





Genetic diversity and morphological variability of Iranian *Silene aucheriana* populations inferred from nrDNA ITS sequences and morphological analysis

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Key words: Caryophyllaceae, Iran, ecology, phenotypic plasticity, Canonical Correspondence Analysis.

Ključne besede: Caryophyllaceae, Iran, ekologija, fenotipska plastičnost, kanonična korelacijska analiza.

Abstract

We conducted a comprehensive study on the diversity of *Silene aucheriana* Boiss. populations by analyzing both morphological data and nrDNA ITS sequences. Maximum parsimony and Bayesian methods on representative material from 15 populations throughout Iran demonstrated that they did not form a monophyletic group. Strong positive correlation occurred between epipetalous filaments length to claw (EFLC), basal leaf length (BLL), basal leaf width (BLW), and plant height (PLH) with elevation. While negative correlation achieved between anthophore length (AnL) with the average air temperature and rainfall. Canonical Correspondence Analysis (CCA) showed that most morphological traits were more correlated to elevation than to average rainfall and temperature. Multivariate analysis of morphological traits with ITS analysis displayed a slight divergence between two types of regions based on their elevation.

Izvleček

Izvedli smo celovito študijo o raznolikosti populacij vrste *Silene aucheriana* Boiss. z analizo morfoloških meritev in zaporedij ITS nrDNA. Največja parsimonija in statistične metode po Bayesu na reprezentativnem materialu iz 15 populacij po vsem Iranu so pokazale, da ne tvorijo monofiletične skupine. Ugotovili smo močno pozitivno korelacijo med dolžino epipetalnih filamentov in žebice (EFLC), dolžino spodnjih listov (BLL), širino spodnjih listov (BLW) in višino rastline (PLH) z nadmorsko višino. Negativno korelacijo smo ugotovili med dolžino antofore (AnL) in povprečno temperaturo zraka ter količino padavin. Kanonična korespondenčna analiza (CCA) je pokazala, da je večina morfoloških lastnosti bolj povezana z nadmorsko višino, kot pa s povprečno količino padavin in temperaturo. Multivariatna analiza morfoloških lastnosti z analizo ITS je pokazala rahlo odstopanje med dvema skupinama regij glede na njihovo nadmorsko višino.

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Introduction

Silene aucheriana Boiss. is in the Caryophyllaceae subgenus *Silene*, section *Auriculatae* (Boiss.) Schischk. (Melzheimer, 1988; Oxelman & Lidén, 1995). This species is found in Anatolia, Iraq, Turcomania and it is widespread in Iran (Melzheimer, 1988). Genetic diversity is traditionally explored via analysis of morphological traits (Rautenberg et al., 2012). This approach includes some restrictions like the effect of environmental factors, giving outputs that present just a fraction of total genetic variability (Živković et al., 2012). Applying an approach integrating morphological and genetic data is generally necessary to unravel species delimitation (Duminil & Di Michele, 2009; Heinrichs et al., 2009). Regarding genus *Silene*, Oxelman (1996) used morphological and molecular markers including Internal Transcribed Spacer DNA (ITS) to study the species relationships in the sect. *Sedoideas*. Populations of *S. aucheriana* are widely distributed in different regions of Iran with different ecological conditions and therefore, it is expected to show some morphological variation (Safaeishakib et al., 2023). The genetic structure based on ISSR data of these studied populations was previously reported by Safaeishakib et al. (2023), which revealed that genetic divergence among populations most likely reflects their climatic conditions. The current morphometric data and internal transcribed spacer (ITS) sequences were performed from two different types of geographical regions. With these data, the aims of the present study are a) to examine the morphological variation in different populations of *S. aucheriana*, b) to investigate the correlation between quantitative morphological traits and some meteorological data, and c) to evaluate which morphological features are more significantly impacted by environmental factors.

Materials and methods

Plant material and Morphological studies

Five samples (for morphological analysis) from each of the fifteen *S. aucheriana* populations were collected from May to early July 2017 from two types of regions with different average temperatures, elevations, and rainfall. For better comprehension, 15 populations were divided into two groups according to their elevation (Table 1, Figure 1). Populations with an elevation cut-off limit higher than 2800 m (mountainous area and dry cold) in one group and those with an elevation cut-off limit lower than 1800 m (semi-arid, and sub-humid) placed in another

group. These cut-off limits were based on the mean elevations of localities (Safaeishakib et al., 2023). The assessment of these climate differences and classification was based on the Köppen-Geiger (KG) climate classification scheme (Hijmans et al., 2005; Peel et al., 2007; Fick and Hijmans, 2017), www.WorldClim.org, and www.weatherunderground.com definitions. Meteorological data on localities including average temperature and rainfall were obtained from www.en.climate-data.org (O'Donnell & Ignizio, 2012; Safaeishakib et al. 2023). Voucher specimens are deposited at the Islamic Azad University Herbarium (IAUH) (Table 1, Figure 1). Seventeen morphological characteristic were measured for each individual material (see Table 2 for characters used). These characters have been used by various authorities in identifying *Silene* species (Chowdhuri, 1957; Coode & Cullen, 1967; Ghazanfar, 1984; Firat & Yildiz, 2016; Townsend et al., 2016). All measurements were done by using a Stereo Microscope (NTB-3A).

Statistical analysis

Statistical analysis was made using software package SPSS 18.0 for Windows (SPSS Inc. Chicago, USA). Morphological results are shown as mean \pm standard error observations (mean \pm SE). Duncan's test followed by a Bonferroni correction ($\alpha' = \alpha/k$ and $\alpha = 0.05$) was used to detect possible differences in the studied populations (Rice, 1989). Differences among mean values obtained were determined at $p < 0.05$ by Duncan's test. The relationship between populations and environmental variation was assessed using canonical correspondence analysis (CCA) (Ter Braak, 1986). Clustering was performed according to the method of Ward with PAST software version 4.06b based on 1000 bootstrap replicates to explore relationships among samples with high variability where data and grouping are unclear (Hammer et al., 2001). Principal Component Analysis (PCA) was constructed using Varimax rotation to obtain variation in morphological traits (Safaei et al., 2016). Pearson's correlation coefficient was analyzed by using GraphPad Prism version 6.04 for Windows (GraphPad Software, La Jolla California USA), to identify the relationships between the seventeen morphological traits and the environmental variables.

