

Leaf macro- and micromorphological traits and phenotypic diversity of *Quercus petraea* subspecies in Eastern Romania

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Abstract

Sessile oak (*Quercus petraea*) is a polytypic species comprising three subspecies (*Q. petraea* subsp. *petraea* – *Qpe*, *Q. petraea* subsp. *dalechampii* – *Qda*, and *Q. petraea* subsp. *polycarpa* – *Qpo*) with distinct ecological requirements, posing significant challenges in morphological differentiation. The integration of macro- and micro-morphological analyses plays a crucial role in clarifying the taxonomic uncertainties. This study aimed to characterize phenotypic diversity and identify key leaf descriptors for distinguishing sessile oak subspecies across three peripheral populations, one reference population, and one sessile oak comparative trail from Eastern Romania. A comprehensive analysis was conducted on 227 sampled trees, utilizing multivariate statistical analysis - encompassing 18 macromorphological and 9 micromorphological leaf descriptors. The results revealed distinct traits of *Qda* and *Qpo*, including shorter leaves with maximal width in the lower half of the lamina, fewer lobes, ovate shapes, a subcordate basal shape, and a higher intercalary vein frequency compared to *Qpe*. Furthermore, *Qpo* could be differentiated from both *Qpe* and *Qda* by its shorter lamina lengths, fewer lobes, greater lobe width ratios, and stellate trichomes with shorter rays. The length of rays of stellate trichomes has emerged as a significant micromorphological descriptor. *Qda* predominated in peripheral populations, likely due to natural selection in drought-affected local ecosystems. This highlights the importance of prioritizing this taxon in breeding programs and conserving it *in situ*, given its remarkable leaf plasticity and adaptability. Additionally, principal component indicated a fairly high level of morphological similarity among the three subspecies. These findings emphasize the critical importance of comprehensive morphological analyses for precise species classification and deeper understanding of sessile oak taxonomy.

Keywords: leaf descriptors; peripheral populations; *Quercus petraea* subsp. *petraea*; *Q. petraea* subsp. *dalechampii*; *Q. petraea* subsp. *polycarpa*; Sessiliflorae Loj.; taxonomic classification

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Introduction

Leaf morphological traits serve as key indicators for distinguishing closely related taxa and for understanding their evolutionary relationships (Meng *et al.*, 2016; Qiu *et al.*, 2023). Taxonomic studies rely on a variety of leaf traits, such as shape, size, margin characteristics, venation patterns, and the presence of specialized structures like hairs or glands, to classify plants into various groups and identify specific species or subspecies (Deng *et al.*, 2017; Ullah *et al.*, 2018).

At the species level, oaks (*Quercus* L., *Fagaceae*) present considerable difficulties in differentiation (Owusu *et al.*, 2015), primarily due to their extensive variability, large number of species, and frequent occurrence of introgressive hybridization (Aas, 1993; Rushton, 1993; Manos *et al.*, 1999; Petit *et al.*, 2003; Curtu *et al.*, 2007). These challenges have prompted frequent revisions in taxonomic classifications, driven by variations in the characteristics taken into account for classification, adopted infrageneric subdivisions, and certain inconsistencies in species delimitation (Nixon, 1993; Grossoni *et al.*, 2021).

In Romania, there are considered 7-9 native *Quercus* species, depending on the taxonomic classification utilised (Georgescu and Morariu, 1948; Beldie, 1952; Stănescu *et al.*, 1997; Șofletea and Curtu, 2007). This discordance emerged due to differing perspectives on how to separate the three representatives of the series *Sessiliflorae* (*Quercus petraea* subsp. *petraea* – *Qpe*, *Q. petraea* subsp. *dalechampii* – *Qda*, *Q. petraea* subsp. *polycarpa* – *Qpo*). Taxonomically, some authors regard them as subspecies (e.g., Stănescu *et al.*, 1997; Clinovschi, 2005; Șofletea and Curtu, 2007), while others classify them as distinct species (e.g., Beldie and Cretzoiu, 1941; Georgescu and Morariu, 1948)

However, at the European level, apart from *Qpe*, the taxonomic status of *Qda* and *Qpo* is intensely debated. According to various taxonomic classifications, *Qda* is described as a distinct species related to *Q. petraea* (Schwarz, 1936; Govaerts and Frodin, 1998; Filipova and Ansenov, 2016) or, in some cases, as a distinct species related to *Q. pubescens* (Camus, 1954; Brullo *et al.*, 1999; Biondi *et al.*, 2004), or even as a hybrid between these two (Jovanovic and Tucovic, 1975; Kleinschmit, 1993). It is noteworthy that in Italy, *Q. dalechampii* is regarded as a distinct species, and subsequent lectotypification has positioned it within *Q. pubescens* aggregate (Di Pietro *et al.*, 2012). Consequently, Kučera (2018) later assigned the name *Q. banatus* to this taxon, previously considered taxonomically related to the sessile oak. Despite new insights into the taxonomic classification of sessile oaks, their taxonomic nomenclature and division into three subspecies of sessile oak has remained unchanged in Romania.

Also, the taxonomic status of *Q. polycarpa* is still a subject of debate, with no clear consensus among taxonomists. While some studies and checklists have highlighted its morphological similarities to *Q. petraea sensu stricto*, considering it a subspecies of *Q. petraea* (Nyman, 1884; Govaerts and Frodin, 1998; Șofletea and Curtu, 2007; Raab-Straube and Raus, 2013), others have reported it as a distinct species (Požgaj and Horváthov, 1986; Roleček, 2005; Matula, 2009). Given these divergent viewpoints, taxonomic classification and discrimination uncertainties within the *Q. petraea* complex remain subject of ongoing debate in the field of taxonomy (Di Pietro *et al.*, 2016; Proietti *et al.*, 2021). Moreover, these historical taxonomic disagreements underscore the intricate nature of species identification and emphasize the importance of sustained research endeavors aimed at achieving clearer distinctions among taxonomic units within the series *Sessiliflorae*.

These three subspecies were considered different in their ecological preferences. *Qda* and *Qpo* are known to require larger quantities of heat (subthermophilic) compared to *Qpe* (mesothermophilic), and they exhibit greater drought resistance (mesoxerophytes-mesophytes) (Șofletea and Curtu, 2007; Demeter *et al.*, 2014). Therefore, their ecological requirements restrict their altitude range compared to common sessile oak. They are often found together in the warmer regions of the country (Georgescu and Ciobanu, 1965; Sanda *et al.*, 2004), especially at the transition to the forest-silvosteppe, as observed in marginal populations at the eastern

distribution limit of sessile oak in Romania (Gafenco *et al.*, 2023). Given the ongoing and anticipated climate aridification, easy identification of sessile oaks could be invaluable for forest management practices. This would aid in selecting the appropriate species for reforestation and in conservation efforts tailored to the changing environmental conditions. Hence, selecting morphological descriptors that can effectively differentiate between entities within the *Q. petraea* complex is still a challenge. In this context, knowledge of both macromorphological and micromorphological characteristics is particularly important in studying the variability of sessile oak subspecies.

At the micromorphological level, the variability of sessile oak in our country is still poorly analysed, although other studies of this kind in Europe have found better differentiation capacity based on micromorphological traits (Fortini *et al.*, 2009). Additionally, micromorphological descriptors can illustrate the modulating effect of stabilizing natural selection and the resolution level of the genotype-environment relationship between populations adapted to normal climatic conditions and those in limiting conditions (Şofletea, 2005).

In this context, the objective of the current study was to assess the macro- and micromorphological variability of the *Sessiliflorae* series at both inter- and intra-specific levels, aiming to characterize phenotypic diversity and identify new, relevant traits, capable of discriminating between the three subspecies.

Materials and Methods

Study sites and tree sampling

The study was conducted in Eastern Romania, where three peripheral sessile oak populations (FUN, OLT, and SAT) were selected along a north-to-south transect (Table 1, Figure 1). Additionally, for comparative analysis, a non-peripheral population (HEL) located in the inner part of the native species' range, within an ecologically optimal zone (classified as mesophilic oak forest according to Gancz *et al.*, 2008), was identified and selected. Within each population, 34-50 sessile oak trees, located at least 30 m apart (usually 50 m), were sampled from the dominant layer. Moreover, a comparative trial with native provenances of sessile oak (FAN) was included in our study, and five trees from 10 provenances in different areas of Romania (Dolhasca, Sascut, Botoşani, Băbeni, Văleni, Beiuş, Făget, Blaj, Lechinţa, and Cluj) from different ecological subregions were also randomly selected and analysed (Figure 1).

