Original Article

Chromosome count of some species of *Cynaroides* Boiss.section of *Centaurea* L. "Aseracaea" genus in Iran

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Abstract

In the present study, the chromosome number of *Cynaroides* section species in Iran is counted andsome of them arereported for the first time in the world based on available sources by preparing the karyotype of chromosome numbers and ideogram analysis of *Cynaroides* section species. For this purpose, after the germination of seeds, the end part of the root was isolated and after staining by squashing method, the mitotic zone was prepared. In this study, it was found that the base chromosome number in the species of *Cynaroides* section, except *C. iranshahrii*, is x= 9, which confirms previous studies and indicates the occurrence of homoploid transformation among the species in this section. The chromosome number of *C. iranshahrii* was 2n= 4x= 32, which indicates the base number of x= 8 for this species; and it was suggested that it will be isolated from this section and transferred to the *Microluphus* section.

Keywords: Asteraceae, *Cynaroides* section, Karyotype, Chromosome counting, *Cynaroides*

INTRODUCTION

Asteraceae family is the largest family of vascular plants in the world with 1600-1700 genera and 23000 species. This family is cosmopolitan and is found almost everywhere on Earth except Antarctica [1]. It has a high diversity in settlements and life forms. Some species of this family have great economic importance as edible vegetables, oil sources, insecticides, and ornamental plants [2]. Rapid diversity has created particular difficulties in understanding phylogenetic relationships in this family. Recently, through studies and phylogenetics, many advances have been made in solving the taxonomic relations and classifications at different levels of this family [1]. The origin of this family has long been debated. One theory considers its origin to be in South America, which had been the earliest time for the evolution of this family [3].

This genus is also known as CompositaeGiseke or SynantheraeJeuss [1]. Recent studies show that Cardueae is a single ancestral family and, based on molecular data, includes five sub-tribes: Centaureinae, Carlininae, Carduinae, Echinopsinae, and Cardopatiinae [4, 5]. The genus *Centaurea* L. in its broad sense is the largest genus of the Centaureinae sub-tribe. This sub-tribe is concentrated mainly in the Mediterranean region and includes 31 genera and approximately 800 species [6]. This sub-tribe consists of a range of tall shrubs to one-year plants, mostly as polycarpic perennials or monocarpic biennials. This sub-family is also identified by achene with lateral-axial umbilicus indentation, a double pappus, and thorn-lessleaves. This sub-tribe is characterized by perennial, biennial, or annual thorn-less plants, rarely shrubs [7]. Capitols are often heterogamous with radially sterile flowers and rarely homogamous. Involucres arewrinkled, thorny, or thornless appendages. The achene has a thick shell, lateral-axial or concave umbilicus, and double pappus [1]. In recent years, a system has been adopted to divide this genus into four natural genera: Centaurea, Psephellus Cass., Cyanus Mill., and RhaponticoidesVaill [7]. The classification of *Centaurea* sections is mainly based on the indicator characteristics derived from the morphology of the appendage of phyllaries, achenes, and pappus [8]. One of the large parts of the *Centaurea*genus is *Cynaroides* Boiss, which mainly consists of large biennial plants and is differentiated from other sections of *Centaurea* genus by features such as large Lanceolate or Oblong, Raceme, Spike, or dichasial cymeas well as long articulated hairs. The members of this section are exclusive species and are found

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only in a very small area, which indicates high species formation rate in this section [8]. They are rarely thorough, thorny, or lacy, with no thorns at the ends and sometimes recurvate. Sometimes, phyllaries are without appendages or with thorn-shaped appendages, flowers are pink or purple, yellow or white, rarely red, central male-female flowers, sterile lateral flowers, numerous, sometimes very few (up to 3 per capita), equal to or shorter than the central flowers, and are usually not radial. The achenes are large, rectangular, or spear-shaped, of various colors, and pappus is rough, almost taller than the achene, sometimes shorter, usually with shorter inner row, and rarely slightly taller [6].
*C. regia*Boiss. subsp. *cynarocephala* Wagenitz was introduced by Negaresh and Rahiminejad in 2016 as a new report for the flora of Iran [9]. In 2014, based on morphological and phytogeographical data, Negaresh and Rahiminejad proved that the two species in this section, *C. gabrieliae* (Bornm.) Wagenitz and *C. wendelboi*Wagenitz, should be isolated and transferred to a new section called Ranjbariane Negaresh [8]. Basically, the basis of measurements proposed for chromosomes in all methods so far depends on the high and low length of the arm length of each chromosome in homologous pairs [10].

In karyotype examination, different chromosome sizes indicate that the karyotype is advanced and has chromosomes of different sizes [11]. Large chromosome size may be the result of replication at different gene sites and in different series, indicating a move toward adaptation. However, the opposite is also true, because transverse gene duplication along with chromosome shortening hasbeen observed in species of some genera [12]. Also, different climates may have increased adaptive differences, leading to the production of new varieties and even new species in plant habitats [13-16].

The asymmetry of the karyotype is probably due to the occurrence of chromosomal structural changes such as chromosomal deletion or unequal shifts, etc. Symmetrical karyotypes are usually more primary and the tendency toward asymmetry can occur through pericentric inversions and uneven displacements of parts of chromosome arms without any change in the number of centromers and independent chromosomes [15].

