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RESEARCH

Glacial relict *Sphagnum fuscum* at the Pyrenean rear-edge: Genetically depauperate and highly differentiated populations

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Abstract
Cold-adapted species often persist at their rear-edge in isolated refugia, where they are especially vulnerable to climate change. These populations frequently harbour unique genetic lineages and local adaptations, making them critical for biodiversity conservation. However, fine-scale intraspecific genetic and ecological data are rarely integrated into conservation planning. For the locally threatened peat moss *Sphagnum fuscum* at its southern rear-edge in the Pyrenees, we aimed to reveal its regional demographic status and genetic structure in order to inform conservation strategies. We genotyped 104 shoots from seven Pyrenean populations using 16 nuclear microsatellite markers. Genetic patterns were analysed using AMOVA, STRUCTURE, PCoA, MSN, and UPGMA and Complete Linkage dendrograms based on Bruvo's distance. We also estimated population sizes and surveyed sporophyte production in the field. Pyrenean populations exhibited extremely low within-population diversity and complete genetic distinctiveness among them. Pyrenean genotypes shared a private allele distinctive from the populations of Canada and Norway. Sporophyte production was widespread, although many populations were monoclonal, probably consistent with regional monoicy. Pyrenean populations likely constitute an evolutionary significant unit, which should be prioritised in conservation planning. Our results provide a basis for designing a genetic rescue by promoting gene flow among these isolated populations.

Keywords Bryophyte conservation · Climate change · Glacial legacy · Peat moss · Refugia · Sexual system

Introduction

Climate changes and associated shifts in species distribution have been recurrent phenomena throughout the Earth's history. In particular, Quaternary glacial-interglacial cycles (2.58 million years ago to the present) have driven repeated altitudinal and latitudinal shifts in species ranges, shaping the current distribution of biodiversity (Hewitt 2000, 2004). In Europe, during the Last Glacial Maximum (LGM, ca. 21,000 years ago), temperate flora retreated to glacial refugia in the northern Mediterranean Basin, from where they expanded during the current interglacial (Taberlet et al. 1998; Hewitt 2000; Eidesen et al. 2013). Conversely, cold-adapted species became widespread across periglacial landscapes that extended during the LGM between southern mountain ranges—such as the Pyrenees, Alps, and Balkans—and the northern ice sheet (Nève and Verlaque 2009). Many of these cold-adapted taxa now exhibit arctic–alpine distributions, thriving at high latitudes and surviving in isolated populations within mountain refugia at lower latitudes (Varga and Schmitt 2008). The latter, "rear-edge" populations, are geographically isolated from the species' main distribution and are often surrounded by unsuitable habitat.

Today, the biosphere is undergoing a biodiversity crisis, with extinction rates reaching unprecedented levels

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(Ceballos et al. 2015). Among the major drivers is anthropogenic climate change, which is already causing shifts in species ranges (Chen et al. 2011; Vitasse et al. 2021; Lenoir and Svenning 2015) and the extinction of particularly vulnerable taxa or populations (Wiens 2016). Many studies have attempted to predict how these rear-edge populations will respond to this rapid climate change (Jiménez-Alfaro et al. 2016; Vilà-Cabrera et al. 2019).

According to the Centre-Periphery Hypothesis (CPH), rear-edge populations are prone to extinction as they remain confined in small, fragmented and isolated enclaves where there is a great degree of environmental stress (Brown 1984; Safriel et al. 1994). In addition, they harbor small effective population sizes and a reduced intra-population genetic variation, but a high inter-population genetic diversity (Safriel et al. 1994; Jiménez et al. 2010). These factors put them at risk from demographic stochasticity and reduce their potential to adapt to new conditions. Indeed, the fossil record shows that extinction commonly occurred at rear-edge populations, particularly during periods of rapid climate change (Davis and Shaw 2001).

However, the existence of microrefugia—small areas with stable, favorable environmental conditions amidst unfavorable regional climates—may enable populations to persist in the rear-edge. Moreover, the performance of rear-edge populations may deviate from the CPH predictions because, as reported in several studies, this hypothesis may overlook the effects of ecological interactions, specific life-traits, and local adaptations (Hampe and Jump 2011; Vilà-Cabrera et al. 2019). As a result, many rear-edge populations may remain stable for the long term despite being close to their climatic tolerance limit, becoming climate relicts, remnants of formerly widespread taxa that now persist in stable refugia (Woolbright et al. 2014).

These climate relicts may have been exposed to severe selective pressures, developing remarkable local adaptations, such as phenological shifts and increased physiological tolerance (Hampe and Petit 2005). In addition, bottlenecks and genetic drift have probably driven the evolution of relicts (Woolbright et al. 2014). Both processes, which are not exclusive, have led to patterns of genetic diversity with high levels of variation and uniqueness (Safriel et al. 1994). Therefore, rear-edge populations on southern refugia (e.g., the Pyrenees, Alps, and the Balkans) might harbor more diversity than populations at the center of their area of distribution (Nève and Verlaque 2009). If this is the case, their uniqueness and antiquity renders them disproportional relevance for the conservation of genetic diversity, phylogenetic history, and evolutionary potential of species (Hampe and Petit 2005). Additionally, understanding rear-edge populations is a key factor to improve predictions of the species range shifts driven by ongoing climate change (Thuiller et al. 2008; Hampe and Jump 2011; Woolbright et al. 2014).

The rear-edge populations are not distributed equally across the geography and landscape. In particular, mires (peat-forming wetlands) are habitats rich in relicts (Hampe and Jump 2011; Hájková et al. 2015; Jiménez-Alfaro et al. 2016). Their harsh abiotic conditions (anoxia, extreme pH, and nutrient-poor soils) may limit the encroachment of competitors, allowing mire specialists to persist, leading to the creation of biotically limited relicts (Hampe and Jump 2011). In addition, in a fine-scale, mires act as climatic buffers generating more stable microclimatic conditions (Fernández-Pascual et al. 2015). Despite their resilience, the current dramatic global warming and the reduction of summer precipitation—which will be of particular significance in the Mediterranean Basin (Liu et al. 2022)—may affect the hydrological conditions of mires. A decline in the water table in mires would threaten them, especially those that occur at the rear-edge of the habitat range (Keith et al. 2014), where most of the relicts occur.

