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PHYLOGEOGRAPHY AND DIVERGENCE DATE ESTIMATES OF A LICHEN SPECIES COMPLEX WITH A DISJUNCT DISTRIBUTION PATTERN[1]

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Disjunct species distributions may result from a combination of geologic events and long-distance dispersal. The foliose lichen species complex *Leptogium furfuraceum-L. pseudofurfuraceum* has an intercontinental disjunction pattern. Populations of this species complex are found in western North America, southern South America, Africa, and southern Europe. We conducted a phylogenetic study to reconstruct the biogeographic history of this species complex using two ribosomal genes (ITS and LSU) and a protein-coding gene (partial *RPB2*). Results indicated that the complex comprises four geographically restricted genetic lineages. A sister relationship was found between populations from the same hemispheres, incongruent with previous data derived from morphological characteristics and geographical classification schemes. Incorporating Bayesian ancestral area reconstruction and Bayesian divergence time estimation, we proposed an evolutionary hypothesis for the species complex. The results suggested that processes of biotic expansion via transoceanic dispersal were responsible for the species divergence and distribution patterns observed today. This study also expands the view that cryptic speciation is not a rare phenomenon among fungi and lichens.

Key words: Collemataceae; cryptic species; disjunction; *Leptogium*; lichens; long-distance dispersal; phylogeography.

The principles and processes governing the geographical distribution of genealogical lineages can be understood through an integrative phylogeographical approach (Avise, 2000). Plants and animals have been well studied in a biogeographical context, but fungi and other cryptogams have been largely overlooked (Hibbett, 2001; Lumbsch et al., 2008), primarily due to limited fossil records and unknown dispersal mechanisms (Hibbett, 2001). In fungi, there has been much recent interest in studying the processes of speciation and biogeography in a phylogenetic context (Printzen and Ekman, 2002; Printzen et al., 2003; Kohn, 2005; Argüello et al., 2007; Buschbom, 2007; Beheregaray, 2008; Moncalvo and Buchanan, 2008; Wirtz et al., 2008). However, biogeographic knowledge of lichen-forming fungi is still very poor because conclusions are based on interpreting distribution maps and correlating distribution patterns with geologic history (Printzen et al., 2003).

Fungal species, including lichens, usually have wider distributions than vascular plants (Lücking, 2003). However, some species have well-defined disjunction patterns, where populations of the same species or closely related taxa present discontinuous distributions (Galloway, 1996; Shaw et al., 2003). Among a variety of disjunction types in lichens, the intercontinental patterns are the most frequent. Many lichen species are only present in western North America and southern Europe (WNA-E), such as *Lecidea holopolia* (Tuck.) Zahlbr., *Leptogium subaridum* P. M. Jørg. & Goward, *Kaernefeltia merrillii* (Du Rietz) A. Thell & Goward and *Waynea californica* Moberg. Another group of species show a South American–African disjunction pattern (SA-AF), for example, *Caloplaca elegantissima* (Nyl.) Zahlbr. and *C. isidiosa* (Vain.) Zahlbr. The intercontinental disjunction is not restricted to lichen species; it also occurs in mosses and genera of seed plants (Tiffney and Manchester, 2001; Printzen et al., 2003; Shaw et al., 2003). Several biogeographic and phylogeographic studies of vascular plants and mosses have pointed out different hypotheses to explain these patterns (Shaw et al., 2003; Hohmann et al., 2006; Milne, 2006; Peng and Wang, 2008). Explanations vary among different species, even within a single group of organisms, suggesting that a single explanation cannot even be applied to closely related organisms. Many studies have concluded that for some taxa, a vicariance model caused by geologic and climatic events during the Tertiary best explains disjunctions (Axelrod, 1975; Brown and Lomolino, 1998; Hileman et al., 2001; McLoughlin, 2001; Tiffney and Manchester, 2001; Milne, 2006). Alternatively, data from other studies have indicated distribution patterns are a result of recent long-distance dispersal (Crespo et al., 2002; Davis et al., 2002; Shaw et al., 2003; Muñoz et al., 2004, Buschbom, 2007).