Changes in chromosome size exhibit different phenotypic traits. Differences in chromosome sizes indicate differences in the types of gene or protein products of them, and differences in the number of chromosomes represent the differences in gene arrangement or gene duplication or both. Also, many morphological and physiological differences indicate differences in the products of gene action that change with environmental effects. In addition, the chromosome number and karyological information are important for studying evolution patterns and are therefore used in the science of taxonomy in addition to morphological features [17].

The study of *C. imperialis* chromosome number was first conducted by Garcia-Jacas et al. (1998) [18]. They reported chromosome number of 2n= 2x= 18 for this species, and Ghaffari and Shahraki (2001) for the second time reported the chromosome number of 2n= 2x= 18 for *C. imperialis* species from the *Cynaroides*section [19].

C. charelii is a tetraploid species from the *Cynaroides* section whose chromosome number has been determined by Constantindis et al. (2002) as 2n= 4x= 36 and its chromosomal base number is x= 9. This is the only report of tetraploid in the *Cynaroides* section [20].

The first chromosome count for species of *C. kurdica*Reichardt, *C. aladaghensis*Wagenitz, and *C. cataonica*Boiss, which was conducted by Romaschenko et al. (2004) showed that the chromosome number of these species is 2n = 2x= 18 [21].

Hayta et al. (2014) counted the chromosome number of 2n= 2x= 18 for the *C. kurdica* species, which was consistent with previous chromosome counts for this species, and its haploid karyotype formula is 6m+ 2sm+ 1m [22].

Using chromosomal information it will be possible to compare species and their populations. Populations belonging to a species each show their own genomic adaptation to the environment in which they are grown. As adaptation differences increase, new varieties and even new species may emerge in plant communities. In determining the kinship relationships between species of a genus, only the number of chromosomes is not sufficient, but also traits such as size, morphology, diversity in coloration, centromere location, and chromosomal behavior must also be known [15].

Given the contents of this research, we are trying to answer the following questions:

1. Can chromosome numbers be helpful in solving taxonomic problems related to *Cynaroides* section?

2. What effect will the data interpretation of karyotype have on the taxonomic position of the *Cynaroides* section?

Materials and Methods

Required materials and equipment

Alpha Bromonaphthalene 0.01%, Glacial Acetic Acid, pure alcohol, 70% alcohol, normal Hydrochloric Acid, Aceto-orcein stain, 45% Acetic Acid, lam, lamel, disposable Petri dishes, filter paper, immersion oil, microscope.

Collection and preparation of herbarium samples

Initially, based on the reported addresses, we referred to the mentioned places during the spring and summer. In addition to collecting the samples from the desired areas, information

such as altitude, geographical coordinates, collection date, and some features of the sample was noted. The collected samples were pressed and dried and then, based on conventional methods, herbarium samples were prepared.

Staining and preparation of mitotic zones

To study the chromosomal number of the species in question, the mitotic zones of meristematic cells at the end of the root that have high dividing power should be studied. For this purpose, the seed samples collected during 2015-2017 were cytogenetically examined in the research laboratory of Shahid Chamran University (Table 1). To observe mitotic division, squashing of the meristematic tissue of the root end was used. At least 10 to 15 mitotic zones for each studied species were prepared. Then, for chromosomal studies, the prepared zones were examined using an Olympus CX31 microscope. To study the number of chromosomes in each array, chromosomal slides prepared in the metaphase stage of mitosis division were first observed using 10, 20, and 40 lenses, and the best metaphase cell was observed with a 100 microscope lens.

Table 1: Samples studied in the present study and their habitat characteristics

Collector	Location altitude (m)	Herbarium number	Habitat	Species name
Negaresh & Kamalnejad	1375	KHAU 150	Ravansar to Kamyaran; 1-3 km after Ravansar	*Centaureaalfonsoi*
Negaresh & Asadbeigi	1100-2000	KHAU 138	Kermanshah, Baneh to Sardasht; 50 km to Sardasht, near Abolhossein village	*C.imperialis*
Negaresh & Kamalnejad	1375	KHAU 118	Kermanshah; 3 km to Ravansar	*C. regia* var. *regia*
Negaresh & Asadbeigi	1250-1300	KHAU 162	Abdanan to Dinarkooh; 10-12 km to Dinarkooh	*C.iranshahrii*
Parishani	785	Herb.Scu 701	Lorestan; Poldokhtar to Khorram Abad, Akharineh	*C.iranshahrii*
Parishani	785	Herb.Scu 702	Lorestan; Poldokhtar to Khorram Abad, Akharineh	*C.behen*

Chromosome counting and karyotype preparation

After obtaining suitable metaphase zones, first, the number of chromosomes in the cells of the studied samples was counted, and karyotypic parameters such as total chromosome length (TL), long arm size (LA), short arm size (SA), long to short arm ratio, short to long arm ratio, and centromere coefficient index (CI), which represents the ratio of the short arm to total chromosome length, were calculated by ImageJ software in micrometers. Other karyotypic parameters such as total form percentage (%TF), relative length of the shortest chromosome (%S), the difference between the percentage of the relative length of the largest and smallest chromosomes (%DRL), the coefficient of asymmetry within chromosomes (A1) and between chromosomes (A2) were also calculated using the following formulas. A Double-sided Stebbins table has been used in this study in order to determine evolution status and study the karyotypic symmetry [13].

Relative length of the shortest chromosome: % S= q/p * 100

Intra-chromosomal asymmetric index: $A2=\frac{SD}{\bar{X}}$

General shape of the karyotype or total percentage of the form: $\%TF=\frac{\sum S}{\sum TL}$

Percentage of the relative length of the long arm: $\%ASK=\frac{\sum L}{\sum TL}$

Relative length range difference: %DRL= RLmax – Rlmin

The chromosomes of each karyotype were classified according to the Levan et al. (1964) method (Table 2) [17]. The type of chromosomes is determined based on the ratio of the length of the long arm to the short arm.

