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ARTICLE

Further contribution to cytotaxonomy of the genus *Silene* L. (Sect. *Auriculatae*, Caryophyllaceae)

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ABSTRACT The present study reports the chromosome numbers of 18 *Silene* L. species subspecies and varieties from the sect. *Auriculatae* for the first time. *S. commelinifolia* var. *isophylla, S. commelinifoilia* var. *ovatifolia, S. araratica, S. meyeri* ssp. *persica, S. nizvana, S. oligophylla, S. persica* and *S. rhynchocarpa* showed 2n = 2x = 24 chromosome number, *S. pseudoaucheriana, S. gynodioica, S. erysimifolia, S. guntensis, S. goniocaula, S. lucida S. microphylla* showed 2n= 4x= 48 and *S. hirticalyx* had 2n=6x=72 chromosome number. The size of the chromosomes varied from 1.53 µm in Ahvan population of *S. commelinifolia* var. *commelinifolia* to 4.97 µm in *S. oligophylla.* The chromosomes were metacentric (m) and sub-metacentric (sm). The species studied differed significantly in total size of the chromosomes, size of the short arms and the long arms indicating the role of quantitative changes of chromosomes in species diversification. The *Silene* species differed in karyotype formulae and symmetry indicating qualitative changes in the chromosomes possibly due to the occurrence of structural changes. Different clustering and ordination methods showed karyotype distinctness of the species studied.
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KEY WORDS

Karyotype
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Silene L. is the largest genus of Caryophyllaceae with about 700 species distributed throughout the northern hemisphere; Europe, Asia and northern Africa (Greuter 1995). *Silene* includes several important weeds, very beautiful horticultural plants and some medicinal species (Swank 1932). About 110 *Silene* species grow in Iran out of which about 35 species are endemic with very limited geographical distribution (Melzheimer 1988). *Silene* species have been placed the in 44 sections (Chowdhuri 1957), but recent molecular studies do not support such sectional classifications particularly for the endemic North American taxa (Oxelman and Lidén 1995; Liden and Berglund 1997; Oxelman et al. 2001; Burleigh and Holtsford 2003). The available literature from the other parts of the world dealing with cytogenetics of *Silene* indicates the importance of such studies (Heaslip 1951; Bari 1973; Melzheimer 1978; 1980; Markova et al. 2006), while similar data is lacking for the species growing in Iran and only recently karyotype details of few *Silene* species has been reported (Sheidai et al. 2009; Gholipour and Sheidai 2010).

The basic chromosome number of *Silene* is *x* = (10) 12, most of the species are diploid (2n = 2x = 24), some are tetraploid (2n = 4x = 48) and hexaploid (2n = 6x = 72). Few species show higher polyploidy levels for e.g. 2n = *c.* 96, 120 and 192 (Bari 1973), while 2n = 3x = 30 is reported for *S. fortunei* (Heaslip 1951).

