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Three systems of molecular markers reveal genetic differences between varieties *sabina* and *balkanensis* in the *Juniperus sabina* L. range

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Abstract

Key message *J. sabina* var. *balkanensis,* that is of hybrid origin, and its maternal progenitor *J. sabina* var. *sabina* are genetically distinct with respect to cpDNA, SNP, and SilicoDArT loci. Mostly non-overlapping distributions of the *sabina* and *balkanensis* varieties are the result of their different climatic requirements.

Context *Juniperus sabina* L. is present in the Eurasian mountains, but its range is severely fragmented. In Europe, two varieties of *J. sabina* occur: var. *sabina* and var. *balkanensis*, the latter being an allotetraploid hybrid between the dip-loid var. *sabina* and a tetraploid ancestor of *Juniperus thurifera* L. The distribution of the two varieties is mostly disjunct.

Aims Assess the taxonomic affiliation and genetic differentiation of the populations of var. *sabina* and var. *balkanensis* in Europe and Asia using cpDNA, SilicoDArT, and SNP markers. Identify climatic niches of both juniper varieties in Europe.

Methods Altogether, 21,134 SilicoDArT, 8,579 SNP, and four cpDNA loci were used. Seven climatic variables were compared in sites inhabited by var. *balkanensis* and the two parental species.

Results The SilicoDArTs and SNPs revealed a pattern of population differentiation that was congruent with the cpDNA analysis. The hybrid var. *balkanensis* occupies habitats with higher temperatures and intermediate levels of precipitation compared to both parental taxa.

Conclusion The low genetic variation and significant genetic differentiation among *J. sabina* populations likely result from the restriction of gene flow imposed by the mountain ranges. The *balkanensis* variety is able to cope with hot and dry climates probably thanks to the admixture of *J. thurifera* genes.

Keywords Climatic niche, cpDNA, Precipitation, SilicoDArT, SNP, Temperature

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1 Introduction

Climate changes, a recurring phenomenon throughout Earth's history, are the primary factors driving species extirpation, diversification, redistribution, and the subsequent structuring of genetic variation within species' ranges (Arenas et al. 2012). The direct result of population extirpation and species range fragmentation is an increase in inter-population distances, which as a consequence impede or even interrupt regional gene flow. When gene exchange is limited, genetic drift can result in the loss of genetic variation, potentially reducing the population's adaptive ability (Dobeš et al. 2017). On the other hand, new mutations and allele frequency changes resulting from the selection and genetic drift drive differentiation of the local populations. As a consequence of these processes, an isolation-by-distance pattern (IBD; Wright 1943), that is revealed by the positive correlation between geographic and genetic distances matrices, can be produced in heterogenous landscapes. In turn, the redistribution of species ranges enforced by climate change can result in the formation of hybrid populations, where individuals from different genetic lineages interbreed and produce mixed progeny (Barton and Hewitt 1989).

The species population histories are often very complex; thus, it is essential to utilize different molecular marker systems to provide reliable data on the extent and nature of genetic differentiation between the studied populations. Application of biparentally inherited nuclear markers and uniparentally inherited chloroplast (cp) or mitochondrial (mt) DNA fragments, which differ in respect to both mutation rate and effective population size, can explain the role of gene flow and selection in the species diversification, the emergence of adaptations, or the maintenance of species distinctness in the face of hybridization and introgression (Rieseberg et al. 1996). The modern genome-wide sequencing and bioinformatics methods allow to study the complexity of genomes and the role of evolutionary processes in shaping the genetic diversity of populations and species on an unprecedented scale so far (Adhikari et al. 2017).

Among terrestrial ecosystems, deserts and xeric shrublands are the most threatened by the present-day climate change, as warming and precipitation decrease can push them to their ecological limits (Tielbörger and Salguero-Gómez 2014; Li et al. 2018).

One of the shrub species adapted to arid and semiarid biomes is *Juniperus sabina* L. (savin juniper), being widely distributed in Eurasia, but its range is strongly fragmented (Farjon 2005; Adams 2014). This juniper is a dioecious, broadly prostrate, sometimes decumbent shrub, reaching up to 1 m in height, and able to cover an area of about 10–12 m in diameter or even more (Farjon

2005). This species exhibits intensive clonal dissemination, with individual shrubs often outcompeting each other. Populations are scattered and typically confined to several hectares in size. According to Adams (2014), two varieties can be distinguished in the *J. sabina* range: *sabina* and *balkanensis* R.P. Adams and A.N. Tashev, although they hardly differ morphologically and are difficult to recognize in the field (Mazur et al. 2021a). The var. *balkanensis* is allotetraploid, likely as a result of the ancient hybridization between var. *sabina* and ancestor of *Juniperus thurifera* L., which are the maternal diploid and paternal tetraploid parents, respectively (Adams et al. 2016). The presence of var. *balkanensis* has been reported in Italy, the Balkan Peninsula, and western Turkey (Adams et al. 2016, 2017, 2018; Rajčević et al. 2022). Individuals of var. *balkanensis* were also found in one population in Spain, where they coexist with var. *sabina* (Farhat et al. 2020a). Today, the two parental species of var. *balkanensis* are sympatric only in the mountains of the Iberian Peninsula and in the Western Alps, while further east only var. *sabina* occurs (Farjon 2005; Adams 2014). *J. thurifera* probably occurred in a wider range in the past (Adams et al. 2016), but today the species can be found in mountains and within loose forests in the western Mediterranean region. The European range of *J. thurifera* includes isolated patches in Spain and southern France. The species is heliophilous and xerothermic, growing under continental and cold Mediterranean climates (Farjon 2005; Adams 2014). *J. sabina* is a mountainous species, inhabiting coniferous forests and invading meadows and pastures, and it can also be found on rocky mountain slopes. The species prefers abundant sunlight and is adapted to a climate with dry and warm summers and relatively cold winters (Farjon 2005; Adams 2014).

In this study, three systems of molecular markers were used to investigate the genetic diversity and differentiation among populations covering the majority of range of *J. sabina*. These were: cpDNA, SNP, and SilicoDArT markers. SNPs and SilicoDArTs were obtained through the DArTseq technology, combining DArT (Diversity Array Technology) with NGS (next-generation sequencing). The DArT method was developed by Diversity Array Technology Pty Ltd. (DArT, Canberra, ACT, Australia) for whole-genome profiling without any prior sequence information (Jaccoud et al. 2001; Kilian et al. 2012). The present study aimed to (1) identify taxonomic affiliation of the *J. sabina* populations from the Romanian mountains and Asian populations from Kyrgyzstan and Georgia based on the cpDNA haplotypes, (2) assess if var. *balkanensis* and var. *sabina* differ in respect of the DArT-seq markers, and (3) to identify the climatic niches of var. *sabina* and var. *balkanensis* in Europe.

2 Materials and methods

2.1 Sampling and DNA isolation

In total, 108 samples of *J. sabina* representing 17 populations were used for cpDNA analyses, and 94 specimens from 14 populations were used for the DArTseq investigation (Appendix Table 1; Fig. 1). The populations are located in different mountains: Cantabrians, Alps, Apennines, Balkans, Apuseni, Carpathians, Crimean, Caucasus, and Tian-Shan. Samples were collected from individuals clearly delimited in the field, usually growing no closer than 30 m from each other. The populations from Spain (SP7), Austria (AU3, AU4), Italy (IT1), Ukraine (CR), and Poland (PL) were previously identified as var. *sabina*, whereas Italian (IT3, IT4), Bulgarian (BG), Croatian (CRO), Greek (GR), and North Macedonian (NM) as var. *balkanensis*, based on the cpDNA markers (Adams et al. 2017, 2018). Populations from Romania (RO1, RO2, RO3) and those from Kyrgyzstan (KYR) and Georgia (GEO) have not been studied so far with molecular markers.

To obtain genomic DNA, the top of the leafy twig was taken from each studied tree. Twigs were preserved in plastic bags with silica gel and stored at room temperature until DNA extraction. Before DNA isolation, the plant tissues were homogenized with a TissueLyser mill (Qiagen, Hilden, Germany). DNA extraction was carried out using a Qiagen DNeasy Mini Kit (Qiagen) following the manufacturer's instructions. The DNA

concentration in each sample was established between 50 and 100 ng μL^{-1} with the help of a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA).

2.2 Molecular analyses

2.2.1 Chloroplast DNA analyses

For cpDNA analyses, four intergenic spacers, *trn*L-*trn*F, *trn*D-*trn*T, *trn*S-*trn*G, and *pet*N-*psb*M (Appendix Table 2), were used. Proportions of the PCR components and amplification program were described by Mazur et al. (2021b). The cpDNA regions were amplified in a Labcycler Basic (SensoQuest, Göttingen, Germany). PCR products were cleaned up with an EPPiC Fast enzymatic mixture (A&A Biotechnology, Gdańsk, Poland) and then sequenced using a BrilliantDye Terminator v. 3.1 Cycle Sequencing kit (NimaGen, Nijmegen, The Netherlands). An ExTerminator Kit (A&A Biotechnology) was used to purify the DNA sequencing reactions. The order of the nucleotides in cpDNA fragments was determined with an ABI PRISM 3500 Genetic Analyser (Thermo Scientific, Waltham, MA, USA). Variable sequences for different loci were deposited in GenBank with the accession numbers listed in Table 3 in the Appendix.

