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Phylogeographic reconstructions can be biased by ancestral shared alleles: The case of the polymorphic lichen *Bryoria fuscescens* in Europe and North Africa

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Abstract

Large phylogeographic studies on lichens are scarce, and none involves a single species within which different lineages show fixed alternative dispersal strategies. We investigated *Bryoria fuscescens* (including *B. capillaris*) in Europe and western North Africa by phenotypically characterizing 1400 specimens from 64 populations and genotyping them with 14 microsatellites. We studied population structure and genetic diversity at the local and continental scales, discussed the post-glacial phylogeography, and compared dispersal capacities of phenotypes with and without soralia. Our main hypothesis is that the estimated phylogeography, migration routes, and dispersal capacities may be strongly biased by ancestral shared alleles. Scandinavia is genetically the richest area, followed by the Iberian Peninsula, the Carpathians, and the Alps. Three gene pools were detected: two partially linked to phenotypic characteristics, and the third one genetically related to the American sister species *B. pseudofuscescens*. The comparison of one gene pool producing soredia and one not, suggested both as panmictic, with similar levels of isolation by distance (IBD). The migration routes were estimated to span from north to south, in disagreement with the assessed glacial refugia. The presence of ancestral shared alleles in distant populations can explain the similar IBD levels found in both gene pools while producing a false signal of panmixia, and also biasing the phylogeographic reconstruction. The incomplete lineage sorting recorded for DNA sequence loci also supports this hypothesis. Consequently, the high diversity in Scandinavia may rather come from recent immigration into northern populations than from an in situ diversification. Similar patterns of ancestral shared polymorphism may bias the phylogeographical reconstruction of other lichen species.

KEYWORDS
bioindicator, climate change, lichen ecology, lichenicolous, *Parmeliaceae*, soredia, speciation

1 | INTRODUCTION

Europe, where glaciations played a major role in shaping genetic diversity and species distributions, is one of the most intensely studied areas in terms of phylogeography (Hewitt, 2011; Schmitt, 2007; Svenning et al., 2008; Weiss & Ferrand, 2008). Through the last 2.5 million years (My), the entire continent has been affected by glacial and interglacial periods (Clark et al., 2009; Zachos et al., 2001). During the most severe glaciations, ice sheets covered Northern Europe and parts of Central Europe, leaving large noncolonized

areas during interglacial periods as the ice retreated (Parducci et al., 2012; Tzedakis et al., 2013). As a consequence, many European taxa currently show "simple" phylogeographic patterns, with glacial refugia close to or in the Mediterranean Basin, especially in the Iberian, Italian, and Balkan peninsulas (Bennet et al., 1991; Feliner, 2011; Habel et al., 2010; Médail & Diadema, 2009; Petit et al., 2003). Northern areas have been recolonized mainly from these regions during interglacial periods (Godfrey, 1999; Taberlet et al., 1998). However, the presence of cryptic northern refugia is still under discussion, not only for polar species but also for forest ecosystems (Birks & Willis, 2008; Magri et al., 2006; Parducci et al., 2012; Tzedakis et al., 2013; Willis & van Andel, 2004; Willis & Whittaker, 2000).

In contrast to plants and animals, the phylogeographic patterns of fungi have been poorly studied across Europe. In lichenized fungi, some studies have been performed in different parts of the continent, most of them detecting indirect signals of long-distance, intercontinental or even bipolar dispersion (Alors et al., 2017; Fernández-Mendoza et al., 2011; Garrido-Benavent et al., 2017; Geml et al., 2010). Werth, Wagner, Gugerli, et al. (2006) and Werth, Wagner, Holderegger, et al. (2006) provided direct evidence of a high dispersal capacity (≥200 m) for *Lobaria pulmonaria*, mainly an epiphytic large foliose lichen producing asexual propagules termed soredia, which comprise both the fungal and the algal partners. Widmer et al. (2012) performed the most extensive phylogeographic study of lichens in Europe to date using the former species, and showed a similar refugial pattern to that established for plants. However, in the case of *Solenopsora candicans*, mainly a Mediterranean lichen that rarely grows in Central Europe but extends into the British Isles, indications were found that glacial refugia existed not only in the southern Mediterranean but also in Central Europe (Fačkovcová et al., 2020). Although lichens are widely recognized to be sensitive to environmental modification (Bates & Farmer, 1992; Estrabou et al., 2011; Nimis et al., 2002; Seed et al., 2013), their ability to persist in microhabitats and their high effective dispersion compared to plant, might affect their phylogeography, leading to patterns that are different from those found in plants.

Bryoria fuscescens is one of three partially cryptic and very closely related species of the *Bryoria fuscescens* species complex (*Parmeliaceae*), the two others being *B. kockiana* and *B. pseudofuscescens*. The three species originated from a common ancestor around 1 My ago (Boluda et al., 2018). *Bryoria fuscescens* is the only species of the complex known to occur outside North America. It has a circumpolar distribution throughout the Northern Hemisphere but is also present at altitude in the East African mountains. The species grows mainly in boreal forests, one of the most affected ecosystems during the most recent glacial events (Hampe & Petit, 2010; Tzedakis et al., 2013), and to a lesser extent on rocks, wood, and soil that were all common European habitats during the Glacial Maxima (Brodo & Hawksworth, 1977; Myllys et al., 2011). It is a species relatively common in Northern Europe, but becomes rarer at more southern latitudes, where it has a fragmented distribution in high and humid mountain areas. As it is sensitive to small environmental modifications it has applications as a bioindicator of forest quality (Esseen et al., 1996, 2017; Myllys et al., 2016).

Bryoria fuscescens is a highly variable species containing chemical and morphological phenotypes that were hitherto considered to belong to eight or more different species (Boluda et al., 2018; Hawksworth et al., 2019; Velmala et al., 2014). Despite this large variability, *B. fuscescens* can be divided into two main phenotypic groups: (1) the *fuscescens* morph which mainly forms pale brown to black thalli, frequently sorediate, with branches of 0.3–0.5 mm in diameter, few epicortical substances (Boluda et al., 2014), and no barbatolic or alectorialic acid; and (2) the *capillaris* morph which mainly forms pale coloured thalli, frequently esorediate, with branches up to 0.4 mm in diameter, usually abundant epicortical substances, and with the above-mentioned acids. Both morphs are widely distributed in Europe and they frequently grow intermixed, indicating that the two morphs are not attributable to environmental plasticity, although some subtle ecological preferences have been detected for each morph (Esseen et al., 2015; Myllys et al., 2016). A putative ongoing speciation process with incomplete lineage sorting for barcoding genetic markers has been proposed to explain such morphological variability within a single species (Boluda et al., 2018).

Bryoria fuscescens has large connected populations in Northern Europe and small isolated populations in southern Europe (Boluda et al., 2018; Brodo & Hawksworth, 1977; Myllys et al., 2011). It is highly sensitive to habitat modification (Esseen et al., 1996, 2017; Myllys et al., 2016) and may currently be under speciation (Boluda et al., 2018). All these characteristics make it a candidate to be used as a model in lichen speciation and phylogeography studies. Additionally, the presence of two intraspecific lineages, one with and another without specialized asexual propagules provide a unique framework in which it is possible to compare within a single species how dispersion is affected by the production or the lack of diaspores.

To study the phylogeography, and dispersal capacities in the *Bryoria fuscescens* complex, 1400 samples were collected across Europe and Western North Africa, including the Mediterranean region, and genotyped with 14 fungal-specific microsatellite markers and eight DNA sequence loci. Our principle hypothesis is that ancestral shared alleles among currently isolated regions can bias the estimated migration routes and dispersal capacities, producing inconsistent results and misleading phylogeographic patterns.

2 | MATERIALS AND METHODS

2.1 | Sampling and phenotype determination

A total of 1400 *Bryoria fuscescens* thalli were collected from 64 populations across Europe, the Mediterranean region, and the Canary Islands (Figure 1 and Table S1). With some exceptions, populations were distant from each other by more than 50 km. An average of 22 specimens per population was collected. In some of the following analyses, populations were clustered according to the main mountain systems or geographic regions into seven groups: Scandinavia

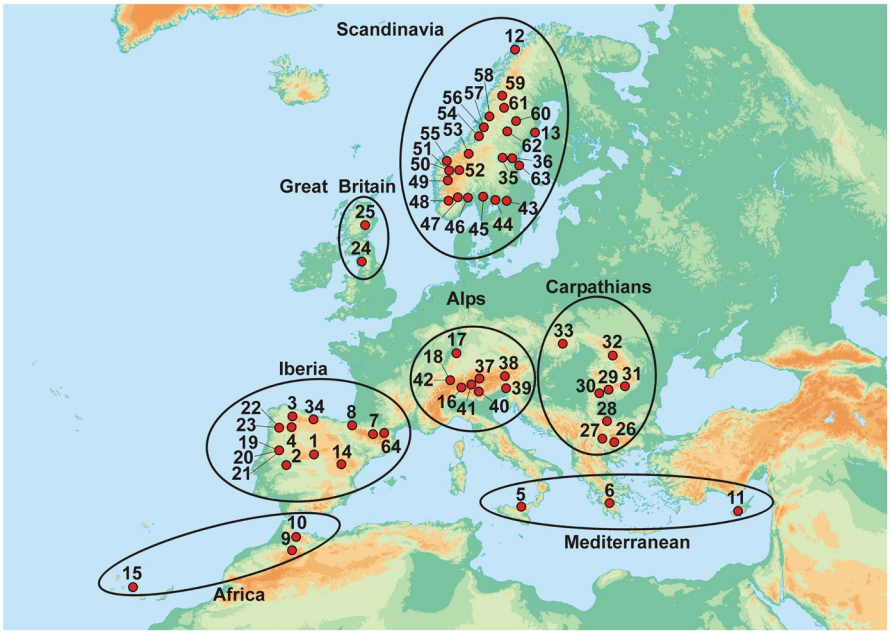

FIGURE 1 Map of all locations of *Bryoria fuscescens* samples, with the region of each population indicated. Map obtained from All-free-download.com

(Scandinavian Peninsula), Great Britain, the Carpathians, the Alps, Iberia (the Iberian Peninsula), the Mediterranean (east and central Mediterranean Region), and Africa (Canary Islands and northern Morocco) (Figure 1).