Table 1. Geographic location and climatic conditions of the sampled populations and of the sessile oak comparative trial

Code	Label	Geographical position			Climatic conditions		De Martonne aridity index
		Latitude (N)	Longitude (E)	Altitude (m)	Annual average temperature (°C)	Annual rainfall (mm)	
<i>Peripheral populations</i>							
FUN	Fundeanu	45°58'	27° 41'	210	9.6	470	24.0
OLT	Olteneşti	46°34'	27° 53'	305	9.2	530	27.6
SAT	Satu Nou	46°58'	27° 49'	300	9.2	535	27.9
<i>Reference population (non-peripheral)</i>							
HEL	Heltiu	46°14'	26° 55'	490	9.2	655	34.1
<i>Comparative trial</i>							
FAN	Fântânele	46°39'	26° 43'	260	9.6	590	30.1

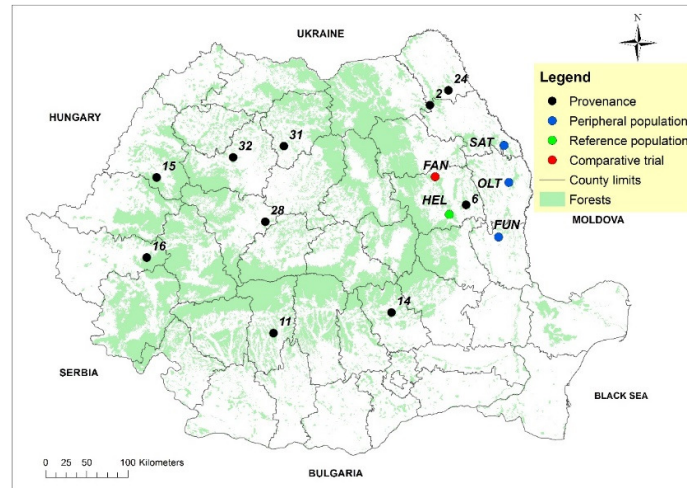


Figure 1. The location of the study area and the selected provenances that were analysed in the FAN comparative trial (2 – Dolhasca, 6 – Sascut, 11 – Băbeni, 14 – Văleni, 15 – Beiuș, 16 – Făget, 24 – Botoșani, 28 – Blaj 31 – Lechința, 32 – Cluj).

In the field, each tree was assigned to a subspecies based on the taxonomic characteristics described in Romanian literature (Beldie and Cretzoiu, 1941; Georgescu and Morariu, 1948; Beldie, 1952; Stănescu *et al.*, 1997; Clinovschi, 2005; Șofletea and Curtu, 2007).

Morphological analysis

The methodology applied for collecting biological samples followed the recommendations from previous morphological studies on oak species (Kremer *et al.*, 2002; Borazan and Babaç, 2003; Curtu *et al.*, 2011; Apostol *et al.*, 2017). The leaves were carefully selected from the inner part of the upper crown to avoid those exposed to direct sunlight. Between 6 and 12 fully developed leaves were collected from each sampled tree branch, following the protocol proposed by Bacilieri *et al.* (1995). In the field, each tree was classified into a subspecies based on the main discriminative characteristics of sessile oaks as presented in Gafenco *et al.* (2023). The collected leaves then underwent a drying and herborization process to facilitate subsequent analysis.

To investigate intraspecific taxonomic variability, both macromorphological and micromorphological descriptors were selected based on international (Bussotti and Grossini, 1997; Bruschi *et al.*, 2000, 2003; Kremer *et al.*, 2002; Viscosi *et al.*, 2009; Fortini *et al.*, 2015; Di Pietro *et al.*, 2016; Yücedağ *et al.*, 2021) and national studies (Curtu *et al.*, 2011; Șofletea *et al.*, 2011; Apostol *et al.*, 2017) across various *Quercus* species.

For macromorphological analysis, a set of 18 phenotypic descriptors was used, encompassing seven dimensional variables, two counted variables, four observed variables, and five transformed variables (Table 2).

Table 2. The foliar macromorphological descriptors analyzed in this study

Foliar macromorphological descriptors		Code
Dimensional variables (measured)	Leaf area (cm ²)	LA
	Leaf perimeter (cm)	LP
	Lamina length (cm)	LL
	Petiole length (cm)	PL
	Lamina width (cm) - measured between the median rib and the tip of the lobe in the area of maximum width	LW
	Sinus width (cm) - below the lobe furthest from the midrib	SW
	Length of lamina at largest width (cm)	WP
Counted variables	Number of lobes	NL
	Number of intercalary veins	NV
Observed variables	Basal shape of the lamina	BSL
	Abaxial laminar pubescence	AB PU
	Adaxial laminar pubescence	AD PU
	Petiole pubescence	PE PU
Transformed variables (calculated)	Lamina shape or obversity: WP/LL·100	OB
	Petiole ratio: PL/(LL+PL) ·100	PR
	Lobe depth ratio: (LW-SW)/LW·100	LDR
	Percentage venation: NV/NL·100	PV
	Lobe width ratio: LW/LL·100	LWR

The seven-dimensional descriptors have been evaluated using the WinFOLIA software (WinFOLIA PRO 2020, Reget Instruments Inc., Canada). The leaf shape evaluation followed the numerical scale (1 - cuneate to 9 - auriculate) proposed by Kremer *et al.* (2002). Concurrently, the degree of pubescence on the petiole and both sides of the lamina was determined using a stereomicroscope (40X), applying the scale (1 - no pubescence to 6 - very pubescent) established by Kissling (1977).

For the micromorphological analysis, a set of nine descriptors was used (Table 3), encompassing descriptors of stomata and specific characteristics of trichomes. Microscopic slides were prepared from leaves previously analyzed from a macromorphological perspective, with six leaves per tree selected for this purpose. These slides were then examined using an Olympus CX43 microscope. Subsequently, the dimensions of the stomata and the length of the rays of the stellate trichomes were obtained using QuickPHOTO MICRO 3.2 software.

Table 3. The foliar micromorphological descriptors analyzed

Foliar micromorphological descriptors	Code
Stomatal density (number · mm ⁻²)	StoD
Stomata rim length (µm)	SRL
Stomata rim width (µm)	SRW
Freedom of stomatal rim (µm)	FSR
Stomatal area index: StoD·SRL	SAI
Number of stellate trichomes	NST
Number of glandular trichomes	NGT
Length of rays of stellate trichomes (µm)	LRS
Number of rays of stellate trichomes	NR

Microscopic slides were obtained by applying a thin layer of nitrocellulose lacquer to the abaxial right side of the leaves, specifically between the veins where the lamina width was maximal. The measurements were conducted on a standard surface area (98278 µm² ≈ 0.1 mm²), and the average values of stomata were derived from measurements taken from 10 randomly selected stomata.

Characteristically, the abaxial surface of sessile oak leaves exhibited both glandular and non-glandular stellate and stellate-bifurcate trichomes (Gafenco, 2023)(Figure 2).

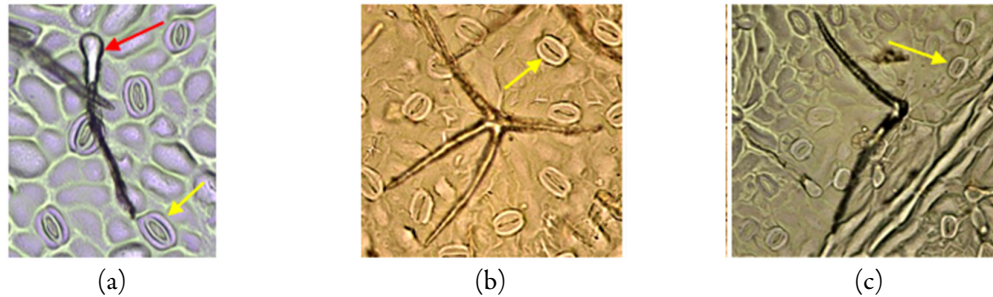


Figure 2. Glandular trichome (a) and non-glandular stellate trichome (b) and stellate-bifurcate trichome (c). The red arrow indicates the distal cell of the glandular trichome and the yellow arrows indicate the stomata.

Statistical analysis

Statistical analysis was conducted using XLFOlia software (Regent Instruments Inc., Canada) to determine the main statistical indicators for each descriptor at the level of each tree. Further statistical processing was performed using R version 4.2.1.

Subsequently, the normality of the data was tested across the entire dataset using the Kolmogorov-Smirnov and Shapiro-Wilk tests (Sheskin, 2003), along with the Levene test for equality of variances (Zar, 2010). Analysis of variance (ANOVA) was used to evaluate differences among the means of the three subspecies. Following ANOVA, the significance of the mean differences between variables was assessed using the Welch test, regardless of whether the group variances were equal or not. Additionally, we employed the Tukey post-hoc test to identify specific significant differences among the subspecies.

To explore the relationships among the three subspecies, we applied the principal component analysis (PCA), a widely used method for taxonomic classification.

Results

Macro- and micromorphological descriptors analysis

Out of the 227 evaluated trees, 164 individuals exhibited phenotypic characteristics specific to *Qpe*, 54 specific to *Qda*, and 9 specific to *Qpo* (Table 4, Figure 3). The latter two subspecies were more prevalent in peripheral populations than in the reference population.

