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Karyological Studies of Six Endemic Species of *Stachys* (Lamiaceae) Subsect. *Fragiles* from Turkey

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Summary Several chromosome numbers are reported for *Stachys* taxa but little information is available about chromosome morphology. Thus, this study aims to investigate the karyotypes of six endemic *Stachys* species from Turkey. Two different chromosome numbers are reported as 2*n*=30 (*Stachys chasmosericea* and *S. pinardii*) and 2*n*=34 (*S. euadenia*, *S. buttleri*, *S. pseudopinardii* and *S. longiflora*). The chromosome types of the studied taxa are metacentric and submetacentric. Karyological features and asymmetry indices (M_{CA}, CV_{CI} and CV_{CL}) of the six endemic species are identified for the first time, and karyological relationships are determined. Also we presented published chromosome data of *Stachys* along with new counts. These data can be used for chromosome number evolution for the genus and helpful for understanding its evolution.

Key words Chromosome, Comparative karyology, *Fragiles*, *Stachys*.

The genus *Stachys* L., one of largest genera in the Lamiaceae, comprises about 300 species. The genus is distributed mainly in the warm temperate regions of the Mediterranean and southwestern Asia as well as North America, South America and southern Africa (Bhattacharjee 1980). It consists of annual and perennial herbs and subshrubs. A great number of *Stachys* species grow in various ecological conditions such as forests, rocky places, on limestone, banks of streams. In Turkey, *Stachys* was represented 115 taxa belonging to 15 sections and two subgenera. Of the 115 taxa, 54 (47%) are endemic to Turkey and are mostly eastern Mediterranean elements (Bhattacharjee 1982, Davis *et al.* 1988, Gemici and Leblebici 1998, Duman 2000, İlçim *et al.* 2008, Dirmenci *et al.* 2011, Akçiçek *et al.* 2012).

The section *Fragilicaulis* R. Bhattacharjee contains 30 taxa and is distributed only in Turkey, North Iraq and West Iran. All species are suffrutescent saxatile perennials and their stems are fragile at base. This section is distinguished from the other section of *Stachys* by this feature. In Turkey, the section has 21 taxa. Endemism rate of this section are 71.4%. A majority of these species are concentrated in Irano-Turanian phytogeographical region (Bhattacharjee 1982, Rechinger 1982, Davis *et al.* 1988, Duman 2000).

Chromosome analysis of the sect. *Eriostomum* was studied in Turkey by Martin *et al.* (2011) and somatic chromosomes numbers of these species were reported as 2*n*=30. Besides, other karyological research conducted on taxa of the genus *Stachys* showed that chromosome numbers were found to be from 2*n*=10 to 2*n*=102 (Pogan *et al.* 1980, Van Loon and Kieft 1980, Bhattacharjee 1982, Strid and Franzen 1983, Papanicolaou 1984, Mulligan and Munro 1989, Baden 1991, Baltisberger 1991a, b, 2006, Falciani and Fiorini 1996, Carr 1998, Wagner *et al.* 1999, Weller and Sakai 1999, Baltisberger and Widmer 2004) (Appendix 1). Generally, chromosome numbers 2*n*=30, 34 and 66. Our reports are congruent with the literature.

Recently, there are some studies on the taxonomy, palynology, karyological, anatomy and micromorphology of *Stachys* (Dirmenci *et al.* 2011, Salmaki *et al.* 2011, Martin *et al.* 2011, Akçiçek *et al.* 2012, Satıl *et al.* 2012, Salmaki *et al.* 2012, Dündar *et al.* 2013). There has been no investigation of karyotype analysis of these endemic species yet. With this study, somatic chromosome numbers of subsect. *Fragiles* were identified. These species are *S. longiflora* Boiss. and Bal., *S. euadenia* P.H. Davis, *S. pinardii* Boiss., *S. buttleri* R.Mill., *S. pseudopinardii* R. Bhattacharjee & Hub.-Mor. and *S. chasmosericea* Ayaşligil & P.H. Davis. Also we determined the karyotype asymmetry indices, statistically correct parameters such as M_{CA}, CV_{CI} and CV_{CL}, and demonstrated the karyologic relationships among *Stachys* taxa. We performed an extensive literature review to show all published chromosome data of *Stachys* using *The Chromosome Counts Database* (CCDB) (Rice *et al.* 2014) and internet resources (*e.g.*, ISI Web of Science, Google Scholar).

