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ARTICLE

Morphological and karyotype diversity in populations of four *Silene* species (Caryophyllaceae)

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ABSTRACT Karyotype and morphometric studies were performed on 14 and 24 Iranian populations of 4 *Silene* (Caryophyllaceae, Sect. *Auriculatae*) species. Phenetic study of 24 populations of *S. commelinifolia*, *S. eremicana*, *S. lucida* and *S. nurensis* from different locations of Iran revealed that a lot of morphological characters as basal and caulinal leaf shape, width and length, capsule shape and condition in calyx, epipetalus stamens to alternate ones, alar pedicel length, lateral pedicel length, epipetalus filament length to claw length and calyx gap length are of taxonomic importance. *S. nurensis* possessed a chromosome number 2n=2x=24, *S. lucida* and *S. eremicana* possessed a chromosome number 2n=4x=48, while *S. commelinifolia* var. *commelinifolia* and *S. commelinifolia* var. *ovatifolia* populations were diploid and tetraploid. The chromosomes were mainly metacentric or sub-metacentric and their size varied from 1.21 µm in *S. nurensis* to 3.96 µm in *S. commelinifolia*. The total size of the chromosomes differed significantly in short and long arm size, indicating the role of quantitative genomic changes in the *Silene* species diversification. The *Silene* species were placed in 1A and 1B classes of Stebbins karyotype symmetry. Presence of B chromosome is recorded for the first time for *S. commelinifolia*. Clustering and ordination methods showed karyotype distinctness in the investigated species.
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KEY WORDS

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The genus *Silene* L. (Caryophyllaceae) is a large genus with worldwide distribution, containing about 700 species. These species are mainly hermaphrodite, although a few species are dioecious or gynodioecious (Bari 1973; Greuter 1995). *Silene* species are mostly distributed throughout the northern hemisphere, Europe, Asia and northern parts of Africa (Greuter 1995) and are annual, biennial, or perennial herbs. Diploid species, which are more frequent have 2*n*=18, 20 and 24. Triploid to hexaploid and even higher polyploidy levels, e.g. 2*n*=*c*. 96, 120 and 192, are known in the genus (Swank 1932; Bari 1973; Oxelman et al. 1997; Heaslip 1951; Bari 1973; Sopova and Sekovski 1982; Zhang 1994). X=9, 10, 12 and 23 are the known basic chromosome numbers in *Silene*.

Available literature about *Silene* cytogenetic studies indicates the importance of such studies in defining the species relationships (Heaslip 1951; Bari 1973; Melzheimer 1978; Markova et al. 2006). But very limited cytological studies have been carried out on the species growing in Iran and only recently some preliminary karyotype and meiotic studies have been reported from the country (Sheidai et al. 2008; Sheidai et al. 2009a, b; Gholipour and Sheidai 2010a, b; Sheidai et al. 2012).

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About 110 *Silene* species grow in Iran from which about 35 species are endemic with very limited geographical distribution (Melzheimer 1988). Chowdhuri (1957) placed the *Silene* in 44 sections, although recent molecular studies do not support such sectional classifications (Oxelman et al. 1997, 2000; Burleigh and Holtsford 2003). The section *Auriculatae* (Boiss.) Schischkin is the largest section of the genus containing about 35 species, of which 21 species are endemic to Iran (Melzheimer 1988). The members of this section are caespitose alpine elements with large flowers placed at the end of short stems. Their inflorescence is unifloral or dichasial. The calyx is very cylindrical-clavate, pubescent or glandular-pubescent. The petals have a conspicuous auricle at the end of the claw.

Species relationships in genus *Silene* sect *Auriculatae* L. based on RAPD and morphological analysis have been studied in Iran (Sheidai et al. 2010).The inter-population morphological and molecular diversity in three *Silene* species in the sect *Auriculatae* L. have been studied in Iran (Sheidai et al. 2012). Morphological and micro-morphological features in seven *Silene* species and subspecies in the sect *Auriculatae* L. and *Inflatae* have been studied in Iran (Tabaripour et al. 2013). Gholipour and Sheidai (2010c) considered *S. eremicana* and *S. goniocaula* as separate *Silene* species in Iran, despite Melzheimer opinion (1988).

Table 1. Voucher details and selected studies for each case (M: morphometry and C: cytology).

