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A statistical overview to the chromosome characteristics of some *Centaurea* L. taxa distributed in the Eastern Anatolia (Turkey)

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Abstract. This study performed statistical analyses based on chromosome micromorphology of 18 *Centaurea* taxa, two of which are endemic. ANOVA, Correlation analysis, Discriminant analysis and Cluster analysis were performed to determine the relationships between taxa based on chromosomal features. In addition, according to the data obtained from these analyses, the relationships between taxa and sections were tried to be interpreted. As a result of the analyses, the taxa *C. drabifolia* Sm. *floccosa* (Boiss.) Wagenitz & Greuter, *C. kotschyi* (Boiss. & Heldr.) Hayek var. *floccosa* (Boiss.) Wagenitz and *C. behen* L. and *C. polypodiifolia* Boiss *pseudobehen* (Boiss.) Wagenitz were located close to each other. These taxa are located in the same sections in the morphological classification. Besides, in the Discriminant analysis, the taxa of *Acrocentron*, *Microlophus*, *Cheirolepis* sections were closely located compared to all other taxa. However, results were not seen to cover all taxa of the sections. This study revealed various chromosomal characteristics of some *Centaurea* taxa distributed in the Eastern Anatolia. It also performed statistical analyses on these data. While determining the relationships between taxa according to chromosomal characteristics, comparing chromosomal formulas and indices collectively and evaluating the relationships was found to be relatively consistent with morphological classification.

Keywords: *Centaurea*, chromosome, endemic, statistical analysis, Turkey.

INTRODUCTION

Members of the Asteraceae family occupy a wide range of habitat types and are found in almost every region except Antarctica. There are 129 genera and 1156 species of the Asteraceae family in Turkey. *Centaurea* L. genus, which is one of the important genera of the Asteraceae family, is accepted as a systematically problematic genus and spreads globally with approximately 700 species in Asia, North Africa, America, and Europe (Güner et al. 2000). In Turkey, the genus *Centaurea* is represented by 238 species. 125 of these species are endemic (Güner et al. 2012). The endemism rate of this genus, which has many endemic species, is approximately 52%. The systematics of the genus *Centaurea* has changed, especially with the development of molecular techniques, and some problems have been solved. In the light of these studies, it is known that Turkey is the essential gene center of the genus *Centaurea* with many rare endemic species.

Species of the *Centaurea* in Turkey can generally grow in very different habitats such as stony calcareous cliffs, vineyards, roadsides, coastlines, steppe, scrub, fallow areas, sandy beaches, forests, dry meadows, rocky slopes. In addition, although many species of this genus have medicinal properties, especially the flowering above-ground parts or only the flower is used to cure many diseases and relieve pain (Yeşilada et al. 2004; Gürbüz & Yeşilada 2007). Many taxonomic, cytotaxonomic, morphological, anatomical and karyological studies have been carried out on *Centaurea* taxa, which are used medically (Haratym et al. 2020; Fattaheian-Dehkordi et al. 2021; Khammar & Djeddi 2012;)

This study was carried out on a statistical evaluation of data obtained from our previous cytological studies (Hayta et al. 2017; Tasar et al. 2018a; 2018b; 2018c).

In the current study, it is aimed to investigate the relationship between taxa, whose karyotype characteristics were revealed using micromorphological chromosome data, and investigate the compatibility and usefulness of this relationship with morphological classification by performing various statistical analyses.

MATERIAL AND METHODS

Material

Plant samples belonging to the genus *Centaurea* were collected and numbered between 2011 and 2012 from their natural habitats in different localities in Elazığ province (Turkey) and its surroundings by field studies. The localities of the taxa are given in Table 1.

Methods

Chromosome measurements

The seeds of the plant samples were sown in petri dishes and germinated in an oven at 20-22 °C. Roots reaching 1–2 cm in length from the germinated seeds were cut and kept in colchicine for 2 hours at room temperature and subjected to pretreatment. Afterward, the root tips were placed in Carnoy fixative (3:1) and kept in the refrigerator at +4 °C for 24 hours and fixed. At the end of the period, root tips were hydrolyzed in 1N HCl in an oven at 60 °C for 5-18 minutes. Root tips removed from hydrolysis were stained with Feulgen stain for 1 hour in a dark environment at room temperature. Then it was washed 2-3 times with tap water. For preparation, the growth meristem part was cut off with a sharp razor blade in a drop of 45% acetic acid on the slide, and the coverslip was closed (Elçi 1982). The photographs of each species' three best somatic cells were taken with a Canon digital camera and an Olympus BX53 microscope with a 100-lens. The naming system of Levan et al. (1964) was used to locate the centromere. The intra-chromosomal asymmetry index (A1) was calculated according to the formula proposed by Zarco (1986). The interchromosomal asymmetry index (A2) and karyotype symmetry nomenclature were made according to Stebbins (1971). Chromosomes measurements of *Centaurea* taxa are given in Table 3.

Data Analyses

Various formulas and indexes were used for analyses based on chromosome characteristics. The measurements were built on haploid datasets. The calculations and abbreviations used in the analysis are as follows. TLC (total length of chromosomes), MTLC (mean of total length of chromosomes), MAX (maximum length of chromosome), MIN (minimum length of chromosomes), MLA (mean of long arms), MSA (mean of short arms), MrV (mean of r-value), MdV (mean of d value), MAR (mean of arm ratio), MCI (mean of chromosome index), MRLC (mean of relative length of chromosomes), DRL (difference of range of relative length), TF% (total form percentage), S% (relative length of shortest chromosome), A1 (intrachromosomal asymmetry index), A2 (interchromosomal asymmetry index), and A (Degree of asymmetry). Both arm ratios were assumed to be equally affected (Adhikary 1974). All karyotype formulas and indexes were determined based on Huziwara (1962) (TF%), Levan et al. (1964) (*r* and *d* values), Zarco (1986) (A1 and A2), Watanabe (1999) (A), Peruzzi & Eroğlu (2013) (CI) as well. The abbreviations were taken from Rezeai et al. (2014) (RLC%, DRL, S%). The formulas are as follows.

Formulas and Indexes

$$r\ value = \frac{Length\ of\ the\ long\ arm\ of\ chromosome}{Length\ of\ the\ short\ arm\ of\ chromosome}$$

$$d\ value = Length\ of\ the\ long\ arm\ of\ chromosome\text{-}Length\ of\ the\ short\ arm\ of\ chromosome$$

$$arm\ ratio = \frac{Length\ of\ the\ short\ arm\ of\ chromosome}{Length\ of\ the\ long\ arm\ of\ chromosome}$$

$$CI = \frac{Length\ of\ the\ short\ arm\ of\ chromosome}{Length\ of\ the\ long\ arm\ of\ chromosome + Length\ of\ the\ short\ arm\ of\ chromosome}$$

Table 1. The localities of studied taxa.

