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The chromosomal evolution and analyses of Turkish *Cousinia* species

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Abstract: In this study, Turkish *Cousinia* species were examined in point of chromosome number and morphology. To our knowledge, this work is the first comprehensive chromosomal study regarding Turkish *Cousinia* as a whole, especially for endemics. As consistent with previous reports, the chromosome numbers are mostly $2n = 26$ and have been determined also as $2n = 24$ for only the members of *Cynaroideae* and *Sphaerocephalae* sections. An interesting result of this paper is that there is not any polyploid species in this taxonomic group and all of them are diploids. Concerning the chromosome evolution of the *Cousinia* species, we concluded that merely dysploidy is effective within the genus and evolutionally a unipolar reduction goes on in the basic chromosome number in speciation (from 13 to 12). In terms of general chromosomal information, the chromosomes of the genus consist of metacentric and submetacentric types and they could be categorized in small category according to Lima De Faria classification (0.67–3.43). According to chromosomal indices used in this paper, while most species have symmetrical chromosomes, fewer have asymmetrical ones. From this point, it could be said that *Cousinia stapfiana* could be assessed as the most evolved taxa in karyomorphology according to the average of all indexes used in here. Besides, *Cousinia bicolor*, *Cousinia birandiana*, and *Cousinia sintenisii* are species having the most primitive chromosome within the genus.

Key words: Asteraceae, chromosome, endemic, karyotype, Turkey

1. Introduction

Cousinia Cass. (Asteraceae, Cardueae) is the 50th largest genus in terms of species number among flowering plants and is represented by approximately 700 species (Frodin, 2004; Ulukuş and Tugay, 2020). The genus is distributed in Western and Central Asia. It has the characteristics of the typical Irano-Turanian phytogeographic region and has also a high endemism rate (Djamali et al., 2012). Most of the endemic plants are distributed in the mountains of Iran, Afghanistan, and Turkmenistan, as well as Turkey (Rechinger, 1986; Knapp, 1987).

The genus was firstly described in 1827 by Cassini, basing it on the species *Carduus orientalis* Adams. In the Flora of Turkey, it is represented by 6 sections and 38 species, 26 of which are endemic (Davis, 1975). At the Turkish Plant List, the number of species in Turkey has been reported as 39, and finally, it reached 40 with the addition of a recently published new one (Tugay, 2012; Tugay et al., 2019).

Previous phylogenetical work informed that the genus is placed within the *Arctium-Cousinia* complex as a natural group but it is not monophyletic alone. As a member of this complex, one of the other genera is *Arctium* L. which includes 27 species. Additionally, *Hypacanthium* Juz. with two species (Tscherneva, 1982) and the monotypic *Schmalhausenia* C.Winkl. *Lipskyella* Juz. are positioned within this complex. Lastly, *Tiarocarpus* Rech.f. has been described as another member of this complex, whereas the genera recognized as separate genera by Häffner (2000) were included in *Cousinia* by Susanna and Garcia-Jacas (2007).

Until now, the palynological, anatomical, and molecular studies have been carried out on the genus *Cousinia* (Ahmad et al., 2011; Ulukuş and Tugay, 2019a, 2019b; Tugay et al., 2019; Ulukuş and Tugay, 2020; Susanna et al., 2003a), It is known that there was a few limited chromosomal studies, especially including Iranian species. However, there is no comprehensive chromosomal information about Turkish populations or taxa. Surprisingly, some researchers report that some chromosomal studies on the genus are unconfirmed or questionable (Susanna et al., 2003b; López-Vinyallonga et al., 2010; Djavadi and Attar, 2010).

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Thus, this paper has two important aims. The first is to expand and clarify knowledge about the chromosomes of the genus. The second is to contribute to the taxonomy of the genus by revealing the chromosome number and morphology, and finally, to make inferences about its chromosomal evolution.

2. Materials and methods

Between 2012 and 2014, many materials belonging to *Cousinia* were collected by our research team from several localities of Turkey. Mature seeds were selected and periodically germinated for chromosomal analyses. Chromosome counts were made on somatic metaphases using the squash technique. Root meristems from germinating seeds collected in the wild were used. Samples were pretreated with 0.002 M 8-hydroxyquinoline at 4 °C for 8 h. The material was fixed with Carnoy for 24 h at low temperatures. Before staining, the material was hydrolyzed with 5N HCl for 1 h at room temperature, stained with 1% aceto-orcein, and mounted in 45% acetic acid. Slides were made permanent in Euparal by means of Bowen's method (1956). At least 10 metaphases were examined per taxa; the best metaphase plates were photographed (100×) with a digital camera (Olympus DP-72), mounted on an Olympus BX53 microscope. Chromosome nomenclature followed that proposed by Levan et al. (1964), with the symbols m and sm designating metacentric and submetacentric chromosomes. Karyotype asymmetry was estimated using the mean centromeric indices, the ratio of the shortest/longest pair, and according to the A_1 and A_2 indices (Zarco, 1986). Moreover, the coefficient of variation of the chromosome length (CV_{CL}), coefficient of variation of the centromeric index (CV_{CI}), karyotype asymmetry index (AI), and mean centromeric asymmetry (M_{CA}) were determined according to the method proposed by Paszko (2006) and then Peruzzi and Eroğlu (2013). Idiograms and karyograms of these taxa were made using the KAMERAM analysis system. Finally, scatter plots were constituted between M_{CA}-CV_{CL} and between A_1-A_2.

3. Results

The karyotype formulae, asymmetry index (AI) values and other karyotype parameters of the studied taxa belonging to the genus *Cousinia* are given in Tables 1 and 2. Also, the metaphase, karyograms, and idiograms of the species are given in Figures 1–6.

Cousinia sect. ***Sphaerocephalae*** Bunge.

Cousinia satdagensis Hub.-Mor.

Turkey: Hakkari, Yüksekova, Esendere, Satdağı, step, 2230 m, 03.08.2013, O.Tugay 8.573 & D.Ulukuş (KNYA).

The chromosome number was determined as $2n = 24$. According to karyotype analysis, all of the chromosomes are metacentric. The basic chromosome number is $x = 12$. This is a first report on the chromosome number and morphology to this species, which is endemic for Turkey.

Cousinia sect. ***Leiocaules*** Bunge.

Cousinia boissieri Buhse

Turkey: Van; Kurubaş Pass, step, 1960 m, 05.08.2012, O.Tugay 8.031, K.Ertuğrul & D.Ulukuş (KNYA).

The chromosome number was determined as $2n = 26$. According to karyotype analysis, it has metacentric and submetacentric chromosomes according to karyotype analysis. The basic chromosome number is $x = 13$. Previous reports on the species are given as $2n = 24$ and $2n = 26$ (Ghaffari et al., 2006; Martin et al., 2015). It has been reported that the *Leiocaules* section includes both basic chromosome numbers ($x = 12, 13$; (Fedorov, 1969; Ghaffari, 1984; Tscherneva, 1985; Susanna et al., 2003b; Sheidai et al., 2006; Djavadi and Attar, 2010).

Cousinia sect. ***Pugioniferae*** Bunge.

Cousinia macroptera C.A.Meyer

Turkey: Ağrı; between Doğubeyazıt and Gürbulak, step, 1500 m, 04.08.2013, O.Tugay 8.591 & D.Ulukuş (KNYA).

The chromosome number was determined as 2n = 26. According to karyotype analyses, the species has metacentric and submetacentric chromosomes. The satellite is located on the short arm of the third chromosome pair. The basic chromosome number is $x = 13$. Previous reports on the species is $2n = 26$ (Afzal-Rafii, 1980). This is a first report on the chromosome morphology to this species.

Cousinia wesheni Post

Turkey: Şanlıurfa; east of Akçakale, step, 430 m, 24.07.2012, O.Tugay 7.776, D.Ulukuş & M.A.Canbulat (KNYA).

The chromosome number of the species was determined as 2n = 26. According to karyotype analysis, all of the chromosomes are metacentric. The basic chromosome number is x = 13. This is a first report on the chromosome number and morphology to this species.

Cousinia sect. ***Cynaroideae*** Bunge.

Cousinia aintabensis Boiss. & Hausskn.

Turkey: Mardin; around the castle, step, 1100 m, 25.07.2012, O.Tugay 7.781, D.Ulukuş & M.A.Canbulat (KNYA).

The chromosome number was determined as $2n = 24$. According to karyotype analysis, it has metacentric and submetacentric chromosomes. The basic chromosome number is $x = 12$. This is a first report on the chromosome number and morphology to this species.

Cousinia arbelensis C.Winkl. & Bornm.

Turkey: Hakkari; between Başkale and Hakkari, 60th km Step, 1820 m, 03.08.2012, O.Tugay 7958 & D.Ulukuş.

