

## Karyological studies of 10 *Cirsium* sect. *Epitrachys* (Asteraceae) species from Turkey

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**Abstract:** Detailed karyotype properties were established for 10 *Cirsium* Mill. sect. *Epitrachys* DC. species distributed in Turkey. The species *Cirsium ligulare* Boiss., *C. sintenisii* Freyn, *C. boluense* P.H.Davis & Parris, *C. eriophorum* (L.) Scop., *C. steirolepis* Petr., *C. baytopae* P.H.Davis & Parris, *C. poluninii* P.H.Davis & Parris, *C. ciliatum* (Murray) Moench subsp. *szovitsii* (K.Koch) Petr., *C. ellenbergii* Bornm., and *C. vulgare* (Savi) Ten. have the somatic chromosome number  $2n = 2x = 34$ , whereas *C. sintenisii* and *C. vulgare* showed  $2n = 4x = 68$ . The chromosome numbers of *C. sintenisii*, *C. boluense*, *C. baytopae*, *C. poluninii*, and *C. ellenbergii* are newly reported here. Karyotype analysis indicated that chromosomes of *Cirsium* taxa generally have median region (m) and rarely median point (M) and submedian region (sm) karyotypes. The findings for each of the analyzed taxa are compared with the results of previous studies.

**Key words:** Chromosome number, *Cirsium*, karyotypes, new count, Turkey

### 1. Introduction

The genus *Cirsium* Mill. (thistle) includes perennial, biennial, and annual members of the family Asteraceae and comprises more than 250 species distributed in Europe; North Africa; East, Central, and Southwest Asia; and North and Central America (Charadze, 1963; Davis and Parris, 1975; Petrak, 1979; Kadereit and Jeffrey, 2007). According to recent studies of the Turkish *Cirsium*, this genus is represented in Turkey by 64 species belonging to 3 sections (Davis and Paris, 1975; Davis et al., 1988; Güner et al., 2000; Daşkın et al., 2006; Yıldız and Dirmenci, 2008; Yıldız et al., 2009a, 2009b, 2011; Arabacı and Dirmenci, 2011; Yıldız, 2012).

Approximately 69% of species of the genus *Cirsium* are diploid  $2n = 34$ , 10% are tetraploid  $2n = 68$ , and 5% are  $2n = 30$  and  $2n = 32$ , while several chromosome numbers such as  $2n = 16$ , 18, 20, 22, 24, 26, 28, 26, 51, and 102 are either very rare or doubtful (Bures et al., 2004). Chromosome studies of the Turkish *Cirsium* species reported that the taxa have  $2n = 32$ , 34, 60, and 68 diploid chromosome numbers (Ozcan et al., 2008, 2011).

A number of anatomical, palynological, molecular, and karyological studies were conducted regarding the members of Asteraceae to clarify the taxonomy of the family from Turkey (Akyalçın et al., 2011; Tabur et

al., 2012; Aydın et al., 2013). The karyology of Turkish *Cirsium* species is currently being studied in order to clarify their taxonomy and make contributions to other multidisciplinary studies on the genus. Ten species from the genus *Cirsium* sect. *Epitrachys* DC. (*C. ligulare* Boiss., *C. sintenisii* Freyn, *C. boluense* P.H.Davis & Parris, *C. eriophorum* (L.) Scop., *C. steirolepis* Petr., *C. baytopae* P.H.Davis & Parris, *C. poluninii* P.H.Davis & Parris, *C. ciliatum* (Murray) Moench subsp. *szovitsii* (K.Koch) Petr., *C. ellenbergii* Bornm., and *C. vulgare* (Savi) Ten.) are karyologically presented in this study.

