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Full Length Research Paper

Intra-specific variations in *Silene*: Morphometry and micromorphometry analyses

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The *Silene* species are annual, biennial or perennial herbs mainly distributed in the northern hemisphere, Europe, Asia and northern Africa. The section, *Inflatae* Boiss., is the smallest section of the genus containing three species and four subspecies in Iran, out of which one subspecies is endemic with very restricted distribution in the western and northern regions. Studies on seed morphology with scanning electron microscope (SEM) have revealed taxonomically useful micro-characters. The present study was performed to study morphological and micromorphological features in seven *Silene* species and subspecies in the sect *Auriculatae* and *Inflatae* growing in Iran, to study their intraspecific variations and to find out if such data can be used in taxonomy of the genus. The grouping obtained by unweighted pair group method with arithmetic mean (UPGMA) dendrogram separated the species of the section *Inflatae* from the species of the section *Auriculatae*. Siahbishe population of *Silene vulgaris* differed in morphological characters and was placed some distance from the other studied populations. It also had the highest values of total haploid chromosome length (50.51 µM), size of the longest chromosome, (5.42 µM), size of the shortest chromosome (2.88 µM) and the mean chromosome length (4.21 µM). Therefore morphological differences of this population may be related to its higher total chromosome length (genome size), compared to those of other populations studied. We also suggested that the Siahbishe population to be considered as a new variety of *S. vulgaris*, based on both morphological and cytological grounds. The seed type in both section are symmetrical reniform, asymmetric reniform and rounded-reniform. In the sect. *Auriculate*, the length of seed ranged from 1.22 to 2.23 mm, while the size of seed width ranged from 0.86 to 1.38 mm. In the sect. *Inflatae*, the length of seed ranged from 1.30 to 1.86 mm and the seed width ranged from 0.80 to 1.42 mm. These two sections differed in some other seed characteristics which are discussed.

Key words: Morphometry, taxonomy, seed characteristics, *Silene*.

INTRODUCTION

The genus *Silene* L. (Caryophyllaceae) consists of about 700 species mainly distributed in the northern hemisphere, Europe, Asia and Northern Africa (Bari, 1973; Greuter, 1995). Based on morphological characters, Chowdhuri (1957) classified the *Silene* species into 22 sections, which was not supported by molecular studies of Oxelman et al. (1995, 1997, 2000) and Burleigh and Holtsford (2003) particularly not for the endemic North American taxa. The *Silene* species are annual, biennial or perennial herbs. Most are diploid having 2n = 2x = 24, or 2n = 2x = 20 chromosome (Swank, 1932; Bari, 1973). However, triploid, tetraploid, hexaploid and higher polyploidy levels, e.g. 2n = *c.* 96, 120 and 192 have been reported (Bari, 1973). Moreover, 2n = 18 is reported for *Silene conica* L. and *Silene lacera* (Steven) Sims. (Sopova and Sekovski, 1982), and 2n = 46 for *Silene firma* Siebold and Zucc (Zhang, 1994).

Thus, x = 9, 10, 12 and 23 are the known basic chromosome numbers in *Silene*.

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Abbreviations: UPGMA, Unweighted pair group method with arithmetic mean; **SEM,** scanning electron microscope.

The section *Inflatae* Boiss. is the smallest section of the genus containing three species and four subspecies in Iran, out of which one subspecies is endemic with very restricted distribution in western and northern regions (Melzheimer, 1980). The members of this section are perennial with unifloral or dichasial inflorescence, vosiculate-planned calyx with 10 to 20 fibrous and the fruit capsules which are often scattered. On the other hand, the section *Auriculatae* (Boiss.) Schischkin, is the largest section of the genus, containing about 35 species in Iran, out of which 21 species are endemic with very restricted distribution in mountainous areas such as Elburz, Zagros and Azarbayejan (Melzheimer, 1988). The members of this section are caespitose plants with large flowers placed at the end of short stems. Their inflorescence is unifloral or dichasial. Calyx is cylindrical-clavate, pubscent or glandular-pubescent. The petals have conspicuous auricule at the end of claws.

Seed coat morphology is surprisingly slightly affected by environmental conditions under which the plant grows (Barthlott, 1984; Özcan, 2002; Gontcharova et al., 2009; Abdel Khalik, 2006; Wada and Reed, 2008; Karihaloo and Malik, 1996; Gandhi et al., 2011; Plazal et al., 2004; Hong et al., 1999). Studies on seed morphology with scanning electron microscopy (SEM) have revealed taxonomically-important microcharacters, such as cell shape (Canne, 1979; Hong et al., 1999; Gandhi et al., 2011, Plazal et al,. 2004), cellular arrangements (Clark and Jernstedt, 1978; Hong et al., 1999), and the protrusions and patterns of the radial walls (Carolin, 1980, Hong et al., 1999). In Caryophyllaceae, seeds are relatively small (usually less than 3 mm long), and the testa is often variously sculptured (Bittrich, 1993). A few SEM studies have been performed on seed morphology in Caryophyllaceae, including *Arenaria* L. (Wofford, 1981; Wyatt, 1984), *Sagina* L. (Crow, 1979) a few local taxa in *Silene* (Melzheimer, 1977), and *Melandrium* Roehl. (Chung and Lee, 1988; Wada and Reed, 2008)

The seeds of eight species of *Silene* in Korea were examined with a SEM to evaluate the systematic significance of seed coat microstructure (Hong et al., 1999). SEM technique was used to investigate the macro and micromorphology of seed in 17 species (12 genera) of *Caryophyllaceae* in Turkey and some important character were identified, including hylar zone, seed surface type, tubercle shape, surface granulation, suture outline and seed type (Yıldız, 2002). The present study was performed to investigate morphological and micromorphological features in seven *Silene* species and subspecies in the sect *Auriculatae* L. and *Inflatae* growing in Iran to illustrate intraspecific variations and to find out if such data is of taxonomic value in the genus *Silene*.