Table 4. Classifications of sampled sessile oak trees based on the main discriminative characteristics

Population	<i>Qpe</i>	<i>Qda</i>	<i>Qpo</i>	Total
FUN	10	18	6	34
OLT	25	23	2	50
SAT	38	11	1	50
HEL	41	2	0	43
FAN*	50	0	0	50
Total	164	54	9	227

*Sessile oak comparative trial

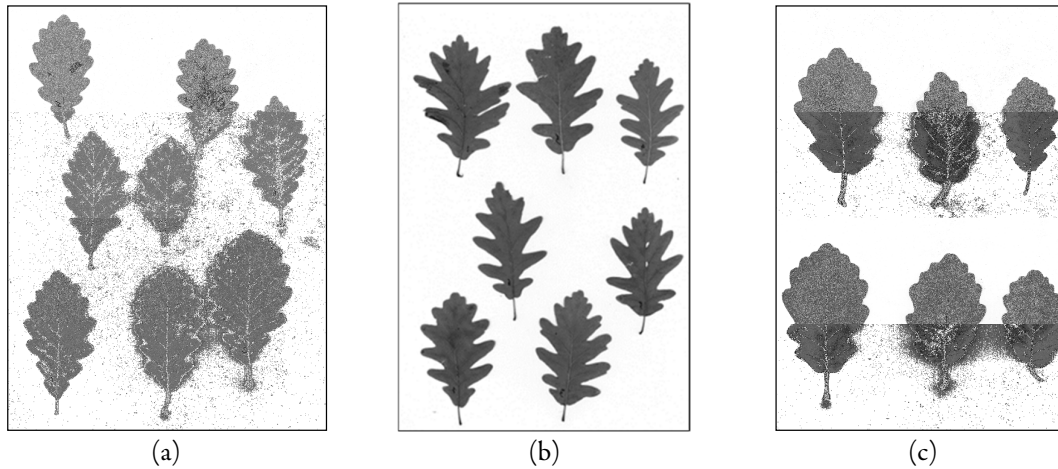


Figure 3. Leaves of *Q. petraea* subspecies: a) subsp. *petraea*, b) subsp. *dalechampii*, c) subsp. *polycarpa*

In the ANOVA analysis conducted among the three sessile oak subspecies, statistically significant differences ($p < 0.05$) were observed in 12 out of the 18 macromorphological descriptors evaluated and only for a single micromorphological descriptor (Table 5).

Table 5. ANOVA analysis between the three sessile oak subspecies

Variable*	<i>Qpe</i>			<i>Qda</i>			<i>Qpo</i>			<i>t_{welch}</i>	<i>p</i>
	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%		
LA	50.2 ^a	±10.0	19.9	49.9 ^a	±9.1	18.2	45.4 ^a	±7.5	16.5	1.67	0.210
LP	46.0 ^b	±6.2	13.4	47.7 ^b	±5.5	11.6	38.5 ^a	±3.0	7.8	29.01	0.000
LL	11.4 ^b	±1.1	9.9	11.2 ^b	±0.9	8.4	9.9 ^a	±0.8	7.6	51.19	0.000
PL	1.7 ^a	±0.3	19.6	1.7 ^a	±0.3	18.1	1.8 ^a	±0.5	28.3	0.63	0.541
LW	3.6 ^a	±0.4	12.2	3.6 ^a	±0.3	9.7	3.4 ^a	±0.3	9.8	1.39	0.270
SW	2.0 ^a	±0.3	16.3	1.8 ^a	±0.4	22.0	2.0 ^a	±0.3	13.7	3.22	0.060
WP	6.4 ^b	±0.8	13.2	5.6 ^a	±0.7	11.6	4.9 ^a	±0.9	19.1	10.22	0.000
NL	13.6 ^a	±1.4	9.9	13.2 ^a	±1.3	9.5	11.1 ^b	±1.3	12.1	16.65	0.000
NV	0.1 ^b	±0.3	242.7	0.4 ^a	±0.6	156.1	0.2 ^{ab}	±0.2	143.2	4.55	0.005
BSL	2.9 ^b	±0.2	8.5	3.1 ^a	±0.4	12.7	3.4 ^e	±0.4	10.8	12.11	0.000
AB PU	2.7 ^b	±0.6	21.0	3.1 ^a	±0.6	20.5	3.3 ^a	±0.4	13.5	11.87	0.000
AD PU	1.4 ^a	±0.4	27.4	1.4 ^a	±0.4	30.9	1.6 ^a	±0.6	38.9	1.07	0.361
PE PU	1.8 ^b	±0.5	26.1	2.1 ^a	±0.5	22.6	1.9 ^{ab}	±0.7	36.5	6.10	0.008
OB	56.1 ^b	±4.6	8.2	50.7 ^a	±5.6	11.0	49.8 ^a	±8.9	17.9	21.96	0.000
PR	13.2 ^a	±2.1	16.0	13.2 ^a	±2.2	16.8	15.2 ^a	±3.2	20.9	1.70	0.165
LDR	44.8 ^b	±8.9	20.0	49.0 ^a	±11.0	22.5	40.4 ^b	±7.8	19.3	4.92	0.001
PV	0.9 ^b	±2.3	246.9	3.1 ^a	±5.2	166.4	1.2 ^b	±1.6	134.4	4.40	0.002
LWR	31.6 ^a	±2.7	8.4	32.3 ^a	±3.0	9.1	34.5 ^b	±2.0	5.7	9.49	0.000
StoD	305.7 ^a	±44.7	14.6	316.8 ^a	±71.5	22.6	310.4 ^a	±77.6	25.0	0.57	0.830
SRW	16.8 ^a	±1.5	8.7	17.3 ^a	±1.2	6.9	16.9 ^a	±1.6	9.3	2.11	0.061
SRL	23.1 ^a	±1.9	8.2	23.3 ^a	±1.9	8.0	23.3 ^a	±2.1	8.9	0.30	0.987
FSR	3.5 ^a	±0.6	16.3	3.6 ^a	±0.4	12.1	3.9 ^a	±0.6	14.4	1.74	0.128
SAI	7030.4 ^a	±1068.9	15.2	7313.3 ^a	±1449.7	19.8	7168.9 ^a	±1516.6	21.2	0.86	0.627
LRS	99.0 ^a	±12.0	12.1	101.1 ^a	±11.8	11.7	91.8 ^b	±10.9	11.9	2.68	0.019
NGT	61.9 ^a	±21.1	34.0	65.2 ^a	±19.8	30.4	60.1 ^a	±27.9	46.4	0.55	0.882
NST	11.4 ^a	±5.5	48.1	12.0 ^a	±5.9	49.3	13.0 ^a	±4.7	36.5	0.578	0.875
NR	2.7 ^a	±0.7	27.5	2.7 ^a	±0.7	25.3	3.0 ^a	±0.8	25.5	0.71	0.767

*The variable codes are presented in Tables 2 and 3. Bold variables show significant differences ($p < 0.05$). SD – standard deviation, CV% - coefficient of variation. Values with different letters are significantly different based on Tukey's post hoc test.

Tukey's post hoc tests indicated that leaves of *Qpe* were clearly differentiated from the other two subspecies, as they were significantly more obovate, had a more cuneate basal shape of the lamina, possessed fewer intercalary veins and exhibited lower abaxial pubescence. In addition, *Qpo* could be differentiated by *Qpe* and *Qda* by its shorter lamina lengths, smaller number of lobes, higher lobe width ratios, and stellate trichomes with shorter rays.

At the subspecies level, relatively similar mean LA values (about 50 cm²) were observed for *Qpe* and *Qda*, which were higher compared to *Qpo* (approximately 45 cm²). Similarly, LP values are notably lower in *Qpo* (38.5 cm), while between *Qpe* and *Qda*, the latter has a mean value with 1.7 cm higher. The LL was smaller for *Qda* and *Qpo* compared to *Qpe* by 1.75% and 13.15%, respectively. The maximum width of the lamina is located in the lower half of the lamina, both *Qda* and *Qpo* were identified having smaller mean WP values than *Qpe* by 12.50% and 23.43%, respectively. The remaining dimensional descriptors (PL, LW, and SW) had similar values for all the three subspecies.

The BSL ranges from wide-cuneate to subcordate for all three subspecies but is notably more prominently subcordate for *Qda* and *Qpo*. Additionally, *Qda* and *Qpo* demonstrated a higher degree of pubescence on the abaxial part of the lamina (Figure 4) and petiole, both exceeding a score of 3 on the evaluation scale for AB PU, while they are nearly at a value of 2 for PE PU.

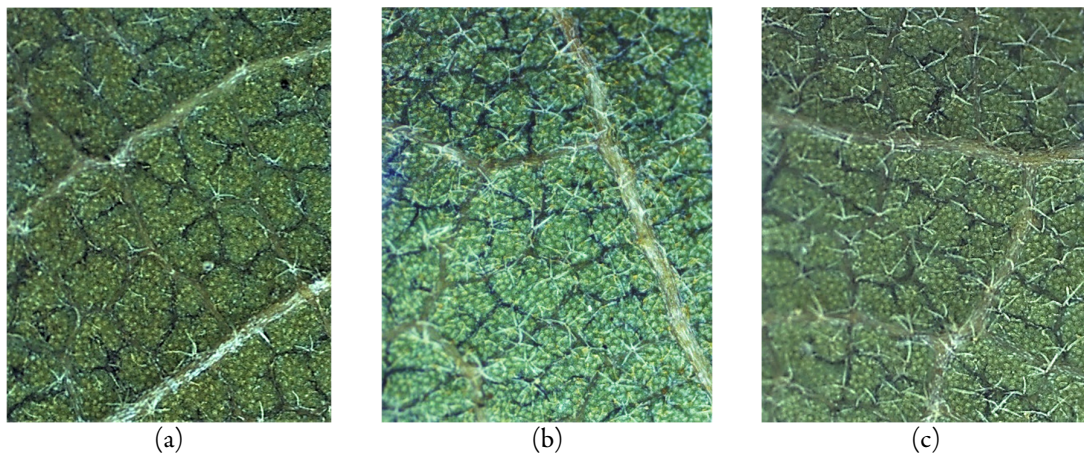


Figure 4. Abaxial pubescence of *Q. petraea* subspecies: a) subsp. *petraea*, b) subsp. *dalechampii*, c) subsp. *polycarpa*

The NL had slightly higher average values for *Qpe* (13.6) than for *Qda* and *Qpo* (11.4). The NV was very low for all three subspecies; however, *Qda* tended to have a higher NV (on average 0.4) than *Qpo* (on average 0.2) and *Qpe* (on average 0.1).