Studies	Locality	Code	Taxon	No.
M, C	West Azerbaijan, Piranshahr to Naghadeh, Gerd Kashaneh, Lik Bin Village, Landi Sheykhan Mountain, 36 41 7.5 N 45 26 27.1 E, 2400 m, 2010/7/2, 890277.	Com14	*S. commelinifolia* var. *ovatifolia*	1
M, C	Tehran, Darakeh Mountain, 1900 m, 2012/6/20, 91287.	Com11	*S. commelinifolia* var. *ovatifolia*	2
M	Tehran, Darakeh Mountain, 35 49 37.3 N 51 22 47.3 E, 1925 m, 2008/6/20, 8768.	Com12	*S. commelinifolia* var. *ovatifolia*	3
M	West Azerbaijan, Urmia, Anhar, Marmisho, 37 29 0.33 N 44 45 0.22 E, 2327 m, 2011/7/19, 900832.	Com10	*S. commelinifolia* var. *ovatifolia*	4
M	West Azerbaijan, Takab, 2008/6/30.	Com13	*S. commelinifolia* var. *ovatifolia*	5
M, C	Mazandaran, Baladeh, Kamarbon, Gosfandsarai-e chai khaksar, 36 14 16.1 N 51 22 17.1E, 2852m, 2011/7/6, 900624.	Com5	*S. commelinifolia* var. *commelinifolia*	6
M	Tehran, Haraz Road, Polor, 35 48 899 N 52 01 643 E, 2405 m, 2007/6/9, 8637.	Com6	*S. commelinifolia* var. *commelinifolia*	7
M,C	West Azerbaijan, Urmia, Anhar, Marmisho, 37 29 03.2 N 44 36 24.7 E, 3007m, 2012/7/2, 91312.	Com7	*S. commelinifolia* var. *commelinifolia*	8
M, C	Hamedan, Alisadr Cave, 2010/6/29.	Com8	*S. commelinifolia* var. *commelinifolia*	9
M, C	Tehran, Touchal, 35 52 572 N, 51 24 131 E, 2700 m, 2008/6/25, 8771.	Com9	*S. commelinifolia* var. *commelinifolia*	10
C	Tehran, Dizin, Gajerah, Velayatroud Village, 36 03 N 51 23 E, 2500 m, 2008/7/15.	Com11	*S. commelinifolia* var. *commelinifolia*	11
C	Lorestan, Azna, Daretakht, Oshtorankuh, 33 20 473 N 49 20 347 E, 2680 m, 2008/8/19.	Com16	*S. commelinifolia* var. *commelinifolia*	12
M, C	Ardabil, Km 30 Ardabil to Kivi, before Neor Lake, 38 00 549 N 48 55 225 E, 2590 m, 2011/7/15, 900701.	Com1	*S.* cf. *commelinifolia*	13
M, C	Ardabil, Neor Lake, 2008/8/19.	Com2	*S.* cf. *commelinifolia*	14
M, C	East Azerbaijan, Sarab, Shalgoon Village, Bozqush Mountain, 37 45 54 N 47 35 31 E, 2650-3000 m, 2012/7/8, 91387.	Com3	*S.* cf. *commelinifolia*	15
M, C	Hamadan, Alvand Mountain, Ganjnameh, 34 43 475 N 48 25 039 E, 2800 m, 2006/6/25, 86105.	Ere	*S. eremicana*	16
M	East Azerbaijan, Sarab, Shalgoon Village, Bozqush Mountain, 37 45 54 N 47 35 31 E, 2650-3000 m, 2012/7/8.	Luc2	*S. lucida*	17
M	Gilan, Kelachay, Rahim Abad, Eshkevarat, Chakol, Boza kuh, 2800-3100 m, 2007/6/29, 86139.	Luc1	*S. lucida*	18
M	East Azerbaijan, Bostan Abad to Miyaneh, km 75, 2006/6/7, 8516.	Luc3	*S. lucida*	19
C	West Azerbaijan Piranshahr to Naghadeh, Km 5, Selve Village, Sepiarez Mountain, 36 50 59.9 N 44 58 24.8 E, 2820 m, 2010/7/1, 890257.	Luc6	*S. lucida*	20
M	West Azerbaijan, Urmia, Silvana, Kuh-e Khalil, 37 22 44.5 N 44 48 3.8 E, 2594 m, 2008/6/4, 8754.	Luc4	*S. lucida*	21
M	West Azerbaijan, Urmia, Silvana, Kuh- e Khalil, 2008/6/4, 87054.	Luc5	*S. lucida*	22
M	Chaharmahal and Bakhtiari, Farsan, Kuhrang, Zardkuh, 32 18 704 N 50 08 574 E, 3300-3400 m, 2008/7/30, 87082.	Nur2	*S. nurensis*	23
M, C	Chaharmahal and Bakhtiari, Farsan, Kuhrang, Zardkuh, 32 18 704 N 50 08 574 E, 3300-3400 m, 2008/7/30, 8782.	Nur1	*S. nurensis*	24
M	Lorestan, Azna, Daretakht, Oshtorankuh, 33 20 473 N 49 20 347 E, 2680 m, 2008/8/19, 87087.	Nur3	*S. nurensis*	25
M	Lorestan, Azna, Daretakht, Oshtorankuh, 33 20 522 N 49 20 427 E, 2535 m, 2008/8/9, 8787.	Nur4	*S. nurensis*	26
M	Kohgiluyeh and Boyer ahmad, Dehdasht, Sarfariab, Joukhaneh, Kuh-e Nir 30 49 30.9 N 50 55 19E, 2980 m, 2011/6/9, 900400.	Nur5	*S. nurensis*	27

Due to Gholipour and Sheidai (2010) biosystematics studies on this section, some species groups were defined. One of this species group is related to *S. commelinifolia.* Based on flora Iranica there are three varieties in this species: *commelinifolia, ovatifolia* and *isophylla*. There are some problems in varieties delimitation. Based on our previous studies there was a probability for misidentifications in this species complex, so there was a necessity for careful population study to define characters range of variation. Recent studies (Sheidai et al. 2012; Gholipour and Sheidai 2010a, b; Sheidai et al. 2009a,

Table 2. Morphological characters and their code.

Code				Character
	x>35	20≤x≤35	x<20	Plant height
	x>55	35≤x≤55	x<35	Basal leaf length
	x>5	2.5≤x≤5	x<2.5	Basal leaf width
		x>0.5	x≤0.5	Length/width Basal
	x>35	20≤x≤35	x< 25	Cauline leaf length
	x>5	2.5≤x≤5	x< 2.5	Cauline leaf width
	x>0.11	0.8≤x≤0.11	x<0.8	Cauline leaf width/length
x>10	5≤x≤10	2≤x≤5	x<2	Alar pedicel length
	x>5	2≤x≤5	x<2	Lateral pedicel length
x>32	21≤x≤32	15≤x≤20	x<15	Calyx length
	x>5	2.5≤x≤5	x<2.5	Calyx tooth length
	x>15	10≤x≤15	x<10	Petal claw length
	x>7	5≤x≤7	x<5	Petal limb length
	x>5	3≤x≤5	x<3	Epipetalus filaments length to claw
		x>1.3	x<1.3	Corona length
	x>10	7≤x≤10	x<7	Capsule length
x>15	11≤x≤15	5≤x≤10	x<5	Antophore length
		x>1.75	x<1.75	Seed length
		x>1.75	x<1.75	Seed width
		x>1.75	x<1.75	Seed width/length
			Caespitose-suffrutescent	Habit
	Linear-lanceolate	Linear	Ob-lanceolate	Basal leaf form
Linear	Lanceolate	Cordate	Ovate	Cauline leaf form
			Present	Cauline leaf indumentums
			Cylindric-clavate	Calyx form
			Compound dichasium	Inflorescence type
			Present	Calyx outside indumentums
		Absent	Present	Calyx inside indumentums
			Parallel	Calyx veins
			Exerted from calyx	Capsule situation to calyx
		Included in calyx	Exerted from calyx	Claw situation to calyx
		Longer than 1/2 limb	Shorter than 1/2 limb	Petal limb division length
		Inconspicuous	Conspicuous	Auricle size
		As long as epipetal	Shorter than epipetal	Alternate filament length
			Absent	Filament indumentum
			Absent	Style indumentum
		Elongate ovate	Oblong- elliptic	Capsule form
			Present	Antophore indumentum

b; Sheidai et al. 2008), showed the polyploidy variations in accessions that could be the main factor in population divergence. So the population divergence of *S. commelinifolia* and related taxa in Iran is considered in the present study.

Materials and Methods

Plant material

Morphological studies were performed on 23 populations of 4 *Silene* species and varieties of the section *Auriculatae.* Karyotype studies were performed in 14 ones (Table 1.). The studied taxa were *Silene commelinifolia* Boiss. var. *commelinifolia*, *S. commelinifolia* Boiss. var. *ovatifolia* Melzh., *S. nurensis* Boiss. & Hausskn, *S. lucida* Chowdhuri and *S.eremicana* Stapf. The vouchers are deposited in the Herbarium of Shahid Beheshti University (HSBU).