Arm ratio: Based on this parameter, the type of chromosomes is determined.

$AR=\frac{L}{S}$

r- value= $\frac{S}{L}$

Table 2: Naming the chromosomes by centromere location based on the Levan method

Chromosome type	Abbreviation	Arms ratio L/S	centromere position
Metacentric	M	1	Exactly the middle
	M	1.7	Middle area
Sub Metacentric	Sm	3	Middle half
Sub telocentric	St	7	End half
Acrocentric	T	7>	End area
Telocentric	T	Up to the infinite	Exactly the end

Using Stebbins et al. (1972) two-sided table, the symmetry of the species is determined. Based on this method the ratio of the smallest chromosome to the largest chromosome is calculated. The class type is then selected from the row corresponding to the arm ratio that is related to the study population [13].

Centromere index: CI=S/(L+S)
Average asymmetry index: A_i= (L–S)/(L+S)

Excel (2007) was used to draw the ideogram of the studied species and Photoshop software was used to draw the karyotype.

Results

In the present study, the chromosome number of 2n= 2x= 18 was obtained for eight studied species; and chromosome number 2n= 4x= 32 was obtained for *C. iranshahrii*; and chromosome number 2n= 4x= 34 was obtained for *C. behen*, among which 5 cases are new in Iran and in the world and have been obtained for the first time. Chromosome counting of Centaurea gabrieliae species from Ranjbarianea section was also examined in this study, which was not successful due to the non-germination of the achenes.

I- *Centaurea* sect. *Cynaroides*Boiss.
C.alfonsoi

This array is a diploid species and 2x= 2n= 18. The first report of chromosome number counting of this array has been done by Negaresh et al. in 2014 that our observations confirmed it. In Tables 3 and 4, karyotype indices for *C. alfonsoi* have been calculated.

Images related to the mitotic zone (Fig 1), Kariogram (Fig 2) and ideogram (Fig 3) of Karyotypic morphology were reported for *C. alfonsoi* Karyogram (Fig 2) and ideogram (Fig 3) of Karyotypic morphology were reported for the first time for *C. alfonsoi.*

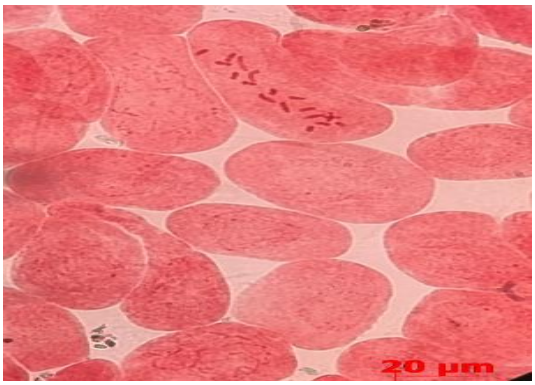

Figure 1: Miotic zone of c. alfonsoi

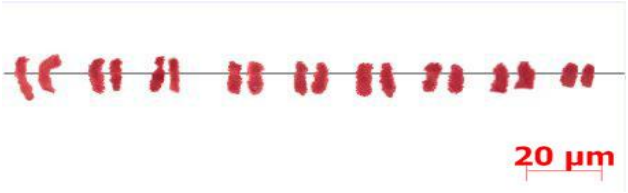

Figure 2: The karyogram drawn for *C. alfonsoi* species

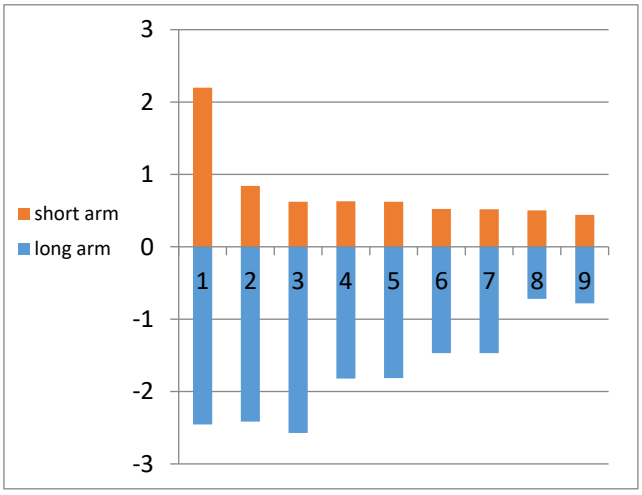

Figure 3: The ideogram drawn for *C. alfonsoi* species

Table 3: Examination of karyotypic traits including long arm length (LA), short arm length (SA), total chromosome length (TL), centromere index (CI), arm ratio (AR), r-value, percentage of relative length range difference (%RL), L/L + S, A_i (average asymmetry), for *C. alfonsoi*

