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Weak Geographical Structure of *Juniperus sabina* (Cupressaceae) Morphology despite Its Discontinuous Range and Genetic Differentiation

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Abstract: In Europe, *Juniperus sabina* L. is a mountainous, rare species that creates small, scattered populations, suggesting their refugial nature. Recently, a new variety of this juniper, *J. sabina* var. *balkanensis* R. P. Adams et A. N. Tashev was described based on genetic studies. We expected morphological differentiation among isolated parts of the species range and between varieties, as was the case with other Mediterranean junipers. Cones, seeds and fragments of shoots from a total of 506 individuals were collected from 24 populations in Europe and for comparisons from three populations from Tian Shan. Almost all of the 16 analysed features significantly differentiated among populations and geographical regions as well as between the varieties, although most groups differed from others only in terms of a single feature. The thickness of cones, the width of shoots and the length of seeds were the most important features for differentiation. The geographical structure of the variation of *J. sabina* was weak, and comparative populations from Tian Shan were clustered with European populations, similar to the findings of a previous study on essential oils. We found slightly different patterns of variation of the two varieties of the species. The little intra-species differentiation could be the result of the long period of contact between nowadays distinct populations and their relatively late separation in the early Holocene.

Keywords: biogeography; biometry; *Juniperus sabina* var. *balkanensis*; *Juniperus sabina* var. *sabina*; Mediterranean region; statistics

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1. Introduction

According to the latest classification, Savin juniper (*Juniperus sabina* L. s.l.) is divided into two species, namely the western *J. sabina* L. (= *J. sabina* var. *sabina* sensu Farjon [1]) and the eastern *J. davurica* Pall., which is further differentiated into three varieties [2]. The species belongs to the monophyletic *Sabina* section, the largest one within the genus *Juniperus* L. [3]. The range of typical *J. sabina* specimens stretches along 6000 km, from the westernmost localities on the Iberian Peninsula to the easternmost ones in Mongolia [4]. In the western part of its distribution range, *J. sabina* grows in several isolated mountainous areas of the Mediterranean region and Central Europe. In Spain, the species can be found in the Iberian System, the Cantabrian Mountains, the Betic System and, albeit more scarcely, in the Pyrenees [5,6], in the Balkans—in the Dynarides, Šar Planina, the Rodopes, Rila Mountains and Kožuf [5,7–9]. In the Apennine Peninsula, this juniper grows in the Central and Southern Apennines [10,11]. It occurs in the Alps [11,12] and, more rarely, in the Carpathians and in the Crimean Mountains [13,14]. *Juniperus sabina* also grows in Turkey—in the Manisa region and in the Black Sea region of the northern part of the country [15,16]. Within its range, *J. sabina* usually creates relatively small, scattered populations, often distant from each other.

The loose, discontinuous type of distribution is characteristic for several junipers of the *Sabina* section, and its development is connected with the genesis of the genus and the geological history of the region of origin. The section most probably emerged in the Tertiary, in the area of the present-day Mediterranean region [3], and the subsequent diversification of junipers was possibly triggered by the arising of dry habitats in this period [17] and by geological events that started with the Arabia-Eurasia collision [18]. The accompanying processes, such as orogeneses, marine regressions and transgressions, as well as diverse climatic conditions caused multiple changes in sea and land shapes and extent, leading to the transformation of the Paratethys into the present Mediterranean Basin [19,20]. These processes often led to the division of plant ranges into parts, in some cases isolated since then. They also initiated more or less intensive processes of speciation in the isolated lineages of the genus *Juniperus* [3]. The shape of the contemporary juniper ranges was ultimately influenced by the Pliocene climate cooling, Pleistocene glacial-deglacial cycles and human pressure in the late Holocene. The large distances between parts of the species range and between individual populations, with a sufficiently long isolation period, could have led to differences between them, e.g., in the case of *J. thurifera* L. [21] or *J. phoenicea* L. [22,23] of the *Sabina* section.