Morphological studies

In total, 38 morphological characters (quantitative and qualitative) were studied (Table 2). Analysis of variance test (ANOVA) was performed to show a significant difference in quantitative morphological characters among the species. For multivariate analyses the mean of quantitative characters was used, while qualitative characters were coded as binary/multistate characters. Standardized variables (mean = 0, variance = 1) were used for statistical analyses. The average taxonomic distance and Manhattan distance were used as dissimilarity coefficients independently in cluster analysis of morphological data (Podani 2000). Grouping of the species based on morphology characteristics was performed using different clustering methods, including un-weighted paired group with arithmetic average (UPGMA), as well as

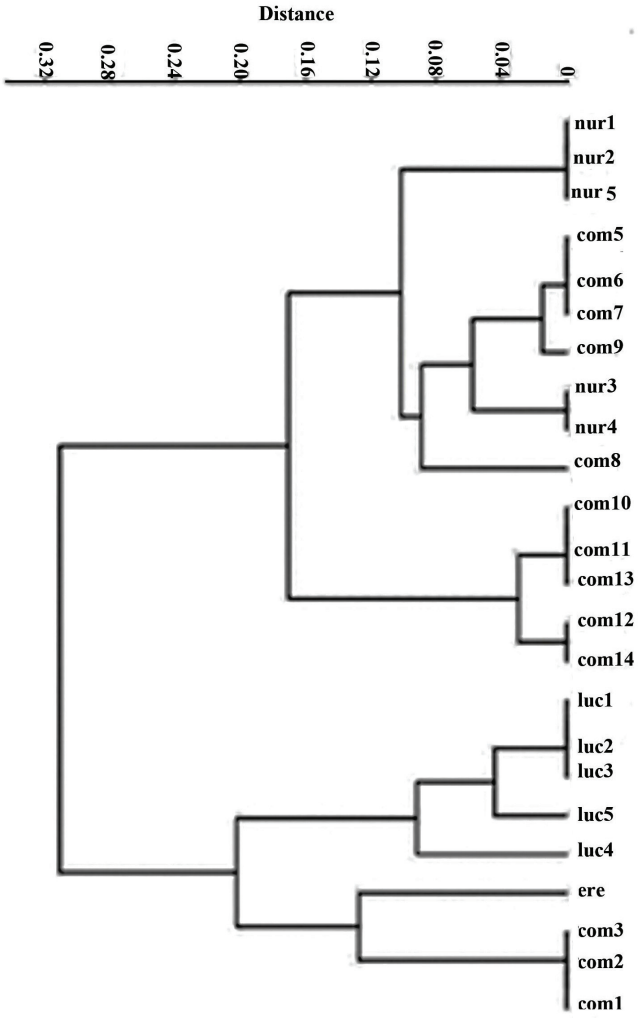

Figure 1. UPGMA dendrogram of studied *Silene* species based on morphological characters. Species code: com5, 6, 7, 8, and 9 = *S. commelinifolia* var. *commelinifolia*; com10, 11, 12, 13 and 14 = *S. commelinifolia* var. *ovatifolia*; com1, 2 and 3 = *S. cf commelinifolia*; nur = *S. nurensis*; luc = *S. lucida* and ere = *S. eremicana*.

principal coordinate analysis (PCO) (Podani 2000). Principal Components Analysis (PCA) and canonical correspondence analysis (CCA) were performed to identify the most variable morphological characters and the plot of the first and second component were used to investigate the species grouping (Podani 2000).

Cytological studies

For karyotype studies freshly grown root tips were collected from the seeds of at least ten randomly selected plants in each species, pretreated with 0.002 mol 8-hydroxyquinolin (1-2 h). Squash technique was used for cytological studies and karyotypic details were studied in at least 5 well prepared metaphase plates as reported earlier (Sheidai and Rashid 2007). The chromosomes were identified according to Levan et al. (1964), karyotype symmetry was determined according to Stebbins (1971). Karyotype parameters as a total form percentage (TF %), coefficient of variation (CV) of the chromosome size as well as A1 indices of Romero- Zarco (1986) were determined (Sheidai and Jalilian 2008).

In order to reveal significant difference, the analysis of variance (ANOVA) followed by the least significant difference test (LSD) was performed on the size of chromosomes, the size of the long arms and the size of the short arms as well as arm ratio among the studied species and populations (Sheidai and Jalilian 2008). Moreover, principal components analysis (PCA) was performed to identify the most variable karyotypic characters. The Karyotypic distinctness of the species studied was checked by using an ordination plot of principal components analysis (PCO) (Sheidai and Jalilian 2008).

Results and Discussion

Morphometry

ANOVA test showed significant differences for almost all quantitative morphological characters studied. UPGMA (Fig. 1) tree, PCA (Fig. 2) and PCO (Fig. 3) plots of morphological characters clearly separated the studied species. However, almost within each species cluster, the populations differed somewhat from each other. PCA analysis of morphological data revealed that three first components comprised about 78% of the total variance (data not shown). In the first component with about 42% of total variance, morphological characters, including basal leaf form, capsule situation to calyx, alternate filament length, basal and cauline leaf length, alar and lateral pedicel length and epipetalus filaments length to claw showed the highest correlation (>0.7). In the second component with about 20% of total variance, basal leaf width, length/width basal and calyx tooth length had the highest correlation (>0.7). In the third component (15% of total variance), cauline leaf form and capsule form had the highest correlation (>0.7).

Based on UPGMA (Fig. 1) dendrogram, PCA (Fig. 2) and PCO (Fig. 3) plot of morphological data, *S. nurensis* populations as Zardkuh and Kuh-e Nir are separated from others by features like plant length, basal leaves length, shape, length and width of caulinar leaves, alar and lateral pedicel length, claw position in calyx, calyx gap length, antophore length and length and width of capsule, although there are some similarities between *S. nurensis* populations and *S. commelinifolia* var. *commelinifolia.*

Populations of Touchal, Mazandaran, Haraz, Marmisho and Alisadr from *S. commelinifolia* var. *commelinifolia* show some differences at varietal level. Populations of *S. commelinifolia* var. *commelinifolia* and *S. commelinifolia* var. *ovatifolia* are grouped in two separate subsets and differed

