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Leaf shape and size variability of *Dryas octopetala* in the Central Great Caucasus (the Kazbegi region, Georgia) based on traditional and geometric morphometrics

Jana EKHVAIA^{1,2} * , Otar ABDALADZE¹ , Tamar JOLOKHAVA^{1,3}  and Arsen BAKHIA^{1,4} 

1 Alpine Ecosystems Research Program, Institute of Ecology, School of Life Sciences and Medicine, Ilia State University, Tbilisi, Georgia

2 Department of Reproductive Biology, Botanical Institute, Ilia State University, Tbilisi, Georgia

3 Ministry of Environmental Protection and Agriculture of Georgia, Science-Research Center of Agriculture, Soil Fertility Division, Tbilisi, Georgia

4 Department of Plants Systematics and Geography, Botanical Institute, Ilia State University, Tbilisi, Georgia

* Correspondence: zhana.ekhvaia@iliauni.edu.ge

ABSTRACT:

While soil pH is known to be a key driver of plant species composition, we still have a poor understanding of the quantification of the responses of leaf traits to different soil types, especially in highly sensitive alpine environments susceptible to global environmental changes. By combining traditional and modern geometric morphometrics we aimed to bridge the existing gap and determine whether certain leaf traits reliably separate evergreen *Dryas octopetala* populations residing in the subalpine-alpine zone of the Kazbegi region, in the eastern part of the Central Great Caucasus in Georgia. To achieve this aim, three populations of *D. octopetala* were analysed through traditional and, for the first time, modern geometric morphometrics. Both methods revealed congruent results indicating significant differences in leaf shape and size in locations with similar climatic conditions but different soil pH environments (acid vs neutral to slightly alkaline) thus suggesting that *D. octopetala* exhibits stronger leaf morphological plasticity (wider and larger leaves vs narrower and smaller leaves) to respond to changes in the soil pH gradient. In addition, modern geometric morphometrics, with its high degree of precision as an accessible and inexpensive method, could be advantageous for researchers and scientists seeking to gain a deeper understanding of plant species' unique responses to varying environmental conditions.

Keywords:

alpine ecosystems, Caucasus, landmarks, soil pH, leaf morphological plasticity

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INTRODUCTION

Alpine ecosystems are facing a threat from climate change, making it crucial to understand how plants respond to global environmental changes (GIGAURI *et al.* 2013; KÖRNER 2021). Plants can adapt to changing climates through a combination of factors. These factors include genotypic variation within the same species, the environment which affects and shapes the plant's ability

to adapt (HAMILTON & AITKEN 2013), and phenotype plasticity which allows different genotypes to produce various physical traits in response to different environmental conditions, often indicating how these traits are associated with geographical and environmental factors across a species' range (MCKNOW *et al.* 2014).

Leaves are the main organs responsible for photosynthesis in plants. Their shape and structure are adapted to environmental conditions (TRAISER *et al.* 2005), as even

minor variations in leaf form can exert a significant impact on a plant's ability to grow and survive in different environments. In the last two decades, numerous studies have found correlations between leaf morphological traits (e.g. leaf shape and size) and environmental factors from global to local scales which have been interpreted as the result of plants' plastic and adaptive responses to varying habitat conditions especially in terms of climatic and soil conditions (ORDOÑEZ *et al.* 2009; MARCYSIAK 2014; PFENNIGWERTH *et al.* 2017; SOUZA *et al.* 2018; LI *et al.* 2021; VARSAMIS *et al.* 2021).

Both traditional morphological measurements and modern geometric morphometric methods have been widely applied in recent leaf morphological analyses. Traditional morphological measurements provide us with leaf morphometrical and functional traits which are an important component of plant functional traits and are closely related to plant biomass and its resource acquisition and utilisation (CORNELISSEN *et al.* 2003). For instance, leaf specific area (SLA) and leaf dry matter content (LDMC) correlate with plant relative growth rate and resource capture and utilisation (WRIGHT *et al.* 2004), while leaf area (LA), leaf blade length (BL) and leaf maximal width (LW) mainly reflect light interception and water stress tolerance (WESTOBY *et al.* 2002). Due to the development of the leaf economics spectrum, the study of leaf functional traits and their relationships with the environment has become a popular subject in ecological research (ZHANG *et al.* 2017) as the relationship between "trait-environment" and "trait-trait" reflects the optimal "adaptation principle" for plant growth and adaptation under natural conditions (DUNBAR-CO *et al.* 2009; BLONDER *et al.* 2016; MURTAZALIEV *et al.* 2020; EKHVAIA *et al.* 2022). However, traditional morphological methods are ineffective for shape analysis and do not allow interpretable graphic representation, while modern geometric morphometrics (GMMs) presents a powerful tool for analysing leaf shape variation. It is based on Cartesian landmark coordinates and uses the relative positions of morphological landmarks to represent each specimen. In contrast to traditional morphological measurements, GMM-based statistical analyses preserve the distances between shapes and by using visualisation tools such as transformation grids illustrate trends in shape changes (MITTEROECKER & GUNZ 2009), and examine allometric relationships (VISCOSI *et al.* 2015) which reflect the covariation of size and shape. Overall, traditional morphological measurements and modern geometric morphometric methods have been increasingly applied to understand how target species adjust their variation to diverse natural conditions (VISCOSI 2015; LIU *et al.* 2018; TUCIĆ *et al.* 2018; AKLI *et al.* 2022; JOVANOVIĆ *et al.* 2022). This, in turn, can improve our understanding of the ability of plants to cope with environmental changes, including climate change (TONIN *et al.* 2020).

