



Research article

Reprint of: Disentangling drivers of plant endemism and diversification in the European Alps - a phylogenetic and spatially explicit approach[☆]



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ABSTRACT

Plant endemism in the European Alps is clustered into particular geographic areas. Two contrasted and non exclusive hypotheses have been suggested to explain these hotspots of endemism: (i) those areas were glacial refugia, where endemism reflects survival-recolonisation dynamics since the onset of Pleistocene glaciations, (ii) those are high elevation mountain areas, where endemism was fostered by local speciation events due to geographic isolation and harsh environmental niches, or by low dispersal ability of inhabiting species.

Here, we quantitatively compared these two hypotheses using data of species distribution in the European Alps (IntraBioDiv database), species phylogenetic relationships, and species ecological and biological characteristics. We developed a spatially and phylogenetically explicit modeling framework to analyze spatial patterns of endemism and the phylogenetic structure of species assemblages. Moreover, we analyzed interrelations between species trait syndromes and endemism.

We found that high endemism occurs in potential glacial refugia, but only those on calcareous bedrock, and also in areas with high elevation. Plant assemblages in calcareous refugia showed phylogenetic overdispersion – a signature of non-selective conservation forces, whereas those located in high mountain areas showed phylogenetic clustering – a signature of recent diversification and environmental filtering. Endemic species were either stress-tolerant, poorly dispersing species, or high elevation specialists with a wide distribution within the European Alps.

While both calcareous refugia and high-elevation hotspots harbour a large portion of plant endemism in the European Alps, the species they host have substantially different characteristics. Our results suggest that hotspots of endemism in calcareous refugia are more important for nature conservation planning, as they host many range restricted endemic species and rather isolated evolutionary lineages.

1. Introduction

Mountain ranges across the world are considered typical examples of endemic-rich regions (Hughes and Atchinson, 2015), but the evolutionary mechanisms and historical factors generating this high endemism are not fully understood. It was early recognized that mountains have a much richer endemic flora than the surrounding lowlands, and also that within mountain systems, there exist specific areas with exceptionally high endemism (de Candolle, 1875; Pawłowski, 1970). Such hotspots of endemism in alpine regions were observed mostly in putative glacial refugia (Pawłowski, 1970; Tribsch and Schönswetter, 2003; Feng et al., 2016) or in high-elevation areas (Aeschmann et al.,

2011; Nagy and Grabherr 2009; Tribsch and Schönswetter, 2003). This suggests that their occurrence is coupled with specific evolutionary dynamics: such regions may exhibit lower extinction rates due to climatic stability and reduced glacial extent, or higher speciation rates and poorer dispersal ability of high-elevation species.

Hotspots of plant endemism in the European Alps (Alps hereafter) have traditionally been explained by the presence of refugia on the periphery of glacial cover during the ice age periods. These refugia are assumed to have promoted long term population persistence of many species during glacial periods, which left imprints in the population structure of survivor species (Alvarez et al., 2009; Schönswetter et al., 2005; Stehlik, 2003). The distribution of glacial refugia has also shaped

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contemporary species distributions and endemism patterns, since some survivors could not recolonize all adjacent regions after the retreat of glaciers (Dullinger et al., 2012). It is often assumed that refugia with different bedrocks hosted different pool of species, as the majority of plant endemics of the Alps show either a clear affinity or a strong intolerance to calcareous bedrock, with endemic flora of calcareous bedrock being generally richer (Schönswetter et al., 2005; Tribsch and Schönswetter, 2003). It is likely that species survival during glacial periods has generated particular patterns of endemism, and also left a particular phylogenetic signature in local floras. We can thus hypothesize that species surviving glacial cycles in refugia were drawn from a pool of “pre-glacial” species, whereas species occurring outside refugia were filtered for ability of fast recolonisation, resulting in relative phylogenetic overdispersion of species assemblages in glacial refugia. Here, we aim at quantitatively testing across the whole Alps whether the areas predicted as inhabitable during glacial periods *sensu* Schönswetter et al. (2005) match the above described patterns of endemism and phylogenetic structure, and whether patterns of endemism in these potential refugia are influenced by other factors, as is refugium bedrock, topography, or geographic region.

In addition to the influence of Pleistocene historical processes, patterns of plant diversity and endemism in the Alps may also be linked to the characteristics of high-elevation ecosystems, such as geographic insularity and availability of free but hostile niches. Indeed, it was observed that endemic diversity in the Alps grows with elevation (Aeschmann et al., 2011; Tribsch and Schönswetter, 2003). This pattern could be explained by two processes that are not mutually exclusive. First, increased speciation rate in high elevation ecosystems (documented on a global scale and reviewed in Hughes and Atchison, 2015) could induce higher endemism in certain plant clades. This may be due to heterogeneity or niches in high mountain environments and specific life histories of mountain species favouring sympatric speciation (Dixon et al., 2009; Roquet et al., 2013a, 2013b), or due to topographic obstacles in high mountain environments stimulating allopatric speciation (Boucher et al., 2016; Comes and Kadereit, 2003). Repeated speciation events in high-mountain floras could then induce a phylogenetic signature of radiating lineages, producing a phylogenetic clustering in local species assemblages. Second, increased plant endemism in high elevations may have resulted from a reduced dispersal potential itself. Adaptation to high-alpine environments may imply stress tolerance, long lifespan, and preference for vegetative spread (Körner, 2003) to the detriment of dispersal capabilities (i.e. insularity syndrome). High endemism in mountains resulting from increased speciation rates or decreased dispersal capacities of high-elevation species can thus be expected to result in specific signatures of phylogenetic clustering in local species assemblages or in the presence of particular trait syndromes that have improved survival in high mountain environments at the expense of dispersal potential. This has never been tested to date.