MATERIALS AND METHODS

Morphological and micro-morphological studies were performed in seven *Silene* species and subspecies from the sect. *Auriculatae* L. and *Inflatae* growing in Iran (Table 1). The species studied are: 1) *Silene aucheriana* Boiss., (2) *Silene sisianica* Boiss. and Buhse, (3) *Silene Peudaucheriana* Melzh., (4) *Silene gynodioica* Ghazanfar, (5) *Silene vulgaris* (Moehnch) Garcke, subsp. *vulgaris*, 6) *Silene pungens* Boiss. and 7) *Silene odontopetala* Fenzl., subsp. *physocalyx* (Ledeb) Borna. and Gauba. subsp. *odontopetala* Fenzl. The voucher specimens are deposited in Herbarium of Shahid Beheshti University (HSBU), Tehran, Iran.

Morphometry

In total, 40 morphological characters were used for morphometry, including quantitative (Table 2) and qualitative characters (Table 3) taken from published materials on *Silene* (Oxelman, 1996) and personal observation in the field. Quantitative morphological characters were randomly measured in at least five plants and the means were used in phonetic analysis. Qualitative characters were coded binary or multistate characters accordingly. Grouping of the species based on morphology characteristics was performed using different clustering methods including unweighted paired group with arithmetic average (UPGMA) and neighbor-Joining (NJ) methods, as well as principal coordinate analysis (PCoA) (Podani, 2000).

Micromorphometry

In total, 17 seed characteristics were used for micromorphometry (Table 4). Seed characters were taken from the studies made by Stearn (1996), Prentice (1978), Hong et al. (1999) and our personal observation in the laboratory. SEM micrographs of seeds were taken by Cam Scan MV 2300 SEM at an accelerating voltage 25.0 kV. Fully mature and undamaged seeds were selected. The seeds were thoroughly vacuum-coated with gold to provide conductivity for the SEM images at Tehran University (Tehran, Iran) Electron Microscopy Unit and examined in three different positions including, surface, ventral and dorsal views using different magnifications.

Seed quantitative morphological characters were randomly measured in at least three plants and the means were used in analysis. Qualitative characters were coded as binary or multistate characters and grouping of the species based on micromorphological characteristics was performed by using PCoA method (Podani, 2000). Statistical analyses were performed using the SPSS ver. 10 (1999) software and NTSYS ver. 2. (1998).

RESULTS AND DISCUSSION

Morphometry

The grouping obtained by UPGMA dendrogram (Figure 1), produced four major clusters. The species of the sect. *Inflatae* are placed in the first three major clusters, separated from the sect. *Auriculatae* which comprised the fourth cluster. The members of these two sections differed in characters like habit form, the basal leaf shape, leaf and stem indumentum as well as capsule form. The populations of *S. vulgaris* form the first major cluster in which Haraz-Panjab, Semnan-Shahdej and Firooz-kooh populations are placed close to each other showing morphological similarities. They differ only in the length of corona in cauline leaf.

Chaloos to Siahbishe population joins the other *S. vulgaris* populations with some distance, differing in

Table 1. *Silene* species studied and their localities.

