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RESEARCH ARTICLE

First evidence of post-glacial contraction of Alpine endemics: Insights from Berardia subacaulis in the European Alps

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Abstract

Aim: Late Quaternary glaciations left an enduring imprint on the distribution of species and their genetic structure. The responses of plants endemic to the Alps can be summarized in three major demographic hypotheses: (i) post-glacial expansion hypothesis; (ii) post-glacial contraction hypothesis; and (iii) long-term stability hypothesis. Here we test these hypotheses and reconstruct the time and extent of demographic responses of an endemic plant to the Late Quaternary climate dynamics.

Location: European Alps.

Taxon: Berardia subacaulis Vill. (Asteraceae).

Methods: We used species distribution models to estimate the paleodistribution of B. subacaulis (Asteraceae) throughout the last 28 Ky and generated genome-wide sequences to estimate current patterns of spatial structure of genetic diversity. We tested five demographic models by integrating the results of the two independent approaches in an Approximate Bayesian Computation framework.

Results: The species has weak genetic differentiation among populations, with two main genetic groups. Species distribution models showed a reduction in potentially suitable areas for B. subacaulis during the post-glacial climate warming and demographic models identified a recent split (2.46 Kya) between the two genetic groups and they slightly supported the post glacial contraction hypothesis.

Main conclusions: Taken together, our results support the post-glacial contraction of an endemic plant, differing from the main pattern observed for endemics species in the European Alps during the Late Quaternary. The different pattern observed in B. subacaulis might be due to several factors, including the less severe effects of glaciations and the environmental heterogeneity of the South Western Alps, combined with some features of the species (i.e. poor dispersal ability, slow growth and microhabitat preferences). Future research in areas where the ice cover was less extensive will contribute to a more complete understanding of the role of climatic changes in shaping the endemics of the European Alps.
1 | INTRODUCTION

Late Quaternary glaciations, in particular the latest glacial cycle (120–0 Kya; Seguinot et al., 2018), left an enduring imprint on biodiversity globally (Bennett & Provan, 2008; Hewitt, 2004; Svenning et al., 2015; Weigelt et al., 2016). The rapid and severe climatic fluctuations during the Late Quaternary induced marked environmental changes, often resulting in dramatic changes in species population sizes, genetic structure and distributional ranges (Bennett & Provan, 2008; Hewitt, 2004; Stewart et al., 2010). The effects of these climatic fluctuations varied according to latitude, topography and intrinsic features of each species, including their ecological requirements and dispersal capabilities (Hewitt, 2004; Stewart et al., 2010). Therefore, different demographic responses and population genetic patterns should be expected in different species depending on the environmental conditions they experienced and their ecological preferences (Bennett & Provan, 2008; Nieto Feliner, 2014; Stewart et al., 2010).

Despite the different patterns expected in response to glaciations, in the European Alps (hereafter Alps) the majority of studies suggests that endemic species survived in refugia during the glaciations, regardless of their ecological requirements. In fact, the Alps were nearly entirely covered by ice (Ehlers et al., 2011) with numerous nunataks (unglaciated peaks) within the ice sheet and larger unglaciated areas at the periphery of the ice sheet (Schönswetter et al., 2005; Van Husen, 1997). Consequently, during glacial maxima, endemic plants contracted their distributional range, surviving in areas both within (i.e. nunataks) and outside (i.e. peripheral or lowland refugia) the ice sheet (Pawłowski, 1970; Schönswetter & Schneeweiss, 2019; Schönswetter et al., 2005; Trisch, 2004; Trisch & Schönswetter, 2003). When the glaciers retreated and new areas became available, these endemic species may have expanded their distributional range, but only moderately (‘post-glacial expansion hypothesis’). The limited extent of post-glacial expansion of such endemics has been explained in terms of reduced colonization ability resulting from dispersal limitation (Dullinger et al., 2012; Essl et al., 2011; Svenning & Skov, 2004, 2007a, 2007b), lower reproductive output than widespread species (Lavergne et al., 2004), and loss of genetic diversity during glacial contraction (Ehrendorfer, 1965; Niklfeld, 1972). In the Alps, this hypothesis has been widely supported in several studies on alpine endemic species based on molecular analyses and species distribution models (Bettin et al., 2007; Schönswetter et al., 2002; Stehlik, 2003; Stehlik et al., 2002; Trisch, 2004).

However, few studies suggest that, in regions of the Alps where the effects of Late Quaternary glaciations were less dramatic (e.g. Maritime and Ligurian Alps), endemics survived in situ via short altitudinal shifts (Casazza et al., 2016; Patsiou et al., 2014). This long-term stability (‘long-term stability hypothesis’) is thought to result from continued moisture availability and complex local topography (Bátori et al., 2017; Scherrer & Körner, 2011; Tzedakis et al., 2002) that allowed in situ survival via short-distance dispersal during climatic shifts, minimizing the extinction of populations and genotypes (Médail & Diadema, 2009; Ohlemüller et al., 2008; Suchan et al., 2019). Moreover, this long-term stability allowed the accumulation of species and especially of old endemic lineages in certain areas, including the southern European mountains (Médail & Diadema, 2009; Ronikier et al., 2012; Tzedakis et al., 2002).

