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Leaf shape differentiation between *Quercus vulcanica* and *Q. frainetto* using geometric morphometrics

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Abstract*:* *Quercus vulcanica* Boiss. & Heldr. ex Kotschy and *Q. frainetto* Ten. are closely related oak species with little genetic differentiation and slight morphological differences in Türkiye possibly as result of hybridization and phenotypic plasticity. While leaf morphology offers strong phenotypic cues for quantitative studies, traditional morphometrics frequently fails to capture these fine-scale differences. The objective of this study is to use geometric morphometrics to measure symmetric and asymmetric leaf shape variation using allometric effects and determine how population-level morphological differentiation, putative hybridization and altitude affect the interspecific differentiation between these two species. A total of 222 leaves from four *Q. vulcanica* and two *Q. frainetto* populations in Türkiye were digitized at 13 homologous landmarks to capture bilateral leaf geometry, enabling the analysis of symmetric and asymmetric shape variation. Generalized Procrustes Analysis, PCA, discriminant analysis and allometric and correlation assessments were performed in MorphoJ and SPSS to evaluate interspecific differences, population-level variation and associations with diameter at breast height and altitude. These analyses revealed that interspecific differentiation between *Q. vulcanica* and *Q. frainetto* is primarily associated with symmetric leaf shape components, particularly lamina width, lobe expansion, and petiole morphology, whereas asymmetric variation showed extensive overlap and lacked taxonomic relevance. 2B-PLS analyses indicated a weak to moderate association between leaf size and symmetric shape variation, especially in *Q. vulcanica*, while asymmetric shape variation was largely independent of size. Population-level comparisons showed clear morphological differentiation with low putative hybridization (2.9–12.2%) based on morphometric intermediacy criteria, and correlations with tree diameter highlighted size-dependent shape changes in *Q. vulcanica*, whereas altitude had little influence on leaf morphology in either species. It will be crucial to conduct future studies that integrate geometric morphometrics with genomic, eco-physiological and experimental methods over wider environmental gradients. Such integrative research will clarify the relative contributions of plasticity, adaptability and developmental constraint to the evolution of oak leaf shapes.

Keywords: hybridization, population-level morphology, oak taxonomy, allometry

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Introduction

With over 900 species spread across the Northern Hemisphere, *Quercus* L. (oak) is one of the biggest and most ecologically significant genera in the Fagaceae family (Khan et al., 2025). Türkiye is a very rich center of diversity for oaks with a total of 24 taxa, four of which are endemic species (Yaltırık, 1998; Koç & Atalay Dutucu, 2024). However, taxonomic classification within the genus remains challenging due to frequent interspecific hybridization potentially associated with weak reproductive isolation among closely related taxa (Işık et al., 2021; Yücedağ et al., 2021). Reliance on highly variable morphological characters within species further complicates reliable diagnosis, especially when fruit traits are absent (Yücedağ et al., 2021).

Among the endemic species found in Türkiye, *Quercus vulcanica* Boiss. & Heldr. ex Kotschy (Kasnak oak) is a relict tree species with significant conservation value due to its fragmented and restricted distribution (Yücedağ et al., 2021; Sarıkaya et al., 2022; Polat et al., 2024). Although it historically occurred at lower elevations, its present populations are mainly confined to high-altitude refugial areas (1200–1800 m), such as the Lakes District and the mountains of Central Anatolia (Öztürk, 2013; Kargıoğlu, 2018; Işık et al., 2021; Yücedağ et al., 2021; Polat et al., 2024). *Q. vulcanica* has been economically important due to its value for timber, veneer and potential barrel production (Koç & Atalay Dutucu, 2024; Polat et al., 2024; Şen et al., 2024). The species is currently classified as Near Threatened (NT) or Least Concern (LC) (Stephan, 2018; Işık et al., 2021; Polat et al., 2024; Sarıkaya et al., 2022).

Quercus frainetto Ten. (Hungarian oak) is a widespread deciduous species native to the Balkan Peninsula, southern Italy and northwestern Türkiye, and commonly occurs up to 1000 m elevation in Anatolia (Yaltırık, 1998; Mauri et al., 2016; Yücedağ et al., 2021). Despite differences in their distribution ranges and ecological preferences, the two species are genetically closely related and their mean genetic differentiation measured with nuclear microsatellite markers (F_{ST}) is very low (0.047) (Yücedağ et al., 2021). Morphological distinction primarily relies on the longer petioles of *Q. vulcanica* and its more regularly spaced leaves along the shoots compared to *Q. frainetto* (Le Hardÿ de Beaulieu & Lamant, 2010; Stephan, 2018; Yücedağ et al., 2021). Both species are considered capable of hybridizing, and it has been proposed that ancient introgression may have occurred when their distributions overlapped more extensively at lower elevations before the heavy exploitation of *Q. vulcanica* forests (Aydınözü, 2004; Yücedağ et al., 2021). The main diagnostically informative traits separating the two species have been found to be quantitative morphological characteristics such as petiole length, petiole ratio calculated as 100 × petiole length / (lamina length + petiole length), and number of lobes (Yaltırık, 1998; Öztürk, 2013; Işık et al., 2021; Yücedağ et al., 2021).

