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Comparative analysis of the three Caucasian oak taxa in Georgia (South Caucasus) based on leaf macromorphological variation

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ABSTRACT

Comprative analysis of three oak taxa (*Q. petraea* subsp. *iberica, Quercus robur* subsp. *imeretina*, and *Q. robur* subsp. *pedunculiflora*) based on the leaf morphological and morphometric analysis have been done. Fifteen statistically significant variables out of the original 24 leaf characters were identified by PCA. The ANOVA *F*-statistics together with Tukey's post hoc range test and PCA analysis allowed the identification of some leaf traits with ability to partly differentiate all three studied taxa from each other. Particularly, the leaf traits related to leaf petiole size and number of lobes and intercalary veins are much higher in *Q. petraea* subsp. *iberica* individuals compared to *Q. robur* subsp. *imeretina* samples, while in *Q. robur* subsp. *pedunculiflora* they are intermediate between these two taxa. Linear regression analysis reveals that statistically significant leaf traits combined in the first and second principal components negatively or positively, but significantly weakly or moderately influenced by ecological factors related to different annual and growth season moisture and temperature conditions.

Keywords: Caucasian oak taxa, Leaf morphometry, Principal Components Analysis, Regression Analysis, Ecological Factors, Leaf traits.

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Introduction

There are more than 500 different oaks species in the worldwide [1]. Among 18 oak species distributed in the Caucasus 7 grows in Georgia. *Pedunculatae* oaks presented by *Q. hartwissiana* Steven*, Q. robur* subsp. *imeretina* (Steven ex Woronow) Menitsky, *Q. robur* subsp. *pedunculifllora* (K. Koch) Menitsky), and *sessiliflorae* oaks with *Q. petraea* subsp. *dshorochensis* (K. Koch) Menitsky*, Q. macranthera* Fisch & C. A. Mey, ex Hohen, *Q. petraea* subsp. *iberica* (Steven ex M. Bieb.) Krasilln. and *Q. pontica* K. Koch.

In the economical and ecological point of view oak in the Georgian forest is considered as one of the most significant species. Oak stands are the most diverse and rich in terms of the floristic composition. Nowadays, they cover 10.5% of the forest area, which is about 241,000 hectares [2]. *Q. petraea* subsp. *iberica* occupies the largest area among the species in the oak genus. It is widely distributed in the forest of Georgia and very like to the most widespread species in Europe - rock oak (*Q. petraea* (Matt.) Liebl.), which replaces our species in the mountains of the North Caucasus [3, 4]. *Q. robur* subsp. *pedunculifllora* occurs at alluvial plains of the river basins of Eastern Georgia [5]. In the western Georgia it is replaced by *Q. robur* subsp. *imeretina*. Both of them belongs to be the group of Q. robur L. [6].

Extensive taxonomy study of oak was made by Krasilnikov [7, 8]. Based on the research he developed the interspecies systematics of some species, where subspecies is the main unit. The monograph of Menitsky „Oaks of Asia" also is important for the phylogenetic studies of the oak genus [9]. The issues of variability, evolution and taxonomy of the roburoid and galiferal Caucasian oaks are widely discussed here.

Hybridization and the presence of hybrid zones is a common fact among the oak species [9-11], which facilitates the high level of phenotypic plasticity and genetic diversity. Therefore, classification of oak genus, assessment of genetic differentiation between species and determination of population structure is quite difficult and a number of studies have been implemented [12-15]. High polymorphism of oak species is a result of introgressive hybridization processes and widespread in the area of coexistence of two or more species, facilitates conditions for conducting of studies for identification of morphological and genetical units and biodiversity on species and population levels [16-18].

Previous implemented studies in Georgia is important for identification of taxonomy data for oak species. Until now most molecular-genetic and morphological studies performed on European and American species [19-21] and there is relatively few information about those species occcured in Georgia [15, 22-24].

