



Leaf differentiation of extinct and remnant species of *Zelkova* in Western Eurasia

Anna K. Jasińska^a , Beata Rucińska^b, Gregor Kozłowski^{c,d} , Laurence Fazan^c, Giuseppe Garfi^e , Salvatore Pasta^e , Sébastien Bétrisey^c, Emanuel Gerber^d, Hajiaga Safarov^{f,g}, Hoàng Văn Sâm^h , Krystyna Boratyńska^a , Steven R. Manchesterⁱ and Adam Boratyński^a 

^aInstitute of Dendrology, Polish Academy of Sciences, Kórnik, Poland; ^bDepartment of Botany, Kazimierz the Great University, Bydgoszcz, Poland; ^cDepartment of Biology and Botanic Garden, University of Fribourg, Switzerland; ^dNatural History Museum Fribourg, Fribourg, Switzerland; ^eInstitute of Biosciences and BioResources, National Research Council (CNR), Palermo, Italy; ^fInstitute of Botany, Azerbaijan National Academy of Sciences, Baku, Azerbaijan; ^gHirkan National Park, Lankaran, Azerbaijan; ^hDepartment of Forest plants, Vietnam National University of Forestry, Hà Nội, Vietnam; ⁱFlorida Museum of Natural History, Gainesville, Florida, USA

ABSTRACT

The genus *Zelkova* (Ulmaceae) has occurred in Europe and Southwest Asia since the Eocene represented mostly by the fossil-species *Z. zelkovifolia*. Currently, relict populations of *Z. abelicea*, *Z. sicula* and *Z. carpinifolia* can be found in Crete, Sicily and the Euxino-Hyrcanian province, respectively. To reveal relationships between extinct and extant *Zelkova* species, we compared fossil Miocene leaves of *Z. zelkovifolia* and leaves of extant species using morphological leaf characteristics and statistical methods (Tukey's test, discrimination analysis, principal component analysis, agglomeration). The fossil leaves of *Z. zelkovifolia* appeared more variable and generally intermediate between all three extant species. The lowest level of significant differences was found between *Z. zelkovifolia* and *Z. abelicea* in the leaves from fertile shoots and between *Z. zelkovifolia* and *Z. sicula* leaves from vegetative long shoots. The intermediate positions of fossil leaves of *Z. zelkovifolia* between leaves of *Z. abelicea*, *Z. sicula* and *Z. carpinifolia* could indicate that the extinct species was an ancestor of all three extant taxa. Consequently, this result suggests divergence between *Z. abelicea*, *Z. sicula* and *Z. carpinifolia* no earlier than the late Miocene and/or during the Pliocene.

ARTICLE HISTORY

Received 13 October 2021
Accepted 17 January 2022

KEYWORDS

biogeography; leaf architecture; plant variation; relict trees; Ulmaceae; vegetation history

Introduction

The taxonomic differentiation of the genus *Zelkova* Spach and its phylogenesis detected using internal transcribed spacers (ITS) of the nuclear DNA (Denk and Grimm 2005), chloroplast DNA (Fukatsu et al. 2012), and a combination of the two (Fineschi et al. 2002; Christe et al. 2014; Maharramova et al. 2015; Naciri et al. 2019) has been strongly influenced by global climate events. Pliocene climate cooling and Pleistocene environmental oscillations significantly reduced the geographic range of the genus, which previously been broadly distributed over the Northern Hemisphere during the Cenozoic (Mai 1995; Kovar-Eder 2003; Magri et al. 2017). In Europe and Western Asia, the genus is today comprised of three remnant species, which are known from a few rare and isolated populations. *Zelkova sicula* Di Pasq., Garfi & Quézel grows in two known localities in Sicily (Garfi et al. 2011, 2017), *Z. abelicea* (Lam.) Boiss. is distributed across five mountain massifs in Crete (Sarlis 1987; Phitos et al. 1995; Kozłowski et al. 2014) and *Z. carpinifolia* (Pall.) K.Koch grows in the Euxino-Hyrcanian province, in Turkey, Armenia, Georgia,

Azerbaijan and northern Iran (Browicz and Zieliński 1982; Browicz 1989).

The remnant *Zelkova* species can be distinguished using the characteristics of fruits and leaves (Wang et al. 2001; Kozłowski and Gratzfeld 2013; Jasińska et al. 2015, 2021). In Europe and Western Asia, the leaves, fruiting twigs, fruits and pollen of extinct species of *Zelkova* have been reported from the Eocene to Pleistocene (e.g., Tralau 1963; Manchester 1989; Velitzelos et al. 2014; Magri et al. 2017). The fossil material most frequently includes leaves, assigned either to the extinct *Z. zelkovifolia* (Unger) Bůžek et Kotl. or to one of the extant *Z. carpinifolia*, *Z. abelicea*, *Z. sicula* or even to the east-Asiatic *Z. serrata* (Thunb.) Makino (Denk and Grimm 2005). The fossil leaves were identified to the species level using the number of marginal teeth and the distance between the lowest tooth and the base of the leaf blade (Follieri et al. 1986; Nakagawa et al. 1998; Worobiec 2014).

