TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2019) 43: 538-550

doi:10.3906/bot-1811-28

Karyomorphological features of Turkish *Centaurea* (subgenus *Cyanus*, Asteraceae) species and its taxonomic importance

Emrah ŞİRİN*, Meryem BOZKURT, Tuna UYSAL, Kuddisi ERTUĞRUL
Department of Biology, Faculty of Science, Selçuk University, Konya, Turkey

Received: 19.11.2018 • **Accepted/Published Online:** 25.02.2019 • **Final Version:** 08.07.2019

Abstract: In this study, the karyomorphology of 20 Turkish *Centaurea* (subgenus *Cyanus*) taxa was examined. The number of chromosomes of 11 taxa belonging to the subgenus *Cyanus* was determined for the first time. As a result of the karyomorphological studies, the number of basic chromosomes was determined to be x = 8, 10, and 12 in annuals and x = 10 and 11 in perennials. The populations are tetraploid in the seven perennial taxa and polyploidy is not rare for this group. On the other hand, all annual taxa are diploid. Considering the asymmetry indices, we can conclude that most taxa have symmetrical karyotypes. The most common karyotype formulas are 40 metacentric chromosomes (m), 20m, and 16m + 4 submetacentric chromosomes, respectively. A satellite was detected in the majority of the taxa, but it was observed to be mainly localized on the short arm of the chromosome. Satellites are located mainly on the second chromosome.

Key words: Asymmetry, chromosome counts, endemic, karyomorphology, metacentric

1. Introduction

Cytotaxonomy is a branch of cytogenetics in which karyological features are systematically evaluated for evolutionary purposes (Siljak-Yakovlev and Peruzzi, 2012). Chromosomes, especially plant chromosomes, are useful materials in nearly any type of cytogenetic research (Guerra, 2012). Because the genetic information of an organism is transmitted through its chromosomes, any changes in numbers (e.g., polyploid or diploid) and structures (e.g., inversion, deletion, or translocation) contribute significantly to plant evolution and speciation; however, to interpret the evolutionary history of a group, the number of chromosomes alone is not enough (Weiss-Schneeweiss and Schneeweiss, 2003) and karyomorphology might supply additional information. In some cases, ecological and morphological data might not be sufficient for analyzing the line of descent among the species. In these situations, cytotaxonomic relationships along with molecular data might be more influential in the analyses (Venora et al., 2008). The karyotype reveals phenotypic appearance in terms of number, size, arm ratio, centromere position, and other basic characteristics of chromosomes (Levin, 2002).

Centaurea, which belongs to the tribe Cardueae, is a large genus with approximately 250 species (Susanna and Garcia-Jacas, 2007) and the highest rate of endemism, with 112 endemics among the total 181 species in Turkey (Uysal, 2012). *Cyanus*, a subgenus, is represented by approximately 25 species worldwide (Hellwig, 2004).

According to recent definitions of *Centaurea* (Susanna and Garcia-Jacas, 2007), the following 3 subgenera are included: *Acrocentron*, *Centaurea*, and *Cyanus*. Sister relationships of *Cyanus* and *Centaurea* are firmly established, but the connections between these subgenera and *Acrocentron* are unclear (Susanna and Garcia-Jacas, 2009). Based on current molecular studies, for the aims of our research, *Cyanus* is considered a subgenus.

Taxonomically, *Cyanus* was first described as a genus by Miller (1754). The group was subsequently reassigned as a section of *Centaurea* by de Candolle (1838) and this was widely accepted by taxonomists (Bentham, 1873; Boissier, 1875; Wagenitz, 1975). It is now generally accepted that *Cyanus* is a subgenus (Hilpold et al., 2014) or, rarely, a group (Wagenitz and Hellwig, 1996; Garcia-Jacas et al., 2001) within *Centaurea*; however, some authors still assert that it is a genus (Greuter, 2003; Bancheva and Greilhuber, 2006).

The *Cyanus* group is distributed across central and southern Europe, North Africa, Anatolia, and the Caucasus, and some species have spread to Iran and Afghanistan (Boršić et al., 2011).

The floret colors are blue or purplish blue in this group, with a few exceptions of taxa that are cream or

* Correspondence: emrahsirin@selcuk.edu.tr

pale pink, which is extremely unusual for the subtribe Centaureinae. In addition, the most peculiar character is the appendages of the phyllaries, which are decurrent to nearly the base and are not spiny (Wagenitz and Hellwig, 1996). This group also shares some important features with the *Jacea* and *Acrocentron* groups. The marginal florets are sterile and without staminodes, and the hilum of the seed is lateral (Garcia-Jacas et al., 2001).

In addition to its morphological characteristics, the *Cyanus* group is characterized by having two types of pollen. According to Wagenitz (1955), two of the eight pollen types in *Centaurea* s.l. are defined within two subgroups of *Cyanus*. Annuals form one subgroup with the *Cyanus* pollen type, while perennials form the other subgroup with the *Montana* pollen type.

The aims of the present study were to reveal the karyomorphological features of species of the subgenus *Cyanus*, solve chromosomal interactions of closely related species, and investigate the degree of chromosomal variation of the studied taxa at the inter- and intraspecies level.

2. Materials and methods

Achenes were collected from various locations in Turkey between 2014 and 2017 (Table 1). Mature achenes were selected and periodically germinated for chromosomal analyses. Chromosomes were counted during somatic metaphase using the squash technique. Primary root meristems were used to obtain metaphase plates. The samples were pretreated with 0.002 M 8-hydroxyquinoline for 8 h at 4 °C and then fixed with Carnoy solution for 24 h at 4 °C. The material was hydrolyzed with 5 N HCl for 30 min at room temperature and then stained with 1% aceto-orcein. Samples were made permanent according to the method of Bowen (1956). At least 10 metaphases for each taxon were examined and the best metaphase image was photographed at 100× magnification using a Olympus DP-72 digital camera attached to an Olympus BX53 microscope.

The chromosome nomenclature of Levan et al. (1964) was followed; m and sm were used to represent metacentric and submetacentric chromosomes, respectively. Karyotype asymmetry was calculated based on the average centromere index (CI), the shortest/longest pairwise rate, and the A1 and A2 indices. The variation in chromosome length (CV_{CL}) and karyotype asymmetry index were calculated according to the method of Paszko (2006), and the mean centromeric asymmetry (M_{CA}) was calculated according to Peruzzi and Eroğlu (2013). The karyograms and idiograms of the taxa were created using the KAMERAM analysis system.

3. Results

3.1. *Centaurea reuteriana* Boiss. var. *reuteriana*

According to our data, this study is the first chromosome count and karyomorphology report of the taxon. The chromosome number of the taxon was identified as 2n = 20 with a diploid set of chromosomes (Figures 1–3; CY1). There are both sm and m chromosomes; the shortest one is 0.98 µm and the longest is 1.59 µm. The asymmetry index is 1.26. The karyotype formula is 16m + 4sm.

3.2. *C. reuteriana* Boiss. var. *phrygia* Bornm.

This investigation is the first chromosome count and morphology report of the taxon. The chromosome number of this taxon is 2n = 22 with a diploid set of chromosomes (Figures 1–3; CY2). There are both sm and m chromosomes; the shortest one is 1.03 µm and the longest is 1.70 µm. The asymmetry index is 0.69. The karyotype formula is 16m + 6sm.

It was interesting for us that two varieties have different chromosomes. However, the chromosome counts we made were from different localities and confirmed this result. We thought of separating the variety as species, but morphological differences were limited. A similar situation was seen in the varieties of *Draba helleriana* Greene (Ward, 1983; Ward and Spellenberg, 1988; Warwick and Al-Shehbaz, 2006).

3.3. *C. lanigera* DC.

