

Evaluation of quantitative morphological characteristics across various populations of Iranian *Silene peduncularis* (Caryophyllaceae)

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Abstract

EVALUATION OF QUANTITATIVE MORPHOLOGICAL CHARACTERISTICS ACROSS VARIOUS POPULATIONS OF IRANIAN *SILENE PEDUNCULARIS* (CARYOPHYLLACEAE).— This study reviews the morphological assessment performed on eight populations of Iranian *Silene peduncularis* section *Sclerocalycinae*. Seventeen distinctive quantitative morphological traits were examined at the population level. The characters exhibiting the highest variation were identified through factor analysis based on Principal Component Analysis (PCA). Three quantitative traits: basal leaf length (BLL), stem leaf length (SLL), and plant height (PLH) were identified as the most effective traits in differentiating among the studied populations. The presented morphometric evaluations revealed that alar pedicel length (APL) is the most variable morphological trait, whereas bracteae length (BRAC) was the least variable. Positive and negative correlations between quantitative parameters and climate variables were revealed. The analysis suggests that elevation significantly influenced the majority of traits.

Keywords: environmental variables; morphology; populations; quantitative traits; *Silene peduncularis*.

Resumen

EVALUACIÓN DE LA VARIACIÓN DE CARACTERÍSTICAS MORFOLÓGICAS CUANTITATIVAS EN VARIAS POBLACIONES IRANÍES DE *SILENE PEDUNCULARIS* (CARYOPHYLLACEAE).— Se presenta la evaluación morfológica realizada en ocho poblaciones iraníes de *Silene peduncularis* sección *Sclerocalycinae*, basada en diecisiete rasgos morfológicos cuantitativos a nivel de población. Los caracteres que exhibieron una mayor variabilidad fueron identificados mediante un análisis factorial basado en el Análisis de Componentes Principales (PCA). Tres rasgos cuantitativos, longitud de la hoja basal (BLL), longitud de la hoja del tallo (SLL) y altura de la planta (PLH) se identificaron como los más efectivos en la diferenciación y caracterización de las poblaciones estudiadas. Las evaluaciones morfométricas presentadas revelaron que la longitud del pedicelo alar (APL) es el rasgo morfológico más variable, mientras que la longitud de las brácteas (BRAC) fue el menos variable. Se observaron correlaciones positivas y negativas entre los parámetros cuantitativos y las variables climáticas. El análisis sugiere que la elevación influyó significativamente en la mayoría de los rasgos.

Palabras clave: características cuantitativas; morfología; parámetros ambientales; poblaciones; *Silene peduncularis*.

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INTRODUCTION

Silene L. (Caryophyllaceae Juss.) is a large and widely distributed genus that includes more than 750 annual or perennial species (Rohrbach, 1868; Rechinger, 1951; Bittrich, 1993; Greuter, 1995) arranged in three subgenera: *Lychnis* L., *Behenantha* (Oth) Torr. & A. Gray, and *Silene* Rohrbach (Oxelman *et al.*, 2000). Subgenus *Lychnis*, which has only weak support as a sister group to the other subgenera, diverges into three main clades and comprises four sections (Jafari *et al.*, 2020). Subgenus *Behenantha* forms a sister group to subgenus *Silene*, exhibits limited resolution at the base and encompasses numerous predominantly small clades, identified as 18 sections. Finally, 11 sections are recognized in subgenus *Silene*, four of which, *Auriculatae*-Boiss., *Sclerocalycinae* (Boiss.) Schischk., *Silene*, and *Siphonomorpha* Oth are broadly defined and subject to considerable debate. *Silene peduncularis* Boiss. belongs to subgenus *Silene* and section *Sclerocalycinae* (Melzheimer, 1988).

Section *Sclerocalycinae* has recently been described in *Flora of Iran* (Assadi *et al.*, 2023) as including perennial plants with a woody stock and an inflorescence made up of a compound or, more rarely, a simple cluster. Flowers in section *Sclerocalycinae* are bisexual; with calyx 15 to 42 mm long, glabrous, cylindrical to club-shaped, 10-veined, at the base of the umbilicus. The base of the flower is hairless or hairy at the bottom and petals are bilobed. *Silene peduncularis* presents a 13 to 18 mm long calyx, with teeth measuring 1.5 to 2.5 mm. Petal lamina ranges from 5 to 8 mm and claw between 7 and 10 mm. Flower base length ranges between 4.5 and 8.5 mm. *Silene peduncularis* blooms from July to August and bears fruit from August to October, with populations growing on rocky mountain slopes at altitudes of 1520–3055 m in the Irano-Turanian mountain element (Melzheimer, 1988). The distribution of *S. peduncularis* is limited to the center, west, northwest of Iran, and Transcaucasia (Boissier, 1867; Melzheimer, 1988; Assadi *et al.*, 2023). This species was also included in *Flora of Turkey* (Coode & Cullen, 1967) without indicating any locality, based on Chowdhuri (1957), and its presence was confirmed by Yildiz & Çirpici (2013). *Silene peduncularis* is not mentioned in *Flora of the U.S.S.R.* (Shishkin, 1936), *Flora Europaea* (Chater & Walters, 1964), or *Flora of Iraq* (Townsend *et al.*, 2016).