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Materials and methods

Stachys specimens were collected from Antalya, Karaman and Mersin provinces between 2012 and 2014 in Turkey. Localities of the collected specimens were presented (Table 1).

For chromosome preparation and karyological observations, we considered Martin *et al.* (2011) and Altınordu *et al.* (2014) respectively. Chromosomes were classified using the nomenclature of Levan *et al.* (1964) as median (m), submedian (sm), subterminal (st) and terminal point (T). For karyotype asymmetry, we measured CV_{CI} (Coefficient of Variation of Centromeric Index), CV_{CL} (Coefficient of Variation of Chromosome Length) (Paszko 2006) and M_{CA} (Mean Centromeric Asymmetry) (Peruzzi and Eroğlu 2013).

A new approach, proposed by Peruzzi and Altınordu (2014), was used to reconstruct the karyological relationships among *Stachys* taxa. To perform cluster analysis, a similarity matrix was created using Gower's (1971) general coefficient similarity to summarize the relationships among taxa (Sneath and Sokal 1973) in Past 3.03 software (Hammer *et al.* 2001, Hammer 2013).

Results

We report here on somatic chromosome numbers of five species and karyological features of six species first. *Stachys chasmosericea* and *S. pinardii* are $2n=30$, *S. euadenia*, *S. buttleri*, *S. pseudopinardii* and *S. longiflora* are $2n=34$ (Fig. 1). *S. buttleri* has the shortest chromosome with a length of 0.90 μm, whereas *S. pinardii* has the longest chromosome with a length of 2.12 μm. Length of the largest haploid chromosome was observed (22.3 μm) in *S. pinardii*. Length of the shortest haploid chromosome was observed (19.01 μm) in *S. longiflora*.

Discussion

In plants, karyotype asymmetry is good evidence for karyotype morphology (Zarco 1986) and one of the most popular, inexpensive and widely used approaches, especially by botanists (Peruzzi and Eroğlu 2013). Researchers have developed a variety of methods for analyzing karyotype asymmetry in chromosome sets. Stebbins (1971), Huziwara (1962), Arano (1963), Greilhuber and Speta (1976), Zarco (1986), Lavania and Srivastava (1992), Watanabe *et al.* (1999), and Paszko (2006)

Table 1. Localities of *Stachys* specimens.

	Localities of taxa and Collector and voucher numbers
S. longiflora	C5 Mersin: Işıktepe villages, Kızıl Dere (Güzel dere) limestone, mouth of cave, 200–250 m, 36°52′ 560″N/34°33′382″E, 13.07.2013, Ö.Güner 2364, Akçiçek & Dirmenci
S. euadenia	C4 Karaman: Ermenek, Hamitseydi Boğaz, Kırboğazi place, limestone crevices, 36°30′202″N, 032°47′219″ E, 1700 m, 12.07.2013, Ö.Güner 2374, Akçiçek & Dirmenci
S. pinardii	C3 Antalya: Road of Feslikan, Geyik Bayırı, Küllien cave, limestone crevices, 230 m, 36°52′657″N/30°28′596″E, 20.04.2013, 24.05.2013, Ö.Güner 2325 & Akçiçek
S. buttleri	C3 Antalya: Düden waterfall, damp rocks, 36°57′49″N/30°3′41″E, 88 m, 24.05.2013, Ö.Güner 2327 & Akçiçek
S. pseudopinardii	C4 Mersin: Silifke, Cennet cave, 0–50 m, 36°27′08″N, 34°06′22″E, 18.08.2013, Ö.Güner 2390 & Akçiçek
S. chasmosericea	C3 Antalya: Manavgat, Beşkonak, east of Yariş, the upper region of Karadağ, Karamuğar (Karapınar), limestone crevices, 37°08′807″N, 031°15′525″E, 825 m, 11.07.2013, Ö.Güner 2371, E. Akçiçek & Dirmenci

