Plant Systematics and Evolution (2020) 306:42
https://doi.org/10.1007/s00606-020-01668-6

ORIGINAL ARTICLE

Phylogeography of *Campanula fenestrellata* s.l. (Campanulaceae) in the northern Adriatic

Ivana Rešetnik[1] · Martina Temunović[2] · Zlatko Liber[1,5] · Zlatko Satovic[3,5] · Sandro Bogdanović[4,5]

Received: 28 November 2019 / Accepted: 25 February 2020

Abstract

Campanula fenestrellata subsp. *fenestrellata* and *C. fenestrellata* subsp. *istriaca* belong to the morphologically and phylogenetically well established amphi-Adriatic group *Campanula* ser. *Garganicae*. The two morphologically differentiated endemic taxa are distributed on the north-eastern Adriatic islands and coast. In order to study the relationships among *C. fenestrellata* s.l. populations and to gain insight into genetic basis of taxonomic separation established on morphology, we examined amplified fragment length polymorphisms (AFLPs) as well as nuclear and plastid DNA sequences (ITS and *trn*L–*trn*F). With the sequence data, no structure and no distinction between the two subspecies were observed. The AFLPs separated populations geographically and inferred high number of most likely clusters. The northern and the southernmost populations were assigned to uniform separate clusters indicating the longer isolation of respective populations, while the admixed populations were evident in the central area where the two taxa geographically overlap, indicating the presence of gene flow. Distinct fragmentation patterns and the existence of several temporally continuous microrefugia during the Quaternary climatic oscillations likely promoted strong genetic differentiation within the species range, while secondary postglacial contacts are reflected in observed admixture. Although the two taxa exhibited differences in ecological preferences, niche conservatism was detected. Overall, phylogeographic reconstruction of *C. fenestrellata* s.l. populations highlights the importance of microrefugia in shaping the intraspecific genetic variation as well as of melting-pot areas in rearranging such variation as a result of a more recent admixture.

Keywords AFLP · Balkan · Environmental niche analyses · Phylogeography · Refugia · Subspecies

Handling Editor: Stanislav Španiel.

Contribution to "Plants of the Balkan Peninsula in Space and Time".

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00606-020-01668-6) contains supplementary material, which is available to authorized users.

✉ Ivana Rešetnik
ivana.resetnik@biol.pmf.hr

[1] Faculty of Science, Department of Biology, University of Zagreb, Marulićev trg 20/II, 10000 Zagreb, Croatia

[2] Faculty of Forestry, Department of Forest Genetics, Dendrology and Botany, University of Zagreb, Svetošimunska cesta 25, 10000 Zagreb, Croatia

[3] Faculty of Agriculture, Department of Seed Science and Technology, University of Zagreb, Svetošimunska cesta 25, 10000 Zagreb, Croatia

[4] Faculty of Agriculture, Department of Agricultural Botany, University of Zagreb, Svetošimunska cesta 25, 10000 Zagreb, Croatia

[5] Centre of Excellence for Biodiversity and Molecular Plant Breeding, Svetošimunska cesta 25, 10000 Zagreb, Croatia

Introduction

Subspecies are defined as groups of populations within a species that share a unique geographical range or habitat, and differ from other such groups in several genetically based traits, e.g. morphology and ecology (Avise and Ball 1990; Hamilton and Reichard 1992). Genetic markers may aid in reconstructing the evolutionary history and thus help in delimiting subspecies, although the patterns observed in molecular-based assessments may differ from those seen in morphological and ecological characters (e.g. Bardy et al. 2011; Caković et al. 2015; Rešetnik et al. 2016b). Moreover, the past range fluctuations can be detected in genetic variation even in the contemporary continuous distribution of closely related taxa (Bardy et al. 2010; Lakušić et al. 2013; Olšavská et al. 2016). The evolutionary history and underlying genetic structure of closely related taxa may be confronted with different habitats that impose particular environmental constraints on them. However, the studies focusing on specific ecological niches of Balkan intraspecific plant taxa are few (Kolář et al. 2016; Frajman et al.

Published online: 19 March 2020

2019). Ecologically, closely related taxa can have a tendency to differ less than expected by chance due to shared environmental constraints and biogeographic history, which is recognized as phylogenetic niche conservatism (Wiens and Graham 2005; Losos 2008; Pyron et al. 2015). Nonetheless, they might differ more ecologically than expected by chance providing the evidence for ecological niche divergence (Warren et al. 2008; McCormack et al. 2010).

The Quaternary refugial character of the Balkan Peninsula promoted the diversification of numerous species groups (e.g. Janković et al. 2016; Španiel et al. 2017; Aleksić et al. 2018), and strong genetic substructure has been shown for several taxa (Slade et al. 2008; Aleksić and Geburek 2010; Surina et al. 2011, 2014; Kutnjak et al. 2014; Rešetnik et al. 2016a; Janković et al. 2019). Although until recently the southern part of the Balkan Peninsula has been regarded as the major refugial area, northern Istria has been considered as local glacial refugium by Médail and Diadema (2009). As other glacial refugia, the north-western Balkan Peninsula also harbours many endemic species, for instance *Campanula tommasiniana* Koch and *Campanula justiniana* Witasek (Campanulaceae), *Asperula borbasiana* (Korica) Korica and *Asperula woloszczakii* Korica (Rubiaceae), *Asplenium hybridum* (Milde) Bange (Aspleniaceae), *Moehringia tommasinii* Marches. (Caryophyllaceae) and *Astragalus glacialis* Lovrić (Fabaceae). Moreover, recent studies show evidence for glacial survival of broad-leaved forests and herbaceous understory species (*Fagus sylvatica* L., Magri et al. 2006; *Cyclamen purpurescens* Mill., Slovák et al. 2012; *Knautia drymeia* Heuff., Rešetnik et al. 2016b) in the westernmost part of the Balkan Peninsula. At the same time, the thermophilous taxa distributed along the northern part of the Adriatic Sea have mostly been shown to exhibit lower levels of genetic diversity indicating their survival in southern part of the Adriatic (Surina et al. 2011; Rešetnik et al. 2016a). Nonetheless, the higher levels of genetic diversity in the northern Adriatic were, for example, observed in *Tanacetum cinerariifolium* (Trevir.) Sch. Bip. (Grdiša et al. 2014), suggesting that refugium for some taxa was also located in the northern part.

One of the thermophilous and chasmophytic species with continuous and abundant distribution along the north-eastern Adriatic coast and islands is *Campanula fenestrellata* Feer. *Campanula fenestrellata* belongs to the morphologically, karyologically and phylogenetically well-supported monophyletic group *Campanula* ser. *Garganicae* Trinajstić distributed in the amphi-Adriatic and Ionian region (Park et al. 2006; Liber et al. 2008; Frajman and Schneeweiss 2009; Bogdanović et al. 2014a, b, 2015, 2019). The group, as currently delimited, includes 12 species, while the sister species of the group is the Albanian endemic *C. comosiformis* (Hayek and Janch.) Frajman and Schneew. The onset of diversification of the group (including *C. comosiformis*) has been suggested to be the upper Burdigalian in the Miocene (Frajman and Schneeweiss 2009), although the Pleistocene climatic fluctuations also influenced the currently observed variation in the group (Bogdanović et al. 2019).

Campanula fenestrellata is composed of two subspecies; *C. fenestrellata* Feer subsp. *istriaca* (Feer) Damboldt that is distributed from Istria to Jablanac (Velebit littoral) and on the northern Adriatic islands and the typical *C. fenestrellata* Feer subsp. *fenestrellata* distributed from Jablanac southwards to Krka river in Dalmatia (Fig. 1). The two subspecies are differentiated morphologically; *C. fenestrellata* subsp. *istriaca* is densely tomentose, basal leaves are serrate, with corolla and capsule usually larger than in typical subspecies, while *C. fenestrellata* subsp. *fenestrellata* is glabrous and with 2-serrate or dentate basal leaves (Damboldt 1965; Bogdanović et al. 2014a, b). The phylogeny of the ser. *Garganicae* inferred with plastid and ITS sequence data unambiguously separated all species in the group although relationships among the species in the group were mostly poorly resolved due to low clade support (Park et al. 2006; Frajman and Schneeweiss 2009; Bogdanović et al. 2014a, b, 2015, 2019). However, the two *C. fenestrellata* taxa always formed a well-supported distinct lineage.

In order to further study the relationships between *C. fenestrellata* s.l. populations and to gain insight into genetic basis of taxonomic separation traditionally established on morphology, we examined amplified fragment length polymorphisms (AFLPs), as well as nuclear (ITS) and plastid DNA sequences (*trn*L–*trn*F). In addition, by employing ecological niche analyses, we assess whether environmental differences between *C. fenestrellata* subsp. *istriaca* and *C. fenestrellata* subsp. *fenestrellata* exist and if they are congruent with obtained genetic and/or morphological differentiation.