2.2.2 Genome-wide genotyping and data filtering

DNA samples of 94 individuals were sent to Diversity Arrays Technology Pty Ltd. in Australia (https://www.diversityarrays.com) for the sequencing and identification
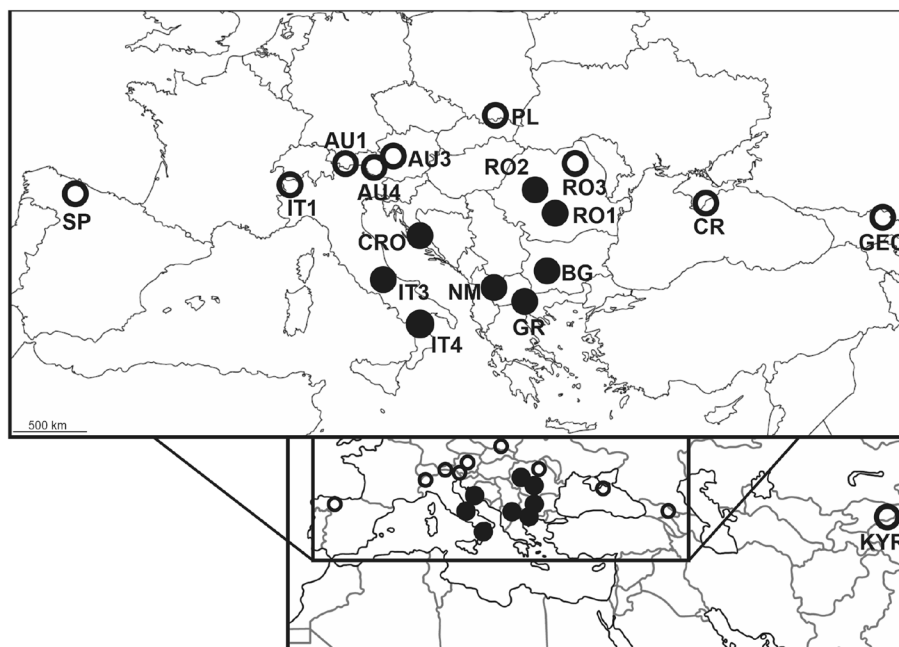

Fig. 1 Locations of *J. sabina* var. *sabina* (open circles) and *J. sabina* var. *balkanensis* (black circles) populations in Europe and Asia. Population codes according to Appendix Table 1

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of genome-wide markers using the DArTseq method. In this method, genome complexity reduction is conducted by applying a combination of restriction enzymes to find and separate the most polymorphic genome regions, which are usually low-copy sequences (Kilian et al. 2012). To detect reproducible DNA polymorphism in *J. sabina*, the combination of *Pst*I and *Mse*I was chosen. The ends of digested fragments created by *Pst*I were ligated to adapters and amplified by PCR and then sequenced on NGS platforms (Kilian et al. 2012; Andrews et al. 2016).

All DNA samples of *J. sabina* sent for DArTseq analyses showed good quality, and we received two types of markers that are available at the Zenodo repository (Jadwiszczak et al. 2023). These were SilicoDArTs, a type of dominant marker, and single nucleotide polymorphisms (SNPs), which are codominant marker systems. In the SilicoDArT loci, markers are scored '1' for presence or '0' for absence, and the marker is assigned as '- ' in the case when counts are too low to score confidently as '1'. In the SNP loci, the reference allele is assigned as '1', and the SNP allele as '0'. The initial SilicoDArT data file comprised 241,216 markers, and the SNP data file included 100,346 markers. Next, data filtering was conducted using the R package dartR version 4.0.2 (Gruber et al. 2018) to reject poor-quality markers. The quality of both marker types was verified based on their reproducibility of 100% and call rate at the threshold of 95% for the SilicoDArTs and 85% for SNPs. Additionally, one ratio parameter at the threshold of 0.05 was also taken into account in the case of the SilicoDArT dataset. Reproducibility specifies the proportion of technical replicate assay pairs for which the marker score is consistent. The call rate is the proportion of samples for which the genotype call is either '1' or '0'. One ratio determines the proportion of samples for which the genotype score is '1'.

2.3 Climatic data

For four sites of var. *sabina*, four var. *balkanensis*, and four localities of *J. thurifera* (Appendix Table 4), seven climatic parameters were analyzed. The parameters were as follows: mean annual dew/frost point temperature (°C) at 2 m above the earth's surface (DFP), maximum (Tmax) and minimum (Tmin) air temperatures (°C) at 2 m above the earth's surface, mean annual soil humidity within the root zone (the layer from the surface 0 cm to 100 cm below grade; 0%—completely water-free soil, 100%—completely saturated soil, RZW), mean annual cloudiness (%; CA), mean daily amount of precipitation at the soil surface over the year (mm day^{-1}; PC), and the mean daily photosynthetically active radiation at the ground surface over the year (W m^{-2}; PAR). All climatic data came from the POWER Release 901 project of the National Aeronautics and Space Administration (NASA) Langley

Research Center (https://power.larc.nasa.gov/data-access-viewer/; accessed 13 November 2022). The data are based upon the Modern Era Retrospective-Analysis for Research and Applications (MERRA-2) assimilation model (Bosilovich 2008). The MERRA model is a general circulation model that shapes estimates of atmospheric variables based on assimilated and optimized observational data (Westberg et al. 2013). NASA provides the POWER data on a global grid with a spatial resolution of 1.0° latitude by 1.0° longitude for the solar radiation parameter, and with a resolution of 0.5° latitude by 0.625° longitude for the six remaining climatic parameters considered in this study.

2.4 Data analyses

2.4.1 CpDNA diversity and phylogenetic analysis

CpDNA haplotypes of 108 J. *sabina* specimens, including both polymorphic sites (parsimony informative sites and singletons) and indels, were established with the help of DnaSP 6.12.03 (Rozas et al. 2017). The haplotype diversity (H_d) was calculated manually according to the formula of Nei (1987):

$$H_d = \left(1 - \sum f_h^{\,2}\right) \times \frac{n}{n-1}$$

where f_h is the frequency of haplotype h in the sample and n is the total number of individuals. Nucleotide diversity (π) was established in MEGA version 11 (Tamura et al. 2021) using the partial deletion option that allowed us to include in the calculations all sites with coverage $\geq 5\%$. Phylogenetic analysis of the cpDNA haplotypes was described in the Appendix.

2.4.2 Polymorphism of DArTseq markers, population differentiation, and structuring

Based on the filtered sets of both types of DArTseq markers, the frequency distributions of polymorphism information content (PIC) values were established. The observed heterozygosity (H_O) and gene diversity (H_S) were estimated for those *J. sabina* populations that included at least 5 individuals. These two indices, as well as the expected heterozygosity of the total population (H_T) and inbreeding coefficient (F_{IS}), were calculated for the SilicoDArT and SNP datasets. For both SilicoDArTs and SNPs, based on the Euclidean genetic distances, the genetic relationships between all juniper individuals were studied using principal coordinate analysis (PCoA) and the hierarchical clustering method utilizing Ward's approach (Ward.D2; Ward 1963). Considering populations with at least 5 specimens, the genetic differentiation was measured with pairwise F_{ST} estimates, and sequential Bonferroni's correction (Rice 1989) was applied to avoid spurious positives. F_{ST} values were also established

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between groups of populations occupying different mountain ranges (Alps, Carpathians, Apennines, Balkans, Tian-Shan) as well as between the European var. *sabina* and var. *balkanensis* and between them and the Kyrgyz population from Tian-Shan. All of the above statistical analyses were performed using the dartR package (Gruber et al. 2018). The number of migrants (Nm) among the mountain ranges and the European and Asian savin junipers was assessed using the formula of Slatkin and Voelm (1991):

$$Nm = \frac{(d - 1)(1 - F_{ST})}{4dF_{ST}}$$

where *N* means the effective size of each population, m is the proportion of migrants arriving in a population per generation, and d indicates the number of populations analyzed. To investigate the correlations between F_{ST} and geographic distance (km) matrices (isolation by distance, IBD), the Mantel test (Mantel 1967) was performed in PAleontological STatistics (PAST) version 4.11 software (Hammer et al. 2001).

For both SNP and SilicoDArT markers, the most likely number of independent genetic groups (K) was assessed using the STRUCTURE 2.3.4 software (Pritchard et al. 2000) with 10 independent runs for K values ranging from 1 to 14. After the initial testing of different run lengths, the length involving 100,000 iterations after 10,000 burn-in periods was chosen. The locality information (LOCPRIOR model) as well as the admixture model with correlated allele frequencies were utilised in the analyses. Additionally, the recessive model was implemented for the SilicoDArTs. The CLUMPAK software was used to sum up and show graphical representations of the STRUCTURE results (Kopelman et al. 2015). The most probable number of genetic clusters was chosen based on the ad hoc statistic ΔK (Evanno et al. 2005). Based on *K* = 2 for the SilicoDArTs, the mean value of the cluster membership coefficient (Q) was calculated for each population across 10 runs.

2.4.3 Climatic niche analysis

In each site, the mean annual values of five climatic parameters recorded within the period 1981–2021 were standardized according to the Z score method. The maximum and minimum values of temperature in each site and each year were also considered and standardized. In the PAST software, the standardized parameter values were used to conduct redundancy analysis (RDA) with 9,999 permutations to study the potential impacts of the climatic parameters (explanatory variables) on the occurrence of var. *sabina*, var. *balkanensis*, and *J. thurifera* (response variables).

3 Results

3.1 Genetic diversity, population differentiation, and structuring

3.1.1 CpDNA markers

The nucleotide sequence lengths were 675 bp, 649 bp, 822 bp, and 878 bp for the *trn*L-*trn*F, *trn*D-*trn*T, *trn*S-*trn*G, and *pet*N-*psb*M fragments, respectively. The total length of the combined cpDNA sequence was 3,024 bp. Altogether, 33 nucleotide substitutions (8 singletons and 25 parsimony informative) and 18 indel positions were found. The length of indels ranged from 1 to 20 bp. Out of 19, 12 haplotypes (H1–H12) were revealed in diploid European and GEO localities, five (H13–H17) in the tetraploid populations, and two (H18, H19) in the Kyrgyz population (Appendix Table 5). The highest number of haplotypes (5) was found in the GEO population, which resulted in the highest H_d (0.86) and π (0.00048). The total sample was characterized by H_d = 0.82 and π = 0.00404.

The BI phylogenetic tree revealed two main clusters of haplotypes. One cluster with 100% bootstrap support included five haplotypes revealed in var. *balkanensis* populations that grouped with those of *J. thurifera* and *J. sabina* × *J. thurifera* triploid hybrids (Appendix Fig. 3). The second cluster with 100% bootstrap support was divided into the var. *sabina* haplotypes from Europe, Georgia, and Azerbaijan, and another group comprising two Kyrgyz haplotypes. The Iranian sample seemed to be an outgroup with respect to all accessions in the cpDNA tree.

3.1.2 DArTseq markers

As a result of filtering, 21,134 SilicoDArT and 8579 SNP markers were retained for the *J. sabina* specimens. Most of the SilicoDArTs (99.99%) showed PIC values that were above 0.1 (Appendix Fig. 4), with a mean value of 0.195. In turn, most of the SNPs (76.78%) showed PIC values below 0.1, with a mean PIC of 0.074.

