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Taxonomic relationships and population differentiation of the south-western Eurasian *Zelkova* species inferred in leaf morphology

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Abstract: The relict tree species *Zelkova abelicea* and *Z. sicula* (Ulmaceae) occur in Crete and Sicily, respectively. *Zelkova abelicea* grows in approximately 40 localities in the mountains of Crete, while *Z. sicula* has been found in only two stands in Sicily.

We compared 25 morphological characters of the leaves of both species and used statistical methods (Tukey's test, discrimination analysis, principal component analysis, agglomeration) to reveal the differences between these two species, their relation to *Z. carpinifolia* (the third *Zelkova* south-west Eurasian species), and between the leaves from different shoot types.

Our study represents the first comparative biometric analysis of the three *Zelkova* species localized in the western edge of the current geographic range of the genus. We found that the species differed from each other both, in terms of leaf form characters and level of leaf variation. *Zelkova carpinifolia* was clearly different from *Z. abelicea,* while the two populations of *Z. sicula*, SIB and SIA, were more similar to *Z. carpinifolia* and *Z. abelicea*, respectively. The latter finding supports the results of recent molecular studies suggesting the hybrid origin of *Z. sicula*.

Zelkova abelicea, *Z. sicula* and *Z. carpinifolia* differ each other in the leaf characteristics and level of phenotypic variation.

Keywords: biogeography, plant variation, relict trees, systematics, taxonomy, Ulmaceae

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Introduction

The genus *Zelkova* includes six species, the three in the Eastern Asia (*Z. serrata* (Thun.) Makino, *Z. sinica* C. K. Schneid. and *Z. schneideriana* Hand.-Mazz.), and three (*Z. carpinifolia* (Pall.) K. Koch, *Z. abelicea* (Lam.) Boiss. and *Z. sicula* Di Pasq., Garfì & Quézel) in the western part Asia and eastern Mediterranean region (Kozlowski & Gratzfeld, 2013; Kozlowski et al., 2014). The East-Asiatic species and Euxino-Hyrcanian *Z. carpinifolia* occurs in the mesic climate (Prilipko, 1961; Browicz & Zieliński, 1982; Kozlowski & Gratzfeld, 2013), whilst Mediterranean *Z. abelicea* and *Z. sicula* live under typical Mediterranean climate conditions, the later with long-lasting summer hot and dry period (Rivas-Martínez et al., 2004). Due to their rarity and conservation status, both insular species are threatened in their natural ranges. According to IUCN red list criteria, *Z. abelicea* is considered endangered (EN), while *Z. sicula* is critically endangered (CR) (Garfì et al., 2017; Kozlowski et al., 2018).

Zelkova abelicea can grow as a tree up to 15 m high or as a shrub, the latter habit frequently resulting from browsing disturbance (Sarlis, 1987; Christensen, 1997; Fazan et al., 2012). It is found in isolated populations on the five mountain ranges of Crete, between 800 and 1800 m a.s.l., mostly on calcium-rich soils issuing from Triassic metamorphic limestone (Søndergaard & Egli, 2006; Goedecke & Bergmeier, 2018). Its distribution extends up to the supra-Mediterranean climate belt (Rivas-Martínez et al., 2004). The lowermost known localities are on north-facing rocky slopes, while the uppermost stands on south-facing slopes (Bosque et al., 2014; Kozlowski et al., 2014; Goedecke & Bergmeier, 2018). The long-lasting isolation of the populations living in the different mountain massifs appears the main cause of their current genetic differentiation (Christe et al., 2014; Naciri et al., 2019). The global population of *Z. abelicea* include approximately 20,000 fructifying trees mostly concentrated in the Lefka Ori (West Crete) (Kozlowski et al., 2014).

Zelkova sicula grows as a shrub of 3–4 (max 8) m high and potentially as a small tree. It is known from two localities where it survives forming two different clonal populations (Garfì et al., 2017), both of them probably having a hybrid origin (Christe et al., 2014). The two populations occur mostly along the bottom of seasonal streams that dry out during summers, between 320 and 525 m a.s.l., respectively (Garfì et al., 2011), in the thermo-Mediterranean climate belt (Rivas-Martínez et al., 2004). Some individuals form flowers and fruits, but they do not produce viable seeds, so the species regenerates only by root suckering (Garfì et al., 2017).

Leaves of trees are basic from the taxonomic point of view (Krüssmann, 1962), despite their relatively high level of variation and susceptibility to influence of environmental conditions (e.g. Marcysiak, 2012a, b, c; Pérez-Harguindeguy et al., 2013). The leaves of plants living in mesic regions are as a rule larger and have thinner epidermis and mesodermis layers, comparing to plants leaving in more xeric regions (Krüssmann, 1962; Lakusic et al., 2010; Zhong et al., 2014). The leaf blade length, width and shape, the number of marginal teeth and secondary veins are species specific, but influenced by their function and position on the vegetative *versus* reproductive shoots and position within tree crown (Mejnartowicz, 1972; Wójcicki, 1997; Wang et al., 2001; Jasińska et al., 2015). The leaf size and shape of specimens exposed to the animal browsing are different when compare to the leaves from undamaged specimens (Zieliński & Güner, 2000; Denk & Grimm, 2005; Kozlowski & Gratzfeld, 2013).

In the genus *Zelkova*, the leaf morphology represents a set of key diagnostic characters, providing basic elements in studies on phylogeny and biogeography (Denk & Grimm, 2005). The species can be distinguished based on fruit and leaf characters

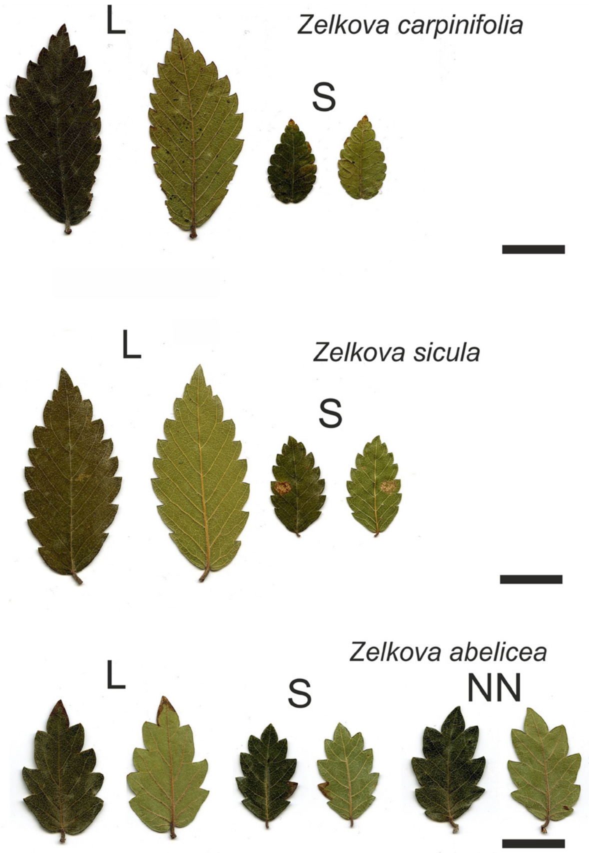

Fig. 1. Scanned leaves of *Zelkova carpinifolia*, *Z. sicula* and *Z. abelicea*: L – leaves from vegetative shoots, S – leaves from fertile shoots, NN – leaves of *Z. abelicea* from non fruiting trees; in left column adaxial (upper) and in right column abaxial (underside) leaf surface in every leaf category; bar = 2 cm

(Quézel et al., 1993; Wang et al., 2001; Fu et al., 2003; Kozlowski & Gratzfeld, 2013). However, except for *Z. carpinifolia* (Jasińska et al., 2015), so far investigations and comparative analyses based on leaf morphometry have not yet been conducted. The *Zelkova* leaves from sterile/vegetative shoots have been usually described as different than those of fertile/fruiting twigs (Fig. 1). The first are usually larger than the latter, and are more regularly serrated (Denk & Grimm, 2005; Rucińska, 2012; Jasińska et al., 2015). However, when considered individually, leaves from vegetative shoots of one species can be sometimes confused with the leaves of fertile shoots of another species. Also, leaves from individuals impacted by external disturbances (e.g. browsing) can even pose problems of taxonomic misidentification. This is the case of most populations of *Z. abelicea*, where permanent browsing induces the prevalence of extremely small and deformed leaves.

Based on the morphological characters of *Z. carpinifolia* used by Jasińska et al. (2015), we compared the all three south-western Eurasian species of *Zelkova* in the aim to: (1) point out leaf characteristics discriminating among species, (2) evaluate the possible compliance of fruiting and vegetative shoots, from one species to another and (3) outline the geographical and taxonomic patterns of morphological differentiation among the three investigated species and the possible consistency with findings issuing from the genetic investigations (e.g. Christe et al., 2014).

