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Morphological variability of leaves of *Sorbus domestica* L. in Croatia

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Abstract

The paper studies the morphological variability of the service tree populations in the continental and Mediterranean regions of Croatia. The inter- and intra-population variability, as well as eco-geographic population differentiation, was established on the basis of eight measured morphological characteristics of two types of leaflets (lateral and terminal) and two derived ratios, using descriptive and multivariate statistical methods. The conducted research established a high variability of morphological characteristics. In comparison with lateral leaflets, terminal leaflets showed a higher degree of variability for the majority of the studied variables. The differences between the trees within populations, as well as the differences between populations, were confirmed for all studied characteristics. The study also confirmed a trend of population variations according to the eco-geographic principle. Populations from climatically different and geographically distant habitats, in other words from the Mediterranean and the continental region, differed in the majority of studied characteristics. In addition to the established pattern of ecotypical variability between the continental and the Mediterranean regions, the clinal variability with regard to altitude and mean annual temperature was also confirmed. Small, although statistically significant clinal variability with regard to the longitude was recorded for two measured characteristics. The conducted study provided insights into the variability of the service tree populations in Croatia, which is a basis for further research that should be implemented in order to produce guidelines for the breeding and conservation of genetic resources of this rare and endangered noble hardwood species.

Keywords: service tree; leaf morphology; population variability; geographical differentiation; ecological differentiation; clinal variability

Introduction

The genus *Sorbus* belongs to the subtribe Pyrinae in the family Rosaceae. Subtribe Pyrinae corresponds to the long-recognized subfamily Maloideae in which the fruit type is generally a pome [1,2]. The genus *Sorbus* encompasses about 250 species widespread in the northern hemisphere [3]. According to Hegi [4], it can be divided into 5 subgenera (*Cormus*, *Aria*, *Torminaria*, *Sorbus* and *Chamaemespilus*), although recent classifications [1,2,5,6] treat genus *Sorbus* in a narrower sense, including only pinnate-leaved species of subgenus *Sorbus* and raising several other subgenera to generic rank [7]. Species of the genus *Sorbus* belong to noble hardwoods [8,9]. Some have valuable and high quality wood, while some have edible fruits, due to which they have been cultivated for centuries. In Croatia they naturally grow in different environmental conditions and are widespread in the mountainous, subalpine and Mediterranean regions [10]. According to Kárpáti [11] there are 14 different taxa of the genus *Sorbus* naturally widespread in Croatia. The most common species in Croatia are: *Sorbus aria* (L.) Crantz, *S. domestica* L., *S. aucuparia* L. and *S. torminalis* (L.) Crantz.

The service tree (*Sorbus domestica*) is an insect pollinated and animal dispersed, rare species, and is of high interest both from silvicultural as well as from nature and gene conservation aspects [12,13]. It has been a very important noble hardwood species since early times and has been grown for its fruits and quality wood [14–17]. It is naturally widespread in southern and central Europe, and to a lesser degree in North Africa, the Crimea and Caucasus. It is most widespread on the Balkan Peninsula and in southern France and Italy [18]. It is difficult to determine the exact boundaries of its natural distribution, as it has been planted and sub-spontaneously spread since antiquity. The service tree most often grows in dry to very dry and depleted habitats, in different exposures and different types of soil. This species typically grows scattered in mixed hardwood forests, i.e. as solitary tree or in small groups, mixed with other species. According to Matić and Vukelić [19] its center of distribution in Croatia is in the Mediterranean zone of the *Quercus ilex* L., as well as in the sub-Mediterranean zone, in association *Querco pubescenti-Carpinetum orientalis* Horvatić 1939.

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In the sub-Mediterranean region, it occurs individually in *Castanea sativa* Mill. [20] and *Pinus nigra* J.F. Arnold forests [21]. It rarely appears in the continental area, only in dry and sunny places within *Querco pubescenti-Ostryetum carpinifoliae* Horvat 1938 association. In addition, in the continental region of Croatia [22], it is also found in the *Quercetum frainetto-cerridis* Rudski (1940) 1949 association, and individually also in termophile *Quercus petraea* (Matt.) Liebl. forests [23].

In Croatia as well in the most of the European countries it is a rare and threatened species due to an overall reduction in the number of individuals, fragmentation and isolation of populations, lack of natural regeneration, and disturbance of the natural metapopulation structure due to human impact [18,24]. Its conservation through both in situ and ex situ methods has therefore been recognized as a priority [10,17,18,25], and one of the key issues for conservation of genetic resources is to gain better knowledge of its genetic variability [24]. However, there is very little insight into the genetic variability of the service tree populations, and the research to date only included a smaller number of populations [12,26–30]. In addition to genetic markers, morphological traits were also used. Although in comparison with molecular markers their significance has recently been on the decrease, morphological traits are still frequently applied [25,31–33]. This is supported by an increasing amount of research combining methods of morphometric and molecular analysis [34–36]. So far only a few studies have been conducted that pertain to the morphology of fruits and leaves of the service tree [37–41].

Due to its small population density and endangered state, in Croatia the service tree is on the list of priority species for the conservation of the genetic resources [10,17]. The objective of this paper was to determine the morphological variability of service tree populations based on the morphometric leaf analysis in the entire area of its distribution in Croatia.