At the population level as well as for the total sample, the values of H_O and H_S were higher for SilicoDArT than for SNP markers (Appendix Table 6). The highest H_O (0.1620) and H_S (0.1382) values for the SilicoDArTs were revealed in the North Macedonian population. The lowest values of these parameters were found in Austrian AU3, 0.0462 and 0.0372, respectively. For the SNPs, H_O values ranged from 0.0110 in AU3 to 0.0579 in RO1 from Romania, while H_S ranged from 0.0196 in AU3 to 0.0843 in Croatia. In general, H_O and H_S values were clearly higher for the tetraploid localities when compared to the diploid populations for both types of DArTseq markers. In the total sample, the average values of H_O and H_S were 0.1160 and 0.0977, respectively, for SilicoDArTs

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and 0.0393 and 0.0607, respectively, for SNPs. The value of the H_T parameter was also higher for SilicoDArT (0.1052) than for SNP (0.0749) markers. The inbreeding coefficient was high and positive ($F_{IS} = 0.3611$) for SNP loci and negative ($F_{IS} = -0.18$) for SilicoDArTs.

In the SilicoDArTs-based PCoA, a clear division of the two main groups was revealed. The first and more diverse group consisted of the individuals deriving from var. *balkanensis* populations, and the diploid specimens were in the second group (Appendix Fig. 5a). The group consisting of the diploid specimens included the clearly distinct Kyrgyz population from Asia. In this PCoA, the first axis explained 6.25%, and the second explained 2.79% of the overall variance. In PCoA based on the SNP markers, three distinct groups were revealed (Appendix Fig. 5b). The first group included individuals from the tetraploid populations, the second group comprised specimens from diploid European populations, and the third group was constituted by the Kyrgyz specimens. The first and second axes showed 9.46% and 5.24% of the total variance, respectively, in the PCoA distribution based on the SNPs.

In the hierarchical dendrogram based on the SilicoDArTs, the first main group included all diploid specimens, and the second group consisted of tetraploids. In the cluster with diploids, European specimens were separated from the Kyrgyz samples (Appendix Fig. 6a). In the SNP-based hierarchical clustering, two main groups were revealed: one with the Kyrgyz specimens and the second with the European specimens, which were divided into diploids and tetraploids (Appendix Fig. 6b).

For the SNP markers, all pairwise population comparisons were statistically significant (F_{ST} from 0.046 to 0.512; Appendix Table 7). In the case of SilicoDArT loci, F_{ST} values ranged from 0 to 0.188. Based on the SNPs, populations from different mountain ranges were significantly genetically differentiated, and the number of migrants was the lowest between Tian-Shan and all remaining groups (Nm = 0.1–0.3; Appendix Table 8). For SilicoDArTs, the highest F_{ST} values were noted between Tian-Shan and var. *sabina* populations from the Alps and Carpathians. Considering three groups of populations, i.e., var. *sabina* in Europe, var. *balkanensis* and the Kyrgyz population, both kinds of DArTseq markers revealed higher genetic differentiation and a lower number of migrants between Kyrgyzstan and var. *sabina* compared to Kyrgyzstan-var. *balkanensis* (Appendix Table 8). Isolation by distance (IBD) was detected for both SNP ($r = 0.93$, $P = 0.004$) and SilicoDArT ($r = 0.83$, $P = 0.010$) markers.

The most likely number of genetic clusters, based on the method designed by Evanno et al. (2005) was $K = 4$ and $K = 5$ for the SilicoDArTs and SNPs, respectively (Appendix Fig. 7). In the case of $K = 4$ for SilicoDArTs, the first group involved var. *sabina* individuals from European populations as well as the Kyrgyz specimens (green color), and the second group consisted of the var. *balkanensis* populations from Romania, Italy, Croatia, and North Macedonia (orange), and third and fourth groups were represented by Greek and Bulgarian specimens (different proportions of violet and orange colors), respectively. When $K = 2$ was considered for SilicoDArTs (Appendix Fig. 7a), the populations were divided into var. *sabina* and var. *balkanensis*. The mean values of cluster 2 membership coefficient indicated that var. *balkanensis* populations likely exhibited an admixture of *J. thurifera* genes, ranging from 16% (Italy) to 20% (Greece) (Appendix Table 9). Based on the SNPs with $K = 5$, the STRUCTURE assigned most Romanian and Italian individuals to one cluster (orange), some Greek and one specimen from North Macedonia to a second cluster (violet), while the remaining var. *sabina* and var. *balkanensis* individuals showed an admixture of at least two gene pools (Appendix Fig. 7b).

3.2 Climatic niches

The RDA clearly differentiated var. *sabina*, var. *balkanensis*, and *J. thurifera* sites ($F = 13.21$, $p = 0.001$; Fig. 2). The first axis in the RDA explained 49% of the variability in the relationship among the occurrence of the three junipers and climatic parameters. This axis separated *J. thurifera* sites from both var. *sabina* and var. *balkanensis*. The second axis was responsible for explaining 46.9% of the variability, and it differentiated var. *sabina* sites from var. *balkanensis* and *J. thurifera* sites. The occurrence of var. *sabina* revealed strong positive correlation with the precipitation (PC), cloudiness (CA), and soil humidity (RZW) parameters and negative correlations with the photosynthetically active radiation (PAR), minimum (Tmin) and maximum (Tmax) temperatures, and dew/frost point (DFP) variables. The very acute angles between PC, CA, and RZW indicated that they were strongly correlated with each other. The *J. thurifera* sites showed positive correlations with PAR, Tmin, and Tmax and negative correlations with the humidity parameters. The var. *balkanensis* sites were positively correlated with PC, CA, RZW, DFP, and Tmax.

4 Discussion

4.1 Distribution and genetic differentiation of the J. sabina varieties

The previous investigations showed that var. *sabina* and var. *balkanensis* were clearly distinct with respect to the cpDNA markers (Adams et al. 2016, 2018; Farhat et al. 2020a). In the present study, these markers were used to identify *J. sabina* populations that have remained
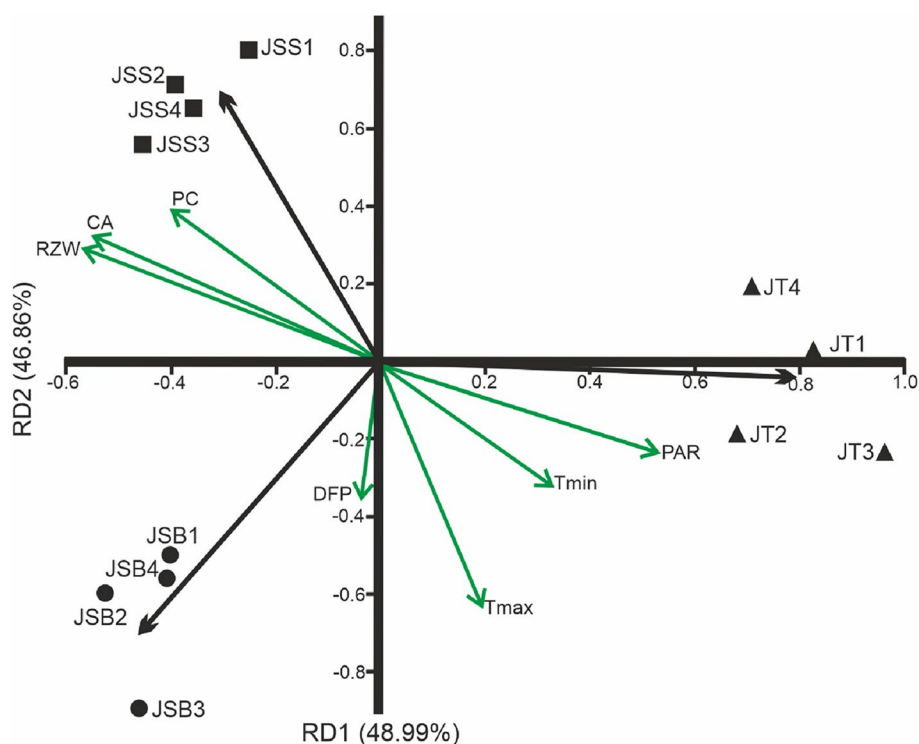
Fig. 2 Redundancy analysis (RDA) plot showing the relationship between var. *sabina* (JSS1-4; squares), var. *balkanensis* (JSB1-4; dots), and *J. thurifera* (JT1-4; triangles) sites and the climatic variables in the studied sites. DFP dew/frost point temperature, Tmax and Tmin maximum and minimum air temperatures, respectively, RZW soil humidity within the root zone, CA cloudiness, PC precipitation, PAR photosynthetically active radiation

genetically indefinite thus far (Mazur et al. 2021a). In the Romanian RO1 from the Southern Carpathians and RO2 from the Apuseni Mts., the cpDNA haplotype H13 was found. This haplotype formed a cluster with other haplotypes found in var. *balkanensis* populations. Thus, the RO1 and RO2 populations belong to var. *balkanensis*, and RO2 is its northernmost population at present. The RO3 population located in the Eastern Carpathians shared the H3 and H4 haplotypes with other var. *sabina* populations from Europe. In the Georgian population, the H3 haplotype was revealed, being most frequent in var. *sabina* populations. The clustering of two haplotypes from Azerbaijan (Hojjati et al. 2018) with other var. *sabina* samples in the BI tree implied that the variety extends at least to the western side of the Caspian Sea. The H18 and H19 haplotypes from Kyrgyzstan formed a distinct subgroup within the diploid cluster of *J. sabina*, suggesting the existence of a distinct variety in this country. It is not excluded that the same Kyrgyz haplotypes were also found in Kazakhstan and Mongolia, as Adams et al. (2016) noted that the Kazakhstan/Mongolia cpDNA cluster was clearly distinct from that from Europe and Azerbaijan.

Examination of cpDNA haplotypes did not reveal a population where the two savin juniper varieties co-occurred. Instead, var. *balkanensis* was found in the Apennine and Balkan Peninsulas, while var. *sabina* was spread from Spain to Georgia. The *sabina* and *balkanensis* varieties coexist in Sierra de Baza in Spain (Farhat et al. 2020a). Haplotypes of var. *balkanensis* were nested with the GenBank accessions of *J. thurifera* and *J. sabina × J. thurifera* triploid hybrids (Farhat et al. 2020b) in the cpDNA tree. This is the consequence of the origin of var. *balkanensis*, which is the allotetraploid hybrid between diploid var. *sabina* and the tetraploid ancestor of *J. thurifera* (Adams et al. 2016; Farhat et al. 2019). The high similarity of the cpDNA fragments of *J. thurifera* and var. *balkanensis* indicates that *J. thurifera* must be the paternal parent, as chloroplasts are most likely transmitted via pollen in Cupressaceae species (Farhat et al. 2019).