Specie	Population code	Locality
S. pungens	pun4	Urumiyeh, Silvana, Khalil-kooh, 37° 22 ´44.5˝ N 44° 48 ´3.8˝ E, 2594 m, 1387/03/15, 8751,A. gholipour
S. pungens	pun3	Tabriz, Ahar, Kaleibar, Qaleh babak, 38° 50´ 32.7˝ N 46 °58 ´35.8˝ E, 2270 m, Gholipour
S. pungens	pun1	Urumiyeh, Anhar to Marmisho Road, Solok, 2300 m, A. Sonboli and M. Kanani
S. pungens	pun2	Urumiyeh , Gholipour
S. odontopetala	od2	Lurestan, Azna, Darreh tacht, Oshtoran kouh, 33° 20´ 473˝ N 49° 20´ 347 ˝E, 2680 m, A. gholipour
S. odontopetala	od5	Tehran, Tochal, 35° 52 ´572˝ N, 51° 24´ 131˝ E, 2800 m, Gholipour
S. odontopetala	od8	Semnan, Chashm 36° 00 ´243˝ N 53° 18 ´647˝ E, 2780 m, Gholipour
S. odontopetala	od6	Neishabor to Mashad, Bujan, Kuh-e Binalod, 36 °15´ 418˝ N 58° 59 ´251 ˝E, 1760 m, A. gholipour
S. odontopetala	od3	Tehran, Dizin, Gajerah, Velaiat roud village,36° 03 ´N 51° 23´ E, 2500 m, Gholipour
S. odontopetala	od9	Semnan, Heikouh, 36° 00´ 243˝ N 53° 18´ 647 ˝E, 2780 m , Gholipour
S. odontopetala	od1	Guilan, Kelachi, Rahim-Abad, Eshkevarat, Chakol, Boza kouh, 2800-3100 m , Gholipour
S. odontopetala	od4	Tehran, Haraz road, Polur, 35° 48´899˝ N 52° 01´ 643˝ E, 2405 m, Gholipour
S. odontopetala	od10	Mazandaran, Sari, Kiasar, Langar, Shahdej peak, 36° 10´ 233˝ N 53° 37´ 647 ˝E,2686 m , Gholipour
S. odontopetala	od7	Tabriz, Ahar, Kaleibar, Babak castle, 38 °50´ 32.7 ˝N 46 °58 ´35.8˝ E, 2270 m, Gholipour
S. vulgaris	vul4	Firoozkooh, Gaduk pass, 35° 40 ´372˝ N 52° 29 ´747 ˝E, 1920 m, Gholipour
S. vulgaris	vul3	Tehran, chalus , Siahbishe, Gholipour
S. vulgaris	vul1	Mazandaran, Haraz road, Panjab village, 1800 m, Gholipour
S. vulgaris	vul2	Mazandaran, Sari, Kiasar, Langar, Shahdej peak, 36° 10´ 233˝ N 53° 37 ´647 ˝E,2686 m, Gholipour
S. aucheriana	auc1	Hamedan, Alvand mountain, 48 °25´ 559 ˝E 34° 43´ 078˝ N.2700m, Koohe
S. aucheriana	auc3	Shahrood, Bstam, Nekarman, Koohe shahvar. 54° 48 ´489 ˝E 36° 32´ 268˝ N.2410m. Gholipour
S. aucheriana	auc2	Semnan, Chashm, Kouh-e Nizva, 35° 55´ 3.2 ˝ N 53° 15´ 58 ˝ E, 2612 m, , 2410m, Gholipour
S. aucheriana	auc4	Esfahan, Damaneh to Khansar, Golestan kooh, 24° E 33° 09´ N. 2640m, Gholipour
S. aucheriana	auc5	Tehran, Reineh, Damavand peak, 35 °54´ 9.2 ˝N 52° 5´ 22.2 ˝ E, 3200 m,
S. aucheriana	auc6	Zanjan, Soltaniieh, Qidar, Arghin, Kuh-e ghalleh bayer, 36° 09´ 014 ˝N, Gholipour
S. aucheriana	auc7	Zanjan, Soltaniyeh, Roostaye Salvar, 48° 43´ 55 ˝E 36° 21´ 96˝ N, 1900m, Gholipour
S. aucheriana	auc8	Semnan to Firuzkouh, Gorsefid, bashm, 35° 45´ 3.8 ˝N 52° 54 ´ 54 ˝ E, 2170-250 m, Gholipour
S. aucheriana	auc13	Tehran , Tuchal, 35° 49´ 41.5 ˝ N 51 °24´ 11.9 ˝, 2227-980 m, Gholipour
S. aucheriana	auc9	Tehran. Dizin. Gajereh.roostaye Velayat rood.51° 23´ E 36 °03´ N. 2600m, Gholipour
S. aucheriana	auc10	Esfahan, Semirom to Shahreza, Mehrgerd, Narmeh, 31° 34´ 194 ˝ N 51 °26´ 887 ˝ E, 2630 m, Gholipour
S. aucheriana	auc11	Orumiyeh. Silvana. Khalil kooh. 44° 47´ 770˝ E 37° 33´ 101 ˝N . 2300m. Gholipour
S. aucheriana	auc12	Tabriz, Saaid abad, Motnegh, Kuh-e Sahand, 37° 47´ 172 ˝ N 46° 30´ 978 ˝ E, 2900m. Gholipour
S. aucheriana	auc17	Lorestan. Ezna. Roostaye Dareh takht. Oshtorankooh. 49° 20´ 347˝ E 33° 20´ 473˝ N. 260m. Gholipour
S. aucheriana	auc14	Ardebil to Khalkhal, Daryacheh Neur, 2850m, Gholipour
S. aucheriana	auc15	Zanjan, Angooran mountain, 47 °25´ 239˝ E 36° 37´ 573 ˝N, 2469m. Gholipour
S. aucheriana	auc16	Gilan, RAHIM abad, Chakol, Bozakooh, 2700m Gholipour
S. pseudaucheriana	pseu	Lorestan, Ezna, Roostaye Dareh takht, Oshtorankooh, 49° 20 ´347˝ E 33 °20 ´473˝ N, 2680m, Gholipour

Table 1. Contd.

S. sisianica	sis1	Urumiyeh to Salmas, Ghoschi pass, 8758 m, Gholipour
S. sisianica	sis2	Tabriz, Marand, Koohe Mishoodagh, 45° 45´ 785˝ E 38° 20´ 160˝ N. 2100m. A. Gholipour
S. sisianica	sis3	Marand, Kuhe Yam, 38 °20 ´10 ˝ N 45° 45´ 785 ˝ E, 1900 m, A. Gholipour
S. ginodioica	gyn	Tabriz, Ahar, Kaleibar, Babak castle, 38° 50´ 32.7 ˝ N 46° 58´ 35.8 ˝ E, 2270, Gholipour

Table 2. Quantitative morphological characters in *Silene* species studied.