For accurate species identification, precise and reliable analytical techniques are required due to the overlapping variance in morphological traits and the possible history of hybridization (Işık et al., 2021). Conventional morphological measurements might not be able to identify hybrids or distinguish slight form changes between species. Despite poor overall genetic differentiation (Yücedağ et al., 2021), recent computational research employing deep learning methods applied to leaf pictures has demonstrated robust phenotypic signals and classification accuracies up to 95%. These verify that leaf morphology is appropriate for reliable quantitative evaluations (Işık et al., 2021).

Geometric morphometric methods (GMMs) have become central to contemporary evolutionary and ecological research by enabling a rigorous quantification of biological form through the geometric decomposition of size and shape (Jovanović et al., 2022a). By capturing the Cartesian coordinates of homologous anatomical landmarks, GMMs retain the full geometry of structures while ensuring invariance to translation, rotation, and scale, thereby overcoming key limitations of traditional morphometrics (Aissi & Beghami, 2022; Qi et al., 2024). The application of Procrustes superimposition further isolates true shape variation from non-shape components, allowing statistically robust visualization and comparison of morphological characteristics with demonstrably higher discriminatory power (Yang et al., 2022; Fortini et al., 2025). This analytical framework has proven particularly powerful in integrative taxonomy and evolutionary biology, where it facilitates species delimitation, detection of hybrids and admixture, and the quantification of inter- and intraspecific divergence (Akli et al., 2022; De Luna-Bonilla et al., 2024; Qi et al., 2024).

The main goal of this study is to analyse how leaf morphology and interspecific differentiation of *Q. vulcanica* and *Q. frainetto* are shaped by allometric growth, population-level morphological differentiation, putative hybridization and altitude. Primarily, this research aims to quantify symmetric and asymmetric components of leaf shape variation in *Q. vulcanica* and *Q. frainetto* using geometric morphometrics and to evaluate their relative taxonomic relevance. Secondarily, it assesses the contribution of symmetric leaf shape variation to interspecific differentiation in contrast to asymmetric variation associated with individual-level or environmental noise. Additionally, it investigates allometric relationships between leaf size and symmetric shape variation and tests whether size–shape coupling differs between the two species. Finally, it examines population-level morphological differentiation, putative hybridization and altitude influences on leaf shape variation.

Material and Methods

Leaf material

This study analysed leaf samples to evaluate whether the recognized taxonomic distinction between *Q. vulcanica* and *Q. frainetto* is reflected in differences in leaf morphology. A total of 222 leaves were collected for this purpose, comprising 135 samples from four separate *Q. vulcanica* populations and 87 samples from two *Q. frainetto* populations in Türkiye (Fig. 1; Table 1). Each leaf was collected from a different individual to ensure statistical independence among samples and to avoid pseudoreplication.

Geometric arrangement of landmarks

All leaf photos were saved in JPEG format and TpsUtil v.1.79 was used to create a TPS file (Rohlf, 2019). TpsDig2 v.2.64 (Rohlf, 2018) was then used to digitize 13 homologous landmarks. The landmark configuration is based on a protocol developed by Viscosi (2015), which is widely accepted in the literature, in order to comprehensively represent the morphological features of oak leaves. To ensure repeatability, all landmarks were selected following this standardized protocol and digitized twice by the same operator with consistent orientation and

Table 1. The characteristics of the study areas and the number of sampled individuals

Population	Altitude (m)	Latitude (°)	Longitude (°)	Exposure	Nr of individuals
VUL/Isparta – Yukarıgökdere	1606	37°44'38"	30°49'31"	S, SE	27
VUL/Afyonkarahisar – Sultandağı	1698	38°18'22"	31°22'19"	NE, NW	40
VUL/Karaman – Karadağ	1985	37°23'33"	33°9'27"	NE, S	34
VUL/Kütahya – Türkmendağı	1522	39°24'23"	30°16'30"	N, NE	34
FRA/Çanakkale – Biga	225	40°12'13"	27°22'26"	SW	41
FRA/Bursa – Mustafakemalpaşa	258	40°03'27"	28°33'29"	N, NE	46