Genomic DNA extraction and amplification

Total genomic DNA was extracted from silica gel dried leaves following the protocol Nucleo Spin™ Plants Kits (Machery-Nagel, Germany) (Doyle & Doyle, 1987). The quality of the extracted DNA was evaluated on a 1% aga-

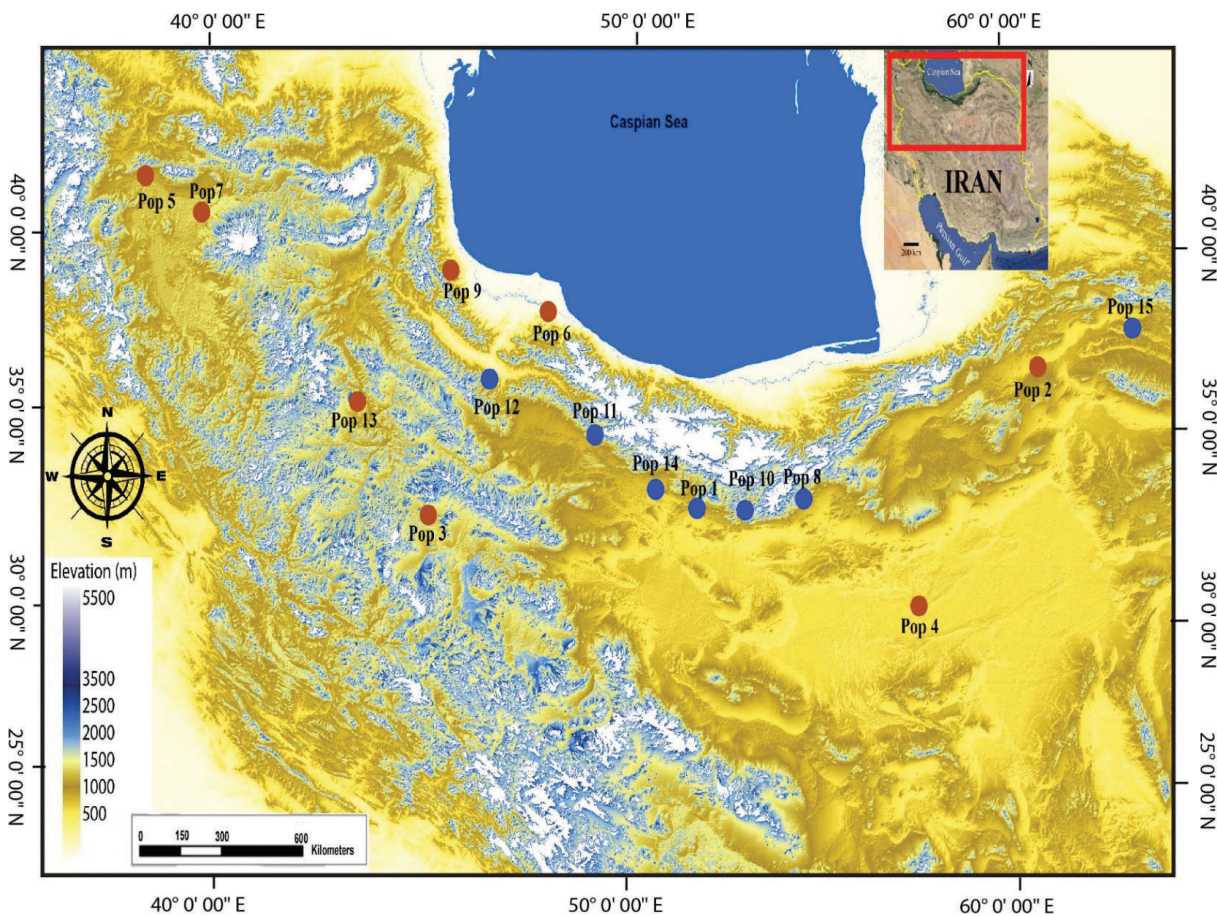


Figure 1: Geographic distribution of *S. aucheriana* populations used in this study. Blue-colored circles reflect elevation cut-off limit higher than 2800 m, lower average temperature, and less rainfall (8.66 °C, 566.34 mm), whereas brown-colored circles show different climate conditions with an elevation cut-off limit lower than 1800 m, higher average temperature, more average rainfall, (15.57 °C, 600 mm). Local names are presented in Table 1. (Map designed using Arc GIS 10.2).

Slika 1: Geografska razširjenost populacij vrste *S. aucheriana* uporabljenih v študiji. Modra barva odraža nadmorsko višinsko mejo nad 2800 m, nižjo povprečno temperaturo in manj padavin (8,66 °C, 566,34 mm), rjava barva pa drugačne podnebne razmere nadmorsko višinsko mejo nižjo od 1800 m, višje povprečne temperature, več povprečne količine padavin, (15,57 °C, 600 mm). Krajevna imena so predstavljena v tabeli 1. (Zemljevid je bil izdelan z Arc GIS 10.2).

rose gel. The concentration of DNA was also estimated through Nano-Drop™ 2020 (Thermo Fisher Scientific, USA) at 260 nm. Amplification of ITS region was carried out by using forward primer AB101 (5'-ACG AAT TCA TGG TCC GGT GAA GTG TTC G-3') and reverse primer AB102 (5'-TAG AAT TCC CCG GTT CGC TCG CCG TTAC-3') (Douzery et al., 1999). The amplification was gained by 35 cycles of 1 min, denaturation (94 °C), 1 min annealing (54 °C), and 2 min, 30-second elongation (72 °C), (Oxelman & Lidén, 1995). Finally, PCR products were evaluated qualitatively by electrophoresis on 1% agarose gel. The concentration of DNA was also estimated through Nano-Drop™ 2020 (Thermo Fisher Scientific, USA) at 260 nm. Sequencing of amplicons was performed on an ABI 3730 DNA Analyzer

(Hitachi-Applied Biosystems, Waltham, Massachusetts, USA). Sequences of the ITS regions for 15 populations were submitted to GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>). In addition to these newly generated sequences, ITS sequences of 37 accessions of other *Silene* species from Safaeishakib et al. (2020) were added to analysis for further comparison (see Table1).

Sequence editing and phylogenetic analysis

Sequences were visually checked and edited with the software tool Sequencher™ version 5.4.6. (Gene Codes Corporation, Ann Arbor, Michigan, USA). New dataset was aligned using the software tool MacClade 4.08

(Maddison & Maddison, 2000). Phylogenetic analyses were carried out with PAUP v. 4.0b10 (Swofford, 2003). The consistency index (CI) and retention index (RI) were estimated to assess the amount of homoplasy present in the data. Maximum parsimony (MP) analyses was performed using the heuristic search option with 1000 random taxon additions and tree bisection and reconnection (TBR) as the branch swapping algorithm (Felsenstein, 1985). The model of molecular evolution for each dataset was selected using the Akaike Information Criterion (AIC) in MrModeltest v. 2.3 (Posada & Crandall, 1998). A Markov Chain Monte Carlo (MCMC) sampling was performed for 4,000,000 generations with sampling occurring at every 100 generations. The posterior probability (PP) values were calculated using MrBayes version. 3.1.2 (Ronquist & Huelsenbeck, 2003). Twenty-five percent of the initial trees was discarded, and the remaining sampled trees were combined into a 50% majority rule consensus tree.

Results

Nuclear ribosomal DNA ITS

The ITS region varied in length from 650 to 840 bp and parsimony-informative characters gained 52. The shortest trees found by PAUP* had 231 steps resulted in CI=0.77, RI=0.65. The (GTR+I+G) was selected as the best-fit model by MrModeltest. The frequencies of nucleotides were estimated as following: A=0.2182, C=0.2955, G=0.2883 and T=0.1980). The majority-rule consensus tree generated via Bayesian inference analysis and is highly consistent with the strict consensus tree inferred from MP analysis. Therefore, only the Bayesian tree is shown. Posterior Probabilities (PP) are indicated by numbers above each branch. Bootstrap supports for this branch retrieved in the Maximum Parsimony (MP) assessment were displayed by the numbers below each branch. The phylogenetic analysis revealed that Tarom (Pop 13) and Silvana (Pop 7) populations are well supported (PP=0.95); furthermore, Dona (Pop 11), Qoshchi (Pop 5) and Heydareh (Pop 3) populations showed close affinity to each other (populations belonging to elevation cut-off limit lower than 1800 m). On the other hand, four populations such as Emamzadeh Hashem (Pop 1), Gaduk (Pop 8), HezarMasjed (Pop 15), and Rineh (Pop 10), belong to elevation cut-off limit higher than 2800 m, and were separately placed close to each other. Other values of posterior probability (PP) and maximum parsimony bootstrap (MP) were achieved from other species of sect. *Auriculatae* like *S. bornmuelleri* and *S. sisianica* (PP=1, PB=90%) (Figure 2).