Material and methods

Plant material

The material for morphometric analysis was collected in 10 natural populations on the territory of Croatia. The study encompassed nine populations from the Mediterranean, and only one population from the continental region due to its small representation and a small number of adult trees that bear fruit (Tab. 1, Fig. 1). Each population was represented with 10 trees, and each tree with 30 healthy and undamaged leaves of short shoots from the external, sunlit part of the tree crown. The leaves were collected in early July 2011. The leaves were scanned and measured using the WinFolia program [42]. A total of 16 characteristics were measured (Fig. 2). The accuracy of measurements was 0.1 mm, and the following morphological characteristics were measured on each leaf, for the terminal and for one lateral leaflet each: leaflet blade area (LA); leaflet blade length (LL); leaflet blade width (LW); distance between the leaflet widest part and the leaflet base (LWP); leaflet blade width at 50% of blade length (LW1); leaflet blade width at 90% of blade length (LW2); angle closed by the main vein and the line defined by the leaflet blade base and a point on the margin, at 10% of blade length (LA1); angle closed by the main vein and the line defined by the leaflet blade base and a point on the margin, at 25% of blade length (LA2). From the measured characteristics the following ratios were derived: leaflet blade width / leaflet blade length (LW/LL); distance between the leaflet widest part and the leaflet base / leaflet blade length (LWP/LL).

Statistical analysis

The measured morphological characteristics were shown by standard descriptive statistical parameters [43]: arithmetic mean (*M*) and coefficient of variation (*CV*). Assumptions of normality were checked using the Shapiro–Wilk test, and the assumption of homogeneity of variance using Levene's test. The relationship between mean values of particular leaf characteristics and geographic longitude, latitude and altitude and the influence of annual mean temperature and annual precipitation on leaf characteristics were tested using Spearman's coefficient, *rs* [43]. Statistically significant differences between the studied objects were established by non-parametric testing using the univariate Kruskal–Wallis analysis of variance (Kruskal–Wallis ANOVA). The analysis was conducted on the entire sample by examining the differences between populations and between the trees within each population. In order to obtain a clearer picture of the variability of the studied populations, the differences in the variability of the two studied eco-geographic regions "continent" and "Mediterranean" were also examined.

In order to establish similarities and differences between the analyzed populations based on the measured morphological characteristics of leaves, cluster and canonical discriminant analyses were used [44]. The conducted cluster analysis resulted in a hierarchical tree, where the weighted pair-group average method (WPGAM) was used to join the clusters, and the Euclidean distance to define the distance between the studied objects. In the cluster analysis, variables were used which contributed mostly to the differentiation of the groups according to the eco-geographic principle. In order to establish which characteristics best discriminate the studied populations, and in order to further elucidate the trend of their differentiation, a discriminant analysis was conducted. All measured variables were introduced into the model except for LW1, due to the occurrence of redundancy. The input data in multivariate statistical methods were previously standardized using the *z*-score method. The above statistical analyses were conducted using the STATISTICA 8.0 statistical program [45].

Results

The results of the conducted descriptive statistical analysis are shown for each investigated population and overall, for all populations together (Tab. 2, Tab. 3). The most variable characteristics with the *CV* above 35% were LA of the terminal leaflet, LA of the lateral leaflet and LW2 of the terminal leaflet. As opposed to that, the lowest degree of variability is present for LWP/LL of the terminal leaflets, and for LA1 and

Tab. 1 Sampled populations.

Region	Locality	Acronym	Latitude	Longitude	Altitude (m)	Precipitation (mm)	Mean annual temperature (°C)
CON	Psunj	1	45°18′32.56″ N	17°09′59.17″ E	335	933	10.3
MED	Istra	2	45°17′10.37″ N	13°50′51.38″ E	375	1139	12.5
MED	Bribir	3	45°08′46.08″ N	14°47′47.14″ E	239	1320	12.6
MED	Rab	4	44°47′00.41″ N	14°41′21.74″ E	79	1169	13.8
MED	Kaprije	5	43°42′00.92″ N	15°41′49.70″ E	46	858	15.0
MED	Kozjak	6	43°34′31.81″ N	16°20′12.16″ E	256	949	12.5
MED	Gata	7	43°27′51.35″ N	16°42′13.06″ E	264	942	14.3
MED	Makarska	8	43°17′24.10″ N	17°01′57.52″ E	112	1008	15.7
MED	Pelješac	9	42°53′09.07″ N	17°30′29.72″ E	244	1186	15.0
MED	Konavle	10	42°30′28.73″ N	18°20′45.82″ E	256	1313	14.9

Climate data for current conditions were obtained from the WorldClim database with a spatial resolution close to a square km (http://www.worldclim.org/). CON – continent; MED – Mediterranean.

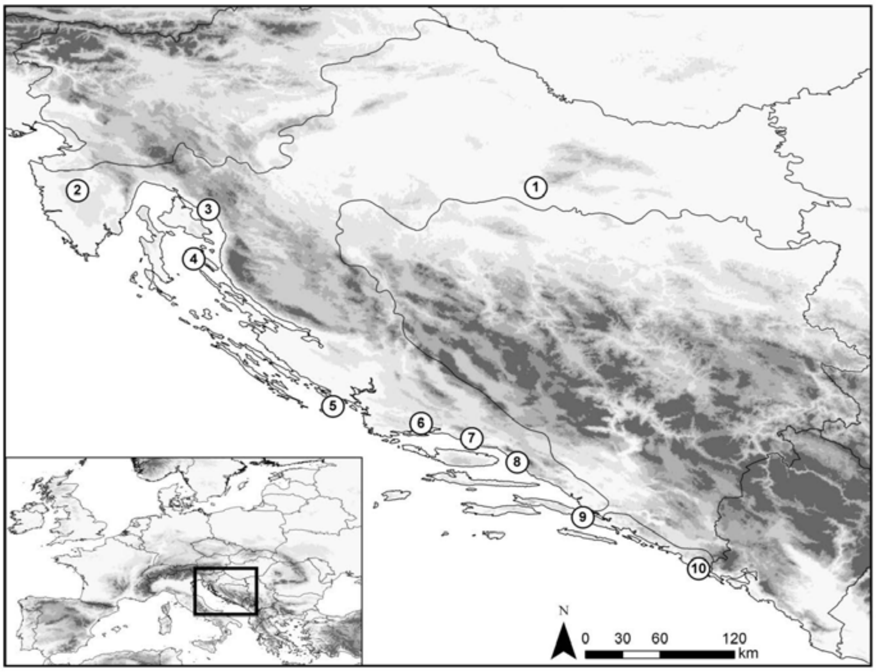

Fig. 1 Location of analysed populations (1 – Psunj; 2 – Istra; 3 – Bribir; 4 – Rab; 5 – Kaprije; 6 – Kozjak; 7 – Gata; 8 – Makarska; 9 – Pelješac; 10 – Konavle).