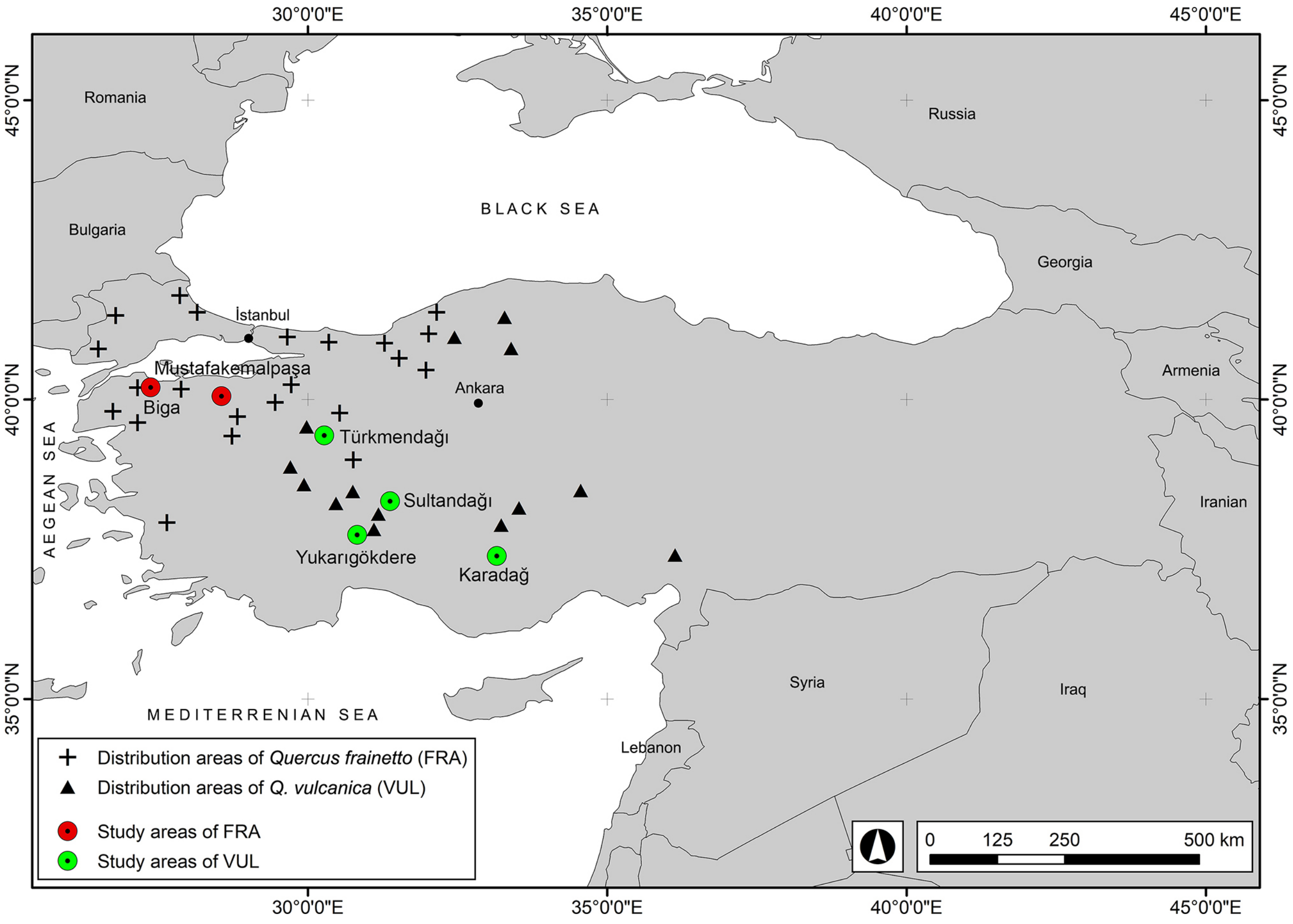
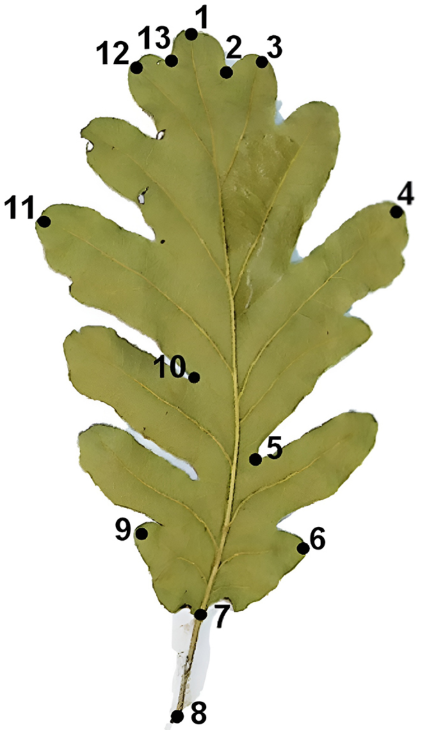


Fig. 1. Distribution areas of the species and sampling sites



Landmark	Description
LM1	blade tip
LM2	base of the apical sinuses of the right - hand leaf apex
LM3	the first lobe immediately above the apex of the right – hand leaf apex
LM4	tip of the lobe at the largest width of the right – hand leaf
LM5	the sinus immediately above the lobes of lm 4
LM6	the first basal lobe of the right – hand leaf, starting from the petiole.
LM7	junction of the blade and petiole
LM8	junction of the petiole and branch
LM9	the first basal lobe of the left – hand leaf, starting from the petiole
LM10	the sinus immediately above the lobes of lm 11
LM11	tip of the lobe at the largest width of the left – hand leaf
LM12	the first of the lobe immediately above the apex of the left – hand leaf apex
LM13	base of the apical sinuses of the left – hand leaf apex

Fig. 2. Landmark configuration of *Q. vulcanica* and *Q. frainetto* leaves showing the positions of the 13 measured traits

placement criteria applied to each leaf. These specific landmarks were selected to capture key aspects of leaf morphology, including the shape and depth of sinuses, the form of lobes and overall leaf blade geometry as they are known to be taxonomically informative and relevant for distinguishing *Q. vulcanica* and *Q. frainetto* (Fig. 2). Prior to landmark digitization, the leaves were oriented in a consistent direction. These landmarks were positioned on the leaf's left and right sides in order to record bilateral structure and thereby facilitate the examination of the leaf's symmetric and asymmetric elements. By capturing these 13 anatomically significant points, the landmark design accurately depicts overall leaf geometry. This makes it possible to perform thorough geometric morphometric analyses of the interspecific differences between *Q. vulcanica* and *Q. frainetto*. Moreover, this uniform arrangement ensures sample comparability and enables robust statistical analysis of variations in leaf shape. The x and y Cartesian coordinates of the points characterizing the general shape of the leaves in the analytic plane were obtained using the landmark digitization.

