Impact of Quaternary climatic oscillations on phylogeographic patterns of three habitat-segregated Cerastium taxa endemic to the Dinaric Alps

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Abstract

Aim: The Balkan Peninsula has served as source for the colonisation of large parts of Europe after the Pleistocene, but the processes driving the intraspecific diversification within the Balkan Peninsula are much less understood. To fill this gap, we investigated the phylogeography and evolutionary history of three habitat-segregated Cerastium taxa endemic to the Dinaric Alps and explored whether they responded differently to the Quaternary climatic oscillations in the Balkan Peninsula.

Location: Europe, Balkan Peninsula, Dinaric Alps.

Taxon: Cerastium (Caryophyllaceae, angiosperms).

Methods: We combined climatic niche comparisons and species distribution modeling (SDM) on the one hand, with cytological (relative genome size, RGS) and genetic / genomic approaches such as amplified fragment length polymorphism (AFLP) fingerprinting, phylogenetic analyses of nuclear ribosomal internal transcribed spacer (ITS) and plastid (trnT–ndhJ) sequences on the other hand, in order to investigate how the Pleistocene glaciations and the Holocene warming influenced the phylogeographic structure of the studied taxa. SDM was used to reconstruct past, present and future habitat suitability.

Results: Despite nonequivalent climatic niches, we detected climatic niche similarity amongst the three Cerastium taxa. Nonhierarchical K-means clustering of AFLP data revealed an optimal split into two geographically segregated groups in all three taxa. Genetic divergence between the northwestern and the southeastern populations can be explained by isolation in northern and southern refugia, as suggested by SDM. The ITS and plastid sequences revealed similar, although more complex, patterns of genetic differentiation, and RGS differentiation was mainly geography-correlated.

Main conclusions: Despite the pronounced habitat segregation amongst the studied taxa, their overall phylogeographic structure is similar, suggesting comparable underlying processes and similar responses to the same historical events. However, we anticipate that the three taxa will respond differently to ongoing climate warming.
1 | INTRODUCTION

Climatic oscillations during the Pleistocene triggered fragmentation and displacements of species’ distributions (Hewitt, 1999, 2004; Keppel et al., 2012). Range contractions and expansions were correlated with glacial and interglacial cycles, and their extent was found to be highly dependent on species’ ecological preferences (Hewitt, 1999; Stewart et al., 2010). Warm-adapted species had their glacial refugia in the Southern European peninsulas (Hewitt, 2004; Nieto Feliner, 2014; Schmitt, 2007), of which the Balkan Peninsula played an important role for the Quaternary recolonisation of Central and Northern Europe (Đurović et al., 2017; Frajman & Oxelman, 2007; Hewitt, 1999; Rešetnik et al., 2016).

The Balkan Peninsula is one of the European centres of plant diversity due to its climatic and topographic complexity, milder influences of Pleistocene glaciations compared to northerly adjacent areas and geographic position on the crossroads of different floristic regions (Hewitt, 2004; Nieto Feliner, 2014; Tomović et al., 2014). In the western Balkan Peninsula, the Dinaric Alps extend between Slovenia in the northwest and Albania in the southeast. They are topographically complex with high-mountain peaks reaching the alpine zone, separated by deep valleys with thermophilous submediterranean vegetation (Kutnjak et al., 2014; Surina et al., 2011). Compared to northerly adjacent areas such as the (European) Alps, the Dinaric Alps were free of a continuous ice sheet during the Pleistocene glaciations (Čvijić, 1917; Penck, 1900), which allowed persistence of many plant species in the area (Kutnjak et al., 2014; Surina et al., 2011). However, the snowline during glacial was about 1000 m lower than today (Kutnjak et al., 2014; Surina et al., 2011). The main centre of diversity (34 species) and endemism (17 species) is the Balkan Peninsula, where extensive polyploidisation has been evidenced (Niketić et al., 2013). Three Cerastium taxa are endemic to the Dinaric Alps, namely, C. dinaricum (Beck and Szyszył, Kutnjak et al., 2014), C. hekuravense Jáv. (Caković et al., 2018) and Edraianthus tenuifolius (Vis.) A. DC. (Surina et al., 2011) in the Dinaric Alps, and also for more widely distributed species, such as Betula nana L., Dryas octopetala L. or Trollius europaeus L. (Espíndola et al., 2012; Stewart & Lister, 2001; Stewart et al., 2010).

Similar range shifts, that is, expansions of warm-adapted species and contractions of cold-adapted species, are observed nowadays in the face of ongoing global warming (Gottfried et al., 2012; Pauli et al., 2012). High-mountain plants in the Mediterranean mountains are of particular concern because they are suffering severe range contractions and changes in community compositions due to global warming (Jiménez-Benavides et al., 2018). For instance, Kutnjak et al. (2014) predict that the suitable habitats of C. dinaricum will be reduced by more than 70% by the year 2080 and the cold-adapted beetle Cryptocephalus barii, endemic to the Orobian Alps in northern Italy, might get extinct in the next 50 years due to global warming (Brunetti et al., 2019).