Materials and methods

Plant material

Fourteen populations of *C. fenestrellata* s.l. (each subspecies represented with seven populations) were sampled across the entire distribution area between 2011 and 2017 (Fig. 1). In addition, for the phylogenetic analyses we used ITS sequences and *trn*L–*trn*F sequences (30 accessions of ser. *Garganicae* and one outgroup) from previous studies (Park et al. 2006; Frajman and Schneeweiss 2009; Bogdanović et al. 2014a, b, 2015, 2019) deposited in GenBank (for details see Online Resource 1).

DNA extraction, ITS and *trn*L–*trn*F sequencing

Extraction of total genomic DNA was performed from silica gel dried leaves using the DNeasy plant mini kit (Qiagen

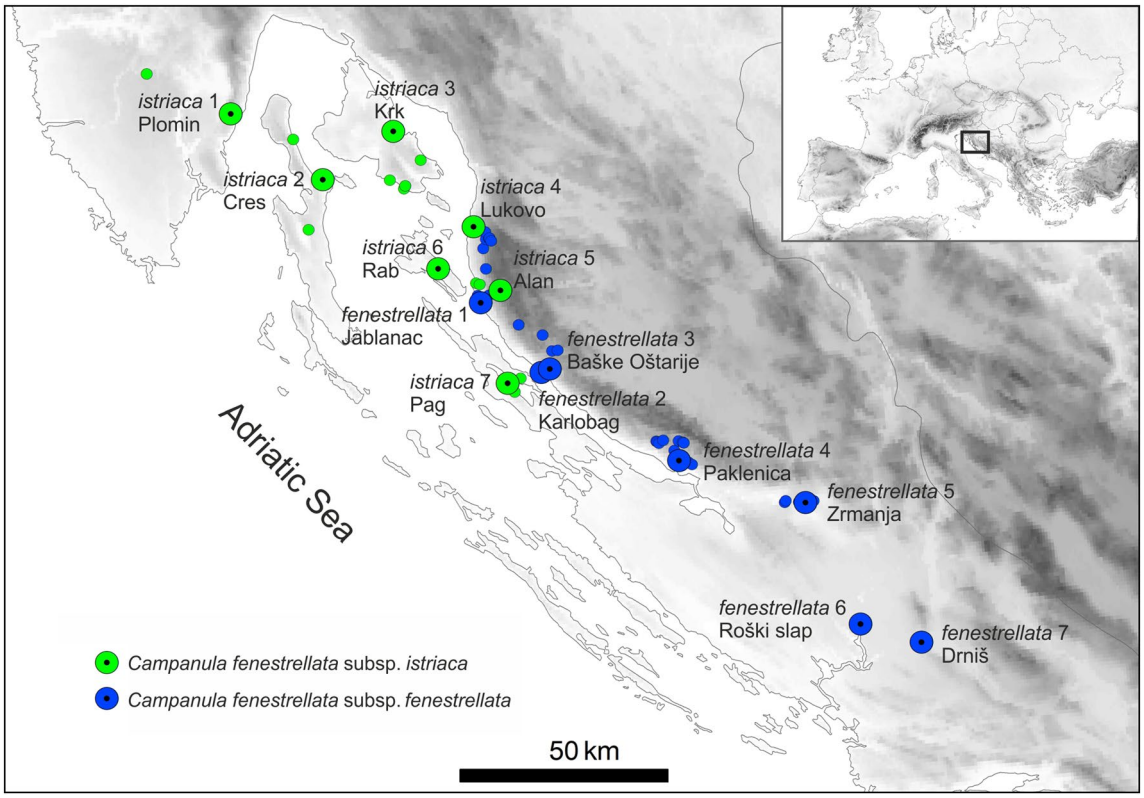

Fig. 1 Distribution of *Campanula fenestrellata* s.l. populations used in environmental niche analyses (small circles) and sampled populations for genetic analyses (large circles). Blue circles correspond to *C. fenestrellata* subsp. *fenestrellata,* and green circles correspond to *C. fenestrellata* subsp. *istriaca.* For details, see Online Resource 1

GmbH, Hilden, Germany), following the manufacturer's instructions. Polymerase chain reactions (PCR) were performed as described in Bogdanović et al. (2015) using the 17SE and 26SE primers (Sun et al. 1994) for the nuclear ITS and c and f primers (Taberlet et al. 1991) for the plastid *trn*L–*trn*F region. The PCR products were purified with the GenElute PCR clean-up kit (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), according to the manufacturer's protocol. The products were sequenced by the Macrogen Inc. (Seoul, Korea) using the same primers as in PCR and the BigDyeTM terminator cycle sequencing kit (Applied Biosystems, Foster City, California) and analysed on an ABI PRISM 3730XL automated sequencer (Applied Biosystems, Foster City, California). Contigs were assembled and edited, and sequences were aligned using Geneious Pro5.3.6 (Drummond et al. 2011; Online Resource 2). Base polymorphisms in the ITS sequences were coded using NC-IUPAC ambiguity codes.

Analyses of sequence data

Two different data sets (ITS and *trn*L–*trn*F) were analysed using maximum parsimony (MP) and Bayesian inference (BI). The trees were rooted using *C. pyramidalis* L. as outgroup. The most parsimonious trees were searched for heuristically with 1000 replicates of random sequence addition, and TBR swapping, as implemented in PAUP* 4.0b10 (Swofford 2003). Bootstrap support values (MPB; Felsenstein 1985) from 1000 replicates were generated using the heuristic search options as above except for random addition sequence with 100 replicates. Bayesian analyses were performed using MrBayes v.3.2.2 (Ronquist et al. 2012) applying the SYM+G substitution model selected based on the Akaike information criterion implemented in MrModelTest (Nylander 2004). The settings for the Markov chain Monte Carlo process included two runs with four chains each for 10^7 generations, sampling trees every 1000th generation. The first 2500 trees (prior to the 2.5×10^6 generation), which was well after the chains, had reached stationarity as judged from plots of the likelihood and from the average standard deviation of split frequencies being < 0.01, were discarded as burn-in. Convergence of the MCMC procedure was assessed by visual inspection of the traces and via effective sample size (ESS) values calculated with the program Tracer v. 1.4 (Rambaut and Drummond 2007).

AFLP fingerprinting

AFLP fingerprinting was performed with five to ten individuals per population (Table 1). The AFLP procedure followed Vos et al. (1995) with several modifications from Carović-Stanko et al. (2011). The four primers for selective PCR were as follows: VIC-EcoRI-ACG+MseI-CGA, FAM-EcoRI-ACA+MseI-CAC, NED-EcoRI-AGA+MseI-CAC and PET-EcoRI-AGC+MseI-CGA. Restriction and ligation, preamplification and selective amplification were performed in a ProFlex™ PCR System (Applied Biosystems^{R}) with a 2 × 96-Well PCR block. Six blank controls (DNA replaced with water) were included to test for contamination, Three DNA samples were replicated within each individual 96-well PCR plate, while two DNA samples were repeated in both 96-well PCR plates. The products were detected by capillary electrophoresis in the Macrogen Inc. (Seoul, Korea) using the ABI 3730xl analyser (Applied Biosystems^{R}).

Analyses of AFLP data

Electropherograms were analysed with GeneMapper 4.0 software (Applied Biosystems^{R}). The error rate estimation and final selection of AFLP alleles were calculated according to Herrmann et al. (2010) using ScanAFLP script. A matrix of 116 individuals was finally produced and analysed as described below. The within-population genetic diversity was assessed by calculating the proportion of polymorphic markers (*%P*), the number of private markers (N_{pr}) and the Shannon's information index (*I*; Shannon and Weaver 1949; Lewontin 1972). The frequency down-weighted marker values (DW; Schönswetter and Tribsch 2005) were calculated using AFLPdat (Ehrich 2006). A neighbour joining tree was constructed based on pairwise distance matrix between individual plants calculated using Dice's coefficient (Dice 1945). Statistical support of the branches was assessed by bootstrap analysis using 1000 replicates (Felsenstein 1985) in PAST ver. 2.01 (Hammer et al. 2001). The analysis of molecular variance (AMOVA; Excoffier et al. 1992) was used to partition the total genetic variance among and within populations as well as among subspecies, among populations within subspecies and within populations in Arlequin ver. 3.5.2.2 (Excoffier and Lischer 2010). The variance components were tested using 10,000 permutations. The genetic structure of *C. fenestrellata* populations was inferred using two Bayesian model-based clustering methods as implemented in STRUCTURE ver. 2.3.4 (Pritchard et al. 2000) and BAPS ver. 5.4 (Corander et al. 2003). In STRUCTURE, ten runs per each *K* were performed by setting the number of clusters (*K*) from 1 to 15 on the Isabella computer cluster at the University of Zagreb, University Computing Centre (SRCE). Each run consisted of a burn-in period of 200,000 steps followed by 10^6 MCMC (Monte Carlo Markov Chain) replicates assuming admixture model and correlated allele frequencies. The choice of the most likely number of clusters (*K*) was carried out by calculating an ad hoc statistic ΔK based on the rate of change in the log probability of data between successive *K* values, as described by Evanno et al. (2005) and implemented in STRUCTURE HARVESTER ver. 0.6.94 (Earl and von Holdt 2012). The population mixture analysis was conducted in BAPS by setting the maximal number of clusters

