Historical and contemporary demography of *Salvia officinalis* (Lamiaceae) as revealed by microsatellite (SSR) markers

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Introduction

Figure 1. *Salvia officinalis* L.

With over 900 species distributed worldwide, the genus *Salvia* is by far the largest and most diverse genus in the Lamiaceae family. In Europe, 36 taxa are described and grouped into seven sections. The section *Salvia*, comprised of 13 species, includes *Salvia officinalis* (Dalmatian sage) (Fig. 1) as the type species. Moreover, due to high content and quality of its essential oils, Dalmatian sage is economically the most important species of the genus. As a typical member of indigenous flora, Dalmatian sage naturally grows along the eastern Adriatic coast and in the central and southern Apennines while some relict populations can be found in continental parts of the Balkan Peninsula. Despite the medicinal as well as historical and cultural importance, the knowledge on genetic diversity and population structure of Dalmatian sage is still very limited.

To perform a comprehensive population genetic research on natural populations from entire distribution area of Dalmatian sage, recently developed microsatellite markers were applied. The goals of this study were to investigate: (1) genetic diversity and population structure, (2) role of geography in structuring genetic variation and (3) demographic history of Dalmatian sage with regard to the paleoclimatic changes in the Mediterranean region.

Materials and methods

A total of 1424 specimens of Dalmatian sage were sampled from 62 locations across the Balkan and Apennine Peninsula (Fig. 2).

Leaf material of 20 to 25 individuals per population was collected and stored in silica gel. Genomic DNA was extracted from dried leaf tissue by using a GenElute Plant Genomic DNA Miniprep Kit (Sigma-Aldrich). Eight microsatellite primers were used for the analysis: SoUZ001, SoUZ002, SoUZ003, SoUZ007, SoUZ011, SoUZ013, SoUZ014, SoUZ019. GENEPOP 4.0 was used to estimate population genetic parameters (the average number of alleles per locus, N_a; the observed heterozygosity, H_O and the expected heterozygosity or gene diversity, H_E) and to test the population genotypic frequencies across all loci for their conformity to Hardy-Weinberg expectations (HWE). The allelic richness, N_{ar}, as the measure of the number of alleles per locus independent of sample size was calculated by FSTAT v. 2.9.3.2 programme package.

Pairwise Cavalli-Sforza and Edwards' chord distances were calculated and the cluster analysis was performed using Fitch-Margoliash algorithm.

A model-based clustering method was applied to infer genetic structure and define the number of clusters using the software STRUCTURE ver. 2.3.3.. Additional Bayesian cluster analyses were performed using TESS, v. 2.3.1. The procedure of TESS also includes a spatial component, such that genotypes from geographically closer locations are considered more likely to belong to the same cluster.