### 2. Materials and methods

#### 2.1. Plant material

All plant specimens were collected from natural habitats during the fruiting season in 2006 and 2007 as a part of a taxonomic revision of the genus *Cirsium* in Turkey. The last 2 authors and Dr T Dirmenci carried out extensive field work and collected a large number of specimens in the scope of this revisionary study. Pertinent reference works from the literature were used to identify the specimens (Boissier, 1875; Petrak, 1910, 1979; Charadze, 1963; Davis and Parris, 1975; Davis et al., 1988; Güner et al., 2000). In addition, they examined many herbarium specimens at AEF, ANK, B, BM, E, EGE, ESSE, G, GAZI, HUB, ISTE, K,

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W, and WU. The voucher specimens were deposited at the Balıkesir University Herbarium. The localities according to the grid system adopted for the *Flora of Turkey and the East Aegean Islands* by Davis (1975), geographical position, altitude, and voucher number of the species are presented in Table 1.

## 2.2. Chromosome analysis

Approximately 100 seeds belong to 10 plant specimens were used for each taxon and 30 root meristems were sampled. Root meristems were used from seeds germinated on moist filter paper in petri dishes at 25 °C. The actively growing root tips were pretreated with aqueous colchicine (0.05%) for 3–3.5 h at room temperature. The material was fixed with Carnoy (1:3 glacial acetic acid–absolute ethanol) for at least 24 h at 4 °C, hydrolyzed in 1 M HCl at 60 °C for 15 min, then rinsed in tap water for 3–5 min, stained in Feulgen for 1 h, and mounted in 45% acetic acid (Elçi, 1982). Digital microphotographs from at least 5 well-spread metaphase plates were taken using an Olympus BX51 microscope and were recorded with an Olympus Camedia C-4000 digital camera. The length of the short arm (s) and long arm (l) of each chromosome was measured from images of selected cells. In addition, total lengths ( $tl = l + s$ ), arm ratios ( $r = l/s$ ), and centromeric indices ( $100 \times s/tl$ ) were determined. The chromosome nomenclature followed that of Levan et al. (1964). The intrachromosomal asymmetry index (A1) was calculated according to the formula given by Romero Zarco (1986), and the interchromosomal asymmetry index (A2) was defined as the ratio of the chromosome length to the mean chromosome length.

The chromosome numbers of the species were identified and checked using the *Flora of Turkey* (Davis and Parris, 1975) and supplements (Davis et al., 1988; Güner et al., 2000). Relevant literature (Boissier, 1875; Petrak, 1910) and the online chromosome number databases, *Index to Plant Chromosome Numbers (IPCN)* (<http://www.tropicos.org/Project/IPCN>) and *Index to Chromosome Numbers in Asteraceae* (<http://www.lib.kobe-u.ac.jp>), were also checked (Goldblatt and Johnson, 1979; Watanabe, 2010).

## 3. Results and discussion

### *Cirsium* sect. *Epitrachys* DC.

#### *Cirsium ligulare* Boiss.

This annual or biennial species grows in *Abies* Mill. forests, deciduous forest openings, watersides, and steppe at altitudes of 300–1800 m. The chromosome number of this species was previously found to be both diploid ( $2n = 34$ ) and tetraploid ( $2n = 68$ ) in Bulgarian and Greek populations (Strid and Franzen, 1981; Kuzmanov and Georgieva, 1983; Kuzmanov et al., 1991). According to our counts, the chromosome number in examined specimens is diploid and  $2n = 34$  in the Turkish population. The chromosome length ranges from 3.8 to 7.6  $\mu\text{m}$ , and the ratio of the longest to shortest chromosome is 2:1. In general, it consists of median region (m) chromosomes, with only one median point (M) chromosome (Table 2; Figures 1 and 2).