Bogs are a specific type of mire characterized by being ombrotrophic, i.e. fed only by precipitation rather than groundwater (minerotrophy) (Lappalainen 1996; Joosten and Clarke 2002). In temperate, boreal, and subarctic regions, the vegetation of bogs is dominated by *Sphagnum* mosses, also known as peat mosses. This genus, of cosmopolitan distribution (Michaelis 2019), has an important role as an ecosystem engineer due to its ability to create an acidic, nutrient-poor, and anoxic habitat that is unsuitable for most other plants, which at the same time improves its fitness outcompeting for light (Breemen 1995). This habitat's extreme conditions boost its potential as a sink of biotically limited relicts (Slowinski 2014). Some *Sphagnum* species form clonal colonies known as hummocks—compact, elevated clumps of moss—which are often composed of one or very few genets (Gunnarsson et al. 2007; Shaw et al. 2023). Hummock formation is particularly common among some ombrotrophic species, as it helps them remain above the water table in nutrient-poor, acidic environments (Daniels and Eddy 1990). Bogs cover vast areas of boreal and subarctic zones, but they are scarce and fragmented in southern Europe, primarily confined to high-altitude mountain systems, where they may be reduced to isolated *Sphagnum* hummocks embedded in fen matrices (minerotrophic mires). Beyond their ecosystem-engineering role, *Sphagnum* mosses are increasingly recognized as a valuable eco-evolutionary system for studying links among genetic variation, functional traits, ecological differentiation, microbiome interactions, and ecosystem processes (Turetsky et al. 2025).

Sphagnum fuscum (Schimp.) Klinggr. (subg. Acutifolia) is a notable example of a *Sphagnum* species capable of

persisting in these marginal environments. It has a wide circumboreal distribution in the Northern Hemisphere but is also found in isolated populations extending into the temperate and sub-meridional zones, restricted to high-elevation mountain ranges in the latter (Kyrkjeeide et al. 2015). *Sphagnum fuscum* is a haploid species that typically forms high and compact hummocks (20,000–60,000 shoots/m^2), which stand above the water table level (Fig. 1 and Fig. S1). The main reproductive mode at the local scale is assexual reproduction, either by vegetative growth or fragmentation (Gunnarsson et al. 2007). Nevertheless, it frequently produces sporophytes in high densities (Daniels and Eddy 1990; Sundberg 2002). Although traditionally described as dioicous, *S. fuscum* has also been reported to be polyoicous (also known as polygamous), i.e., individuals of the same species may be either monoicous or dioicous (Pujos 1994).

In the Pyrenees, *S. fuscum* reaches one of the southernmost rear-edges, which is highly isolated from the core distribution area. Other isolated localities are known from the French Massif Central (230 km) and a single population in the Cantabrian Mountains in Spain (370 km). There are only 10 known populations in the Pyrenees that inhabit mire systems at margins of alpine lakes, rivulets or springs, mostly above 2000 m. Due to its scarcity in this region, *S. fuscum* has been assessed as Vulnerable in the old French administrative region of Midi-Pyrénées (Sánchez et al. 2015), as Endangered in Catalonia (Sáez et al. 2019), and as Critically Endangered in the Iberian Peninsula and Spain (Sérgio et al. 2007; Garilleti and Albertos 2012). However, it lacks regional assessment across political borders, which would be biogeographically more coherent. Apart from the currently known occurrences in the Pyrenees, many crucial aspects for the conservation of this species in the region remain largely unexplored—including population size, reproductive capacity, genetic diversity, phylogenetic relationships with populations from other regions, and recent evolution associated with glacial history.

This study aims to assess the conservation status of *S. fuscum* in the Pyrenees by integrating novel genetic and demographic data. Specifically, our objectives are to: (1) reveal the genetic structure of *S. fuscum* in the Pyrenees; (2) compare the genetic profile of Pyrenean populations with that of central regions of the range (Canada and Norway); (3) study the occurrence of sexual reproduction in the Pyrenean populations; (4) update the distribution range and estimate the abundance of *S. fuscum* in the Pyrenees; and (5) integrate the previous points to address science-based conservation strategies for the Pyrenean populations of this species. Beyond its species-specific focus, this study aims to emphasize the broader need for detailed ecological and genetic assessments of rear-edge populations, which are often overlooked despite their high conservation value and unique evolutionary trajectories.



Fig. 1 *Sphagnum fuscum* hummock from Estibèra showing high density of sporophytes. Numerous reddish, subspherical capsules (sporophytes) are visible, most of which have already shed their calyptra. The sporophytes are elevated by pseudopodia (gametophyte structures) embedded within pale green perichaetial leaves, which contrast sharply with the typically brown capitula of *S. fuscum*

Materials and methods

Sampling sites

Between 2019 and 2021 we sampled seven (out of ten) populations of *S. fuscum* in the Pyrenees (Fig. 2; Table 1). This selection covers the maximum geographical and altitudinal amplitude of its regional distribution (Fig. 2). At least one shoot was collected from every hummock occurring at Cauterès (CAU), Coma de Vaca (CDV), Era Planhòla (EPL), Ferreroles (FER), Formiguera (FMP), and Plan deth Horn (PDH). For Estibèra (EST), which is the largest population, a sample of 28 hummocks were randomly selected. A total of 104 shoots were dried and preserved at room temperature.

For logistic reasons, it was not possible to visit neither Pessons nor Comapedrosa before the genotyping was completed. We could not include Basses d'en Gumbau (BGO) because this population was found only after the study had been completed.

Distributional, demographic and reproductive characteristics

To update the distribution range of *S. fuscum* we performed extensive surveys along the Pyrenees to find more populations in suitable sites, complemented by an in-depth review of the existing literature. To delineate the extent of the distribution range, we employed the Extent of Occurrence (EOO) metric as defined by the IUCN (2012). The EOO was estimated using a minimum convex polygon—the smallest polygon in which no internal angle exceeds 180° and that encompasses all known occurrence sites— generated with the online tool GeoCAT (https://geocat.iucnredlist.org/), based on confirmed populations.

We recorded the number of hummocks in each population as a proxy for population size, following the approach of Garilleti and Albertos (2012). For each hummock, we measured its surface area, and its height above the water table. We also assessed sporophyte presence at the population level.