Geometric morphometric analysis

A generalized Procrustes superimposition was applied independently to each leaf in order to accommodate for differences in size, position and orientation between photos (Slice, 2007). The Procrustes-aligned coordinates were then subjected to principal component analysis (PCA). Leaf regions exhibiting pronounced variation were further examined to assess allometric effects and group differentiation. In addition, Two-Block Partial Least Squares (2B-PLS) analysis was conducted to statistically examine covariation between leaf shape and size. Significant variations in leaf form between the two species were also tested using discriminant analysis through the cross-validation tool. All analyses were performed using MorphoJ software (Klingenberg, 2011). Furthermore, Pearson correlation analysis was separately performed to determine the associations of leaf shape (symmetric and asymmetric components) with both altitude and diameter at breast height (DBH) of trees by using SPSS version 25 (Corp IBM, 2017). Potential confounding effects between altitude and DBH were not explicitly accounted for in this analysis.

Results

Generalized Procrustes analysis of overall leaf shape

Generalized Procrustes Analysis (GPA) based on the full coordinate matrix revealed clear yet subtle interspecific differentiation between *Q. vulcanica* and *Q. frainetto* (Fig. 3a). The Procrustes distance between species' consensus shapes was 0.021, and a permutation test with 10,000 iterations indicated that the observed shape difference was statistically significant ($p < 0.001$). Despite having a largely similar foliar architecture, the two taxa were systematically differentiated by displacements of eight landmarks (LM1, LM3-LM9), primarily associated with lobe depth, mid-lamina expansion and basal sinus morphology. Whereas *Q. frainetto* had a longer lamina and deeper lobation, *Q. vulcanica* had a wider lamina with shallower lobes.

With 92.4% of the Procrustes variance, the symmetric component accounted for the majority of the overall shape variation. When only symmetric variation was taken into account, interspecific variations were more noticeable (Fig. 3b). The Procrustes distance of the symmetric component between species was 0.019 ($p < 0.001$), indicating that most taxonomic distinction is primarily associated with fixed morphological characteristics rather than stochastic asymmetry. At basal and mid-lamina landmarks, symmetric variations were concentrated, indicating conserved developmental pathways specific to each species.

Asymmetric variation accounted for 7.6% of the total shape variance and showed extensive overlap between species (Fig. 3c). The Procrustes distance between species for the asymmetric component was low (0.004; $p = 0.284$), indicating that asymmetric shape deviations are more consistent with individual-level developmental noise (small, random deviations in leaf shape during individual development, not under genetic or species-level control) rather than species identity. Asymmetry was most pronounced along distal lobes and marginal regions, with no evidence of directional asymmetry in either species (DA test: $F = 1.07$, $p = 0.342$). These results demonstrate that the asymmetric component contributes mainly to intra-specific variation.

GPA demonstrates that most interspecific differentiation resides in the symmetric shape domain whereas asymmetry contributes predominantly to within-species variability. These results confirm that *Q. vulcanica* and *Q. frainetto* can be morphometrically discriminated based on leaf shape despite sharing a broadly similar foliar pattern, particularly when symmetric shape components are considered.

Leaf-level principal component analysis

Leaf-level principal component analysis (PCA) revealed clear patterns of shape variation between *Q. vulcanica* and *Q. frainetto* for the symmetric component, whereas no distinct separation was observed for the asymmetric component. For the symmetric dataset (Fig. 4a), PC1 and PC2 explained 40.21% and 29.75% of the total shape variation, respectively, accounting for a cumulative 69.96%. The scatterplot showed a pronounced differentiation between the two species primarily along PC1, with only partial overlap of the confidence ellipses, indicating substantial interspecific divergence in symmetric leaf shape. Transformation grids and wireframe graphs associated with PC1 illustrated that this variation was primarily associated with differences in lamina width, lobe expansion, and the relative elongation of the petiole–blade junction. Additionally, PC2 further captured variation related to the curvature and relative displacement of lateral lobes.