LA2 of the lateral leaflets. Likewise, from Tab. 2 and Tab. 3 it is evident that terminal leaflets in comparison with the lateral ones are more variable for the majority of studied variables.

The greatest mean blade surface of the lateral leaflet, as well as the highest values for LW, LW1 and LW2, and for mean LWP were noted in the continental population Psunj. The smallest mean surface and the shortest and narrowest lateral leaflets were characteristic of the Makarska and Kaprije populations, which were also the populations with the lowest measured values for LA1 and LA2. The highest values for the last two characteristics pertained to the Pelješac population.

The highest mean values for LA and LL of terminal leaflets were observed in the continental Psunj population. The lowest mean values for the said characteristics, and for LW, were found in the Kaprije population. The highest values for LA1 and LA2, and the lowest values for LW2 were proper to the Rab population. On the other hand, the lowest values

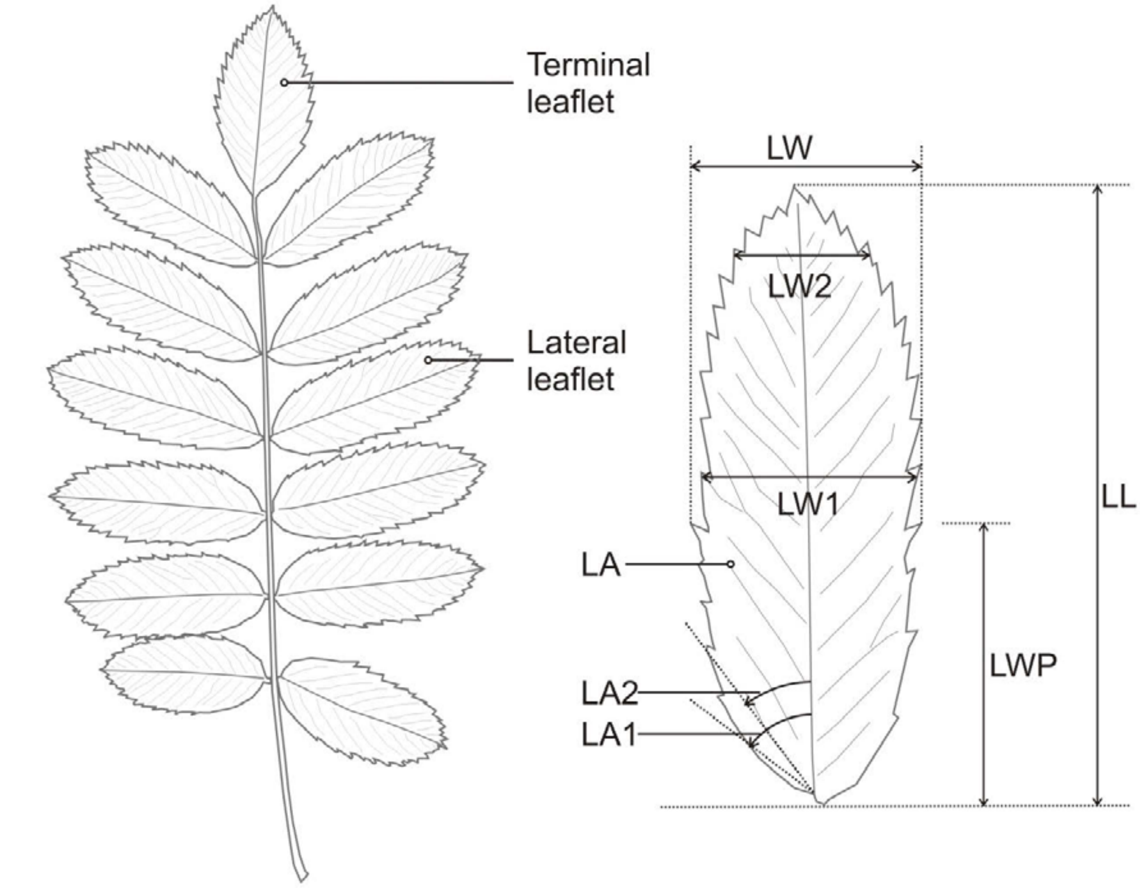

Fig. 2 Measured leaflet traits. LA – leaflet blade area; LL – leaflet blade length; LW – leaflet blade width; LWP – distance between the leaflet widest part and the leaflet base; LW1 – leaflet blade width at 50% of blade length; LW2 – leaflet blade width at 90% of blade length; LA1 – angle closed by the main vein and the line defined by the leaflet blade base and a point on the margin, at 10% of blade length; LA2 – angle closed by the main vein and the line defined by the leaflet blade base and a point on the margin, at 25% of blade length.

for the LA1 and LA2 characteristics were observed in the Makarska population, and the widest leaflets at 90% of the blade length were present in the South Dalmatian populations of Pelješac and Gata. The greatest mean leaflet blade width was recorded in the Psunj and Gata populations, and the greatest distance from the leaflet blade base to the point of its greatest width in the Psunj and Konavle populations.

Using Spearman's correlation coefficient, a high positive correlation was found between altitude and the five measured variables on lateral leaflets (LA, LL, LW, LW1, LW2). On the other hand, for four measured variables (LA, LW, LW1, LW2) a negative correlation was found with the average annual monthly temperature (Tab. 4). Furthermore, Tab. 5 showed the leaf blade length of the terminal leaflet to be positively correlated with altitude, and negatively correlated with the average annual temperature. From the same table it could be concluded that only two measured variables demonstrated a weaker dependence on geographical latitude, namely LWP and LW2. The LWP/LL ratio in terminal leaflets was negatively correlated with geographical latitude and altitude, and positively correlated with geographical longitude and average annual temperature (Tab. 5).