This study is the first chromosome count and karyomorphology report of the species. The chromosome number of this taxon is 2n = 20 with a diploid set of chromosomes (Figures 1–3; CY3). There are both sm and m chromosomes; the shortest is 0.94 µm and the longest is 1.51 µm. The asymmetry index is 1.601. The karyotype formula is 16m + 4sm.

3.4. *C. nigrofimbria* (K.Koch) Sosn.

According to our data, this is the first chromosome count and karyomorphology report of the species. This taxon is distinguished from the others by its blackish cilia. The chromosome number is 2n = 20 with a diploid set of chromosomes (Figures 1–3; CY4). The chromosomes consist of both sm and m chromosomes; the shortest one is 1.13 µm and the longest is 1.86 µm. The asymmetry index is 1.08. The karyotype formula is 18m + 2sm.

3.5. *C. woronowii* Bornm.

This investigation is the first chromosome count and morphology report of the species. *C. woronowii* is distinguished from the others by the linearity of the lobes of marginal flowers. Its chromosome number is 2n = 20 with a diploid set of chromosomes (Figures 1–3; CY5). The chromosomes consist of both sm and m chromosomes; the shortest one is 1.07 µm and the longest is 2.33 µm. The asymmetry index is 2.41. The karyotype formula is 16m + 4sm.

Table 1. Locations of the studied taxa.

Taxa	Taxa Accesions	Locality	Collection Number	Endemic to Turkey
C. reuteriana var. *reuteriana*	CY1	[C2] Muğla: Köyceğiz, Sandras Mountain, 1750 m, 29.06.2015 (KNYA)	EŞ-574-MŞ	
C. reuteriana var. *phrygia*	CY2	[A4] Karabük: Keltepe, 1800 m, 09.07.2015 (KNYA)	EŞ-582-MY	+
C. lanigera	CY3	[B5] Aksaray: Hasan Mountain, 1979 m, 29.06.2016 (KNYA)	EŞ-659-MŞ	+
C. nigrofimbria	CY4	[A8] Rize: İkizdere, Cimil Plateau, 1803 m, 13.07.2016 (KNYA)	EŞ-669-MŞ	
C. woronowii	CY5	[A8] Artvin: Hatila Valley National Park, 500 m, 11.06.2016 (KNYA)	EŞ-640-MŞ	
C. eflanensis	CY6	[A4] Karabük: Safranbolu - Bartın road, 1078 m,	EŞ-654-MŞ	+
C. thirkei	CY7	[C2] Denizli: Tavas, Kazıkbeli Pass, 1260 m, 28.04.2016 (KNYA)	EŞ-601-MŞ	
C. cheiranthifolia var. *cheiranthifolia*	CY8	[A9] Ardahan: Hanak, Aydere Village, 2326 m, 14.07.2016 (KNYA)	EŞ-672-MŞ	
C. cheiranthifolia var. *purpurascens*	CY9	[A9] Ardahan: Posof, Ilgar Pass, 2437 m, 14.07.2016 (KNYA)	EŞ-671-MŞ	
C. bourgaei	CY10	[C3] Antalya: Elmalı, Kızlar Sivrisi, 1900 m, 29.06.2015 (KNYA)	EŞ-572-MŞ	
C. pichleri subsp. *pichleri*	CY11	[B6] Kayseri: Pınarbaşı, Şirvan Mountain, 2078 m, 30.06.2016 (KNYA)	EŞ-661-MŞ	
C. pichleri subsp. *extrarosularis*	CY12	[C4]Konya: Konya-Beyşehir road, 1270 m, 27.06.2015 (KNYA)	EŞ-568-MŞ	+
C. triumfettii subsp. *axillaris*	CY13	[A7] Gümüşhane: Torul, Büyükçit Village, 1480 m, 13.06.2016 (KNYA)	EŞ-651-MŞ	
C. huetii	CY14	[B7] Sivas: Divriği, Göl Mountain, 1926 m, 01.07.2016 (KNYA)	EŞ-666-MŞ	
C. mathiolifolia	CY15	[C2] Burdur: Tefenni-Korkuteli road, 1351 m, 28.04.2016 (KNYA)	EŞ-599-MŞ	+
C. germanicopolitana	CY16	[A4] Karabük: Eflani, Kavak Village, 920 m, 03.08.2015 (KNYA)	EŞ-586-MŞ	+
C. depressa	CY17	[B6] Sivas: Gürün, Böğrüdelik Pass, 1800 m, 30.06.2016 (KNYA)	EŞ-663-MŞ	
C. pinardii	CY18	[B3] Afyon: Dazkırı, Sarıkavak Village, 864 m, 29.04.2016 (KNYA)	EŞ-602-MŞ	
C. tchihatcheffii	CY19	[B4] Ankara:Gölbaşı, 950 m, 28.05.2015 (KNYA)	EŞ-556-MŞ	+
C. cyanus	CY20	[B1]Manisa: Spil Mountain, 647 m, 30.04.2016 (KNYA)	EŞ-604-MŞ	

3.6. *C. eflanensis* (Kaya & Bancheva) Şirin & Ertuğrul, comb. nova

≡ *Cyanus eflanensis* Kaya & Bancheva, Novon 19: 175 (2009)

Cyanus was accepted as a section in the *Flora of Turkey* by Wagenitz (1975) and as a genus in *A Checklist of the Flora of Turkey* (Uysal, 2012) but has been considered as a subgenus in this study; therefore this species needs a new combination. The chromosome number of the taxon is identified as 2n = 20 with a diploid set of chromosomes (Figures 1–3; CY6). There are both sm and m chromosomes; the shortest one is 0.99 µm and the longest is 1.42 µm. The asymmetry index is 0.679. The karyotype formula is 18m + 2sm.