The Caucasus region is an important ecological area with complex bioclimatic, bedrock, and geomorphological conditions. Its rich topography and varied soil composition (MACHARASHVILI *et al.* 2019) make it an ideal subject for evaluating the impact of climate change on natural populations. While it is widely known that soil pH influences plant species composition at higher elevations (3000–3900 m a.s.l.) in the Central Great Caucasus (JOLOKHAVA *et al.* 2020, 2021; KIKVIDZE *et al.* 2020), our understanding of the impact of soil pH on leaf shape and size variability in this region remains limited. This knowledge gap requires further investigation, especially in an alpine environment sensitive to global environmental changes (ABDALADZE *et al.* 2015). Therefore, the general goal of this study was to contribute to the limited knowledge of the variation in plant leaf traits within the range of species distribution by quantifying the differences in leaf shape and size through both traditional and modern geometric morphometrics. For this study, we chose an evergreen species, *D. octopetala*, which inhabits the subalpine-alpine zone of the Kazbegi region in the eastern part of the Central Great Caucasus. Our specific goals were to examine intraspecific variation in the leaf shape and size of *D. octopetala* in three different locations where this plant is a dominant species. To control the variation in environment, we compared climatic data for these locations and measured soil pH as an integral indicator of soil fertility. We then compared the results obtained from the traditional and modern approaches to identify those leaf traits which can reliably differentiate between the studied populations.

MATERIAL AND METHODS

Material. *Dryas octopetala* L., commonly known as a White Dryad, is a circumpolar, arctic-alpine flowering plant species in the family Rosaceae residing in areas characterised by harsh climatic conditions. It is an important constituent of the alpine heaths of calcareous temperate mountains in Europe, in Asia south to Japan, Altai and the Caucasus, and in western North America south to the southern Rocky Mountains (HULTÉN & FRIES 1986). In terms of biomass, *D. octopetala* is one of the most important plant genera in the Arctic, along with sedges (*Carex*) and willows (*Salix*) (MURAOKA *et al.* 2015). It is a small prostrate evergreen semi-shrub which forms large colonies and has a life expectancy of over 500 years (DE WITTE *et al.* 2012). It also forms ectomycorrhizal (ECM) symbiotic relationships with fungi (BJORBÆKMO *et al.* 2010). Its stems are woody and tortuous, with short, horizontal rooting branches. The leaves are dark green, glabrous or with simple hairs above, and densely white-tomentose beneath. The stipules are lanceolate, adnate with the petiole below, green at first, becoming brown and scarious. The flowers are white and have eight petals. The style is persistent on the fruit with

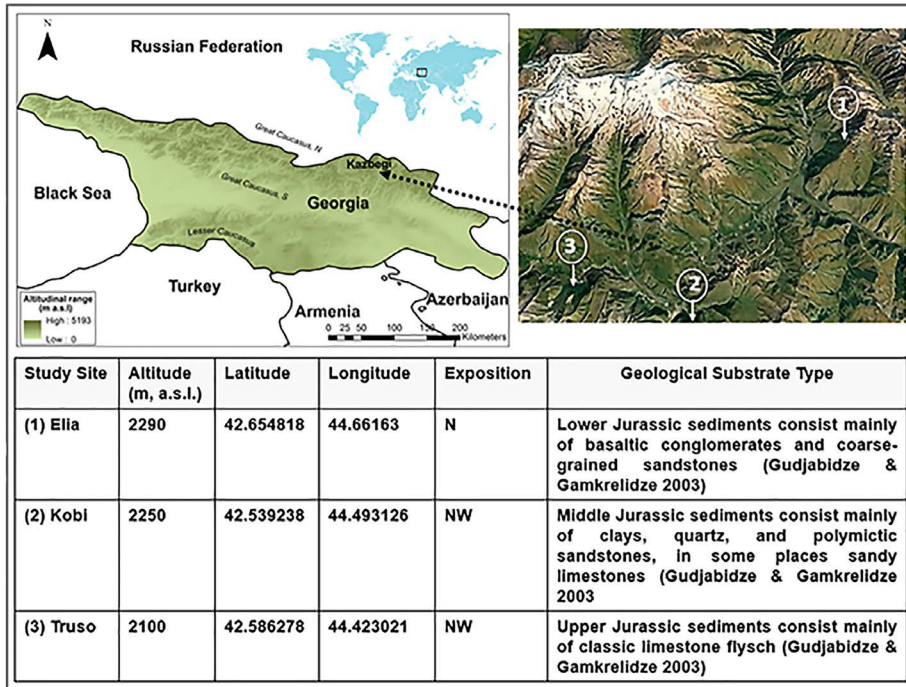


Fig. 1. The sampling locations of ever-green *Dryas octopetala* populations in the Kazbegi region (the Central Great Caucasus, Georgia) with geographical and geological characterisation of the studied sites.

white feathery hairs, functioning as a wind-dispersal agent.

In the Caucasus ecoregion, *D. octopetala* is known as a Caucasian Dryad or *Dryas caucasica* Juz. which is considered an endemic Caucasian species. Now, according to KEW's database (<https://powo.science.kew.org/>), it is considered a synonym of *D. octopetala* subsp. *caucasica* (Juz.) Hülten. However, the WFO's databases (<https://www.worldfloraonline.org/>) still identify it as an independent species. The area occupied is not large and covers skeletal and stony calcareous soils on steep (20–50°) slopes with north and north-west aspects mainly in the alpine belt (2500–3000 m a.s.l.), but it also extends down to the sub-alpine belt up to 2050–2100 m (DAVLIANIDZE *et al.* 2018).

Study area and sampling procedure. The study was conducted in the Kazbegi region, situated in the extreme eastern part of the Central Great Caucasus range (Fig. 1). Despite its relatively small area (only 1081.7 km²; NAKHUTSRISHVILI *et al.* 2005), the region is characterised by complex topography, diverse soil types, and high richness of plant communities (NAKHUTSRISHVILI & ABDALADZE 2017a, b). The region is home to more than 1100 vascular plant species, which make up 27% of the total number of plants (about 4100 species; DAVLIANIDZE *et al.* 2018) recorded in Georgia. The study area is located in the montane to alpine zone and belongs to the geomorphological zone of the Tergi-Arguni interridge isoclinal depression characterised by high tectonic and geomorphological activities (BONDYREV *et al.* 2015). The

landscape morphology is diverse and shaped by various types of sediment, including quaternary fluvial and glacial sediments, Tertiary and Quaternary volcanic rocks, and Jurassic sediments (LEBEDEV *et al.* 2014). The soil cover mainly consists of montane forest-meadow and montane meadow soils of more than ten types, subtypes, and genera (HANAUER *et al.* 2017; MACHARASHVILI *et al.* 2019).