We thus argue that patterns of endemism in the Alps could be explained both by local survival-recolonization dynamics following glaciations, and by dynamics of speciation and dispersal in high-elevation ecosystems. In this paper: (i) We quantitatively compare the relative importance of potential glacial refugia on different bedrocks on one side, and elevation on the other side, for patterns of plant endemism in the Alps. To do this, we use two measures of endemism, namely the proportion of endemic species and range size of endemic species, using a grid-based species occurrence data in the Alps (IntraBioDiv; Gugerli et al., 2008). (ii) We test whether the phylogenetic structure (richness-standardized phylogenetic diversity; Faith, 1992) of species assemblages differs between those different types of hotspots, according to hypothesized evolutionary processes. Importantly, we developed here a novel method based on Bayesian imperfect detection framework in order to overcome difficulties when calculating community phylogenetic indices from non-completely resolved phylogenies (Molina-Venegas and Roquet, 2014; Rangel et al., 2015). (iii) To shed more light on processes forming endemism in high elevations, we explore how

endemism and range size of endemics are related to species elevational optimum (Landolt et al., 2010), ecological and functional characteristics related to high elevation adaptations and evolutionary distinctiveness of species (Isaac et al., 2007).

2. Methods

2.1. Study region

We focus on the European Alps, which corresponds to the great mountain range system stretching from south-eastern France to Slovenia. The tree line in the European Alps lies at cca. 2000 m a. s. l. and upper limits of vascular plant occurrence lie in 3500–4500 m a.s.l., differing by region (Ozenda and Borel, 2003; Tribsch and Schönswetter, 2003). The European Alps belong to one of the coldest biomes on the planet at its highest elevations (Körner, 2011); nevertheless, this region appears to be relatively species rich, with a fairly high rate of plant endemism (about 13%; Aeschmann et al., 2004; Pawlowski, 1970). The Alps thus constitute a well known hotspot of biodiversity in Europe (Väre et al., 2003).

2.2. Species distribution and environmental data

Species distribution data originate from the mapping of the flora across the Alps produced by the IntraBioDiv consortium (IBD; Gugerli et al., 2008). This dataset contains census and expert based presences and absences of all plant species occurring above the tree line on a regular grid with cells of 20' longitude and 12' latitude (ca. 25 × 22 km). This grid was used for all subsequent spatial analyses. The restriction of the species pool to species occurring clearly above the treeline may be considered problematic for example for interpreting species richness across the dataset (but see Taberlet et al., 2012). Nevertheless, while investigating evolutionary processes, such restriction removes potential noise generated by lowland species that likely have reduced evolutionary histories related to mountains. We also excluded gymnosperms and ferns, because some of our working hypotheses may not be extended to them, trait definition for angiosperms are not easily applicable to those groups and sampling efforts for ferns was low compared to angiosperms.

To quantify which grid cells might serve as glacial refugia during glacial cycles, we overlaid the IBD grid with distribution of potential siliceous and calcareous refugia based on combination paleoclimatic model with geological data (adapted from Schönswetter et al., 2005) and estimated whether each grid cell contained calcareous, siliceous, none, or both types of potential refugia. The potential refugia, adapted from Schönswetter et al. (2005), are estimated for maximum of the last glacial period (110,000–12,000 years before present), but they can also be considered a proxy information for distribution of refugia in previous glacial periods. These potential refugia are mostly peripheral (along southwestern, southern, eastern and northern borders of the Alps), likely favouring isolation of plant populations for thousands of years (Schönswetter et al., 2005). The calcareous refugia are the ones lying on limestone or dolomitic bedrock, whereas siliceous refugia lie on variety of acidic bedrocks like granite or gneiss. Larger coherent areas of bedrock types not falling into these two categories are relatively rare within the Alps (Schönswetter et al., 2005; Tribsch and Schönswetter 2003). To separate the effect of refugia from the effect of bedrock itself, we also estimated whether each grid cell contained calcareous (limestone or dolomite) or siliceous bedrock (granite, diorite or gneiss) based on dominant parent material map (PARMADO) from European Soil database (resolution 1 × 1 km). To quantify the topography of grid cells, we calculated their mean elevation and their difference between highest and lowest elevation (elevation range, hereafter) based on Global digital elevation model by US Geological Survey (resolution 30" × 30", cca 1 × 1 km). All calculations were performed using the statistical environment R (R Core Team, 2016) and the R

libraries raster (Hijmans, 2016), rgdal (Bivand et al., 2016) and spatialEco (Evans, 2016). All data used for our analyses are accessible in Dryad repository under accession number (to be completed after attribution of doi).

2.3. Phylogenetic data

A genus-level phylogeny was built for the Alpine flora using the workflow proposed by Roquet et al. (2013a, 2013b). We downloaded from Genbank three conserved chloroplastic regions (rbcL, matK and ndhF) plus eight regions for a subset of families or orders (atpB, ITS, psbA-trnH, rpl16, rps4, rps4-trnS, rps16, trnL-F), which were aligned separately by taxonomic clustering. All sequence clusters were aligned with three programs (MUSCLE, Edgar, 2004; MAFFT, Katoh et al., 2005; Kalign, Lassmann and Sonnhammer, 2005a), then the best alignment for each region was selected using MUMSA (Lassmann and Sonnhammer, 2005b) and depurated with TrimAl (Capella-Gutiérrez et al., 2009) after visual checks. DNA matrices were concatenated to obtain a supermatrix. Maximum-likelihood phylogenetic inference analyses were conducted with RAxML (Stamatakis et al., 2008) applying a supertree constraint at the family-level based on Davies et al. (2004) and Moore et al. (2010). 100 independent tree searches were performed. The 100 ML trees obtained were dated by penalized-likelihood using r8s (Sanderson, 2003) and 25 fossils for calibration extracted from Smith and Beaulieu (2010) and Bell et al. (2010).

To deal with unknown within-genera structures, we simulated 10 scenarios of within-genera random branchings for each of 100 general-level trees using a Yule process as implemented in the R library apTreeshape (Bortolussi et al., 2012). This resulted in 1000 trees that represent a distribution of possible hypotheses about evolutionary relations in our dataset *sensu* Rangel et al. (2015).