Cerastium (Caryophyllaceae) is a genus of flowering plants including approximately 200 species (Jalas et al., 1993; Niketić et al., 2013). The main centre of diversity (34 species) and endemism (17 species) is the Balkan Peninsula, where extensive polyploidisation has been evidenced (Niketić et al., 2013). Three Cerastium taxa are endemic to the Dinaric Alps, namely, C. decalvans Schloss. and Vuk. subsp. decalvans, C. dinaricum and C. grandiflorum Waldst. and Kit. (Figures 1a–c and 2a–c). These are caespitose perennial palaeotetraploids with 36 chromosomes, with the exception of northern populations of C. grandiflorum, for which 72 chromosomes have been reported (Niketić et al., 2013, and references therein). They have largely overlapping distributions and often occur on the same mountains but—according to our field observations and previous, mostly phytocoenological, reports (Horvat, 1931; Horvat et al., 1974; Jalas et al., 1993; Kutnjak et al., 2014; Lakušić, 1968; Niketić, 2012; Šilić, 1973)—have different habitat preferences and corresponding morphological adaptations (Figure 1d–e and Table S1 in the Supporting Information), despite their shared preference for calcareous scree and rocky habitats. The most thermophilous is narrow-leaved and rocky habitats. The most thermophilous is narrow-leaved and rocky habitats. 

How individual species respond to climate change depends largely on their adaptations and environmental tolerances (Stewart et al., 2010). Thermophilous species expanded their distributions during interglacials, including the Holocene, and retracted to refugia at lower altitudes and latitudes during glacials. From there, some of them significantly extended their ranges in the Holocene (Hewitt, 1999), as suggested for the Balkan endemic Edraianthus tenuifolius (A.DC.) A.DC. (Glasnović et al., 2018) as well as widespread Asplenium ceterach L. (Trewick et al., 2002) and Euphorbia seguieriana Neck. (Frajman et al., 2019). In contrast, cold-adapted species likely had more continuous distributions during glacials and were forced into high-altitude refugia during interglacials and the Holocene (Hewitt, 1999; Stewart et al., 2010), as suggested for Cerastium dinaricum Beck and Szyszyl (Kutnjak et al., 2014), C. hekuravense Jáv. (Caković et al., 2018) and Edraianthus tenuifolius (Vis.) A. DC. (Surina et al., 2011) in the Dinaric Alps, and also for more widely distributed species, such as Betula nana L., Dryas octopetala L. or Trollius europaeus L. (Espíndola et al., 2012; Stewart & Lister, 2001; Stewart et al., 2010).

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**KEYWORDS**
AFLP, Balkan Peninsula, glacial refugia, ITS, phylogeography, species distribution modelling, trnT-ndhJ
compared to the other two taxa and is of high conservation concern (Annex II of the European Habitats Directive).

Comparison of modelled past distributions and inferred phylogeographic patterns as applied in this study can shed light on how taxa with divergent habitat requirements in the Dinaric Alps responded to past climatic oscillations. In light of the divergent habitat preferences of the three Cerastium taxa observed in the field, we assume that they responded differently to climatic oscillations (cf. Stewart et al., 2010). We hypothesise that the distribution of C. dinaricum was larger during the Last Glacial Maximum (LGM), similarly as it was suggested for other cold-adapted plant species (Espíndola et al., 2012; Stewart & Lister, 2001; Stewart et al., 2010). On the other hand, the distribution of mesophilous C. decalvans and especially that of thermophilous C. grandiflorum likely shrunk during cold stages, as suggested for temperate species (Frajman et al., 2019; Glasnović et al., 2018; Trewick et al., 2002). Conversely, in face of anthropogenic global warming, we expect high population losses for cold-adapted C. dinaricum, possible distribution expansion for thermophilous C. grandiflorum and potential upward migration for mesophilous C. decalvans. The impact of the ongoing climate change on their future distributions, along with the present-day patterns of genetic variation, may have important conservation implications (Avise, 2009).

In addition to applying species distribution modelling (SDM) to address the hypotheses outlined above, we explore whether the three species exhibit similar phylogeographic patterns or if these patterns differ according to their climatic niche preferences. Deep genetic splits amongst geographically defined groups have been observed in cold-adapted C. dinaricum (Kutnjak et al., 2014) due to pronounced elevational range shifts during the Quaternary climate oscillations. We hypothesise weaker genetic differentiation due to latitudinal Quaternary range shifts and gene flow amongst more continuously distributed populations in thermophilous C. grandiflorum and mesophilous C. decalvans. In this line, we also explore whether the inferred phylogeographies in the three Cerastium species follow the general patterns revealed in other codistributed species, in which the main phylogeographic split was along the

