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Karyomorphological Study of Eight *Centaurea* L. Taxa (Asteraceae) from Turkey

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Abstract: *Centaurea* L. (Asteraceae) is one of the largest genera in Turkey. The chromosome number and morphology of 8 taxa belonging to the genus *Centaurea* were investigated using an image analysis system. Among these taxa, the chromosome numbers were as follows: $2n = 18$ in *C. cariensis* Boiss. subsp. *niveotomentosa* (Hub.-Mor.) Wagenitz, *C. lycaonica* Boiss. & Heldr., *C. virgata* Lam., and *C. polyclada* DC.; $2n = 24$ in *C. cyanus* L.; $2n = 36$ in *C. virgata* and *C. cariensis* Boiss. subsp. *cariensis*; $2n = 40$ in *C. urvillei* DC. subsp. *urvillei*; $2n = 54$ in *C. tuzgoluensis* Aytaç & H.Duman. In 5 taxa mixoploidy was observed (*C. lycaonica*, *C. urvillei* subsp. *urvillei*, *C. cariensis* subsp. *cariensis*, *C. virgata*, and *C. tuzgoluensis*). Based on karyotypic data for this genus, 3 basic chromosome numbers in *Centaurea* taxa were observed ($x = 9, 10, \text{ and } 12$). Furthermore, ideograms are given for all the *Centaurea* taxa studied.

Key Words: Image analysis system, *Centaurea*, Compositae, karyotype, Turkey

Türkiye'den Sekiz *Centaurea* L. (Asteraceae) Taksonu Üzerinde Karyomorfolojik Çalışmalar

Özet: *Centaurea* L. (Asteraceae) Türkiye'nin en büyük cinslerinden birisidir. *Centaurea* cinsine ait sekiz taksonun kromozom sayısı ve morfolojisi Görüntü Analiz Sistemi kullanılarak incelendi. Çalışılan sekiz taksonda kromozom sayıları *C. cariensis* Boiss. subsp. *niveotomentosa* (Hub.-Mor.) Wagenitz, *C. lycaonica* Boiss. & Heldr., *C. virgata* Lam. ve *C. polyclada* DC.'da $2n = 18$, *C. cyanus* L.'da $2n = 24$, *C. virgata* ve *C. cariensis* subsp. *cariensis*'de $2n = 36$, *C. urvillei* DC. subsp. *urvillei*'de $2n = 40$ ve *C. tuzgoluensis* Aytaç & H.Duman'de $2n = 54$ olarak sayıldı. Bununla birlikte beş taksonda miksploidi gözlemlendi (*C. lycaonica*, *C. urvillei* subsp. *urvillei*, *C. cariensis* subsp. *cariensis*, *C. virgata* ve *C. tuzgoluensis*). Elde edilen karyotip verilerine göre *Centaurea* taksonlarında üç farklı temel kromozom sayısı belirlendi ($x = 9, 10 \text{ ve } 12$). Yine çalışılan *Centaurea* taksonlarına ait idiyogramlar verildi.

Anahtar Sözcükler: Görüntü Analiz Sistemi, *Centaurea*, Compositae, karyotip, Türkiye

Introduction

Turkey is one of the main centres of diversity for the genus *Centaurea* L., particularly the south-western and eastern parts of the country, (Wagenitz, 1986). It is also the third largest genus in terms of the number of species in Turkey. In the Mediterranean and Irano-Turanian regions of Turkey 189 taxa have been reported

(Wagenitz, 1975; Davis et al., 1988; Wagenitz et al., 1998; Güner, 2000; Duran & Duman, 2002; Türkoğlu et al., 2003; Uzunhisarcıklı et al., 2005; Vural et al., 2006). A considerable proportion of these taxa are endemic to Turkey or localised to a limited area, even to a single mountain. There are 112 endemic *Centaurea* taxa and the endemism value of the genus in Turkey is about 60%.

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Generally, *Centaurea* localisation in Turkey is as follows: stony calcareous cliffs, vineyards, roadsides, seashores, gypsum fields, open woods and shrubs, waste places, steppes, fallow fields, maquis, sandy beaches, forests, dry meadows, rocky slopes, and maritime limestone cliffs (Türkoğlu et al., 2003).