We expected that the leaf morphological characteristics used by Jasińska et al. (2015, 2021) for morphometric investigations of the remnant Western Asian *Zelkova* spp. would be effective in comparisons between extant *Z. carpinifolia*, *Z.*

abelicea and *Z. sicula* and their extinct potential ancestor, *Z. zerkovifolia*. In this study, we conduct a biometrical comparison of fossil leaf assemblages of *Z. zerkovifolia* from Anatolia and Greece to data collected from extant *Zelkova* species from the Mediterranean region and Western Asia.

Materials and methods

Plant material

The fossil leaves used in this study were collected from early and late Miocene deposits, and except for one, all were determined to be *Z. zerkovifolia* (Supplementary material Table S1). Part of the leaves came from fertile shoots (S-type), while the remaining leaves came from undetermined shoots (N-type). The leaves from Turkey and Greece represent two different samples, ZELA and ZELB, respectively (Figure 1).

We used 38 fossil leaves of *Z. zerkovifolia* from the Güvem area in Ankara Province (Turkey), collected by Baki Kasaplıgil in the 1960s and dated to 19.7 ± 0.6 Ma, currently stored at the University of California Museum of Paleontology (UCMP) in Berkeley (Wilson et al. 1997; Denk et al. 2017). Four additional leaves were taken from the plates by Velitzelos et al. (2014), for which original fossils are stored at the University of Athens.

Data concerning *Z. carpinifolia* (Jasińska et al. 2015), *Z. abelicea* and *Z. sicula* (Jasińska et al. 2021) leaves were used as comparative material (Table 1).

Measurements

The leaf characteristics (Table 2) and measurement procedures were adopted from Jasińska et al. (2015, 2021).

Fossil leaf characteristics were taken from images with an added scale using ImageJ software (National Institutes of Health). Every fossil leaf was scaled separately, and only leaves with at least 15 unambiguous characters were considered (Table 2; Supplementary material Figure S2). Because of different levels of preservation, not all characteristics could be taken from every fossil leaf. In this case, the lacking data were filled with averages during analyses.

Statistics

The average values of fossil leaf characteristics were compared to those of extant species using Tukey's T test. Principal component analysis (PCA) was used to determine the position of the *Z. zerkovifolia* population, comparing them to the populations of extant European and Western Asian *Zelkova* species. Stepwise progressive discrimination analysis was used to compare the single leaves of *Z. zerkovifolia* to the average for individuals of extant species (Sokal and Rohlf 2003; Marramà and Kriwet 2017). STATISTICA 9 PL software (StatSoft Poland, Kraków) was used for calculations.