FIGURE 1 Habit of Cerastium grandiflorum (a), C. decalvans subsp. decalvans (b) and C. dinaricum (c) from the Dinaric Alps with their corresponding habitats (d–f) and the species-specific leaf indumentum and bracts in insets (g–i)
**FIGURE 2** Genetic differentiation in *Cerastium grandiflorum* (a), *C. decalvans* subsp. *decalvans* (b) and *C. dinaricum* (c). NeighborNet diagrams of AFLP profiles complemented with bootstrap values >50% derived from NJ analyses (d–f); geographic distribution of phylogeographic groups according to nonhierarchical K-means clustering of amplified fragment length polymorphism (AFLP) profiles (g–i)—clustering at K = 2 is indicated by black lines and clustering at K = 3 is illustrated by colour coding (blue, green and yellow); NeighborNets of ITS sequences and geographic distribution of ribotype groups (j–l); statistical parsimony network of plastid DNA sequences and geographic distribution of haplotype groups (m–o). Population numbers correspond to Table S2. Projection in the maps: WGS84.
Neretva river area in Bosnia and Herzegovina, and the southern populations exhibited higher levels of genetic diversity and reciprocal differentiation (Caković et al., 2015; Falch et al., 2019; Kutnjak et al., 2014; Lakušić et al., 2013; Mereda et al., 2011; Španiel et al., 2017). To address these questions, we employ an integrative approach, combining phylogenetic methods (using sequences of the nuclear ribosomal internal transcribed spacer, ITS, plastid trnT–ndhJ sequences and amplified fragment length fingerprinting, AFLP) and relative genome size (RGS) measurements. The employed genetic markers have different pathways of inheritance, which may help uncovering species’ evolutionary histories. Specifically, whereas AFLPs show the overall nuclear differentiation and are less influenced by stochastic factors affecting single genes, ITS sequences are more strongly influenced by lineage sorting, gene flow and concerted evolution (Álvarez & Wendel, 2003). On the other hand, maternally inherited plastomes have half the effective population size of nuclear genes and are more strongly influenced by genetic drift (Frajman et al., 2009; Masel, 2011). Whereas phylogenetic methods and SDM are commonly used in biogeographic studies, also RGS variation amongst populations can show a geographic pattern, both in the distribution of different ploidy levels (Kolář et al., 2009; Sonnleitner et al., 2010) and different genome sizes within the same ploidy (Kutnjak et al., 2014; Šmarda & Bureš, 2010), thus adding value to resolving species phylogeographic histories.

2 | MATERIALS AND METHODS

2.1 | Study area and sampling

Plant material was collected and dried in silica gel during field excursions between 2004 and 2012. Herbarium vouchers are deposited in BEO, BEOU, IB and ZA. Voucher numbers and collecting details are given in Table S2. The distribution of the studied populations is presented in Figure 2d–f. The phylogenetic data for C. dinaricum are from Kutnjak et al., (2014), whereas the SDM was redone with additional populations sampled in Albania. In the case of C. decalvans, we only included tetraploid populations belonging to C. decalvans subsp. decalvans endemic to the Dinaric Alps, as other populations are mostly of higher ploidies (Niketić et al., 2013) and genetically divergent (Niketić et al., in press). For simplicity we refer to the here analysed populations as C. decalvans (and not as C. d. subsp. decalvans) hereafter.

2.2 | Climatic niche comparison amongst species

To characterise and compare the climatic niches of the three taxa, we first performed the PCA-env approach (Broennimann et al., 2012). Briefly, species occurrence densities obtained with the kernel smoothing function were plotted onto a gridded PCA environmental space (here a 1000 x 1000 grid) defined by the first two PC axes. PCA-env was based on 10 climatic variables selected for SDM (Table 1; see variable selection below) and was calibrated on the entire study area corresponding approximately to the geographic area potentially accessible to the three Cerastium taxa during their evolutionary history (i.e. potential current and LGM distribution; Barve et al., 2011; Guisan et al., 2014). Available background climate was depicted by extracted values of the climatic variables from 10,000 random points across the study area. This allowed us to carry out all niche comparisons in the common available background climate encompassing the entire known and potential distribution of the three co-occurring sister species (see, e.g., Broennimann et al., 2014; Molina-Henao & Hopkins, 2019).

We measured the observed niche overlap in the environmental space using Schoener’s D metric (Rödder & Engler, 2011; Schoener, 1968) and performed niche equivalency and similarity tests (Broennimann et al., 2012; Warren et al., 2008) for all species pairs. The former assesses whether the two considered climatic niches are equivalent/identical, while the latter tests whether they are more similar than expected by chance. Significance of both tests was evaluated using 1000 repetitions simulating the null distributions of D values, which were compared with the observed D values (Broennimann et al., 2012; Warren et al., 2008). All analyses were performed using the R package ‘ecospat’ (Di Cola et al., 2017). We tested and visualised the climatic niche differences along each variable amongst the three taxa using ANOVA, Tukey’s HSD tests and boxplots in R.

2.3 | Species distribution modelling

We modelled the potential habitat suitability for each taxon for the present, the LGM (~21 kya) and the future (2080, RCP8.5 climate change scenario). Two General Circulation Models (GCMs) were used for the past and for the future (CCSM4 and MPI-ESM-P for LGM; CCSM4 and MPI-ESM-LR for 2080). A consensus model for each time period was obtained by averaging the two GCM predictions. Georeferenced species occurrence data were compiled from literature, herbarium labels and our own field observations. We obtained 292 occurrences of C. decalvans, 25 of C. dinaricum and 213 of C. grandiflorum as input data for SDM, covering the entire distributions of all three taxa (Table S3). The SDM was based on the same set of climatic variables used for niche