Within each population, specimens of all phenotypes growing on twigs, branches, rocks, and trunks, and with different orientations and heights, were collected to minimize clonality. Samples were air dried and then frozen at −20°C until further treatment. Each specimen was examined at ×65 magnification using a Nikon SMZ-1000 stereomicroscope, and the following characteristics were recorded: occurrence and type of soralia (absent, tuberculate or fissural); and presence/absence of pseudocyphellae, apothecia, and the lichenicolous fungus *Raesaenenia huuskonenii*. In addition, the chemotype of each specimen was determined by thin layer chromatography (TLC), following Orange et al. (2010), using the solvent system C (200 ml toluene/30 ml acetic acid) and silica gel 60 F254 aluminum sheets (Merck). The same lichen fragment used for TLC was also used for DNA extraction to ensure an absolute correlation between genotype and chemotype (Boluda et al., 2015).

2.2 | DNA extraction and PCR conditions

A single terminal branch per specimen was processed for DNA extraction with the DNeasy 96 Plant Kit (Qiagen). Each specimen was genotyped with 18 fungus-specific microsatellite markers (Bi01–Bi16, Bi018 and Bi019), following Nadyeina et al. (2014). Fragment lengths were obtained on an ABI PRISM® 3130 Genetic Analyser (Life Technologies) using fluorescently labelled primers. Electropherograms were analysed with the internal size standard LIZ-500 and GeneMapper 3.7 (Applied Biosystems).

For phylogenetic analyses, 34 specimens representing the most disparate phenotypes and geographic regions were selected (Table S2) and sequences from 101 specimens from Velmala et al. (2014) were downloaded from GenBank (https://www.ncbi.nlm.nih.gov). Eight fungal markers were used: three loci that are commonly employed in lichenized-fungal barcoding (ITS, IGS and GAPDH) and five that we defined for this study (FRBi13, FRBi15, FRBi16, FRBi18, and FRBi19; Table S3). The new markers are specific to *Bryoria* sect. *Implexae* (Boluda et al., 2018), to which the complex belongs, and were obtained from the microsatellite flanking sequences (Chatrou et al., 2009; Devkota et al., 2014; Zardoya et al., 1996). The flanking regions of the 18 microsatellite markers were checked upstream and downstream in the 454 pyrosequencing contigs used to obtain microsatellites markers in Nadyeina et al. (2014, deposited in the NCBI Sequence Read Archive under Accession no. SRR1283191; http://www.ncbi.nlm.nih.gov/sra). The variability in each region was assessed by counting the number of variable sites in contigs supported by two to 16 copies. From the 36 regions, the five most variable (corresponding to microsatellites Bi13, Bi15, Bi16, Bi18 and Bi19) were selected and primers were designed with Primer 3 Plus (Untergasser et al., 2007). The markers FRBi15 and FRBi16 were previously used in Boluda et al. (2018). Additionally, the algal partner ITS loci of the 35 selected samples were sequenced using specific primers (Tables S2 and S3).

PCR conditions and sequencing methods were as in Boluda et al. (2018), except for markers FRBi18 and the algal ITS, for which the same conditions as for the fungal ITS were used but with an annealing temperature of 50°C.

2.3 | Genetic diversity and population dynamics

For each population and each group, several parameters were calculated. Allelic richness (AR) and private allelic richness (PAR) were estimated using a rarefaction approach with ADZE (Szpiech et al.,

2008). Nei's unbiased haploid diversity (uh) was calculated using GenAlEx v.6.41 (Peakall & Smouse, 2006). Levels of linkage disequilibrium were determined using the index rBarD (unbiased measure of linkage disequilibrium), which corresponds to the index of association (IA) but is independent of the number of included loci (Agapow & Burt, 2001). rBarD was calculated with clone-corrected data within populations and among loci with the R package poppr v.2.1.1 (Kamvar et al., 2014, 2015), which is appropriate for haploid species with both clonal and sexual reproductive strategies. The null model of no linkage between loci was performed with 999 permutations. It is expected to be 0 if populations are freely recombining (sexual reproduction) and significantly greater than 0 if alleles are associated (asexual reproduction). To check for putative drastic recent population size variations, the Microsoft Excel macro KGTESTS (Bilgin, 2007) was used. A significant negative value in the *K*-test indicates expansion, whereas a significant positive value indicates constant population sizes. For all the above-mentioned tests, a Benjamini correction was applied with an adjusted level of 0.001 (Benjamini & Yekutieli, 2001). Genetic differentiation among groups (F_{CT}), populations (F_{ST}) and individuals were tested with an analysis of molecular variance (AMOVA) over loci with Arlequin v.3.5 (Excoffier & Lischer, 2010). The same AMOVA was run taking into account allele sizes, following Slatkin (1995) and Michalakis and Excoffier (1996), in order to compute R_{ST} and R_{CT} and compare them with F_{ST} and F_{CT}, respectively. The significance values were obtained using a nonparametric permutation process with 20,022 permutations.

2.4 | Genetic structure

Two approaches were applied to delimit genetic pools using microsatellite data: discriminant analysis of principal components (DAPC: Jombart et al., 2010) and structure v.2.3.4 (Falush et al., 2003; Pritchard et al., 2000). DAPC, unlike structure, does not assume that markers are unlinked and that populations are panmictic. DAPC analysis was performed with the R package adegenet v.2.0.1 (Jombart, 2008; Jombart & Ahmed, 2011; Jombart et al., 2010), using the microsatellite data of Table 1 and Table S4.

structure was run on the same data set, performing 100,000 burnin generations and 100,000 iterations, and using a *k* value from 1 to 10 with 20 replicates for each *k*. Runs of each *k* value were combined with clumpp v.1.1.2 (Jakobsson & Rosenberg, 2007) and structure harvester (Earl & von Holdt, 2012). The Δ*K* method (Evanno et al., 2005) was used to find the *k* value that best fitted our data.

2.5 | Spatial analyses

An isolation-by-distance test (IBD) was carried out using the R package adegenet (Jombart, 2008; Jombart & Ahmed, 2011; Jombart et al., 2010). The Edward's genetic distances and the Euclidean geographic distances were used for correlation using a Mantel test with the mantel.randtest function in adegenet. The results were plotted

TABLE 1 Differences between obtained and analyzed microsatellite markers (SSRs)

	SSRs obtained		SSRs used in the analyses	
Locus	Number of specimens	Number of alleles	Number of specimens	Number of alleles
Bi01	1384	22	Discarded	Discarded
Bi02	1123	6	Discarded	Discarded
Bi03	1391	5	1359	5
Bi04	1388	8	1359	7
Bi05	1359	14	1359	10
Bi06	1366	22	1359	21
Bi07	1368	6	1359	6
Bi08	1385	5	1359	5
Bi09	597	3	Discarded	Discarded
Bi10	1393	5	1359	3
Bi11	1391	12	1359	10
Bi12	1399	22	1359	21
Bi13	1359	18	1359	18
Bi14	1391	4	1359	3
Bi15	1071	3	Discarded	Discarded
Bi16	1360	6	1359	6
Bi18	1359	9	1359	9
Bi19	1388	8	1359	6

Note: Specimens with missing data in any marker, as well as microsatellite sizes not corresponding to expected values between the repeated motifs, were discarded before analyses.

with the "kde2d" function in the R package *MASS* (Venables & Ripley, 2002) for a better visualization of the local density points.

Possible asymmetric patterns of gene flow between neighbouring pairs of geographic areas, as well as gene flow between Scandinavia (the region that shares the highest number of alleles) and each of the remaining areas, were estimated with MIGRATE-N 3.2.6 (Beerli & Palczewski, 2010). The mutation-scaled immigration rate M ($M = m/\mu$, where m = immigration rate and μ = mutation rate) was estimated assuming an unspecified μ, set as identical in all populations. An unconstrained migration model was used to estimate M and Θ (genetic diversity; $\Theta = 2N_e\ \mu$, where N_e = effective population size) for each pair of populations separately, using a uniform prior for both ($M = 0$–1000; $\Theta = 0, 100$) divided into 1500 bins. Four Metropolis-coupled Monte-Carlo chains were set with four different temperatures (1, 1.2, 3, and 1,000,000) and run for two million generations, recording every 200th step (10,000 steps for each run), after a burn-in period of 2500 generations. Asymmetric rates of immigration between regions were compared using M (mode of the posterior distribution across all loci) and the number of immigrants per generation (2Nm), which is the product of M and Θ of the recipient population (Moeller et al., 2011).

To compare the spatial distribution of alleles with that expected under panmixia, a spatial analysis of shared alleles was conducted (SAShA; Kelly et al., 2010). This analysis is particularly appropriate for species with low genetic diversity and high gene flow, as expected in lichens (Alors et al., 2017; Brodo & Hawksworth, 1977; Galloway & Aptroot, 1995). The geographically restricted alleles were identified according to Widmer et al. (2012). For each allele, the geographic x and y centroids were calculated. Then, using a bootstrap method subsampling the coordinates x and y N times (N = the number of individuals carrying a given allele), the null distribution and the 95% confidence intervals were determined. Alleles with a centroid falling outside of the 95% confidence intervals were considered as geographically restricted. The observed and expected frequencies of restricted alleles at different geographic distances were compared following Alors et al. (2017). The expected distribution under panmixia was determined with 1000 nonparametric permutations of the allele-by-location data sets (Alors et al., 2017).

2.6 | Potential distribution estimation

The potential distribution was mapped using MAXENT v.3.3.3a (Philips et al., 2006). Grids of the current global climatic conditions (spatial resolution of 30 arcsec, about 1 km^2), Mid-Holocene (6000 years ago [ya], spatial resolution of 2.5 min), Last Glacial Maximum (22,000 ya, 2.5 min), and Last Interglacial (approx. 130,000 ya, 30 arcsec) were obtained from the WORLDCLIM database (Hijmans et al., 2005), together with the 19 environmental variable layers BIO1 to BIO19. The different layers of each BIO variable were merged to form a single layer per variable using DIVA-GIS (Hijmans et al., 2004). BIL layer format was transformed to Esri.asc using DIVA-GIS, whereas tiff format was converted using the R packages tiff and raster (Hijmans & Jacob, 2012; R Core Team, 2013; Urbanek, 2015, https://cran.r-project.org/web/packages/tiff/tiff.pdf). To select the most relevant set of environmental variables and discard auto-correlated ones, the R package MaxentVariableSelection was used. Two Maxent analyses were run for each data set, one with all the variables and another with the most relevant ones. Each analysis was run 10 times, and the median value of all runs was plotted. For past potential distribution analyses, ArcGIS was used to build the input text SWD (samples with data) format.