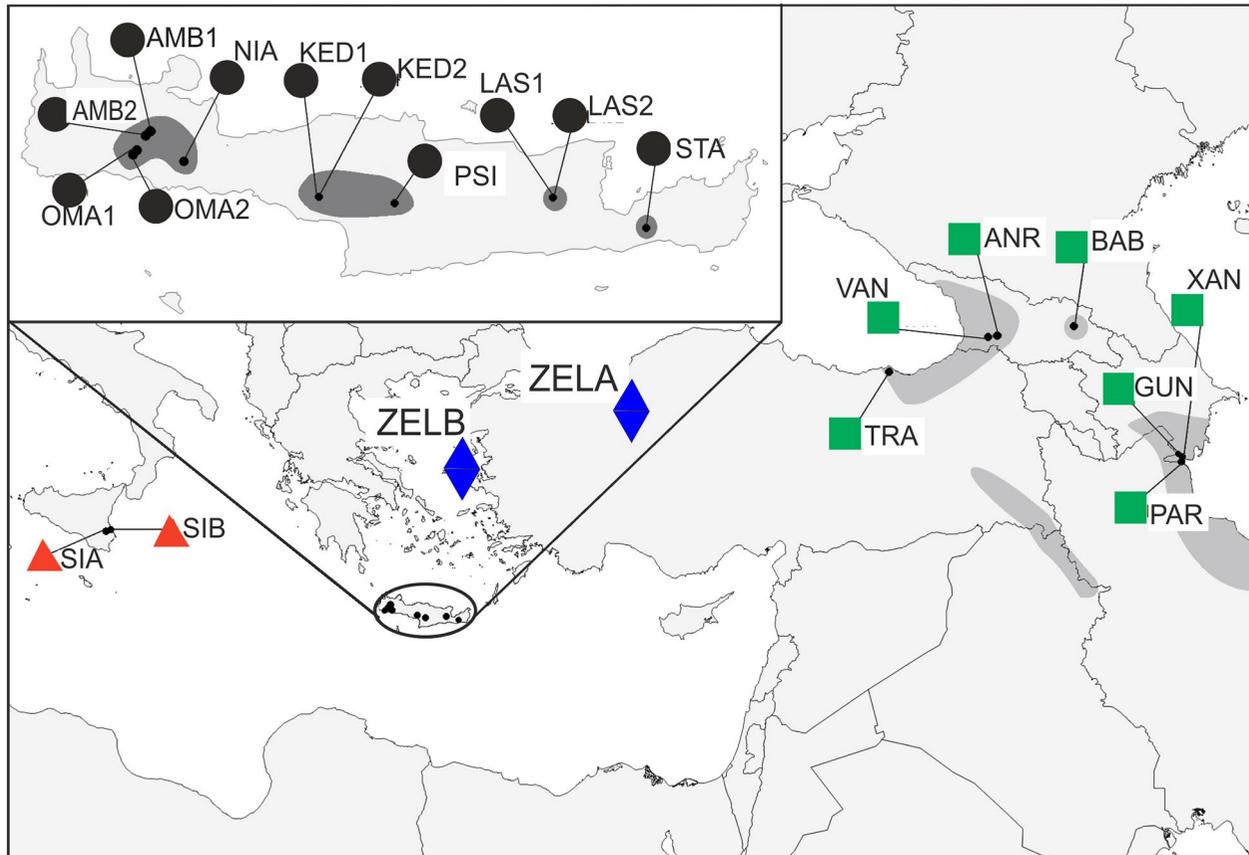


Figure 1. Geographic position of fossil samples of *Zelkova zerkovifolia* and distribution of referenced populations of *Z. carpinifolia*, *Z. abelicea* and *Z. sicula* (acronyms as in Table 1); shaded – simplified areas of distribution of *Z. carpinifolia* (after Browicz and Zieliński 1982) and *Z. abelicea* (after Kozłowski and Gratzfeld 2013).

Table 1. Sampled populations of *Zelkova sicula* and *Z. abelicea*; material of *Z. carpinifolia* from paper by Jasińska et al. (2015, 2021); N – number of individuals; fructifying individuals: L – vegetative shoot, S – fertile shoot; NN – not fructifying individuals; AMT – annual mean temperature; APR – annual precipitation.

Species	Locality	Code	N	Leaf type (number of leaves)	Longitude E [°]	Latitude N [°]	Altitude [m]	AMT [°C]	APR [mm]	
<i>Z. zelkovifolia</i>	Turkey, Güvem area	ZELA		S(18) NN(20)	40.50	32.40	1200			
	Greece, Chios (incl. 1 leaf from Elassona)	ZELB		NN(4)	38.24	26.09	130			
<i>Z. sicula</i>	Italy, Sicily 1 (1991)	SIA	9	L(75), S(90)	14.861	37.171	510	15.05	482	
	Italy, Sicily 2 (2009)	SIB	12	L(117), S(119)	15.045	37.210	330	16.35	518	
<i>Z. abelicea</i>	Greece, Crete, Omalos 1	OMA1	20	NN(200)	23.912	35.316	1231	11.96	917	
	Greece, Crete, Omalos 2	OMA2	10	L(99), S(100)	23.903	35.309	1265	12.48	899	
	Greece, Crete, Ambelitsias 1	AMB1	20	NN(200)	23.986	35.367	1269	11.96	917	
	Greece, Crete, Ambelitsias 2	AMB2	3	L(20), S(26)	23.981	35.355	1429	10.85	950	
	Greece, Crete, Niato	NIA	22	NN(220)	24.155	35.288	1221	12.26	945	
	Greece, Crete, Mt. Kedros	KED1	9	L(90), S(88)	24.627	35.188	1293	12.04	1021	
	Greece, Crete, Mt. Kedros	KED2	3	NN(30)	24.627	35.188	1293	12.04	1021	
	Greece, Crete, Rouvas	PSI	7	NN(70)	24.929	35.179	1334	10.76	1015	
	Greece, Crete, Kéfala 1	LAS1	3	L(30), S(30)	25.538	35.170	1196	12.58	906	
	Greece, Crete, Kéfala 2	LAS2	3	NN(29)	25.538	35.170	1196	13.95	857	
	Greece, Crete, Afendis Staviomenas	STA	20	NN(200)	25.888	35.081	1151	15.80	694	
	<i>Z. carpinifolia</i>	Turkey, Trabzon	TRA	7	S	39.866	40.950	76	14.46	981
		Georgia, Ajameti Nature Reserve	ANR	20	L, S	42.763	42.143	150	13.59	1303
Vani		VAN	16	L, S	42.565	42.089	100	14.40	1402	
Georgia, Babaneuri Nature Reserve		BAB	17	L, S	45.371	42.081	470	12.50	696	
Azerbaijan, Xanbulan		XAN	10	L, S	48.800	38.661	40	14.22	1086	
Azerbaijan, Parakand		PAR	10	L, S	48.803	38.650	40	14.22	1086	
Azerbaijan, Güneşli	GUN	10	L, S	48.469	38.805	650	12.58	612		

Results

The fossil material was considerably variable, with N-type leaves revealing the highest values of variation coefficient among the entire compared material of extant species. The fossil leaves of the S-type were smaller and less variable than leaves of the N-type (Table 2).

Among the investigated characteristics, only LA did not differ significantly between fossil leaves and all types of leaves of the remnant species of the Mediterranean and Euxino-Hyrcanian regions (Table 3). Additionally, the leaves of *Z. zelkovifolia* from fertile shoots revealed lower levels of statistically significant differences between *Z. abelicea* S- and N-type leaves and *Z. sicula* S-type leaves. The N-type fossil leaves had the lowest level of difference from leaves of *Z. sicula* from long shoots (Table 3).

The two compared samples of *Z. zelkovifolia* representing different assemblages from different periods of the Miocene and coming from distant areas appeared similar in the PCA concerning the N-type of leaves. This analysis also indicated the close relation of fossil N-type leaves to the populations of *Z. carpinifolia*, placing them between the L- and S-type leaves of this species. Additionally, the N-type fossil leaves were close to the *Z. sicula* L-type leaves (Figure 2 (1)). The fossil S-type leaves appeared the most similar to *Z. abelicea* S-type leaves. Single fossil leaves from undetermined shoots (ZEL_N) were positioned among individuals of *Z. carpinifolia*

S- and L-type leaves, from fertile shoots (ZEL_S) among individuals of *Z. carpinifolia* S-type leaves, *Z. sicula* L-type leaves and *Z. abelicea* L-type leaves (Figure 2 (2)).