When examining the average values of OB, it has been observed that for *Qda* and *Qpo* the values are near 50% while for *Qpe* it was 56%. Furthermore, the average values of LDR varied between 40% for *Qda* and 49% for *Qpo*, with *Qpe* having an intermediate average value for the lobe depth ratio (44%). Significant differences were observed for LWR, with average values ranging between 31.6% and 34.5% for *Qpe* and *Qpo*, respectively. The percentage of venation exhibited very high variability (CV% > 100) (Table 5), with mean values ranging from 0.9% for *Qpe* to 3.1% for *Qda*.

Regarding micromorphological descriptors (Figure 5), only LRS displayed significant differences among the three subspecies (Table 5). Specifically, *Qpe* and *Qda* had trichomes with average lengths exceeding 100 μm, while those of *Qpo* measured less than 100 μm, on average.

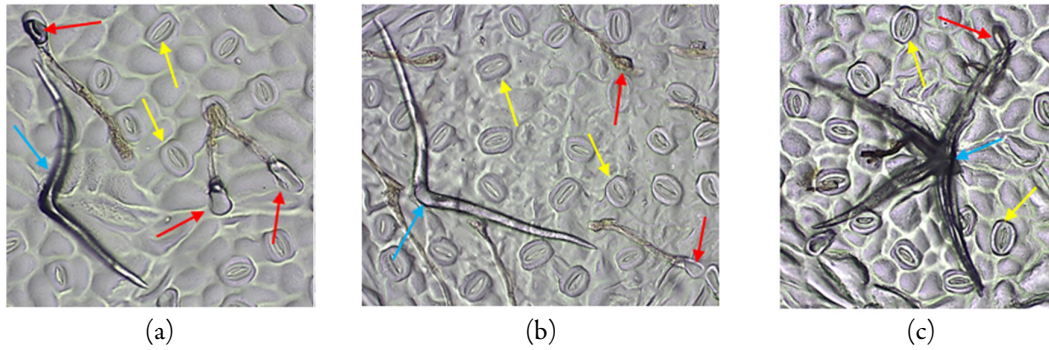


Figure 5. Micromorphological descriptors of *Q. petraea* subspecies: a) ssp. *petraea*, b) subsp. *dalechampii*, c) subsp. *polycarpa*. The red arrow indicates the distal cell of the glandular trichome, the yellow arrows indicate the stomata, and the blue arrows indicate the non-glandular trichomes.

Significant differences ($p < 0.05$) in morphological leaf descriptors were observed among the peripheral populations, reference population, and FAN comparative trial for all three subspecies (Tables 6, 7, and 8). Specifically, *Qpe* displayed the lowest average dimensional descriptors in the FUN population and the highest descriptors in the SAT population. Overall, at the dimensional variable level, all populations exhibited medium-to-low variability ($CV\% < 20\%$) (Table 6). In accordance with the dimensional characteristics of leaves, individuals from the SAT population exhibited a higher average NL value than those from the FUN population, which had the lowest NL value. In comparison to the HEL population and the FAN comparative trial, the leaves of *Qpe* from the peripheral population exhibited higher degrees of pubescence on the abaxial surface and petiole, along with the presence of intercalary veins (Table 6). Across all three subspecies, the AD PU was significantly lower than the AB PU. Notably, all leaves examined in the OLT population were glabrous on the adaxial surface (AD PU = 1). At the interpopulation level, the lowest values of OB were found in the leaves from the OLT and SAT populations (Table 6). For *Qpo* identified in the SAT population, the average value of OB was below 30%, indicating an ovate leaf shape. Less frequently, OB values around 30% were also found for *Qda*.

In terms of micromorphological descriptors, the average values of StoD were higher in the peripheral populations compared to the reference population, where the lowest StoD values were recorded (Table 6). Despite the absence of significant differences between the three subspecies regarding StoD, certain differences were noted at the population level (Table 6). Specifically, the FUN population, with the highest StoD, demonstrated a lower mean SAI compared to the SAT population, which was distinguished by stomata with longer lengths. Conversely, the HEL population with the lowest mean StoD also displayed the lowest mean SAI.

Table 6. ANOVA analysis between the four populations and the FAN comparative trial for *Qpe*

Variable*	FUN			OLT			SAT			HEL			FUN			<i>t_{welch}</i>	<i>p</i>
	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%		
LA	45.4	±7.0	15.5	47.7	±8.8	18.4	55.9	±9.4	16.7	48.6	±10.5	21.6	49.5	±9.7	19.5	5.28	0.001
LP	43.6	±4.7	10.8	44.2	±5.1	11.4	48.5	±5.8	12.0	46.2	±7.3	15.8	45.2	±5.7	12.6	3.08	0.024
LL	10.8	±1.1	9.9	11.0	±0.9	8.3	11.8	±0.9	7.9	11.5	±1.2	10.8	11.4	±1.2	10.6	3.85	0.009
PL	1.6	±0.3	19.8	1.8	±0.3	18.5	1.7	±0.3	17.4	1.8	±0.4	23.0	1.7	±0.3	17.8	1.33	0.273
LW	3.4	±0.3	9.3	3.5	±0.4	12.0	3.9	±0.4	11.1	3.5	±0.4	12.0	3.6	±0.4	11.6	5.67	0.001
SW	1.9	±0.2	9.1	2.0	±0.2	11.8	2.1	±0.4	16.7	1.9	±0.3	18.5	1.9	±0.3	15.0	3.01	0.026
WP	6.3	±0.6	9.3	6.1	±0.7	11.2	6.4	±0.9	14.6	6.6	±0.8	12.5	6.5	±0.9	13.6	2.51	0.053
AB PU	2.8	±0.6	21.6	3.6	±0.4	9.8	2.7	±0.4	16.3	2.5	±0.3	14.0	2.5	±0.4	16.5	43.12	0.000
AD PU	1.6	±0.3	17.8	1.0	±0.1	6.1	1.5	±0.4	25.0	1.4	±0.3	23.7	1.5	±0.4	27.6	38.17	0.000
PE PU	1.7	±0.3	18.9	2.3	±0.4	18.8	2.0	±0.2	11.2	1.6	±0.4	25.5	1.6	±0.4	27.8	15.33	0.000
NL	14.1	±1.2	8.8	13.8	±1.4	10.4	14.0	±1.6	11.2	13.6	±1.4	10.2	13.3	±1.0	7.8	1.24	0.306
BSL	2.8	±0.4	12.4	3.1	±0.3	8.7	2.9	±0.2	6.9	3.0	±0.2	6.7	2.9	±0.3	9.3	1.74	0.157

NV	0.1	±0.1	164.6	0.3	±0.4	135.2	0.3	±0.5	147.7	0.0	±0.1	366.3	0.0	±0.0	0.0	8.32	0.000
OB	58.0	±2.4	4.2	54.9	±3.4	6.1	53.7	±6.3	11.7	57.7	±3.8	6.6	56.9	±3.7	6.6	5.14	0.001
PR	12.5	±2.2	17.1	13.9	±2.7	19.2	12.8	±1.6	12.2	13.6	±2.3	17.3	13.1	±1.9	14.8	1.47	0.226
LDR	43.9	±5.1	11.7	43.7	±6.3	14.4	44.3	±10.6	23.9	45.6	±10.3	22.5	45.1	±8.3	18.5	0.26	0.904
PV	0.6	±1.0	165.7	2.0	±2.7	133.7	2.4	±3.7	153.2	0.2	±0.6	371.2	0.0	±0.0	0.0	8.74	0.000
LWR	31.9	±3.0	9.4	32.1	±2.0	6.3	32.8	±2.4	7.3	30.3	±2.4	7.8	31.3	±2.9	9.2	5.59	0.001
StoD	319.5	±59.6	18.7	297.8	±61.5	20.7	320.3	±17.4	5.4	282.2	±27.6	9.8	315.0	±49.9	15.9	13.37	0.000
FSR	3.7	±0.2	5.9	3.3	±0.5	15.7	4.0	±0.4	10.3	3.6	±0.7	18.5	3.3	±0.5	14.4	10.59	0.000
SWR	17.2	±0.9	5.1	17.0	±1.2	7.1	17.8	±1.2	7.0	16.5	±1.7	10.3	16.3	±1.3	8.0	5.22	0.001
SRL	22.5	±1.8	8.0	23.7	±1.8	7.5	23.8	±1.4	5.8	23.0	±2.4	10.6	22.3	±1.4	6.5	3.90	0.008
LRS	96.2	±10.7	11.2	101.0	±11.3	11.2	105.0	±12.9	12.3	95.5	±12.5	13.0	96.8	±9.6	9.9	3.32	0.018
NGT	70.6	±24.0	34.0	66.9	±22.7	34.0	67.0	±21.2	31.7	57.4	±20.2	35.1	57.6	±19.1	33.1	1.74	0.157
NST	14.2	±7.8	55.1	12.2	±4.9	40.3	12.3	±5.4	43.6	10.9	±5.4	50.1	10.3	±5.2	51.0	0.88	0.486
NR	3.2	±0.8	24.4	2.8	±0.7	24.8	2.7	±0.7	26.1	2.7	±0.8	29.1	2.6	±0.7	28.6	1.04	0.395
SAI	7122.4	±1074.6	15.1	7048.4	±1372.7	19.5	7627.2	±570.5	7.5	6475.8	±890.8	13.8	7004.4	±1111.8	15.9	11.64	0.000