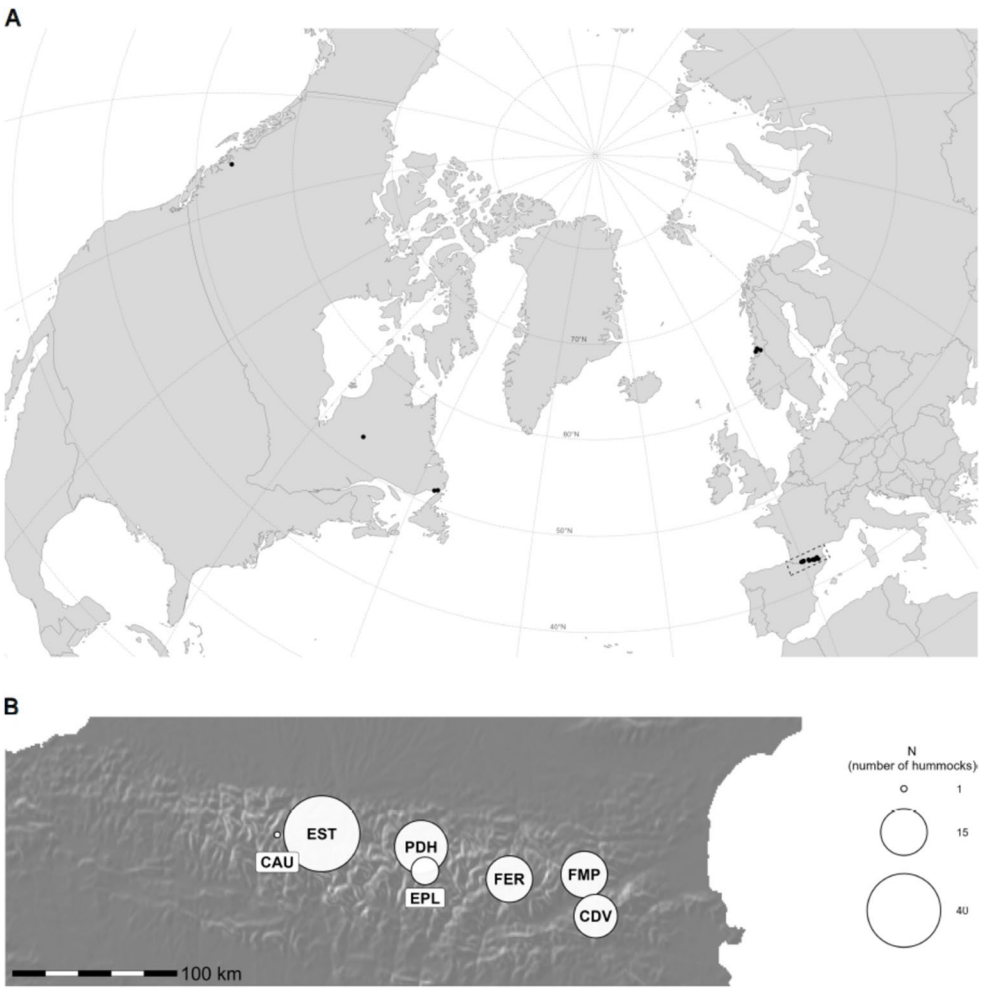


Fig. 2 Distribution of studied *Sphagnum fuscum* populations. (**A**) Sample sites included in this study. Genotypes from Norway and Canada are compiled from Kyrkjeeide, M. et al. (2023). In Kyrkjeeide, M. et al. (2023) the coordinates of the sampling site in British Columbia (western Canada) were not added, so the point in this map is the centroid of the province. (**B**) Pyrenean sampling sites. The size of the circles symbolises the number of hummocks of the populations

Table 1 *Sphagnum fuscum* populations in the Pyrenees. *Site*, toponym; *Code*, abbreviation of the site (* genotyped population); *First report*, year of the first report for the site; *Author(s)*, author(s) and reference of the first report; *Elevation*, metres above sea level (m); *Number of hummocks*, total number of hummocks at each site, that can be a proxy of the population size ([a] counts on original report; [b] own counts); *NaturalProtected Area* (NPA), name of the NPA; *UICN Management Category*, category of protection of the NPA according to the IUCN System (UNEP-WCMC and IUCN, 2022); *Country*, State

Site	Code	Author(s)	Elevation (m)	Number of hummocks	Natural Protected Area	Management Category	Country	Latitude	Longitude
Val de Cauterès	CAU*	Hugonnot, in Corriol 2004	1620	1[a, b]	Parc National des Pyrénées	II	France	42.844012	−0.167969
Nhèuvièlha (Estanh Cap de Long)	ECL	Corriol 2004	2180	3–7[a]	Parc National des Pyrénées	II	France	42.862814	0.178369
Nhèuvièlha (Varètja)	VAR	Corriol 2004	2220	11[a]	Aire d'adhésion du Parc National des Pyrénées	V	France	42.869081	0.150478
Nhèuvièlha (Estibèra)	EST*	Gauthier, 1990	2280	43[b]	Réserve Naturelle Nationale du Néouvielle	Unknown	France	42.849262	0.163702
Nhèuvièlha (Gorguet)	GOR	Chouard (1929) in Corriol 2004	2220	NA	Réserve Naturelle Nationale du Néouvielle	Unknown	France	42.817769	0.116675
Plan deth Horn	PDH*	Pérez-Haase et al., 2012; 2019	1970	20[b]	Site of Community Interest Alt Pallars	Unknown	Spain	42.777337	0.903436
Era Planhòla	EPL*	Gauthier, 1991	1840	5[b]	Parc Nacional d'Aigüestortes i Estany de Sant Maurici	V	Spain	42.646006	0.932356
Comapedrosa	CMP	S. Riba, comm. pers.	2250	7[b]	Parc Natural Comunal de les Valls del Comapedrosa	Unknown	Andorra	42.580886	1.442205
Ferreroles	FER*	Lazare et al., 2005	2170	15[b]	Non-protected	Non-protected	Andorra	42.601465	1.56029
Pessons (Els Estanys)	EES	Lazare et al., 2005	2410	NA	Ramsar Site and UNESCO's Cultural Landscape of Vall del Madriu-Perafita-Claror	Unknown	Andorra	42.487072	1.654789
Pessons (Els Collells)	ECO	Lazare et al., 2005	2325	10[b]	Non-protected	Non-protected	Andorra	42.517692	1.696025
Formiguera	FMP*	Hugonnot 2012	1455	15[b]	Parc naturel régional des Pyrénées Catalanes	V	France	42.626449	2.117656
Desert del Carlit (Serra de les Llebres*)	LLE	SCOP SAGNE, 2005	2100–2200	NA	Parc naturel régional des Pyrénées Catalanes	V	France	42.554042	1.985058
Desert del Carlit (Basses d'en Gombau)	BGO	A. Pérez-Haase, pers. obs.	2140	15[b]	Parc naturel régional des Pyrénées Catalanes	V	France	42.557563	1.970705
Coma de Vaca	CDV*	Pladevall-Izard et al., 2023	2180	13[b]	Parc Natural de les Capçaleres del Ter i del Freser	V	Spain	42.398786	2.203188

DNA extraction and genotyping

We used the upper 1.5–2.5 cm of the dried shoots (capitulum and first branches) for DNA extraction, which was carried out using the E.Z.N.A. SP Plant DNA Kit (https://omegabiotek.com/) following the manufacturer's instructions. Extractions were performed at the laboratories of the Botanical Institute of Barcelona (IBB).

For genotyping, we used sixteen microsatellites (Table S1) that have already been proven to perform successfully for *Sphagnum fuscum* (Shaw et al. 2008; Stenøien et al. 2011; Kyrkjeeide et al. 2015, 2016). Electrophoresis-based genotyping was performed at CD Genomics (https://www.cd-genomics.com/), using GeneMapper™ Software 5 (Applied Biosystems).