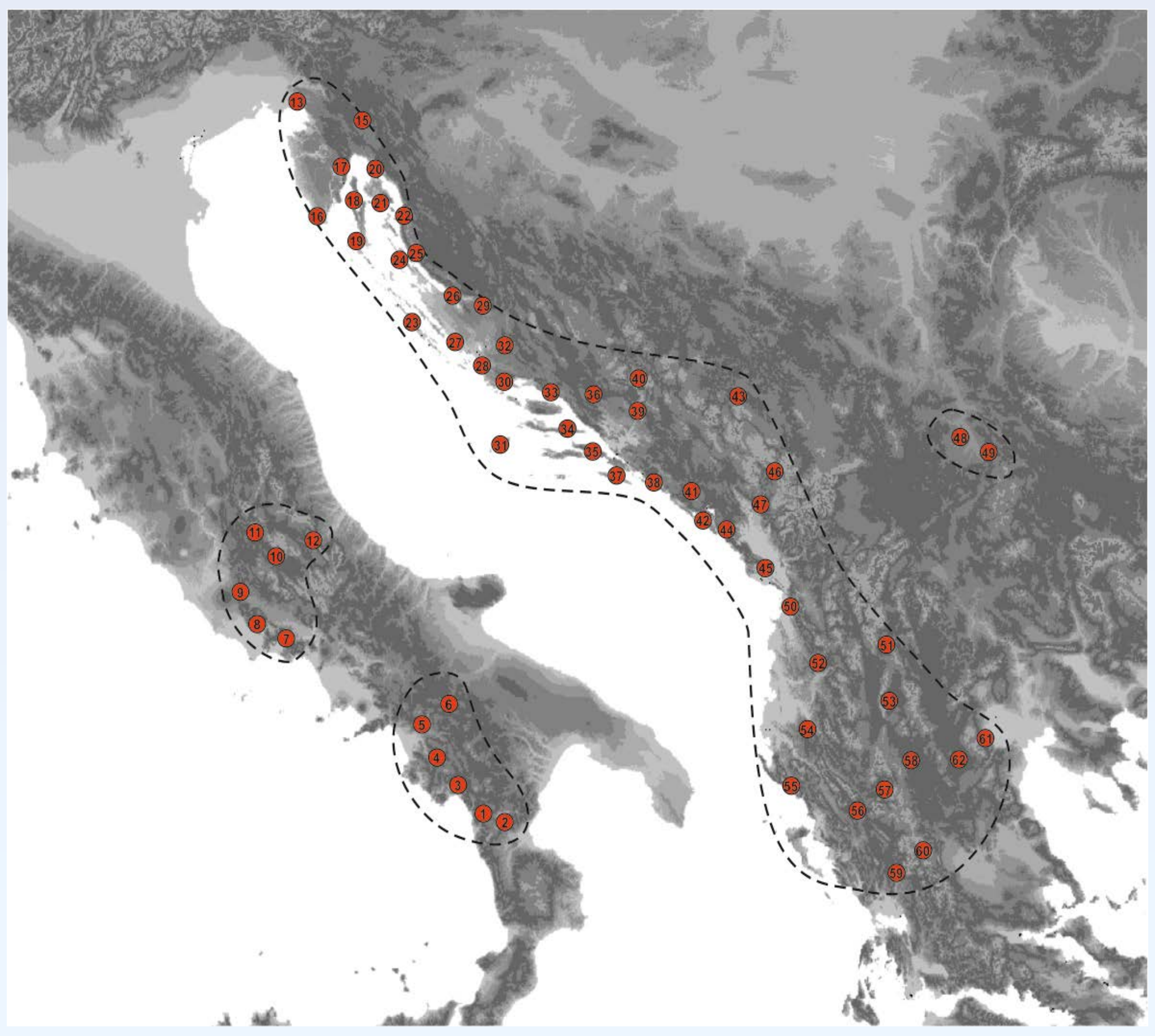

Figure 2. Location of the 62 populations of *S. officinalis* included in this study. The geographical distribution of the species is shown in dashed line.

Results

The allelic richness (N_{ar}) varied from 1.35 (P12) to 10.30 (P38). A total of 24 private alleles was observed in 17 populations. The highest number of private alleles was observed in populations P55 and P60. Observed and expected heterozygosity ranged from 0.08 (P12) to 0.83 (P44) and from 0.08 (P12) to 0.83 (P44), respectively. In general, the highest levels of allelic richness and expected heterozygosity were detected in populations from southern coastal region of the western Balkan Peninsula, while considerably lower levels were observed in Apennine populations, inland Balkan populations and populations from northern Adriatic coastal region. On the unrooted Fitch-Margoliash tree all populations grouped together in accordance with geographical position of the collecting sites (Fig. 3).

Using STRUCTURE, the highest ΔK was observed for K = 2 followed by that for K = 6 (Fig. 4). In TESS, DIC values reached a plateau at K = 6, in concordance with STRUCTURE analysis. The results of both analyses are shown in Fig. 5.