Recently, genetic analyses of *J. sabina* from the Balkan and Apennine Peninsulas have revealed the presence of *J. thurifera*-like plastid DNA in some populations, interpreted as a probable trace of ancient hybridisation between the two species. The populations bearing the divergent plastid DNA were accommodated in a new variety, *J. sabina* var. *balkanensis* R. P. Adams et A. N. Tashev [24]. Its occurrence has, so far, been confirmed for several countries on the Balkan Peninsula, western Turkey and central and southern Italy [25–27]. The authors of the diagnosis stated that the new variety is morphologically cryptic, but they listed several morphological features allowing to distinguish *J. sabina* var. *balkanensis* from the typical *J. sabina* var. *sabina*, such as, among others, the coarser foliage, more acute leaf tips and mostly reniform (bi-lobed) cones with 1–2 seeds, opposed to mostly ovate cones with 1–2–3 seeds for the typical variety [24].

The main goal of this study was to verify the hypothesis of morphological differentiation of *J. sabina* in the European part of its geographic range, using biometry and statistical comparative analyses. We expected that isolation between populations of *J. sabina* in the mountain massifs of Europe could be a reason for the morphological differentiation of the species. We also wanted to check whether the two varieties, *J. sabina* var. *sabina* and the recently described *J. sabina* var. *balkanensis*, could be distinguished by morphological characteristics.

2. Materials and Methods

2.1. Material

Samples from 24 European populations of *J. sabina*, 1 from the Aegean region of Anatolia and, for comparisons, 3 from the easternmost part of the species range in Tian Shan, were collected (Table 1, Figure 1). Populations of both varieties were sampled, namely *J. sabina* var. *sabina* and var. *balkanensis*, and their location was determined based on previous research results by Adams et al. [25]. As in the population sample from Sierra de Baza (SP4), specimens of both varieties were found (analyses by Adams and Ronikier, unpublished), the sample collected from this population was divided into two parts: SP4-s, comprising individuals of var. *sabina*, and SP4-b, with individuals of var. *balkanensis*. Populations from the Carpathians and Tian Shan were not assigned to any variety and were considered indefinite (Table 1). Several populations were sampled in each of the following geographical regions: the Alps, the Apennines, the Balkan Peninsula, the Iberian Peninsula, the Carpathians and Tian Shan. The Crimean Mountains and the Aegean region were represented by one population each (Table 1).

Table 1. Sampled populations of *J. sabina* with their geographic location, variety assignment and number (No) of analysed individuals (i), cones and seeds (c) and shoots (s).

Locality	Acronym	Geographical Region	Variety	No i/c/s	Lat. N [°]	Long. E [°]	Alt. [m]
Austria, Alps, Ötztal, Sölden	AU1	Alps	<i>sabina</i>	10/50/50	46.994	11.012	1300
Austria, Alps, Ötztal, Zwischenstein	AU2	Alps	<i>sabina</i>	22/110/110	46.935	11.039	1650
Austria, Alps, Jedl	AU3	Alps	<i>sabina</i>	23/109/115	47.116	13.464	1400
Austria, Alps, Obermauern, Burg	AU4	Alps	<i>sabina</i>	32/158/160	47.006	12.433	1400
Italy, Alps, Vall d'Aosta, Les Combes	IT1	Alps	<i>sabina</i>	26/130/130	45.689	7.166	1250
Switzerland, Alps, Ausserberg	SW	Alps	<i>sabina</i>	27/135/135	46.310	7.870	1000
Spain, Sierra de Gudar, Valdelinares	SP1	Iberian Peninsula	<i>sabina</i>	25/250/115	40.367	−0.6	1800
Spain, Sierra de Gudar, Valdelinares	SP2	Iberian Peninsula	<i>sabina</i>	19/93/93	40.367	−0.6	1800
Spain, Sierra de Gudar, Valdelinares	SP3	Iberian Peninsula	<i>sabina</i>	18/90/90	40.367	−0.6	1600
Spain, Sierra de Baza	SP4	Iberian Peninsula	<i>sabina/balkanensis</i>	14/225/75	37.467	−2.733	2000
Spain, Sierra de Albarracín	SP5	Iberian Peninsula	<i>sabina</i>	24/240/112	40.5	−1.567	1800
Spain, Sierra de Moncayo	SP6	Iberian Peninsula	<i>sabina</i>	24/240/120	41.767	−1.667	1900
Spain, Leon, Los Barrios de Luna	SP7	Iberian Peninsula	<i>sabina</i>	30/297/300	42.88	−5.87	1150
Ukraine, Crimea, Chatyrdag	CRI	Crimea	<i>sabina</i>	15/120/75	44.567	34.217	1250
Turkey, Sipil Dağı above Manisa	TU	Aegean region	<i>balkanensis</i>	26/260/130	38.567	27.417	1450
Italy, Majella, Provincia di Chieti, Valle del Solle	IT2	Apennines	<i>balkanensis</i>	4/16/20	41.928	14.221	1600
Italy, Majella, Provincia di Chieti, slopes of Colle Bandiera	IT3	Apennines	<i>balkanensis</i>	7/35/35	42.107	14.188	1400
Bulgaria, Rila Park, (Beli Iskar)	BG	Balkans	<i>balkanensis</i>	10/48/50	42.263	23.5406	1200
Croatia, Gračac	CRO	Balkans	<i>balkanensis</i>	18/90/90	44.248	15.810	728
Greece, Mount Tsena (Notia)	GR	Balkans	<i>balkanensis</i>	17/80/85	41.140	22.2461	1460
North Macedonia, Mavrovo	NM1	Balkans	<i>balkanensis</i>	11/54/55	41.635	20.686	1700
North Macedonia, Galichnik	NM2	Balkans	<i>balkanensis</i>	24/120/120	41.594	20.665	1450
Romania, Buila-Vânturarița Park (Pietreni)	RO1	Carpathians	indefinite	13/59/65	45.2075	24.0803	1000
Romania, Apuseni Mountains (Posaga)	RO2	Carpathians	indefinite	29/141/145	46.488	23.366	900
Romania, Oriental Carpathians (Bicaz)	RO3	Carpathians	indefinite	14/69/70	46.8258	25.855	800
Kyrgyzstan, Central Tian-Shan, Tosor	KYR1	Tian Shan	indefinite	8/37/39	43.006	77.364	2400
Kyrgyzstan, Central Tian-Shan, Naryn	KYR2	Tian Shan	indefinite	8/36/41	41.587	76.443	2360
Kyrgyzstan, Central Tian-Shan, Naryn	KYR3	Tian Shan	indefinite	8/40/40	41.495	76.426	2283