The studied area included three typical sites of a dwarf semi-shrub community dominated by *D. octopetala* located on the northern (N) and north-western (NW) slopes of (1) the Kuro Range in the vicinity of Mount Elia (hereafter Elia), (2) in the vicinity of the village of Kobi (hereafter Kobi) near the river of the Bidara gorge, and (3) the Truso gorge (hereafter Truso) on the bank of the river Tergi (Fig. 1). Geologically, all three studied sites were represented by Jurassic rocks of different ages. Currently, (1) the Elia site is represented by Lower Jurassic sediments consisting mainly of basaltic conglomerates and coarse-grained sandstones (GUDJABIDZE & GAMKRELIDZE 2003), (2) the Kobi site is characterised by Middle Jurassic sediments mainly composed of clay quartz and polymorphic sandstones, and in some places, sandy limestones (GUDJABIDZE & GAMKRELIDZE 2003), and (3) the Truso site is represented by Upper Jurassic sediments mainly comprising classic limestone flysch (GUDJABIDZE & GAMKRELIDZE 2003). In addition, the Truso site is distinct from the others due to the abundance of travertines emerging from the mineral springs predominant in this area (NAKHUTSRISHVILI *et al.* 2005).

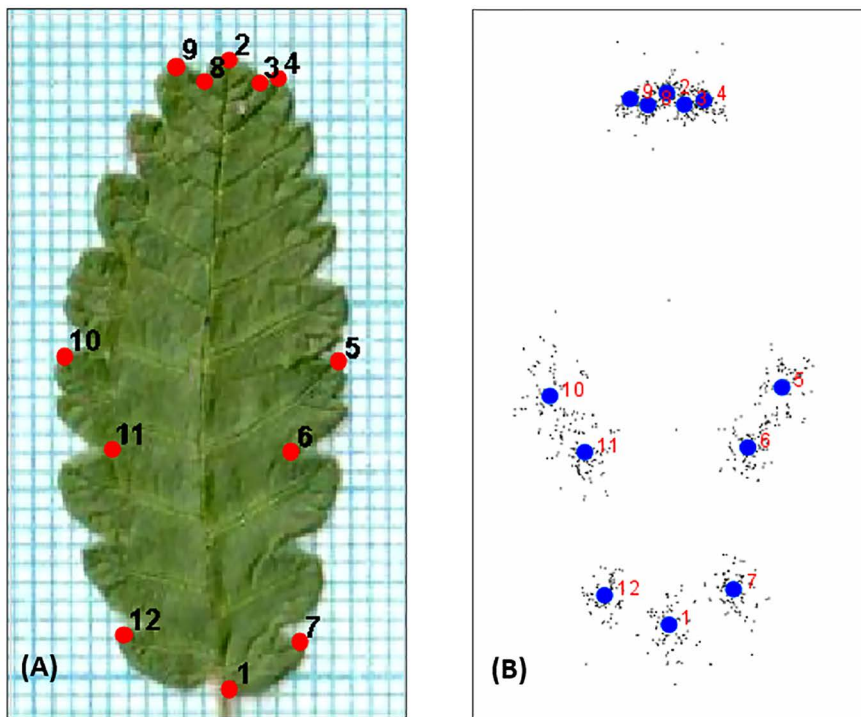


Fig. 2. (A) The configuration of *Dryas* leaves from the Kazbegi region (the Central Great Caucasus, Georgia) along with 12 specified landmarks: 1 – the junction of the blade and the petiole; 2 – the apex of the leaf blade; 3 and 8 – the blade of the apical sinuses of the blade tip (right and left side); 4 and 9 – the tip of the lobe immediately above the apex of the leaf blade (right and left side); 5 and 10 – the tip of the lobe at the largest width of the blade (right and left side); 6 and 11 – the base of the sinus immediately beneath the lobe of the landmarks 5 and 10 (right and left side); 7 and 12 – the first basal lobe of the blade (right and left side), (B) the results of generalised Procrustes analysis of the leaf shape, involving 12 landmarks in two dimensions and a total of 90 observations of studied individuals.

Three populations of the target species were sampled in the Kazbegi region during August 2023. A total of 90 leaves, 30 per population, were collected. To avoid collecting leaves from the same genet, six prominent individuals were chosen from each population with at least 3 meters between them. Five undamaged and infection-free leaves were collected from the central part of each of the six individuals' stems at five different shoots. The fresh leaf material was scanned, and images were used both for traditional morphological measurements and modern geometric morphometric methods (GMMs). For modern geometric morphometry, 12 landmarks were recorded on the left and right sides of each leaf (Fig. 2). The first 2 landmarks were unpaired and distributed along the midrib of the leaves, while the other landmarks (landmarks 3-12) were paired and distributed symmetrically on both sides of the leaves. The coordinates of the landmarks for each leaf were recorded using tpsDig and tpsUtil software (ROHLF 2015). Traditional measurements, such as leaf blade length, BL (cm), leaf blade width, BW (cm), and leaf area, LA (cm²) were calculated using ImageJ (RUEDEN *et al.* 2017). Additionally, the scanned leaves were dried in a dry oven (Vent-Line 180 Prime, Poland) at 80°C for 48 hours, and were then weighed to obtain the leaf dry mass, LDM (g). The ratio between the fresh LA (cm²) and LDM (g) was used to calculate the specific leaf area, SLA (cm² g⁻¹).

Soil samples were taken from 0–20 cm depth, using a special drill (Dormer Standard Soil Auger). Five sub-samples were taken from each plot of 1 × 1 m in size and were mixed to produce one soil sample per plot. In

total, 45 samples (15 per population) were processed according to the method proposed by THOMAS (1996) and PANSU (2006).