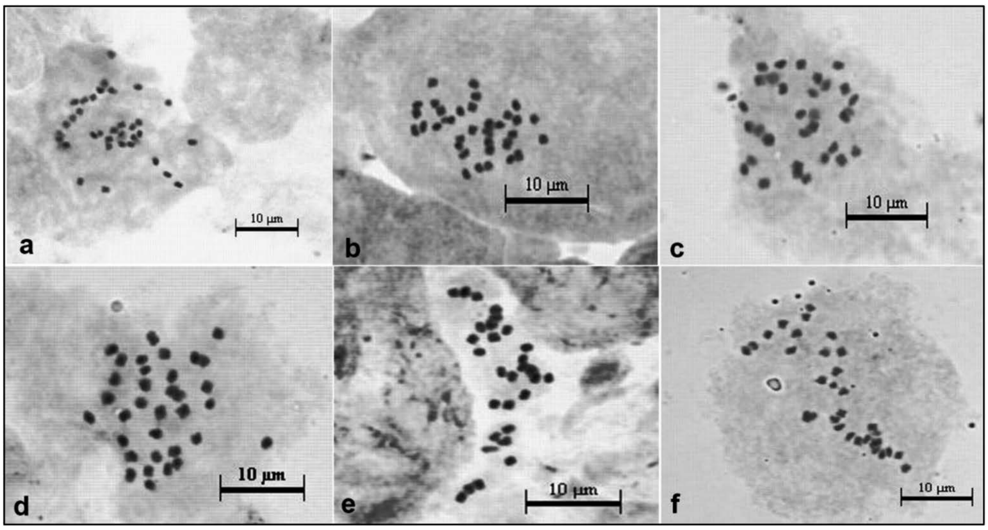

Fig. 1. Metaphase plates of *Stachys*. a: *S. longiflora*; b: *S. euadenia*; c: *S. buttleri*; d: *S. pseudopinardii*; e: *S. chasmosericea*; f: *S. pinardii*.

have developed a variety of methods for analyzing the karyotype variation of a chromosome complement (Zuo and Yuan 2011). For this aim, we considered three of them (M_{CA}, CV_{CI} and CV_{CL}) and discarded TF%, AsK%, AsI%, Syi, A1, CG (for details and references see Peruzzi and Eroğlu 2013; Peruzzi and Altınordu 2014). Lowest CV_{CI} values indicate chromosome complements with the most homogeneous centromere position, observed in *S. euadenia*. On the other hand, the most heterogeneous centromere position occurs in *S. chasmosericea*. Among species, CV_{CL} values range between 11.6 and 17.44, and *S. buttleri* shows highest interchromosomal asymmetry with 17.44 value. As to intrachromosomal asymmetry (M_{CA}), *S. buttleri* has highest value as 22.34 and *S. euadenia* has lowest value as 14.63. Detailed karyotype asymmetry indices and variability of symmetry indices among species are given (Table 2). Also, variabilities of CV_{CL} and CV_{CI} values for each taxon are illustrated by the boxplots (Fig. 2).

To reconstruct the karyological relationships among *Stachys* taxa, we used six quantitative features such as characters; chromosome number (2*n*), basic chromosome number (*x*), THL, M_{CA}, CV_{CL} and CV_{CI} (Peruzzi and Altınordu 2014), and formed karyological tree according to these characters. Moreover, we superimposed the idiograms onto this tree for clear relationships (Fig. 3).

According to literature, there are several data on the basis of chromosome count for *Stachys* taxa (Appendix 1). However, chromosome morphologies could not be analysed because of their short lengths which were not suitable for karyotype analysis. As such, there is little data on the karyology of the genus *Stachys*. Khadivi-Khub and Aghaei (2014) investigated *Stachys lavandulifolia* Vahl from four Iranian populations. Chromosome number of this species reported as $2n=60$. The presence of 60 chromosomes in all populations corresponds to tetraploidy ($2n=4x=60$) levels. The same taxa were investigated by Asghari Zakaria and Zare (2013) and chromosome numbers reported as $2n=68$, tetraploidy level. When Turkish and Iranian *Stachys* taxa were compared based on chromosome number, it was revealed that our studied taxa were diploid while Iranian taxa were tetraploid. When compared with their chromosome morphology, for each study, chromosome lengths varied from 1.30 to 1.48 μm and 0.93 to 2.45 μm, respectively. The lengths of chromosomes in the present study are

Table 2. Karyological features of studied taxa.