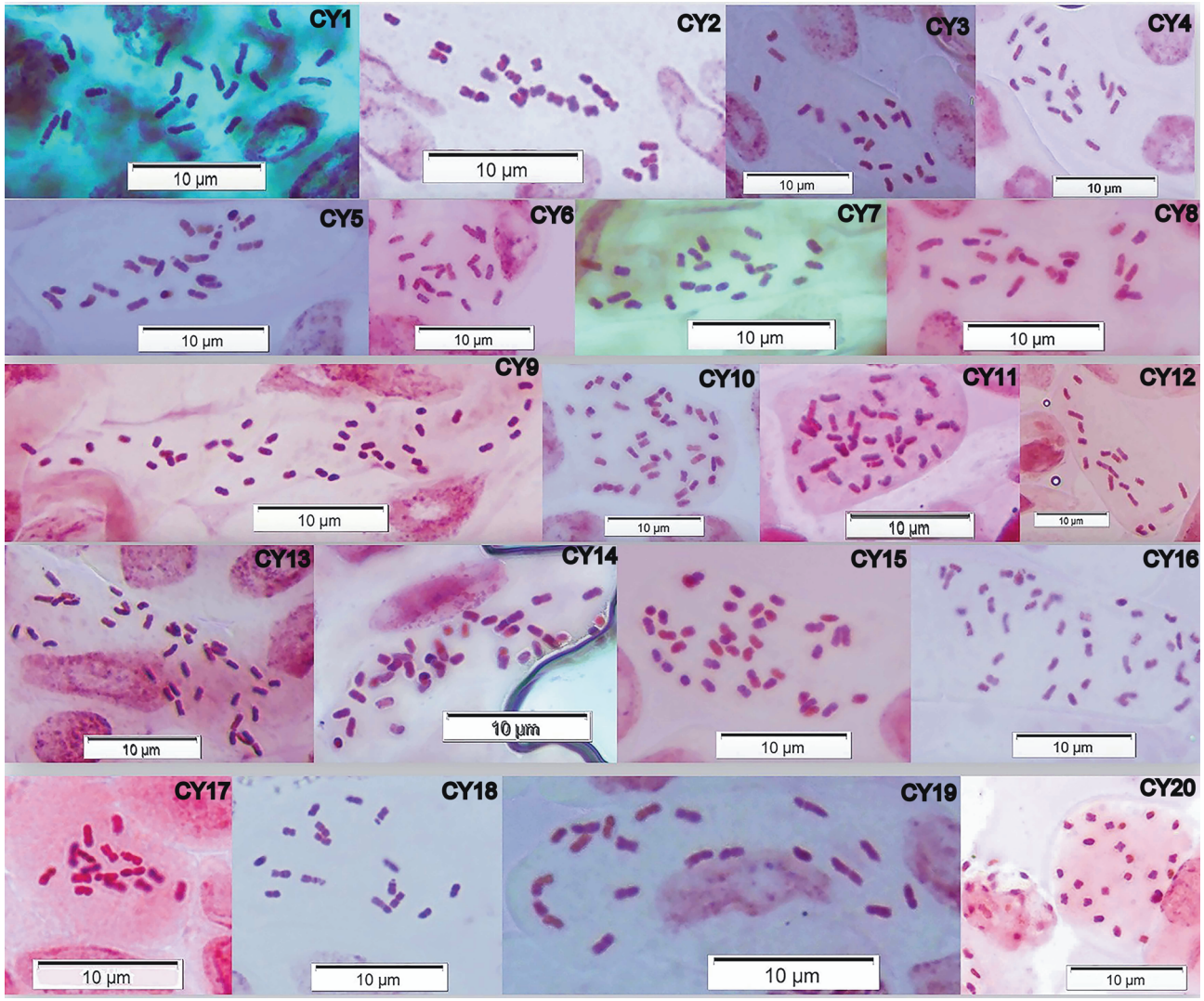

Figure 1. Mitotic metaphase chromosomes of taxa belonging to subgen. *Cyanus*. CY1: *C. reuteriana* var. *reuteriana*, CY2: *C. reuteriana* var. *phrygia*, CY3: *C. lanigera*, CY4: *C. nigrofimbria*, CY5: *C. woronowii*, CY6: *C. eflanensis*, CY7: *C. thirkei*, CY8: *C. cheiranthifolia* var. *cheiranthifolia*, CY9: *C. cheiranthifolia* var. *purpurascens*, CY10: *C. bourgaei*, CY11: *C. pichleri* subsp. *pichleri*, CY12: *C. pichleri* subsp. *extrarosularis*, CY13: *C. triumfettii* subsp. *axillaris*, CY14: *C. huetii*, CY15: *C. mathiolifolia*, CY16: *C. germanicopolitana*, CY17: *C. depressa*, CY18: *C. pinardii*, CY19: *C. tchihatcheffii*, CY20: *C. cyanus*. Scale bar: 10 µm.

3.7. *C. thirkei* Sch.Bip.

The chromosome number of this taxon has been previously reported as 2n = 20 + 1B (Bancheva and Greilhuber, 2006). According to the results of our analysis, chromosome B was not identified in the Turkish samples. The chromosome number of the taxon was instead identified as 2n = 22 with a diploid set of chromosomes (Figures 1–3; CY7). These are m chromosomes; the shortest one is 0.64 µm and the longest is 1.11 µm. The asymmetry index is 0.954. The karyotype formula is 22m.

3.8. *C. cheiranthifolia* Willd. var. *cheiranthifolia*

Olšavská et al. (2013) reported that the previous counts for this taxa were 2n = 18, 32, and 40, but the 18 and 32 counts were noted as misdiagnosed or miscounted. In our study, the chromosome number is 2n = 20 with a diploid set of chromosomes (Figures 1–3; CY8). These are m chromosomes; the shortest one is 1.41 µm and the longest is 2.52 µm. The asymmetry index is 0.925. The karyotype formula is 20m.

3.9. *C. cheiranthifolia* Willd. var. *purpurascens* (DC.) Wagenitz

The chromosome number of this taxon was identified as 2n = 4x = 40, which is tetraploid level (Figures 1–3; CY9). These are m chromosomes; the shortest one is 0.8 µm and the longest is 1.25 µm. The asymmetry index is 0.2. The karyotype formula is 40m.

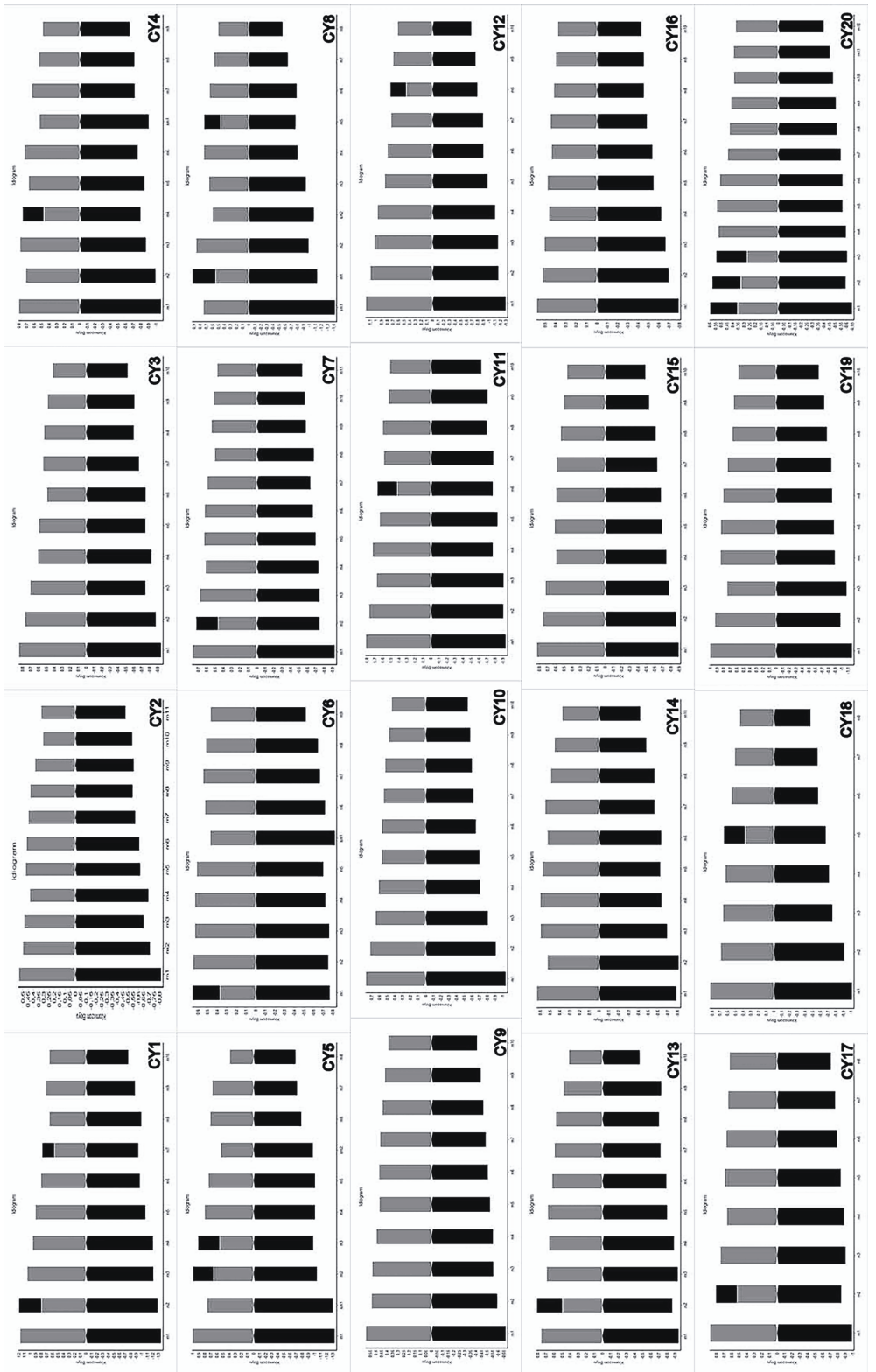

Figure 2. Idiograms of taxa belonging to subgen. *Cyanus*. CY1: *C. reuteriana* var. *reuteriana*, CY2: *C. reuteriana* var. *phrygia*, CY3: *C. lanigera*, CY4: *C. nigrofimbria*, CY5: *C. woronowii*, CY6: *C. eflanensis*, CY7: *C. thirkei*, CY8: *C. cheiranthifolia* var. *cheiranthifolia*, CY9: *C. cheiranthifolia* var. *purpurascens*, CY10: *C. bourgaei*, CY11: *C. pichleri* subsp. *pichleri*, CY12: *C. pichleri* subsp. *extrarosularis*, CY13: *C. triumfettii* subsp. *axillaris*, CY14: *C. huetii*, CY15: *C. mathiolifolia*, CY16: *C. germanicopolitana*, CY17: *C. depressa*, CY18: *C. pinardii*, CY19: *C. tchihatcheffii*, CY20: *C. cyanus*.