The results of the Kruskal–Wallis ANOVA are shown in Tab. 6 for lateral leaflets, and in Tab. 7 for terminal leaflets. All populations differed significantly for all studied variables. Moreover, for each individual population it was established that the trees differed with a high level of significance based on all of the studied variables ($P < 0.0001$). The differences in the variability of the two studied eco-geographic regions "continent" and "Mediterranean" were also confirmed.

From the cluster on Fig. 3 it could be concluded that at the greatest distance, the Mediterranean populations of the service tree are joined by the Psunj continental population. Application of cluster analysis revealed grouping of populations according to the eco-geographic principle.

The results of the discriminant analysis suggest that the differentiation between the studied populations is significant: Wilks' $\lambda = 0.00259$; $F\,(126.602) = 5.6454$; $P < 0.0001$. For 14 variables and 10 groups, the canonical analysis provided nine discriminant functions. From the mean values of canonical variables it could be determined that the first function differentiates to the largest extent the Kaprije population from the Rab population, and from all other studied populations (Tab. 8). Tab. 9 shows that this differentiation is most

Tab. 2 Descriptive statistical parameters for measured morphological traits – lateral leaflet.

	LA (cm²)		LL (cm)		LW (cm)		LWP (cm)		LW1 (cm)		LW2 (cm)		LA1 (°)		LA2 (°)		LW/LL		LWP/LL	
Population	***M***	***CV***	***M***	***CV***	***M***	***CV***	***M***	***CV***	***M***	***CV***	***M***	***CV***	***M***	***CV***	***M***	***CV***	***M***	***CV***	***M***	***CV***
Psunj	5.07	35.2	4.48	18.4	1.48	18.4	2.11	28.8	1.40	18.5	0.75	21.6	43.93	12.9	29.06	13.2	0.33	13.9	0.47	21.4
Istra	4.20	29.3	4.18	15.9	1.34	18.5	1.62	24.6	1.24	18.9	0.57	22.0	46.12	10.1	29.76	13.1	0.32	15.7	0.39	20.8
Bribir	3.91	30.5	4.04	17.4	1.30	17.3	1.83	22.9	1.22	17.7	0.61	24.0	42.84	11.7	28.15	13.6	0.32	14.7	0.46	16.1
Rab	3.62	33.1	3.88	18.1	1.24	18.6	1.76	24.2	1.18	18.8	0.50	23.6	44.55	11.7	28.65	12.9	0.32	14.1	0.45	16.6
Kaprije	2.92	41.0	3.54	23.0	1.08	19.9	1.75	30.4	1.03	20.3	0.55	26.0	39.98	15.6	26.80	16.4	0.31	18.8	0.49	17.2
Kozjak	4.39	29.4	4.32	16.4	1.37	16.7	1.74	24.9	1.28	17.3	0.58	24.7	45.14	13.0	29.55	13.3	0.32	15.7	0.40	20.3
Gata	3.94	27.9	3.82	15.6	1.33	16.5	1.91	24.1	1.27	16.2	0.69	24.3	46.92	9.8	30.38	12.1	0.35	13.2	0.50	19.7
Makarska	2.97	33.3	3.66	16.7	1.09	21.2	1.76	23.4	1.03	22.1	0.51	25.9	39.70	11.6	26.04	15.4	0.30	18.3	0.48	17.9
Pelješac	3.85	27.2	3.80	15.6	1.30	15.6	1.66	24.2	1.24	15.8	0.68	20.8	47.78	9.3	30.83	11.3	0.35	13.7	0.44	20.7
Konavle	4.04	29.1	4.17	16.3	1.24	18.2	1.85	28.1	1.19	18.3	0.64	25.6	44.09	10.6	27.43	14.0	0.30	17.7	0.44	20.4
Total	3.89	35.3	3.99	18.8	1.28	20.1	1.80	26.9	1.21	20.3	0.61	27.1	44.11	13.0	28.67	14.4	0.32	16.3	0.45	20.6

Tab. 3 Descriptive statistical parameters for measured morphological traits – terminal leaflet.

	LA (cm²)		LL (cm)		LW (cm)		LWP (cm)		LW1 (cm)		LW2 (cm)		LA1 (°)		LA2 (°)		LW/LL		LWP/LL	
Population	***M***	***CV***	***M***	***CV***	***M***	***CV***	***M***	***CV***	***M***	***CV***	***M***	***CV***	***M***	***CV***	***M***	***CV***	***M***	***CV***	***M***	***CV***
Psunj	4.35	32.8	3.79	17.1	1.72	19.5	1.84	21.1	1.63	18.9	0.63	27.3	40.71	18.6	32.71	17.6	0.46	16.9	0.49	13.7
Istra	3.43	38.3	3.43	18.5	1.49	22.9	1.63	19.8	1.41	21.5	0.49	27.3	41.01	15.5	31.89	14.3	0.43	14.6	0.48	12.5
Bribir	3.53	40.1	3.36	19.3	1.55	23.5	1.73	21.2	1.49	23.6	0.56	34.7	41.15	17.2	31.94	15.3	0.46	15.3	0.51	9.8
Rab	3.63	36.4	3.35	17.3	1.66	22.3	1.60	19.2	1.57	21.1	0.38	46.3	46.78	19.2	36.34	17.4	0.50	19.0	0.48	12.5
Kaprije	2.12	48.9	2.74	24.9	1.12	25.1	1.50	25.8	1.07	26.0	0.48	38.0	35.24	19.0	27.96	16.1	0.41	16.7	0.55	11.6
Kozjak	3.69	36.5	3.61	17.6	1.56	21.4	1.76	17.6	1.50	21.4	0.49	31.6	36.36	24.0	31.05	16.4	0.43	15.7	0.49	11.2
Gata	3.82	34.2	3.25	17.5	1.72	20.4	1.64	17.4	1.65	20.5	0.71	34.9	45.35	14.2	36.00	13.4	0.53	16.1	0.51	14.4
Makarska	2.64	38.4	3.14	19.7	1.31	22.8	1.68	21.1	1.25	22.9	0.44	36.9	31.93	18.8	27.10	15.3	0.42	16.4	0.54	10.5
Pelješac	3.74	33.3	3.33	15.3	1.66	21.8	1.77	17.8	1.60	22.0	0.70	32.1	42.55	13.3	33.43	13.2	0.50	16.5	0.53	11.6
Konavle	3.48	31.7	3.53	19.1	1.45	20.2	1.87	21.9	1.39	20.2	0.58	35.2	38.60	20.8	29.14	19.7	0.42	21.4	0.53	12.1
Total	3.44	40.5	3.35	20.2	1.52	24.9	1.70	21.3	1.46	24.8	0.55	39.3	39.97	21.1	31.76	18.4	0.46	18.9	0.51	13.0

