Received: 11 June 2020 | Revised: 13 October 2020 | Accepted: 18 October 2020

DOI: 10.1111/jbi.14033

RESEARCH ARTICLE

Journal of Biogeography | WILEY

Phylogenetic diversity of two geographically overlapping lichens: isolation by distance, environment, or fragmentation?

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Abstract

Aim: Phylogenetic diversification is a precursor to speciation, but the underlying patterns and processes are not well-studied in lichens. Here we investigate what factors drive diversification in two tropical, morphologically similar macrolichens that occupy a similar range but differ in altitudinal and habitat preferences, testing for isolation by distance (IBD), environment (IBE), and fragmentation (IBF).

Location: Neotropics, Hawaii, Macaronesia.

Taxon: *Sticta andina*, *S. scabrosa* (Peltigeraceae).

Methods: We analysed 395 specimens from 135 localities, using the fungal ITS barcoding marker to assess phylogenetic diversification, through maximum likelihood tree reconstruction, TCS haplotype networks, and Tajima's D. Mantel tests were employed to detect structure in genetic vs. geographic, environmental, and fragmentation distances. Habitat preferences were quantitatively assessed by statistical analysis of locality-based BIOclim variables.

Results: *Sticta andina* exhibited high phenotypic variation and reticulate phylogenetic diversity across its range, whereas the phenotypically uniform *S. scabrosa* contained two main haplotypes, one unique to Hawaii. *Sticta andina* is restricted to well-preserved andine forests and paramos, naturally fragmented habitats due to disruptive topology, whereas *S. scabrosa* thrives in lowland to lower montane zones in exposed or disturbed microsites, representing a continuous habitat. *Sticta scabrosa* showed IBD only across its full range (separating the Hawaiian population) but not within continental Central and South America, there exhibiting a negative Tajima's D. *Sticta andina* did not exhibit IBD but IBE at continental level and IBF in the northern Andes.

Main conclusions: Autecology, particularly preference for either low or high altitudes, indirectly drives phylogenetic diversification. Low diversification in the low altitude species, *S. scabrosa*, can be attributed to rapid expansion and effective gene flow

Funding information
Bundesministerium für Bildung und Forschung, Grant/Award Number: FK 01DN17006; Center for International Cooperation of the Freie Universität Berlin, Grant/Award Number: FM Ex3-2017-013; Division of Environmental Biology, Grant/Award Number: 1354884

Handiling Editor: Alain Vanderpoorten

across a more or less continuous niche due to disturbance tolerance. In contract, high diversification in the high altitude species, *S. andina*, can be explained by niche differentiation (IBE) and fragmentation (IBF) caused by the Andean uplift.

KEYWORDS
Brazil, drift, Galapagos, Mexico, Puerto Rico

1 | INTRODUCTION

This past year, the world celebrated the 250th birthday of Alexander von Humboldt [1769–1859], whose work laid the foundation for the field of biogeography. Humboldt's travel to South America (Venezuela, Colombia and Ecuador), with Aimé Bonpland [1773–1858], between 1799 and 1803, and particularly the exploration of the northern Andes, resulted in a pioneering model of altitudinal zonation in the tropics in the *Essai sur la Géographie des Plantes* (von Humboldt & Bonpland, 1805). Lichens played an integral role in this concept: '*These stony and icy tops that the eye distinguishes barely above the clouds, are covered only by mosses and lichenous plants*'. (von Humboldt & Bonpland, 1805:14). The authors also recognized lichen communities as one of their 15 plant physiognomies in the tropics (von Humboldt & Bonpland, 1805:31). During their exploration of the Colombian and Ecuadorian Andes, Humboldt and Bonpland must have come across the two most common species of the genus *Sticta* (Peltigeraceae), although their actual names, *S. andina* and *S. scabrosa*, were only recently established (Moncada et al., 2020). As we demonstrate in this paper, these two taxa exemplify the critical role of autecology in driving phylogenetic diversity, specifically the connection between altitudinal zonation and biogeography, first established by Humboldt. They thus represent an excellent case study to explore links between geo- and biodiversity in lichen fungi.

Phylogenetic diversification and speciation are often related to key innovations that allow a lineage to conquer a new area or niche space (Heard & Hauser, 1995; Schluter, 2000; Hagen & Kadereit 2003; Coyne & Orr, 2004; Kraichak et al., 2015). However, while a key innovation may explain the success of a lineage in terms of abundance and occupied range, it does not directly cause diversification or radiation into separate lineages. The latter also requires mechanisms of phylogenetic diversification, population fragmentation and reproductive isolation (Kisel & Barraclough, 2010).

Populations are generally expected to follow the classic model of isolation by distance (IBD; Wright, 1943), comparable to the concept of regional equilibrium (Hutchinson & Templeton, 1999). In this model, gene flow is assumed to be limited by distance and hence geographically more distant individuals are expected to be also genetically more different. In contrast, isolation by environment (IBE) denotes a correlation between genotypes and environmental parameters, with the consequence of gene flow being limited between ecological niches, even in close proximity. IBD appears to be more frequent in plants and IBE more common in animals (Sexton et al., 2014: figure 1). Reasons include indirect versus direct fertilization in plants versus animals and largely stochastic dispersal in plants compared to active movement and migration in animals. The survey by Sexton et al. (2014) did not consider fungi (including lichens), because of the few studies available and because mechanisms potentially facilitating IBE are demonstrably rare in fungi. The detection of isolation patterns in fungi is also challenging because the largely stochastic dispersal of typically small diaspores, including vegetative propagules in lichens (Tripp et al., 2016), causes a considerably amount of noise in population structure (Buschbom, 2007). Since fungi behave more like plants in terms of reproduction and usually lack specific fertilization mechanisms such as vectorized pollinization, one would assume IBD to be the predominant mechanism driving phylogenetic diversification in these organisms. Indeed, the few available studies suggest that fungi mostly diversify through IBD (Allen et al., 2018; Branco et al., 2015; Liti et al., 2009).

While there are various ways for populations to become effectively isolated, either geographically or ecologically (Coyne & Orr, 2004; Losos & Ricklefs, 2009; Ryan et al., 2007; Wiens & Graham, 2005), geographic isolation is generally based on plate tectonics: fragmentation of land masses subsequently separated by water bodies, creation of oceanic islands through volcanic hot spots, and the uplift of mountain ranges along collision zones that generate a topographically disrupted terrain (Mila et al., 2010). Mountain uplift will act selectively upon lineages in dependence of their autecology, particularly their altitudinal preferences, leading to a third mechanism of isolation besides IBD and IBE, namely isolation by fragmentation (IBF; Hutchinson & Templeton, 1999). Lineages preferring high altitudes and occurring in disrupted terrain possibly diversify through IBF, whereas lineages occupying lowland regions with plane to undulate terrain likely diversify through IBD (Sexton et al., 2014). Also, lineages adapted to more illuminated conditions and tolerating disturbances may be less susceptible to IBF than those restricted to well-preserved, closed vegetation. Thus, autecology will variously affect IBF and IBD and lead to different population structures, even if other factors such as lineage age and geographic range are comparable (Cannon et al., 2009; Malhi et al., 2013; Morley, 2011; Shimizu-Kimura et al., 2017).

