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CYTOTAXONOMIC STUDY OF SOME SPECIES OF SECTION *CYNAROIDES* BOISS. OF THE GENUS *CENTAUREA* L. (ASTERACEAE) IN IRAN

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Abstract

The genus Centaurea is one of the largest genera of the Asteraceae family, having 400 to 700 species in the world and having high taxonomic complexity. The aim of this study was to determine the chromosome number of 5 species of the section Cynaroides as the largest section of genus Centaurea in Iran. For this purpose, after germination of the seeds, we separated the terminal part of the root and prepared mitotic zone after coloring through crushing. In this study, it was found that thechromosomalbase number in the species of section Cynaroides is x = 9; it confirms the previous studies and suggests that hemoploidy transformation is occurring among the species of this section. Chromosomal count of the species of *C. kabirkuhensis, C. rahiminejadii, C. ravansarensis, C. shahuensis, C. regia* var. cynarocephala was performed for the first time. In addition, we determed karyological traits for all taxa under study for the first time.
Keywords: Asteraceae, section *Cynaroides*, karyotype, chromosomal count, sinaroid.

Introduction

Asteraceae is the largest strain of flowering plants, with over 1600 genera, including about 23,000 species, regardless of apomictic species. According to the latest classification by Jeffrey in 2007, the Asteraceae family includes 24 tribes.

Recent taxonomic studies on this genus have revealed that the number of its species in Iran has increased to 106 species belonging to 31 sections (Negaresh and Rahiminejad, 2015). *Centaurea* is the most complex genus of Asteraceae family and is an abnormal taxon**.**

There are many plants family in the world that are affected by many factors. (Alla Sharafi *et al*., 2019) The sunflower family is the most abundant species among vascular plants (Funk, 2009). Because of the size of this family and its importance, several taxa of this family have been studied cytogenetically and this family probably has the highest number of chromosomes among flowering plants. The haploid chromosome number in the chicory family ranges from 2 to 120, and contains many polyploid species, some of which are very rare. Among species with a determined chromosomal number, species with haploid numbers of x = 9, 10,12,17 and18 constitutes 50% of the total family. The most commonly observed haploid chromosome number is x = 9, probably the basic chromosome number in this family. Ancestral haploid chromosome number in this family is proposed also to be x = 9. Variation in chromosome number has led to processes by Polyploidy (hybrids derived from two diploid plants with anomalous genomes). It includesamphiploidy (Polyploidy plant that behaves like a diploid plant), Aneuploidy changes (a kind of polyploidy in whichthe differences in chromosome number are not in the multiplication of the original set but in one or more chromosomes) and the loss of the chromosome. Polyploidy is found in species with relatively humid habitats, herbaceous perennials and shrub species that occupy relatively suitable habitats (Jeffrey, 2007).

Prior to the present research, only chromosomal reports of species of the section *Cynaroides* in Iran was related to *C. imperialis* Hausskn. exBornm and *C. alfonsoi* (Ghaffari and Shahraki, 2002, Negaresh *et al.*, 2014). Given the introduction of new species or the exclusion of some species of this section in recent years and that most of the species in this section are exclusive to Iran, cytological studies on this section can be beneficial for many species. They can lead to the reporting of new chromosome numbers for the first time and provide valuable information for solving taxonomic problems in this section.

Centaurea

Centaurea is a taxonomically complex genus because it contains many species that show a high degree of morphological diversity. Hybridization is also seen in this genus due to the absence of reproductive barriers (Susanna and Garcia-Jacas, 2009; Negaresh and Rahiminejad, 2018). To address this systematic problem, further studies are needed on this genus, including karyological, morphological, cytogenetic, and molecular studies (Valles *et al*., 2005).

One-, two-year-old plants or perennials without thorns are rarely thorny. Heterogamous capitol, wrinkled fillaries, rarely leaf-like, with appendages, thorny or without thorns are rarely without appendages. Flowers are blue, pink, yellow or rarely white. Infertile flowers are usually radial. The achenes are rectangular, elongated and rarely conical. The color of the achenes is brownish-black, smooth and rarely serrated. Papus is doubly (Susanna and Garcia-Jacas, 2009).