In contrast, PCA of the asymmetric component (Fig. 4b) explained 58.33% of the total variation along PC1 and 18.87% along PC2 (cumulative 77.20%), yet the two species showed strong overlap in multivariate space. The absence of distinct

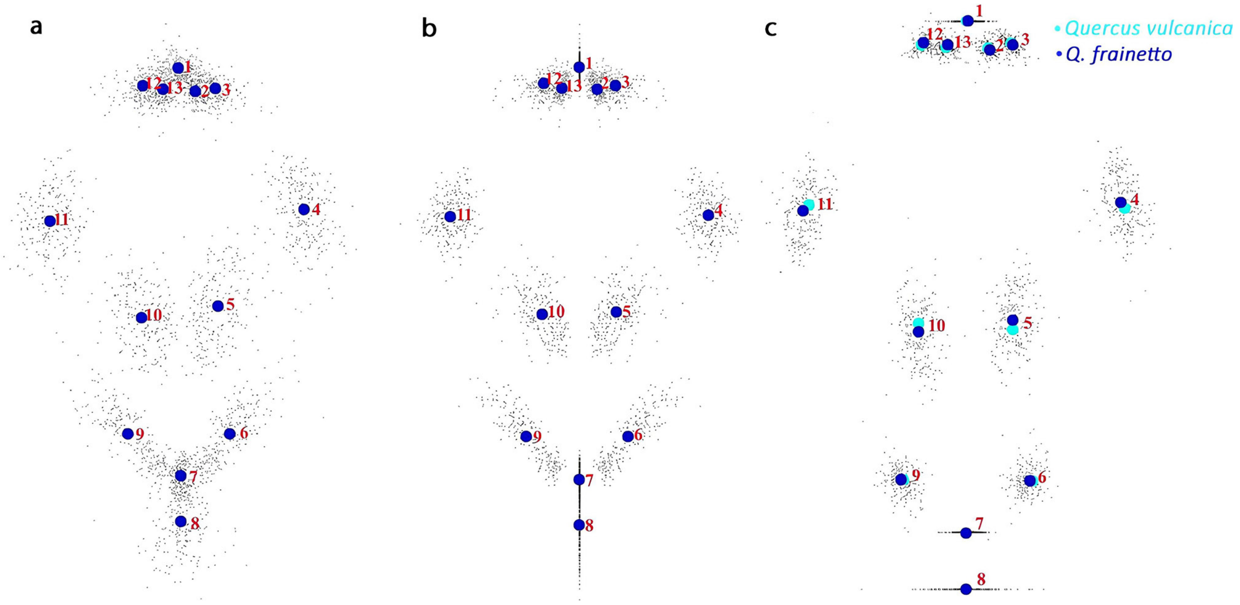


Fig. 3. Leaf shape variation in *Q. vulcanica* and *Q. frainetto* from generalized Procrustes analysis of full (a), symmetric (b) and asymmetric (c) datasets

clustering suggests that asymmetric shape variation is largely shared between *Q. vulcanica* and *Q. frainetto* and likely reflects individual-level developmental or environmental noise rather than taxonomically informative variation. Consistently, the corresponding transformation grids and wireframes indicated minor, localized deformations with no consistent species-specific asymmetry pattern across the examined landmark configuration.

Overall, symmetric shape components provided clear discriminatory power between the two oak species, whereas asymmetric components did not contribute significantly to their morphological separation.

Size–shape relationships based on 2B-PLS analysis

2B-PLS analysis was performed to evaluate the covariation between leaf size (log centroid size) and both symmetric and asymmetric components of leaf