IBF has been much less frequently studied than IBD and IBE (Sexton et al., 2014), and mostly in a context of anthropic land use changes and conservation (Ghazoul & McLeish, 2001; Saeki et al., 2018). Mountain ranges provide an environment highly susceptible to IBF, and effects of within-lineage phylogenetic diversification and speciation at high altitudes have been shown for a broad range of organisms, mostly plants and birds (Boucher et al., 2016;

Givnish, 2010; Givnish et al., 2014, 2015; Hughes & Atkinson, 2015; Küper et al., 2004; Losos & Ricklefs, 2009; Madriñan et al., 2013; Ryan et al., 2007). Demonstrating effects of IBF in mountain ranges is challenging, as first a spatial model for patterns of fragmentation has to be established.

Here we use the two aforementioned species in the genus *Sticta*, *S. andina* and *S. scabrosa*, to test for evidence of IBD, IBE and IBF, in correlation with altitudinal zonation and habitat preferences. These lichens largely disperse by vegetative propagules and, although the symbiotic nature of such propagules leads to larger distribution ranges due to quick establishment and subsequent re-propagation (Tripp et al., 2016), the relatively large size of the propagules causes dispersal limitations, making these taxa excellent study objects to analyse scale-dependent phylogenetic diversification patterns. Both species were previously identified with the broadly defined name *S. weigelii* (Galloway, 1994, 1998a,b, 2007), but are only distantly related to each other and not closely related to *S. weigelii* s. str. (Moncada et al., 2020). The two species have largely congruent geographic ranges, being broadly distributed in the Neotropics and Hawaii, and *S. andina* is also known from the Azores; both are comparatively abundant lichens. Their main differences are found in their autecology: *S. andina* is a tropical montane species found in well-preserved, upper montane cloud forest and paramo (usually above 2,500 m), whereas *S. scabrosa* occurs in tropical lowland to lower montane forest (usually below 1,500 m), often extending into open and anthropic vegetation, locally with a 'weedy' character. We therefore hypothesized that *S. scabrosa* follows IBD, whereas *S. andina* exhibits IBF. Both taxa are well-sampled at global level, representing the two largest species-level clades in the currently available global *Sticta* ITS phylogeny, with 164 and 180 OTUs, respectively, and therefore constitute an excellent model to approach this question.

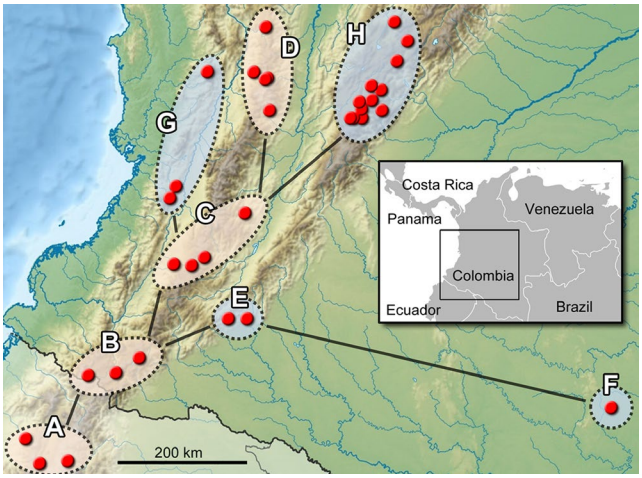

FIGURE 1 Model of population migration and fragmentation developed for *Sticta andina* in the northern Andes, based on the reconstruction of the final Andean uplift (Hoorn et al. 2010). Clusters were defined based on sample proximity and possible migration routes between clusters are indicated by connecting lines

2 | MATERIAL AND METHODS

2.1 | ITS barcoding

In this study, we focus on the fungal ITS barcoding marker (Schoch et al., 2012), which is highly effective in delimiting species in the genus *Sticta* and produces phylogenies congruent with other ribosomal and protein markers (Magain & Sérusiaux, 2015; Simon et al., 2018; Widhelm et al. 2018). Congruence with other markers and with phenotype characters of the ITS-based lineages also preclude the possibility of ITS paralogy; in general, reported cases of presumed ITS paralogy in fungi mostly do not reflect gene duplication but hybridization (Lücking & Hawksworth, 2018). The exclusive use of ITS sequences allowed to place the samples in a broad context including numerous species (Moncada et al., 2014). The *S. andina* complex initially comprised 20 OTUs, all sampled in Colombia, believed to comprise three species informally labelled 'andina', 'colombiana' and 'paramuna' (Moncada, Lücking, et al., 2014); for this study, 144 ITS sequences were newly generated, resulting in a total of 164 OTUs from Mexico, Costa Rica, Colombia, Ecuador, Brazil, the Azores and Hawaii. *Sticta scabrosa* was initially based on 10 OTUs from Colombia and the Dominican Republic (Moncada, Lücking, et al., 2014), while the current set included 165 new accessions, for a total of 175 OTUs from Mexico, Costa Rica, the Dominican Republic, Puerto Rico, Colombia, Brazil, Argentina, Galapagos and Hawaii (Appendix S1). DNA extraction, sequencing and sequence assembly was performed at the Field Museum, the University of Liège and the Botanischer Garten und Botanisches Museum, Freie Universität Berlin, with methodological details described elsewhere (Lücking et al., 2017; Magain & Sérusiaux, 2015; Moncada, Lücking, et al., 2014; Simon et al., 2018).

2.2 | Phylogenetic analysis

To delimit the two species and to verify their position in the global *Sticta* phylogeny based on previous analyses (Moncada et al., 2014; Widhelm et al. 2018), the sequences of *S. andina* and *S. scabrosa* were aligned with 338 ITS sequences of *Sticta* from GenBank, using *Lobaria pulmonaria* as outgroup (Appendix S2). The alignment was assembled in BIOEDIT 7.2.5 (Hall, 1999) and sequences were in alignment with MAFFT 7.244 (Katoh et al., 2002, 2009) using the [-- auto] option. The final alignment included 677 ingroup OTUs and was 626 bases long. Phylogenetic analysis was performed using maximum likelihood in RAxML 8.2.8 on the CIPRES Science Gateway (Miller et al., 2010; Stamatakis, 2015; Stamatakis et al., 2008), applying the GTR-Gamma model and 1,000 bootstrap replicates. The resulting tree was visualized in FIGTREE 1.4.2 (Drummond & Rambaut, 2007) and was used to delimit the two lineages. Subsequently, two subtrees were generated for each lineage, with the most closely related species as outgroup: for *S. andina*, we used 164 ingroup and six outgroup sequences (*S. phyllidiata*, *S. squamifera*), with a total alignment length of 543 sites (Appendix S3), whereas for *S. scabrosa*, we used

175 ingroup and six outgroup sequences (*S. pseudobeauvoisii*), with a total alignment length of 550 sites (Appendix S4).

2.3 | ITS haplotype network and expansion analysis

ITS haplotype networks were elaborated for both *S. andina* and *S. scabrosa* using POPART 1.7 (Leigh & Bryant, 2015) on the separate species alignments (Appendix S3, S4) but excluding the outgroup sequences; we thereby employed the Templeton-Crandall-Sing method or TCS (Clement et al., 2002) and coded the main geographic origins of the specimens, and in the case of *S. andina* for Colombia also the departments.

To test for haplotype structure in terms of a potential recent expansion (selective sweep), we employed Tajima's D on subsets of the ITS alignments for both species with only specimens from continental Central and South America included. The test was performed using DnaSP 6 (Rozas et al., 2017). To evaluate the effect of terminal and internal gaps, we analysed both the original (raw alignments) and subalignments where sites with internal gaps (indels) were removed, sequences with long terminal gaps were removed and the remaining alignments were trimmed or short remaining terminal gaps in constant sites were filled with the corresponding bases. This resulted in two sets of alignments: *andina* continental original (158 terminals, 543 sites), *andina* continental edited (147 terminals, 511 sites), *scabrosa* continental original (78 terminals, 548 sites) and *scabrosa* continental edited (62 terminals, 503 sites).