Lichens can be dispersed either by sexual (microscopic individual spores of the mycobiont) or vegetative propagules (contain both symbionts) such as isidia or soredia. The vegetative propagules are better suited to the establishment of new individuals, but spores are more effective as long-distance propagules (Buschbom, 2007). Some lichen species with an intercontinental disjunction distribution pattern rarely produce sexual reproductive structures, and the vegetative propagules are their main means of dispersal. This fact might suggest that

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long-distance dispersal is not common and that historical fragmentation is the most likely explanation for their current distribution (Buschbom, 2007). However, the fact that morphological variability in disjunct lichen species is rare to absent suggests these taxa have a more recent origin (Liu et al., 2009). Some disjunct distributions in phylogenetically related fungal species has been explained as vicariance events (O'Donnell et al., 1998; Nilsson et al., 2003). Nevertheless, Moncalvo and Buchanan (2008) and Liu et al. (2009) used molecular substitution rates to estimate divergence times and reported that some taxa diverged far more recently than can be explained by continental separation. These results have provided new insights into dispersal capabilities and patterns of fungal gene flow, which were not available until the development of molecular markers given the limited availability of fungal and lichens fossil records. The reconstruction of ancestral area distribution and estimates of diversification dates has not yet been assessed in lichen species with an intercontinental disjunction pattern. Therefore, the historical events and processes that explain their current distribution remain elusive.

This paper focuses on the phylogenetic and phylogeographic study of the lichen species complex *Leptogium furfuraceum-L. pseudofurfuraceum* (Collemataceae, Lecanorales). On the basis of morphological differences (i.e., spore shape and size) and geographical distribution patterns, Jørgensen (1997) pointed out that *L. pseudofurfuraceum* P. M. Jørg. & A. K. Wallace is a species distinct from *L. furfuraceum* (Harm.) Sierk. *Leptogium furfuraceum* is found in southern Europe and eastern Africa (Swinscow and Krog, 1988; Aragón et al., 2005), whereas *L. pseudofurfuraceum* is limited to western North and Central America (Jørgensen, 1997). Years later when more specimens were studied, Aragón et al. (2005) and Jørgensen and Nash (2004) found that both species show a great morphological and anatomical similarity when sterile. This observation suggested that the species complex corresponds to a single species. However, molecular markers had not been used to evaluate the level of genetic differentiation within and among this species complex. The entire complex was demonstrated to be monophyletic in a family level phylogenetic study (Otálora et al., 2010).

Our main goals were as follows: (1) to distinguish whether the *Leptogium furfuraceum* complex corresponds to several monophyletic species or whether it is a single, widely distributed, monophyletic taxon; (2) to assess the level of molecular divergence and estimate the divergence time between disjunct populations; and (3) to reconstruct ancestral area distribution to elucidate the biogeographic history of the species complex. To reach these objectives, we investigated the phylogenetic relationships between individuals of both species, and we determined the population structure and genetic variability of the complex using two ribosomal sequences and one protein-coding gene: internal transcribe spacer (ITS), large subunit (LSU) and fragment of the gene coding for the second largest subunit of DNA-dependent RNA polymerase II (*RPB2*) spanning regions 5–7 (Liu et al., 1999). Each of these markers has been widely used to investigate variation in closely related species (Argüello et al., 2007; Buschbom, 2007; Otálora et al., 2008). We further employed a Bayesian procedure implementing a relaxed clock model for estimating divergence time throughout our species complex phylogeny with the software BEAST (Drummond and Rambaut, 2008) using molecular evolution rates obtained from the literature. This methodology allows simultaneous analyses of multiple data sets/partitions with different substitution models taking into account phylogenetic uncertainty. Finally, we used a Bayesian approach to elucidate the biogeographical history of these species by inferring the ancestral area distribution states with the software SIMMAP (Huelsenbeck et al., 2003) in which a geographical area is mapped onto a phylogeny and is optimized on nodes (Baker et al., 2006).

MATERIALS AND METHODS

Taxon sampling—*Leptogium furfuraceum* is an epiphytic species restricted to the Old World, where it occurs in some regions of southern Europe (Croatia, France, Portugal, and Spain) and Africa (Ethiopia, Kenya, and Tanzania). We included 14 specimens representing populations from Croatia, Ethiopia, Kenya, Portugal, and Spain. The distribution of *L. pseudofurfuraceum* was previously thought to be restricted to western North America. However, fertile specimens were recently collected in the province of Salta in Argentina, which extends the distribution of this species from North America to South America. We selected 10 specimens of *L. pseudofurfuraceum* from Arizona, New Mexico, Mexico, and Argentina. Table 1 provides the collection locality, voucher specimen locations, and GenBank accession numbers. *Leptogium saturninum*, *Collema undulatum* and *C. furfuraceum* were selected as outgroup taxa based on results from previous Collemataceae phylogeny (Otálora et al., 2010).