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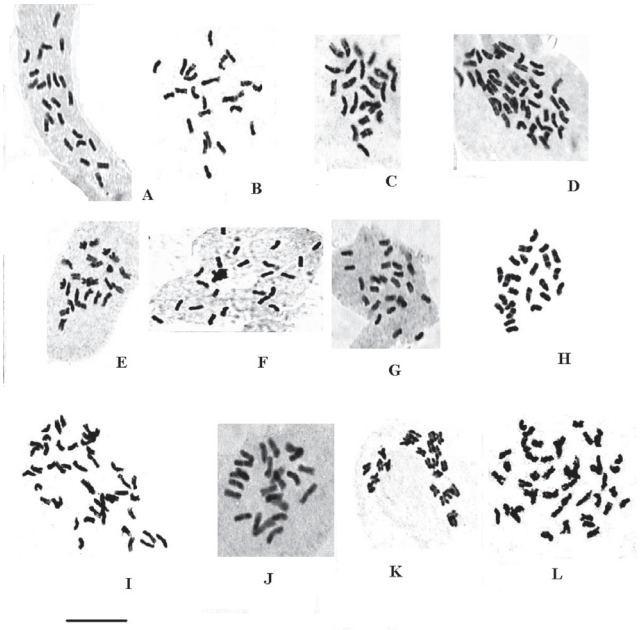
The section *Auriculatae* (Boiss.) Schischkin is the largest section of the genus containing about 35 species in Iran, out of which 21 species are endemic with very restricted distribution in mountainous areas such as Elburz, Zagros and Azarbayejan (Melzheimer 1988). The members of this section are caespitose plants with large flowers placed at the end of short stems. Their inflorescence is unifloral or dichsial. Calyx is cylindrical–clavate, pubescent or glandular–pubescent. The petals have conspicuous auricule at the end of claw. The present study reports the chromosome number of 18 *Silene* species from the sect. *Auriculatae* growing in Iran and also provides karyotype details of 16 diploid populations of 8 *Silene* species for the first time.

Materials and Methods

Plant material

Chromosome numbers were determined in 24 populations of 18 *Silene* species, subspecies and varieties from the sect. *Auriculatae* L. growing in Iran. The species studied are: 1- *S. commelinifolia* Boiss. var. *commelinifolia*, 2- *S. commalinifolia* var. *isophylla* Bornm., 3- *S. commelinifolia* var. *ovatifolia* Melzh., 4- *S. aucheriana* Boiss., 5- *S. nizvana* Melzh., 6- *S. oligophylla* Melzh., 7- *S. meyeri* Fenzl ex Boiss. ssp. *persica* Melzh, 8- *S. rhynchocarpa* Melzh., 9- *S. persica* Boiss., 10- *S. araratica* Schisck., 11- *S. pseudoaucheriana* Melzh., 12- *S. gynodioica* Ghazanfar; 13- *S. lucida* Chowdhuri; 14- *S. erysimifolia* Stapf., 15- *S. guntensis* B. Fedtsch. 16- *S. goniocaula* Boiss., 17- *S. hirticalyx* Boiss.; and 18- *S. microphylla*

Figure 1. Representative somatic metaphase cells in *Silene* species studied. A= Metaphase cell showing 2n = 24 in *S. araratica*, B = Metaphase cell showing 2n = 48 in Bozghosh population of *S. aucheriana,* C = Metaphase cell showing 2n = 24 in Darake population of *S. commelinifolia* var. *ovatifolia,* D = Metaphase cell showing 2n = 24 in *S. gynodioica*, E = Metaphase cell showing 2n = 24 in *S. commelinifolia* var. *commelinifolia*, F = Metaphase cell showing 2n = 24 in *S. oligophylla*, G = Metaphase cell showing 2n = 24 in *S. meyeri* ssp. *Persica,* H = Metaphase cell showing 2n = 24 in Polor population of *S. persica*, I = Metaphase cell showing 2n = 24 in *S. pseudoaucheriana*, J = Metaphase cell showing 2n = 24 in *S. nizvana*, K = Metaphase cell showing 2n = 24 in *S. commelinifolia* var. *isophylla*, L = Metaphase cell showing 2n = 48 in Neor population of *S. lucida*. Scale bar = 10 µm.



Boiss. The voucher specimens are deposited in Herbarium of Shahid Beheshti University (HSBU).

Cytological studies

For karyotypic 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 hrs.) and fixed in ethanol: acetic acid (3:1) for 24 hrs. The fixed tips were then washed thoroughly in distilled water and macerated in 60°C 1N HCl for about 5 min. Squash technique was used for cytological studies with 2% aqueous aceo-orcein as the stain. The somatic chromosome number and karyotype details were studied in at least 5 well-prepared metaphase plates. The chromosomes were photographed by digital camera and measured by Image Tools3 software (Sheidai and Rashid 2007).