2.4 | Environmental data and phenotype analysis

The 339 OTUs representing *Sticta andina* and *S. scabrosa* were georeferenced and all samples were coded for selected phenotype features: presence or absence of apothecia, isidia and phyllidia in *S. andina*, and lobe surface configuration in *S. scabrosa* (for detailed explanations and illustrations of these features, see Moncada et al., 2020 and figures displayed in the results section below). Morphological characters of the specimens of *S. andina* and *S. scabrosa* were assessed at the Universidad Distrital Francisco José de Caldas (Bogotá), the Field Museum (Chicago), the Université de Liège and the Botanischer Garten und Botanisches Museum, Freie Universität Berlin, using standard microscopical techniques described in Moncada (2012) and Ranft et al. (2018). For all specimens, we also recorded altitude (Appendix S5). Using the georeference data, we assigned values for 19 climate variables to 337 specimens, using the bioclimatic variables BIOclim1 to BIOclim19 and the altitude layer (Appendix S6) from Worldclim in 2.5 arc minutes (http://www.worldclim.org; Fick & Hijmans, 2017; Hijmans et al., 2005), as well as the Landsat tree cover layer (http://glcf.umd.edu/data/landsatTreecover). Two specimens had to be excluded: for one (from Colombia), no precise georeference data could be obtained, and the other (from the Azores) did not have a close enough grid match among the Worldclim grid cells. In the case of Hawaii, we also analysed 56 additional, older collections housed at HAW for altitudinal and ecological differences. Differences in altitude, climate data and tree cover between the two taxa were statistically compared using the nonparametric Mann–Whitney U and the more sensitive Kolmogorov–Smirnov tests in STATISTICA™ 6.0.

2.5 | Isolation by distance, environment and fragmentation

In order to test for isolation by distance (IBD), environment (IBE) and/or fragmentation (IBF), we elaborated distance matrices for each of the parameters for both species under different scenarios, assessing the data globally, on a continental scale (to filter effects of rare long-distance dispersal e.g. to island biota), and for the northern Andeas, focusing on a region with disruptive topology. Based on georeferenced data for all samples, pairwise geographic distances for samples within each species were calculated using Batch Distance Computations (Morse, 2011). Pairwise genetic distances based on the ITS barcoding marker were computed from the above species alignments using Kimura's two-parameter distance computed in DNADist 3.69 (Felsenstein, 1993, 2008). Correlations between pairwise geographic and genetic distances were tested for each species using a simple Mantel test as employed in zt (Bonnet & Van de Peer, 2002), with 10,000 permutations. The tests were employed both at global level (all known samples) and for continental samples in Central and South America only.

In order to evaluate isolation by environment, we used the complete set of environmental variables (Appendix S6) for the subset of continental (Central and South America) samples of *Sticta andina* and *S. scabrosa*. For each species, we performed principal component analysis (PCA) on the environmental variables and extracted the Euclidean distance matrices from the PCA as proxy for environmental distances between samples. We employed a partial Mantel test to the matrices, taking into account geographic distances as third matrix. The utility of the Mantel test, and particularly the partial Mantel test, for matrix correlations has been challenged (Guillot & Rousset, 2013), but remains the method of choice in lieu of alternatives. While the Mantel test theoretically overcomes the issue of interdependency in matrix correlations, it continues to artificially inflate the degrees of freedom relating to statistical power, resulting in weak correlations receiving significant p values and so potentially overestimating the influence of underlying parameters. This applies in particular to the partial Mantel test, which aims at filtering spatial autocorrelation. Recently, Crabot et al. (2019) developed a spatially constrained permutation procedure to replace the partial Mantel test. However, this promising approach can currently only be applied to site data (e.g. Cañedo-Argüelles et al., 2020, in which each site is unique and therefore the spatial vector contains no duplicates. For specimen data in which more than one specimen share the same site, this method is currently not applicable.

To assess a potential effect of isolation by fragmentation in *Sticta andina* in the northern Andes, we developed a migration model for that species following reconstruction of the final uplift

of the northern Andes (Hoorn et al., 2010). In Colombia, the Andes divide into three mountain ranges (Cordillera Occidental, Central and Oriental), which allows to hypothesize migration of altoandine species from south to north and subsequent fragmentation of populations along each of these three ranges. We consequently arranged the Ecuadorian and Colombian samples of the species into eight spatial clusters (A–H), allowing for dispersal parallel to each mountain range. 'Fragmentation distances' between each cluster were then calculated as the total of dispersal lines connection two clusters (Figure 1), resulting in distance scores between 0 (samples within the same cluster) and 4 (between clusters F and D, G or H). In this case, a simple Mantel test was applied to the distance matrices.

All distance matrices are available in a zipped file (Appendix S7).

3 | RESULTS

3.1 | Phylogeny and phenotype

Sticta andina and *S. scabrosa* formed unrelated clades in the global *Sticta* ITS phylogeny (Figure 2; Appendix S8). Both in the global and in the separate analysis, *Sticta andina* consisted of two small and three large, unsupported subclades (bootstrap support values below 70), with slight differences in terminal positions, whereas the internal topology of *S. scabrosa* showed five mostly unsupported subclades, two of which were essentially comb-shaped (Figure 3; Appendix S8–S10). Due to lack of terminal resolution in the global ITS data set (due to the higher frequency of indels), the species most closely related to *S. scabrosa*, *S. laselvae* and *S. pseudobeauvoisii*, appear nested within *S. scabrosa* in the global tree, but in smaller data sets and multilocus analyses form a sister clade (Moncada, Reidy, et al., 2014; Widhelm et al. 2018).

Sticta andina and *S. scabrosa* can be considered geographically congruent, as they exhibited rather similar distribution ranges, predominantly across the Neotropics and Hawaii, with *S. scabrosa* also including the Caribbean (Dominican Republic and Puerto Rico) and the Galapagos Islands, whereas *S. andina* was further present in the Azores (Figure 4). *Sticta andina* included both apotheciate and isidiate to phyllidiate specimens, as well as sun and shade forms with higher or lower concentration of brown melanine pigments (Figures 3a, 5a-f). *Sticta scabrosa* was uniformly phyllidiate but, besides most specimens having an uneven lobe surface, featured two additional, unique surface morphodemes in the Hawaiian subspecies, either faveolate-pitted or papillose (Figures 3b, 5g-l).