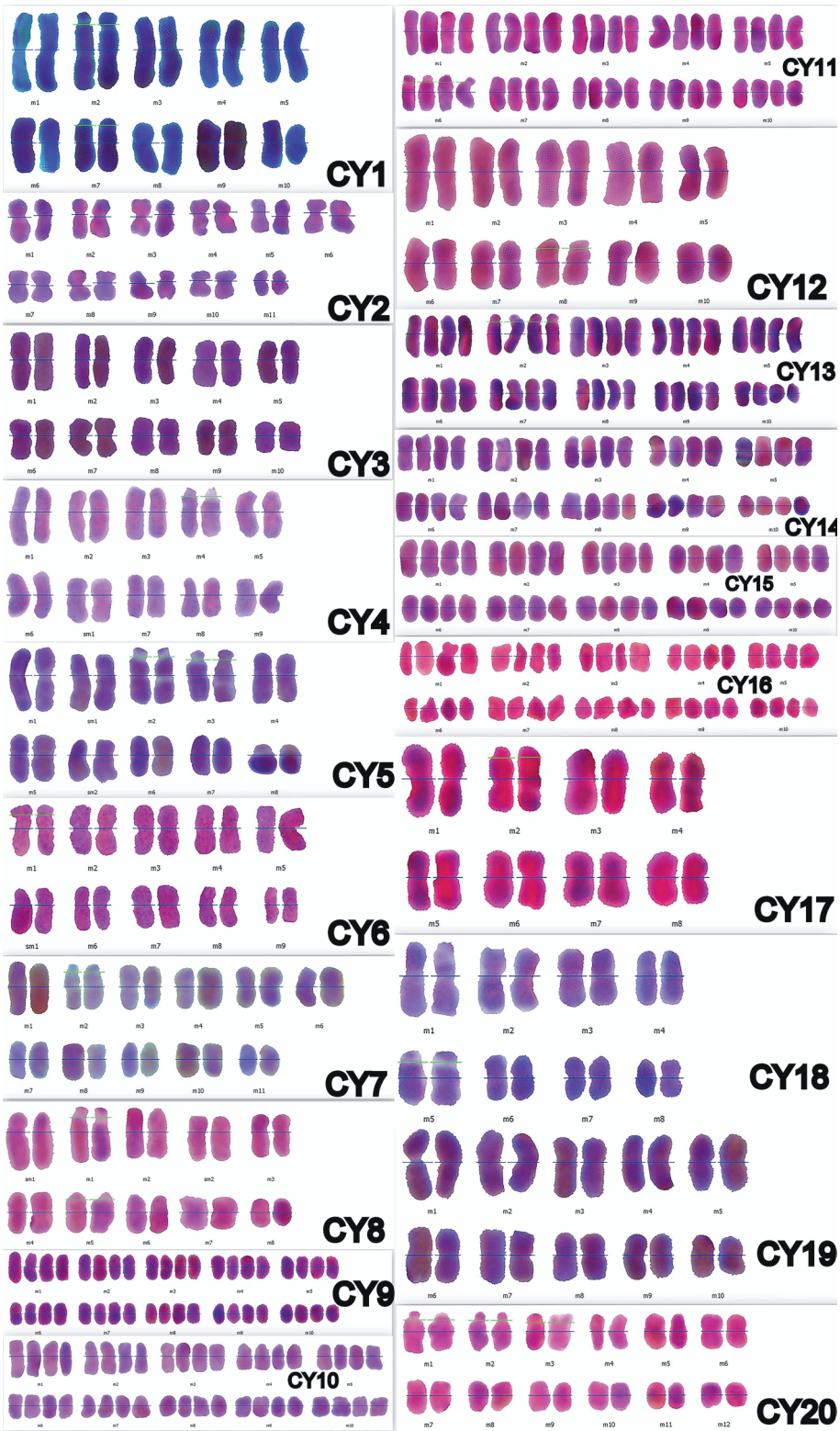

Figure 3. Karyograms of taxa belonging to subgen. *Cyanus*. CY1: *C. reuteriana* var. *reuteriana*, CY2: *C. reuteriana* var. *phrygia*, CY3: *C. lanigera*, CY4: *C. nigrofimbria*, CY5: *C. woronowii*, CY6: *C. eflanensis*, CY7: *C. thirkei*, CY8: *C. cheiranthifolia* var. *cheiranthifolia*, CY9: *C. cheiranthifolia* var. *purpurascens*, CY10: *C. bourgaei*, CY11: *C. pichleri* subsp. *pichleri*, CY12: *C. pichleri* subsp. *extrarosularis*, CY13: *C. triumfettii* subsp. *axillaris*, CY14: *C. huetii*, CY15: *C. mathiolifolia*, CY16: *C. germanicopolitana*, CY17: *C. depressa*, CY18: *C. pinardii*, CY19: *C. tchihatcheffii*, CY20: *C. cyanus*.

3.10. *C. bourgaei* Boiss.

According to our data, this is the first chromosome count and karyomorphology report of the species. *C. bourgaei* is separated from the others by its 3 to 4 pairs of lateral segments in the rosette leaves. The chromosome number is 2n = 4x = 40, which is tetraploid level (Figures 1–3; CY10). These are m chromosomes; the shortest one is 0.98 µm and the longest is 1.81 µm. The asymmetry index is 0.521. The karyotype formula is 40m.

3.11. *C. pichleri* Boiss. subsp. *pichleri*

The number of chromosomes in *C. pichleri* was previously reported to be 2n = 4x = 44 (Bancheva and Greilhuber, 2006). The chromosome number of the taxon is now identified as 2n = 4x = 40, which is tetraploid level (Figures 1–3; CY11). These are m chromosomes; the shortest one is 1.14 µm and the longest is 1.74 µm. The asymmetry index is 0.53. The karyotype formula is 40m.

3.12. *C. pichleri* Boiss. subsp. *extrarosularis* (Hayek & Siehe) Wagenitz

This investigation is the first chromosome count and morphology report of the taxon. The chromosome number of this taxon is 2n = 20 with diploid sets of chromosomes (Figures 1–3; CY12). These are m chromosomes; the shortest one is 1.3 µm and the longest is 2.46 µm. The asymmetry index is 0.429. The karyotype formula is 20m.

It was interesting for us that two subspecies have different ploidy levels. However, the chromosome counts we made from different localities confirmed this result. We thought of separating the subspecies as species but morphological differences were limited.