So, there is the less information about Caucasus oak, and there is a need to conduct studies for clarification of its taxonomical status and presented article is partly addressed to this issue. Therefore, research aim is to conduct a comparative analysis of three Caucasian oak taxa (*Quercus robur* subsp. *imeretina, Q. robur* subsp. *pedunculiflora and Q. petraea* subsp. *iberica*) based on leaf morphometric study.

Materials and Methods

Study Object and Study Area

The research object of the presented article are: *Q. robur* subsp. *imeretina*, *Q. robur* subsp. *pedunculiflora* and *Q. petraea* subsp. *iberica*. The field surveys were conducted in 2017-2019 years in the three regions of Georgia; Imereti (Western Georgia), Shida Kartli and Kakheti (Eastern Georgia). Only natural stands were included in the sampling.

Individuals of *Q. robur* subsp. *imeretina* presented by 10 locations and 80 trees were collected within the range of heir distribution in Imereti region (western Georgia), while the samples of *Q. petraea* subsp. *iberica* (9 locations, 85 trees) and *Q. robur* subsp. *pedunculiflora* (5 locations, 65 trees) were collected in different regions of eastern Georgia (Table 1). 5 healthy leaves were collected from each tree and the distance between them was at least 50 m. The total number of investigated leaves taken only from adult trees was 1150.

Slope declination, exposition, forest type, stand density, height and diameter of oak trees were scored for each study site (data not shown) according to forest taxation protocols [25-27]. The locations of investigated oak tax were presented in Fig. 1.