Data analysis. In modern geometric morphometry, the Cartesian X and Y coordinates of the specified landmarks were used for the following analysis using MorphoJ (KLINGENBERG 2011). Generalised Procrustes Analysis (GPA) was carried out to define “shape” information in a landmark array disregarding size, location, and orientation (KLINGENBERG *et al.* 2012). For this purpose, all configurations were standardised to the size of the unit centroid and rotated to generate minimal differences from previously established landmarks (LIU *et al.* 2018). Procrustes ANOVA was carried out to extract leaf-level shape variation for the detection of deviations by using the Procrustes distance. Canonical Variates Analysis (CVA) was used to visualise any differences between the populations growing in different edaphic environments, such as varying soil pH levels. The statistical differences in the average forms were evaluated by means of permutation tests (1000000 iterations per test) such as Mahalanobis distance to emphasise the distance between the individuals within the samples, and Procrustes distance to describe the distances between populations. Discriminant Function Analysis (DFA) was further performed to determine whether populations inhabiting different soil environments (acid soils vs neutral to slightly alkaline soils) differ from each other based on leaf shape variation. The DFA was carried out using classification tables (p-value < 0.0001 for a test

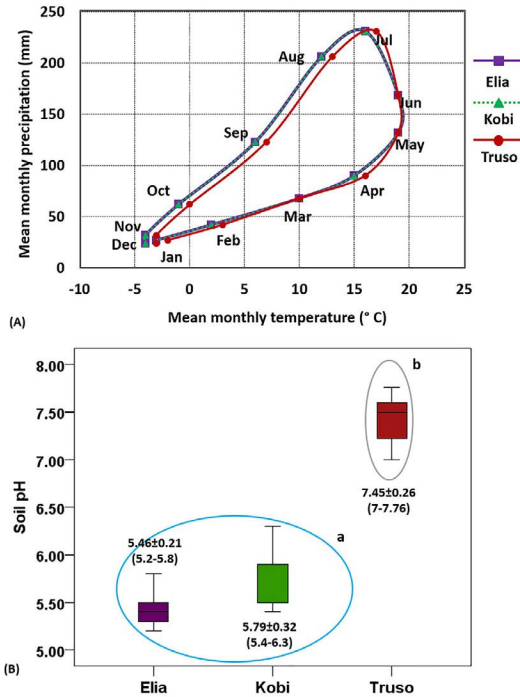


Fig. 3. Environmental characterisation of the study sites of three *Dryas octopetala* populations in the Kazbegi region (the Central Great Caucasus, Georgia). (A) The climadiagram illustrates the mean monthly temperatures and monthly precipitation. The annual mean daily maximum temperature (t , °C) is 7.25°C for the Elia and Kobi populations, and 8°C for the Truso population; the annual mean daily minimum temperature (t , °C) is -1.92°C for the Elia and Kobi populations and -1.16°C for the Truso population. The mean annual precipitation (mm) is equal for all three populations and amounts to 1205 mm. (B) The boxplots show the descriptive statistics for the soil pH level for each study site. These descriptive statistics include the mean, standard deviation (SD), minimum-maximum range, and F -test statistics. To compare the means among each pair of study sites, Tukey’s B post hoc test was utilised. Accordingly, populations which share at least one letter within columns are not significantly different at the 0.001 probability level, based on Tukey’s B Multiple Range Test results.

with 1000000 permutations), which were complemented with Cross-Validation to verify whether the populations were correctly classified. Additionally, SPSS ver. 21.0 (<https://www-01.ibm.com/>) was used to analyse the leaf size trait variability based on the measurements obtained from the traditional morphometric methods. Descriptive statistics for each measured leaf characteristic and soil pH level were produced, listing the minimum (min), maximum (max), mean, and standard deviation (SD). One-way analyses of variance (ANOVA) and Tukey’s B multiple-range comparisons were used to analyse the differences in the studied leaf traits and soil pH levels among three different locations of the target species within the studied area. Bivariate correlation analyses using Pearson’s correlation were conducted to evaluate the effects of soil pH on leaf trait variability. The Pearson correlations were carried out with an estimation of a 95% confidence interval of bivariate parameters based on 10000 bootstrap iterations.

RESULTS

Leaf shape and size variation were investigated both by geometric and traditional morphometric analysis which included 90 individuals of three *D. octopetala* populations from the Kazbegi region (the Central Great Caucasus, Georgia) all growing in a relatively small geographical area (only, 1081.7 km², NAKHUTSRISHVILI & ABDALADZE 2017b; Fig. 1). Firstly, we studied these three locations and found the climatic conditions to be very similar (Fig. 3A). However, the three sites differed signifi-

cantly in terms of the soil pH gradient (Fig. 3B). The Elia and Kobi populations were found on acid soils with pH levels of 5.46 ± 0.21 and 5.79 ± 0.32 , respectively, while the Truso population was located in an area with neutral to slightly alkaline soil with $pH = 7.45 \pm 0.26$.

Geometric morphometry was initiated with the Generalized Procrustes Analysis considering the centroid size as the variables, and the Procrustes coordinates as the shape variables. Subsequently, the configurations were rotated to a concentrated distribution around 12 landmarks (Fig. 2A) based on the row coordinate matrix (Fig. 2B). Procrustes ANOVA analysis was applied to assess the relative amount of variation among the individuals. Statistically significant differences ($p < 0.0001$, Fig. 4) were observed for both centroid size and shape in the *D. octopetala* samples, with higher variability from the consensus for landmarks (LMs) 1, 7, 10 and 11 (Fig. 4).

The results of the Canonical Variate Analysis (CVA) showed significant differentiation among the means of the studied *D. octopetala* populations caused by leaf shape variation, which was confirmed by strong test statistics (Goodall’s $F=11.01^{***}$, Pillai’s trace 1.23^{***} ; Tab. 2B). The first two canonical variates (CV1 and CV2) accounted for 100% of the total variation in the leaf shape (Table 1A). Among these, CV1 was the most influential variate accounting for 90.96% of the variability, thus demonstrating the clear separation of the Truso population from the Elia and Kobi populations and overlapping between the latter two (Fig. 5A). At the same time, CV2 exerted a relatively lesser effect, explaining 9.04% of the variability, and was not indicative of a clear pattern

Table 1. The results of canonical variate analysis (CVA) of three *Dryas octopetala* populations from the Kazbegi region (the Central Great Caucasus, Georgia) based on leaf shape variation obtained from 12 specified landmarks with eigenvalues, percentage of leaf shape variation explained by the two canonical varieties (CVs), global tests, and Mahalanobis and Procrustes distances among populations.