Discussion

The high level of morphological variation of fossil leaves of *Z. zelkovifolia* compared to the extant species could result from the random character of the sample, which includes every possible leaf, independent of shady vs sun-exposed position on the tree crown and position on the shoot, which strongly influence the leaf variation (Jentys-Szaferowa 1970; Staszkievicz 1997; Boratyńska et al. 2021). The leaves of extant species were sampled from the central parts of the shoots on portions of the tree crown exposed to high levels of sunlight, which reduced their variability (Jasińska et al. 2015, 2021). On the other hand, the high level of variation among fossil leaves could also indicate the higher diversity of ancestral *Zelkova* compared to the extant taxa. The lineage(s) leading to the *Zelkova* species currently occurring in Europe and Western Asia were influenced by drastic climate change during the formation of the current Mediterranean climate. Consequently, their geographic ranges were strongly reduced, in the case of *Z. sicula* to only two clones (Christe et al. 2014; Garfi et al. 2017; 2021) and in *Z. abelicea* to several populations in the mountain massifs on Crete (Kozłowski et al. 2014). Past climate change could have also reduced the level of

Table 2. Average values of leaf characters of *Zelkova zelkovifolia*; S – fertile shoot leaves, NN – leaves from unknown type of shoots; N – number of leaves measured, M – arithmetic mean (shaded column), Min – minimum, Max – maximum, V – variation coefficient.

Leaf character	Code	Leaf type									
		S					NN				
		N	M	Min	Max	V	N	M	Min	Max	V
Leaf blade surface (cm ²)	A	18	1.53	0.40	7.00	100.34	22	8.57	1.59	16.43	63.02
Leaf blade length (cm)	BL	18	2.10	1.06	5.18	49.86	24	5.29	2.20	9.36	37.85
Leaf blade maximum width (cm)	BW	18	0.93	0.52	2.14	39.24	24	2.38	1.01	3.24	32.33
Leaf blade width in 50% of length (cm)	BW_50	18	0.80	0.45	1.61	36.03	24	2.17	0.92	3.47	33.35
Leaf blade width in 90% of length (cm)	BW_90	18	0.27	0.16	0.44	26.10	21	0.66	0.15	1.05	41.40
Distance between midrib and denticle apex at maximal width of leaf blade. along the lateral vein (cm)	LLV	7	0.73	0.40	1.50	49.63	24	1.63	0.76	2.56	34.56
Distance between midrib and denticle apex below maximal width of leaf. along the lateral vein (cm)	LV	7	0.70	0.34	1.46	52.30	24	1.53	0.71	2.54	35.87
Distance between midrib and indentation between LLV and LV (cm)	LI	7	0.54	0.28	1.13	51.57	24	1.30	0.63	2.12	36.29
Distance from basis to maximal width of leaf (cm)	LWP	18	0.74	0.24	1.89	63.12	24	2.14	0.70	3.70	43.18
Angle of leaf blade apex (°)	AA	10	38.80	22.13	54.74	27.10	16	39.19	25.38	64.74	26.44
Angle of leaf blade basis (°)	AB	13	82.31	50.08	131.22	29.53	22	90.04	41.02	136.73	26.84
Number of leaf denticles on one side of leaf blade	TN	12	7.83	5	12	30.71	23	10.13	7.00	16.00	23.68
Length of longer side of blade (cm)	LHL	6	2.56	1.44	5.22	53.81	16	5.24	2.16	8.14	35.04
Length of shorter side of blade (cm)	SHL	6	2.52	1.44	5.18	54.40	17	5.11	2.19	7.92	34.41
Asymmetry of leaf blade [100× (LHL-SHL/LHL)]	LA	6	1.56	0.12	2.41	89.22	16	2.06	0	4.89	64.59
Leaf blade width in 90% / maximum width [100×(BW_90/BW)]	W_1	18	31.60	12.8	42.81	30.85	21	27.61	13.82	48.13	34.24
Leaf blade width in 50% / maximum width [100×(BW_50/BW)]	W_2	18	86.83	71.39	94.58	9.70	24	90.96	75.17	99.58	6.57
Leaf blade width in 90% / width in 50% of length [100×(BW_90/BW_50)]	W_3	18	36.44	14.18	57.26	29.98	21	29.79	15.48	48.34	30.95
Position of leaf maximal width [100×(LWP/BL)]	LBW	18	33.88	17.12	49.09	25.84	24	39.72	27.28	52.01	16.88
Serration depth [(LLV + LV)/2-LI]	DI	5	0.18	0.08	0.35	52.19	24	0.28	0.06	0.47	37.35
Leaf blade length/width (BL/BW)	LS	18	2.22	1.78	3.54	17.94	24	2.22	1.46	3.71	22.36

variation *via* adaptation of the species currently growing on the Mediterranean islands of Sicily and Crete (Jasińska et al. 2021).