#### *Cirsium sintenisii* Freyn

*C. sintenisii* is an endemic species that grows in *Abies* and *Pinus* L. forest openings, field margins, roadsides, and

**Table 1.** Localities and voucher numbers of *Cirsium* taxa investigated.

Taxa	Locality	Voucher number
<i>C. ligulare</i>	A3 Bolu: 25 km from Mudurnu to Göynük, waterside, 700 m, 22.08.2007	Yıldız 16604 & Arabacı
<i>C. sintenisii</i>	A4 Kastamonu: North of Ilgaz mountain, 1650 m, 23.08.2006	Yıldız 16408 & Dirmenci
<i>C. boluense</i>	A3 Bolu, Kartalkaya, mountain meadow, 1900–2000 m, 22.08.2007	Yıldız 16612 & Arabacı
<i>C. eriophorum</i>	A2(A) Bursa: Uludağ: 1800 m, 21.08.2007	Yıldız 16591 & Arabacı
<i>C. steirolepis</i>	B1 Çanakkale: Between Bayramiç and Yenice, <i>Pinus</i> forest openings, 350 m, 31.07.2007	Yıldız 16496 & Dirmenci
<i>C. baytopae</i>	A1 (E) Kırklareli: Saray, Güngörmez village, <i>Quercus-Carpinus</i> forest openings, 100 m, 02.08.2007	Yıldız 16528 & Dirmenci
<i>C. poluninii</i>	B5 Yozgat: Akdağmadeni, 1–2 km west of Oluközü village, 1200 m, 11.08.2006	Yıldız 16232 & Dirmenci
<i>C. ciliatum</i> subsp. <i>szovitsii</i>	A8 Erzurum: Oltu, east of Başbağlar village, steppe, 1500–1600 m, 16.08.2006	Yıldız 16293 & Dirmenci
<i>C. ellenbergii</i>	B6 Kahramanmaraş: Göksun, 1 km west of Çardak village, steppe, 1300 m, 02.09.2006	Yıldız 16430 & Dirmenci
<i>C. vulgare</i>	B6 Sivas: 5 km east of Zara, steppe, 1200 m, 11.08.2006	Yıldız 16236 & Dirmenci

**Table 2.** Somatic chromosome number (2n), ploidy level, karyotype formula, ranges of chromosome length, total karyotype length (TKL), and asymmetry indexes (A<sub>1</sub>, A<sub>2</sub>) of Romero Zarco (1986) for the studied *Cirsium* taxa.

Taxa	2n	Ploidy level	Karyotype formula	Chromosome length range (µm)	TKL (µm)	A <sub>1</sub>	A <sub>2</sub>
<i>C. ligulare</i>	34	2x	M+16m	3.80–7.60	91.40	0.22	0.19
<i>C. sintenisii</i>	34	2x	M+16m	5.50–12.70	138.95	0.18	0.23
	68	4x	M+2sm+31m	3.70–8.80	203.95	0.24	0.20
<i>C. boluëense</i>	34	2x	sm+16m	5.00–7.54	105.7	0.25	0.21
<i>C. eriophorum</i>	34	2x	M+16m	6.00–11.69	138.77	0.16	0.21
<i>C. steirolepis</i>	34	2x	17m	5.00–8.92	113.85	0.25	0.20
<i>C. baytopae</i>	24	2x	12m	4.40–9.15	75.5	0.21	0.20
<i>C. poluninii</i>	34	2x	M+16m	3.60–6.40	83.8	0.21	0.16
<i>C. ciliatum</i> subsp. <i>szovitsii</i>	34	2x	M+sm+15m	4.90–10.60	122.05	0.21	0.21
<i>C. ellenbergii</i>	34	2x	2M+15m	3.50–6.40	85.4	0.18	0.20
<i>C. vulgare</i>	34	2x	M+16m	3.55–6.70	82.9	0.20	0.17
	68	4x	3M+31m	4.25–6.60	169.05	0.21	0.11

steppe at altitudes of 500–1700 m. The seeds were collected from Kastamonu Province, which is also the locality of the type specimen of the species (Table 1). This species occurs as both diploid and tetraploid with  $2n = 34, 68$  chromosome numbers. To our knowledge, this is the first chromosome count report for this species. In addition to normal cells with  $2n = 2x = 34$ , some polyploid cells with  $2n = 4x = 68$  were also observed. It can be concluded that the polyploidy level derived from a mechanism of abnormal cell division. Metaphase chromosome length ranges from 5.50 to 12.70 µm, and the longest-to-shortest chromosome ratio is 2.3:1. The karyotype comprises 16 median region (m) chromosomes and 1 median point (M) chromosome in diploids. In tetraploids, the chromosome length varies between 3.70 and 8.80 µm. The longest-to-shortest chromosome ratio is 2.4:1, and the karyotype consists of 31 median region (m) chromosomes, 2 submedian region (sm) chromosomes, and 1 median point (M) chromosome (Table 2; Figures 1 and 2).