Genetic analysis

We evaluated the levels of genetic diversity of the Pyrenean populations by calculating several metrics at the population level: the proportion of polymorphic loci (*PPL*), number of alleles per locus (*A*), number of multilocus genotypes

(hereafter MLGs), number of private alleles per MLG, and haploid diversity per population (*H*). We computed all these parameters using GenAlEx v. 6.5 (Peakall and Smouse 2012).

To examine the genetic structure of the Pyrenean populations, we applied multiple analytical approaches. First, we conducted a hierarchical analysis of molecular variance (AMOVA) (Excoffier et al. 1992) to partition genetic variation between and within populations. We ran the AMOVA in GenAlEx, with 999 permutations.

Next, we carried out a Bayesian cluster analysis using Structure v. 2.3.4 (Pritchard et al. 2000), implementing an admixture model with correlated allele frequencies. For each *K* value ranging from 1 to 10, we conducted 10 replicate runs, with a burn-in of 100,000 steps followed by 500,000 iterations per run. We identified the most likely number of clusters (*K*) using the ad hoc statistic Δ*K* (Evanno et al. 2005).

To assess the robustness of the inferred genetic structure, we performed additional analyses under alternative conditions. Specifically, we repeated the analyses (i) after removing a highly variable locus (locus 4) that was nearly private to most multilocus genotypes, and (ii) using this reduced allele dataset without locus 4 in combination with the LOCPRIOR model.

To further explore genetic relationships without relying on model-based assumptions as in Structure, we also calculated a pairwise dissimilarity matrix based on Bruvo's distance among the Pyrenean MLGs using "poppr" R package v. 2.9.4. We visualized this matrix as a heatmap and applied hierarchical clustering using both average linkage (UPGMA) and a Complete Linkage dendrogram, via the R package "pheatmap" v.1.0.12 (Kolde 2019) in R v. 4.3.1. Additionally, we constructed a minimum spanning network (MSN) based on Bruvo's distance using the "poppr" package.

At a broader geographical scale, we examined the genetic structure of *S. fuscum* by performing a Principal Coordinates Analysis (PCoA) based on a Bruvo's distance dissimilarity matrix. This analysis included additional genotypes from Canada and Norway (available in Kyrkjeeide et al. 2023). We calculated the dissimilarity matrix using "poppr" package and the PCoA using the "ade4" R package v. 1.7.23. To integrate the data from Kyrkjeeide et al. (2023) with our own dataset, we manually aligned microsatellite loci. We excluded three polymorphic loci (4, 10, and 28) due to missing data in the Norwegian and Canadian samples (Supplementary Table 2). Conversely, we incorporated locus 56, which is monomorphic in the Pyrenean populations but polymorphic across regions.

Results

Distributional and demographic characteristics and sexual reproduction

The extensive surveys we performed along the Pyrenees during the period 2019–2021 only allowed us to locate three additional populations: Coma de Vaca (for more details about the discovery; please see Pladevall-Izard et al., 2023), in the Spanish side of the Pyrenees; Basses d'en Gumbau (BGO), in the French side, and Comapedrosa in Andorra (CMP) (Table 1).

The ten populations known to date are located in central and eastern Pyrenees, four in France (two in the Hautes-Pyrénées Department and two in the Pyrénées-Orientales Department), four in Andorra and three in Spain (all in Catalonia), in an altitudinal range between 1455 m and 2410 m (Table 1). The EOO of *S. fuscum* in the Pyrenees was 3,000.36 km^2.

Sphagnum fuscum formed small populations, typically comprising no more than 15 hummocks per site (Table 1). We estimated less than 200 hummocks across the Pyrenees, based on a median of 11 hummocks per population in sites lacking direct censuses. The median surface area of hummocks was 0.75 m^2 although some reached substantially larger dimensions, of up to 12 m^2 in Ferreroles. The most common growth form was on hummocks elevated 2–60 cm above the water table (Fig. S1). Most hummocks appeared to show an ombrotrophic tendency based on their elevated position above the water table, although some lower hummocks occurred close to the water table and may therefore have been more influenced by minerotrophic conditions.

Sporophyte production was frequent in the Pyrenean populations and was observed in five of the seven sites studied (Fig. 2), including Cauterès, where only a single hummock was present. Moreover, sporophyte production within populations varied among hummocks, with some showing no signs of sexual reproduction, whereas others produced sporophytes at varying densities.

Genetic diversity

Of the 16 microsatellite loci, nine (56.25%) were polymorphic for the Pyrenean populations, yielding 28 alleles (Table 2). Locus 4 was particularly variable, with nine alleles, while the remaining polymorphic loci each harboured two to three alleles each. More than half of the alleles (54%) were private to a single population.

We identified 10 MLGs, all private to their respective populations; thus, no MLGs were shared among populations (Table 2). Five populations were monoclonal, each

Table 2 Pyrenean *Sphagnum fuscum* genotypes based on nine variable microsatellites *Population*, population codes as in Table 1; MLG, code for every different multi-locus genotype; *n*, is the number of gametophytes that belong to each MLG; Loci, are the nine variable microsatellites for *S. fuscum* Pyrenean populations and its numeration corresponds to the originally published nomenclature (Supplementary Table 1)

Population	MLG	*n*	Locus 4	Locus 7	Locus 9	Locus 10	Locus 20	Locus 22	Locus 28	Locus 30	Locus 68
CAU	CAU	3	**189**	188	**182**	219	295	103	239	131	228
EST	EST1	6	**199**	188	184	231	295	103	239	127	228
	EST2	21	**187**	188	184	231	**289**	103	239	131	228
	EST3	1	**201**	188	184	231	295	103	239	127	228
PDH	PDH1	10	**185**	188	**186**	231	292	103	**231**	127	228
	PDH2	8	**191**	192	184	231	292	103	**229**	131	228
EPL	EPL	11	**195**	192	184	219	292	**97**	239	131	228
FER	FER	17	**193**	188	184	231	292	103	239	131	228
FMP	FMP	17	197	192	184	231	292	103	239	131	228
CDV	CDV	10	197	188	184	231	295	103	239	131	**232**

Private alleles are highlighted in bold

Table 3 Diversity parameters

Population	H	MLG	*n*	Private Alleles
CAU	0	CAU	3	2
EST	0.13 ± 0.06	EST1	6	1
		EST2	21	2
		EST3	1	1
PDH	0.27 ± 0.09	PDH1	10	3
		PDH2	8	2
EPL	0	EPL	11	2
FER	0	FER	17	1
FMP	0	FMP	17	0
CDV	0	CDV	10	1
Mean ± sd	0.057 ± 0.02			1.5 ± 0.85

Population, population codes as in Table 1; *H*, haploid diversity per population; MLG, code for every different multi-locus genotype; *n*, number of gametophytes that belong to each MLG; *Private alleles*, number of alleles that are unique to each respective MLG. At the bottom, mean ± standard deviation of *H* and the number of private alleles per population

composed of a single MLG. Only two populations (Plan deth Horn and Estibèra) contained more than one MLG (two and three MLGs, respectively). In Estibèra (EST), the multilocus genotype EST3 was a singleton that differed from EST1 by a single tandem repeat at the highly variable locus 4. Formiguera was the only population lacking private alleles. The haploid diversity was obviously null in the monoclonal populations. In Plan deth Horn, the two MLGs occurred in a 10:8 ratio, yielding higher diversity than in Estibèra, where the three MLGs were present but one (EST2) predominated (21:6:1) (Table 3).