Material and methods

Plant material and measurement

We analyzed both known populations of *Z. sicula* and 11 populations of *Z. abelicea*. From fruiting trees, leaves were collected separately from sterile long-shoots (L) and fertile dwarf-shoots (S). In case of lack of fruiting specimens of *Z. abelicea*, the non-fruiting, possibly browsed trees were also sampled. In this case, the leaves from not injured parts of tree crowns were gathered without distinguishing between types of shoots (NN). From each individual, up to 10 leaves per shoot-type were sampled (Table 1). All the material was collected from the sunny side of the tree crowns, 1–3 m above ground level, from the central parts of the twigs. The leaves were pressed, dried as herbarium material, and conserved in this state until measurement. Data on *Z. carpinifolia* from Jasińska et al. (2015) were included in the analyses for comparison. In total, 3325 leaves from 361 individuals representing 20 populations were analyzed (Fig. 2).

A number of leaf characteristics with diagnostic/discriminant value were selected, including 16 simple traits (e.g. blade length and width, number of teeth and secondary veins, angle of leaf blade apex and base, etc.) and 9 proportional characters (e.g. length/width ratio, number of nerves/number of teeth ratio, asymmetry of leaf blade, etc; see Table 2 for the complete list and codes and electronic supplementary materials, Fig. S1 for some details).

Table 1. Studied populations of *Z. sicula*, *Z. abelicea* and *Z. carpinifolia*

Species	Locality	Code	N	Leaf type (number of leaves)	Longitude E [°]	Latitude N [°]	Altitude [m]
Z. sicula	Italy, Sicily 1	SIA	9	L(75), S(90)	14.861	37.171	510
	Italy, Sicily 2	SIB	12	L(117), S(119)	15.045	37.210	330
Z. abelicea	Greece, Lefka Ori, Omalos 1	OMA1	20	NN(200)	23.912	35.316	1231
	Greece, Lefka Ori, Omalos 2	OMA2	10	L(99), S(100)	23.903	35.309	1265
	Greece, Lefka Ori, Ambelitsias 1	AMB1	20	NN(200)	23.986	35.367	1269
	Greece, Lefka Ori, Ambelitsias 2	AMB2	3	L(20), S(26)	23.981	35.355	1429
	Greece, Lefka Ori, Niato	NIA	22	NN(220)	24.155	35.288	1221
	Greece, Psiloritis, Mt. Kedros	KED1	9	L(90), S(88)	24.627	35.188	1293
	Greece, Psiloritis, Mt. Kedros	KED2	3	NN(30)	24.627	35.188	1293
	Greece, Psiloritis, Rouvas	PSI	7	NN(70)	24.929	35.179	1334
	Greece, Lasithi, Kéfala 1	LAS1	3	L(30), S(30)	25.538	35.170	1196
	Greece, Lasithi, Kéfala 2	LAS2	3	NN(29)	25.538	35.170	1196
	Greece, Thripiti, Afentis Stavromenas	STA	20	NN(200)	25.888	35.081	1151
*Z. carpinifolia**	Turkey, Trabzon	TRA	7	S	39.866	40.950	76
	Georgia, Ajametis Nature Reserve	ANR	20	L, S	42.763	42.143	150
	Georgia, Vani	VAN	16	L, S	42.565	42.089	100
	Georgia, Babaneuri Nature Reserve	BAB	17	L, S	45.371	42.081	470
	Azerbaijan, Xabulan	XAN	10	L, S	48.800	38.661	40
	Azerbaijan, Parakand	PAR	10	L, S	48.803	38.650	40
	Azerbaijan, Güneshli (*Z. hyrcana*)	GUN	10	L, S	48.469	38.805	650

* after Table 1 in Jasińska et al. (2015).

N – number of sampled individuals; leaf-types collected from fructifying individuals: L vegetative shoot, S fertile shoot; NN – leaf collected from non-fructifying individuals.

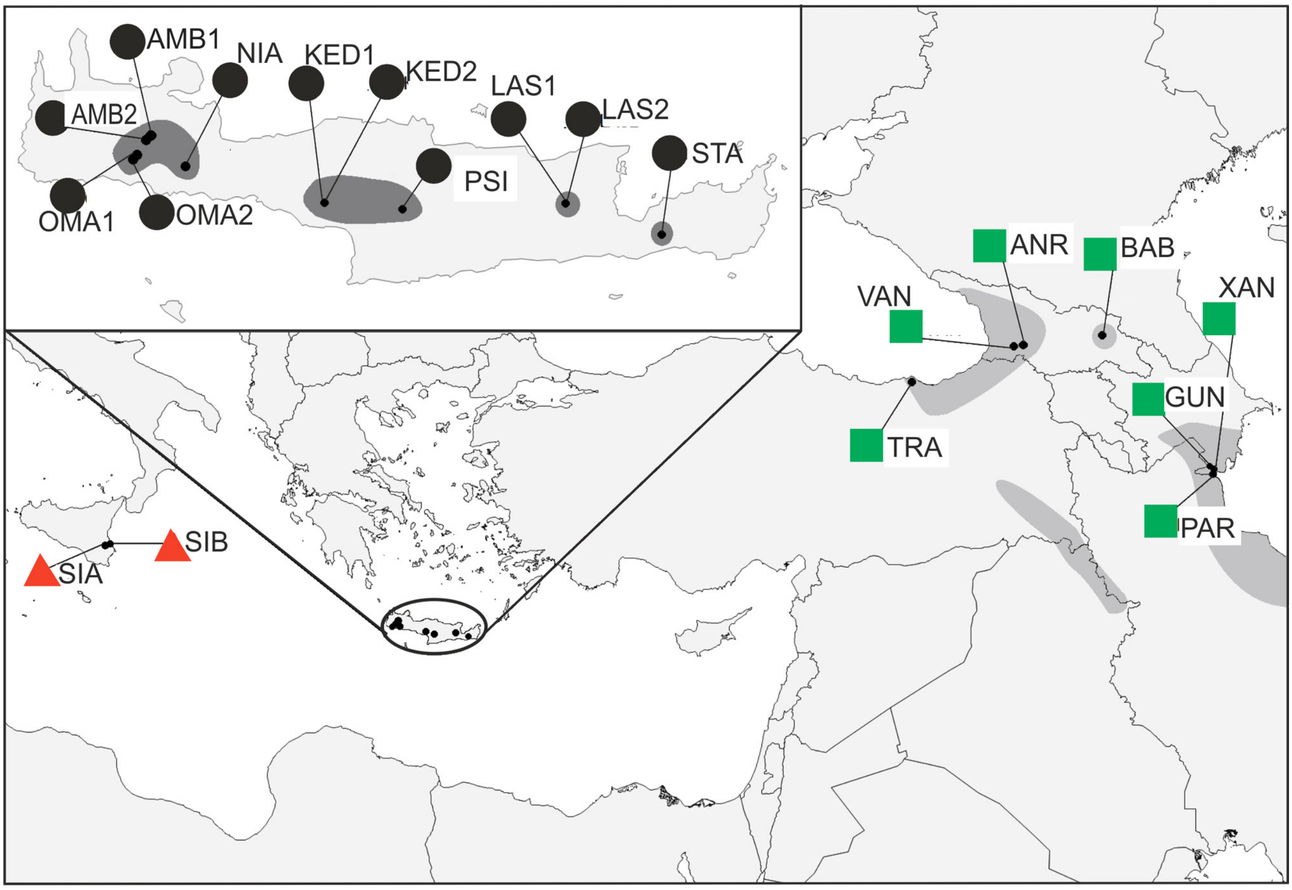

Fig. 2. Geographic distribution of studied populations of *Zelkova carpinifolia*, *Z. abelicea* and *Z. sicula* (acronyms as in Table 1); shaded – area of distribution of *Z. carpinifolia* in Asia (after Browicz & Zieliński 1982a, simplified) and *Z. abelicea* on Crete (after Kozlowski & Gratzfeld 2013, simplified)

Leaf characteristics were measured on scanned images, following the method described in Jasińska et al. (2015). Measurements were performed on scanned images using WinFolia software (Regent Instrument).

Statistics

The statistical distribution of each leaf character was assessed for data symmetry and unimodality using the Shapiro-Wilk test (Shapiro & Wilk, 1965). Data with skewed distributions were log-transformed. The homoscedasticity of variances was verified using the Brown-Forsythe test to ensure the appropriateness of parametric statistical tests in multivariate analyses (Zar, 1999; Sokal & Rohlf, 1995).

Possible interactions between characters were determined using Pearson's correlation coefficient (Garren, 1998). The differences in the mean values of characters between populations and species were tested using Tukey's *post hoc* honestly significant differences *T*-test and the *t*-test for independent samples with Cochran-Cox correction for non-homogeneous variances (Zar, 1999; Sokal & Rohlf, 1995; Stanisz, 2007). The similarities among species, populations and leaves based on L-, S- and NN-types of shoots were evaluated using a discrimination function analysis followed by agglomeration with Ward's method on the Euclidean and Mahalanobis distances (Sokal & Rohlf, 1995; Stanisz, 2007).

The proportional characters (LA, PV, W_1, W_2, AP, LBW, DI, and LS, see Table 2), the simple traits not included within the recalculated characters (AA, AB) and the traits important for the differentiation between species (BL, BW, TN) were used in the multivariate analyses. All other characters were excluded due to their strong correlations with each other ($r \leq |0.95|$). The data were standardized before analyses (Sokal & Rohlf, 1995). STATISTICA 9 PL software (StatSoft Poland, Kraków) was used for calculations.