Taxa	Localities	Voucher specimen
C. aggregata Fisch. & C.A.Mey. ex DC. subsp. *aggregata*	B7 Elazığ; Sivrice, Gözeli village, Kuşakcı mountain slopes, 1550 m. 28.06.2012, Based on Grids (Turkey): A6, A7, A8, A9, B6, B7, B8, B9, C5, C6, C10	Taşar 1001
C. virgata Lam.	B7 Elazığ; Koçkale village on the Elazig-Bingöl road, mountainous area, 1380 m. 21.06.2012, Based on Grids (Turkey): A2, A3, A4, A5, A7, A8, A9, B2, B3, B4, B5, B6, B7, B8, B9, B10, C2, C3, C4, C5, C6, C10	Taşar 1003
C. balsamita Lam.	B7 Elazığ; Sürsürü district, roadside, 1050 m. 02.07.2012, Based on Grids (Turkey): A2, B7, B8, B9, C4, C6, C8	Taşar 1006
C. behen L.	B7 Elazığ; Keban road, Beşik village entrance, roadside, 1090 m. 15. 07. 2012, Based on Grids (Turkey): B6, B7, B8, B9, C6, C7, C8, C9, C10	Taşar 1009
C. polypodiifolia Boiss. var. *pseudobehen* (Boiss.) Wagenitz	B7 Elazığ; Çemişgezek, Danbüken, In the village of Avşan, 1090 m. 16.07.2012 , Based on Grids (Turkey): B6, B7	Taşar 1010
C. polypodiifolia Boiss. var. *polypodiifolia*	B7 Elazığ; Baskil, Kumtarla village, hills, 1090 m. 14.07.2012, Based on Grids (Turkey): A8, A9, B6, B7, B8, B9, C9, C10	Taşar 1012
C. carduiformis DC. subsp. *carduiformis* var. *carduiformis*	B7 Elazığ; Keban, Pınarlar village on Arapgir road, field edges, 1430 m. 20-07-2012, Based on Grids (Turkey): A3, A4, A5, A6, A8, B3, B4, B5, B6, B7, C4, C5	Taşar 1013
C. urvillei DC. subsp. *armata* Wagenitz	B7 Elazığ; Baskil, radiolink station surroundings, 1350 m. 16.06.2011, Based on Grids (Turkey): A2, A4, A5, B1, B2, B6, B7, C5, C6	Taşar 1015
C. urvillei DC. subsp. *hayekiana* Wagenitz	B7 Elazığ; Baskil, Kayabeyli village, slopes, 1460 m. 13.06.2011, Based on Grids (Turkey): B6, B7, C3	Taşar 1017
C. urvillei DC. subsp. *urvillei*	B7 Elazığ; Harput, Rocky areas around Anguzlu Baba Tomb, 1400 m. 13.06.2011, Based on Grids (Turkey):A1, A2, A3, A4, A5, B1, B2, B6, B7, C1, C2, C3, C4, C5, C6,C7	Taşar 1014
C. cynarocephala Wagenitz	B7-Elazığ; Sivrice, Gözeli village, Kuşakçı mountain, 1750 m. 23.06.2012, Based on Grids (Turkey): C8	Taşar 1020
C. kurdica Reichardt	B7 Elazığ; Baskil roadway, 23. km. roadsides, 1280 m. 13.07.2011, Based on Grids (Turkey): B7, B8, C8	Taşar 1022
C. derderiifolia Wagenitz	B7 Elazığ; Baskil, Haroğlu mountain lower slopes, 1350 m. 22.07.2011, Based on Grids (Turkey): B6, B7	Taşar 1024
C. drabifolia Sm. *floccosa* (Boiss.) Wagenitz & Greuter	B7 Elazığ; Baskil, Haroğlu mountain, behind the TV station, rocky area, 1950 m. 22.07.2011, Based on Grids (Turkey): A4, A5, A6, B2, B3, B4, B6, B7, C3, C4	Taşar 1025
C. kotschyi (Boiss. & Heldr.) Hayek var. *floccosa* (Boiss.) Wagenitz	B7 Elazığ; Baskil, Yukarı Kuluşağı village Kuzucuk hamlet mountainous region, 1350 m. 26.08.2012, Based on Grids (Turkey): B6, C6	Taşar 1026
C. saligna (K.Koch) Wagenitz	B7 Elazığ; Palu, Baltasi village, the hills behind the military post, 1450 m. 17.07.2012, Based on Grids (Turkey): B6, B7, B8, B9, C9, C10	Taşar 1027
C. iberica Trev. ex Sprengel	B7 Elazığ; Sürsürü District, 1067 m. 22.06.2011, Based on Grids (Turkey): A1, A2, A3, A4, A5, A6, A7, A8, B1, B2, B3, B4, B8, B9, C2, C3, C4, C5, C6, C7, C8, C9, C10	Taşar 1028
C. solstitialis L. subsp. *solstitialis*	B7 Elazığ; Sürsürü District, 1067 m. 22.06.2011, Based on Grids (Turkey): A1, A2, A3, A4, A5, A7, A8, B1, B3, B4, B5, B6, B7, B8, B9, C1, C2, C3, C4, C5, C6, C8, C9	Taşar 1029

$$RLC\% = \frac{total\ length\ of\ each\ chromosome}{total\ length\ of\ chromosomes} \text{ x } 100$$

DRL = ($maximum$ $relative$ $length$) - ($minimum$ $relative$ $length$)

$$TF\% = \frac{total\ length\ of\ short\ arms}{total\ length\ of\ chromosomes} \text{ x } 100$$

$$S\% = \frac{length\ of\ shortest\ chromosome}{length\ of\ longest\ chromosome} \text{ x } 100$$

$A = \left(\frac{1}{n}\right)\sum Ai, \quad Ai = \frac{li-si}{li+si}$, (*li = lengths of a long arm, si = lengths of a short arm, n = haploid chromosome number).*

$A1 = 1 - \frac{\sum_{i=1}^{n} \frac{b_i}{B_i}}{n}$ (*n = number of homologous chromosome pairs, b_i = the average length of short arms in every homologous chromosome pair, B_i = the average length of long arms in every homologous chromosome pair).*

$A2 = \frac{s}{\bar{x}}$ (*S = standard deviation of chromosome lengths, = mean of chromosome lengths).*

A data matrix was constructed according to 17 chromosomal traits in Table 4. The discriminant analysis (LDA) was used based on the data matrix. Next, the

Table 2. The sections of the studied *Centaurea* taxa.