Tab. 4 Correlation between lateral leaflet traits and geographical (altitude, latitude, longitude) and climatic (annual precipitation, annual mean temperature) data.

	LA	LL	LW	LWP	LW1	LW2	LA1	LA2	LW/LL	LWP/LL
Latitude	0.32 (0.37)	0.47 (0.17)	0.46 (0.18)	0.05 (0.88)	0.30 (0.40)	−0.04 (0.91)	−0.16 (0.65)	0.07 (0.85)	0.23 (0.53)	−0.02 (0.95)
Longitude	0.14 (0.70)	−0.03 (0.93)	−0.02 (0.96)	0.39 (0.27)	0.16 (0.66)	0.59 (0.07)	0.03 (0.93)	−0.01 (0.99)	0.03 (0.94)	0.13 (0.71)
Altitude	**0.88 (0.00)**	**0.72 (0.02)**	**0.83 (0.00)**	0.13 (0.72)	**0.80 (0.01)**	**0.64 (0.05)**	0.52 (0.13)	0.60 (0.07)	0.43 (0.22)	−0.36 (0.31)
Annual mean temp.	**−0.82 (0.00)**	**−0.90 (0.00)**	**−0.84 (0.00)**	−0.13 (0.71)	**−0.73 (0.02)**	−0.30 (0.40)	−0.22 (0.54)	−0.38 (0.28)	−0.36 (0.30)	0.39 (0.27)
Annual precipitation	−0.04 (0.91)	0.08 (0.83)	−0.14 (0.70)	−0.12 (0.75)	−0.19 (0.60)	−0.05 (0.88)	0.15 (0.68)	0.02 (0.96)	−0.12 (0.74)	−0.50 (0.14)

Spearman's correlation coefficient (level of significance); significance values are bolded.

Tab. 5 Correlation between terminal leaflet traits and geographical (altitude, latitude, longitude) and climatic (annual precipitation, annual mean temperature) data.

	LA	LL	LW	LWP	LW1	LW2	LA1	LA2	LW/LL	LWP/LL
Latitude	−0.16 (0.63)	0.51 (0.11)	−0.08 (0.82)	−0.25 (0.47)	−0.13 (0.71)	−0.39 (0.23)	0.37 (0.26)	0.44 (0.14)	−0.16 (0.64)	**−0.72 (0.01)**
Longitude	0.49 (0.13)	−0.16 (0.63)	0.34 (0.30)	**0.65 (0.03)**	0.36 (0.27)	**0.67 (0.03)**	−0.43 (0.19)	−0.36 (0.27)	0.22 (0.52)	**0.64 (0.03)**
Altitude	0.11 (0.74)	**0.73 (0.01)**	0.09 (0.80)	0.35 (0.29)	0.10 (0.78)	0.20 (0.56)	0.37 (0.26)	0.41 (0.22)	−0.06 (0.85)	**−0.63 (0.04)**
Annual mean temp.	−0.48 (0.16)	**−0.82 (0.00)**	−0.49 (0.15)	−0.17 (0.64)	−0.44 (0.20)	−0.15 (0.69)	−0.24 (0.50)	−0.35 (0.32)	−0.27 (0.45)	**0.83 (0.00)**
Annual precipitation	−0.08 (0.83)	0.18 (0.63)	−0.08 (0.83)	0.33 (0.35)	−0.07 (0.85)	0.04 (0.91)	0.36 (0.31)	0.15 (0.68)	0.17 (0.64)	−0.10 (0.79)

Spearman's correlation coefficient (level of significance); significance values are bolded.

Tab. 6 Kruskal–Wallis ANOVA – lateral leaflet.

Analysis	df	Test	LA	LL	LW	LWP	LW1	LW2	LA1	LA2	LW/LL	LWP/LL
Regions	1	*H*	171.8***	113.4***	174.5***	92.2***	177.5***	221.6***	0.4^{NS}	5.1*	18.5***	9.4**
		χ^2	120.0***	70.5***	125.7***	43.4***	120.5***	166.8***	0.7^{NS}	8.5**	13.4**	2.5^{NS}
Populations	9	*H*	580.4***	431.0***	592.0***	191.1***	551.8***	682.4***	593.9***	414.1***	320.9***	396.2***
		χ^2	404.3***	333.4***	416.6***	120.1***	390.2***	522.5***	433.2***	348.3***	240.9***	254.0***

*** $P < 0.001$; ** $0.001 < P < 0.01$; * $0.01 < P < 0.05$; ^{NS} $P > 0.05$.

Tab. 7 Kruskal–Wallis ANOVA – terminal leaflet.