Collectors: Alexandru Badaru RO2; Adam Boratyński: AU1, AU2, IT1, SW, SP1, SP2, SP4, SP7, UA, TU; Krystyna Boratyńska: AU1, AU2, IT1, SW, SP1, SP2, SP4, SP7, TU; Fabio Conti IT2; Valter di Cecco IT3; Grzegorz Iszkuło CRI; Ewelina Klichowska: KYR1, KYR2, KYR3; Piotr Kosiński SP3, SP5, SP6; Amelia Lewandowska NM1, NM2; Andrzej Lewandowski CRI; Marcin Nobis: KYR1, KYR2, KYR3; Arkadiusz Nowak: KYR1, KYR2, KYR3; Katarzyna Marcysiak AU3, AU4, IT2, IT3, GR, NM1, NM2, RO1, RO2, RO3; Małgorzata Mazur AU3, AU4, IT2, IT3, BG, CRO, GR, RO1, RO2, RO3; Juliusz Szysler BG; Anna Wróbel: KYR1, KYR2, KYR3.

Figure 1. Localization of the *J. sabina* populations. Acronyms as in Table 1. Symbols mean classes of the length of cone (CL) of populations. Light grey CL ≤ 6.00 mm. Dark grey 6.00 < CL ≤ 6.50 mm. Black CL > 6.50 mm.

A sample from each population consisted of 10–30 individuals, depending on the size of the population, the presence of cone-bearing specimens and the availability of individual shrubs in a difficult to reach mountainous terrain, and therefore, in extremely small populations, fewer specimens were sampled. One sample from each individual comprised at least five properly developed, not injured, ripe cones and small parts of shoots. To avoid collecting the same genet, shrubs clearly delimited in the field, usually growing no closer than 30 m from each other, were treated as separate individuals.