Section	Taxa
Acrolophus	*C. aggregata* subsp. *aggregata, C. virgata*
Acrocentron	*C. urvillei* subsp. *hayekiana*, *C. urvillei* subsp. *urvillei, C. carduiformis* subsp. *carduiformis* var. *carduiformis, C. urvillei* subsp. *armata*
Stizolophus	*C. balsamita*
Microlophus	*C. behen*, *C. polypodiifolia* var. *pseudobehen*, *C. polypodiifolia* var. *polypodiifolia*
Cynaroides	*C. cynarocephala, C. kurdica*
Cheirolepis	*C. derderiifolia, C. drabifolia* subsp. *floccosa, C. kotschyi* var. *floccosa, C. saligna*
Calcitrapa	*C. iberica*
Mesocentron	*C. solstitialis* subsp. *solstitialis*

Table 3. Chromosomes measurements of *Centaurea* taxa (Ch. No: Chromosome No, C: Total length of the chromosome, L: Length of the long arm, S: Length of the short arm, CP: Centromeric position).

Ch. No	C	L	S	CP	Ch. No	C	L	S	CP
	C. aggregata subsp. *aggregata*					*C. urvillei* subsp. *hayekiana*			
1	4.74	2.37	2.37	M	1	6.07	3.04	3.04	M
2	4.74	2.47	2.26	m	2	4.54	2.54	2	m
3	4.68	2.53	2.16	m	3	3.86	2.43	1.43	sm
4	4.32	2.95	1.37	sm	4	3.75	1.93	1.82	m
5	4.32	2.95	1.37	sm	5	3.68	2.18	1.5	m
6	4.21	2.95	1.26	sm	6	3.68	2.32	1.36	sm
7	3.89	2.37	1.53	m	7	3	1.64	1.36	m
8	3.68	2.32	1.37	m	8	2.89	1.64	1.25	m
9	3.37	1.68	1.68	M	9	2.57	1.29	1.29	M
10	3.05	1.58	1.47	m	10	2.29	1.14	1.14	M
	C. urvillei subsp. *urvillei*					*C. behen*			
1	5.28	2.64	2.64	M	1	4.04	2.57	1.46	sm
2	4.66	2.56	2.1	m	2	3.96	2.14	1.82	m
3	4.24	2.8	1.44	sm	3	3.5	1.89	1.61	m
4	4.08	2.04	2.04	M	4	3.46	1.93	1.54	m
5	4	2.28	1.72	m	5	3.43	1.71	1.71	M
6	3.58	2.12	1.46	m	6	3.21	1.61	1.61	M
7	3.4	2.12	1.28	m	7	3.14	1.57	1.57	M
8	2.92	1.66	1.26	m	8	3.07	1.75	1.32	m
9	2.88	2.16	0.72	sm					
10	2.8	2.08	0.72	sm					
	C. polypodiifolia var. pseudobehen					*C. polypodiifolia var. polypodiifolia*			
1	4.4	2.32	2.08	m	1	4.66	2.61	2.05	m
2	4.04	2.38	1.66	m	2	3.8	1.9	1.9	M
3	4.12	2.92	1.2	sm	3	3.07	1.83	1.24	m
4	3.84	2.56	1.28	sm	4	2.59	1.59	1	m
5	3.6	2.04	1.56	m	5	2.37	1.32	1.05	m
6	3.44	2.16	1.28	m	6	2.37	1.66	0.71	sm
7	3.44	1.72	1.72	M	7	2.12	1.22	0.9	m
8	3.26	1.63	1.63	M	8	1.93	1.24	0.68	sm
	C. carduiformis subsp. *carduiformis*					*C. urvillei* subsp. *armata*			
1	6.11	3.63	2.49	m	1	4.74	2.37	2.37	M
2	5.18	3.15	2.03	m	2	4.74	2.47	2.26	m

Ch. No	C	L	S	CP	Ch. No	C	L	S	CP
3	4.55	2.93	1.63	sm	3	4.68	2.53	2.16	m
4	4.31	2.49	1.82	m	4	4.32	2.95	1.37	sm
5	4.25	2.39	1.86	m	5	4.32	2.95	1.37	sm
6	4.22	2.65	1.57	m	6	4.21	2.95	1.26	sm
7	4.01	2.8	1.2	sm	7	3.89	2.37	1.53	m
8	3.52	2.09	1.43	m	8	3.68	2.32	1.37	m
9	3.3	2.15	1.15	sm	9	3.37	1.68	1.68	M
10	2.88	1.57	1.31	m	10	3.05	1.58	1.47	m
		C. cynarocephala					*C. kurdica*		
1	6.41	3.62	2.79	sm	1	5.81	3.36	2.44	m
2	5.12	3.5	1.62	M	2	5.17	3.01	2.16	m
3	4.99	3.09	1.9	m	3	4.71	2.36	2.36	M
4	4.35	3.21	1.15	sm	4	4.76	2.86	1.91	m
5	4.29	2.15	2.15	M	5	4.4	2.7	1.69	m
6	4.22	2.62	1.6	m	6	4.18	2.58	1.6	m
7	3.91	2.38	1.53	m	7	4.09	2.7	1.4	sm
8	3.68	2.26	1.41	m	8	4.06	3.03	1.2	sm
9	3.18	1.79	1.38	m	9	3.91	2.41	1.5	m
		C. derderiifolia					*C. drabifolia* subsp. *floccosa*		
1	2.48	1.46	1.02	m	1	4.42	2.21	2.21	M
2	2.4	1.4	1	m	2	3.84	2.53	1.32	sm
3	2.3	1.39	0.9	m	3	3.84	2.53	1.32	sm
4	2.1	1.31	0.79	m	4	3.37	2.37	1	sm
5	2.06	1.03	1.03	M	5	3.28	2.21	1.08	sm
6	2.07	1.16	0.9	sm	6	3.06	1.85	1.21	m
7	1.98	1.21	0.77	m	7	3	1.79	1.21	m
8	1.9	1.08	0.82	m	8	2.89	1.79	1.11	m
9	1.85	1.1	0.76	m	9	2.76	1.61	1.16	m
10	1.81	1.27	0.53	sm	10	2.84	2	0.84	sm
11	1.67	1.03	0.64	m	11	2.54	1.49	1.05	m
12	1.54	0.84	0.7	m	12	2.21	1.11	1.11	M
13	1.52	1.03	0.48	sm	13	2.13	1.18	0.95	m
14	1.47	0.74	0.73	m	14	2.03	1.13	0.89	m
15	1.35	0.77	0.58	m	15	1.95	1.16	0.79	m
16	1.35	0.77	0.58	m	16	1.89	1.05	0.84	m
17	1.29	0.65	0.65	M	17	1.79	0.95	0.84	m
18	1.13	0.52	0.61	m	18	1.79	1	0.79	m
		C. kotschyi var. *floccosa*					*C. saligna*		
1	4.93	3.48	1.44	sm	1	6.08	3.44	2.65	m
2	3.81	2.56	1.26	sm	2	5.26	2.95	2.31	m
3	3.81	2.26	1.56	m	3	5.1	2.55	2.55	M
4	3.26	1.63	1.63	M	4	4.66	3.03	1.63	sm
5	3.1	1.8	1.3	m	5	4.52	2.52	2	m
6	3	1.8	1.2	m	6	4.02	2.18	1.84	m
7	2.9	1.8	1.1	m	7	3.9	2.52	1.38	sm
8	2.85	1.74	1.11	m	8	3.47	2.02	1.45	m
9	2.74	1.7	1.04	m	9	3.18	1.98	1.2	m
10	2.56	1.59	0.96	m					
11	2.41	1.33	1.07	m					
12	2.37	1.19	1.19	M					