The chromosome number was determined as $2n = 24$. According to karyotype analysis, all of the chromosomes

are metacentric. The satellite is located on the short arm of the fifth chromosome pair. The basic chromosome number is $x = 12$. This is a first report on the chromosome number and morphology to this species.

Cousinia birecikensis Hub.-Mor.

Turkey: Şanlıurfa; Çiftlik Village, step, 650 m, 24.07.2012, O.Tugay 7.771, D.Ulukuş & M.A.Canbulat (KNYA).

The chromosome number was determined as $2n = 24$. According to karyotype analysis, all chromosomes are metacentric. The basic chromosome number is $x = 12$. This is a first report on the chromosome number and morphology to this species, which is endemic for Turkey.

Cousinia eriocephala Boiss. & Hausskn.

Turkey: Van; Bahçesaray, Krapit Pass, stony place, step, 3010 m, 20.08.2011, O.Tugay 7.215, K.Ertuğrul & T.Uysal (KNYA).

The chromosome number was determined as $2n = 24$. According to karyotype analysis, it has metacentric and submetacentric chromosomes. The satellite is located on the short arm of the sixth chromosome pair. This is a first report on the chromosome number and morphology to this species, which is endemic for Turkey.

Cousinia grandis C.A.Meyer

Turkey: Van; Gürpınar, Hamurkesen Village, step, 1940 m, 05.08.2012, O.Tugay 8.051, K.Ertuğrul & D.Ulukuş (KNYA).

The chromosome number was determined as $2n = 24$. According to karyotype analysis, it has metacentric and submetacentric chromosomes. The basic chromosome number is $x = 12$. This is a first report on the chromosome number and morphology to this species.

Cousinia calocephala Jaub. & Spach subsp. ***calocephala***

Turkey: Van; Toprakkale, step, 1760 m, 05.08.2012, O.Tugay 8.053, K.Ertuğrul & D.Ulukuş (KNYA).

The chromosome number was determined as $2n = 24$. According to karyotype analysis, it has metacentric and submetacentric chromosomes. The basic chromosome number is $x = 12$. Previous reports on the species are given as $n = 12$ and $2n = 24$ (Ghaffari, 1987; Ghaffari et al., 2000). This is a first report concerning with the chromosome morphology to this species.

Cousinia sect. ***Cousinia***

Cousinia aucheri DC.

Turkey: Elazığ; Baskil, Mount Haroğlu, step, 1500 m, 26.07.2012, O.Tugay 7.819, D.Ulukuş &

M.A.Canbulat (KNYA).

The chromosome number was determined as $2n = 26$. According to karyotype analyses, the species has metacentric and submetacentric chromosomes. The basic chromosome number is $x = 13$. This is a first report on the chromosome number and morphology to this species, which is endemic for Turkey.

Cousinia bicolor Freyn & Sint.

Turkey: Bitlis; Ahlat, step, 1800 m, 02.08.2013, O.Tugay 8.571 & D.Ulukuş (KNYA).

The chromosome number was determined as $2n = 26$. According to karyotype analysis, all of the chromosomes are metacentric. The basic chromosome number is $x = 13$. This is a first report on the chromosome number and morphology to this species, which is endemic for Turkey.

Cousinia birandiana Hub.-Mor.

Turkey: Konya; Karapınar, Meke Salt flats, stony place, step, 980 m, 21.07.2012, O.Tugay 7.721 & D.Ulukuş (KNYA).

The chromosome number was determined as $2n = 26$. According to karyotype analysis, all of the chromosomes are metacentric. The basic chromosome number is $x = 13$. This is a first report on the chromosome number and morphology to this species, which is endemic for Turkey.

Cousinia brachyptera DC.

Turkey: Erzurum; east of Horasan, step, 1600 m, 14.08.2013, O.Tugay 8.607 & D.Ulukuş (KNYA).

The chromosome number was determined as $2n = 26$. According to karyotype analysis, all of the chromosomes are metacentric. The basic chromosome number is $x = 13$. This is a first report on the chromosome number and morphology to this species.

Cousinia decolorans Freyn & Sint.

Turkey: Sivas; Divriği, northern slopes, step, 1450 m, 28.07.2012, O.Tugay 7.866, D.Ulukuş & M.A.Canbulat (KNYA).

The chromosome number was determined as $2n = 26$. According to karyotype analysis, all of the chromosomes are metacentric. The satellites are located on the short arm of the second and eighth pairs of chromosomes. The basic chromosome number is $x = 13$. This is a first report on the chromosome number and morphology to this species, which is endemic for Turkey.

Cousinia eleonorae Hub.-Mor.

Turkey: Sivas; Kangal, step, 1600 m, 28.07.2012, O.Tugay 7.855, D.Ulukuş & M.A.Canbulat

(KNYA).

The chromosome number was determined as $2n = 26$. According to karyotype analyses, the species has metacentric and submetacentric chromosomes. The basic chromosome number is $x = 13$. This is a first report on the chromosome number and morphology to this species, which is endemic for Turkey.

Cousinia ermenekensis Hub.-Mor.

Turkey: Karaman; Ermenek; Tekeçatı, Yumru hill, *Quercus* clearings, 1450 m, 04.11.2012, O.Tugay 8.145 & D.Ulukuş (KNYA).

The chromosome number was determined as $2n = 26$. According to karyotype analyses, the species has metacentric and submetacentric chromosomes. The basic

chromosome number is x = 13. This is a first report on the chromosome number and morphology to this species, which is endemic for Turkey.

Cousinia euphratica Hub.-Mor.

Turkey: between Malatya and Elazığ, step, 750 m, 27.07.2012, O.Tugay 7.848, D.Ulukuş & M.A.Canbulat (KNYA).

The chromosome number was determined as $2n$ = 26. According to karyotype analyses, the species has metacentric and submetacentric chromosomes. The basic chromosome number is x = 13. This is a first report on the chromosome number and morphology to this species, which is endemic for Turkey.

Cousinia halysensis Hub.-Mor.

Turkey: Konya; Kulu, Lake Tuz, step, 1020 m, 17.08.2012, O.Tugay 8.120 & D.Ulukuş (KNYA).

The chromosome number was determined as $2n$ = 26. According to karyotype analysis, all of the chromosomes are metacentric. The basic chromosome number is x = 13. This is a first report on the chromosome number and morphology to this species, which is endemic for Turkey.

Cousinia humilis Boiss.

Turkey: Kayseri; between Pınarbaşı and Gürün, Ziyaret Pass, step, 1900 m, 25.08.2013, O.Tugay 8.617 & D.Ulukuş (KNYA).

The chromosome number was determined as $2n$ = 26. According to karyotype analysis, all of the chromosomes are metacentric. The satellite is located on the short arm of the third chromosome. The basic chromosome number is x = 13. This is a first report on the chromosome number and morphology to this species, which is endemic for Turkey.

Cousinia iconica Hub.-Mor.

Turkey: Konya; northwestern slopes, step, 1080 m, 16.09.2012, O.Tugay 8.144 & D.Ulukuş (KNYA).

The chromosome number was determined as $2n$ = 26. According to karyotype analysis, all of the chromosomes are metacentric. The basic chromosome number is x = 13. This is a first report on the chromosome number and morphology to this species, which is endemic for Turkey.

Cousinia intertexta Freyn & Sint.

Turkey: Elazığ; Keban, western slopes, step, 1080 m, 26.07.2012, O.Tugay 7.805, D.Ulukuş & M.A.Canbulat (KNYA).

The chromosome number was determined as $2n$ = 26. According to karyotype analysis, all of the chromosomes are metacentric. The satellite is located on the short arm of the second chromosome. The basic chromosome number is x = 13. This is a first report on the chromosome number and morphology to this species, which is endemic for Turkey.

Cousinia nabelekii Bornm.

Turkey: Hakkari; Kocaniş, step, 2450 m, 03.08.2013, O.Tugay 8.583 & D.Ulukuş (KNYA).

The chromosome number was determined as $2n$ = 26. According to karyotype analyses, the species has metacentric and submetacentric chromosomes. The basic chromosome number is x = 13. This is a first report on the chromosome number and morphology to this species, which is endemic for Turkey.

Cousinia sintenisii Freyn

Turkey: Elazığ; Pertek road, step, 920 m, 26.07.2012, O.Tugay 7.794 & D.Ulukuş (KNYA).

The chromosome number was determined as $2n$ = 26. According to karyotype analysis, all of the chromosomes are metacentric. Also, the species has B chromosome. The basic chromosome number is x = 13. This is a first report on the chromosome number and morphology to this species, which is endemic for Turkey.

Cousinia sivasica Hub.-Mor.