Studies of different *Centaurea* species have concentrated on their morphology and anatomy (Font et al., 2002), palynology (Villodre & Garcia-Jacas, 2000; Garcia-Jacas et al., 2000; Garcia-Jacas et al., 2006), phytochemistry (Reyhan et al., 2004; Karamenderes et al., 2006), and cytology (Morales, 1974; Lopez, 1981; Ghaffari & Chariat-Panahi, 1985; Hellwig, 1994; Garcia-Jacas et al., 1996; Garcia-Jacas et al., 1997; Garcia-Jacas et al., 1998a, 1998b; Garcia-Jacas, 1998; Dekui, 2001; Romaschenko et al., 2004; Martin et al., 2006; Tan & Vural, 2007).

Cytological studies on the taxa of the genus *Centaurea* in Turkey show that the somatic chromosome number varies from $2n = 16$ to 54 (Wagenitz, 1975; Davis et al., 1988; Güner, 2000; Gömürgen & Adıgüzel, 2001;

Romaschenko et al., 2004; Martin et al., 2006). *Centaurea* is the largest genus in Turkey. To date, the somatic chromosome numbers of 46 taxa have been reported. The purpose of the present study was to add to our karyological knowledge of this highly endemic genus in Turkey.

The chromosome number and morphology of 8 taxa of the genus *Centaurea* in Turkey were studied using an image analysis system. Additionally, the chromosome morphology of all other taxa and the chromosome number of *C. lycanica* Boiss. & Heldr., *C. cariensis* Boiss. subsp. *cariensis*, *C. cariensis* subsp. *niveotomentosa* (Hub.-Mor.) Wagenitz, and *C. tuzgoluensis* Aytaç & H.Duman were determined for the first time.

Materials and Methods

The taxa examined were *Centaurea lycanica*, *C. cyanus* L., *C. urvillei* DC. subsp. *urvillei*, *C. cariensis* subsp. *cariensis*, *C. cariensis* subsp. *niveotomentosa*, *C. polyclada* DC. *C. virgata* Lam., and *C. tuzgoluensis* (Table 1).

Table 1. The karyotyped *Centaurea* taxa, and their diploid chromosome numbers and localities.

Taxon	2n	Locality	Vouchers
<i>C. lycanica</i>	18	Konya: Konya-Şeydişehir road, Yatağan village junction, 1400 m, 29.06.2004, Serpentine stony places	A.Duran 6664 & M.Dinç
<i>C. cyanus</i>	24	Balıkesir: Edremit, Kızılköçü village, Kazdağı, Gölcük, 400 m, 22.07.2006, <i>Pinus nigra</i> and <i>Quercus</i> sp. clearings	A.Duran 7299 & M.Dinç
<i>C. urvillei</i> subsp. <i>urvillei</i>	40	İzmir: İzmir-Aydın road, Pınarbaşı junction, 45 km, 150 m, 10.06.2006, road side	M.Dinç 2664
<i>C. cariensis</i> subsp. <i>cariensis</i>	36	Muğla: Eski Kale road, Yılanlı Mountain, around the transmitters, 1360 m, 23.07.2006, <i>Pinus nigra</i> and <i>Quercus</i> sp. clearings	A.Duran 7304 & M.Dinç
<i>C. cariensis</i> subsp. <i>niveotomentosa</i>	18	Muğla: Fethiye-Korkuteli road, c. 43 km, 100 m, 25.07.2006, road side, marly places	A.Duran 7327 & M.Dinç
<i>C. polyclada</i>	18	Balıkesir: Edremit, Kızılköçü village, Kazdağı, Gölcük locality, 400 m, 22.07.2006, <i>Pinus nigra</i> and <i>Quercus</i> sp. clearings	A.Duran 7300 & M.Dinç
<i>C. virgata</i>	36	Bursa: Harmancık-Dursunbey road, 14 km, 450 m, 21.07.2006, <i>Pinus brutia</i> clearings	A.Duran 7290 & M.Dinç
	18	Burdur: Dirmil passage, protected area, 1630 m, 25.07.2006, clearings	A.Duran 7332 & M.Dinç
<i>C. tuzgoluensis</i>	54	Aksaray: Eskil, Osmanın Çardağı surroundings, 932 m, 28.06.2004	A.Duran 6640