| Table 1 | Bioclimatic variables with their definitions (following worldclim.org) used for the climatic niche comparisons and species distribution modelling |
|---------|---------------------------------------------------------------------------------------------|
| bio3    | Isothermality                                                                               |
| bio4    | Temperature seasonality                                                                      |
| bio6    | Minimum temperature of coldest month                                                      |
| bio8    | Mean temperature of wettest quarter                                                       |
| bio9    | Mean temperature of driest quarter                                                        |
| bio12   | Annual precipitation                                                                       |
| bio14   | Precipitation of driest month                                                             |
| bio15   | Precipitation seasonality                                                                  |
| bio18   | Precipitation of warmest quarter                                                          |
| bio19   | Precipitation of coldest quarter                                                          |
comparisons (Table 1). The 10 climatic variables were taken at a resolution of 30 arc-seconds (~1 km) from the WorldClim 1.4 database (worldclim.org) as predictors and were selected based on their ecological relevance for the studied species, avoiding highly correlated (|r| < 0.85) ones. The selected variables (Table 1) depict temperature and precipitation dimensions of the study area and likely shape the distribution limits of the studied taxa. SDMs for each species were built using three modeling algorithms (GAM, Maxent and Random forest) implemented in the R platform ‘biomod2’, version 3.3–7.1 (Thuiller et al., 2009). For each species and each algorithm, we used the same modelling settings: 10,000 pseudo-absences randomly generated throughout the entire study area and three independent replicated runs with randomly chosen 70% of the occurrences used for model calibration and the remaining 30% for model evaluation.

The current ensemble model for each species was based on AUC-weighted-average (Araujo & New, 2007) including only models with AUC >0.9 (Fielding & Bell, 1997) and then hindcasted to the LGM and projected onto future climate for 2080.

### 2.4 | DNA extraction and AFLP analyses

Total genomic DNA was extracted from silica gel-dried tissue (10 mg) with DNeasy 96 Plant Kit (QIAGEN) following the manufacturer’s protocol.

AFLP fingerprinting was performed for 44 populations scattered across the entire distribution ranges of the studied taxa: 22 populations of *C. decalvans*, 11 of *C. dinaricum* and 12 of *C. grandiflorum*, using 1 to 18 individuals per population, in total including 268 individuals. The AFLP procedure followed Vos et al., (1995) with the modifications described in Kutnjak et al., (2014). Raw data were collected and aligned with the internal size standard using GeneScan v.3.7 (Applied Biosystems). The GeneScan files were imported into GENOGRAPHER v.1.6 for scoring. The data were exported as a presence/absence matrix. Nonreproducible markers and those scored as present for only a single individual were removed from the dataset. Replicates were used to calculate the error rate according to Bonin et al., (2004).

A Neighbour Joining (NJ) analysis was conducted and bootstrapped (1000 pseudo-replicates) with TREECON v.1.3b (Van de Peer & De Wachter, 1997). A NeighborNet was produced using SplitsTree4 v.12.3 (Huson & Bryant, 2006) and TCS 1.21 (Clement et al., 2000). Sequence gaps were treated as fifth character state in TCS and indels longer than 1 bp were reduced to a single character prior to the analysis. Indels were deleted in *C. decalvans* and *C. grandiflorum* in poly-A (alignment positions 1311–1320, 2045–2051 and 473–484, respectively) and poly-T (alignment positions 1707–1713, 1876–1891, 1913–1921, 2052–2062 and 1416–1425, 1815–1830, respectively) prior to TCS analyses.

### 2.5 | Sequencing and analyses of sequence data

The ITS and plastid trnT-ndhJ regions were sequenced for one individual per population for 12 populations of *C. decalvans*, 11 of *C. dinaricum* (data for this species taken from Kutnjak et al., 2014) and 8 of *C. grandiflorum*, as described in Kutnjak et al., (2014). We chose this sequencing strategy as preliminary analyses showed no intrapopulational variation. Contigs were assembled, edited and sequences aligned using Geneious Pro 5.5.9 (Kearse et al., 2012). GenBank numbers of sequences are given in Table S2.

NeighborNets of ITS sequences and statistical parsimony networks of plastid sequences were produced using SplitsTree4 12.3 (Huson & Bryant, 2006) and TCS 1.21 (Clement et al., 2000). Sequence gaps were treated as fifth character state in TCS and indels longer than 1 bp were reduced to a single character prior to the analysis. Indels were deleted in *C. decalvans* and *C. grandiflorum* in poly-A (alignment positions 1311–1320, 2045–2051 and 473–484, respectively) and poly-T (alignment positions 1707–1713, 1876–1891, 1913–1921, 2052–2062 and 1416–1425, 1815–1830, respectively) prior to TCS analyses.

### 2.6 | Genome size measurements

For estimation of RGS and ploidy level, we used flow cytometry (FCM) of 40,6-diamidino-2-phenylindole (DAPI)-stained nuclei using a Partec (Münster, Germany) CyFlow Space flow cytometer as described by Greilhuber & Ebert, (1994). Forty-four populations of three *Cerastium* species from the Dinaric Alps were analysed, 21 of *C. decalvans*, 11 of *C. dinaricum* (data from Kutnjak et al., 2014) and 12 of *C. grandiflorum*. The number of measurements per population yielding high quality FCM histograms is given in Table S2. The data were visualised using the R ‘ggplot2’ package (Wickham & Chang, 2016) using R 2.13.1 (R Core Team, 2018). We compared the RGS values between the two main genetic groups revealed by K-means clustering of AFLP data (see Section 3) in all three studied taxa using Kruskal–Wallis H and Mann–Whitney U tests in R 2.13.1. In addition, we compared RGS between the two tetraploid genetic groups of *C. grandiflorum*.