	Long arm	Short arm	Total chromosome length	Centromere index	Arm ratio	r-value	%RL	L/L+S	A_i
1	2.455	2.199	4.654	0.472	1.11	0.895	%20	0.52	0.05
2	2.415	0.842	3.257	0.258	2.93	0.348	%14.53	0.74	0.48
3	2.573	0.622	3.195	0.194	4.13	0.241	%14.25	0.8	0.61
4	1.82	0.629	2.449	0.256	2.89	0.345	%10.92	0.74	0.48
5	1.813	0.622	2.435	0.255	2.91	0.343	%10.86	0.74	0.48
6	1.469	0.552	1.991	0.25	2.82	0.355	%8.88	0.73	0.47
7	1.469	0.518	1.987	0.26	2.83	0.352	%8.86	0.73	0.47
8	0.72	0.503	1.223	0.411	1.43	0.698	%5.45	0.58	0.17
9	0.781	0.44	1.221	0.36	1.43	0.563	%5.44	0.63	0.27

Table 4: Calculation of total form percentage (%TF), relative length of the shortest chromosome (%S), percentage of the relative length of the long arm (%ASK), intra-chromosomal symmetry index (A1), inter-chromosomal symmetry index (A2), karyotype formula (KF), Relative range difference (%DRL), Stebbins symmetry index (SA), total genome length (TL), for *C. alfonsoi*

	TL	SA	A_2	%DRL	FK	A_1	%ASK	%S	%TF
C.alfonsoi	43.23	2B	0.38	3.43	3m+5sm+1st	0.58	69.21	26.23	29.72

C.regia* var. *regia

This array was diploid and its chromosome number was 2n= 2x= 18. The distribution range of this array is southeastern Anatolia, Syria, Iran, and Iraq. The first chromosome count report for this array has been provided by Negaresh and Rahiminejad (2018), and this report is the second report for this array in the world [6]. The figures related to the mitotic zone (Fig 4), karyogram (Fig 5), and ideogram (Fig 6) of this species are on below In Tables 5 and 6, karyotypic indices for *C. regia* var *regia* species are calculated. Karyogram and ideogram of Karyotypic morphology were reported for the first time for *C. regia* var. *regia*.

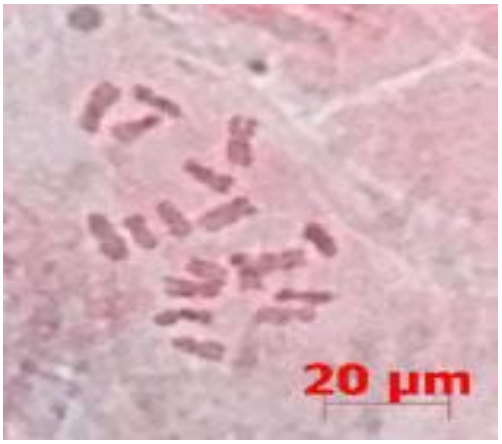

Figure 4: Miotic zone of c. regia var regia

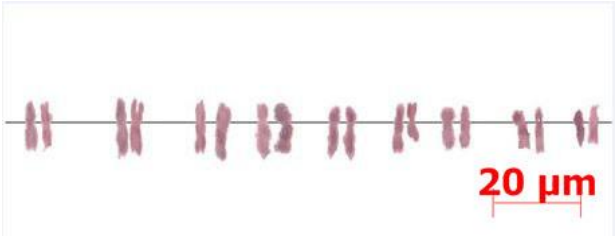

Figure 5: The karyogram drawn for *C. regia* var. *regia* species

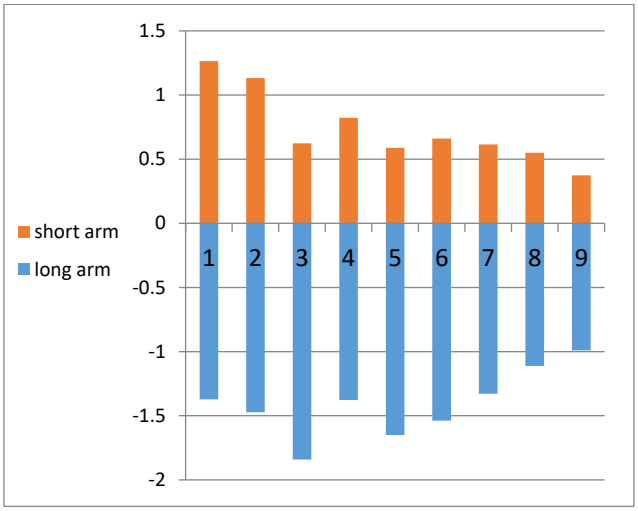

Figure 6: The idiogram drawn for *C.regia* var. *regia*

Table 5: Examination of karyotypic traits including long arm length (LA), short arm length (SA), total chromosome length (TL), centromere index (CI), arm ratio (AR), r-value, percentage of relative length range difference (%RL), L/L + S, A_i (average asymmetry), for *C. regia* var. *regia*

	Long arm	Short arm	Total chromosome length	Centromere index	Arm ratio	r-value	%RL	L/L+S	Ai
1	1.372	1.264	2.636	0.479	1.08	0.92	13.64	0.52	0.01
2	1.473	1.132	2.605	0.435	1.3	0.76	13.49	0.56	0.13
3	1.841	0.623	2.464	0.252	2.95	0.33	12.78	0.74	0.49
4	1.377	0.823	2.2	0.374	1.67	0.59	11.41	0.62	0.25
5	1.65	0.588	2.20	0.266	2.95	0.35	11.41	0.74	0.49
6	1.538	0.661	2.199	0.3	2.32	0.42	11.36	0.69	0.39
7	1.328	0.614	1.942	0.316	2.16	0.46	10.06	0.68	0.36
8	1.112	0.549	1.661	0.33	2.02	0.49	8.61	0.66	0.33
9	0.99	0.374	1.364	0.274	2.64	0.37	7.05	0.72	0.45

