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New chromosome counts of genus *Stachys* (Lamiaceae) from Turkey

Esra MARTİN[1,*], Özlem ÇETİN[1], Ekrem AKÇİÇEK[2], Tuncay DİRMENCİ[2]

[1]Selçuk University, Ahmet Keleşoğlu Faculty of Education, Department of Biology Education, 42090 Konya - TURKEY

[2]Balıkesir University, Necatibey Faculty of Education, Department of Biology Education, 10100 Balıkesir - TURKEY

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Abstract: Somatic chromosome numbers of 26 *Stachys* L. taxa (14 species, 11 subspecies, and 1 varietas), collected from different localities in Turkey, were counted for the first time, except for *Stachys byzantina* C.Koch and *S. thirkei* C.Koch. In this study, all of the *Stachys* taxa determined were somatic with chromosome numbers counted as 2n = 30. This research has made a contribution to the taxonomic revision of the genus *Stachys* in Turkey.

Key words: Chromosome number, *Eriostomum*, Labiatae, *Stachys*, Turkey

Türkiye'den *Stachys* cinsinde (Lamiaceae) yeni kromozom sayıları

Özet: Türkiye'den farklı lokalitelerden toplanan 26 *Stachys* L. (14 tür, 11 alttür ve 1 varyete) taksonunun somatik kromozom sayısı *Stachys byzantina* C.Koch ve *S. thirkei* C.Koch hariç ilk kez sayıldı. Bu çalışmada bütün *Stachys* taksonlarının diploid kromozom sayısı 2n = 30 olarak belirlendi. Çalışma Türkiye'deki *Stachys* cinsinin taksonomik revizyonuna katkı sağlamıştır.

Anahtar sözcükler: Kromozom sayısı, *Eriostomum*, Labiatae, *Stachys*, Türkiye

Introduction

Stachys L. (Lamiaceae), one of the largest genera of the Lamiaceae, contains about 300 taxa. It is a cosmopolitan genus centred in the warm temperate regions of the Mediterranean and southwestern Asia, with secondary centres in North and South America and southern Africa. The genus has not been reported from Australia or New Zealand. There are 2 main centres of diversity, as assessed by the number of species. One is confined to southern and eastern Anatolia, Caucasia, northwestern Iran, and northern Iraq, and the other to the Balkan Peninsula. The majority of the species grow in rocky places, mainly on limestone and other basic rocks (Bhattacharjee, 1974, 1980).

Stachys was revised by Bhattacharjee for the *Flora of Turkey* (Bhattacharjee, 1982). Since then, 13 new taxa have been described from Turkey. *Stachys* has

* E-mail: esramartin@gmail.com

84 species (110 taxa) belonging to 2 subgenera and 15 sections in Turkey. Of the 110 taxa, 54 (49%) are endemic to Turkey (Davis et al., 1988; Duman, 2000; Dinç & Doğan, 2006; İlçim et al., 2008; Daşkın et al., 2009; Akçiçek, 2010). The endemic taxa are mostly eastern Mediterranean elements.

Section *Eriostomum* (Hoffmanns. & Link) Dumort. is fairly homogeneous in its overall character resemblances and has a wide range throughout Europe, Asia, and part of northern Africa. This section is divided into 3 subsections. Of these, subsect. *Spectabiles* R.Bhattacharjee is mainly oriental and Irano-Turanian in distribution, while subsect. *Creticae* R.Bhattacharjee and subsect. *Germanicae* R.Bhattacharjee grow widely throughout Europe and Asia (Bhattacharjee, 1982). The section has 20 species (32 taxa) in Turkey. Of the taxa, 12 (37%) are endemic to Turkey.

Karyological investigations made on taxa of the genus *Stachys* showed that chromosome numbers in these taxa were found to be from 2n = 10 to 2n = 102 (Pogan et al., 1980; Van Loon & Kieft, 1980; Bhattacharjee, 1982; Strid & Franzen, 1983; Papanicolaou, 1984; Mulligan & Munro, 1989; Baden, 1991; Baltisberger, 1991a, 1991b; Baltisberger & Baltisberger, 1995; Falciani & Fiorini, 1996; Carr, 1998; Wagner et al., 1999; Weller & Sakai, 1999; Baltisberger & Widmer, 2004; Baltisberger, 2006; Marhold, 2006).