Character	Character state				
	1	2	3	4	5
Plant height	x<20	20≤x<40	x>40		
Basal leaf length	x<20	20≤x<35	35≤x<55		
Basal leaf width	x<2.5	2.5≤x<5	5≤x<7.5	x>7.5	
Length leaf cauline	x<20	20≤x<40	40≤x<60	x>60	
Cauline leaf width	x<2.5	2.5≤x<5	5≤x<7.5	7.5≤x<10	x>10
Alar pedicel length	Absent	x<10	10≤x<20	20≤x<30	x>30
Calyx length	x<10	10≤x<20	20≤x<30		
Calyx tooth length	x<1.5	1.5≤x<3	x>3		
Corona length	x<5	5≤x<10	x>10		
Petal claw length	x<2	2≤x<4	x>4		
Petal limb length	x<4	4≤x<8	8≤x<12		
Epipetal filament alternate	x<1	1>x	X=1		
Length of linkage of filament stamen to corona	x<1	1≤x<2	2≤x<3	x>3	
Capsule length	x<5	5≤x<15	10≤x<15	x>15	
Antophore length	x<4	4≤x<8	8≤x<12	x>12	
Seed length	x≤1.5	x>1.5			
Seed width	x≤1	x>1			
Width/length seed	x≤1.5	x>1.5			
Coronal length	x≤1.3	x>1.3			

characters like capsule shape and basal leaf length and width, length of stamen filament linkage to corona, length/width seed and pedicel length. According to Flora Iranica, these populations with ovate-oblanceolate leaves are from the subsp. *vulgaris*. Our previous cytological study in the same populations (Sheidai et al., 2011) showed uniform karyotype features in these populations including somatic chromosome number and polyploidy level (2n = 2x = 24), karyotype formulae (12 m) and karyotype symmetry (1A class of Stebbins' classification). However, they differed somewhat in total chromosome length and the mean chromosome length. Siahbishe population, which differed in morphological characters and is placed with

Table 3. Qualitative morphological characters in *Silene* species.

Character	*S. vulgaris*	*S. aucheriana*	*S. pseudaucheriana*	*S. sisianica*	*S. ginodioica*
Basal leaf form	Linear- oblanceolate	Linear-lanceolate, linear, Oblanceolate	Oblanceolate	Oblanceolate, Linear-lanceolate	Oblanceolate
Cauline leaf form	Ovate- oblanceolate	Oblanceolate, Linear, Lanceolate	Lanceolate	Lanceolate	Lanceolate
Cauline and leaf indumentums	Absent	Present	Present	Present	Present
Calyx veins	Reticulate	Parallel	Parallel	Parallel	Parallel
Inflorescence type	Compound dichasium	Compound dichasium	Compound dichasium	Compound dichasium	Tyrsoide
Calyx form	Campanulate	Cylindric-clavate	Cylindric-clavate	Cylindric-clavate	Cylindric-clavate
Claw situation to calyx	Included in calyx	Exerted from calyx, Included in calyx	Included in calyx	Included in calyx, Exerted from calyx	Exerted from calyx
Calyx ring	Without ring	Without ring	Without ring	Without ring	Without ring
Calyx outside indumentums	Absent	Present	Present	Present, Absent	Present
Calyx inside indumentums	Present	Absent, Present	Absent	Absent	Absent
Capsule form	Globular -ovate, oblong- elliptic	Elongate ovate	Elongate ovate	Elongate ovate	Elongate ovate
Capsule situation to calyx	Exerted from calyx, Included in calyx	Included in calyx	Included in calyx	Included in calyx	999
Antophore indumentums	Present, Absent	Present, Absent	Present	Present	999
Corona	Absent	Absent, Present	Present	Present	Present
Auricle size	Inconspicuous	Absent, Inconspicuous, Conspicuous	Conspicuous	Conspicuous	Conspicuous
Claw and filament indumentums	Absent	Absent, Present	Present	Present	Present
Style indumentums	Absent	Present, Absent	Absent	Present	Absent
Testa cell projections	Present	Absent	Absent	999	999
Suture outline	Serrate	Serrate, smooth	serrate	999	999
Auricle	Present	Absent, Present	Present	Present	Present

Table 4. Seed quantitative characters in *Silene* species.

Specie	Locality	Pop code	SL	SW	R	TL	TW	TR	HL	HW	HR	TB
S. aucheriana	Neur	Auch1	1.63	1.03	1.58	152.41	60.81	2.51	277.00	130.82	2.12	Absent
S. aucheriana	Kooh shahvar	Auch3	1.50	0.95	1.58	224.31	93.93	2.39	300.10	151.29	1.98	Absent
S. aucheriana	Angooran	Auch2	1.86	1.17	1.59	280.00	110.00	2.55	400.16	156.15	2.56	Absent
S. aucheriana	Tochal	Auch13	1.56	0.99	1.58	257.03	65.26	3.94	300.45	251.45	1.19	Absent
S. aucheriana	Chakol	Auch4	1.82	1.22	1.49	240.00	80.00	3.00	375.14	232.40	1.61	Absent
S. aucheriana	Oshtorankooh	-	1.95	1.10	1.77	320.00	130.00	2.46	478.46	252.42	1.90	Absent
S. pudaucheriana	Oshtorankuh	Pud1	2.23	1.38	1.62	350.00	110.00	3.18	298.30	147.00	2.03	Absent
S. sisianica	Ghoshchi	Sis1	1.22	0.86	1.42	196.04	62.74	3.12	253.29	153.58	1.65	Absent
S. odontopetala	Oshtorankuh	Od2	1.47	0.97	1.51	140.01	90.10	1.55	181.61	153.45	1.18	153.31
S. odontopetala	Dizin	Od3	1.36	0.80	1.71	120.01	80.10	1.50	226.88	192.56	1.18	-
S. odontopetala	Chakol	Od1	1.43	1.04	1.38	155.94	69.00	2.26	147.43	144.06	1.00	95.6

Table 4. Contd.

