

Karyological Studies in Some Species of the Genus *Silene* (Caryophyllaceae)

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Summary Karyology of the 6 *Silene* species from Turkey was investigated. Diploid chromosome numbers and the basic chromosome number of the studied taxa were obtained as $2n=24$ and $x=12$ respectively. The karyotype analysis of *S. viscosa*, *S. eremitica*, *S. skorpilii* and *S. confertiflora* were carried out for the first time. Also the chromosome numbers of *S. eremitica* and *S. confertiflora* were determined for the first time. The largest chromosomes were observed in *S. otites* and the smallest ones were observed in *S. viscosa*. *S. otites* shows the highest A_1 index. *S. skorpilii* represents the highest interchromosomal asymmetry coefficient (A_2). The karyologic results of the study and the analysis of chromosomal morphology of each taxon were shown as tables.

Key words Caryophyllaceae, Chromosome, Karyology, *Silene*.

Silene L. (Caryophyllaceae) comprises about 750 species, of which about half occur in the Mediterranean area to which no less than 40% are endemic (Greuter 1997). *Silene* is one of the species-rich genera in Turkey. It is represented by 31 sections and 154 taxa which belong to 135 species in “Flora of Turkey” (Coode and Cullen 1967, Davis *et al.* 1988, Tan and Vural 2000). After the publication of the last supplement of “Flora of Turkey” 7 species and 1 subspecies have been described (Aytaç 1998, Duran and Menemen 2003, Deniz and Düsen 2004, Özgökçe *et al.* 2005, Genç *et al.* 2007, Bağcı *et al.* 2007, Bağcı 2008, Tugay and Ertugrul 2008). Therefore the genus is represented by 162 taxa that belong to 142 species in Turkey and 48% of them are endemic to Turkey. In *Silene*, some studies on molecular genetic studies (Popp and Oxelman 2001), pollen morphology studies (Yıldız 2006), chemical studies (Larhsini *et al.* 2003, Dötterl *et al.* 2005), and studies on the chromosome number and morphology (Guardia 1987, Colombo and Marcenó 1990, Westerbeigh and Saura 1994, Nersesian-Anoush and Goukasian-Anahit 1995, Yıldız and Çirpici 1996, Šioroký *et al.* 2001, Yıldız and Minareci 2008) have been conducted. The chromosome numbers of the genus *Silene* are reported as $2n=20$, 24 and 48 in 36 taxa in the Turkish Flora (Davis *et al.* 1988, Güner *et al.* 2000).

Many karyological and taxonomical studies have been performed on *Silene* sections. Prentice (1978, 1979, 1980, 1988) and Nigtevecht and Prentice (1985) karyologically and taxonomically studied the section *Elisanthe* (Fenzl) Fenzl. Moreover, Wringley (1986) studied section *Otites* (Adans.) Otth. karyologically and taxonomically. There are 6 *Silene* species that take place in *Chloranthae* (Rohrb.) Schischk., *Tataricae* Chowdhuri and *Otites* sections in Turkey. Of these, 4 *Silene* species have had chromosome number determined. As far as the species of the present study are concerned, the diploid chromosome number $2n=24$ has been found in *S. viscosa* (L.) Pers., *S. paphlagonica* Bornm., *S. skorpilii* Vel. and *S. otites* Wibel (Fedorov 1974, Moore, 1982, Yıldız and Çirpici 1996, Greuter 1997). Investigated species are *S. viscosa* and *S. paphlagonica* from section

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Chloranthae, *S. eremitica* Boiss. and *S. skorpii* Vel. from section *Tataricae*, *S. otites* and *S. confertiflora* Chowdh. from section *Otites*. In the present study, we report the results of a study on 6 related species. All these species were comparatively investigated in terms of karyology. Such studies are important for our understanding of taxonomy and evolution in/inter the group concerned.

Materials and methods

We examined 6 species of *Silene* sections *Chloranthae*, *Tataricae* and *Otites* from Turkey. Karyological observations were based on the material collected between 2005 and 2006 from natural populations (Table 1). Mature seeds were sampled in the field as well as from specimens at Marmara University, Faculty of Science and Letters Herbarium (MUFE), Faculty of Science, the University of Istanbul (ISTF) and Herbarium of Gazi University (GAZI). In order to establish the karyotype the seeds were germinated in humidified petri dishes at room temperature in the laboratory. When root tips had grown to a length of 0.5–1.0 cm, they were exposed to a preliminary treatment of 0.5% colchicine between 1–5 h and were kept in aceto-orcein for 10–16 h. Subsequently, the root tips were treated in a 3 : 1 absolute alcohol: glacial acetic acid mixture. Finally, aceto-orcein squash method was applied to the root tips. The preparations obtained were examined and photographed by using an Olympus triocular microscope with D-plan 100–1.25 160/0.17 oil immersion objective and NFK X 3.3 LD 125 lens.