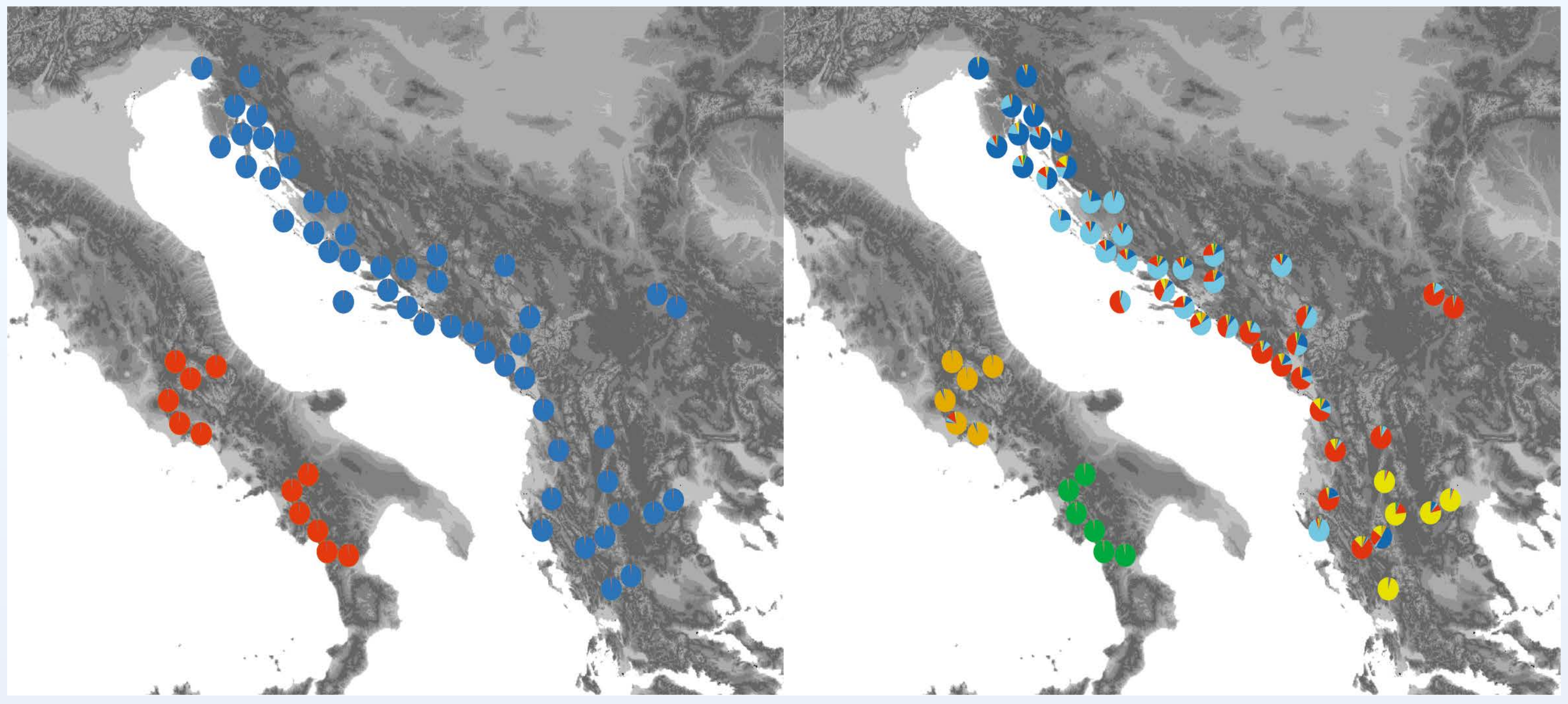

Figure 4. Best number of genetic clusters (K) obtained for *S. officinalis* using STRUCTURE (K = 2 and K = 6). Pie charts show averaged values of the different runs for the proportion of membership to each genetic cluster. Different colours indicate different genetic clusters.