Analysis	df	Test	LA	LL	LW	LWP	LW1	LW2	LA1	LA2	LW/LL	LWP/LL
Regions	1	*H*	134.4***	127.6***	91.9***	39.1***	85.3***	74.3***	3.6^{NS}	11.5**	0.2^{NS}	51.5***
		χ^2	102.1***	85.8***	62.8***	32.7***	61.3***	53.3***	2.6^{NS}	8.3**	0.1^{NS}	31.3***
Populations	9	*H*	604.1***	446.2***	694.3***	253.1***	699.9***	686.3***	812.4***	776.3***	589.1***	427.0***
		χ^2	380.9***	253.4***	447.3***	179.2***	450.7***	406.0***	506.2***	474.1***	421.7***	277.4***

*** $P < 0.001$; ** $0.001 < P < 0.01$; * $0.01 < P < 0.05$; ^{NS} $P > 0.05$.

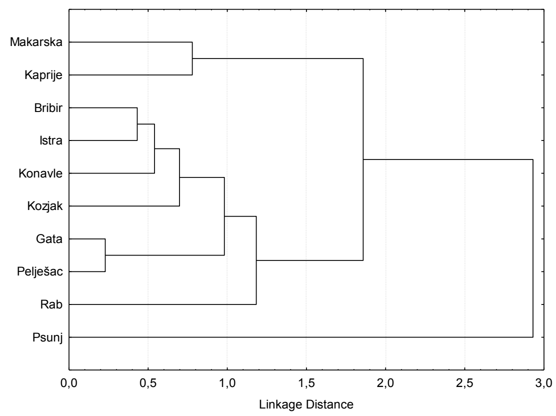

Fig. 3 Horizontal hierarchical tree diagram.

contributed to by the variables LA and LA2 of the terminal leaflet. The first function contains 27.1% of the explained variability. The second function, which together with the first accounts for 50.2% of the variability, best differentiates the Rab population from other populations. The separation of this island population from the others is mostly contributed to by LA and LL of the terminal leaflet. The third and fourth functions, together with the first two, account for 79.9% of total variability. The third function differentiates to the largest extent the Kozjak population from the Bribir, Konavle and Pelješac populations, and the fourth differentiates the Psunj population from the Mediterranean populations. Fig. 4 shows projections of canonical variables for the first two functions. The separation of island populations of Kaprije along the first axis and of Rab along the second axis is clearly visible.

Discussion

The obtained results confirmed the existence of significant variability in the measured morphological characteristics of the service tree leaves. The significant variability of morphological characteristics of the service tree leaves in three populations on the Balkan Peninsula was also found by [25], although the variation trend in this study is somewhat greater, which could be explained by greater heterogeneity of the habitats in which the populations were sampled. In addition, the said study included a considerably smaller number of studied variables, hence the results are not entirely comparable.

The significant variability of morphological characteristics of leaves was also observed in other species of the *Sorbus* genus [3,11,46–52]. Such a great diversity is partly the result of natural hybridization [53]. That can be completely ruled out for the service tree because it is the only species of the *Sorbus* genus for which there are no known hybrids with related species [54].

Leaf variability is greatly influenced by various microclimate factors, their position on the shoot and tree, and the type of shoot on which they develop [50,55–57]. In this study, those sources of variability were attempted to be reduced by collecting all leaves from the external sunlit part of the tree crown from short fertile shoots. Regardless of that, one cannot leave out the impact of microhabitat conditions that could influence the phenotypic variability of leaves. Although for the above reasons the results of morphometric research are frequently not comparable, it is evident that in relation to some other species with small population density and scattered distribution, such as, for instance, the wild service

Tab. 8 Means of canonical variables.

Group	Root 1	Root 2	Root 3	Root 4	Root 5	Root 6	Root 7	Root 8	Root 9
Psunj	−0.1793	−1.7667	−1.3351	−2.1284	0.1710	−0.7297	0.1715	−0.0508	0.1901
Istra	−0.2137	−0.0838	−0.2433	0.4478	−1.3816	0.7185	0.9199	0.4807	0.3278
Bribir	0.1067	0.4028	1.0846	−0.3313	−0.5539	0.0154	0.7572	−0.8426	−0.4439
Rab	−2.8624	2.8465	0.8044	−0.7201	0.0907	−0.0090	−0.4576	0.0699	0.1437
Kaprije	3.7862	1.4071	0.0967	−0.5782	−0.2611	0.4442	−0.6665	−0.0101	0.0897
Kozjak	−0.8088	0.1598	−3.2768	0.9791	−0.1061	0.3179	−0.4342	−0.2428	−0.2175
Gata	−0.5008	−1.6263	0.9359	−0.2391	1.2512	1.4259	−0.0809	0.2948	−0.1971
Makarska	1.0207	1.2566	−0.2571	0.8726	1.4876	−0.8841	0.7862	0.2971	0.0117
Pelješac	−0.2302	−1.5001	1.1678	1.2547	0.1569	−0.2990	−0.4316	−0.6292	0.4672
Konavle	−0.1183	−1.0958	1.0228	0.4429	−0.8546	−1.0000	−0.5639	0.6329	−0.3717

Tab. 9 Standardized coefficients for the canonical variables.