Ch. No	C	L	S	CP	Ch. No	C	L	S	CP
13	2.37	1.19	1.19	M					
14	2.3	1,26	1.04	m					
15	2,22	1.19	1.04	m					
16	2.22	1.56	0.67	sm					
17	2.07	1.04	1.04	M					
18	1.44	0.89	0.56	m					
		C. iberica					*C. solstitialis* subsp. *solstitialis*		
1	2.95	1.84	1.11	m	1	3.53	2.39	1.14	sm
2	2.76	1.68	1.08	m	2	2.81	1.58	1.22	m
3	2.42	1.49	0.93	m	3	2.72	1.47	1.25	m
4	2.29	1.31	0.98	m	4	2.28	1.22	1.06	m
5	2.25	1.26	0.99	m	5	2.25	1.36	0.89	m
6	2.01	1.2	0.82	m	6	2.23	1.12	1.12	M
7	1.97	0.99	0.99	M	7	2.17	1.42	0.75	sm
8	1.84	1.06	0.78	m	8	1.81	1.17	0.64	sm
9	1.84	1.24	0.61	sm					
10	1.57	0.84	0.73	m					

cluster analysis was made using the Manhattan distance index to determine the relationships between *Centaurea* taxa's chromosome properties (Romesburg 2004). In addition, the Pearson correlation coefficient (r) analysis was performed to see strong and weak relationships between chromosome traits. At the same time, Shapiro - Wilk normality test was performed. Then, the one-way analysis of variance (ANOVA) was performed to determine whether the difference between the data was statistically significant. All the analyses were carried out with PAleontoSTatistics (PAST) (Hammer et al. 2001).

RESULTS

Chromosome micromorphological features of 18 *Centaurea* taxa were specified, and statistical analyses were performed on them using formulas created using various chromosome features. Mitotic metaphase chromosome images of *Centaurea* taxa are given in Figure 1, and karyotype features are given in Table 4. One way ANOVA test, which is one of the analyses made according to the chromosome characteristics of the taxa, is given in Table 5. According to the values obtained with the formulas using the micromorphological chromosome features of taxa, the data show a normal distribution according to the Shapiro-Wilk test ($p>0.05$) and the residual plot graph is shown in Figure 2 accordingly. Then, according to the one-way ANOVA test p-value, the difference between taxa was statistically significant ($p<0.05$) (Table 5).

Correlation analysis

According to the correlation analysis, there are relations between the r-values of chromosomal data according to the significance level less than $p < 0.05$. Particularly a high positive relationship among MTLC-MLA-MSA, MrV-A1-A, MAR-A1-A, MRLC-A2, and a strong negative relations among TLC-MRLC, TLC-A2, MCL-A1, MCL-A, MCI-MAR-MrV and MrV- TF% values (Figure 3).

Discriminant analysis (LDA)

According to LDA (Table 6-7, Figure 4), the first two components explained most of the variation according to chromosome data between the taxa. While the first two components explain 93.56% and 4,34% of the variance, respectively, these characters explained 97.9% of the total variation. The variation most affected were TLC, MRLC, MCI, and DRL%. Similarly, since some variables (such as A, A1) have lower values than calculations, the effects on variation in LDA have been low.

In addition, with the results obtained with the chromosomal characters determined by the formulas, taxa grouped according to sections (given groups) were regrouped (predicted group) by discriminant analysis and distributed into groups with an accuracy of around 5.5%. In other words, when the sections determined according to the morphology of the taxa were regrouped with the characters determined according to the chromosomal formulas in the analysis, the overlap was around 5.5%.