DNA sequencing—DNA isolation, PCR amplification of ITS and LSU sequences, PCR product purification, PCR sequencing reactions, and automated sequencing were performed according to Otálora et al. (2008) and Otálora et al. (2010). The *RPB2* fragment was amplified using primers fRPB2-5F and fRPB2-7R (Liu et al., 1999) following the methodology of Buschbom and Mueller (2006). The purified PCR products were sequenced using the same amplification primers, as well as RPB2-6F and RPB2-6R (Liu et al., 1999) for the *RPB2* fragment. The sequences were aligned manually using the program MacClade 4.01. (Maddison and Maddison, 2001). Nucleotide sequences for *RPB2* were translated to amino acid to facilitate the alignment. Ambiguously aligned regions in ITS were excluded from the alignment.

Phylogenetic analyses—Phylogenetic relationships were inferred using maximum parsimony (MP) and Bayesian Markov chain Monte Carlo (MCMC) analyses. Individual MP analysis were performed on ITS and LSU separately in the program PAUP* version 4.0b10 (Swofford, 2002). For the MP analysis, a heuristic search with 1000 random addition sequence replicates was run with tree-bisection-reconnection (TBR) branch swapping and MULTREES on. All character states were treated as unordered and equally weighted, and gaps were treated as a fifth character. To evaluate the relative robustness of the clades found in the most parsimonious trees, we performed bootstrap analyses (Felsenstein, 1985) with heuristic searches as described for MP analysis on 1000 bootstrap data sets. Two random addition sequences (RAS) per bootstrap replicate were specified based on the high resolving power of the original data when 1000 RAS were implemented.

The evolutionary models for Bayesian analyses were selected using the Akaike information criterion (AIC) as implemented in the program Modeltest 3.06 (Posada and Crandall, 1998). Two optimal models of nucleotide substitution were selected for ITS regions, one for ITS1 and ITS2 (GTR+G) and the other for 5.8S (GTR+I+G). The TrN model plus proportion of invariable sites (TrN+I) was used for LSU. For each codon position of the *RPB2* region, a model was selected (GTR+I, K81 and HKY+G for the first, second and third codon, respectively). Individual data sets were analyzed using the program MrBayes 3.0 (Ronquist and Huelsenbeck, 2003). Two runs with five millions generations starting from an initial random tree and employing four simultaneous chains were executed. A tree was saved every 100th generation. The first 5000 trees were discarded as "burn-in". Using PAUP* 4.0b10, the majority rule consensus trees were assembled with the remaining sampled trees and posterior probabilities calculated for each node. The phylogenetic trees were drawn using the program TreeViewPPC 1.5.3 (Page, 1998). The combinability of the single-locus data sets was assessed by visual inspection of individual bootstrap values (Mason-Gamer and Kellogg, 1996; Wiens, 1998). Clades supported by bootstrap values ≥70% were compared between individual data partitions. A conflict was considered significant when one data partition supported a monophyletic group with bootstrap values ≥70% and the other data partition supported the same group as nonmonophyletic with bootstrap values ≥70%. Because no significant conflicts were detected, we assumed that the three data

TABLE 1. List of taxa included in this study, with country of origin, voucher location and number (ASU= Arizona State University, MA = R.J.B. Madrid, OL = University of Oslo), and GenBank accessions for the three gene sequences.

Taxon (ID number)	Geographic origin	Voucher	GenBank accession		
			nrITS	nrLSU	*RPB2*
Collema furfuraceum	Spain, Madrid	MA-16260	GQ396263	EU982608	GQ396261
C. undulatum	Spain, Málaga	MA-16036	DQ466044	EU982595	GQ396262
Leptogium furfuraceum 1	Croatia, Potomje	MA-16284	EU982636	EU982659	—
L. furfuraceum 2	Portugal, Marvao	MA-16248	EU982639	EU982662	—
L. furfuraceum 3	Spain, Toledo	MA-16280	EU982634	EU982594	GQ396247
L. furfuraceum 4	Portugal, Guarda	MA-16285	EU982637	EU982660	GQ396255
L. furfuraceum 5	Portugal, Abrumosa-a-velha	MA-16286	EU982638	EU982661	GQ396254
L. furfuraceum 6	Spain, Albacete	MA-09431	EU982655	EU982678	—
L. furfuraceum 7	Spain, Madrid	MA-16282	EU982635	EU982658	—
L. furfuraceum 8	Spain, Jaén	MA-16283	EU982633	EU982657	GQ396246
L. furfuraceum 9	Spain, Albacete	MA-16281	EU982632	EU982656	GQ396248
L. furfuraceum 10	Kenya, Mt Kenia	OL-151589	EU982640	EU982663	GQ396257
L. furfuraceum 11	Ethiopia, Arusia province	OL-97639	EU982643	EU982666	—
L. furfuraceum 12	Kenya, Mt Kenia	OL-151590	EU982642	EU982665	GQ396259
L. furfuraceum 13	Ethiopia, Bale	OL-97627	EU982641	EU982664	GQ396258
L. furfuraceum 14	Ethiopia, Arusia province	OL-97635	EU982644	EU982667	—
L. pseudofurfuraceum 1	USA, Arizona	ASU-N38938	EU982649	EU982672	GQ396250
L. pseudofurfuraceum 2	Mexico, Baja California	ASU 515222	EU982654	EU982677	—
L. pseudofurfuraceum 3	USA, Arizona	ASU-515352	EU982653	EU982676	GQ396252
L. pseudofurfuraceum 4	USA, Arizona	ASU-505643	EU982652	EU982675	—
L. pseudofurfuraceum 5	USA, Arizona	ASU-515744	EU982651	EU982674	GQ396251
L. pseudofurfuraceum 6	USA, Nuevo Mexico	ASU-533859	EU982650	EU982673	—
L. pseudofurfuraceum 7	USA, Arizona	ASU- N43230	EU982648	EU982671	—
L. pseudofurfuraceum 8	Argentina, Salta	MA-16291	EU982645	EU982603	GQ396249
L. pseudofurfuraceum 9	Argentina, Salta	MA-16292	EU982646	EU982669	GQ396256
L. pseudofurfuraceum 10	Argentina, Salta	MA-16293	EU982647	EU982670	GQ396253
L. saturninum	Spain, Jaén	MA-16295	EU982679	EU982680	GQ396260
L. saturninum	France, Meres les vals	MA-16024	DQ466043	EU982610	—