#### *Cirsium boluëense* P.H.Davis & Parris

It is a biennial species that grows in subalpine meadows and steppes between 1800 and 2000 m in altitude. According to literature, this is the first chromosome count for this Turkish endemic species. This species is diploid with  $2n = 34$  chromosome number, and it consists of 16 median region (m) chromosomes and 1 submedian region (sm) chromosome. Metaphase chromosome length ranges from 5 to 7.54 µm, and the longest-to-shortest chromosome ratio is 1.5:1 (Table 2; Figures 1 and 2).

#### *Cirsium eriophorum* (L.) Scop.

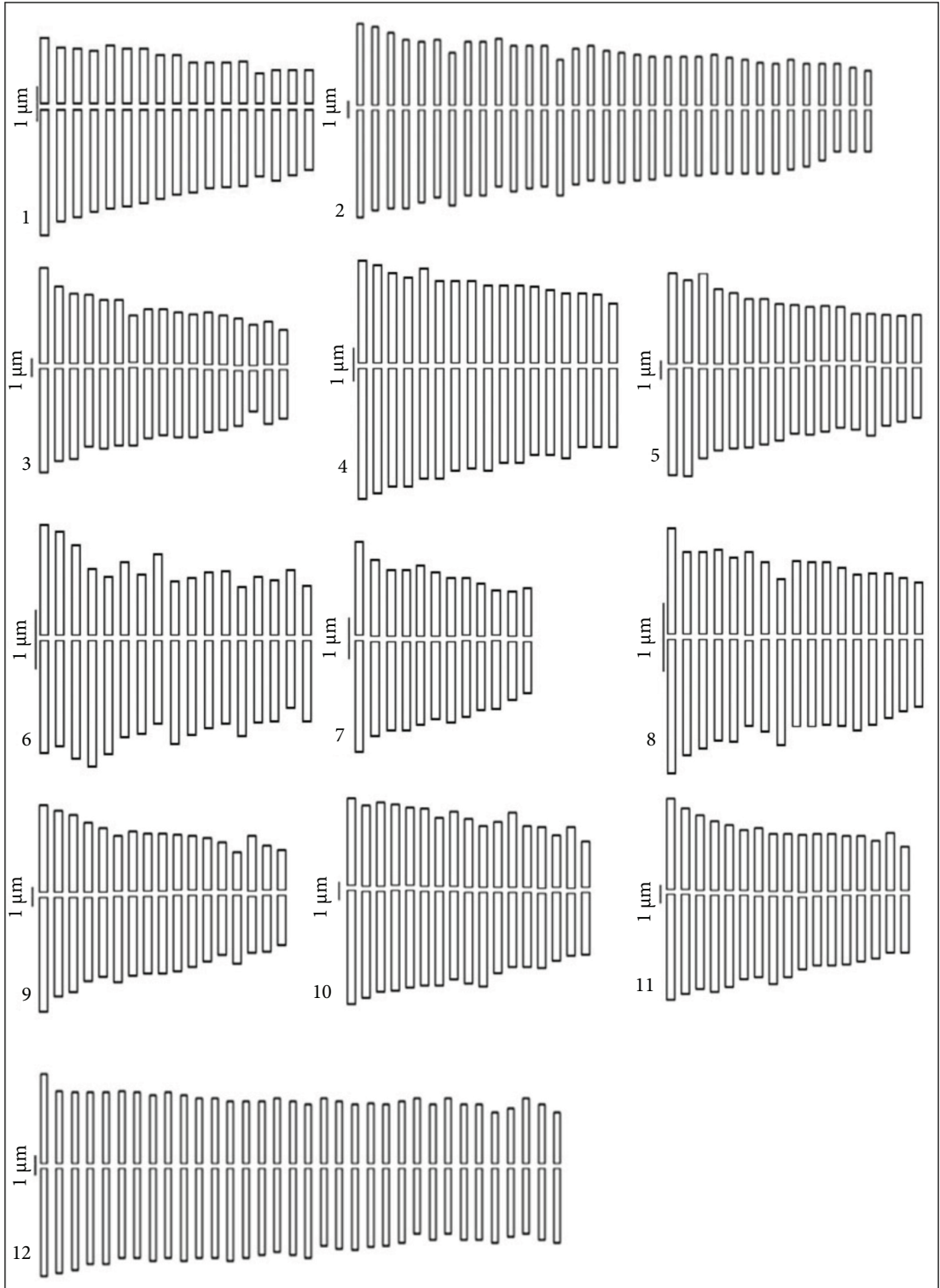
It is a biennial species that grows in forest openings, roadsides, and open places at altitudes of 1700–1800 m. There are many counts given for this species. All previous counts and our results are well in agreement with the diploid chromosome number  $2n = 34$  (Czapik, 1958; Moore and Frankton, 1962; Majovsky et al., 1970; Sz.-Borsos, 1970; Ferakova, 1972; Morton, 1977; Dobeš and Vitek, 2000; Bures et al., 2004; Daşkın et al., 2006). According to our micromorphological studies, the metaphase chromosome length ranges from 6 to 11.69 µm and the ratio of the longest to shortest chromosome is 1.9:1. It consists of 16 median region (m) chromosomes and 1 median point (M) chromosome (Table 2; Figures 1 and 2).