In the present study, somatic chromosome numbers of *Stachys* (sect. *Eriostomum*) were determined. We believe this study will play a positive role in resolving the morphologically unsolved problems of this taxonomically complex genus.

Materials and methods

Karyological observations were made on metaphase cells of root tips obtained from germinating seeds. Root tips were pretreated for 16 h in α-monobromonaphthalene at 4 °C and washed and fixed in Carnoy's solution (3:1, absolute ethanol:glacial acetic acid) overnight. The root tips were hydrolysed for 10 min in 1 N HCl at room temperature, and washed and stained in 2% aceto-orcein for 2 h. Chromosome counts were conducted using Bs200Pro image analysis software (Martin et al., 2009; Dirmenci et al., 2010; Hamzaoğlu et al., 2010).

Results and discussion

The somatic chromosome number of 26 taxa and the chromosome number of the *Stachys* (Lamiaceae) genus of the *Eriostomum* section were determined (Figures 1-5). The endemism state of the taxa is given in the Table. In this karyological study on the taxa of the genus *Stachys*, the chromosome morphology and types could not be determined due to problems during the processes of staining and hydrolisation. Based on the data obtained, the features of the metaphase chromosomes of 26 taxa are given below.

Subgen.: *Stachys*

Sect. *Eriostomum* (Hoffmanns. & Link) Dumort.

A: subsect. *Germanicae*

Stachys germanica subsp. *heldreichii*

C2 Muğla: Ortaca, 1 km from Dalyan to Okçular, field sides, 36°50′407″N, 028°39′425″E, 5 m, 16.08.2009, *Akçiçek* 5374 & *Dirmenci*.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

Stachys germanica subsp. *tymphaea*

A1 Kırklareli: 13 km from Dereköy to Kırklareli, forest openings, 41°50′439″N, 027°18′435″E, 460 m, 21.06.2009, *Akçiçek* 5289 & *Dirmenci*.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

Stachys thracica

A1 Kırklareli: 6 km from Armutveren to Sarpdere, forest openings, 41°52′586″N, 027°34′671″E, 380 m, 21.06.2009, *Akçiçek* 5291 & *Dirmenci*.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

Stachys alpina subsp. *macrophylla*

B2 Balıkesir: Dursunbey: Alaçam Mountains, Gölcük, Çamaşırlıkdere, shadow, 800 m, 01.08.2008, *Akçiçek* 5218.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