Alternatively, some cold-adapted endemics attained larger distributions during glacial maxima by surviving and expanding in ice-free areas (Birks, 2008). When glaciers retreated and temperatures increased, these cold-adapted endemics contracted their distributional ranges (‘post-glacial contraction hypothesis’), thus mountain chains might serve as current refugial areas. To the best of our knowledge, the hypothesis of post-glacial contraction has never been demonstrated for endemics in the Alps, while it has been supported for endemic species in areas less affected by glaciations, such as mountains in the central Iberian Peninsula and the Japanese Alps (Ikeda et al., 2008; Ikeda & Setoguchi, 2007; Martín-Bravo et al., 2009; Peredo et al., 2009). In the central Iberian Peninsula and the Japanese Alps, given the limited ice extent during the Last Glacial Maximum (LGM), some cold-adapted endemics underwent range expansions during glacial maxima, and contracted their range into high-altitude refugia as mountain forests recovered during post-glacial (Ikeda et al., 2008; Martín-Bravo et al., 2009). The post-glacial contraction of such endemics has been mainly explained by an increase in competition of larger plants and/or their intolerance to warmer temperatures (Birks, 2008).

An ideal study area to test the three competing hypotheses, which, to the best of our knowledge, have never been explicitly tested before in Alpine endemics, are the South Western European Alps (hereafter SW Alps). Contrary to the rest of the Alps, in the SW Alps the effects of glaciations were less severe: in fact, the LGM (~24 Kya BP in the SW Alps, Seguinot et al., 2018) temperature depression was moderated by the Mediterranean Sea influence (Seguinot et al., 2018). The SW Alps are characterized by high local climatic heterogeneities, as a consequence of the close proximity of the Mediterranean and Alpine climates and high topographic heterogeneity (Casazza et al., 2005, 2008; Fauquette et al., 2018). This climatic heterogeneity results in the variety of phylogeographical patterns detected in this region, where both the post-glacial expansion and the long-term stability (mainly by altitudinal shift) hypotheses have been proposed (Casazza et al., 2016; Médail & Diadema, 2009). Moreover, the limited extent of the glacial sheet in this area may have allowed cold-adapted species to expand during glaciation, as expected by the post-glacial contraction hypothesis, even if this
pattern has never been detected. Consequently, the SW Alps are an ideal study area for investigating all three hypotheses outlined above. Here, we chose *Berardia subacaulis* Vill., an Alpine endemic widely distributed in the SW Alps, to test the three alternative hypotheses. On the basis of its current distributional range, Susanna and García-Jacas (2009) hypothesized that *B. subacaulis* survived the glacial period in refugia in the southern part of the SW Alps, seemingly supporting the post-glacial expansion hypothesis. Alternatively, Guerrina, Conti, et al. (2016) suggested that *B. subacaulis* likely survived *in situ*, in line with the long-term stability hypothesis, but these authors used paleoclimatic data at a coarse scale, hence they might have underestimated climate-change impacts compared to finer-scale data (Franklin et al., 2013). Moreover, the authors of the mentioned studies on *B. subacaulis* did not use molecular data or demographic inference to explicitly test alternative hypotheses of post-glacial colonization.

Here we test the three competing hypotheses described above (Figure 1) by integrating species distributions models (SDMs) with thousands of genome-wide polymorphisms in an Approximate Bayesian Computation framework. We first reconstruct spatial dynamics through the Late Quaternary climatic cycles using a high-resolution paleoclimatic data set spanning the last 28 Ky (from Upper Pleniglacial to Holocene - Maiorano et al., 2013; Theodoridis et al., 2017), and quantify genetic variation in *B. subacaulis* using a reduced representation library approach. We finally integrate the results of the two independent approaches to reconstruct the recent demographic history of *B. subacaulis*. The results of this study should allow us to better understand the processes that shaped the distribution of Alpine endemic species and their responses to post-glacial warming.

### 2 MATERIALS AND METHODS

#### 2.1 Study species

*Berardia subacaulis* Vill. belongs to a monospecific genus endemic to the SW Alps (Figure 2), it is an element of the Tertiary paleo flora (Ozenda, 2009), which went almost completely extinct during Late Quaternary glaciations (Ozenda, 2009). The split between the lineage leading to *B. subacaulis* and its sister lineage was recently confirmed by molecular phylogenetic analyses as dating to the Late Oligocene (Herrando-Moraira et al., 2019). *Berardia subacaulis* is a diploid, acaulescent, perennial herb growing on calcareous screes and stony slopes at high altitudes (between 1700 and 2700 m), currently restricted to the SW Alps (Figure 2). Capitula are mainly solitary, pollinated by a wide array of insects, whose visits remain scarce (Guerrina, Casazza, et al., 2016). The flowers are self-compatible but protandrous, favouring cross-fertilization first and allowing for self-fertilization when cross-fertilization does not occur (Guerrina, Casazza, et al., 2016). Pappus is characterized by short hairs, which combined with the acaulescence of the plant, seems to be less efficient. On the basis of the poorly efficient plume, we categorized the dispersal mode of *B. subacaulis* as trichometeorochochory (dispersal distance of ~15 m for the 99% of the seeds of a plant, classification of Vittoz & Engler, 2007). Information on the generation times of *B. subacaulis* is scarce. It is known that seed dormancy is absent and that from the germination it might occur from 40 to 80 days.
on average for having the first pair of true leaves (personal communication, M. Mucciarelli). The species generation time is estimated around 5 years (personal communication, C. Voisin & M. Rome).