#### *Cirsium steirolepis* Petr.

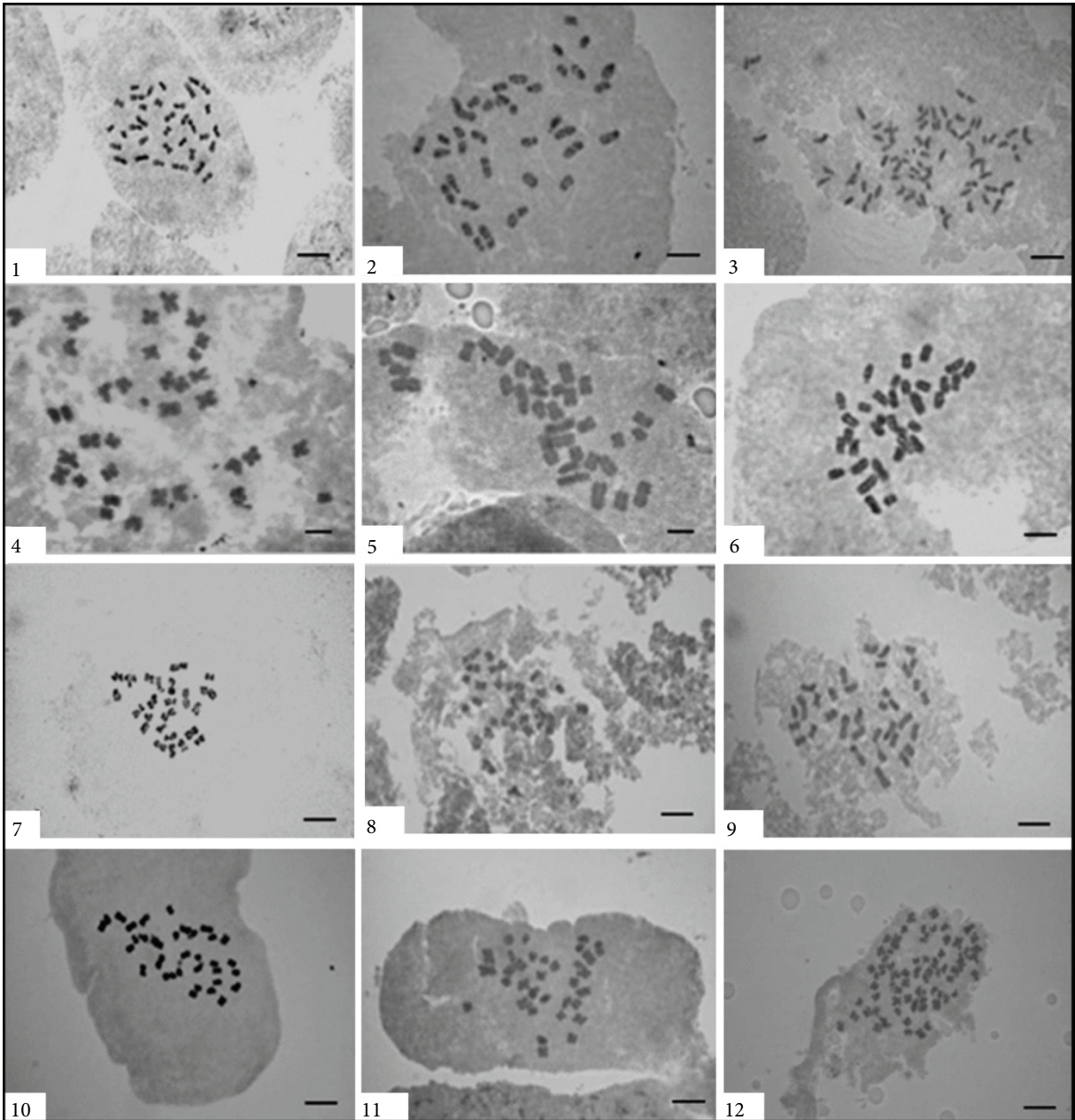
This annual or biennial endemic species grows in *Pinus brutia* Ten. and *Quercus* L. forest openings between 300 and 1400 m. In our study, the somatic chromosome number is determined as  $2n = 34$  for this species. According to our knowledge, this is the second report after Snogerup (1995) and it is in agreement with the previous count. The karyotype of this species is uniformly median region (m), the chromosome length ranges from 5 to 8.92 µm, and the longest-to-shortest chromosome ratio is 1.8:1 (Table 2; Figures 1 and 2).

#### *Cirsium baytopae* P.H.Davis & Parris

This is a Thrace species and grows in deciduous forest openings at altitudes of 50–380 m. To our knowledge, this is the first count for this species. The somatic chromosome



**Figure 1.** Haploid idiograms. 1- *Cirsium ligulare*, 2- *C. sintenisii*, 3- *C. sintenisii*, 4- *C. boluense*, 5- *C. eriophorum*, 6- *C. steirolepis*, 7- *C. baytopae*, 8- *C. poluninii*, 9- *C. ciliatum* subsp. *szovitsii*, 10- *C. ellenbergii*, 11- *C. vulgare*, 12- *C. vulgare*.



**Figure 2.** Somatic metaphases in *Cirsium* taxa. 1- *Cirsium ligulare*, 2- *C. sintenisii*, 3- *C. sintenisii*, 4- *C. boluense*, 5- *C. eriophorum*, 6- *C. steirolepis*, 7- *C. baytopae*, 8- *C. poluninii*, 9- *C. ciliatum* subsp. *szovitsii*, 10- *C. ellenbergii*, 11- *C. vulgare*, 12- *C. vulgare*.

number is determined as  $2n = 24$  and all of them are median region (m). The chromosome length ranges from 4.4 to 9.15  $\mu\text{m}$ , and the ratio of the longest to shortest chromosome is 2.1:1 (Table 2; Figures 1 and 2).