The chromosome number of *S. peduncularis* has been reported as $2n = 24$ ($\times = 12$) (Yildiz *et al.*, 2009). A recent molecular study by Bahmani *et al.* (2020) based on nuclear ITS and chloroplast *rps16* markers, found no evidence to support the existence of subspecies in *S. peduncularis*. This study also revealed that *S. peduncularis* and *S. bupleuroides* L. are placed in the same clade in the phylogeny of the section.

An investigation into the morphological and genetic diversity of 15 populations of *S. aucheriana* Boiss. using the ITS molecular marker showed that various regions of Iran, characterized by distinct climatic conditions, exhibit notable morphological diversity within this species (Safaeishakib *et al.*, 2024b). Furthermore, morphological studies on *S. latifolia* Poiret also showed population divergence, suggesting the impact of two distinct climate zones on the variation of morphological traits (Brothers *et al.*, 2016). This study seeks to identify the most variable quantitative traits and distinctive morphological characteristics among populations of *S. peduncularis*, as well as to assess the relationship between the observed variation and some environmental parameters: average temperature and rainfall and elevation. To achieve these aims, several statistical multivariate methods have been employed to assess morphological variation in 17 morphological characters from eight populations of *S. peduncularis* and their correlation to environmental variables.

MATERIALS AND METHODS

Site selection and plant materials

In June to July 2017, specimens from eight populations of *S. peduncularis* were collected in different regions of Iran: West, Center, and Northwest, which is the center of distribution for this species (see Figs. 1 and 2).

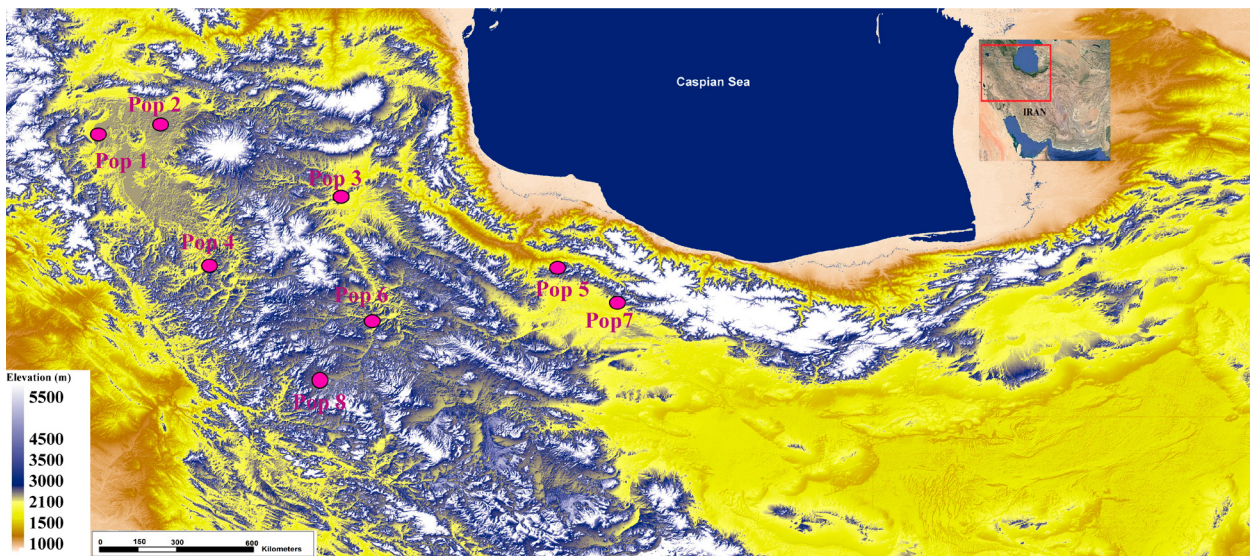


Figure 1. Topographic map showing the location of *Silene peduncularis* populations collected for this study in Iran. (map designed using ArcGIS v10.2).



Figure 2. *Silene peduncularis* and its habitat: (A), in E-Azerbaijan Yam mountain; (B), plant natural habitat (W-Azerbaijan, Qoshchi pass). Photos by M. Safaeishakib.

In sampling at each location, a 100 m distance between individuals was considered and five individuals were taken per population. Populations were selected based on *Flora Iranica* (Melzheimer, 1988), as well as on their variation in the chosen environmental characteristics. Voucher specimens were deposited in the Islamic Azad University Herbarium (IAUH) (Table 1).

Table 1. *Silene peduncularis* populations and their localities. Note: Elev.: Elevation (m); Ave. T: Average temperature (°C); Ave. R: Average rainfall (mm).

Pop.	Locality	Elev.	Longitude	Latitude	Ave. T	Ave. R	Voucher No.
Pop. 1	W-Azerbaijan, Qosh-chi pass	2100	45° 81' 75" E	37° 36' 58" N	15.80	321	IAUH000015372
Pop. 2	E- Azerbaijan Yam mountain	2450	46° 45' 65" E	37° 64' 52" N	10.7	467	IAUH000015055
Pop. 3	Zanjan, Tarom	1640	48° 56' 89" E	36° 67' 16" N	15.45	386	IAUH000015056
Pop. 4	Kurdistan, Zarrineh	1700	46° 64' 39" E	35° 47' 72" N	13.2	49	IAUH000015057
Pop. 5	Alborz, Dizin	2870	51° 39' 30" E	35° 64' 80" N	7.33	591	IAUH000015058
Pop. 6	Hamedan, Barfiin, Heydareh	1520	48° 89' 70" E	34° 78' 23" N	13.32	443	IAUH000015059
Pop. 7	Larijan-Rineh	2850	52° 73' 33" E	36° 43' 33" N	11.82	1044	IAUH000015060
Pop. 8	Lorestan, Oshto-rankoh	3055	48° 12' 00" E	33° 46' 67" N	12.4	482	IAUH000015061