Variable	Root 1	Root 2	Root 3	Root 4	Root 5	Root 6	Root 7	Root 8	Root 9
LA (L)	0.9607	−0.3301	0.2895	−0.9026	2.0930	−2.3776	−1.1210	2.4495	3.6619
LL (L)	−0.3935	0.4109	−0.7690	2.4826	−2.6921	4.0833	−1.9575	−1.0498	−1.8182
LW (L)	−0.7555	0.1171	0.4401	−0.8716	−0.5677	−1.2285	4.2401	−2.7855	−2.7462
LWP (L)	0.0149	0.0591	−0.0507	−0.4673	0.9063	1.7129	−0.3905	1.4461	−0.4035
LW2 (L)	−0.0975	−0.7883	−0.1014	−0.3782	−0.4304	−1.0843	−0.6703	−0.2338	0.4153
LA1 (L)	−1.8414	−0.9390	0.6613	1.3615	1.0481	−0.2163	0.8478	0.4587	−0.9758
LA2 (L)	2.2469	0.8464	−1.4940	0.3234	−1.3781	3.4529	−3.2407	1.4874	1.9857
LA (T)	3.2526	3.0222	−1.6701	−1.5856	−0.4709	1.3460	−1.8390	−4.0411	−0.3768
LL (T)	−1.9632	−3.1055	−1.1687	−1.4346	0.2784	−1.5162	1.5048	4.0930	0.2817
LWP (T)	−0.9656	1.3053	−0.5452	0.4422	−0.6263	−2.1794	−2.0141	−2.1899	−1.1312
LW (T)	−1.6244	−1.0533	3.6385	2.5348	1.3450	0.7757	2.3837	1.8309	1.6821
LW2 (T)	0.4442	−1.1338	0.0306	0.2490	0.1044	0.8587	0.4909	0.4439	−0.5901
LA1 (T)	0.4346	0.0288	4.5761	−0.3418	−1.6680	0.7575	1.6140	1.0963	0.9003
LA2 (T)	−2.2314	0.3295	−6.6073	−1.4161	0.5844	−2.4842	−4.0212	−1.7860	−2.4515
Eigenvalue	2.7368	2.3416	1.9738	1.0268	0.7629	0.5835	0.3800	0.2199	0.0902
Cumulative proportion of explained variation	0.2706	0.5020	0.6972	0.7987	0.8741	0.9318	0.9693	0.9911	1.0000

tree (*Sorbus torminalis*) [50,51] and the wild pear (*Pyrus pyraster* /L./ Burgsd.) [58], the service tree populations have a high degree of morphological variability.

Based on the Kruskal–Wallis ANOVA, statistically significant differences were confirmed for all studied variables on the intra- and inter-population level. Similar results were obtained by [25] for three populations of the service tree on the Balkan Peninsula. As the service tree is a rare species with a small population density and a high degree of fragmentation and isolation, and in line with population genetics theories, a decreased genetic diversity and high degree of differentiation are to be expected [18]. The studies of genetic diversity of the service tree populations conducted to date on a relatively small sample did not completely confirm this expected variation pattern, and genetic variability was similar to the variability of widely distributed species, although subpopulations were found to be genetically more different than the widely distributed species, but less than what was expected for fragmented and isolated populations [18,26]. A very similar variation pattern was obtained by [59] in their genetic research of the populations of the wild service tree in France. By partitioning the total variance to sources of variability for four of the eight studied morphological characteristics, [25] found a greater inter-population variability in comparison with the intra-population one. Contrary to that, in the studies of leaf variability of widely distributed

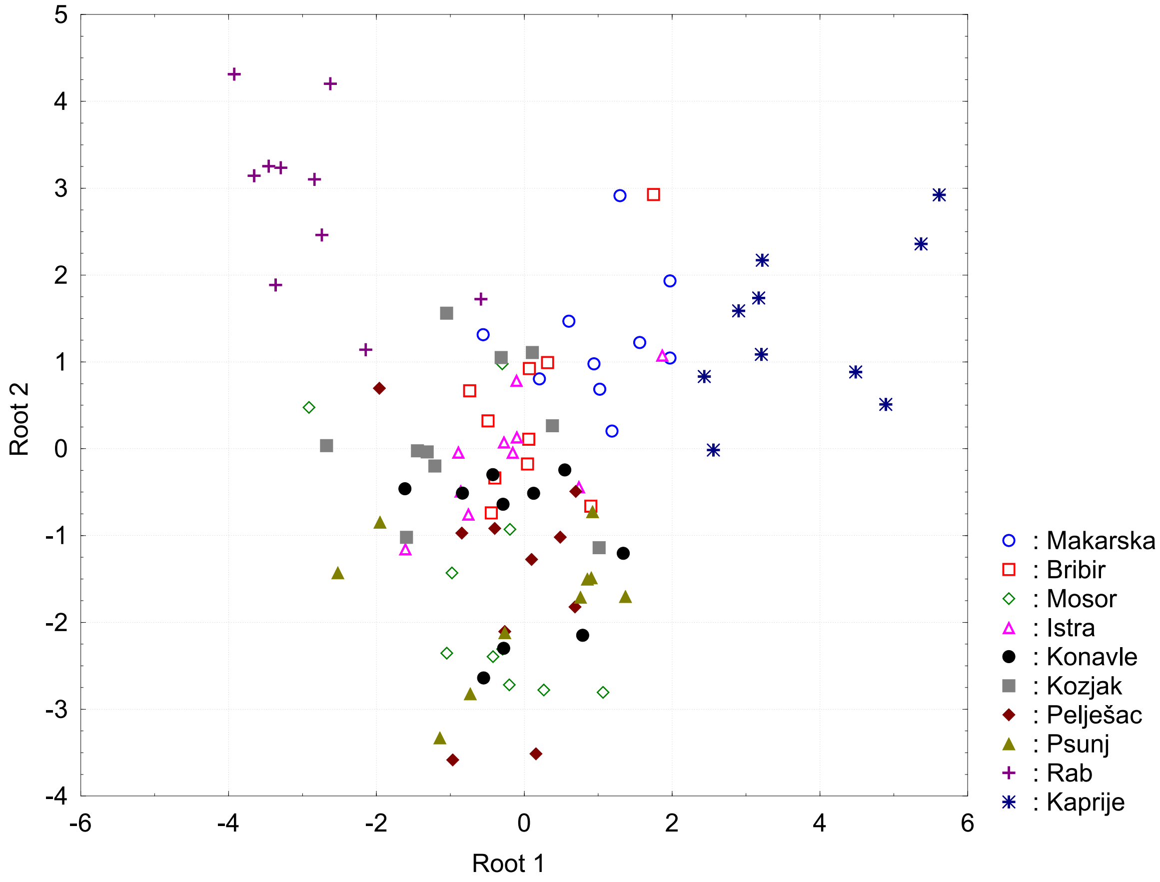

Fig. 4 Scatterplot of the canonical scores for 10 studied populations of *Sorbus domestica*.

woody species by methods of classic morphometric analysis, it is common for a greater share in the overall variability to be accounted for by the variability of trees within populations [25,31,33,51]. Although the service tree populations in Croatia demonstrated a high degree of interpopulation variability, the study also found a high variability between trees within populations, which suggested a high gene flow between populations. Similar research on other scattered forest tree species has demonstrated that the genetic system of rare species is well adjusted for the small number of units [18].