The internal topology of *Sticta andina* lacked distinct correlations between clades and morphodemes or geography, but exhibited some partial structure (Figure 3a, Appendix S9). Thus, clades I, IV and V were predominantly phyllidiate, with some apotheciate morphs nested within, whereas clades II and III mostly included isidiate specimens, with some apotheciate and phyllidiate specimens intermingled. Some small subclades with peculiar propagule types (verruciform or dark isidia or small squamiform phyllidia) were found in clades II, III

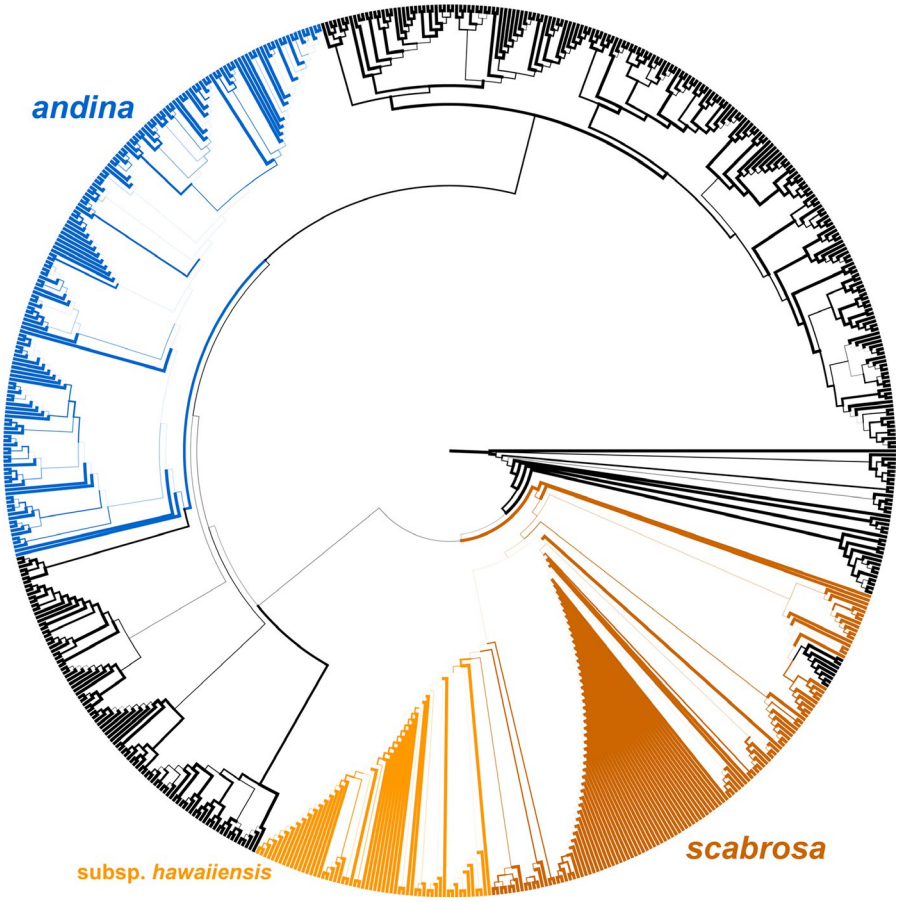

FIGURE 2 Circle cladogram of the genus *Sticta* based on the ITS fungal barcoding locus, showing the position of the target clades (*S. andina*, *S. scabrosa*) in the global phylogeny of the genus. Branch thickness is proportional to bootstrap support (all thickened branches have a bootstrap support of 70% or higher). See Appendix S8 for fully labeled phylogram

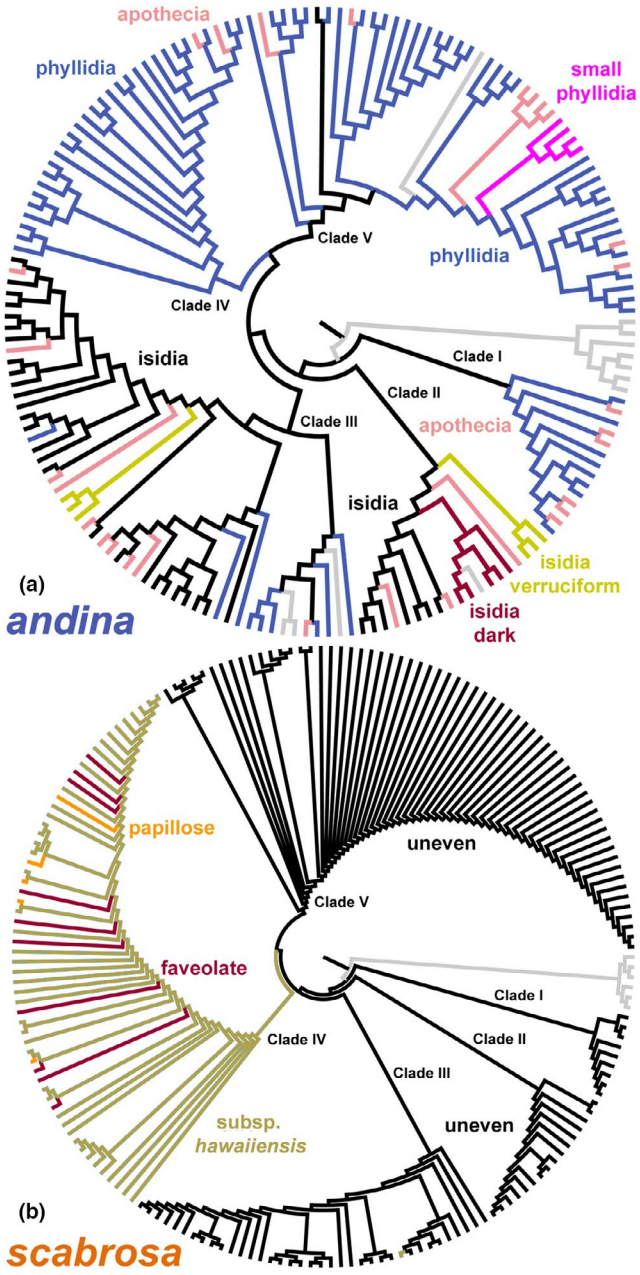

FIGURE 3 Individual circle cladograms of *Sticta andina* (A) and *S. scabrosa* (B) based on the ITS fungal barcoding locus, depicting reproductive morphodemes in *S. andina* and main distribution and lobe surface morphodemes in *S. scabrosa*. See Appendices S9 and S10 for fully labeled phylograms

and V. In *S. scabrosa*, subspecies *hawaiiensis* formed a terminal clade (clade IV), but within that clade, the three surface morphodemes were not phylogenetically clustered (Figure 3b, Appendix S10).

3.2 | ITS haplotype network and expansion analysis

The ITS-based TCS haplotype networks for the two species showed substantial differences. *Sticta andina* exhibited a highly reticulate network with numerous interconnected haplotypes; while some haplotypes were more frequent than others, and no haplotype was particularly dominant (Figure 6; see also Appendix S3). There was no immediately obvious correlation between haplotypes and distribution ranges, although some patterns were discernible. Thus, specimens from the northern Andes in Colombia (Cundinamarca to Risaralda) represented 21 haplotypes, whereas all specimens from Hawaii and the Azores belonged to a single haplotype identical to one of the northern Andes haplotypes. Specimens from the eastern flanks of the Andes in southern Amazonian Colombia (Caqueta, Putumayo, Vaupes) formed three unique haplotypes, whereas specimens from Mexico and Ecuador corresponded to several different haplotypes clustered in different portions of the network, shared with or related to northern Andean haplotypes. Specimens from Brazil were also homogeneous and shared a haplotype with the northern Andes.

In contrast, *Sticta scabrosa* formed a rudimentary network with two dominant and several minor haplotypes (Figure 6; see also Appendix S4). One dominant haplotype and its only satellite was exclusive to Hawaii, whereas all specimens from the Neotropics corresponded to the other main haplotype and its various satellites. Almost all haplotypes were present in Colombia, and also Puerto Rico and the region around southern Brazil and northern Argentina exhibited more than one haplotype. Specimens from Costa Rica and the Galapagos Islands were genetically uniform and corresponded to the predominant Colombian–Puerto Rican–Brazilian haplotype.