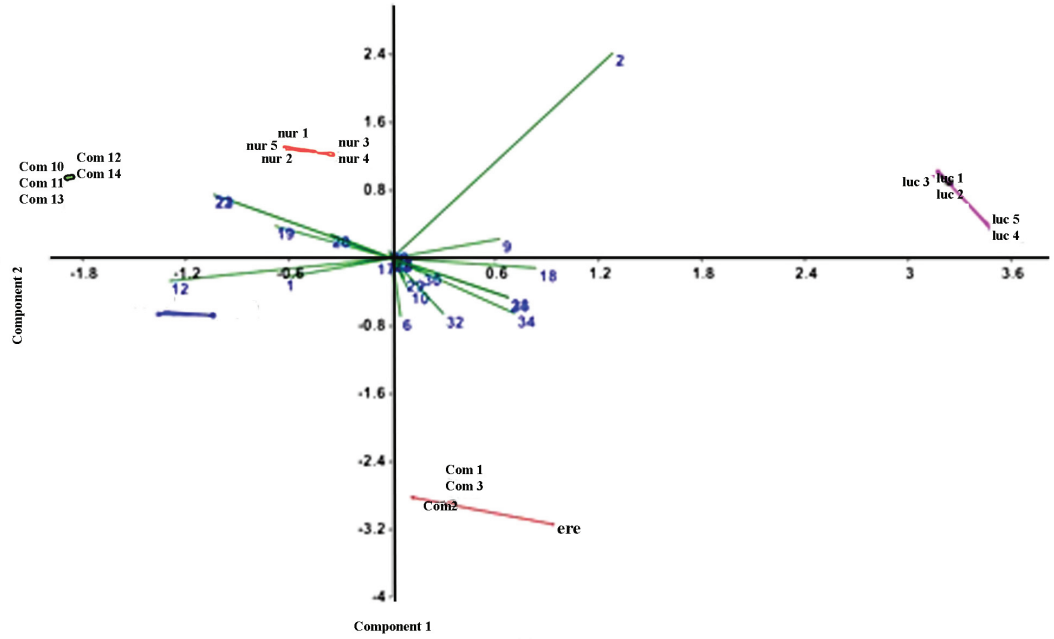

Figure 2. PCA plot of studied *Silene* species based on morphological characters. Species code: com5, 6, 7, 8, and 9 = *S. commelinifolia* var. *commelinifolia*; com10, 11, 12, 13, and 14 = *S. commelinifolia* var. *ovatifolia*; com1, 2, and 3= *S. cf commelinifolia*; nur = *S. nurensis*; luc = *S. lucida* and ere = *S. eremicana.*

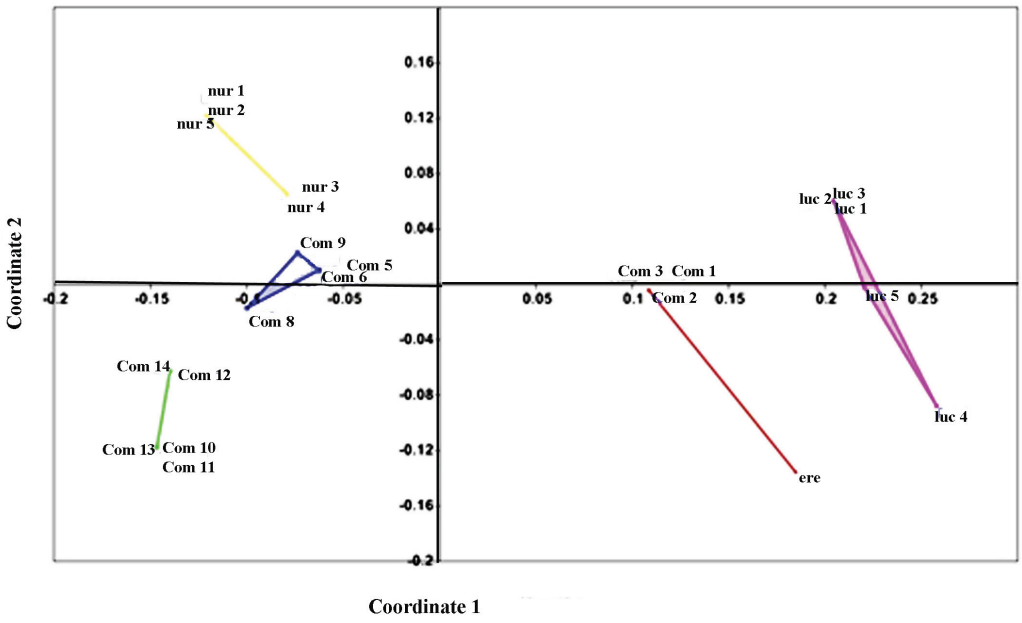

Figure 3. PCO plot of *Silene* species based on morphological characters. Species code: com5,6,7,8,9 = *S. commelinifolia* var. *commelinifolia*, com10,11,12,13,14 = *S. commelinifolia* var. *ovatifolia*, com1,2,3= *S. cf commelinifolia*, nur = *S. nurensis*, luc = *S. lucida* and ere = *S. eremicana*.

in features such as basal leaf width, width to length of basal leaves, shape and width of caulinar leaves, width to length of caulinar leaves, alar and lateral pedicel.

Our morphological observations of Alvand population from *S. eremicana* showed that it is the same plant that is identified as *S. commelinifolia* var. *isophylla*, so as Gholipour and Sheidai (2010c) had suggested it should be considered as synonym for *S. commelinifolia* var. *isophylla*. Kivi, Neor and

Table 3. Karyotype features of the *Silene* species studied.

Kf	St	C.V	A1	X	TF%	L/S	S(µm)	L(µm)	T.L(µm)	2n	Code	Locality	Species
12m	1A	16.00%	0.76	1.99	43.05%	1.83	1.43	2.61	23.91	24	Com7	Marmisho	*S. commelinifolia* var. *commelinifolia*
12m	1A	18.90%	0.83	2.07	45.36%	1.96	1.37	2.69	24.83	24	Com9	Touchal	*S. commelinifoli* var. *commelinifolia*
12m	1B	19.61%	0.81	2.03	44.64%	2.09	1.31	2.74	24.41	24	Com5	Mazan-daran	*S. commelinifoli* var. *commelinifolia*
12m	1A	18.72%	0.76	1.99	43.03%	1.96	1.38	2.70	23.92	24	Com15	Dizin	*S. commelinifoli* var. *commelinifolia*
24m	1B	21.57%	0.78	1.99	43.68%	2.49	1.18	2.94	47.82	48	Com8	Alisadr	*S. commelinifoli* var. *commelinifolia*
24m	1B	19.06%	0.76	2.06	43.14%	2.18	1.34	2.92	49.34	48	Com16	Osh-torankuh	*S. commelinifoli* var. *commelinifolia*
11m+1Sm	1A	15.89%	0.70	1.84	40.94%	1.72	1.35	2.33	22.10	24	Com11	Darakeh	*S. commelinifoli* var. *ovatifolia*
24m	1B	20.50%	0.79	2.73	43.78%	2.39	1.66	3.96	65.63	48	Com2	Neor	*S. cf commelinifolia*
24m	1B	16.49%	0.77	2.21	43.20%	2.05	1.49	3.05	52.96	48	Com1	Kivi	*S. cf commelinifolia*
24m	1B	18.79%	0.74	2.44	42.73%	2.24	1.66	3.71	58.64	48	Com3	Bozqush	*S. cf commelinifolia*
12m	1A	17.22%	0.73	1.74	42.04%	1.89	1.21	2.28	20.93	24	Nur1	Zardkuh	*S. nurensis*
24m	1B	18.08%	0.80	2.05	44.50%	2.13	1.41	2.99	49.31	48	Luc6	Sepiarez	*S. lucida*
-	-	-	-	-	-	-	-	-	-	48	ere	Alvand	*S. eremicana.*
-	-	-	-	-	-	-	-	-	-	48	Com14	Piranshahr	*S. commelinifolia*

Abbreviations: TL = Total chromatin length (µm), L = Size of the longest chromosome pair (µm), S = Size of the shortest chromosome pair (µm), L/S = Ratio of the longest to shortest chromosome, TF = Total form percentage, X = Mean chromatin length (µm), A1 = Romero-Zarco indices, CV = Coefficient of variation, ST = Stebbins' symmetry class, KF = Karyotypic formulae.