2.4. Species ecological and biological features

For each species, we extracted from Flora Indicativa (Landolt et al., 2010) the following ecological indicator values and biological traits:

inverted values of Landolt's T (expert based ordinal scale classification of species elevation, ranging from 1 for lowland to 5 for alpine species) as species level information about its elevational optimum domain,

- CSR strategy *sensu* Grime (1977) depicting stress tolerance (S), ruderal strategy (R) and competitive capacity (C) of each species (S and R were coded as independent ordinal variables with values between 0 a 3 referring to amount of “S” and “R” in three letter characteristic of a species, C is a linear combination of S and R and thus was not used separately; e.g. for CSS species, stress tolerance = 2 and ruderal strategy = 0),
- species dispersal capacity (a value of 1 was attributed to anemo- or zoochoric species, and 0 to those with a different dispersal strategy),
- vegetative reproduction (a value of 1 was attributed to species with the ability of any vegetative reproduction, and 0 to species with completely non-vegetative reproduction),
- cushion life form *sensu* Aubert et al., 2014; a value 1 was attributed to species with vegetative reproduction forming tussocks or cushions, 0 for all other species)
- sexuality of the species (a value of 1 was given to species only capable of sexual reproduction, and 0 for species with facultative or obligate asexual seed generation mechanism, as is apomixis or cleistogamy)
- Raunkiaer plant life-forms (a set of binary variables coding for species being a therophyte, geophyte, hemicryptophyte, chamaephyte or phanerophyte)

Species evolutionary distinctiveness (Isaac et al., 2007) was estimated as a so-called fair proportion measurement (as implemented in R

package picante; Kembel et al., 2010) averaged across all 1000 phylogenetic trees. The evolutionary distinctiveness describes whether species are positioned in strongly or weakly branching parts of a phylogenetic tree (sometimes referred as “bushy” or “stemmy” subtrees), and the inverse value of evolutionary distinctiveness may be considered a species-level measure of diversification rate (Jetz et al., 2012).

Endemism distribution and its relationships with potential glacial refugia and topography

Species were classified as endemic or non-endemic to the Alps based on the Flora Alpina (variable referred to as endemic status hereafter; Aeschimann et al., 2011). To explore the relationship between spatial patterns of endemism, potential glacial refugia, bedrock and topography, we modeled the ratio of the number of endemics to the total number of plant species within each grid (proportion of endemics, hereafter) by a binomial process with the following predictors: mean elevation, elevation range, the presence of calcareous or siliceous bedrock, and presence of potential calcareous or siliceous refugia in grid cell. In order to locate region-specific patterns, spatial smoothing was added as an additional term to the model. To control for overdispersion, a random effect from a Gaussian distribution was added. This was done by means of a generalized additive model (GAM), fitted with Bayesian inference. The MCMC sampling was performed using a JAGS sampler (Plummer, 2003; Plummer, 2016), using modified BUGS code generated by the jagam procedure in the R library mgcv (Wood, 2011). The model code is provided in Appendix D in Supplementary material. We ran the model on 5 chains for 70000 iterations, with a 20000 generation burn-in period and we thinned the resulting chains by 50. The convergence of the model was checked visually and by means of the Gelman-Rubin statistic (Gelman and Rubin, 1992).

The endemic rarity was defined as $\log(1/\text{species number of occurrences in IBD data})$ for all species endemic to the Alps. For each grid cell, we calculated the mean rarities of all occurring endemic species (variable further referred to as mean endemic rarity). To investigate links between mean endemic rarity and the presence of potential refugia and topography, we fitted a GAM similar to the one for proportion of endemics. To deal with the fact that mean endemic rarity is poorly estimated in grid cells with fewer endemics, we added a layer of hierarchy and modeled mean endemic rarity of each grid cell as a latent variable representing the mean of a Gaussian distribution from which rarities of different species in grid cell are drawn. The distribution of mean endemic rarity was modeled as a function of mean elevation, elevation range, presence of calcareous and siliceous bedrock, potential calcareous and siliceous refugia and spatial spline as predictors, using the Gaussian error structure. The model code is provided in Appendix D in Supplementary material. The JAGS sampler setup and convergence checking procedure was identical to the one used for modeling species endemism.

The GAM framework is useful for discovering region-specific patterns, does not rely on any pre-defined relationships, and provides easily interpretable visualizations. However, this framework may not necessarily be the most suitable way to control for spatial autocorrelation (Dormann et al., 2007). Because of that, we also tested other methods to account for spatial autocorrelation, namely spatial generalized least square models and conditional autoregressive models. These alternative methods provided qualitatively similar results to those obtained with the spatial GAM (see Appendix A in Supplementary material), and thus only GAM results are presented and discussed in the main text.

Phylogenetic diversity and its relation to potential glacial refugia and topography

We quantified the phylogenetic structure in each grid cell by calculating phylogenetic diversity (Faith, 1992) standardized for species richness effects (ses.PD), as implemented in R package PhyloMeasures (Tsirogianis and Sandel, 2017). This standardization was used to remove implicit interdependence between species richness and phylogenetic diversity, and resulting values are further interpreted in this way.

The link between ses.PD and the presence of potential refugia and topography was explored with a bayesian GAM similar to the one for proportion of endemism or mean endemic rarity. In order to control for phylogenetic uncertainty, we performed ses.PD calculations for each of 1000 phylogenetic trees within each grid cell. The “real” value of ses.PD was modeled as a latent variable representing mean parameter of Gaussian distribution from which ses.PDs of different trees are drawn. The distribution of the “real” ses.PD per grid cell was modeled as a function of mean elevation, elevation range, presence of calcareous or siliceous bedrock, potential calcareous or siliceous refugia and a spatial spline as predictors, using a Gaussian error structure. For the model code, see Appendix D in Supplementary material. The sampler setup and convergence checking was identical as for the previous Bayesian models.

Endemism-elevation relationships and its link to species traits and ecological properties

In order to explore relationships between species endemism and their elevational niche, we modeled species-level endemic status (endemism to the Alps coded as a binary variable) with a non-spatial generalized linear model (GLM) with a binomial error term, and used each species’ elevational optimum (Landolt’s T) as a predictor. To evaluate how the other species characteristics affected this endemism-elevation relationship, we fitted the same model with additional predictors: evolutionary distinctiveness, ecological strategies and species traits (see above “Species ecological and biological features”). All predictors were standardized prior to analyses. The important predictors were selected using forward-backward model selection based on Akaike’s information criterion (AIC), as implemented in stepAIC procedure in R, starting from a full model containing all variables. In order to tease apart the relative effects of different species features, partial correlations for non-parametric Kendall’s correlation coefficient were computed using the package ppcor in R (Kim, 2015). To explore relationships among endemics rarity, elevational optimum and other species characteristics, we fitted models similar to the ones used for endemic status. In this case we used Gaussian error structure in the GLM and parametric Pearson correlation coefficients for estimating partial correlations.