A great number of seeds taken from at least 5 specimens belonging to each taxon were germinated for the study. Then, 20 well-spread metaphase plates of each species were analyzed karyologically; measuring long arm (L), short arm (S) and total chromosome length (TCL); relative lengths (%); arm ratios (L/S); arm index and location of the centromeres that were used to construc-

Table 1. The location and habitats of studied specimens in *Silene*

Taxa	Specimen location and habitat	Voucher
<i>S. viscosa</i>	B4 Konya: Karapınar, Nasuhpınar village, salty meadow, 1020 m, 37°46'153"N, 33°35'203"E, 24 Jun 1997	GAZI 7809
<i>S. paphlagonica</i>	A4 Çankırı: Ilgaz mountain, Çankırı-Kastamonu city border, south slopes, glade area, 1850–1870 m, 41°03'925"N, 33°44'999"E, 15 July 2006	MUFE 12093
<i>S. eremitica</i>	Kars: Igdir, Turkey-Iran border, Subası field, salty-sand soil, 800 m, 39°42'558"N, 44°40'579"E, 19 July 1956	ISTF 16076
<i>S. skorpii</i>	A1(E) Edirne: From Edirne to Lalapasa, 0.5–1 km, hills, 150 m, 41°40'584"N, 6°35'029"E, 18 July 2006	MUFE 12387
<i>S. otites</i>	Kayseri: Gesi vineyards-Ildem, slope area, treeless fields, rocky steppe, 1200–1250 m, 38°46'929"N, 35°37'504"E, 04 July 2005	MUFE 12001
<i>S. confertiflora</i>	C6 Hatay: Dörtöyl, Topaktas plateau, ascent of Mıgır summit, 1100–1150 m, 36°49'265"N, 36°20'156"E, 10 July 2006	MUFE 12381

Table 2. Karyological features of *Silene* taxa. Long arm (L), short arm (S), mean chromosome length (CL), total haploid chromosome length (TCL), \pm st. dev. (SD)

Taxa	L (SD)	S (SD)	CL (SD)	TCL (SD)	Mean arm ratio L/S (SD)
<i>S. viscosa</i>	0.90 (0.2)	0.68 (0.1)	1.58 (0.3)	19.00 (2.7)	1.33 (0.2)
<i>S. paphlagonica</i>	0.99 (0.2)	0.69 (0.1)	1.69 (0.3)	20.25 (2.9)	1.48 (0.4)
<i>S. eremitica</i>	0.92 (0.2)	0.78 (0.1)	1.70 (0.3)	20.34 (3.3)	1.19 (0.3)
<i>S. skorpii</i>	1.50 (0.3)	1.12 (0.3)	2.61 (0.5)	31.33 (4.2)	1.42 (0.5)
<i>S. otites</i>	2.16 (0.3)	1.48 (0.4)	3.64 (0.6)	43.71 (3.1)	1.55 (0.5)
<i>S. confertiflora</i>	1.00 (0.2)	0.78 (0.2)	1.78 (0.2)	21.38 (2.8)	1.33 (0.3)

tion of the ideograms. Relative chromosome length and arm ratio were calculated according to Krikorian *et al.* (1983). Absolute chromosome length and the centromeric index were used for comparison of the karyotypes. Chromosome morphology was determined according to Levan *et al.* (1964). Karyotype asymmetry was calculated according to the indexes suggested by Romero Zarco (1986);

$$A_1 = 1 - \frac{\sum_{i=1}^n \frac{bi}{Bi}}{n}, \quad A_2 = \frac{s}{\bar{X}}$$

where b =length of short arm, B =length of long arm, n =number of chromosome pairs, X =mean chromosome length, and s =standard deviation of mean chromosome length.

Results

This study concerned 6 taxa of which 2 of them are endemic to Turkey. Chromosome numbers and the karyotype of those taxa were determined. The karyomorphology of *S. viscosa*, *S. eremitica*, *S. skorpilii* and *S. confertiflora* are here described for the first time. The caryological data are given in Table 2. The number of somatic chromosomes was found to be $2n=24$ for 6 *Silene* species (Fig. 1). The basic chromosome number is $x=12$.