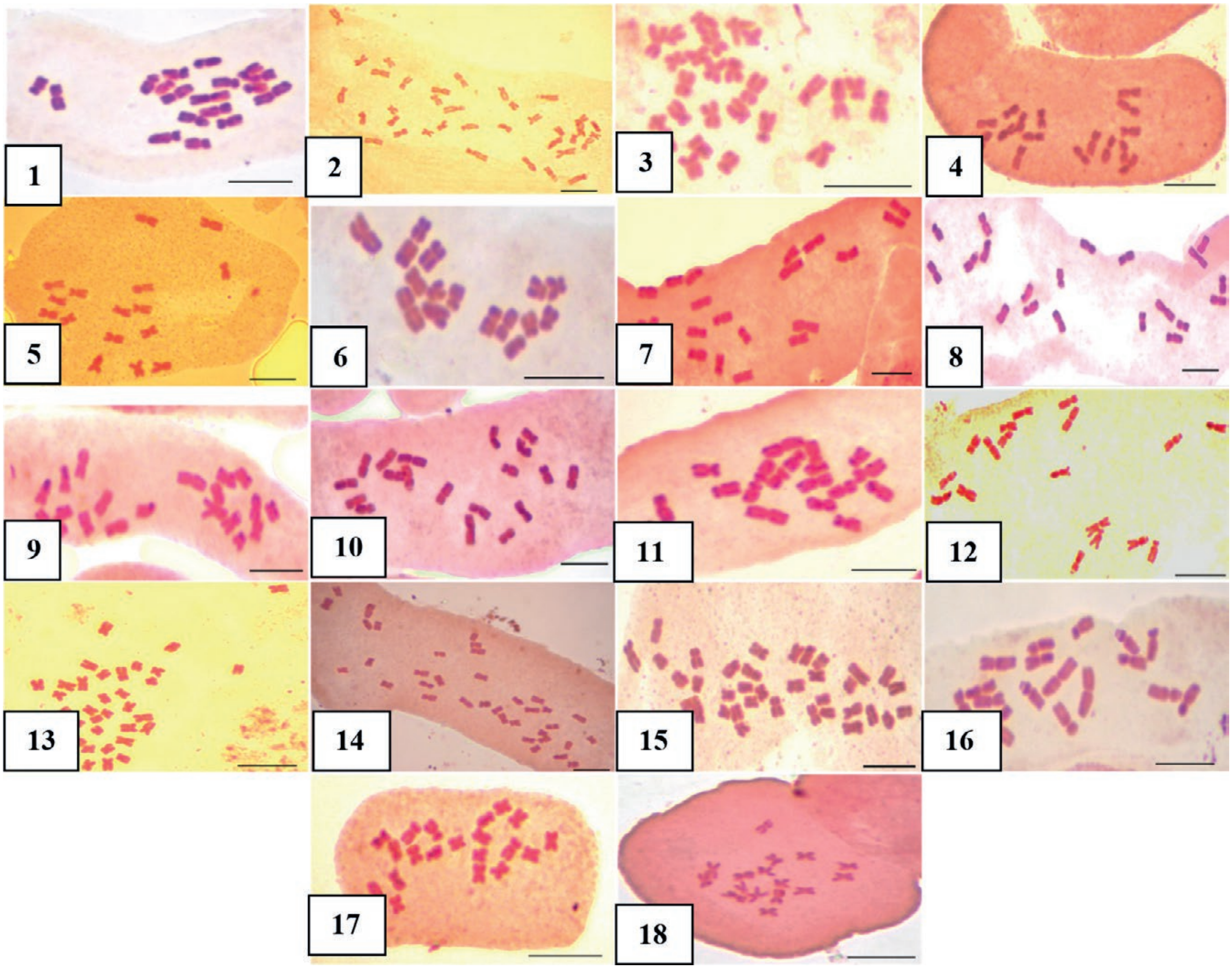

Figure 1. Mitotic metaphase chromosomes of *Centaurea* taxa (1. *C. aggregata* subsp. *aggregata*, 2. *C. virgata*, 3. *C. balsamita*, 4. *C. behen*, 5. *C. polypodiifolia* var. *pseudobehen*, 6. *C. polypodiifolia* var. *polypodiifolia*, 7. *C. carduiformis* subsp. *carduiformis* var. *carduiformis*, 8. *C. urvillei* subsp. *armata*, 9. *C. urvillei* subsp. *hayekiana*, 10. *C. urvillei* subsp. *urvillei*, 11. *C. cynarocephala*, 12. *C. kurdica*, 13. *C. derderiifolia*, 14. *C. drabifolia* subsp. *floccosa*, 15. *C. kotschyi* var. *floccosa*, 16. *C. saligna*, 17. *C. iberica*, 18. *C. solstitialis* subsp. *solstitialis,* Scale bars: 10 µm).

Cluster analysis

According to the Cluster analysis results made according to the UPGMA algorithm and Manhattan distance index, the taxa are divided into 3 main groups (Figure 5). These groups are also divided into subgroups among themselves. The *Stizolophus* and *Cynaroides* sections were found together in Group 1, the *Microlophus* section in Group 2, and the *Cheirolepis* section in Group 3.

Especially *C. drabifolia* subsp. *floccosa* and *C. kotschyi* var. *floccosa*; *C. behen* and *C. polypodifolia* var. *pseudobehen*; *C. urvillei* subsp. *armata* and *C. aggregata* subsp. *aggregata* stand out as closely related taxa. Within these relationships, *C. urvillei* subsp. *armata* and *C. aggregata* subsp. *aggregata* taxa were found to be close to each other in terms of chromosomal characteristics, although they were in different sections.

DISCUSSION

This study investigated 18 taxa belonging to 8 sections of genus *Centaurea* (Table 2). Among the investigated taxa, *C. derderifolia*, *C. saligna* are endemic to Turkey. No statistical study of this scale has been encountered based on chromosome characteristics on the genus. In some studies, the cluster analysis data can yield similar trees with the morphological classification of the taxa (Açar & Satıl 2019; Arabaci et al., 2021; Dirmenci et al. 2019; Genç et al. 2021).

Table 4. Karyotype characteristics of *Centaurea* taxa (TLC: Total Lenght of Chromosomes, MTLC (Mean of Total Length of Chromosomes, MAX: Maximum Length of Chromosome, MIN: Minimum Length of Chromosome, MLA: Mean of Long Arms, MSA: Mean of Short Arms, MrV: Mean of r Value, MdV: Mean of d Value, MAR: Mean of Arm Ratio, MCI: Mean of Chromosome Index, MRLC: Mean of Relative Length of Chromosomes, DRL: Difference of Range of Relative Length, TF%: Total Form Percentage, S%: Relative Length of Shortest Chromosome, A_1: Intrachromosomal Asymmetry Index, A_2: Interchromosomal Asymmetry Index).