In the hierarchical clustering and PCoA based on the SilicoDArT and SNP markers, var. *balkanensis* and var. *sabina* specimens from Europe were also clearly separated. However, the position of the Kyrgyz individuals depended on the marker used. Using SilicoDArTs, the Kyrgyz genotypes formed a subcluster in the diploid

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group. In the SNP-based analyses, the Kyrgyz individuals grouped independently to both types of European populations. In the STRUCTURE clustering based on the SNPs ($K=5$) and SilicoDArTs ($K=4$), the Kyrgyz population was classified with var. *sabina* from Europe. In general, each clustering for SilicoDArTs revealed very high contribution of var. *sabina* genes in all studied populations, but when $K=2$ was considered var. *balkanensis* populations were characterized by up to 20% of *J. thurifera* admixture. This result was congruent with the hybridization scenarios between diploid var. *sabina* and tetraploid *J. thurifera* that assumed backcrossing of tetraploid hybrid progeny with diploid maternal plants (Farhat et al. 2019). As an effect, var. *balkanensis* clusters with var. *sabina* in the nuclear internal transcribed spacer (ITS) region analyses (Adams et al. 2016). For $K=4$, the SilicoDArTs showed some level of differentiation within var. *balkanensis* group because the Greek and Bulgarian populations had the admixture of a unique gene pool. The cpDNA analysis showed that the Greek and Bulgarian populations shared the H16 haplotype that was absent in other var. *balkanensis* populations. We were not able to find a biological sense of any clustering ($K=2$, 3, or 5) based on the SNPs because the suggested groupings did not reveal taxonomic or geographical patterns. These discrepancies between the SilicoDArT and SNP marker analyses likely resulted from the lower informativeness of SNPs (PIC=0.074) compared to SilicoDArTs (PIC=0.195). In DArTseq technology, the SNP locus is considered biallelic, with the most common nucleotide acting as the reference allele and the second nucleotide representing an alternate allele. Any failure in calling SNP loci is referred to as missing data (Gruber et al. 2018). Calling failure can appear when the SNP state is ambiguous by consensus (Gruber et al. 2018), which seems to be more likely in polyploid than diploid genomes. It was found that the SilicoDArTs from the diploid genomes were more useful than SNPs in the determination of putative progenitors of polyploid *Aegilops* L. and *Triticum* L. species (Edet et al. 2018).

The distribution of *J. sabina* is confined to the mountain ranges; thus, a restricted gene flow was expected among the populations over the species range. As it was presumed, the highest F_{ST} and lowest number of migrants (Nm=0.1–0.3) were noted between the Kyrgyz population from Tian-Shan and all other mountain ranges. Based on both types of DArT markers, the lowest F_{ST} and highest Nm were revealed between var. *balkanensis* populations located in the Apennines, Carpathians, and Balkans. However, we suppose that this result can indicate shared ancestry rather than actual gene exchange among them. In general, F_{ST} values were higher for SNPs compared to SilicoDArTs but most of the F_{ST} values were statistically significant at both mountain group and population levels. Consequently, the significant isolation-by-distance (IBD) pattern was revealed by both kinds of markers. Significant IBD strongly implies spatially restricted gene flow due to the limitation of pollen and seed movements. This limitation can result from the presence of mountain ranges as geographical barriers. Meta-analysis of 179 plant species from Central Europe and the European Alps revealed significantly higher genetic differentiation among the populations inhabiting the mountains compared to those from the lowlands, and among the populations from the alpine zone compared to those from the subalpine areas (Reisch and Rosbakh 2021).

4.2 Climatic niches of the J. sabina varieties

The influence of climate on juniper distribution is evident, as their diversification during the Tertiary was linked to progressive climate drying (Farjon 2005). The current range of *J. sabina* spreads from Spain to Mongolia but it is strongly fragmented, and the varieties *sabina* and *balkanensis* show nearly completely nonoverlapping distributions. This geographical disjunction can result from different climatic requirements of var. *sabina* and var. *balkanensis*. Our RDA analysis revealed that var. *sabina* was present in areas with the greatest cloudiness and precipitation but the lowest temperatures. In contrast, var. *balkanensis* occurred in the sites with the highest maximum temperature and dew/frost point. With respect to the precipitation, var. *balkanensis* occupies the areas with lower humidity than that in the var. *sabina* sites. Analyses of bioclimatic factors in the Balkan Peninsula showed a substantial drought tolerance of var. *balkanensis* as two populations from the Balkan Mts. experienced at least 20% less precipitation than the sites located in the Dinaric Mts. (Rajčević et al. 2022). This tolerance seems to be an effect of *J. thurifera* genes because *J. thurifera*, that is the paternal ancestor of var. *balkanensis* (Adams et al. 2016), inhabits sites with the highest photosynthetic radiation and lowest precipitation.

The almost allopatric distribution of the two varieties likely dates back to at least the last Pleistocene glaciation. The *balkanensis* variety could have survived the glacial maximum in the refugia located in the Iberian, Apennine, and/or Balkan Peninsulas. However, during the Holocene warming, its northward spread was likely impeded by the harsh climate of the Pyrenees and the Alps. The *sabina* variety as a mountainous species can easily cope with relatively cold winters with abundant snowfall; thus, its populations could have survived the Pleistocene in different mountain ranges, including the periphery of the

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Alps (Schönswetter et al. 2005). After the glacial retreat, refugial populations of var. *sabina* recolonized the upper mountain elevations. In a consequence of the long-lasting geographical separation of var. *sabina* and var. *balkanensis*, being a result of their different climatic adaptations, the clear genetic differentiation in the cpDNA, SNP, and SilicoDArT loci was shown (Adams et al. 2016; this study). The long-term isolation caused the genetic differentiation of several mountainous plants. It was shown that the populations of *Campanula alpina* Jacq. inhabiting the Carpathians and the Alps as well as *C. alpina* populations from the different Carpathians ranges were genetically differentiated (Ronikier et al. 2008). Genetic differentiation was also found between the populations of *Pinus mugo* Turra from the Sudetes, Carpathians, and Alps (Boratyńska et al. 2014).

In the face of current climate deterioration, the conclusions regarding the further fate of *J. sabina* are uncertain. The species has survived many climatic changes thus far. On the one hand, based on SNPs and SilicoDArTs, var. *sabina* appears to be less genetically diverse, making it potentially more vulnerable to climate warming than the allotetraploid var. *balkanensis.* It was found that the hybrid populations having mixed gene combinations could be more resilient to climate change than the parental taxa (Brauer et al. 2023; Turbek and Taylor 2023). On the other hand, it was reported that var. *sabina* intensively overgrew pastures in Spain (García-Cervigón et al. 2017) and in the Alps, and it was even proposed to cut down bushes to stop this process (Reinhard 2017). In the Carpathians and the Dynaric Mts., the shepherds burn bushes of var. *balkanensis* to prevent the overgrowth of grassy pastures by the junipers (Łuczaj and Ronikier, pers. inf.). Climate change, but also the removal of shrubs by humans, can force both varieties of *J. sabina* to move to higher altitudes, to the subnival zone in the mountains. In our opinion, the juniper populations in the low mountain ranges are at the greatest risk.

4.3 Genetic resources

At the population level, the values of the diversity parameters H_O and H_S for both types of DArT markers were up to fivefold higher in var. *balkanensis* populations compared to var. *sabina* populations. The clear distinction of heterozygosity measures between the diploid and tetraploid junipers was rather expected, as previous molecular analyses revealed that 75% of var. *balkanensis* individuals and 47% of var. *sabina* were polymorphic with respect to the nuclear ITS regions (Adams et al. 2018; Farhat et al. 2020a). One of the most surprising results in our study was the quite low H_T values assessed using both SNP (0.0749) and SilicoDArT (0.1052) markers, although *J. sabina* individuals

originated from 14 populations spread over a distance of 6600 km. The H_T parameter was similar or even higher in small-scale studies of other species: the palm *Mademia argun* (Mart.) Wurttenb. ex H.Wendl. from Sudan (0.036 and 0.127 for SNPs and SilicoDArTs, respectively; Elshibli and Korpelainen 2021) or the flowering tree *Trema orientalis* (L.) Blume in Uganda (0.40 and 0.06 for SNPs and SilicoDArTs, respectively; Nantongo et al. 2022). There are three possible explanations for this phenomenon. First, the low H_T value for SNP markers in *J. sabina* resulted from inbreeding, as F_{IS} was equal to 0.3611. Based on both SilicoDArT and SNP markers, the *T. orientalis* population was not inbred (Nantongo et al. 2022). Second, the low diversity level of the savin juniper could be a consequence of the lower PIC values of both markers compared to other investigations. The average PIC values were 0.17 for SNPs and 0.22 for SilicoDArTs in *T. orientalis* (Nantongo et al. 2022). Low PIC value of the DArT markers can result, among others, from the presence of null alleles, i.e., alleles that are not sequenced due to the mutation (Andrews et al. 2016). The third possible explanation is the much higher rate of molecular evolution per unit time in angiosperm than in gymnosperm species (De La Torre et al. 2017). Contrary to the variation of DArT markers, the cpDNA diversity was higher in var. *sabina* (H_d=0–0.86, π=0–0.00048) populations compared to var. *balkanensis* (H_d=0–0.60, π=0–0.00009). This phenomenon can likely be explained by the mixing of different phylogenetic lineages or survival in the isolated glacial refugia; however, this is a topic of our next study.

5 Conclusions

The SilicoDArTs and SNPs showed that the genetic variation in the savin juniper populations spread over a large area was low. Moreover, the parameters of genetic diversity were much lower in var. *sabina* populations compared to var. *balkanensis*. As genetic diversity is a prerequisite for populations to cope with environmental changes, the var. *sabina* populations, especially those from the lower mountain elevations, can be at a higher risk than var. *balkanensis* in the face of ongoing climate warming. The var. *sabina* inhabits sites with high precipitation and low temperatures; thus, it may suffer from rising temperatures and subsequent drying of arid and semiarid habitats in the future. As moisture excess during winter-spring season at high altitudes can limit the upward expansion of *J. sabina* (García-Cervigón et al. 2018), the species' genetic resources should be preserved by *ex-situ* conservation measures. Successful *ex-situ* germination efforts were undertaken in the Kórnik Arboretum of the Institute of Dendrology of the Polish Academy of Sciences where var. *sabina* from the Pieniny Mts. is preserved.