Karyotypes were determined from somatic tissue samples (cells at the metaphase stage of mitotic division), using the squash technique. Root meristems from germinating seeds of each taxon collected in the wild were used. First, root tips were pre-treated with α -monobromonaphthalene at 4 °C for 16 h. Then, root tips were fixed in Carnoy's solution for 24 h at 4 °C. Before staining, the material was hydrolysed with 1N HCl for 13 min at room temperature. Root tips were stained with 2% aceto-orcein and then were squashed and mounted on slides with 45% acetic acid. For all chromosome counts, a minimum of 10 plates (representing 10 different individuals of each species) were examined for each taxon; however, the 10 individuals examined came from the same locality for all taxa, except for *C. virgata*. Permanent slides were made using the standard liquid nitrogen method (Martin et al., 2006). Photographs were made with a light microscope. Ideograms were prepared with measurements made on enlarged microphotographs of 5 well-spread metaphase plates of different individuals. The classification of chromosomes, length of the long and short arms, arm ratio, centromeric index, and relative chromosomal length were measured with an image analysis program (BS200Pro) running on a personal computer. The classification of chromosomes as median (m), submedian (sm), subterminal (st), and terminal point

(T) was based on the analysis of metaphase chromosomes (Levan et al., 1964; Paszko, 2006).

Results

The karyomorphology of 8 taxa belonging to the genus *Centaurea*, in 4 taxa, were studied for the first time. The chromosome numbers of the taxa studied are as follows: $2n = 18$ for *C. cariensis* subsp. *niveotomentosa*, *C. lycaonica*, *C. virgata*, and *C. polyclada*; $2n = 24$ for *C. cyanus*; $2n = 36$ for *C. virgata* and *C. cariensis* subsp. *cariensis*; $2n = 40$ for *C. urvillei* subsp. *urvillei*; $2n = 54$ for *C. tuzgoluensis*. Three basic chromosome numbers were observed ($x = 9$ for *C. cariensis* subsp. *niveotomentosa*, *C. lycaonica*, *C. virgata*, and *C. polyclada*, $x = 10$ for *C. urvillei* subsp. *urvillei*, and $x = 12$ for *C. cyanus*). The detailed karyomorphological features are presented in Table 2.

Section: *Phalolepis* (Cass.) DC.

Centaurea lycaonica Boiss. & Heldr.

The karyotype formula is $n = 9 = 5m + 4sm$ (Figure 1). The chromosome number and morphology are reported for the first time. Metaphase chromosome length ranges from 3.52 to 1.71 μm , total haploid chromosome length is 22.81 μm .

Table 2. Chromosomal comparison of the 8 taxa of *Centaurea* (AR: arm ratio; CI: centromeric index; THC: total length of haploid complement; M: median; SM: submedian; T: Terminal point).

Taxon	2n	Ploidy level	Chromosome sizes (μm)	AR	CI	THC (mm)	M	SM	T
<i>C. lycaonica</i>	18		1.71-3.52	1.75	4.19	22.81	5	4	–
<i>C. cyanus</i>	24		1.42-2.34	1.55	3.35	21.00	8	4	–
<i>C. urvillei</i> subsp. <i>urvillei</i>	40	4x	3.04-7.12	1.99	1.75	97.70	9	10	1
<i>C. cariensis</i> subsp. <i>cariensis</i>	36	4x	1.61-2.88	1.61	2.13	39.42	13	5	–
<i>C. cariensis</i> subsp. <i>niveotomentosa</i>	18		2.51-3.81	1.75	4.09	28.18	4	5	–
<i>C. polyclada</i>	18		1.50-3.12	1.55	4.37	17.51	6	3	–
<i>C. virgata</i>	36	4x	1.74-2.99	2.02	1.93	40.20	9	8	1
	18		1.49-3.04	1.49	4.48	17.82	7	2	–
<i>C. tuzgoluensis</i>	54	6x	0.78-1.73	1.78	1.33	31.86	14	13	1

Section: *Cyanus* (Mill.) DC.

Centaurea cyanus L.

The karyotype formula is $n = 12 = 8m + 4sm$ (Figure 1). The chromosome morphology is reported for the first time. Metaphase chromosome length ranges from 2.34 to 1.42 μm ; total haploid chromosome length is 21.00 μm .

Section: *Acrocentron* (Cass.) DC.