The genus Centaurea is in its broadest sense the largest genus of subfamily Centaureinae, with 400 to 700 species in the world. Wagnitz in Flora Iranica considered 89 species and 28 parts of this genus, of which 70 species are dispersed in Iran and 22 species are endemic. According to recent taxonomic studies on this genus, it was found that the number of its species for Iran has increased to 106 species belonging to 31 sections. Centuarea is the most complex genus of the family Asteraceae and is a polyphyletic taxon; but in recent years, a system has been adopted to divide it into four genera: Centaurea s. str, Psephellus Cass., Cyanus Mill and Rhaponticoides Vaill (Negaresh and Rahiminejad, 2015).

Features of section Cynaroides

This section has 37 species worldwide. Iran has 19 species, 3 subspecies and 4 varieties of this section, mainly found in the Zagros Mountains, from southwest to northwest and central parts. Having 16 exclusive species, and 10 species, 1 subspecies and 2 varieties in Kermanshah province and the Shaho Mountains are considered as major centers for the *Cynaroides* section. Turkey has 18 species, 3 subspecies and 2 varieties. Most species in Turkey grow in the southern part of the country. A number of species are grown in the Amanus Mountains on the border between Turkey and Syria and are exclusive to the region (Negaresh and Rahiminejad, 2018).

Two-year-old plants are of thick straight roots, sometimes turnip-rooted, plant height 20–250 cm, with right stem, sometimes branched above, rarely branching from base to top, lower part of stem without crack or withscattered long-articulatedown, upper part with felt-like or small downs. The leaves are slender, hard, when drying are paper-like, battle-axe-like or heart-shaped, or in the form of a violin, sometimes oval, rectangular or lanceolate. The basal leaves are generally very large, sometimes hook-like, sometimes withering at flowering.The upper and middle stalks are often continuous; the upper leaves are increasingly smaller and covered with long-articulate downs.The capitols are small to numerous, large to very large, sometimes medium in size, often arranged in cluster inflorescences, sometimes arranged in crown inflorescences or double-sided cyme without a peduncle, with long peduncle, oval-shaped collar, egg-shaped spherical, hard appendages, sometimes Paper-like, triangular to oval, oval to circular, often non-extended, often with eyelashes that eventually become thorn or small thorn.

Indices of Karyotype measurement and comparison

Nowadays, we can obtain a number of data and information through chromosomal studies, chromosome number, karyotype structure and chromosome staining (Graphodatsky *et al.*, 2011; Hany *et al*., 2019). One of the most important and widely used cases of evolutionary theory, especially for botanists, is the problem of karyotype asymmetry. The standard karyotype is the relative length and arm rate (long arm / short arm ratio) when the chromosomes are easily coupled (Stebbins, 1971). Karyotypes are designed for cross-species comparison when there is little variation between genotypes. However, these measurements are difficult to use when the chromosomes do not mate or to compare relative taxa that have different karyotypes.

Comparing karyotypes, both chromosome length and centromere position are examined. Thus, each chromosome presents two concepts by a chain of observations. If the chromosomes within a genotype are very similar, the compaction of errors may cause problems in determining the type of chromosomes. Specifically, the introduction of these two concepts allows simultaneously some of these ambiguities to be resolved and makes it easier to identify chromosome pairs. Comparison of species' chromosomes can be related to the presence and number of small, medium and large chromosomes, so the species under study may include similar and dissimilar chromosomes. Comparison of longitudinal relationship between species shows that the total content of DNA of members of a taxonomic group is similar, like one genus (Stebbins, 1971). When the chromosomes are enough large and recognizable, different species and even genotype within species can produce different karyotypes (Qusti *et al*., 2019). These methods provide a wide range of common methods (Levan *et al.*, 1964; Stebbins, 1971). for presenting this data and comparing chromosomes between taxa.

In order to confirm the similarity of the genotypes, more and more detailed botanical and molecular tests are needed to verify the validity of this subject. The mechanism of reduction in the number of chromosomes has been identified with greater confidence than reduction in chromosome size; it has been shown in numerous instances that it results in asymmetric karyotypes with varying sizes, which is a result of the evolution of the species under study (Romaschenko *et al.*, 2004).

