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Doç. Dr. Sevinç KIRAN

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ARALIK 2025

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COMPARATIVE ANALYSIS OF LEAF MORPHOLOGY IN FOUR *QUERCUS* SPECIES

Fahrettin ATAR[1] , Deniz GÜNEY[2] , İbrahim TURNA[3]

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1. INTRODUCTION

The genus *Quercus*, one of the most prominent and ecologically influential components of temperate-zone forests in the Northern Hemisphere, is notable for its wide distribution and high species diversity. Globally comprising more than 500 species, the main centers of diversification of this genus are the Sierra Madre Occidental of Mexico and East and Southeast Asia (Govaerts et al., 1998; Denk et al., 2017). The representation of approximately 250 species in the Americas and around 125 species in Asia and Malaysia, together with more limited yet ecologically critical populations in Europe, North Africa, and Macaronesia, reflects the complex biogeographic history of *Quercus* (Govaerts et al., 1998). Türkiye also constitutes an important center of diversity for this genus, hosting 18 native oak species (Yaltırık, 1984). The broad morphological and genetic variation observed in oaks is closely associated with weak reproductive barriers among many species (Bacilieri et al., 1995; Manos et al., 1999; Samuel, 1999). Extensive hybridization is commonly observed among species within the same section, and in many regions, species occur as mosaic-like mixed populations. While this pattern supports the high adaptive capacity of oaks, it also results in complex variation structures that complicate taxonomic classification. The ability of oaks to rapidly adapt to diverse climatic and edaphic conditions, together with their root system characteristics, leaf litter production, and contributions to soil organic matter cycling, has led to their recognition as "ecosystem engineer" species (McShea and Healy, 2002; Johnson et al., 2019). These functional roles within ecosystems place the genus *Quercus* in a critical position with respect to both biodiversity and forest dynamics. The extensive root systems of oak species and their litterfall processes, which enrich soil organic matter, make substantial contributions to the maintenance of nutrient cycling and soil fertility in forest ecosystems (Crow, 1988; Johnson et al., 2009). Therefore, the genus *Quercus* represents a fundamental component not only in terms of woody plant diversity but also in sustaining key ecosystem functions.

In the biogeographic regions of Europe, Anatolia, and the Caucasus, oaks constitute one of the principal components of forest cover. The phylogenetic and morphological diversity of *Quercus* species in Western Eurasia is of key importance for understanding the paleoclimatic history of the region and the evolutionary development of forest structure (Denk and Grimm, 2010). Anatolia is particularly notable for the dominance of oak species within maquis, pseudomaquis, and broadleaved forest formations. Similarly, the occurrence of different oak species across both humid and relatively dry microclimates in the Caucasus demonstrates the broad environmental tolerance of this genus (Yaltırık, 1984). Across Europe, oaks are regarded as structural elements of many forest types and play a major role in maintaining habitat diversity and continuity (Mölder et al., 2019).

Oaks also have strategic economic importance. Species such as *Quercus robur*, *Q. petraea*, and *Q. cerris* have long been preferred for furniture, veneer, construction, and shipbuilding due to their high-density, durable, and long-lasting wood. In addition, acorns represent one of the primary food resources for wildlife and directly influence the population dynamics of many mammal and bird species. Oak forests further provide critical habitats for a wide range of insects, fungi, and lichens, thereby making a strong contribution to biological diversity within forest ecosystems (Smallwood and Peters, 1986; Eaton et al., 2016). For these reasons, oaks represent one of the central tree groups in European and Western Asian forestry in terms of both economic value and habitat-enhancing functions.