Centaurea urvillei DC. subsp. *urvillei*

The karyotype formula is $n = 20 = 9m + 10sm + 1st$ (Figure 1). The chromosome morphology is reported for the first time. Metaphase chromosome length ranges from 7.12 to 3.04 μm ; total haploid chromosome length is 97.70 μm .

Section: *Acrolophus* (Cass.) DC.

Centaurea cariensis Boiss. subsp. *cariensis*

The karyotype formula is $n = 18 = 13m + 5sm$ (Figure 1). The chromosome number and morphology are reported for the first time. Metaphase chromosome length ranges from 2.88 to 1.61 μm ; total haploid chromosome length is 39.42 μm .

Centaurea cariensis subsp. *niveotomentosa* (Hub.-Mor.) Wagenitz

The karyotype formula is $n = 9 = 4m + 5sm$ (Figure 1). The chromosome number and morphology are reported for the first time. Metaphase chromosome length ranges from 3.81 to 2.51 μm ; total haploid chromosome length is 28.18 μm .

Centaurea polyclada DC.

The karyotype formula is $n = 9 = 6m + 3sm$ (Figure 1). The chromosome morphology is reported for the first time. Metaphase chromosome length ranges from 3.12 to 1.50 μm ; total haploid chromosome length is 17.51 μm .

Centaurea virgata Lam.

Bursa locality: The karyotype formula is $n = 18 = 9m + 8sm + 1st$ (Figure 1). The chromosome morphology is reported for the first time. Metaphase chromosome length ranges from 2.99 to 1.74 μm ; total haploid chromosome length is 40.20 μm .

Burdur locality: The karyotype formula is $n = 9 = 7m + 2sm$ (Figure 1). The chromosome morphology is reported for the first time. Metaphase chromosome

length ranges from 3.04 to 1.49 μm ; total haploid chromosome length is 17.82 μm .

Centaurea tuzgoluensis Aytaç & H.Duman

The karyotype formula is $2n = 54 = 14m + 12sm + 1st$ (Figure 1). The chromosome number and morphology are reported for the first time. Metaphase chromosome length ranges from 1.73 to 0.78 μm ; total haploid chromosome length is 31.86 μm .

The ideograms for all taxa are shown in Figure 2.

Discussion

The chromosome morphology of the studied *Centaurea* taxa all differed from each other. The shortest chromosome was 0.78 μm (*C. tuzgoluensis*) and the longest chromosome was 7.12 μm (*C. urvillei* subsp. *urvillei*). *C. virgata* (found in Burdur) had the smallest arm ratio (1.49) and *C. virgata* (found in Bursa) had the largest arm ratio (2.02). According to the centromeric index, the *C. tuzgoluensis* taxon had the smallest index value (1.33) and the *C. virgata* taxon (found in Burdur) had the largest (4.48). *C. polyclada* had the shortest total length of haploid complement (17.51 μm) and *C. urvillei* subsp. *urvillei* had the longest (97.70 μm).

The somatic chromosome number and morphology of the species *Centaurea lycaonica* were determined in the present study. Tetraploid cells $2n = 4x = 36$ are present in this species. Tetraploidy is common for the taxa *Centaurea* (Garcia-Jacas et al., 1998a).

The diploid chromosome number of the species *Centaurea cyanus* was previously reported as $2n = 24$ (Georgiadis & Christodoulakis, 1984; Strid, 1987; Hellwig, 1994). Morales (1974) reported the diploid chromosome number of this species as $2n = 18$. Hence, it is possible to consider that *C. cyanus* has different somatic chromosome numbers. These cytogenetic diversions may have an effect on the distinct occurrence of infraspecific variation.