2.7 | Phylogenetic reconstruction

Alignments were performed using MAFFT v.7 (Katoh & Standley, 2013), as described in Boluda et al. (2018). Aligned matrices were deposited in TreeBASE under Accession nos. TB2:S20593 (ITS, IGS and GAPDH), TB2:S20588 (FRBi13), TB2:S20589 (FRBi15), TB2:S20590 (FRBi16), TB2:S20591 (FRBi18), and TB2:S20592 (FRBi19). PartitionFinder (Lanfear et al., 2012) was used to detect possible intra-locus substitution model variability, resulting in the splitting of the fungal ITS region into ITS1, 5.8S and ITS2, and each codon position was coded separately in GAPDH. Substitution models were selected with jModeltest 2.0 (Darriba et al., 2012), using the Akaike information criterion (AIC; Akaike, 1974). The best-fit models were the following: ITS1 = TIM2, 5.8S = K80, ITS2 = TIM2ef + G, IGS = TrN + I, GAPDH first codon position = TrN + I, GAPDH second codon position = F81 + I, GAPDH third codon position = TPM3uf, FRBi13 = TrN, FRBi15 = TPM3uf + I, FRBi16 = TPM3uf + G, FRBi18 = HKY, and FRBi19 = TPM3uf + I. To detect possible topological conflicts among loci, the CADM test (Campbell et al., 2011; Legendre & Lapointe, 2004) was performed using the function CADM.global implemented in the ape package in R (Paradis et al., 2004). The five FRBi loci were not congruent, and separate phylogenetic reconstructions were estimated for each. ITS, IGS, and GAPDH loci were concatenated in a single matrix, removing the specimens with more than one missing locus. Data sets were analysed using maximum likelihood (ML) in RAXML v.8.2.10 (Stamatakis, 2006, 2014) and Bayesian reconstruction with MRBAYES v.3.2.1 (Ronquist & Huelsenbeck, 2003). The parameters used in the analyses were the same as those described in Boluda et al. (2018). TRACER v.1.5 (Rambaut et al., 2014) was used to ensure that ESS values were above 200.

To deal with tree topology conflicts, a species tree using multispecies coalescent (MSC; Yang & Rannala, 2014) and the eight loci mentioned above was reconstructed with STACEY v.1.2.2 (Jones, 2016; Jones et al., 2015) implemented in BEAST v.2.4.5 (Bouckaert et al., 2014). The parameters used in this reconstruction were as in Gerlach et al. (2019). A GTR substitution model for each locus with a relaxed exponential molecular clock was used. The species tree prior was set to the birth–death model, with a collapse height (ε) of 1×10^{-4} and a collapse weight (ω) of 0.5 and with parameters alpha and beta set to 1. Two independent runs of 200 million, storing every 100,000th tree, were combined using LogCombiner (Bouckaert et al., 2014), discarding the first 25% of the generations. All the phylogenetic trees were drawn with FIGTREE v.1.4 (Rambaut, 2009).

3 | RESULTS

3.1 | Genetic diversity

To avoid including putative mutations from the microsatellite flanking regions, which may have a different evolutionary pattern than the single sequence repeats (SSRs), all amplified microsatellites with unexpected allele sizes according to its repetitive motif were treated as missing data. After removing microsatellites with more than 10% missing data, then any specimens with any missing locus, a total of 14 microsatellites (out of 18) and 1359 specimens (out of 1400) were kept for further analyses (Table 1 and Table S4). Missing data were related to loci, but not to phenotypes or geographic origins.

The cleaned dataset contained 946 multilocus genotypes, with an average of nine alleles per locus (Table S4). The unbiased haploid diversity ranged from 0 for completely clonal populations, such as those from localities 22 and 23, to 0.59 for population 57 from Scandinavia, with a mean of 0.41 (Table 2). Allelic richness (AR) ranged from 0 to 4.0 for the same populations, with a mean value of 2.93. Private allelic richness (PAR) ranged from 0 (e.g., populations 2, 4, 5, 8) to 0.21 (population 10 from Morocco), with a mean of 0.03. As expected, PAR values were low in groups where geographically close populations are found, as for example in Scandinavia. However, at the regional scale, Scandinavia had the highest AR and PAR values, whereas Great Britain displayed the lowest values, with no private alleles (Table 3). The Alps, Iberia and Carpathian populations contained similar levels of allelic richness, but the Carpathian populations lacked private alleles. The African populations, despite having small sample sizes, exhibited high levels of private alleles. AR and PAR values for each substratum are shown in Table S5. Specimens from twigs and trunks showed similar PAR values. Much lower levels were found for specimens from rocks as they did not have a single allele that was not also found on individuals from trees.

Significant levels of linkage (rBarD; adjusted $p < .001$) were found in 36 of the 64 populations (56%), suggesting that the species displays a high level of clonal reproduction, even when apothecia are present (in 47.2% of the populations exhibiting apothecia). In the remaining 28 populations despite apothecia being less frequent (17.9%; Table 2) rBarD levels were not significant. While apothecia were more common in Scandinavian material, rBarD did not have lower levels than in other regions where apothecia were not observed. The *K*-test showed signs of recent population expansions in 12 localities: 9 (Africa); 17, and 37 (Alps); 20, 21, 22, and 23 (Iberian Peninsula); 30 (Carpathians); and 44, 45, 50, and 62 (Scandinavia).

AMOVA analysis (Table S6) revealed that most of the genetic variation occurs within populations (76.81%), with a lower amount between populations (20.89%) and an even lower proportion between regions as defined in Figure 1 (2.30%). The permutation test showed that differences among populations ($F_{ST} = 0.232$, $p < .001$) and regions ($F_{CT} = 0.023$, $p \leq .035$) were statistically significant. These indices were lower when allele size was considered ($R_{ST} = 0.208$, $p < .001$; $R_{CT} = 0.012$, $p > .05$; Table S6). The genetic differentiation between the *fuscescens* and *capillaris* morphs was high in both analyses ($F_{ST} = 0.272$ and $R_{ST} = 0.208$) and statistically significant ($p < .001$ for both).

3.2 | Gene pool detection

STRUCTURE output, using the ΔK method (Evanno et al., 2005), suggests that our data best fit two gene pools (Figure S1). DAPC analysis, however, indicated three as the most reasonable number of gene pools (DAPC *k3* in Figure S1), splitting the blue STRUCTURE genepool into two groups. Both analyses produced the same clustering independently whether a clone-corrected input was used or not. The third DAPC group, containing only 18 specimens, may be masked in STRUCTURE because this program is sensitive to unbalanced population sizes. STRUCTURE becomes uninformative at $k \geq 3$, while DAPC becomes uninformative at $k \geq 4$ (Figure S1), and new putative gene pools are formed dividing further the same cluster – the red one in STRUCTURE and the orange one in DAPC. DAPC analysis is more appropriate than STRUCTURE for species with a high probability of linkage disequilibrium or asexual reproduction, as it seems to be the case here. With a similar clustering as the one suggested by STRUCTURE with $k = 2$, DAPC $k = 3$ was selected as the number of clusters that best fits our data. Based on the retained discriminant functions of DAPC, the probabilities of group membership were high (gene pool 1 = 0.99, gene pool 2 = 0.98, and gene pool 3 = 0.86). Gene pool 1 is equivalent to the blue STRUCTURE cluster. Gene pool 2 gathers individuals from both the red and green clusters of STRUCTURE, as it is expected given the high levels of admixed specimens they contain. Gene pool 3 was not detected in STRUCTURE in any of the analyses from $k = 2$ to $k = 6$, probably due to the unbalanced gene pool sizes.

3.3 | Gene pool phenotypes and distribution

Morphological and chemical information for specimens and their gene pool assignments according to DAPC *k3* is given in Table S7. Gene pool 1 (blue in Table S7) contains 331 specimens, which lack soralia and fumarprotocetraric acid with the exception of two sorediate specimens (0.6%) and three specimens containing that acid (0.9%). Alectorialic and barbatolic acids are common, although norstictic, and psoromic acids can also be present (7.8%, and 11.2%, respectively). This group comprises 287 *capillaris* (87%) and 44 *fuscescens* morphs (13%). Gene pool 2 (orange), with the highest genetic and phenotypic diversity, contains 1008 specimens, with 130 *capillaris* (13%) and 878 *fuscescens* morphs (87%). Extrolite composition comprises all acids observed in the species, and fumarprotocetraric acid is present in 63% of the specimens. Gene pool 3 (green) contains only 18 homogeneous specimens, all of which are *capillaris* morphs with only alectorialic and barbatolic acids and without soralia. No other relationships between gene pools and the studied morphological characteristics were detected. The lichenicolous fungus *Raesaenenia huuskonenii* showed a similar distribution among phenotypes (5.2% thalli infected in the *fuscescens* morph vs. 4.2% in the *capillaris* morph;

TABLE 2 Results of the analyses for each population: number of specimens (*n*), number of non-clonal specimens, percentage of polymorphic loci, unbiased haploid genetic diversity (uh), unbiased measure of linkage disequilibrium (rBarD, in bold = significant values with $p < .001$), rarefied allelic richness (AR), rarefied private allelic richness (PAR), number of loci with negative values in the *K*-test (in bold = significant values with $p < .001$, suggesting a recent population expansion), and putative population disturbances (– indicates well-preserved, more or less uniform forest)