Bozqush populations of S. *cf commelinifolia* are grouped with *S. eremicana* due to the similarity in features as shape, width and length of caulinar leaves, length of lateral pedicel, length of calyx gap and capsule shape. Same populations show some similarities in some features as plant size, shape, width and length of basal leaves, calyx length, antophore hair type, petal blade, claw and corona length, alternate to epipetalus stamens and alar pedicel length to *S. commelinifolia*.

Kuh-e Khalil, Gilan, Miyaneh and Bozqush populations of *S. lucida* are grouped in a separate set. These populations show some deviation from the type specimen of *S. lucida* as was described in flora Iranica in plant size, basal and caulinar leaves shape, width and length of basal and caulinar leaves, pedicel length, bract length and width, calyx length, length of calyx gap, length of corona, claw and capsule width and length. These are considered here as *S. cf lucida*.

Cytology

Details of karyotypic analyses in the *Silene* species studied are presented in Table 3 and Fig. 4. In *S. commelinifolia* five studied accessions of Marmisho, Touchal, Mazandaran, Dizin and Darakeh chromosome counts were 2n=2x=24 which is in concordance with previous results (Gholipour and Sheidai 2010a). Six studied accessions of Alisadr, Oshtorankuh, Neor, Kivi, Bozqush and Piranshahr chromosome counts were 2n=4x=48 for the first time. So *S. commelinifolia* showed two ploidy levels. In *S. eremicana* the Alvand population showed 2n=4x=48 which is in agreement with the previous counts (Gholipour and Sheidai 2010a). Sepiarez population of *S. lucida* had chromosome number 2n=4x=48 which is in concordance with the previous result (Gholipour and Sheidai 2010b). *S. nurensis* from Zardkuh population showed 2n=2x=24 chromosome number which is recorded for the first time.

The chromosomes were mostly metacentric (m), but a pair of sub metacentric (sm) chromosome was observed in the Darakeh population of *S. commelinifolia* (Table 3). The size of the longest chromosome varied from 2.28 µm in Zardkuh population of *S. nurensis* to 3.96 µm in Neor population of *S. cf commelinifolia* (Table 3), while the size of shortest chromosomes varied from 1.21 µm in Zardkuh population of *S. nurensis* to 1.66 µm in Neor and Bozqush populations of *S. cf commelinifolia*. The highest haploid total chromatin length as well as mean chromosome length occurred in population of *S. cf commelinifolia* (65.63 and 2.73 µm respectively), while the lowest value of the same occurred in Zardkuh population of *S. nurensis* (20.93 and 1.74 µm, respectively). The highest value of chromosomes size variation (CV= 21.57) occurred in Alisadr population of *S. commelinifolia* while the lowest CV (15.89) occurred in Darakeh population of *S. commelinifolia*.

So in higher CV values, variation in chromosomes sizes is more and karyotype is more asymmetric. Alisadr population has the highest CV values this indicated the highest values

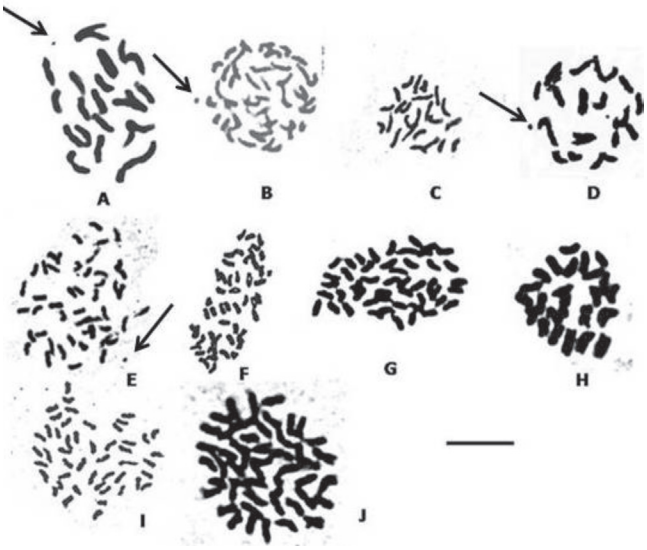

Figure 4. Representative somatic cells. A–C = Somatic metaphase cell in Marmisho, Oshtorankuh and Mazandaran population of *S. commelinifolia* var. *commelinifolia*, respectively. D = Somatic metaphase cell in Darakeh population of *S. commelinifolia* var. *ovatifolia*. E-G = Somatic metaphase cell in Bozqush, Neor and Kivi population of *S. cf commelinifolia*. H = Somatic metaphase cell in Zardkuh population of *S. nurensis*. I = Somatic metaphase cell in Bozqush population of *S. lucida*. J = Somatic metaphase cell in Alvand population of *S. eremicana*. A, B, D and E = arrows show B- chromosomes. Scale bar = 10 μm.

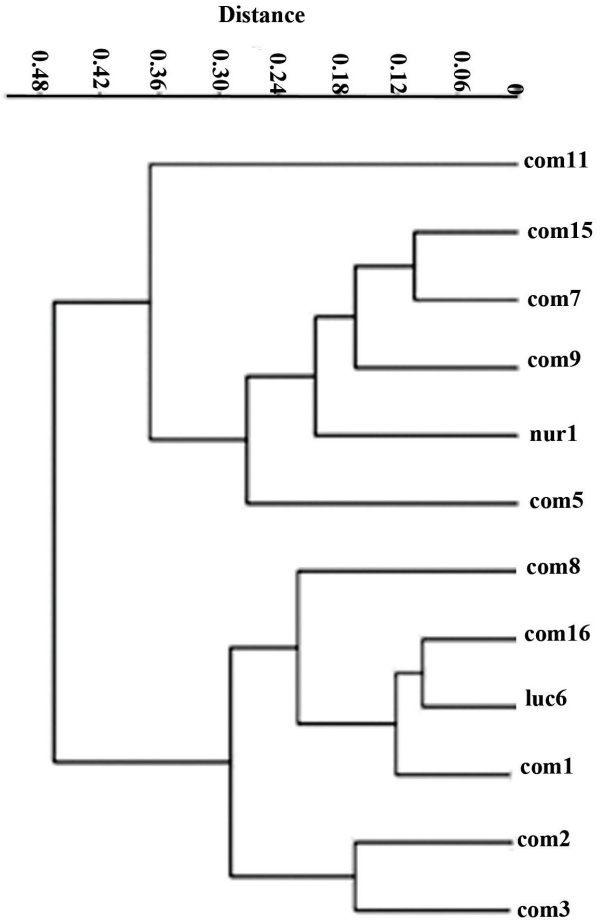

Figure 5. UPGMA clustering of *Silene* species based on karyotype data. Species abbreviations: com5, com 7, com 8, com 9, com 15 and com 16 = *S. commelinifolia* var. *commelinifolia*; com 11 = *S. commelinifolia* var. *ovatifolia*; com 1, com 2 and com 3 = *S. cf commelinifolia*; nur1 = *S. nurensis*, luc6 = *S. lucida*.