Genetic structure

The AMOVA showed that most genetic variation (81%) was explained by differences among populations, rather than within them (19%) ($\Phi_{PT} = 0.808$, $p = 0.001$).

Using STRUCTURE, Bayesian clustering analysis identified nine as the best supported number of genetic clusters (K), according to the Evanno ΔK method (Fig. S2). These clusters corresponded well with the nine main MLGs, with EST3 grouping with EST1. The Evanno ΔK also showed two secondary peaks at $K = 3$ and $K = 7$, which represent alternative suboptimal hierarchical solutions and likely reflect deeper levels of genetic structure within the dataset (Fig. S2; Fig. 3).

The deletion of locus 4 entailed the fusion of the singleton MLG EST3 with the other minority allele EST1, resulting in nine MLGs for the reduced dataset. The additional analysis under the same parameters with the reduced dataset identified $K = 5$ as the most supported number of genetic clusters (Fig. S2). These clusters corresponded to (i) EST2, (ii) EST1–CAU–CDV, (iii) PDH2–FMP, (iv) EPL, and (v) PDH1. FER showed an admixed ancestry, being primarily

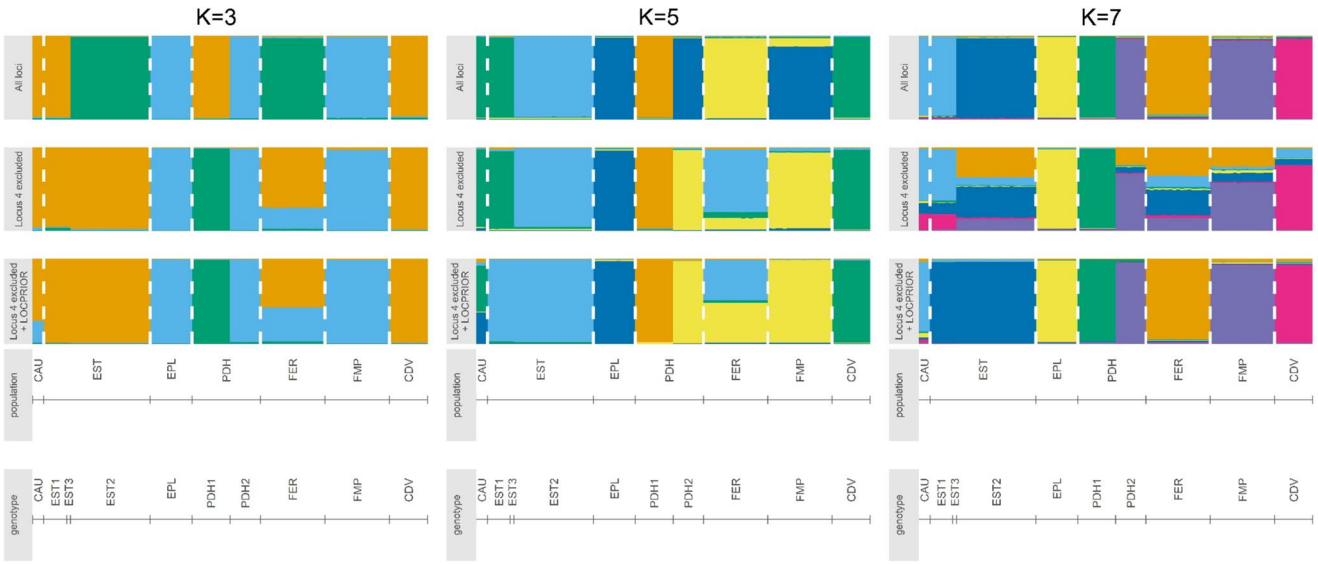


Fig. 3 Comparison of STRUCTURE plots for $\boldsymbol{K}=3$, $\boldsymbol{K}=5$ and $\boldsymbol{K}=7$ under three analytical settings. The first row includes all loci, the second excludes locus 4, and the third excludes locus 4 while implementing the LOCPRIOR model. Each vertical bar represents a multilocus genotype (MLG), and its width is proportional to the number of samples sharing that MLG. Vertical dashed lines separate populations. The two lower panels identify the populations (upper panel) and the corresponding MLGs (lower panel), with populations arranged from west to east. Colours indicate the estimated ancestry proportions in each inferred genetic cluster. Colours were aligned across analytical settings within each K to facilitate comparisons, although perfect colour correspondence among analyses was not always possible. According to Evanno's ΔK, $K=3$ and $K=7$ represented suboptimal hierarchical solutions when all loci were included, whereas $K=5$ was optimal after excluding locus 4. However, assignment patterns differed among analytical settings for the same K values

assigned to the EST2 cluster while also displaying contributions from the PDH2–FMP and EST1–EST3–CAU–CDV clusters (Fig. 3). When assuming the LOCPRIOR model with the reduced dataset we identified $K=5$ again as the best supported aggrupation. However, the identity of the groups differed slightly from the LOCPRIOR free model. These clusters corresponded to (i) EST1-EST2, (ii) CDV, (iii) PDH2–FMP, (iv) EPL, and (v) PDH1. As in the LOCPRIOR model-free analysis, FER displayed an admixed ancestry, with approximately equal assignment to the EST1–EST2 and PDH2–FMP clusters. CAU also showed evidence of admixture, being mainly assigned to the CDV cluster while exhibiting a smaller contribution from the EPL cluster (Fig. 3).

The UPGMA dendrogram based on Bruvo's distance matrix grouped the MLGs into two main clusters: one containing CAU, CDV, FER, FMP, EST1, EST2 and EST3 and another comprising only EPL and PDH2, (Fig. 4). Notably, PDH1 was sister to both groups and did not cluster with PDH2 despite both MLGs being found in the same population. Although UPGMA grouped EPL and PDH2 together, pairwise distances indicated that FER and FMP are more closely related to EPL and PDH2, respectively, than EPL and PDH2 are to each other.

The Complete Linkage method provided a contrasting topology using the same distance matrix. This algorithm partitioned the MLGs in two groups: one containing CAU, EST2, CDV, EST1 and EST3, and the other comprising EPL, PDH2, FER and FMP (Fig. 4). This latter cluster resolved the incongruence observed in the UPGMA regarding these MLGs.