Region	Population number	*n*	Nonclonal specimens	Polymorphic loci (%)	uh mean (stand. error)	rBarD	AR	PAR	*K*-test	Additional population information
Africa	9	22	9	36	0.127 (0.059)	−0.048	1.642 (0.307)	0.076 (0.076)	**14**	–
	10	21	12	86	0.440 (0.064)	**0.297**	2.642 (0.414)	0.214 (0.152)	5	–
	15	22	16	100	0.559 (0.053)	**0.097**	3.428 (0.571)	0.129 (0.088)	8	–
Mean		22	12	74	0.375 (0.223)		2.570 (0.132)	0.140 (0.041)		
Alps	16	20	20	100	0.573 (0.033)	**0.126**	3.285 (0.398)	0.071 (0.071)	4	Apothecia present, recreational area
	17	14	6	71	0.255 (0.066)	**0.221**	2.071 (0.245)	0.071 (0.071)	**14**	Apothecia present
	18	23	16	100	0.519 (0.046)	**0.170**	3.285 (0.398)	0.000 (0.000)	5	Apothecia present
	37	24	24	100	0.477 (0.059)	**0.057**	3.571 (0.551)	0.069 (0.069)	**12**	Apothecia present
	38	25	23	93	0.428 (0.059)	0.030	2.642 (0.289)	0.000 (0.000)	5	–
	39	21	17	86	0.428 (0.059)	0.028	2.785 (0.408)	0.008 (0.008)	8	–
	40	20	19	100	0.480 (0.035)	**0.222**	3.214 (0.394)	0.071 (0.071)	9	Recreational area
	41	21	20	93	0.399 (0.059)	0.001	2.642 (0.341)	0.000 (0.000)	9	–
	42	21	16	100	0.405 (0.049)	**0.258**	2.714 (0.411)	0.068 (0.068)	10	–
Mean		21	18	94	0.440 (0.052)		2.912 (0.382)	0.040 (0.040)		
Carpathians	26	23	21	100	0.505 (0.046)	**0.267**	3.357 (0.570)	0.000 (0.000)	7	Apothecia present, recreational area
	27	23	21	93	0.451 (0.063)	0.016	3.071 (0.412)	0.000 (0.000)	8	Human activities and constructions
	28	23	18	100	0.466 (0.041)	**0.291**	3.214 (0.350)	0.002 (0.002)	6	Recreational area
	29	23	23	100	0.494 (0.057)	**0.144**	3.571 (0.531)	0.008 (0.008)	10	Apothecia present, human constructions
	30	22	20	86	0.337 (0.072)	0.031	2.857 (0.553)	0.008 (0.008)	**12**	Apothecia present
	31	23	20	100	0.056 (0.048)	**0.168**	3.785 (0.612)	0.000 (0.000)	5	Apothecia present
	32	21	19	93	0.380 (0.068)	0.037	2.642 (0.357)	0.000 (0.000)	7	Apothecia present
	33	21	17	79	0.362 (0.073)	0.056	2.428 (0.309)	0.000 (0.000)	8	Recent fire, recreational area
Mean		22	20	94	0.381 (0.059)		3.116 (0.462)	0.002 (0.002)		
Great Britain	24	9	5	64	0.317 (0.079)	0.010	2.071 (0.286)	0.000 (0.000)	10	Planted forest
	25	23	14	71	0.375 (0.072)	0.021	2.214 (0.317)	0.002 (0.002)	7	–

(Continues)

TABLE 2 (Continued)

Region	Population number	*n*	Nonclonal specimens	Polymorphic loci (%)	uh mean (stand. error)	rBarD	AR	PAR	*K*-test	Additional population information
Mean		26	10	68	0.346 (0.076)		2.143 (0.302)	0.001 (0.001)		
Iberian peninsula	1	20	11	93	0.492 (0.058)	**0.191**	2.642 (0.307)	0.014 (0.014)	5	–
	2	20	10	79	0.336 (0.079)	0.026	2.500 (0.402)	0.000 (0.000)	10	–
	3	20	18	100	0.535 (0.056)	**0.056**	3.642 (0.520)	0.080 (0.071)	11	–
	4	15	4	50	0.163 (0.050)	−0.051	1.500 (0.138)	0.000 (0.000)	11	Saxicolous specimens
	7	22	21	100	0.486 (0.057)	**0.171**	3.285 (0.450)	0.005 (0.005)	9	–
	8	22	14	100	0.492 (0.050)	**0.203**	3.500 (0.521)	0.000 (0.000)	8	Apothecia present
	14	19	16	100	0.525 (0.038)	**0.209**	3.214 (0.350)	0.003 (0.003)	7	–
	19	23	13	100	0.366 (0.053)	−0.025	2.642 (0.289)	0.000 (0.000)	10	–
	20	9	4	71	0.276 (0.059)	0.077	1.928 (0.195)	0.000 (0.000)	**14**	Saxicolous specimens
	21	10	5	86	0.356 (0.057)	0.057	2.000 (0.148)	0.000 (0.000)	**14**	Saxicolous specimens
	22	23	1	0	0.000 (0.000)	NA	1.000 (0.000)	0.000 (0.000)	**14**	Apothecia present, village close
	23	23	1	0	0.000 (0.000)	NA	1.000 (0.000)	0.000 (0.000)	**14**	Near pastures
	34	22	20	100	0.494 (0.063)	**0.216**	3.285 (0.518)	0.070 (0.064)	6	–
	64	25	14	57	0.202 (0.065)	0.083	2.071 (0.370)	0.000 (0.000)	11	–
Mean		20	11	74	0.337 (0.049)		2.443 (0.301)	0.012 (0.011)		
Mediterranean	5	22	16	93	0.466 (0.071)	0.022	3.142 (0.430)	0.000 (0.000)	10	–
	6	21	15	100	0.553 (0.046)	**0.081**	3.642 (0.341)	0.098 (0.079)	11	–
	11	22	12	79	0.363 (0.068)	**0.032**	2.285 (0.354)	0.000 (0.000)	9	Sparse and dry forest
Mean		22	14	91	0.461 (0.062)		3.023 (0.375)	0.033 (0.026)		
Scandinavia	12	21	20	93	0.411 (0.071)	0.019	3.000 (0.363)	0.000 (0.000)	9	Planted forest
	13	13	11	71	0.386 (0.075)	0.013	2.357 (0.324)	0.000 (0.000)	8	Apothecia present
	35	16	15	86	0.364 (0.060)	0.016	2.500 (0.402)	0.000 (0.000)	10	Apothecia present
	36	22	21	100	0.409 (0.063)	**0.138**	3.357 (0.487)	0.000 (0.000)	10	–
	43	25	19	100	0.404 (0.062)	**0.097**	3.000 (0.377)	0.000 (0.000)	11	–
	44	20	19	93	0.416 (0.072)	0.017	3.142 (0.442)	0.071 (0.071)	**12**	Recreational area
	45	25	17	100	0.330 (0.053)	**0.091**	3.000 (0.432)	0.000 (0.000)	**13**	–
	46	23	21	100	0.481 (0.057)	0.047	3.357 (0.487)	0.076 (0.061)	8	–
	47	24	22	100	0.480 (0.063)	0.042	3.642 (0.607)	0.059 (0.059)	10	–
	48	23	23	100	0.522 (0.056)	**0.082**	3.642 (0.560)	0.186 (0.134)	11	Farm close
	49	24	22	100	0.519 (0.051)	**0.111**	3.857 (0.404)	0.089 (0.069)	11	–

(Continues)

TABLE 2 (Continued)

Region	Population number	*n*	Nonclonal specimens	Polymorphic loci (%)	uh mean (stand. error)	rBarD	AR	PAR	*K*-test	Additional population information
	50	25	24	100	0.455 (0.062)	**0.127**	3.285 (0.437)	0.000 (0.000)	**12**	Apothecia present
	51	11	10	100	0.549 (0.053)	**0.173**	3.000 (0.347)	0.001 (0.001)	6	Apothecia present, trees on a bog
	52	25	25	93	0.427 (0.065)	0.024	3.000 (0.444)	0.000 (0.000)	7	Competition with *Bryoria fremontii*
	53	25	22	100	0.479 (0.048)	**0.071**	3.000 (0.331)	0.000 (0.000)	8	Apothecia present, close to crops
	54	23	19	100	0.581 (0.040)	**0.206**	3.642 (0.427)	0.014 (0.010)	5	Apothecia present
	55	24	22	86	0.388 (0.073)	0.045	2.857 (0.442)	0.019 (0.013)	7	–
	56	22	21	100	0.580 (0.051)	**0.210**	3.571 (0.521)	0.002 (0.002)	7	–
	57	24	22	100	0.588 (0.037)	**0.185**	4.000 (0.419)	0.017 (0.012)	5	Apothecia present, human activities close
	58	21	19	100	0.516 (0.052)	**0.096**	3.428 (0.250)	0.000 (0.000)	11	Competition with *Bryoria fremontii*
	59	25	16	100	0.493 (0.038)	**0.323**	3.357 (0.414)	0.000 (0.000)	8	Apothecia present, competition with *Bryoria fremontii*
	60	24	21	100	0.483 (0.058)	**0.272**	3.571 (0.571)	0.000 (0.000)	8	Apothecia present
	61	22	18	100	0.596 (0.046)	**0.214**	3.642 (0.464)	0.064 (0.064)	4	Apothecia present
	62	24	13	93	0.286 (0.059)	**0.289**	2.714 (0.338)	0.059 (0.059)	**12**	Apothecia present, recreational area
	63	25	22	93	0.330 (0.069)	0.030	2.928 (0.450)	0.000 (0.000)	10	Forest between houses
Mean		22	19	96	0.459 (0.057)		3.234 (0.430)	0.026 (0.022)		

Khi^2 statistics = 0.33, $p > .05$) and was present whatever the detected extrolites. However, this fungus was observed in gene pools 1 and 2 but not on the reduced number of specimens in gene pool 3.

Geographic distribution of the gene pools (Figure 2) shows that gene pool 3 (green) is restricted to northern Scandinavia, whereas gene pools 1 (blue) and 2 (orange) are distributed across the entire continent with an identical overlapping pattern. Gene pool 1 was, however, not detected in Cyprus, Great Britain, Portugal or Sicily, but this result must be treated with caution due to the lower sampling density in those regions. No specimen of gene pool 1 was, however, detected in populations growing on rocks (4, 20, and 21).