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Appendix

Table 1 Population names, geographical locations, and number of *J. sabina* individuals studied in respect of the DArTseq markers and cpDNA

	Country	Population code	Mountain area	Latitude N	Longitude W/E	Number of samples	
						DArTseq	cpDNA
Europe							
1	Spain	SP7	Cantabrians	42.88	− 5.87	4	6
2	Austria	AU3	Alps	47.11	13.46	6	5
3		AU4	Alps	47.00	12.43	3	5
4	Romania	RO1	Southern Carpathians	45.20	24.08	7	6
5		RO2	Apuseni	46.48	23.33	7	5
6		RO3	Eastern Carpathians	46.82	25.85	9	6
7	Italy	IT1	Alps	45.68	7.16	–	7
8		IT3	Apennines	42.10	14.18	6	6
9		IT4	Apennines	39.91	16.28	8	6
10	Croatia	CRO	Balkans	44.24	15.81	10	8
11	North Macedonia	NM	Balkans	41.59	20.66	5	6
12	Greece	GR	Balkans	41.14	22.24	9	6
13	Bulgaria	BG	Balkans	42.26	23.54	9	5
14	Poland[a]	PL	Outer Western Carpathians	49.40	20.42	-	5
15	Ukraine	CR	Crimean	44.56	34.21	4	4
Asia							
16	Georgia	GEO	Caucasus	42.37	42.62	–	7
17	Kyrgyzstan	KYR	Tian-Shan	43.00	77.36	7	15
						94	108

[a] In Poland, the natural population of *J. sabina* var. *sabina* is located in the Pieniny Mts. (the range of Outer Western Carpathians). The present study involves individuals originated from the Pieniny Mts. but grown in the Kórnik Arboretum of the Institute of Dendrology of the Polish Academy of Sciences (52.24 N, 17.09 E)

Table 2 Sequences of chloroplast (cpDNA) primer pairs used in the *J. sabina* study (see Adams et al. 2016 and references therein)

cpDNA fragment	Primer F	Primer R
*trn*L-*trn*F	CGAAATCGGTAGACGCTACG	ATTTGAACTGGTGACACGAG
*trn*D-*trn*T	ACCAATTGAACTACAATCCC	CTACCACTGAGTTAAAAGGG
*trn*S-*trn*G	GCCGCTTTAGTCCACTCAGC	GAACGAATCACACTTTTACCA
*pet*N-*psb*M	AACGAAGCGAAAATCAATCA	AAAGAGAGGGATCGTATGGA

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Table 3 GenBank accessions of *J. sabina*, *J. thurifera*, and *J. sabina* ✕ *thurifera* triploid hybrids used in the construction of phylogenetic tree from the Bayesian inference analysis using the concatenated cpDNA sequences

Species	Herbarium specimen	GenBank number				References
		*trn*L-*trn*F	*trn*D-*trn*T	*trn*S-*trn*G	*pet*N-*psb*M	
J. sabina	SP7-1	OQ849616	OQ818713	OQ863623	OQ830574	This study
	SP7-5	OQ849617	OQ818714	OQ863624	OQ830575	This study
	SP7-9	OQ849618	OQ818715	OQ863625	OQ830576	This study
	SP7-12	OQ849619	OQ818716	OQ863626	OQ830577	This study
	SP7-20	OQ849620	OQ818717	OQ863627	OQ830578	This study
	SP7-22	OQ849621	OQ818718	OQ863628	OQ830579	This study
	AU3-1	OQ849622	OQ818719	OQ863629	OQ849696	This study
	AU3-2	OQ849623	OQ818720	OQ863630	OQ830580	This study
	AU3-4	OQ849624	OQ818721	OQ863631	OQ830581	This study
	AU3-10	OQ849625	OQ818722	OQ863632	OQ830582	This study
	AU3-14	OQ849626	OQ818723	OQ863633	OQ830583	This study
	AU4-2	OQ849627	OQ818724	OQ863634	OQ830584	This study
	AU4-5	OQ849628	OQ818725	OQ863635	OQ849697	This study
	AU4-6	OQ849629	OQ818726	OQ863636	OQ830585	This study
	AU4-9	OQ849630	OQ818727	OQ863637	OQ830586	This study
	AU4-19	OQ849631	OQ818728	OQ863638	OQ830587	This study
	RO1-1	OQ830612	OQ818729	OQ863639	OQ849699	This study
	RO1-2	OQ830613	OQ818730	OQ863640	OQ849700	This study
	RO1-5	OQ830614	OQ818731	OQ863641	OQ849701	This study
	RO1-8	OQ830615	OQ818732	OQ863642	OQ849702	This study
	RO1-11	OQ830616	OQ818733	OQ863643	OQ849703	This study
	RO1-15	OQ830617	OQ818734	OQ863644	OQ849704	This study
	RO2-1	OQ830618	OQ818735	OQ863645	OQ849705	This study
	RO2-2	OQ830619	OQ818736	OQ863646	OQ849706	This study
	RO2-7	OQ830620	OQ818737	OQ863647	OQ849707	This study
	RO2-11	OQ830621	OQ818738	OQ863648	OQ849708	This study
	RO2-21	OQ830622	OQ818739	OQ863649	OQ849709	This study
	RO3-1	OQ849632	OQ818740	OQ863650	OQ830588M	This study
	RO3-2	OQ849633	OQ818741	OQ863651	OQ830589	This study
	RO3-7	OQ849634	OQ818742	OQ863652	OQ830590	This study
	RO3-12	OQ849635	OQ818743	OQ863653	OQ830591	This study
	RO3-16	OQ849636	OQ818744	OQ863654	OQ830592	This study
	RO3-18	OQ849637	OQ818745	OQ863655	OQ849697	This study
	IT1-1	OQ849638	OQ818746	OQ863656	OQ830593	This study
	IT1-4	OQ849639	OQ818747	OQ863657	OQ830594	This study
	IT1-10	OQ849640	OQ818748	OQ863658	OQ830595	This study
	IT1-11	OQ849641	OQ818749	OQ863659	OQ830596	This study

Species	Herbarium specimen	GenBank number				References
		*trn*L-*trn*F	*trn*D-*trn*T	*trn*S-*trn*G	*pet*N-*psb*M	
	IT1-16	OQ849642	OQ818750	OQ863660	OQ830597	This study
	IT1-25	OQ849643	OQ818751	OQ863661	OQ830598	This study
	IT1-26	OQ849644	OQ818752	OQ863662	OQ830599	This study
	IT2-1	OQ830623	OQ818753	OQ863663	OQ849659	This study
	IT2-6	OQ830624	OQ818754	OQ863664	OQ849660	This study
	IT3-2	OQ830625	OQ818755	OQ863665	OQ849661	This study
	IT3-5	OQ830626	OQ818756	OQ863666	OQ849662	This study
	IT3-8	OQ830627	OQ818757	OQ863667	OQ849663	This study
	IT3-10	OQ830628	OQ818758	OQ863668	OQ849664	This study
	IT4-2	OQ830629	OQ818759	OQ863669	OQ849665	This study
	IT4-5	OQ830630	OQ818760	OQ863670	OQ849666	This study
	IT4-10	OQ830631	OQ818761	OQ863671	OQ849667	This study
	IT4-12	OQ830632	OQ818762	OQ863672	OQ849668	This study
	IT4-15	OQ830633	OQ818763	OQ863673	OQ849669	This study
	IT4-21	OQ830634	OQ818764	OQ863674	OQ849670	This study
	CRO-2	OQ830635	OQ818765	OQ863675	OQ849671	This study
	CRO-3	OQ830636	OQ818766	OQ863676	OQ849672	This study
	CRO-4	OQ830637	OQ818767	OQ863677	OQ849673	This study
	CRO-6	OQ830638	OQ818768	OQ863678	OQ849674	This study
	CRO-9	OQ830639	OQ818769	OQ863679	OQ849675	This study
	CRO-12	OQ830640	OQ818770	OQ863680	OQ849676	This study
	CRO-17	OQ830641	OQ818771	OQ863681	OQ849677	This study
	CRO-29	OQ830642	OQ818772	OQ863682	OQ849678	This study
	NM1-1	OQ830643	OQ818773	OQ863683	OQ849679	This study
	NM1-3	OQ830644	OQ818774	OQ863684	OQ849680	This study
	NM1-5	OQ830645	OQ818775	OQ863685	OQ849681	This study
	NM1-9	OQ830646	OQ818776	OQ863686	OQ849682	This study
	NM2-17	OQ830647	OQ818777	OQ863687	OQ849683M	This study
	NM2-24	OQ830648	OQ818778	OQ863688	OQ849684	This study
	GR1-1	OQ830649	OQ818779	OQ863689	OQ849688	This study
	GR1-3	OQ830650	OQ818780	OQ863690	OQ849710	This study
	GR1-4	OQ830651	OQ818781	OQ863691	OQ849711	This study
	GR1-9	OQ830652	OQ818782	OQ863692	OQ849712	This study
	GR1-10	OQ830653	OQ818783	OQ863693	OQ849713	This study
	GR1-13	OQ830654	OQ818784	OQ863694	OQ849689	This study
	BG2-1	OQ830655	OQ818785	OQ863695	OQ849690	This study
	BG2-2	OQ830656	OQ818786	OQ863696	OQ849691	This study
	BG2-3	OQ830657	OQ818787	OQ863697	OQ849685	This study