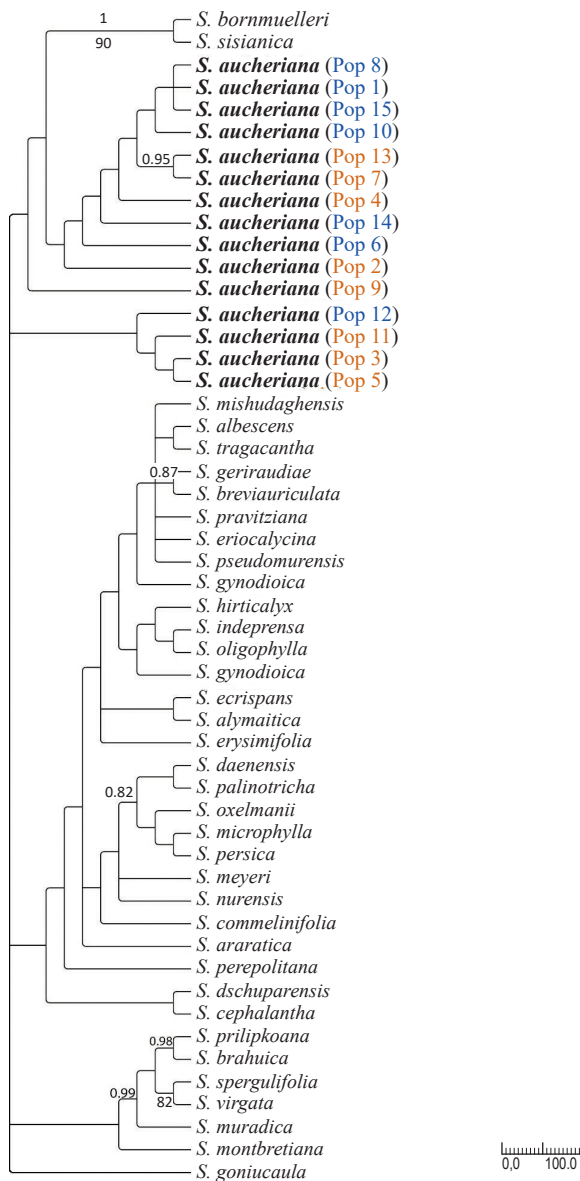


Figure 2: Bayesian majority-rule consensus tree derived from nuclear ribosomal DNA internal transcribed spacer sequences (ITS). Posterior probability (≥ 0.90) and MP bootstrap support ($\geq 70\%$) values are presented on above and below branches. Blue color reflects elevation cut-off limit higher than 2800 m, lower average temperature, and less rainfall (8.66 °C, 566.34 mm), whereas brown color shows different climate conditions with an elevation cut-off limit lower than 1800 m, higher average temperature, more average rainfall (15.57 °C, 600 mm). Local names are presented in Table 1.

Slika 2: Konsenzno drevo Bayesovega večinskega pravila, izpeljano iz regije ITS jedrne ribosomalne DNK. Vrednosti posteriorne verjetnosti ($\geq 0,90$) in podpora s samovzorčenjem analize MP ($\geq 70\%$) so predstavljene na zgornjih in spodnjih vejah. Modra barva odraža nadmorsko višinsko mejo nad 2800 m, nižjo povprečno temperaturo in manj padavin (8,66 °C, 566,34 mm), rjava barva pa drugačne podnebne razmere z nadmorsko višinsko mejo nižjo od 1800 m, višje povprečne temperature, večjo povprečno količino padavin (15,57 °C, 600 mm). Krajevna imena so predstavljena v tabeli 1.

Statistical analysis

Correlation coefficients demonstrated that correlations were mostly either non-significant or null. However, significant correlations in some relations were observed, for example, a strong positive correlation occurred between epipetalous filaments length to claw (EFLC), basal leaf length (BLL), basal leaf width (BLW), and plant height (PLH) with elevation. Similarly, positive correlation between seed width (SEW) and average air temperature was also obtained. The correlations between anther length (AnL) with the average air temperature and rainfall were mainly negative and non-significant at $p < 0.05$ level respectively (Figure 3). Although no significant differences in a single trait were observed in all populations, basal leaf width (BLW) in Silvana (Pop 7) displayed a noteworthy variation compared to the other populations. Moreover, basal leaf length (BLL) trait in Qoshchi (Pop 5), petal limb length (PLL) in HezarMasjed and Klishom (Pop 15, Pop 9), petal limb division length (PLDL) in Klishom (Pop 9), epipetalous filaments length to claw (EFLC) in Tochal (Pop 12), and capsule length (CAPL) in Golestan Kooh (Pop 4) revealed a significant difference among the studied populations. Noticeably, meteorological data exhibited significant differences with all quantitative traits (Table 3).

Cluster analysis and principal component analysis of morphological traits

Morphological variation in 17 studied traits (using PCA) showed populations from Zangoleh (Pop 6), Emamzadeh Hashem (Pop 1), Rineh (Pop 10), Gaduk (Pop 8) were clustered at the first component while the second component clustered populations from Tarom (Pop 13), Silvana (Pop 7), Qoshchi (Pop 5), Heydareh (Pop 3), Tochal (Pop 12), Dona (Pop 11), Golestan Kooh (Pop 4), Gorsfid (Pop 2). The populations of Dizin (Pop 14), HezarMasjed (Pop 15), and Klishom (Pop 9) were positioned in the third dimension (Figure 4).

Cluster analysis based on Ward’s method showed that populations of Rineh (Pop10), Zangoleh (Pop 6), Emamzadeh Hashem (Pop 1), were placed together with similar environmental conditions while populations from Silvana (Pop 7), Qoshchi (Pop 5), Heydareh (Pop 3), Dona (Pop 11), and Tochal (Pop 12) were located at close distance (Figure 5). These results were in agreement with the PCA plot result. In order to estimate the relative importance of each eco-geographical trait populations distribution, CCA was used, and showed that most of the evaluated traits are influenced by elevation (Figure 6).