Table 1 Molecular diversity revealed by AFLP markers in 14 *Campanula fenestrellata* s.l. populations

Taxon	Locality	*n*	*P%*	N_{pr}	*I*	DW
Campanula fenestrellata Feer subsp. *fenestrellata* 1	Croatia, Jablanac	10	33.27	0	0.243	42.73
Campanula fenestrellata Feer subsp. *fenestrellata* 2	Croatia, Karlobag	6	29.70	1	0.239	39.69
Campanula fenestrellata Feer subsp. *fenestrellata* 3	Croatia, Velebit, Baške Oštarije	6	40.23	7	0.333	72.55
Campanula fenestrellata Feer subsp. *fenestrellata* 4	Croatia, Velebit, Velika Paklenica	10	26.32	5	0.199	89.09
Campanula fenestrellata Feer subsp. *fenestrellata* 5	Croatia, Zrmanja, Kudin most	8	32.52	3	0.239	61.02
Campanula fenestrellata Feer subsp. *fenestrellata* 6	Croatia, NP Krka, Roški slap	5	28.95	5	0.236	71.88
Campanula fenestrellata Feer subsp. *fenestrellata* 7	Croatia, Drniš	7	28.57	10	0.226	99.04
Campanula fenestrellata subsp. *istriaca* (Feer) Damboldt 1	Croatia, Istra, Plomin	10	33.27	7	0.241	105.88
Campanula fenestrellata subsp. *istriaca* (Feer) Damboldt 2	Croatia, island Cres, Merag	10	35.53	1	0.254	70.76
Campanula fenestrellata subsp. *istriaca* (Feer) Damboldt 3	Croatia, island Krk, Vrbnik	10	33.27	7	0.242	67.48
Campanula fenestrellata subsp. *istriaca* (Feer) Damboldt 4	Croatia, Lukovo	8	36.28	1	0.270	77.71
Campanula fenestrellata subsp. *istriaca* (Feer) Damboldt 5	Croatia, Alan	8	38.72	0	0.295	43.57
Campanula fenestrellata subsp. *istriaca* (Feer) Damboldt 6	Croatia, island Rab, Kamenjak	10	36.09	3	0.247	69.30
Campanula fenestrellata subsp. *istriaca* (Feer) Damboldt 7	Croatia, island Pag, Kolan	8	32.52	1	0.237	49.58

n sample size; *P%* proportion of polymorphic bands; N_{pr} number of private bands; *I* Shannon's information index; *DW* frequency down-weighted marker values

(*K*) to 15 with 10 runs per each *K*. Results of the mixture analyses were used as input for the following admixture analyses (Corander and Marttinen 2006), with the default settings in order to detect admixture between clusters.

Environmental niche analyses

Occurrence data for both taxa were collected from the Flora Croatica Database (Nikolić 2019), including herbarium data, observations and literature georeferenced points. The initial data set (172 occurrence points for subspecies *fenestrellata* and 126 for subspecies *istriaca*) was first checked for duplicates, precision and taxonomic accuracy and then reduced to include only unique georeferenced points with the highest precision (i.e. GPS coordinates and points georeferenced with at least 1:25.000 topographic map) and unambiguous taxonomy. This data set was complemented with our own GPS sampling points of 14 populations used for phylogenetic analyses. In total, we obtained 40 unique occurrence points for *C. fenestrellata* subsp. *fenestrellata* and 21 for *C. fenestrellata* subsp. *istriaca* used for the subsequent niche analyses. To characterize and compare the environmental niche of the two closely related taxa, we selected eight out of initially 26 tested environmental variables based on their ecological relevance for the studied taxa and low collinearity (we removed highly correlated variables with $|r| > 0.85$ and the variance inflation factor VIF below recommended threshold of 10). Selected environmental variables were used at the 1 km^2 resolution and included: Temperature Seasonality (bio4), Temperature Annual Range (bio7), Mean Temperature of Wettest Quarter (bio8), Mean Temperature of Driest Quarter (bio9), Precipitation of Driest Month (bio14), and Precipitation of Coldest Quarter (bio19) from the WorldClim database (www.worldclim.org), Hargreaves climatic moisture deficit (CMD) from the ClimateEU database (https://sites.ualberta.ca/~ahamann/data/climateeu.html), and Soil pH in H_2O at 30 cm depth (pH) from the SoilGrids database (https://soilgrids.org). To assess the environmental niche differences or similarity between *C. fenestrellata* subsp. *fenestrellata* and *C. fenestrellata* subsp. *istriaca,* we first used the environmental principal component approach (PCA-env) following Broennimann et al. (2012). Background available environment was depicted by extracted environmental data from 5000 random points across the study area (distribution area of the whole ser. *Garganicae*). We then calculated the observed niche overlap between the two taxa in the environmental space using the Schoener's *D* metric which ranges from 0 (no overlap) to 1 (identical niches) (Schoener 1968; Warren et al. 2008) and performed the niche equivalency and similarity tests described by Warren et al. (2008) and implemented in the ecospat R package (Di Cola et al. 2017). The observed niche overlap *D* values were then compared to the null distributions of *D* values simulated using 1000 replicates (Warren et al. 2008; Broennimann et al. 2012). In the niche equivalency test, if the observed *D* value is below the 95% confidence limits of the null distribution the two niches are not statistically equivalent, while in the similarity test higher observed *D* value than 95% confidence limits of the null distribution indicates that the two niches are more similar than expected by chance, indicating niche conservatism (Warren et al. 2008; Broennimann et al. 2012).

Results

Phylogenetic analyses of ITS and *trn*L–*trn*F

The characteristics of the ITS and *trn*L–*trn*F data sets analysed using MP and BI are summarized in Online Resource 3. The sequence data of both data sets confirmed the distinct position of *C. fenestrellata* within the ser. *Garganicae* (ITS: 1 PP/99 BS; *trn*L–*trn*F: 1 PP/91 BS; Fig. 2). However, no structure and no distinction between the *C. fenestrellata* subsp. *fenestrellata* and *C. fenestrellata* subsp. *istriaca* were observed. None of the other species belonging to ser. *Garganicae* were resolved as sister taxa of *C. fenestrellata* s.l. All other species within the group received high support from both maximum parsimony and Bayesian analyses and the relationships among them are congruent with the results obtained in Bogdanović et al. (2019).

AFLP data

A total of 551 polymorphic fragments were scored in 116 individuals. Nineteen fragments were excluded because they were present or absent in a single individual. The global mismatch error rate was 2.5%. The percentage of polymorphic markers varied among the populations with the highest proportion in population Baške Oštarije (40.23%) and the lowest in population Paklenica (26.32%) (Table 1). Similarly, Shannon's information index (I) ranged from 0.199 (Paklenica) to 0.333 (Baške Oštarije) with an average of 0.250 (Table 1, Fig. 4a). Out of 532 polymorphic markers, 51 were private. The population with the most private alleles (10) was Drniš, while no such alleles were found in the Alan and Jablanac populations. The frequency down-weighted marker values (DW) varied greatly and ranged from 39.69 in Karlobag population to 105.88 in Plomin population (Table 1, Fig. 4b).

The NJ analysis separated most of the analysed populations with high bootstrap support. The northern and the southernmost populations receiving maximal support, while the populations in the centre of distribution having lower support and with Jablanac population receiving no support and Alan population separated into two clusters