The minimum spanning network placed FER in a central position, consistent with its relative even distance to the remaining MLGs, as seen in the heatmaps. Three main branches radiated from this central position: one comprising FMP, EPL and PDH2; another including EST2 and CAU; and a third comprising CDV, EST1, EST3 and PDH1 (Fig. 5). Overall, the network exhibited a branched topology with no clear separation into discrete genetic groups.

At an extra Pyrenean scale, the resolution of Pyrenean genotypes decreased when aligned with the dataset from Kyrkjeeide et al. (2023), reducing the number of distinct MLGs from 10 to eight (i.e., EST1 merged with EST3, and FMP with PDH2). The PCoA's first two axes accounted for 40,3% of the corrected variance in the Bruvo's distance matrix (Fig. 6). Pyrenean, Norwegian, and Canadian genotypes were clearly separated in the ordination plot. The primary axis (21,5%, corrected variance) differentiated the Pyrenean populations from the Norwegian-Canadian group. Locus 56 contributed strongly to this separation, being fixed at 206 bp in the Pyrenees, while alleles in Canadian and Norwegian populations ranged from 209 to 215 bp. Interestingly, a single Norwegian MLG (sample 211) clustered near the Pyrenean group, sharing similar negative scores.

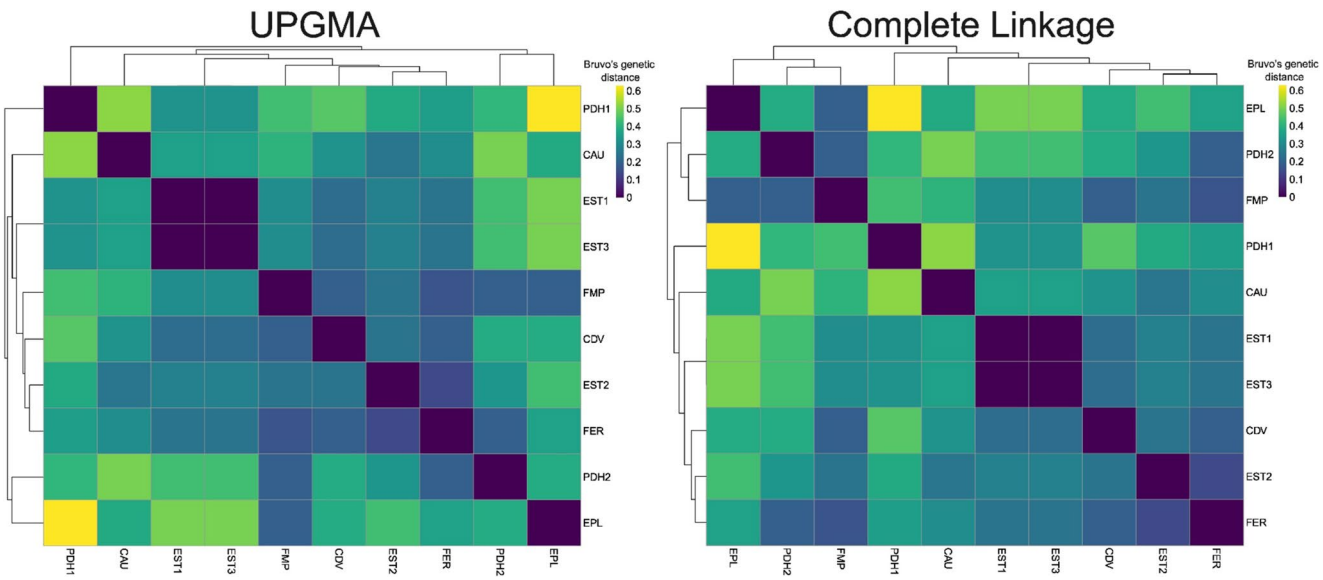


Fig. 4 Heatmaps of pairwise Bruvo's genetic distances among multilocus genotypes (MLGs) of *Sphagnum fuscum* populations in the Pyrenees. Hierarchical clustering was performed using UPGMA (left) and Complete Linkage (right) algorithms. Warmer colors (yellow) indicate greater genetic distances, whereas cooler colors (dark blue) indicate higher genetic similarity. The two algorithms mainly differ in the position of PDH1 and FMP, the first being very distant to most of the other MLGs while the latter is the most equidistant to all the other MLGs

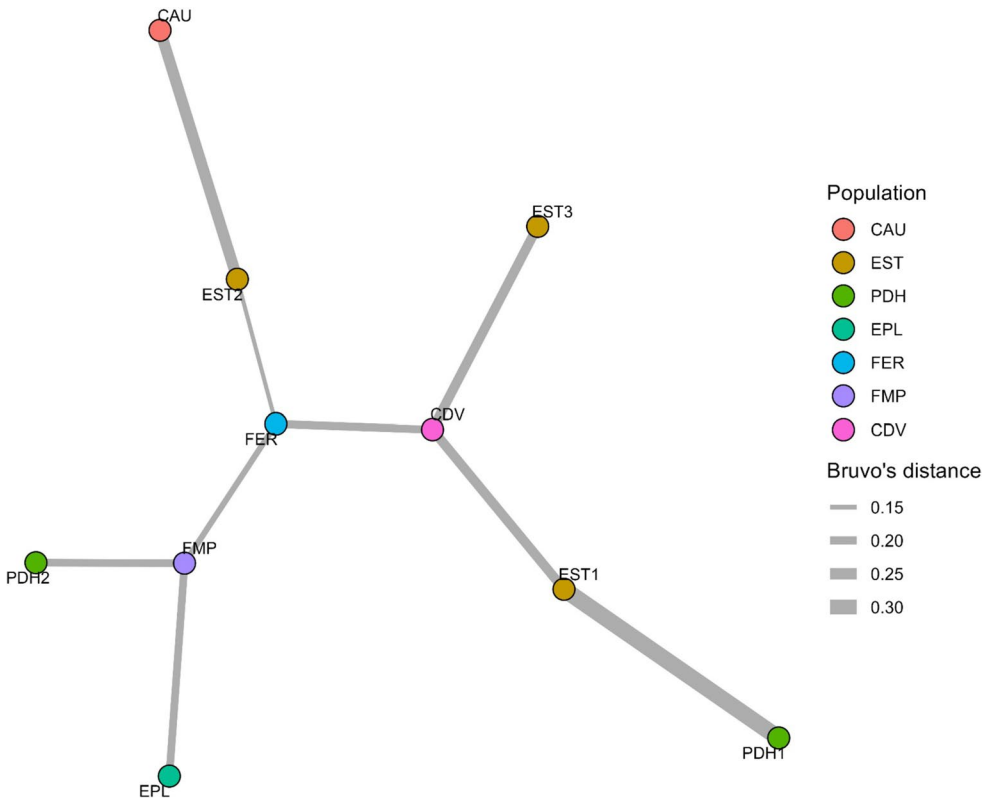


Fig. 5 Minimum Spanning Network of multilocus genotypes (MLGs) from the Pyrenees. Nodes represent MLGs and are coloured according to the population to which they belong. For Estibèra (EST) and Plan deth Horn (PDH), the labels correspond to the nomenclature used throughout the analyses. The width of the links between MLGs is proportional to the genetic distance between them, measured using Bruvo's distance. The size of the dots does not correspond to their relative abundance

The secondary axis (18.8%, corrected variance) mainly reflected internal structure within each region, and suggested a moderate differentiation between Norwegian and Canadian samples, with the former showing higher average scores and limited overlap with the latter.