To evaluate the geographical and morphological multi-character relationships among populations and species, the Mantel test (Mantel, 1967) was performed between the matrices of Mahalanobis and geographical distances using the software Barrier 2.2 (Manni et al., 2004) and PopTools v. 3.2.5 (Hood, 2010). The geographical distances between populations were determined using MapInfo Professional 10.5 PL software (Pitney Bowes) based on their geographic coordinates.

Table 2. Average values of leaf characters of *Z. carpinifolia* (after Jasińska et al. 2015), *Z. abelicea* and *Z. sicula* (original data)

Leaf character	Code	*Zelkova carpinifolia*														
		L					S					NN				
		N	M	Min	Max	V	N	M	Min	Max	V	N	M	Min	Max	V
Leaf blade surface (cm^2)	A	712	12.28	3.21	37.90	35.02	768	4.37	1.15	11.61	37.27					
Leaf blade circumference (cm)	P	712	19.59	9.70	36.73	20.66	768	11.21	3.96	21.38	21.68					
Leaf blade length (cm)	BL	713	5.83	2.54	10.62	16.06	769	3.43	1.13	6.26	21.72					
Leaf blade maximum width (cm)	BW	713	2.97	1.44	5.53	20.35	769	1.82	0.59	2.92	18.26					
Leaf blade width in 50% of length (cm)	BW_50	713	2.78	1.31	5.28	20.28	769	1.68	0.48	2.79	19.14					
Leaf blade width in 90% of length (cm)	BW_90	713	0.77	0.26	2.30	27.64	769	0.51	0.15	1.19	25.92					
Distance between midrib and denticle apex at maximal width of leaf blade. along the lateral vein (cm)	LLV	713	2.29	1.05	4.33	19.15	769	1.31	0.34	2.30	24.13					
Distance between midrib and denticle apex below maximal width of leaf. along the lateral vein (cm)	LV	713	2.13	1.01	4.07	19.56	768	1.21	0.35	2.26	24.58					
Distance between midrib and indentation between LLV and LV (cm)	LI	713	1.85	0.88	3.65	18.99	768	1.04	0.33	1.89	23.19					
Distance from basis to maximal width of leaf (cm)	LWP	713	2.64	0.92	5.48	21.15	769	1.43	0.09	3.04	31.58					
Angle of leaf blade apex (°)	AA	710	38.59	13.21	98.73	16.10	768	42.97	18.19	88.15	19.07					
Angle of leaf blade basis (°)	AB	708	99.34	48.92	168.63	9.75	766	99.92	49.33	160.55	11.20					
Number of leaf denticles on one side of leaf blade	TN	712	10.19	6	16	11.19	769	8.84	5	15	14.81					
Number of lateral veins of one side of leaf blade	NVT	712	9.45	5	15	11.66	768	8.19	4	15	15.24					
Length of longer side of blade (cm)	LHL	713	5.79	2.62	10.58	16.84	768	3.48	1.18	7.60	23					
Length of shorter side of blade (cm)	SHL	713	5.98	2.83	10.82	16.97	768	3.55	1.19	7.88	22.2.					
Asymmetry of leaf blade [100× (LHL − SHL / LHL)]	LA	713	3.06	0.00	12.67	65.28	768	2.03	0.00	10.31	63.6					
Ratio of number of nerves to number of teeth [100× (NVT / TN)]	PV	712	92.86	66.67	100.00	4.05	768	92.80	44.44	120.00	4.0					
Leaf blade width in 90% / maximum width [100× (BW_90 / BW)]	W_1	713	26.09	14.12	71.09	15.77	769	27.89	13.91	58.04	14.6					
Leaf blade width in 50% / maximum width [100× (BW_50 / BW)]	W_2	713	93.82	78.68	100.00	2.57	769	92.11	65.70	100.00	3.4					
Leaf blade width in 90% / width in 50% of length [100× (BW_90 / BW_50)]	W_3	713	27.82	14.87	74.18	15.52	769	30.25	14.68	63.36	13.3					
Leaf blade surface to circumference ratio (A / P)	AP	712	0.60	0.33	1.05	16.86	769	0.38	0.14	0.63	14.9					
Position of leaf maximal width [100× (LWP / BL)]	LBW	713	44.97	23.44	67.26	10.73	769	40.84	4.15	60.06	14.6					
Serration depth [(LLV + LV) / 2 − LI]	DI	713	0.36	0.14	0.73	25.17	769	0.22	0.04	0.53	32.1					
Leaf blade length/width (BL / BW)	LS	713	2.00	1.08	3.09	12.47	769	1.89	1.12	2.99	12.6					

L – vegetative shoots, S – fructifying shoots, NN – undetermined shoot from browsed specimens; N – number of leaves measured; M – arithmetic mean value, Min – minimum, Max – maximum, V – variation coefficient; shaded columns indicate mean values.

Table 2 continued

Leaf character	Code	*Zelkova abelicea*														
		L					S					NN				
		N	M	Min	Max	V	N	M	Min	Max	V	N	M	Min	Max	V
Leaf blade surface (cm^2)	A	249	3.29	0.55	9.08	35.06	244	1.80	0.53	4.55	32.80	949	1.51	0.10	17.51	112.56
Leaf blade circumference (cm)	P	249	8.68	3.89	15.09	19.78	244	6.10	3.21	10.11	19.93	949	5.12	1.36	22.24	57.68
Leaf blade length (cm)	BL	249	2.81	1.32	4.71	19.88	244	1.98	1.00	3.38	19.98	949	1.58	0.38	5.42	53.26
Leaf blade maximum width (cm)	BW	249	1.59	0.60	2.94	19.41	244	1.22	0.66	1.96	17.53	949	1.00	0.31	4.47	58.36
Leaf blade width in 50% of length (cm)	BW_50	249	1.49	0.56	2.62	19.56	244	1.14	0.62	1.82	16.96	949	0.93	0.27	3.82	56.59
Leaf blade width in 90% of length (cm)	BW_90	249	0.49	0.18	1.04	23.27	244	0.40	0.17	0.78	20.90	949	0.32	0.07	1.14	42.59
Distance between midrib and denticle apex at maximal width of leaf blade. along the lateral vein (cm)	LLV	249	1.10	0.35	2.00	18.95	244	0.82	0.38	1.40	19.02	949	0.66	0.19	2.72	56.00
Distance between midrib and denticle apex below maximal width of leaf. along the lateral vein (cm)	LV	249	0.99	0.33	1.76	19.33	244	0.74	0.30	1.26	20.91	949	0.59	0.16	2.38	54.96
Distance between midrib and indentation between LLV and LV (cm)	LI	249	0.82	0.23	1.45	19.53	244	0.62	0.29	1.09	19.72	949	0.49	0.14	2.10	54.21
Distance from basis to maximal width of leaf (cm)	LWP	249	1.22	0.35	2.32	21.12	244	0.80	0.30	1.66	28.91	949	0.68	0.15	2.28	49.19
Angle of leaf blade apex (°)	AA	248	47.92	23.91	76.41	11.20	243	48.80	27.54	73.63	10.83	948	53.28	27.39	97.72	9.90
Angle of leaf blade basis (°)	AB	248	89.38	54.15	138.72	11.24	244	89.79	54.02	127.71	9.63	948	88.87	38.22	153.65	13.45
Number of leaf denticles on one side of leaf blade	TN	249	5.16	3.00	8.00	13.73	244	4.77	3.00	7.00	12.14	949	4.73	3.00	8.00	12.89
Number of lateral veins of one side of leaf blade	NVT	249	5.12	3.00	8.00	13.33	244	4.68	3.00	7.00	11.82	949	4.66	3.00	8.00	12.89
Length of longer side of blade (cm)	LHL	249	2.81	1.33	4.72	19.78	244	1.99	1.02	3.39	19.75	949	1.59	0.38	5.81	53.48
Length of shorter side of blade (cm)	SHL	249	2.85	1.34	4.76	19.49	244	2.01	1.03	3.43	19.73	948	1.62	0.40	5.85	53.47
Asymmetry of leaf blade [100× (LHL − SHL / LHL)]	LA	249	1.32	0.00	5.33	46.05	244	1.20	0.00	5.67	27.87	948	1.67	0.00	10.51	37.87
Ratio of the number of nerves to the number of teeth [100× (NVT / TN)]	PV	249	99.35	80.00	100.00	1.16	244	98.25	75.00	100.00	2.91	948	98.62	71.43	100.00	2.00
Leaf blade width in 90% / maximum width [100× (BW_90 / BW)]	W_1	249	31.20	18.33	49.68	13.10	244	32.61	14.73	53.53	14.77	949	34.75	17.54	63.21	19.84
Leaf blade width in 50% / maximum width [100× (BW_50 / BW)]	W_2	249	93.66	74.99	100.00	5.56	244	93.27	76.68	100.00	2.00	949	94.21	65.55	100.00	2.41
Leaf blade width in 90% / width in 50% of length [100× (BW_90 / BW_50)]	W_3	249	33.31	20.17	54.10	12.19	244	34.94	15.81	57.74	13.96	949	36.84	18.26	68.06	18.65
Leaf blade surface to circumference ratio (A / P)	AP	249	0.36	0.14	0.62	16.97	244	0.28	0.16	0.45	15.56	949	0.22	0.07	0.79	51.06
Position of leaf maximal width [100×(LWP / BL)]	LBW	249	43.49	24.57	58.94	7.25	244	39.92	21.79	59.64	12.58	949	43.85	22.86	64.68	8.01
Serration depth [(LLV + LV) / 2 − LI]	DI	249	0.22	0.11	0.49	25.38	244	0.15	0.05	0.32	27.52	949	0.14	0.03	0.69	63.68
Leaf blade length/width (BL / BW)	LS	249	1.79	1.27	2.36	12.27	244	1.62	1.07	2.24	10.68	949	1.62	1.11	2.41	10.68

L – vegetative shoots, S – fructifying shoots, NN – undetermined shoot from browsed specimens; N – number of leaves measured; M – arithmetic mean value, Min – minimum, Max – maximum, V – variation coefficient; shaded columns indicate mean values.