To ascertain that analyses were not biased by a phylogenetic structure in the dataset, we re-ran the final models obtained above by the stepAIC procedure within a phylogenetic regression framework, as implemented in the package phylolm (Ho and Ane, 2014). We obtained an Ornstein-Uhlenbeck alpha = 0.193 in the binomial model of endemic status and Pagel’s lambda = 0.037 in the Gaussian model of endemics rarity. These values showed that the phylogenetic signal was weak in both cases, making non-phylogenetic analyses appropriate with our data set (for results of phylogenetic comparative models see Appendix C in Supplementary material).

3. Results

Endemism distribution and its relationships with potential glacial refugia and topography

Table 1

Mean effect size (ES), lower and upper bounds of the 95% credibility intervals of the effects sizes of spatial models in which the proportion of endemics and the mean endemics rarity, as well as species-richness standardized phylogenetic diversity (ses.PD) are explained. Model estimates with credibility intervals not overlapping with 0 are given in bold.

	proportion of endemics			mean endemic rarity			ses.PD		
	lower	ES	upper	lower	ES	upper	lower	ES	upper
intercept	-3.102	-2.962	-2.822	-4.483	-4.368	-4.253	-0.171	0.162	0.501
calc. bedrock	0.173	0.245	0.318	0.051	0.107	0.164	0.247	0.487	0.727
calc. refugia	0.135	0.195	0.255	0.020	0.068	0.116	0.379	0.575	0.770
silic. bedrock	-0.057	0.025	0.108	-0.142	-0.077	-0.012	-0.471	-0.225	0.021
silic. refugia	-0.140	-0.070	-0.002	-0.064	-0.008	0.048	0.000	0.255	0.511
mean elevation	0.185	0.256	0.327	-0.008	0.048	0.103	-0.596	-0.335	-0.077
elevation range	0.096	0.161	0.226	-0.067	-0.015	0.036	-0.046	0.201	0.447

The proportion of endemics tended to be significantly (in the sense that Bayesian 95% credibility interval did not overlap 0) larger in grid cells that contained calcareous bedrock (effect size = 0.245; (see Table 1 for 95% credibility intervals, and Appendix A in Supplementary material for the results of alternative spatial models). This effect was further augmented by the presence of potential calcareous refugia (ES = 0.195). The proportion of endemics was not affected by the presence of siliceous bedrock and was even significantly lower in cells containing potential siliceous refugia (ES = -0.070). The proportion of endemics increased significantly with mean elevation (ES = 0.256) and elevation range (ES = 0.161) in grid cells; for instance, an increment of 1000 m in mean elevation has an effect comparable to presence of potential calcareous refugia (Fig. 1A). The spatial component of the model showed that the proportion of endemics declined towards the north-western edge of the Alps, and to a lower extent toward the eastern edge of the Alps (Fig. 1 B).

The mean endemic rarity was significantly positively associated with the presence calcareous bedrock (ES = 0.107) and of potential calcareous refugia (ES = 0.068; see Table 1 for 95% credibility intervals). The effect of siliceous bedrock was negative (ES = -0.077) and the effect of potential siliceous refugia was not distinguishable from 0. Mean endemic rarity was influenced neither by mean elevation nor by the elevation range (fig. 1C). Similar as for the proportion of endemics, we found a decline of mean endemic rarity towards the north-western corner of the Alps (Fig. 1D).

Phylogenetic diversity and its relation to potential glacial refugia and topography

Grid cells containing calcareous bedrock presented systematically larger ses.PD (ES = 0.487, see Table 1 for 95% credibility intervals) and this effect was further augmented in potential calcareous refugia (ES = 0.575). Effect of siliceous bedrock on ses.PD is not distinguishable from 0, but potential siliceous refugia exhibited marginally significant increase of ses.PD (ES = 0.255). Large ses.PD in calcareous bedrock, calcareous refugia and possibly siliceous refugia indicates that these areas host species assemblages separated by longer evolutionary branches than the rest of dataset. Sites with a higher mean elevation hosted species assemblages with a relatively lower ses.PD (ES = -0.335); a decrease in 500 m of mean elevation is comparable to the presence of potential calcareous refugia (Fig. 2a). This indicates that areas of higher mean elevation in the Alps host species assemblages that are more closely related than the rest of the dataset. Visualization of the spatial component of the model displayed particular areas with high (Savoy Prealps, Julian Alps) or low ses.PD (Ötztal and Rhaetian Alps). These spatial effects were, however, weak in comparison to the effects of the linear terms of the model (see Fig. 2b).

Endemism-elevation relationship and its link to species traits and ecological properties

The elevational optimum of species (summarized with Landolt’s T) was a significant positive predictor of species endemic status (tau = 0.086; Fig. 3A, see Table 2 for p-values). When mixed with other species trait predictors, the relationship with elevation became weaker