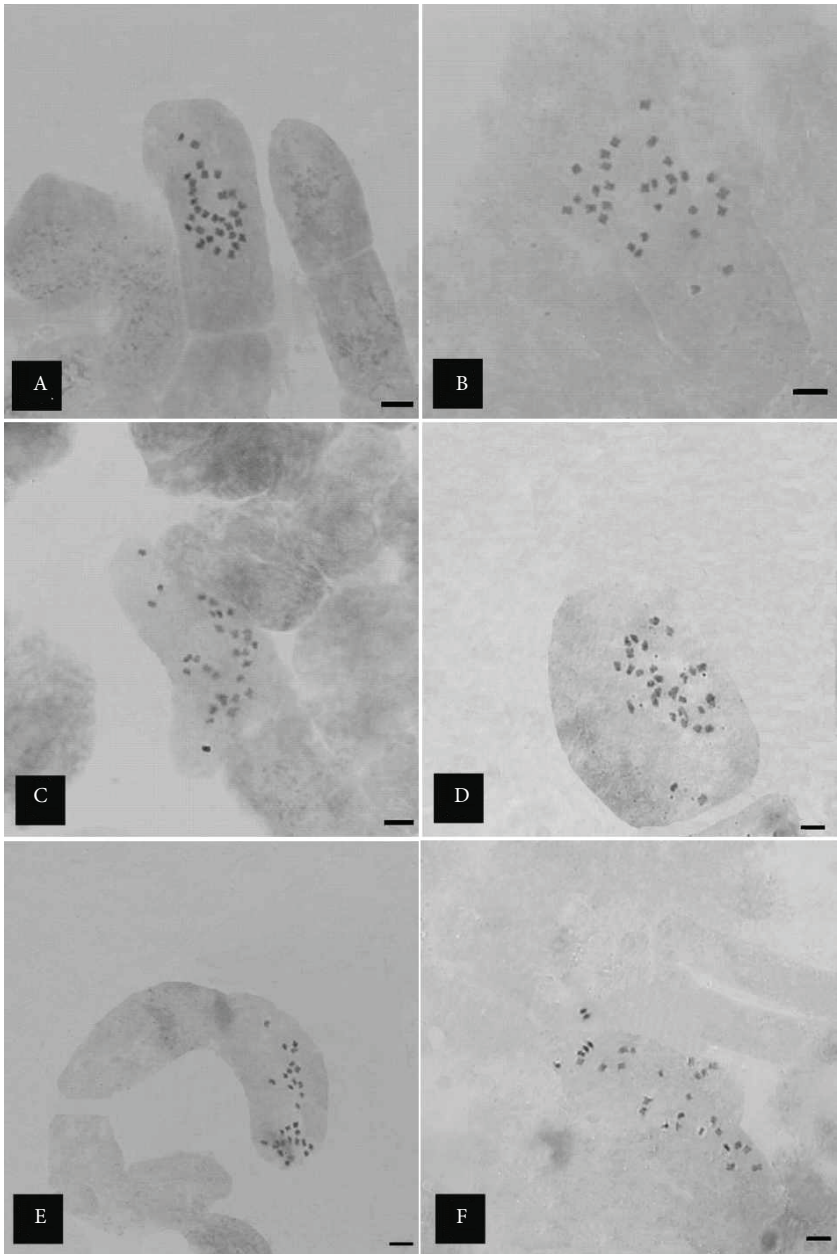

Figure 1. Metaphase chromosomes in the studied taxa: A) *Stachys germanica* subsp. *heldreichii*, B) *Stachys germanica* subsp. *tymphaea*, C) *Stachys thracica*, D) *Stachys alpina* subsp. *macrophylla*, E) *Stachys balansae* subsp. *balansae*, F) *Stachys huber-morathii*; bar: 5 µm.

Stachys balansae subsp. *balansae*

A8 Rize: İkizdere, 6 km from Başköy to Cermaniman, rocky slopes, 2450 m, 01.09.2008, *Akçiçek* 5223.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

Stachys huber-morathii

A5 Çorum: 22 km from Çorum to Osmancık, Kırkdilim village, *Quercus* openings, 40°43′685″N, 034°53′889″E, 1050 m, 29.06.2008, *Akçiçek* 5175 & *Dirmenci*.