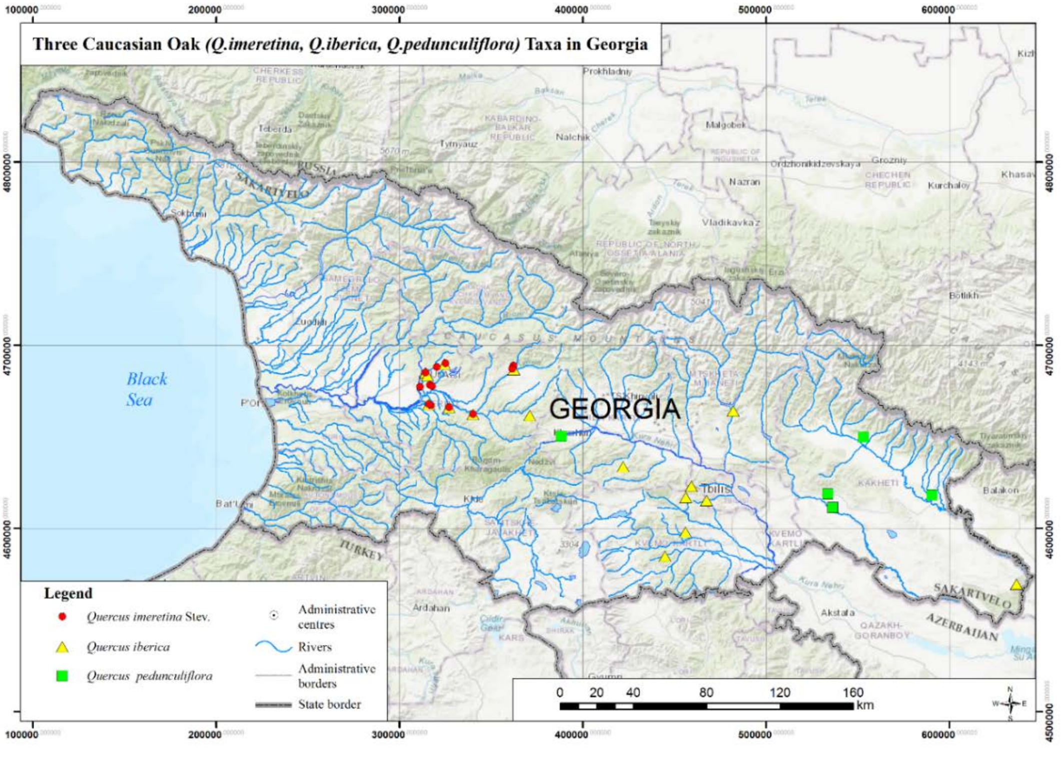

Fig. 1. *Ampuls with the diagnostic test system*

Climatic data

Ecological characterization of investigated oak taxa according to study sites was done in table 1. Climatic data for the last 34 years (1985-2019) of hourly weather model simulation for each selected localities were obtained from ‘Meteoblue’ (2019) (https://www.meteoblue.com/en/weather/history-climate/climatemodelled/) that have a spatial resolution of approximately 30 km. We used annual and growth season precipitation amount (mm), sum of mean daily maximum temperatures (° C), number of sunny, partly cloudy and precipitation days for both periods, and mean daily maximum temperatures (° C) for each month of growth season to characterize the climatic heterogeneity of the environments in different locations of the Caucasian oak taxa in Georgia (South Caucasus) within the range of distribution. Growth season includes climatic data corresponding to May, June, July, August and September, respectively.

Table 1. *Ecological characterization of three Caucasian oak taxa in Georgia (South Caucasus) within the range of distribution. Data were obtained from the ‘Meteoblue’ (2019) (https://www.meteoblue.com/en/weather/historyclimate/climatemodelled/) for the last 34 years (1985-2019). * In case of Q. robur subsp. imeretina for the study location from Ajameti Managed Reserve (Imereti, western Georgia) samples were taken from different altitudes from 144 m to 205 m a.s.l.; accordingly, all ecological data were done for each altitude in the corresponding column.*

Abbreviation	Population	Altitude (m a.s.l.)	Latitude/ Longitude	Annual precipitation (mm) (mm)	Sum of annual mean daily maximum t (°C)	Annual sunny days	Annual partly cloudy days	Annual precipitation days	Growth season precipitation (mm)	Sum of growth season mean daily maximum t (°C)	Growth season sunny days	Growth season partly cloudy days	Growth season precipitation days	April mean daily maximum t (°C)	May mean daily maximum t (°C)	June mean daily maximum t (°C)	July mean daily maximum t (°C)	August mean daily maximum t (°C)
Q. petraea subsp. *iberica*																		
1	Zestaponi (Sviri), western Georgia	205	42°1291′/ 42°9084′	815	229	86.1	148.1	168.7	315	153	42.3	90.4	84.3	20	23	26	29	29
2	Chiatura (Darkveti), western Georgia	533	42°3217/ 43°3312′	815	187	86.1	148.1	168.7	315	132	42.3	90.4	84.3	16	20	23	25	26
3	Mount. „Shavi Mta“, Kakheti, eastern Georgia	813	41°2671′/ 46°6303′	279	191	152.4	148.6	73.2	128	143	92.3	73.2	36.7	17	23	18	31	30
4	Vicinity of Betania Monastery, southwest of Tbilisi, eastern Georgia	835	41°6899′/ 44°6089′	262	185	124.1	159.5	84.7	134	138	70	86.7	43.6	16	20	25	28	27
5	Mtskheta (Vicinity of vil. Didgori), eastern Georgia	1600	41°7589′/ 44°9088′	229.6	125	124.1	159.5	84.7	101	108	70	86.7	43.6	11	15	20	23	22
Q. robur subsp. *imeretina*																		
1	Ajameti Managed Reserve, western Georgia	144	42°7745′/ 42°′ 14165	1193	222	89.7	131.9	179	449	146	42.5	80.6	91	19	22	25	27	28
		171	42°7871′/ 42°13584′	1193	222	89.7	131.9	179	449	146	42.5	80.6	91	19	22	25	27	28
		205	42°1291′/ 42°9084′	815	229	86.1	148.1	168.7	315	153	42.3	90.4	84.3	20	23	26	29	29
2	Terjola (Vicinity of Brolis-kedi), western Georgia	159	42°2257′/ 42°79093′	1584	214	106.2	128.1	175.6	555	147	55.3	75.9	85.5	19	23	25	27	28
3	Zestaponi (vil. Chognari), western Georgia	168	42°236′/ 42°7759′	1584	213	106.2	128.1	175.6	555	147	55.3	75.9	85.5	19	23	25	27	28