(A) Variation among groups, scaled by the inverse of within-group variation		
Eigenvalue	CV1	CV2
	6.07	0.6
Explained (%)	90.96	9.04
Cumulative (%)	90.96	100
(B) Global tests for the determination of significant differences among the means of studied populations		
Goodall's F	11.01 ($p < 0.0001$)	
Pillai's trace	1.23 ($p < 0.0001$)	
(C) Mahalanobis distances among studied populations		
Kobi	Elia	Kobi
	2.01 ($p < 0.0005$)	
Truso	4.84 ($p < 0.0001$)	5.53 ($p < 0.0001$)
(D) Procrustes distances among studied populations		
Kobi	Elia	Kobi
	0.03 (n.s.)	
Truso	0.09 ($p < 0.0001$)	0.1 ($p < 0.0001$)

in the distribution of the studied populations across the study locations (Fig. 5A). Furthermore, the leaf shape, as quantified by both the Mahalanobis and Procrustes distances, differed between the populations (Table 1C, D). The highest and most significant values were obtained between the populations Kobi and Truso (Mahalanobis distance (MD) = 5.53***, Procrustes distance (PD) = 0.1***), followed by Elia and Truso (MD = 4.84***, PD = 0.09***), while between Elia and Kobi the distances were relatively low and non-significant for Procrustes distance (MD = 2.01***, PD = 0.03 n.s.; Table 1D). The morphological variability of leaf shape explained by CV1 was mainly visible at (1) the junction of the blade and the petiole (LM 1), (2) the first basal lobe of the blade on the right side (LM7), (3) the tip of the lobe at the largest width of the lobe on the left (LM10), and (4) the base of the sinus immediately beneath the lobe of LM10 (LM11) (Fig. 5B). The main changes in shape along CV1 involved the general narrowing of the leaf blade on the left (LM 10, 11) and the asymmetrical elongation of the first basal lobe of the blade mainly on the right (LM 1, 7). These morphological characteristics were generally present in the Truso population (Fig. 5B).

To assess the degree of separation between each pair of *D. octopetala* populations, a discriminant function analysis (DFA) was performed. The results obtained were in agreement with the CVA. The multiple pairwise comparisons between each pair of populations showed

that the largest significant differentiation in leaf shape variation was observed between Kobi and Truso ($T^2 = 726.49^{***}$), followed by Elia and Truso ($T^2 = 365.85^{***}$), with the smallest being shown between Elia and Kobi ($T^2 = 95.32^{***}$). Additionally, the cross-validation values for each pair indicated that the most accurate classification was obtained for the Kobi-Truso pair comparison, where 100% of Kobi and 96.9% of Truso individuals were correctly classified (Fig. 6C). When comparing Elia and Truso – 96.37% of Elia and 87% of Truso individuals were correctly classified (Fig. 6B), while a comparison of Elia and Kobi showed that only 87% of Elia and 56% of Kobi individuals were correctly classified (Fig. 6A).

Further, the leaf size trait variability was analysed using traditional morphometric methods. Four leaf parameters were taken into account for the analysis: leaf blade length (BL), leaf blade width (BW), leaf area (LA), and leaf specific area (SLA). The findings were consistent with the geometric morphometry, indicating a high degree of intraspecific variation of leaf traits across populations. The one-way ANOVA *F*-statistics and Tukey's B Post Hoc Test demonstrated significant differences in the mean values of all the studied leaf traits ($p \leq 0.0001$, Table 2). All the leaf traits followed a general trend, with the Truso individuals having smaller and narrower leaves than the Elia and Kobi samples (Fig. 7). Additionally, Tukey's multiple comparisons between each pair of populations revealed statistically highly reliable differences in the means ($p \leq 0.001$, Fig. 7), clearly distinguishing two groups - the Elia and Kobi populations growing on acid soils with similar sizes in one group (larger and wider leaves) and the Truso population inhabiting neutral to slightly alkaline soil in the another (smaller and narrower leaves). Subsequently, the total variance was divided into inter-population and intra-population variance, with the results demonstrating much higher inter-population variance than intra-population variance for all the traits, ranging between 95.83% and 98.65% (Table 2).

Bivariate correlations were used to investigate the relationship between the leaf size traits and soil pH levels. Pearson's correlations were carried out to estimate a 95% confidence interval of bivariate parameters based on 10000 bootstrap iterations. Based on the analysis of these correlations with 10000 bootstrap iterations, the evidence suggests that soil pH levels significantly impact on the measured leaf traits. The individuals growing on acid soils, such as the Elia and Kobi populations, had larger and wider leaves compared to the Truso samples which were located on neutral to slightly alkaline soil and had smaller and narrower leaves. The BW exhibited the highest significantly negative correlation with soil pH, with a mean of -0.72^{**} and a 95% confidence interval ranging from -0.81 to -0.61 . The correlation with LA was the second-highest, with $r = -0.68^{**}$ and a 95% confidence interval between -0.76 and -0.58 . The corre-

Table 2. The one-way ANOVA results for the leaf traits of three *Dryas octopetala* populations from the Kazbegi region (the Central Great Caucasus, Georgia). **SS** - sum of squares; **df** – degrees of freedom; **MS**- mean squares; **F** – *F*-test statistics; asterisks indicate the overall significance of the *F*- test statistics at $p < 0.0001$.