The chromosomes were identified according to Levan et al. (1964), karyotype symmetry was determined according to Stebbins (1971), while other karyotype parameters like total form percentage (TF %), coefficient of variation (CV) of the chromosome size as well as A1 and A2 indices of Romero-Zarco (1986) were determined (Sheidai and Jalilian 2008).

Statistical analyses

In order to reveal significant difference, the analysis of variance (ANOVA) followed by the least significant difference test (LSD) were performed on the size of chromosomes, size of the long arms and size of the short arms as well as arms ratio among the species and populations studied (Sheidai and Jalilian 2008). Moreover, principal components analysis (PCA) was performed to identify the most variable karyotypic characters. SPSS ver. 9 (1998), NTSYS ver. 2.1 (1998) and DARwin ver. 5. (2008) was used for statistical analysis.

In order to group the species studied based on similarity in their karyotypic features, UPGMA (Unweighted Paired Group with Arithmetic Average) and Neighbor Joining (NJ) clustering methods as well as ordination based on principal components analysis (PCA) and principal coordinate analysis (PCO) were performed. NTSYS Ver. 2.02 (1998) was used for clustering and PCO analyses. Standardized karyotypic data (mean = 0, variance = 1) were used to determine taxonomic distance among the species which were used in clustering (Sheidai and Jalilian 2008). Cophenetic correlation was estimated to determine the goodness of fit of the clusters to the original data (Sheidai and Jalilian 2008).

Results

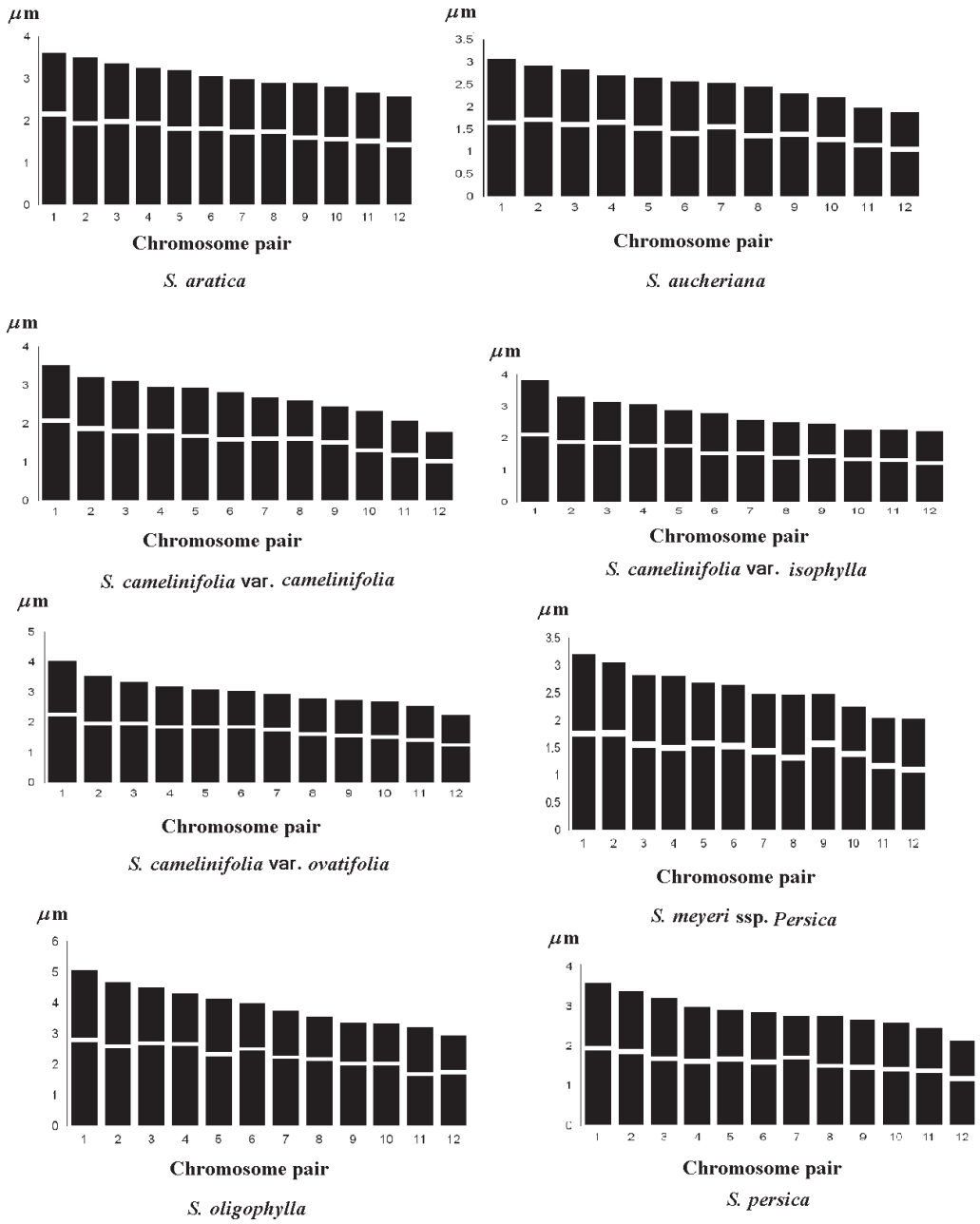
The somatic chromosome number and details of karyotypes of the *Silene* species studied are presented in Figsures 1-4. Nine species, subspecies and varieties studied showed 2n = 2x = 24 chromosome number, while the other species showed 2n = 4x = 48 and 2n = 6x = 72 chromosome number.