Tajima's D resulted negative for both *Sticta andina* and *S. scabrosa*, both for the original data and alignments edited for gaps. However, the test was not significant for *S. andina* in either case (original: Tajima's D = −1.23581, $p > 0.10$; edited: Tajima's D = −1.31471, $p > 0.10$), whereas it was marginally significant for *S. scabrosa* for the original data (Tajima's D = −1.68416, $0.10 > p > 0.05$) and significant for the edited data (Tajima's D = −1.91481, $p < 0.05$), suggesting some evidence of a recent expansion in that species, depending on how the data are treated.

3.3 | Ecological differentiation

Occurrences of *Sticta andina* and *S. scabrosa* differed significantly in 13 bioclimatic variables for both statistical tests, including all temperature variables, and an additional five precipitation variables for the more sensitive Kolmogorov–Smirnov test (Table 1). Mean annual temperature in the grids with occurrence of *S. andina* was five degrees lower than in those corresponding to *S. scabrosa*. Temperature seasonality as well as mean temperature in the warmest and coldest month and the wettest, driest, warmest and coolest quarter was also significantly higher in grids with occurrence of *S. scabrosa*. Significant differences were further found in precipitation seasonality and precipitation in the driest month and driest quarter (Table 1). Mean tree cover in the grids with *Sticta scabrosa* was not significantly different with either test (Table 1).

A marked difference was found in altitudinal preferences, both for the altitudinal grid layer and the actually measured altitude at the

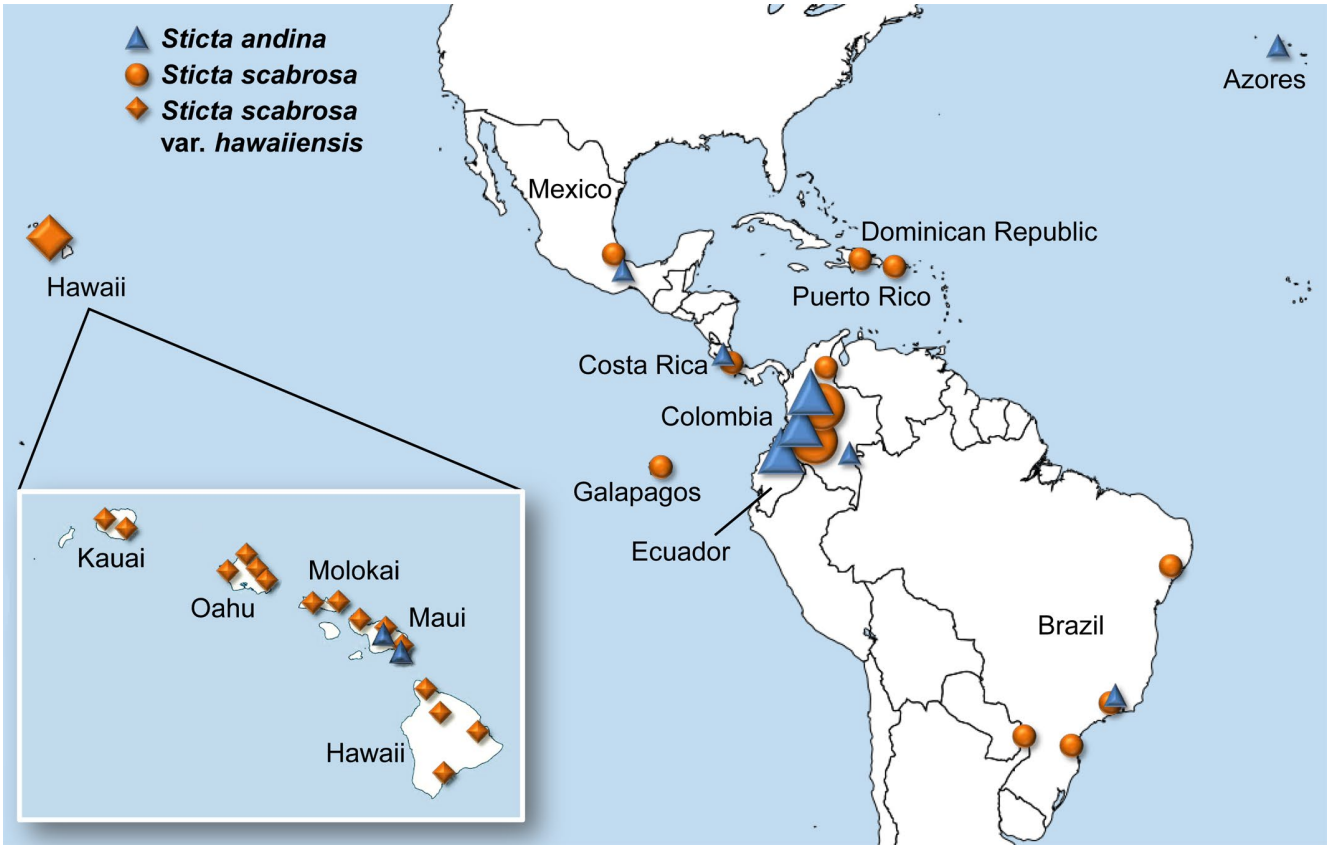

FIGURE 4 Global distribution of *Sticta andina* and *S. scabrosa* based on sequenced specimens included in this study. Individual symbols may indicate more than one locality in close proximity and larger symbols indicate numerous localities in close proximity relative to scale. Inset with Hawaii enlarged to show locality details for both species, including non-sequenced specimens. Map derived from Wikimedia Commons (Robinson projection)

sample localities (Table 1). *Sticta andina* occurrences exhibited a mean of 2,834 m (measured) and 2,242 m (grid layer data), whereas *S. scabrosa* occurrences showed a mean of 920 m (measured) and 840 m (grid layer data). This characterized *S. andina* as a tropical–subtropical, upper montane to alpine and *S. scabrosa* as a tropical lowland to lower montane species. Both species showed little overlap in their altitudinal ranges, with an optimum for *S. scabrosa* below 1,000 m and for *S. andina* between 2,500 and 3,500 m (Figure 7). In Hawaii, *S. andina* was only found in well-preserved montane forest, mostly on the island of Maui in Haleakalā National Park and the Kipahulu and Makawao Forest Reserves, whereas *S. scabrosa* has an almost weedy character, being found on all major islands and often in disturbed or anthropic vegetation (Figure 4). In the Azores, *S. andina* was extremely rare, being confined to little disturbed cloud forest on the island of Pico; it was not found on the nearby island of Faial, which also has remnants of these natural forests (Purvis & James, 1993).

3.4 | Isolation by distance, environment and fragmentation

At global level, *S. scabrosa* showed a highly significant correlation between genetic and geographic distances (IBD), whereas such a correlation was absent in *S. andina* (Table 2). At continental level (Central and South America), correlation between genetic and geographic distances were not detected in either *S. andina* or *S. scabrosa*. Therefore, the effect of IBD at global level in *S. scabrosa* is based on the genetically deviating metapopulation in Hawaii, recognized as separate subspecies. While not showing patterns of IBD at either global or continental level, *S. andina* exhibited a highly significant correlation between genetic and environmental distances (IBE) at the continental level and also a highly significant correlation between genetic and fragmentation distances (IBF) in the northern Andes (Table 2).

Both taxa exhibited a similar range of Kimura distances, mostly between 0 and 0.015, with *S. scabrosa* including some instances of slightly higher values, suggesting that both lineages were of similar evolutionary age.