### 2.2 Genetic analyses

#### 2.2.1 DNA extraction

DNA was extracted from 95 individuals of 19 populations (5 individuals per population; Figure 2 and Table S4), covering the entire distributional range of the species. Extractions were performed using a modified CTAB method (Doyle & Doyle, 1990) and concentrated to a minimum of 30 ng/μl for samples. DNA quality was visualized on 0.8% agarose gels and quantity was assessed using a QuBit 2.0 fluorometer (2.0, Life Technologies). The extracted DNA was sent to the Cornell Institute for Genomic Diversity for genotyping-by-sequencing (GBS; Elshire et al., 2011). Individual DNA samples were digested with the restriction enzyme EcoT22I. Single-end sequencing was carried out using one lane of an Illumina HiSeq 2000, producing raw reads that were 101 bp long.

#### 2.2.2 SNPs discovery and genotyping

To demultiplex the raw data and recover the individual samples in the Illumina library, the sequences were processed with the program process_radtags in Stacks 1.35 (Catchen et al., 2011, 2013) and the single-end reads were then merged into a single file per individual. We used Trimmomatic v0.33 (Bolger et al., 2014) to trim low quality or uncalled bases from raw reads, remove potential adapter contamination and discard reads that were less than 85 bp (i.e. the length of the trimmed reads) long after low quality base trimming. Because of the lack of a reference genome for *B. subacaulis*, a de novo assembly of the filtered reads was performed with de_novo_map.pl in Stacks 1.35 using the following parameters: a minimum number of identical raw reads required to create a stack (m) 10; the number of mismatches allowed between loci when processing a single individual (M) 4; and the number of mismatches allowed between loci when building the catalog (n) 8. At this stage, we eliminated four individuals, each one of them belonging to a different population, due to low sequence coverage.

Finally, we used the POPULATIONS program in Stacks 1.35 to create data matrices of sequences and single nucleotide polymorphisms (SNPs) in fasta and vcf format, respectively, for all subsequent molecular analyses.

#### 2.2.3 Population genetic structure

The assignment of individuals to population clusters was conducted using the program STRUCTURE version 2.3.4 (Pritchard et al., 2000). Since STRUCTURE assumes that the investigated loci are unlinked, we used the package Gonospy v0.1 (https://github.com/spyrothestodoridis/gonospy) to randomly select only one SNP per locus and repeated the analysis five times to account for biases in the SNP selection process, as routinely done (Theodoridis et al., 2017). We used a model of admixture to determine the number of population clusters (K) with a burn-in of 50,000 and 100,000 iterations. We performed five replicate runs for each value of the number of population clusters, K, between 1 and 10. The average and standard deviation (SD) of the natural log probability across replicates were used to calculate deltaK (Evanno et al., 2005) using Structure Harvester (Earl & vonHoldt, 2012). For the best K value, the results of the five randomly selected SNP repetitions and five replicated STRUCTURE runs per repetition were combined using the ‘greedy’ algorithm within CLUMPP v1.1.2n (Jakobsson & Rosenberg, 2007) and plotted using DISTRUCT (Rosenberg, 2004). To estimate partitioning of genetic variance among the groups determined with STRUCTURE, among populations within groups, within populations and within individuals we used an analysis of molecular variance (AMOVA; Excoffier et al., 1992) in Arlequin 3.5 (Excoffier & Lischer, 2010), where the total variance is partitioned into components analogous to F-statistics. AMOVA tests were conducted using 10,000 permutations between groups on the three matrices identifying two groups as the best partition (see STRUCTURE results). Because principal component analysis (PCA) is free from many of the population genetic assumptions underlying STRUCTURE (Gao et al., 2007; Jombart et al., 2009), we further performed a PCA to visualize the major axes of variation applied to the five random SNP matrices using the *dudi.pca* function implemented in ‘ade4’ package (Dray & Dufour, 2007) in R (R Development Core Team, 2008).

Finally, we used TreeMix to estimate the maximum likelihood tree for the populations of *B. subacaulis*. TreeMix uses as input
a set of population allele frequencies and assumes biallelic, unlinked sites (Pickrell & Pritchard, 2012). Therefore, the analysis was repeated on each of the random SNP matrices (with only one random SNP per locus, excluding SNPs with more than two nucleotide states across populations) and for each SNP repetition we ran 1,000 bootstrap replicates. A consensus of the trees across all SNP repetitions and bootstrap replicates was computed in R using ‘phangorn’ (Schliep, 2011) and ‘ape’ (Paradis et al., 2004) packages.