Zelkova zelkovifolia is the hypothetical ancestor of the *Zelkova* species currently occurring in the Mediterranean and Western Asian regions (Kovar-Eder 2003; Denk and Grimm 2005). The leaves we have at our disposal came from the early Miocene deposits in Anatolia (Wilson et al. 1997; Denk et al. 2017) and late Miocene deposits in Greece (Velitzelos et al. 2014). The position of fossil N-type leaves among individuals of *Z. carpinifolia* N- and L-type leaves could be interpreted as confirmation of their origin from *Z. zelkovifolia*. The

positioning of S-type fossil leaves among individuals of all three extant species also supports their origin from *Z. zelkovifolia*. The high level of differences between leaves of *Z. carpinifolia* and *Z. abelicea* support their early divergence, in contrast with much younger and possibly hybrid origin *Z. sicula*, as suggested basing on the cpDNA markers (Christe et al. 2014).

Mesothermic vegetation with *Z. zelkovifolia* was one of the frequent zonal forests in Europe around the Tethys and in Anatolia during the Miocene (Biltekin et al. 2015; Velitzelos et al. 2014; Denk et al. 2017; Magri et al. 2017). The species occurred in places with yearly precipitation of approximately

Table 3. Results of Tukey's and/or Kruskal-Wallis tests: P-level of differences between leaf characters of *Zelkova zelkovifolia* (ZEL), compared to *Z. carpinifolia* (CAR), *Z. abelicea* (ABE) and *Z. sicula* (SIC) depending on the leaf type (L, S and NN); differences significant at $p \leq 0.01$ bolded; character codes as in Table 2.

Character code	ZEL_S						ZEL_N							
	CAR_L	CAR_S	ABE_L	ABE_S	ABE_NN	SIC_L	SIC_S	CAR_L	CAR_S	ABE_L	ABE_S	ABE_NN	SIC_L	SIC_S
A	0.000	0.026				0.000		0.000	0.000	0.000	0.000	0.000		0.000
BL	0.000	0.000				0.000		0.000	0.000	0.000	0.000	0.000	0.001	0.000
BW	0.000	0.000	0.002			0.000		0.001	0.002	0.000	0.000	0.000		0.000
BW_50	0.000	0.000	0.000			0.000	0.022	0.000	0.005	0.000	0.000	0.000		0.000
BW_90	0.000	0.000	0.001			0.000				0.016	0.000	0.000		0.000
LLV	0.000					0.000		0.000	0.046	0.000	0.000	0.000		0.000
LV	0.000					0.000		0.000	0.026	0.000	0.000	0.000		0.000
LI	0.000	0.018				0.000		0.000	0.036	0.000	0.000	0.000		0.000
LWP	0.000	0.000	0.049			0.000		0.006	0.000	0.000	0.000	0.000		0.000
AA					0.000					0.009	0.002	0.000		0.003
AB	0.012	0.008				0.030								
TN	0.000		0.000	0.000	0.000		0.000		0.005	0.000	0.000	0.000	0.000	0.000
LHL	0.000					0.044			0.000	0.000	0.000	0.000	0.021	0.000
SHL	0.000					0.038			0.000	0.000	0.000	0.000	0.026	0.000
LA														
W_1						0.002						0.001		
W_2	0.000					0.017	0.012							
W_3	0.000	0.031				0.000						0.002		
LBW	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.008						
DI	0.000					0.018		0.013			0.000	0.000		
LS		0.005	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000