Centaurea Taxa	TLC	MTLC	MAX	MIN	MLA	MSA	MrV	MdV	MAR	MCI	MRLC	DRL	TF%	S%	A_1	A_2	A
C. aggregata subsp. *aggregata*	41.00	4.10	2.95	1.26	2.42	1.68	1.44	0.74	1.52	41.18	10.01	4.10	0.41	0.43	0.270	0.10	0.179
C. urvillei subsp. *hayekiana*	36.33	3.63	3.03	1.14	2.02	1.62	1.24	0.40	1.27	44.58	10.01	10.43	0.45	0.38	0.182	0.10	0.109
C. urvillei subsp. *urvillei*	37.84	3.78	2.64	0.72	2.25	1.54	1.46	0.71	1.68	39.43	10.01	6.55	0.41	0.27	0.368	0.10	0.187
C. virgata	63.52	3.53	4.63	0.63	2.23	1.29	1.73	0.94	1.83	38.06	5.56	6.88	0.37	0.14	0.348	0.06	0.266
C. balsamita	33.95	2.61	2.49	0.64	1.62	0.99	1.62	0.63	1.68	38.42	7.69	7.72	0.38	0.26	0.356	0.08	0.238
C. behen	27.81	3.48	2.57	1.32	1.89	1.58	1.20	0.31	1.21	45.68	12.50	3.47	0.45	0.51	0.147	0.13	0.091
C. polypodiifolia var. *pseudobehen*	30.14	3.77	2.32	1.20	2.22	1.55	1.43	0.67	1.49	41.42	12.50	3.77	0.41	0.52	0.267	0.13	0.177
C. polypodiifolia var. *polypodiifolia*	22.91	2.86	2.61	0.68	1.67	1.19	1.40	0.48	1.51	40.66	12.50	11.93	0.42	0.26	0.299	0.12	0.168
C. carduiformis subsp. *carduiformis* var. *carduiformis*	42.33	4.23	3.63	1.15	2.58	1.65	1.57	0.93	1.60	38.96	10.00	7.63	0.39	0.32	0.352	0.10	0.221
C. urvillei subsp. *armata*	41.00	4.10	2.95	1.37	2.42	1.68	1.44	0.74	1.52	41.18	10.00	4.10	0.41	0.46	0.271	0.10	0.179
C. cynarocephala	40.15	4.46	3.62	1.38	2.74	1.73	1.59	1.01	1.66	38.73	11.11	8.06	0.39	0.38	0.349	0.11	0.184
C. kurdica	41.09	4.57	3.36	1.20	2.78	1.80	1.54	0.98	1.63	38.72	11.11	4.63	0.40	0.36	0.345	0.11	0.226
C. derderiifolia	32.27	1.79	1.46	0.58	1.04	0.75	1.39	0.29	1.42	42.28	5.55	4.17	0.42	0.40	0.248	0.06	0.212
C. drabifolia subsp. *floccosa*	49.63	2.75	2.53	0.79	1.66	1.09	1.52	0.57	1.54	40.38	5.55	5.31	0.40	0.31	0.305	0.06	0.206
C. kotschyi var. *floccosa*	50.36	2.79	3.48	0.56	1.66	1.13	1.47	0.53	1.48	41.24	5.56	6.91	0.41	0.16	0.277	0.06	0.191
C. saligna	40.19	4.46	3.44	1.20	2.58	1.89	1.36	0.69	1.42	41.91	11.11	7.21	0.42	0.35	0.266	0.11	0.154
C. iberica	21.90	2.19	1.84	0.61	1.29	0.90	1.43	0.39	1.45	41.38	10.00	6.29	0.41	0.33	0.281	0.10	0.177
C. solstitialis subsp. *solstitialis*	19.80	2.47	2.39	0.64	1.46	1.01	1.45	0.45	1.49	40.95	12.5	8.71	0.41	0.27	0.287	0.12	0.185

Table 5. One way ANOVA test results.

Test for equal means

	Sum of sqrs	df	Mean square	*F*	*p* (same)
Between groups:	45343.3	16	2833.96	351.6	8.149E-179
Within groups:	2329.64	289	8.06104		Permutation *p* (n=99999)
Total:	47672.9	305			1E-05
omega^2:	0.9483				

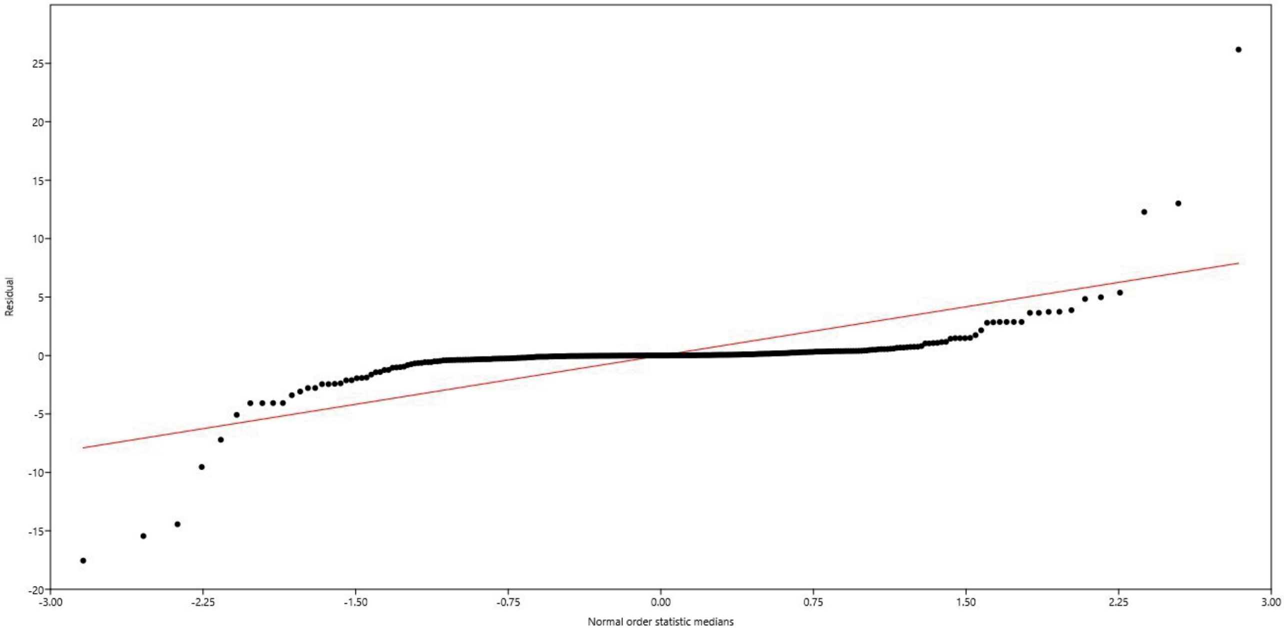

Figure 2. Shapiro - Wilk normality test(p=0.4809>0.05)-Residual plot.