2.2.4 Genetic diversity statistics

The simplest measure of genetic diversity at a locus is the number of alleles (allelic richness) and a simple measure of genetic distinctiveness is the number of unique alleles in a population (private allelic richness) (Kalinowski, 2004). Following a rarefaction approach to account for sample size differences across lineages (Kalinowski, 2004), allelic richness and private allelic richness per locus were estimated in the genetic groups identified by STRUCTURE. Following the approach of Theodoridis et al. (2017), we used the full sequence of each locus as recovered in the Stacks packages.

2.3 Species distribution modelling

2.3.1 Species occurrence data and environmental layers

Occurrence data were retrieved from the database “SILENE” of the Conservatoire Botanique National Méditerranéen de Porquerolles (CBNMED) and “FLORE” of the Conservatoire Botanique National Alpin de Gap (CBNA). We gathered a total of 1942 occurrences for the species from CBNMED, from CBNA and from our field surveys. To increase the accuracy of the distribution models, we used only data collected with a GPS instrument during the field surveys; a final data set of 1184 presence records was used in the analyses.

To train SDMs and estimate current climatically suitable habitats, we first extracted the current monthly mean temperature (tmean) and precipitation (prec) from the WorldClim data set (version 1.4) at 30-s (c. 1 km) spatial resolution (Hijmans et al., 2005). Using the monthly climatic values and the function biovars in the package ‘dismo’ (Hijmans et al., 2012), we derived a set of 17 climatic variables (Table S1). We then performed a pairwise Pearson correlation among bioclimatic predictors aimed at reducing multicollinearity and minimizing model overfitting, retaining only predictors that were not highly correlated (r ≤ |0.80|): see recommendation of Elith et al., 2006) and that are physiologically important for plant species. Three bioclimatic variables were retained for further analyses: mean temperature of driest quarter (BIO9); mean temperature of warmest quarter (BIO10); and precipitation seasonality (BIO15). Because B. subacaulis grows only on specific calcareous substrates, for all the time slices we added a layer reporting the presence/absence of suitable substrate, based on the global lithological map dataset GLiM (Hartmann & Moosdorf, 2012). The GLiM represents the rock types (437 different combinations of lithological classes and subclasses) of the Earth surface with 1,235,400 polygons and an average scale 1:3,750,000 (Hartmann & Moosdorf, 2012). The lithological classification used in GLiM consists of three levels. The first level contains lithological classes comparable to previously applied definitions in global lithological maps, while the additional two levels describe more specific rock attributes (Hartmann & Moosdorf, 2012). We used the first level of classification, because the information about second and the third levels are not always present. We extracted the classes of the first level of classification for the 1184 occurrences of B. subacaulis. Then, we calculated the percentage of occurrences on the different lithological classes and excluded those rarest and not matching the soil requirement (i.e. calcareous rocks) of the species (e.g. acid volcanics; Table S3).

To reconstruct the potential past distributional ranges of B. subacaulis, we used a paleoclimate dataset of monthly mean temperature and precipitation data from Maiorano et al. (2013) described in Singarayer and Valdes (2010) and downscaled it at 1 km spatial resolution using the delta method (see Patsiou et al., 2014 for the downscaling method of the paleoclimate dataset). These paleoclimate layers were used to derive the three bioclimatic variables described above. The final paleoclimate dataset covered a period of 28 Ky at 1 ka (from 1 to 21 Kya) and 4 ka intervals (from 21 to 28 Kya).

2.3.2 Species distribution modelling

Species occurrence data and the four selected variables (three bioclimatic variables plus the soil) were used to train and project SDMs through time using the BIOMOD2 package (Thuiller et al., 2009) implemented in R. We applied five SDM techniques: generalized boosting models (GBM—Ridgeway, 1999), generalized linear models (GLM—McCullagh & Nelder, 1989), classification tree analysis (CTA—Breiman et al., 1984), flexible discriminant analysis (FDA—Hastie et al., 1994), and Maxent (Phillips et al., 2006). These techniques belong to three different method families (i.e. regression methods—GLM; classification methods—CTA and FDA; and machine learning algorithms—GBM and Maxent; Barbet-Massin et al., 2012; Marmion et al., 2008).

Because most modelling techniques implemented in BIOMOD2 require that species distribution data are represented as presence and absence, we generated ten different sets of pseudo-absences. For each SDM technique, the number of pseudo-absences (Table S2) was selected according to the recommendation of Barbet-Massin et al. (2012). The predictive performance of the models
was evaluated for each pseudo-absence run by repeating a split-sample cross-validation 10 times, using a random subset (70%) of the initial dataset each time to calibrate the models, while the remaining 30% was used to evaluate the models. Three different evaluation measures were calculated: area under the receiver operating characteristic curve (AUC), true skill statistic (TSS) and Kappa statistic (Thuiller et al., 2009). To further transform continuous probability values from model outputs to binary presence-absence we used the threshold that maximized TSS. For the final ensemble projections, we employed ‘majority’ combinations (i.e. at least three out of five techniques predicting suitable areas) to produce binomial maps of presence and absence through time. To exclude potential suitable areas that were covered by ice, we further filtered these presence-absence maps using reconstructed ice margins spanning a period from 28 to 15 Kya (Ehlers et al., 2011; Rolland et al., 2020).