sets were congruent and could be combined. Thus, additional MCMC analyses were conducted on the combined data set. The partitioned Bayesian analysis of the combined data set was conducted by applying the previously determined models to each data partition.

Molecular dating—Estimated ages of divergence were obtained using a Bayesian inference framework as implemented in BEAST v. 1.4.8 (Drummond et al., 2002; Drummond and Rambaut, 2008) following the approach described in Drummond et al. (2006) under a relaxed-clock model. The rate in each branch was drawn from either an exponential or lognormal distribution. This approach tests evolutionary hypotheses accounting for phylogenetic uncertainty with 95% confidence intervals for the parameter estimate based on the posterior probability distribution over the parameter state space. In the absence of fossil evidence in Lecanorales, the molecular evolution rates for ITS (2.52×10^{-9}) and LSU (6.50×10^{-10}) reported for a group of Ascomycetes (Takamatsu and Matsuda, 2004), were used to estimate the time to the most recent common ancestor (MRCA) for all clades resulting from the Bayesian phylogeny of the combined ITS and LSU data set. Because a rate of molecular evolution cannot be assigned to each partition, a weighted average was calculated. An independent model of nucleotide substitution was applied to each data set (ITS and LSU). The GTR model of nucleotide substitution, with a gamma distribution rate of variation among sites, and six rate categories was applied to the ITS partition, while a TrN model of nucleotide substitution with invariant sites was applied to the LSU partition. The Yule prior to simulate the process of speciation was employed. Two independent analyses were run for 200 million generations. Results from two runs (with the first 10 000 000 discarded as burn-in and parameter values sampled every 1000 generations) were combined and the effective sample size for parameter estimates and convergence were checked using the program Tracer v. 1.3 (Rambaut and Drummond, 2003).

Biogeographic analyses—The historical biogeography of the *L. furfuraceum* complex was estimated by assessing ancestral distribution areas (Baker et al., 2006) using the program SIMMAP v.1 beta 2.3 (Huelsenbeck et al., 2003; Bollback, 2006). SIMMAP implements a post-tree analysis for the stochastic mapping of characters (areas) to infer character evolution (Nielsen, 2002; Huelsenbeck et al., 2003). It summarizes the area maps by calculating posterior predictive *P* values from posterior probabilities (PP). The option "multiple mapping" was used over the last 18 000 trees resulting from Bayesian analysis. For each Bayesian tree sampled with SIMMAP during the ancestral area reconstruction, we carried out 1000 draws. Four states were used for the area distribution character: 0 = South America, 1 = Europe, 2 = Africa, 3 = North America.

RESULTS

Sequence variation and phylogenetic analyses—ITS and LSU sequences were obtained for all 28 samples, but only 17 sequences were obtained for *RPB2*. Difficulties in gene sequence alignment were not experienced because little sequence variation was detected. After the exclusion of ambiguous alignment regions, the final lengths of the ITS, LSU, and partial *RPB2* alignments were 499, 1092, and 1118 characters, respectively. All North American specimens had a 25-bp deletion at position 15 of the nrITS-1 region, which was treated as a unique event. A total of 138, 58, and 99 sites were parsimoniously informative for ITS, LSU, and *RPB2*.