Turkey: Sivas; Ulaş, step, 1400 m, 28.07.2012, O.Tugay 7.889, D.Ulukuş & M.A.Canbulat (KNYA).

The chromosome number was determined as $2n$ = 26. According to karyotype analyses, the species has metacentric and submetacentric chromosomes. The basic chromosome number is x = 13. This is a first report on the chromosome number and morphology to this species, which is endemic for Turkey.

Cousinia stapfiana Freyn & Sint.

Turkey: Ankara; Beytepe, step, 1022 m, 27.08.2012, O.Tugay 8.131 & M.A.Canbulat (KNYA).

The chromosome number was determined as $2n$ = 26. According to karyotype analyses, the species has metacentric and submetacentric chromosomes. The basic chromosome number is x = 13. This is a first report on the chromosome number and morphology to this species, which is endemic for Turkey.

Cousinia urumiensis Bornm.

Turkey: Hakkari; between Başkale and Hakkari, step, 1820 m, 04.08.2012, O.Tugay 8.017, K.Ertuğrul & D.Ulukuş (KNYA).

The chromosome number was determined as $2n$ = 26. According to karyotype analysis, all of the chromosomes are metacentric. Our counts are in full agreement with the previous report (Sheidai et al., 2012).

Cousinia woronowii Bornm.

Turkey: Kars; between Kağızman and Iğdır, step, 1150 m, 07.08.2012, O.Tugay 8.097, K.Ertuğrul & D.Ulukuş (KNYA).

The chromosome number was determined as $2n$ = 26. According to karyotype analysis, all of the chromosomes are metacentric. The satellite is located on the short arm of the second chromosome pair. The basic chromosome number is x = 13. This is a first report on the chromosome number and morphology to this species, which is endemic for Turkey.

Table 1. Karyotype formula according to Levan et al. (1964) and characteristic parameters of the studied *Cousinia* taxa. PL-ploidy level; R-range; SC-the shortest chromosome length; LC-the 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 centromeric index; TCL-the total chromosome length of the haploid complement; m-metacentric and sm-submetacentric; CF: Chromosome formula; Satellite: S; B-chromosome: B; SD-standard deviation.

Taxa	PL		R (SC-LC) (µm)	Ratio LC/SC	p (µm) mean (±SD)	q (µm) mean (±SD)	CL (µm) mean (±SD)	TCL	CI mean (±SD)	CF/S/B	Section
	2n	*x*									
Cousinia satdagensis (OT8573)	24	12	0.81 - 1.27	1.559	0.45 (±0.06)	0.54 (±0.08)	0.99 (±0.13)	11.918	45 (±0.02)	24m	***Sphaerocephalae***
C. boissieri (OT8031)	26	13	0.89 - 2.39	2.7	0.60 (±0.20)	0.82 (±0.18)	1.42 (±0.37)	18.48	41 (±0.04)	16m + 10sm	***Leiocaules***
C. macroptera (OT8591)	26	13	1.08 - 1.76	1.639	0.60 (±0.08)	0.76 (±0.12)	1.36 (±0.18)	17.643	44 (±0.03)	24m + 2sm (4m+2m^{SAT}+2sm+18m)	***Pugioniferae***
C. wesheni (OT7776)	26	13	1.02 - 1.77	1.727	0.63 (±0.11)	0.76 (±0.13)	1.39 (±0.23)	18.006	45 (±0.02)	26m	***Pugioniferae***
C. aintabensis (OT7781)	24	12	0.95 - 2.38	2.49	0.65 (±0.20)	0.90 (±0.28)	1.55 (±0.44)	18.556	42 (±0.04)	20m + 4sm	***Cynaroideae***
C. arbelensis (OT7958)	24	12	0.67 - 1.18	1.769	0.40 (±0.05)	0.50 (±0.09)	0.90 (±0.14)	10.849	45 (±0.02)	24m (8m+2m^{SAT}+14m)	***Cynaroideae***
C. birecikensis (OT7771)	24	12	0.88 - 1.56	1.766	0.54 (±0.08)	0.63 (±0.11)	1.16 (±0.19)	13.941	46 (±0.02)	24m	***Cynaroideae***
C. eriocephala (OT7215)	24	12	1.04 - 1.92	1.844	0.64 (±0.14)	0.82 (±0.20)	1.47 (±0.30)	17.605	44 (±0.05)	20m + 4sm (2m+2sm+8m+2m^{SAT}+8m+2sm)	***Cynaroideae***
C. grandis (OT8051)	24	12	0.87 - 1.33	1.52	0.46 (±0.06)	0.60 (±0.09)	1.06 (±0.14)	12.77	43 (±0.03)	22m + 2sm	***Cynaroideae***
C. calocephala (OT8053)	24	12	0.87 - 1.90	2.184	0.55 (±0.13)	0.72 (±0.18)	1.27 (±0.30)	15.23	43 (±0.04)	20m + 4sm	***Cynaroideae***
C. aucheri (OT7819)	26	13	0.85 - 1.18	1.392	0.45 (±0.05)	0.55 (±0.07)	1.00 (±0.10)	12.94	45 (±0.03)	24m + 2sm	***Cousinia***
C. bicolor (OT8571)	26	13	0.81 - 1.31	1.617	0.49 (±0.05)	0.57 (±0.08)	1.06 (±0.13)	13.785	46 (±0.02)	26m	***Cousinia***
C. birandiana (OT7721)	26	13	1.12 - 2.04	1.833	0.70 (±0.12)	0.82 (±0.14)	1.52 (±0.26)	19.795	46 (±0.02)	26m	***Cousinia***
C. brachyptera (OT8607)	26	13	0.89 - 1.55	1.737	0.57 (±0.08)	0.68 (±0.11)	1.24 (±0.19)	16.17	46 (±0.02)	26m	***Cousinia***
C. decolorans (OT7866)	26	13	1.12 - 1.98	1.771	0.66 (±0.10)	0.79 (±0.14)	1.45 (±0.23)	18.877	46 (±0.03)	26m (2m+2m^{SAT}+10m+2m^{SAT}+10m)	***Cousinia***
C. eleonorae (OT7855)	26	13	1.11 - 1.92	1.735	0.62 (±0.10)	0.81 (±0.13)	1.43 (±0.20)	18.563	43 (±0.03)	24m + 2sm	***Cousinia***
C. ermenekensis (OT8145)	26	13	1.47 - 3.43	2.329	0.86 (±0.28)	1.18 (±0.26)	2.04 (±0.53)	26.568	42 (±0.04)	18m + 8sm	***Cousinia***
C. euphratica (OT7848)	26	13	0.72 - 1.32	1.842	0.42 (±0.05)	0.51 (±0.11)	0.94 (±0.15)	12.165	45 (±0.03)	24m+2sm	***Cousinia***
C. halysensis (OT8120)	26	13	0.97 - 1.90	1.962	0.60 (±0.10)	0.74 (±0.15)	1.34 (±0.24)	17.463	45 (±0.02)	26m	***Cousinia***
C. humilis (OT8617)	26	13	0.99 - 2.43	2.455	0.67 (±0.15)	0.85 (±0.19)	1.52 (±0.32)	19.711	44 (±0.03)	26m (4m+2m^{SAT}+20m)	***Cousinia***
C. iconica (OT8144)	26	13	1.20 - 1.89	1.579	0.69 (±0.09)	0.83 (±0.11)	1.52 (±0.20)	19.758	45 (±0.02)	26m	***Cousinia***
C. intertexta (OT7805)	26	13	0.82 - 1.55	1.884	0.55 (±0.10)	0.64 (±0.09)	1.18 (±0.19)	15.382	46 (±0.02)	26m (2m+2m^{SAT}+22m)	***Cousinia***
C. nabelekii (OT8583)	26	13	0.96 - 2.00	2.078	0.57 (±0.14)	0.80 (±0.16)	1.36 (±0.28)	17.695	41 (±0.04)	22m + 4sm	***Cousinia***
C. sintenisii (OT7794)	26	13	0.91 - 1.80	1.972	0.56 (±0.10)	0.64 (±0.12)	1.20 (±0.21)	15.621	47 (±0.02)	26m (26m+2B)	***Cousinia***

Table 1. (Continued).