2.4 | Estimation of demographic history

To test for alternative demographic responses of B. subacaulis to the Late Quaternary glaciations, we integrated the genomic data and the results of SDMs in a statistical phylogeography framework using the ABC procedure (Cornuet et al., 2014; Patiño et al., 2015; Ray et al., 2010; Wegmann & Excoffier, 2010). We evaluated three main alternative demographic scenarios corresponding to each one of the three proposed hypotheses: (i) a post-LGM expansion model, characterized by expansion in population sizes after the LGM and divergence time during the last glaciation (‘post-glacial expansion model with old divergence’); (ii) a post-LGM contraction model, characterized by reduction in population sizes after the LGM followed by the split between the two genetic groups (‘post-glacial contraction model with recent divergence’); and (iii) a standard neutral model representing two lineages that kept constant population size during and after the LGM (‘long-term stability model’). In order to further test the effect of divergence time between the two genetic groups, we evaluated two additional hypotheses: (iv) a post-LGM expansion model, characterized by expansion in population sizes after the LGM followed by the split between the two genetic groups (‘post-glacial expansion model with recent divergence’); and (v) a post-LGM contraction model, characterized by reduction in population sizes after the LGM and a divergence time between the two main genetic groups during the last glaciation (‘post-glacial contraction model with old divergence’). A total of five demographic models were tested (Figure S5).

Following Theodoridis et al. (2017), we used the program fastsimcoal v. 2.5.0.2 (Excoffier et al., 2013) to perform \(6 \times 10^5\) coalescent simulations of genomic diversity under each of the five models for the demographic history of B. subacaulis. Current population sizes and time intervals of demographic changes were fixed across all simulations and models, while divergence times, growth rates and mutation rates were randomly drawn from uniform prior distributions (Table 2).

Current population sizes were based on census population sizes (personal observations in the field) and converted to effective population sizes with reference to population sizes of other perennial plants (Gossmann et al., 2010). The time interval for post-glacial demographic changes (expansion and contraction hypotheses) was inferred by visually identifying changes in the number of suitable cells predicted by the SDMs and according to geological evidence (Rolland et al., 2020) and vegetation reconstruction (Brisset et al., 2015). In fact, in the slopes (~2300 m a.s.l.) where B. subacaulis occurs, it has been showed that the main deglaciation took place at 15.3–14.2 Kya (Rolland et al., 2020) and that the meso-thermophilous flora increased in the Preboreal (~11 Kya - Brisset et al., 2015). Similarly, SDMs identified Preboreal as the end of habitat suitability loss for B. subacaulis (Figure 4, Figure S1). We thus identified as time interval the period between 24 Kya (corresponding to the maximum of LGM in the SW Alps—Seguinot et al., 2018) and 11 Kya.

The divergence times between the two main genetic groups of B. subacaulis were sampled from uniform prior distributions. The time interval for the recent divergence models was defined between 11 and 0 Kya, while for the old divergence models it was defined between 120 and 24 Kya. For the long-term stability hypothesis, divergence time was sampled from the interval between 120 and 0 Kya. Time was expressed in number of generations by assuming an average generation time of 5 years (personal communication, C. Voisin & M. Rome).

Growth rates were sampled from uniform priors for all hypotheses. Changes in population growth rates were estimated by using changes in the mean habitat suitability of SDMs at the beginning and the end of the selected time interval. The bounds (\(r\)) of the prior distributions for growth rates were obtained using the formula of Excoffier et al. (2013),

\[
\frac{\log \left( \frac{N_0}{N_t} \right)}{t} = r
\]

which assumes exponential population growth, where \(N_0\) and \(N_t\) are the population sizes, expressed in mean habitat suitability, at the beginning and at the end of the time interval respectively. For all SDMs, we estimated the lower and upper bounds of \(r\) as follows. The lower bound was inferred from the single SDM output that showed the smallest change (i.e. \(N_0 - N_t\)) in mean habitat suitability during the interval. The upper bound was inferred from the single SDM output that showed the largest change of mean habitat suitability during the interval. Since all SDMs showed a reduction in mean habitat suitability between 24 and 11 Kya (i.e. contraction; see Results), growth-rate priors for the expansion hypothesis were defined by using the additive inverse (i.e. by changing the sign) of the estimated lower and upper bounds. Mutation rates were sampled from a uniform distribution with a lower bound of 0.05 \(\times 10^{-8}\) and an upper bound of \(10 \times 10^{-8}\) substitutions/site/year, based on previously published estimates for other annual and perennial plants (Gossmann et al., 2010; Ossowski et al., 2010).