In Türkiye, the genus *Quercus* constitutes one of the most important forest components in terms of both species richness and spatial extent. According to data from the General Directorate of Forestry, oak forests account for approximately 30% of the country's total forest area and exhibit a wide distribution (OGM, 2021). Extensive oak communities dominated by different species occur in Central Anatolia, the Aegean, Marmara, and Eastern Anatolia regions. Oak forests are of high silvicultural importance, particularly due to their functions in erosion control, regulation of water

regimes, conservation of soil fertility, and provision of fuelwood in rural areas. Moreover, the difficulties encountered in species identification and the high degree of morphological variation among oaks in Türkiye render this group increasingly critical from both scientific research and applied forestry perspectives (Yaltırık, 1984; Kaya and Raynal, 2001; Atalay, 2014).

Oak species exhibit remarkable diversity in leaf morphology due to their broad ecological tolerances and high phenotypic plasticity. This variability represents a fundamental indicator both for taxonomic discrimination among species and for assessing the adaptive responses of populations to environmental conditions. Comprehensive morphometric studies on *Quercus petraea* and *Q. robur*, two widespread European species, have demonstrated that traits such as petiole length, lobe depth, sinus angles, and leaf width show statistically robust differences between the two species (Kremer et al., 2002). Similar studies conducted in Türkiye have likewise revealed that leaf shape and size ratios provide high discriminatory power for classification in species such as *Q. robur*, *Q. petraea*, *Q. frainetto*, and *Q. hartwissiana* (Borazan and Babaç, 2003).

One of the major factors complicating the taxonomy of the genus *Quercus* is the widespread occurrence of natural hybridization and introgressive individuals. The formation of hybrid zones in sympatric areas by many species renders the biological species concept controversial for this genus (van Valen, 1976) and leads to blurred morphological boundaries among taxa. In both Europe and Western Asia, the presence of individuals exhibiting intermediate traits of parental species clearly illustrates the role of hybridization in generating taxonomic uncertainty (Muir and Schlötterer, 2005; Curtu et al., 2007). Consequently, leaf characters are regarded as indispensable indicators for evaluating phenotypic variation and identifying hybrid individuals in oaks (Stace, 1989; Jensen et al., 1984).

In recent years, advances in morphometric approaches—particularly geometric morphometrics—have enabled high-resolution analyses of leaf shape, providing a powerful tool for both species delimitation and the identification of environmental adaptation patterns. Studies on Chinese oaks have demonstrated that geometric morphometrics achieves high classification accuracy in discriminating closely related species and reliably reveals shape variation associated with habitat differences (Viscosi et al., 2009; Liu et al., 2018). Research conducted on populations originating from geographically distinct regions has further confirmed the presence of significant variation in quantitative traits such as leaf length, width, and area, with populations from Azerbaijan providing a strong example of this pattern (Aliyeva et al., 2021). Morphometric analyses are also of critical importance for the detection of hybrid individuals; studies from European forests have reported the successful discrimination of hybrids using both classical and geometric metrics (Bruschi et al., 2000; Viscosi et al., 2009). Collectively, these findings demonstrate that leaf morphology constitutes a key tool in the genus *Quercus* for taxonomic discrimination as well as for elucidating hybridization processes and environmental adaptation mechanisms.

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Nevertheless, studies on oak leaf morphology have largely focused on a limited number of species groups and, in Europe, have concentrated predominantly on the *Q. robur–Q. petraea* species pair. In regions with high species diversity such as Türkiye and the Caucasus, comprehensive morphometric studies comparing multiple oak species within a unified methodological framework remain scarce. Research conducted in Türkiye has likewise often been restricted to specific populations and therefore does not fully capture the broad species diversity present in the region (Borazan and Babaç, 2003).

The comparison of four ecologically and taxonomically important species—*Q. cerris*, *Q. hartwissiana*, *Q. petraea*, and *Q. robur*—within a single dataset addresses a clear gap in the existing literature. The majority of morphometric studies on oak leaves have focused primarily on leaf shape, outline, and size. Research based on geometric morphometric methods has demonstrated that leaf shape reflects interspecific differentiation with high resolution and is particularly effective in distinguishing sympatric species (Viscosi et al., 2009; Liu et al., 2018). However, the combined evaluation of functional traits such as vein angle alongside classical and geometric morphometric measurements remains rare. Vein angle may be considered a physiological indicator potentially associated with water transport, photosynthetic efficiency, mechanical stability, and growth orientation of the leaf. Therefore, the assessment of vein angle together with classical morphometric parameters such as leaf length, width, and area in these four oak species is expected to provide a valuable contribution to the literature. Given the high phenotypic plasticity and complex interspecific relationships within the genus *Quercus* (Denk and Grimm, 2010), such a comprehensive interspecific analysis is likely to yield important insights into both oak systematics and species-specific ecological adaptations.