C. sivasica (OT7889)	26	13	1.39 - 2.91	2.096	0.84 (±0.19)	1.14 (±0.22)	1.98 (±0.39)	25.692	42 (±0.04)	24m + 2sm	***Cousinia***
C. stapfiana (OT8131)	26	13	1.01 - 3.42	3.399	0.82 (±0.30)	1.17 (±0.34)	1.99 (±0.60)	25.867	41 (±0.05)	20m + 6sm	***Cousinia***
C. urumiensis (OT8017)	26	13	0.89 - 1.62	1.828	0.52 (±0.09)	0.65 (±0.13)	1.17 (±0.21)	15.187	44 (±0.02)	26m	***Cousinia***
C. woronowii (OT8097)	26	13	1.10 - 2.32	2.104	0.70 (±0.17)	0.87 (±0.19)	1.56 (±0.34)	20.301	44 (±0.03)	24m + 2sm ($2m+2m^{SAT}+6m+2sm+14m$)	***Cousinia***
C. agrıdaghensis (OT8058)	26	13	0.87 - 1.65	1.9	0.59 (±0.10)	0.69 (±0.12)	1.29 (±0.21)	16.728	46 (±0.02)	26m ($4m+2m^{SAT}+20m$)	***Cousinia***
C. davisiana (OT8147)	26	13	1.07 - 2.23	2.077	0.66 (±0.14)	0.86 (±0.21)	1.52 (±0.31)	19.708	44 (±0.05)	20m + 6sm	***Stenocephalae***
C. ramosissima (OT7773)	26	13	1.34 - 2.43	1.813	0.76 (±0.16)	1.09 (±0.21)	1.85 (±0.33)	24.097	41 (±0.04)	18m + 8sm ($2m+2sm+2m^{SAT}+2m+2m^{SAT}+10m+6sm$)	***Stenocephalae***
C. stenocephala (OT7778)	26	13	1.05 - 1.92	1.824	0.61 (±0.11)	0.79 (±0.18)	1.39 (±0.27)	18.13	44 (±0.04)	22m + 4sm	***Stenocephalae***

Table 2. Karyotypes of *Cousinia* taxa using different methods of evaluating karyotype asymmetry. A_1-intrachromosomal asymmetry index; A_2-interchromosomal asymmetry index; CV_{CL}-relative variation in chromosome length; CV_{CI}-relative variation in centromeric index; AI-karyotype asymmetry index; M_{CA}-mean centromeric asymmetry.

Taxa		A_1	A_2	CV_{CL}	CV_{CI}	AI	M_{CA}
C. satdagensis	SP1	0.167	0.135	13.479	3.838	0.517	9.09
C. boissieri	L1	0.283	0.261	26.13	10.093	2.637	15.49
C. macroptera	P1	0.201	0.134	13.429	7.712	1.036	11.76
C. wesheni	P2	0.171	0.169	16.949	4.019	0.681	9.35
C. aintabensis	CY1	0.264	0.286	28.63	10.156	2.908	16.12
C. arbelensis	CY2	0.187	0.16	15.966	4.803	0.767	11.11
C. birecikensis	CY3	0.138	0.16	16.003	4.358	0.697	7.69
C. eriocephala	CY4	0.203	0.203	20.3	11.96	2.428	12.32
C. grandis	CY5	0.226	0.13	12.976	6.627	0.86	13.2
C. calocephala	CY6	0.225	0.237	23.674	9.241	2.188	13.38
C. aucheri	C1	0.173	0.1	10.029	6.972	0.699	10
C. bicolor	C2	0.133	0.118	11.836	4.645	0.55	7.54
C. birandiana	C3	0.152	0.169	16.874	3.391	0.572	7.89
C. brachyptera	C4	0.157	0.149	14.905	4.147	0.618	8.8
C. decolorans	C5	0.16	0.156	15.558	5.83	0.907	8.96
C. eleonorae	C6	0.232	0.143	14.255	6.87	0.979	13.28
C. ermenekensis	C7	0.273	0.258	25.824	10.392	2.684	15.68
C. euphratica	C8	0.167	0.158	15.825	6.366	1.007	9.67
C. halysensis	C9	0.173	0.18	18.05	5.219	0.942	10.44
C. humilis	C10	0.205	0.214	21.389	6.481	1.386	11.84
C. iconica	C11	0.17	0.129	12.912	4.574	0.591	9.21
C. intertexta	C12	0.141	0.156	15.649	4.222	0.661	7.56
C. nabelekii	C13	0.286	0.207	20.669	9.226	1.907	16.78
C. sintenisii	C14	0.122	0.175	17.475	3.565	0.623	6.66
C. sivasica	C15	0.263	0.197	19.721	9.153	1.805	15.15
C. stapfiana	C16	0.294	0.304	30.379	12.752	3.874	17.58
C. urumiensis	C17	0.196	0.182	18.219	5.396	0.983	11.11
C. woronowii	C18	0.192	0.216	21.581	7.637	1.648	10.82
C. agrıdaghensis	C19	0.145	0.161	16.131	4.123	0.665	7.81
C. davisiana	S1	0.192	0.208	20.825	9.128	1.901	13.15
C. ramosissima	S2	0.29	0.177	17.705	10.677	1.89	17.83
C. stenocephala	S3	0.215	0.194	19.422	8.279	1.608	12.85

Cousinia agridaghensis Tugay, Ertuğrul & Ulukuş

Turkey: Ağrı, Doğubeyazıt, step, 1690 m, 06.08.2014, O.Tugay 8.058, K.Ertuğrul & D.Ulukuş (KNYA).

The chromosome number was determined as $2n = 26$. According to karyotype analysis, all of the chromosomes are metacentric. The satellite is located on the short arm of the third chromosome pair. The basic chromosome number is $x = 13$. In the original publication of the species, chromosome counts based on samples with the same collection number were incorrectly given as $2n = 24$ by our project team; therefore, incorrect evaluations were given regarding the chromosome morphologies. Thanks to

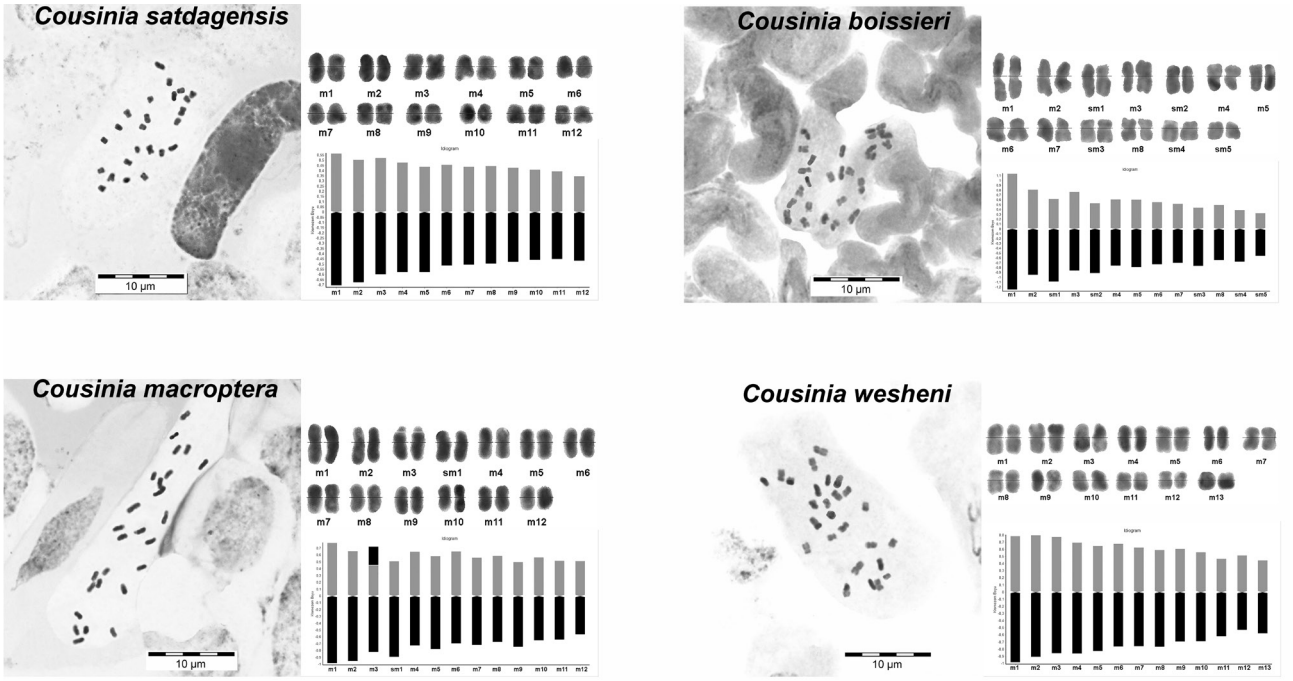

Figure 1. Mitotic metaphase chromosomes, karyograms, and idiograms of the taxa belonging to the *Sphaerocephalae, Leiocaules*, and *Pugioniferae* sections of the studied *Cousinia* genus.