We simulated the same amount of diploid loci that was used to estimate the genetic diversity statistics in B. subacaulis, maintaining the
same size of the empirical data set (see the ‘Genetic diversity statistics’ section). For each simulated dataset, we calculated the same two summary statistics used in the genomic diversity analysis of the empirical data set: allelic richness and private allelic richness for the two genetic groups (as in François et al., 2008), for a fixed sample size of 15 individuals (30 sequences) as point estimate. We chose allelic richness and private allelic richness because they explicitly account for different sample sizes among genetic groups. Moreover, commonly used summary statistics of genetic diversity calculated from data generated by Reduced Representation Libraries (such as RADseq) are known to deviate considerably from real values, thus considered inappropriate for demographic inferences (Arnold et al., 2013). Nevertheless, we additionally calculated expected heterozygosity for each genetic group using the full sequence of each simulated locus following Nei (1973). To estimate the posterior probabilities of the five models (i.e. the fit between observed and simulated data under each model), we first calculated the Euclidean distance between the observed and simulated summary statistics (standardized across all simulations for each model), following Beaumont et al. (2002). We then adopted the standard rejection procedure proposed by Pritchard et al. (1999), as follows. For each of the five models, we retained the top 1% (i.e. 6000) simulations with the smallest Euclidean distances to the observed summary statistics. The retained simulations for each model were combined and the final 30,000 simulations were ordered by ascending Euclidean distances recomputed on summary statistics standardized with common mean and standard deviation (Patiño et al., 2015; Ray et al., 2010). The posterior probability of each model was then computed as the proportion of simulations executed under the respective model that were included in the set of the top 1% (i.e. 600) simulations closest to the observed data (Estoup et al., 2004; Miller et al., 2005). To estimate the parameters of the model with the higher posterior probability, we used the neural network approach implemented in R package ‘abc’ (Csilléry et al., 2012). Postsampling adjustment and parameter estimation were carried out using the 6000 top simulations. We report the mode of the posterior distribution and the 95% highest posterior density (HPD) interval for each parameter.

3 | RESULTS

3.1 | Genetic analyses

3.1.1 | Genotyping

The genomic library yielded 234.2 million single-end Illumina reads of 101 bp length for 95 B. subacaulis individuals. After the trimming with Trimmomatic and quality-control filtering, we excluded four individuals (one individual for each of the following populations: population 4, population 6, population 16, population 18) containing excessively

![Figure 3](https://example.com/figure3.png)

**Figure 3** (a) Plot of posterior probabilities for the assignment of each sampled individual of Berardia subacaulis to its cluster based on STRUCTURE analysis: two genetic clusters were identified (k = 2). (b) Allelic richness (mean number of alleles per locus) and private allelic richness (mean number of private alleles per locus) of the two genetic groups of B. subacaulis as functions of sample size (i.e. rarefaction approach; Kalinowski, 2004). Vertical bars show standard error.
high amounts of missing data (more than 80%) and we retained a matrix with a total of 2,246,647 reads of 85 bp length. The matrix consisted of 4035 RADSeq loci comprising 423,120 aligned nucleotide positions and 12,668 variable sites using a 60% threshold of missing data per locus.

### 3.1.2 Population genetic structure

For three out of the five replicates, STRUCTURE analyses identified $K = 2$ (Figure 3a, Figure S3) as the most probable number of genetic

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**TABLE 1** Summary table of the analysis of molecular variance (AMOVA) for *Berardia subacaulis*.

| Source of variation          | df | Sum of squares | Variance components | Percentage of variation |
|------------------------------|----|----------------|---------------------|------------------------|
| $k = 2$ Among groups         | 1  | 955.855        | 13.13308            | 18.59                  |
| Among populations within groups | 17 | 4889.752       | 26.89926            | 38.07                  |
| Within populations           | 163| 4993.025       | 30.63206            | 43.35                  |
| Total                        | 181| 10838.632      | 70.66440            |                        |

Fixation index $Fst$ 0.50070**

** Indicate a significant difference $P \leq 0.001$.

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**FIGURE 4** Overview of the post-glacial contraction model with recent divergence for cold-adapted endemics (see Figure 1) supported by statistical phylogeographic analyses of *Berardia subacaulis*: (a) synoptic maps with overall visual representation of habitat suitability for *B. subacaulis* in the SW Alps predicted in three time intervals corresponding to the time of demographic transition for the species, the maps are based on mean habitat suitability among the five methods used for hindcasting, with red indicating high and blue indicating low values; gray background corresponds to unsuitable areas; black line represent the distributional range of *B. subacaulis*; the extent of LGM (white area) is reported in the map relative to the time interval 24–11 Kya; (b) the predicted demographic fluctuations in *B. subacaulis*; (c) mean annual temperatures (dotted gray line) for the pixels where the species is present and mean habitat suitability (black line) in the study area across the 24 time slices.

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AMOVA shows that most of the genetic variance is due to diverged a clade sister to CS populations (Figure S4). Nevertheless, evident in the PCA (Figure S2). These results are also corroborated SDS
TSS value excellent model performance for all modelling techniques (average predicted using the classification of Araújo et al. (2005), indicated ex-

3.1.3 | Genetic diversity statistics

We obtained estimates of allelic richness and private allelic richness across the two lineages identified by STRUCTURE for subsamples of 2 to 30 sequences (15 diploid individuals) and 4035 loci. The CS populations had on average the highest allelic richness and the highest number of private alleles across most investigated sample sizes (Figure 3b).