Selection of morphological characteristics

The morphological characteristics used in the study were chosen following *Flora Iranica* (Melzheimer, 1988) and *Flora of Turkey* (Coode & Cullen, 1967). Seventeen quantitative morphological characters were chosen and assessed across the collected specimens (Table 2). Character selection was based on their proven usefulness for the identification and characterization of *Silene* species (Melzheimer, 1988; Yildiz, 2006; Firat & Yildiz, 2016; Gholipour, 2018; Bahmani *et al.*, 2020; Safaeishakib *et al.*, 2020, 2024b; Assadi *et al.*, 2023).

Table 2. Quantitative variables applied in morphometric analyses.

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

Evaluating local climate patterns

Environmental parameters (elevation, average temperature and rainfall) were chosen for this study on *S. peduncularis* because they often significantly influence morphology, growth, development, and adaptation in *Silene* and other taxa (Prentice, 1986; Jolivet & Bernasconi, 2007; Brothers *et al.*, 2016; Safaeishakib *et al.*, 2024a, b). Each population was positioned by a GPS (including its altitude), and average temperature and rainfall were obtained from www.en.climate-data.org, which provides regional climate averages rather than data for specific points (Table 1).

Statistical analysis

In this study, the following software was employed: IBM SPSS statistics v18 (Green & Salkind, 2016), PAST software v4.06b (<https://past.en.lo4d.com>), GraphPad Prism v6.04 (www.graphpad.com), and Minitab v14 (www.minitab.com).

Duncan's test was utilized to compare the means and analyze the patterns of change in the quantitative traits across the studied populations. This was conducted alongside a Bonferroni correction ($\alpha' = \alpha/k$, where $\alpha = 0.05$), as described by Rice (1989). Principal Components Analysis (PCA) was performed to see the distribution pattern of populations based on average values of morphological traits among the studied populations (Podani, 2000; Safaei *et al.*, 2016). Relationship between collected populations and environmental variation was evaluated using Canonical Correspondence Analysis (CCA) (Ter Braak, 1986). A cluster analysis of morphological data was conducted with Ward's method, with 1000 bootstrap replicates using squared Euclidean distance after standardization of measurements to 0–1 range (Hammer *et al.*, 2001). Pearson's correlation coefficient was also tested. Quantitative variables were assessed for normality at the 95% confidence level using the Kolmogorov-Smirnov test and the dataset was transformed using Box-Cox transformations, employing a Lambda value of -0.05). The morphological results are presented as mean $\pm$ standard error observations (mean $\pm$ SE). To identify potential differences in the studied populations, significant differences among the mean values were determined at a significance level of $p < 0.05$. Furthermore, standard deviation (SD), and coefficient of variation (CV) for populations are provided. Factor analysis was conducted to determine what has the greatest impact or influence on the overall morphological characters among the studied populations.

RESULTS

Morphological description of *S. peduncularis*

Based on the data obtained from the eight populations (Fig. 1), *S. peduncularis* is a perennial herb with a woody base that presents glabrous branches, rarely viscid above (Fig. 2). Stems are erect, 25–80 cm long, pale green; basal leaves 15–55 $\times$ 4–7 mm, stem leaves 25–70 $\times$ 3–8 mm, linear-lanceolate, lanceolate or narrowly ovate, acute. Stems and leaves glabrous (except for the leaf margins near the base). The inflorescence is racemose or dichasial, pauciflora, with very long pedicels ranging 10–50 mm; axillary flowers, anthophore 5–7 mm long, glabrous; claws 11–12 mm long, petals pink, paracorolla lobes 4–5 mm, lamina 7–9 mm, bracts small, 2–5.5 mm. Calyx 12–17 $\times$ 3–5 mm, obconic-cylindrical, leathery, base ringed, glabrous; calyx's teeth are triangular, alternately sharp. Capsule 8–13 mm, oblong, seeds reniform.

Morphometrical analyses

All quantitative characters exhibited wide-ranging values of coefficient of variation (CV), from 7.04% (bracteae length, BRAC) to 43.96% (alar pedicel length, APL). Duncan's test revealed no significant differences in any single trait across all populations (Table 3).

Table 3. Duncan's means of the traits, assessed from eight populations of *Silene peduncularis*. The data are analysed as Mean $\pm$ SD (Standard deviation). Mean values with the same letters are not significantly different at ($P < 0.05$). Note: SE: standard error; CV: coefficient of variation; Sig.: significance level. Details of each acronym/trait are provided in Table 2. Value in bold indicate the most variable trait.