4	Kutaisi (Saghorie Forest), western Georgia	173	42°2257'/ 42°7118'	1584	213	106.2	128.1	175.6	555	147	55.3	75.9	85.5	19	23	25	27	28
5	Tkibuli (Vicinity of vil. Orpiri),	318	42°3250'/ 42°8190'	1584	199	106.2	128.1	175.6	555	147	55.3	75.9	85.5	18	22	24	26	26
	western Georgia																		
6	Tkibuli (Vicinity of Khresili), western Georgia	392	42°3447/ 42°8759'	1594	193	181	128.1	175.6	565	137	55.3	75.9	85.5	17	22	24	25	26
7	Chiatura (Vicinity of vil. Zodi), western Georgia	604	42°3239'/ 43°3184'	1178	192	99.1	144.8	175.3	548	141	53.9	83.5	90.1	17	22	25	27	27
Q. robur subsp. *pedunculiflora*																			
1	Lagodekhi (vil. Heretiskari), eastern Georgia	225	41°7102/ 46°0874'	204	192	141.2	156.2	62.1	83	55	81	82.9	27.2	16	21	26	29	29
2	Kvareli (vil. Gremi), eastern Georgia	401	41°9999/ 45°6425'	983	225	88	158.1	170.8	535	160	53.9	90.9	91.6	19	24	29	31	31
3	Sagarejo (Vicinity of vil. Manavi), eastern Georgia	542	41°6549/ 45°437'	270	211	150.5	148.7	73.9	150	156	88.5	75.7	38.2	18	23	28	31	31
4	Khashuri (vil.Osiauri), eastern Georgia	670	41°9982/ 43°6523'	432	181	67.7	183	142	179	133	38.6	106	68.3	16	20	23	26	26

Leaf parameters

In leaf morphological characterization was based on descriptor list for Oak species (Fig. 2) according to Kremer et al. [28]. All leaves were digitized; morphometric parameters were measured using the program ImageJ1.47v (https://imagej.net/Plugins). Material taken from the field was processed into herbarium specimens.