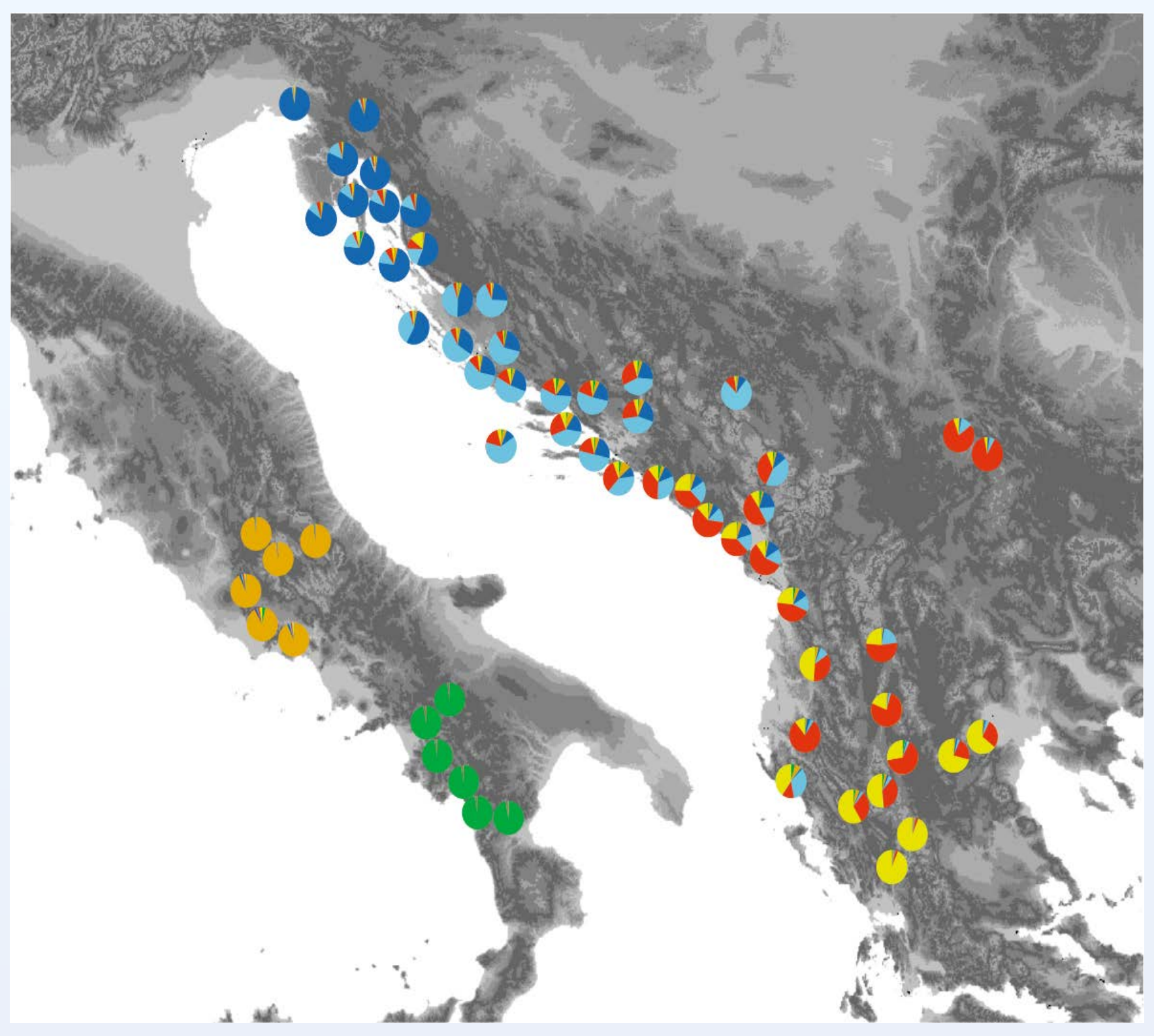

Figure 5. Genetic structure of *S. officinalis* populations, inferred with the Bayesian clustering algorithm implemented in TESS (K=6).