2.2. Methods

2.2.1. Biometry

The collected material was dried without pressing before measurement. Subsequently, 5 to 10 healthy and undamaged cones and 5 to 10 1-year-old lateral branchlets from each

individual were measured. The traits for analyses were chosen based on previous examinations by different authors, performed for other junipers of the *Sabina* section [21,28,29]. To date, one of the features used to determine the size of the cones was cone diameter (CD), calculated as the average of two measurements of the width of the cone at its widest point, perpendicular to each other. We separately analysed the width (CW) and thickness of the cone (CTh), mainly because var. *balkanensis* is distinguished from var. *sabina* by having bi-lobed cones [24], indicating that the width of the cone should be greater than the thickness and that the CW/CTh ratio should be greater than 1. We also included the characteristic CD in the analyses; in total, we analysed 16 characteristics. Six of them were measured under the Delta Optical SZ-630 microscope: cone length (CL), cone width (CW), cone thickness (CTh), seed length (SL), seed width (SW) and thickness of the last ramification shoot with leaves (ST). The Delta Optical DLTCamViewer software (Delta Optical, Nowe Osiny, Poland) was used to obtain digital images and to measure elements. Leaves were counted (LN) under the microscope on the last 5-mm long section of the shoot. Seed number (SN) was counted manually. Eight ratios were calculated with the measured/counted characteristics (Table 2).

Table 2. Morphological characteristics of *J. sabina* cones, seeds and shoots, with codes and descriptive statistics. M—arithmetic mean, Me—median, Min—minimum value, Max—maximum value, CV—variation coefficient. K-W—significance of Kruskal-Wallis test for populations.

Characteristic	Code	All Data					Var. <i>Balkanensis</i>		Var. <i>Sabina</i>		K-W
		M	Me	Min	Max	CV	M	CV	M	CV	
Length of cone [mm]	CL	6.22	6.19	3.90	10.00	10.06	6.51	9.81	6.08	9.32	0.000
Width of cone [mm]	CW	6.30	6.26	3.40	9.81	13.52	6.77	11.71	6.03	12.66	0.000
Thickness of cone [mm]	CTh	5.58	5.53	3.20	8.84	13.46	6.01	11.72	5.34	12.28	0.000
Number of seeds	SN	2.18	2.00	1.00	6.00		3 *	2 **	2 *	2 **	0.000
Length of seed [mm]	SL	4.47	4.48	2.60	6.60	8.55	4.75	8.33	4.35	7.76	0.000
Width of seed [mm]	SW	3.33	3.32	1.70	5.46	12.24	3.30	13.18	3.33	11.74	0.000
Number of leaves	LN	17.62	18.00	8.00	32.00	15.38	17.95	12.48	18.02	15.49	0.000
Thickness of the last ramification shoot with leaves [mm]	ST	0.87	0.88	0.50	1.25	9.70	0.91	8.51	0.85	7.92	0.000
Diameter of cone [(CW + CTh)/2] [mm]	CD	5.94	5.90	3.35	9.17	13.29	6.39	11.50	5.68	12.27	0.000
Ratio of cone length/cone diameter	CL/CD	1.06	1.05	0.73	1.64	9.49	1.03	8.38	1.08	9.44	0.000
Ratio of cone width/ cone thickness	CW/CTh	1.13	1.11	0.97	1.77	4.90	1.13	4.75	1.13	4.81	0.000
Ratio of cone length /seed length	CL/SL	1.40	1.38	0.89	2.18	8.01	1.38	7.92	1.40	7.51	0.000
Ratio of cone diameter/seed width	CD/SW	1.83	1.76	1.03	3.91	16.42	1.99	13.01	1.75	16.83	0.000
Ratio of cone diameter/thickness of shoot	CD/ST	6.96	6.83	3.91	14.91	29.94	7.08	28.63	6.81	16.36	0.000
Ratio of seed length of seed /seed width	SL/SW	1.37	1.33	0.76	2.46	15.87	1.48	12.95	1.33	10.18	0.000
Ratio of leaves number/thickness of shoot	LN/ST	20.64	20.00	7.02	52.00	11.70	20.04	12.38	21.55	16.16	0.000

*—median; **—mode.

2.2.2. Statistical Analyses

Two types of data sets were investigated: (1) comprising all cases, i.e., each single measurement of the cone and shoot, and (2) consisting of the average values of individuals. Descriptive statistics and properties were tested for non-transformed feature values, standardized values and logarithmically transformed values [30]. Unimodality and frequency distributions of values of features were checked using Shapiro-Wilk's test, and the distribution measures, skewness and kurtosis were calculated [31,32]. The descriptive statistics were arithmetic means, medians, modes, the lowest and highest values of characteristics, standard deviations, and coefficients of variation, which were compiled for all data, for data of each variety, each geographic region, and each population, as well as for sets of means of individuals of each of the above groups. Mutual correlations of characteristics were examined using Spearman's rank correlation coefficient as values of many characteristics were not normally distributed [33].