Table 6: Calculation of total form percentage (%TF), relative length of the shortest chromosome (%S), percentage of the relative length of the long arm (%ASK), intra-chromosomal symmetry index (A1), inter-chromosomal symmetry index (A2), karyotype formula (KF), Relative range difference (%DRL), Stebbins symmetry index (SA), total genome length (TL), for C.regiavar. regia

	TL	SA	A_2	%DRL	FK	A_1	%ASK	%S	%TF
C.regia var. regia	39.23	3A	0.15	1.27	2m+7sm	0.44	65.5	51.71	36.79

C. imperialis

This species is native to Iran and Iraq. This array is diploid and its chromosome number is 2x= 2n= 18, which is emphasized by previous reports for this species by Garcia-Jacas et al. (1998) and the report by Ghaffari and Shahraki (2001) [19]. Karyotypic indices for *C. imperialis* are calculated in Tables 7 and 8. The karyogram and ideogram of karyotypic morphology for *C. imperialis* were reported for the first time. Images of the mitotic zone (Fig 7), karyogram (Fig 8), and ideogram (Fig 9) of this species are on below.

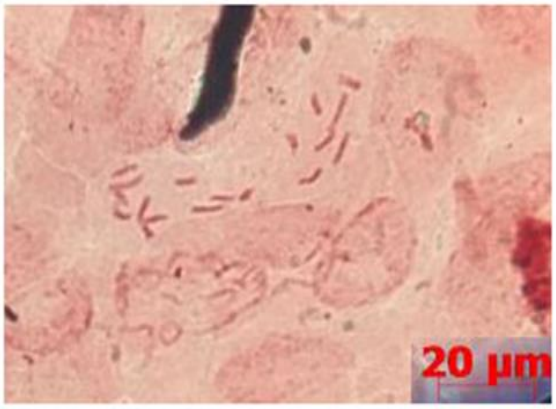

Figure 7: Miotic zone of c. imperialis

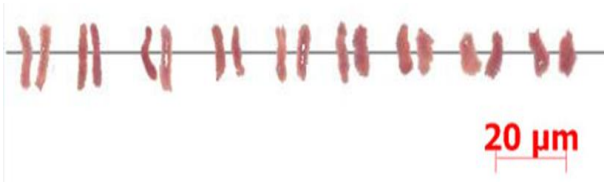

Figure 8: The karyogram drawn for *C. imperialis* species

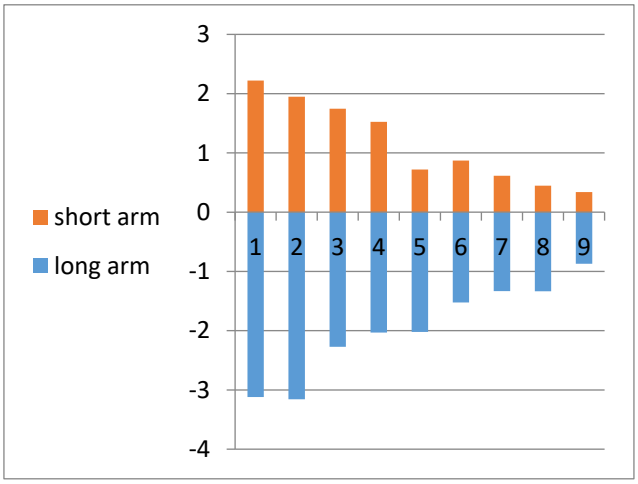

Figure 9: The ideogram drawn for *C. imperialis* species

Table 7: Examination of karyotypic traits including long arm length (LA), short arm length (SA), total chromosome length (TL), centromere index (CI), arm ratio (AR), r-value, percentage of relative length range difference (%RL), L/L + S, A_i (average asymmetry), for *C. imperialis*

	Long arm	Short arm	Total chromosome length	Centromere index	Arm ratio	r-value	%RL	L/L+S	A_i
1	3.12	2.221	5.341	0.415	1.4	0.711	19.01	0.58	0.16
2	3.158	1.948	5.106	0.381	1.62	0.616	18.17	0.61	0.23
3	2.272	1.745	4.017	0.433	1.3	0.768	14.29	0.56	0.13
4	2.032	1.524	3.556	0.428	1.33	0.75	12.65	0.57	0.14
5	2.021	0.72	2.741	0.262	2.8	0.356	9.75	0.73	0.47
6	1.542	0.87	2.394	0.27	1.74	0.57	8.52	0.63	0.27
7	1.333	0.615	1.947	0.315	2.16	0.461	6.93	0.68	0.36
8	1.336	0.446	1.782	0.25	2.99	0.333	6.34	0.74	0.49
9	0.871	0.339	1.21	0.445	1.8	0.389	4.3	0.71	0.43

Table 8: Calculation of total form percentage (%TF), relative length of the shortest chromosome (%S), percentage of the relative length of the long arm (%ASK), intra-chromosomal symmetry index (A1), inter-chromosomal symmetry index (A2), karyotype formula (KF), Relative range difference (%DRL), Stebbins symmetry index (SA), total genome length (TL), for C. imperialis

	TL	SA	A_2	%DRL	FK	A_1	%ASK	%S	%TF
C. imperialis	55.78	2A	0.41	4.13	4m+5sm	0.39	61.9	28.27	38.34