POP.	PLH	BLL	BLW	SLL	SLW	APL	CL	CTL
1	600 $\pm$ 5.00 ^b	45 $\pm$ 1 ^{b,c}	5.6 $\pm$ 1.52 ^a	50 $\pm$ 1 ^b	6.00 $\pm$ 2 ^d	35 $\pm$ 1 ^b	14.6 $\pm$ 2.5 ^b	2.25 $\pm$ 0.2 ^{a,b}
2	400 $\pm$ 1 ^a	26.66 $\pm$ 1.04 ^a	4.5 $\pm$ 0.5 ^a	33.3 $\pm$ 7.6 ^a	3.5 $\pm$ 0.5 ^{a,b}	36.6 $\pm$ 1.5 ^b	14.3 $\pm$ 2.08 ^b	2.25 $\pm$ 0.2 ^{a,b}
3	450 $\pm$ 1 ^a	28.6 $\pm$ 9.2 ^a	4 $\pm$ 1 ^a	33.5 $\pm$ 5.6 ^a	4 $\pm$ 1 ^{a,b,c}	22.3 $\pm$ 2.5 ^{a,b}	13.6 $\pm$ 1.5 ^b	3 $\pm$ 0.5 ^{b,c}
4	426 $\pm$ 2.5 ^a	51.3 $\pm$ 3.2 ^c	5.5 $\pm$ 0.5 ^a	40 $\pm$ 5 ^{a,b}	5.5 $\pm$ 0.5 ^{c,d}	15 $\pm$ 5 ^a	15 $\pm$ 1 ^b	2.5 $\pm$ 0.5 ^{b,c}
5	376 $\pm$ 2.5 ^a	47.6 $\pm$ 2.5 ^c	5 $\pm$ 0.5 ^a	50 $\pm$ 1 ^b	5 $\pm$ 1 ^{b,c,d}	15 $\pm$ 5 ^a	13.6 $\pm$ 1.5 ^b	3.2 $\pm$ 0.2 ^c
6	386 $\pm$ 4.9 ^a	38.3 $\pm$ 3.2 ^a	5 $\pm$ 1 ^a	32.3 $\pm$ 2.5 ^a	3 $\pm$ 1 ^a	11.6 $\pm$ 3 ^a	12.3 $\pm$ 2.5 ^b	3.16 $\pm$ 0.7 ^{b,c}
7	410 $\pm$ 1 ^a	38 $\pm$ 1 ^a	6 $\pm$ 2.6 ^a	29 $\pm$ 1 ^a	3.5 $\pm$ 0.5 ^{a,b}	15 $\pm$ 5 ^a	4.8 $\pm$ 0.7 ^a	1.5 $\pm$ 0.5 ^a
8	430 $\pm$ 7.5 ^a	44.6 $\pm$ 5.5 ^c	5.3 $\pm$ 0.7 ^a	35.6 $\pm$ 5.5 ^a	5.3 $\pm$ 0.7 ^{b,c,d}	23.3 $\pm$ 7.6 ^{a,b}	16 $\pm$ 3 ^b	2.8 $\pm$ 0.7 ^{b,c}
Sig.	1	0.27	0.10	0.09	0.28	0.06	0.06	0.12
Min.	376	26	4	29	3	11	4.80	1.50
Max.	600	51.3	6	50	6	36.6	16	32
Mean	434.75	40	5.11	37.9	4.47	21.72	13.02	2.58
SE mean	25.10	3.12	0.22	2.84	0.39	3.37	1.23	0.2
CV (%)	16.33	22.08	12.47	21.20	24.81	43.96	26.86	22.36

Table 3. (Continued)

POP.	PCL	PLL	PLDL	ESA	EFLC	CAPL	AnL	BRAC	CW
1	11.5 $\pm$ 0.5 ^c	7.5 $\pm$ 0.5 ^b	3.5 $\pm$ 0.5 ^{c,d}	1.5 $\pm$ 0.5 ^b	0.9 $\pm$ 0.1 ^a	10.6 $\pm$ 2.08 ^b	6 $\pm$ 1 ^a	4 $\pm$ 1 ^a	4.5 $\pm$ 0.5 ^a
2	12 $\pm$ 1 ^{c,d}	7.6 $\pm$ 0.7 ^{b,c}	4 $\pm$ 0.5 ^d	1.05 $\pm$ 0.1 ^{a,b}	1.2 $\pm$ 0.2 ^a	10 $\pm$ 2 ^b	6 $\pm$ 1 ^a	4 $\pm$ 1 ^a	4.5 $\pm$ 0.5 ^a
3	12 $\pm$ 0.5 ^{c,d}	7.5 $\pm$ 0.5 ^b	4.2 $\pm$ 0.2 ^d	1.1 $\pm$ 0.3 ^{a,b}	1.2 $\pm$ 0.2 ^a	12 $\pm$ 1 ^b	5 $\pm$ 0.5 ^a	4 $\pm$ 1 ^a	3.5 $\pm$ 0.5 ^a
4	12 $\pm$ 0.5 ^{c,d}	8 $\pm$ 0.5 ^{b,c}	4 $\pm$ 0.5 ^d	1 $\pm$ 0 ^{a,b}	1.5 $\pm$ 0.5 ^a	10 $\pm$ 1 ^b	5.5 $\pm$ 0.5 ^a	4.5 $\pm$ 0.5 ^a	3.5 $\pm$ 0.5 ^a
5	11 $\pm$ 0.5 ^c	8.5 $\pm$ 0.5 ^{b,c}	4.4 $\pm$ 0.3 ^d	1 $\pm$ 0 ^{a,b}	1.5 $\pm$ 0.5 ^a	9.8 $\pm$ 0.2 ^b	5.5 $\pm$ 0.5 ^a	4.3 $\pm$ 1.5 ^a	3.5 $\pm$ 0.5 ^a
6	6 $\pm$ 1 ^a	4.8 $\pm$ 0.7 ^a	2 $\pm$ 0.5 ^{a,b}	1 $\pm$ 0.5 ^{a,b}	0.83 $\pm$ 0.2 ^a	6 $\pm$ 1 ^a	10 $\pm$ 1 ^c	3.66 $\pm$ 1.2 ^a	4.3 $\pm$ 0.7 ^a
7	7.5 $\pm$ 0.5 ^b	4 $\pm$ 1 ^a	1.5 $\pm$ 0.5 ^a	0.8 $\pm$ 0.2 ^a	1 $\pm$ 0.5 ^a	4 $\pm$ 1 ^a	5.5 $\pm$ 0.5 ^a	4 $\pm$ 0.5 ^a	4 $\pm$ 1 ^a
8	13 $\pm$ 0.5 ^d	8.8 $\pm$ 0.7 ^c	2.8 $\pm$ 1.2 ^{b,c}	1.16 $\pm$ 0.2 ^{a,b}	1 $\pm$ 0.5 ^a	12 $\pm$ 1 ^b	8 $\pm$ 1 ^b	4.5 $\pm$ 0.5 ^a	4.5 $\pm$ 0.5 ^a
Sig.	0.10	0.07	0.11	0.10	0.08	0.08	1	0.36	0.06
Min.	6.00	4.00	1.50	0.80	0.83	4	5	3.66	3.50
Max.	13	8.8	4.4	1.5	1.5	12	10	4.5	4.5
Mean	10.62	7.08	3.3	1.07	1.14	9.3	6.43	4.12	4.03
SE mean	0.88	0.61	0.38	0.07	0.09	1.004	0.6	0.10	0.16
CV (%)	23.42	24.51	32.83	18.63	22.44	30.54	26.40	7.04	11.76