Table 2 continued

Leaf character	Code	*Zelkova sicula*														
		L					S					NN				
		N	M	Min	Max	V	N	M	Min	Max	V	N	M	Min	Max	V
Leaf blade surface (cm^2)	A	192	6.71	2.08	12.41	27.01	209	2.32	1.03	4.42	20.20					
Leaf blade circumference (cm)	P	192	13.51	6.74	18.73	17.41	209	7.72	5.11	10.72	12.04					
Leaf blade length (cm)	BL	192	4.19	2.09	6.09	19.32	209	2.39	1.49	3.50	13.48					
Leaf blade maximum width (cm)	BW	192	2.33	1.44	3.36	12.07	209	1.40	0.92	1.92	10.24					
Leaf blade width in 50% of length (cm)	BW_50	192	2.20	1.12	3.19	11.86	209	1.31	0.79	1.91	10.69					
Leaf blade width in 90% of length (cm)	BW_90	192	0.56	0.31	1.22	16.48	209	0.41	0.17	0.75	13.75					
Distance between midrib and denticle apex at maximal width of leaf blade. along the lateral vein (cm)	LLV	192	1.78	1.13	2.55	12.91	209	1.11	0.70	1.82	11.48					
Distance between midrib and denticle apex below maximal width of leaf. along the lateral vein (cm)	LV	192	1.63	1.04	2.46	13.20	209	1.01	0.56	1.71	11.37					
Distance between midrib and indentation between LLV and LV (cm)	LI	191	1.39	0.81	2.04	12.61	209	0.84	0.47	1.45	12.57					
Distance from basis to maximal width of leaf (cm)	LWP	192	1.79	0.56	3.25	24.09	209	1.02	0.44	1.86	16.73					
Angle of leaf blade apex (°)	AA	191	40.94	26.75	77.07	17.12	209	48.71	26.71	77.66	14.54					
Angle of leaf blade basis (°)	AB	192	97.37	60.36	132.96	8.51	209	90.43	56.31	127.51	7.77					
Number of leaf denticles on one side of leaf blade	TN	192	7.13	5.00	9.00	7.50	209	5.82	3.00	8.00	5.85					
Number of lateral veins of one side of leaf blade	NVT	192	6.85	4.00	9.00	9.64	209	5.49	3.00	8.00	8.59					
Length of longer side of blade (cm)	LHL	192	4.18	2.08	6.01	19.00	209	2.40	1.48	3.50	13.39					
Length of shorter side of blade (cm)	SHL	192	4.23	2.10	6.08	19.03	209	2.38	1.45	3.47	13.27					
Asymmetry of leaf blade [100× (LHL − SHL / LHL)]	LA	192	1.15	0.00	7.18	39.74	209	0.90	0.00	6.58	41.45					
Ratio of number of nerves to number of teeth [100× (NVT / TN)]	PV	192	96.07	75.00	100.00	4.10	209	94.52	66.67	100.00	6.29					
Leaf blade width in 90% / maximum width [100× (BW_90 / BW)]	W_1	192	24.25	13.40	39.62	14.19	209	29.22	12.92	52.27	11.06					
Leaf blade width in 50% / maximum width [100× (BW_50 / BW)]	W_2	192	94.58	77.34	100.00	1.90	209	93.43	77.03	100.00	1.96					
Leaf blade width in 90% / width in 50% of length [100× (BW_90 / BW_50)]	W_3	192	25.68	13.52	43.33	14.68	209	31.31	14.00	55.99	11.52					
Leaf blade surface to circumference ratio (A / P)	AP	192	0.49	0.31	0.67	12.04	209	0.30	0.20	0.43	10.08					
Position of leaf maximal width [100× (LWP / BL)]	LBW	192	42.45	24.76	56.47	7.44	209	42.45	22.76	57.66	5.20					
Serration depth [(LLV + LV) / 2 − LI]	DI	192	0.33	0.13	1.53	18.98	209	0.22	0.11	0.34	16.74					
Leaf blade length/width (BL / BW)	LS	192	1.82	1.00	2.69	16.27	209	1.72	1.16	2.45	13.77					

L – vegetative shoots, S – fructifying shoots, NN – undetermined shoot from browsed specimens; N – number of leaves measured; M – arithmetic mean value, Min – minimum, Max – maximum, V – variation coefficient; shaded columns indicate mean values.

Results

Variation and correlation of characters

Most of the leaf characters showed normal or nearly normal distributions and homoscedastic variances. Non-normal distributions (Shapiro-Wilk test, $P \leq 0.01$) were found for a few characters and populations (electronic supplementary materials, Table S1). The unimodality, normal distribution, and homoscedasticity of the variances of most data after transformation and standardization allowed the application of the multivariate analyses.

Among species, morphological variation was the lowest in *Z. sicula* for the leaves of both L- and S-type shoots, and the highest in *Z. carpinifolia*. At the species level, the S-type leaves of *Z. sicula* were less variable than the L-type leaves. The characters of the NN-type leaves of *Z. abelicea* were the most variable, with coefficients of variation generally more than twice as high as those of the S- and L-type leaves (Table 2). The most variable characters in all species and types of shoots ($V > 25\%$) were the blade surface (LA), and asymmetry (A) in *Z. abelicea* and *Z. carpinifolia*. The lowest level of variation ($V < 7–10\%$) concerned the nerve/teeth ratio (PV) and the blade/width ratios (W_2 and LBW) in every species (Table 2).

Most of the characters describing the size of the leaf blade, as well as the number of veins and teeth, were significantly correlated in the L- and S-type leaves in every species, as well as in the NN-type leaves of *Z. abelicea*. These variables were also positively correlated when the L-type and S-type leaves of all species were taken into account together (Supplementary material, Table S2). In the L- and S-type leaves of every species, the strongest correlations ($r = 0.95–1.00$) were detected in each of the possible combinations between pairs related to blade size (A, P, BL, BW, BW_50) and margin (LLV, LV and LI). Consequently, the individual characters from these pairs were omitted in the multivariate analyses as redundant.

Differences between L-, S- and NN-type leaves

The leaves from L-type shoots were significantly ($P<0.01$) larger than those from S-type (Table 3). PV was the only character that did not differ between L-type and S-type leaves in every species. AB was similar in the two types of leaves in *Z. carpinifolia* and *Z. abelicea*, as were LA and W_2 in *Z. abelicea* and *Z. sicula*. A lack of differences between L- and S-type

Table 3. Level of differences between L-, S- and NN-types of leaves within *Z. carpinifolia* (after Jasińska et al. 2015), *Z. abelicea* and *Z. sicula* detected in the t-Student test; differences significant at p ≤ 0.01 bolded; character codes as in Table 2