C. iranshahrii

This array is tetraploid and its chromosome number has been determined as 2n= 4x= 32. This array is tetraploid and its base chromosome number is x= 8. Given the chromosome counting list provided by Watanabe for the Sunflower family in 2008, this is the first chromosome number reported for this taxon [23]. Karyotypic indices for *C. iranshahrii* have been calculated in Tables 9 and 10. Images of the mitotic zone (Fig 10). The karyogram (Fig 11), the ideogram (Fig 12) of karyotypic morphology were reported for *C. iranshahrii* for the first time

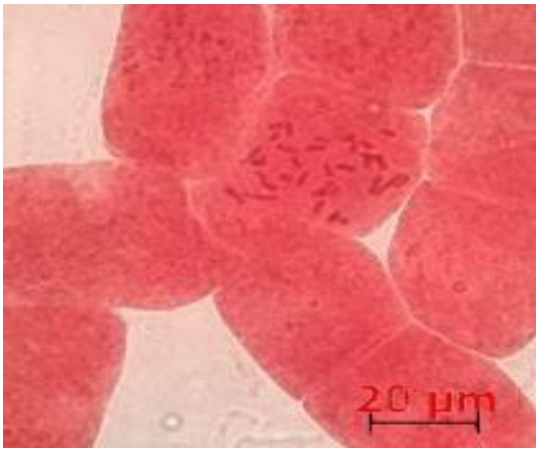

Figure 10: Miotic zone of c. iranshahrii

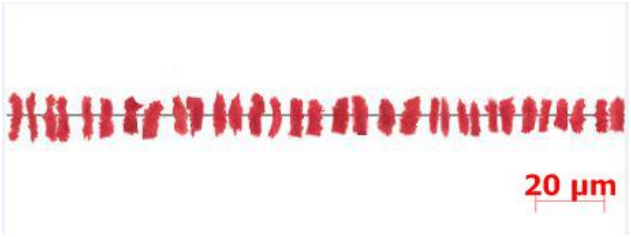

Figure 11: The karyogram drawn for *C. iranshahrii* species

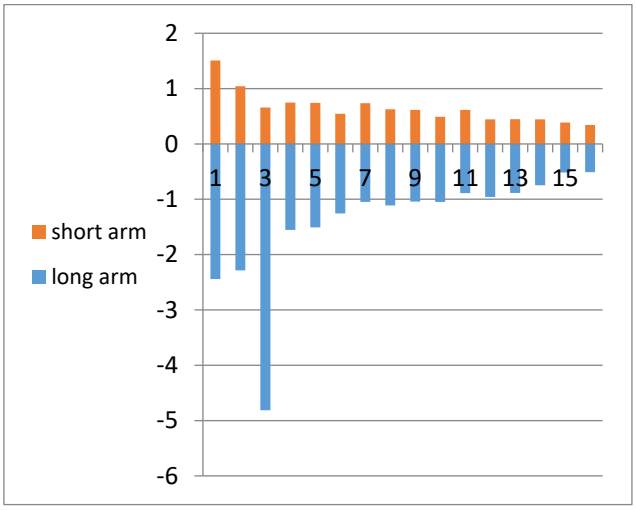

Figure 12: The ideogram drawn for *C. iranshahrii* species

Table 9: Examination of karyotypic traits including long arm length (LA), short arm length (SA), total chromosome length (TL), centromere index (CI), arm ratio (AR), r-value, percentage of relative length range difference (%RL), L/L + S, A_i (average asymmetry), for *C. iranshahrii*

	Long arm	Short arm	Total chromosome length	Centromere index	Arm ratio	r-value	%RL	L/L+S	A_i
1	2.442	1.508	3.95	0.381	1.61	0.617	13.22	0.61	0.23
2	2.285	1.042	3.327	0.313	1.63	0.456	11.14	0.68	0.37
3	4.813	0.657	2.47	0.265	2.75	0.362	8.27	0.73	0.46
4	1.554	0.747	2.301	0.324	2.08	0.48	7.73	0.67	0.35
5	1.508	0.742	2.132	0.329	2.03	0.492	7.13	0.7	0.35
6	1.256	0.545	1.801	0.302	2.3	0.433	6.03	0.69	0.39
7	1.049	0.737	1.786	0.412	1.42	0.702	5.98	0.58	0.17
8	1.112	0.626	1.738	0.36	1.77	0.562	5.82	0.63	0.27
9	1.042	0.615	1.657	0.371	1.69	0.59	5.54	0.62	0.25
10	1.049	0.491	1.54	0.318	2.13	0.468	5.15	0.68	0.36
11	0.86	0.615	1.475	0.416	1.39	0.715	4.93	0.58	0.16

12	0.959	0.443	1.402	0.315	2.16	0.461	4.69	0.68	0.36
13	0.886	0.447	1.333	0.335	1.98	0.504	4.46	0.66	0.33
14	0.747	0.443	1.19	0.372	1.68	0.593	3.98	0.62	0.25
15	0.517	0.386	0.903	0.427	1.33	0.746	3.02	0.57	0.14
16	0.51	0.342	0.852	0.401	1.49	0.67	2.85	0.59	0.19

Table 10: Calculation of total form percentage (%TF), relative length of the shortest chromosome (%S), percentage of relative length of the long arm (%ASK), intra-chromosomal symmetry index (A1), inter-chromosomal symmetry index (A2), karyotype formula (KF), Relative range difference (%DRL), Stebbins symmetry index (SA), total genome length (TL), for *C. iranshahrii*

	TL	SA	A_2	%DRL	FK	A_1	%ASK	%S	%TF
C. imperialis	59.52	2A	0.4	3.09	8m+8sm	0.4	62.44	21.56	37.49

***Centaurea*sect. *microluphus*(Cass.) DC.**

C. behen

According to the present study, this array is tetraploid and its chromosome number is equal to 2n= 4x= 34. According to available sources, the first report of chromosome counting of this array was provided by Ghaffari and Shahraki (2001) and the second report by Romaschenko et al. (2004), and therefore, the present report is the third report for this array that confirms the previous results [19, 21]. Images of the mitotic zone (Fig 13), karyogram (Fig 14), and ideogram (Fig 15) of this species are on In Tables 11 and 12, the karyogram and ideogram of karyotypic morphology were reported for *C. behen* for the first time.