S. odontopetala	Tochal	Od5	1.30	1.07	1.21	132.89	49.81	2.67	149.03	112.86	1.32	102.86
S. odontopetala	Binalood	Od6	1.36	0.98	1.39	90.01	90.00	1.00	292.66	100.01	2.93	118.32
S. pungens	Orumiye	Pun1	1.60	1.20	1.32	100.01	70.01	1.47	418.52	96.95	4.32	999
S. vulgaris	Siahbishe	Vul3	1.86	1.42	1.30	140.01	120.00	1.17	387.56	131.78	2.94	46.51
S. vulgaris	Firuzkuh	Vul4	1.47	1.20	1.22	120.00	90.00	1.33	137.19	123.18	1.11	38.42
S. vulgaris	Shahdej	Vul2	1.82	1.32	1.41	150.00	90.00	1.67	-	-	-	39.58

SL, Seed length (cm); SW, seed width (cm); R, length/width ratio; TL, testa cell length (µM); TW, testa cell width (µM); TR, ratio of testa cell length/ width; HL, hilum length (µM); HW, hilum width (µM); HR, ratio of hilum length/width; TB, Tubercul height (µM).

some distance from the other *S. vulgaris* populations, also has the highest values of total haploid chromosome length (50.51 µm), size of the longest chromosome, (5.42 µM), size of the shortest chromosome (2.88 µM) and the mean chromosome length (4.21 µM). Therefore, morphological differences of this population may be related to its higher total chromosome length (genome size) compared to those of other populations studied. Hence, we suggest that the Siahbishe population be considered as a new variety of *S. vulgaris*, based on both morphological and cytological grounds.

The second major cluster is formed by populations of *S. odontopetala*. Binalud population joins the other *S. odontopetala* populations with some distance. According to the subspecies description provided in Flora Iranica, this population belongs to the *S. odontopetala* sub*. odontopetala*. The other populations show similarity and form a group together. According to the subspecies description provided in Flora Iranica, Touchal population belongs to the *S. odontopetala* subsp. *physocalyx*. Therefore, the populations showing morphological similarities to it are considered to be *S. odontopetala* subsp. *physocalyx*. The third major cluster is formed by populations of *S. pungens* in which Anhar to Marmisho road population is placed far from the other three populations studied due to difference in characters like calyx length and alar pedicel length. Unfortunately, these populations have not been studied from karyotype point of view and we are not able to compare morphological differences with those characters. Separation of the species studied in the sect. *Inflatae* from the sect. *Auriculatae* is well supported in morphological tree. Furthermore, inclusion of different popula-tions of each species in the sect. *Inflatae* indicates proper identification of these three species and possible lack of introgression or any other pollen exchange among them as no overlap was observed in morphological characteristics in these species.

The forth major cluster is formed by the populations of *S. aucheriana*, *S. sisianica*, *Silene pseudaucheriana* and *S. gynodioica* from the sect. *Auriculatae*. *S. sisianica* shows morphological similarity to *S. gynodioica* and are placed close to each other. Affinity between these species has been suggested in Flora Iranica too. Two populations of Mishodagh and Marand in *S. sisianica* show some morphological differences and join each other with some distance. Moreover, the populations of *S. aucheriana* show close affinity in morphological characters and are placed close to each other. They mainly differ in some of their morphological quantitative characters.

Micromorphometry

SEM images of the mature seeds in the studied species are presented in Figures 2A to W The seed type in both section are symmetrical reniform, asymmetric reniform and rounded-reniform (Table 4). In the sect. *Auriculate*, the length of seed ranged from 1.22 mm in the Ghoshchi population of *S. sisianica* to 2.23 mm in Oshtorankuh population of *S. pseudaucheriana*, while the size of seed width, ranged from 0.86 mm in Ghoshchi population of *S. sisianica* to 1.38 mm in Oshtorankuh population *of S. pseudaucheriana*. In the sect. *Inflatae*, the length of seed ranged from 1.30 mm in the Tochal population of *S. odontopetala* to 1.86 mm in Siahbishe population of *S. vulgaris*, while the seed size of width ranged from 0.80 mm in Dizin population of *S. odontopetala* to 1.42 mm in Siahbishe population of *S. vulgaris.* Furthermore, in the section, the seed length/width ratio ranged from 1.21 in Tochal population of S*. odontopetala* to 1.71 in Dizin population of *S. odontopetala* while, in the sect. *Auriculate*, this ratio ranged from 1.42 in Oshtorankuh population of *S. aucheriana* to 1.77 in Ghoshchi