The Principal Component Analysis (PCA) revealed that the initial two axes collectively accounted for 56.8% of the total variance (37.4% and 19.4% for the first and second components, respectively) (Fig. 3).

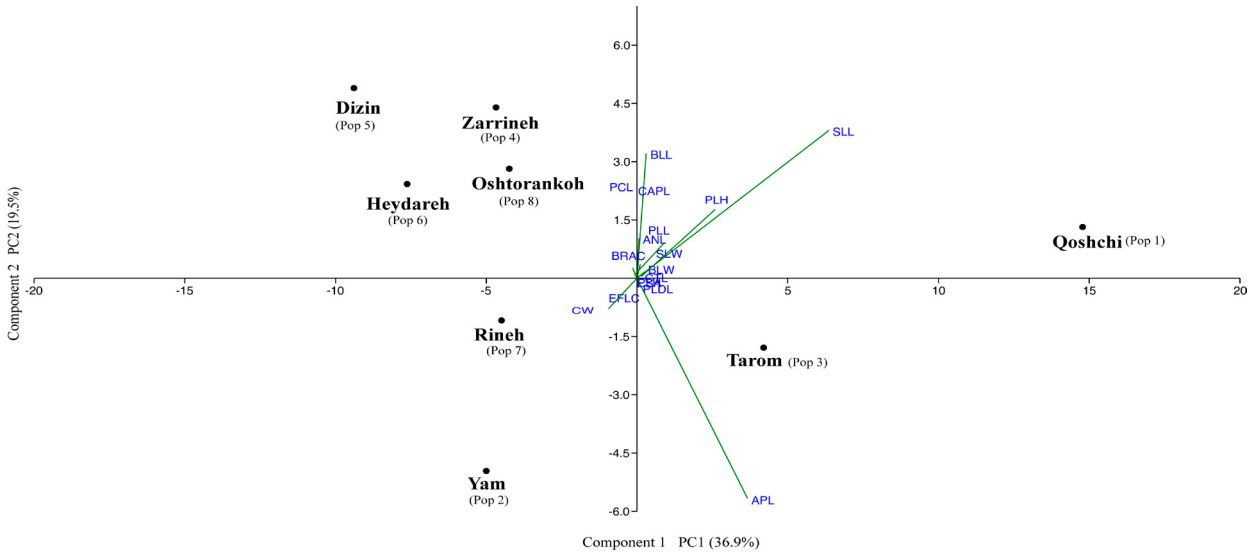


Figure 3. Principal Component Analysis (PCA) performed on 17 morphological traits recorded in the studied populations. Trait names as in Table 2.

This test also suggested that characters like basal leaf length (BLL), alar pedicel length (APL), calyx width (CW), stem leaf length (SLL), and plant height (PLH) could be used in population delimitation (Table 4).

Table 4. Factor analysis of the characteristics in *Silene peduncularis* populations. Details of each acronym/trait are provided in Table 2. Values in bold indicate the most influential traits.