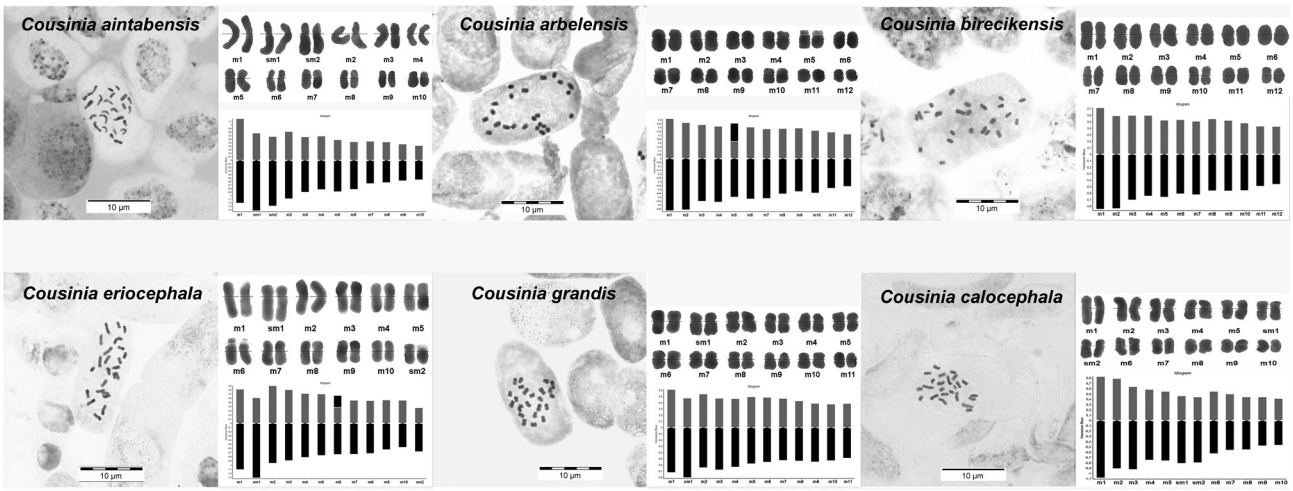

Figure 2. Mitotic metaphase chromosomes, karyograms, and idiograms of the taxa belonging to the *Cynaroideae* section of the studied *Cousinia* genus.

better and clearer metaphase appearances in the ongoing chromosomal studies on the species, this error has been eliminated and accurate information has been obtained.

Cousinia sect. ***Stenocephalae*** Bunge.

Cousinia davisiana Hub.-Mor.

Turkey: Karaman; Ermenek; stony place, step, 1400 m, 04.11.2012, O.Tugay 8.147 & D.Ulukuş (KNYA).

The chromosome number was determined as $2n$ = 26. According to karyotype analyses, the species has metacentric and submetacentric chromosomes. The basic chromosome number is x = 13. This is a first report on the chromosome number and morphology to this species, which is endemic for Turkey.

Cousinia ramosissima DC.

Turkey: Şanlıurfa; Çiftlik Village, step, 595 m, 24.07.2012, O.Tugay 7.773, D.Ulukuş & M.A.Canbulat (KNYA).

The chromosome number was determined as $2n$ = 26. According to karyotype analysis, it has metacentric and submetacentric chromosomes. The satellites are located

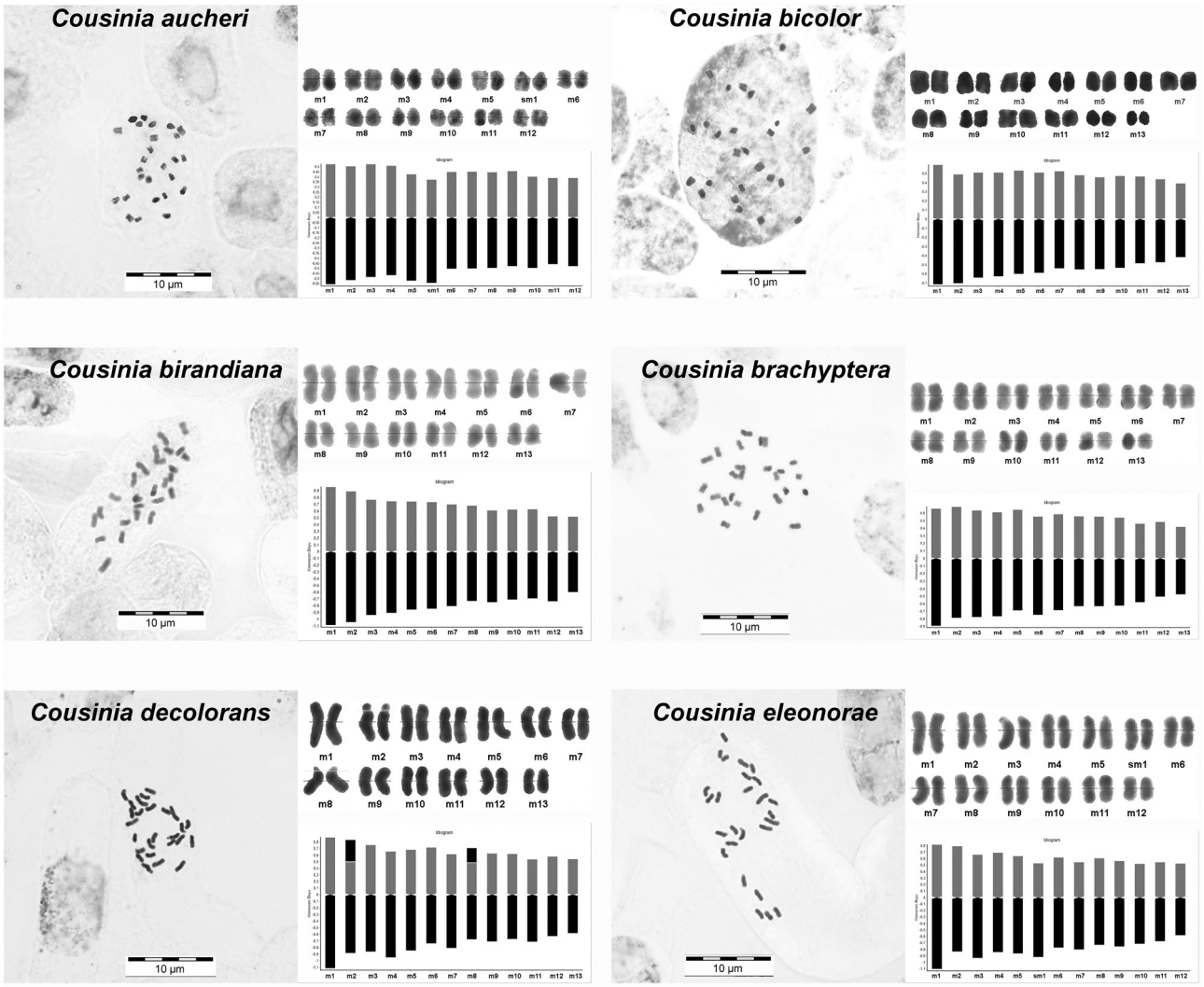

Figure 3. Mitotic metaphase chromosomes, karyograms, and idiograms of the taxa belonging to the *Cousinia* section of the studied *Cousinia* genus.

on the short arm of the third and fifth chromosome pairs. This is a first report on the chromosome number and morphology to this species.

Cousinia stenocephala Boiss.

Turkey: Şanlıurfa; Akçakale, Dereli Village, step, 430 m, 24.07.2012, O.Tugay 7.778, D.Ulukuş & M.A.Canbulat (KNYA).

The chromosome number was determined as $2n$ = 26. According to karyotype analyses, the species has metacentric and submetacentric chromosomes. The basic chromosome number is x = 13. This is a first report on the chromosome number and morphology to this species.

4. Discussion

The findings point to the focus of the discussion mainly on basic chromosome number and karyomorphology, since polyploidy is not observed in the chromosomal evolution of the genus.