3.2 | Species distribution modelling

Under current climate conditions, AUC, TSS and KAPPA, interpreted using the classification of Araújo et al. (2005), indicated excellent model performance for all modelling techniques (average TSS value = 0.8587, SD = 0.0341; average AUC values = 0.9589, SD = 0.0212; average KAPPA value = 0.8735, SD = 0.0306; Table S5).

The results of ABC-based analyses supported the post-glacial contraction of B. subacaulis with a recent divergence between the genetic groups (Figure 4b; posterior probability under the standard rejection procedure 0.38), followed by the post-glacial expansion model with recent divergence (posterior probability under the standard rejection procedure 0.33), the long-term stability model of constant population sizes (posterior probability under the standard rejection procedure 0.20). The results of ABC-based analyses provided low support for the post-glacial expansion model with old divergence (posterior probability under the standard rejection procedure 0.09), and no support for the post-glacial expansion model with old divergence (posterior probability under the standard rejection procedure 0).

The demographic parameter estimates under the model with the highest posterior probability (i.e. the post-glacial contraction model with recent divergence; Table 2) are the following: the split between the NW and CS groups was estimated at 2.46 Kya (2.07–2.81 Kya 95% HPD); the estimated mutation rate was $4.2 \times 10^{-8}$ (3.9–4.4 95% HPD); and the estimated contraction rates during period 24–11 Kya was $1.07 \times 10^{-4}$ (0.78–1.35 95% HPD).

4 | DISCUSSION

In this study, we investigated the response of the endemic B. subacaulis to the Late Quaternary climatic oscillation. Combining several independent lines of evidence in an ABC modeling framework, we were able to discern among three main competing hypotheses (i.e. post glacial contraction, post glacial expansion and long-term stability) and to provide, for the first time, empirical evidence of post-glacial demographic contraction and a recent split between the two genetic groups for an endemic plant in the European Alps during the Late Quaternary. However, considering the high species diversity and environmental heterogeneity in the SW Alps, and that the present study is based only on a single species, further research is needed to draw general conclusions about the regional Quaternary history of endemic species in the Alps.

The statistical phylogeographic approach used in our study provided strong support for a recent divergence between the two genetic groups (Figure 4b, Table 2), while it only slightly supported the post-glacial demographic contraction of B. subacaulis (Figure 4b, Table 2) compared to the post-glacial expansion model. However, in line with the post-glacial demographic contraction, SDMs (Figure 4a,c, Figure S1) showed a reduction in potentially suitable areas for B. subacaulis during the post-glacial climate warming, suggesting that B. subacaulis started to contract in this period, while it likely attained a larger distribution during the glaciations (Guerrina, Conti, et al., 2016). In this scenario, the post-glacial isolation due to range contraction likely led to the recent divergence between the two main genetic groups.

In B. subacaulis we observed a weak genetic subdivision of populations, supported by the low number of genetic groups (the
NW group and the CS group; Table 1, Figure 3, Figures S2 and S4). Usually, in a scenario of post-glacial range expansion, a strong phylogeographic structure is expected as result of high differentiation among refugial populations due to the genetic drift (Comes & Kadereit, 1998). Moreover, low levels of genetic diversity are expected in the populations at the wave front of expansion (Excoffier & Ray, 2008; Hewitt, 2000). Differently, we recorded low genetic diversity values in populations scattered over the CS group (Table S7). The weak phylogeographic structure we detected in B. subacaulis may be explained mainly by a long-lasting period of connectivity among populations either in a scenario of extensive in situ survival and low degree of geographical isolation (Casazza et al., 2016) or in a scenario of expansion during the glacial cycles followed by a short period of interglacial isolation, thus preventing the accumulation of among-populations genetic differentiation (Garcia et al., 2011; Gibbard & van Kolfschoten, 2004; Kropf et al., 2003; Stewart et al., 2010). This latter scenario is likely the one observed in B. subacaulis, as supported by its recent split between the two genetic groups (2.46 Kya; Table 2, Figure 4b), by SDMs and by ABC analysis. However, in some species, the small population sizes in interglacial refugia may be more strongly affected by genetic drift, potentially overcoming temporal effects and generating deep phylogeographic structure (Bonatelli et al., 2014), but this does not seem to be the case for B. subacaulis, likely because of its very recent split between the two genetic groups and/or probably limited recent genetic drift.

Taken together, our results support the post-glacial range contraction hypothesis. These results contrast with the dominant pattern observed for endemics in the Alps, based on molecular analyses and SDMs, where it is hypothesized that cold-adapted species, both endemic (e.g. Androsace alpina) and not endemic (e.g. Phyteuma globularifolium), primarily contracted their range, retreating into refugia during cold periods (Schönswetter et al., 2002, 2003, 2004, 2005; Tribsch & Schönswetter, 2003). In fact, in most of the Alps, the massive ice extent during the LGM prevented cold-adapted endemics to reach or to survive in most of the potentially suitable areas, forcing them to retract in nunataks, when available, or to migrate to peripheral refugia (Schönswetter et al., 2005), occupied mainly by trees (Ravazzi, 2002) and steppe vegetation adapted to a cold and dry climate (Janská et al., 2017). Therefore, the reduced availability in suitable habitats may have resulted in range contractions for most of the cold-adapted endemics in the Alps.