*The variable codes are presented in Tables 2 and 3. Bold variables show significant differences ($p < 0.05$). SD – standard deviation, CV% - coefficient of variation

The highest NGT and NST values were identified in the peripheral populations (FUN, OLT, and SAT), while the lowest values were observed in the HEL population. In the FAN comparative trial, NST values were similar to those of the HEL population; however, the coefficients of variation at the infraspecific level were notably higher (over 40%) (Table 6), indicating a considerable level of variability in this regard. Additionally, stellate trichomes exhibited longer rays in peripheral populations compared to the reference population (Table 6).

For *Qda*, micromorphological descriptors did not produce significant differences between populations; however, unlike the other two subspecies, the PR descriptor generates significant differences between populations, with a higher PR value characteristic of peripheral populations (Table 7).

Table 7. ANOVA analysis between the four analysed populations for *Qda*.

Variable*	FUN			OLT			SAT			HEL			<i>t</i> _{welch}	<i>p</i>
	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%		
LA	45.9	±8.8	19.1	49.4	±8.3	16.7	56.7	±7.2	12.8	55.6	±13.8	24.8	3.19	0.146
LP	47.9	±7.2	15.0	46.4	±4.9	10.7	50.0	±2.9	5.9	49.1	±3.2	6.5	1.72	0.277
LL	10.9	±1.1	9.9	11.1	±0.9	7.7	11.5	±0.7	6.1	12.3	±0.5	4.0	2.96	0.137
PL	1.8	±0.3	17.9	1.6	±0.3	15.6	1.7	±0.4	23.0	1.6	±0.0	0.0	2.04	0.135
LW	3.4	±0.4	11.4	3.6	±0.3	7.3	3.8	±0.3	8.2	3.6	±0.4	10.0	2.37	0.211
SW	1.7	±0.4	25.6	1.9	±0.3	16.6	2.1	±0.5	22.4	1.8	±0.3	15.7	1.54	0.335
WP	5.6	±0.6	10.2	5.7	±0.5	8.8	5.4	±1.0	18.6	6.1	±0.1	1.2	5.21	0.009
AB PU	2.9	±0.7	22.8	3.4	±0.6	16.9	2.8	±0.2	8.5	2.2	±0.0	0.0	61.39	0.000
AD PU	1.8	±0.3	16.0	1.0	±0.0	0.0	1.6	±0.3	21.7	1.2	±0.0	0.0	57.75	0.001
PE PU	1.9	±0.2	11.8	2.3	±0.5	22.1	1.9	±0.3	16.1	1.4	±0.5	36.7	4.14	0.102
NL	12.9	±1.5	11.4	13.6	±0.8	6.2	12.8	±1.5	11.4	12.8	±1.8	13.9	1.01	0.474
BSL	3.2	±0.5	16.1	3.2	±0.4	11.2	2.9	±0.2	7.0	3.0	±0.3	9.4	2.00	0.233
NV	0.3	±0.5	172.9	0.4	±0.6	134.3	0.5	±0.9	176.8	0.3	±0.4	141.4	0.21	0.888
OB	52.1	±4.5	8.7	51.5	±3.5	6.8	46.8	±8.9	19.0	49.5	±2.6	5.3	1.06	0.443
PR	14.0	±2.6	18.4	12.7	±1.6	12.2	13.1	±2.7	20.3	11.6	±0.4	3.7	4.54	0.030
LDR	51.4	±12.4	24.1	48.9	±7.8	15.9	45.3	±15.0	33.2	49.5	±3.5	7.1	0.35	0.789
PV	2.6	±4.8	186.6	3.1	±4.3	138.6	4.3	±7.9	180.7	2.2	±3.0	141.4	0.15	0.928
LWR	31.4	±3.0	9.5	32.7	±2.4	7.4	33.7	±3.5	10.4	29.0	±1.8	6.1	2.59	0.166
StoD	340.7	±78.3	23.0	294.3	±69.5	23.6	335.7	±49.6	14.8	255.5	±51.6	20.2	2.03	0.228
FSR	3.6	±0.4	9.7	3.4	±0.4	13.2	3.9	±0.3	8.4	3.5	±0.4	10.2	3.71	0.119
SRW	17.2	±1.3	7.8	16.9	±1.0	6.2	18.0	±1.0	5.7	18.2	±0.4	2.3	4.29	0.061

Variable*	FUN			OLT			SAT			HEL			<i>t_{welch}</i>	<i>p</i>
	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%		
SRL	23.3	±2.3	9.8	23.0	±1.8	7.8	23.5	±1.4	6.0	24.4	±1.0	4.1	0.77	0.557
LRS	99.5	±11.5	11.6	102.4	±12.5	12.2	101.2	±12.4	12.3	99.7	±6.9	6.9	0.16	0.920
NGT	68.3	±18.1	26.5	61.9	±20.4	33.0	68.3	±23.2	34.0	58.5	±12.0	20.5	0.47	0.718
NST	11.6	±6.2	53.4	12.2	±5.9	48.7	12.1	±6.1	50.8	12.5	±6.4	50.9	0.03	0.993
NR	2.6	±0.7	26.8	2.9	±0.7	25.4	2.5	±0.5	20.3	2.5	±0.7	28.3	0.81	0.551
SAI	7887.3	±1741.7	22.1	6700.0	±1209.9	18.1	7857.2	±794.8	10.1	6208.5	±1006.2	16.2	3.67	0.098

*The variable codes are presented in Tables 2 and 3. Bold variables show significant differences (*p*<0.05).

SD – standard deviation, CV% - coefficient of variation.

However, for *Qpo*, the populations differed significantly in terms of pubescence (*p*<0.05 for AB PU, AD PU, and PE PU), stomatal density, stomatal area index, length of stellate trichome rays, and number of stellate trichomes (Table 8).

Table 8. ANOVA analysis between the three populations for *Qpo*

Variable*	FUN			OLT			SAT			<i>t_{welch}</i>	<i>p</i>
	Med	SD	CV%	Med	SD	CV%	Med	SD	CV%		
LA	44.3	±8.7	19.6	45.2	±4.1	9.0	52.5	-	-	0.04	0.844
LP	38.1	±3.2	8.3	37.9	±2.5	6.5	42.1	-	-	0.01	0.923
LL	9.7	±0.9	8.9	10.0	±0.3	2.8	10.6	-	-	0.49	0.491
PL	1.7	±0.6	31.7	1.7	±0.1	4.3	2.5	-	-	0.13	0.720
LW	3.3	±0.3	10.3	3.6	±0.2	6.0	3.8	-	-	1.49	0.240
SW	1.9	±0.3	14.5	2.2	±0.3	12.9	2.2	-	-	1.34	0.281
WP	5.1	±0.8	14.9	5.3	±0.1	1.3	3.0	-	-	0.18	0.676
AB PU	3.2	±0.3	10.7	3.9	±0.1	3.6	2.8	-	-	15.77	0.001
AD PU	1.8	±0.6	30.2	1.0	±0.0	0.0	1.0	-	-	13.46	0.001
PE PU	1.8	±0.7	38.7	2.5	±0.3	11.3	1.2	-	-	4.57	0.043
NL	10.7	±1.1	10.3	12.0	±2.3	19.5	11.7	-	-	0.55	0.492
BSL	3.4	±0.4	13.2	3.6	±0.1	2.0	3.2	-	-	0.63	0.435
NV	0.1	±0.1	159.5	0.3	±0.4	141.4	0.2	-	-	0.51	0.509
OB	52.5	±4.8	9.1	52.6	±2.2	4.2	28.3	-	-	0.00	0.984
PR	15.0	±3.6	23.8	14.2	±0.2	1.5	19.1	-	-	0.30	0.589
LDR	41.2	±8.0	19.5	37.5	±12.0	32.1	42.0	-	-	0.16	0.701
PV	0.8	±1.2	158.4	2.2	±3.1	141.4	1.7	-	-	0.40	0.553
LWR	34.0	±2.2	6.5	35.5	±1.1	3.2	35.8	-	-	1.63	0.217
StoD	335.0	±80.8	24.1	233.5	±0.7	0.3	317.0	-	-	9.46	0.005
FSR	3.8	±0.4	9.5	3.7	±0.8	21.3	4.9	-	-	0.03	0.867
SRW	16.7	±1.8	10.6	16.7	±0.1	0.8	18.8	-	-	0.00	0.946
SRL	23.3	±2.5	10.8	22.8	±0.4	1.6	24.8	-	-	0.24	0.628
LRS	88.8	±10.2	11.5	104.3	±5.0	4.8	85.2	-	-	8.04	0.010
NGT	58.3	±25.3	43.3	76.5	±44.5	58.2	38.0	-	-	0.30	0.603
NST	12.5	±4.9	39.4	12.5	±6.4	50.9	17.0	-	-	0.00	1.000
NR	3.3	±0.6	18.8	2.0	±0.0	0.0	3.7	-	-	24.80	0.000
SAI	7672.2	±1378.3	18.0	5312.5	±98.3	1.9	7862.0	-	-	17.32	0.000

*The variable codes are presented in Tables 2 and 3. Bold variables show significant differences (*p*<0.05).