The aim of this study is to compare the main morphological characteristics of leaves from *Quercus cerris*, *Q. hartwissiana*, *Q. petraea*, and *Q. robur* in order to elucidate structural differences among these species.

2. MATERIAL AND METHOD

The study was conducted on natural oak species located on the Kanuni Campus of Karadeniz Technical University (Figure 1). Accordingly, *Q. cerris*, *Q. hartwissiana*, *Q. petraea*, and *Q. robur* were selected as representative species. Leaf samples were collected at a minimum of 50 leaves per individual. The samples were taken from the outer, light-exposed portions of the tree crowns.

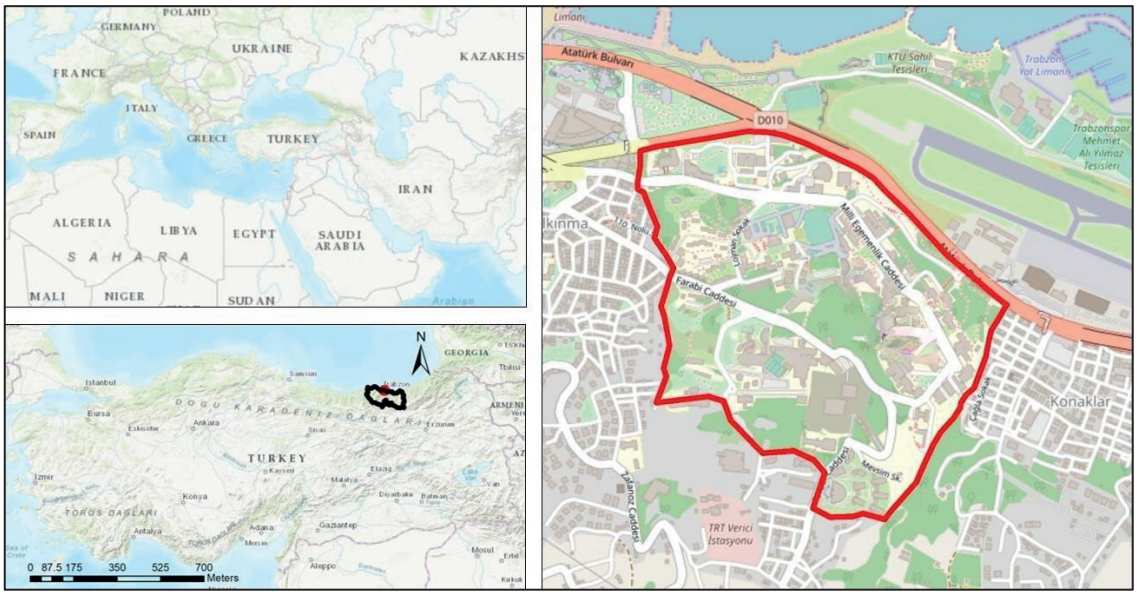

Figure 1. Geographical location of the study area

In this study, morphological traits including leaf length, leaf width, leaf area, leaf vein angle, and leaf shape index were measured. Leaf vein angle was obtained by measuring three different sections of the leaf, namely the basal, median, and apical parts. The ratio of leaf length to the corresponding leaf width was used to calculate the leaf shape index (Jeong et al., 2011; Wang and Zhang, 2012). Leaf samples were scanned at a resolution of 1200 dpi using a flatbed scanner (Hewlett-Packard Scanjet G4010). Subsequent measurements were performed using Image Analysis Software (ImageJ). This software has been widely applied in various scientific studies to analyze variation in leaf traits (Bayramzadeh et al., 2008; Atar, 2022).