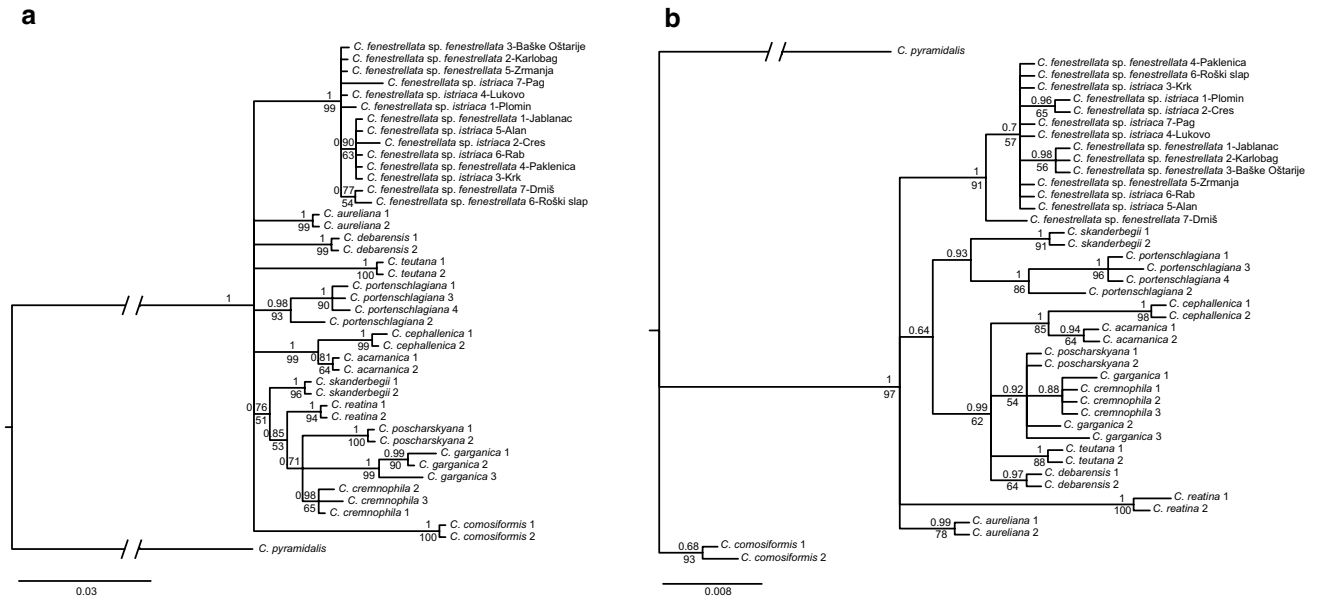

Fig. 2 Phylogenetic trees of the species belonging to *Campanula* ser. *Garganicae* based on **a** ITS data set, and **b** *trn*L–*trn*F data set from Bayesian analyses. Values above branches are Bayesian posterior probabilities (PP), and values below branches are maximum parsimony (BS) bootstrap percentages (only shown if at least 50%)

(Fig. 3). The results of the one-way AMOVA showed that 39.00% of the total genetic diversity was attributable to among-population component. AMOVA's ϕ_{ST} equalled 0.39 ($P < 0.0001$) suggesting an extensive interpopulational differentiation ranging from 0.169 (between populations Jablanac and Alan) to 0.539 (between populations Paklenica and Roški slap) (Online Resource 4). Two-way AMOVA revealed that only 3.14% of the total genetic diversity could be explained by differences between subspecies (subsp. *istriaca* vs subsp. *fenestrellata*; $\phi_{CT} = 0.031$; $P = 0.026$).

The STRUCTURE analysis resulted in an optimal partition ($\Delta K = 13.27$) with eight clusters ($K = 8$; Fig. 4c; Online Resource 5), separating the Plomin, Paklenica and Zrmanja populations into individual clusters, population pairs Cres-Krk, Rab-Lukovo, Jablanac-Pag and Roški Slap-Drniš forming other four separate clusters, populations Alan and Karlobag showing a range of admixture proportions and Baške Oštarije population indicating separate cluster with admixed pattern. The second best partition with two clusters ($K = 2$; $\Delta K = 8.51$; Online Resource 5) separated Paklenica-Roški Slap-Drniš populations from the rest, with Plomin, Cres, Baške Oštarije and Zrmanja populations showing high admixture proportions. In BAPS, the best partition received log marginal likelihoods of − 20,760.32 suggesting the existence of six clusters (Plomin-Cres-Krk, Lukovo-Rab, Jablanac-Pag-Baške Oštarije, Paklenica, Zrmanja, Roški Slap-Drniš) with the admixture present in the overlapping region of the two subspecies (Alan, Karlobag; Fig. 4d).

Environmental niche analyses

The first two PCA-env axes cumulatively explained 77.3% of the environmental variation in the data. PC1 accounted for 41.5% of the variation and was mainly influenced by temperature variables (bio4, bio7 and bio8) and also had a negative relationship with bio19. PC2 which accounted for 35.8% of the variation primarily reflected moisture availability gradient (negative correlation with CMD and positive with bio14), followed by a negative relationship with bio9 and pH (Fig. 5a). The niche overlap in environmental space between the two *C. fenestrellata* subspecies was low following Rödder and Engler (2011) classification of niche overlap, with *D* value = 0.35 (Fig. 5d). The environmental niche of *C. fenestrellata* subsp. *istriaca* was a subset of the wider niche of *C. fenestrellata* subsp. *fenestrellata*. According to niche equivalency test, niches of the two taxa were significantly not equivalent ($P = 0.001$). However, niche similarity test showed that niches of the two taxa are more similar than random ($P = 0.02$), indicating niche conservatism (Fig. 5).

Discussion

Campanula fenestrellata s.l. clearly represents a highly supported lineage (ITS: 1 PP/99 BS; *trn*L–*trn*F: 1 PP/91 BS; Fig. 2) within the *C. garganica* group. However, the DNA sequence data are inconclusive regarding the relationships of *C. fenestrellata* s.l. with other members of the group which is in line with previous phylogenetic studies (Park

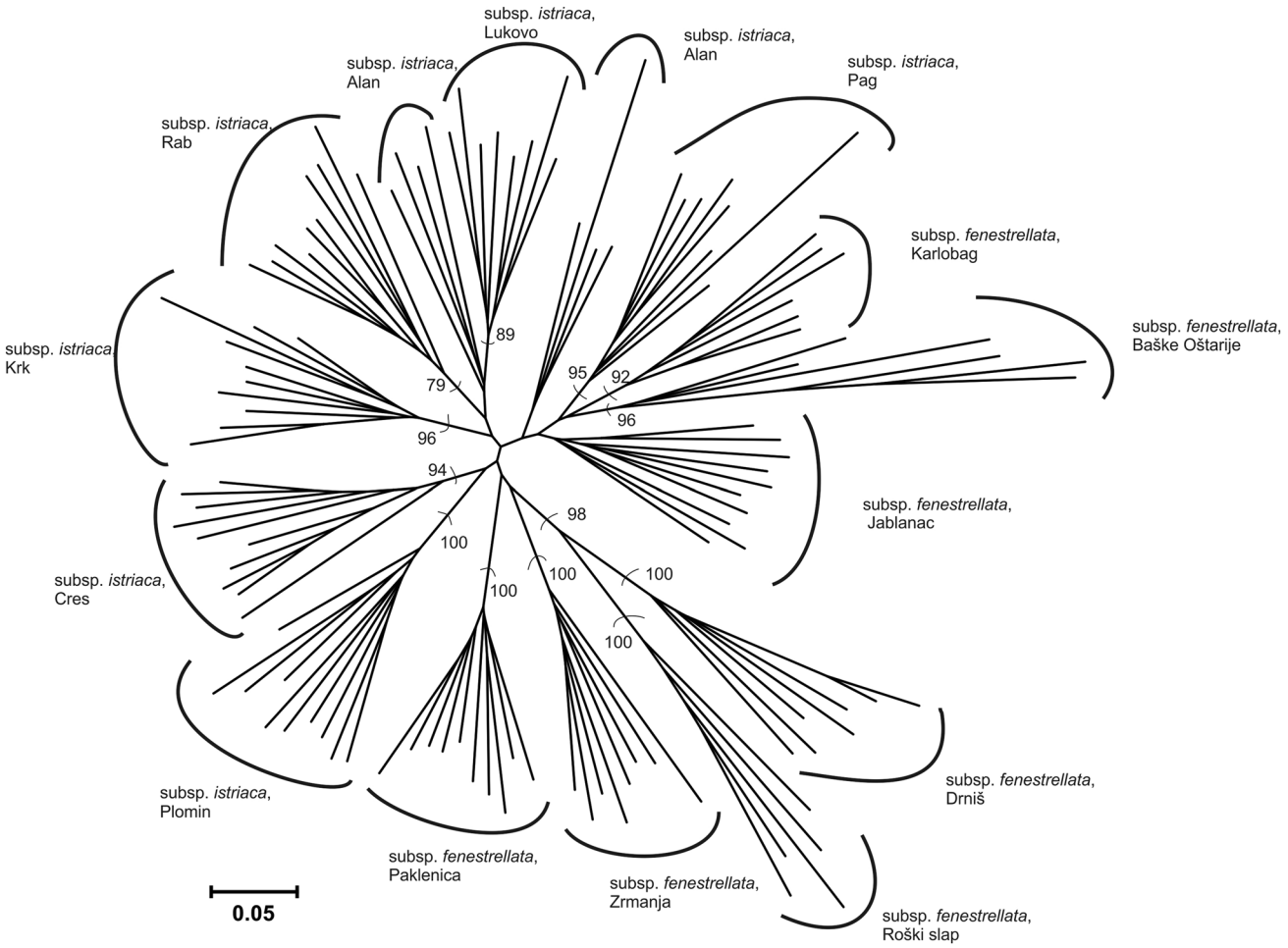

Fig. 3 Neighbour-joining tree based on the AFLP data of *Campanula fenestrellata* s.l. populations inferred from Dice genetic distances between individuals. Bootstrap values (BS) > 50% are indicated for major branches

et al. 2006; Frajman and Schneeweiss 2009; Bogdanović et al. 2014a, b, 2015, 2019). Moreover, neither cpDNA nor ITS data provide any support for separation of respective subspecies, inferring only some geographically based groupings (populations from Drniš and Roški slap with 0.77 PP/54 BS in ITS data set, populations from Jablanac, Karlobag and Baške Oštarije with 0.98 PP/56 BS and Plomin and Cres with 0.96 PP/65 BS in plastid data set; Fig. 2).