Karyotypes

S. viscosa (Figs. 1A, 2A). All chromosome pairs at the mitotic metaphase phase were metacentric. The chromosome length ranges between 1.23 and 2.10 μm . The total haploid chromosome length was measured as 19.00 μm (Table 2).

S. paphlagonica (Figs. 1B, 2B), Endemic. At mitotic metaphase, 11 pairs of metacentric and 1

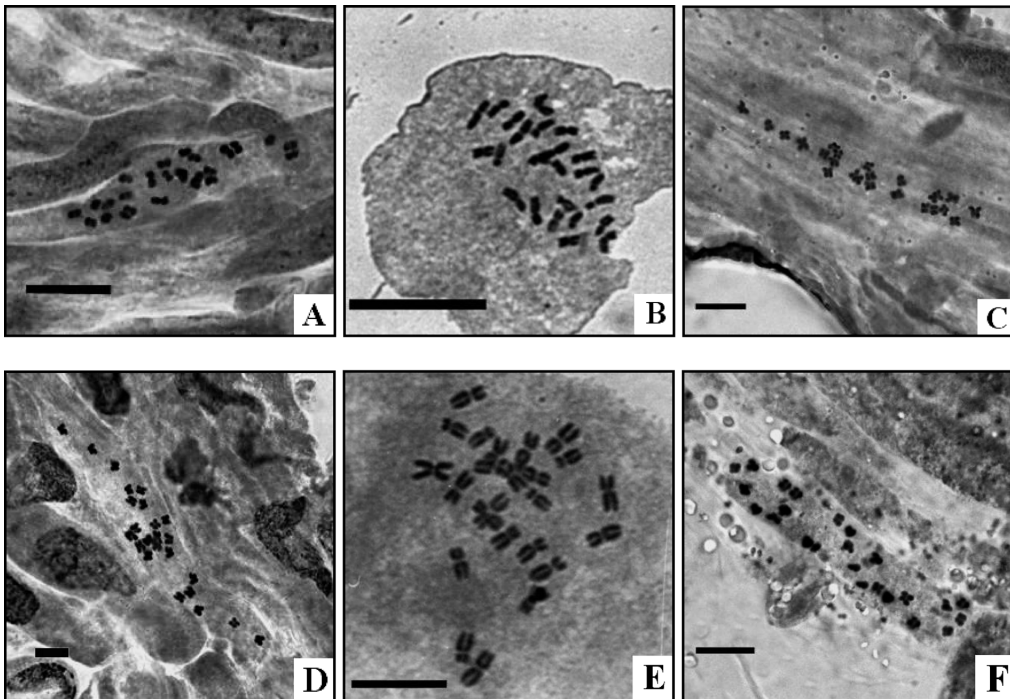


Fig. 1. Mitotic metaphase chromosomes of *Silene* species, A, *S. viscosa*; B, *S. paphlagonica*; C, *S. eremitica*; D, *S. skorpilii*; E, *S. otites*; F, *S. confertiflora*. Scale bar=10 μm .