#### *Cirsium poluninii* P.H.Davis & Parris

It is a biennial species that grows on steppe, *Pinus nigra* J.F.Arnold forest openings, and roadside between 1000 and 1750 m. Our count in examined specimens was

$2n = 34$ . To the extent of our knowledge, this is the first chromosome count for this Turkish endemic species. The chromosome length ranges from 3.6 to 6.4  $\mu\text{m}$ , the longest-to-shortest chromosome ratio is 1.8:1, and it consists of 16 median region chromosomes (m) and 1 median point (M) chromosome (Table 2; Figures 1 and 2).

*Cirsium ciliatum* (Murray) Moench subsp. *szovitsii* (K.Koch) Petr.

This is a biennial taxon and Irano-Turanian element that grows on roadsides, steppe, fallow fields, and ditches between 1500 and 2500 m. Previous studies reported 2 different chromosome counts. The chromosome number of this taxon was reported by Tonian (1982) from Armenia as  $2n = 4x = 68$  and by Ozcan et al. (2008) from Turkey as  $2n = 2x = 34$ . Our count, based on Turkish plants, is in accordance with that of Ozcan et al. (2008), with a diploid chromosome number of  $2n = 2x = 34$ . The chromosome length ranges from 4.9 to 10.6  $\mu\text{m}$  and the ratio of the longest chromosome to the shortest is 2.2:1. The karyotype consists of 15 median region chromosomes (m), 1 submedian region (sm) chromosome, and 1 median point (M) chromosome (Table 2; Figures 1 and 2).

#### ***Cirsium ellenbergii* Bornm.**

It is a biennial species and Irano-Turanian element that grows on limestone, steppe, and fallow fields at altitudes of 1200–3000 m. The chromosome number of this species is determined as  $2n = 2x = 34$ . To our knowledge, this is the first count for this rare endemic species. According to our study, the chromosome length ranges from 3.5 to 6.4  $\mu\text{m}$ , and the ratio of the longest to shortest chromosome is 1.8:1. It consists of 15 median region (m) and 2 median point (M) chromosomes (Table 2; Figures 1 and 2).

#### ***Cirsium vulgare* (Savi) Ten.**

This species has a wide distribution range, especially in the northern hemisphere, from sea level to 2000 m. In this study, the chromosome number is determined as  $2n = 34, 68$ . Several chromosome counts of  $2n = 34, 56\text{--}60, 60, 68, \text{ and } 102$  were given previously in the literature for this species (Tischler, 1950; Fedorov, 1969; Fernandes and Queiros, 1971; Talavera, 1974; Morton, 1977; Agapova et al., 1990; Kuzmanov et al., 1991; Dempsey et al., 1994; Krasnikov et al., 2003; Ozcan et al., 2008, 2011; Nouroozi et al., 2010). *C. vulgare* is a widespread species and is distributed in various habitats. It has a mixed reproduction biology system that involves both self- and cross-pollination (Powell et al., 2011). Hence, various chromosome numbers are seen in this species. According to our counts the chromosome length ranges from 3.55 to 6.7  $\mu\text{m}$ , the longest/shortest chromosome ratio is 1.9:1, and the karyotype consists of 16 median region (m) chromosomes and 1 median point (M) chromosome at the diploid level. In tetraploids, the chromosome length ranges from 4.25 to 6.6  $\mu\text{m}$ , and the ratio of the longest-to-shortest chromosome is 1.6:1. The karyotype consists of 31 median region (m) and 3 median point (M) chromosomes (Table 2; Figures 1 and 2).