Results of our AFLP based genetic analyses also revealed that genetic structure within *C. fenestrellata* s.l. does not reflect taxonomic treatment and separation into two subspecies, but rather indicates a more complex structure possibly driven by ecological factors (Figs. 3, 4). The majority of analysed populations are separated with high bootstrap support; the northern and the southernmost populations receiving maximal support, while the populations in the centre of distribution having lower support, Jablanac population receiving no support and Alan population separated into two clades (Fig. 3). Interestingly, the Jablanac and Alan populations belonging to separate subspecies, *C. fenestrellata* subsp. *fenestrellata* and *C. fenestrellata* subsp. *istriaca*, respectively, exhibited the lowest ϕ_{ST} value, while the populations Paklenica and Roški slap, both belonging to *C. fenestrellata* subsp. *fenestrellata,* had the highest ϕ_{ST} value (Online Resource 4). However, the geographic distance of the former population pair is smaller than the latter population pair (Fig. 1). Both the STRUCTURE and BAPS analyses of AFLP data revealed the high number of most likely clusters, $K = 8$ and $K = 6$, respectively, with representative populations of each identified gene pool located in proximity of each other (Fig. 4c, d; Online Resource 5). The unique and least admixed clusters are found within northern and southern populations while the central ones, which are located within the sympatric area of the two subspecies, are the most admixed (Figs. 1, 4c, d). Moreover, the morphologically based taxonomical separation into two subspecies is not evident at $K = 2$ either which separated southern populations into one gene pool, the central ones in a separate gene

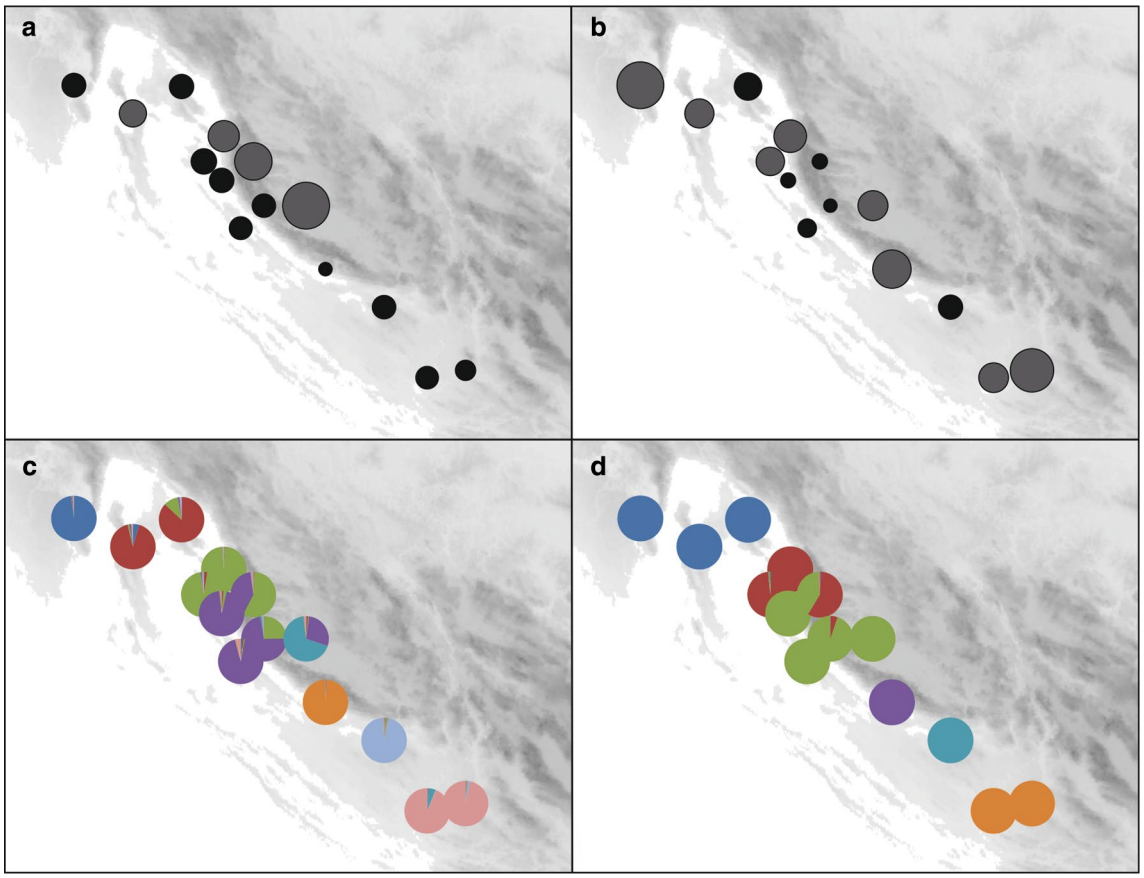

Fig. 4 AFLP variation of *Campanula fenestrellata* s.l. populations. **a** Shannon's information index (I). **b** Frequency down-weighted marker values (DW). In **a** and **b**, the size of the circles is proportional to the depicted values (dark grey colour represents values above average across populations and black represents values below average). **c** Bayesian analysis of the population structure using the software STRUCTURE assuming $K=8$. **d** Bayesian multilocus assignment method using the software BAPS assuming $K=6$. In **c** and **d**, the proportions of the ancestry of each population in each of the defined gene pools are colour-coded

pool and the northern populations exhibiting highly admixed structure (Online Resource 5).

The unique gene pools occurring in the northern part of *C. fenestrellata* distribution correspond to *C. fenestrellata* subsp. *istriaca* populations. Plomin population, located on the mainland in Istria, today is connected with the rest of the *C. fenestrellata* s.l. populations only through the populations on the islands of Cres and Krk (Fig. 1). The Plomin population also has a high number of private bands and the highest DW frequency (Table 1), which is indicative of a longer and isolated in situ persistence (Schönswetter and Tribsch 2005; Grdiša et al. 2014; Olšavská et al. 2016). Similar pattern is visible among the southern populations corresponding to *C. fenestrellata* subsp. *fenestrellata* that are situated on the mainland along the deep karst canyons: Paklenica, Zrmanja and Krka (Roški slap and Drniš). The population from Paklenica canyon and two populations situated along the Krka river system formed two unique gene pools and are also characterized with the high number of private bands and the high DW frequency (Table 1). The distinctiveness of these populations is likely fostered by specific locations situated in deep karst canyons which limit the seed dispersal possibilities and hence reduce the gene flow towards other populations. The canyons in Dinaric karst were shown to limit gene flow in e.g. *Campanula secundiflora* s.l. (Janković et al. 2016), *Tanacetum cinerariifolium* (Trevir.) Sch. Bip. (Grdiša et al. 2014) and *Fraxinus angustifolia* Vahl. (Temunović et al. 2012). Surprisingly, the Zrmanja population did not exhibit high DW values as populations in Krka valley and Paklenica population, although it formed a unique cluster in both STRUCTURE and BAPS analyses (Fig. 4c, d).

The central distribution area of *C. fenestrellata* s.l. is most numerous in populations, and throughout the coastal and north Velebit area between Alan and Baške Oštarije, overlapping and mixed populations of the two subspecies occur (Fig. 1, personal observations I. Rešetnik and S. Bogdanović). These populations show quite admixed pattern comprising larger fractions of different gene pools (pops. Alan, Karlobag, Baške Oštarije) revealed both by