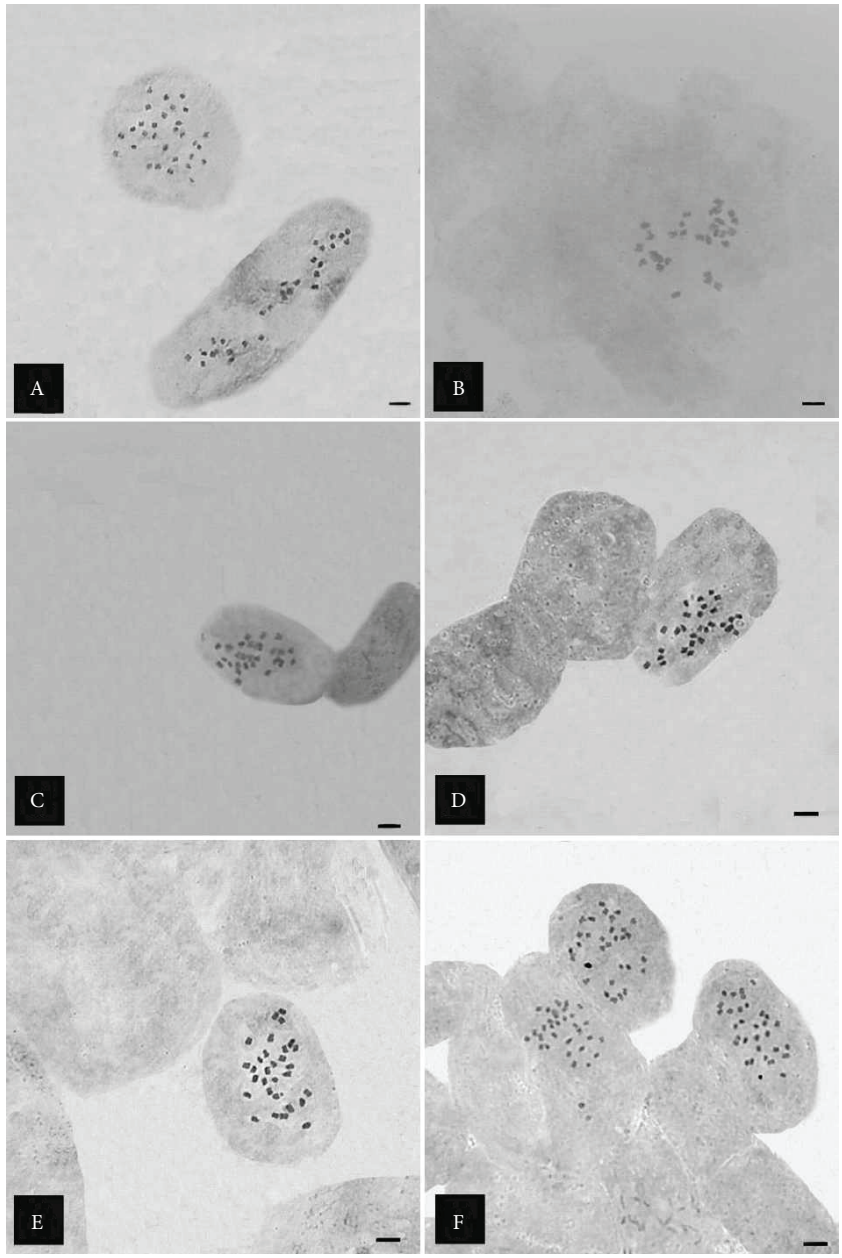

Figure 2. Metaphase chromosomes in the studied taxa: A) *Stachys obliqua*, B) *Stachys libanotica* var. *minor*, C) *Stachys sericantha*, D) *Stachys tmolea*, E) *Stachys cretica* subsp. *cassia*, F) *Stachys cretica* subsp. *garana*; bar: 5 μm.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

Stachys obliqua

B2 Balıkesir: Dursunbey, between Aşağı Musalar village and Çamaşırlıkdere, forest openings, 900 m, 25.07.2007, *Akçiçek* 4772 & *Dirmenci*.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

Stachys libanotica var. *minor*

C6 Hatay: 2 km from Yayladağı to Yeditepe village, *Pinus brutia* forest, 35°55′528″N, 036°02′675″E, 500 m, 20.07.2009, *Akçiçek* 5319 & *Dirmenci*.