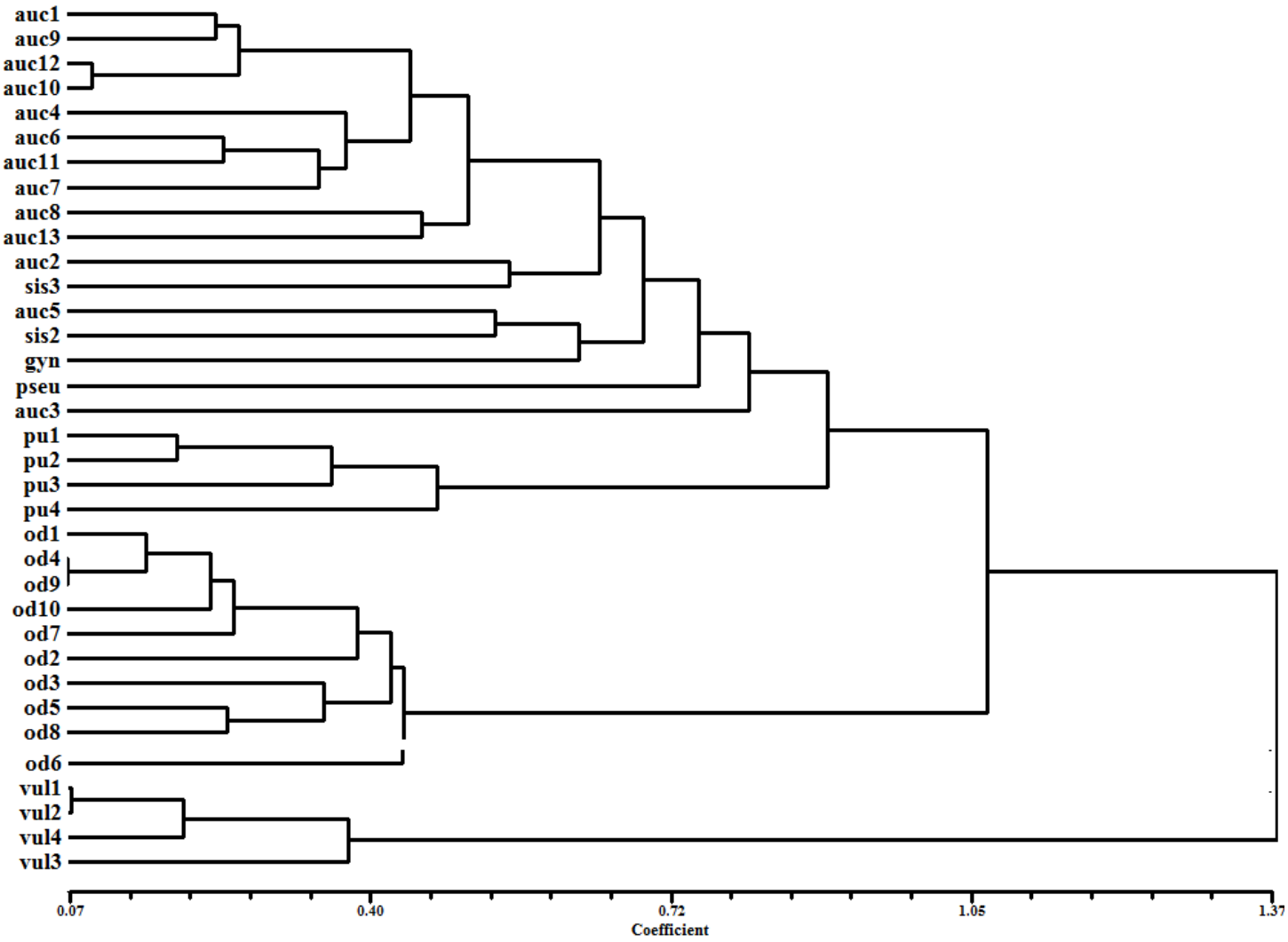

Figure 1. UPGMA dendrogram of morphological data. auc, *Silene aucheriana*; sis, *S. sisianica*; gyn, *S. ginodioica*; pseu, *S. pseduaucheriana*; pu, *S. pungens*; od, *S. odontopetala* and vul, S. vulgaris. The population's code is according to Table 1.

population of *S. sisianica*.

The suture outline (Table 4) was v-shape in *S. aucheriana* (except in Chakol and Oshtorankuh populations), *S. sisianica*, *S. pudaucheriana*, *S. vulgaris*, and S. *odontopetala* (except Chakol, Dizin and Tochal populations). It was u-shape in *S. pungens*, Dizin and Tochal populations of *S. odontopetala* and Chakol and Oshtorankuh populations of *S. aucheriana*. The only aperture in the seeds of *Silene* seeds is called hilar zone. The size of hilar zone length varied from 137.19 µM in Firuzkuh population of *S. vulgaris* sect. *Inflatae* to 418.52 µM in Orumiyeh population of *S. pungens* sect. *Inflatae*. The width of hilar zone greatly varied from 96.95 µM in Orumiyeh population of *S. pungens* sect. *Inflatae* to 252.42 µM in Oshtorankuh population of *S. aucheriana* sect. *Auriculatae*. The cell of two side of hilar zone are similar in shape for some of the species including *S. sisianica* (sect. *Auriculatae*), Tochal population of *S. odontopetala* (sect. *Inflatae*) and Orumiyeh population of *S. Pungens* (sect. *Inflatae*), while they were different in shape for the other species.

The t-test analysis performed (data not shown) for quantitative seed characters between members of two sections showed significant difference for characters like ratio of length/width, length and width of seed surface, the ratio of these characters, length and width of hilar zone. Moreover, analysis of variance (ANOVA) test performed (data not shown) for quantitative seed characters showed significant difference for the same characters among three species of *S. aucheriana*, *S. odontopetala* and *S. vulgaris*. PCoA grouping of the species based on micromorphometry data (Figure 3) also separated the species of the sections *Inflatae* and *Auriculatae* into almost distinct groups showing the use of micromorphometry in taxonomy of the group.