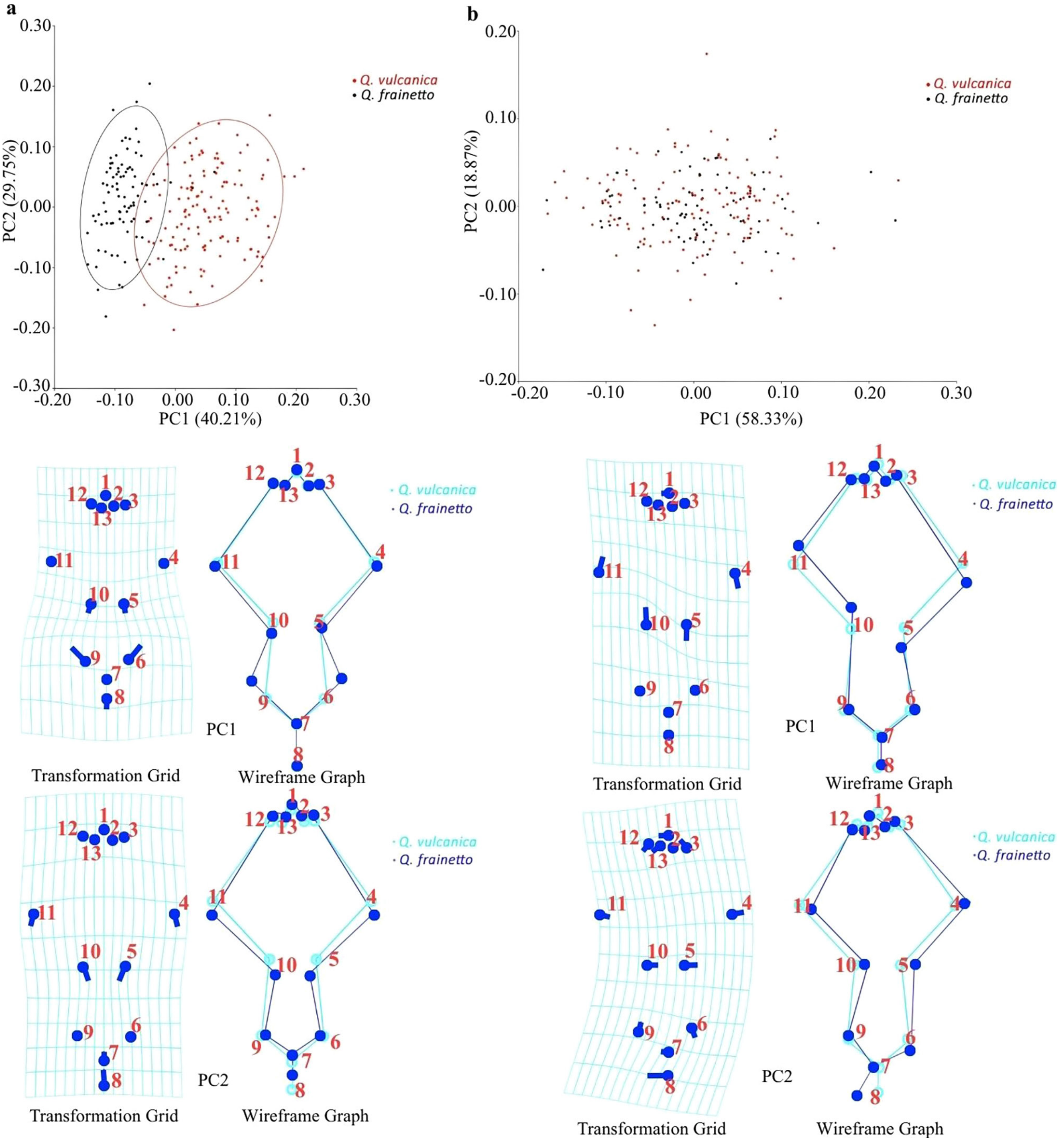


Fig. 4. Leaf-level PCA of symmetric (a) and asymmetric (b) shape components in *Q. vulcanica* and *Q. frainetto* showing PC1–PC2 scatter plots with ellipses and corresponding shape transformations

shape in *Q. vulcanica* and *Q. frainetto*. For the symmetric component (Fig. 5a), the scatterplots suggested a weak to moderate association between centroid size and symmetric shape variation with considerable overlap between species and no pronounced size-dependent separation pattern. *Q. vulcanica* showed a higher dispersion toward bigger sizes, but *Q. frainetto* was comparatively more concentrated around intermediate size values despite overlap between individuals of both species in multivariate space. The transformation grids indicate subtle shape adjustments associated with increasing leaf size, including slight widening of the lamina and minor positional shifts in the lateral, distal and basal regions.

On the other hand, there was a weak and diffuse correlation between log centroid size and asymmetric shape variation in the 2B-PLS scatterplot for the asymmetric component (Fig. 5b). There was no discernible directional trend, and individuals of both species were closely mixed throughout the size gradient. Only modest, localized deformations were visible in the relevant transformation grids and wireframe visualizations, indicating that asymmetry-related shape variation is mostly independent of leaf size and is driven by small-scale, non-systematic deviations.

Overall, these results demonstrate that leaf size is strongly coupled with symmetric shape variation in both *Q. vulcanica* and *Q. frainetto*, whereas asymmetric variation shows little to no size dependence and does not contribute significantly to interspecific differentiation.

Pairwise leaf shape comparisons revealed significant Procrustes and Mahalanobis distances for the pairs of most populations, indicating clear morphological differentiation ($p < 0.01$). The highest shape distinction was determined between FRA_Çanakkale-Biga and VUL_Isparta-Yukarıgökdere, FRA_Çanakkale-Biga and VUL_Afyonkarahisar–Sultandağı, FRA_Bursa-Mustafakemalpaşa and VUL_Afyonkarahisar–Sultandağı. However, VUL_Kütahya-Türkmendağı and VUL_Karaman-Karadağ showed the lowest distances and no significant difference ($p > 0.05$), suggesting strong shape similarity between these intraspecific groups. These results show pronounced intergroup shape variation with limited overlap except for a small number of closely related group pairs (Table S1). Lastly, according to discriminant scores (Fig. 6), of 222 leaf samples, 125 (56.3%) were identified as *Q. vulcanica*, 78 (35.1%) as *Q. frainetto*, and 19 (8.6%) as putative hybrids or introgressive forms. *Q. vulcanica* was sampled from

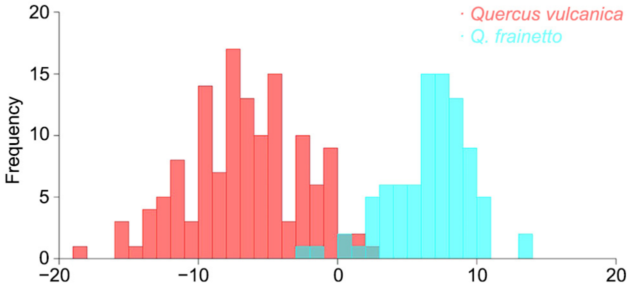


Fig. 6. Discriminant analysis based on the shapes of *Q. vulcanica* and *Q. frainetto* leaves