Trait		SS	DF	MS	Proportion of intra- and inter-population variance (%)	F***
Blade Length (cm)	Between populations	9.654	2	4.827	97.38	32.27***
	Within populations	11.578	87	0.133	2.62	
	Total	21.232	89			
Blade Width (cm)	Between populations	1.458	2	0.729	98.65	53.17***
	Within populations	1.193	87	0.014	1.36	
	Total	2.651	89			
Leaf Area (cm ²)	Between populations	19.76	2	9.88	97.43	37.76***
	Within populations	22.763	87	0.262	2.57	
	Total	45.523	89			
Leaf Specific Area (cm ² g ⁻¹)	Between populations	0.045	2	0.023	95.83	24.77***
	Within populations	0.08	87	0.001	4.17	
	Total	0.125	89			

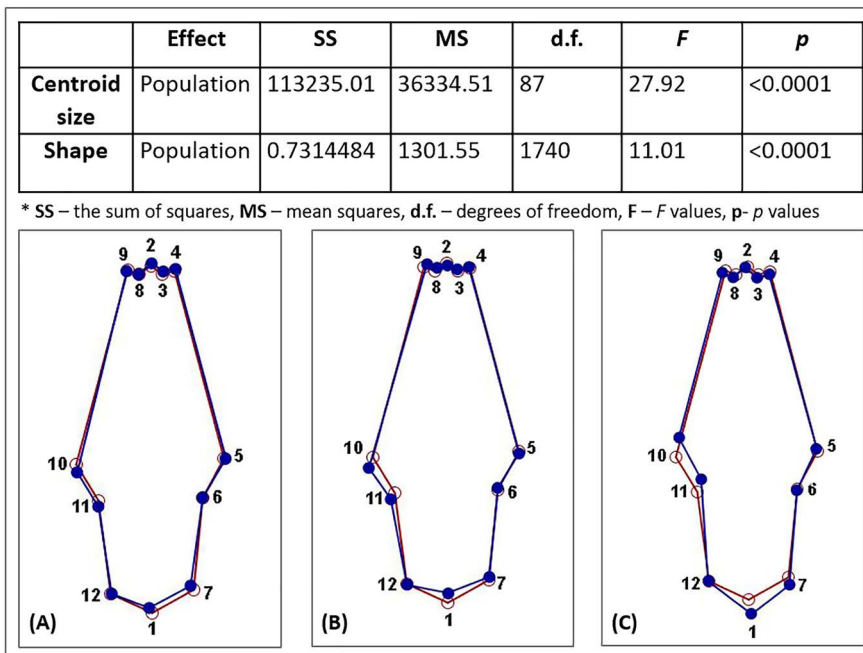


Fig. 4. The Procrustes ANOVA results of the *Dryas octopetala* individuals from the Kazbegi region (the Central Great Caucasus, Georgia). The individual mean wire-frame of 12 landmark configurations for each studied population is depicted in panels (A) for Elia, (B) for Kobi, and (C) for Truso. The red outline represents the starting shape, while the blue outline represents the target shape of the landmark configuration for each population.

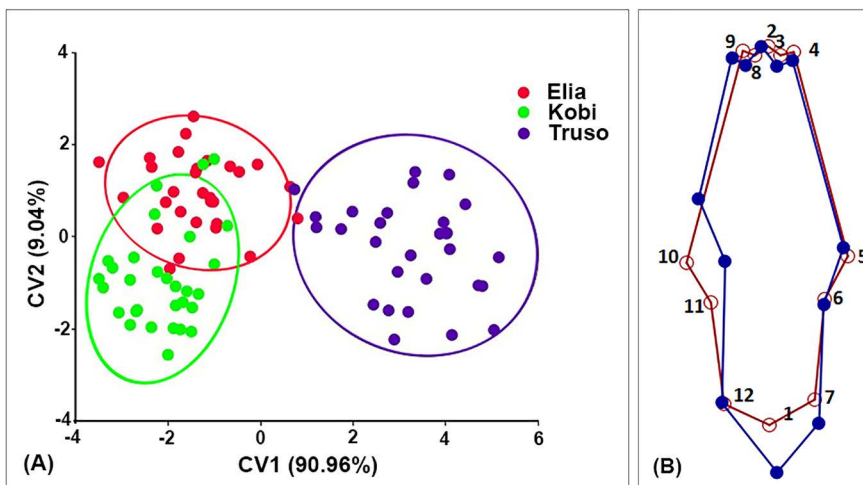


Fig. 5. The results of canonical variate analysis (CVA) of the *Dryas octopetala* populations from the Kazbegi region (the Central Great Caucasus, Georgia): (A) – ordination of the studied *D. octopetala* populations along CV1 with 95% confidence ellipses, and (B) – displays the canonical variate shape changes derived from CV1.