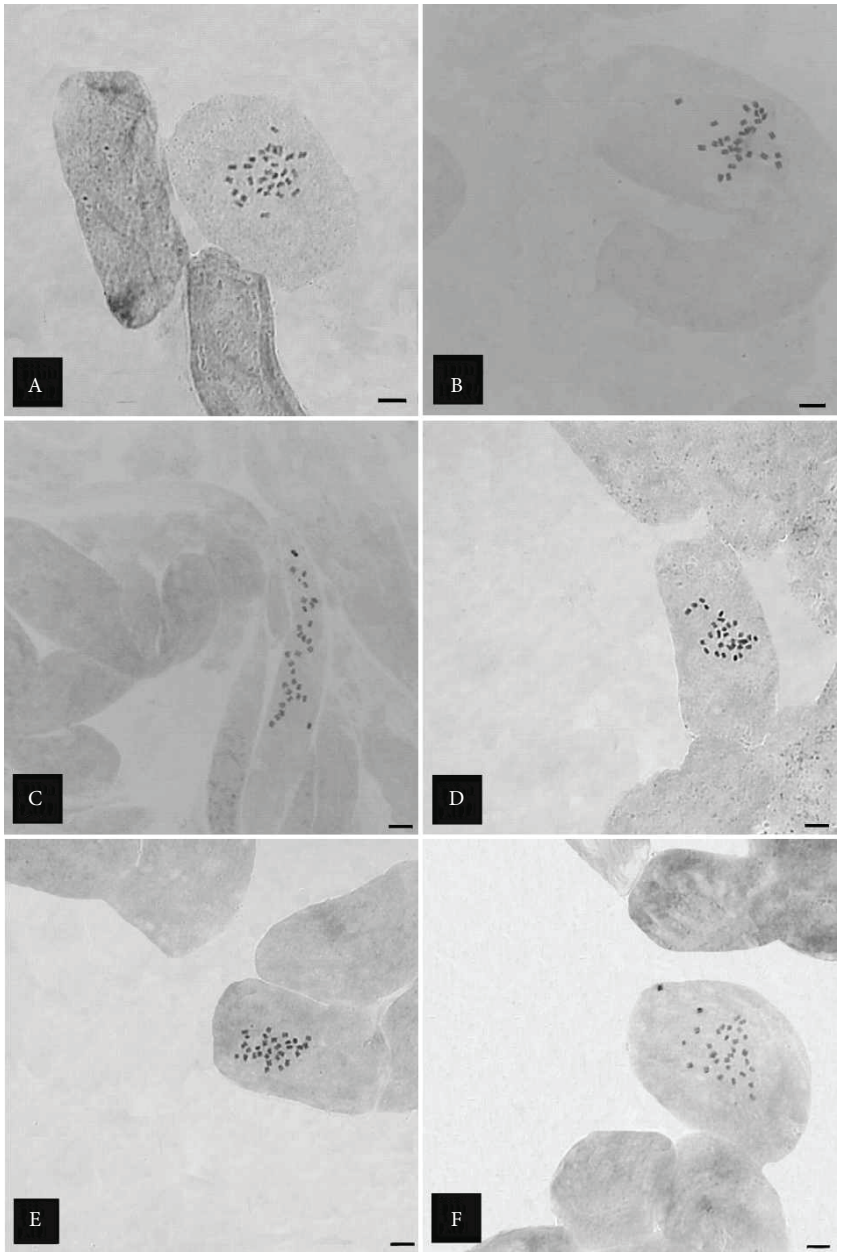

Figure 3. Metaphase chromosomes in the studied taxa: A) *Stachys cretica* subsp. *lesbiaca*, B) *Stachys cretica* subsp. *bulgarica*, C) *Stachys cretica* subsp. *vacillans*, D) *Stachys cretica* subsp. *smyrnaea*, E) *Stachys cretica* subsp. *anatolica*, F) *Stachys cretica* subsp. *kutahyensis*; bar: 5 µm.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

Stachys sericantha

C3 Antalya: Kemer, Ovacık village, *Pinus brutia* forest, 36°39′273″N, 030°25′899″E, 1200 m, 08.06.2007, *Akçiçek* 4624.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

Stachys tmolea

B1 Balıkesir: Kazdağı, Kartal Çimeni hill, steppe, 1750 m, 27.07.2007, *Akçiçek* 4779 & *Dirmenci*.

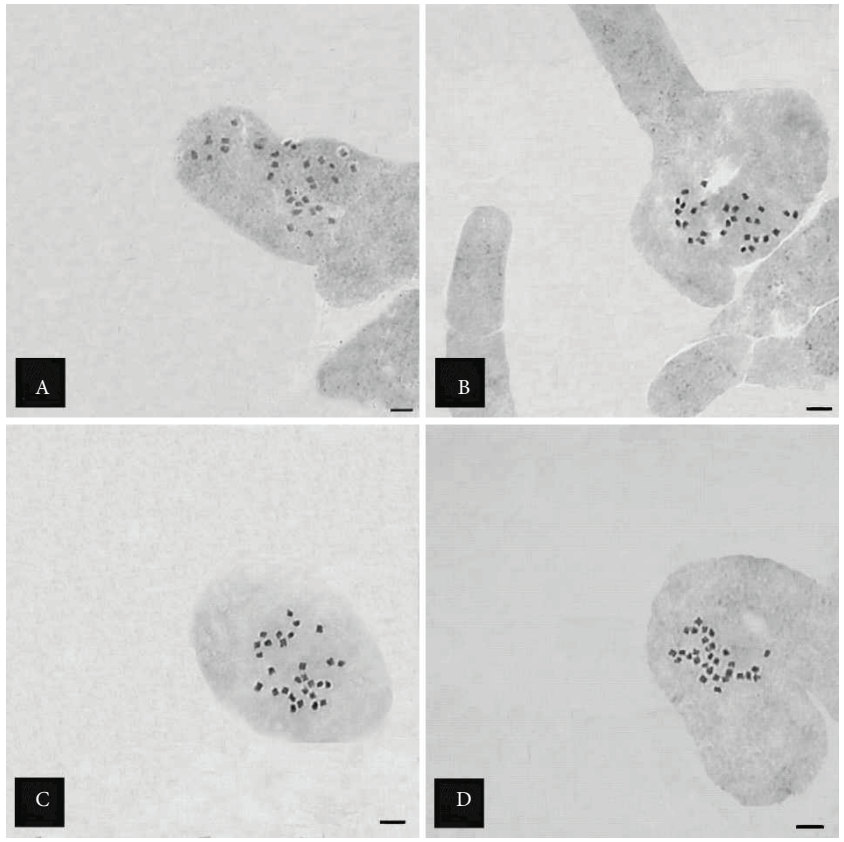

Figure 4. Metaphase chromosomes in the studied taxa: A) *Stachys byzantina*, B) *Stachys vuralii*, C) *Stachys thirkei*, D) *Stachys spectabilis*; bar: 5 µm.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