The chromosome number of *Centaurea urvillei* subsp. *urvillei* determined in the present study is $2n = 2x = 40$, which is the same as previously reported (Georgiadis & Christodoulakis, 1984; Garcia-Jacas et al., 1997). Moreover, tetraploidy ($2n = 4x = 80$) is herein reported for the first time. *Centaurea kunkelii* Garcia-Jacas is an endecaploid with $2n = 11x = 110$, the highest ploidy level

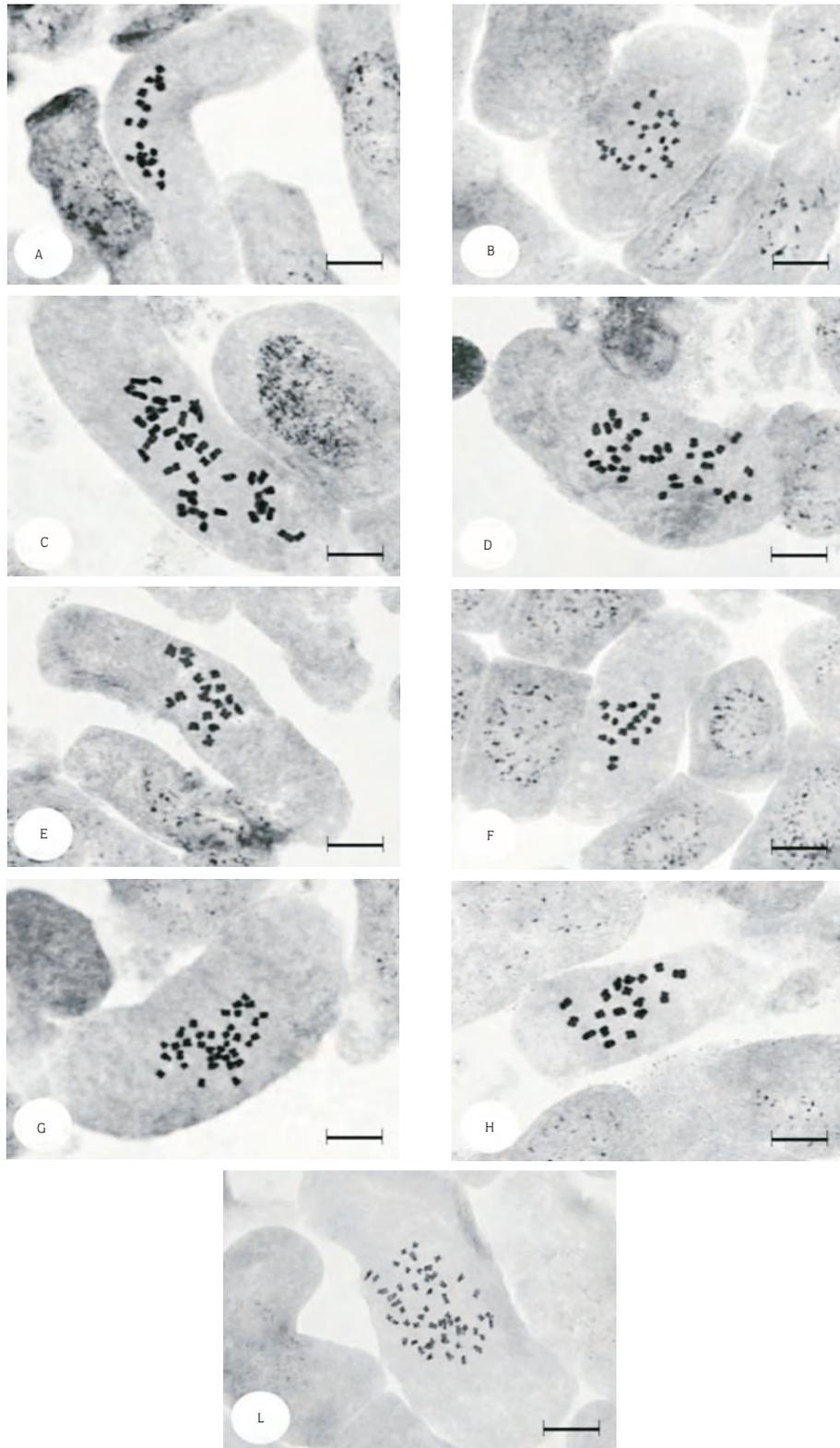


Figure 1. Microphotographs of diploid metaphase chromosomes. A: *Centaurea lycaonica* ($2n = 18$); B: *C. cyanus* ($2n = 24$); C: *C. urvillei* subsp. *urvillei* ($2n = 40$); D: *C. cariensis* subsp. *cariensis* ($2n = 36$); E: *C. cariensis* subsp. *niveotomentosa* ($2n = 18$); F: *C. polyclada* ($2n = 18$). G: *C. virgata* (Bursa locality: $2n = 36$); H: *C. virgata* (Burdur locality: $2n = 18$); I: *C. tuzgoluenis* ($2n = 54$) (scale bar: $10 \mu\text{m}$).