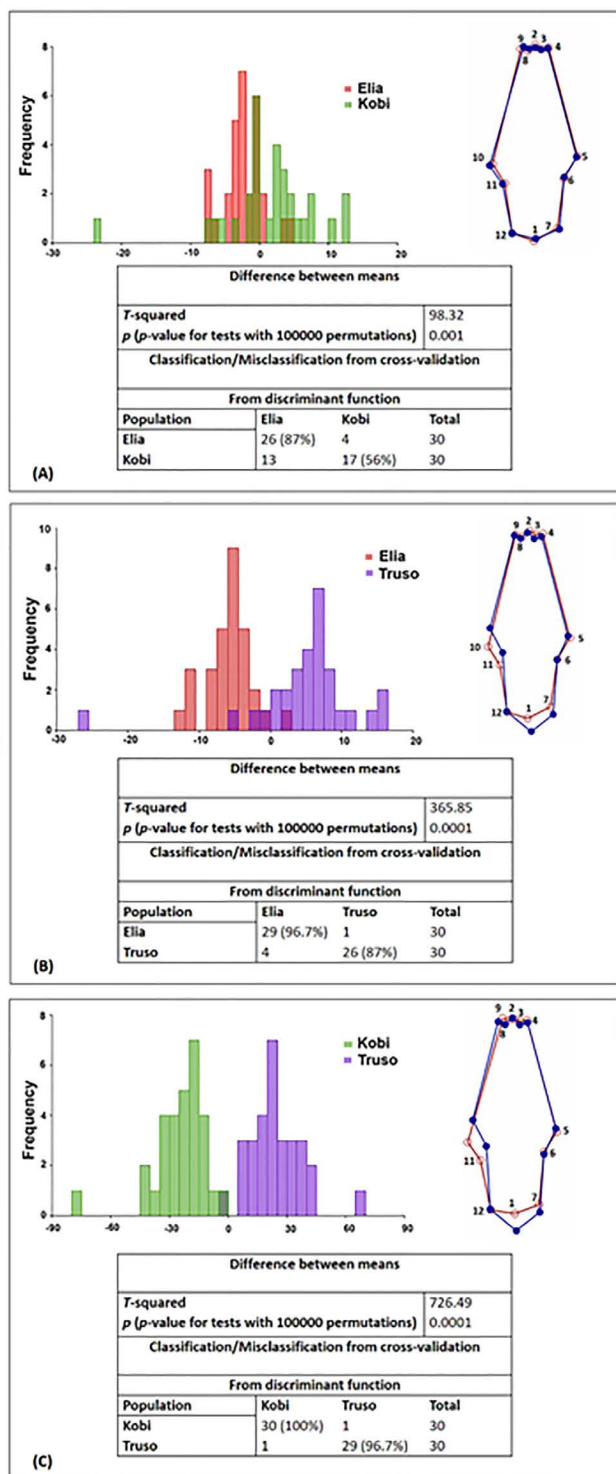


Fig. 6. The results of the discriminant function analysis (DFA) of the leaf shape variation as a potential morphological characteristic for the distinction between pairs of the *Dryas octopetala* populations from the Kazbegi region (the Central Great Caucasus, Georgia): (A) leaf shape difference between means and in landmark configuration between the Elia (red) and Kobi (green), (B) – between the Elia (red) and Truso (violet), and (C) between the Kobi (green) and Truso (violet) populations, respectively.

lation between soil pH level and BL as well as SLA were relatively lower, with both cases showing a significantly negative correlation of -0.64^{**} . The 95% confidence interval ranged from -0.73 to -0.54 in the case of BL and soil pH level, and between -0.74 and -0.53 for SLA and soil pH, respectively.

DISCUSSION

Plant-environmental interactions play a crucial role in the succession of plant communities, which occur due to mutual interactions between plants, the climate, and soil. In alpine regions, plants have adapted to survive harsh conditions characterised by extreme temperatures, wind, and limited water availability and soil resources which lead to various specific morphological adaptations expressed, for instance, in leaf shape and size variation. In this study, we aimed to determine whether certain leaf traits reliably separate *D. octopetala* populations through an analysis of traditional and, for the first time, modern geometric approaches and also to identify which environmental factors exert an impact on these traits. Despite covering a relatively small area of the studied region (only, 1081.7 km², NAKHUTSRISHVILI & ABDALADZE 2017b) our results demonstrated a significant variation among the studied populations in leaf shape and size variation revealed by both methods. The Elia and Kobi populations with larger and wider leaves were unified in one group, while the Truso population characterised by narrower and smaller leaves clearly differed from the former two and formed a separate cluster. Environmentally, the studied populations were situated in sites with very similar climatic conditions, but differing soil environments which could serve to explain the observed strong and negative correlation between the soil pH and measured leaf traits. As the soil becomes more alkaline, all the studied traits decrease. Both, the Elia and Kobi populations with larger and wider leaves were grown on acidic soils supported by substrates' type where they were found. The Elia population was found on igneous rock consisting of basaltic conglomerates and coarse-grained sandstones, while the Kobi population was primarily located in areas composed of clay quartz and polymorphic sandstones (GUDJABIDZE & GAMKRELIDZE 2003). In contrast, the Truso population with narrower and smaller leaves inhabited sites mainly consisting of classic limestone flysch (GUDJABIDZE & GAMKRELIDZE 2003) and was characterised by a neutral to slightly alkaline soil with the highest pH among the studied sites supported by limestone's alkaline nature (AMATYA *et al.* 2021). Changes in leaf traits in locations with similar climatic conditions but different soil environments might suggest that *D. octopetala* has stronger leaf morphology plasticity in response to changes in soil pH, thus indicating that soil nutrient content determines the observed variation. Soil pH is considered a master variable which affects all the other properties of the soil

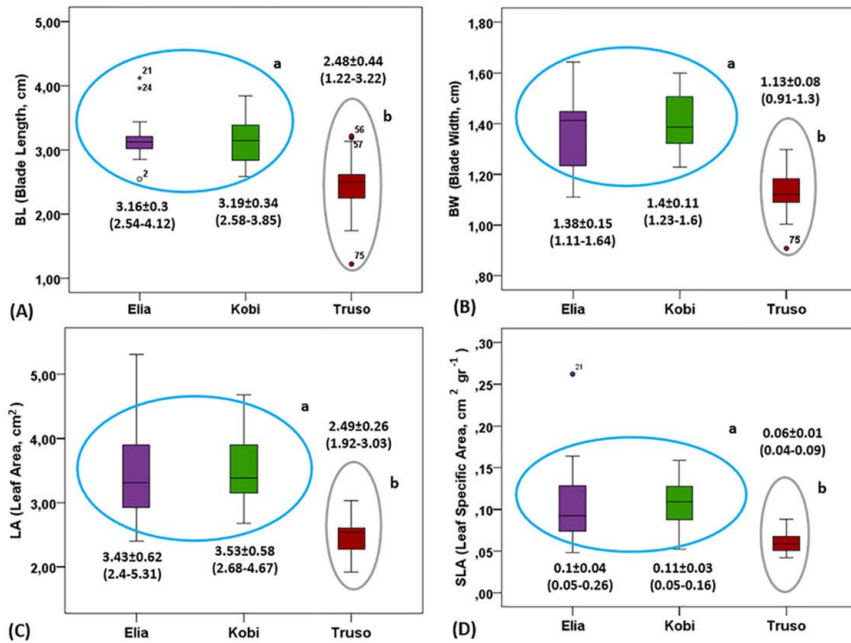


Fig. 7. Boxplots with descriptive statistics including the mean, standard deviation (SD), and minimum-maximum range for each studied leaf trait (A - Blade length; B - Blade width; C - Leaf area; D - Leaf Specific Area) of three *Dryas octopetala* populations in the Kazbegi region (the Central Great Caucasus, Georgia). Tukey's B post hoc test was used to compare the means among each pair of populations. The results indicate that populations sharing at least one letter within columns are not significantly different at the 0.001 probability level, as indicated by Tukey's B Multiple Range Test.