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Species	Herbarium specimen	GenBank number				References
		*trn*L-*trn*F	*trn*D-*trn*T	*trn*S-*trn*G	*pet*N-*psb*M	
	BG2-5	OQ830658	OQ818788	OQ863698	OQ849686	This study
	BG2-11	OQ830659	OQ818789	OQ863699	OQ849687	This study
	PL-1	OQ849614	OQ818790	OQ863700	OQ830600	This study
	PL-8	OQ849645	OQ818791	OQ863701	OQ830601	This study
	PL-13	OQ849646	OQ818792	OQ863702	OQ830602	This study
	PL-19	OQ849647	OQ818793	OQ863703	OQ849692	This study
	PL-22	OQ849648	OQ818794	OQ863704	OQ849693	This study
	CR1-1	OQ849649	OQ818795	OQ863705	OQ830603	This study
	CR1-2	OQ849650	OQ818796	OQ863706	OQ830604	This study
	CR1-3	OQ849651	OQ818797	OQ863707	OQ849694	This study
	CR2-1	OQ849652	OQ818798	OQ863708	OQ830605	This study
	GEO-1	OQ849653	OQ818799	OQ863709	OQ830606	This study
	GEO-3	OQ849654	OQ818800	OQ863710	OQ830607	This study
	GEO-7	OQ849655	OQ818801	OQ863711	OQ849695	This study
	GEO-12	OQ849656	OQ818802	OQ863712	OQ830608	This study
	GEO-18	OQ849657	OQ818803	OQ863713	OQ830609	This study
	GEO-21	OQ849658	OQ818804	OQ863714	OQ830610	This study
	GEO-24	OQ849615	OQ818805	OQ863715	OQ830611	This study
	KYR2-1	OQ849599	OQ818806	OQ863716	OQ849714	This study
	KYR2-3	OQ849600	OQ818807	OQ863717	OQ849715	This study
	KYR4-1	OQ849601	OQ818808	OQ863718	OQ849716	This study
	KYR4-2	OQ849602	OQ818809	OQ863719	OQ849717	This study
	KYR4-3	OQ849603	OQ818810	OQ863720	OQ849718	This study
	KYR4-7	OQ849604	OQ818811	OQ863721	OQ849719	This study
	KYR5-1	OQ849605	OQ818812	OQ863722	OQ849720	This study
	KYR5-2	OQ849606	OQ818813	OQ863723	OQ849721	This study
	KYR5-5	OQ849607	OQ818814	OQ863724	OQ849722	This study
	KYR5-10	OQ849608	OQ818815	OQ863725	OQ849723	This study
	KYR6-1	OQ849609	OQ818816	OQ863726	OQ849724	This study
	KYR6-2	OQ849610	OQ818817	OQ863727	OQ849725	This study
	KYR6-6	OQ849611	OQ818818	OQ863728	OQ849726	This study
	KYR6-7	OQ849612	OQ818819	OQ863729	OQ849727	This study
	KYR6-8	OQ849613	OQ818820	OQ863730	OQ849728	This study
	14316 (BAYLU)	LC420908	LC420907	LC420909	LC420906	Hojjati et al. 2018
	14317 (BAYLU)	LC420918	LC420917	LC420919	LC420916	Hojjati et al. 2018
	101434 (TARI)	LC420997	LC420996	LC420998	LC420995	Hojjati et al. 2018
J. thurifera	JT2	MN248727	MN248711	MN831462	MT114139	Farhat et al. 2020b
	JT3	MN248726	MN248712	MN831467	MT114140	Farhat et al. 2020b
	JT5	MN248731	MN248713	MN831464	MT114141	Farhat et al. 2020b

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Species	Herbarium specimen	GenBank number				References
		*trn*L-*trn*F	*trn*D-*trn*T	*trn*S-*trn*G	*pet*N-*psb*M	
	JT9	MN278730	MN248716	MN831463	MT114142	Farhat et al. 2020b
	JT16	MN248729	MN248715	MN831466	MT114143	Farhat et al. 2020b
	JT39	MN248728	MN248714	MN831465	MT114144	Farhat et al. 2020b
J. sabina × J. thurifera triploid hybrid	JTS8	MN248732	MN248717	MN831468	MT114145	Farhat et al. 2020b
	JTS14	MN248733	MN248718	MN831469	MT114146	Farhat et al. 2020b
	JTS19	MN248734	MN248719	MN831470	MT114147	Farhat et al. 2020b

Table 4 Locations of *J. sabina* and *J. thurifera* populations analyzed in respect of seven climatic parameters

Species	Country	Population	Latitude N	Longitude W/E	Altitude m	References
J. sabina var. sabina	Austria	JSS1	46.994	11.012	1300	Mazur et al. (2021a)
	Italy	JSS2	45.689	7.166	1250	
	Austria	JSS3	47.116	13.464	1400	
	Ukraine	JSS4	44.567	34.217	1250	
J. sabina var. balkanensis	Italy	JSB1	42.107	14.188	1400	Mazur et al. (2021a)
	Bulgaria	JSB2	42.263	23.540	1200	
	Greece	JSB3	41.140	22.246	1460	
	Romania	JSB4	45.207	24.080	1000	
J. thurifera	Spain	JT1	41.08	−3.33	1100	A. Boratyński (pers. inf.)
	Spain	JT2	41.18	−3.62	1100	
	Spain	JT3	40.17	−1.97	1300	
	Spain	JT4	41.77	−2.84	1020	

Table 5 Genetic variation of the concatenated *trn*L-*trn*F, *trn*D-*trn*T, *trn*S-*trn*G, and *pet*N-*psb*M cpDNA fragments in 17 populations of *J. sabina*

Pop	N_H	Hd	π	Haplotypes																		
				H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19
SP7	2	0.34	0.00011	1	–	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
AU3	2	0.40	0	–	–	4	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–
AU4	2	0.40	0	–	–	4	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
RO1	1	0	0	–	–	–	–	–	–	–	–	–	–	–	–	6	–	–	–	–	–	–
RO2	1	0	0	–	–	–	–	–	–	–	–	–	–	–	–	5	–	–	–	–	–	–
RO3	3	0.73	0.00020	–	3	2	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
IT1	1	0	0	–	–	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
IT3	2	0.40	0	–	–	–	–	–	–	–	–	–	–	–	–	–	5	–	–	1	–	–
IT4	1	0	0	–	–	–	–	–	–	–	–	–	–	–	–	6	–	–	–	–	–	–
CRO	2	0.25	0.00009	–	–	–	–	–	–	–	–	–	–	–	–	–	7	1	–	–	–	–
NM	1	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	6	–	–	–	–	–
GR	2	0.53	0	–	–	–	–	–	–	–	–	–	–	–	–	4	–	–	2	–	–	–
BG	2	0.60	0	–	–	–	–	–	–	–	–	–	–	–	–	3	–	2	–	–	–	–
PL	3	0.80	0.00027	–	–	2	–	–	–	–	–	–	–	1	2	–	–	–	–	–	–	–
CR	2	0.50	0	–	–	3	–	–	1	–	–	–										
GEO	5	0.86	0.00048	–	–	3	–	–	–	1	1	1	1	–	–	–	–	–	–	–	–	–
KYR	2	0.13	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	14	1
		0.82	0.00404	1	3	30	2	1	1	1	1	1	1	1	2	15	27	1	4	1	14	1

N_H number of haplotypes, H_d haplotype diversity, π nucleotide diversity, *H1–H19* symbols of haplotypes. Population codes according to Table 1

Table 6 Diversity parameters of the SilicoDArT and SNP markers in the *J. sabina* populations

	Population code	SilicoDArT		SNP	
		H_O	H_S	H_O	H_S
1	SP7	–	–	–	–
2	AU3	0.0462	0.0372	0.0110	0.0196
3	AU4	–	–	–	–
4	RO1	0.1588	0.1334	0.0579	0.0728
5	RO2	0.1569	0.1326	0.0570	0.0698
6	RO3	0.0644	0.0548	0.0200	0.0433
7	IT3	0.1461	0.1261	0.0497	0.0794
8	IT4	0.1482	0.1243	0.0524	0.0620
9	CRO	0.1517	0.1318	0.0505	0.0843
10	NM	0.1620	0.1382	0.0564	0.0784
11	GR	0.1590	0.1346	0.0502	0.0822
12	BG	0.1576	0.1338	0.0563	0.0783
13	CR	–	–	–	–
14	KYR	0.0812	0.0613	0.0316	0.0738
Average		0.1160	0.0977	0.0392	0.0607
Total population					
H_T		0.1052		0.0749	
F_{IS}		-0.1800		0.3611	

H_O observed heterozygosity, H_S gene diversity, H_T expected heterozygosity of the total population, F_{IS} inbreeding coefficient. At the population level, the estimations were not conducted for SP7, AU4, and CR because of a small number of individuals per sample. Population codes according to Table 1

Table 7 Heat map for pairwise genetic differentiation (F_{ST}) values between the *J. sabina* populations based on SNP (above diagonal) and SilicoDArT (below diagonal) markers. Population codes according to Table 1

	AU3	RO1	RO2	RO3	IT3	IT4	CRO	NM	GR	BG	KYR
AU3	X	0.189*	0.172*	0.114*	0.209*	0.178*	0.148*	0.224*	0.198*	0.181*	0.512*
RO1	0.041*	X	0.081*	0.171*	0.096*	0.089*	0.066*	0.079*	0.097*	0.097*	0.411*
RO2	0.016*	0	X	0.148*	0.088*	0.084*	0.06*	0.074*	0.097*	0.093*	0.410*
RO3	0	0.086*	0.058*	X	0.192*	0.176*	0.156*	0.186*	0.192*	0.176*	0.460*
IT3	0.004	0	0	0.060*	X	0.078*	0.057*	0.079*	0.102*	0.101*	0.401*
IT4	0.046*	0	0	0.104*	0	X	0.048*	0.080*	0.099*	0.094*	0.430*
CRO	0	0	0	0.052*	0	0	X	0.046*	0.088*	0.078*	0.384*
NM	0.022*	0	0	0.078*	0	0	0	X	0.076*	0.086*	0.396*
GR	0.046*	0	0	0.102*	0	0	0	0	X	0.107*	0.399*
BG	0.032*	0	0	0.082*	0	0	0	0	0	X	0.407*
KYR	0.188*	0.146*	0.123*	0.158*	0.113*	0.152*	0.101*	0.119*	0.142*	0.131*	X

$F_{ST} = 0$
$0 < F_{ST} \leq 0.02$
$0.02 < F_{ST} \leq 0.03$
$0.03 < F_{ST} \leq 0.05$
$0.05 < F_{ST} \leq 0.1$
$0.1 < F_{ST} \leq 0.2$
$0.2 < F_{ST} \leq 0.3$
$0.3 < F_{ST} \leq 0.4$
$0.4 < F_{ST} \leq 0.5$
$F_{ST} > 0.5$