Taxa	CV_{CI}	CV_{CL}	M_{CA}	THL	2*n*	*x*
Stachys longiflora	15.79	14.38	21.51	19.01	34	17
S. euadenia	7.41	15.4	14.63	19.31	34	17
S. buttleri	10.93	17.44	22.34	19.64	34	17
S. pseudopinardii	10.89	15.37	16.63	22.11	34	17
S. chasmosericea	16.87	11.6	18.32	19.1	30	15
S. pinardii	11.87	16.38	20.53	22.3	30	15

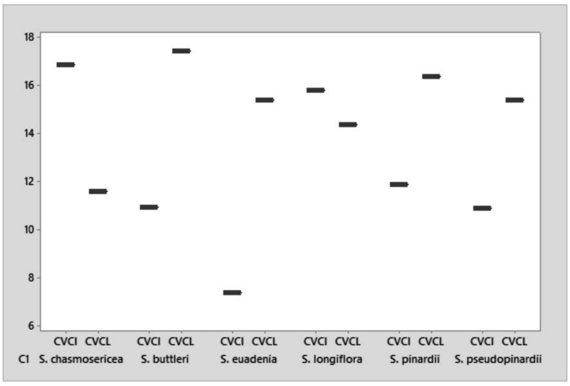

Fig. 2. Variabilities of CV_{CL} and CV_{CI} values among taxa.

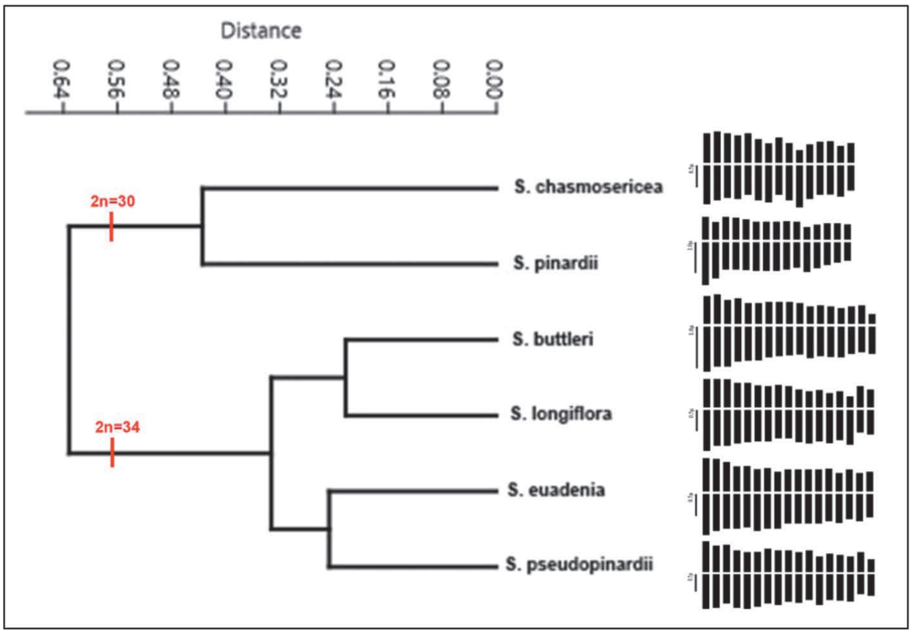

Fig. 3. Idiograms superimposed onto karyological tree.

congruent with these reports and vary between 0.90 and 2.12 μm. Khadivi-Khub and Aghaei (2014) reported the CV_{CI} index between 1.22 and 3.44. In this study CV_{CI} index was found between 7.41 and 16.87. Lowest CV_{CI} values implies chromosome complements with the most homogeneous centromere position, so our studied species have more heterogeneous centromere positions than Iranian *Stachys*. Also, low CV_{CL} values imply low interchromosomal asymmetry. We found CV_{CL} values between 11.6 and 17.44. Khadivi-Khub and Aghaei (2014) reported the CV_{CL} index between 5.12 and 14.20. Thus, we can say that Iranian species is more symmetrical than Turkish species.