in chromosome size variation. The ANOVA and LSD tests revealed a significant difference ($p < 0.05$) for total size of the chromosomes, the size of the short arms and the long arms among the species and populations studied, indicating the role of quantitative genomic changes in the *Silene* species diversification. In PCO diagram (Fig. 7), this population is located far from other populations due to the difference in karyotype features. Total form percentage (TF%) varied from 40.94% in Darakeh to 45.36% in Touchal population of *S. commelinifolia* (Table 3); a higher value of TF% indicates the presence of relatively more symmetrical karyotype. Comparisons of karyotype symmetries based on Stebbins classification (1971) showed that Marmisho, Touchal, Dizin and Darakeh populations of *S. commelinifolia* and Zardkuh population of *S. nurensis* were of A1 class. Mazandaran, Alisadr, Oshtorankuh, Kivi, Neor and Bozqush accessions of *S. commelinifolia* and Sepiarez from *S. lucida* belonged to 1B class which is considered relatively primitive in Stebbins's system. Therefore, it seems that the *Silene* species studied are having symmetrical karyotypes.

Among the species placed in A1 class, Touchal population of *S. commelinifolia* shows a higher value of the A1 index (0.83) of Romero-Zarco index (A1) and, therefore, has a relatively more asymmetrical karyotype. All these results indicate the role of both quantitative and qualitative changes in the genome during the *Silene* species diversification. Lower intra-chromosomal asymmetry index (A1) was observed in Darakeh population (0.7%). The smaller the A1 values the more the frequency of metacentric chromosomes and the more is the symmetry of karyotype.

Different clustering methods, PCA and PCO plot of the *Silene* species based on karyotype data produced similar results (Figs. 5, 6 and 7). In UPGMA dendrogram of karyotype data (Fig. 5) two major clusters were formed; in the first major cluster 2n = 2x = 24 populations and in second one 2n = 4x = 48 populations were located. The first major cluster contains most *S. commelinifolia* populations with Zardkuh population of *S. nurensis* which show similarities and overlaps in morphological features too (Atazadeh 2013). In this sub-cluster, Darakeh population of *S. commelinifolia* var. *ovatifolia* was located far from other populations. Marmisho, Dizin and Touchal populations of *S. commelinifolia* var. *commelinifolia* showed the highest similarities.

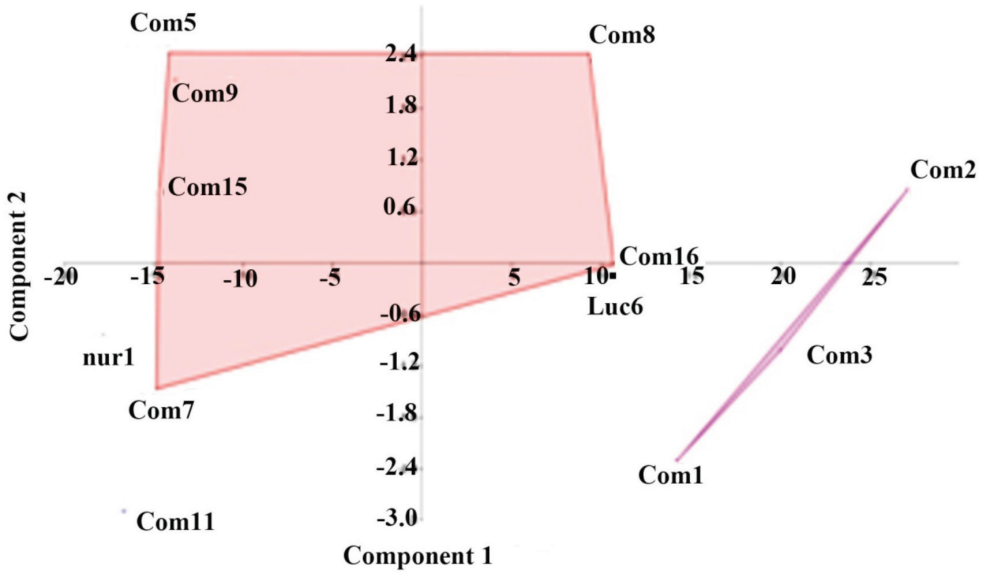

Figure 6. PCA plot of *Silene* species based on karyotype data. Species abbreviations: com 5, com 7, com 8, com 9, com 15 and com 16 = *S. commelinifolia* var. *commelinifolia*; com 11 = *S. commelinifolia* var. *ovatifolia*; com 1, com 2 and 3 = *S. cf commelinifolia*; nur 1 = *S. nurensis*; luc 6 = *S. lucida*.

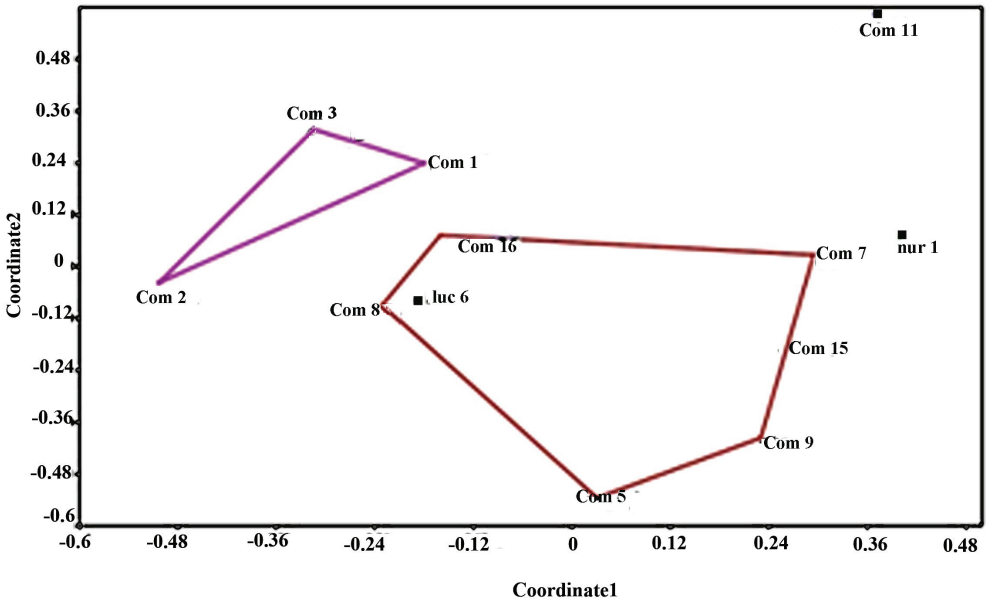

Figure 7. PCO plot of *Silene* species based on karyotype data. Species abbreviations: com 5, com 7, com 8, com 9, com 15 and com 16 = *S. commelinifolia* var. *commelinifolia*; com 11 = *S. commelinifolia* var. *ovatifolia*; com 1, com 2 and 3 = *S. cf commelinifolia*; nur 1 = *S. nurensis*; luc 6 = *S. lucida*.