4 | DISCUSSION

The two studied species, *Sticta andina* and *S. scabrosa*, differed strongly in their phylogenetic structure. *Sticta scabrosa* had a predominant haplotype throughout most of its range except Hawaii. The high number of haplotypes in Colombia (9) and southern Brazil and

FIGURE 5 Reproductive and morphological variation in *Sticta andina* (a–f) and *S. scabrosa* (g–l). a. Brown thallus with concolorous, marginal phyllidia (Colombia, *Lücking & Moncada 34003*). b. Dark brown thallus with darker, marginal isidia (Hawaii, *Moncada et al. 6983*). c. Damp, olive-green thallus with concolorous, marginal phyllidia showing underside with thick, dark brown tomentum and white cyphellae (Colombia, *Lücking et al. 39466*). d. Brown thallus with predominantly laminal phyllidia (Colombia, *Lücking & Moncada 35263*). e. Thallus with apothecia only (Colombia, *Lücking & Moncada 35345*). f. Sterile, brown thallus with rather thick, dark brown tomentum (Colombia, *Lücking & Coca 39391*). g–j. Typically olive brown thallus (in I damp) with concolorous, marginal phyllidia (g, Costa Rica, *Lücking 34607*; h, Brazil, *Lücking et al. 40073*; i, Brazil, *Lücking et al. 40042*; j, Hawaii, *Moncada et al. 6917*). k. Damp, olive-green thallus with marginal and laminal phyllidia and minute surface papillae (Hawaii, *Moncada et al. 7017*). l. Brown thallus with distinctly foveolate lobe surface (Hawaii, *Moncada et al. 7009*)

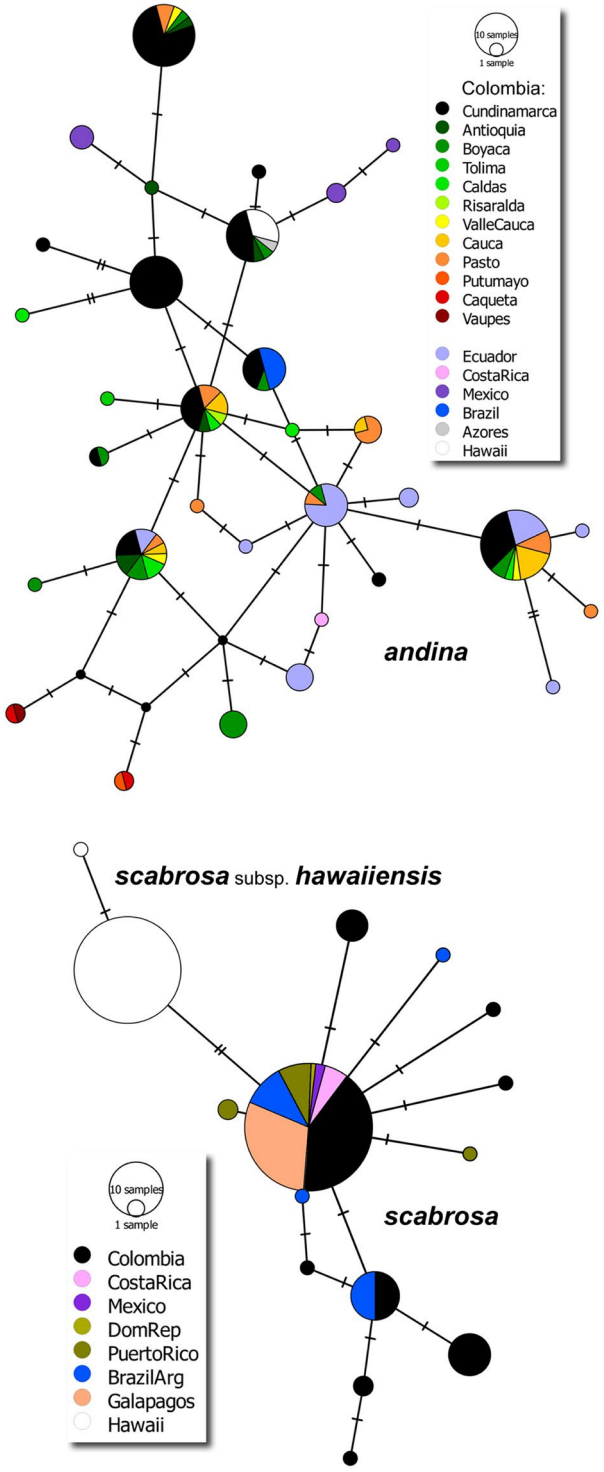

FIGURE 6 TCS haplotype networks for *Sticta andina* and *S. scabrosa* (*including* subsp. *hawaiiensis*) based on the ITS fungal barcoding locus

northern Argentina (4), compared to the low number in Mexico (1), Costa Rica (1), Dominican Republic (1), Puerto Rico (2), Galapagos (1) and Hawaii (1), together with the more terminal position of specimens from Central America, the Caribbean, Galapagos and Hawaii, were consistent with the interpretation of continental South America being the evolutionary centre of this species, although this hypothesis needs to be tested with additional markers and specimens. The genetic structure of this species within its continental range, with a single dominant haplotype and several infrequent satellite haplotypes, indicates a recent, rather a fast expansion consistent with its ecological interpretation as a somewhat 'weedy' species; however, our analysis using Tajima's D also showed that this test is sensitive to how missing data and indels are treated by editing the underlying alignment prior to analysis. For instance, a single gap within a polymorphic site will cause exclusion of that site from the computation of Tajima's D.

Besides the main haplotype in *Sticta scabrosa*, the only other distinctive and predominant haplotype was the one in Hawaii, representing subspecies *hawaiiensis* (Moncada et al., 2020). In contrast to the rather uniform morphology of the neotropical populations, the Hawaiian material featured three distinct morphodemes: the common morphodeme with uneven lobe surface, a second morphodeme characterized by faveolate to pitted lobes especially towards the tips and a third morphodeme with surface papillae. The common morphodeme occurred throughout Hawaii, whereas the faveolate-pitted morphodeme was found on Kauai and rarely on Maui, and the papillose morphodeme was exclusive to Maui.

The phenomenon of morphological disparity combined with a rather uniform genotype is proper to island biotas and has been well-documented for plant radiations, such as the Hawaiian lobeliads in the family Campanulaceae and the Silversword alliance in the family Asteraceae (Baldwin et al., 1991; Baldwin & Sanderson, 1998; Barrier et al., 2001; Carlquist et al., 2003; Givnish et al., 2009). For lichens, it was recently shown for the genera *Lobariella* and *Pseudocyphellaria* in Hawaii, with closely related species developing distinctive morphologies (Lücking et al., 2017; Moncada, Reidy, et al., 2014).

In contrast to *Sticta scabrosa*, *S. andina* had a much larger number of frequent haplotypes, interconnected in reticulate fashion. The highest number of haplotypes was found in the northern Andes (Colombia and Ecuador), decreasing towards North America (Mexico) and South America (Brazil), suggesting that the centre of distribution of this species may lie in the northern Andes, supported by the more terminal position of specimens outside this area. In addition to greater phylogenetic diversity, *Sticta andina* also exhibited greater phenotype diversity, particularly in the nature of the vegetative propagules, including various types of isidia and phyllidia.