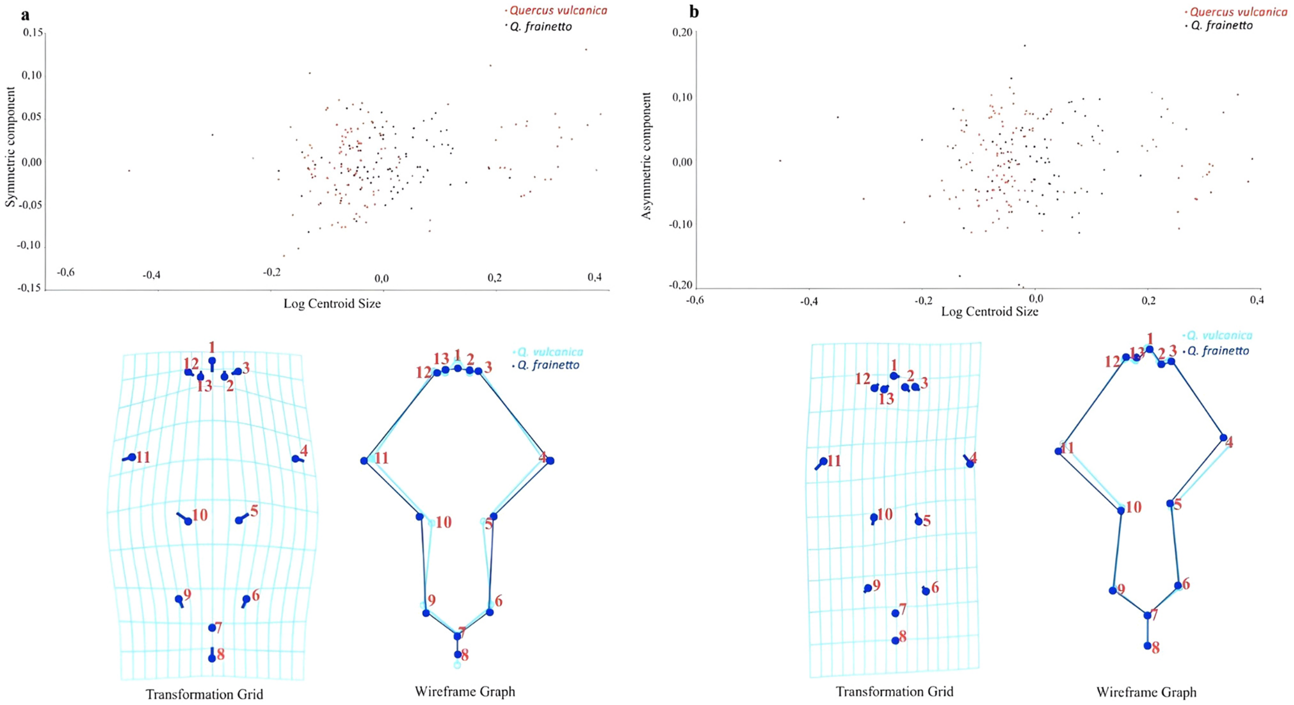


Fig. 5. Scatterplots from 2B-PLS analysis relating log centroid size to symmetric (a) and asymmetric (b) leaf-shape components of *Q. vulcanica* and *Q. frainetto*

Table 2. Associations of leaf shapes of the species with both altitude and diameter at breast height based on Pearson's correlation test

	Altitude (m)	Diameter at breast height (cm)
Q. vulcanica		
Symmetric components	−0.164ns	0.179*
Asymmetric components	−0.055ns	0.236**
Q. frainetto		
Symmetric components	−0.180ns	−0.111ns
Asymmetric components	0.085ns	0.028ns

four populations (n = 25–36 per site), yielding 10 hybrid individuals, while *Q. frainetto* was represented by two populations (n = 36–42), with 9 hybrids detected. Putative hybrid frequencies identified by intermediate leaf morphologies were low, ranging from 2.9% to 12.2% based on morphometric intermediacy criteria. The difference between the means of *Q. vulcanica* and *Q. frainetto* was found to be significant ($T^2 = 670.99$, $p < 0.0001$). A cross-validation test showed that 91.79% of *Q. vulcanica* individuals and 95.40% of *Q. frainetto* individuals were correctly classified.

According to Pearson's correlation test, in *Q. vulcanica*, symmetric and asymmetric shape components had significant positive correlations with diameter at breast height whereas no significant relationships were determined with altitude. In contrast, for *Q. frainetto*, neither symmetric nor asymmetric shape components were significantly correlated with altitude or diameter at breast height (Table 2).

Discussion

The study revealed that symmetric components mainly accounted for interspecific differences in leaf morphology between *Q. vulcanica* and *Q. frainetto*, which have traits under tight genetic control that are conserved during development while asymmetric variation mostly reflects noise at the individual or environmental level (Liu et al., 2018; Li et al., 2021). This is corroborated by PCA results in the present study, which demonstrate distinct interspecific separation along symmetric axes, with differentiation being driven by lamina width, lobe expansion and petiole length. In contrast, asymmetric variation lacks taxonomic significance and shows remarkable overlap (Slice, 2007; Klingenberg, 2011). In line with other research (Işık et al., 2021; Yücedağ et al., 2021) highlighting petiole length and lobe number as important discriminators, these results show that species-specific and stable leaf traits offer reliable morphometric markers for taxonomic identification. Studies involving landmark-based geometric morphometrics proved to be useful in highlighting relationships between leaf size and shape variation, and between phylogenetic patterns and hybridization events (Fortini et al., 2025). Leaf fluctuating asymmetry, which reflects both genetic variability and species' buffering capacity, has been highlighted in other oak studies as well (Li et al., 2022; Qi et al., 2024). Moreover, Yang et al. (2022) reported that geometric morphometric methods provided higher classification accuracy and more detailed information about leaf shape compared to traditional ones.