Further analyses were performed with the set of log-transformed values of individuals means. The homoscedasticity of variances of traits with normal distributions was verified with Brown-Forsythe's test [34]. Analysis of variance (ANOVA), followed by the Tukey's test, was performed for the traits with normal distributions and homoscedastic variations, and the Kruskal-Wallis test by ranks was done for the others [33]. These tests were conducted between populations, geographical regions and between two varieties and

the groups of indefinite samples from the Carpathians and Tian Shan. The aim of these analyses was to delimit differences between the given groups, to find the best features to characterize them and to determine which groups differed by which characteristics. The results were illustrated via box-and-whiskers plots of chosen traits most important for differentiation, and shown in a common results table. The traits with normal distributions and homoscedastic variations were then used in the two-factor nested mixed ANOVA, for populations nested in regions.

The least mutually correlated, normally distributed characteristics, which, at the same time, most significantly influenced the sample differentiation in the ANOVA, were chosen for the discrimination analysis [35]. The analysis of discrimination from the multivariate statistics package of the STATISTICA 13 software (StatSoftPolska, Kraków, Poland) was performed to verify the level of differences and detect the geographic patterns, for the following groups: (1) two varieties: *sabina* and *balkanensis* and two indefinite groups: Carpathians and Tian Shan, (2) geographical regions, (3) all populations, (4) populations of each variety (two separate analyses) [32]. Results of these analyses were shown on the scatterplots between the first two discriminant variables [33]. Finally correlations between mean values of features and longitude, latitude and altitude of populations were calculated using the Spearman's rank correlation coefficient.

STATISTICA 13 software was used in all the above analyses.

3. Results

3.1. Morphological Characteristics

The obtained values of features, in the set of all measurements, were not normally distributed, both for non-transformed and standardized data. Most of the distributions were right-skewed and leptokurtic, and the distributions of the measured characteristics were closer to normal compared to the ratios. After log-transformation, only CL and CD/ST were normally distributed. In the set of all individual means, the characteristics CW, SW, LN and CD were normally distributed, whereas the other distributions were right-skewed. However, the characteristics CL, CW, CTh, SL, SW, ST, CD, CL/CD, CL/SL, CD/ST, SL/SW and LN/ST were log-normally distributed (see Supplementary Material, Table S1). Characteristics were moderately variable. The coefficient of variation (CV) of most characteristics took values between 10 and 20% (Table 2), with few exceptions. The ratio CW/CTh was the least variable, with the CV for all data equalling 4.90%, and in samples from 2.72% (SP6) to 8.17% (AU3). Ratio of leaves number/thickness of shoot (LN/ST) was the most variable feature, with a CV equalling 18.07% (from 8.25% in KYR1 to 22.79% in KYR2) (see Supplementary Material, Table S2).

Almost full correlations were found between the three features describing the width of the cone, namely CW, CTh and CD, and they were also highly correlated with CL, CL/CD, CD/SW and CD/ST, at $p < 0.01$. The parameter SL was significantly related to CL, whereas CW/CTh was not significantly correlated with any other parameter, and LN, ST and CL/SL were only correlated with one or two parameters each. Ratios were usually highly correlated to the characteristics involved; however CL/CD was not significantly correlated with CL, CL/SL was only weakly correlated with SL, and SL/SW was highly related to SW but weakly to SL.

3.2. Differences between Varieties, Regions and Populations

Comparisons between the varieties *sabina* and *balkanensis* and two indefinite groups of populations, namely Carpathians and Tian Shan, showed that all characteristics except seed width (SW) were responsible for differentiation, at $p < 0.05$. Most differences were found between the two varieties (Table 3). The box-and-whisker graphs of means of varieties and means of populations showed the considerably greater variation of population means compared to the means of varieties, and the substantial variation of means of populations within regions (Figure 2a–c). According to the analyses performed to compare regions, the Iberian Peninsula and Crimea differed from other regions in terms of numerous features,

whereas the Apennines and Tian Shan differed less (Table 4). The comparisons between populations again showed that all characteristics differentiated them significantly, but the post hoc tests pointed out that most populations differed from others only in terms of a single feature, at $p < 0.05$. The greatest number of differences between pairs of samples was caused by CTh (177, whereas CW caused 169 and CD 163), CD/ST (168), CL (123) and ST (117); the least important characteristics in this context were CW/CTh (31 differences), SW (51) and SL/SW (52). Among features analyzed in the nested ANOVA, that is: CW, SL, SW, ST, CL/CD, CD/ST and LN/ST; all of them were important for differentiation of populations, and only SL, ST and LN/ST differentiated regions (Table S3).