Character code	*Z. carpinifolia*			*Z. abelicea*						*Z. sicula*		
	L	S	$P_{L/S}$	L	S	NN	$P_{L/S}$	$P_{L/NN}$	$P_{S/NN}$	L	S	$P_{L/S}$
A	12.28	4.48	**0.0000**	3.29	1.81	1.51	**0.0000**	**0.0000**	0.1548	6.64	2.32	**0.0000**
P	19.60	11.21	**0.0000**	8.68	6.12	5.12	**0.0000**	**0.0000**	**0.0116**	13.39	7.72	**0.0000**
BL	5.83	3,42	**0.0000**	2.81	1.98	1.58	**0.0000**	**0.0000**	**0.0009**	4.14	2.39	**0.0000**
BW	2.97	1.82	**0.0000**	1.59	1.23	1.00	**0.0000**	**0.0000**	**0.0024**	2.33	1.40	**0.0000**
BW_50	2.78	1.68	**0.0000**	1.49	1.45	0.93	**0.0000**	**0.0000**	**0.0021**	2.20	1.31	**0.0000**
BW_90	0.77	0.51	**0.0000**	0.49	0.40	0.32	**0.0019**	**0.0000**	**0.0010**	0.56	0.41	**0.0000**
LLV	2.29	1.31	**0.0000**	1.10	0.82	0.66	**0.0000**	**0.0000**	**0.0020**	1.77	1.11	**0.0000**
LV	2.13	1.22	**0.0000**	0.99	0.74	0.59	**0.0000**	**0.0000**	**0.0015**	1.63	1.01	**0.0000**
LI	1.85	1.04	**0.0000**	0.82	0.63	0.49	**0.0000**	**0.0000**	**0.0003**	1.38	0.84	**0.0000**
LWP	2.64	1.43	**0.0000**	1.22	0.80	0.68	**0.0000**	**0.0000**	0.0393	1.76	1.02	**0.0000**
AA	38.59	42.97	**0.0001**	47.90	49.03	53.28	0.4599	**0.0000**	**0.0010**	41.43	48.69	**0.0019**
AB	99.34	99.92	0.7153	89.31	89.85	88.91	0.8374	0.8675	0.6588	98.04	90.41	**0.0027**
TN	10.19	8.84	**0.0000**	5.16	4.78	4.73	0.0409	**0.0087**	0.7275	7.10	5.82	**0.0000**
NVT	9.45	8.19	**0.0000**	5.12	4.68	4.66	0.0157	**0.0039**	0.8510	6.82	5.49	**0.0000**
LHL	5.79	3.48	**0.0000**	2.81	1.99	1.59	**0.0000**	**0.0000**	**0.0010**	4.13	2.38	**0.0000**
SHL	5.98	3.55	**0.0000**	2.85	2.02	1.62	**0.0000**	**0.0000**	**0.0013**	4.17	2.40	**0.0000**
LA	3.06	2.03	**0.0001**	1.32	1.20	1.67	0.3829	0.0161	**0.0000**	1.18	0.90	0.0403
PV	92.86	92.80	0.9192	99.35	99.12	98.62	0.0542	0.0210	0.4102	95.93	94.55	0.3815
W_1	26.09	27.89	**0.0042**	31.19	32.71	34.75	0.2373	**0.0016**	0.0941	24.20	29.23	**0.0000**
W_2	93.82	92.11	**0.0001**	93.67	93.30	94.21	0.5417	0.3161	0.0430	94.53	93.42	0.0527
W_3	27.82	30.25	**0.0002**	33.31	35.03	36.84	0.1812	**0.0016**	0.1400	25.64	31.32	**0.0000**
AP	0.60	0.38	**0.0000**	0.36	0.29	0.22	**0.0000**	**0.0000**	**0.0000**	0.49	0.30	**0.0000**
LBW	44.97	40.84	**0.0000**	43.48	39.99	43.85	**0.0053**	0.6129	**0.0011**	42.15	42.46	0.7193
DI	0.36	0.22	**0.0000**	0.22	0.15	0.14	**0.0000**	**0.0000**	0.2916	0.32	0.22	**0.0000**
LS	2.00	1.89	**0.0048**	1.79	1.62	1.62	**0.0033**	**0.0012**	0.8953	1.79	1.72	0.3988

leaves was detected in 9 characters for *Z. abelicea*, 5 characters for *Z. sicula*, and only 2 characters in *Z. carpinifolia*. The leaves sampled from non-fruiting individuals of *Z. abelicea* (NN-type) were more different from the L-type leaves (20 characters different at P ≤ 0.01) than from the S-type leaves (12 characters different at P ≤ 0.01, Table 3).

In *Z. sicula*, the highest discrimination power between individuals was observed for basic traits related to leaf blade size and proportion (BL, LS, AP), and edge (TN), with partial Wilks' λ values of 0.30, 0.61, 0.69 and 0.68, respectively. The leaves specifically representing either L-type or S-type shoots formed different groups in the space between the first two discrimination variables, and the individuals formed compact groups (Fig. 3). U_1 was responsible for nearly 70% of the total variation, while U_2 contributed for about 23%; here too, both axes were mainly affected by basic characters associated to blade size and edge (respectively BL, AA, LS, DI, and BW, AP, BL).

Within *Z. abelicea*, the level of difference between L- and S-type leaves was lower than in *Z. sicula*. NN-type leaves were dispersed between L- and S-type leaves when collected from the same populations. Additionally, the NIA, STA and AMB_1 populations, represented exclusively by NN-type leaves, were well separated from all the others (Fig. 4A). The first two discrimination variables accounted for more than 87% of the total variation and both were largely determined by blade width (BW) and blade edge (DI) features. The individuals on the scatter plot formed rather dispersed groupings reflecting the distribution at population level. The few individuals represented by L- and NN-type leaves were positioned at the margins of the cloud and were separated from those of the individuals represented by S-type leaves (Fig. 4B).

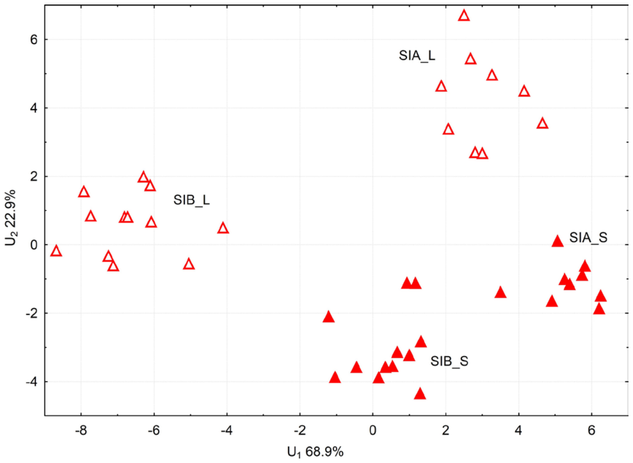

Fig. 3. Differentiation of individuals from the two populations (SIA and SIB) of *Zelkova sicula* based on the comparison between the leaves from vegetative shoots (SIA_L, SIB_L) and fertile shoots (SIA_S, SIB_S)

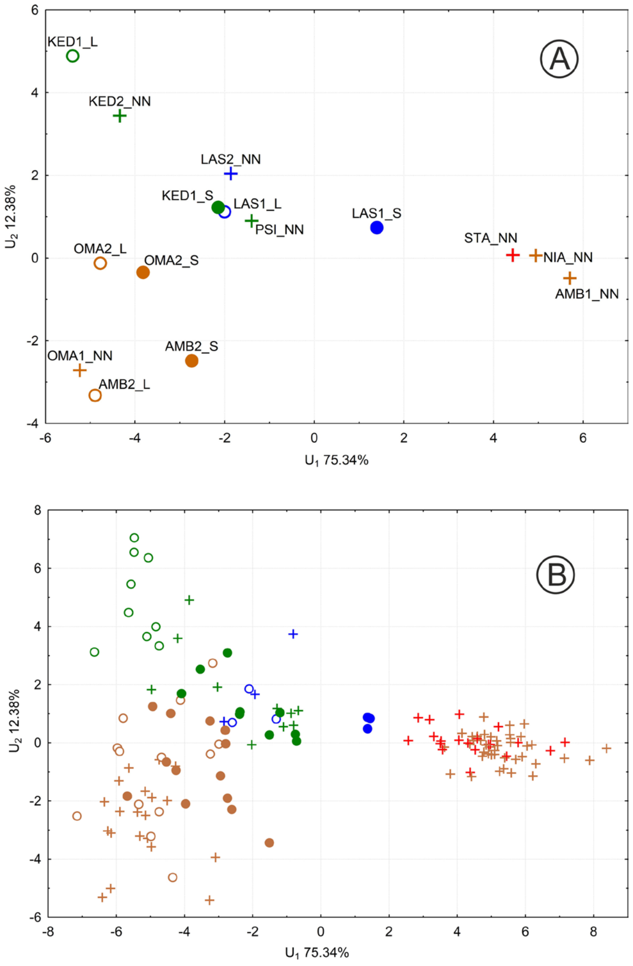

Fig. 4. Differentiation of populations (A) and individuals (B) of *Z. abelicea* based on the comparison between the leaves from vegetative shoots (L, ○), fertile shoots (S, ●) and non-fruiting individuals (NN, +); population acronyms as in Table 1; samples from Lefka Ori (Omalos, Ambelitsias, Niato) in brown, Psiloritis (Kedros, Rouvas) in green, Lasithi (Dikti Oros, Kefala) in blue, Thripiti (Afentis Stavromenas) in red

Differences among species

Most of the characters revealed species-specific mean values and, to a lesser degree, differed between populations within species. Despite differences, the ranges of character variation overlapped (Table 2). Student's *t*-test with Cochran-Cox correction and Kruskal-Wallis tests detected statistically significant (P≤0.01) differences between pairs of species for most of leaf characters and between the majority of the possible combinations of species and leaf types. The lowest number of differences was found in W_2 and LBW (3 and 4 differences, respectively). On the other hand, A, P, BL, BW, BW_50, LWP, TN, NVT, LHL and SHL differed at statistically significant levels (P≤0.01) between all possible pairwise species and leaf-type combinations (Table 4). The species with the fewest significantly different characters were *Z. abelicea* L-type and *Z. sicula* S-type leaves.