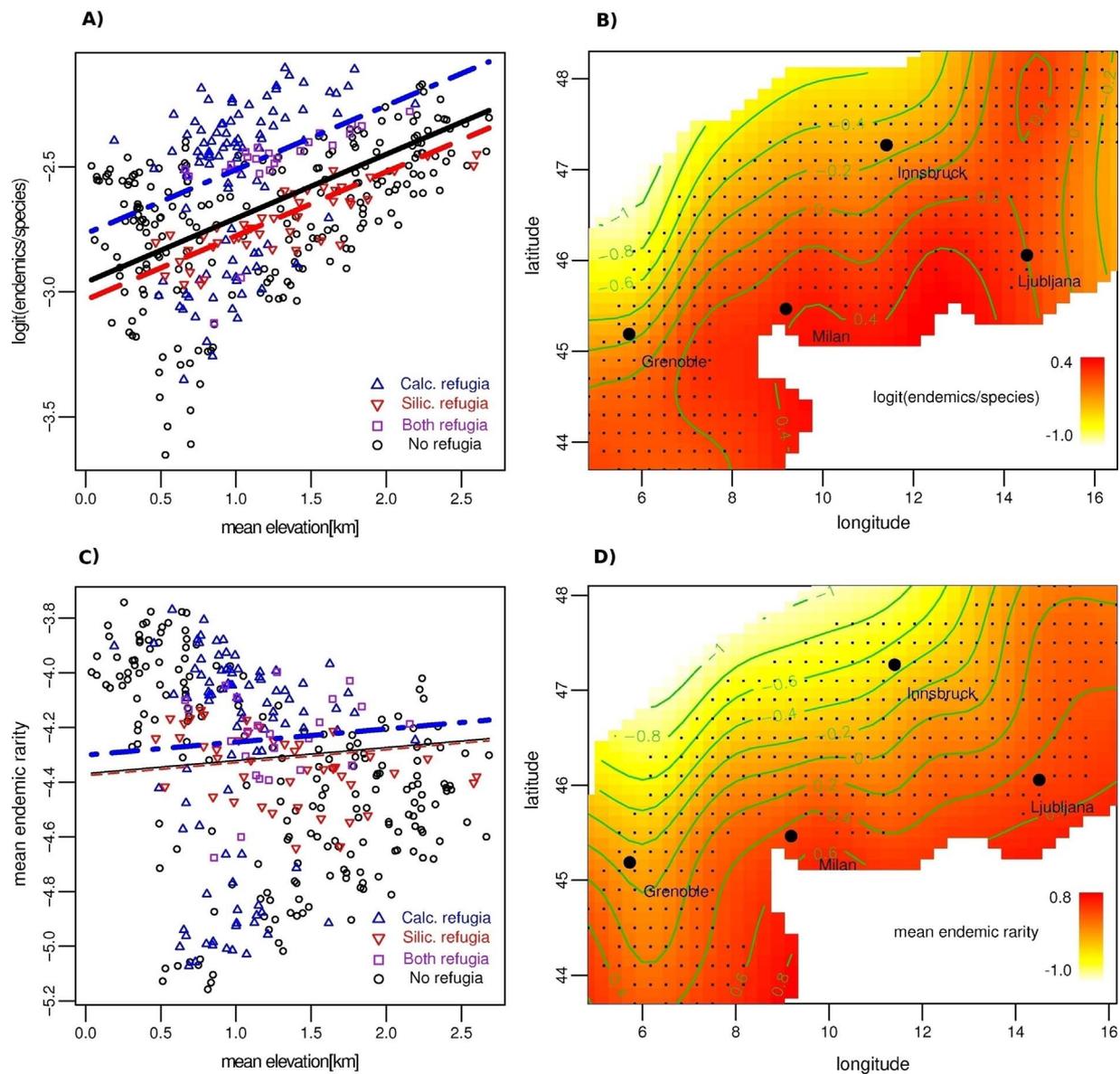


Fig. 1. Spatial models depicting the geographic patterns of proportion of endemics (A, B) and the geographic patterns of mean rarity of endemics (C, D). Panels (A) and (C) show relationship of these two indices with mean elevation for grid cells without refugia (black, solid line, plotted in bold if effect of mean elevation is significant), with calcareous refugia (blue, double dashed line, plotted in bold if effect of calcareous refugia is significant) or with siliceous refugia (red, single dashed line, plotted in bold if effect of siliceous refugia is significant). Note that y-values of points are adjusted to account for the effect of not-displayed model variables and for the mean effect of smooth model component per group. Panels (B) and (D) represent the smooth component of each model, showing geographic areas with overall higher or lower proportion of endemics or mean endemic rarity when simultaneously accounting for parametric model components. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

but remained significant ($\tau = 0.032$). Concerning other species-level predictors, the endemic status is significantly linked to poor dispersal capacity ($\tau = -0.137$), sexuality ($\tau = 0.080$), and stress tolerance ($\tau = 0.100$; Fig. 3A, Table 2). Evolutionary distinctiveness was negatively related to endemic status ($\tau = -0.054$), and its inclusion improved model AIC, but this effect did not reach statistical significance ($p = 0.134$). The cushion life form was positively related to endemic status ($\tau = 0.064$); the inclusion of this trait also improved the model AIC, but it did not reach statistical significance ($p = 0.110$) either.

Endemics rarity was significantly negatively linked with elevational optimum ($R = -0.110$) and the strength of this relationship remained similar in a model that included species traits and ecological predictors ($R = -0.102$; Table 2, Fig. 3B). Endemics rarity was further associated with sexuality ($R = 0.068$) and geophyte life form ($R = 0.144$). Dispersal capacity ($R = -0.084$) had a weak but significant negative effect on rarity ($p = 0.029$), but this would turn marginally non-significant if

accounting for phylogenetic correction ($p = 0.097$; see Appendix C in Supplementary material). Stress tolerance ($R = 0.046$) and hemi-cryptophyte life form ($R = 0.084$) were positively related to endemics rarity and improved model fit, yet these effects did not reach significance ($p = 0.088$, $p = 0.104$; Fig. 3B, Table 2).

4. Discussion

In this study, we quantitatively compared the importance of endemism hotspots in potential glacial refugia and in areas with high elevation. We showed that the phylogenetic structure of plant assemblages occurring in these two types of hotspots is substantially different and reflects their contrasting evolutionary histories. Moreover, we explored so far undocumented interrelations between plant endemism, elevation, species ecological strategies, biological traits and evolutionary distinctiveness.