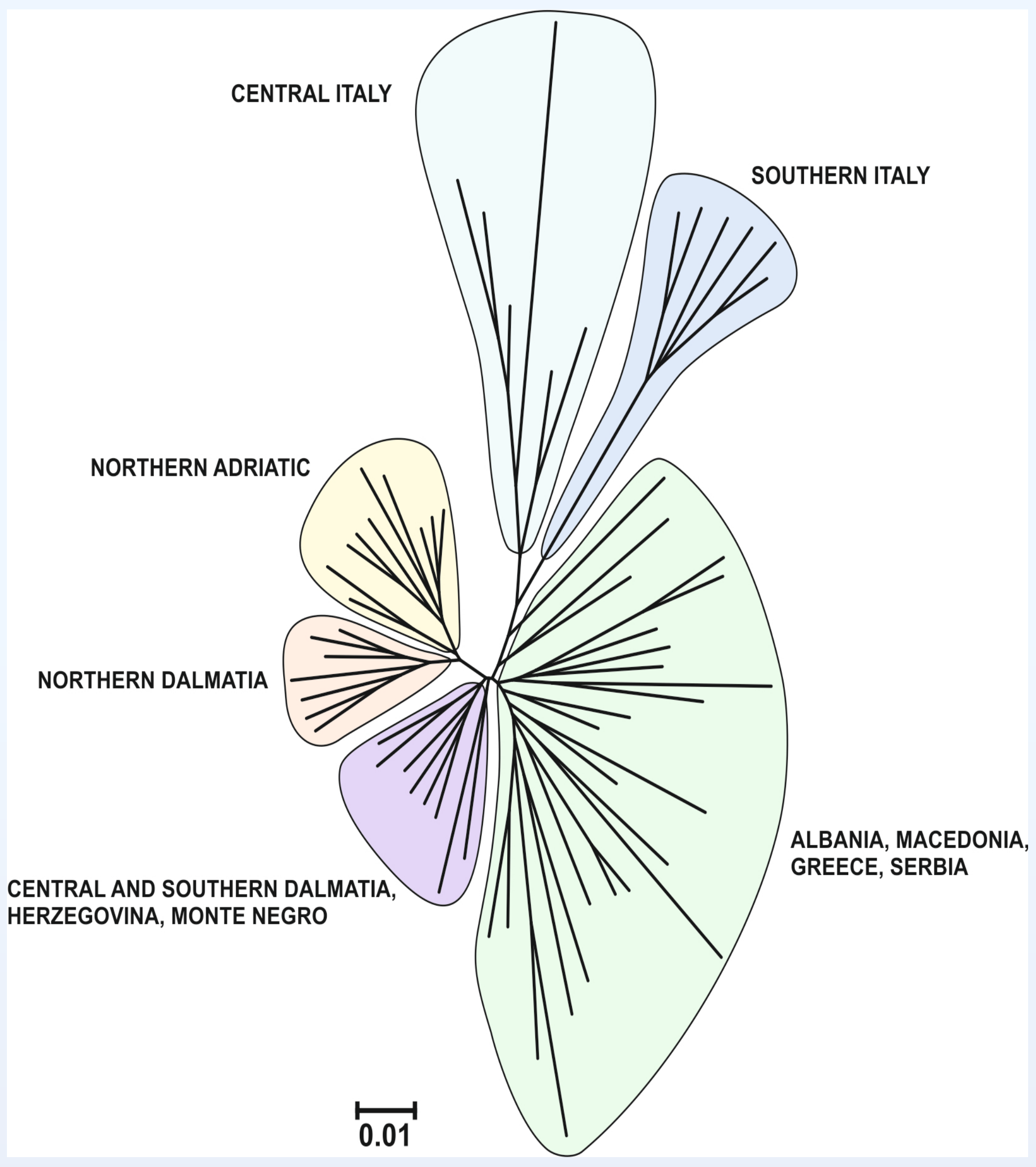

Figure 3. An unrooted Fitch-Margoliash tree based on Cavalli-Sforza's chord distances among 62 populations of *S. officinalis*.

Discussion

The analysis of 62 populations of Dalmatian sage using eight microsatellite markers revealed very high values of genetic diversity in populations from southern parts of western Balkan coastal region, while noticeable lower levels of genetic diversity were observed in populations from central parts of Balkan Peninsula, northern Adriatic coastal region and Apennine Peninsula. In addition, the highest number of private alleles was also detected in populations characterized by high levels of genetic variability suggesting their long lasting presence in studied locations.

The results suggest that the eastern Adriatic coastal region, from middle Dalmatia to Albania, could be considered as the refugium area during the Last Glacial Maximum (LGM). More recently, after the climate got warmer (i.e. during the last ~15 000 years) Dalmatian sage spread to the north and inland to the east (Fig. 6).

Consequently, uniform genetic structure and low levels of genetic variability can today be observed in populations from these regions. Moreover, populations from central part of distribution area are characterized not only by very high levels of genetic diversity and numerous private alleles, but also by very low population differentiation suggesting that this geographic region is the center of the origin of this species, at least in Balkan Peninsula.

Two groups of Apennine populations are highly differentiated not only from the populations from Balkan Peninsula, but also from each other. A plausible explanation for such finding is that the geographical isolation of Apennine populations dates long before the LGM (i.e. ~ 25 000 – 15 000 ybp), leaving a very long period of time at disposal for genetic differentiation. The exact moment when Apennine populations diverged from each other and from Balkan populations remains unknown, but it is possible that it dates as far as 5.3 Ma, at the end of the Messinian salinity crisis, when the Apennine and Balkan peninsulas were joined for the last time. Also, in populations from southern Apennines notable number of private alleles was found, suggesting that this area was an Apennine peninsula refuge during the LGM (Fig. 6).