Table 3. Characteristics differing significantly two varieties of *J. sabina*: *sabina* and *balkanensis* and two indefinite groups of populations: Carpathians and Tian Shan, according to results of Kruskal-Wallis' test; character codes as in Table 2.

	CL, CD, CW, CTh, SL, LN, ST, CL/CD, CD/SW, LN/ST		
Carpathians			
	CL, CD, CW, CTh, SL, ST, CL/CD, CD/SW, CD/ST, SL/SW	ST, CW/CTh, CD/ST, LN/ST	
Tian Shan			
	CL, CD, CW, CTh, SL, ST, CL/CD, CL/SL, CD/SW, SL/SW, LN/ST	LN, ST, SL/SW, LN/ST	LN, ST, CW/CTh, CL/SL, CD/ST
var. <i>balkanensis</i>			
	var. <i>sabina</i>	Carpathians	Tian Shan

Table 4. Characteristics differing significantly geographical regions of *J. sabina* (Table 1) according to results of Kruskal-Wallis' test, character's codes as in Table 2.

Crimea	CL, CD, CW, CTh, SW SW, LN, ST, CL/CD, CW/CTh, CD/SW, CD/ST, SL/SW, LN/ST	CD, CW, CTh, CL/CD, CD/ST					
Alps	CL, CD, CW, CTh, SL, SW, ST, CL/SL, CD/SW, CD/SN, SL/SW, LN/ST	CL, CD CW, CTh, SL, ST, CD/SW, SL/SW	CL, CTh, SN, SL, ST, SL/SW				
Aegean Region	CL, CD, CW, CTh, SL, LN, CL/CD, CL/SL, CD/SW, SL/SW, LN/ST	CL, CD CW, CTh, SL, CL/CD, CD/SW	CL, CD CW, CTh, SL, SW, LN, ST	SL/SW			
Balkans	CL, CD CW, CTh, SL, LN, ST, CL/CD, CD/SW, LN/ST	CL, CD CW, CTh, SL, SW, LN, ST, CL/CD, CD/SW, LN/ST	CL, CD CW, CTh, SL, SW, ST, CL/CD, CW/CTh	SW, LN, SL/SW	LN, LN/ST		
Carpathians	CL, CD CW, CTh, LN, ST, CL/CD, CD/SW, LN/ST	CL, CD, CW, CTh, SL, LN, CL/CD, CD/SW, CD/SW	CL, CD CW, CTh, CD/SW, CD/ST, LN/ST	SL, ST, CL/SL, CD/SW	LN, ST, CL/SL, CD/SW	ST, CD/SW, LN/ST	
Tian Shan	CL, CD (CW), CTh, CL/CD, CD/ST	CL, CD, CW, CTh, CL/CD, CD/SW	CL, CD, CW, CTh, CL/SL	SL, ST, CL/SL, CD/SW, SL/SW	CL/SL, SL/SW	LN, ST, LN/ST	-
Apennines	Iberian Peninsula	Crimea	Alps	Aegean Region	Balkans	Carpathians	Tian Shan

(a)

(b)

(c)

Figure 2. Box-and whiskers plot of characters important for inter-specific differentiation of *J. sabina*: (a) CTh-cone thickness; (b) SL-seed length; (c) ST-shoot thickness. Point-mean. Box-standard error. Whiskers-1.96 standard error. Empty box-and-whiskers-for populations. Filled box-and whiskers-for varieties and indefinite groups. Populations acronyms as in Table 1.