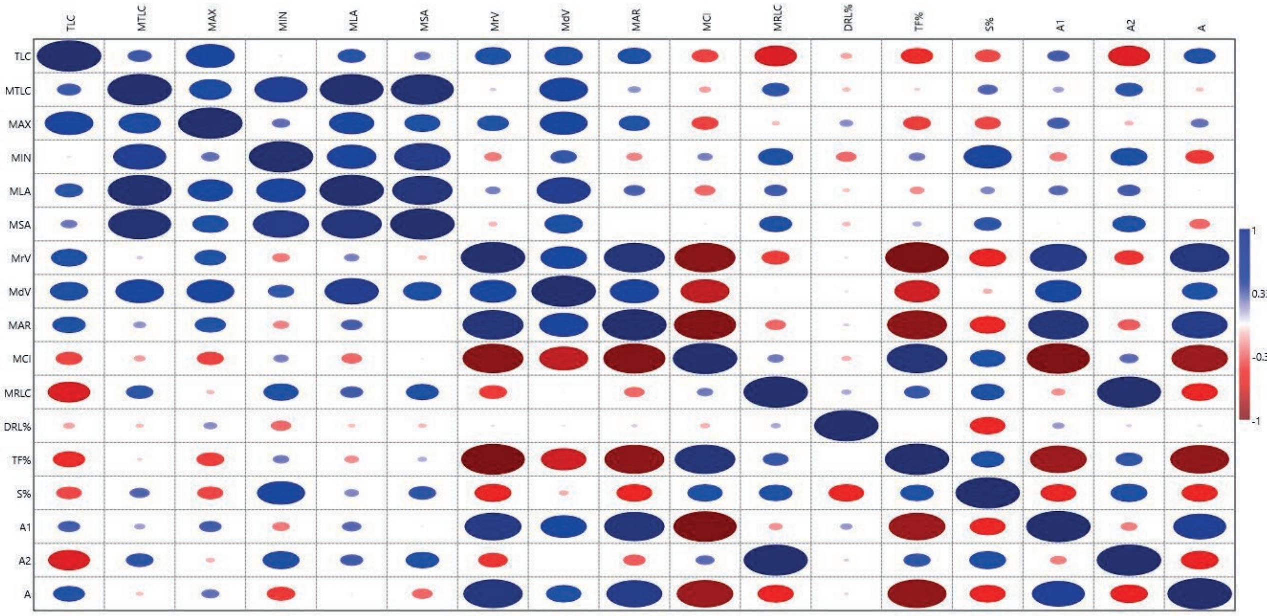

Figure 3. Correlation analysis between karyotype characteristics ((TLC: Total Lenght of Chromosomes, MTLC (Mean of Total Length of Chromosomes, MAX: Maximum Length of Chromosome, MIN: Minimum Length of Chromosome, MLA: Mean of Long Arms, MSA: Mean of Short Arms, MrV: Mean of r Value, MdV: Mean of d Value, MAR: Mean of Arm Ratio, MCI: Mean of Chromosome Index, MRLC: Mean of Relative Length of Chromosomes, DRL: Difference of Range of Relative Length, TF%: Total Form Percentage, S%: Relative Length of Shortest Chromosome, A1: Intrachromosomal Asymmetry Index, A2: Interchromosomal Asymmetry Index).

Wagenitz (1975) divided the genus *Centaurea* into 34 sections in Flora of turkey. Wagenitz (1975) also stated that the genus in Flora of Turkey is taxonomically difficult and noted that much more studies are needed. In addition, he emphasized that it is especially important to obtain cytological data. There are many taxonomic dif-

Table 6. Discriminant analysis (LDA) of *Centaurea* taxa showing the eigenvalues of the total variance.

PC	Eigenvalue	% variance
1	185.98	93.56
2	8.6347	4.344
3	2.3673	1.191

Table 7. Discriminant analysis (LDA) of *Centaurea* taxa showing the given (morphological sections) and predicted groups (after the analyses).

Taxa	Given group	Classification
C. aggregata subsp. *aggregata*	1	5
C. urvillei subsp. *hayekiana*	2	5
C. urvillei subsp. *urvillei*	2	5
C. virgata	1	5
C. balsamita	3	4
C. behen	4	7
C. polypodiifolia var. *pseudobehen*	4	7
C. polypodiifolia var. *polypodiifolia*	4	7
C. carduiformis subsp. *carduiformis* var. *carduiformis*	2	5
C. urvillei subsp. *armata*	2	5
C. cynarocephala	5	6
C. kurdica	5	6
C. derderiifolia	6	5
C. drabifolia subsp. *floccosa*	6	5
C. kotschyi var. *floccosa*	6	5
C. saligna	6	5
C. iberica	7	4
C. solstitialis subsp. *solstitialis*	8	8

ficulties in the genus, which has a large number of taxa according to the Flora of Turkey. Therefore, studies on the genus containing many such species will provide important data. In this study, taxa from 8 sections were discussed. Relationships between taxa were tried to be revealed based on cytological data.

In the cluster analysis, which is one of the analyses, although the grouping to cover the whole section was not fully formed, some taxa could be located close to each other in the same section. However, as seen in the discriminant analysis, individuals belonging to the same section in a certain way could not be found in the same spot. In addition, some taxa were close in both discriminant analysis and cluster analysis, and this result was also found to be consistent with morphological classification. This result shows us a strong relationship between these taxa, such as C. *drabifolia* subsp. *floccosa* and *C. kotschyi* var. *floccosa* and *C. behen* and *C. poly-*