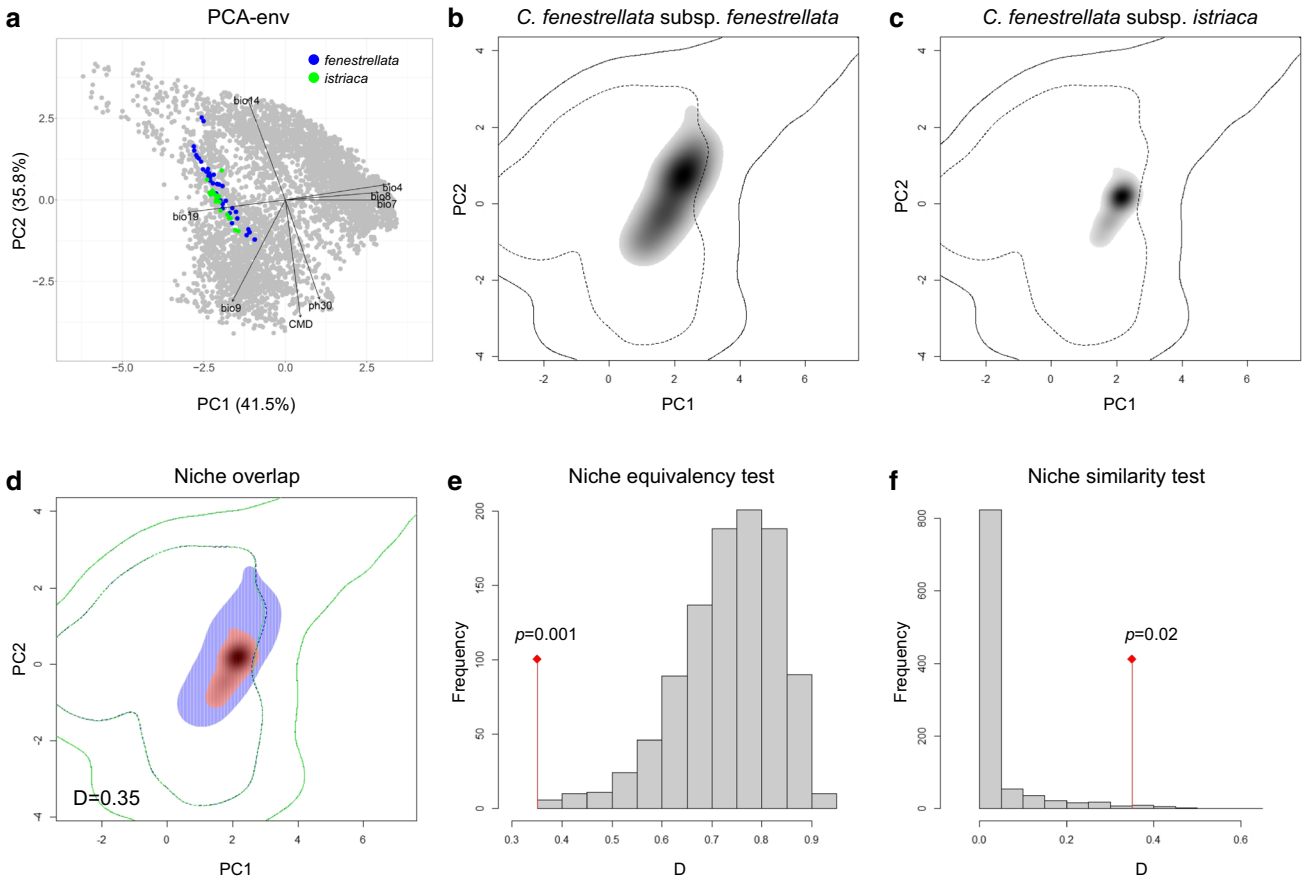

Fig. 5 Environmental niche analyses results. **a** Environmental principal component analysis (PCA-env) with the contribution of the environmental variables on the first two PC axes. **b** Niche space of *Campanula fenestrellata* subsp. *fenestrellata*. **c** Niche space of *Campanula fenestrellata* subsp. *istriaca*. Grey shading shows the density of the occurrence of each subspecies. **d** Niche overlap between *C. fenestrellata* subsp. *fenestrellata* and *C. fenestrellata* subsp. *istriaca* as measured with Schoener's *D*. In **b**–**d** solid and dashed contour lines depict 100% and 50% of the available environmental space, respectively. **e** Niche equivalency test. **f** Niche similarity test. In both tests, observed niche overlap *D* value (red vertical line) is compared against simulated null distribution using 1000 replicates (grey bars). *p*—significance of the tests

STRUCTURE and BAPS analyses of AFLP data (Fig. 4c, d). The existence of introgressive forms and hybrids between the two subspecies in this area was postulated by Degen (1938) and Damboldt (1965); however, based on morphological diagnostic characters (glabrous leaves in *C. fenestrellata* subsp. *fenestrellata* vs. densely tomentose leaves in *C. fenestrellata* subsp. *istriaca*), the two subspecies are easily recognizable. Admixed pattern is indicative of more extensive gene flow that is also collaborated with the highest levels of gene diversity found in these populations (Fig. 4a). However, these observed patterns are mostly accompanied by low levels of private bands and DW (Table 1, Fig. 4b). The specific structure of these populations could be a consequence of the admixture of divergent lineages through secondary contact after postglacial colonization (Petit et al. 2003; Bardy et al. 2010).

Such high genetic divergence occurring in relatively small geographic area in the north-eastern Adriatic is indicative of several temporally continuous glacial microrefugia during Pleistocene glacials. The majority of recent studies on herbaceous taxa distributed along the eastern Adriatic coast suggested only two to three most likely genetic clusters (e.g. Surina et al. 2011; Mereďa et al. 2011; Grdiša et al. 2014; Rešetnik et al. 2016a) while for *C. fenestrellata* s.l. six and eight clusters were designated (Fig. 4). Moreover, the comparison of genetic diversity parameters such as Shannon's information index (*I*) with other Balkan species revealed higher values obtained for *C. fenestrellata* s.l. (Grdiša et al. 2014, 2019). Genetic divergence of the northernmost (Plomin, Cres, Krk) and southernmost (Paklenica, Zrmanja, Roški slap, Drniš) populations is likely caused by both geographic isolation preventing contemporary gene flow and long-term in situ persistence. This is consistent with the

view of two separated, northern and more southern, local glacial refugia along eastern Adriatic coast (Médail and Diadema 2009). However, the observed high DW values in some central populations (Rab, Lukovo and Baške Oštarije) and high number of private bands (Baške Oštarije) are possible indications of some central populations also surviving in situ by tracking suitable microhabitats during the cold period (Schönswetter and Tribsch 2005; Grdiša et al. 2014). It is likely that these populations were more successful in expanding their areal and mixing through secondary postglacial contact than the northern populations, which were left separated by postglacially formed sea, or the southern populations trapped within the deep karst canyons.

Although the morphological distinction of *C. fenestrellata* subsp. *istriaca* and *C. fenestrellata* subsp. *fenestrellata* was not supported with genetic evidence, the two taxa show differences in their ecological preferences. Despite the fact that the niche of *C. fenestrellata* subsp. *istriaca* was only a subset of the wider environmental niche of *C. fenestrellata* subsp. *fenestrellata*, the observed niche overlap between the two taxa was quite low (0.35 as measured with *D* metrics) and the niche equivalency test confirmed that niches of the two subspecies are not identical (Fig. 5). These results indicate significant ecological differentiation between the two taxa and some unique niche features of each subspecies. For example, *C. fenestrellata* subsp. *istriaca* occupies narrower environmental space occurring in warmer and drier sites compared to *C. fenestrellata* subsp. *fenestrellata* which has a broader niche, tolerating wider temperature range and generally occurring in colder and more humid sites. It should be noted that the observed environmental niche differences may simply be driven by different environmental conditions available in the distribution range of each subspecies. However, this is not likely, because both niche tests were performed in the common available background environment, encompassing the whole known distribution area of the ser. *Garganicae*. Nevertheless, niche similarity test did not provide evidence for significant niche divergence, but has rather supported niche conservatism for the two subspecies because the observed niche overlap was still higher than expected by chance. This suggests common ancestral ecological traits of the two subspecies, which is in line with the expectations for niches of closely related taxa (Wiens and Graham 2005; Wiens et al. 2010; Pyron et al. 2015).

Substantial morphological and/or ecological distinction not supported by genetic differentiation has been observed for other closely related plant species suggesting recent divergence and short-term evolutionary events rather than long-term divergence which could have been detected in phylogeny (Peterson 2011; Kreuzer et al. 2014; Albarrán-Lara et al. 2019). Therefore, even though the two subspecies are lacking genetic diversification, based on the observed unique ecological niche features and the clear and simple morphological discrimination, we favour the taxonomic recognition of two subspecies. Such a treatment would be consistent with the treatment in the Flora Croatica Database (Nikolić 2019) and with the available morphological evidence from our previous studies of *Campanula* ser. *Garganicae* (Bogdanović et al. 2014a, b, 2015, 2019).

Overall, our phylogeographic reconstruction suggests distinct fragmentation patterns during the climatic oscillations that promoted genetic differentiation within the species range. However, the highest levels of genetic diversity were found towards the centre of the species' range due to high admixture among previously allopatric lineages supporting the view that the areas hosting populations with the highest levels of genetic diversity do not strictly overlap with areas of long-term stability (i.e. refugia; Petit et al. 2003). Therefore, the data presented here highlight the importance of microrefugia in shaping the intraspecific genetic variation as well as of melting-pot areas in rearranging such variation as a result of a more recent admixture (Petit et al. 2003).

Acknowledgements This study was partially financed by the HAZU project "Genetic diversity of Croatian endemic *Campanula*".

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Information on Electronic Supplementary Material

Online Resource 1. Checklist of taxa, collection details, voucher information and GenBank accession numbers of *Campanula* species analysed in this study.
Online Resource 2. ITS and *trn*L-*trn*F alignments.
Online Resource 3. Characteristics of the *trn*L-*trn*F and ITS data sets analysed using maximum parsimony (MP) and Bayesian inference (BI).
Online Resource 4. The AFLP pairwise population F_{st} for fourteen populations of *C. fenestrellata* s.l.
Online Resource 5. a The most probable value of Evanno's delta K for 116 individuals of the fourteen populations of *Campanula fenestrellata* s.l., **b** the Bayesian analysis of the population structure using the software STRUCTURE assuming K=2, the proportions of the ancestry of each population in each of the defined gene pools are color-coded.