In addition to significant inter- and intra-population variability, descriptive statistical methods and the application of the analysis of variance have also contributed to establish differences between the studied biogeographic regions "continent" and "Mediterranean". Although the research included only one service tree population from the continental region, the observed differentiation was significant for the majority of studied characteristics. This observed pattern of morphological divergence between two different biogeographic regions is also visible from the results of descriptive analysis. The observed differentiation was mostly manifest in the characteristics: leaflet blade area (LA), leaflet blade width (LW), leaflet blade width at 50% of blade length (LW1) and leaflet blade width at 90% of blade length (LW2). In other words, Mediterranean populations were characterized by smaller leaflets in comparison with the Psunj continental population. Such differences in variability were also found in other woody species that occurred both in the continental and in the Mediterranean regions. In the study of morphological diversity of the *Quercus pubescens* in Croatia, [60] found a similar ecological–geographical pattern of population variations. Likewise, for the populations of the *Fraxinus angustifolia* Vahl in Croatia, through ecological modeling [61] found a clear ecological divergence between the continental and the Mediterranean populations, which was also clearly reflected in their genetic structure. The observed trend of separation of the service tree populations on the ecological–geographical principle was also confirmed by the applied cluster analysis.

The canonical discriminant analysis additionally clarified the trend of population differentiation. As the share of each of the first four discriminant functions is relatively large, the results of this analysis additionally confirm a high degree of divergence between populations. The first two functions, each of which accounts for about 25% of overall variability, undoubtedly suggest the separation of the two island populations of Rab and Kaprije. Although the distance from the mainland to the respective islands is relatively small, such isolation could have conditioned the genetic structure of these populations. From the results of the descriptive statistical analysis it is visible that these two populations are distinguished in terms of variables that describe more closely the shape of the blade base of the terminal leaflet. The Rab population was characterized by the highest values for LA1, while on the other hand the Kaprije population was characterized by the lowest value for the said characteristics. In other words, the Rab population had the most rounded blade base of the terminal leaflet, while the Kaprije population had the most pointed one. Although it is an accepted rule that with increased distance from the island to the mainland, genetic variability is reduced and thus genetic differentiation of populations is increased [62], the *CV*s for individual studied characteristics in these two populations are extremely high, which suggests high population heterogeneity. Moreover, for the Kaprije population, the highest *CV*s were observed for the majority of studied characteristics. Such results also suggest a certain gene flow between the island and mainland populations, and the explanation for it should be sought in pollination and seed dispersal.

On the other hand, the service tree has been a highly valued species since the times of the Roman Empire, planted and cultivated for its fruits and good quality wood [17,18]. Considering the above, one could not exclude the impact of man on the variability of service tree populations, although the samples in this research were collected in populations considered to be natural. In addition, according to Matić and Vukelić [19], in Croatia, in addition to forest areas, the service tree also occurs cultivated, in orchards, along vineyards, roads, etc. Because of that, there is also a certain possibility of gene introgression into natural populations via pollen and seed of cultivated service trees from orchards, which can affect their variability.

In addition to the established pattern of ecotypical variability between the continental and the Mediterranean regions, the clinal variability with regard to altitude was also confirmed. With increased altitude, LW, LW1, LW2 and LL also increase. As opposed to that, for the same characteristics a negative correlation was found with regard to the mean annual temperature. This variation trend is associated with ecological conditions in which the studied populations occur. The populations in the Mediterranean region on lower altitudes are relatively close to the sea and are exposed to higher temperatures on average. At the same time they have smaller leaflet surfaces, and shorter and narrower lateral leaflets. With increased altitude and reduced mean annual temperature, the values for the said measured characteristics increase. This form of ecological divergence of the service tree populations in Croatia is also confirmed by their phytosociological characteristics. The trees of the Rab and Kaprije island populations and of the Makarska population phytosociologically belong to the association *Fraxino orni-Quercetum ilicis* Horvatić (1956) 1958. Other Mediterranean populations phytogeographically belong more or less to the sub-Mediterranean vegetation zone, where the service tree is most frequently represented as an element in the *Querco pubescenti-Carpinetum orientalis* Horvatić 1939 association. Small, although statistically significant clinal variability with regard to the longitude was recorded only for two measured characteristics of terminal leaflets: distance between the leaflet widest part and the leaflet base (LWP) and leaflet blade width at 90% of blade length (LW2). It is also interesting that the LWP/LL ratio in terminal leaflets shows clinal variability. With decreased latitude and increased longitude this ratio increases. Small clinal variability with regard to latitude [50] and altitude [63] was also found for the wild service tree populations in Poland and in Croatia.

The conducted study provides insights into the variability of the service tree populations in Croatia, which is a basis for further research that should be implemented for the breeding and conservation of genetic resources of this rare and endangered noble hardwood species. In order to confirm the conclusions reached on the variability of the service tree populations obtained by morphometric methods, the research also needs to be extended to molecular methods.

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Authors' contributions

The following declarations about authors' contributions to the research have been made: concept of the study: IP, MI; field work: IP, ILJ; leaf measurement: ILJ; statistical analysis: IP; writing the manuscript: IP, DK, MI.

Competing interests

No competing interests have been declared.

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