The size of the longest chromosome varied from 2.97 µm in Damavand population of *S. aucherianai* to 4.97 µm in *S. oligophylla*, while the size of shortest chromosomes varied from 1.53 µm in Ahvan population of *S. commelinifolia* var. *commelinifolia* to 2.75 µm in *S. oligophylla*. The chromosomes were mainly metacentric (m) and sub-metacentric (sm; Fig. 2).

The highest haploid total and mean chromosome length occurred in *S. oligophylla* (45.67 & 3.8 µm respesctively), while the lowest values of the same parameters occurred in Damavand population of *S. aucheriana* (28.95 & 2.41 µm, Table 1). The highest value of the ratio of longest to shortest chromosome occurred in Ahvan population of *S. commelinifolia* var. *commelinifolia* (2.16) while the lowest value of the same occurred in *S. nizvana* (1.53).

The highest value of coefficient of variation (CV) (33.00) for the size of chromosomes occured in Damavand population of *S. aucheriana* indicating the highest degree of size variation among its chromosomes, while the least CV value (16.00) occured in *S. rhynchocarpa*. Total form percentage value (TF%) varied from 41 in *S. commelinifolia* var. *isophylla*

Figure 2. Representative idiograms of *Silene* species studied.



and *S. oligophylla* to 45.5 in *S. rhynchocarpa* and *S. meyeri* ssp. *persica*, a higher value of TF% indicates the presence of relatively more symmetrical karyotype. The species studied also differ in their karyotype formula.

The *Silene* species were placed in 1A and 1B classes of Stebbins 1971 karyotype classification which are considered relatively primitive in this system. Ahvan population of *S. commelinifolia* var. *commelinifolia* shows the highest asymmetric karyotype among the species studied as it stands in 1B class of Stebbins classification. Among the species placed in 1A class, Damavand population of *S. aucheriana* shows a higher value of Romero-Zarco A1 index (0.33) and has relatively a more asymmetrical karyotype.