Discussion

The findings of this study provide a comprehensive understanding of the genetic structure of *Sphagnum fuscum* in the Pyrenees and contribute valuable information on the

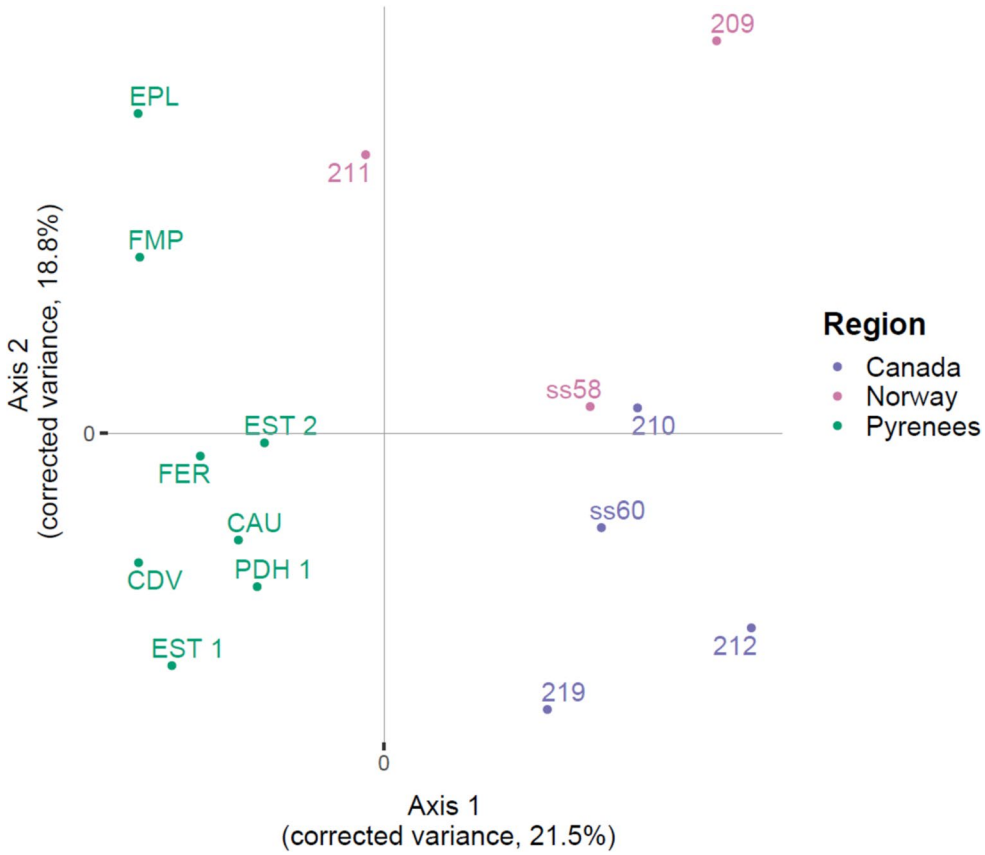


Fig. 6 Principal Coordinates Analysis based on pairwise Bruvo's distance between MLGs. The PCoA shows the coordinates of the Pyrenean, Canadian, and Norwegian populations for the first two axes, which account for 40.3% of the corrected variance of the dissimilarity matrix

demographic and reproductive context of these populations. These insights are particularly relevant for informing conservation strategies for this species at this rear-edge.

Genetic diversity and structure

On a large geographic scale, Pyrenean populations are genetically distinct from those in Norway and Canada, showing a fixed private allele, and would therefore be considered as an independent evolutionarily significant unit (ESU). This is another evidence that rear-edge populations of plant species harbour unique genetic lineages, both in the Pyrenees (De Lafontaine et al. 2013; López-Pujol et al. 2013) and elsewhere (e.g., Gugger et al. 2011; Erichsen et al. 2018; Worth et al. 2021; Balao et al. 2024). However, broader phylogeographic analyses with a more expanded sampling will be needed to assess the global uniqueness of the Pyrenean lineage and to investigate whether similar patterns occur in other rear-edge areas within the entire distribution range of *S. fuscum*. Interestingly, the Pyrenean ESU as a whole holds notable levels of genetic diversity (mostly to differences among populations; see below), as indicated by the wide area occupied by Pyrenean MLGs in the PCoA ordination.

Sphagnum fuscum populations in the Pyrenees exhibit extremely low intrapopulation genetic diversity and strong geographic structuring, with each population harbouring unique MLGs. Only a few studies have assessed the genetic structure of endangered *Sphagnum* species. Terracciano et al. (2012) worked on four populations of *Sphagnum palustre* in the Italian Peninsula (also a rear-edge area for this species) and found that 82% of genetic variation was partitioned among populations according to an AMOVA—remarkably similar to the 81% observed in our Pyrenean populations. In contrast, Scandinavian populations of *Sphagnum angermanicum* showed only 6% variation among populations (Gunnarsson et al. 2005)—what reflects a leading-edge dynamic, which is a radically different biogeographical context. As proposed by Stenøien et al. (2011), *S. angermanicum* populations in Northern Europe may have originated from relatively recent (<40,000 years) west-to-east colonisation events from North America. Low genetic diversity within and among populations is expected in such leading-edge dynamics due to strong founder effects (Hewitt 2000). On the other hand, *S. fuscum* (this work) and *S. palustre* (Terracciano et al. 2012) show higher among-population differentiation in the Mediterranean Basin rear-edge (Italy, the Pyrenees), which is consistent with the persistence of small and isolated populations.

This strong differentiation among populations is accompanied by extremely low within-population diversity—a concerning finding according to the small population paradigm (Ellstrand and Elam 1993; Frankham 1996). This outcome

was not entirely unexpected, given the high degree of habitat fragmentation and the small size of populations. Similar patterns of reduced within-population diversity have been reported in other peatland mosses with fragmented distributions, such as *Hamatocaulis vernicosus* (Manukjanová et al. 2020) and *Scorpidium revolvens* (Kophimai et al. 2014).

Overall, the different approaches used to infer hierarchical genetic structure did not converge on a consistent interpretation of the relationship among MLGs. Given the available data and results, we conclude that Pyrenean populations are characterized by strong genetic fragmentation. Such fragmentation does not translate into a hierarchical structure that could reflect geographic or environmental distances. Indeed, genetic distances between MLGs occurring within the same population, as in Plan deth Horn and Estibèra, were often greater than those observed between MLGs from different populations.