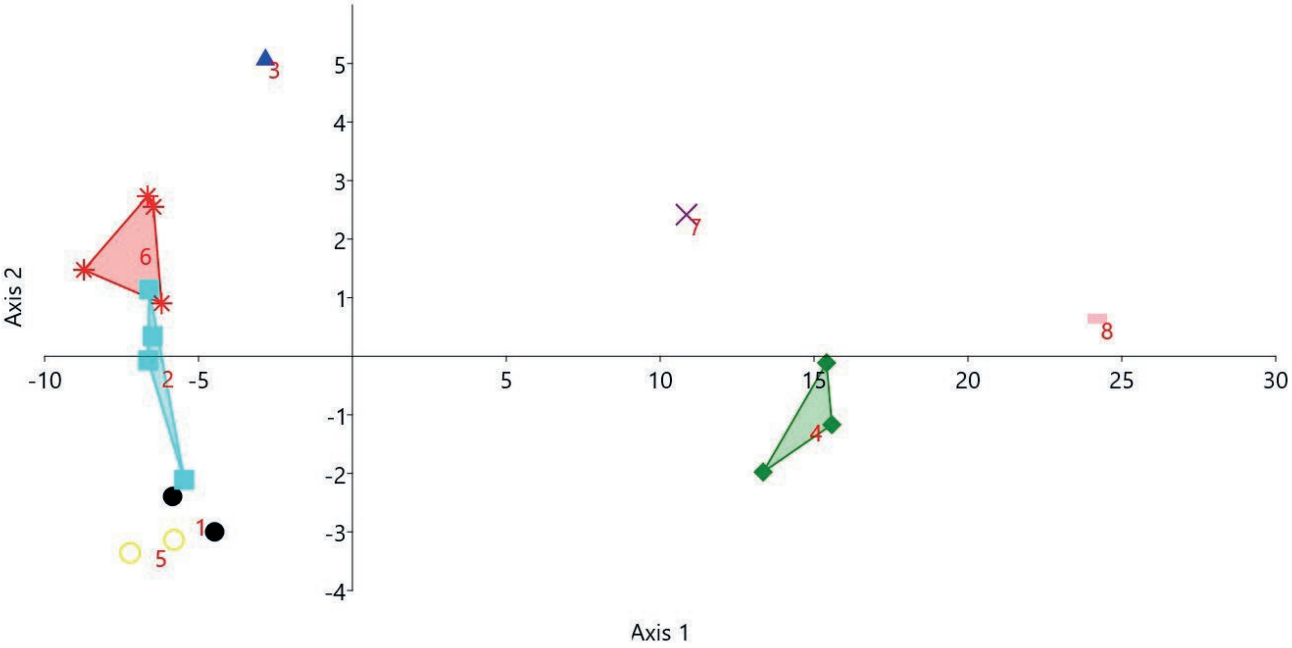

Figure 4. Discriminant analysis scatter plot diagram (Different colors and numbers refer to different sections, Black(1): *Acrolophus*, Aqua (2): *Acrocentron*, Blue(3): *Stizolophus*, Green(4): *Microlophus*, Yellow(5): *Cynaroides*, Red(6): *Cheirolepis*, Purple(7): *Calcitrapa*, Pink(8): *Mesocentron*).

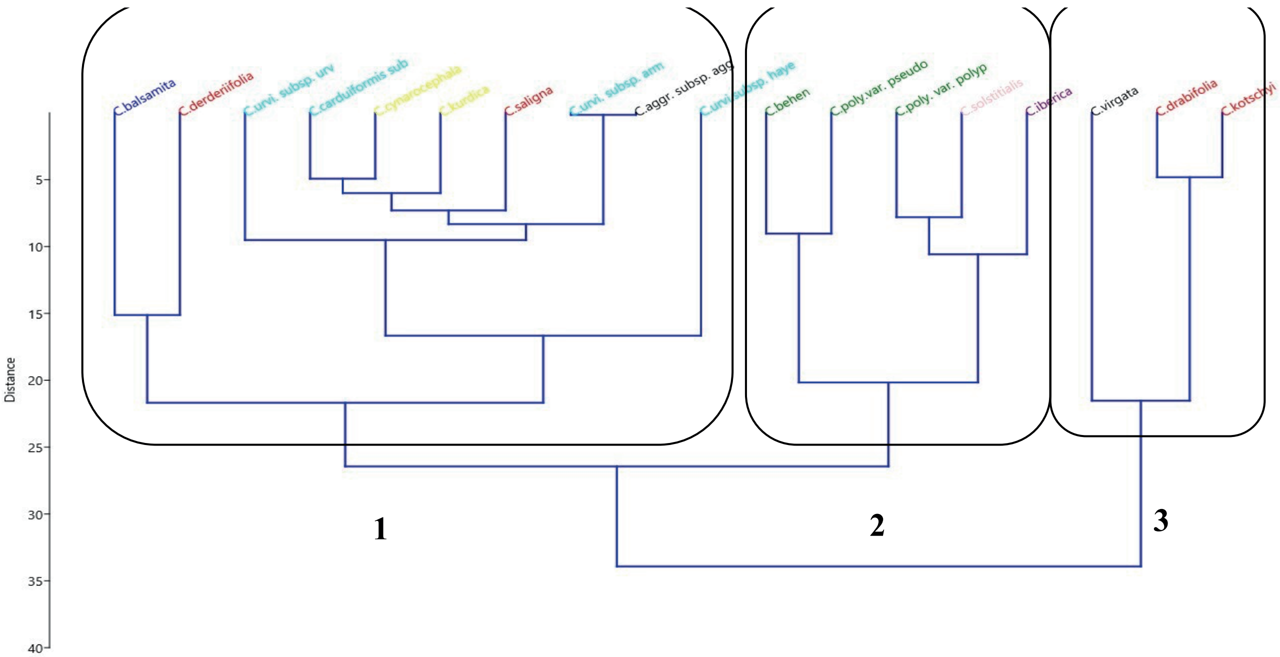

Figure 5. Cluster analysis according to karyotype characteristics show that 3 main groups (Same colored taxa are located in the same section).

podifolia var. *pseudobehen*. Also these taxa are located in the same sections in the morphological classification. In addition, in the discriminant analysis, the taxa of *Acrocentron*; *Microlophus*; *Cheirolepis* sections were located close to each other according to the diagram compared to all other taxa (Figure 4).

On the discriminant analysis, there was a 5.5% overlapping classification between the sections in which they were classified according to the taxa morphological classification and the new groups that emerged according to the relations between them as a result of the analyses made according to the chromosomal formulas (Table 7). Accordingly, most of the taxa in the *Cherloides* section were found in the *Cynaroides* section. Again, according to the analysis, Sections 1 and 3, *Acrolophus* and *Stizolophus* sections were not included in the new grouping. As a result, other sections are sufficient to classify these taxa. However, the most important result is that the morphological classification and the classification made according to the data series obtained from the chromosomal formulas show similarity at a rate of 5.5%. Moreover, it has no linear relationship in taxonomic terms. Genç et al. (2021) reached the same conclusion in their study. Accordingly, chromosomal formulas are not suitable for evaluating together with each other to draw a meaningful conclusion. However, it would be more appropriate to compare taxa one by one.

Cluster analysis can be a helpful tool in classifying taxa. Accordingly, sections of *Acrocentron*, *Microlophus*, *Cynaroides* were grouped with each other. This result was also relatively similar in the discriminant analysis. In general, when this analysis is performed according to morphological data, while there is a more directly proportional grouping, according to the data using the formulas obtained with chromosomal micromorphological data, although there is consistency in some sections, there may be a possibility that the classification to be made using these data in classifying taxa in general terms may be incorrect.

In conclusion, there is no general overlap between morphological classification and chromosomal micromorphological-based classification. In addition, there were taxa located close to each other in both morphological data and chromosomal micromorphological data. Undoubtedly, these taxa are estimated to be closely related to each other. However, the results obtained from the formulas were seen as characters while creating data sets, and analyses were made in that way. The validity of this needs to be investigated better with more data and evaluation of taxa from different perspectives. This study also provides important data for this situation.

This study revealed various chromosomal characteristics of *Centaurea* taxa distributed in Eastern Anatolia. It also performed statistical analyses on these data and revealed that comparing chromosomal calculations separately in taxa would be more beneficial in morphological classification than classifying them together into analysis structures.

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