The different pattern observed in B. subacaulis might be due to several factors. First, the SW Alps were characterized by a greater availability of ice-free terrain. In fact, here the Mediterranean Sea mitigated the climate during LGM, maintaining temperatures some degrees higher than in the rest of the Alps (Seguinot et al., 2018) and, consequently, the ice cover was less extensive (Federici et al., 2012). Second, the SW Alps were characterized by relatively high precipitation (Janská et al., 2017), which, combined with the ice-free areas, might have allowed B. subacaulis to persist or even expand in most of the climatically suitable areas at high altitude during the LGM, while other cold-adapted endemics distributed in other parts of the Alps restricted their ranges because of the dryness of glacial steppes beyond the ice margins (Schmitt, 2007; Schönswetter et al., 2005).

Third, after the LGM, the increase in temperatures and the reduction in precipitation may have reduced available areas at low elevations in the southern parts of SW Alps, while, as deglaciation proceeded, newly available (i.e. ice-free) areas were available northernmost. However, the poor dispersal abilities (~15 m per year - Vittoz & Engler, 2007) and the slow developmental growth of B. subacaulis, combined with the microhabitat preferences (i.e. calcareous screes), may have prevented post LGM colonization of these northernmost areas, resulting in range contraction on screes, characterized by low aboveground competition (Lavergne et al., 2004) and stones coverage assuring the moisture necessary for plant growth (Körner, 2003; Pérez, 1998). Similarly, in other alpine systems that were less affected by glaciation, for instance in central Iberian Peninsula and in Japan, cold-adapted endemics attained larger distributions during glacial maxima and contracted when temperature increased (i.e. Phyllocladus japonica and Potentilla matsumurae-Ikeda & Setoguchi, 2007; Ikeda et al., 2008; Senecio boissieri-Ikeda & Setoguchi, 2008).

Our findings thus provide support to the idea that the response of cold-adapted endemics to past climate change depends not only on their ecological features, but also on where they occurred (Bennett & Provan, 2008; Nieto Feliner, 2014; Stewart et al., 2010), shedding light on the complexities of organisms’ response to the Late Quaternary climatic oscillations.

In general, low levels of genetic diversity (Appendix 1, Table S7), as observed in B. subacaulis populations, are expected in species with small geographic range (Hamrick & Godt, 1989) and are detected in other Tertiary species like Haberlea rhodopensis (Petrova et al., 2015) and Ramonda myconi (Dubreuil et al., 2008), endemic to other southern European mountains. Loss of genetic diversity in these species was hypothesized to result from severe genetic bottlenecks, likely as a consequence of range contractions during the cold periods (Dubreuil et al., 2008; Petrova et al., 2015). Contrary to the above-mentioned Tertiary species, B. subacaulis contracted its range after the LGM (Figure 4) and likely during other warm periods, such as the Last Interglacial (LIG, ~120 Kya; Guerrina, Conti, et al., 2016). As a consequence, its low genetic diversity may result from losses that occurred not only during the recent post-glacial contraction but also during the adverse warm periods. Moreover, because of the strong reduction in population sizes during bottlenecks (Nei et al., 1975), the mixed-mating system of B. subacaulis (Guerrina, Casazza, et al., 2016) might have favoured self-fertilization to ensure reproduction.

In conclusion, as opposed to the main response suggested for endemic species, attaining narrower distributions during glacial maxima (Pawlowski, 1970; Schönswetter & Schneeweiss, 2019; Schönswetter et al., 2005; Tribsch, 2004; Tribsch & Schönswetter, 2003), our results provide the evidence of post-glacial range
contraction of an endemic plant in the European Alps through Late-Quaternary climatic oscillations. Future research in areas where the ice cover was less extensive, such as the SW Alps (the richest centre of endemism in the Alps; Aeschimann et al., 2011), will contribute to a more complete understanding of the role of climatic changes in shaping the highly endemic biota of the European Alps.

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CONFLICT OF INTEREST
The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT
The Fasta file, as exported from STACKS and used for all subsequent molecular analyses, is available on FigShare https://doi.org/10.6084/m9.figshare.14270246.v1

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**BIOSKETCH**

**Maria Guerrina** is currently a postdoctoral scholar. This work is part of her PhD project, focused on the biogeographical history and conservation of species endemic to the South-western Alps. The project was carried out in collaboration between the University of Genoa and the University of Zurich.

**Authors Contribution:** Gabriele Casazza and Luigi Minuto designed the study. Samples were collected by Maria Guerrina, Gabriele Casazza and Luigi Minuto. Maria Guerrina did the molecular work and analysed the data. Spyros Theodoridis conducted the GBS analyses and the ABC-based analyses. Gabriele Casazza gave conceptual advice on analyses of the data and interpretation of the results. Gabriele Casazza, Luigi Minuto and Elena Conti supervised the project. Luigi Minuto obtained funding. Maria Guerrina wrote the first draft, and Elena Conti, Gabriele Casazza, Luigi Minuto and Spyros Theodoridis improved successive versions. All authors read and approved the final manuscript.

**SUPPORTING INFORMATION**

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