Given this genetic landscape, we recommend treating each unique MLG—except for EST1 and EST3, which could be considered as a single unit—as a separate conservation unit to preserve the full genetic diversity of the Pyrenean ESU.

Advantages and limitations of microsatellite markers

The choice of microsatellite markers may seem unexpected given the current availability of reduced-representation genomic approaches. However, because the primers employed in this study had already been developed, microsatellites represented a substantial economic advantage over genomic approaches, particularly at the time the project was initiated. Additionally, other studies showed that microsatellites produced genetic structure results largely concordant with those obtained with RADseq (Mesak et al. 2014; Camacho-Sánchez et al. 2020; Sunde et al. 2020). Microsatellites therefore remain a valuable cost-effective alternative for genetic structure studies conducted under limited funding.

In addition, the availability of previous studies using the same, or partially overlapping, set of markers in *Sphagnum* facilitated direct comparisons with published datasets. For example, this compatibility allowed us to compare the Pyrenean genotypes with those reported from Norway and Canada by Kyrkjeeide et al. (2023).

The limitations of microsatellite markers, however, are evident in our results. STRUCTURE analyses proved highly sensitive to marker selection, as the exclusion of locus 4 resulted in substantial changes in both the ΔK distribution and the inferred clustering solutions (Fig. 3 and Fig. S2). Such sensitivity highlights the need for caution when interpreting these results.

Despite these limitations, the microsatellite dataset was sufficient to reveal the main genetic patterns characterizing the Pyrenean populations. Nevertheless, the application of genome-wide markers would be highly valuable for future research, as it would enable more detailed inferences regarding the evolutionary history of these rear-edge populations.

Sexual reproduction

The occurrence of sporophytes in several populations provides evidence that sexual reproduction takes place, despite the absent, or low, within genetic diversity of the populations. Nevertheless, in Estibèra and Plan deth Horn—sites where more than one MLG coexists and sporophytes have been observed—no evidence of recombinant offspring was found. This finding might indicate a limited contribution of sexual reproduction to local dynamics, supporting the results of Gunnarsson et al. (2007).

The presence of sporophytes in apparently monoclonal populations is noteworthy and may be consistent with monoicy. This possibility is particularly interesting because *Sphagnum fuscum* is generally regarded as a dioicous species. The only exception we found in the literature is the work of Pujos (1994), who suggested that *S. fuscum* may be polyoicous. However, this statement was made only briefly and without presenting the observations or data supporting that conclusion.

Our results do not allow us to determine the sexual system of the studied populations, as surveys were not designed to assess sex expression. The possibility of monoicy was not initially considered. Once this issue emerged during the interpretation of our results, we examined our herbarium material for evidence of gametangia, but none were detected. We suspect that this may be related to the timing of collection, as sampling was conducted in early-mid summer, whereas gametangia production in *Sphagnum* is thought to occur during late summer and autumn (Pujos 1994). Targeted surveys focusing on gametangia production would be necessary to clarify the sexual system of Pyrenean populations and could have important implications for their conservation.

Conservation implications

The extremely small size of most Pyrenean populations makes them highly vulnerable to stochastic events and local extinctions. We recommend implementing intensive demographic monitoring to track trends, identify major threats (e.g., overgrazing; Pérez-Haase 2019), and enable timely in situ interventions. Monitoring should include hummock counts, surface measurements, assessments of reproductive status, and evaluations of desiccation impacts. This

emphasis on local habitat monitoring is also supported by recent evidence showing that *S. fuscum* growth and productivity are strongly associated with climatic conditions, peat properties, and vascular plant cover (Santelmann et al. 2025). During our extensive sampling, we observed that the primary threat to conservation is cattle overgrazing. However, due to unusually long dry spells in recent summers and the likelihood of their recurrence in the future, we expect desiccation to cause mortality at the tops of the hummocks.

In addition, we advocate for demographic rescue through population reinforcements to reverse the precarious demographic status, particularly at sites showing signs of hummock degradation. Propagating Pyrenean MLGs *ex situ* from minimal material may not only support reinforcement efforts but also safeguard their genetic identity against potential local extinctions. While substantial experience exists in *Sphagnum* propagation—especially in the context of large-scale peatland restoration in the boreal regions—these methods have been developed under ecological conditions quite different from those of the Pyrenean mires (Caporn 2018; Rochefort and Lode 2006). Pyrenean mires are smaller and host other endangered species, and are further limited in the availability of suitable microhabitats for the establishment of *S. fuscum.* These constraints make population reinforcement a considerable technical challenge.

From a genetic conservation perspective, we stress the importance of preserving the distinctiveness of the Pyrenean populations. In the absence of robust ecological, genomic, and fitness data, any attempt to introduce extra-Pyrenean genetic material is considered premature and potentially harmful.

Facilitating gene flow among Pyrenean populations could be explored as a potential future conservation strategy. Based on the small population paradigm, this type of genetic rescue has shown success in increasing population size and vigour in various taxa (Frankham 2015). However, in the case of clonal organisms such as *Sphagnum fuscum*, the goal would not merely be to establish multiple MLGs in close proximity—since hummocks may vegetatively persist over long timespans—but to promote effective crossing between MLGs. If successful, this could yield sexually produced diverse offspring with potentially higher fitness.

Before any field implementation is considered, this hypothesis should first be evaluated under controlled conditions given the evolutionary uncertainties involved in a genetic rescue. In the absence of robust guarantees regarding the effectiveness and monitorability of the genetic rescue programme, we advocate for a precautionary approach—i.e., favouring population reinforcement using local genotypes. This recommendation is consistent with recent conservation guidelines proposed for *Pedicularis palustris*, another European mire specialist plant (Mikulášková et al. 2025).

Fortunately, the vast majority of known *S. fuscum* populations in the Pyrenees are located within protected natural areas. Implementing genetic rescue strategies such as those discussed here, however, would require substantial coordination between conservation authorities across national boundaries (Andorra, Spain, and France). Recent initiatives (e.g., Interreg POCTEFA FLORALAB, or FLORALAB+ projects) offer a promising framework for such cooperation. We strongly support such collaborative approaches, which are essential for the effective conservation of biodiversity in a biogeographic region that clearly transcends administrative borders.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10592-026-01839-x.

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Author contributions JC-B was responsible for data analysis, laboratory work, and manuscript writing. NG-J advised on the design and interpretation of the molecular analyses. JL-P advised on the design of the study's molecular component, and contributed to the review and editing of the manuscript. EP-I significantly contributed to field sampling, and the review and editing of the manuscript. AP-H (corresponding author) coordinated the project, was responsible for its conceptual design, participated in field sampling, oversaw laboratory work and data analysis, and contributed to manuscript writing.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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