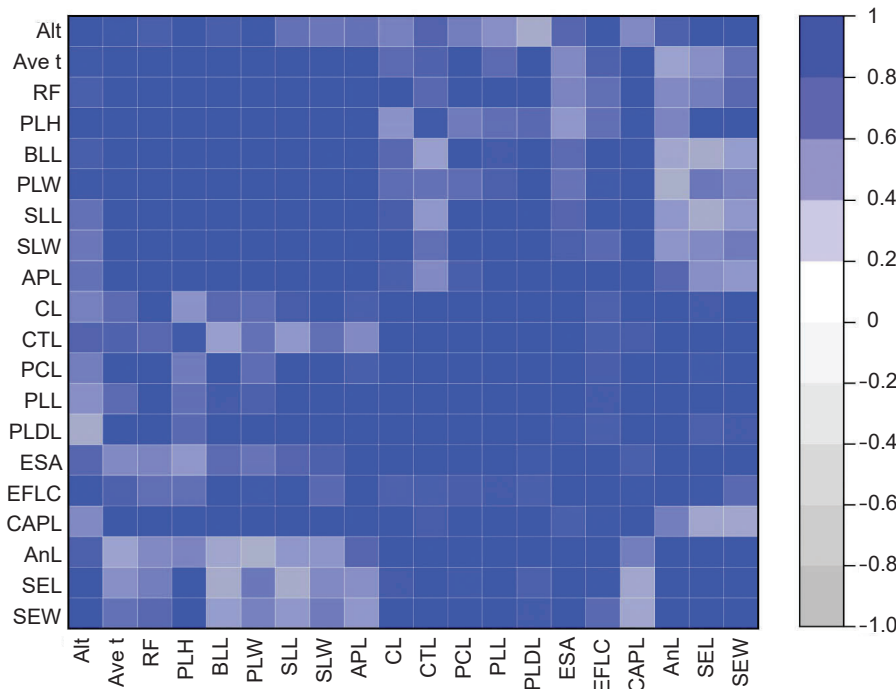


Figure 3: Correlation analysis between eco-geographical variables and morphological traits achieved from populations of *S. aucheriana*. The abbreviations of traits are presented in Table 2. Note: Ave t: average temperature; RF: rainfall; Alt: Altitude.

Slika 3: Korelacijska analiza med ekološko-geografskimi spremenljivkami in morfološkimi znaki, pridobljenimi iz populacij vrste *S. aucheriana*. Okrajšave znakov so predstavljene v tabeli 2. Opomba: Ave t: povprečna temperatura; RF: padavine; Alt: nadmorska višina.

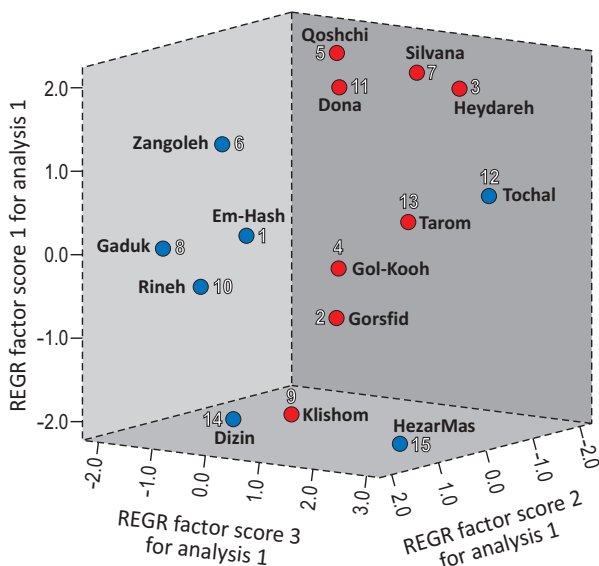


Figure 4: Principal component analysis (PCA) of the morphometric data. Blue-colored circles reflect elevation cut-off limit higher than 2800 m, lower average temperature, and less rainfall (8.66 °C, 566.34 mm), whereas brown-colored circles show different climate conditions with an elevation cut-off limit lower than 1800 m, higher average temperature, more average rainfall, (15.57 °C, 600 mm).

Slika 4: Analiza glavnih komponent (PCA) morfometričnih podatkov. Modro obarvani krogi odražajo mejno nadmorsko višino nad 2800 m, nižjo povprečno temperaturo in manj padavin (8,66 °C, 566,34 mm), medtem ko rjavi krogi prikazujejo različne podnebne razmere z mejno nadmorsko višino, nižjo od 1800 m, višjo povprečno temperaturo, večjo povprečno količino padavin (15,57 °C, 600 mm).

Discussion

Cluster analysis and PCA based on morphological traits indicated Klishom's population (Pop 9) is close to Dizin (Pop 14), and HezarMasjed (Pop 15) populations belonging to elevation cut-off limit higher than 2800 m. While the genetic structure study among 15 populations of *S. aucheriana* (Safaishakib et al., 2023) showed slight gene flow has occurred in Klishom's population (Pop 9) and revealed that this population is grouped with populations at elevation cut-off limit lower than 1800 m, more average rainfall, and higher average temperature (600 mm, 15.57 °C). From the morphological point of view and based on the results of the sequence, we found that the population of Tochal (Pop 12) (belonging to the elevation cut-off limit higher than 2800 m) is placed close to the populations from the elevation cut-off limit lower than 1800 m. However, this result was not obtained based on ISSR sequencing (Safaishakib et al., 2023). In this study, the molecular data achieved from *S. aucheriana* populations exhibited an almost consistent pattern that nearly aligned with the results obtained from the morphological analysis. Taxonomically, *S. aucheriana*

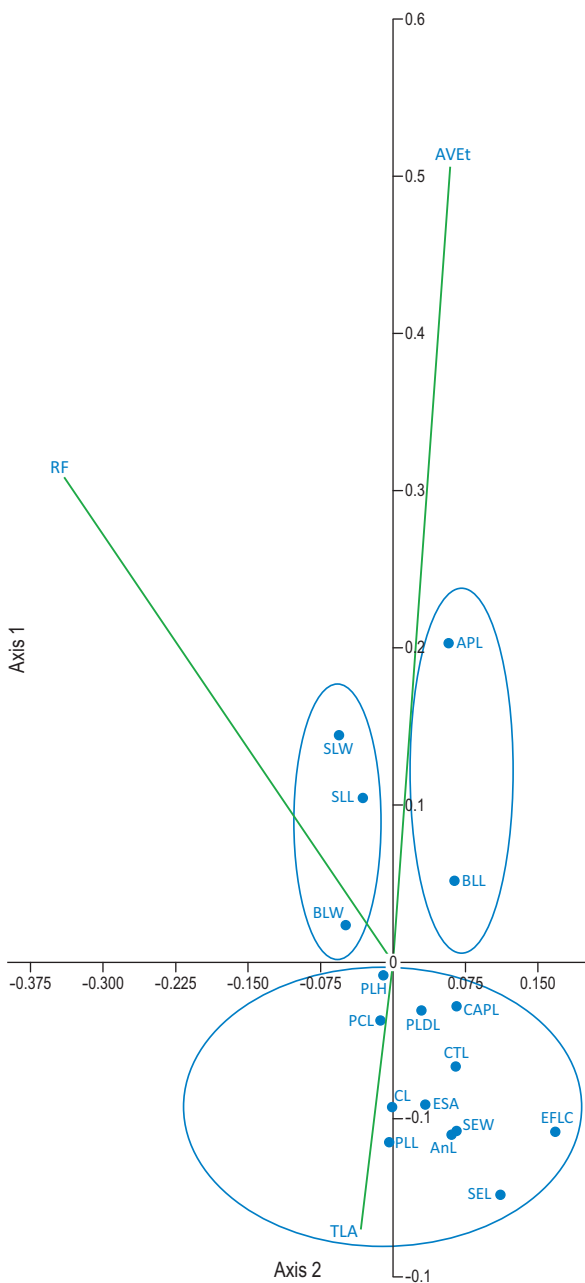


Figure 5: Canonical correspondence analysis (CCA) was carried out with seventeen morphological data and three geographical traits of fifteen accessions of *S. aucheriana* collected from different locations. The abbreviation of traits is presented in Table 2. Note: AVE t: average temperature; RF: rainfall; ALT: Altitude