References

Albarrán-Lara AL, Petit RJ, Kremer A, Caron H, Peñaloza-Ramírez JM, Gugger PF, Dávila-Aranda PD, Oyama K (2019) Low genetic differentiation between two morphologically and ecologically distinct giant-leaved Mexican oaks. Pl Syst Evol 305:89–101. https://doi.org/10.1007/s00606-018-1554-8

Aleksić JM, Geburek T (2010) Mitochondrial DNA reveals complex genetic structuring in a stenoendemic conifer *Picea omorika* [(Panč.) Purk.] caused by its long persistence within the refugial

Balkan region. Pl Syst Evol 285:1–11. https://doi.org/10.1007/s00606-009-0250-0
Aleksić JM, Škondrić S, Lakušić D (2018) Comparative phylogeography of capitulate *Campanula* species from the Balkans, with description of a new species, *C. daucoides*. Pl Syst Evol 304:549–575. https://doi.org/10.1007/s00606-018-1490-7
Avise JC, Ball RM (1990) Principles of genealogical concordance in species concepts and biological taxonomy. In: Futuyma DJ, Antonovics J (eds) Oxford surveys in evolutionary biology. Oxford University Press, New York, pp 45–68
Bardy KE, Albach DC, Schneeweiss GM, Fischer MA, Schönswetter P (2010) Disentangling phylogeography, polyploid evolution and taxonomy of a woodland herb (*Veronica chamaedrys* group, Plantaginaceae s.l.) in southeastern Europe. Molec Phylogen Evol 57:771–786. https://doi.org/10.1016/j.ympev.2010.06.025
Bardy KE, Schönswetter P, Schneeweiss GM, Fischer MA, Albach DC (2011) Extensive gene flow blurs species boundaries among *Veronica barrelieri*, *V. orchidea* and *V. spicata* (Plantaginaceae) in southeastern Europe. Taxon 60:108–121
Bogdanović S, Brullo S, Rešetnik I, Lakušić D, Satovic Z, Liber Z (2014a) *Campanula skanderbegii*: molecular and morphological evidence of a new *Campanula* species (Campanulaceae) endemic to Albania. Syst Bot 39:1250–1260. https://doi.org/10.1600/036364414x682571
Bogdanović S, Brullo S, Rešetnik I, Šatović Z, Liber Z (2014b) *Campanula teutana*, a new isophyllous *Campanula* (Campanulaceae) from the Adriatic region. Phytotaxa 162:1–17. https://doi.org/10.11646/phytotaxa.162.1.1
Bogdanović S, Rešetnik I, Brullo S, Shuka L (2015) *Campanula aureliana* (Campanulaceae), a new species from Albania. Pl Syst Evol 301:1555–1567. https://doi.org/10.1007/s00606-014-1171-0
Bogdanović S, Rešetnik I, Jeričević M, Jeričević N, Brullo S (2019) Molecular and morphological survey on *Campanula cremnophila* (Campanulaceae), a new isophyllous species from Croatia. Pl Syst Evol 305:687–703. https://doi.org/10.1007/s00606-019-01599-x
Broennimann O, Fitzpatrick MC, Pearman PB, Petitpierre B, Pellissier L, Yoccoz NG, Thuiller W, Fortin M, Randin C, Zimmermann NE, Graham CH, Guisan A (2012) Measuring ecological niche overlap from occurrence and spatial environmental data. Global Ecol Biogeogr 21:481–497. https://doi.org/10.1111/j.1466-8238.2011.00698.x
Caković D, Stešević D, Schönswetter P, Frajman B (2015) How many taxa? Spatiotemporal evolution and taxonomy of *Amphoricarpos* (Asteraceae, Carduoideae) on the Balkan Peninsula. Organisms Diversity Distrib 15:429–445. https://doi.org/10.1007/s13127-015-0218-6
Carović-Stanko K, Liber Z, Politeo O, Strikić F, Kolak I, Milos M, Satovic Z (2011) Molecular and chemical characterization of the most widespread *Ocimum* species. Pl Syst Evol 294:253–262. https://doi.org/10.1007/s00606-011-0471-x
Corander J, Marttinen P (2006) Bayesian identification of admixture events using multi-locus molecular markers. Molec Ecol 15:2833–2843. https://doi.org/10.1111/j.1365-294X.2006.02994.x
Corander J, Waldmann P, Sillanpaa MJ (2003) Bayesian analysis of genetic differentiation between populations. Genetics 163:367–374
Damboldt J (1965) Zytotaxonomische revision der isophyllen Campanulae in Europa. Bot Jahrb Syst 84:302–358
Degen A (1938) Flora Velebitica III. kötet. A Magyar Tudományos Akadémia Kiadása, Budapest
Di Cola V, Broennimann O, Petitpierre B, Breiner FT, Damen M, Randin C, Engler R, Pottier J, Pio D, Dubuis A, Pellissier L (2017) ecospat: an R package to support spatial analyses and modeling of species niches and distributions. Ecography 40:774–787. https://doi.org/10.1111/ecog.02671
Dice LR (1945) Measures of the amount of ecologic association between species. Ecology 26:297–302
Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A (2011) Geneious v5.4. Available at: http://www.geneious.com/
Earl DA, von Holdt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservatio Genet Resources 4:359–361. https://doi.org/10.1007/s12686-011-9548-7
Ehrich D (2006) AFLPdat: a collection of R functions for convenient handling of AFLP data. Molec Ecol Notes 6:603–604. https://doi.org/10.1111/j.1471-8286.2006.01380.x
Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molec Ecol 14:2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x
Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molec Ecol Resources 10:564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x
Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitocondrial DNA restriction sites. Genetics 131:479–491
Felsenstein J (1985) Confidence-limits on phylogenies—an approach using the bootstrap. Evolution 39:783–791. https://doi.org/10.2307/2408678
Frajman B, Schneeweiss GM (2009) A campanulaceous fate: the Albanian stenoendemic *Asyneuma comosiforme* in fact belongs to isophyllous *Campanula*. Syst Bot 34:595–601. https://doi.org/10.1600/036364409789271173
Frajman B, Záveská E, Gamisch A, Moser T, Schönswetter P, STEPPE Consortium (2019) Integrating phylogenomics, phylogenetics, morphometrics, relative genome size and ecological niche modelling disentangles the diversification of Eurasian *Euphorbia seguieriana* sl (Euphorbiaceae). Molec Phylogen Evol 134:238–252. https://doi.org/10.1016/j.ympev.2018.10.046
Grdiša M, Liber Z, Radosavljević I, Carović-Stanko K, Kolak I, Satovic Z (2014) Genetic diversity and structure of Dalmatian pyrethrum (*Tanacetum cinerariifolium* Trevir./Sch./Bip., Asteraceae) within the Balkan Refugium. PLoS ONE 9:e105265. https://doi.org/10.1371/journal.pone.0105265
Grdiša M, Radosavljević I, Liber Z, Stefkov G, Ralli P, Chatzopoulou PS, Carović-Stanko K, Šatović Z (2019) Divergent selection and genetic structure of Sideritis scardica populations from southern Balkan Peninsula as revealed by AFLP fingerprinting. Sci Rep 9:12767. https://doi.org/10.1038/s41598-019-49097-x
Hamilton CW, Reichard SH (1992) Current practice in the use of subspecies, variety, and forma in the classification of wild plants. Taxon 41:485–498. https://doi.org/10.2307/1222819
Hammer Ø, Harper DAT, Ryan PD (2001) PAST: paleontological statistics software package for education and data analysis. Palaeontol Electronica 4:9
Herrmann D, Poncet BN, Manel S, Rioux D, Gielly L, Taberlet P, Gugerli F (2010) Selection criteria for scoring amplified fragment length polymorphisms (AFLPs) positively affect the reliability of population genetic parameter estimates. Genome 53:302–310. https://doi.org/10.1139/g10-006
Janković I, Šatović Z, Liber Z, Kuzmanović N, Radosavljević I, Lakušić D (2016) Genetic diversity and morphological variability in the Balkan endemic *Campanula secundiflora* s.l. (Campanulaceae). Bot J Linn Soc 180:64–88. https://doi.org/10.1111/boj.12359
Janković I, Satovic Z, Liber Z, Kuzmanović N, Di Pietro R, Radosavljević I, Nikolov Z, Lakušić D (2019) Genetic and morphological data reveal new insights into the taxonomy of

Campanula versicolor s.l. (Campanulaceae). Taxon 68:340–369. https://doi.org/10.1002/tax.12050

Kolář F, Fuxová G, Záveská E, Nagano AJ, Hyklová L, Lučanová M, Kudoh H, Marhold K (2016) Northern glacial refugia and altitudinal niche divergence shape genome-wide differentiation in the emerging plant model *Arabidopsis arenosa*. Molec Ecol 25:3929–3949. https://doi.org/10.1111/mec.13721

Kreuzer M, Tribsch A, Nyffeler R (2014) Ecological and genetic differentiation of two subspecies of *Saussurea alpina* in the Western Alps. Alpine Bot 124:49–58. https://doi.org/10.1007/s00035-014-0128-9