B: subsect. *Creticae*

Stachys cretica subsp. *cassia*

C6 Osmaniye: Amanos Mountains, Yarpuz, roadsides, 850 m, 09.07.2007, *Akçiçek* 4758 & *Dirmenci*.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

Stachys cretica subsp. *garana*

B9 Muş: 20 km from Varto to Hınıs, steppe, 39°12′252″N, 041°39′258″E, 1770 m, 12.08.2007, *FÖ* 9889.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

Stachys cretica subsp. *lesbiaca*

A1 Çanakkale: 1 km from Çan to Yenice, roadsides, 40°00′266″N, 027°03′394″E, 300 m, 11.06.2007, *Akçiçek* 4645 & *Dirmenci*.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

Stachys cretica subsp. *bulgarica*

A1 Tekirdağ: 24 km from Şarköy to Malkara, *Quercus* openings, 40°44′742″N, 027°04′747″E, 250 m, 20.06.2009, *Akçiçek* 5287 & *Dirmenci*.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

Stachys cretica subsp. *vacillans*

C3 Antalya: Kemer, Ovacık village, *Pinus brutia* forest, 1240 m, 11.09.2008, *Akçiçek* 5244 & *Dirmenci*.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

Stachys cretica subsp. *smyrnaea*

C2 Muğla: Between Marmaris and Datça, rocky slopes, 36°51′603″N, 028°14′017″E, 120 m, 09.06.2007, *Akçiçek* 4638 & *Dirmenci*.

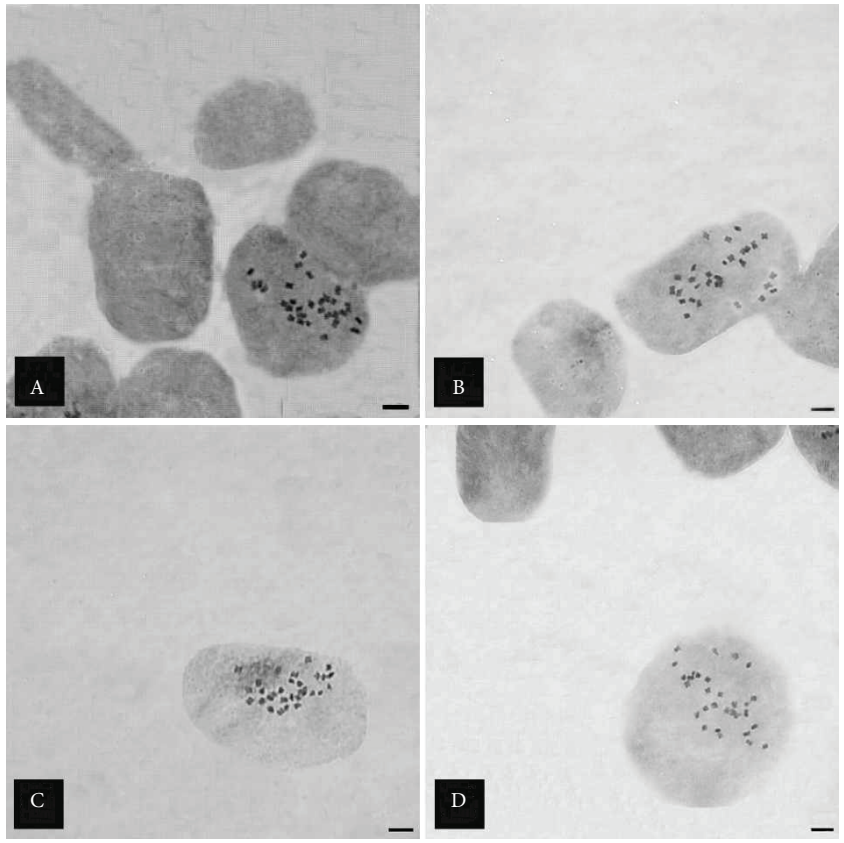

Figure 5. Metaphase chromosomes in the studied taxa: A) *Stachys longispicata*, B) *Stachys viticina*, C) *Stachys huetii*, D) *Stachys bayburtensis*; bar: 5 µm.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