3.13. *C. triumfettii* subsp. *axillaris* (Čelak.) Stef. & T.Georgiev

Diploids and tetraploids have been reported for the *C. triumfettii* complex, with two basic chromosomes (x = 10, x = 11) (Dostál, 1976). In addition to diploid chromosomes, there are two tetraploid counts from Southeast Europe with x = 11 (2n = 4x = 44) (Guinochet, 1957; Lovric, 1982). One of the tetraploid counts was conducted for *C. graminifolia* (*C. triumfettii* var. *seusana*) and was 2n = 4x = 40 (Guinochet, 1957). For synonyms of *C. triumfettii*, there is a diploid count of the basic chromosome numbers in *C. pindicola* from Mediterranean taxa with x = 10 (2n = 2x = 20) (Morales, 1974). According to the results of our analysis, the chromosome number for the species is 2n = 4x = 40, which is tetraploid level (Figures 1–3; CY13). These are m chromosomes; the shortest one is 0.87 µm and the longest is 1.7 µm. The asymmetry index is 0.915. The karyotype formula is 40m.

3.14. *C. huetii* Boiss.

The chromosome number of this species was previously (as *Cyanus atratus* (Willd.) Holub) determined to be 2n = 4x = 40 + 2B (Tonjan, 1968) and our counts confirmed these numbers; however, the B chromosomes were not observed. The chromosome number of this taxon is 2n = 4x = 40, which is tetraploid level (Figures 1–3; CY14). These are m chromosomes; the shortest one is 0.79 µm and the longest is 1.42 µm. The asymmetry index is 0.935. The karyotype formula is 40m.

3.15. *C. mathiolifolia* Boiss.

The chromosome number of this taxon was previously determined to be 2n = 4x = 40 + 2B (Tonjan, 1968) and our counts confirmed these numbers; however, the B chromosomes were not observed in Turkish samples. The taxon has tetraploid chromosomes (Figures 1–3; CY15). These are m chromosomes; the shortest one is 0.95 µm and the longest is 1.72 µm. The asymmetry index is 0.458. The karyotype formula is 40m.

3.16. *C. germanicopolitana* Bornm.

According to our data, this is the first chromosome count and karyomorphology report of the species. The chromosome number of this taxon is 2n = 4x = 40, which is tetraploid level (Figures 1–3; CY16). These are m chromosomes; the shortest one is 0.8 µm and the longest is 1.37 µm. The asymmetry index is 0.77. The karyotype formula is 40m.

3.17. *C. depressa* M.Bieb.

This species is distinguished from the other taxa by having the longest pappus. The chromosome number has been previously reported to be 2n = 16 (Bakhshi Khaniki, 1995; Garcia-Jacas et al., 1997) and our data verify this count. The taxon has a diploid set of chromosomes (Figures 1–3; CY17). These are m chromosomes; the shortest one is 1.33 µm and the longest is 1.87 µm. The asymmetry index is 0.363. The karyotype formula is 16m.

3.18. *C. pinardii* Boiss.

The species is separated from other taxa by its lack of pappus. The number of chromosomes in *C. pinardi* was previously reported to be 2n = 16 (Romaschenko et al., 2004) and our data verify this count. The taxon has a diploid set of chromosomes (Figures 1–3; CY18). These are m chromosomes; the shortest one is 0.91 µm and the longest is 1.83 µm. The asymmetry index is 0.867. The karyotype formula is 16m.

3.19. *C. tchihatcheffii* Fisch. & C.A.Mey.

Centaurea tchihatcheffii is a local endemic taxon with diploid set of chromosomes that were previously reported as 2n = 20 (Gömürgen and Adıgüzel, 2001). Our data verify this count (Figures 1–3; CY19). These are m chromosomes; the shortest one is 1.22 µm and the longest is 2.16 µm. The asymmetry index is 0.74. The karyotype formula is 20m.

3.20. *C. cyanus* L.

Centaurea cyanus L. is separated from other taxa by having the smallest achene. The number of chromosomes has been previously reported to be 2n = 24 (Arohonka, 1982;

Bancheva and Greilhuber, 2006; Martin et al., 2009); our data verify this count (Figures 1–3; CY20). These are m chromosomes; the shortest one is 0.77 µm and the longest is 1.24 µm. The asymmetry index is 0.488. The karyotype formula is 24m.

4. Discussion

The number, size, and asymmetry of chromosomes are important characteristics that help explain the phylogenetic relationships of species (Eroğlu et al., 2013). The importance of karyology in the systematic characterizations of several genera of Centaureinae has been verified using the links between karyological, morphological, and molecular data (Wagenitz and Hellwig, 1996; Hellwig, 2004).

According to Garcia-Jacas and Susanna (1992), among the species in the *Acrocentron* section, those with x = 11 are more primitive than those with x =10. In addition, Wagenitz and Hellwig (1996) adopted this characteristic for perennial species of *Cyanus*.

Although most of the taxa have diploid sets of chromosomes, some have tetraploid sets. When taxa karyotypes are examined, the redundancy of m chromosomes draws attention.

The most common karyotype formulas are 40m, 20m, and 16m + 4sm, respectively. The other identified formulas are 24m, 22m, 18m + 2sm, 16m + 6sm, 16m, and 14m + 2sm (Table 2). According to the classification of Lima-De-Faria (1980), species within the subgenus *Cyanus* have small chromosomes with an average length (CL_m) ranging from 0.87 to 1.95 µm (Table 2). These values are lower than those found in subgenus *Centaurea* taxa (1.61–3.28 µm) based on the study of Uysal et al. (2017). Values of total chromosomal length (TCL) range from 9.54 to 39.92 µm. The obtained data are proportional to the level of ploidy.

While *C. cheiranthifolia* var. *purpurascens*, which is a polyploid species, has the highest TCL value, *C. thirkei* has the lowest TCL value and it is a diploid species (Table 2). The centromeric index (CI) might be considered to be an important value for distinguishing close relatives (Uysal et al., 2017). The CI values of the taxa are between 41 and 48, and the results are relatively higher than those of previous studies on *Centaurea* (Benamara-Bellagha et al., 2016; Uysal et al., 2017).

Karyotype asymmetry is a good indicator of the general morphology of karyotype of plants. The changes in the characteristics of a genome are often associated with the evolution of advanced plants. A different method by which to measure karyotype asymmetry has been proposed that considers intrachromosomal asymmetry (A1) and interchromosomal asymmetry (A2) indices (Zarco, 1986).

Our A1 and A2 values were lower than those of previous reports on *Rhaponticoides* and *Centaurea* taxa (Uysal et al., 2015, 2016); therefore, the genus includes more symmetric karyotypes and fewer derived species.

According to the A2 index, values were distributed between 0.09 and 0.21 (Table 3). In particular, the lowest value was detected in *C. cheiranthifolia* var. *purpurascens* and the highest was detected in *C. reuteriana* var. *reuteriana*. In addition, the karyotypes of all taxa are symmetrical. These findings show that chromosomal exchanges (crossovers) within the subgenus are limited.

Satellites have been detected in one or two pairs of chromosomes in 12 of the taxa analyzed (not *C. cyanus*) and were observed to be located on the short arm of the chromosomes (Table 4).

According to the karyogram, these satellites are located mainly on the 2nd chromosome, although they are also at times found on the 1st, 3rd, 4th, 5th, 6th, 7th, and 8th chromosomes. Having a single satellite with a single location on the 2nd chromosome does not mean that there is a relationship among *C. thirkei*, *C. triumfettii* subsp. *axillaris*, and *C. depressa*. Satellites might be sound chromosomal markers, but they do not always provide information for determining interspecific relationships (Uysal et al., 2017).

The chromosomes of *Cyanus* belong to types 4A and 4B (Stebbins, 1971).

M_{CA} and CV_{CL} are the most suitable parameters for measuring intra- and interspecies asymmetry (Peruzzi and Altınordu, 2014). *Centaurea pinardii* has the highest CV_{CL} value at 21.08, which distinguishes it from other species. *Centaurea eflanensis* has the lowest CV_{CL} value at 9.88, and the length of the chromosomes is highly similar. The M_{CA} values of the taxa of the lower subgenus of *Cyanus* range from 4.0 to 18.46. The highest value is observed in *C. reuteriana* var. *reuteriana*, while the lowest value is observed in *C. cheiranthifolia* var. *purpurascens*.