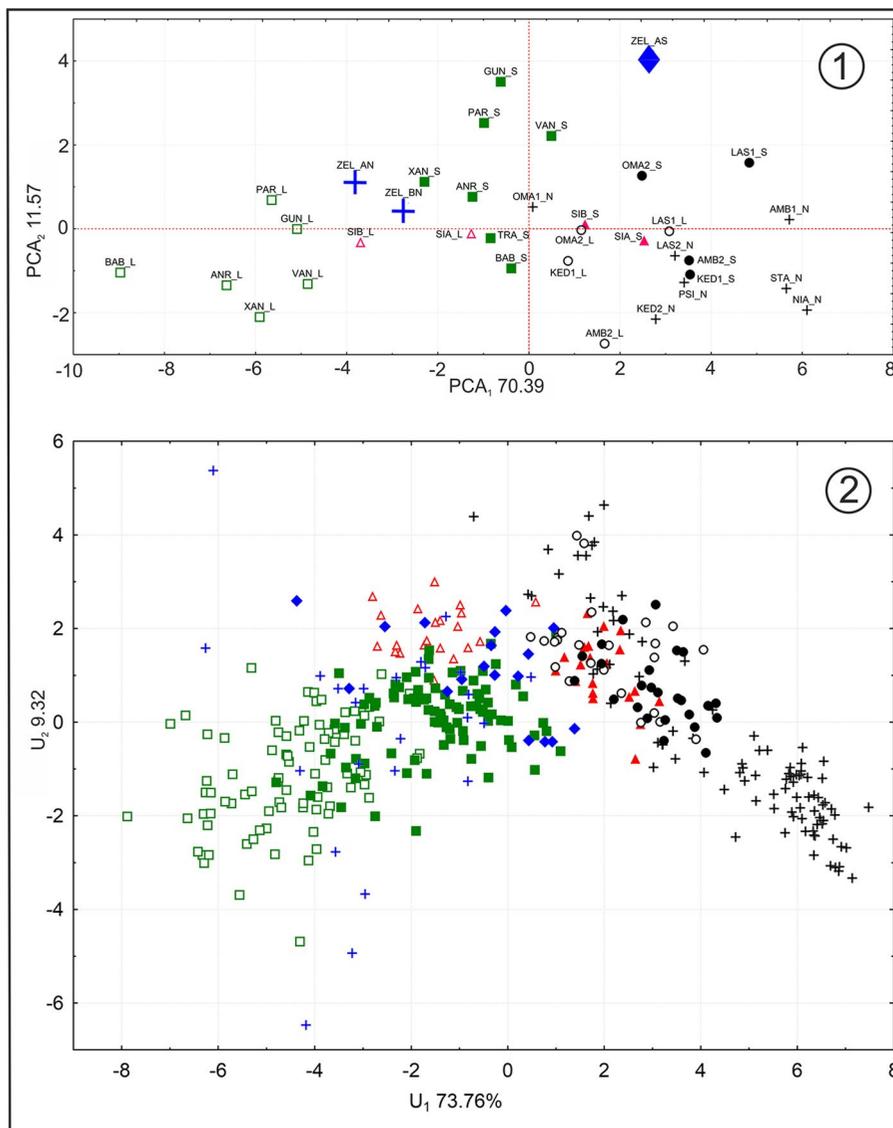


Figure 2. Position of extinct *Zelkova zelkovifolia* among extant *Z. carpinifolia*, *Z. abelicea* and *Z. sicula* based on the leaf characters: 1 – PCA of populations (acronyms as in Table 1; L – leaves from long-shoots, S – leaves from fertile shoots, N – for *Z. zelkovifolia* leaves from undetermined type of shoots, for *Z. abelicea* from non-fructifying individuals; influence of particular character to dispersion of compared data explained in Fig. S3); 2 – discrimination analysis of particular leaves of *Z. zelkovifolia* and individuals of *Zelkova carpinifolia*, *Z. abelicea* and *Z. sicula* (the species, leaf type symbols and colors as in 1).

1000–1200 mm, more or less evenly distributed over the year and with mild temperatures (Follieri et al. 1986; Kovar-Eder 2003). Pliocene climate cooling, the formation of Mediterranean climate type and Pleistocene climate oscillations reduced the occurrence of *Zelkova* to the Euxino-Hyrcanian floristic province and/or to Mediterranean islands (Kovar-Eder 2003). *Zelkova carpinifolia* currently grows in the Euxino-Hyrcanian region, with temperatures and precipitation resembling those of the Miocene (Table 1).

The climate of Mediterranean islands has higher yearly average temperatures when comparing to the mainland area. Additionally, on the islands are lower temperatures and higher precipitation during late autumn, winter and early spring than on the mainland Mediterranean (Lionello 2012). *Zelkova abelicea* and *Z. sicula* currently growing under Mediterranean climates had to adapt to these conditions. *Zelkova abelicea* ‘escaped’ to oro-Mediterranean locations with relatively high precipitation (Kozłowski et al. 2014), while *Z. sicula* persisted in hydrological microrefugia at low elevation in the thermo-Mediterranean climate, with very low summer precipitation (Garfi et al. 2021). It should be expected that adaptation of the two latter species to the Mediterranean-type climate would have caused more xeromorphic characters of their leaves, manifested in smaller leaf size, a reduced number of teeth and shallower serration, when compared to *Z. carpinifolia*. Indeed, the Miocene *Z. zelvovifolia* leaves have a higher number of teeth and relatively shallower serration than *Z. sicula* and *Z. abelicea* but lower than *Z. carpinifolia* (compare data from Jasińska et al. 2015, 2021 to the present Table 2). This trend could reflect the direction of the evolution of *Zelkova* leaves following climate change during the Neogene (Wolfe 1995; Milne and Abbott 2002; Iszkuło and Myślicka 2015). However, the results described above should be treated with caution, as we were only able to analyze a limited number of fossil leaves.

Conclusions

The fossil leaves of *Z. zelvovifolia* appeared more variable and generally intermediate between all three extant species. The fewest number of differences were found between the fertile leaf shoots of *Z. zelvovifolia* and *Z. abelicea* and between *Z. zelvovifolia* leaves from undetermined-type shoots and *Z. sicula* leaves from vegetative long shoots.

The intermediate position of the fossil leaves of *Z. zelvovifolia* between the leaves of *Z. abelicea*, *Z. sicula* and *Z. carpinifolia* may indicate that this extinct species was the ancestor of all three surviving taxa. Consequently, this could suggest divergence between *Z. abelicea*, *Z. sicula* and *Z. carpinifolia* during the late Miocene and throughout the Pliocene. Future molecular studies on extant *Zelkova* species are needed to verify this thesis.

Acknowledgments

We thank T. Denk (Swedish Museum of Natural History) for sharing the fossil material and for his thoughtful comments.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Fondation Franklinia and by the Institute of Dendrology of the Polish Academy of Sciences.