*Values statistically significant after applying the sequential Bonferroni's correction

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Table 8 Heat map for pairwise genetic differentiation (F_{ST}) values between the mountain ranges and varieties of *J. sabina* based on SNP (above diagonal) and SilicoDArT (below diagonal) markers

		J. sabina var. *sabina*		*J. sabina* var. *balkanensis*			Tian-Shan
		Alps	**Carpathians**	**Apennines**	**Balkans**	**Carpathians**	
J. sabina* var. *sabina	**Alps**		0.114* (1.3)	0.137* (1.1)	0.094* (2.4)	0.123* (1.3)	0.524* (0.2)
	Carpathians	NA		0.143* (1.0)	0.102* (1.7)	0.118* (1.2)	0.460* (0.1)
J. sabina* var. *balkanensis	**Apennines**	0.056* (3.2)	0.060*, (2.6)		0.026* (7.8)	0.048* (3.7)	0.407* (0.2)
	Balkans	0.023* (9.4)	0.026* (7.5)	NA		0.026* (7.8)	0.432* (0.2)
	Carpathians	0.042* (4.2)	0.031* (5.2)	NA	NA		0.398* (0.3)
	Tian-Shan	0.197* (0.6)	0.158* (0.6)	0.112* (1.3)	0.074* (2.5)	0.095* (1.6)	

	J. sabina var. *sabina* (Europe)	*J. sabina* var. *balkanensis* (Europe)	Kyrgyzstan (Asia)
***J. sabina* var. *sabina* (Europe)**		0.090* (2.3)	0.514* (0.2)
***J. sabina* var. *balkanensis* (Europe)**	0.073* (2.9)		0.370* (0.4)
Kyrgyzstan (Asia)	0.188* (0.9)	0.069* (3.0)	

NA	$0 < F_{ST} \leq 0.02$	$0.02 < F_{ST} \leq 0.03$	$0.03 < F_{ST} \leq 0.05$	$0.05 < F_{ST} \leq 0.1$	$0.1 < F_{ST} \leq 0.2$	$0.2 < F_{ST} \leq 0.3$	$0.3 < F_{ST} \leq 0.4$	$0.4 < F_{ST} \leq 0.5$	$F_{ST} > 0.5$

Number of migrants (Nm) per generation is given in parentheses. *NA* F_{ST} not available. *FST values are statistically significant after applying the sequential Bonferroni's correction

Table 9 The values of membership coefficient Q in the particular populations of *J. sabina* based on the *K* = 2 clustering using the SilicoDArT markers. Population codes according to Table 1

	J. sabina variety	Q values	
		Cluster 1	**Cluster 2**
SP7	*sabina*	0.99	0.01
AU3	*sabina*	1.00	0.00
AU4	*sabina*	0.98	0.02
RO1	*balkanensis*	0.81	0.19
RO2	*balkanensis*	0.83	0.17
RO3	*sabina*	0.99	0.01
CR	*sabina*	0.99	0.01
IT3	*balkanensis*	0.84	0.16
IT4	*balkanensis*	0.84	0.16
CRO	*balkanensis*	0.83	0.17
NM	*balkanensis*	0.82	0.18
GR	*balkanensis*	0.80	0.20
BG	*balkanensis*	0.81	0.19
KYR	variety from Asia	1.00	0.00

Phylogenetic analysis of the cpDNA haplotypes

Phylogenetic analysis was conducted in the program MrBayes 3.2.7a (Ronquist et al. 2012), which performs Bayesian inference (BI) using a variant of Markov chain Monte Carlo to approximate the posterior probabilities of the tree. In this analysis, the combined cpDNA fragments of three *J. sabina* individuals from Azerbaijan and Iran [14316 (BAYLU), 14317 (BAYLU), 101434 (TARI); Hojjati et al. 2018], six *J. thurifera* (JT2, JT3, JT5, JT9, JT16, JT39), and three *J. sabina* × *J. thurifera* triploid hybrids (JTS8, JTS14, JTS19; Farhat et al. 2020b) were also included (Appendix Table 3). The HKY substitution model (Hasegawa et al. 1985), established in MEGA based on the lowest AIC value, was used in the construction of the BI tree. In MrBayes, two independent runs of 10 million generations each were carried out. Each run consisted of three heated chains and one cold chain. Trees were sampled every 1000 generations. After excluding 25% of the first runs as burn-in, a majority-rule consensus tree with posterior probabilities (PPs) from two runs was obtained.
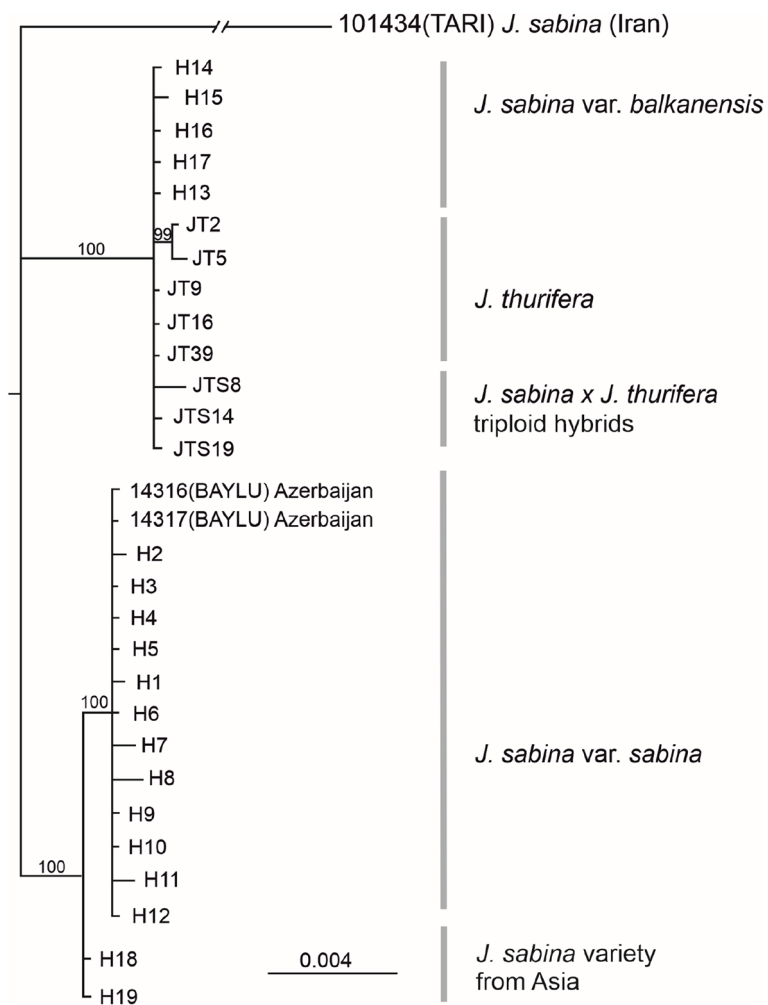
Fig. 3 Bayesian tree based on the concatenated *trn*L-*trn*F, *trn*D-*trn*T, *trn*S-*trn*G, and *pet*N-*psb*M cpDNA fragments of *J. sabina*, *J. thurifera*, and *J. sabina*×*J. thurifera* triploid hybrids
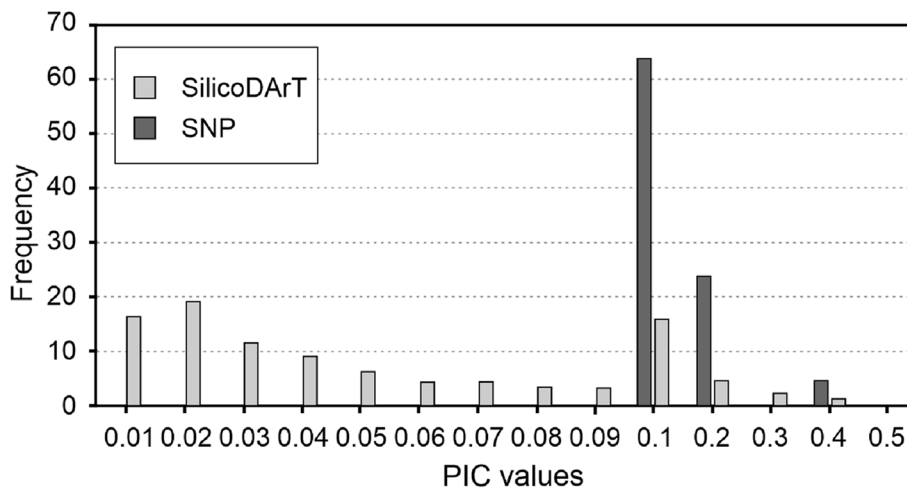