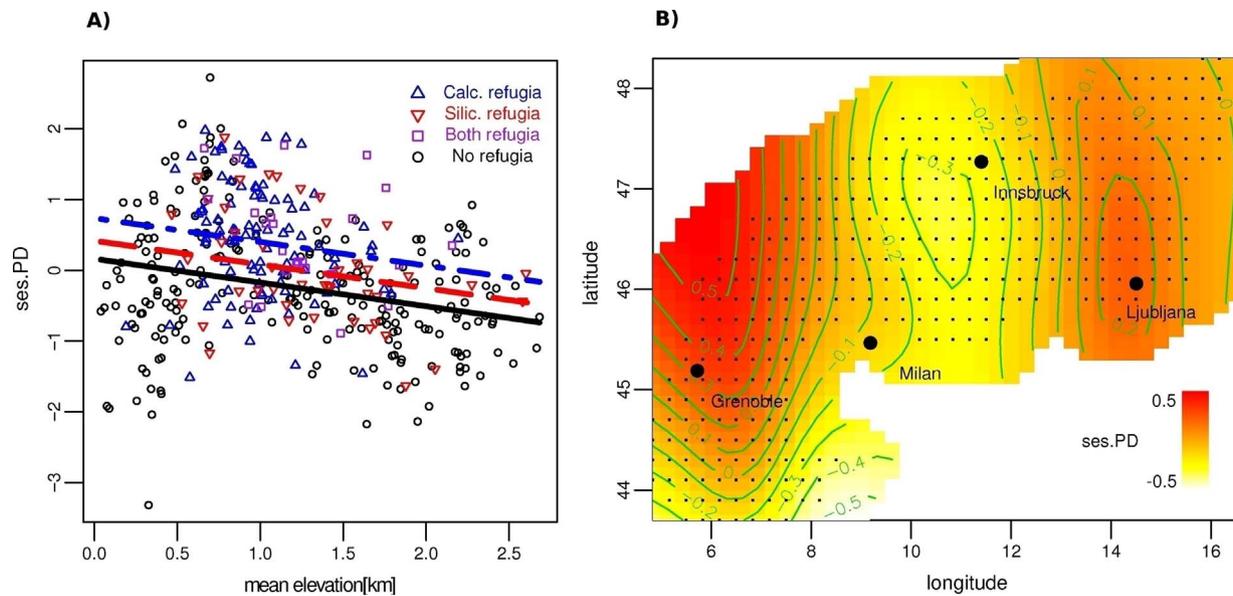


Fig. 2. Spatial model depicting geographic patterns of species-richness standardized phylogenetic diversity (ses.PD). Panel (A) shows the relationship of ses.PD with mean elevation (black, solid line, plotted in bold if effect of mean elevation is significant) and the occurrence of calcareous (blue, double dashed line, plotted in bold if effect of calcareous refugia is significant), siliceous refugia (red, single dashed line, plotted in bold if effect of siliceous refugia is significant). Note that y-values of points are adjusted to account for the effect of not-displayed model variables and for the mean effect of smooth model component per group. Panel (B) represents the smooth component of the model, showing geographic areas with overall higher or lower ses.PD when simultaneously accounting for parametric model components. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.1. Hotspots of endemism in calcareous refugia and areas with high elevation

Areas of the Alps comprising potential calcareous glacial refugia hold a substantially high proportion of alpine endemics, and these endemics are typically narrowly distributed (with high rarity of endemics) within the Alps. This is partly caused by generally higher endemism in calcareous areas, but potential calcareous refugia host even larger proportion and rarer endemics than calcareous areas in general in our data. It suggests that potential calcareous refugia indeed host species that did not manage to recolonize larger areas after the retreat of glaciers (Dullinger et al., 2012). Increasing endemism with elevation has an effect per 1000 elevation meters comparable to the presence of calcareous refugia. However, high-elevation endemics are typically widespread within the Alps (with low rarity), in contrast to narrowly distributed endemics of potential calcareous refugia. While high endemism in glacial refugia and high elevations was previously reported from the Alps (Aeschmann et al., 2011; Tribsch and Schönswetter, 2003) and other mountain systems across the world (Feng et al., 2016; Mráz et al., 2016; Nagy and Grabherr, 2009), we quantitatively measured and tested those effects within a spatially explicit modeling framework. We show that endemism in potential calcareous glacial refugia and in high elevations are of comparable importance in the Alps, suggesting that similar patterns might be found in other mountain ranges affected by Pleistocene glaciation dynamics. Our quantitative approach does not only compare importance of refugia and elevation gradient for formation of endemic hotspots in the Alps, but also allows us to shed light on exceptions from this general trend.

Interestingly, we found that the overall patterns of high proportion of endemics and the high rarity of endemics in potential calcareous refugia are not paralleled in potential siliceous refugia. The mean rarity of endemic species occurring in potential siliceous refugia is comparable to the siliceous areas outside the refugia and the proportion of alpine endemics in potential siliceous refugia is even significantly lower than outside refugia. This suggests that high endemism previously reported in some potential siliceous refugia (Tribsch and Schönswetter, 2003) could at least in some cases rather be attributed to the other predictors used in our model than to favourable conditions during

glacial periods, i.e. those areas might be endemic-rich due to topographic roughness or because they lie in region that is overall richer in endemics (which is modeled by spatial autocorrelation in our model). A possible reason why potential siliceous refugia are not richer in endemics than non-refugial siliceous areas is that siliceous refugia are typically interconnected with non-refugial siliceous areas in the central Alps, in contrast to calcareous refugial and non-refugial areas, which are scattered at the margins of the Alps. Hence, most siliceous specialists species that survived glacial periods may have been able to recolonize broader areas after glacier retreat due to lower spatial isolation of habitats on siliceous bedrock (Alvarez et al., 2009; Dullinger et al., 2012). Following this hypothesis, postglacial migration might have erased patterns of endemism, even though the presence of siliceous refugia may be still visible in the spatial genetic structures of some particular species (Schönswetter et al., 2005; Stehlik, 2003).

Spatial models of proportion of endemics and mean endemic rarity suggest that the region of the north-western Alps hosts a lower proportion of endemics and also fewer rare endemics. A possible explanation is that the impact of glaciers was relatively stronger in this area due to combination of lower elevation and higher glacial extent towards the north-western part of the Alps (see maximum extent of glaciers in Schönswetter et al., 2005), making potential refugia in this area uninhabitable. This explanation is in agreement with studies tracking glacial refugia using intraspecific genetic patterns (Schönswetter et al., 2005), counts of endemic species (Tribsch and Schönswetter, 2003), or species rarity (Taberlet et al., 2012); all of these studies found weak or no support for the existence of glacial refugia in the north-western Alps.