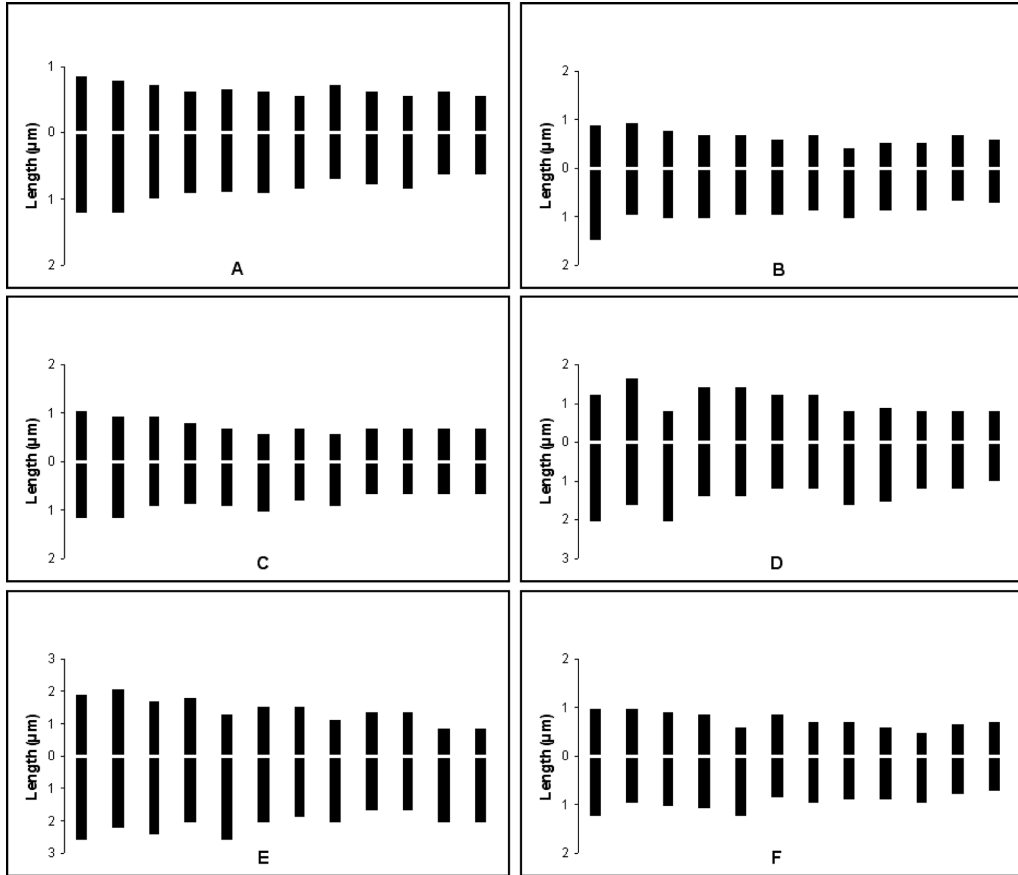


Fig. 2. Idiogram of *Silene* species, A, *S. viscosa*; B, *S. paphlagonica*; C, *S. eremitica*; D, *S. skorpilii*; E, *S. otites*; F, *S. confertiflora*.

pair of submetacentric chromosomes were observed. The chromosome length ranges between 1.40 and 2.43 μm . The total haploid chromosome length was measured as 20.25 μm (Table 2).

S. eremitica (Figs. 1C, 2C). Eleven pairs of metacentric and 1 pair of submetacentric chromosomes were observed at mitotic metaphase. The chromosome length varied between 1.44 and 2.28 μm . The total haploid chromosome length was measured as 20.34 μm (Table 2). It shows the lowest intrachromosomal asymmetry coefficient (A_1) (Table 3, Fig. 3).

S. skorpilii (Figs. 1D, 2D), Endemic. At mitotic metaphase, 10 pairs of metacentric and 2 pairs of submetacentric chromosomes were observed. The chromosome length ranges between 1.87 and 3.32 μm . The total haploid chromosome length was measured as 31.33 μm (Table 2). It represents the highest interchromosomal asymmetry coefficient (A_2) (Table 3, Fig. 3).

S. otites (Figs. 1E, 2E). At mitotic metaphase phase, 8 pairs of metacentric and 4 pairs of submetacentric chromosomes were observed. The chromosome length ranges between 2.98 and 4.55 μm . The total haploid chromosome length was measured as 43.71 μm (Table 2). This species shows the highest intrachromosomal asymmetry coefficient (A_1) (Table 3, Fig. 3).

S. confertiflora (Figs. 1F, 2F). Ten pairs of metacentric and 2 pairs of submetacentric chromosomes were observed at mitotic metaphase. The chromosome length ranges between 1.50 and 2.25 μm . The total haploid chromosome length was measured as 21.38 μm (Table 2). It shows the lowest interchromosomal asymmetry coefficient (A_2) (Table 3, Fig. 3).

Table 3. Comparison of chromosome properties of *Silene* taxa. (m) metacentric, (sm) submetacentric, (KF) karyotype formula, (CI) centromeric index, (A_1) asymmetry coefficient intrachromosomal and (A_2) asymmetry coefficient interchromosomal

Taxa	Chrom. number ($2n$)	Chromosome length (μm)			KF	CI	A_1	A_2
		Mean	Max	Min				
<i>S. viscosa</i>	24	1.58	2.10	1.23	24m	43.3	0.229	0.167
<i>S. paphlagonica</i>	24	1.69	2.43	1.40	22m+2sm	41.1	0.286	0.176
<i>S. eremitica</i>	24	1.70	2.28	1.44	22m+2sm	46.1	0.132	0.169
<i>S. skorpilii</i>	24	2.61	3.32	1.87	20m+4sm	42.6	0.231	0.181
<i>S. otites</i>	24	3.64	4.55	2.98	16m+8sm	40.3	0.308	0.152
<i>S. confertiflora</i>	24	1.78	2.25	1.50	20m+4sm	43.7	0.206	0.138