3.4 | Analyses of potential distribution

The most relevant and independent environmental variables, BIO1, 2, 4, 8–12, 15, 18 and 19, were used in Maxent, but predicted distributions were very similar if all variables were used. AUC values for clusters 1, 2 and 3 were 0.98, 0.98 and 0.96, respectively, indicating a highly supported predicted distribution. *Bryoria fuscescens* is generally absent from lowlands, with the exception of the highly oceanic areas of Norway and northern parts of Great Britain (Figure S2).

Some unsampled areas in our study were predicted as adequate for *B. fuscescens*, such as the Lebanese and Turkish mountains, the Ethiopian highlands, the Balkan Peninsula and the Caucasus, in some of which the species is indeed recorded. On the other hand, the boreal region, from Sweden to Russia, was not predicted as suitable for the species, although it actually exists there. As no populations were collected from that area, their possibly specific environmental conditions could not be considered by Maxent.

Gene pools 1 and 2 do not show significant differences in their potential distributions, whereas the Scandinavian-endemic gene pool 3 is mainly centred on the oceanic parts of Norway, southern Iceland, and the Alps (Figure S2). The four most significant bioclimatic variables

TABLE 3 Rarefied allelic richness (AR) and rarefied private allelic richness (PAR) for each region, with standard deviation in brackets

	Region	Number of specimens	AR	PAR	Number of specimens	AR	PAR
North	Great Britain	32	2.642 (0.439)	0.000 (0.000)	588	7.500 (1.207)	1.357 (0.452)
	Scandinavia	556	7.428 (1.170)	1.357 (0.452)			
Central	Alps	189	5.357 (1.014)	0.357 (0.199)	641	7.071 (1.442)	0.857 (0.274)
	Carpathians	179	5.428 (0.976)	0.000 (0.000)			
	Iberia	273	5.571 (0.976)	0.285 (0.125)			
South	Africa	65	4.571 (0.947)	0.428 (0.227)	130	5.571 (1.087)	0.500 (0.251)
	Mediterranean	65	4.214 (0.575)	0.071 (0.071)			

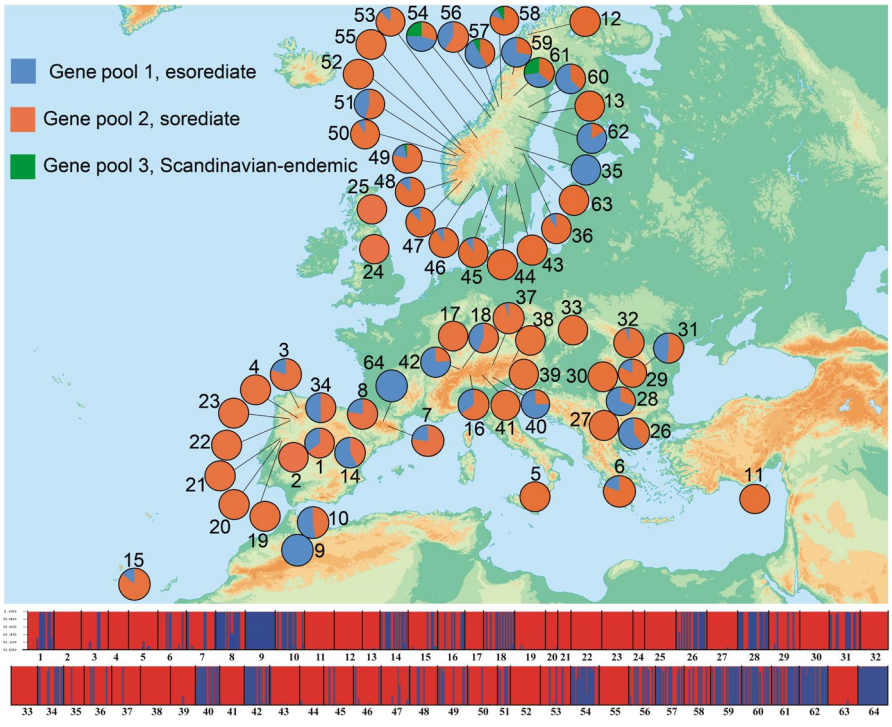

FIGURE 2 Distribution of the gene pools predicted by DAPC analysis across the entire study area. Numbers refer to population identifiers. STRUCTURE output at $k = 2$ ordered by populations is indicated at the bottom, showing that genetic introgression only occurs on few specimens. Gene pool 3 (green) identified by DAPC clusters is together with gene pool 1 (blue) in STRUCTURE analyses

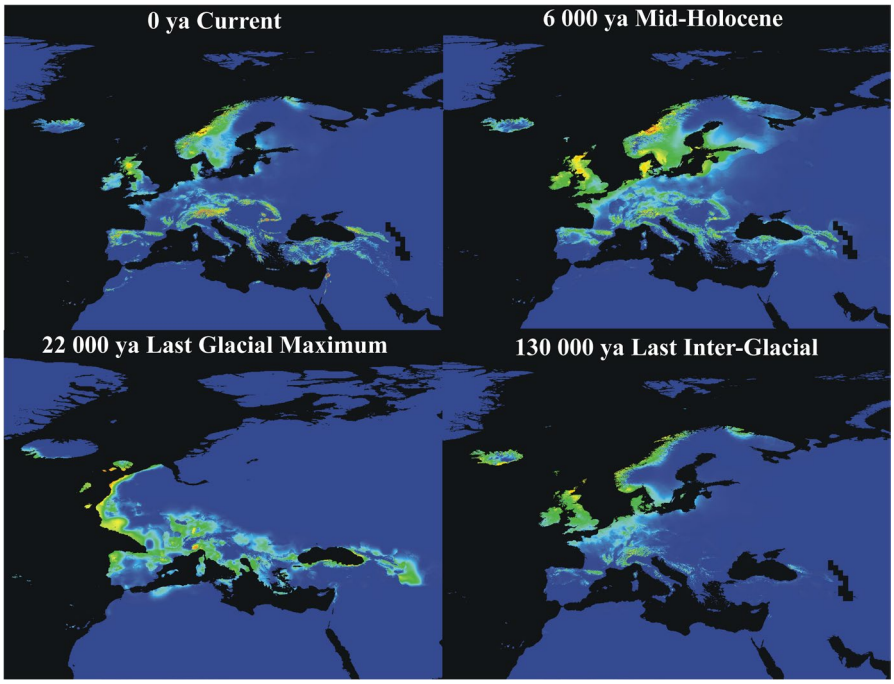

FIGURE 3 Potential distribution maps suggested by Maxent for *Bryoria fuscescens* during the most significant past climatic events in Europe. Ya, years ago

contributing to the prediction were, in order of importance: cluster 1: BIO10, BIO8, BIO1 and BIO19; cluster 2: BIO10, BIO1, BIO19 and BIO8; and cluster 3: BIO2 (twice as important as the next variable), BIO15, BIO8 and BIO18. Variables BIO 1, 2, 10 and 8 are related to temperature, whereas BIO 15, 18 and 19 are related to precipitation.

Analyses of past bioclimatic maps showed that the potential distribution pattern was different during the Last Interglacial period (around 130,000 ya), when the climate was warmer (Figure 3). During that period, the distribution shrank in southern Europe and especially in the southeast in the Balkans, Carpathians, Turkey, and Caucasus. Apart from Iceland, no new adequate land extension was identified. The potential distribution evidently underwent the strongest alteration during the Last Glacial Maximum (22,000 ya) with *Bryoria* distribution was reduced to lower elevations and latitudes (Figure 3). No suitable habitats were inferred in Scandinavia at that time, while the west coast of Central Europe and the lowlands of the Alps were suggested as the most suitable areas. During the Mid-Holocene (6000 ya) the potential distribution was estimated to be similar to the current distribution, although with a slightly larger area of occurrence.

3.5 | Spatial analyses

The isolation by distance analysis showed a positive correlation for gene pool 2 (sorediate, $r = .246$, $p = .001$), but this was only marginally significant for gene pool 1 (esorediate, $r = .147$, $p = .054$; Figure 4). In both cases, the most distant pairs of populations were not the most genetically isolated, the most genetically isolated occurring at various geographic distances.

The estimation of the migration rates (M) among the 38 tested putative migration routes, and the effective population size (Θ) of the recipient populations, are both shown in Table S8. Comparisons of migration intensity among regions using the 2Nm value ($M \times \Theta$) are shown in Figure 5. A high migration rate from Scandinavia to the southern areas is shown, with the main migration route going from Scandinavia to Central-Southern Europe.

From the 946 multilocus genotypes of the data set, 130 (13.7%) were identified as geographically restricted ($p < .05$, Figure S3). The mean observed geographic distance between restricted alleles was 1690 km, while the expected distance under panmixia was 1712 km ($p < .001$), just 22 km more. This suggests that *B. fuscescens* populations are close to a complete panmixia across the study area.

3.6 | Phylogenetic analysis

The phylogenetic reconstruction (Figure S4) showed the same topology as the one obtained in Boluda et al. (2018), with the three species of the *Bryoria fuscescens* complex supported and originating from a tritomy. Both morphs of *B. fuscescens* appeared intermixed. The 34 phenotypically variable specimens included from this study revealed that individuals L54.19, L54.20 and L58.08 cluster with the cryptic species *B. pseudofuscescens*, whereas all the other samples match with *B. fuscescens*. GAPDH marker had no successful amplifications in specimens from gene pool 3 (L54.19, L54.20, L58.08, and L58.17), even after repeated DNA extractions and the use of different PCR conditions.

The gene-tree reconstruction of the eight loci revealed topological conflicts between all trees except those from ITS, IGS, and GAPDH (Figure 6). Consequently, each FRBi marker was analysed separately. The coalescent STACEY species tree (Figure 6) improved the overall resolution, but it did not resolve the *capillaris* and *fuscescens* morphs as monophyletic or even paraphyletic clades. Algal ITS sequences showed that all samples contained the *Trebouxia simplex* group as

the algal partner. Two algal lineages were distinguished, one for gene pools 1 and 2 (with five haplotypes) and the other for gene pool 3 (with two haplotypes). Specimen L58.17, with genetic characteristics intermediate between *B. fuscescens* and *B. pseudofuscescens*, contained both lineages, as revealed by the presence of peaks of equal intensity for variable sites in the electropherogram (confirmed twice).