The data were analyzed using the SPSS statistical software package version 26.0. For each species, descriptive statistical parameters were calculated to assess the degree of variation in each measured trait. Analysis of variance (ANOVA) was employed to determine whether statistically significant differences existed among species with respect to the measured morphological characters. In addition, Duncan's multiple range test was applied to identify homogeneous groups.

3. RESULTS

Descriptive statistics and analysis of variance (ANOVA) results for leaf length, leaf width, leaf vein angle, leaf area, and leaf shape index of *Quercus cerris*, *Q. hartwissiana*, *Q. petraea*, and *Q. robur* are presented in Table 1.

Table 1. The results of analysis of variance and descriptive statistics concerning the values of leaf characteristics

Leaf Characters	Species	Mean	SD	SE	Minimum	Maximum	*P*-value (F)
LL (cm)	*Q. Cerris*	10.52	1.28	0.24	7.79	14.22	0.000 (F: 8.392)
	Q. hartwissiana	10.30	0.99	0.18	8.25	12.13	
	Q. petraea	11.70	1.13	0.22	9.33	13.89	
	Q. robur	10.42	1.17	0.24	8.27	12.44	
LW (cm)	*Q. Cerris*	5.72	0.90	0.17	4.31	8.21	0.036 (F: 2.955)
	Q. hartwissiana	5.93	0.73	0.13	4.47	7.40	
	Q. petraea	6.22	0.67	0.13	5.23	7.58	
	Q. robur	6.30	0.81	0.17	4.73	8.27	
LVA (°)	*Q. Cerris*	43.48	4.68	0.88	35.34	54.61	0.000 (F: 34.711)
	Q. hartwissiana	44.22	4.44	0.82	36.36	55.62	
	Q. petraea	35.69	3.63	0.71	28.38	41.28	
	Q. robur	48.05	4.78	0.99	36.92	56.63	
LA (cm^2)	*Q. Cerris*	32.95	8.42	1.59	22.63	60.80	0.000 (F: 16.840)
	Q. hartwissiana	40.86	6.96	1.29	26.05	55.57	
	Q. petraea	48.16	7.96	1.56	32.98	62.52	
	Q. robur	38.36	8.54	1.78	25.71	61.73	
LSI	*Q. Cerris*	1.85	0.22	0.04	1.48	2.32	0.000 (F: 7.161)
	Q. hartwissiana	1.75	0.19	0.03	1.39	2.19	
	Q. petraea	1.89	0.17	0.03	1.60	2.33	
	Q. robur	1.66	0.16	0.03	1.36	1.99	

LL: Leaf length, LW: Leaf width, LVA: Leaf vein angle, LA: Leaf area, LSI: Leaf shape index, SD: Standard deviation, SE: Standard error

The results indicate that statistically significant differences were detected among the oak species for all examined leaf morphological characters ($p < 0.05$). With respect to leaf length, the highest mean value was recorded in *Q. petraea* at 11.70 cm, with minimum and maximum values ranging from 9.33 to 13.89 cm. Mean leaf length was determined as 10.52 cm (7.79–14.22 cm) in *Q. cerris*, 10.42 cm (8.27–12.44 cm) in *Q. robur*, and 10.30 cm (8.25–12.13 cm) in *Q. hartwissiana*. The analysis of variance for this parameter was significant ($F = 8.392$; $p < 0.001$), indicating that *Q. petraea* was distinguished from the other species particularly in terms of both mean value and range of variation. In terms of leaf width, the highest mean value was measured in *Q. robur* at 6.30 cm, with values ranging between 4.73 and 8.27 cm. The mean leaf width was 6.22 cm (5.23–7.58 cm) in *Q. petraea*, 5.93 cm (4.47–7.40 cm) in *Q. hartwissiana*, and 5.72 cm (4.31–8.21 cm) in *Q. cerris*. These interspecific differences were found to be statistically significant ($F = 2.955$; $p = 0.036$), demonstrating that leaf width varies depending on species.