When the M_{CA}–CV_{CL} distribution graph was examined, we observed that annual taxa were separated from perennials (except *C. cyanus*) (Figure 4).

Uysal et al. (2015) identified the AI value in some examined *Centaurea* species at between 1.71 and 3.64. Our results are somewhat consistent with these values, and AI ranged from 0.2 to 2.41. Symmetrical karyotypes are found in these taxa, with the most found in *C. cheiranthifolia* var. *purpurascens* and the fewest found in *C. woronowii*. For all indices, we can assume that the studied taxa are characterized by both symmetrical karyotypes and superiority of m chromosomes. Considering the various indices used to characterize chromosomes, we found that AI might be preferred and shows a better correlation when compared to other indices. We presume that AI is more significant in distinguishing closely related species.

In conclusion, all of the indices used in this research were found to have a positive contribution to explaining the chromosomal characteristics of various *Cyanus* taxa, contrary to some of the criticisms in the report of

Table 2. Karyotype formulas according to Levan et al. (1964) and characteristic parameters of the studied *Cyanus* taxa.

Taxa	2n	X	PL	R (SC- LC (µm)	Ratio LC/ SC	*p* (µm) mean (±SD)	*q* (µm) mean (±SD)	CL (µm) mean (±SD)	TCL	CI mean (±SD)
**C. reuteriana* var. *reuteriana*	20	10	2×	0.98-1.59	1.61	0.53 (±0.08)	0.77 (±0.12)	1.31 (±0.17)	13.073	41 (±0.04)
**C. reuteriana* var. *phrygia*	22	11	2×	1.03-1.70	1.65	0.63 (±0.10)	0.76 (±0.11)	1.39 (±0.20)	15.294	46 (±0.02)
**C. lanigera*	20	10	2×	0.94-	1.611	0.53	0.67	1.20	11.986	44
				1.51		(±0.05)	(±0.15)	(±0.18)		(±0.05)
**C. nigrofimbria*	20	10	2×	1.13-	1.641	0.66	0.83	1.49	14.879	44
				1.86		(±0.11)	(±0.12)	(±0.20)		(±0.03)
**C. woronowii*	20	10	2×	1.07-	2.17	0.75	0.98	1.73	17.315	43
				2.33		(±0.18)	(±0.21)	(±0.35)		(±0.05)
**C. eflanensis*	20	10	2×	0.99-	1.43	0.57	0.71	1.28	12.754	44
				1.42		(±0.07)	(±0.08)	(±0.13)		(±0.03)
^C. thirkei	22	11	2×	0.64-1.11	1.727	0.38 (±0.05)	0.49 (±0.09)	0.87 (±0.13)	9.544	44 (±0.03)
^C. cheiranthifolia var. cheiranthifolia	20	10	2×	1.41-2.52	1.79	0.88 (±0,19)	1.06 (±0,18)	1.95 (±0.37)	19.487	45 (±0.02)
**C. cheiranthifolia* var. *purpurascens*	40	10	4×	0.80-1.25	1.57	0.48 (±0.06)	0.52 (±0.07)	1.00 (±0.13)	39.92	48 (±0.01)
**C. bourgaei*	40	10	4×	0.98-1.81	1.84	0.59 (±0.10)	0.72 (±0.15)	1.30 (±0.25)	26.01	45 (±0.01)
^C. pichleri subsp. *pichleri*	40	10	4×	1.14-1.74	1.533	0.65 (±0.09)	0.79 (±0.10)	1.44 (±0.18)	28.82	45 (±0.02)
**C. pichleri* subsp. *extrarosularis*	20	10	2×	1.30-2.46	1.899	0.86 (±0.18)	0.97 (±0.19)	1.84 (±0.37)	18.373	47 (±0.01)
^C. triumfettii subsp. *axillaris*	40	10	4×	0.87- 1.70	1.95	0.63 (±0.11)	0.78 (±0.14)	1.41 (±0.24)	28.188	44 (±0.02)
^C. huetii	40	10	4×	0.79-1.42	1.79	0.53 (±0.07)	0.62 (±0.12)	1.15 (±0.18)	23.062	46 (±0.03)
**C. mathiolifolia*	40	10	4×	0.95- 1.72	1.81	0.62 (±0.11)	0.69 (±0.12)	1.31 (±0.23)	26.224	47 (±0.01)
**C. germanicopolitana*	40	10	4×	0.80-1.37	1.703	0.46 (±0.06)	0.57 (±0.11)	1.03 (±0,17)	20.576	45 (±0.02)
^C. depressa	16	8	2×	1.33-1.87	1.405	0.71 (±0.08)	0.84 (±0.08)	1.55 (±0.16)	12.423	46 (±0.02)
^C. pinardii	16	8	2×	0.91-1.83	2.017	0.61 (±0.11)	0.71 (±0.17)	1.32 (±0.28)	10.59	47 (±0.02)
^C. tchihatcheffii	20	10	2×	1.22-2.16	1.77	0.78 (±0.13)	0.89 (±0.15)	1.66 (±0.26)	16.631	47 (±0.02)
^C. cyanus	24	12	2×	0.77-1.24	1.61	0.47 (±0.08)	0.54 (±0.07)	1.01 (±0.14)	12.157	47 (±0.02)

PL: Ploidy level; R: range; SC: shortest chromosome length; LC: longest chromosome length; *p*: mean length of the short arm; *q*: mean length of the long arm; CL: mean length of the chromosome; CI: mean centromere index; TCL: total chromosome length of the haploid complement; m: metacentric; sm: submetacentric.

* New chromosome counts; ^ different from previous literature.

Paszko (2006). According to Garcia-Jacas et al. (1996), the boundary between primitive and derived groups in the subtribe Centaureinae can be fixed at x = 12. The basic chromosome numbers at x = 12 and below (e.g., 8 and 9) are found in the most advanced groups. Thus, we can presume that *Cyanus* is one of the most advanced groups within the subtribe.

There is a tendency within the subtribe Centaureinae toward a decrease in basic chromosome numbers (Garcia-Jacas et al., 1996). Dysploidy and polyploidy play an important role in the evolution of the subtribe (Hellwig, 2004). In addition, there is an increasing tendency toward disploidy to be able to adapt to arid habitats (Bigazzi and Selvi, 2003; Uysal et al., 2017). We can also interpret the

Table 3. Karyotypes of *Cyanus* taxa using different methods of evaluating karyotype asymmetry.

Taxa	A1	A2	CV_{CL}	AI	M_{CA}	Stebbins
C. reuteriana var. reuteriana	0.299	0.133	13.298	1.268	18.46	4A
C. reuteriana var. *phrygia*	0.161	0.145	14.46	0.697	9.35	4A
C. lanigera	0.187	0.154	15.371	1.601	11.66	4A
C. nigrofimbria	0.205	0.137	13.731	1.081	11.40	4A
C. woronowii	0.235	0.204	20.36	2.414	13.29	4B
C. eflanensis	0.194	0.099	9.888	0.679	15.15	4A
C. thirkei	0.196	0.147	14.661	0.954	12.64	4A
C. cheiranthifolia var. *cheiranthifolia*	0.172	0.188	18.83	0.925	9.27	4B
C. cheiranthifolia var. *purpurascens*	0.079	0.128	12.8	0.2	4	4A
C. bourgaei	0.176	0.19	19.03	0.521	9.92	4A
C. pichleri subsp. *pichleri*	0,18	0,126	12,607	0,53	9.72	4A
C. pichleri subsp. *extrarosularis*	0.114	0.2	19.995	0.429	6.01	4A
C. triumfettii subsp. *axillaris*	0.197	0.171	17.072	0.915	10.63	4A
C. huetii	0.137	0.155	15.546	0.935	7.82	4A
C. mathiolifolia	0.104	0.178	17.788	0.458	5.34	4A
C. germanicopolitana	0.178	0.167	16.739	0.77	10.67	4A
C. depressa	0.153	0.102	10.167	0.363	8.38	4A
C. pinardii	0.12	0.211	21.085	0.867	7.57	4A
C. tchihatcheffii	0.119	0.156	15.648	0.74	6.62	4A
C. cyanus	0.121	0.14	14.036	0.488	6.93	4A

A1: Intrachromosomal asymmetry index; A2: interchromosomal asymmetry index; CV_{CL}: relative variation in chromosome length; AI: karyotype asymmetry index; M_{CA}: mean centromeric asymmetry; Stebbins: types, classification of karyotypes in relation to their degree of asymmetry according to Stebbins (1971).