Traits	Factor 1	Factor 2	Factor 3	Factor 4
PLH	2.3177	-2.8594	-1.8496	0.091385
BLL	3.318	-0.04827	1.1421	-1.4022
BLW	0.33714	-0.1118	0.66779	-0.30094
SLL	1.8052	-1.5357	-0.69479	2.9962
SLW	0.2128	-0.17953	-0.09053	-0.05185
APL	-1.0941	-3.2838	1.7885	-0.48143
CL	0.25173	-0.94245	-2.2728	-1.3102
CTL	0.13207	-0.099	-0.43959	-0.14965
PCL	0.12479	-0.8649	-0.78006	-0.74683
PLL	0.1472	-0.50098	-0.90184	-0.36468
PLDL	0.012825	-0.29564	-0.62269	0.20714
ESA	0.028911	-0.06964	-0.00892	-0.02777
EFLC	0.052582	-0.0496	-0.06289	0.003571
CAPL	0.022367	-0.85111	-1.7982	-0.69202
AnL	0.43745	-0.13236	0.12988	-1.2375
BRAC	0.1994	-0.1707	0.083463	-0.29659
CW	0.15098	-0.21911	0.3612	-0.40006

Therefore, alar pedicel length (APL) was identified as the most effective trait in differentiating among the populations, especially in the Tarom population (Pop. 3). The trait epipetalous filaments length to petal claw length (EFLC) and calyx width (CW) contributed to the distinctiveness of the Rineh and Yam populations (pops. 7 and 2, respectively). Basal leaf length (BLL), stem leaf length (SLL), and plant height (PLH), all vegetative traits, were especially relevant for the differentiation of the Qoshchi population (Pop. 1). Finally,

petal claw length (PCL) and bracteae length (BRAC) equally contributed to the differentiation of the four populations (Zarrineh, Dizin, Heydareh, Oshtorankoh) (i.e., pops. 4, 5, 6, and 8) (Fig. 3).

The multivariate CCA indicated that traits such as calyx tooth length (CTL) and plant height (PLH) are influenced by average air temperature, whereas anthophore length (AnL), calyx width (CW), and basal leaf width (BLW) are affected by rainfall. Conversely, other traits including stem leaf length (SLL), basal leaf length (BLL), bracteae length (BRAC), and epipetalous filaments length to petal claw length (EFLC) show associations with elevation (Fig. 4).

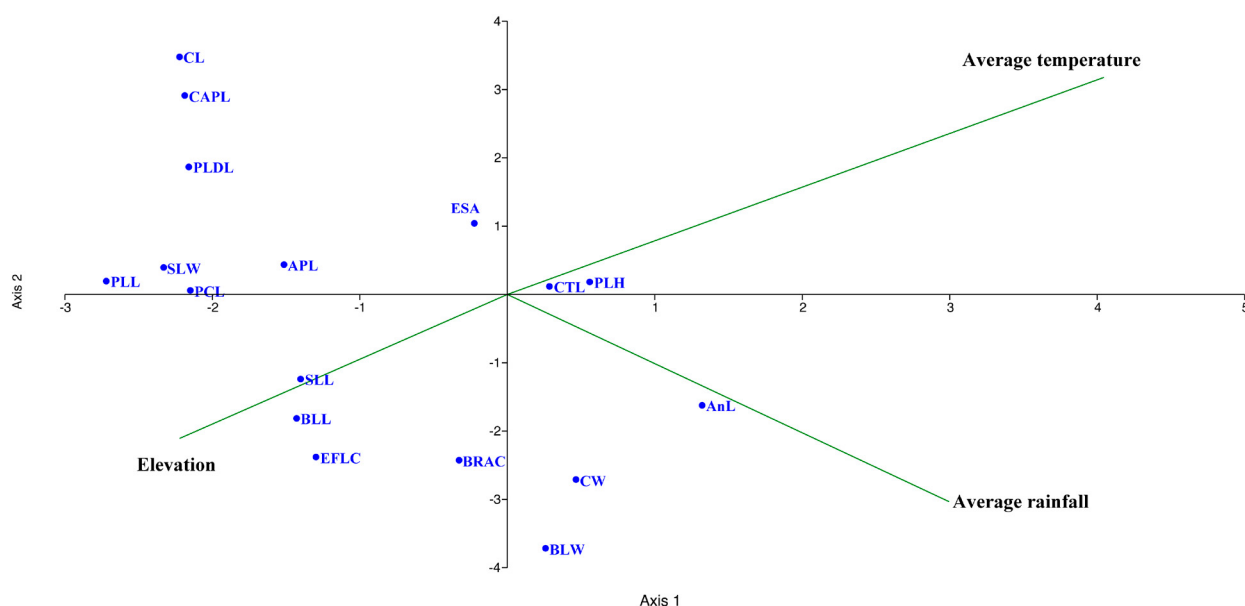


Figure 4. Canonical Correspondence Analysis (CCA) of *Silene peduncularis* based on 17 morphological characteristics and three environmental traits recorded in the studied populations. Trait names as in Table 2.

The clustering analysis using Ward Linkage (Fig. 5) largely confirmed the results of the PCA test. This analysis also highlights the clear differentiation of the Qoshchi population (Pop. 1) based on morphometric data.