One of the most important results is that Turkish Cousins do not have a wide variation in terms of basic chromosome number and only two different basic chromosome numbers have been determined for Cousins placed within 6 different sections. However, in previous studies, the basic chromosome numbers are reported at intervals varying in the range of x = 9–13 (Susanna et al., 2003b; Ghaffari et al., 2006). Reports from other countries or geographies for the sections of Cousins where the species distributed also in Turkey mostly overlap with our results. Additionally, we confirm again that the polyploidy does not have any role in the chromosomal evolution of *Cousinia* taxa as previously mentioned by Lopez-Vinyallonga et al. (2009 and 2010). According to

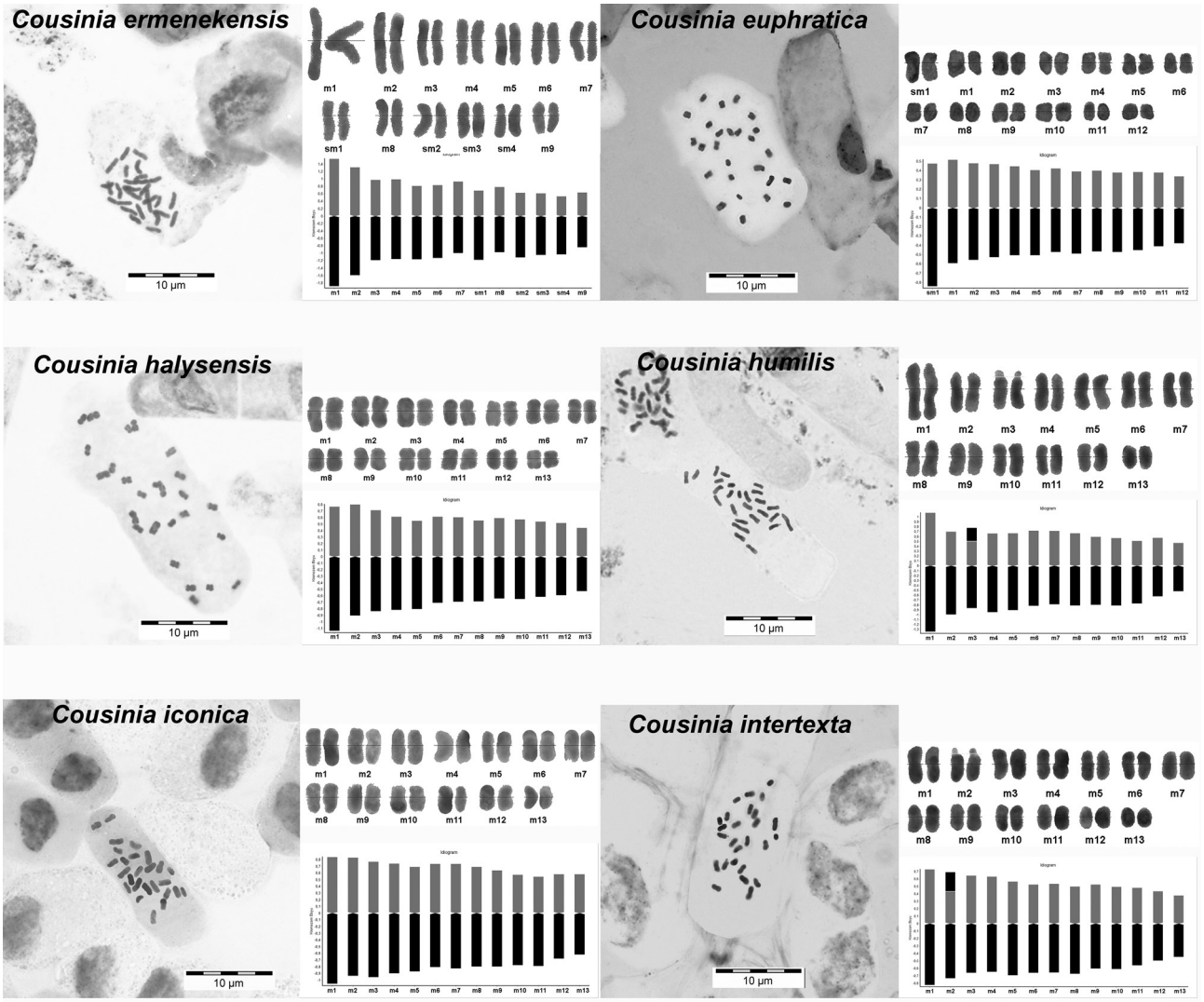

Figure 4. Mitotic metaphase chromosomes, karyograms, and idiograms of the taxa belonging to the *Cousinia* section of the studied *Cousinia* genus.

our observations, it could be possible that hybridization is more frequent in groups specially having small heads and we saw that a broad variation seen in their central flowers and stylus color may be a good indicator morphologically but it remains unclear chromosomally in the studied taxa due to insufficient taxonomic knowledge and the existence of several morphologically very similar species. Concerning the chromosome evolution of the *Cousinia* species, we concluded that merely dysploidy is effective within the genus and evolutionally a unipolar reduction goes on in the basic chromosome number in speciation of Turkish taxa (from 13 to 12). Similarly, Susanna et al. (2003b) hypothesized that descending dysploidy moving down from $x = 13$ has independently and frequently occurred in *Cousinia* s. str., which seems to be a highly plausible scenario.

This paper is one of the most comprehensive and thorough studies, perhaps the most important on the chromosome structure and morphology of Turkish Cousins. Considering the chromosome morphology and karyotypes of the genus, it can be deduced that the chromosomes are evaluated as evolutionarily primitive. Chromosomes of most species are entirely composed of metacentric type chromosomes (Table 1). In addition, the indices indicate that symmetrical chromosomes are dominant for the majority of species. In addition, satellites were determined in a few species and the presence of B chromosome was detected in one species (*Cousinia sintenisii*).

In Turkey, the members of the *Cousinia* section constitute the most crowded subgroup of the genus *Cousinia*. According to previous reports, it is known that

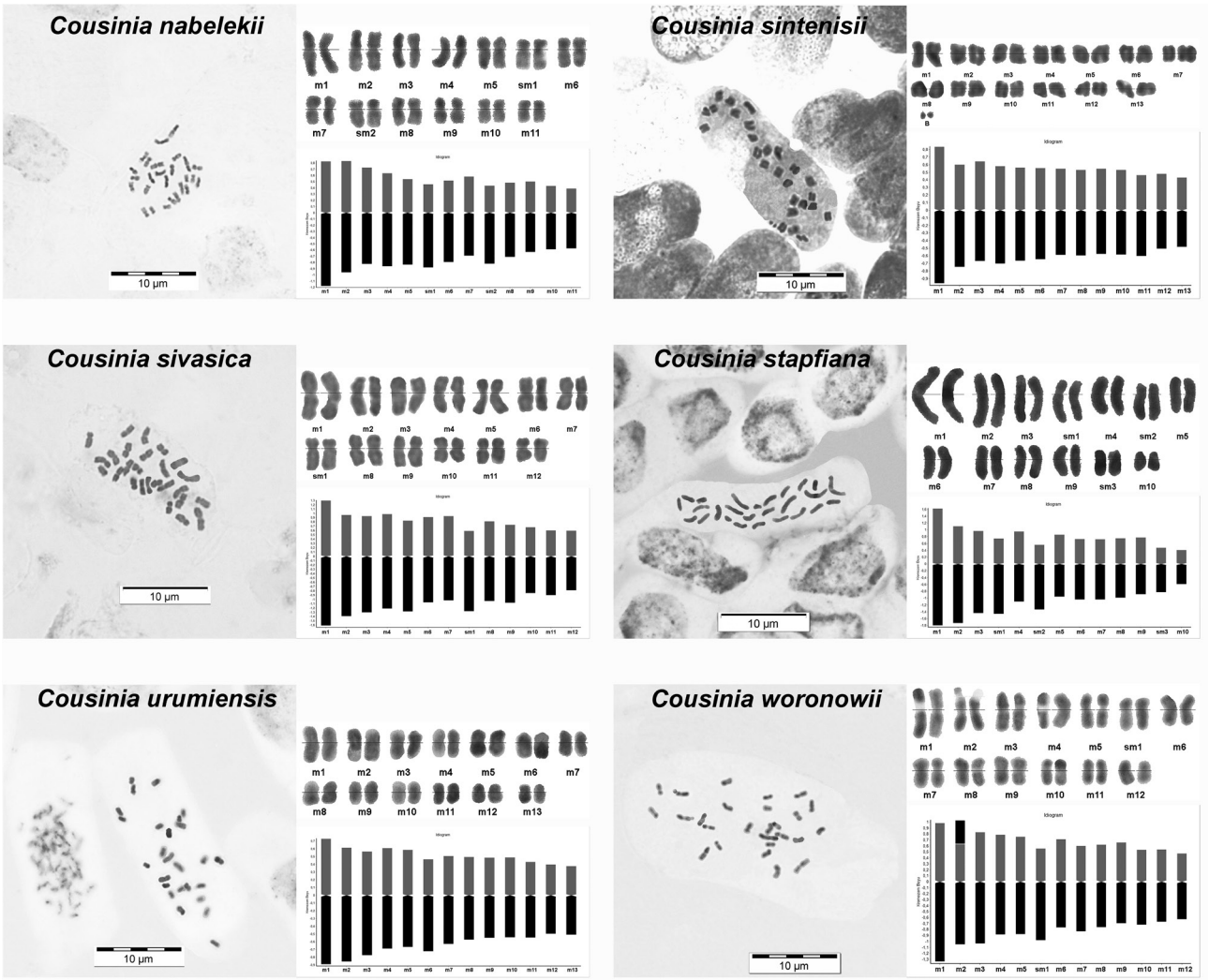

Figure 5. Mitotic metaphase chromosomes, karyograms, and idiograms of the taxa belonging to the *Cousinia* section of the studied *Cousinia* genus.