4.2. Calcareous refugia as species museums and high elevations as cradles

Areas with potential calcareous refugia contain systematically higher richness-standardized phylogenetic diversity (ses.PD), which suggests that they host a higher proportion of species with long and isolated branches than other sites in the Alps. The ses.PD in potential calcareous refugia is higher also in comparison with calcareous areas outside refugia. Such a phylogenetic signature may result from a random selection of refugial survivors from the pre-glacial species pool

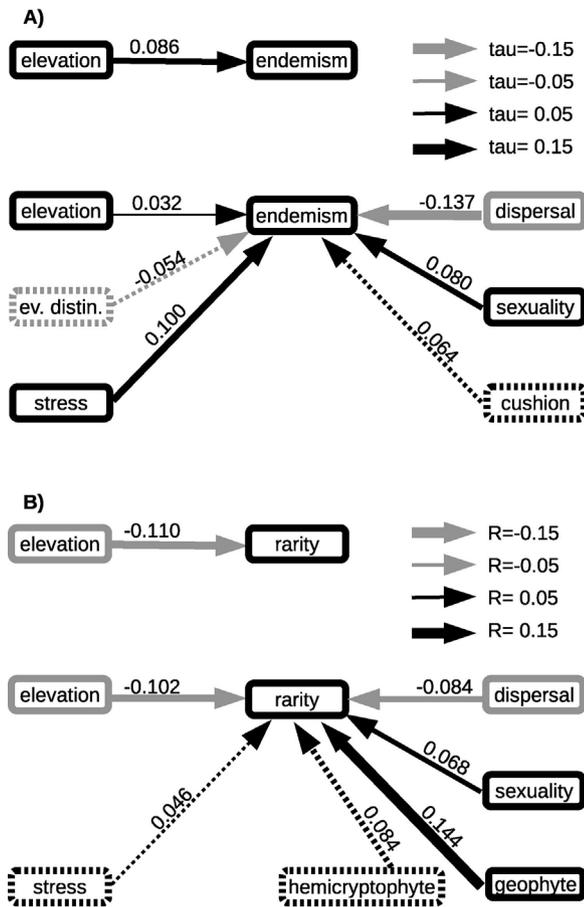


Fig. 3. Relationship of elevational optimum (Landolt's T) and species endemic status (A) or the rarity of endemic species (B) in elevation only models and in models with elevation and the other species level predictors included. Tau is the partial Kendall correlation in binomial model of endemic status (A), R is the partial Pearson correlation in Gaussian model (B) of endemics rarity. All listed predictors contribute to model fit as measured by AIC, predictors significant at $p < 0.05$ appear with solid arrows, non-significant with dashed.

combined with selective re-colonizations of previously glaciated sites by well-dispersing species, resulting in assemblages at sites outside refugia being more clustered than in refugia. In addition, resource competition in refugia during glacial periods could have also contributed to the competitive exclusion of related species *sensu* Webb et al. (2002). The ses.PD in glacial refugia might have also been augmented by allopatric speciation among isolated glacial refugia, which would result in sister species seldom sharing one refugial site (Pigot and Etienne, 2015; Warren et al., 2014). With the current resolution of phylogenetic data and without evidence for past migration from and to the Alps it is not possible to estimate the relative importance of those three processes. Still, the observed evolutionary overdispersion in potential calcareous refugia strongly supports the idea that those areas constitute museums of phylogenetic diversity of the Alpine flora *sensu* Stenseth (1984), and served as islands of suitable condition during glacial periods. The long-term conservation of this local flora since glacial periods onwards has thus made them important zones for the protection of plant phylogenetic diversity in the Alps. For potential siliceous refugia, we found a pattern of phylogenetic overdispersion similar to potential calcareous refugia, but only marginally significant. It supports the interpretation that potential siliceous refugia also hosted species during glacial periods. Although the patterns of endemism were erased by postglacial migrations, the imprint of glacial survival may still be detectable in the phylogenetic structure of plant assemblages.

Areas in the Alps with high mean elevation exhibit significantly lower ses.PD than other sites. This means that with increasing

Table 2

Effect size (ES), p-values and correlation coefficients (tau and R) of models explaining endemic status and endemics rarity species level characteristics. Model results are shown with species altitudinalelevational optima (Landolt's T) as a single predictor, and in combination with all other species characteristics. N stands for predictors that did not pass the stepwise AIC optimization procedure and thus did not contribute to the model. Bold are significant model terms ($p < 0.05$).

	endemic status			endemics rarity		
	tau	ES	p	R	ES	p
elevational optimum alone	0.086	0.297	< 0.001	-0.11	-0.194	0.012
elevational optimum	0.032	0.179	0.043	-0.102	-0.206	0.009
evolutionary distinctiveness	-0.054	-0.132	0.134	N	N	N
ruderal strategy	N	N	N	N	N	N
stress strategy	0.1	0.231	0.009	0.046	0.144	0.088
dispersal	-0.137	-0.297	< 0.001	-0.084	-0.183	0.029
sexuality	0.08	0.258	0.023	0.068	0.193	0.01
vegetative reproduction	N	N	N	N	N	N
cushions	0.064	0.122	0.11		N	N
chamaephyte	N	N	N	N	N	N
geophyte	N	N	N	0.144	0.245	0.003
hemicryptophyte	N	N	N	0.084	0.136	0.104
phanerophyte	N	N	N	N	N	N
therophyte	N	N	N	N	N	N

elevation, species are more likely co-occur with their close relatives, and that this pattern is consistent throughout the Alps. Two classes of processes may explain this pattern. First, the high alpine flora could be strongly shaped by environmental filtering *sensu* Webb et al. (2002) by which related species tend to share adaptations to similar climatic environments and are, thus, jointly sorted across climatic gradients. Second, this first process may have almost certainly been amplified by recent and repeated speciations in clades that have largely diversified in alpine environments (Boucher et al., 2016; Mansion et al., 2012; Molina-Venegas et al., 2015; Roquet et al., 2013a, 2013b). Clearly, environmental filtering alone cannot explain the phylogenetic clustering of high alpine floras, since we found additional evidence that species richness increases with mean elevation (see Appendix B in Supplementary material) and increased filtering should decrease species richness. Our results are thus in line with the extensive evidence that plant clades have rapidly diversified into high-mountain environments in general (reviewed in Hughes and Atchison, 2015). Given increasing evidence that speciation in alpine plants widely occur by allopatric speciation with little niche shift (Boucher et al., 2016; Comes and Kadereit, 2003), the observed signature of phylogenetic clustering suggests that migration during post-glacial recolonization caused closely related species to co-occur in similar mountain environments, a process known as secondary sympatry (Pigot and Tobias, 2013).

Our analyses thus show that high elevation and refugial hotspots of endemism in the Alps are of similar importance, but were formed by different evolutionary processes. It suggests that in the Alps, but possibly also in other temperate mountains affected by Pleistocene glaciation dynamics, endemism should not be considered an indicator of one particular evolutionary process as is glacial survival, altered dispersal dynamics or faster speciation.