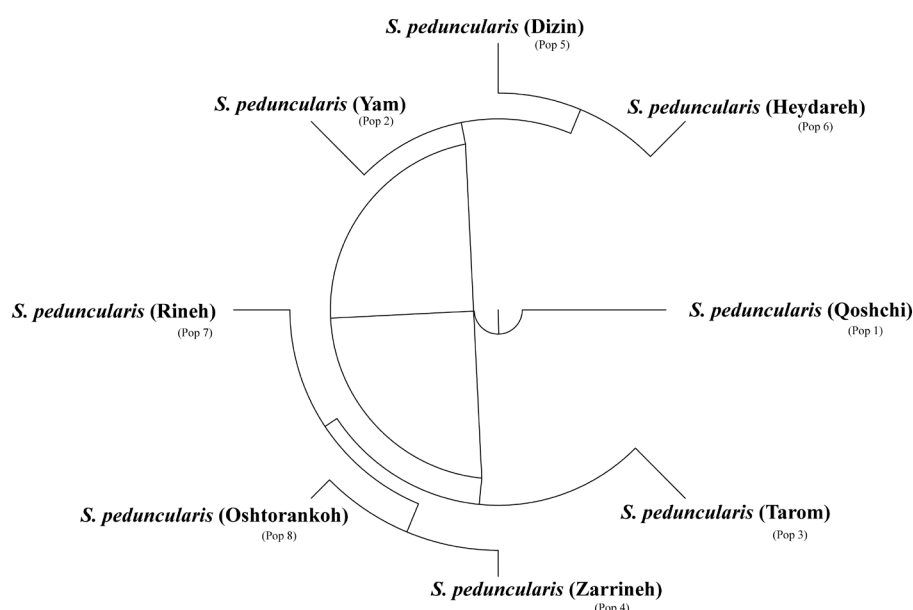


Figure 5. Dendrogram produced by the cluster analysis of *Silene peduncularis* populations based on morphological data and using Ward's method.

Significant correlations ($P < 0.05$) were observed between some of the chosen variables; for example, petal limb division length (PLDL) with petal claw length (PCL), or petal limb length (PLL) with calyx length (CL). Regarding the influence of meteorological variables on plant morphology, positive correlations were found between plant height (PLH), calyx tooth length (CTL), and epipetalous stamens to epipetalous alternate (ESA) with average temperature. Conversely, epipetalous filament length to petal claw length (EFLC) showed a negative correlation with average temperature, as did bract length (BRAC). Significant negative correlations were observed between calyx length (CL) and capsule length (CAPL) with average rainfall. In contrast, basal leaf width (BLW) exhibited a positive correlation with calyx width (CW) concerning average rainfall. Moreover, basal leaf width (BLW), basal leaf length (BLL), and bracteae length (BRAC) showed a strong positive correlation with elevation, whereas calyx tooth length (CTL) exhibited a strong negative correlation with elevation (Fig. 6). The Box-Cox plot provides a visual representation of the transformation parameters that optimize the normality of the data distribution (Fig. 7).

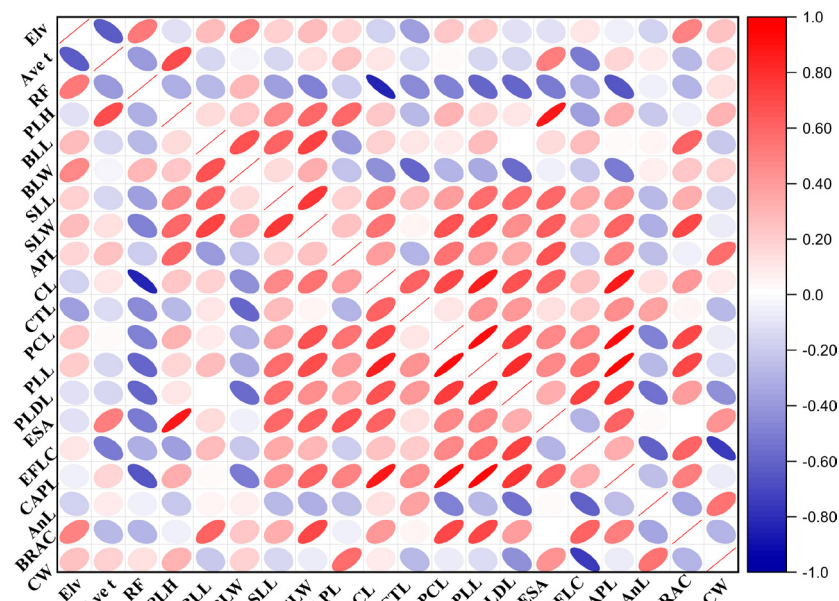


Figure 6. Correlation analysis of quantitative traits of *Silene peduncularis*. Different colors and shapes are used in accordance with the amount of correlation. Details of each acronym/trait are provided in Table 2.