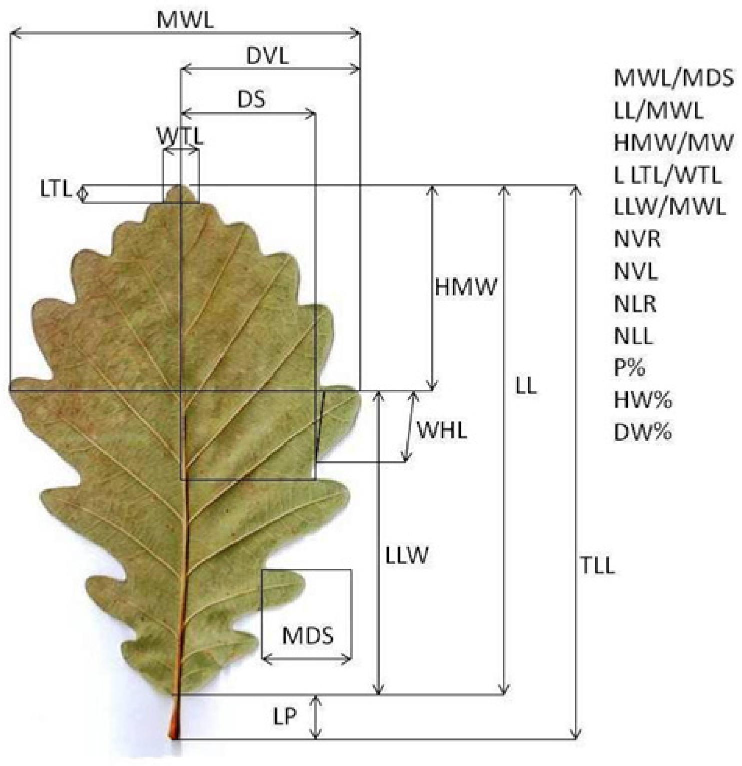

Fig. 2. *Leaf parameters*

Data analysis

Analysis of variance (ANOVA) together with Tukey's HSD post hoc range test (P< 0.001) was used to analyze leaf macromorphological differences in the investigated Caucasian oak taxa. At first, a principal component analysis (PCA) was used to remove highly correlated variables and replace the entire data file with a smaller number of uncorrelated variables. PCA was then carried out with the selected variables, and these were reduced to three principal components representing most of the information in the original dataset. A principal component (PC) solution was determined based on scree plot and Kaiser criterion (all eigenvalues greater than 1). Regression analysis were conducted to evaluate the correlation between principal components (PCs) and ecological factors (r) and proportion of the variance (r^2, %) for a dependent variables (PCs) that explained by independent variables or ecological factors in the regression model.

The statistical package SPSS ver. 21.0 (https://www-01.ibm.com/) was used for ANOVA with multiple comparisons, while PCA and regression analysis were performed by software PAST [29].

Results

Leaf trait variability comparison among the three Caucasian oak taxa

15 statistically significant variables out of the original 24 macromorphological leaf traits were identified by PCA. The ANOVA *F*-statistics together with Tukey's post hoc range test (p <0.001; Table 3) allowed the identification of some studied leaf traits with ability to differentiate all three studied taxa from each other. Particularly, mean values of the leaf petiole length (LP), number of intercalary veins on both sides (NVR, NVL) and percentage of leaf petiole in total leaf length (P, %) (Table 2) are much higher in *Q. petraea* subsp. *iberica* individu-

Table 2. *Means (AV), Standard Deviations (SD) and F values of leaf traits in three Caucasian oak taxa. Comparisons among oak taxa were done using one-way ANOVA analyses with Tukey's HSD test; asterisks indicate overall significance of the F-statistics (* – p < 0.05; ** – p < 0.01, *** – p<0.001), and the letters indicate significant differences among the means at p<0.001 according to Tukey's HSD test.*

Leaf traits	Oak taxa			*F*
	Q. petraea subsp. *iberica*	*Q. robur* subsp. *imeretina*	*Q. robur* subsp. *pedunculiflora*	
LP (Length of petiole)	1.44±0.4^{a}	0.29±0.14^{b}	0.94±0.28^{c}	682.14***
HMW (Height of maximal width)	4.82±1.04^{a}	3.86±0.95^{b}	4.13±0.17^{b}	31.78***
MDS (Maximal depth of sinus)	1.42±0.54^{a}	1.72±0.51^{b}	1.6±0.49^{ab}	11.48***
WHL (Width of the most handing lobe)	1.5±0.35^{a}	1.75±0.42^{b}	1.77±0.38^{b}	16.09***
DVL (Distance of the principal vein to top of the most handing lobe)	3.03±0.85^{a}	2.72±0.58^{b}	2.88±0.61^{ab}	7.49***
WTL (width of the terminal lobe)	0.61±0.24^{a}	0.89±0.46^{b}	0.5±0.36^{a}	25.41***
LTL (length of the terminal lobe)	0.95±0.33^{a}	1.16±0.37^{b}	0.82±0.4^{a}	31.29***
NLR (Number of lobes on the right side)	7.75±1.48^{a}	5.68±1.3^{b}	6.43±1.75^{b}	150.81***
NLL (Number of lobes on the left side)	7.7±1.56^{a}	5.68±1.23^{b}	6.2±1.6^{b}	143.32***
NVR (Number of intercalary on the right side)	8.17±1.48^{a}	4.5±1.18^{b}	6.69±1.31^{c}	298.22***
NVL (Number of intercalary on the left side)	8.12±1.52^{a}	4.56±1.06^{b}	6.33±1.48^{c}	278.76***
TLL (Total leaf length (LL+LP))	11.31±1.63^{a}	9.46±1.33^{b}	10.33±1.7^{c}	55.41***
P (Length of petiole x 100/ total leaf length)	12.85±3.69^{a}	3.12±1.33^{b}	9.03±2.15^{c}	632.55***
HMW/MWL	0.83±0.2^{a}	0.71±0.19^{b}	0.73±0.22^{ab}	12.81***
LLW/MWL	0.11±0.05^{a}	0.17±0.1^{b}	0.09±0.07^{a}	30.81***