Slika 5: V kanonično korespondenčno analizo smo vključili sedemnajst morfoloških in tri geografske znake petnajstih nabirkov vrste *S. aucheriana*, nabranih na različnih nahajališčih. Okrajšave znakov so predstavljene v tabeli 2. Opomba: AVE t: povprečna temperatura; RF: količina padavin; ALT: nadmorska višina.

is distinct from *S. montbretiana*, while they have been treated synonymous in *Flora of Turkey* and *Flora of Iraq* (Coode & Cullen 1967; Townsend et al., 2016). Mel-

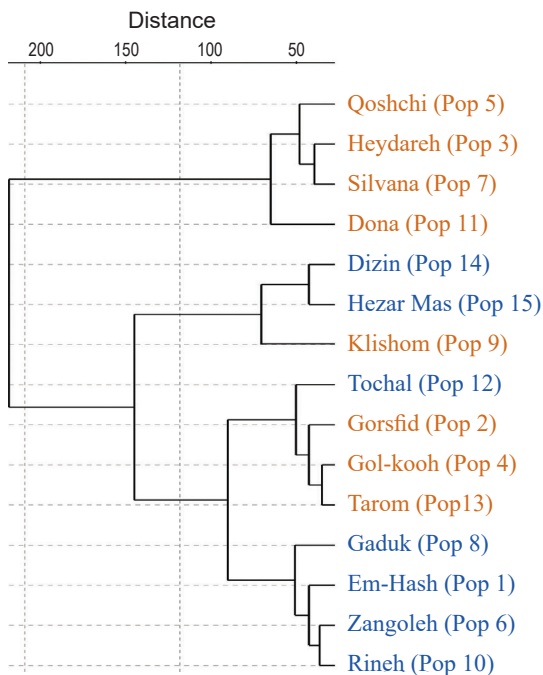


Figure 6: Cluster analysis of *S. aucheriana* populations using Ward's linkage based on morphological data. Blue color reflects elevation cut-off limit higher than 2800 m, lower average temperature, and less rainfall (8.66 °C, 566.34 mm), whereas brown color shows different climate conditions with an elevation cut-off limit lower than 1800 m, higher average temperature, more average rainfall, (15.57 °C, 600 mm).

Slika 6: Klastrska analiza populacij vrste *S. aucheriana* z Wardovo metodo povezovanja na osnovi morfoloških podatkov. Modra barva odraža nadmorsko višinsko mejo nad 2800 m, nižjo povprečno temperaturo in manj padavin (8,66 °C, 566,34 mm), rjava barva pa drugačne podnebne razmere z nadmorsko višinsko mejo, nižjo od 1800 m, višjo povprečno temperaturo, večjo povprečno količino padavin (15,57 °C, 600 mm).

zheimer (1988) has separated Iranian *S. aucheriana* from the Turkish *S. montbretiana* on the basis of leaf characters (Ghazanfar & Edmondson, 2013).

Differences in habitat can be reflected in morphological features, so different environmental conditions cannot be ignored in population diversity studies (Thorpe, 1987; Peppe et al., 2011). Elevation is one of the important environmental factors that affect population differentiation and genetic diversity. High elevation, especially in alpine regions, has significant effect on plants. Recently, plant-based ecology studies have raised many fundamental questions of morphological variation as populations have obvious adaptation strategies by changing their morphological and physiological characteristics (Royer et al., 2005; Verheijen et al., 2013). The ordination techniques are useful in the elucidation of cluster pattern as well as in the detection of the eco-geographical variation (Mucino, 1982). In this regard, CCA showed basal leaf length

and alar pedicel length traits affected by average temperature. Additionally, stem leaf width, stem leaf length and basal leaf width appear to be strongly affected by rainfall. While other traits such as epipetalous filaments length to claw, seed length, anthophore length, seed width, calyx tooth length, calyx length, plant height, petal limb division length, capsule length, and epipetalous stamens are affected mainly by elevation. Additionally, based on the morphological observations in the collected samples of *S. aucheriana* populations, we noticed two traits of basal leaf width and calyx length are more variable than other traits. Some studies have shown that genetic diversity in *Silene* species is more representative of geography and climate than taxonomy; for example, in *S. latifolia*, divergence of populations showed that two kinds of climate regions were involved that resulted in variation of morphological characters (Brothers et al., 2016; Durović et al., 2017; Del Valle et al., 2019). Similarly, the study conducted by Karrenberg et al. (2018) utilized environmental data consisting of 19 bioclimatic variables to analyze populations of *S. dioica* and *S. latifolia*. The findings suggest that speciation has been driven by ecological divergence.

Conclusions

The present study intended to examine the genetic and morphological diversity of two different groups of *S. aucheriana* populations, with a focus on elevation cut-off limits above 2800 m and below 1800 m. Cluster analysis of molecular data was shown to be similar to morphological traits results. Accordingly, Emamzadeh Hashem (Pop 1), Gaduk (Pop 8), Zangoleh (Pop 6), and Rineh (Pop 10) populations belong to elevation cut-off limit higher than 2800 m, and were separately placed close to each other. Subsequently Silvana (Pop 7), Qoshchi (Pop 5), Heydareh (Pop 3), and Dona (Pop 11) populations were located in another group (elevation cut-off limit lower than 1800 m) at close distance. The phylogenetic results of nrDNA ITS revealed that the two populations of Tarom (Pop 13), and Silvana (Pop 7) formed a well-supported branch as a monophyletic group (PP=0.95). Similarly, in terms of morphological analyses, apart from the Tochal (Pop 12) and Klishom (Pop 9), other populations were placed together based on their elevation grouping. Having such a relatively clear-cut grouping from both methods could suggest the existence of a genetic background supporting morphological diversity. Some morphological variables such as basal leaf length, and alar pedicel length showed a positive correlation with average air temperature while negative cor-





relation was seen between capsule length, and petal limb division length with elevation. The evaluation conducted by CCA demonstrated that elevation had a stronger correlation with most of the morphological traits as compared to average rainfall and temperature. Among all investigated traits, only basal leaf length (BLL), basal leaf width (BLW), petal limb length (PLL) and epipetalous filaments length to claw (EFLC), and capsule length (CAPL) displayed a significant difference among the studied populations. Two quantitative traits, basal leaf width and calyx length, were variable compared to seventeen other quantitative traits.

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Table 1: Localities, climatic details, and voucher specimens of *S. aucheriana* populations based on nrDNA ITS data used for this study. In different regions, GPS was used to determine the location (longitude, latitude, and elevation). Average rainfall and temperature were obtained from www.en.climate-data.org. Other taxa (non highlighted cells) of sect. *Auriculatae* are listed for comparison.

Table 1: Nahajališča, podnebne lastnosti in vavčerski vzorci populacij vrste *S. aucheriana* na osnovi podatkov regije ITS nrDNA, uporabljenih v tej študiji. V različnih regijah smo za določanje lokacije (zemljepisna dolžina, širina in nadmorska višina) uporabili GPS. Povprečno količino padavin in temperaturo smo pridobili iz www.en.climate-data.org. Drugi taksoni (neosensčene celice) iz sekcije *Auriculatae* so navedeni za primerjavo.