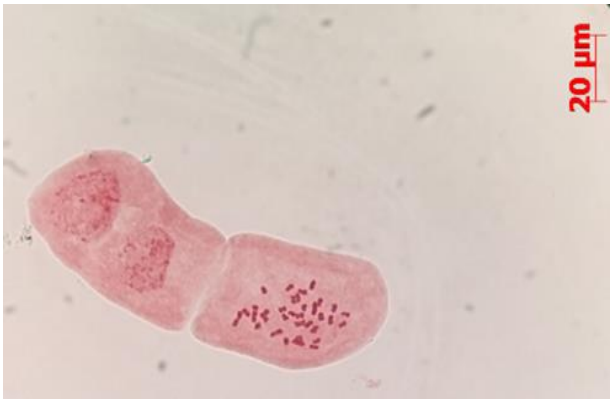

Figure 13: Miotic zone of c. behen

Figure 14: The karyogram drawn for *C. behen* species

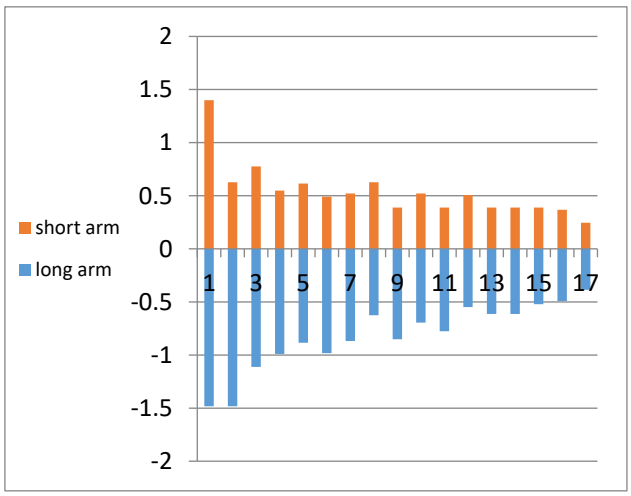

Figure 15: The ideogram drawn for *C. behen* species

Table 11: Examination of karyotypic traits including long arm length (LA), short arm length (SA), total chromosome length (TL), centromere index (CI), arm ratio (AR), r-value, percentage of relative length range difference (%RL), L/L + S, Ai (average asymmetry), for C. behen

	Long arm	Short arm	Total chromosome length	Centromere index	Arm ratio	r-value	%RL	L/L+S	A_i
1	1.483	1.4	2.883	0.485	1.05	0.94	12.47	0.52	0.16
2	1.483	0.626	2.109	0.296	2.36	0.42	9.12	0.7	0.4
3	1.112	0.776	1.888	0.411	1.43	0.69	8.16	0.58	0.17
4	0.99	0.549	1.539	0.356	1.8	0.55	6.65	0.64	0.28

5	0.885	0.614	1.499	0.409	1.44	0.69	6.48	0.66	0.33
6	0.982	0.491	1.473	0.333	2.53	0.5	6.37	0.59	0.18
7	0.868	0.521	1.389	0.375	1.66	0.6	6.01	0.62	0.24
8	0.626	0.626	1.252	0.5	1.05	1	5.41	0.5	0
9	0.852	0.389	1.241	0.313	2.19	0.45	5.36	0.68	0.37
10	0.694	0.521	1.215	0.428	1.33	0.75	5.25	0.57	0.14
11	0.776	0.388	1.164	0.333	2	0.5	5.03	0.66	0.33
12	0.549	0.506	1.055	0.479	1.08	0.92	4.56	0.52	0.04
13	0.614	0.388	1.002	0.387	1.58	0.63	4.33	0.61	0.22
14	0.614	0.388	1.002	0.387	1.58	0.63	4.33	0.61	0.22
15	0.521	0.388	0.909	0.426	1.34	0.74	3.93	0.57	0.14
16	0.491	0.368	0.859	0.428	1.33	0.74	3.71	0.57	0.14
17	0.386	0.246	0.632	0.389	1.56	0.63	2.73	0.61	0.22

Table 12: Calculation of total form percentage (%TF), relative length of the shortest chromosome (%S), percentage of relative length of the long arm (%ASK), intra-chromosomal symmetry index (A1), inter-chromosomal symmetry index (A2), karyotype formula (KF), Relative range difference (%DRL), Stebbins symmetry index (SA), total genome length (TL), for *C. behen*

	TL	SA	A_2	%DRL	FK	A_1	%ASK	%S	%TF
C. imperialis	43.57	2A	0.35	2.25	13m+ 4sm	0.35	59.09	21.92	38.23

Discussion and Conclusion

According to the monograph by Negaresh and Rahiminejad (2018), given the morphological characteristics of *C. iranshahrii*, it belongs to the *Cynaroides* section, but in the present study, the base chromosome number for this species is x= 8, and since the base chromosome number in the *Cynaroides* section is x= 9, this species should probably be isolated from this section and transferred to the *Microluphus* section, which has the base chromosome number of x= 8 [6]. In this regard, molecular studies should also be performed to prove that this species should be isolated from *Cynaroides* section.