Cytological studies

Chromosomal information and karyotypic information are very important in examining different populations. Cytogenetic studies of plant species and their associated populations, especially wild and exclusive plants, are of great importance for providing quantitative information on plant evolutionary history, determining interspecific affinities, determining karyological characteristics, and so on (Hesamzadeh, Hejazi and ZiaeiNasab, 2007).

Numerous cytogenetic studies have been performed in different sections of the genus Centaurea in different parts of the world including Iran. We refer in the following to some of these studies and their results summarized in Tables 1, 2.

Negaresh et al. introduced in 2014 the species *C. alfonsoi* for Iranian flora and reported its chromosome number being 2n = 2x = 18 (Negaresh et al., 2014).

Negaresh and Rahiminejad (2018) reported a chromosome number for the species *C.regia* var *regia*, the chromosome number of 2n = 2x = 18.

Hayta *et al.* (2014) counted the chromosomal number 2n = 2x = 18 for the species *C. kurdica*, which corresponded to previous chromosomal counts for this species and its haploid karyotype formula is 6m + 2sm + 1m.

Aksoy *et al.* (2016) determined the chromosome number of 12species of the genus *Centaurea* containing 6 species of section Cynaroides. They counted for the first time the chromosome number of *C. zafferii* Negaresh, *C. haussknechtii* Boiss., *C. gigantea* Sch. Bip. ex. It was 2n = 2x = 18. Also the karyotype formula of *C. haussknechtiis* equal to 4 sm + 5m, that of *C. zafferii* 2m + 7sm and that of *C. gigantea* is 2m + 7sm.

A study of the chromosome number of five species of the genus *Centaurea* belonging to five different sections of this genus by Kocygit *et al.* (2013) showed that the chromosome number of species *C. arifolia* Boiss. of the section *Cynaroides* is 2n = 2x = 18. So its base chromosomal number corresponds to the base chromosome number assigned to the species whose chromosome number has already been determined.

Materials and Methods

Collection and preparation of herbarium samples

The first step in this research is field operations and collection of plant samples. So, based on reported addresses, these sites were referred to during the spring and summer seasons. We pressed and dried the collected samples and

thenprepared the herbarium samples according to conventional methods**.**

Staining and preparation of mitotic zones

To study the chromosome number of the species in question, we need to study the mitotic zones of root apical meristematic cells that are of high division ability. For this purpose, we studied cytogenetically the seed samples collected during 2015-17 at the research laboratory of Shahid Chamran University (Table 1). For observation of mitosis division, we used squashing method of root apical meristematic tissue.

To study the number of chromosomes in each array, we first visualized the chromosomal slides prepared in the metaphase phase of mitosis using 10, 20 and 40 lenses; we observed the best metaphase cells with 100 microscope lenses.

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

Name of species	Habitat	Herbarium number	Site height (meter)	Collector
C. regia var. *cynarocephala*	Kermanshah, three kilometers to Ravansar	KHAU 113	1375 meters	Negaresh and Kamalnejad
C. kabirkuensis	Ilam, Abdanan, towards Dinarkuh, 10-12 kilometers toDinarkuh	KHAU 132	1250-1300 meters	
C. shahuensis	Kermanshah, Ravansar towardsJavanrood, 5 km after Ravansar	KHAU 174	1415 meters	Negaresh and Kamalnejad
C. ravansarensis	Kermanshah, Ravansar, 2 km from Ravansar towards Kamyaran	KHAU 174	1345 meters	Negaresh and Kamalnejad
C. rahiminejadii	Kermanshah, Ravansar towardsKamyaran, 1-3 km after Ravansar	KHAU 28689	1375 meters	Negaresh and Kamalnejad

Chromosome counting and karyotype preparation

After obtaining appropriate metaphase zones, the number of chromosomes was first counted in the cells of the samples under study. Karyotypic parameters such as total length of chromosome (TL), long arm size (LA), short arm size (SA), long to short arm ratio, ratio of short arm to long arm and the index of centromere coefficient (CI) which represents the ratio of short arm to the total length of chromosome, was calculated by Image J software in micrometer units. Other karyotypic parameters such as percentage of total form (% TF), relative length of shortest chromosome (% S), percentage difference of relative length of the largest and smallest chromosomes (% DRL), asymmetric intra-chromosomal (A1) and inter-chromosomal (A2) coefficient were calculated through the following formulas. For determining the evolution and studying karyotype symmetry, we used two-way table of Stebbins (Stebbins, 1971).