ORCID

Anna K. Jasińska  <http://orcid.org/0000-0003-4661-027X>

Gregor Kozłowski  <http://orcid.org/0000-0003-4856-2005>

Giuseppe Garfi  <http://orcid.org/0000-0003-0466-4288>

Salvatore Pasta  <http://orcid.org/0000-0003-3265-9072>

Hoàng Văn Sâm  <http://orcid.org/0000-0001-7266-439X>

Krystyna Boratyńska  <http://orcid.org/0000-0002-9325-1452>

Adam Boratyński  <http://orcid.org/0000-0003-0678-4304>

References

- Biltekin D, Popescu SM, Suc JP, Quézel P, Jiménez-Moreno G, Yavuz N, Çağatay MN. 2015. Anatolia: A long-time plant refuge area documented by pollen records over the last 23 million years. *Rev Palaeobotany Palynol.* 215:1–22.
- Boratyńska K, Jasińska AK, Sobierajska KI, Salva Catarineu M, Romo A, Boratyński A. 2021. Morphology supports the geographic pattern of genetic differentiation of *Pinus sylvestris* (Pinaceae) in the Iberian Peninsula. *Plant Biosyst.* 1–9. <https://doi.org/10.1080/11263504.2021.1922532>.
- Browicz K, Zieliński J. 1982. *Zelkova* Spach. In: Davis PH, editor. *Flora of Turkey and the East Aegean Islands*, vol. 7. Edinburgh: University Press; p. 648–649.
- Browicz K. 1989. Chorology of the Euxinian and Hyrcanian element in the woody flora of Asia. *Pl Syst Evol.* 162(1–4):305–314.
- Christe C, Kozłowski G, Frey D, Bétrisey S, Maharramova E, Garfi G, Pirtintos S, Naciri Y. 2014. Footprints of past intensive diversification and structuring in the genus *Zelkova* (Ulmaceae) in south-western Eurasia. *J Biogeogr.* 41(6):1081–1093.
- Denk T, Grimm GW. 2005. Phylogeny and biogeography of *Zelkova* (Ulmaceae *sensu stricto*) as inferred from leaf morphology, ITS sequence data and the fossil record. *Botanical J Linnean Soc.* 147(2):129–157.
- Denk T, Güner TH, Kvaček Z, Bouchal JM. 2017. The early Miocene flora of Güvem (Central Anatolia, Turkey): a window into early Neogene vegetation and environments in the Eastern Mediterranean. *Acta Palaeobotanica.* 57(2):237–338.
- Fineschi S, Anzidei M, Cafasso D, Cozzolino S, Garfi G, Pastorelli R, Salvini D, Turchini D, Vendramin GG. 2002. Molecular markers reveal a strong genetic differentiation between two European relic tree species: *Zelkova abelicea* (Lam.) Boissier and *Z. sicula* Di Pasquale, Garfi et Quézel (Ulmaceae). *Conserv Genetics.* 3(2):145–153.
- Follieri M, Magri D, Sadori L. 1986. Late Pleistocene *Zelkova* extinction in Central Italy. *New Phytol.* 103(1):269–273.
- Fukatsu E, Watanabe A, Nakada R, Isoda K, Hirao T, Ubukata M, Koyama Y, Kodani J, Saito M, Miyamoto N, et al. 2012. Phylogeographical structure in *Zelkova serrata* in Japan and phylogeny in the genus *Zelkova* using the polymorphisms of chloroplast DNA. *Conserv Genet.* 13(4):1109–1118.
- Garfi G, Carimi F, Fazan L, Gristina AS, Kozłowski G, Livreri Console S, Motisi A, Pasta S. 2021. From glacial refugia to hydrological microrefugia: Factors and processes driving the persistence of the climate relict tree *Zelkova sicula*. *Ecol Evol.* 11(6):2919–2936.