Table 4. Results of Student's t test and Kruskal-Wallis tests: P-level of differences between leaf characters of *Z. carpinifolia* (CAR), *Z. abelicea* (ABE) and *Z. sicula* (SIC) depending on the leaf type (L, S or NN); differences significant at p ≤ 0.01 bolded; character codes as in Table 1

Character code	CAR_L					CAR_S					ABE_L		ABE_S		ABE_NN	
	ABE_L	ABE_S	ABE_NN	SIC_L	SIC_S	ABE_L	ABE_S	ABE_NN	SIC_L	SIC_S	SIC_L	SIC_S	SIC_L	SIC_S	SIC_L	SIC_S
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.02	**0.00**	0.00	0.00	0.00
BL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BW	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
BW_50	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
BW_90	0.00	0.00	0.00	0.00	0.00	0.52	**0.00**	**0.00**	0.04	**0.00**	0.03	**0.00**	**0.00**	0.69	**0.00**	**0.01**
LLV	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**	**0.00**	0.81	**0.00**	**0.00**	**0.00**	**0.00**
LV	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.69	**0.00**	**0.00**	**0.00**	**0.00**
LI	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.61	**0.00**	**0.00**	0.00	0.00
LWP	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AA	**0.00**	**0.00**	**0.00**	0.10	**0.00**	**0.00**	**0.00**	**0.00**	0.39	**0.00**	**0.00**	0.68	**0.00**	0.86	**0.00**	**0.01**
AB	0.00	0.00	0.00	0.54	**0.00**	**0.00**	**0.00**	**0.00**	0.39	**0.00**	**0.00**	0.66	**0.00**	0.81	**0.00**	0.45
TN	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
NVT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**	0.04	**0.00**	**0.00**	**0.00**	**0.00**
LHL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SHL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LA	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.02	**0.00**	**0.00**	0.38	**0.01**	0.88	**0.01**	**0.00**	**0.00**
PV	0.00	**0.00**	**0.00**	**0.00**	0.11	**0.00**	**0.00**	**0.00**	**0.00**	0.21	**0.00**	**0.00**	0.04	0.02	**0.01**	**0.01**
W_1	**0.00**	**0.00**	**0.00**	0.04	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.11	**0.00**	0.08	**0.00**	**0.01**	**0.00**	**0.00**
W_2	0.79	0.26	0.27	0.14	0.48	**0.01**	0.02	**0.00**	**0.00**	0.01	0.17	0.69	0.03	0.83	0.48	0.09
W_3	**0.00**	**0.00**	**0.00**	0.03	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.24	**0.00**	0.09	**0.00**	**0.01**	**0.00**	**0.00**
AP	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.12	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.36	**0.00**	**0.00**
LBW	0.08	**0.00**	0.08	**0.00**	0.02	**0.00**	0.47	**0.00**	0.16	0.04	0.16	0.20	0.08	0.03	0.04	0.03
DI	**0.00**	**0.00**	**0.00**	0.03	**0.00**	0.62	**0.00**	**0.00**	**0.00**	0.86	**0.00**	0.72	**0.00**	**0.00**	**0.00**	**0.00**
LS	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.05	**0.00**	**0.00**	0.14	**0.00**	0.98	0.29	0.02	0.11	0.02	0.09

Zelkova carpinifolia populations appeared to be the most distinct from those of *Z. abelicea* and *Z. sicula*, regardless of the leaf character. On the contrary, *Z. abelicea* showed no evident distinctions among L-type, S-type and NN-type leaves. Only some populations represented by exclusive NN-type leaves (NIA, STA and AMB1) were separated from the other populations (Fig. 5A). Both populations of *Z. sicula*, when represented by S-type leaves, were placed between populations of *Z. carpinifolia* and *Z. abelicea* closer to the later species. However, when *Z. sicula* was represented by L-type of leaves, it was separated from *Z. carpinifolia* and *Z. abelicea*. With respect to the discriminant variable U_1 (responsible for approximately 70% of the total variation), samples from L-type shoots of *Z. sicula* were more similar to the *Z. carpinifolia* S-type leaves, whereas those from S-type shoots resembled leaves from *Z. abelicea* L-type shoots (Fig. 5A). Conversely, with concern to the discriminant variable U_2 (explaining about 10% of the total variation) all samples of *Z. sicula* resembled leaves of *Z. abelicea*. The variable U_1 was determined mostly by BL, TN, AP, BW, DI, AA, W_1, LS, AB and LA; interestingly, variance in U_2 was explained by a sub-sample of the same characters (TN, DI, AP, W_1 and BW).

The discrimination between individuals showed greater dispersion and distinctness among *Z. carpinifolia* trees than among *Z. abelicea* trees, independently on the leaf type (Fig. 5B). The individuals of *Z. sicula* also formed a dispersed group, but 22 individuals predominantly represented by L-type shoots from the SIB population entered the 95% confidence interval of *Z. carpinifolia*, while 7 individuals of *Z. sicula* entered the 95% confidence interval of *Z. abelicea*. The latter specimens of *Z. sicula* were represented by S-type leaves and were mostly sampled from the SIA population.

The agglomeration of the populations based on Mahalanobis distances according to Ward's method revealed a prominent separation of all populations of *Z. carpinifolia* based on L-type leaves, which formed the most distant group (Fig. 5C). The second group of populations was not homogenous and consisted of three sub-groups. The first one was composed of populations of *Z. abelicea*, including six populations with NN-type leaves, two with L-type leaves and one with S-type leaves. The second subgroup included populations of *Z. carpinifolia* based on S-type leaves, with the exception of TRA and BAB. The third subgroup was the most heterogeneous at the species level and comprised six populations of *Z. abelicea* (three based on S-type, two on L-type and one on NN-type

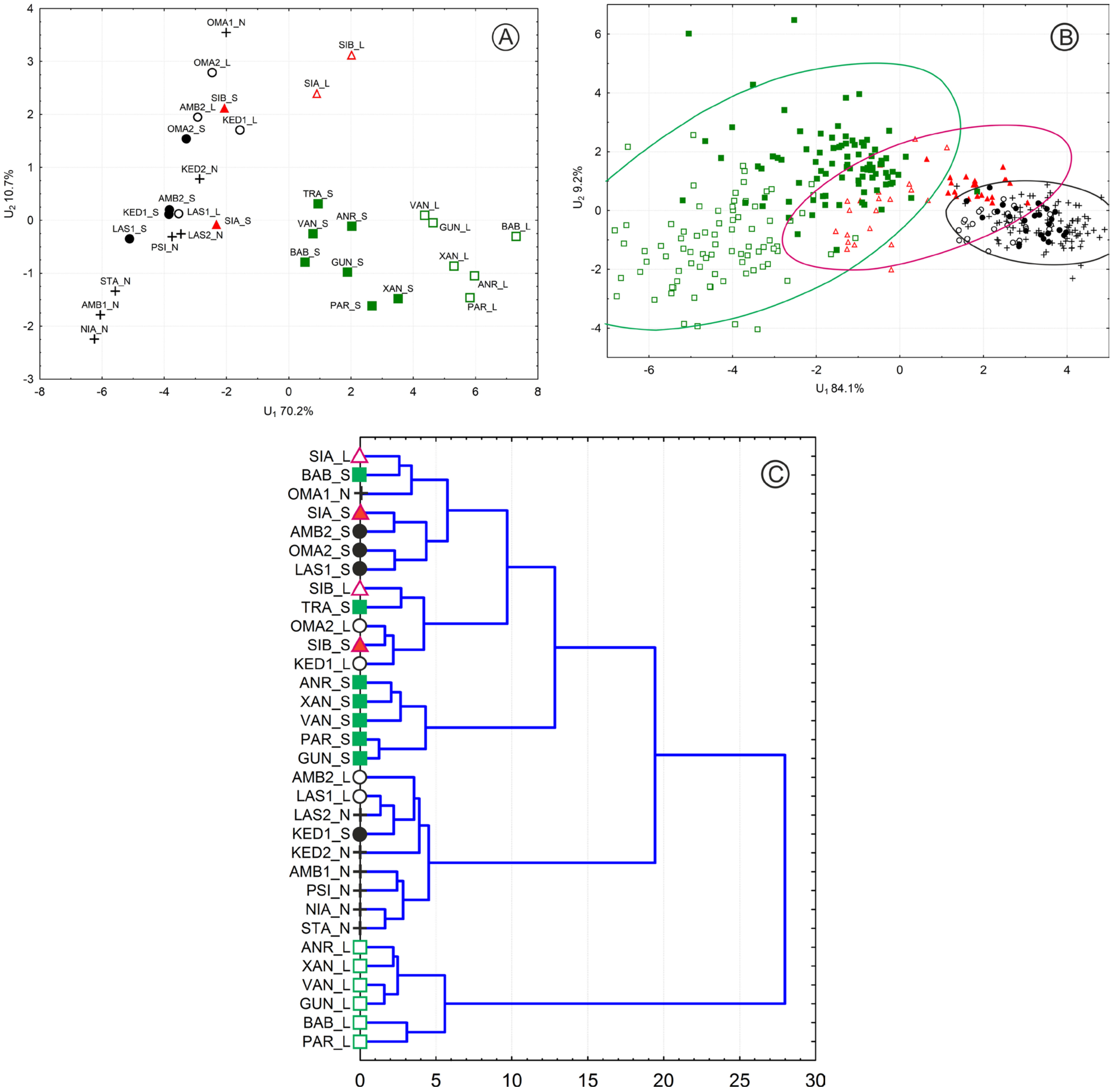

Fig. 5. Differentiation of the populations of *Zelkova carpinifolia*, *Z. abelicea* and *Z. sicula* based on L-, S- and NN-shoot leaves: *Z. carpinifolia*: L-type, S-type; *Z. abelicea*: L-type, S-type, + – NN-type; *Z. sicula*: L-type, S-type (population acronyms as in Table 1); A: discrimination between populations, B: discrimination between individuals; C: agglomeration of the populations based on the Euclidean distances

shoots), two populations of *Z. carpinifolia* with S-type leaves, and all populations of *Z. sicula*.