SD – standard deviation, CV% - coefficient of variation

Principal component analysis

The PCA analysis was conducted without redundant variables, revealing an overlap among the three subspecies (Figure 6). However, a stronger association with the assessed variables was observed in *Qpo*, displaying negative values on factor 1. This tendency is likely due to the lower values of the dimensional variables LP, LL, LW, and WP, which exert a significant and positive contribution to factor 1 (Figure 7).

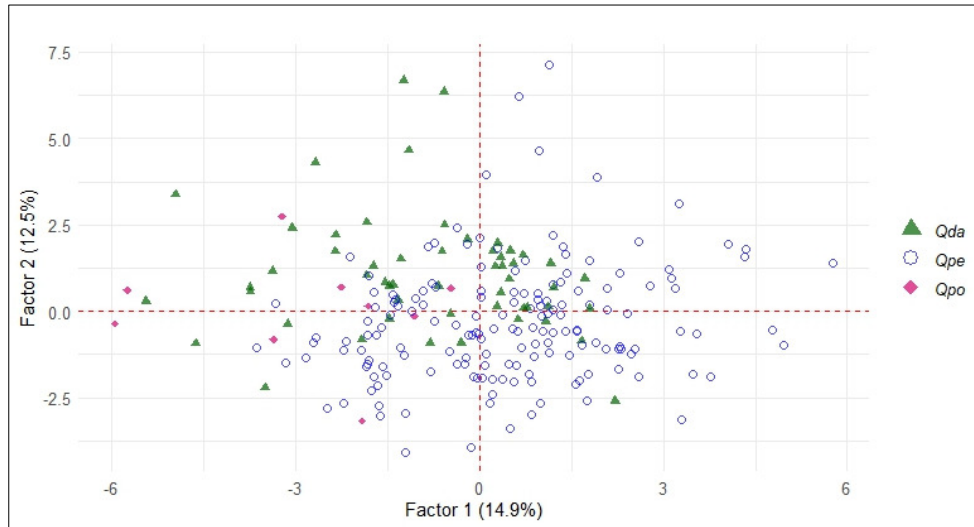


Figure 6. PCA plot with the full set of variables included in the analysis

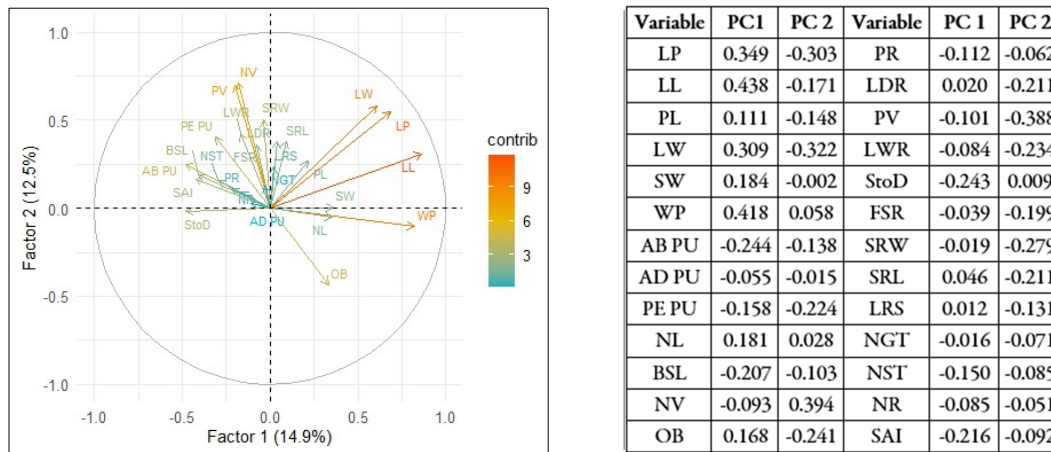


Figure 7. Variable contributions and loadings on the first two principal components

When analysing only the variables previously identified to exhibit significant differences between the three subspecies through ANOVA, the two main components accounted for 24.5% (Component 1) and 19.20% (Component 2) of the total variance (Figure 8). However, even with this consideration, distinct separation of the subspecies was not achieved. Along Component 1, the most discriminative variables were LL and WP, which showed lower values for *Qpo*. Conversely, the presence of intercalary veins and the percentage of venation have been found as the distinguishing traits for *Qda* individuals at the level of Component 2. Additionally, variables AB PU and BSL made nearly zero contribution to Factor 2 but exhibited a higher contribution to Factor 1 (Figure 9).

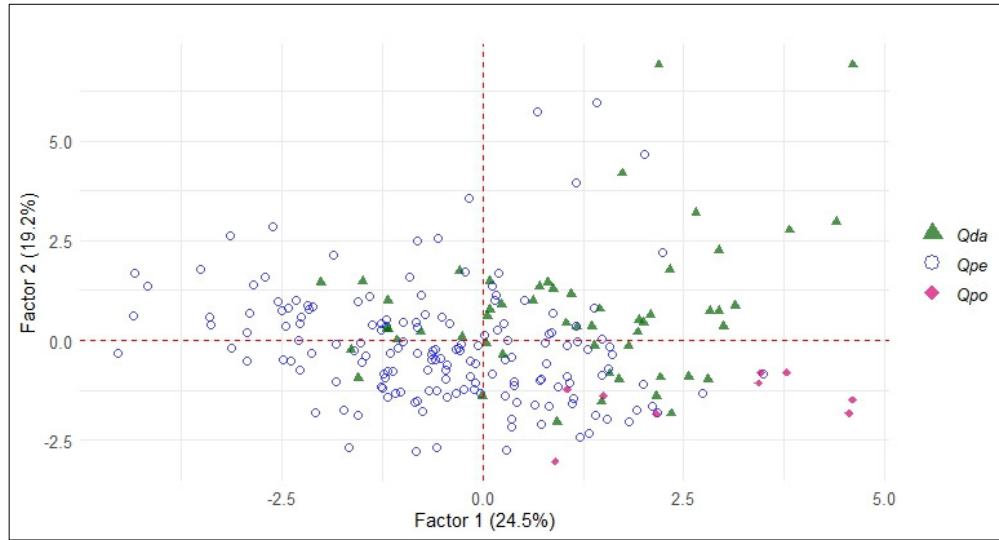


Figure 8. PCA graph including the set formed by the significant variables of ANOVA

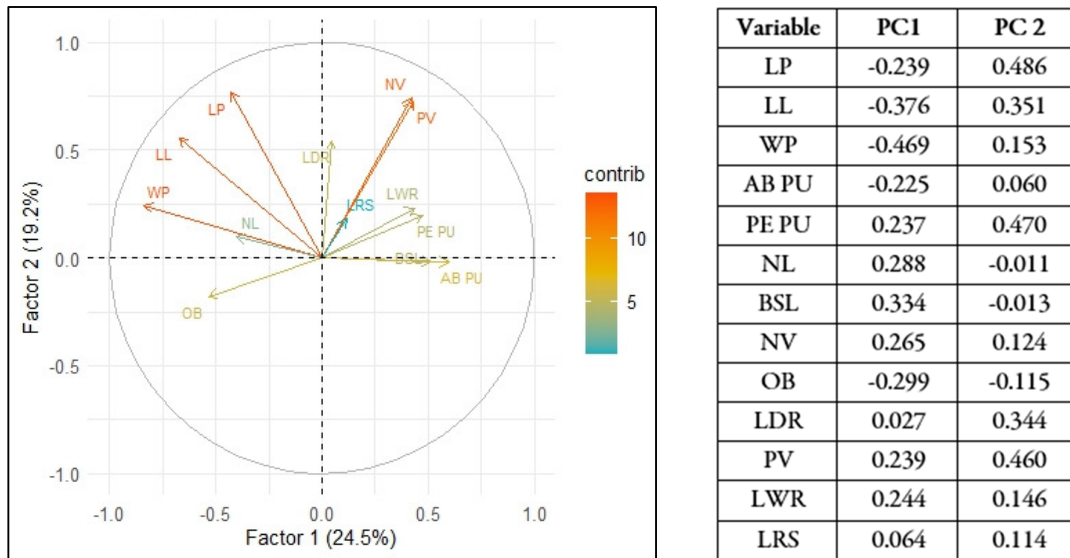


Figure 9. Variable contributions and loadings on the first two principal components

Discussion

Taxonomists still encounter challenges in selecting effective morphological descriptors to differentiate entities within the *Q. petraea* complex. Leaf morphological attributes have long been recognized as essential criteria for species identification and classification.