ecosystem (DAHLGREN 2006) including nutrient solubility and availability as well as microbial activity and plant growth (GENTILI *et al.* 2018; CROWTHER *et al.* 2019). Growing in acid soils gave the Elia and Kobi populations the advantage of being more successful in terms of biomass allocation as most micronutrients are more available to plants in these conditions than in neutral-alkaline soils (LONČARIĆ *et al.* 2008). However, the smaller and narrower leaves in the Truso population are beneficial in a relatively resource-poor environment such as neutral to slightly alkaline soils. Although alkaline soils increase the availability of most macronutrients, they also tend to reduce the availability of phosphorus and micronutrients, which negatively affect plant growth and fitness (AMATYA *et al.* 2021; ZLATIĆ 2023). Additionally, the well-known associations of *D. octopetala* with numerous ECM fruit bodies (RYBERG *et al.* 2009) are more successful in acidic soils than in alkaline soils as fungal growth is maximal in low pH or slightly acidic soils (LAVELLE & SPAIN 2005). As a result, the Truso population's remarkable plastic response to the soil pH gradient significantly reduced leaf size and shape variation. Furthermore, for all the leaf trait values, ANOVA analysis revealed that inter-population variation exceeded 95% (Table 2) indicating the response of the populations to the variation in site conditions, and showing that leaf traits can be linked with geographical and environmental variables across a species' range (EKHVAIA *et al.* 2018; TUCIĆ *et al.* 2018; LI *et al.* 2021; ABBELD-JALIL & BEGHAMI 2022).

However, more evidence from extensive investigations with additional populations and other edaphic

factors are required, examining the other chemical and physical soil properties which determine a soil's sustainability for plant growth, including molecular markers to explore genotypic and phenotypic variation, in order to draw firmer conclusions regarding the intraspecific leaf shape and size variation of *D. octopetala*.

CONCLUSIONS

In conclusion, this study (1) demonstrates a large phenotypic leaf shape and size variation in *D. octopetala* populations in the Kazbegi region (the Central Great Caucasus, Georgia), (2) shows a clear relationship between soil pH and all leaf traits, indicating that *D. octopetala* is well adapted to a rather wide range of soil pH levels, and (3) encourages the use of plant traits at the intraspecific level as a tool to understand the future ecological transformations of vegetation in changing environments. The remarkable correlation of soil pH with leaf shape and size variation confirms that phenotypic variance could play a crucial role in the adaptation of plants to different soil environments (acid soils vs neutral to slightly alkaline soils) resulting in a strong leaf morphology plasticity (wider and larger leaves vs narrower and smaller leaves) along the soil pH gradient. This requires further investigations so as to identify the leading environmental drivers, which along with soil pH, influence plant trait variability in alpine areas. Importantly, the combination of traditional and geometric morphometrics disclosed clear and observable morphological variations among the three studied populations, making it particularly suitable for detecting leaf

shape and size differences which may reflect the plasticity of *D. octopetala* in different soil environments. Such an accessible and inexpensive approach, especially modern geometric morphometrics with its high degree of precision and statistical significance, could be advantageous for researchers and scientists seeking to gain a deeper understanding of plant species' unique responses to varying environmental conditions.

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REZIME

Promenljivost oblika i veličine listova *Dryas octopetala* na Centralnom Velikom Kavkazu (region Kazbegi, Gruzija) na osnovu tradicionalne i geometrijske morfometrije

Jana EKHVAIA, Otar ABDALADZE, Tamar JOLOKHAVA i Arsen BAKHIA

Iako je poznato da je pH zemljišta ključni pokretač sastava biljnih vrsta, još uvek slabo razumemo kvantifikaciju odgovora osobina lista na različite tipove zemljišta, posebno u visoko osetljivom alpskom okruženju podložnom globalnim promenama životne sredine. Kombinovanjem tradicionalne i moderne geometrijske morfometrije želeli smo da doprinesemo postojećoj praznini i utvrdimo da li postoje osobine listova koje pouzdano razdvajaju populacije zimzelenih *Dryas octopetala* koje žive u subalpsko-alpskoj zoni regiona Kazbegi, istočnog dela Centralnog Velikog Kavkaza u Georgia. U tom cilju analizirane su tri populacije *D. octopetala* kroz tradicionalnu i, po prvi put, geometrijsku morfometriju. Obe metode su otkrile kongruentne rezultate koji ukazuju na značajne razlike u obliku i veličini listova na lokacijama sa sličnim klimatskim uslovima, ali različitim pH okruženjima tla (kiselih naspram neutralnih do blago alkalnih) što sugeriše da *D. octopetala* ima jaču plastičnost morfologije listova (širi i veći listovi u odnosu na uže i manje listove) da reaguju na promene u pH gradijentu zemljišta. Pored toga, geometrijska morfometrija kao pristupačna i jeftina metoda sa visokim stepenom preciznosti mogla bi biti korisna za istraživače i naučnike koji žele da steknu dublje razumevanje jedinstvenih odgovora biljnih vrsta na različite uslove životne sredine.

Ključne reči: alpski ekosistemi, Kavkaz, oznake, pH zemljišta, plastičnost morfologije listova

ORCID:

Jana EKHVAIA
Otar ABDALADZE
Tamar JOLOKHAVA
Arsen BAKHIA

<https://orcid.org/0000-0001-7104-1561>
<https://orcid.org/0000-0001-8140-0900>
<https://orcid.org/0000-0003-3868-0526>
<https://orcid.org/0009-0009-3809-1124>