4.3. High elevation endemism is shaped by dispersal

Above we discussed that areas with high elevation and calcareous refugia are important hotspots of endemism and here we focus more closely on processes forming endemism in high elevations. Accordingly with spatial models, our species-level analysis provides evidence that

species endemic status is positively related to elevational optimum and that endemic species with high elevational optimum have larger distribution ranges (lower rarity) than endemic species from lower elevations. However, once other predictors are included, the effect of species elevational optima on endemism becomes much weaker. The major pattern arising from this analysis is that poorly dispersing and/or stress tolerant endemics tend to be over-represented with increasing elevation. In addition, endemics with restricted distribution ranges (high rarity) seem to be rather poor-dispersing and stress tolerant species, although this last effect is weaker and marginally significant. The relationship between endemism, elevation optimum, dispersal and stress tolerance is in line with previous findings (Aeschimann et al., 2012; Hobohm, 2008; Mráz et al., 2016), and suggests that some of the high-elevation species are endemic, because of their poor colonizing capabilities that are linked to their ecological and trait characteristics. These species are specialists of stressful habitats, such as of rocky outcrops or high-elevation habitats, possibly within glacial refugia, e.g. in Dolomites, Julian Alps or south-western Alps. Typical examples of narrowly distributed high-elevation endemics that are stress tolerant and poor dispersers are: *Rhizobotrya alpina*, *Braya alpina*, *Draba ladina*, *Sempervivum dolomiticum*, *Saxifraga florulenta*, *Myosotis gallica*, *Cardaminopsis pedemontana*, *Linaria tonzigii* or *Moehringia concarenana*.

Species life-forms and reproduction strategies also partly explained species endemic status and the rarity of endemic species. Cushion plants are more likely alpine endemics (e.g. *Androsace alpina*), and geophytes are more likely rare endemics (e.g. *Allium kermesinum*) than expected by chance. Our results also indicate that sexually reproducing species are more likely to be endemic and rare than asexual ones. This may appear surprising given that asexual lineages tend to create small but distinct populations, typically considered a species (e.g. *Alchemilla* or *Sorbus*). This result is thus likely an artefact derived from the fact that, in large compilations such as IntraBioDiv (Gugerli et al., 2008) or Flora Alpina (Aeschimann et al., 2004), asexual and taxonomically problematic species are typically merged to relatively broadly aggregated species definitions. For instance, the genus *Alchemilla* has only 5 species distinguished in the IntraBioDiv data. To shed more light on endemism-sexuality relations, it would be necessary to explicitly control for the species definition, e.g. by including data about species population structures, and perhaps by tackling the issue of species delimitation with modern sequencing techniques. However, given the large number of species of the Alps, this is likely a very demanding, if currently not impossible, task.

In contrast to endemics that are stress-adapted, poorly dispersing or having endemism-related life form, there is another large portion of species whose endemic status is best explained by elevational optimum itself. Such endemics are typically widespread within the Alps (have low rarity), and are likely well adapted to environments such as the subnival zone, that are relatively common within the Alps, but rare or non-existing in other European mountain systems. These species are thus expected to have a dispersal capacity sufficient to spread across the Alps. Yet, their dispersal capacity is not sufficient for colonizing and maintaining viable populations in isolated patches of suitable habitats outside the Alps. Typical examples of well-dispersing, widespread high-elevation endemics are for example: *Festuca interdecens*, *Cerastium pedunculatum*, *Crepis terglouensis*, *Adenostyles leucophylla*, *Erigeron neglectus* or *Artemisia genepi*.

Surprisingly, the evolutionary distinctiveness of species is only weakly linked to endemic status; one would have expected that endemism should be common in species with little evolutionary distinctiveness resulting from repeated recent speciation events. Our analysis on the relationship between endemic status and evolutionary distinctiveness may have been partly weakened by the use of genus-level phylogenies, as recent speciation events would be most apparent at terminal branches, where we are missing accurate information. But if speciation events in general were a main driving force of high mountain endemism patterns, it is unlikely that missing information at the species

level would completely remove the signal. The weak relationship between endemic status and evolutionary distinctiveness is interesting, because according to other results from our study, the flora of high elevation areas (including non-endemic species) shows a pattern of phylogenetic clustering – a possible indication of faster and recent diversification. Combining our findings, we hypothesize that the high-elevation flora may indeed have faster speciation rate, as was reported in studies from mountains all around the world (reviewed in Hughes and Atchison, 2015). However, even the youngest speciation events in our phylogeny are older than the last glaciation period (all terminal branch length estimates are longer than 110,000 years) and most of them is even older than the onset of glaciation-deglaciation cycles in the Quaternary (77% of terminal branch length estimates are longer than 2.58 million years). This suggests that although species may speciate faster in high elevations, the relationship between speciation and endemism is further erased by massive glacial-interglacial migrations and extinctions (see Kadereit et al., 2004). The temporal dynamics of the evolution of new species and drivers of endemism need to be further studied with a well-dated species-level phylogeny of the Alps and possibly beyond such as including the Carpathians and the Pyrenees or the Balkanic mountain systems.

5. Conclusions

Our analyses demonstrate that important hotspots of endemism in the European Alps are situated in potential calcareous glacial refugia, rather than siliceous ones, but also in areas with high elevation: an elevational change of 1000 m contributes to endemism similarly as the presence of potential calcareous refugium. Potential calcareous refugia harbour a large portions of endemics that are rare within the Alps, and plant assemblages with higher phylogenetic diversity than random expectations. It suggests that these areas indeed served as safe harbours for distinct evolutionary lineages during the periods of glaciation. The assemblages of high-elevation hotspots carry a signature of phylogenetic clustering, which indicates a combination of strong environmental filtering and faster speciation rate. More detailed analyses of high elevation endemism reveal that a large portion of endemics in the Alps are range restricted species with poor dispersal ability, and another large portion of endemics are high elevation specialists that are widespread within the Alps. Our results show that endemic hotspots both in calcareous refugia and high elevations are of high importance, but they are formed by contrasting evolutionary processes. In line with this, future studies of endemism and diversification in the Alps or other mountain ranges affected by past glaciation should consider that endemism in such areas is formed by interplay of migration and different diversification processes acting on different timescales, rather than one dominant force. From a point of view of nature conservation, calcareous refugia in the Alps deserve more attention than high elevation hotspots, because they host primarily range-restricted endemics and phylogenetically distinct species retaining a substantial evolutionary history overall.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ppees.2017.08.003>.

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