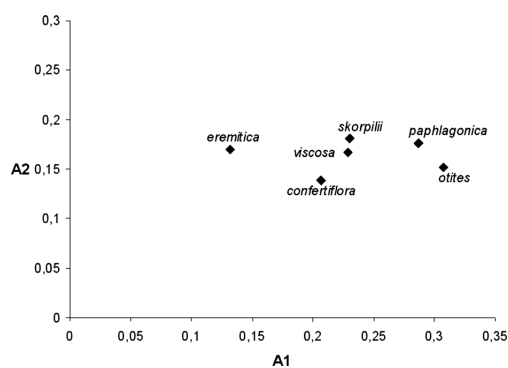


Fig. 3. Dispersion graphic of asymmetry coefficient, A_1 and A_2 , of *Silene* species.

Discussion

Chromosome numbers and karyological characters of studied 6 *Silene* species that belong to sections *Chloranthae*, *Tataricae* and *Otites* were determined. This study confirmed the chromosome numbers of *S. viscosa*, *S. paphlagonica*, *S. skorpilii* and *S. otites* (Fedorov 1974, Moore 1982, Yıldız and Çırpıcı 1996, Greuter 1997), which are all diploid with $2n=24$ and basic chromosome number is $x=12$. Chromosomal counts of 2 new species were added to the genus namely *S. eremitica* and *S. confertiflora* were all shown to have $2n=24$.

The chromosome number of approximately 320 *Silene* taxa has this far been determined (*ca.* 750 species of *Silene* worldwide). Previous studies on the chromosome numbers of the *Silene* genus have been reported as $2n=20, 24, 30, 48, 72, 96$. Studies (Darlington and Wylie 1955, Löve and Löve 1961, Fedorov 1974, Löve 1978a, Löve 1978b, Moore 1982, Davis 1988, Güner *et al.* 2000, Yıldız and Gücel 2006, Martin *et al.* 2008) have proven that this genus has 2 different basic chromosome numbers as $x=10$ and 12. The diploidy percentage is about 80% among the karyologically studied taxa of *Silene*. The somatic chromosome number of our studied species is in accordance with earlier studies. However, the karyotype analyses of those species are here conducted for the first time. Melzheimer (1977), in a biosystematic revision of the genus *Silene* in the Balkans, determined the same chromosome number $2n=24$ for all the *Silene* species that he studied, which is fully compatible with the data obtained in our study.

Some *Otites* section's species do not have spread in Turkey. The species are *S. borysthenica* (Gruner) Walters, *S. wolgensis* (Willd.) Besser ex Sprengel and *S. exaltata* Friv. Their chromosome number were determined the same chromosome number as $2n=24$ but the karyomorphological features of these have not been reported (Chater *et al.* 1993, Greuter 1997). So, these species must not compare with our study's species.

Chromosome dimensions are comparable among the species which belong to the same section. Badr *et al.* (1987) observed that *S. nocturna* L. has smaller chromosomes than *S. biappendiculata* Rohrb. in section *Scorpiodeae* (Rohrb.) Chowh. In addition, Yıldız and Çırpıcı (1996) observed that *S. olympica* Boiss. has smaller chromosomes than *S. marschallii* Meyer in section *Lasiostemones* Boiss., *S. alba* subsp. *eriocalycina* (Boiss.) Walters has smaller chromosomes than *S. noctiflora*

L. in section *Elisanthe* and *S. gallica* L. has smaller chromosomes than *S. bellidifolia* Jacq. in section *Silene*. It was determined that *S. viscosa* had smaller chromosomes than *S. paphlagonica* from sect. *Chloranthae*, *S. eremitica* had smaller chromosome *S. skorpili* from sect. *Tataricae*, and *S. confertiflora* had smaller chromosomes than *S. otites* from sect. *Otites*. Although, there are no major differences in chromosome dimensions among the species which have similar morphological features and belong to the same sections, there are large differences in mean chromosome length between the species which differ largely morphologically. The mean chromosome length of *S. marschallii* and *S. olympica* is $2.009\ \mu\text{m}$ and $1.592\ \mu\text{m}$ respectively and these 2 species have obvious differences morphologically in section *Lasiostemones* (Yıldız and Çirpici 1996). In section *Silene*, *S. gallica* and *S. bellidifolia* have less morphological differences in comparison to differences between *S. marschallii* and *S. olympica* that have less differences of mean chromosome length which are $1.47\ \mu\text{m}$ and $1.49\ \mu\text{m}$ respectively (Yıldız and Çirpici 1996). Thus, differences of chromosome length are positively proportional to the morphological differences among species of *Silene*.