The combined matrix (ITS+LSU+*RPB2*) analysis produced 99 equally most parsimonious trees (score = 792 steps, hit = 998 times) with a CI of 0.81 and RI of 0.91. The strict consensus tree was congruent with the Bayesian tree topology. Nine internodes had bootstrap support ≥70%, and eight had posterior probabilities ≥0.95 (Fig. 1). The *Leptogium furfuraceum* complex formed a well-supported clade, and within the complex, two well-supported groups were distinguishable. The first group was comprised of all the northern hemisphere specimens (*L. furfuraceum* from Europe and *L. pseudofurfuraceum* from North American), and the second group comprised all the southern hemisphere specimens (*L. furfuraceum* from Africa and *L.*

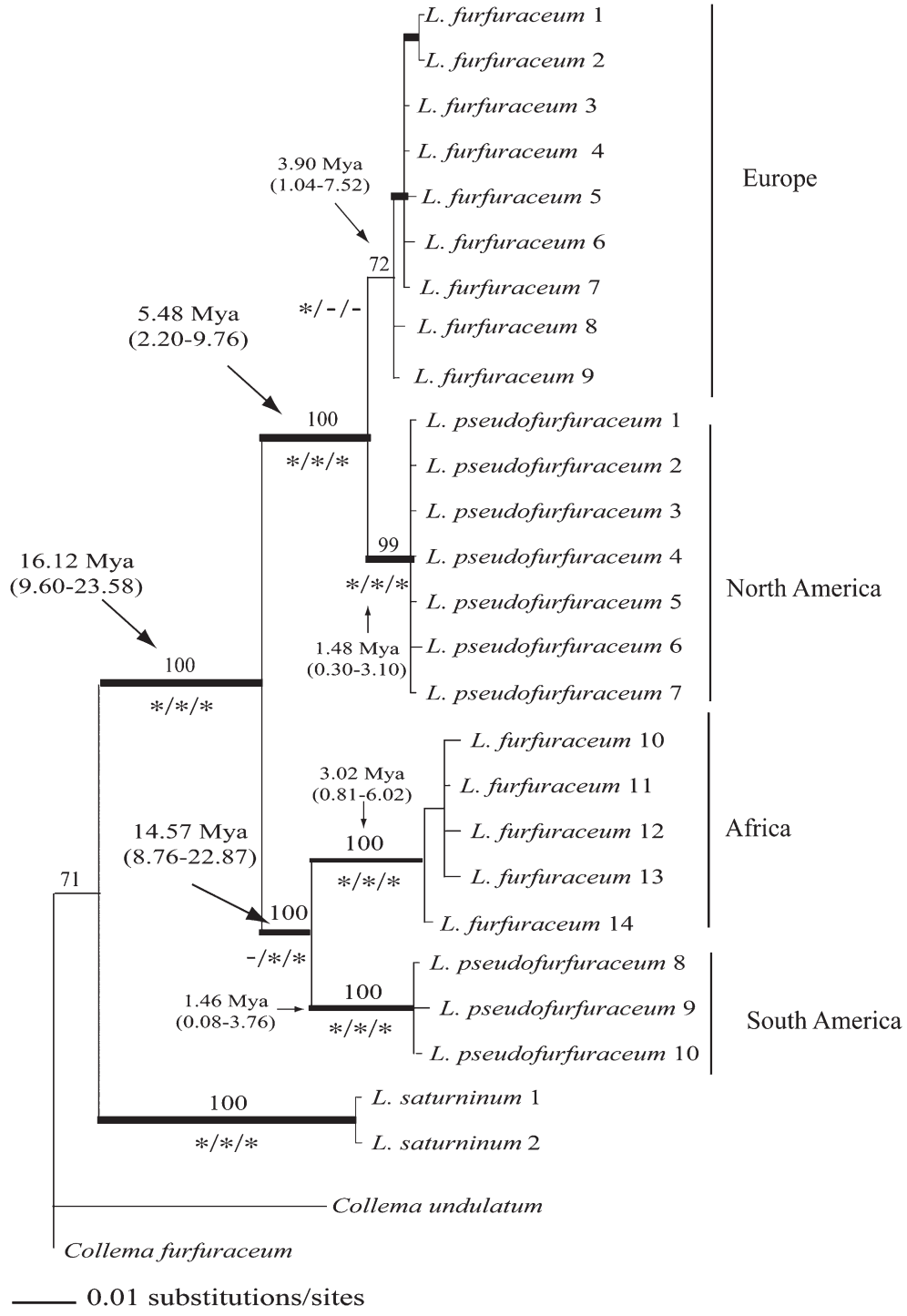

Fig. 1. The 50% majority rule consensus tree from Bayesian analysis based on ITS, LSU, and *RPB2* sequences of the 26 specimens in Table 1. Thickened branches represent significant posterior probabilities ≥0.95. Numbers above branches indicate bootstrap support of the combined analysis based on 1000 replicates. The symbols below branches indicate the bootstrap support of individual data sets in the following order: ITS/LSU/*RPB2*, an asterisk represents values up to 70%, and a dash represents values below 70%. Names correspond to current lichen species and numbers with the identification of specimens. Divergence times were estimated by the software BEAST v. 1.4.8 (Drummond and Rambaut, 2008) under an uncorrelated relaxed clock model. Numbers followed by an arrow are the mean posterior estimates of divergence time for each branch. Numbers in parentheses represent the 95% highest posterior density intervals for the divergence time estimates.

pseudofurfuraceum from South America). Two sister clades were evident within the first group; one was well supported by both posterior probability and bootstrap values that corresponded to *L. pseudofurfuraceum* from North America, and the second clade was comprised of *L. furfuraceum* from Europe only supported by bootstrap. Two well-supported sister clades