The two species exhibited quite different patterns of long-distance dispersal (LDD), highlighting the role of stochastic dispersal effects. In *Sticta scabrosa*, the remote population in Hawaii constituted a unique and exlusive haplotype, evidently the result of natural LDD in the recent past, leading to a phylogenetically isolated subspecies (Moncada et al., 2020). In contrast, *Sticta andina*, had a single haplotype present in Hawaii and the Azores, identical with one of the main Colombian haplotypes, which suggests recent and perhaps anthropic LDD to both archipelagos. This is comparable to the situation found in *Pseudocyphellaria hawaiiensis*, which occurs with identical haplotypes in the Americas and in Hawaii, where it is largely restricted to the extensive, introduced conifer forests on Maui (Moncada, Reidy, et al., 2014). Notably, Hawaiian *Sticta andina* was also almost

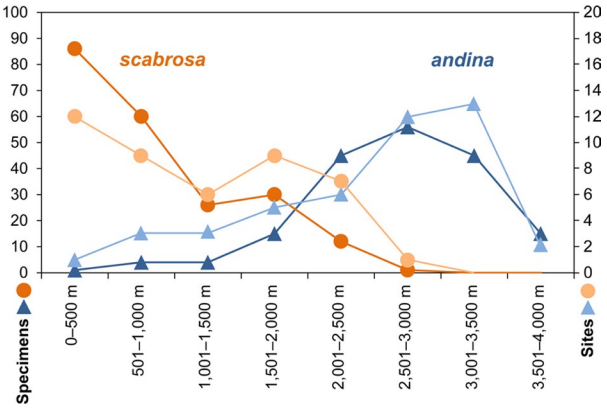

FIGURE 7 Altitudinal zonation of *Sticta andina* and *S. scabrosa* based on all samples, indicating total number of samples and number of different sites per altitudinal range (in steps of 500 m)

exclusively found in that habitat. Hawaiian conifer forests are exclusively composed of introduced taxa, including species of *Araucaria* (from Brazil and Argentina, New Caledonia, Australia), *Pinus* (North America and Mexico, Europe), *Cryptomeria* (Asia), *Sequoia* (North America) and *Cupressus* (North America south to El Salvador, Europe). Of particular interest for the accidental dispersal of associated lichens is *Cupressus lusitanica* Mill., native to Mexico and northern Central America but widely naturalized in Central and South America including at high altitudes in Colombia (Little & Skolmen, 1989).

In *Sticta andina*, there was no significant correlation between genetic and geographic distances, neither at global nor at continental level, although at continental level, the correlation was marginally significant, indicating a weak effect of IBD. However, maximum genetic distances were found already at small geographic distances, corresponding to the scale of topographical isolation in the highly distrupted terrain of the northern Andes. This pattern was closest to the case III model defined by Hutchinson and Templeton (1999), suggesting a pattern of IBF. Indeed, our postulated migration model for *S. andina* in the northern Andes, based on reconstruction of the final Andean uplift (Hoorn et al., 2010), supported a significant effect of IBF. At continental level, *S. andina* also displayed a significant effect of IBE, suggesting that genetically coherent populations of this species have evolved ecologically differentiated niche preferences along altitudinal and vegetation gradients in the highly dissected terrain of the continental range of the species. At global level, *Sticta scabrosa* followed the case I model of Hutchinson and Templeton (1999), with a strong effect of

TABLE 1 Statistical comparison of bioclimatic parameters, altitude and tree cover between occurrences of *Sticta andina* and *Sticta scabrosa*, using a more sensitive Kolmogorov-Smirnov test (left columns) and a less sentitive Mann-Whitney U test (right columns) in comparison

		Mean		Std. Dev.		*p*-level	Z	*p*-level
		andina	*scabrosa*	*andina*	*scabrosa*			
bio1	**Annual Mean Temp**	15.5	20.6	3.5	3.1	**< 0.001**	−11.7	**< 0.001**
bio2	**Mean Diurnal Temp Range**	10.3	9.3	2.0	1.3	**< 0.001**	3.5	**< 0.005**
bio3	**Isotermality**	8.3	7.2	0.8	1.0	**< 0.001**	9.7	**< 0.001**
bio4	**Temp Seasonality**	45	116	44	70	**< 0.001**	−11.0	**< 0.001**
bio5	**Max Temp Warmest Month**	21	27	4.1	3.1	**< 0.001**	−11.5	**< 0.001**
bio6	**Min Temp Coldest Month**	9.3	14.2	3.4	3.4	**< 0.001**	−11.2	**< 0.001**
bio7	**Temp Annual Range**	12.4	13.0	2.6	2.0	**< 0.001**	−6.3	**< 0.001**
bio8	**Mean Temp Wettest Quarter**	15.4	21	3.7	3.1	**< 0.001**	−11.7	**< 0.001**
bio9	**Mean Temp Driest Quarter**	15.3	20	3.5	3.7	**< 0.001**	−10.4	**< 0.001**
bio10	**Mean Temp Warmest Quarter**	16.0	22	3.7	3.3	**< 0.001**	−11.9	**< 0.001**
bio11	**Mean Temp Coldest Quarter**	14.8	19.1	3.4	3.2	**< 0.001**	−10.5	**< 0.001**
bio12	**Annual Prec**	1827	1930	736	916	**< 0.005**	−1.2	0.2276
bio13	**Prec Wettest Month**	254	257	117	144	**< 0.050**	0.0	0.9147
bio14	**Prec Driest Month**	61	79	33	43	**< 0.001**	−3.7	**< 0.005**
bio15	**Prec Seasonality**	40	33	14	17	**< 0.001**	4.4	**< 0.001**
bio16	**Prec Wettest Quarter**	673	683	324	396	**< 0.005**	0.4	0.6870
bio17	**Prec Driest Quarter**	229	295	112	153	**< 0.001**	−3.3	**< 0.050**
bio18	**Prec Warmest Quarter**	457	452	155	145	**< 0.001**	0.0	0.9709
bio19	**Prec Coldest Quarter**	498	528	363	432	**< 0.001**	0.0	0.8890
	Tree Cover	4.3	4.2	4.3	4.1	> 0.100	0.0	0.6457
	Altitude Layer	2,242	840	731	715	**< 0.001**	12.5	**< 0.001**
	Altitude Measured	2,834	920	606	664	**< 0.001**	15.0	**< 0.001**

Significant *p* values are highlighted.

TABLE 2 Mantel test of genetic vs. geographic, environmental, and fragmentation distances in *Sticta andina* and *S. scabrosa*. Significant correlations are highlighted.

Species	Test range	Geographic distances (simple Mantel test)	Environmental distances (partial mantel test)	Fragmentation distances (simple mantel test)
andina	global	R = −0.0298, $p = 0.2781$		
	continental	R = 0.0705, $p = 0.0772$	**R = 0.1436, $p = 0.0001$**	
	continental (log)	R = −0.0047, $p = 0.4530$	**R = 0.1597, $p = 0.0001$**	
	Andes	**R = 0.1725, $p = 0.0001$**	**R = 0.1741, $p = 0.0001$**	**R = 0.1779, $p = 0.0001$**
scabrosa	global	**R = 0.5345, $p = 0.0001$**		
	continental	R = −0.0534, $p = 0.2609$	R = 0.0189, $p = 0.3127$	
	continental (log)	R = −0.0309, $p = 0.2935$	R = 0.0092, $p = 0.4379$	

Significant correlations are highlighted.