In this study, we conducted an integrated macro- and micromorphological analysis focused on sessile oaks within the *Q. petraea* complex, employing a dataset consisting of 18 macromorphological and nine micromorphological descriptors.

Observations of leaf macromorphology in the *Q. petraea* complex indicated slight differences among the three subspecies.

This study also revealed slight morphological differences among the same subspecies across the populations examined. Given that these morphological differences were observed in populations growing under different environmental conditions, it is likely that they are ecologically determined. Notably, in each subspecies (except *Qpo*), leaf dimensions were reduced in the peripheral populations. This suggests that trees growing in more limiting conditions tend to have smaller leaves compared to those in optimal environments. Similar responses in leaf size have been documented in other studies (Uribe-Salas *et al.*, 2008). These patterns underscore the potential adaptive significance of leaf size as a response to environmental stressors.

In terms of dimensional characters, *Qda* and *Qpo* are characterised by shorter leaves, with maximum width located in the lower half of the lamina. However, leaf dimensions are influenced by shading, as Ponton *et al.* (2004) found average lengths of 8.8 cm for leaves fully exposed to light. It should be noted that the average value obtained for *Qda* (11.2 cm) was noticeably higher than the values reported for *Q. pubescens* in similar studies, a species that some studies consider to be more closely related morphologically (Camus, 1954; Brullo *et al.*, 1999; Di Pietro *et al.*, 2012). For instance, in native stands, *Q. pubescens* has an average lamina length around 8.0-8.4 cm (Şofletea *et al.*, 2011; Enescu *et al.*, 2013), whereas international studies report values ranging from 6.9 to 9.4 cm (Bruschi *et al.*, 2000; Di Pietro *et al.*, 2016), sometimes even below 6 cm (Franjić *et al.*, 2006; Jerše and Batič, 2007).

Variability in leaf dimensions within populations reflects dynamic responses to resource availability, where larger leaves are typically associated with resource-rich environments and smaller leaves with resource-limited conditions (Kleiman and Aarssen, 2007). The observed reduction in leaf dimensions observed in both the FUN and OLT populations may represent an adaptive response to minimize transpiration in more xerophytic environments (Ogaya and Peñuelas, 2007; Soheili *et al.*, 2023). This phenomenon is particularly significant, given the proximity of the FUN population to the external steppe (Apostol *et al.*, 2015; Gafenco *et al.*, 2023). Also, the increased prevalence of *Qda* in peripheral populations, particularly in FUN and OLT, may be attributed to the influence of natural selection. This selection likely favored *Qda* due to its adaptation to local xerophytic conditions, which are more pronounced in peripheral areas (Martonne aridity indices < 28.0) compared to the HEL reference population (Martonne aridity index = 34.1).

Furthermore, the more xerophytic and thermophilic nature of *Qda* and *Qpo* (Constantinescu, 1973) can explain their higher pubescence, particularly on petiole and abaxial leaf surfaces, which are important adaptive traits in drier climatic conditions (Morales *et al.*, 2002). Most morphological studies on the sessile oak have traditionally focused only on the abaxial surface of the leaf (Dupouey and Badeau, 1993; Bruschi *et al.*, 2000, 2003; Kremer *et al.*, 2002; Fortini *et al.*, 2015), considering the upper surface to be glabrous (Gellini *et al.*, 1992), although some studies indicate a low degree of pubescence (Milletti *et al.*, 1982). Similarly, petiole pubescence is a less frequently assessed trait in taxonomic oak studies but it has proven utility in certain situations (Fortini *et al.*, 2013). Compared to the values reported in other studies (Bruschi *et al.*, 2003), the mean PE PU in our study was approximately two times higher. Such variations in pubescence may have ecological and adaptive implications, reflecting their ability to cope with different environmental conditions.

The basal shape of the lamina is widely regarded as a diagnostic feature in distinguishing species within the *Quercus* genus (Beldie, 1952; Mátyás, 1971; Schwarz, 1993; Christensen, 1997). Numerous studies have confirmed the significance of BSL's in oak species, such as sessile oak from pedunculate oak (Jurkšiene and Baliuckas, 2014), pedunculate oak from greyish oak (Apostol *et al.*, 2017), or in distinguishing between pedunculate oak and the pair of sessile oak-pubescent oak (Şofletea *et al.*, 2011).

The average number of lobes observed for the three subspecies falls within the established literature ranges (Beldie, 1952; Şofletea and Curtu, 2007), with *Qda* and *Qpo* having fewer lobes. In a study by Fortini *et al.* (2013), an average of 12.39 lobes was recorded for *Q. petraea*, which aligns as an intermediate value compared to our findings. Similarly, Dupouey and Badeau (1993) reported a comparable value of 13.2. Yücedağ and Gailing (2013) identified similar NL values ranging between 12.3 and 14.1 in oak populations from Turkey.

Notably, NL values were the lowest in the FUN population, which had a higher proportion of trees assimilating *Qda* and *Qpo* characteristics.

Regarding the number of intercalary veins, the obtained values in our study are notably lower compared to those reported by Fortini *et al.* (2015) (of 2.86) or Yücedağ and Gailing (2013) (of 0.8 – 1.5). However, Ponton *et al.* (2004) documented NV values for sessile oak ranging from 0.3 to 2.3. Conversely, some studies have indicated either the absence of intercalary veins in oaks (Wigston, 1975) or their limited presence (Scotti-Saintagne *et al.*, 2004). Notably, *Qda* and *Qpo* exhibit a higher NV compared to *Qpe*, reflecting distinct adaptation strategies. This trait is commonly associated with species thriving in dry and warm climates, as supported by plant physiological studies (Scoffoni *et al.*, 2011; Zhu *et al.*, 2012; Sack and Scoffoni, 2013; de Boer *et al.*, 2016), which aligns with our findings given the predominance of *Qda* and *Qpo* in more xerophytic areas.

Regarding the OB, *Qpe* leaves predominantly exhibited an obovate shape, while the other two subspecies showed an elliptic-ovate shape. This aligns with the average WP values, which were less than half of the mean LL value for *Qda* and *Qpo*, whereas, for *Qpe*, WP exceeded half of the mean LL value. These distinctions are characteristic of sessile oak differentiation and are well-documented in the literature (Georgescu and Morariu, 1948; Beldie, 1952; Clinovschi, 2005; Şofletea and Curtu, 2007). In the study by Fortini *et al.* (2015), the OB value was reported as 49.2%, which closely resembles our findings for *Qda* and *Qpo*.

Additionally, based on the average LDR values, it has been observed that the leaves generally ranged from pinnate-lobed (*Qpo*) to pinnate-fidate (*Qda*). This aligns with morphological descriptions found in the specialized literature (Georgescu and Morariu, 1948; Beldie, 1952; Şofletea and Curtu, 2007). For instance, Fortini *et al.* (2013) reported a higher LDR of 52.4% for *Qpe*. In the same study, Fortini *et al.* (2013) also found a significantly higher value for PV compared to our findings, specifically 23.4%. Conversely, Fortini *et al.* (2015) reported a lower LWR average value for sessile oak (27.47%). Regarding LWR, it was observed that *Qpe* has narrower leaves, whereas *Qpo* exhibits the widest ones.

Significant differences in leaf micromorphology were identified among the three subspecies, particularly regarding LRS.

Stomatal density serves as a phenotypic descriptor crucial for understanding the long-term adaptation and short-term response of plant species to changing environmental conditions (Hetherington and Woodward, 2003). It also provides insights into the species' adaptation when introduced outside their native range (Du *et al.*, 2021). The average StoD values observed in this study are comparable to recent findings for sessile oak in Northern Turkey (Yücedağ *et al.*, 2019), where values of 333 stomata-mm² were reported, and in Northern and Central Italy by Bruschi *et al.* (2000), where 335 stomata-mm² were observed. However, in a subsequent, more comprehensive study, Bruschi *et al.* (2003) analyzed both unexposed and exposed leaves and found average StoD values ranging between 329 and 498.

Typically, in regions with more limited climatic conditions – characterized by higher average annual temperatures, lower average annual precipitation, and intense evapotranspiration – it is expected that stomata numbers would be lower compared to more temperate hilly areas. However, contrary to this expectation, StoD values from peripheral populations exceeded those of the HEL reference population and, in some instances, even surpassed those of the comparative trial. A similar trend was observed by Şofletea *et al.* (2001) in their analysis of high-altitude submesotherm sessile oak from Poiana Braşov, where StoD was 321, lower compared to populations from hilly areas where StoD reached 394. Moreover, in a comparative study of StoD between *Q. petraea* and *Q. robur* species, Yücedağ *et al.* (2019) found higher StoD in populations thriving in drier conditions. Additionally, Bruschi (2010) demonstrated that oak species with higher stomata numbers but smaller individual stomata tend to exhibit greater resistance to water stress conditions. These findings align with a large body of literature indicating that plants in water-limited (xeric) environments often exhibit increased stomatal density as an adaptive response to enhance water-use efficiency (Abrams, 1994; Mediavilla

and Escudero, 2004; Bussotti *et al.*, 2015). While stomatal density is influenced by climatic factors, it is predominantly under genetic control (Wang *et al.*, 2014).