### 2.7 | Correlation between genetic, geographic and environmental distances

To test for correlations between pairwise population genetic, geographic and environmental distances (we use the latter term as it is commonly used in the literature even if the distances are
exclusively based on climatic variables), we used simple and partial Mantel tests implemented in the R Package ‘vegan’ (Oksanen et al., 2017) and assessed their significance with 9999 permutations. Genetic distances (Gen) were based on calculated pairwise \( \Phi_{ST} \) values between populations using an Analysis of Molecular Variance (AMOVA) in the programme FAMD 1.31 (Schlüter & Harris, 2006). We used latitude and longitude coordinates of populations to estimate geographic distances (Geo) and extracted values of corresponding climatic variables (Table 1) for each population. Further, we calculated environmental Euclidean distances (Env) between population pairs. We then applied simple Mantel tests to test for isolation by distance (IBD; correlations between Gen and Geo) for isolation by environmental distance (IBED; correlations between Gen and Env; Mendez et al., 2010) amongst the populations of each species. Finally, we applied partial Mantel tests to assess if correlations between genetic and environmental differentiation remain significant while controlling for geographic distances (Gen, Env | Geo).

3  | RESULTS

3.1  | Climatic niche comparison amongst species

The PCA of climatic variables (Figure 3a) indicated partial overlap of the clusters of *C. decalvans* and *C. grandiflorum*, which were more divergent along PC2, whereas along PC1 *C. dinaricum* was placed within *C. decalvans* and *C. grandiflorum* at one extreme. PC1 explained 37.17% of the variance and mainly reflected the precipitation gradient (bioclimatic variables bio14, bio18, bio12; Table 1) and the minimum temperature of the coldest month (bio6) in the study area. PC2 explained 32.91% of the variance corresponding to the temperature gradient (bioclimatic variables bio4, bio8, bio9) and the precipitation of the coldest quarter (bio19). *Cerastium dinaricum* had the narrowest climatic niche as assessed visually in the climatic niche space (Figure 3), whereas occurrences of *C. decalvans* and *C. grandiflorum* were scattered along both axes indicating higher climatic variability. However, all three taxa were on the margin of the available climatic space concerning precipitation (Figure 3).

The observed niche overlap (D values) in climatic niche space was 0.24 between *C. dinaricum* and *C. grandiflorum*, 0.26 between *C. dinaricum* and *C. decalvans*, and 0.66 between *C. decalvans* and *C. grandiflorum* (Figure 3b–d). The niche equivalency test showed that the niches of the three species were not equivalent in the pairwise comparison (\( p < 0.01 \)), indicating climatic niche differences amongst them. However, the niche similarity test showed that all species pairs occupy more similar climatic niches than expected by chance, because the observed D values were always significantly higher (\( p < 0.01 \)) than the simulated null distributions (Figure S1).

ANOVA and the Tukey HSD tests (\( p < 0.05 \)) in the pairwise comparisons between the species showed significant differences in each climatic niche variable (Tables S4 and S5) as depicted also in the boxplots (Figure S2), which indicated higher climatic variability in *C. decalvans* and *C. grandiflorum* compared to *C. dinaricum* and revealed low to broad overlaps amongst the species. Some

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**FIGURE 3** Climatic niche comparisons amongst the three *Cerastium* taxa from the Dinaric Alps. (a) Climatic niche space summarised by the first two axes of the principal component analysis based on 10 bioclimatic variables. Coloured points: species’ occurrences; grey points: available background climate depicted by 10,000 randomly distributed points across the study area. Pairwise niche overlap using D metrics between (b) *C. decalvans* and *C. grandiflorum* (D = 0.66), (c) *C. dinaricum* and *C. decalvans* (D = 0.26) and (d) *C. dinaricum* and *C. grandiflorum* (D = 0.24). In b–d niches are represented as shading indicating the occurrence density of each species; solid grey area indicates niche overlap between the species pairs, and solid and dashed lines delimit 100% and 50% of the available climatic space. In c–d the niche of *C. dinaricum* is nested within the wider niche of the other taxon.
general trends were visible: *C. grandiflorum* grows in warmest habitats, followed by *C. decalvans* and *C. dinaricum*, which tolerates the lowest winter temperatures; *C. grandiflorum* and *C. decalvans* tolerate a similar and much broader yearly temperature variation compared to *C. dinaricum*. *Cerastium dinaricum* and *C. grandiflorum* occur in areas with higher annual precipitation compared to *C. decalvans*, whereas the highest mean precipitation in the warmest quarter and in the driest month was in the areas of *C. dinaricum* followed by *C. decalvans*. Finally, *C. grandiflorum* occurs in the summer-driest and winter-wettest areas, thus tolerating the highest annual variability of precipitation of all species.

3.2 | Species distribution modelling

Mean AUC scores across three replicated runs showed that performance of all models using current climate was excellent (average AUC > 0.9 with SD ranging from 0.0 to 0.08 in all cases except for Maxent in *C. dinaricum*). Across the species, models were on average most accurate in predicting the distribution of *C. decalvans* (AUC = 0.97, SD = 0.03 across methods). Current modelled habitat suitability agreed with the actual distribution of the three *Cerastium* taxa, but also some additional suitable areas, where no occurrence points were present, were indicated (Figure 4a–c).