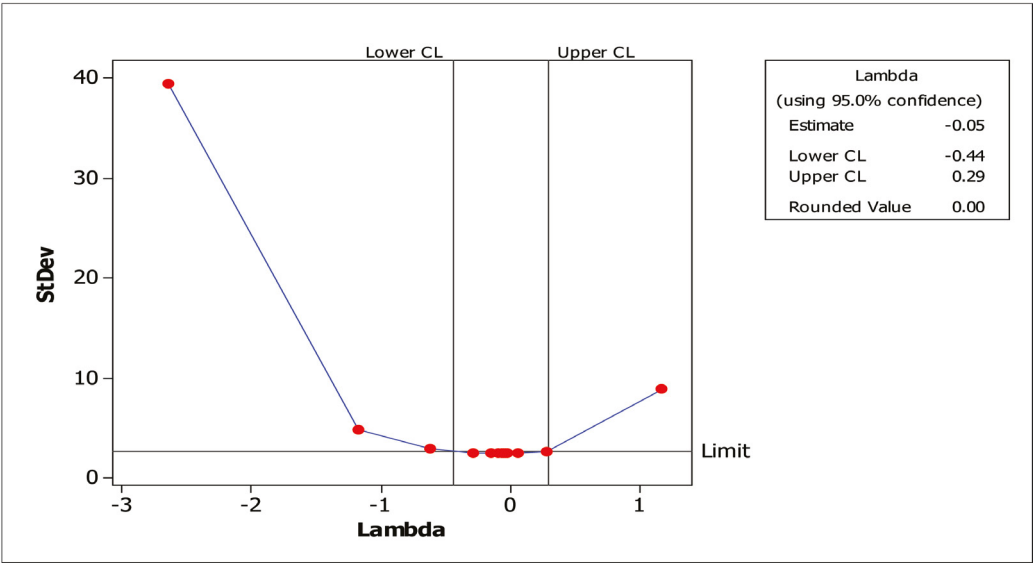


Figure 7. Box-Cox plot of estimated lambda value based on standard deviations at 95% confidence for 17 morphometric variables.

DISCUSSION

In a comparable paper conducted by Kephart *et al.* (1999), leaf width was identified as the most effective trait for distinguishing between montane and coastal *Silene douglasii* Hook. populations. In contrast, this study highlights three traits: basal leaf length (BLL), stem leaf length (SLL), and plant height (PLH), as the most effective for population differentiation.

Based on the findings of these two studies, it has been established that most vegetative characteristics of leaves are effective in differentiating between populations. However, Miladin *et al.* (2022) also highlighted that climate factors play a significant role in shaping both vegetative and floral traits in European and Mediterranean *Silene*. This suggests that while leaf traits are crucial for population differentiation, environmental conditions can further influence these traits, potentially affecting how populations adapt and evolve in response to their specific climates. Thus, understanding the interplay between vegetative characteristics and climate factors is essential for a comprehensive view of population differentiation in these species.

Based on the study of morphological variability of Iranian *S. aucheriana* (Safaeishakib *et al.*, 2024b) two quantitative traits, basal leaf width (BLW) and calyx length (CL) were introduced as the most variable traits at the population level. In the current study, alar pedicel length (APL) emerged as the most variable morphological trait. Both studies revealed a negative correlation between calyx tooth length (CTL) and elevation. Conversely, basal leaf width (BLW) and basal leaf length (BLL) exhibited a positive correlation with elevation in both species. Additionally, the research on *S. aucheriana* indicated that anthophore length (AnL) displayed a negative correlation with average air temperature and rainfall. For *S. peduncularis*, calyx length (CL) and the ratio of epipetalous filament length to petal claw length (EFLC) were found to have negative correlations with average rainfall and average air temperature, respectively. Furthermore, when comparing the positive correlations between the two species and environmental parameters, it was observed that alar pedicel length (APL) in *S. aucheriana* populations positively correlated with average air temperature, while in *S. peduncularis* both epipetalous stamens to epipetalous alternate (ESA) and plant height (PLH) exhibited positive correlations with this parameter. Regarding average rainfall, both species demonstrated a positive correlation with basal leaf width (BLW).

Canonical Correspondence Analysis (CCA) revealed a strong correlation between elevation and most morphological traits, indicating that elevation is a key factor influencing morphological variation. In contrast, average air temperature showed a weaker correlation with morphological traits. Additionally, Ward's cluster analysis and Principal Component Analysis (PCA) yielded similar results, supporting the findings of the CCA.

In this study, divergence could not be detected, and only the Qoshchi population (Pop. 1) is slightly distinct from other populations concerning morphological traits. Furthermore, this population distinguishes itself as the westernmost and nearest to the Caucasus region which is close to the border (see Figs. 1, 3, and 5). This distinct positioning is likely influenced by environmental factors affecting the morphology of the plants (Bonanomi *et al.*, 2016). However, quantitative morphology in this study could not effectively distinguish species or subspecies among the collected populations, as it requires further investigations. In general, differences in population could stem from a combination of ecological context, environmental variability, genetic diversity, phenotypic plasticity, sampling and methodological factors. Further research exploring these aspects could provide deeper insights into the morphological variability of these species (Sultan, 2003; Richards *et al.*, 2019). Future studies could benefit from exploring the genetic basis of the observed morphological differences and how these traits correspond to environmental gradients in a broader geographical context. Additionally, examining the potential effects of other environmental variables like soil type could provide a more comprehensive understanding of how *Silene* species adapt to its habitat. Thus, a pertinent question arises: What role do genetic factors play in the morphological variation observed among different populations of *Silene*, and how might future studies integrate both morphological assessments and genetic analyses to elucidate the adaptive strategies of this species in response to environmental changes?

CONCLUSIONS

The current study examined quantitative morphological characteristics across various populations of *S. peduncularis*, focusing on exploring correlations among these traits within different populations and their associations with climatic factors. The variability analysis highlighted alar pedicel length (APL) as the most variable trait, contrasting with the stability of bracteae length (BRAC). So, it was found that the majority of morphological traits are more significantly influenced by elevation rather than by rainfall and average air temperature. Additionally, quantitative morphology was unable to effectively differentiate between varieties or subspecies within the collected populations.

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DECLARATION OF COMPETING INTEREST

The authors of this article declare that they have no financial, professional or personal conflicts of interest that could have inappropriately influenced this work

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