Only the features: CTh, SL, ST, LN and CL/SL were at the same time weakly correlated, had the log-normal distributions and the strong impact on the differentiation of juniper groups. They were therefore used in the discrimination analyses. The grouping of individuals on the plot in the analysis performed for two varieties and two indefinite groups showed similar variations of the variety *balkanensis* and the Carpathian group, and the Tian Shan group covered a part of the variation of the variety *sabina* (Figure 3a). The differences between the two varieties were mostly influenced by ST and CTh. In the DA performed for regions, the grouping their means (Figure 3b) confirmed the relations of Carpathians to the variety *balkanensis*, represented on this plot by the Balkans and Aegean regions. However, the Apennines, belonging also to var. *balkanensis*, were closer to the Iberian Peninsula and to the Alps. This grouping was mostly influenced by the factors ST and SL. The results of the discrimination analysis performed among all populations showed a slight separation of the samples BG, CRO, GR, RO1, RO2 and TU, as well as a mixed group containing all other samples (not shown). However, on the scatter-plots based on the results of the discrimination analyses made separately for the populations of var. *sabina* and var. *balkanensis* and showing means of populations, their grouping had a weak geographical reference. The ‘sabina’ populations from Spain were divided into two groups, and the southernmost populations clustered together. Populations from the Alps formed the loose group, with IT1 from the Aosta Valley as the liaison between other groups (Figure 4a). On the second plot, the ‘balkanensis’ populations from the Balkans were scattered, but the Apennine populations and the only one from Spain grouped together (Figure 4b). All the above discussed scatter-plots showed the means of populations or regions. Plots illustrating the individuals means in DA performed for regions, all populations, and separately for populations of the two varieties, showed a homogeneous clouds (not shown). The only exception was DA of varieties.

Figure 3. Scatterplots showing results of discrimination analysis of *J. sabina*. (a) All individuals means. The variety *sabina*—green circles. The variety *balkanensis*—red squares. Tian Shan mountains—black triangles. The Carpathians—blue diamonds. Confidence ellipses with $p < 0.05$ colours of lines as above. (b) Means of regions (Table 1). In square brackets—codes of characters the most strongly connected with the discriminant variable.

The analysis of correlations of feature values with geographic coordinates showed that only the number of leaves (LN) correlated positively with altitude ($r = 0.57$), and negatively with latitude ($r = -0.67$), with $p < 0.01$.

Figure 4. Scatterplots showing results of discrimination analysis of *J. sabina* (a) Samples means of the variety *sabina*. (b) Samples means of the variety *balkanensis*. In square brackets-codes of characters the most strongly connected with the discriminant variable. Samples acronyms as in Table 1.

4. Discussion and Conclusions

4.1. Morphological Characteristics

The results of our measurements were generally consistent with the data in monographs [4,17], although in a study by Amaral Franco [36], both cone width (4–6 mm) and shoot width (0.6–0.8 mm) were smaller than the values obtained in our research. These differences can be explained by the low number of specimens measured by the latter author. All characteristics selected in our study were useful, but the ratios showed several high correlations and were often non-parametric, limiting their use in multivariate analyses. Among the traits of cone width, namely CW, CTh and CD, the characteristic CTh played the greatest role in differentiating the analysed groups, but the differences among these three features were small.

The variability of our data was very similar to published data for *J. excelsa* [29] and *J. phoenicea* [23].

4.2. Intra-Species Differentiation

Our analyses showed statistically significant differences among populations and geographical regions, and between varieties of *J. sabina*, but the structure of this differentiation did not meet our expectations. The greatest differentiation was found among populations, and they did not cluster in any explicable way. Only populations of the two varieties analysed separately tended to show a weak geographical structure. This result, together with the statistically significant differences between the two varieties, indicates slightly different patterns of variation of these varieties. On the other hand, clustering of regions could imply the stronger effect of geographic location on differentiation, compared to the taxonomic status. Importantly, it should be noted that it was not possible to classify a given population as a variety based on the values of the features. The found differences between populations can only be treated as a certain tendency, e.g., to a greater number of seeds for *J. sabina*.

The diagnostic value of the two features CW/CTh and SN, proposed by Adams et al. [24] as differentiating varieties, was not confirmed. The ratio of the two measures of cone width (CW/CTh) was the least variable characteristic with a biased distribution of its values, not significant in any comparisons. Seed number (SN) did not differ significantly between the two varieties, and its value was greater for the variety *balkanensis* (one to six seeds, with a median of three) than for the variety *sabina* (one to five seeds, with a median of two), contrary to the findings of Adams et al. [24]. As the comparisons made

by Adams et al. [24] were based on the first discoveries of the variety *balkanensis* in Greece and Bulgaria, their conclusion perhaps could only be applied to specimens of these two populations.