In general, the present findings reveal intraspecific

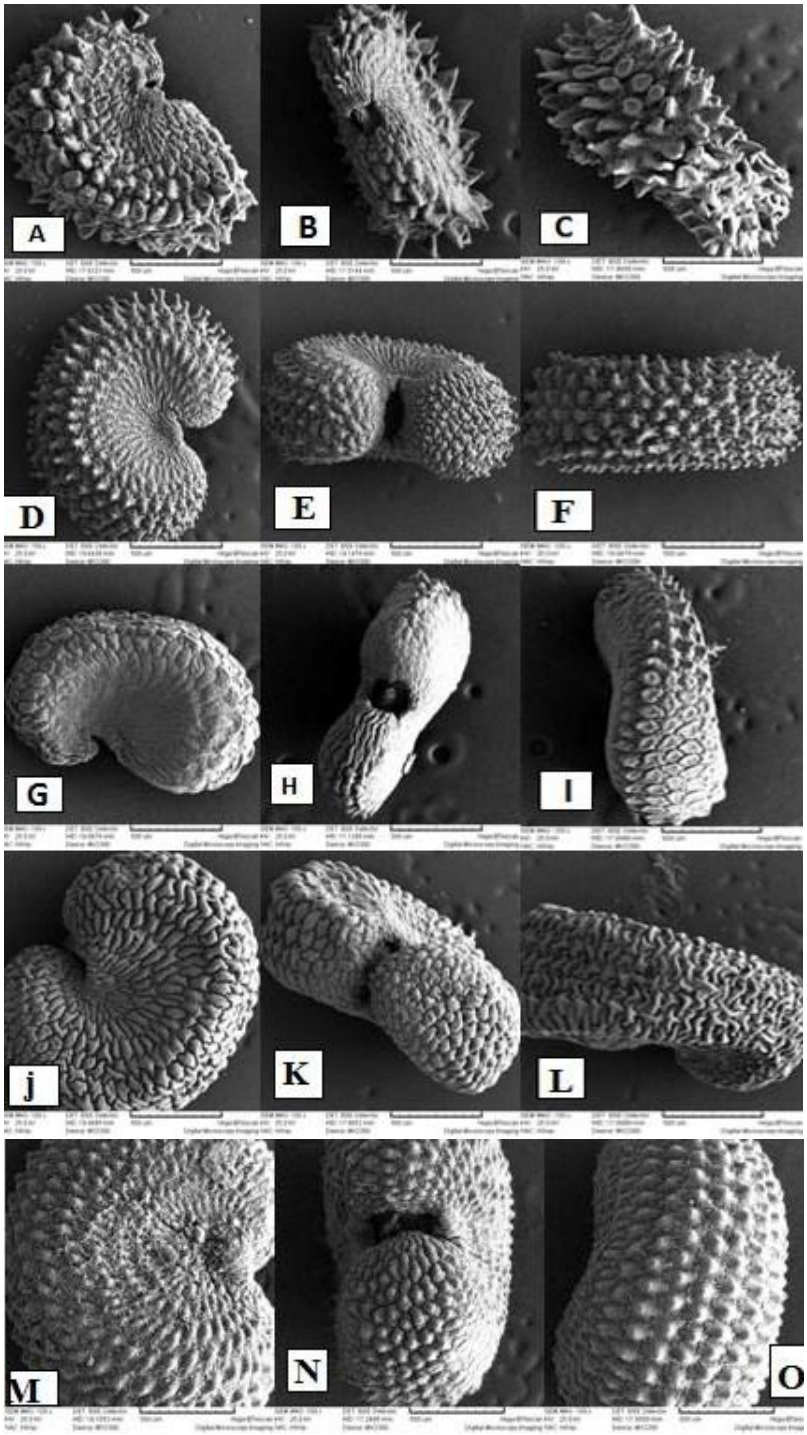

Figure 2. Representative SEM of seeds in *Silene* species studied. **A,** Surface view in Oshtorankooh population of *S. odontopetala*. **B.** Ventral view in Oshtorankooh population of *S. odontopetala*. **C.** Dorsal view in Oshtorankooh population of *S. odontopetala*. **D.** Surface view in Binalud population of *S. odontopetala*. **E.** Ventral view in Binalud population of *S. odontopetala*. **F.** Dorsal view in Binalud population of *S. odontopetala*. **G.** Surface view in Dizin population of *S. odontopetala*. **H.** Ventral view in Dizin population of *S. odontopetala*. **I.** Dorsal view in Dizin population of *S. odontopetala*. **J.** Ventral view in Orumiyeh population of *S. pungens*. **K.** Dorsal view in Orumiyeh population of *S. pungens*. **L.** Surface view in Orumiyeh population of *S. pungens*. **M.** Surface view in Siahbisheh population of *S. vulgaris*. **N.** Ventral view in Siahbisheh population of *S. vulgaris*. **O.** Dorsal view in Siahbisheh population of *S. vulgaris*.