emerged from the southern hemisphere clade, one corresponding to African and the other to South American specimens. The resolving characteristics of ITS, LSU, and *RPB2* were complementary. The ITS data resolved relationships among northern hemisphere specimens, while LSU and *RPB2* both resolved the monophyly of the entire complex as well as the relationships among southern hemisphere lineages. The monophyly of European specimens was only supported by the nrITS and combined data sets (Fig. 1). In general, phylogeny reconstruction indicated *L. furfuraceum* and *L. pseudofurfuraceum* are not monophyletic species, and populations of *L. furfuraceum* from Europe are more closely related to *L. pseudofurfuraceum* from North America than to African *L. furfuraceum.* Furthermore, *L. pseudofurfuraceum* populations from South America are more closely related to African *L. furfuraceum* (Fig. 1).

Molecular dating—The methodology of Arnedo and Fernández (2007) was followed to compare the Bayes factors of trees obtained under the different molecular clock models (uncorrelated log normal distribution compared to the exponential distribution). The best-fitting model was the relaxed molecular clock using uncorrelated exponential distribution. Therefore, this model was used to estimate lineage divergence times. Bayesian age estimates for the each node within the *L. furfuraceum* complex are shown in Fig. 1. The split between the European and North American clades and between the African and South American lineages was estimated at 5.48 (2.20–9.76) and 14.57 (8.76–22.87) million years ago (Mya), respectively. The divergence between the northern hemisphere and southern hemisphere was estimated at 16.12 (9.60–23.58) Mya.

Biogeographic analyses—The hierarchical Bayesian reconstruction of the ancestral areas state is shown in Fig. 2. It indicates that the ancestor of the northern hemisphere lineages had European origins with a posterior probability of 0.90. In contrast, neither the ancestor of the southern hemisphere lineage nor ancestor of the entire complex was resolved. In the case of southern hemisphere lineage, both South America and Africa are equally probable ancestors. However, for the entire complex the ancestral region with the highest probability is Africa (Fig. 2).

