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

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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.

Introduction
The taxonomic differentiation of the genus Zelkova Spach and its phylogenesis detected using internal transcribed spacer (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., Garfì & Quézel grows in two known localities in Sicily (Garfì et al. 2011, 2017), Z. abelicea (Lam.) Boiss. is distributed across five mountain massifs in Crete (Sarlis 1987; Phitos et al. 1995; Kozlowski 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 Zielinski 1982; Browicz 1989).

The remnant Zelkova species can be distinguished using the characteristics of fruits and leaves (Wang et al. 2001; Kozlowski 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) Büzèk 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 (Folieri 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. zelkovifolia. In this study, we conduct a biometrical comparison of fossil leaf assemblages of Z. zelkovifolia 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. zelkovifolia (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. zelkovifolia 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. zelkovifolia 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. zelkovifolia 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.
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] |
|-----------------|-----------------------------------|-------|-----|-------------------------------|-----------------|----------------|--------------|-----------|----------|
| Z. zelkovifolia | Turkey, Güvem area                | ZELA  | 18  | NN(20)                        | 40.50           | 32.40          | 1200         |           |          |
|                 | Greece, Chios (incl. 1 leaf from Elssona) | ZELB  | 4   | NN(4)                         | 38.24           | 26.09          | 130          |           |          |
| Z. sicula       | 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      |
| Z. abelicea     | Greece, Crete, Omalos 1           | OMA1  | 20  | NN(200)                       | 23.912          | 35.316         | 1231         | 11.96     | 917      |
|                 | Greece, Crete, Omalos 2           | OMA2  | 10  | S(99), S(100)                 | 23.903          | 35.309         | 1265         | 12.48     | 899      |
| Z. carpinifolia | 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 | 35.170 | 1196 | 13.95 | 857 |
| Azerbaijan, Xanbulan | XAN | 10 | L, S | 48.803 | 38.650 | 40 | 14.22 | 1086 |
| Azerbaijan, Gunesli | GUN | 10 | L, S | 48.469 | 38.650 | 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 shaddy vs sun-exposed position on the tree crown and position on the shoot, which strongly influence the leaf variation (Jentys-Szaferowa 1970; Staszkiewicz 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 (Kozlowski et al. 2014). Past climate change could have also reduced the level of
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 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 character | S | N | M | Min | Max | V | NN | N | M | Min | Max | V |
|-----------------|----------|------|------|-------|------|-------|----------|------|-------|------|-------|----------|------|-------|------|-------|----------|------|-------|------|-------|----------|------|
| Leaf blade surface (cm²) | A | 18 | 1.53 | 1.00 | 7.00 | 100.34 | 22 | 8.57 | 1.59 | 16.43 | 37.85 |          |   |      |      |      |          |   |      |      |      |          |   |
| 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 blade (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 |          |   |      |      |      |          |   |      |      |      |          |   |
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 or NN); differences significant at p ≤ 0.01 bolded; character codes as in Table 2.

| Character code | 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 | 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 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 |
| BW             | 0.000 | 0.000 | 0.002 | 0.000 | 0.000  | 0.001 | 0.002 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 |
| BW_50          | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 |
| BW_90          | 0.000 | 0.000 | 0.001 | 0.000 | 0.000  | 0.001 | 0.046 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 |
| LLV            | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 |
| LV             | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.026 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 |
| LI             | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.036 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 |
| LWP            | 0.000 | 0.000 | 0.049 | 0.000 | 0.000  | 0.006 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 |
| AA             | 0.012 | 0.008 | 0.000 | 0.000 | 0.000  | 0.009 | 0.002 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 |
| AB             | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 |
| TN             | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 |
| LHL            | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 |
| SHL            | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 |
| LA             | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 |
| W_1            | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 |
| W_2            | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 |
| W_3            | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 |
| LBW            | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 |
| DI             | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000  | 0.000 | 0.000 |
| LS             | 0.000 | 0.000 | 0.000 | 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 at low elevation in the thermo-Mediterranean climate, with very low summer precipitation (Garfì 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. zelkovifolia 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. zelkovifolia 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. zelkovifolia and Z. abelicea and between Z. zelkovifolia leaves from undetermined-type shoots and Z. sicula leaves from vegetative long shoots.

The intermediate position of the fossil leaves of Z. zelkovifolia 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.

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