Fig. 4 Frequency distribution of polymorphic information content (PIC) values for SilicoDArT and SNP markers retained after quality analyses in the set of 94 *J. sabina* individuals
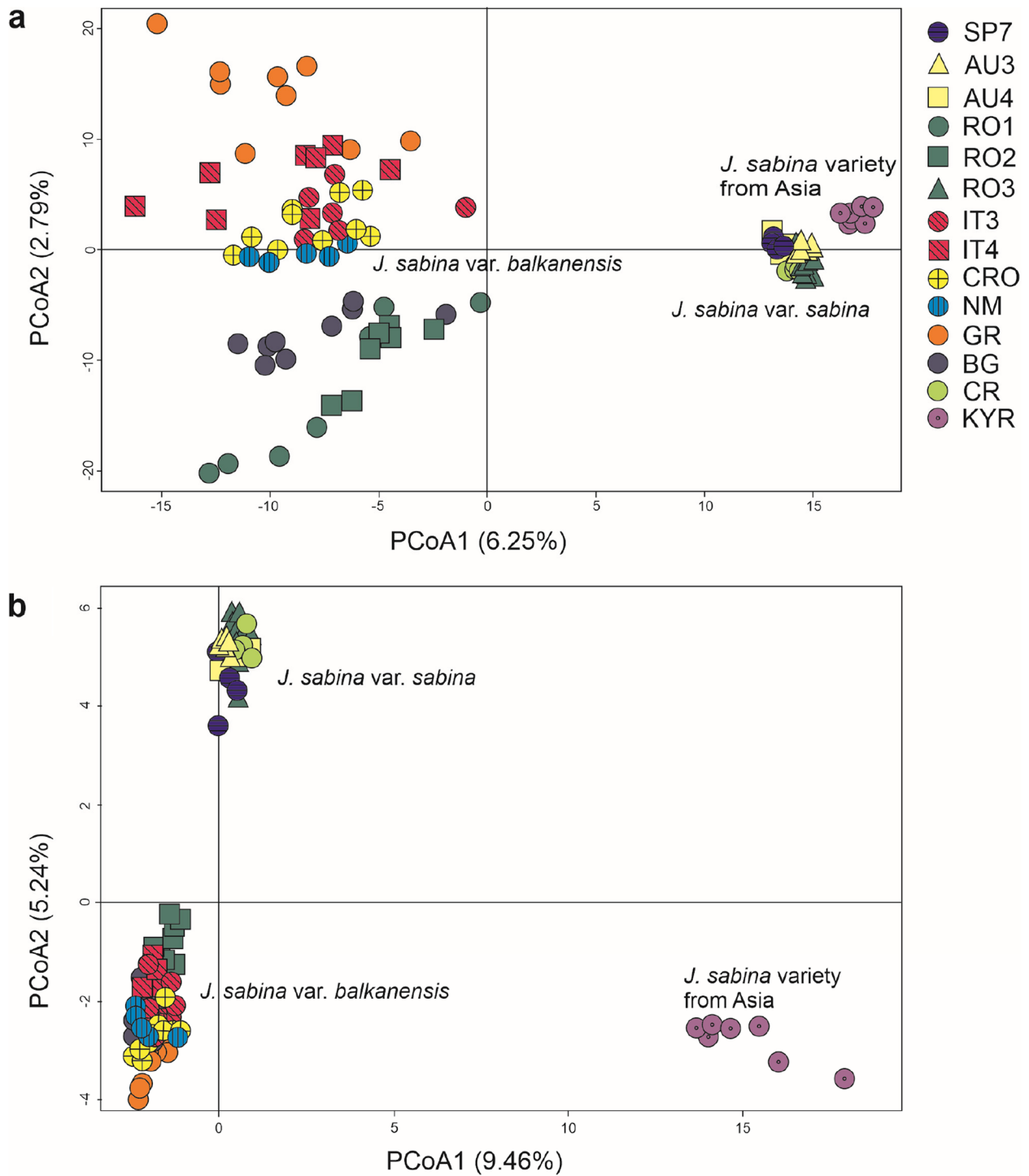
Fig. 5 Principal Coordinates Analysis (PCoA) revealing the Euclidean genetic distances between 94 *J. sabina* individuals based on the SilicoDArT (**a**), and SNP (**b**) markers. Population codes according to Appendix Table 1
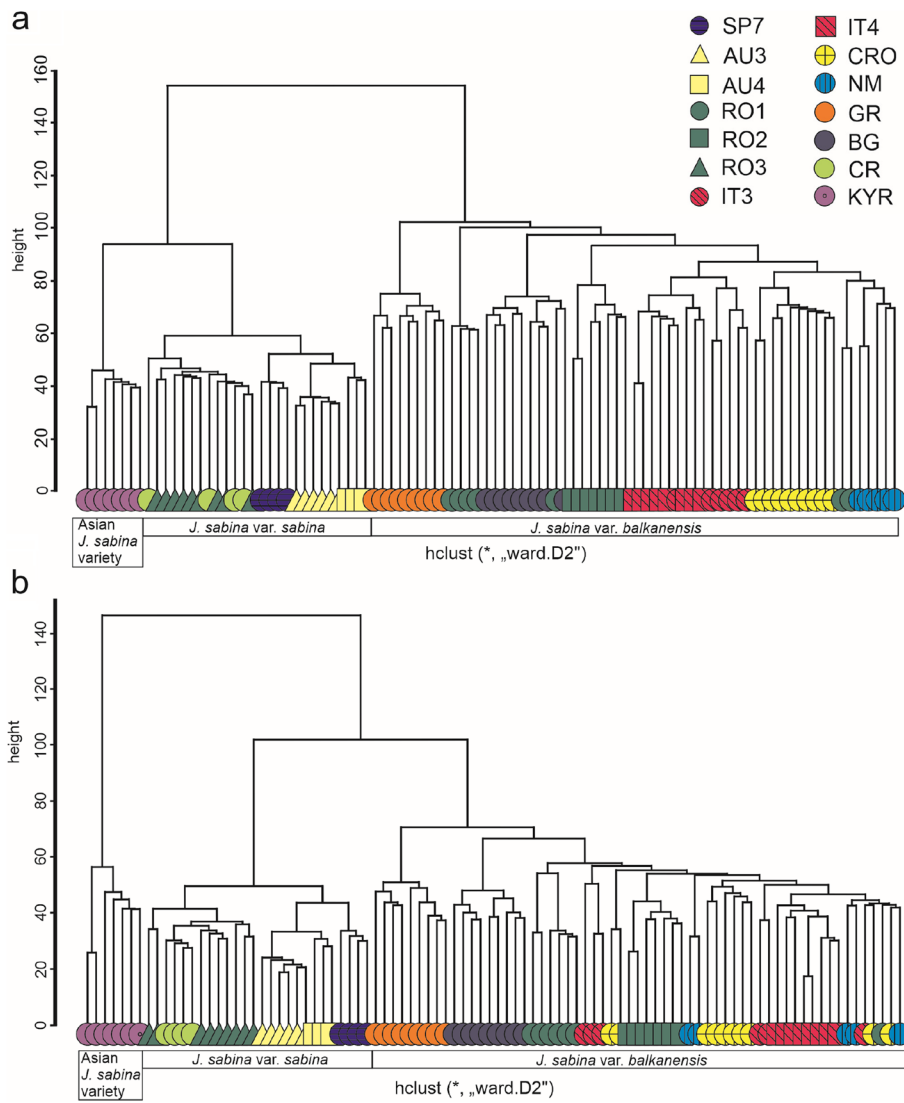
Fig. 6 Results of the hierarchical cluster analyses of 94 *J. sabina* individuals, utilizing Ward's approach for SilicoDArT (**a**), and SNP (**b**) markers. Population codes according to Appendix Table 1
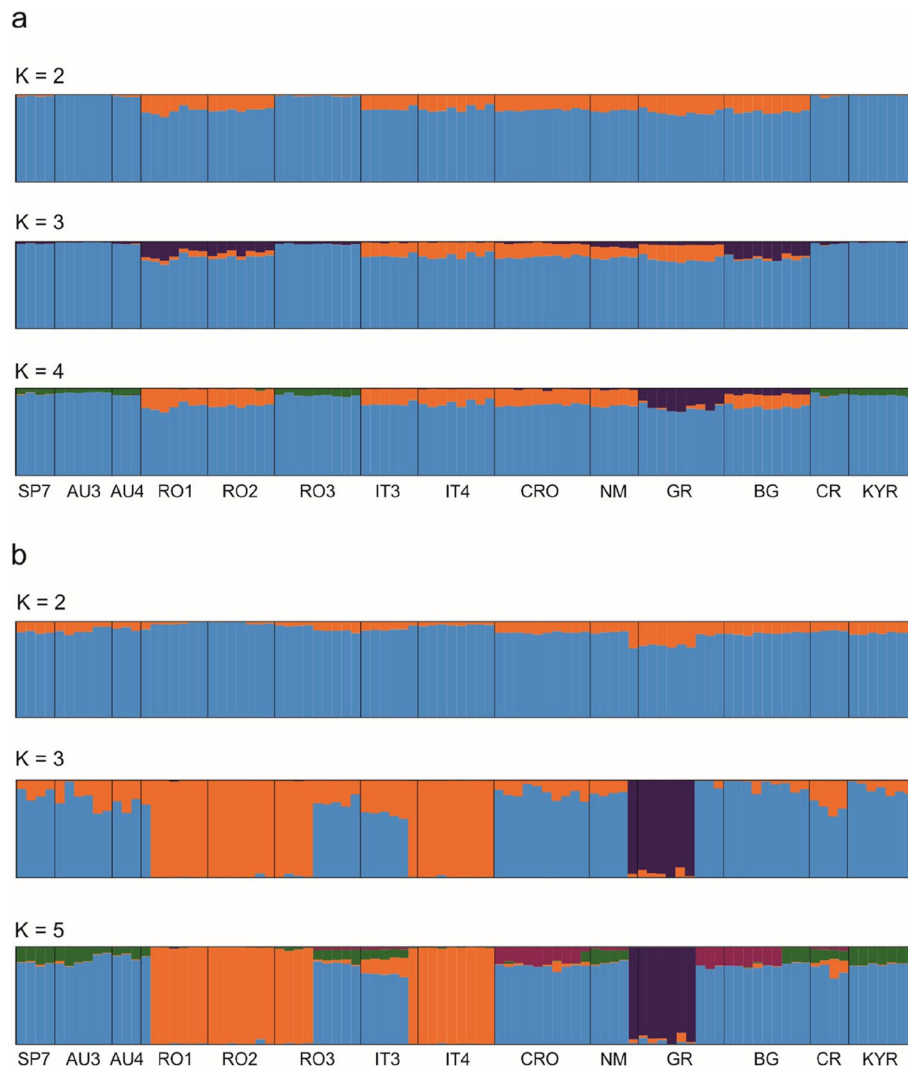
Fig. 7 Bayesian clustering of 94 *J. sabina* individuals from 14 populations based on **a** SilicoDArT loci with $K=2$, $K=3$, and $K=4$, and **b** SNP loci with $K=2$, $K=3$, and $K=5$. Population codes according to Appendix Table 1

Authors' contributions
Conceptualization: Adam Boratyński, Katarzyna Marcysiak, Małgorzata Mazur, Katarzyna Jadwiszczak; methodology: Katarzyna Marcysiak, Katarzyna Jadwiszczak, Agnieszka Bona; formal analysis and investigation: Katarzyna Jadwiszczak, Agnieszka Bona; writing—original draft preparation: Katarzyna Jadwiszczak, Katarzyna Marcysiak, Agnieszka Bona, Małgorzata Mazur; writing—review and editing: Katarzyna Jadwiszczak, Agnieszka Bona, Katarzyna Marcysiak, Małgorzata Mazur, Adam Boratyński; funding acquisition: Katarzyna Marcysiak. All authors read and approved the final manuscript.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors gave their informed consent to this publication and its content.

Competing interests
The authors declare that they have no conflict of interest.

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