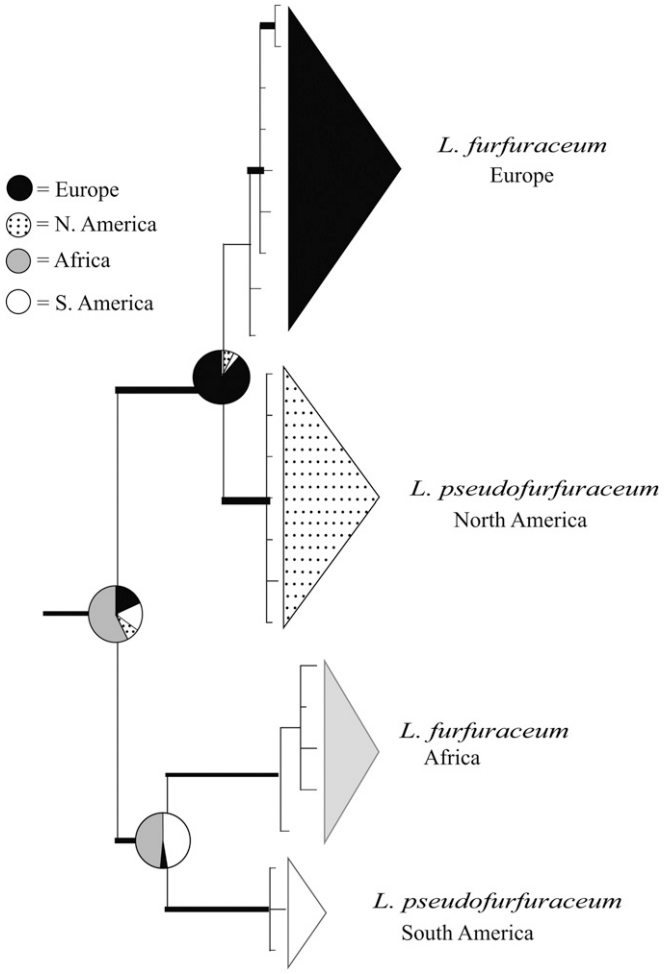

Fig. 2. Ancestral area reconstruction of *Leptogium furfuraceum* complex species using a Bayesian approach (SIMMAP). Reconstruction is shown on each corresponding node by large pie charts. On the left is the key to the character states for each taxon.

DISCUSSION

The phylogeny from combined maximum parsimony and Bayesian analyses of two ribosomal genes and a protein gene provided several insights into the evolution of a *Leptogium* species complex with a disjunctive distribution pattern. The *L. furfuraceum-L. pseudofurfuraceum* complex comprises four genetic lineages, which have a strict correlation to geographical distribution. The European populations of *L. furfuraceum* are phylogenetically more closely related to North American *L. pseudofurfuraceum* than to the African *L. furfuraceum*. Although these conclusions disagree with the initial morphological species delimitations (Jørgensen, 1997) based on spore size, they are not dissimilar to recent taxonomical observations regarding these species. Several authors have confirmed the high degree of similarity between North American and European taxa (Jørgensen and Nash, 2004; Aragón et al., 2005).

The African and South American populations represent two different lineages, which are distinct taxonomic units from *L. furfuraceum* s.s. (= Europe) and *L. pseudofurfuraceum* s.s. (= North America). Our results support the hypothesis that each of four entities could be considered as cryptic species. These lineages are geographically isolated from each other, which could explain the absence of gene flow within the complex (Grube and Kroken, 2000; Kroken and Taylor, 2001). However, because the populations are not sympatric, we cannot demonstrate a total genetic isolation, and these results could also be explained as population variations within a widely distributed species (Bickford et al., 2006). Nevertheless, we consider each of the monophyletic entities (Europe, Africa, North America, and South America) as a cryptic species based on the robustness of the clades and the number and features of the markers used. Many authors have pointed out that cryptic speciation seems to be a common phenomenon in fungi. In the last 10 years, it has been suggested that due to rapid fungal speciation and scanty morphological characters, many morphological species comprise multiple genetically isolated lineages (Kauserud et al., 2006; Liu et al., 2009). Our result is a new example in lichens where phylogenetic species recognition based on genetic variation could be also used to distinguish cryptic species within morphologically uniform lineages as it is now widely used in fungal lineages (Kauserud et al., 2006).

The well-supported sister relationship between the species of North America and Europe (Fig. 1) indicates that these two lineages share a common recent ancestor, which occupied Europe (Fig. 2). This result could be consistent with a Madrean-Tethyan hypothesis of the *L. furfuraceum* complex disjunction of the northern hemisphere, which has been suggested to explain the western North American and European Mediterranean disjunctions in several species (Axelrod, 1975; Jørgensen, 1983; Hileman et al., 2001; Tiffney and Manchester, 2001; Valiente-Banuet et al., 2006). However, the estimates of divergence dates for these lineages, using rates of molecular evolution suggests that the sister relationships of the European and North American populations are too young to be explained by this model (Fig. 1). The breakup of Laurasia took place approximately 100 Mya in the middle Cretaceous, and the estimated divergence date of these two lineages is 5.48 (2.20–9.76) Mya. Therefore, the most plausible explanation for this disjunction is rare episodes of transoceanic dispersal. The Bayesian ancestral reconstruction for area distribution shows Europe as the ancestral northern hemisphere range (Fig. 2), suggesting that the migration route was from Europe to North America.

Similarly, the South American lineages share a recent common ancestor with African lineages, which is in accordance with previous hypotheses of strong cryptogamic floristic affinities and close biogeographic relationships between southern South America and Africa (Lücking, 2003; Heinrichs et al., 2006). Divergence date estimates for these lineages support long-distance migration events as the most likely explanation for their current geographic distribution. Our results suggest these lineages diverged earlier than those of the northern hemisphere, approximately 15 Mya during the Late Tertiary when global cooling and drying transitions occurred. This period was suitable for dispersal of many organisms (Brown and Lomolino, 1998; Couvreur et al., 2008). The known populations of the *Leptogium furfuraceum* species complex from Africa and South America inhabit montane and upper montane, well-preserved forest ecosystems (i.e., 1800–3200 m a.s.l. in Tanzania, Kenya, Ethiopia, and northern Argentina). Although we sampled specimens from the documented populations of Africa and South America, we presume a large portion of the lichen flora of both continents remains unknown. Therefore, the estimated divergence date of the African and South American lineages must be viewed with caution; it may have been overestimated because of limited geographic sampling in the entire species range. In spite of this, our approach allowed us to select long-distance dispersal and to rule out the vicariance hypothesis as the most likely reason for current distributions. In fact, long-distance dispersal is congruent with the rare to absent morphological variation found among lineages in the *L. furfuraceum* complex.