Kutnjak D, Schönswetter P, Dullinger S, Kuttner M, Niketić M, Frajman B (2014) Escaping to the summits: phylogeography and predicted range dynamics of *Cerastium dinaricum*, an endangered high mountain plant endemic to the western Balkan Peninsula. Molec Phylogen Evol 78:365–374. https://doi.org/10.1016/j.ympev.2014.05.015

Lakušić D, Liber Z, Nikolić T, Surina B, Kovačić S, Bogdanović S, Stefanović S (2013) Molecular phylogeny of the *Campanula pyramidalis* species complex (Campanulaceae) inferred from chloroplast and nuclear non-coding sequences and its taxonomic implications. Taxon 62:505–524. https://doi.org/10.12705/623.1

Lewontin RC (1972) The apportionment of human diversity. Evol Biol 6:381–398

Liber Z, Kovačić S, Nikolić T, Likić S, Rusak G (2008) Relations between western Balkan endemic *Campanula* L. (Campanulaceae) lineages: evidence from chloroplast DNA. Pl Biosystems 142:40–50. https://doi.org/10.1080/11263500701872283

Losos JB (2008) Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. Ecol Lett 11:995–1007. https://doi.org/10.1111/j.1461-0248.2008.01229

Magri D, Vendramin GG, Comps B, Dupanloup I, Geburek T, Gömöry D, Latalowa M, Litt T, Paule L, Roure JM, Tantau I, Van Der Knaap WO, Petit RJ, De Beaulieu J (2006) A new scenario for the Quaternary history of European beech populations: palaeobotanical evidence and genetic consequences. New Phytol 171:199–221. https://doi.org/10.1111/j.1469-8137.2006.01740.x

McCormack JE, Zellmer AJ, Knowles LL (2010) Does niche divergence accompany allopatric divergence in *Aphelocoma* jays as predicted under ecological speciation? Insights from tests with niche models. Evolution 64:1231–1244. https://doi.org/10.1111/j.1558-5646.2009.00900.x

Médail F, Diadema K (2009) Glacial refugia influence plant diversity patterns in the Mediterranean Basin. J Biogeogr 36:1333–1345. https://doi.org/10.1111/j.1365-2699.2008.02051.x

Mereďa P, Hodálová I, Kučera J, Zozomová-Lihová J, Letz DR, Slovák M (2011) Genetic and morphological variation in *Viola suavis* s.l. (Violaceae) in the western Balkan Peninsula: two endemic subspecies revealed. Syst Biodivers 9:211–231. https://doi.org/10.1080/14772000.2011.603903

Nikolić T (ed) (2019) Flora Croatica database, University of Zagreb, Faculty of Science. Availble at: http://hirc.botanic.hr/fcd. Accessed 10 Sep 2019

Nylander JAA (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University. Available at: www.abc.se/~nylander/

Olšavská K, Slovák M, Marhold K, Štubňová E, Kučera J (2016) On the origins of Balkan endemics: the complex evolutionary history of the *Cyanus napulifer* group (Asteraceae). Ann Bot (Oxford) 118:1071–1088. https://doi.org/10.1093/aob/mcw142

Park J-M, Kovačić S, Liber Z, Eddie WM, Schneeweiss GM (2006) Phylogeny and biogeography of isophyllous species of *Campanula* (Campanulaceae) in the Mediterranean area. Syst Bot 31:862–880. https://doi.org/10.1600/036364406779695924

Peterson AT (2011) Ecological niche conservatism: a time-structured review of evidence. J Biogeogr 38:817–827. https://doi.org/10.1111/j.1365-2699.2010.02456.x

Petit R, Aguinagalde I, de Beaulieu JL, Bittkau C, Brewer S, Cheddadi R, Ennos R, Fineschi S, Grivet D, Lascoux M, Mohanty A, Müller-Starck G, Demesure-Musch B, Palmé A, Martín JP, Rendell S, Vendramin GG (2003) Glacial refugia: hotspots but not melting pots of genetic diversity. Science 300:1563–1565. https://doi.org/10.1126/science.1083264

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Pyron RA, Costa GC, Patten MA, Burbrink FT (2015) Phylogenetic niche conservatism and the evolutionary basis of ecological speciation. Biol Rev 90:1248–1262. https://doi.org/10.1111/brv.12154

Rambaut A, Drummond AJ (2007) Tracer v1.4. Available at: http://beast.bio.ed.ac.uk/tracer

Rešetnik I, Baričevič D, Batîr Rusu D, Carović-Stanko K, Chatzopoulou P, Dajić-Stevanović Z, Goncearuc M, Grdiša M, Gregurаš D, Ibraliu A, Jug-Dujaković M, Krasniqi E, Liber Z, Murtić S, Pećanac D, Radosavljević I, Stefkov G, Stešević D, Šoštarić I, Šatović Z (2016a) Genetic diversity and demographic history of wild and cultivated/naturalised plant populations: evidence from Dalmatian sage (*Salvia officinalis* L., Lamiaceae). PLoS ONE 11:e0159545. https://doi.org/10.1371/journal.pone.0159545

Rešetnik I, Frajman B, Schönswetter P (2016b) Heteroploid *Knautia drymeia* (Caprifoliaceae) includes *K. gussonei* and cannot be separated into diagnosable subspecies. Amer J Bot 103:21300–21313. https://doi.org/10.3732/ajb.1500506

Rödder D, Engler JO (2011) Quantitative metrics of overlaps in Grinnellian niches: advances and possible drawbacks. Global Ecol Biogeogr 20:915–927. https://doi.org/10.1111/j.1466-8238.2011.00659.x

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542. https://doi.org/10.1093/sysbio/sys029

Schoener TW (1968) Anolis lizards of Bimini: resource partitioning in a complex fauna. Ecology 49:704–726

Schönswetter P, Tribsch A (2005) Vicariance and dispersal in the alpine perennial *Bupleurum stellatum* L. (Apiaceae). Taxon 54:725–732. https://doi.org/10.2307/25065429

Shannon CE, Weaver W (1949) The mathematical theory of communication. University of Illinois Press, Urbana

Slade D, Škvorc Ž, Ballian D, Gračan J, Papeš D (2008) The chloroplast DNA polymorphisms of White Oaks of section *Quercus* in the Central Balkans. Silvae Genet 57:227–234. https://doi.org/10.1515/sg-2008-0035

Slovák M, Kučera J, Turis P, Zozomová-Lihová J (2012) Multiple glacial refugia and postglacial colonization routes inferred for a woodland geophyte, *Cyclamen purpurascens*: Patterns concordant with the Pleistocene history of broadleaved and coniferous tree species. Biol J Linn Soc 105:741–760

Španiel S, Marhold K, Zozomová-Lihová J (2017) The polyploid *Alyssum montanum-A. repens* complex in the Balkans: a hotspot of species and genetic diversity. Pl Syst Evol 303:1443–1465. https://doi.org/10.1007/s00606-017-1470-3

Sun Y, Skinner DZ, Liang GH, Hulbert SH (1994) Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. Theor Appl Genet 89:26–32. https://doi.org/10.1007/bf00226978

Surina B, Schönswetter P, Schneeweiss GM (2011) Quaternary range dynamics of ecologically divergent species (*Edraianthus serpyllifolius* and *E. tenuifolius*, Campanulaceae) within the Balkan refugium. J Biogeogr 38:1381–1393. https://doi.org/10.1111/j.1365-2699.2011.02493.x

Surina B, Schneeweiss GM, Glasnović P, Schönswetter P (2014) Testing the efficiency of nested barriers to dispersal in the Mediterranean high mountain plant *Edraianthus graminifolius* (Campanulaceae). Molec Ecol 23:2861–2875. https://doi.org/10.1111/mec.12779

Swofford DL (2003) PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Pl Mol Biol 17:1105–1109. https://doi.org/10.1007/bf00037152

Temunović M, Franjić J, Satovic Z, Grgurev M, Frascaria-Lacoste N, Fernández-Manjarrés JF (2012) Environmental heterogeneity explains the genetic structure of Continental and Mediterranean populations of *Fraxinus angustifolia* Vahl. PLoS ONE 7:e42764. https://doi.org/10.1371/journal.pone.0042764

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M (1995) AFLP: a new technique for DNA fingerprinting. Nucl Acids Res 23:4407–4414. https://doi.org/10.1093/nar/23.21.4407

Warren DL, Glor RE, Turelly M (2008) Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. Evolution 62:2868–2883. https://doi.org/10.1111/j.1558-5646.2008.00482.x

Wiens JJ, Graham CH (2005) Niche conservatism: integrating evolution, ecology, and conservation biology. Annual Rev Ecol Evol S 36:519–539. https://doi.org/10.1146/annurev.ecolsys.36.102803.095431

Wiens JJ, Ackerly DD, Allen AP, Anacker BL, Buckley LB, Cornell HV, Damschen EI, Davies TJ, Grytnes JA, Harrison SP, Hawkins BA (2010) Niche conservatism as an emerging principle in ecology and conservation biology. Ecol Lett 13:1310–1324. https://doi.org/10.1111/j.1461-0248.2010.01515.x