In section *Chloranthae*, *S. viscosa* and *S. paphlagonica* are very similar species morphologically in Flora of Turkey (Coode and Cullen 1967). Also those 2 species are found to be the very similar by means of chromosome mean value. On the other hand, morphologically dissimilar species *S. otites* and *S. confertiflora* in *Otites* section were found to be dissimilar once, by means of mean chromosome length. Additional differences have been found by means of other morphological, palynological and molecular (PCR-PAPD) as reported by Çirpici *et al.* (2008). We observed the similarities or dissimilarities of those species karyologically and other results of the revision of these sections (Çirpici *et al.* 2008) are parallel to the study of Coode and Cullen (1967).

Yıldız and Çirpici (1996) worked on *S. paphlagonica* and *S. otites* karyologically. They observed chromosomal features of *S. otites* as follows; chromosome number is $2n=24$; chromosome length (mean) is $4.36\ \mu\text{m}$; maximum chromosome length is $6.17\ \mu\text{m}$; minimum chromosome length is $3.23\ \mu\text{m}$; 16 chromosomes are metacentric and 8 chromosomes are submetacentric. We have the same results about the chromosome number and the karyotypic formula. Our results are presented in Table 3.

Chromosomal features of *S. paphlagonica* were found by Yıldız and Çirpici (1996) to be as follows; chromosome number is $2n=24$; mean chromosome length is $1.25\ \mu\text{m}$; maximum chromosome length is $1.65\ \mu\text{m}$; minimum chromosome length is $1.18\ \mu\text{m}$; all chromosomes are metacentric. Our results are presented in Table 3. Differences of centromeric position between the studies of us and Yıldız and Çirpici (1996) are due to small differences of arm ratios of those chromosomes (e.g. If arm ratio is 1.7 that chromosome is metacentric, if arm ratio is 1.8 that chromosome is submetacentric). Those kinds of differences are observed frequently on the same taxa which especially have small chromosomes and/or unobvious centromeres.

All studied *Silene* species are perennials in sections *Chloranthae*, *Tataricae* and *Otites*. Badr *et al.* (1987) suggested that perennial *Silene* species have larger chromosomes than annual ones. Our results confirm the suggestion of Badr *et al.*, Yıldız and Çirpici (1996) determined mean chromosomal length of annual species *S. gallica* L. and *S. bellidifolia* Jacq. as $1.47\ \mu\text{m}$ and $1.49\ \mu\text{m}$ respectively. These values are lower than mean chromosome length of *Silene* species in our study.

In studied species, only metacentric and submetacentric chromosomes exist. According to mean arm ratios (L/S), it may be said that generally they have metacentric chromosomes (Table 2). The size of the chromosomes ranged between 1.23 and $4.55\ \mu\text{m}$ (Table 3). *S. eremitica* presents the most symmetric karyotype, which is reflected in its highest mean centromeric index and lowest intrachromosomal asymmetry coefficient (A1); both characters clearly differentiate this species from the remaining taxa. *S. skorpilii* shows the highest interchromosomal asymmetry coefficient (A2), which reflects the amplitude of its chromosome size (Table 3, Fig. 3). Generally, The 6 *Silene* species that are studied exist in different sections of neighbouring region's flora. In European flora,

S. viscosa and *S. skorpilii* and in Iranian flora *S. eremitica* belong to *Sclerocalycina* (Boiss.) Schischk. section. According to the results of our study, general chromosomal features of this species are very similar with Turkish endemic *S. paphlagonica*. These species that have similar morphologic features may be collected under 1 section. On the contrary, chromosomal features of *S. otites* and *S. confertiflora* in sect. *Otites* are very different from each other. In fact, in revision study of Chowdhuri (1957), *S. confertiflora* took place in *Holopetelae* section. It was exhibited that, general morphologic, pollen and seed features of these 2 species are different from each other (Çırpıcı *et al.* 2008). Also, Coode and Cullen (1967) who performed the *Silene* taxonomy in Turkey flora noted that this species is taken into the same section just because general appearance is similar to *S. otites*. Studies performed show that sections of Turkey flora should be reviewed.

We believe that the results of the present study are important for understanding the differentiation of similar taxa and their taxonomic position. Such results will be the base for future biosystematic studies in *Silene*.

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