According to previous reports, the most common ploidy levels of *Stachys* species are diploid. Martin *et al.* (2011) studied chromosome numbers of 26 *Stachys* taxa from Turkey and reported the chromosome numbers of all taxa as $2n$=30 and diploid (Appendix 1). Chehregani-Rad *et al.* (2012) investigated 13 populations of *Stachys inflata* Benth. from Iran and reported chromosome numbers of $2n$=$2x$=16 and $2n$=$4x$=32, both diploid and tetraploid. Also, *S. aspera* Michx., *S. hispida* Pursh, *S. pilosa* Nutt. and *S. turcomanica* Trautv. were reported at tetraploid level (Mulligan and Munro 1989; Chuksanova and Kaplanbekova 1971). In the present study, six endemic *Stachys* were studied and reported as $2n$=30 and 34 as diploid.

In conclusion, our results showed that the basic chromosome numbers of studied *Stachys* taxa are x=15 and x=17. Chromosome numbers correspond to the diploid level and when compared with available studied taxa from other countries, the karyotype of Turkish taxa is more asymmetrical than others.

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Appendix 1.

Taxa	$2n$	References	Taxa	$2n$	References
Stachys aculeolata Hook.	$2n=46$ and 52	Morton 1993.	*S. candida* Bory and Chaub.	$2n=34$	Baltisberger and Lenherr 1984.
S. aegyptiaca Pers.	$2n=34$	CCDB	*S. canescens* Bory and Chaub.	$2n=34$	Baltisberger and Lenherr 1984.
S. affinis Bunge	$2n=16$	Krestovskaya and Vassiljeva 1998.	*S. chamissonis* Benth.	$2n=64$	Mulligan and Munro 1989.
S. agraria Schltdl. and Cham.	$2n=32$	Mulligan and Munro 1989.	*S. chinensis* Bunge ex Benth.	$2n=$c.80	Sokolovskaya *et al.* 1986.
S. ajugoides Benth.	$2n=66$	Mulligan and Munro 1989.	*S. chrysantha* Boiss. and Heldr.	$2n=34$	Baltisberger and Lenherr 1984.
S. albanica Markgr.	$2n=34$	Baltisberger and Lenherr 1984.	*S. circinata* L'Hér.	$x=10$	Ruíz de Clavijo 1991.
S. albens A. Gray	$2n=66$	Mulligan and Munro 1989.	*S. coccinea* Ortega	$x=42$	Spellenberg 1986.
S. alopecuros (L.) Benth.	$2n=16$	Baltisberger 1991.	*S. cordata* Riddell	$2n=34$	Mulligan and Munro 1989.
S. alpina L.	$2n=30$	Strid and Franzen 1983.	*S. corsica* Pers.	$2n=18$	Verlaque *et al.* 1992.
S. angustifolia M. Bieb.	$2n=34$	Sekovski and Jovanovska 1983.	*S. cretica* L.	$2n=30$	Baltisberger 1987.
S. anisochila Vis. and Pancic	$2n=34$	Baltisberger and Lenherr 1984.	*S. discolor* Benth.	$2n=16$	Gagnidze and Gviniashvili 1997.
S. annua (L.) L.	$2n=34$	Uhríková and Schwarzová 1980.	*S. drummondii* Benth.	$2n=$c.80	Mulligan and Munro 1989.
S. arvensis (L.) L.	$2n=10$	Ruíz de Clavijo 1990.	*S. eplingii* J. B. Nelson	$2n=34$	Mulligan and Munro 1989.
S. aspera Michx.	$2n=68$	Mulligan and Munro 1989.	*S. euboica* Rech.	$2n=34$	Baltisberger and Lenherr 1984.
S. atherocalyx K. Koch	$2n=34$	Guinochet and Lefranc 1981.	*S. floccosa* Benth.	$x=15$	Gill 1984.