For the L-type leaves, the intermediate position of the *Z. sicula* SIA population between *Z. carpinifolia* and *Z. abelicea* was visible on the scatter plots between the possible combinations of U_1 and U_2 with the discriminant variable U_3, while SIB was placed separately. The variable U_4 also resulted in a separate position for *Z. sicula* (Fig. S2).

The analysis of Mahalanobis and geographic distances based on L-type leaves showed that the main geographic barrier (a) was surprisingly between the two known populations of *Z. sicula*, the next two barriers separated respectively the Mediterranean and the Transcaucasian species (b), and the two Mediterranean insular relicts (c) (Fig. 6). On the basis of S-type leaves, the stronger separation (a) was between *Z. carpinifolia* and the two Mediterranean relatives, the next (b) was again, interestingly, between the two populations of *Z. sicula*, and the third (c) distinguished between the Colchic and the Hyrcanian populations of *Z. carpinifolia*.

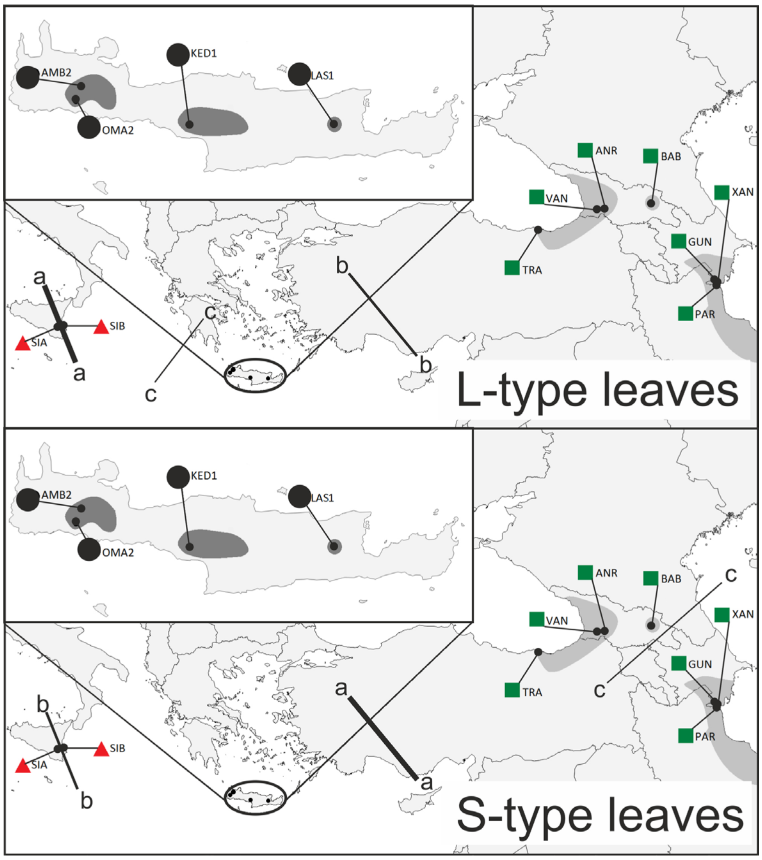

Fig. 6. Morphological barriers between sampled populations from the data on L- and S-type of leaves, detected using Barrier 2.2 of the Mahalanobis' distances between populations; thickness of the lines corresponds to the importance of the barrier: the thickest (a) represent the most important barriers, and the thinnest lines (c) represent the least important barriers; population acronyms as in Table 1

Discussion

Value of leaf morphometric analyses within the genus *Zelkova*

Our study represents the first biometrical comparison of all the three south-west Eurasian *Zelkova* species made on a broad range of material and a large set of leaf characters. So far, only a few basic leaf characters have been included in the description of *Zelkova* species (Tutin, 1964; Zieliński, 1979; Browicz & Zieliński, 1982b; Christensen, 1997), mainly concerning leaf length (our BL) and leaf width (our BW), and sometimes the number of secondary veins (NTV) and the number of teeth (TN) (Table 5). With respect to the data reported in the literature, our data usually appeared less variable. These differences may issue from the more standardized procedure of plant material collection, we adopted with respect to previous measurements. Our data came from a large amount of leaves sampled from the central parts of the shoots, from parts of tree crowns exposed to the sun, which reduced the level of their variation. Conversely, the literature data were likely based on limited herbarium specimens and may not have distinguished between L- and S-type leaves. Additionally, the majority of herbarium specimens are most often collected from the best-developed, 'nicest' tree twigs, which consequently provided larger leaves. The higher minimal values found in our data probably resulted from omission of leaves from the basal parts of the shoots.

When describing the leaves, we used several additional characters that have not been reported before. We present many data on leaf blade size and their edge (A, P, BL, BW, LLV, LV, LI, LWP, AA, AB, TN, NVT, DI) and certain characters calculated as proportions. Such data hitherto have been published only for *Z. carpinifolia* (Jasińska et al., 2015) but are original for *Z. sicula* and *Z. abelicea*. These results may be valuable in the determination of fossil materials, including those belonging to extinct taxa. For instance, based on only a few morphological traits Nakagawa et al. (1998) suggested to ascribe to *Z. sicula* or its ancestor fossil remains originally reported as *Z. carpinifolia*. Thus, our analytical approach may reduce uncertainty, providing useful additional information for the reliable reconstruction of the spatial-temporal differentiation in *Zelkova* in the Mediterranean regions and south-western Asia.

Table 5. Average values and range of variation of BL, BW, NVT and TN of *Zelkova carpinifolia* (after Jasińska et al., 2015, simplified), *Z. abelicea* and *Z. sicula* issuing from the present study (bolded), compared to literature data; character codes as in Table 1

Species	BL [cm]	BW [cm]	NVT	TN	Source of data
Z. carpinifolia	4–6				Grossheim, 1930
	4–8	2–4	4–8		Lozina-Lozinskaya, 1951
	(1.5–)2–3.5(4.6)		(4–)6–7(–9)		Prilipko, 1961
	to 10–12 (S shoot)				Prilipko, 1961
	(1.5–)2–8(–9)	(1–)1.5–4(–4.5)	6–10(–12)		Browicz & Zieliński, 1982b
	to 8(–10)	to 4(–4.5)			Zieliński, 1979
	2–5(–6)	(0.8–)1.5–2(–2.5)			Anşin & Gerçek, 1991
			(3–) 6–12		Wang et al., 2001
			to 12		Denk & Grimm, 2005
	5.83(2.54–10.62)	**2.97(1.44–5.53)**	**9.45(5–15)**	**10.19(6–16)**	**L shoot**
	3.43(1.13–6.26)	**1.82(0.59–2.92)**	**8.19(4–15)**	**8.84(5–15)**	**S shoot**
Z. abelicea	1.2–2.5				Krüssmann, 1962
	to 2.5			7–9	Tutin, 1964
	(0.6–)1–4(–4.8)	(0.4–)0.7–3(–4.3)	(3–)4–6(–7)		Christensen, 1997
	1–5	0.5–3.5		6–8	Quézel et al., 1993
	1–3.5	0.5–2.5		3–6	Di Pasquale et al., 1992
	2–5			7–9	Sarlis, 1987
	1–5	0.5–2.5		3–6	Søndergaard & Egli, 2006
			3–8		Wang et al., 2001
			<10		Denk & Grimm, 2005
	2.81(1.32–4.71	**1.59(0.60–2.94)**	**5.12(3–8)**	**5.16(3–8)**	**L shoot**
	1.98(1.00–3.38)	**1.22(0.66–1.96)**	**4.68(3–7)**	**4.77(3–7)**	**S shoot**
	1.58(0.38–5.42)	**1.00(0.33–4.47)**	**4.66(3–8)**	**4.73(3–8)**	**NN shoot**
Z. sicula	1–3	0.5–2.5		3–6	Quézel et al., 1993
	2.7(1–5)	1.8(0.5–5)		5–8	Di Pasquale et al., 1992
	4.19(2.09–6.09)	**2.33(1.44–3.36)**	**6.85(4–9)**	**7.13(5–9)**	**L shoot**
	2.39(1.49–3.50)	**1.40(0.92–1.92)**	**5.49(3–8)**	**5.82(3–8)**	**S shoot**