This study shows that  $2n = 2x = 34$  is the most frequent chromosome number in the examined species of *Cirsium* sect. *Epitrachys*. In addition, the  $2n = 4x = 68$  chromosome number is also seen in 2 taxa (Table 2). The ploidy levels are  $2n = 2x = 34$  and  $4x = 68$  in rare endemic species *C.*

*sintenisii* and in the widespread (especially in the northern hemisphere) weed *C. vulgare*. Similarly, diploid and tetraploid cytotypes and also some aneuploid series ( $2n = 60$ ) were reported by Ozcan et al. (2008) from Turkish populations for *C. vulgare*. This may be related to their reproductive biology, such as self- and cross-pollination. *C. vulgare* also shows morphological variations in terms of stem, leaf, and capitula size. In addition, several chromosome numbers such as  $2n = 34, 56\text{--}60, 60, 68, \text{ and } 102$  were reported from different localities for this species. *C. ligulare* from Bulgarian and Greek populations ( $2n = 34, 68$ ) and *C. ciliatum* subsp. *szovitsii* collected from Armenia ( $2n = 68$ ) had different chromosome numbers than ours. Specimens collected from various geographical regions can have different chromosome numbers. The diverging karyological results might result from intraspecific karyological differentiation. Similar situations were reported by Hayırlioğlu-Ayaz and Beyazoğlu (2000), Inceer and Beyazoglu (2004), and Ozcan et al. (2008).

To the extent of our knowledge, the chromosome numbers of *C. sintenisii*, *C. boluense*, *C. baytopae*, *C. poluninii*, and *C. ellenbergii* are new counts to science. During the revisionary study of Turkish *Cirsium* species, the distribution of *C. bulgaricum* DC. in Anatolia (as given by Davis and Parris, 1975, p. 380) could not be confirmed (Yıldız, 2012). Except for the type specimen, the Anatolian specimens belong to *C. poluninii*. Therefore, the chromosome number of *C. bulgaricum* ( $2n = 34$ ) given by Ozcan et al. (2011) from an Anatolian specimen was probably for *C. poluninii*, which would be in agreement with the chromosome number of the *C. poluninii* that we examined here.

The main karyotype details of the species examined in this study are presented in Table 2 and shown in Figures 1 and 2. The chromosomes generally consist of median region (m) karyotypes. Moreover, the chromosomes of 7 taxa (*C. ligulare*, *C. sintenisii* ( $2x$ ), *C. eriophorum*, *C. poluninii*, *C. ciliatum* subsp. *szovitsii*, *C. ellenbergii*, and *C. vulgare*) include median point (M), and 3 taxa (*C. sintenisii* ( $4x$ ), *C. boluense*, and *C. ciliatum* subsp. *szovitsii*) have submedian region (sm) chromosomes.

Chromosome lengths show wide variations. The mean chromosome length varies between 4.8 and 8.1  $\mu\text{m}$ . *C. vulgare* has the smallest chromosome length, ranging from 3.55 to 6.70  $\mu\text{m}$  (mean: 4.88  $\mu\text{m}$ ) at diploid level and between 4.25 and 6.60  $\mu\text{m}$  (mean: 4.97  $\mu\text{m}$ ) at tetraploid level. The longest chromosome length seen at diploid level of *C. sintenisii* ranged from 5.50 to 12.70  $\mu\text{m}$  (mean: 8.17  $\mu\text{m}$ ), and that of *C. eriophorum* was between 6.00 and 11.69  $\mu\text{m}$  (mean: 8.16  $\mu\text{m}$ ) (Table 2).

The intrachromosomal asymmetry index (A1) varies from 0.16 in *C. eriophorum* to 0.25 in *C. boluense* and *C. steirolepis*. Interchromosomal asymmetry index (A2)

ranges from 0.11 at tetraploid level in *C. vulgare* to 0.23 at diploid level in *C. sintenisii* (Table 2).

According to previous studies by Ozcan et al. (2008, 2011) on Turkish *Cirsium* species, chromosome numbers of 36 taxa (18 of them were new counts) were established as  $2n = 32, 34, 36,$  and  $68$ . According to our knowledge, there is no detailed karyotype analysis on Turkish *Cirsium* species in the literature. In our study, chromosome numbers (5 of them are new counts) and detailed karyotype analysis

of 10 taxa are given. Further studies will determine the karyotype properties and implications on the systematics of the genus *Cirsium* from Turkey.

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