Species/Populations	Locality	Longitude/ Latitude	Elevation cut- off limits (m)	Elevation (meter)	Average temp (°C)	Average rainfall (mm)	Voucher No.	GenBank No. (ITS) (Safaeishakib et al. 2020)
<i>S. aucheriana</i> (Pop 1)	Haraz-Emamzadeh Hashem mountain (Em-ha)	52° 02' 08"E 35° 50' 04"N	> 2800	3050	6.8	374	IAUH000015357	OL662942
<i>S. aucheriana</i> (Pop 2)	Semnan-Firozkooh to Sorkheh, Gorsfid	53° 31' 59"E 35° 15' 19"N	< 1800	1251	17.40	103	IAUH000015358	OL662988
<i>S. aucheriana</i> (Pop 3)	Hamedan-Heydareh	48° 27' 45"E 34° 46' 41"N	< 1800	1115	13.32	443	IAUH000015359	OL662990
<i>S. aucheriana</i> (Pop 4)	Esfahan-Khansar, Golestan Kooh	50° 27' 12"E 33° 07' 35"N	< 1800	1723	13.52	222	IAUH000015360	OL662995
<i>S. aucheriana</i> (Pop 5)	W-Azerbaijan, Qoshchi (to Salmas)	45° 03' 32"E 37° 59' 38"N	< 1800	1780	15.80	321	IAUH000015361	OL663837
<i>S. aucheriana</i> (Pop 6)	Chalou-Zangoleh pass	51° 07' 06"E 36° 28' 15"N	> 2800	2840	6.5	1660	IAUH000015362	OL663847
<i>S. aucheriana</i> (Pop 7)	W-Azerbaijan-Silvana	44° 51' 02"E 37° 23' 57"N	< 1800	1720	19.49	982	IAUH000015363	OL663850
<i>S. aucheriana</i> (Pop 8)	Firozkooh-Gaduk pass	52° 55' 05"E 35° 49' 29"N	> 2800	2840	8.5	530	IAUH000015364	OL681878
<i>S. aucheriana</i> (Pop 9)	Rudbar-Klishom village	49° 25' 27"E 36° 46' 32"N	< 1800	1780	12.9	907	IAUH000015365	OL663851
<i>S. aucheriana</i> (Pop 10)	Larijan-Rineh	52° 10' 07"E 35° 52' 52"N	> 2800	2889	11.82	1044	IAUH000015366	OL664062
<i>S. aucheriana</i> (Pop 11)	Chalou road-Dona village	51° 22' 09"E 36° 11' 46"N	< 1800	1790	16.73	1440	IAUH000015367	OL664563
<i>S. aucheriana</i> (Pop 12)	Tehran-Tochal	51° 24' 20"E 35° 49' 12"N	> 2800	3010	9.38	429	IAUH000015368	OL664578
<i>S. aucheriana</i> (Pop 13)	Zanjan-Tatrom	48° 49' 18"E 36° 39' 29"N	< 1800	1010	15.45	386	IAUH000015369	OL665118
<i>S. aucheriana</i> (Pop 14)	Tehran-Dizin	51° 25' 02"E 36° 02' 57"N	> 2800	2823	7.33	591	IAUH000015370	OL665145
<i>S. aucheriana</i> (Pop 15)	Khorasan Razavi-HezarMasjed mountain	60° 38' 21"E 36° 04' 23"N	> 2800	2950	10.3	370	IAUH000015371	MK587512

<i>S. albescens</i> Boiss	Fares, Nurabad to kurkan	1800	TARI 45940	MK587506
<i>S. anaratica</i> Schischk.	W. Azarbajjan, Razi village	2500	TARI 68855	MK583448
<i>S. bornmuelleri</i> Freyn.	E. Azarbajjan, Arasbaran	1104	TARI 125	MK883601
<i>S. breviauriculata</i> Ghazn.	N. Khorasan, Jajarm	1010	IAUH 14608	MK583510
<i>S. brabuica</i> Boiss.	Zahedan, Nikshahr,	552	TARI 43057	MK580974
<i>S. cephalantha</i> Boiss.	E. Azarbajjan , Sabalan	3160	TARI 397	MK580973
<i>S. commelinifolia</i> Boiss.	Tehran, Tochal	1914	IAUH 0051	MK583467
<i>S. crispans</i> Litw.	Gorgan, Maravehetapeh	980	TARI 55549	MK583446
<i>S. dschuparensis</i> Bornm.	Kerman, Rayen	3200	IAUH 12456	MK601678
<i>S. daenensis</i> Melzh.	Kerman, Rayen, Bab Zangi	4400	IAUH 595	MK580980
<i>S. eriocalycina</i> Boiss.	Lorestan, Nozhian waterfall	1397	IAUH 0053	MK587509
<i>S. erysimifolia</i> Stapf.	Arak, 20km saveh	1750	TARI 8227	MK583471
<i>S. eymatica</i> Bornm.	Semnan, Hirkuh	2700	TARI 40671	MK583462
<i>S. gertraudiae</i> Melzh.	R. Khorasan Torbat sefid	1700	TARI 23528	MK580978
<i>S. gonioacuta</i> Boiss.	Hamedan, Alvand mount	3300	IAUH 0052	MK601677
<i>S. gynodioica</i> Ghazanfar.	Zanjan, Ghyedar road	2203	IAUH 0054	MK583514
<i>S. hirticalyx</i> Boiss & Hausskn.	Kermanshah, Pavch	1000	TARI 86101	MK587507
<i>S. indoprensa</i> Schischk.	N. Khorasan, Shah Jahan	1400	TARI 48622	MK580641
<i>S. mishudaghensis</i> . Gho & Parsa.	E. Azarbajjan, Mishudaghi	1900	TARI 73034	MK605250
<i>S. micropbylla</i> Boiss.	Yazd, Shirkooh	1978	IAUH 03494	MK581021
<i>S. montbretiana</i> Boiss.	Lorestan, Noorabad	1900	TARI 64398	MK601680
<i>S. muradica</i> Schischk.	W. Azerbajjan, Razi village	2020	TARI 9920	MK883602
<i>S. meyeri</i> Fenzl.	Gorgan, Tilabad	1000	TARI 49253	MK583466
<i>S. nurensis</i> Melzh.	Shahr-e Kord, Sabzkoh	2820	TARI 49254	MK587514
<i>S. oligophylla</i> Melzh.	W. Azerbajjan, Firouzabad	1180	TARI 34262	MK587516
<i>S. oxelmannii</i> Gholipour.	Lorestan, Drehtakht village	2500	IAUH 0054	MK583460
<i>S. palinotricha</i> Fenzl ex Boiss.	Semnan, Shapesand	1950	TARI 29671	MK583512
<i>S. pravitziana</i> Rech.	Isfahan, 30 km from Ardestan	850	TARI 11531	MK587513
<i>S. persepolitana</i> Melzh.	Faris, Firuzabad	2000	TARI 41296	MK580637
<i>S. persica</i> Boiss.	Shahrekd, Mount kalar	3400	TARI 57395	MK587510
<i>S. prilipkoana</i> Schischk.	W. Azarbajjan, Maku	1380	TARI 7515	MK587511
<i>S. pseudonurensis</i> Melzh.	Tehran, Garmdarreh	1700	TARI 33352	MK587515
<i>S. spergulfolia</i> Willd.	W Azarbajjan, Silvana, Khalilkooh	2300	IAUH 0056	MK580621
<i>S. sisanica</i> Boiss.	W. Azarbajjan, Khoi	2150	TARI 30255	MK583449
<i>S. sojakeri</i> Melzh.	Semnan, Tilabad	1000	TARI 29581	MK580620
<i>S. tragacantha</i> Fenzl ex Boiss.	Isfahan, Samerom	3000	TARI 31697	MK587508
<i>S. virgata</i> Stapf.	Sanandaj, Bavaneh village	1720	TARI 78812	MK583468

Table 2: The morphological characters used for morphometric analyses among populations of *S. aucheriana* (description of each character is given separately in the Results).