In the second major cluster, Oshtorankuh and Alisadr of *S. commelinifolia* var. *commelinifolia* and Sepiarez population of *S. lucida* and Neor, Kivi and Bozqush populations of *S*. cf *commelinifolia* show more similarity and are placed together. In this cluster Oshtorankuh and Alisadr populations are more similar to *S. lucida*. Based on PCA (Fig. 6) and PCO (Fig. 7) plots, almost populations of *S. commelinifolia* varieties and *S. nurensis* and *S. lucida* composed a definite group. *S. nurensis* populations and Neor, Kivi and Bozqush population of *S*. cf. *commelinifolia* and *S. commelinifolia* var. *ovatifolia* are

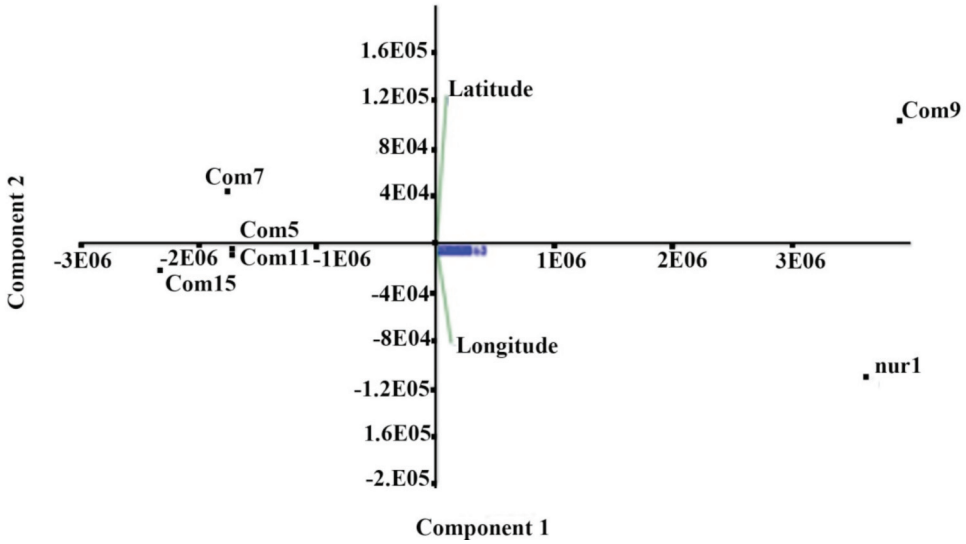

Figure 8. CCA plot of *Silene* species based on longitude and latitude for 2n = 24 populations. Species abbreviations: nur 1 = *S. nurensis*; com 9, com 7, com 15 and com 5 = Touchal, Marmisho, Dizin and Mazandaran populations of *S. commelinifolia* var. *commelinifolia*; com 11 = Darakeh populations of *S. commelinifolia* var. *ovatifolia.*

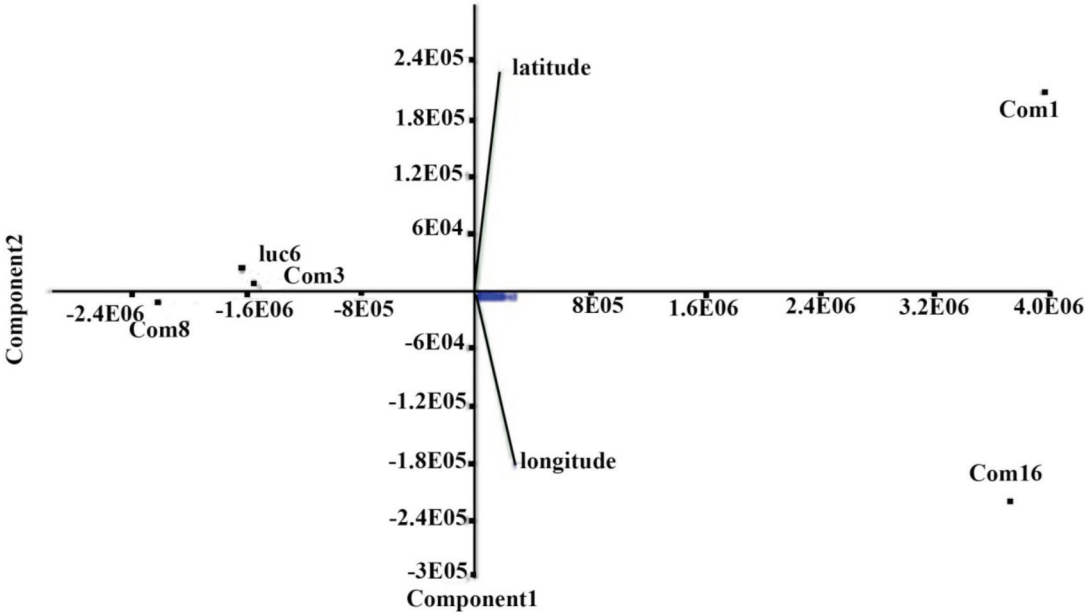

Figure 9. CCA plot of *Silene* species based on longitude and latitude for 2n=48 populations. Species abbreviations: luc 6 = *S. lucida*; com 16 and com 8 = Oshtorankuh, Alisadr populations of *S. commelinifolia* var. *commelinifolia;* com 1 and 3= Kivi, Bozqush populations of *S. cf commelinifolia.*

considered as a separate group. In PCA ordination diagram taxa are clearly separated (Fig. 6).

In populations of *ovatifolia* and *commelinifolia* varieties, two ploidy levels were observed. In *S. commelinifolia* var. *commelinifolia* populations a high karyotypic variation were observed due to two ploidy levels. CCA plot based on latitude and longitude for 2n = 2x = 24 accessions (Fig. 8) it was evident that longitude is efficient in *S. nurensis* separation and latitude in separation of Touchal population of *S. commelinifolia* var. *commelinifolia*. For 2n = 4x = 48 populations, PCA plot (Fig. 9) based on latitude and longitude Oshtorankuh population of *S. commelinifolia* var.

commelinifolia by longitude and Kivi population of *S. cf commelinifolia* by latitude was separated. In the correlation analysis of karyotypic features there was a significant positive correlation between L, S, TL and L/S.

Polyploidy is abundant in most perennial plants which result in adaptation of genomic variation. In *Silene* polyploidy has a great role in speciation and evolution of taxa (Sheidai et al. 2012; Gholipour and Sheidai 2010a, b; Sheidai et al. 2009a, b; Sheidai et al. 2008). Polyploidy is a way for stabilization of equilibrium polymorphism by an increase in heterozygosity. Polyploidy provides gene pool richness and can be used in environmental changes and evolution (Sheidai 2002).