Differences among leaf types and their ecological significance

One main result of our study is, that as in *Z. carpinifolia* (Jasińska et al., 2015, Fig. 3), also in the two Mediterranean species the leaves from the vegetative long shoots are significantly larger, than those from fruiting shoots. This feature is indeed characteristic of every *Zelkova* species (Kozlowski & Gratzfeld, 2013) and is also found in many other tree species (Jentys-Szaferowa, 1970; Staszkiewicz, 1997, and literature cited herein). However, in *Z. abelicea* differently than in the other two species, the strongest distinction was observed not at level of shoot-type but rather among populations (Figs. 2A–C). This particularity could mirror the life-history of each population and/or the remarkable environmental gradient characterizing the whole distribution range of the Cretan species. As a matter of fact, the western populations host the majority of plants with a normal tree habit (Kozlowski et al., 2014), whose leaves can develop free from browsing disturbance. This condition allows the "normal" development of the leaf blades, preserving the features typical for the species in term of size and proportional traits. Additionally, the western populations can enjoy of more mesic-humid climate, progressively shifting towards a significant increase of dryness at the easternmost side of the island (Goedecke & Bergmeier, 2018). In tree species, this environmental pattern usually associates with a general decrease of leaf size and related characters (Marcysiak, 2012a, b, c; Pérez-Harguindeguy et al., 2013). Therefore, it can be suggested that when different leaf-types from such diverse populations are compared all together, the influence of population distinctiveness may prevail on the value of the diagnostic traits at shoot type level.

In many taxa, the leaves from vegetative shoots are more variable than leaves from fructifying shoots (Jentys-Szaferowa, 1970; Staszkiewicz, 1997). Actually, this pattern was revealed for *Z. sicula*, and had also been described in previous researches for *Z. carpinifolia* (Lozina-Lozinskaya, 1951; Wang et al., 2001; Denk & Grimm, 2005; Jasińska et al., 2015). It is also worth mentioning that though the S-, L- and NN-type leaves significantly enlarged multivariate range of variation of *Z. abelicea*, but they did not form clearly distinct monotypic groups, as occurred in *Z. carpinifolia* (Jasińska et al., 2015) and *Z. sicula*.

As in *Z. carpinifolia* (Jasińska eta al., 2015), a broader apex angle of S-type than of L-type leaves was found in *Z. sicula* but not in *Z. abelicea*. This is quite unusual and rather inconsistent with a more general rule (Jentys-Szaferowa, 1970; Wójcicki, 1997; Staszkiewicz, 1997 and literature cited herein).

Concerning the Cretan species, the NN-type leaves deserve some additional discussion. They exhibited the highest values of variation coefficients in most leaf characters, which once again could be interpreted as the direct reaction of plants to ovine and caprine overgrazing. Actually, it is likely that many of these undetermined leaves came from the largely widespread browsed specimens (Kozlowski et al., 2014; Kairis et al., 2015). Heavy browsing involves a significant reduction in leaf blade size and directly impacts the values of other diagnostic characters. In fact, only some simple qualitative/descriptive features (AA, AB, TN and NTV) or quantitative proportional characters (PV, W_1, W_2, W_3, LBW, LS) seemed to be relatively less affected by browsing. In this case, our samples including leaves with very different life-history, probably mirror the current 'anomalous' situation, i.e. the direct effects of overbrowsing. Hence, biting/browsing disturbance represents an important element of evaluation when using the morphometric approach to classify taxonomically unknown leaf samples.

Variation of leaf characters and taxonomic relationships among species

It is noteworthy that the molecular differences already pointed out among the three investigated species (Fineschi et al., 2002; Christe et al., 2014) were corroborated by the differences detected using the leaf characters of our study. In the data set we used, only one character of the leaves (W_2) did not differentiate between *Z. carpinifolia* and *Z. abelicea*, independent of the L-, S- or NN-type leaves of the latter species. However, no leaf character considered separately proved effective to distinguish between these taxa. Simple traits, such as the length (BL) and width (BW) of the leaf blade, number of denticles (TH) and lateral veins (NVT), generally had high diagnostic values in *Z. carpinifolia* and *Z. abelicea* (Table 2), but only when used jointly allowed distinct between them, irrespective of whether the leaves were from vegetative or fruiting shoots. It should be stressed yet, that the S-type leaves of *Z. carpinifolia* are somewhat similar to the L-type leaves of *Z. abelicea* leaves.

With regard to *Z. sicula*, it should be pointed out that leaf morphological characters situated this species between *Z. carpinifolia* and *Z. abelicea*, but simultaneously emphasized the high level of multivariate differences between their two known populations. Interestingly, the characters of the S-type leaves placed both populations of *Z. sicula* close to *Z. abelicea*, whereas those of the L-type leaves separated the two Mediterranean insular relicts, but with the Sicilian species entering the 95% confidence intervals of *Z. carpinifolia* (Fig. 5B). This could be interpreted as an additional support of the probable hybrid origin of *Z. sicula*, as already suggested by Christe et al. (2014).

An intriguing history of lineage separation

Our morphometric analyses provided some interesting results concerning the geographic distinctiveness at inter- and intra-specific level of the three south-western Eurasian *Zelkova* species. Firstly, leaf features from vegetative shoots, and to a lesser extent also from fruiting shoots, clearly situated apart the Euxino-Hyrcanian species from the two Mediterranean taxa at least on the population level. Among the investigated species, *Z. carpinifolia* is the only one still growing under temperate and humid climatic conditions (Kvavadze & Connor, 2005), with short dry season similar to the Cenozoic (Tertiary) subtropical environments (Mai, 1995; Kovar-Eder, 2003), where the genus originated and spread throughout Eurasia (Manchester, 1989; Akhmetiev, 2010; Kan-Kan et al., 2016; Zhang et al., 2017). Conversely, both the insular *Z. abelicea* and *Z. sicula* occur under typical Mediterranean climates, with 3–4 months of summer drought. It can be assumed that along their evolutionary history the two Mediterranean *Zelkova* species developed adaptive traits, such as leaf size reduction and other associated/derived characters (Marcysiak 2012b; Pérez-Harguindeguy et al., 2013), which resulted in higher fitness to the more xeric climate experienced in their respective refugial areas. Most evidently, the discriminant value of such traits is so remarkable that on its basis the two different biogeographic units could be easily separated.

With concern to intra-specific variability, our results also emphasized the high levels of multivariate distance between the two only known populations of *Z. sicula*. This finding is consistent with the results of previous genetic investigations, which showed that the Sicilian populations have a different single plastid haplotype, so that they have been guessed as two different clones (Christe et al., 2014). This could explain the relatively low degree of intra-population variation of leaf characters detected in our study. Their separation could result of the severe isolation of each population due to the complete lack of sexual reproduction (Garfì et al., 2011). Alternatively, it possibly origins from two independent hybridization events, as suggested for *Pinus uliginosa* Neumann (Boratyńska et al., 2015). In the case of *Z. sicula*, the hybridization could take place only after the emersion of the Hyblaean Plateau (South-Eastern Sicily), between different parental individuals close to the ancestral species of *Z. abelicea* and *Z. carpinifolia* (Fineschi et al., 2002; Christe et al., 2014). Interestingly, the Hyblaean Plateau was still below sea level until early Pleistocene, when punctual uplift driven mainly by magmatic processes triggered its stepwise emergence (Henriquet et al., 2019). The Hyblaean Plateau represented an island separated from Sicily until late Pleistocene, when it was finally connected to Sicily (Longhitano & Colella, 2003). Consequently, both the putative ancestors of *Z. sicula* were able to colonize this area only afterwards, hence their co-occurrence, hybridization and large-scale extinction shall happened no earlier than before 2–3 MA.

The populations of *Z. abelicea* from the different mountain massifs in Crete exhibited high levels of genetic differentiation, which was explained by their long-lasting spatial isolation (Christe et al., 2014; Naciri et al., 2019). Unfortunately, in our analysis we could obtain material from only some representatives of the Cretan populations. Additionally, our material was heterogeneous because of NN-type of leaves. Despite that, the geographic trends of differentiation among populations and individuals through leaf morphometry were rather consistent with patterns issued from genetic analysis. This picture, however, needs further validation by including samples from all known populations and using leaves from the same type of shoots, as it has been done for *Z. carpinifolia*.

Conclusions

The morphological leaf characters allowed us to distinguish among *Zelkova* species in the Mediterranean region and south-western Asia, especially when at least 3–4 characters were used simultaneously. The leaves from vegetative long shoots appeared to be more suitable for discriminating among species than other leaf types. Also, leaf biometric analyses proved to be effective in discriminating the investigated species according to their current biogeographic range. The two known populations of the relict species *Z. sicula* (Garfì et al. 2021) differed from each other strongly in terms of leaf characters, but each population revealed the lowest level of phenotypic variation, probably as a result of their clonal origin. Moreover, *Z. sicula* appeared to be an intermediate between its Mediterranean relative *Z. abelicea* and the Euxino-Hyrcanian *Z. carpinifolia*, supporting previous hypotheses about its hybrid origin.

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