A pronounced differentiation among species was observed with respect to leaf vein angle, and differences for this trait were highly significant ($F = 34.711$; $p < 0.001$). The highest mean vein angle was recorded in *Q. robur* at 48.05°, with values ranging from 36.92° to 56.63°. The mean vein angle was 44.22° (36.36–55.62°) in *Q. hartwissiana*, 43.48° (35.34–54.61°) in *Q. cerris*, and markedly lower in *Q. petraea*, with a mean of 35.69° (28.38–41.28°). These results indicate that vein angle represents a highly discriminative morphological trait among the studied species.

Significant differences were also detected among species in terms of leaf area ($F = 16.840$; $p < 0.001$), with the highest mean value observed in *Q. hartwissiana* at 40.86 cm^2. This was followed by *Q. robur* (38.36 cm^2; 25.71–61.73 cm) and *Q. petraea* (38.16 cm^2; 32.98–62.52 cm), whereas *Q. cerris*

exhibited the lowest mean leaf area at 32.95 cm² (22.63–60.80 cm). Such variation in leaf area may be interpreted as reflecting species-specific morphological responses to environmental conditions.

Regarding the leaf shape index (LSI), the highest mean value was recorded in *Q. petraea* (1.89), with values ranging from 1.60 to 2.33. Mean LSI values were 1.85 (1.48–2.32) in *Q. cerris*, 1.75 (1.39–2.19) in *Q. hartwissiana*, and 1.66 (1.36–1.99) in *Q. robur*. These differences were statistically significant ($F = 7.161$; $p < 0.001$), indicating that leaf shape constitutes a strong indicator for the morphological differentiation of oak species. Overall, all examined leaf morphological traits exhibited clear and significant variation among the oak species, demonstrating that these characteristics can be effectively used to reveal morphological and ecological differences among taxa. The results of the Duncan multiple range test, conducted to identify species groupings based on the examined leaf characters, are presented in Figure 2.