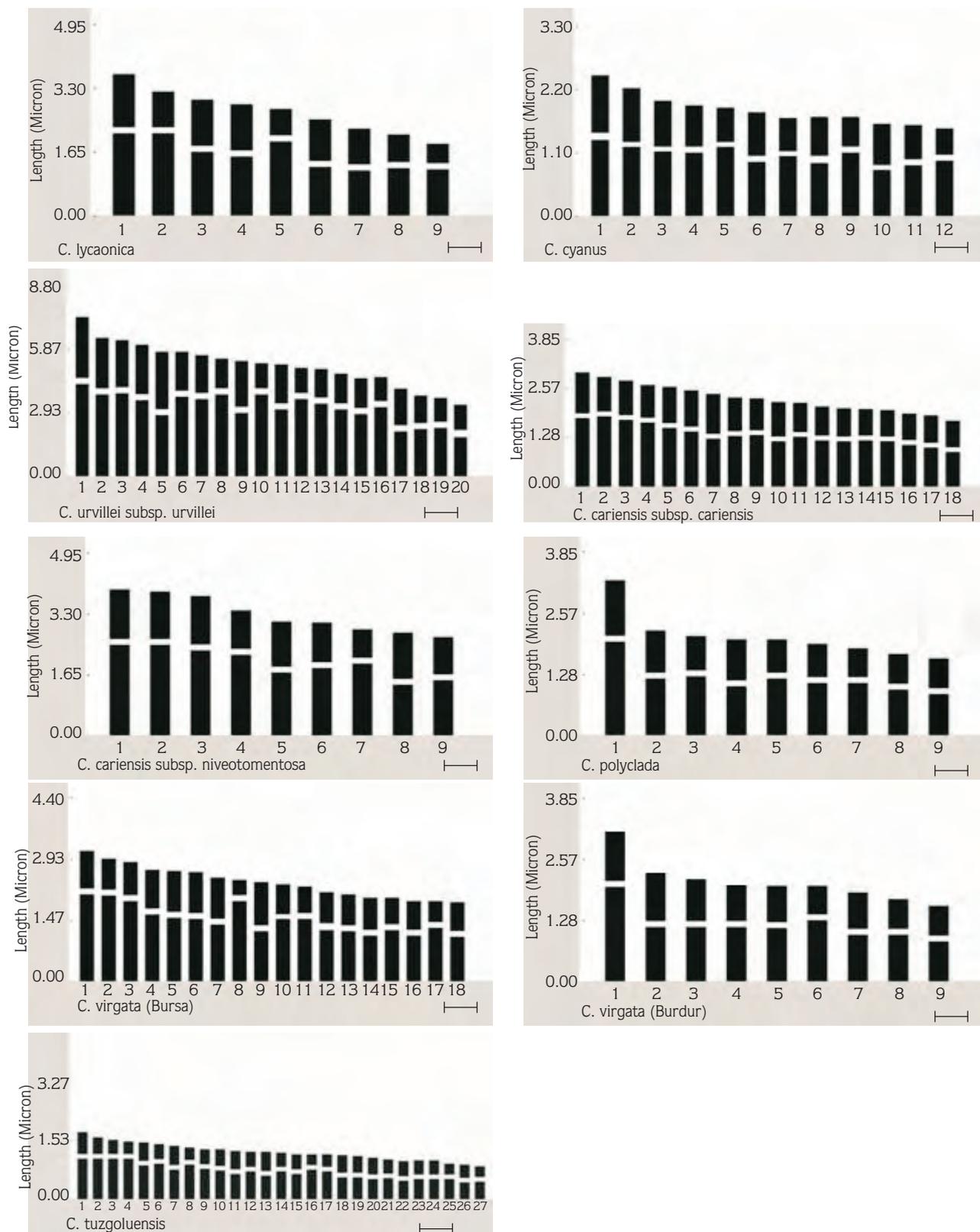


Figure 2. Haploid ideograms of taxa belonging to *Centaurea* (scale bar: 10 μ m).

hitherto known in the genus *Centaurea*. On the basis of morphological evidence (especially some features of the cypselas) and polyploidy, a hybrid origin of *C. kunkelii* between species of sections *Acrocentron* and *Chamaecyanus* has been hypothesised (Garcia-Jacas, 1998). The polyploidy agrees with the cytological studies of other taxa of the species *Centaurea*.