there are two different (x = 12 and 13) basic chromosome numbers in this section. According to our results, Turkish *Cousins* (Sect. *Cousinia*) have only x = 13 basic chromosome number. Similar results were reported for *Cousinia aleppica* by López-Vinyallonga et al. (2010). Therefore, a divergence or contradiction regarding this section arises because of the presence of the other basic chromosome number, x = 12, which is reported from East neighbors of Turkey because all of the chromosomal counts from Irano-Turanian geography (except for *Cousinia congesta*) indicate the existence of x = 12 basic chromosome number (Susanna et al., 2003b; Gaffari et al., 2006). Thus, Turkish *Cousins* appear to be evolutionarily isolated from Iran-Turanian species in terms of basic chromosome number. The fact that all taxa are endemic to Turkey, except for two species (except *C. aleppica* and *C. urumiensis*) reinforces this situation. As a conclusion, it can be said that the main gene center of this section would be Anatolian steppes, and considering that the basic chromosome number is in the ancestral position, it can be thought that this section might have evolved from Anatolian steppes towards Far East.

At the end of this study, except only the members of the *Cynaroideae* and *Sphaerocephala* sections, the fact that all sections and their taxa handled in here contain the same ancestral number strengthened the scenario regarding the chromosomal evolution of the genus, which was first established by Susanna et al. (2003b). In terms of species and section richness, the Irano-Turanian region, especially the Iranian flora draws attention to the genus *Cousinia*, but the fact that the basic chromosome numbers of distributed in these areas are mostly 11 or 12 (Susanna et al., 2003b; Ghaffari et al., 2006), which turns our course to another direction as regarding the

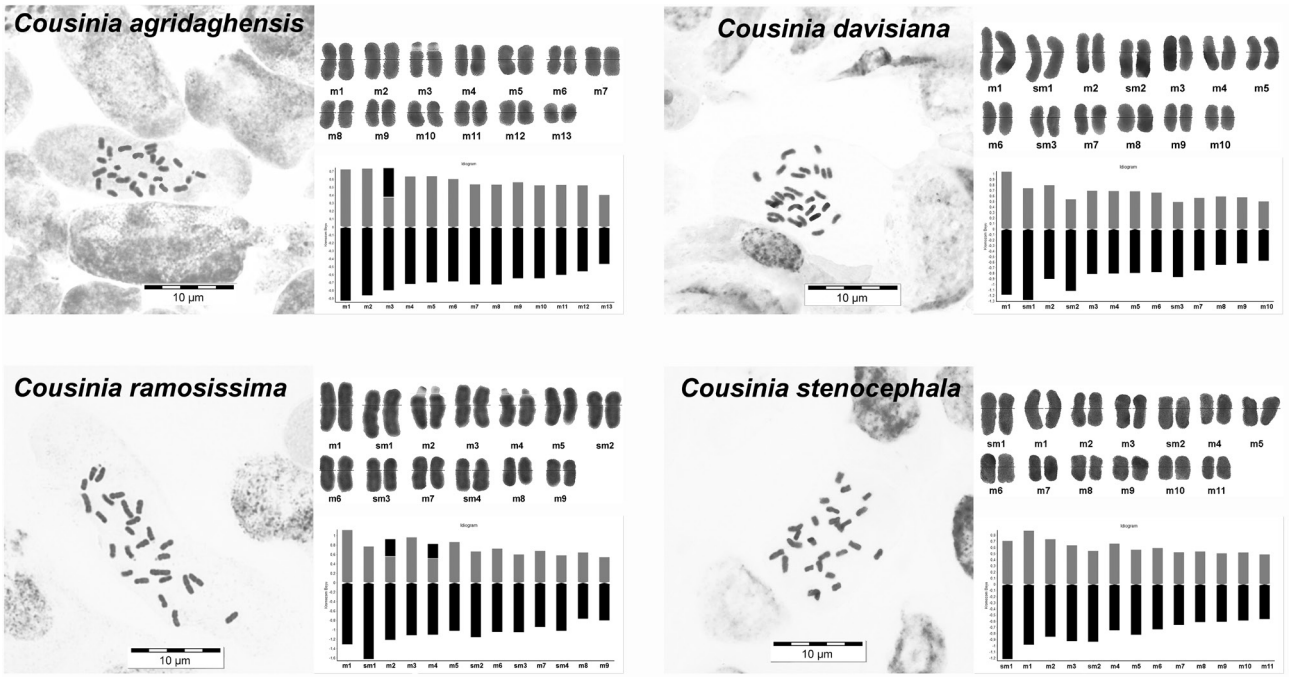

Figure 6. Mitotic metaphase chromosomes, karyograms, and idiograms of the taxa belonging to the *Cousinia* and *Stenocephalae* sections of the studied *Cousinia* genus.

main gene center. As a result, contrary to popular belief, we think that the main gene center for cousins may be Anatolian steppes rather than the Irano-Turanian, at least for this *Cousinia* section. Additionally, we assume that Irano-Turanian regions are the main differentiation gene centers for this genus. Last molecular approaches point out the presence of a few nonmonophyletic sections (*Stenocephalae*, *Albidae*, and *Cousinia*) within the genus; in fact, they are known very well traditionally based on the morphologic aspect (Kalouti et al., 2022). However, many scientists have recently reported that it is clear that there is a conflict or disagreement among morphological and molecular characters (Atazadeh et al., 2021; Kalouti et al., 2022). Additionally, our chromosomal data indicate that these sections include a variation in terms of the basic chromosome number. At least, except for the Albidae section, which is not distributed in Turkey, it can be said that these sections display a divergence or separation in terms of basic chromosome numbers between Anatolia (only $x = 13$) and the neighbors ($x = 12$ and 13).

The analyses based on the ITS gene region, which try to explain the phylogenetic relationships of the different sections of the genus *Cousinia*, indicate that the subcategories formed on the basis of morphology are not natural and point to the existence of nonmonophyletic sections or subsections (Susanna et al., 2003a; Lopez-Vinyallonga et al., 2009; Atazadeh et al., 2021; Kalouti et al., 2022). This reveals the existence of a mismatch between morphology and molecular data in cousins. Similarly, it is not possible to make a distinction at the subgenus or section level in terms of chromosomal data. Thus, it is not possible to talk about a sinapomorphy in terms of the basic chromosome number of Cousins. This incompatibility and homoplasy (among morphology, chromosomal data, and geneology) had been associated with various reasons (Susanna et al., 2003a; Lopez-Vinyallonga et al., 2009; Mehregan and Assadi, 2016) which are listed by the presence of interspecific hybridization, the occurrence of the intermediate forms (Mehregan and Kadereit, 2009), as well as homoploid hybrid speciation (Lopez-Vinyallong et al., 2009) and incomplete lineage sorting (Zhang et al., 2015).

It is seen that the indices used for chromosomal asymmetry (A_1, A_2, AI, CV_{CI}, CV_{CL}, and M_{CA}) are mostly compatible with each other and give similar information about the chromosomal evolution of Cousins (Table 2; Figures 7 and 8). According to our findings, M_{CA} and CV_{CL} values displayed largely correlation unlike the general acceptance. The karyotypes of sect. *Cousinia* express that the members have moderately variable chromosomes in point of M_{CA}, CV_{CL}, and CV_{CI} as well as karyotype formulae (Table 2). Within the section, all chromosomes of eleven species are metacentric. In the remaining species, mostly metacentric chromosomes are seen to dominate, but the presence of submetacentric chromosomes has been determined too. Unlike these,