Different clustering methods and PCO ordination of the *Silene* species based on karyotypic data produced similar results (Figs. 2 and 3). UPGMA dendrogram showed the highest cophenetic correlation (r>0.80). In general two major clusters were formed; the first major cluster is comprised of

Figure 3. UPGMA clustering of *Silene* species based on karyotypic data. Species code: com1-5 = *S. commelinifolia* Ahvan population, Shahrood population, Solok population, Touchal population and Darake population respectively, auch1-4 = Shahrood population, Neor population, Damavand population and Anguran population of *S. aucheriana* respectively, mpers = *S. meyeri* ssp. *persica,* rhyn = *S. rhynchocarpa,* nizvan = *S. nizvana*, oligo = S. *oligophylla*, persica = *S. persica*, ara = *S. araratica*.

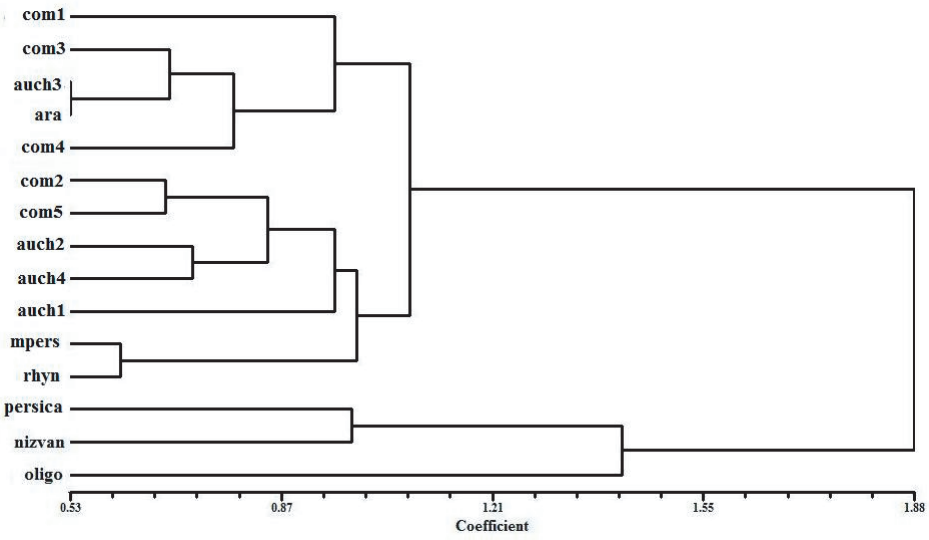
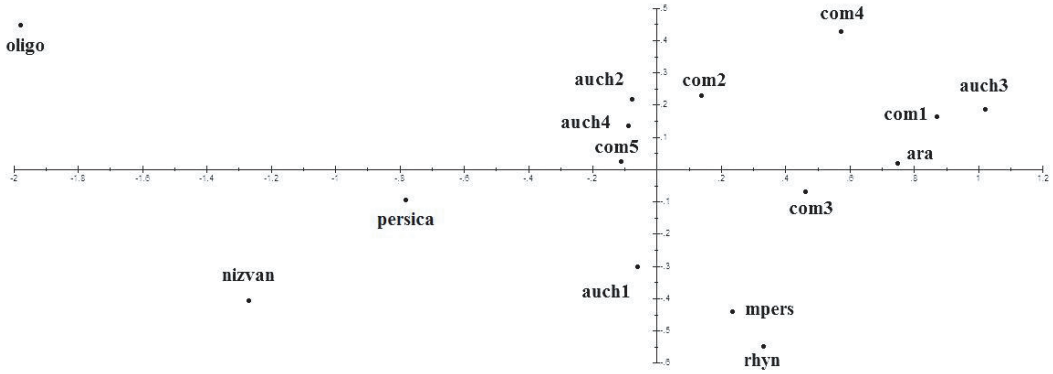


Figure 4. PCA plot of *Silene* species based on karyotypic data. Species code as in Fig. 2.



two sub-clusters. Three populations of *S. commelinifolia* var. *commelinifolia* (com1, com3 and com4 in Fig. 2), show more similarity and are grouped together in the first sub-cluster, while populations of *S. commelinifolia* var. *isophylla* and *S. commelinifolia* var. *ovatifolia* (com2 & com5 in Fig. 2) are placed in the second sub-cluster. Similarly three populations of *S. aucheriana* (auch 1, auch 2 & auch 4 in Fig. 2) show more similarity and are placed together, while Damavand population of *S. aucheriana* (auch3 in Fig. 2) differs in its karyotype features and is placed in another sub-cluster.