B-chromosomes

Marmisho, Mazandaran, Darakeh, Oshtorankuh, Alisadr and Bozqush populations of *S. commelinifolia* has 0 to 3 B chromosomes (Fig. 4) which are recorded for the first time. B chromosomes are smaller than "A" chromosomes. Such subsidiary chromosomes are found in more than 1300 plant species (Camacho et al. 2000). Evidences showed the adaptive success of organisms with B chromosomes under stress condition. Plants with B chromosomes are more tolerant to dryness and environmental stresses than those lacking such chromosomes (Sheidai 2002).

Conclusion

In morphological results we found some efficient diagnostic features as was mentioned before for studied species and varieties separation. Based on PCA and PCO ordination diagrams of morphological character names of the specimens of Payame Nour University, Sari Branch Herbarium, Alzahra Herbarium and Shahid Beheshti University Herbarium were corrected. The position of Neor, Kivi and Bozqush populations were defined due to results of morphological and karyotype studies and seemed to be a new sub-species of *S. commelinifolia*. Kuh-e Khalil, Gilan, Miyaneh and Bozqush populations here referred to as *S.* cf. *lucida* seemed to be a new species of *Silene* for Iran but not exactly *S. lucida*. PCA & PCO ordination graphs and dendrograms provided by karyotype features indicated the clear separation of species and varieties.

Karyotype analysis of *S. commelinifolia* indicated that there are two ploidy levels in this species as 2n = 2x = 24 (which is in concordance with previous findings of Gholipour and Sheidai 2010a) and 2n = 4x = 48 which is recorded for the first time. B chromosome is recorded for the first time for this species in the world.

As there are two ploidy levels in each variety of *S. commelinifolia* a great morphological variation is also observed in these populations. Polyploidy has a great effect on the phenotype of the organism. Morphological and genomic differences between populations of these two varieties seemed to be more than variety here we propose further phylogenetic and molecular studies to clarify the rank of these taxa.

References

Atazadeh N (2013) Population divergence of *Silene commelinifolia* in Iran. MSc Thesis, University of Alzahra, Iran.

Bari EA (1973) Cytological studies in the genus *Silene* L. New Phytol 72:833-838.

Burleigh JG, Holtsford TP (2003) Molecular systematics of the eastern North American *Silene* (Caryophyllaceae): Evidence from nuclear ITS and chloroplast trnL intron sequences. Rhodora 105:76–90.

Camacho JPM, Sharbel TF, Beukeboom LW (2000) B- chromosome evolution. Phil Trans of the R Soc Lond, B 355:163-178.

Chowdhuri PK (1957) Studies in the genus *Silene*. Notes from the Royal Botanic Garden. Edinburgh 22:221-287.

Gholipour A, Sheidai M (2010c) A taxonomic study of *Silene goniocaula* complex in Iran. Rostaniha 11(1):83-86.

Gholipour A, Sheidai M (2010b) Further contribution to cytotaxonomy of the genus *Silene* L. (Sect. *Auriculatae*, Caryophyllaceae). Acta Biol Szeged 54:111-115.

Gholipour A, Sheidai M (2010a) Karyotype analysis and new chromosome number reports in *Silene* L. species (Sect. *Auriculatae*, Caryophyllaceae). Biologia 65:23-27.

Greuter W (1995) *Silene* (Caryophyllaceae) in Greece: A subgeneric and sectional classification. Taxon 44:543-581.

Heaslip MB (1951) Some cytological aspects in the evolution of certain species of the plant genus *Silene*. Ohio J Sci 51:62-70.

Levan A, Fredga K, Sandberg A (1964) Nomenclature for centromeric position on chromosomes. Hereditas 52:201- 220.

Markova M, Martina L, Zluvova J, Janousek B, Vyskot B (2006) Karyological analysis of an interspecific hybrid between the dioecious *Silene latifolia* and the hermaphroditic *Silene viscose*. Genome 42:373-379.

Melzheimer, V. 1988. Caryophyllaceae in: Flora Iranica, Rechinger, K. H. (ed.), AkademischeDruck-U. Verlagsanstalt, Graz-Austria 163:353-508.

Melzheimer V (1978) Notes on cytology of several species of the genus *Silene* (Caryophyllaceae) from central Greece and from Crete. Pl Syst Evol 130:203-207.

Oxelman B, Lidén M, Berglund D (1997) Chloroplast *rps*16 intron phylogeny of the tribe Sileneae (Caryophyllaceae). Pl Syst Evol 206:411–420.

Podani J (2000) Introduction to the Exploration of Multivariate Data. English translation. Backhuyes Publishers, Leiden, p. 407.

Romero-Zarco C (1986) A new method for estimating karyotype asymmetry. Taxon 35:526 530.

Sheidai M, Jalilian N (2008) Karyological studies of some species and populations of *Lotus* L. in Iran. Acta Bot Croat 67:42-52.

Sheidai M, Rashid S (2007) Cytogenetic study of some *Hordeum* L.species in Iran. Acta Biol Szeged 51:107-112.

Sheidai M (2002) Cytogenetic. Tehran, Adena publisher (in Persian).

Sheidai M, Nikoo M, Gholipour A (2008) Cytogenetic variability and new chromosome number reports in *Silene* L. species (Sect. *Lasiostemones*, Caryophyllaceae). Acta Biol Szeged 52(2):313-319.

Sheidai M, Enayatkhani M, Bahmani F, Gholipour A (2009a) Cytological study in the genus *Silene* (sec. Sclerocalycinae). Cytologia 74:437-442.

Sheidai M, Bahmani F, Enayatkhani M, Gholipour A (2009b) Contribution to cytotaxonomy of *Silene*: Chromosome pairing and unreduced pollen grain formation in sec. *Sclerocalycinae*. Acta Biol Szeged 53: 87-92.

Sheidai M, Gholipour A, Noormohammadi Z (2010) Species relationship in the genus *Silene* L. Section *Auriculatae* (Caryophyllaceae). Based on morphology and RAPD analysis. Acta Biol Szeged 54(1):25-31.

Sheidai M, Eftekharian R, Gholipour A, Noormohammadi Z (2012) Population Diversity and Polyploidy Incidence in 3 *Silene* Species. A Cytologi-

cal Approach. Cytologia 76(4):1-8.
Sopova M, Sekovski Z (1982) Chromosome atlas of some Macedonian angiosperms. III. Godishen Zbornik Bioloshki Fakultet na Univerzitetot Kiril i Metodij 35:145-161.
Stebbins GL (1971) Chromosomal Evolution in Higher Plants. Edward Arnold, London.
Swank GR (1932) The Ethnobotany of the Acoma and Laguna Indians. MA Thesis, University of New Mexico.
Zhang Y-x (1994) Studies on chromosomes of some plants from Guandi Mountain, Shanxi. J. Wuhan Bot Res 12(2):201-206.