- Garfi G, Carimi F, Pasta S, Rühl R, Trigila S. 2011. Additional insights on the ecology of the relic tree *Zelkova sicula* di Pasquale, Garfi et Quézel (Ulmaceae) after the finding of new population. *Flora*. 206(5):407–417.
- Garfi G, Pasta S, Fazan L, Kozłowski G. 2017. *Zelkova sicula*. The IUCN Red List of Threatened Species. 2017:e.T61678A86134112. <http://dx.doi.org/10.2305/IUCN.UK.2017-3.RLTS.T61678A86134112.en>.
- Iszkuło G, Myślička K. 2015. Is climate warming advantageous for plants with untoothed leaves? *Basic Appl Ecol*. 16(5):386–393. <http://dx.doi.org/10.1016/j.baee.2015.04.011>.
- Jasińska AK, Rucińska B, Kozłowski G, Bétrisey S, Safarov H, Boratyńska K, Boratyński A. 2015. Morphological differentiation of leaves in the relict tree *Zelkova carpinifolia* (Ulmaceae). *Dendrobiology*. 74:109–122.
- Jasińska AK, Rucińska B, Kozłowski G, Fazan L, Garfi G, Pasta S, Bétrisey S, Gerber E, Boratyńska K, Boratyński A. 2021. Leaf variation of *Zelkova abelicea* and *Z. sicula*: the relict Mediterranean species. *Dendrobiology*. 85:60–77.
- Jentys-Szaferowa J. (Ed.). 1970. Zmienność liści i owoców drzew i krzewów w zespołach leśnych Białowieżskiego Parku Narodowego [Variability of the leaves and fruits of trees and shrubs in forest associations of the Białowieża National Park]. *Monographiae Botanicae*. 32:5–238. [e-ISSN: 2392-2923, p-ISSN: 0077-0655], [in Polish with English summaries]
- Kovar-Eder J. 2003. Vegetation dynamics in Europe during the Neogene. In: Reumer JWF, Wessels W, editors. *Distribution and migration of tertiary mammals in Eurasia*. A volume in honour of Hans de Bruijn. Rotterdam: DEINSEA; vol. 10; p. 373–392. [ISSN 0923-9308].
- Kozłowski G, Frey D, Fazan L, Egli B, Bétrisey S, Gratzfeld J, Garfi G, Pirintzos S. 2014. The Tertiary relict tree *Zelkova abelicea* (Ulmaceae): distribution, population structure and conservation status. *Oryx*. 48(1):80–87. DOI: .
- Kozłowski G, Gratzfeld J. 2013. *Zelkova* - an ancient tree. Global status and conservation action. *Natural History Museum Fribourg, Fribourg*. 32. ISBN 978-2-9701096-2-4.
- Lionello P. 2012. (Ed.) *The climate of the Mediterranean region. From the past to the future*. Amsterdam: Elsevier; p. 502.
- Magri D, Di Rita F, Aranbarri J, Fletcher W, González-Sampériz P. 2017. Quaternary disappearance of tree taxa from Southern Europe: Timing and trends. *Quat Sci Rev*. 163:23–55. <http://dx.doi.org/10.1016/j.quascirev.2017.02.014>.
- Maharramova E, Safarov HM, Kozłowski G, Borsch T, Muller LA. 2015. Analysis of nuclear microsatellites reveals limited differentiation between Colchic and Hyrcanian populations of the wind-pollinated relict tree *Zelkova carpinifolia* (Ulmaceae). *Am J Bot*. 102(1):119–128.
- Mai DH. 1995. *Tertiäre Vegetationsgeschichte Europas*. Jena: Gustav Fischer; pp. :691. ISBN 3-334-60456-X.
- Manchester SR. 1989. Systematics and fossil history of the Ulmaceae. In: Crane PR, Blackmore S, editors. *Evolution, systematics, and fossil history of the Hamamelidae*, Vol., 2. Oxford: Clarendon Press; p. 221–251.
- Marramà G, Kriwet J. 2017. Principal component and discriminant analyses as powerful tools to support taxonomic identification and their use for functional and phylogenetic signal detection of isolated fossil shark teeth. *PLoS One*. 12 (11):e0188806.
- Milne RI, Abbott RJ. 2002. The origin and evolution of tertiary relict floras. *Adv Botanical Res*. 38:281–314. [http://dx.doi.org/10.1016/S0065-2296\(02\)38033-9](http://dx.doi.org/10.1016/S0065-2296(02)38033-9).
- Naciri Y, Christe C, Bétrisey S, Song Y-G, Deng M, Garfi G, Kozłowski G. 2019. Species delimitation in the East Asian species of the relict tree genus *Zelkova* (Ulmaceae): A complex history of diversification and admixture among species. *Mol Phylogenet Evol*. 134:172–185.
- Nakagawa T, Garfi G, Reille M, Verlaque R. 1998. Pollen morphology of *Zelkova sicula* (Ulmaceae), a recently discovered relict species of the European Tertiary flora: description, chromosomal relevance, and palaeobotanical significance. *Rev Palaeobot Palynol*. 100(1–2):27–37.
- Phitos D, Strid A, Snøgerup S, Greuter W. (Eds.). 1995. *The Red Data Book of rare and threatened plants of Greece*. Michalis K, Athens: WWF Greece; p. 527. ISBN: 960-7506-04-9.
- Sarlis PG. 1987. *Zelkova abelicea* (Lam.) Boiss., an endemic species of Crete (Greece), *Zelkova abelicea* (Lam.) Boiss., specie endemica di Creta (Grecia). *Webbia*. 41(2):247–255.
- Sokal RR, Rohlf FJ. 2003. *Biometry. The principles and practice of statistics in biological research*. 3rd ed. New York: W. H. Freeman and Co; p. 887.
- Staszkiwicz J. (Ed.). 1997. *Zmienność wybranych gatunków drzew i krzewów [Variability of selected shrub and tree species]*. *Fragmenta Floristica et Geobotanica, Series Polonica, Supplementum 2*. [In Polish with English summaries].
- Tralau H. 1963. Asiatic dicotyledonous affinities in the Cainozoic flora of Europe. *Kungliga Svenska Vetenskapsakademiens Handlingar Series*. 4(9):5–87.
- Velitzelos D, Bouchal JM, Denk T. 2014. Review of the Cenozoic floras and vegetation of Greece. *Rev Palaeobot Palynol*. 204:56–117.
- Wang YF, Ferguson DK, Zetter R, Denk T, Garfi G. 2001. Leaf architecture and epidermal characters in *Zelkova*, Ulmaceae. *Botanical J Linnean Soc*. 136 (3):255–265.
- Wilson M, Tankut A, Gulec N. 1997. Tertiary volcanism of the Galatia Province, NW Central Anatolia, Turkey. *Lithos*. 42(1–2):105–121. (97)00039-X
- Wolfe JA. 1995. Paleoclimatic estimates from tertiary leaf assemblages. *Annu Rev Earth Planet Sci*. 23(1):119–142. <http://dx.doi.org/10.1146/annurev.earth.23.1.119>.
- Worobiec G. 2014. Late Neogene leaf assemblage from Bełchatów Lignite Mine (central Poland). *Acta Palaeobotanica*. 54(2):249–277.