als compared to *Q. robur* subsp. *imeretina* samples, while in *Q. robur* subsp. *pedunculiflora* they are intermediate between these two taxa. Additionally, Tukey's post hoc multiple comparisons at $p<0.0001$ identified some traits shared between *iberica-pedunculiflora* and *imeretina-pedunculiflora* sample pairs. The mean differences of sizes in terminal lobes (WTL, LTL) and ratios between dimensions of terminal lobe and lamina (HMW/MWL, LLW/MWL) are not significantly different between *iberica-pedunculiflora* sample pairs, while traits related to the height of maximal width and the most handing lobe (HMW, WHL), and number of lobes on the both sides (NLR, NLL) do not show significant differences among *imeretina* and *pedunculiflora* individuals (Table 2).

The PCA gave congruent results and revealed that the first three principal components account for 74.55% of the total variation in the dataset (40.27, 18.11, and 16.17%, respectively; Table 3). The first PCA is mainly influenced by variables expressing leaf size and lobes and veins number and mostly differentiates *Q. petraea* subsp. *iberica* and *Q. robur* subsp. *imeretina* samples with partly overlapping of individuals from *Q. robur* subsp. pedunculiflora and *Q. robur* subsp. *imeretina* group in the middle part of the plot (Fig.1). The second PCA is influenced dimensions related to maximal depth of sinus and width of the most handing lobe, while the third PCA is related to sizes in terminal lobes, and ratio between the length and width of lamina (Table 3) but both provided no further subdivision.Linear re-

Table 3. *Eigenvalues, percentages of explained variance and cumulative percentage of explained variance, contribution of the variables to the first three principal components values of each leaf character in 3 Caucasian oak taxa. Redundant variables: LL (Length of lamina); MWL (Maximal width of lamina); DS (Distance of the principal vein to the sinus); HW (height of maximal width x 100/ total leaf length); LLW (Length of lamina from base to the widest part (LL-HMW); DW (Height of maximal width x 100/ total leaf length); HMW/MWL; LTL/WTL; LLW/MWL*

Total variance explained	PC1	PC2	PC3
Eigenvalues	6.04	2.72	2.43
Percentage of explained variance	40.27	18.11	16.17
Cumulative percentage of variance	40.27	58.38	74.55
Leaf character	Eigenvalues		
LP (Length of petiole)	0.31		
HMW (Height of maximal width)			0.5
MDS (Maximal depth of sinus)		0.5	
WHL (Width of the most handing lobe)		0.47	
DVL (Distance of the principal vein to top of the most handing lobe)		0.48	
WTL (width of the terminal lobe)			0.34
LTL (length of the terminal lobe)			0.4
NLR (Number of lobes on the right side)	0.36		
NLL (Number of lobes on the left side)	0.36		
NVR (Number of intercalary on the right side)	0.34		
NVL (Number of intercalary on the left side)	0.36		
TLL (Total leaf length (LL+LP))	0.26	0.27	
P (Length of petiole x 100/ total leaf length)	0.29		
HMW/MWL			0.4
LLW/MWL			0.33