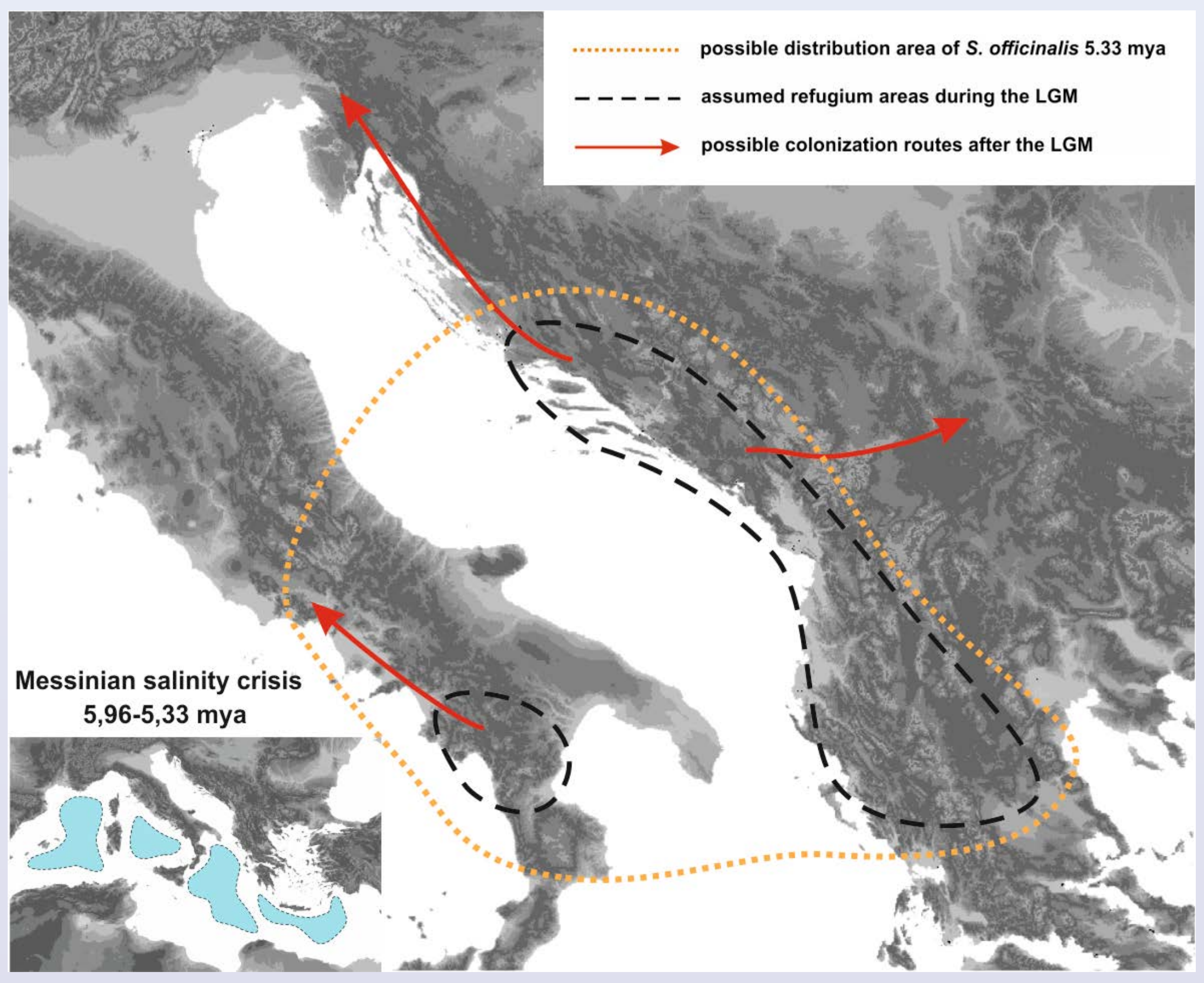

Figure 6. Possible historical demographic fluctuations of *S. officinalis* in Apennine and Balkan Peninsula. In the bottom left corner: assumed water bodies (in blue) in the Mediterranean basin during the Messinian salinity crisis.

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