In addition, in this study, similarities were observed in karyotypic studies of *C. iranshahrii* and *C. behen* (in terms of %TF and %S). In terms of %TF or percentage of total karyotype form, *C. imperialis* species with the highest amount has more symmetrical karyotype, and *C. alfonsoi* species with the lowest amount has more asymmetric karyotype. In terms of A1, *C.behen* species shows the lowest value, that according to this criterion and its karyotype, it is more symmetrical; and C. alfonsoi species has the highest amount that as a result, this species is shown to be more asymmetric. In terms of A2, *C. imperialis*with the highest amount is considered to be more asymmetric. The karyotypic formula of *C. iranshahrii* species is 8m+ 8sm, *C.behen* species is 13m+ 4m, *C. imperialis* is 4m+ 5sm, *C. regia* var. *regia* is 2m+ 7sm, and *C. alfonsoi* species is 3m+ 5sm+ 1st. The karyotypic formula of all species except *C. alfonsoi* is a combination of metacentric and sub-metacentric chromosomes. Therefore, it can be said that the *C. alfonsoi* species (due to the presence of a sub-telocentric chromosome pair) is more asymmetric than other species. The lowest %S is in *C. iranshahrii* species (21.56). The highest length of the long arm (3.12 microns) was observed in *C. imperialis* species and the lowest length of the long arm was observed in *C. behen* species (0.386 microns). Also, the highest length of the short arm was obtained for *C. imperialis* species (2.221 microns) and the lowest length was obtained in *C.behen* species (0.246 microns). The highest amount of %ASK was calculated in *C. alfonsoi* species (69.21) and the lowest amount in *C. behen* species (59.09). There was not much difference between *Cynaroides* section species in terms of %ASK parameter.

As mentioned, chromosome length and centromere position are two important factors in measuring chromosomes. Regarding the *C. iranshahrii* species which is a tetraploid species and is very similar to *C. behen* species, the difference between these two species is in the larger size of *C. iranshahrii* chromosomes than the *C. behen*. These species are tetraploid and differ from their diploid relatives in many respects caused by the high number of chromosomes. The cells of tetraploid species are larger than those of diploid species. Although the size of different limbs of the species does not increase with increasing the number of chromosomes, another polyploidy effect is on growth rate. Polyploid species usually have slower growth rates than their diploid ancestors, such as slower flowering rates. Studies show that polyploidy that occurs in nature is highly correlated with hybridization, whether between different species or different subspecies or among the offspring of similar species. In the *Centaurea* genus, there is significant hybridization between different species, that the reason for

the formation of tetraploid species of this genus can be the high amount of hybridization. There is still a lot of controversy about *C. behen* species because, given the chromosome number of this species, it is suggested that *C. behen* species is the result of hybridization between two species with a base chromosome number of x= 8 and x= 9.

In terms of the total genome content, the species existing in Kermanshah province are more similar to each other compared to *C. iranshahrii*and *C. imperialis* species that have a more different distribution range (in terms of total genome length and distribution range).

There are also morphological similarities between the studied species, for example,*C. imperialis*is very similar to *C. regia*var. regia in terms of the shape of the involucrate, the texture and the position of the appendages, and the number of laces. However, the difference of *C. regia*var. regia is due to the density of long articulated grayish hairs on the stem.

The *C. alfonsoi* species has the karyotypic formula of 3m+ 5sm+ 1st. This species has a pair of sub-telocentric chromosomes and considering that Negaresh has introduced this species as a new species in 2014, so it can be said that this species is newer and more advanced compared to other species in this section. In the monograph by Negaresh and Rahiminejad (2018) the *C. imperialis* species is morphologically similar to *C. regia* var. *regia*species. According to the present study, there is probably a greater affinity between the two *C. imperialis* and *C. kabirkuhensis* species. Two important distribution areas of *Cynaroides* section are Turkey and Iran. Since Turkey is closer to the Mediterranean region, so the origin of this section is probably in Turkey, and since the number of species of this sector is higher in Iran, Iran is probably the place of species formation and diversification of this section. Morphological studies by Negaresh and Rahiminejad and a series of observations show some hybrid characteristics between the species. In addition, despite the great diversity in morphological and pollination characteristics, they show no difference in chromosome number. The base chromosome number in the *Cynaroides* section suggests the possibility of a widespread evolution event by hemoploidy. Thisevidence suggests that hemoploidy along with pollination may have played an important role in species formation in this section. Thus, hemoploidyhas been found in the center of diversity in western Iran while tetraploidy has occurred in remote areas (Greece). Finally, confirming the previous suggestions, hemoploidy in the *Cynaroides*section is consistent with the evolution suggested by morphological studies and nuclear DNA sequencing. In this study, it was found that the base chromosome number in the species of *Cynaroides*section, except *C. iranshahrii*, is x= 9, which confirms previous studies and indicates occurrence of transformation through hemoploidy among the species of this section.

According to the obtained results, in order for further investigation in this field, it is suggested to future researchers to conduct molecular, pollen, and seed studies on *Cynarides*section species, and it is also suggested to examine the environmental factors affecting the growth of the studied species as well as the species existing in this section.

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