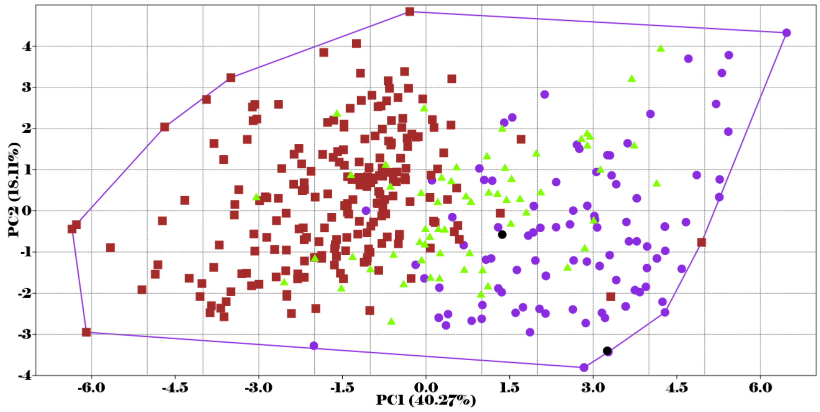

Fig. 3. *Principal component analysis (PCA) plot based on studied leaf traits of the three Caucasian oak taxa individuals in Georgia within the range of distribution with different moisture conditions and temperatures. Violet circles indicate samples of Q. petraea subsp. iberica; dark brown squares - Q. robur subsp. imeretina and light green triangles – Q. robur subsp. pedunculiflora.*

gression analysis based on ecological factors related to different annual and growth season moisture and temperature conditions allowed to identify statistically significant linear influence on differentiation of the leaf trait values ($p < 0.0001$, Table 4) between the investigated Caucasian oak taxa in Georgia. All the trait values explained by PCs (PC1, PC2) negatively or positively but significantly weakly or moderately ($r \leq 0.3$-0.6) correlate with all ecological factors included in the presented study. Among temperature conditions, the highest negatively significant influence was accounted for mean daily maximum t (°C) in April (r=-0.48 for PC1, r=-0.33 for PC2, Table 4), and significantly positive influence

Table 4. *Results of regression analysis (r^2 – coefficient of determination of the proportion of variance for the dependent first two principal components (PC1, PC2) that explained by independent ecological factors in the regression model; r_{xy} – coefficient of correlation between principal components (PC1, PC2) and ecological factors; ^{ns} not significant; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).*

Trait	PC1		PC2	
	r^2 (%)	r_{xy}	r^2	r_{xy}
Latitude	21	-0.46 ***	13	-0.37 ***
Longitude	26	0.51 ***	7	0.27 ***
Altitude (m a,s,l,)	13	0.37 ***	8	0.29 ***
Annual Precipitation	35	-0.60 ***	17	-0.41 ***
Sum of annual mean daily maximum t (°C)	6	-0.23 ***	2	-0.15 **
Annual sunny days	1	0.03^{ns}	1	0.12 *
Annual partly cloudy days	31	0.55 ***	6	0.25 ***
Annual precipitation days	24	-0.49 ***	13	-0.36 ***
Growth season precipitation (mm)	32	-0.57 ***	18	-0.42 ***
Sum of growth season mean daily maximum t (°C)	1	-0.11 *	1	-0.09^{ns}
Growth season sunny days	13	-0.36 ***	6	-0.24 ***
Growth season partly cloudy days	0	0.06^{ns}	0	0.008^{ns}
Growth season precipitation days	23	-0.48 ***	11	-0.33 ***
April mean daily maximum t (°C)	23	-0.48 ***	12	-0.34 ***
May mean daily maximum t (°C)	9	-0.29 ***	3	-0.17 *
June mean daily maximum t (°C)	6	-0.25 ***	3	-0.17 ***
July mean daily maximum t (°C)	1	-0.12 *	3	-0.19 ***
August mean daily maximum t (°C)	5	0.22 ***	3	0.16 ***