Table 4. Satellite locations on the chromosomes.

Taxa	Satellite numbers	Satellite position	Chromosome location of satellite
C. reuteriana var. *reuteriana*	2	First and seventh chromosomes	m
C. nigrofimbria	1	Fourth chromosome	m
C. woronowii	1	Thirth and fourth chromosomes	m
C. eflanensis	1	First chromosome	m
C. thirkei	1	Second chromosome	m
C. cheiranthifolia var. *cheiranthifolia*	2	Second and seventh chromosomes	m
C. pichleri subsp. *pichleri*	1	Sixth chromosome	m
C. pichleri subsp. *extrarosularis*	1	Eighth chromosome	m
C. depressa	1	Second chromosome	m
C. pinardii	1	Fifth chromosome	m
C. cyanus	3	First, second and thirth chromosomes	m

identified differences in ploidy level (diploid/tetraploid) in the subspecies of *C. cheiranthifolia* and *C. pichleri* as a reflection of the changes in response to different ecological conditions. *Centaurea cyanus* can be distinguished by its karyomorphology and by having a different basic chromosome number.

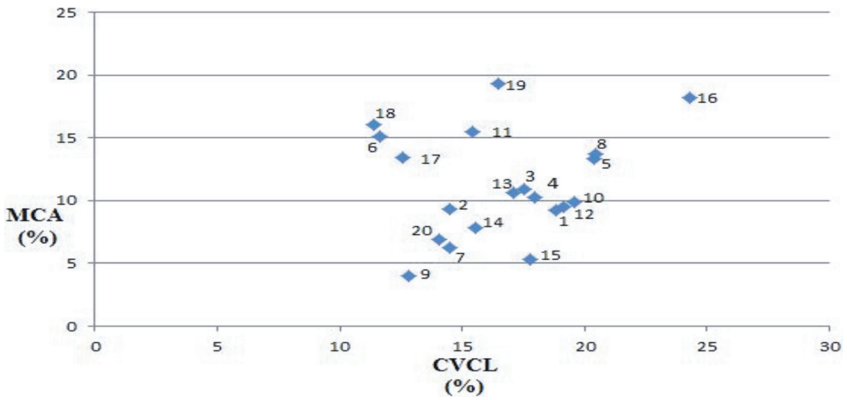

Figure 4. Mean centromeric asymmetry (M_{CA}) versus chromosome length change variation (CV_{CL}) parameters belonging to subgenus *Cyanus* taxa. 1: *C. reuteriana* var. *reuteriana*, 2: *C. reuteriana* var. *phrygia*, 3: *C. lanigera*, 4: *C. nigrofimbria*, 5: *C. woronowii*, 6: *C. eflanensis*, 7: *C. thirkei*, 8: *C. cheiranthifolia* var. *cheiranthifolia*, 9: *C. cheiranthifolia* var. *purpurascens*, 10: *C. bourgaei*, 11: *C. pichleri* subsp. *pichleri*, 12: *C. pichleri* subsp. *extrarosularis*, 13: *C. triumfettii* subsp. *axillaris*, 14: *C. huetii*, 15: *C. mathiolifolia*, 16: *C. germanicopolitana*, 17: *C. depressa*, 18: *C. pinardii*, 19: *C. tchihatcheffii*, 20: *C. cyanus.*

Many ploidy levels (3x, 4x, 6x) have been reported within *Centaurea* (Romaschenko et al., 2004; Uysal et al., 2009a, 2009b), and the ploidy ratio supports the formation of a very broad scale of TCL within the subgenus *Cyanus*. Similar reports on the positive correlation of TCL with genome size in many genera of the family Asteraceae have been published (Garnatje et al., 2004; Olanj et al., 2013); therefore, it can be presumed that *C. cheiranthifolia* var. *purpurascens* (tetraploid) and *C. thirkei* (diploid) have the largest and smallest genome sizes, respectively, among the studied taxa.

Our study suggests that dysploidy and polyploidy play important roles in the evolution of the *Cyanus* taxa at the subgenus and species levels. Differences in the karyotype formula and asymmetry indices suggest that structural changes might contribute to the diversity of the species studied. The basic chromosome numbers are x = 10 and 11 for perennials and x = 8, 10, and 12 for annuals. While there are two different basic chromosomes in the 16 perennial taxa, three different basic chromosome numbers were identified in the four annual taxa, which might indicate that perennials are more primitive than annuals in terms of chromosomal evolution.

Acknowledgments

We thank Ela Nur Şimşek Sezer and Ahmet Emre Ceylan for technical assistance. This study was produced from the PhD thesis of Emrah Şirin. This work was supported by the Scientific Investigation Projects Coordinator of Selçuk University (Project No. 15101001).

References

Arohonka T (1982). Chromosome counts of vascular plants of the island Seili in Nauvo, southwestern Finland. Turun Yliopiston Julkaisuja: Sarja A II, Biologica, Geographica, Geologica 3: 1-12.

Bakhshi Khaniki G (1995). Karyological studies in some taxa of the genus *Centaurea* (Asteraceae) in the Iran. Cell & Chromosome 18 (1): 16-33.

Bancheva S, Greilhuber J (2006). Genome size in Bulgarian *Centaurea* s.l. (Asteraceae). Plant Systematics and Evolution 257: 95-117.

Benamara-Bellagha M, Baziz K, Pustahija F, Khalfallah N, Siljak-Yakovlev S (2016). Cytogenetic characterization and nuclear DNA content of three North African endemic *Centaurea* species. Plant Biosystems 150: 501-511.

Bentham G (1873). Notes on the classification, history, and geographical distribution of Compositae. Botanical Journal of the Linnean Society 13: 335-577.

Bigazzi M, Selvi F (2003). Chromosome variation in Anatolian species of *Nonea* Medik. (Boraginaceae), with special reference to endemics and *N. persica*. Caryologia 56: 509-519.

Boissier E (1875). Flora orientalis, sive enumeratio plantarum in Oriente a Graecia et Aegypto ad Indiae fines hucusque observatarum. Basel, Switzerland: H. Georg (in Latin).

Boršić I, Susanna A, Bancheva S, Garcia-Jacas N (2011). *Centaurea* sect. *Cyanus*: nuclear phylogeny, biogeography, and life-form evolution. International Journal of Plant Sciences 172: 238-249.

Bowen C (1956). Freezing by liquid carbon dioxide in making slides permanent. Stain Technology 31: 87-90.

De Candolle A (1838). Prodromus VI. Paris, France: Treuttel & Wurtz (in Latin).

Dostál J (1976). *Centaurea* L. Flora Europaea 4: 254-301.

Eroğlu H, Şimşek N, Koç M, Hamzaoğlu E (2013). Karyotype analysis of some *Minuartia* L. (Caryophyllaceae) taxa. Plant Systematics and Evolution 299: 67-73.