The southern and northern hemisphere lineages are two independent, well-supported clades (Fig. 1), suggesting that, since the ancestor diverged, two independent dispersal ranges (southern and northern hemispheres) were available and the ancestor expanded into these two regions. The Bayesian reconstruction of ancestral area generated a 63% probability that the ancestor of the entire complex occupied African lands. According to divergence date estimates, the ancestral population of the complex split 16.12 (9.60–23.58) Mya. This date coincides with the division of African and South American lineages, possibly due to an overestimation of the southern hemisphere divergence date (as discussed earlier). The expansion from Africa to Europe occurred in the Tertiary when climatic oscillations produced favorable conditions for several large-scale organismal migrations (Brown and Lomolino, 1998).

Events of biotic expansion by long-distance dispersal have been reported in several spore-dispersed organisms. In lichens, recent genetic population and ecological studies have revealed that broad and disjunct distributions are the result of dispersal among suitable habitats in the past or even in the present (Crespo et al., 2002; Muñoz et al., 2004; Buschbom, 2007). Lichen propagules can be dispersed via global movements or high-altitude air masses (Muñoz et al., 2004). In particular, the ascospores (sexual propagules) are actively ejected from the asci and are able to survive harsh conditions of high altitudes (i.e., UV radiation and low temperatures) (Buschbom, 2007). Despite the fact that the European and African lineages do not produce sexual reproductive structures (in Europe only one specimen with apothecia has been reported; Aragón et al., 2005), the data indicate these taxa from Old World lineages have more genetic variability than New World lineages (Fig. 1). This fact could indicate that the Old World populations must have produced sexual propagules in the past, which were effectively established, but for unknown reasons might have recently experienced a reduced ability to produce sexual spores. The lost and reduced ability to produce sexual propagules is a phenomenon previously reported in other cryptogams (Frahm, 2008). Most of the genetic variation in fungal populations is promoted by sexual reproduction; however, high levels of genetic diversity have been found in sterile Ascomycetes species (Berbee et al., 2003; Geiser et al., 1998; Cassie and Piercey-Normore, 2008). The genetic variability reported for the European and African lineages may be explained by an historical gene flow throughout the populations, followed by recent reduction of sexual reproduction. The estimated date of divergence within European and African (Old World) populations is approximately 3.02 and 3.90 Mya, respectively, while within the New World, it is approximately 1.46 Mya for North America and 1.48 Mya for South America (Fig. 1). Neither New World nor Old World populations share a common ancestor (Fig. 1); however, they have similar reproductive structure characteristics suggesting that this feature is homoplasic among the complex. The fact that populations characterized by the production of sexual reproductive structures (North America and South America) have less genetic diversity, also could corroborate that spores are less successful than vegetative propagules for their establishment (Buschbom, 2007).

By incorporating Bayesian ancestral area reconstruction and Bayesian divergence date estimation, we proposed a hypothesis of phylogeography and divergence times for a foliose lichen species complex with a disjunct distribution pattern. Molecular dating techniques have the potential to estimate the time of origin of any biological lineage, while the ancestral areas reconstruction techniques propose the most possible historical biogeographic scenarios. The phylogenetic methods, including historical biogeography applied in this study have provided new insights into evolutionary patterns and processes of speciation in lichens, evidenced by the *Leptogium furfuraceum* species complex. The molecular data generated in this work contributes to the growing body of evidence that many lichen species are genetically complex, even under asexual reproductive strategies. In addition, the *Leptogium furfuraceum* species complex lends further support to the longstanding hypothesis that isolated events of long-distance dispersal have occurred repeatedly in the past, giving rise to the origin of new species and their subsequent migration (Crespo et al., 2002; Muñoz et al.,

2004; Moncalvo and Buchanan, 2008). This study also expands the view that cryptic speciation is not a rare phenomenon among fungi (Kroken and Taylor, 2001; Molina et al., 2004; Matute et al., 2006) and suggests that the absence of morphologic variability in disjunct populations should be evaluated in light of cryptic speciation.

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