Tabela 2: Morfološki znaki, uporabljeni za morfometrične analize med populacijami vrste *S. aucheriana* (opis vsakega od znakov je podan posebej v Rezultatih).

No	Quantitative morphological characters	No	Quantitative morphological characters
1	Plant height (PLH; cm)	10	Petal limb length (PLL; mm)
2	Basal leaf length (BLL; mm)	11	Petal limb division length (PLDL; mm)
3	Basal leaf width (BLW; mm)	12	Epipetalous stamens to alternate (ESA; mm)
4	Stem leaf length (SLL; mm)	13	Epipetalous filaments length to claw (EFLC; mm)
5	Stem leaf width (SLW; mm)	14	Capsule length (CAPL; mm)
6	Alar pedicel length (APL; mm)	15	Anthophore length (AnL; mm)
7	Calyx length (CL; mm)	16	Seed length (SEL; mm)
8	Calyx tooth length (CTL; mm)	17	Seed width (SEW; mm)
9	Petal claw length (PCL; mm)		

Table 3: The Duncan’s means of the traits, assessed from fifteen populations of *S. aucheriana*. Note: The data are reported as Mean ± SD (Standard deviation). Mean values with the same letters are not significantly different at ($p < 0.05$) according to Duncan’s test followed by Bonferroni’s correction ($\alpha' = \alpha/k$, $\alpha = 0.05$). The abbreviation of traits is presented in Table 2. AvgT: average temperature; RF: rainfall; Sig: significance level. Highlighted cells show significant differences.

Tabela 3: Duncanove povprečne vrednosti znakov, ocenjenih iz petnajstih populacij vrste *S. aucheriana*. Opomba: podatki so navedeni kot povprečje ± SD (standardni odklon). Srednje vrednosti z istimi črkami se statistično značilno ne razlikujejo ($p < 0,05$) glede na Duncanov test, ki mu sledi Bonferronijeva korekcija ($\alpha' = \alpha/k$, $\alpha = 0,05$). Okrajšave znakov so predstavljene v tabeli 2. AvgT: povprečna temperatura; RF: padavine; Sig: stopnja statistične značilnosti. Osenčene celice kažejo statistično značilne razlike.

Pop	SLL	Sig.	SLW	Sig.	APL	Sig.	CL	Sig.	CTL	Sig.	PCL	Sig.
1	22.8±8.90 ^{d,fg}	0.57	0.725±0.43 ^{g,f}	0.34	9.97±6.11 ^{b,c,d}	0.81	10.55±1.75 ^g	0.37	0.42±0.29 ^d	0.35	6±2.00 ^f	0.34
2	14.2±7.70 ^{e,fg}	0.44	1.37±0.89 ^{d,e,f}	1	7.25±5.53 ^{c,d,e,f}	0.86	12.75±2.33 ^{d,e,f}	0.66	1.05±0.58 ^{b,c}	0.80	6.17±1.11 ^{e,f}	0.37
3	18.45±5.83 ^{e,f}	0.62	2.13±1.03 ^d	0.54	5.96±5.36 ^d	0.74	9.8±3.03 ^g	0.27	1.11±0.76 ^{b,c}	0.87	6.42±1.94 ^{e,f}	0.47
4	15.2±7.50 ^{e,fg}	0.47	2.96±1.31 ^c	0.45	7.27±5.30 ^{c,d,e}	0.93	15.12±0.47 ^{a,b,c}	0.59	0.85±2.31 ^{b,c}	0.93	8±2.31 ^{b,c,e}	0.77
5	36.35±1.65 ^a	0.25	3.95±1.01 ^a	0.32	17.35±1.59 ^{a,c,e,f}	0.45	11.6±3.45 ^{c,e,fg}	0.61	1.1±0.57 ^{b,c}	0.87	8.57±2.93 ^{a,b,c}	0.72
6	32.4±1.37 ^{a,b}	0.83	3.37±1.27 ^{a,b,c}	0.34	13.05±6.41 ^{a,b}	0.55	16.55±3.10 ^a	0.28	0.89±0.64 ^{b,c}	0.93	9.92±1.64 ^a	0.42
7	34.8±8.10 ^a	0.37	3.45±1.07 ^{a,b,c}	0.33	10.65±5.41 ^{a,c,d,e,f}	0.78	11.65±2.65 ^{e,fg}	0.21	1±0.75 ^{b,c}	0.87	6.92±1.11 ^{d,e,f}	0.54
8	10.95±4.46 ^{f,fg}	0.77	1.15±0.92 ^{e,g,f}	1.04	5.87±3.35 ^{c,d,e}	0.73	13.5±2.62 ^{c,d,e}	0.66	1.72±0.82 ^a	0.12	9.35±1.51 ^{a,b}	0.55
9	27.6±1.23 ^{b,c}	0.53	3.8±2.39 ^{a,b}	1	11.97±8.43 ^{b,c}	1.35	15.9±0.52 ^{a,b}	0.43	0.89±0.52 ^{b,c}	0.93	9.47±2.45 ^a	0.54
10	26.2±1.09 ^{b,c,d,e}	0.52	1.79±0.82 ^{d,e}	0.45	4.6±4.64 ^c	0.65	16.55±3.03 ^a	0.28	1.17±0.65 ^{b,c}	0.88	7.97±1.55 ^{b,c,d}	0.77
11	19.42±1.08 ^{d,e}	0.65	1.75±1.02 ^{d,e}	0.45	7.15±4.42 ^{c,d,e}	0.12	14.55±2.53 ^{b,c,d}	0.61	1.2±0.50 ^b	0.90	8.7±2.27 ^{a,b,c}	0.69
12	26.5±9.80 ^{a,b,c}	0.53	1.95±1.11 ^{d,e}	0.48	12.65±1.16 ^{b,d,c}	0.59	13.77±2.63 ^d	0.34	1.18±0.55 ^{b,c}	0.89	8.97±1.80 ^{a,b}	0.66
13	30±1.14 ^{a,b,c}	0.47	3.05±1.30 ^{b,c}	0.43	6±4.82 ^{d,e,c,f}	0.75	11.4±2.98 ^{f,fg}	0.59	0.82±0.42 ^{b,c}	0.93	9.35±1.70 ^{a,b}	0.55
14	14.15±8.27 ^{e,fg,c}	0.44	1.57±1.23 ^{d,e}	0.49	4.2±4.49 ^{d,e,c,f}	0.63	13.37±3.25 ^{c,d,e,f}	0.65	1.12±0.49 ^{b,c}	0.87	7.42±1.70 ^{c,d,e}	0.69
15	12±3.20 ^{f,g}	0.45	0.48±0.28 ^g	0.23	2.63±2.16 ^{d,c,e,f}	0.54	13.15±1.92 ^{c,d,e,f}	0.64	0.76±0.41 ^d	0.93	7.36±1.87 ^{c,d,e}	0.67