4 | DISCUSSION

4.1 | Dispersal capacities and false panmixia

Two major gene pools are present in *Bryoria fuscescens*, one without soralia for 99.4% of the specimens (gene pool 1), and the second one predominantly with soralia (68.3% of the specimens, gene pool 2). Soralia produce soredia, which are joint propagules comprising both the algal and fungal partners; soredia measure around 15–60 μm in diameter and have a rather limited dispersal capacity (Walser, 2004; Werth, Wagner, Gugerli, et al., 2006; Werth, Wagner, Holderegger, et al., 2006). However, as they are produced by billions in the populations, a priori improbable long-distance dispersion should occur with relative frequency. Small broken-off branches may also act as dispersal agents in both gene pools, but continental-scale displacements should be considered highly exceptional and only mediated by birds, which also can distribute soredia over long distances (Bailey, 1976). The rarity and doubtful functionality of apothecia, make ascospores unlikely to be an effective dispersal method for both gene pools. According to the isolation by distance analysis, which showed similar dispersal capacities for the two gene pools (Figure 4), soredia can only be interpreted as inefficient dispersal agents over large

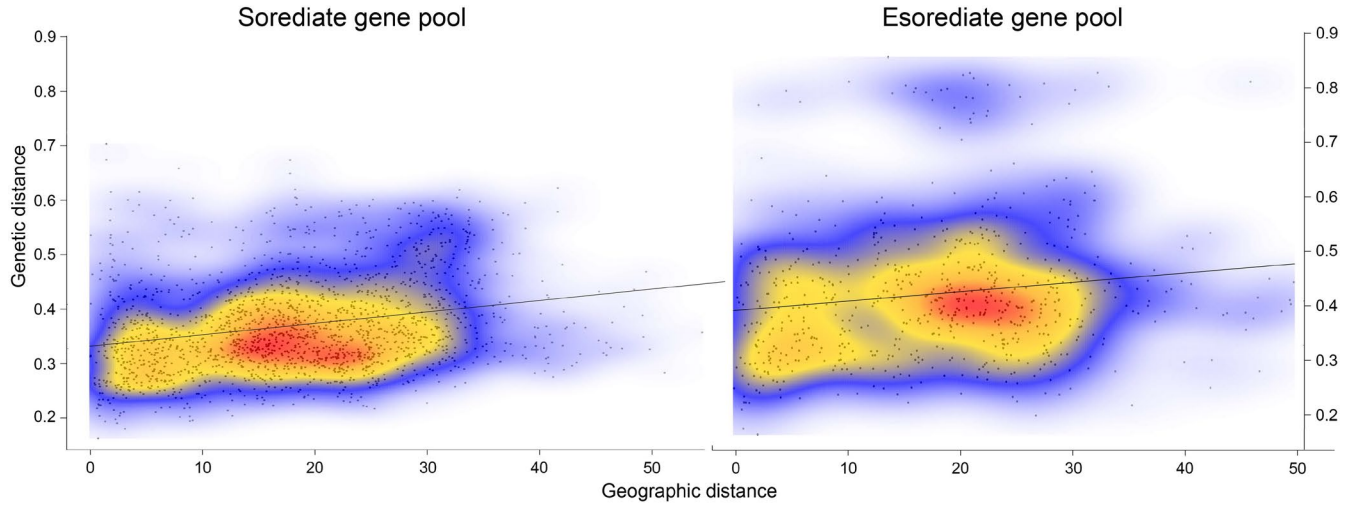

FIGURE 4 Isolation by distance plots for pairs of populations in gene pool 1 (esorediate, $r = .147$, $p = .054$) and gene pool 2 (sorediate, $r = .246$, $p = .001$)

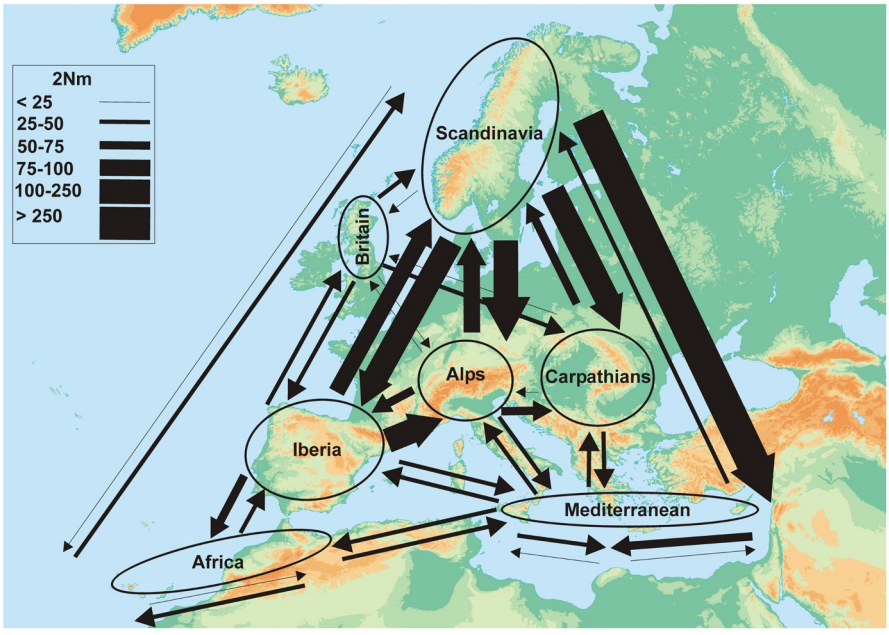

FIGURE 5 Migration patterns inferred from Migrate analysis. Arrows represent the number of immigrants per generation ($2Nm = M \times \Theta$). Arrow width is proportional to the degree of immigration into a region

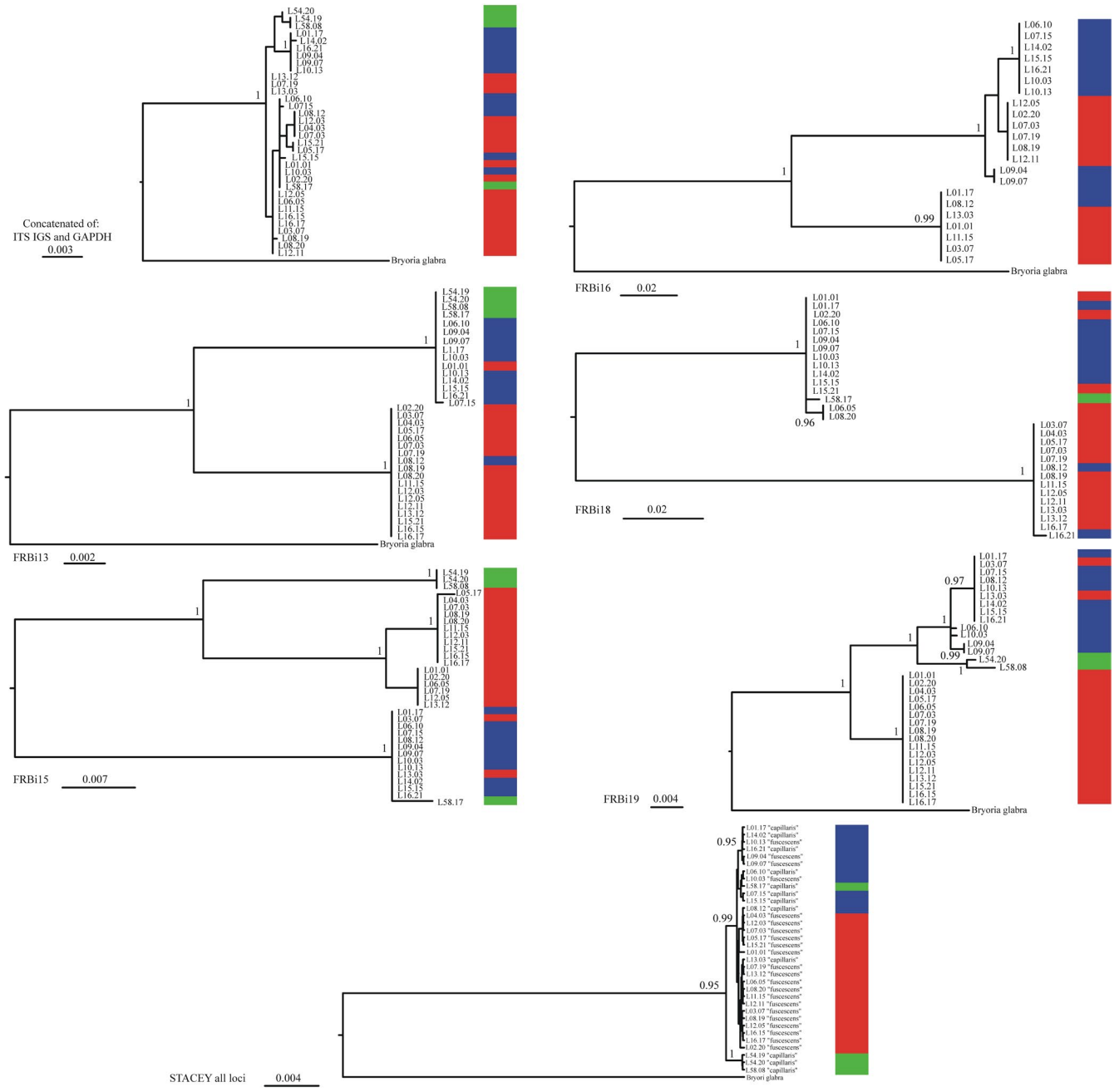

FIGURE 6 Bayesian and coalescence (STACEY) phylogenetic tree reconstruction of 35 selected specimens using a concatenated DNA matrix of ITS, IGS and GAPDH, and the five microsatellite flanking regions FRBi13, 15, 16, 18 and 19. The DAPC *k*3 gene pool assignment for each specimen is indicated with the lateral colour bars. Gene pool 1, blue; gene pool 2, red; gene pool 3, green. Morphospecies are indicated in the STACEY tree

distances. This assumption is striking and contraintuitive, especially considering that in other taxa, sorediate species did not show stronger population differentiation than species with ascospores (Lagostina et al., 2021). That indicate that soredia may be as efficient as spores when not only the dispersion but also the establishment of the propagules is considered (spores mortality may be higher as they need to find the appropriate photobiont, while soredia do not).