Although the differences between the varieties were weak, the indefinite samples from Tian Shan, according to our results, belonged to the variety *sabina*, whereas the variation of the Carpathian samples suggests their affinities to the variety *balkanensis*. These proposals, of course, require confirmation by genetic analyses, but the identification of samples from Tian Shan seems obvious. The *balkanensis* variety is the result of the ancient hybridisation between *J. sabina* and the ancestor of *J. thurifera* [24,27] and was found only on territories occupied by *J. thurifera* at present or in the past [28,37,38]. The identification of the Carpathian samples as var. *balkanensis* would be consistent with the proposal of Adams et al. [24].

Despite the differences discussed above, the geographical structure of the variation of *J. sabina* was weak and it could only be detected on the scatterplots of geographical regions and of populations of each variety. Besides, populations from the easternmost stands in Tian Shan, 4800 km from the Alps, did not show any specific features and grouped with the European populations. A similar geographic structure was obtained by Adams et al. [39] on the basis of research on essential oils.

4.3. Possible Reasons of the Weak Geographical Structure of *J. sabina* Morphology

The weak morphological differences between *J. sabina* var. *sabina* and *J. sabina* var. *balkanensis* were partly expected, as the latter has been defined as morphologically cryptic [24]. Still, for several junipers in the Mediterranean region, a morphological intra-specific differentiation was recognised, which made us expect such variability in *J. sabina*. Intra-specific morphological differentiation, leading to discrimination between taxa, has been detected in the *J. oxycedrus* complex [40,41], *J. thurifera* [21] and *J. phoenicea* s.l. [23,42]. The variation of the Mediterranean junipers has been linked to their Tertiary and Pleistocene migrations [21,28,37,38] and long-term isolations [23,43]. The lack of geographic diversity of *J. sabina*, in view of the considerable distances between its populations, is puzzling. Based on the typical Mediterranean juniper species mentioned above, it is distinguished by its vast range, reaching Central Asia, and its mountainous nature [17]. Its dispersed type of the geographic range suggests the refugial characteristic of the distribution of the species, resembling those of alpine plant species, which were related to their migration after the end of the Pleistocene [44]. Morphological examinations of these Angiosperms based on vegetative traits revealed weak geographic signals, with climate as the possible cause of their intra-species variability [45–47]. However, a study of the complex *Pinus mugo*, based on vegetative features [48], as well as of *P. sylvestris* from the refugial areas, using both vegetative and generative morphological characteristics [49], found geographical differentiations. The homogeneity of the morphology of *J. sabina* could be the result of the long period of contact between nowadays distinct populations and their relatively late separation.

The species is extremely light-demanding, well adapted to a climate with dry and warm summers and relatively cold winters (with average temperatures of -6.5 to 32 °C), tolerant of snowfall, and resistant to drought [6,15,17,50,51]. Shrubs die when shaded by higher species of trees, such as spruce (personal observation made in the Alps and the Eastern Carpathians). It is therefore likely that the species occupied a larger territory during the Late Glacial and the Early Holocene. Because juniper species cannot be distinguished based on pollen and because of the scarcity of macrofossils, it is difficult to trace the history of these species [52–54]. The widespread occurrence of the species could have been promoted by the dryness of the climate during the Younger Dryas and again in the Preboreal and Boreal periods [52], with an increase in seasonality and with the highest precipitation in winter [55], and by the significantly lower treeline in the mountains [53,56]. The pressure of deciduous forests developing during the Atlantic period could have limited the occurrence of junipers, which was found, for example, on the Iberian Peninsula [52].

The survival of the species was possible because of the plasticity of its response to changing climatic conditions [57]. The subsequent drying of the climate once again favoured the spread of juniper, although human activity also increased during this period, especially pastoralism, which could have had an impact on juniper [55].

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/d13100470/s1>, Table S1: Normality statistics of characteristics (characteristic codes—Table 2) for data, means of individuals (MI) and logarithms (log), of varieties and regions (Table 1), Table S2: Mean values (M) and coefficient of variations (CV) of characteristics (codes as in Table 2) for all populations (acronyms—Table 1), Table S3: Results of two-factor nested mixed analysis of variance between populations and regions, for characters meeting assumptions.

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