Pop	Elevation	Sig.	AveT	Sig.	RF	Sig.	PLH	Sig.	BLL	Sig.	BLW	Sig.
1	2.715±00	.000	11.69±00 ^j	.000	12.91±00 ^m	.000	180.5±40 ^{d,e,f}	0.75	22.45±7.86 ^{c,d,e,f}	0.78	0.56±0.33 ^d	0.85
2	1.31±00	.000	17.4±00 ^b	.000	10.75±00 ^o	.000	226±40.70 ^{c,d,e}	0.86	15.05±5.18 ^{g,h}	0.52	0.75±0.46 ^{c,d}	0.93
3	3.3±00	.000	11.32±00 ^j	.000	32±00 ^c	.000	283±0.58 ^{a,b,c}	0.66	24.75±8.15 ^{b,d,e}	0.63	1.96±1.37 ^{b,c,d}	0.93
4	2.46±00	.000	10.52±00 ^k	.000	16.66±00 ^j	.000	247.5±52.8 ^{a,b,c,d}	0.85	19.1±7.83 ^{e,f,g}	0.67	2.47±1.33 ^{a,b,c,d}	0.93
5	2.1±00 ^g	.000	51.8±00 ^a	.000	31±00 ^f	.000	268.5±53.24 ^{a,b,c}	0.75	37.25±8.96 ^a	0.03	3.47±1.16 ^b	1
6	2.32±00	.000	15.2±00 ^f	.000	90.41±00 ^a	.000	191±40.89 ^{d,e,c}	1.64	26.5±1.22 ^{b,d}	0.52	2.04±1.39 ^{b,c,d}	0.93
7	2.3±00 ^k	.000	9.49±00 ^m	.000	39.83±00 ^d	.000	283.5±62.68 ^{a,b}	0.65	28.3±6.38 ^{b,c}	0.42	7.85±1.25 ^a	0.00
8	2.22±00	.000	12.81±00 ^g	.000	11.31±00 ⁿ	.000	199±55.71 ^{c,d,e}	0.86	11.55±5.79 ^h	0.38	0.72±0.45 ^{c,d}	0.93
9	1.94±00	.000	10.4±00 ^l	.000	25±00 ^g	.000	158.5±37.17 ^{e,f}	1.33	29.1±1.28 ^b	0.42	3.25±2.08 ^{b,c}	0.93
10	2.01±00	.000	11.82±00 ^h	.000	13.66±00 ^l	.000	195±53.90 ^{c,d,e}	0.85	18.9±7.69 ^{e,f,g}	1.32	1.32±1.05 ^{b,c,d}	0.93
11	2.51±00	.000	15.73±00 ^d	.000	90.08±00 ^b	.000	313.75±6.51 ^a	0.47	16.62±0.36 ^{f,g,h}	0.56	1.36±0.77 ^{b,c,d}	0.93
12	1.91±00	.000	16.38±00 ^c	.000	18.33±00 ⁱ	.000	231±57.93 ^{d,e,c}	0.93	27.15±9.72 ^{b,d}	0.46	1.36±1.19 ^{b,d}	0.93
13	2.1±00	.000	15.45±00 ^c	.000	56.41±00 ^c	.000	227.5±51.79 ^{d,e}	0.93	22±8.98 ^{d,e,f}	0.85	1.7±0.86 ^{b,c,d}	0.93
14	2.72±00	.000	7.33±00 ⁿ	.000	16.25±00 ^k	.000	168±44.79 ^{e,f}	0.67	15.2±1.12 ^{g,h}	0.53	1.79±1.48 ^{b,c,d}	0.93
15	2.91±00	.000	11.8±00 ⁱ	.000	21.25±00 ^h	.000	110.5±22.35 ^f	0.40	18.25±8.18 ^{f,g}	0.63	0.53±0.27 ^d	0.84

Pop	PLL	Sig.	PLDL	Sig.	ESA	Sig.	EFLC	Sig.
1	4.47±1.22 ^{e,f}	0.73	1.25±0.61 ^{f,g}	0.82	4.47±0.73 ^d	0.57	1.25±0.61 ^{b,c}	0.93
2	4.77±2.01 ^{d,e}	0.61	1.91±1.20 ^f	0.68	4.7±0.86 ^d	0.58	0.53±0.30 ^{b,c}	0.93
3	4.87±1.38 ^{d,e}	0.63	1.95±0.63 ^f	0.68	3.7±0.42 ^c	0.75	0.5±0.30 ^{b,c}	0.93
4	5.78±1.28 ^{b,c,d}	0.70	2.46±1.01 ^{d,e}	0.69	6.31±1.37 ^b	1.88	0.84±0.50 ^{b,c}	0.93
5	3.75±0.85 ^f	0.47	2.65±1.08 ^{c,d,e}	0.68	5±1.71 ^{c,d}	0.56	0.81±0.32 ^{b,c}	0.93
6	6.27±1.95 ^{b,c}	0.37	2.65±1.08 ^{c,d,e}	0.68	7.52±3.04 ^a	0.56	0.76±0.41 ^{b,c}	0.93
7	4.75±0.76 ^{d,e}	0.61	2.52±0.96 ^{d,e}	0.68	5.4±0.78 ^{b,c,d}	0.51	1.05±0.75 ^b	0.93
8	5.32±2.02 ^{d,e}	0.73	2.92±1.57 ^{c,d}	0.72	7.9±2.01 ^a	0.2	0.94±0.41 ^{b,c}	0.93
9	7.82±1.87 ^a	0.04	5.21±0.88 ^a	0.03	7.44±2.22 ^a	0.29	0.68±0.30 ^{b,c}	0.93
10	6.5±1.51 ^{b,c}	0.77	4.02±1.62 ^a	0.16	5.9±1.35 ^{b,c}	0.48	0.32±0.18 ^c	0.93
11	4.92±1.26 ^{d,e}	0.65	2.46±0.69 ^{d,e}	0.69	5.05±0.87 ^{c,d}	0.54	0.55±0.27 ^{b,c}	0.93
12	6.29±1.26 ^{b,c}	0.06	3.5±1.04 ^{a,c}	0.49	7.87±1.57 ^a	0.21	2.33±3.26 ^a	0.002
13	4.59±1.14 ^{e,f}	0.6	2.26±0.81 ^{d,e}	0.68	3.34±1.33 ^c	0.15	0.52±0.45 ^{b,c}	0.93
14	4.97±1.37 ^{d,e}	0.66	2.08±0.81 ^{d,e,f}	0.67	5.9±1.72 ^{b,c}	0.48	1.07±0.76 ^b	0.93
15	2.24±0.86 ^g	0.008	0.65±0.28 ^g	0.10	3.6±1.16 ^c	0.32	1.02±0.64 ^{b,c}	0.93