was shown for August (r=0.22 for PC1, r=0.16 for PC2, Table 4), respectively. Furthermore, the significantly negative influence of annual sum of mean daily maximum t (°C) on leaf trait variability was relatively weakly higher than for the growth season (Table 4). Site (latitude, longitude, altitude) and moisture conditions, and number of partly cloudy and precipitation days moderately (r ≥0.3-0.6, p<0.0001, Table4) influenced on leaf trait variation. Among them, the most influenced were annual (r=-0.6*** for PC1 and r=-0.41, *** for PC2, Table 4) and growth season (r=-0.57*** for PC1 and r=-0.42, *** for PC2, Table 4) precipitation; they explained 32-35 % (Table 4) of the proportion of variance for the leaf trait variation related to leaf size and lobes and veins number. Particularly, with the increase of precipitation these parameters in the investigated oak taxa were decreased. Additionally, it was shown that growth season sunny days' number (r=-0.36 for PC1, r=-0.24 for PC2, Table4) was negatively and moderately significantly influenced on leaf trait variation compared to the number of annual sunny days (not significant for PC1 and significantly weak for PC2, Table 4), respectively.

Discussion

Our univariate (ANOVA with Tukey's multiple comparisons) and multivariate (PCA) analysis allowed the identification some leaf traits with ability to partly differentiate studied taxa from each other. Particularly, the leaf traits related to leaf petiole size and number of lobes and intercalary veins are much higher in *Q. petraea* subsp. *iberica* individuals compared to *Q. robur* subsp. *imeretina* samples, while in *Q. robur* subsp. *pedunculiflora* they are intermediate between these two taxa and share more pronounce similarity with the first one. The sharing/convergence of leaf traits between *pedunculatae* and *sessiliflorae* oaks is not an occasional phenomenon. As in the case of other sympatric and closely related oak species, this could be explained with the incomplete reproductive isolation that characterize oaks in general [13, 30] and overlapping morphological variation due to ecological adaptation [15, 31, 32]. Conversely, *Q. petraea* s.l. and *Q. robur* s.l. generally grow in different ecological niches and areas in the Caucasus, with the second species occurring exclusively in lowland, mesophylous forests. The obtained morphological differences in leaf sizes related to petiole size together with number of lobes and veins recorded between the *Q. petraea* subsp. *iberica* and *Q. robur* subsp. *imeretina* samples might therefore reflect isolation and strong ecological adaptation to different environments [15, 28]. As frequently mentioned, no absolute diagnostic character discriminating between oak taxa could be detected. As the authors [33] noted, *Q. robur* s. l. and *Q. petraea* s. l. offspring can be discriminated in experimental or natural conditions, whenever shaded or not by surrounding adult trees, and preferably using leaves from the first flush. Masking the species differences could result from non-random mating in small, fragmented woodland populations. Hybridization and introgression between the species could also have played a significant role [34].

Furthermore, according to our results there is a linear dependence between ecological factors (annual and growth season precipitation and temperature conditions) and the leaf trait values. There is a correlation between macromorphological features explained by first two principal components and studied ecological factors. Leaf trait variability obtained in our study negatively or positively but significantly weakly or moderately correlate with all ecological factors. Particularly, with the increase of precipitation, leaf size and number of veins de-crease. It is also important that the number of sunny days during the growing season, negatively and moderately significantly influenced on leaf trait variation. Based on the obtained data we can assume, that the investigated oak taxa (*Quercus robur* subsp. *imeretina*, *Q. robur* subsp. *pedunculiflora*, *Q. petraea* subsp. *iberica)* in the different climatic conditions give us different results depending on the humidity indicators and the ecotope where they are distributed.

More extensive investigations with additional morphological descriptors (e.g., flower organs, cupule scales, pubescence, trichome shape) together with molecular markers, the self-ecology of each taxon are needed to clearly assess the true taxonomic status of these oaks and understand the existent oak biodiversity in the Caucasus to assist conservation of this important biome.

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