S. persica, S. nizvana and *S. oligophylla* form the second major cluster showing more differences from the other species studied. PCA and PCO ordination (Fig. 3) support the clustering results.

Discussion

The results obtained support the earlier report on *S. araratica* (Nersesian and Goukasian 1995), while the chromosome numbers of *S. commelinifolia* var. *isophylla, S. commelinifoilia* var. *ovatifolia, S. meyeri* ssp. *persica, S. aucheriana*, *S. nizvana*, *S. oligophylla*, *S. persica*, S. *rhynchocarpa*, *S. pseudoaucheriana*, *S. gynodioica*, *S. erysimifolia*, *S. guntensis*, *S. goniocaula, S. lucida*, *S. microphylla* and *S. hirticalyx* are new to science. *S. aucheriana* showed both 2n = 2x = 24 and 2n = 4x = 48 chromosome numbers.

Polyploidy is of limited occurrence in the genus *Silene* reported in some of arctic and subarctic species and in some species endemic to North America (Oxelman et al. 2000;

Popp and Oxelman 2007). The present study shows that about 30% of the species in the section *Auriculatae* growing in Iran are polyploid.

ANOVA and LSD tests showed significant differences ($p < 0.01$) for total size of the chromosomes, size of the short arms and the long arms among the species; subspecies and varieties studied, indicating the role of quantitative genomic changes in the *Silene* species diversification.

Pearson correlation determined among the karyotypic features showed a positive significant correlation between the mean chromosomes length and the size of the short and long arms of the chromosomes ($r > 0.70$, $p < 0.01$). Therefore the significant quantitative change in the chromosomes has been occurred in the size of both chromosomes arms during the species diversification.

Difference of the species in TF%, karyotype formulae and symmetry of karyotype indicate the occurrence of structural changes in their chromosomes which is also supported by PCA analysis given bellow.

PCA analysis (data not given) showed that the first 3 components comprise about 81% of the total variation. In the first component with about 66% of total variance, the mean chromosome length, size of the short arms and long arms as well as total length of the chromosomes are the most variable characters ($r > 0.80$), supporting the results of ANOVA stated earlier.

In the second factor with about 9% of total variance, the ratio of the long arm to the short arm of the chromosomes pair number 4, 7, 8 and 9 are most variable characters ($r > 0.65$), supporting our earlier suggestion about the role of qualitative changes in the *Silene* species diversification. In the third factor with about 5% of total variance, the ratio of the long arm to the short arm of the chromosomes pair numbers 1, 11 and 12 are most variable characters ($r > 0.60$). Clustering and PCO ordination of the *Silene* species indicate karyotypic distinctness of the *Silene* species studied.

Morphological studies performed on the section, supports karyotype analysis (Sheidaei et al. 2010). Separation of the *S. oligophylla*, *S. persica* and *S. nizvana* from the other species studied are supported by morphological analysis. Close affinity of *S. oligophylla* and *S. nizvana* has also been shown by both karyotype and morphological analyses. Close affinity between *S. araratica* and *S. commelinifolia* var. *commelinifolia* and *S. commelinifolia* var. *ovatifolia* due to shared morphological characters like reticulate calyx veins, large conspicuous auricle, claw placed in calyx, is also observed in both karyotype and morphological analyses. Therefore, karyotype features can be used in both species delimitation and illustrating species relationships in the genus *Silene*.

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