IBD (Sexton et al., 2014). However, this effect was exclusively caused by the genetically deviating Hawaiian metapopulation. Excluding this metapopulation and restricting the analysis to continental Central and South America only, no effect of IBD or IBE was discernable.

Given that both species are geographically congruent, their different evolutionary histories may be explained by their autecology, specifically their different altitudinal and habitat preferences. Largely confined to undisturbed andine forests and paramos, the high altitude species *Sticta andina* apparently underwent small-scale fragmentation and partial isolation (IBF) in the recent past, due to the topographically disrupted terrain preventing effective genetic exchange between populations across parallel mountain ranges. In addition, altitudinal and vegetation gradients fostered small-scale niche differentiation between genetically coherent populations, potentially a precursor stage towards speciation. In *S. scabrosa*, the situation is quite different. Since this species is confined to lower altitudes and quite tolerant towards disturbance, it can easily establish in diverse lowland habitats, from exposed microsites in closed forest to secondary and anthropogenic vegetation. This results in nearly continuous niche distribution, facilitating effective gene exchange also over larger distances. In such a situation, apparently only prolonged isolation of the disjunct Hawaiian metapopulation lead to genetic differentiation.

Our findings also show that both deterministic and stochastic patterns may determine phylogenetic diversification and speciation in opposed fashion. Although the effects of IBE and IBF in *Sticta andina* and their absence in *S. scabrosa* can be predicted by deterministic factors of geology and habitat preferences, stochasticity lead to the fact that *S. scabrosa* apparently reached a remote location such as Hawaii much earlier than *S. andina* and hence evolved a genetically distinct subspecies.

To our knowledge, this appears to be the first case were IBF has been demonstrated in lichen fungi; in the few other studies available on lichens,for example, *Cetraria aculeata*, *Cetradonia linearis* and *Lobaria pulmonaria* (Walser et al., 2005; Fernández-Mendoza et al., 2011; Allen et al., 2018), IBD was the predominant pattern detected. In plants and animals, where IBE appears to be the main pattern besides IBD (Sexton et al., 2014), IBF has also been demonstrated for high altitude lineages, such as andine orchids and paramo plants (Givnish, 2010; Givnish et al., 2014, 2015; Küper et al., 2004; Madriñan et al., 2013). A recent study on plants of the Cape Floristic Region (Verboom et al., 2015) found effects of IBF to be predominant among higher altitude lineages, as opposed to IBD combined with IBE in lower-altitude species.

Our findings also lend support to the notion that key innovations alone do not cause phylogenetic diversification but require additional steps of isolation. Given that *Sticta andina* and *S. scabrosa* have comparable, rather wide geographic ranges, both with similar abundances, one would consider them as equally 'successful' in evolutionary terms. Thus, both likely acquired key innovations leading to their range expansions compared to other species, although the nature of these is not known (though likely physiological). However, only in *S. andina* we found a level of genetic, phenotypic and ecological diversification potentially leading to subsequent speciation across its continental range in Central and South America, whereas *S. scabrosa* is genetically, morphologically and ecologically more homogeneous within the same area and is not likely to further speciate, except for long-distance, long-term isolation, as is the case with the Hawaiian subspecies.

In conclusion, *Sticta andina* and *S. scabrosa* constitute an excellent case study to highlight the link between altitudinal and habitat preferences, that is, autecological features and geographical metapopulation structure, which relates to species biogeography. The early work of von Humboldt and Bonpland (1805), largely performed in the region where the two lichens have their centres of distribution, already hinted at such a connection, although at the time the underlying evolutionary mechanisms were far from being recognized.

ACKNOWLEDGEMENTS

Funding for field and laboratory work for this study was provided by a grant from the National Science Foundation (NSF) to The Field Museum: DEB-1354884 'Collaborative Research: Evolution, Diversification, and Conservation of a Megadiverse Flagship Lichen Genus' (PI HTL, CoPI RL). For recent collections gathered in Colombia (Bogotá), BM and RL are also grateful for the financial support from the Bundesministerium für Bildung und Forschung (BMBF; *Kooperation mit dem Botanischen Garten Bogotá und der Universidad del Norte Barranquilla*; ColBioDiv, FK 01DN17006; *Pilotprojekt Kooperation mit dem Botanischen Garten Bogotá*; Förderkennzeichen:

01DN13030) and the Center for International Cooperation of the Freie Universität Berlin (CIC; FM Ex3-2017–013). The following colleagues and students are thanked for providing additional material or aiding in the logistics of field work in Colombia: Wilson Álvaro, C. J. Arango, M. Cáceres, L. F. Coca, M. Dal Forno, L. Ferraro, M. A. Herrera-Campos, L. Katib, Y. Lozano, N. Marín, R. Peláez, S. Silano, D. F. Simijaca, E. Soto-Medina, A. Suárez, L. Tisnes, J. M. Torres and L. Vargas. Galapagos material of *Sticta* was examined as part of the Galapagos Lichen Inventory by the Charles Darwin Foundation (CDF) and the Galapagos National Park (DPNG) as part of a national biodiversity assessment program 'Biodiversidad Genética del Ecuador' led by the Instituto Nacional de Biodiversidad del Ecuador (INABIO). The authors are indebted to our colleagues and collaborators at these institutions, especially Danny Rueda, Daniel Lara Solís, Galo Quedaza and Victor Carrión (DPNG), Arturo Izurieta and Maria-José Barragan Paladines (CDF), and Diego Inclán and Rosa Batallas (INABIO) for research permits and logistical support. This publication is contribution number 2249 of the Charles Darwin Foundation for the Galapagos Islands. All material used in this study was collected under the corresponding permits in collaboration with REPP (Mexico), JAMD (Puerto Rico), BM (Colombia), FB (Galapagos), EG (Brazil) and CWS (Hawaii) Specimens from Kauai (Hawaii) were collected under Special Use Permit K2013-090cc of the Division of State Parks. Due diligence under the Nagoya Protocol is currently only required for material used in this study collected in Mexico and Ecuador (Galapagos) on or after 12–10–2014. The U.S.A. (Puerto Rico, Hawaii) has not signed the protocol, Brazil and Colombia are not yet parties and according to the Decreto 1376 de 2013 and the Resolución 1348 de 2014, molecular work for basic research such as taxonomy ('*investigación científica no comercial*') does not constitute access to genetic resources and therefore does not require due diligence with regard to the Nagoya Protocol. The material in Ecuador (Galapagos) was collected prior to 12–10–2014. The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All newly generated data used in this study are available as electronic supplementary material (Appendices S1–S10; https://doi.org/10.5061/dryad.bvq83bk78). DNA sequences have been submitted to Genbank (https://www.ncbi.nlm.nih.gov/genbank; see voucher information in Appendix S1) and original data sources (bioclim variables, tree cover layer) are given in the Methods section (including links).

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Author contributions: BM, HTL and RL conceived the study. All authors contributed to the collection of specimens and data and to parts of the manuscript. BM, FB, CWS, REPP and EG provided area-specific data for Colombia, Galapagos, Hawaii, Mexico and Brazil; BM, JAMD, NM, BPH and ES generated the sequence data and assisted in the global phylogenetic analysis. BM and RL performed the main data analyses and spearheaded the writing of the manuscript.

SUPPORTING INFORMATION

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

How to cite this article: Moncada B , Mercado-Díaz JA , Magain N , et al. Phylogenetic diversity of two geographically overlapping lichens: isolation by distance, environment, or fragmentation?. *J Biogeogr.* 2020;00:1–14. https://doi.org/10.1111/jbi.14033