SDMs hindcasted to the LGM revealed three main common areas of high suitability throughout the Dinaric Alps in each species (Figure 4d–f), overlapping the most in the central Dinaric Alps. In general, all species, but most pronouncedly thermophilous *C. grandiflorum*, were predicted to have undergone substantial range contraction during the LGM compared to the present. Under the RCP8.5 climate change scenario for 2080, our results indicated the strongest decrease in habitat suitability for *C. dinaricum*, followed by *C. decalvans*, which was also predicted to suffer large range contractions, while the lowest decrease in habitat suitability and shift of favourable habitat towards the northwest was forecasted for *C. grandiflorum* (Figure 4g–i).

3.3 | AFLP data

In *C. decalvans* 212 fragments ranging from 40 to 350 bp were scored for 74 individuals, in *C. dinaricum* 151 fragments ranging from 65 to 457 bp were scored for 148 individuals and in *C. grandiflorum* 287 fragments ranging from 30 to 420 bp were scored for 55 individuals.

![Figure 4](image-url) Species distribution models for the three *Cerastium* taxa from the Dinaric Alps under current (a–c), LGM (d–f) and future (g–i) climatic conditions. Species occurrences of *C. grandiflorum* (a), *C. decalvans* (b) and *C. dinaricum* (c) are indicated by yellow dots, and grey shading corresponds to habitat suitability, ranging from 0 (white: no suitability) to 1 (black: maximum suitability). Projection: WGS84
The error rate before the exclusion of low-quality fragments was 0.30%, 2.2% and 0.15%, respectively.

Nonhierarchical K-means clustering revealed an optimal split in all three taxa into two genetically defined groups. The main genetic break was in the area of the Neretva river in *C. decalvans* and *C. dinaricum*, coinciding with a large distribution gap in *C. dinaricum*, and in the northern Dinaric Alps in *C. grandiflorum*, separating the two northernmost octoploid populations from all tetraploid populations (Figure 2d–f). At K = 3, in all three taxa, the southern group was divided into two groups, separated by the Neretva river in *C. grandiflorum* (Figure 2d) and a distribution gap in Bosnia and Herzegovina in *C. dinaricum* (Figure 2f). In *C. decalvans* the border was less clear and positioned within the continuous distribution (Figure 2e). The genetic differentiation in *C. decalvans* was shallow as displayed by the star-like NeighborNet (Figure 2h) and the lack of support at basal nodes in the MCC tree (Figure S3). In contrast, NeighborNets revealed a pronounced divergence illustrated by long splits in *C. grandiflorum* (Figure 2i) and *C. grandiflorum* (Figure 2g), reflecting the results of the K-means clustering and the high support for basal nodes in the MCC trees (Figures S4 and S5).

Spatial reconstruction of evolutionary dynamics using relaxed random walks (Figure S6) suggested that the starting points of diversification for all three taxa were in approximately central parts of their present-day distributions (Figure S6a–c). From there, lineages spread almost evenly towards northwest and southeast but with different dynamics. By half of the time from the start of the divergence, *C. decalvans* (Figure S6e) and *C. grandiflorum* (Figure S6d) have reached almost complete present-day distributions, where they persisted thereafter (Figure S6g,h,j,k). In contrast, dispersal of *C. dinaricum* was delayed (Figure S6f) and advanced more rapidly only during the last quarter of the diversification (Figure S6l).

### 3.4 | Sequence data

The alignments of the ITS sequences were 783 bp long for *C. grandiflorum*, 785 for *C. decalvans* and 792 for *C. dinaricum*. NeighborNets revealed divergence amongst four, only partly geographically segregated groups of populations in *C. grandiflorum* (Figure 2j) and *C. decalvans* (Figure 2k). In *C. dinaricum* the southernmost populations 27–32 were connected with a short split with the northernmost populations 22–25 and with a longer split with the geographically intermediate population 26 (Figure 2l).

The alignments of plastid sequences (trnT–ndhJ) were 2243 bp long for *C. grandiflorum*, 2219 for *C. decalvans* and 2108 for *C. dinaricum*. Statistical parsimony analyses retrieved seven haplotypes in *C. grandiflorum* (Figure 2m), eight in *C. decalvans* (Figure 2n) and five in *C. dinaricum* (Figure 2o). Geographically adjacent populations often shared the same or similar haplotypes (evident especially in *C. dinaricum*, Figure 2o), and the southern populations were genetically more divergent than the northern ones in *C. decalvans* (Figure 2n) and *C. grandiflorum* (Figure 2m), without a clear geographic pattern.

### 3.5 | Genome size variation

Mean RGS ranged from 0.29 to 0.59 in *C. grandiflorum* (Figure 5a), from 0.27 to 0.34 in *C. decalvans* (Figure 5b) and from 0.30 to 0.33 in *C. dinaricum* (Figure 5c). In uniformly tetraploid *C. decalvans* RGS variation was continuous; in *C. dinaricum* RGS differed only slightly amongst populations. In contrast, the northernmost populations of *C. grandiflorum* (33 and 34) were RGS-octoploid (RGS 0.59 and 0.50, respectively), whereas all other populations were RGS-tetraploid (RGS ranging from 0.33 to 0.35; Figure 5a and Table S2). Differences in RGS between the two main K-means groups in all three studied taxa were significant (Table S6), whereas differences in RGS between the two K-means groups in tetraploid *C. grandiflorum* were not (p > 0.05).