Stachys cretica subsp. *anatolica*

B2 Kütahya: Yoncalı, Esentepe, roadsides, steppe, 1000 m, 06.08.2009, *Akçiçek* 5373.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

Stachys cretica subsp. *kutahyensis*

B2 Kütahya: 24 km from Tavşanlı to Harmancık, forest openings, 850 m, 06.07.2007, *Akçiçek* 4726.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

Stachys byzantina

A4 Çankırı: Ilgaz Mountain pass, forest openings, 41°03′55″N, 033°44′59″E, 1850 m, 10.07.2009, *E.Erdoğan* 1007 & *S.Selvi*.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

Stachys vuralii

A4 Bartın: Kurucaşile, *Pinus brutia* forest clearings, 100 m, 04.08.2007, *Yıldız* 16553, *Dirmenci* & *Brauchler*.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

Stachys thirkei

B2 Kütahya: Domaniç, Kocayayla, *Pinus nigra* forest openings, 39°51′765″N, 029°39′207″E, 1500 m, 12.07.2008, *Akçiçek* 5201.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

C: subsect. *Spectabiles*

Stachys spectabilis

B8 Erzurum: 6 km from Pasinler to Erzurum, 39°58′650″N, 041°38′180″E, 1680 m, 12.08.2007, *Dirmenci* 3539.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

Table. *Stachys* taxa in section *Eriostomum* section and their endemism state.

No.	Taxon name	Endemism
	Subsect. *Germanicae* R.Bhattacharjee	
1	*Stachys germanica* L. subsp. *heldreichii* (Boiss.) Hayek	
2	*Stachys germanica* L. subsp. *tymphaea* (Hausskn.) R.Bhattacharjee	
3	*Stachys thracica* Davidov	
4	*Stachys alpina* L. subsp. *macrophylla* (Albov) R.Bhattacharjee	
5	*Stachys balansae* Boiss. & Kotschy subsp. *balansae*	
6	*Stachys huber-morathii* R.Bhattacharjee	Endemic
7	*Stachys obliqua* Waldst. & Kit.	
8	*Stachys libanotica* Benth. var. *minor* Boiss.	Endemic
9	*Stachys sericantha* P.H.Davis	Endemic
10	*Stachys tmolea* Boiss.	Endemic
	Subsect. *Creticae* R.Bhattacharjee	
1	*Stachys cretica* L. subsp. *cassia* (Boiss.) Rech. f.	
2	*Stachys cretica* L. subsp. *garana* (Boiss.) Rech. f.	
3	*Stachys cretica* L. subsp. *lesbiaca* Rech. f.	Endemic
4	*Stachys cretica* L. subsp. *bulgarica* Rech. f.	
5	*Stachys cretica* L. subsp. *vacillans* Rech. f.	
6	*Stachys cretica* L. subsp. *smyrnaea* Rech. f.	Endemic
7	*Stachys cretica* L. subsp. *anatolica* Rech. f.	Endemic
8	*Stachys cretica* L. subsp. *kutahyensis* Akçiçek	Endemic
9	*Stachys byzantina* C.Koch	
10	*Stachys vuralii* Yıldız, Dirmenci & Akçiçek	Endemic
11	*Stachys thirkei* C.Koch	
	Subsect. *Spectabiles* R.Bhattacharjee	
1	*Stachys spectabilis* Choisy ex DC.	
2	*Stachys longispicata* Boiss. & Kotschy	
3	*Stachys viticina* Boiss.	
4	*Stachys huetii* Boiss.	Endemic
5	*Stachys bayburtensis* R.Bhattacharjee & Hub.-Mor.	Endemic

Stachys longispicata

B7 Elazığ: Karakoçan, deep ground, 1000 m, 20.08.2008, *Yıldız* 16968.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

Stachys viticina

C6 Hatay: Yayladağı, Gözlekçiler village, moist places, 400 m, 21.07.2008, *Akçiçek* 5187 & *Dirmenci*.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

Stachys huetii

B8 Erzurum: Palandöken, Tortum, rocky slopes, 40°06′133″N, 041°21′222″E, 2780 m, 10.08.2007, *FÖ* 9692.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

Stachys bayburtensis

A8 Bayburt: 36 km from Bayburt to Aşkale, Kop Mountain, Kop village, scree, 2030 m, 04.09.2008, *Akçiçek* 5241 & *Dirmenci*.