The chromosome number and morphology of 2 *Centaurea cariensis* subspecies were determined in the present study. The somatic chromosome number of *C. cariensis* subsp. *cariensis* is $2n = 36$ and is $2n = 18$ for *C. cariensis* subsp. *niveotomentosa*. The karyotype formula of the 2 *C. cariensis* subspecies is different. The chromosome length of *C. cariensis* subsp. *cariensis* is between 1.61 and 2.88 μm versus between 2.51 and 3.81 μm for subsp. *niveotomentosa*. The total haploid chromosome length of *C. cariensis* subsp. *cariensis* is 39.42 μm versus 28.18 μm for subsp. *niveotomentosa*. The 2 subspecies are separated by their leaf morphology (Wagenitz, 1975). Wagenitz (1975) reported that *C. cariensis* subsp. *cariensis* has divided leaves, whereas *C. cariensis* subsp. *niveotomentosa* has undivided leaves. According to our morphological observations, the Fethiye population of *C. cariensis* subsp. *niveotomentosa* has individuals with divided and undivided leaves, suggesting that leaf morphology does not distinguish the 2 subspecies; however, the chromosomal data we obtained from our karyotype analysis show that these subspecies are distinct taxa.

Among the taxa studied, *C. polyclada* has the smallest total haploid chromosome length: 17.51 μm . Additionally, the chromosome morphology of *C. polyclada* was determined for the first time. The chromosome number determined in the present study is the same as previously reported (Ghaffari & Chariat-Panahi, 1985; Garcia-Jacas et al., 1997).

In the present study *Centaurea virgata* from 2 different localities (Bursa and Burdur) was examined. The chromosome number and morphology of the specimens from each locality are quite different. The chromosome number of this species is $2n = 18$ (Ghaffari & Chariat-Panahi, 1985); however, we determined that the chromosome number of the Burdur specimens is the same, but is $2n = 36$ for the Bursa specimens. The karyotype formula for the Bursa specimens is $9m + 8sm + 1st$ versus $7m + 2sm$ for the Burdur specimens. The chromosome length of the Bursa specimens ranges

between 1.74 and 2.99 μm versus 1.49 and 3.04 μm for the Burdur specimens. *C. virgata* from Burdur has some morphological differences from those mentioned in the *Flora of Turkey*. The general appearance of the species, branching, and the terminal spin length of the phyllaries are different. These differences are supported by cytological data. While *C. virgata* from Burdur is diploid, *C. virgata* from Bursa is tetraploid. Our forthcoming cytotaxonomic and morphologic studies of this species will determine the systematic final position of *C. virgata* from Burdur.

The somatic chromosome number and morphology of the species *C. tuzgoluensis* were determined in the present study and it has the smallest centromeric index (1.33) and shortest chromosome length (0.78 μm).

In the cytological study of *Centaurea aziziana* Rech.f. (section *Phalolepis*) the somatic chromosome number was reported as $2n = 2x = 18$ (Garcia-Jacas et al., 1998b). In the species *C. lycaonica* of the above-mentioned section, the somatic chromosome number is $2n = 18$. The only difference between the present study's observations and those previously reported is the existence of tetraploidy.

The karyomorphology of *Centaurea tchihatcheffii* Fisch. & C.A.Mey., which belongs to the section *Cyanus*, has been previously reported (Gömürgen & Adıgüzel, 2001). The somatic chromosome number is $2n = 20$ and the karyotype formula is $9m + 1sm$. In a study conducted by Tan & Vural (2007) the somatic chromosome number was determined as $2n = 20$. Findings regarding the somatic chromosome number and morphology of *C. cyanus* differ from those of *C. tchihatcheffii*.

In the present study karyomorphological evaluation of 8 taxa belonging to the genus *Centaurea* was conducted. The somatic chromosome numbers of 4 taxa were determined for the first time. Moreover, the karyomorphology of these 8 taxa are presented for the first time. We think that the present study will spur additional study of the genus *Centaurea*.

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