### 3.6 | Correlation amongst genetic, geographic and environmental distances

Populations of all analysed taxa showed significant IBD patterns (Table 2), which was most pronounced in *C. dinaricum*. In addition, there was a clear and significant correlation between genetic and environmental differentiation (IBED) amongst populations of *C. dinaricum*,...
even after controlling for geographic distance. IBED was also significant for populations of C. decalvans but not after correction for geographic distance.

4 | DISCUSSION

Despite nonequivalent climatic niches and relatively low to moderate niche overlap amongst the three studied Cerastium taxa (Figure 3 and Figures S1 and S2), which are reflected in their adaptations to different habitats (Table S1), they all exhibited fairly similar patterns of AFLP divergence (Figure 2d–i). In line with our field observations and elevational distribution patterns (Table S1) Cerastium grandiflorum is the most thermophilous and tolerates the highest climatic variability; C. decalvans is more mesophilous and spans a narrower fraction of the climatic space compared to C. grandiflorum. The climatic niche of cold-adapted C. dinaricum is the most restricted and nested within the niches of the other two taxa at one extreme regarding temperature and humidity, indicating specialisation (Vamosi et al., 2014). Although the three species do not occupy identical climatic niches, the niche similarity test suggests more similar climatic niches than expected by chance, thus indicating climatic niche conservatism, which is expected for niches of closely related species (Pyron et al., 2015; Warren et al., 2008; Wiens et al., 2010). Occurrence under similar and partially shared macroclimatic conditions may also explain the observed congruence in the phylogeographical patterns. The three species often co-occur in adjacent, only microclimatically divergent habitats, which may have—alongside the low precision of Worldclim data in mountainous areas (Hijmans et al., 2005)—contributed to the pronounced climatic niche overlap. However, as potentially divergent microclimatic conditions could not be identified with the relatively coarse spatial resolution of the selected climatic variables, the observed niche overlaps may be an overestimate.

| Species          | Factor        | r      | p        |
|------------------|---------------|--------|----------|
| C. grandiflorum  | Gen +Geo      | 0.606  | 0.0001** |
|                  | Gen +Env      | 0.063  | 0.3213   |
|                  | Gen +Env| Geo    | -0.032  | 0.5708   |
| C. decalvans     | Gen +Geo      | 0.629  | 0.0001** |
|                  | Gen +Env      | 0.295  | 0.0134*  |
|                  | Gen +Env| Geo    | 0.088   | 0.2314   |
| C. dinaricum     | Gen +Geo      | 0.728  | 0.0001** |
|                  | Gen +Env      | 0.677  | 0.0001** |
|                  | Gen +Env| Geo    | 0.280   | 0.0412   |

*p < 0.05.* **p < 0.01.

Bold indicates significant correlations.

4.1 | A strong phylogeographic break in the central Dinaric Alps

Nonhierarchical K-means clustering of AFLP data revealed an optimal split in the three taxa into two groups with northwest to southeast differentiation as well as elevated genetic diversity in the southern part of the distribution area (Figure 2d–f), confirming the ‘refugia-within-refugia’ hypothesis (Gómez & Lunt, 2007). Despite the pronounced habitat differentiation amongst the species (Niketić, 2012; Table S1), the deepest genetic split in C. decalvans and C. dinaricum as well as within the tetraploid populations of C. grandiflorum is positioned roughly in the same area, that is, along the Neretva river valley (Figure 2d–f). This valley has been inferred as a phylogeographic barrier for several other plant as well as animal species (e.g., Caković et al., 2015; Falch et al., 2019; Frajman & Oxelman, 2007; Kryštufek et al., 2007; Lakušić et al., 2013; Surina et al., 2014). These studies also revealed that the areas further south harbour deeper genetic breaks, which was also the case in our taxa. In a few other plant groups (e.g., Đurović et al., 2017), more northerly positioned genetic breaks were inferred, similarly to the barrier between octoploid and tetraploid populations of C. grandiflorum (Figure 1d). In the latter species, the presence of different ploidies poses a barrier to gene flow, which is theoretically possible from lower into higher-ploids via unreduced gametes but considered rare (Kolář et al., 2017).

It remains unclear which historical factors conferred the congruency across phylogeographies of ecologically different species from different plant families. Lakušić et al., (2013) and Kutnjak
et al., (2014) suggested divergence triggered by adaptation to two ecologically divergent glacial refugia, that is, the more arid and continental northern Adriatic basin, which fell dry during Pleistocene glaciations, and the climatically divergent southern Adriatic. Alternatively, Falch et al., (2019) suggested that the divergence observed in Euphorbia myrsinites L. rather resulted from postglacial northward colonisation accompanied by strong genetic drift. Our results suggest that suitable climatic conditions for the species occurred on both sides of the Neretva valley during the LGM. This is in contrast to the hypothesis of two ecologically divergent glacial refugia as suggested by Lakusić et al., (2013) and Kutnjak et al., (2014) and probably implies that this deep and canyon-like valley has acted as a strong physical barrier for unrelated species with contrasting habitat requirements such as lowland and alpine biota. In the same line, heterogeneous environments with a mosaic of habitats enabled local persistence of different lineages in many plant species in the Dinaric Alps during the Pleistocene glaciations (e.g., Kutnjak et al., 2014; Ronikier et al., 2012; Suchan et al., 2019; Surina et al., 2011).