Garcia-Jacas N, Susanna A (1992). Karyological notes on *Centaurea* sect. *Acrocentron* (Asteraceae). Plant Systematics and Evolution 179: 1-18.

Garcia-Jacas N, Susanna A, Garnatje T, Vilatersana R (2001). Generic delimitation and phylogeny of the subtribe Centaureinae (Asteraceae): a combined nuclear and chloroplast DNA analysis. Annals of Botany 87: 503-515.

Garcia-Jacas N, Susanna A, İlarslan R (1996). Aneuploidy in the Centaureinae (Compositae): is n = 7 the end of the series? Taxon 45: 39-42.

Garcia-Jacas N, Susanna A, İlarslan R, İlarslan H (1997). New chromosome counts in the subtribe Centaureinae (Asteraceae, Cardueae) from West Asia. Botanical Journal of the Linnean Society 125: 343-349.

Garnatje T, Vallès J, Garcia S, Hidalgo O, Sanz M et al. (2004). Genome size in *Echinops* L. and related genera (Asteraceae, Cardueae): karyological, ecological and phylogenetic implications. Biology of the Cell 96: 117-124.

Gömürgen A, Adıgüzel N (2001). Chromosome numbers and karyotype analysis of *Centaurea tchihatcheffii* Fisch. et Mey. (Compositae, Cardueae). Ot Sistematik Botanik Dergisi 8: 83-86.

Greuter W (2003). The Euro+ Med treatment of Cardueae (Compositae)—generic concepts and required new names. Willdenowia 33: 49-61.

Guerra M (2012). Cytotaxonomy: The end of childhood. Plant Biosyst. 146: 703-710.

Guinochet M (1957). Contribution à l'étude caryologique du genre *Centaurea* L. sens. lat. Le Bulletin de la Société d'histoire naturelle d'Afrique du Nord 48: 282-300 (in French).

Hellwig F (2004). Centaureinae (Asteraceae) in the Mediterranean – history of ecogeographical radiation. Plant Systematics and Evolution 246: 137-162.

Hilpold A, García-Jacas N, Vilatersana R, Susanna A (2014). Taxonomical and nomenclatural notes on *Centaurea*: a proposal of classification, a description of new sections and subsections, and a species list of the redefined section *Centaurea*. Collectanea Botanica 33: e001.

Kaya Z, Bancheva S (2009). A new species of *Cyanus* (*Centaurea* p.p.) sect. *Napuliferi* (Asteraceae) from Turkey. Novon 19 (2): 175-177.

Levan A, Fredga K, Sandberg A (1964). Nomenclature for centromeric position on chromosomes. Hereditas 52: 201-220.

Levin D (2002). The Role of Chromosomal Change in Plant Evolution. New York, NY, USA: Oxford University Press.

Lima-De-Faria A (1980). Classification of genes, rearrangements and chromosomes according to the chromosome field. Hereditas 93: 1-46.

Lovric A (1982). Reports. Taxon 31: 774-775.

Martin E, Dinç M, Duran A (2009). Karyomorphological study of eight *Centaurea* L. taxa (Asteraceae) from Turkey. Turkish Journal of Botany 33: 97-104.

Miller P (1754). The gardeners dictionary, abr, John & James Rivington, London.

Morales F (1974). Reports. Taxon 23: 805.

Olanj N, Sonboli A, Riahi H, Osaloo S (2013). Karyomorphological study of nine *Tanacetum* taxa (Asteraceae, Anthemideae) from Iran. Caryologia 66: 321-332.

Olšavská K, Perny M, Löser C, Stimper R, Hodalova I (2013). Cytogeography of European perennial species of *Cyanus* (Asteraceae). Botanical Journal of the Linnean Society 173: 230-257.

Paszko B (2006). A critical review and a new proposal of karyotype asymmetry indices. Plant Systematics and Evolution 258: 39-48.

Peruzzi L, Altınordu F (2014). A proposal for a multivariate quantitative approach to infer karyological relationships among taxa. Comparative Cytogenetics 8: 337-349.

Peruzzi L, Eroğlu H (2013). Karyotype asymmetry: again, how to measure and what to measure? Comparative Cytogenetics 7 (1): 1-9.

Romaschenko K, Ertuğrul K, Susanna A, Garcia-Jacas N, Uysal T et al. (2004). New chromosome counts in the *Centaurea jacea* group (Asteraceae, Cardueae) and some related taxa. Botanical Journal of the Linnean Society 145: 345-352.

Siljak-Yakovlev S, Peruzzi L (2012). Cytogenetic characterization of endemics: past and future. Plant Biosystems 146: 694-702.

Stebbins GL (1971). Chromosomal Evolution in Higher Plants. London, UK: Edward Arnold Ltd.

Susanna A, Garcia-Jacas N (2007). The tribe Cardueae. In: Kadereit J, Kubitzki K (editors). Compositae. The Families and Genera of Vascular Plants. Heidelberg, Germany: Springer-Verlag, pp. 123-147.

Susanna A, Garcia-Jacas N (2009). Cardueae (Carduoideae). In: Funk VA, Susanna A, Stuessy TF, Bayer BJ (editors). Systematics, Evolution, and Biogeography of Compositae. Vienna, Austria: IAPT, pp. 293-313.

Tonjan Z (1968). The chromosome numbers of some species of the genus *Centaurea*. Biologicheskii Zhurnal Armenii 21: 86-96 (in Russian).

Uysal T (2012). *Centaurea* L. In: Güner A, Aslan S, Ekim T, Vural M, Babaç MT (editors). Türkiye Bitkileri Listesi (Damarlı Bitkiler). İstanbul, Turkey: Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği, pp. 127-140 (in Turkish).

Uysal T, Bozkurt M, Şimşek E, Ertuğrul K, Tugay O (2015). Karyological studies of four endemic *Centaurea* L. species. Caryologia 68: 339-346.

Uysal T, Bozkurt M, Tugay O, Ertuğrul K, Şimşek Sezer E et al. (2017). Karyomorphology of Turkish species in *Centaurea* sections *Centaurea* and *Phalolepis* (Asteraceae) and implications for taxonomy. Plant Biosystems 151: 949-964.

Uysal T, Ertuğrul K, Susanna A, Garcia-Jacas N (2009a). New chromosome counts in the genus *Centaurea* (Asteraceae) from Turkey. Botanical Journal of the Linnean Society 159: 280-286.

Uysal T, Köse Y, Yücel E, Ertuğrul K (2009b). New chromosome counts in *Centaurea* section *Phalolepis* (Asteraceae) from Turkey. Belgian Journal of Botany 142: 41-46.

Uysal T, Şimşek Sezer E, Bozkurt M, Tugay O, Ertuğrul K et al. (2016). Karyomorphological study of five Turkish endemic *Rhaponticoides* Vaill. (Asteraceae, Cardueae) species. Caryologia 69: 207-214.

Venora G, Ravalli C, Cremonini R (2008). The karyotype as a tool to identify plant species: *Vicia* species belonging to *Vicia* subgenus. Caryologia 61: 300-319.

Wagenitz G (1955). Pollenmorphologie und Systematik in der Gattung Centaurea L. s. 1, Flora oder Allgemeine Botanische Zeitung 142 (2): 213-279

Wagenitz G (1975). Centaurea L. Á In: Davis, PH (ed.), Flora of Turkey and the east Aegean Islands. Vol. 5

Ward DE (1983). Chromosome counts from New Mexico and southern Colorado. Phytologia 54: 302-308.

Ward DE, Spellenberg R (1988). Chromosome counts of angiosperms from New Mexico and adjacent areas. Phytologia 64: 390-398.

Warwick SI, Al-Shehbaz IA (2006). Brassicaceae: chromosome number index and database on CD-ROM. Plant Systematics and Evolution 259 (2-4): 237-248.

Zarco CR 1986. A new method for estimating karyotype asymmetry. Taxon 35 (3): 526-530.