With the exception of Scandinavia, today *B. fuscescens* forms rather isolated populations across Europe, especially in the southernmost regions where it is restricted to small areas on mountains. With this island-like distribution, geographic distance should act as an efficient dispersal barrier. Still, our results (Figure S3) yield a strong panmictic signal. If propagule dispersion is the main factor explaining panmixia, as suggested by MIGRATE (Figure 5), closer populations should be genetically much more similar than distant ones, at least at the continental scale. Such a tendency was however only subtle in the isolation by distance analysis (Figure 4). If birds act as vectors for the dispersal of *Bryoria* fragments and soredia, migration might be much more probable over short distances than over longer ones, which is not the case, therefore excluding birds as significant vectors

of dispersion. On the other hand, if ancestral shared haplotypes exist, remote pairs of populations should not be much more differentiated than closer ones, as indeed observed in the isolation by distance analyses (Figure 4; Muir & Schlötterer, 2005). Additionally, no significant difference was detected in this regard between specimens producing soredia and those lacking such propagules (Figure 4). Finally, the presence of ancestral shared haplotypes was also detected at the DNA sequence level, as shown by the incomplete lineage sorting in FRBi markers (Figure 6; Hartl & Clark, 2006; Maddison, 1997). All these results therefore support the hypothesis of ancestral shared alleles rather than that of an effective panmixia.

Haplotypes originating in small populations, as in glacial refugia, can easily be fixed, or can, at least, spread throughout a population (Hartl & Clark, 2006). Then, after ice retraction, such populations colonized wide areas, dispersing the previously restricted alleles at the continental level, as demonstrated in spatial expansions (Klopfstein et al., 2006). With past human activities and climate warming, the populations became isolated, and the Central and Southern European lowlands acted as effective dispersal barriers. Putative ancestral alleles, once spread over Europe as a result of rapid population expansions, are currently present in distant isolated populations. As it is not possible to distinguish between current migration and ancestral shared alleles, Muir and Schlötterer (2005) have shown that such analyses can be misleading and overestimate dispersal capacities.

The presence of ancestral shared haplotypes has previously been suggested for other lichens of the same family to explain the existence of similar genotypes in different continents (Fernández-Mendoza et al., 2011; Printzen et al., 2003). This hypothesis is strongly supported here by the existence of infraspecific lineages displaying different reproductive strategies but still comparably low levels of isolation by distance. The ancestral shared allele hypothesis is further supported by the R_{ST} values being systematically lower than the F_{ST} values, with the same pattern occurring for R_{CT} and F_{CT}. This indicates that populations harbour alleles that differ greatly in their number of repetitions, and/or that similar alleles can be found everywhere.

Bryoria fuscescens is a young species (Boluda et al., 2018), and therefore expected to be prone to ancestral polymorphism and incomplete lineage sorting among populations, even from distant regions (Charlesworth et al., 2005; Naciri & Linder, 2015). However, the recent origin implies that it quickly colonized distant areas, such as North America and Central Africa, indicating a high dispersal capacity. We suggest that the pattern of panmixia we observe today is probably the result of a combination of a widely distributed ancestral polymorphism and efficient dispersal capacities, even for lineages that currently do not produce any known dispersal propagules. The proportion of ancestral shared alleles thus appears to be high enough to mask any migration signals and to bias phylogeographic reconstructions (see section below).

4.2 | Post-glacial recolonization

The origin of *B. fuscescens* is estimated at approximately one million years ago (Boluda et al., 2018), indicating that the current genetic distribution must have been strongly affected by glacial cycles, including the Last Glacial Maximum (LGM) around 22,000 ya (Clark et al., 2009, 2012). The LGM potential distribution map predicts putative refugia along the coasts of the British Isles, the north-western Iberian Peninsula, and the lowlands of the Pyrenees and the Alps (Figure 3). This parallels the high levels of private alleles detected in the Alps and Iberia, but not the low diversity in the few samples from Great Britain, or the high Scandinavian richness.

Great Britain was expected to show remnants of the high genetic diversity harboured there during the LGM, but ancient human clearance of the British forests (Woodbridge et al., 2014), and air pollution in the last 250–300 years, may have led to the extinction of the haplotypes that originated there (Hawksworth, 1972). However, as this alteration affected Central Europe in the same intensity (Kaplan et al., 2009) but regional endemic haplotypes are still present, the Great Britain diversity could be strongly subestimated by our limited sampling missing the endemic haplotypes, especially in the relict native pine woods of Scotland where more studies should be carried out.

Eastern Europe and the Carpathians have previously been found to be inadequate for *Bryoria* during the LGM, probably owing to their drier environment (Petit et al., 2003; Tzedakis et al., 2013). This may explain the unexpected absence of private alleles in this area (Table 3), suggesting that the original populations became extinct and that the current ones originate from recent colonization. However, it is also conceivable that strong demographic bottlenecks have resulted in the loss of rare and local alleles in Carpathian populations.

Three nonexclusive reasons may explain the high diversity found in Scandinavia. First, according to our past potential distribution map and the literature, Scandinavia was covered by ice during the LGM (Ehlers & Gibbard, 2004) and while some rock outcrops may have acted as refugia, major colonization by *Bryoria* must have occurred after the ice-sheet retreat from different source populations. Therefore, the richness of this region could be the result of admixture, as suggested for other organisms (Petit et al., 2003). For instance, the current Scandinavian populations could have come mainly from British refugial populations, which would then explain why its alleles appears also in Scandinavia, but they could equally have come also from other places not sampled in this study. Although not shown in the past potential distribution maps, a possible contribution of Russia to the Scandinavian diversity has to be considered, especially from refugial forests in western Russia, close to Moscow (Normand et al., 2011). Many boreal forest species are known to have colonized Northern Europe from this area, such as Norway spruce (Parducci et al., 2012; Tollefsrud et al., 2008, 2009), beetles of the genus *Pytho* (Painter et al., 2007), the brown bear (Taberlet & Bouvet, 1994), and possibly the lichen *Lobaria pulmonaria* (Widmer et al., 2012). This could also be the case for *Bryoria fuscescens*, as it is frequently linked to Norway spruce forests. Samples from Russian forests would, however, be needed to test this hypothesis.

The second explanation is that Scandinavia was a cryptic refugium according to the classic view that genetically rich areas typically indicate glacial refugia (Hewitt, 2000). While some studies suggest northern glacial refugia for tree species (Bhagwat & Willis,

2008; Parducci et al., 2012), it seems that the northern tree limit was at 49°N during the LGM (Killis & Andel, 2004; Killis et al., 2000; Tzedakis et al., 2013). Therefore, a Scandinavian refugium for *Bryoria* as an epiphytic species would hardly be expected. Conversely, *Bryoria* could have persisted on small shrubs, tundra soil, and especially rock surfaces on cliff faces or even nunataks, as is currently the case in Canada and Greenland (Brodo & Hawksworth, 1977).

A third explanation could be the extent of human-mediated deforestation. While central and southern Europe have been strongly affected during the last centuries or millennia, Scandinavia remained as the least deforested European region (Kaplan et al., 2009). That could result in a reduction of the original diversity of the southern populations while the northern ones reminded largely intact.

The main migration routes identified were between Scandinavia and Central-Southern Europe (Figure 5), especially from north to south, a direction that does not match with the estimated ice cover and the species past distribution. Considering the ancestral shared alleles hypothesis explained above, however, allows us to suggest a scenario that reconciles the low IBD, the recorded panmixia, estimated past species distribution and our present knowledge about the extent of ice cover. That scenario is of: migration routes from the British, southern European and Russian refugia (Figure 3) where haplotypes originated, northwards to Scandinavia. Under such a scenario, the ancestral haplotypes currently shared between Scandinavia and the Iberian Peninsula would be falsely detected by MIGRATE as evidence of recent migration. Similarly, a colonization from a Russian refugium to Scandinavia and the eastern Mediterranean would explain the strong migration routes detected by MIGRATE between these areas, that also share the same haplotypes. Shared haplotypes between all these areas, which are isolated today, would misleadingly be identified as recent migration in our analyses as the genetic imprint produced by migration or shared ancestral alleles among areas is indistinguishable (see section "Dispersal capacities and false panmixia"; Muir & Schlötterer, 2005). Consequently, the north to south continental migrations suggested by our analyses would be misleading as to how ancestral populations recolonized the continent. Considering the boreal preferences of the *Bryoria* complex, it can be suggested that recent migration might be much lower than estimated and that postglacial recolonization occurred most probably the other way around.

We therefore conclude that Northern Europe must have been recolonized by *B. fuscescens* haplotypes from western, southern and perhaps eastern glacial refugia, a scenario that may explain the high diversity in Scandinavia, whether or not putative northern refugia existed there. This is in agreement with some hypotheses suggesting that genetically richest areas do not always indicate glacial refugia, but rather multiple colonizations from different refugia (Petit et al., 2003).

4.3 | Sexuality

Although considered an asexual lichen, *Bryoria fuscescens*, shows a high genetic variability, and completely clonal populations were not detected, with the exception of those on rocks. Populations growing on isolated rock outcrops seem to be more susceptible to extinction than those growing on trees in forests. Therefore, repetitive extinction–colonization events may inhibit the development of genetically richer saxicolous populations.

In general, clonality levels were lower in Scandinavia, where the species is most abundant and apothecia are more common (Table 2). The lowest detected genetic diversity was in Portugal, in populations with large apotheciate specimens. Although they look suitable for *Bryoria*, the sampled Portuguese habitats showed signs of recent deforestation, indicating that they represent modern colonization events, perhaps, following earlier local extinctions.

