, & Crisci, 1997; Lopez Laphitz et al., 2015a). Also, *Quinchamalium* has been reported to have floral dimorphism, with plants that present pin flowers and plants with thrum flowers (Lopez Laphitz et al., 2015a; Riveros, Arroyo, & Humaña, 1986). In this study, we observed that the plants with thrum flowers are present exclusively in the Mountain lineage. Therefore, we propose that the presence of only pin flowers in Desert–Matorral plants could be associated with selection by different pollinators. In heterostylous species, the absence of one morph in populations of a different geographic area seems to be a derived condition (Hodgins & Barrett, 2008; Perez-Barrales, Simon-Porcar, Santos-Gally, & Arroyo, 2014). Differences in pollinators could have been important in the establishment of genetic barriers between ancestral populations that have resulted in the evolution of the Mountain and Desert–Matorral lineages. But also, these lineages are currently separated by *Nothofagus* forests that generally isolate the open environments of the high-Andean Mountain populations from the lower Desert–Matorral populations.

Conclusions

In conclusion, we therefore propose the existence of two cryptic species within the *Quinchamalium chilense* genus, the Mountain and Matorral–Desert lineages that relate to climatic and morphological differences. The former inhabits southern elevated regions with higher precipitation and has relatively wider leaves and thrum flowers, whereas the latter is found in generally northern, more arid areas, and has narrower leaves and only pin flowers. Although it is relevant to consider these as cryptic species, due to it being important in conservation as they represent genetically divergent entities, we agree that at this moment they should not be given formal species names to avoid 'taxonomic inflation' (Isaac, Mallet, & Mace, 2004; Shepherd et al., 2015).

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Disclosure statement

No potential conflict of interest was reported by the authors.

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