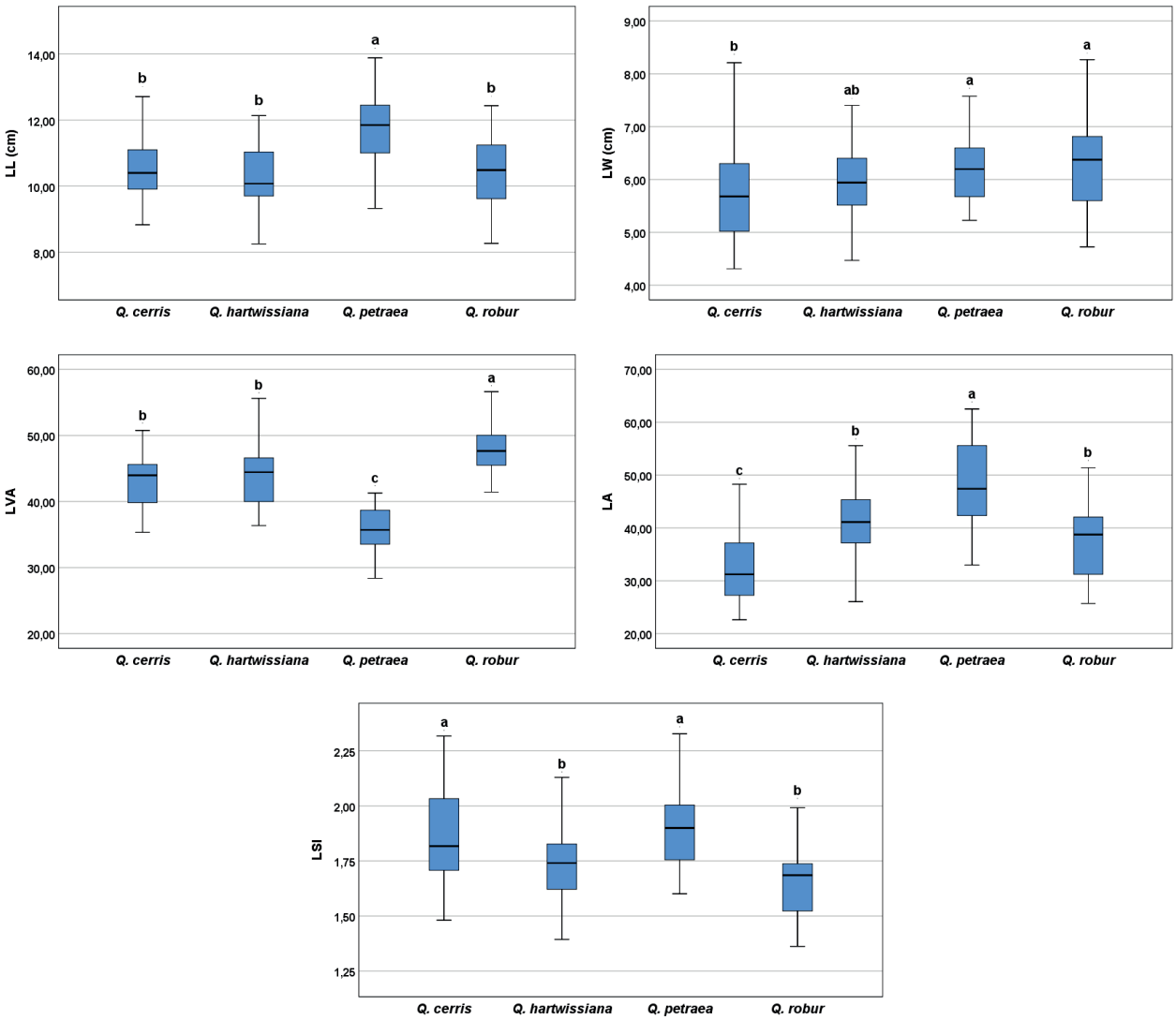

Figure 2. Duncan test results for leaf characters according to species

The results of Duncan's multiple range test presented in Figure 2 reveal clear and consistent groupings among the oak species with respect to the examined leaf morphological characters. For leaf length (LL), *Q. petraea* was statistically placed in the highest group (a), whereas *Q. cerris*, *Q. hartwissiana*, and *Q. robur* were grouped together (b), with no significant differences detected among these three species. This pattern indicates that *Q. petraea* is distinctly separated from the other species in terms of leaf length.

With respect to leaf width (LW), *Q. robur* and *Q. petraea* formed the highest group (a), while *Q. cerris* was assigned to a lower group (b). *Q. hartwissiana* occupied an intermediate position (ab), overlapping with both groups. This result suggests a gradual, rather than discrete, differentiation among species for leaf width. Species separation was more pronounced for leaf vein angle (LVA). *Q. robur* formed the uppermost group (a) with the highest values, whereas *Q. cerris* and *Q. hartwissiana* clustered together in an intermediate group (b). In contrast, *Q. petraea* constituted a distinct lower group (c) with the smallest vein angle values. This clear separation supports the use of vein angle as a strong morphological indicator for species discrimination. In terms of leaf area (LA), *Q. petraea* occupied the highest group (a), followed by *Q. hartwissiana* and *Q. robur* in an intermediate group (b), while *Q. cerris* was positioned in the lowest group (c). These results demonstrate that leaf area varies markedly among species. For the leaf shape index (LSI), Duncan test results indicated that *Q. cerris* and *Q. petraea* were grouped together in the upper group (a) with higher index values, whereas *Q. hartwissiana* and *Q. robur* formed a second group (b) characterized by lower values.