S. balansae Boiss. and Kotschy ssp. *balansae*	$2n=30$	Martin *et al.* 2011.	*S. floridana* Shuttlew. ex Benth.	$2n=34$	Mulligan and Munro 1989.
S. bayburtensis R. Bhattacharjee and Hub.-Mor.	$2n=30$	Martin *et al.* 2011.	*S. germanica* L.	$2n=30$	Pogan *et al.* 1980.
S. beckeana Dörfl. and Hayek	$2n=34$	Baltisberger and Lenherr 1984.	*S. gilliesii* Benth.	$x=16$	De Fernandes and De Sarmıento 1973.
S. bergii G. A. Mulligan and D. B. Munro	$2n=34$	Mulligan and Munro 1989.	*S. glutinosa* L.	$2n=34$	Villa 1978.
S. betoniciflora Rupr.	$2n=16$	Astanova 1984.	*S. heraclea* All.	$2n=30$	Baltisberger 1988.
S. bullata Benth.	$2n=66$	Mulligan and Munro 1989.	*S. hyssopifolia* Michx.	$2n=34$	Mulligan and Munro 1989.
S. byzantina K. Koch	$2n=30$	Gill 1981.	*S. hissarica* Regel	$2n=30$	Astanova 1981.
S. hispida Pursh	$2n=68$	Mulligan and Munro 1989.	*S. mollissima* Willd.	$2n=34$	Baltisberger 1991.
S. huber-morathii R. Bhattacharjee	$2n=30$	Martin *et al.* 2011.	*S. spinosa* L.	$x=17$	De Montmollin, 1984.
S. huetii Boiss.	$2n=30$	Martin *et al.* 2011.	*S. spinulosa* Sm.	$2n=18$	CCDB
S. inflata Benth.	$2n=32$	Cartier 1983.	*S. spreitzenhoferi* Heldr.	$2n=34$	Baltisberger 2006.
S. ionica Halácsy	$2n=34$	Baltisberger and Lenherr 1984.	*S. spruneri* Boiss.	$2n=34$	Baltisberger and Lenherr 1984.
S. iva Griseb.	$2n=34$	Baltisberger and Lenherr 1984.	*S. stebbinsii* G. A. Mulligan and D. B. Munro	$2n=66$	Mulligan and Munro 1989.
S. komarovii Knorring	$2n=32$	Gurzenkov 1973.	*S. stricta* Greene	$2n=66$	Mulligan and Munro 1989.
S. latidens Small	$x=17$	Mulligan and Munro 1989.	*S. swainsonii* Benth.	$2n=34$	Baltisberger, 2006.
S. lavandulifolia Vahl	$2n=60$	Chuksanova and Kaplanbekova 1971.	*S. tenuifolia* Willd.	$2n=34$	Mulligan and Munro 1989.
S. leucoglossa Griseb.	$2n=34$	Baltisberger and Lenherr 1984.	*S. tetragona* Boiss. and Heldr.	$2n=34$	Baltisberger and Lenherr 1984.
S. libanotica Benth. var. *minor* Boiss.	$2n=30$	Martin *et al.* 2011.	*S. thirkei* K. Koch	$2n=30$	Falciani and Fiorini 1996.
S. macrantha (K. Koch) Stearn	$2n=32$	Baltisberger 1989.	*S. thracica* Davidov	$2n=30$	Martin *et al.* 2011.
S. macrostachys (Wender.) Briq.	$2n=16$	Pogosyan 1974.	*S. tmolea* Boiss.	$2n=30$	Martin *et al.* 2011.
S. maritima Gouan	$2n=34$	Baltisberger 1991.	*S. tournefortii* Poir.	$x=15$	Montmollin 1982.
S. marrubiifolia Viv.	$2n=16$	Contandriopoulos 1962.	*S. turcomanica* Trautv.	$2n=60$	Chuksanova and Kaplanbekova 1971.
S. melissifolia Benth.	$x=15$	Saggoo 1983.	*S. tymphaea* Hausskn.	$2n=30$	Krestovskaya and Vassiljeva 1998.
S. menthifolia Vis.	$2n=34$	Baltisberger 1991.	*S. viticina* Boiss.	$2n=30$	Martin *et al.* 2011.
S. menthoides Kotschy and Boiss.	$x=17$	CCDB	*S. vuralii* Yıldız, Dirmenci and Akçiçek	$2n=30$	Martin *et al.* 2011.
S. mexicana Benth.	$x=32$	Gill 1981.			