No significant differences were observed among the three subspecies regarding stomata dimensions. On average, the stomata in this study are longer and wider compared to those reported by Fortini *et al.* (2013), where stomata measured 13.1 μm in length and 11.29 μm in width in sessile oak stands. However, these dimensions align closely with those documented by Şofletea *et al.* (2001) in the Felmer sessile oak forest, where stomata measured 22.4 μm in length and 15.2 μm in width. Furthermore, no differences were found among the three subspecies for FSR. Bruschi *et al.* (2000) reported nearly double the value at 7.13 μm for this descriptor, whereas Fortini *et al.* (2009) reported 4.8 μm .

Given the variation in both stomata dimensions and density, some micromorphological studies on oaks (Bruschi *et al.*, 2000, 2003; Bruschi, 2010), have calculated the stomatal area index. This index represents the product of stomatal density and length (Ashton and Berlyn, 1994), and is considered an approximate indicator of the total potential stomatal opening at the leaf surface (Roussel *et al.*, 2009).

Comparing the three-oak subspecies, the average values for SRL were similar, while StoD was slightly higher for *Qda*. The SAI was also slightly higher in this subspecies. Previous micromorphological studies on oaks have reported higher average SAI values, ranging from 7828 to 11343 (Bruschi *et al.*, 2003).

Regarding trichome characteristics, all three subspecies display a similar number of NGT, with peripheral populations showing a greater abundance. This observation is consistent with previous studies (Mediavilla *et al.*, 2019; Hernandez and Park, 2022), which indicates that plants exposed to stress conditions, such as drought, tend to produce a denser layer of non-glandular trichomes as a mechanism to mitigate transpiration and reduce water loss.

We found that LRS varied significantly among the three subspecies, with longer lengths observed in the peripheral populations. In plants, especially those grown in drier areas, longer lengths of NGT are often associated with an increased tolerance to water stress, as noted earlier.

Additionally, NGT and LRS are known for their high discriminatory power between *Q. petraea* and *Q. pubescens*. The latter species typically exhibits LRS values exceeding 300 μm , whereas NGT values are significantly smaller (Bruschi *et al.*, 2000).

Considerable research has focused on taxonomic differentiation among closely related oak species, employing various multivariate analyses, such as PCA, discriminant analysis, or cluster analysis.

Multivariate methods for discriminating between species within the genus *Quercus* have typically been tested and validated primarily at the interspecific level, with fewer studies focusing on infraspecific differentiation. At the interspecific level, studies have successfully discriminated between sessile oak and pedunculate oak (Bacilieri *et al.*, 1996; Kremer *et al.*, 2002; Boratynski *et al.*, 2008; Yücedağ *et al.*, 2013; Jurkšiene and Baliuckas, 2014), sessile oak and downy oak (Dupouey and Badeau, 1993), sessile oak and Hungarian oak (Fortini *et al.*, 2015), and various species within the subgenus *Lepidobalanus* (Viscosi *et al.*, 2009). Notably, these studies typically involve a limited number of variables contributing to interspecific differentiation. In contrast, studies focusing on discriminating between infraspecific units, such as pedunculate oak and grayish oak (Apostol *et al.*, 2017) have found that discriminative variables differ from those used at the interspecific level and often involve a larger set of variables.

In a previous study on the morphological variability of sessile oak in our country (Stuparu *et al.*, 2003), discriminant analysis using a set of six leaf descriptors highlighted lobe shape and sinus depth as the most effective variables for differentiating between oak subspecies, but with no significant correlations observed between them.

Among the micromorphological descriptors evaluated, only the LRS significantly contributed to the discrimination between the three subspecies. Micromorphological descriptors have proven highly valuable in distinguishing taxa within the genus *Quercus*, particularly at the interspecific level (Bruschi *et al.*, 2000; Fortini

et al., 2009). At the infraspecific level, discriminant functions have successfully differentiated between *Q. brantii* var. *belangeri* and *Q. brantii* var. *brantii*, although complete separation was not achieved (Panahi *et al.*, 2012).

PCA analysis yielded very good results in differentiating between *Q. petraea* and *Q. robur* (Kremer *et al.*, 2002), between *Q. petraea* and *Q. pyrenaica* (López de Heredia *et al.*, 2009), as well as between *Q. frainetto* and *Q. pubescens* (Fortini *et al.*, 2015). In Romania, in a study conducted for four species of the genus *Quercus* (sessile oak, pedunculate oak, downy oak, and Hungarian oak), as well as their hybrids (Şofletea *et al.*, 2011), PCA highlighted the separation between sessile oak and pedunculate oak, as well as Hungarian oak, but not with downy oak.

To assess leaf variability in mixed stands of sessile and pedunculate oak, Kremer *et al.* (2002) employed four categories of morphological descriptors (five measurable, two countable, two observable, and five calculated variables) and three multivariate statistical analysis methods, including PCA, thus enabling a clear distinction between the two populations corresponding to the two species.

On the other hand, Borazan and Babaç (2003) studied leaf morphometric variability in several oak species (*Q. robur*, *Q. petraea*, *Q. pubescens*, *Q. virgiliana*, and *Q. cerris*), using PCA with 16 morphological characters. They found that clear separation among species within the *Lepidobalanus* subgenus was not achieved, suggesting high variability potentially due to hybridization events. Similarly, Enescu *et al.* (2013), in their study on *Q. pubescens* and *Q. virgiliana*, reported a single morphological group identified through PCA.

Our results are consistent with those obtained in a recent study conducted in Italy by Proietti *et al.* (2021), where multivariate analysis methods, including cluster analysis and PCA, were used to discriminate between *Q. petraea*, *Q. pubescens*, and *Q. dalechampii*. Both methods indicated that *Q. petraea* and *Q. dalechampii* emerged into a joint group that overlapped almost completely, suggesting that individuals of *Q. dalechampii* morphologically resemble *Q. petraea* based on leaf and twig traits. Enescu *et al.* (2012) noted similar challenges in differentiation *Q. pubescens* and *Q. virgiliana* due to their morphological resemblances in leaf and fruit traits. More recently, Jovanović *et al.* (2023) employed morphometric methods to analyse sessile oak leaves, aiming to elucidate relationships among individuals through cluster analysis.

However, numerous studies investigating the genetic diversity within the *Quercus* genus supported the view that *Q. dalechampii* may not be a distinct species, but rather a part of the *Q. pubescens* complex, as evidenced both by morphological analyses (Di Pietro *et al.*, 2020a) and molecular data (Di Pietro *et al.*, 2020b; 2021).

Conclusions

Expanding the range of characteristics to encompass both macro- and micromorphological leaf traits is essential for advancing the plant taxonomy. While such assessments may not provide exhaustive information, they offer valuable insights for forest management, particularly in challenging environments like peripheral populations.

Through phenotypic analyses, we have identified key leaf descriptors that play a significant role in distinguishing between the three subspecies of sessile oak. Specifically, *Qda* and *Qpo* exhibit distinct characteristics compared to *Qpe*, including shorter leaves with maximum width in the lower half of the lamina, fewer lobes, an ovate shape, and a subcordate leaf base, consistent with previous literature. Moreover, the higher frequency of intercalary veins in subthermophilic sessile oaks suggests a potential adaptation to dry and warm climates. Our study emphasizes the importance of LRS in differentiating the three subspecies based on micromorphological leaf analysis.

The PCA analysis further suggests that these three taxa are likely intraspecific taxonomic units within the *Q. petraea* complex rather than distinct species. Thus, further research is essential to determine the placement of *Qda* populations from Romania within the *Quercus* complex. This will help refine the species' classification and guide conservation and management strategies tailored to their genetic and ecological characteristics.

Peripheral populations exhibited greater phenotypic variability, as evidenced by the diverse morphologies of their leaves. Evaluating this variability at the interpopulation level underscores the importance of conserving populations with high morphological diversity, such as the FUN and OLT, in their natural habitats (*in situ*). Furthermore, the significant plasticity and adaptability of *Qda* leaf traits in response to climate change highlight the need to prioritize this taxon in conservation and breeding programs.

Overall, this research enhances our understanding of species' adaptive strategies within the Sessiliflorae series and provides valuable insights for informed conservation and management efforts.

Authors' Contributions

Conceptualization: IMG, ENA, and BIP; Data curation IMG; Formal analysis: IMG; Funding acquisition: IMG and ENA; Investigation: IMG, ENA, BIP, EC, DMG, and NŞ; Methodology: IMG, DMG and NŞ; Project administration, IMG and ENA.; Resources: IMG and ENA; Software: IMG; Supervision: IMG, ENA, EC, DMG and NŞ; Validation: IMG, ENA, BIP, EC, DMG and NŞ; Visualization: IMG and BIP; Writing—original draft preparation: IMG; Writing—review and editing: IMG, ENA, BIP, EC, DMG, and NŞ; All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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