Overall, the Duncan test results corroborate the findings of the analysis of variance, indicating clear interspecific differentiation for parameters such as leaf length, vein angle, and leaf area, while leaf width and leaf shape index exhibit more gradual and overlapping groupings. This pattern suggests that the examined morphological characters differ in their discriminatory power for separating oak species.

4. DISCUSSION AND CONCLUSION

The genus *Quercus* is considered one of the most taxonomically complex tree genera due to weak reproductive barriers and widespread hybridization in sympatric distribution areas. This situation often results in indistinct morphological boundaries among species and considerable interspecific overlap (Bacilieri et al., 1995; Kremer and Petit, 1993). In this context, leaf morphology has long been used as a fundamental and practical tool for the discrimination of oak species; however, it has been emphasized that single characters are frequently insufficient, and that the combined evaluation of multiple morphological parameters provides more reliable results (Jensen et al., 1984; Kremer et al., 2002).

In the present study, *Q. cerris*, *Q. hartwissiana*, *Q. petraea*, and *Q. robur* differed significantly with respect to leaf length, leaf width, leaf vein angle, leaf area, and leaf shape index. In particular, the clear separation of *Q. petraea* from the other species in terms of leaf length indicates that this trait may serve as a strong indicator for species discrimination. Similarly, large-scale studies conducted across Europe have reported that leaf size–based distinctions between *Q. petraea* and *Q. robur* can be observed consistently across different geographical regions (Kremer et al., 2002). However, the partial overlap observed among species for certain traits, such as leaf width, suggests that these characters may be more sensitive to environmental factors and phenotypic plasticity.

Leaf vein angle emerged as one of the parameters providing the clearest interspecific separation in this study. The occurrence of wider vein angles in *Q. robur* and markedly narrower angles in *Q. petraea* indicates that this character conveys a distinct morphological signal for species differentiation. These findings are consistent with previous studies emphasizing the importance of venation patterns and leaf architecture in oak species discrimination (Viscosi et al., 2009; Borazan and Babaç, 2003). Viscosi et al. (2009), using geometric morphometric approaches, demonstrated that venation structure and leaf shape constitute strong components supporting interspecific differentiation.

The results obtained for leaf area also point to species-specific ecological strategies. The larger leaf areas observed in *Q. petraea* and *Q. hartwissiana* suggest that these species may have developed strategies aimed at enhancing light capture and increasing photosynthetic surface area. In contrast,

the lower leaf area values recorded for *Q. cerris* indicate that this species may be adapted to different ecological conditions. Such interspecific differentiation in leaf area is consistent with studies emphasizing the influence of environmental conditions and local adaptation on leaf morphology in *Quercus* species (Bruschi et al., 2000; Yücedağ and Gailing, 2013).

Differences observed in the leaf shape index further indicate that species are differentiated in terms of leaf proportions and overall leaf architecture. However, the presence of overlapping values for this parameter in some species suggests that a single ratio-based trait may not be sufficient for reliable species identification. Indeed, Borazan and Babaç (2003) reported that characters related to leaf shape and size among white oaks may exhibit wide variation due to hybridization and gene flow, thereby necessitating the use of multivariate analyses.

The results of Duncan's multiple range test are consistent with the findings of the analysis of variance, showing that certain traits—particularly leaf length, vein angle, and leaf area—produce clearer groupings among species, whereas leaf width and leaf shape index display more gradual and overlapping patterns. This pattern aligns with the effects of hybridization and shared ancestral variation on morphological characters within the genus *Quercus* (Bacilieri et al., 1995; Yücedağ and Gailing, 2013), and further underscores the need to use multiple morphological indicators jointly rather than relying on single traits for species discrimination.

Overall, this study demonstrates that leaf morphological characters provide robust information supporting interspecific differentiation in oaks, while also showing that the overlaps observed in certain traits are a natural consequence of the well-documented genetic and ecological complexity of the genus *Quercus*. Therefore, species discrimination based on leaf morphology should involve the combined evaluation of as many characters as possible and, where appropriate, be supported by molecular data.

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