According to Pizarro et al. (2019), *Bryoria fuscescens* should be considered a heterothallic species, meaning that it cannot inbreed. Hence, the presence of specimens with apothecia would be expected to considerably increase the genetic diversity within populations. In our study, however, populations with apothecia did not show higher genetic diversity than populations lacking them, and 15 of the 22 apotheciate populations displayed no molecular signals of sexual reproduction. Putative sexual reproduction was furthermore detected in eight non-apotheciate populations (33, 38, 39, 46, 47, 52, 55, 54), demonstrating that apothecia may not be an important factor in enhancing genetic diversity. Such a discrepancy between expectations and observations might be due to sexual reproduction signals in today's populations being the result of ancient sexual reproductions, or being hidden by ancestral shared alleles (see section "Dispersal capacities and false panmixia"). This hypothesis is supported by the observation of Brodo and Hawksworth (1977) in which when apothecia are found, they do not always contain spores and so are only exceptionally likely to lead to effective sexual reproduction between individuals of *capillaris* and *fuscescens* morphs.

4.4 | Population diversity

Private alleles were detected in isolated populations as might be expected, such as in the Canary Islands, Morocco and Greece, where geographic barriers may impede the spread of local haplotypes. However, other geographically isolated populations, such as that from Cyprus, do not contain any private allele. Possible explanations are ancestral haplotype retention and, more likely, recent colonization events from continental Europe. On the other hand, nearby populations that are expected to be connected by migrants, such as populations 16, 17, 37, 40 and 42 in the Alps and 46, 47, 48 and 49 in Scandinavia, contained private alleles in addition to the high genetic structure found in these areas (Table 2), suggesting an ancient presence of the populations.

In many populations where evidence of human activities was recorded, such as recreation areas, roads and altered forests, allelic richness was not lower than the average (2.93), for instance in populations 12, 16, 26–29, 40, 44, 48, 53, and 57. While forest management decreases the genetic diversity of many lichen species (Jüriado et al., 2011), it may be maintained in *Bryoria* because the high light

requirements of this species match the conditions recorded in road margins or cleared forests (Arseneau et al., 1997; Campbell & Coxson, 2001). Areas close to roads or at the margin of the forests, usually contain a larger *Bryoria* community than inside forests, especially in dense ones. Lichens are usually very sensitive to an excess of nutrients; nevertheless, unpaved dusty roads or isolated trees in nutrient-rich pastures seemed to have only a limited negative impact on *Bryoria* in rainy areas such as Scandinavia. Populations 27, 29, 39, 48, 53, and 57, in which anthropogenic eutrophication was obvious, contained higher allelic richness than the average, suggesting that under certain conditions, a modest addition of extra nutrient contribution is not unfavorable. Furthermore, human activities, especially leading to open forests, may increase dispersal distances (Werth, Wagner, Gugerli, et al., 2006; Werth, Wagner, Holderegger, et al., 2006; Zoller et al., 1999), thereby enhancing population growth and genetic diversity.

4.5 | Phenotypic plasticity

Bryoria fuscescens is a recent species with two main morphologies that, in the past, were treated as distinct species, the *fuscescens* and *capillaris* morphs (Boluda et al., 2018; Brodo & Hawksworth, 1977; Myllys et al., 2011). Gene pool 1 from our study can be considered to represent the *capillaris* morph group, lacking soralia and fumarprotocetraric acid but frequently containing barbatolic and alectorialic acids, although 13% of these specimens had the dark *fuscescens* appearance and lacked the last two acids. Gene pool 2 matches the *fuscescens* morph group, with the exception of 13% that had *capillaris* phenotypes – with or without soralia and containing alectorialic, barbatolic, and fumarprotocetraric acids (Table S7). There is a tendency for the nonmatching phenotypes to be included in the *capillaris* gene pool if they were southern *fuscescens* morphs (e.g., Moroccan populations) and in the *fuscescens* gene pool if they were northern *capillaris* morphs (e.g., Scandinavian Peninsula).

Myllys et al. (2016) found that the *fuscescens* morph was more abundant in northern areas of Finland while the *capillaris* morph becomes rarer when going north. On the contrary, in southern European areas, the *fuscescens* morph is much more common than the *capillaris* one. However, the Moroccan populations, while composed solely of *fuscescens* morphs, contain many specimens genetically belonging to the *capillaris* gene pool. The increasing frequency of *fuscescens* morphs in northern Finland might be related to more open and illuminated forests. Indeed, the basal portion of the *fuscescens* morph thallus, which is often protected from light when growing on trunks, has frequently paler or whiter colours (Brodo & Hawksworth, 1977; Hawksworth, 1972). This suggests a partial environmental plasticity within gene pools, with individuals producing more melanic pigments for light protection in areas exposed to direct sunlight, but becoming more pallid in Scandinavian shadowy forests. On the other hand, Esseen et al. (2017) found that the *capillaris* morphs often dominate well-lit tree-tops in north-eastern Sweden, whereas the *fuscescens* morphs were mainly found on more shaded lower branches mixed with a few *capillaris* morphs. This pattern is not completely in agreement with our observations in Norway, where *capillaris* morphs were usually abundant in lower shadowy canopies, reflecting other ecological factors discussed by Esseen et al. (2017) as the colonization, production, and ventilation hypotheses.

4.6 | A current speciation process

When a species experiences a phenotypic diversification that is faster than the evolution of the genes used in the phylogeny, ancestral shared polymorphisms are expected (Charlesworth et al., 2005; Naciri & Linder, 2015; Vanderpoorten & Shaw, 2010). Genetic data may indicate the presence of polyphyletic species in such cases, even if it is truly monophyletic (Naciri & Linder, 2015; Petit & Excoffier, 2009). Microsatellites, because of their high mutation rates, can reveal lineages that would not be seen using genes or loci with lower mutation rates (Jarne & Lagoda, 1996). When microsatellite gene pools delimit phenotypes clearly, they are frequently considered as distinct species (Edwards et al., 2008; Hrbek et al., 2014; Lagostina et al., 2018). In *B. fuscescens*, the two gene pools are only partially (~87%) linked to the *fuscescens* and *capillaris* morphs. This suggests that the two morphs are not distinct species, in agreement with the conclusions of Boluda et al. (2018). However, since the correlation is so high the two morphs may be considered as being in the process of speciation.

The *Bryoria* species studied here belongs to a wider complex comprising three taxa (*B. fuscescens*, *B. kockiana* and *B. pseudofuscescens*) that all differentiated around one million years ago (Boluda et al., 2018). These three species are so closely related, that they are at the limit of being interpreted as one single species. Consequently, it is not appropriate to recognize the *fuscescens* and *capillaris* morphs as separate species since the level of differentiation between them is below that distinguishing the other two accepted species. The ancestral taxon of these three species most probably lacked soralia, as they only appear in *B. fuscescens* gene pool 2. We hypothesize that this ancestral taxon may have first appeared in north-western North America, the region that harbours the largest number of *Bryoria* species in the world, and where *B. glabra*, the sister species of the complex, is more common. At some point, around 1 million years ago (Boluda et al., 2018), the ancestral species reached Europe. The Atlantic and Siberian barriers would subsequently have impeded migrations, gradually removing the ancestral shared alleles among continents and leading to a speciation event giving rise to what we now know as *Bryoria fuscescens* in Europe, and as *B. kockiana* and *B. pseudofuscescens* in North America. Isolation produced a strong decrease in shared microsatellite alleles among species, but a slighter decrease in alleles for loci with lower mutation rates such as the ones being used as standard barcoding genes. However, some ancestral shared alleles or intermediate lineages must have remained between North American and European lineages, as demonstrated in our data by the genetically intermediate Scandinavian-endemic gene pool 3. Nevertheless, the Trøndelag area, where genepool 3 is found, is known for its biogeographic connections with North

America, and Norwegian populations would then be the results of a migration event or more probably vicariance (Printzen et al., 2003). According to these results, *B. pseudofuscesens* is then no longer a North American endemic species. This gene pool is also of interest as it retains different haplotypes of the algal partner compared to that found in *B. fuscescens*.

In Europe, *Bryoria fuscescens* has differentiated into two lineages, one sorediate and mainly composed by *fuscescens* morphs, and another esorediate, mainly containing *capillaris* morphs. Subsequently, sorediate specimens from Europe evidently reached North America, establishing at least one known sorediate population of *B. fuscescens* morph *fuscescens* (Boluda et al., 2018).

The recent *fuscescens* and *capillaris* morphs, although slightly specialized in different niches (Esseen et al., 2017; Myllys et al., 2016), can generally be found growing together and even intermixed. This indicates that, at least in many environments, these phenotypes are not in direct competition as an increase in fitness of one of the two morphs would have resulted in either the elimination of intermediate phenotypes, or an ecological niche differentiation. As the group may be considered asexual, hybridization is not probable and the phenotypes can be expected to become more isolated with time, allowing a full speciation into two morphologically and genetically well delimited species in the future.

Interestingly, the North American *Bryoria pseudofuscescens* also seems to be experiencing an ongoing speciation process towards one morph similar to *capillaris*, with pale colours, no soralia and barbatolic and alectorialic acids, and another morph like *fuscescens*, usually with dark colours and frequently containing fumarprotocetraric acid but lacking soralia (Boluda et al., 2018; Brodo & Hawksworth, 1977; Velmala et al., 2014). However, this group has only been poorly studied by Velmala et al. (2014) and Boluda et al. (2018), and additional phenotypic and genetic analyses are needed to test whether those two morphs appeared before or after the speciation of *B. fuscescens* and *B. pseudofuscescens*. A parallel open issue is to test whether sexuality was ancestral in this group and subsequently evolved into at least two mainly asexual species (*B. fuscescens* and *B. pseudofuscescens*). This would mean that particular morphs are ancestral and persisted through the speciation process, while the alternative hypothesis would be to assume that parallel morphs appeared after speciation and are examples of phenotypic convergence.

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AUTHOR CONTRIBUTIONS

Research design: Carlos G. Boluda, David L. Hawksworth, Victor J. Rico and Christoph Scheidegger Sampling: Carlos G. Boluda, Victor J. Rico, Christoph Scheidegger and David L. Hawksworth. Data analysis: Carlos G. Boluda. Writing the manuscript: Carlos G. Boluda, Victor J. Rico, Yamama Naciri and David L. Hawksworth.

CONFLICT OF INTEREST

Carlos Galan Boluda, Víctor Jimenez Rico, Yamama Naciri, David Hawksworth and Christoph Scheidegger confirm they have not any potential source of conflict of interest.

DATA AVAILABILITY STATEMENT

All data generated and used in this study are provided in the text, tables or Supporting Information.

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SUPPORTING INFORMATION

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