Disjoint areas of high suitability separated by regions of low suitability were modelled in all three Cerastium species for the LGM. This suggests the existence of separate Pleistocene refugia (Figure 4d–f), which were more isolated in C. dinaricum and C. grandiflorum as compared to C. decalvans. As hypothesised, areas of high suitability increased for C. dinaricum and decreased for C. grandiflorum, whereas for C. decalvans they were rather displaced. The persistence in isolated refugia may have triggered genetic divergence, which was further enhanced by the disjunct distribution of C. dinaricum in the Holocene. The presence of barriers to gene flow is also reflected in RGS differences amongst the groups of populations, further supporting the pattern revealed by AFLPs. In C. grandiflorum the northwest-to-southeast genetic divergence was likely enhanced by reproductive isolation due to octoploidy of the two northernmost populations (Figure 5a; Khalaf & Stace, 2000; Niketić et al., 2013). Taking into account the morphological similarity of tetraploid and octoploid populations of C. grandiflorum, the sharing of haplotype and ribotypes between both ploidies, as well as the moderate divergence inferred by AFLPs (Figure 2), we argue that the octoploids are of autopolyploid origin.

The suitable areas modelled for the LGM were largest and relatively continuous in C. decalvans (Figure 4e), suggesting that gene flow was possible between the refugia and that expansion from refugia was accompanied by secondary contact and admixture. This hypothesis is supported by shallow AFLP differentiation (Figure 2h) and continuous RGS variation (Figure 5b). Late Pleistocene onset of genetic divergence within the three species is consistent with dating analyses of Caryophyllaceae performed by Frajman et al., (2009), where the divergence between C. arvense L. and C. holosteoides Fr., closely related to our taxa (Frajman, unpublished data), was dated to the Pleistocene, when also our three species likely originated and started to diversify.

The contrast phylogeographical patterns inferred for our study species are not congruent with those obtained for ecologically divergent Edraianthus species in the same area (Surina et al., 2011). However, the strongest phylogeographic structure identified by AFLPs and plastid sequences was evident in cold-adapted C. dinaricum, similarly as inferred for the equally cold-adapted E. serpyllifolius (Surina et al., 2011). This suggests that previously isolated populations did not get into secondary contact in the Holocene and continued diverging due to climate warming, resulting in a highly fragmented distribution. This divergence will likely get even more pronounced in the future, following our predictions of decreased future habitat suitability for this species due to ongoing climate change (Figure 4i). Decrease of areas with suitable habitats is predicted also for C. decalvans (Figure 4h), which will likely migrate upward, and some of its populations might persist in microclimatically suitable habitats in river gorges at lower elevations. Contrary, C. grandiflorum is predicted to increase its distribution in the face of global warming, which will likely facilitate gene flow amongst currently disjoint populations (Figure 4g).

4.2 | Complex patterns of genetic differentiation revealed by plastid and nuclear ITS sequences

In line with the AFLP results, genetic differentiation in northwest–southeast direction was inferred also by ITS sequences (Figure 2j–l). Differentiation was less pronounced north of the Neretva river, with the exception of the divergent octoploid populations of C. grandiflorum. South of the Neretva, more ribotypes were found, especially in C. decalvans. In contrast, plastid sequences revealed high diversity on small geographic scales with a star-like structure in C. decalvans and C. grandiflorum (Figure 2m–n), whereas in C. dinaricum haplotypes were more widespread and the haplotype network linear (Figure 2o).

Differences in diversity patterns inferred by the three markers are likely the result of differences in their inheritance (see Introduction). Our data clearly evidence hybridisation and chloroplast capture (Frajman et al., 2009; Rieseberg & Soltis, 1991). For instance, the haplotype in population 44 of C. grandiflorum was 17 mutational steps away from the haplotype found in the geographically closest studied population 42 (Figure 2g) but was close to the haplotypes of syntopic C. decalvans. A similar scenario was suggested in the case of co-occurring C. sylvaticum and C. subtriflorum (Skubic et al., 2018) and is in line with observations that hybridisation within Cerastium is relatively common (Khalaf & Stace, 2000).

5 | CONCLUSIONS

Our study highlights that habitat-segregated but co-occurring species in the Dinaric Alps with similar macroclimatic niches responded similarly to glaciations and occupied geographically overlapping refugia, resulting in congruence of phylogeographic patterns. These refugia likely provided a wide range of microclimatically diverse habitats, which, however, could not be identified with the coarse climatological data used. In all species IBD was the main driver of
genetic divergence, partly accompanied by RGS differentiation. Genetic drift, hybridisation and partly environmental divergence (IBED) likely also shaped the observed genetic patterns. Even though the main phylogeographic divergence is congruent across the three species, projections of future habitat suitability differ. Thermophilous C. grandiflorum, which seems to tolerate drought the best, will thus be least affected amongst the three species by climate warming and might extend its range to northerly allied areas, for which an increase in habitat suitability has been predicted. On the other hand, cold-adapted C. dinaricum is certainly the most endangered, and extinction of several of its small populations is highly probable. Our results highlight the importance of exploring biogeographic patterns in habitat-segregated, sympatric taxa using an array of complementary methods.

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DATA AVAILABILITY STATEMENT
Geographic data needed to replicate the niche comparisons and species distribution modelling are in Table S3 of the Supporting Information, whereas the GenBank accession numbers for the sequences used in phylogenetic analyses are in Table S2 of the Supporting Information.

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**BIOSKETCH**

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**SUPPORTING INFORMATION**

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

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