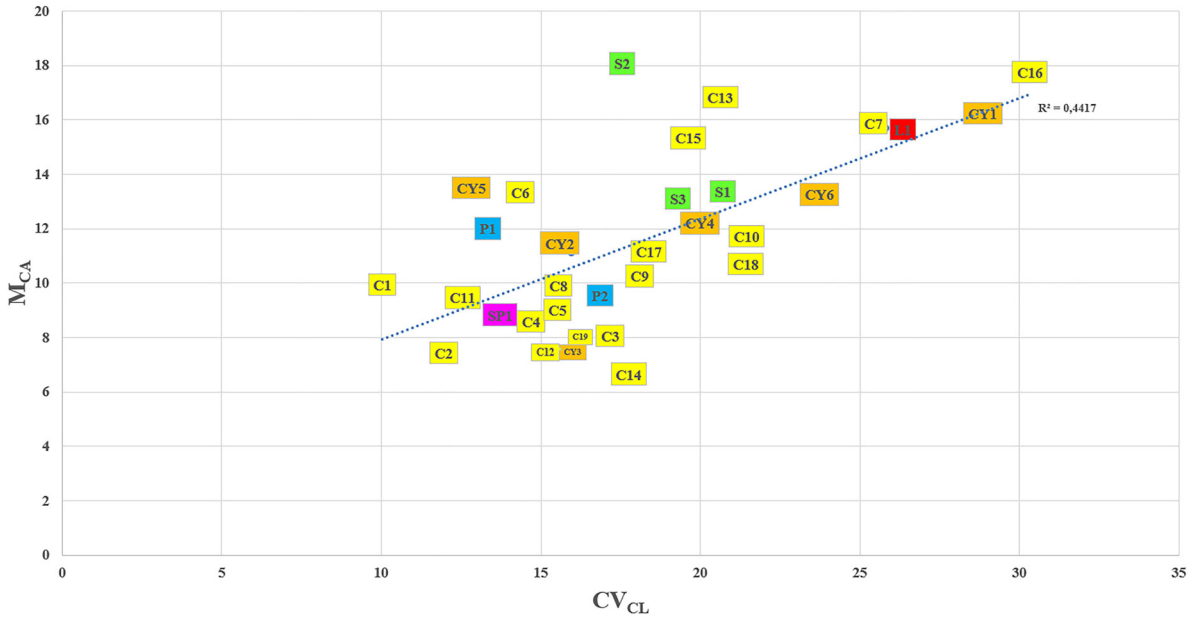

Figure 7. Scatter plot between M_{CA} and CV_{CL} for the studied *Cousinia* taxa.

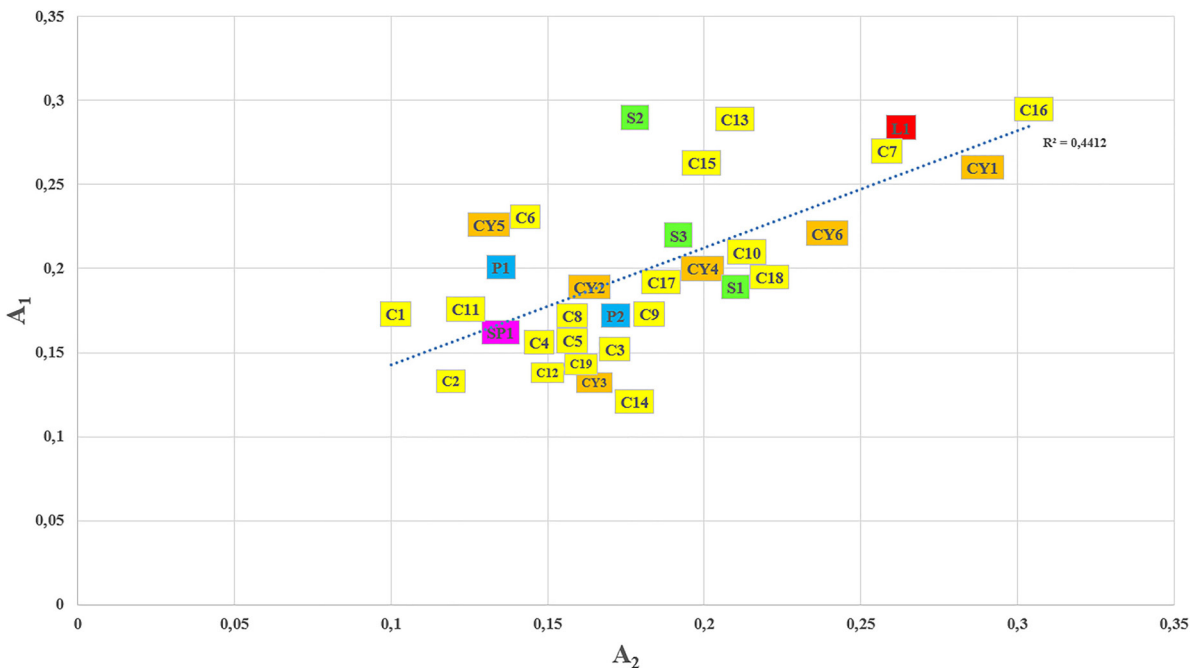

Figure 8. Scatter plot between A_1 and A_2 for the studied *Cousinia* taxa.

the taxa display a broad variation in TCL values and this suggests that deletions or insertions that would be caused to chromosomal rearrangements within this section. Thus, the rearrangements may have been effective in the evolution of the species. *C. stapfiana* (AI: 3.874, CI: 41, CV_{CI}: 12.752, M_{CA}: 17.58) appears to be the most evolved species in terms of both karyotype formula and indices. *C. bicolor*, *C. birandiana* (AI), and *C. sintenisii* (M_{CA}) have the most stable karyotype and the least variable chromosomes.

An important part of the *Leiocaules* section is located in Iran, and the majority of them are endemic species. According to the previous chromosomal studies (Ghaffari, 1984; Tscherneva, 1985; Djavadi, 2005; Susanna et al., 2003b; Ghaffari et al., 2006) and the data produced indicate the existence of two different basic chromosome numbers, x = 12 and 13. In our country, the section is

represented by a single species; *C. boissieri* has localized in East Anatolia and our chromosomal data overlaps with previous information (Fedorov, 1969; Martin et al., 2015). The species includes the moderately chromosomal variation and it has an asymmetric karyotype.

The species of *Cynaroideae* section comprises more than 50 species and they are distributed mainly in the eastern part of Turkey and the western part of Iran. We accept the previous views (Mehregan and Kadereit, 2008; Djavadia and Attar, 2010) that *Cousinia* sect. *Cynaroideae* Bunge seems to be monophyletic. Finally, this group has a uniform basic chromosome number, $x = 12$. The species have relatively stable chromosomes, and only a few have submetacentrics in their karyotypes. However, the chromosomal variation is high in the majority of species and asymmetric chromosomes are dominant. At this point, the most primitive and also similar taxa are *C. arbelensis* and *C. birecikensis* in chromosome morphology. The high similarity seen in their chromosomes reveals the need for a taxonomic judgment. In this section, the most derived species is *C. aintabensis* and the most variable karyomorphology belongs to her with high M_{CA}, CV_{CI} and CV_{CL} index.

Cousinia sect. *Stenocephalae* Bunge is a group that has been studied in detail about its chromosomes. In our country, this group is represented by three species. The basic chromosome number and morphologies were given for the first time and they are in line with previous reports. *C. ramosissima* has very characteristic chromosomes with satellites which are located on the short arm of the third and fifth chromosome pairs. According to our counts and those of other scientists (Afzal-Rafii, 1980; Tscherneva, 1985; Ghaffari, 1986; Ghaffari and Chariat-Panahi, 1985; Ghaffari and Djavadi, 1998; Djavadi, 2005; Ghaffari et al., 2006; Djavadi, 2007; Djavadi and Attar, 2010), the section has common basic number of $x = 13$. Interestingly, *Stenocephalae* is a section that contains the most similar species among *Cousinia* in terms of chromosome structure and morphology. All species display similar karyotypes and especially close chromosomal indices. The taxa have moderately chromosomal variation and the majority have asymmetric and evolved chromosomes. In fact, this relationship observed might be connected with close morphological relationships among the discussed taxa.

One of the groups represented by a single species in Turkey is the *Sphaerocephalae* section. *C. satdagensis* is a locally endemic species and the basic chromosome number is $x = 12$ according to our findings. Chromosome counts related to the Iranian endemic *C. sphaerocephalae* in the same section have been reported as $x = 13$ (Djavadi and Ghaffari, 1999). Thus, the presence of two different basic chromosome numbers for this section raises suspicion and needs confirmation, since the previous counts were poor images based on meiosis. Regarding its karyotype, it could be said that the species has primitive karyomorphology and almost few chromosomal variation. This situation can be internalized by the fact that the species spreads in a very narrow area and its population remains fairly isolated.

The first chromosomal reports and our results about the *Pugioniferae* section, which is represented by two species in our country, overlap, and the basic chromosome number is $x = 13$. The taxa of this section, most of which are known from Iran, can be considered primitive in terms of chromosome number and morphology, and their chromosomal variations are rather limited. The karyotypes are mostly composed of metacentric and symmetrical chromosomes. The taxa can be directly separated based on their karyotype. *Cousinia macroptera* are seen as more evolved compared to the remaining ones due to including satellite and also higher M_{CA}, CV_{CI}, and AI.

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