2B-PLS analyses showed a weak to moderate correlation between symmetric shape variation and leaf size notably in *Q. vulcanica* with larger leaves tending to have slightly shorter petioles and somewhat wider laminae, suggesting a potential link between leaf shape and eco-physiological function (Jovanović et al., 2022a; Yang et al., 2022; Jovanović et al., 2023; Fortini et al., 2025). These size-related changes may moderately influence heat regulation and water-use efficiency, as smaller and more lobed leaves could reduce boundary layer resistance and improve hydraulic performance under Mediterranean drought conditions (Aissi & Beghami, 2022; Jovanović et al., 2022b; Fortini et al., 2025). However, the absence of a significant allometric tendency in *Q. frainetto*, as indicated by the 2B-PLS results, suggests a more developmentally stable (i.e., canalized) leaf morphology in comparison to *Q. vulcanica*. In this context, canalization refers to reduced phenotypic sensitivity of shape to size variation, meaning that leaf shape remains relatively invariant despite changes in overall leaf size (Liu et al., 2018; Jovanović et al., 2022a). These size-dependent shape alterations suggest that growth and developmental programs interact to generate species differences, supplying an extra morphological discrimination axis along with static traits.

Significant Procrustes and Mahalanobis distances were found also between intraspecific pairs of populations in their comparisons with modest differentiation between a few closely related populations, indicating small morphological overlap. A low putative hybridization rate (2.9–12.2%) based on morphometric intermediacy criteria was seen in the current study. However, according to Kremer et al. (2002) and Akli et al. (2022), interspecific gene flow between related oak species does not compromise the general integrity of the species. These results support the application of symmetric leaf traits as taxonomic markers, indicating that selective pressures preserve different morphologies and that low-frequency hybridization only slightly contributes to intraspecific variation.

Leaf morphology appears to be influenced by environmental factors although these effects do not seem to fully override inherent morphological patterns. Subtle leaf shape variation is probably influenced by adaptations to Mediterranean drought such as decreased leaf size, increased indumentum and changed stomatal density, particularly in marginal or distal lamina regions (Yavas et al., 2024). The underlying symmetric developmental patterns, however, do not seem to be compromised by these environmentally induced alterations (Gailing et al., 2018; Aissi & Beghami, 2022; Hegedüs et al., 2025). Elevation-induced environmental gradients have little impact on leaf morphology, as seen by the lack of a meaningful correlation between altitude and leaf shape in both *Q. vulcanica* and *Q. frainetto*. Instead, these traits seem to be more heavily influenced by allometric processes and developmental limitations. Similar evidence from the Euganean Hills in northern Italy indicated that even when a potential third oak species is present, morphological differences are sufficient for taxonomic distinction, emphasizing the stability of leaf traits under environmental variation (Proietti et al., 2021). Analyses of variance for shape and centroid size in European oaks also revealed significant correlations between leaf shape and environmental variables while local hybridization events explained some unexpected difference, yet leaf form remained largely conservative (Fortini et al., 2025). Likewise, Jovanović et al. (2023) showed that intraspecific variability of *Q. petraea* leaves' size and shape at a small spatial scale is influenced by local habitat conditions (altitude, slope and aspect).

All these results suggest that growth allometry has influenced the evolution of leaf morphology in *Q. vulcanica* and *Q. frainetto*. Additionally, species-specific leaf form is shaped by plastic reactions to environmental conditions. While asymmetric variation and low-frequency putative hybrids contribute to within-species variability, symmetric variation emerges as the primary driver of interspecific differentiation. The present study illustrates the ability of geometric morphometrics to resolve small morphological variations in closely related oak taxa by combining PCA, 2B-PLS, population-level and allometric analysis. Moreover, it offers a strong framework for examining the ecological, evolutionary and developmental factors that influence leaf morphology in oaks and other taxonomically difficult plant groups. Integrative approaches combining leaf morphology and environmental data, as shown in studies on Mexican scrub oaks, further support the utility of morphometrics for clarifying taxonomic relationships in complex oak groups (De Luna-Bonilla et al., 2024).

Conclusion

This study shows that a strong and comprehensive foundation for resolving minute but biologically significant leaf-shape differences between *Q. vulcanica* and *Q. frainetto* is provided by geometric morphometrics. The results of the study exhibit that while asymmetric variation and low-frequency putative hybrids contribute to within-species diversity, symmetric shape components linked to growth allometry are the principal drivers of interspecific differentiation. The relevance of selective pressures in preserving species-specific leaf architecture is highlighted by the existence of distinct morphometric boundaries in the face of environmental variation and restricted gene flow. It will be crucial to conduct future studies that integrate geometric morphometrics with genomic, eco-physiological and experimental methods over wider environmental gradients. Such integrative research will clarify the relative contributions of plasticity, adaptability and developmental constraint to the evolution of oak leaf shapes. These studies will enhance our knowledge of how tree species react morphologically to continuous environmental change in addition to improving taxonomic resolution in taxonomically challenging plant species.

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