The results of the karyological analysis reveal that the somatic chromosome number is 2n = 30.

There are 3 subsections of section *Eriostomum* belonging to the genus *Stachys*. The subsection *Germanicae* consists of the taxa of *Stachys germanica* subsp. *heldreichii*, *S. germanica* subsp. *tymphaea*, *S. thracica*, *S. alpina* subsp. *macrophylla*, *S. balansae* subsp. *balansae*, *S. huber-morathii*, *S. obliqua*, *S. libanotica* var. *minor*, *S. sericantha*, and *S. tmolea*, which grow naturally in different localities in Turkey. For instance, 10 taxa of this subsection grow in the cities of Muğla, Kırklareli, Balıkesir, Çorum, Rize, and Antalya. It has been stated that all of the taxa of the subsection have the same chromosome number, although they grow in different localities.

The subsection *Creticae*, belonging to section *Eriostomum* in the genus *Stachys*, consists of the taxa of *Stachys cretica* subsp. *cassia*, *S. cretica* subsp. *garana*, *S. cretica* subsp. *lesbiaca*, *S. cretica* subsp. *bulgarica*, *S. cretica* subsp. *vacillans*, *S. cretica* subsp. *smyrnaea*, *S. cretica* subsp. *anatolica*, *S. cretica* subsp. *kutahyensis*, *S. byzantina*, *S. vuralii*, and *S. thirkei*. These taxa, some of which are endemic for Turkey, also grow in very different localities in the country, i.e. Osmaniye, Muş, Çanakkale, Tekirdağ, Antalya, Kütahya, Çankırı, and Bartın. The somatic chromosome number of the taxa was counted as 2n = 30 as in the subsection *Creticae*.

Spectabiles, another subsection of *Eriostomum* in the genus *Stachys*, contains the species *S. spectabilis*, *S. longispicata*, *S. viticina*, *S. huetii*, and *S. bayburtensis*. This subsection was studied based on specimens collected from the cities of Erzurum, Elazığ, Hatay, and Bayburt. The karyological results of this subsection are in agreement with those of the other 2 subsections. On the whole, 26 taxa of the subsections *Germanicae*, *Creticae*, and *Spectabiles*, all of the section *Eriostomum* in the genus *Stachys* and growing naturally in Turkey, were found to have a chromosome number of 2n = 30. These karyological results, in part, make a contribution to the revision of this section. More informative karyological results would be obtained if the chromosome morphology of the taxa could be studied. However, chromosome morphologies could not be analysed because the lengths of the chromosomes were not suitable for karyotype analysis and the centromere positions could not be observed. There is little data on the karyology of the genus *Stachys*, and these data state only the chromosome numbers of the taxa.

The somatic chromosome number of the taxa of *Stachys argolica* Boiss., *S. candida* Bory & Chaub., *S. canescens* Bory & Chaub., *S. spreitzenhoferi* subsp. *virella* D.Perss., and *S. swainsonii* Benth. was reported as 2n = 34 (Marhold, 2006), while our results showed that the chromosome number of all studied taxa of *Stachys* was determined as 2n = 30.

In a study stating the chromosome numbers of 10 angiosperm species, including a species from the family Lamiaceae, *Stachys palaestina*, the species were reported as having a somatic chromosome number of 2n = 34 (Baltisberger & Widmer, 2004).

Among the taxa of *Stachys* in our study, *S. byzantina* and *S. thirkei* were the only taxa whose somatic chromosome numbers were mentioned previously in the literature (Mulligan & Munro, 1989; Falciani & Fiorini, 1996), with those earlier findings agreeing with our results of 2n = 30.

Stachys cretica and *S. germanica* were reported as having a chromosome number of 2n = 30 (Strid & Franzen, 1983; Papanicolaou, 1984; Baltisberger & Baltisberger, 1995), as in our study. However, within the flora of Turkey, these species have subspecies

whose somatic chromosome numbers were reported for the first time in this study.

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