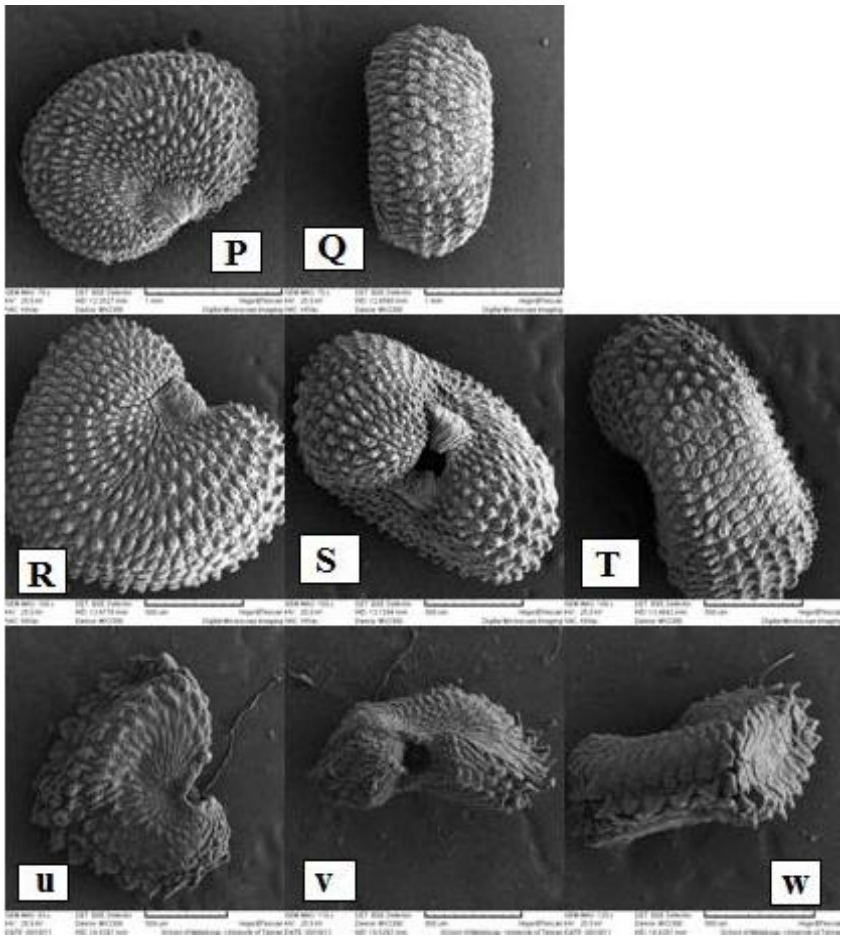

Figure 2 Contd**.** Representative SEM of seeds in *Silene* species studied. **P.** Surface view in Shahdej population of *S. vulgaris*. **Q.** Dorsal view in Shahdej population of *S. vulgaris*. **R.** Surface view in Firuzkuh population of *S. vulgaris*. **S.** Ventral view in Firuzkuh population of *S. vulgaris*. **T.** Dorsal view in Firuzkuh population of *S. vulgaris*. **U.** Surface view in Chakol – bozakuh population of *S. odontopetala*. **V.** Ventral view in Chakol – bozakuh population of *S. odontopetala.* **W.** Dorsal view in Chakol – bozakuh population of *S. odontopetala*. The scale for all photos except for P and Q is 500 µM. For P and Q it is 1 mm.

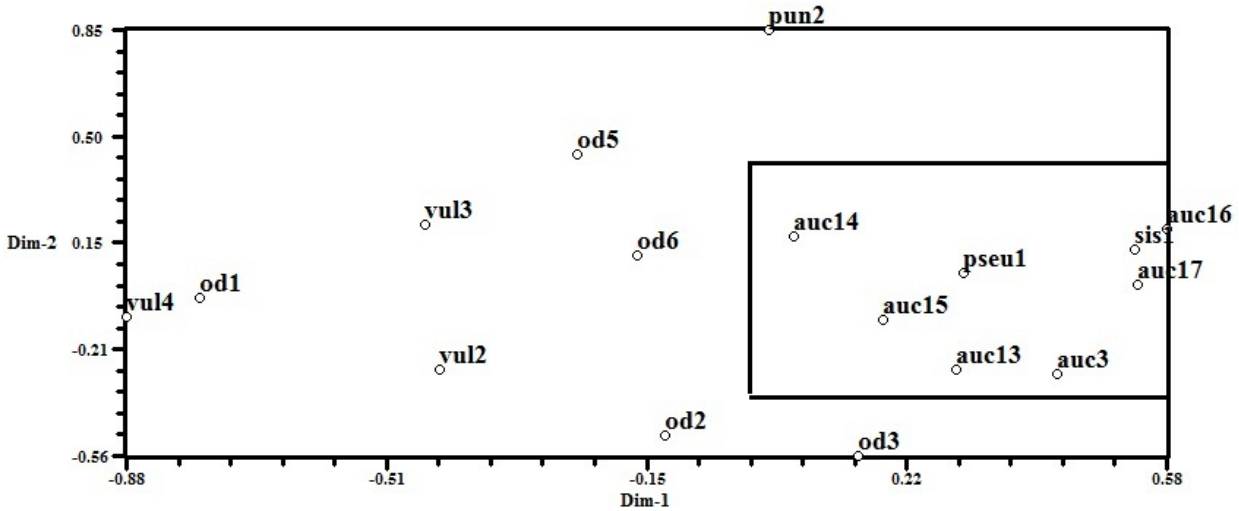

Figure 3. PcoA plot of micromorphometry data.

morphological and micromorphometry variations in the species of the sections *Inflatae* and *Auriculatae*, thus showing the validity in separating the two sections from each other.

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