Excel (2007) was used for drawing the ideogram of species under study and Photoshop software was used for drawing the karyotype.

Results and Discussion

C. regiavar* var. *cynarocephala

It is a diploid array with a chromosome number of 2n=2x=18. This is the first report of the chromosome number for this taxon, according to a list of chromosome counting provided by Mr. Watanabe for the sunflower family in 2008. Table 2 and Table 3 show the calculation of karyotypic indices for *C. regia* var. cynarocephala. Karyogram and ideogram of the karyotypic morphologywere for the first time reported for *C. regia* var. *cynarocephala*

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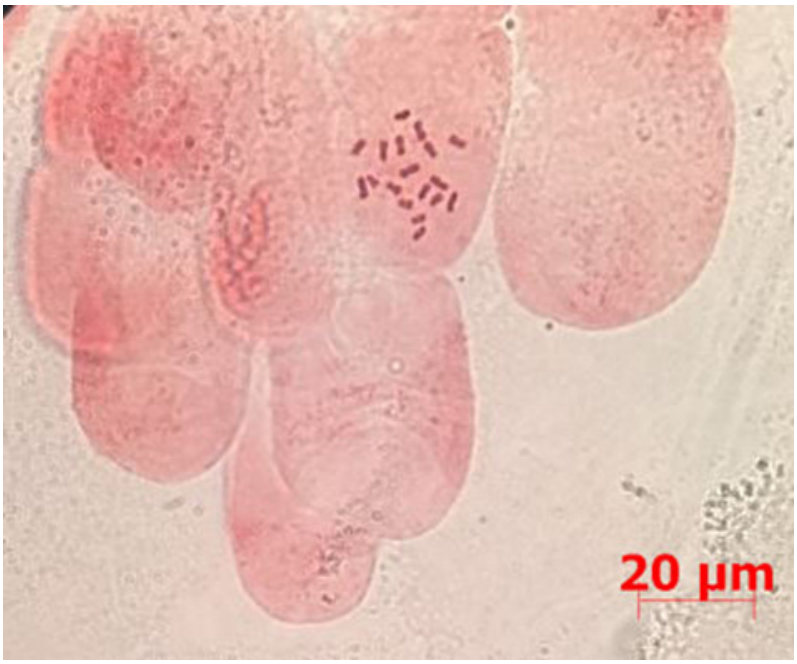


Fig. 1: Mitotic zone of *C. regia* var. *cynarocephala.*

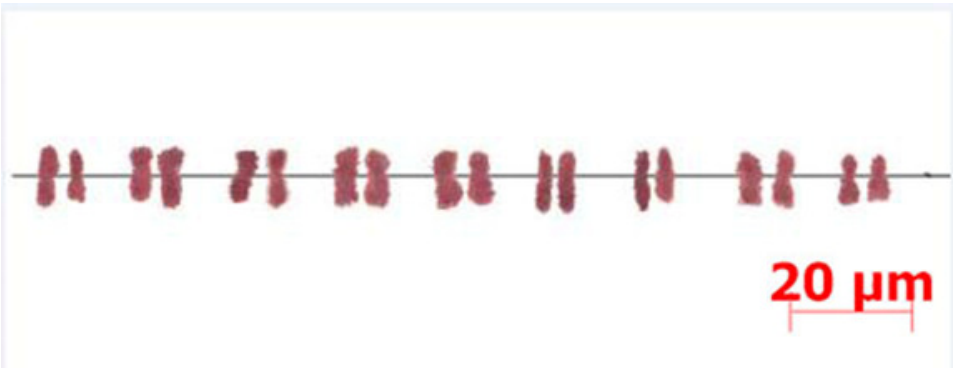


Fig. 2: Karyogram drawn for *C. regia* var. *cynarocephala.*

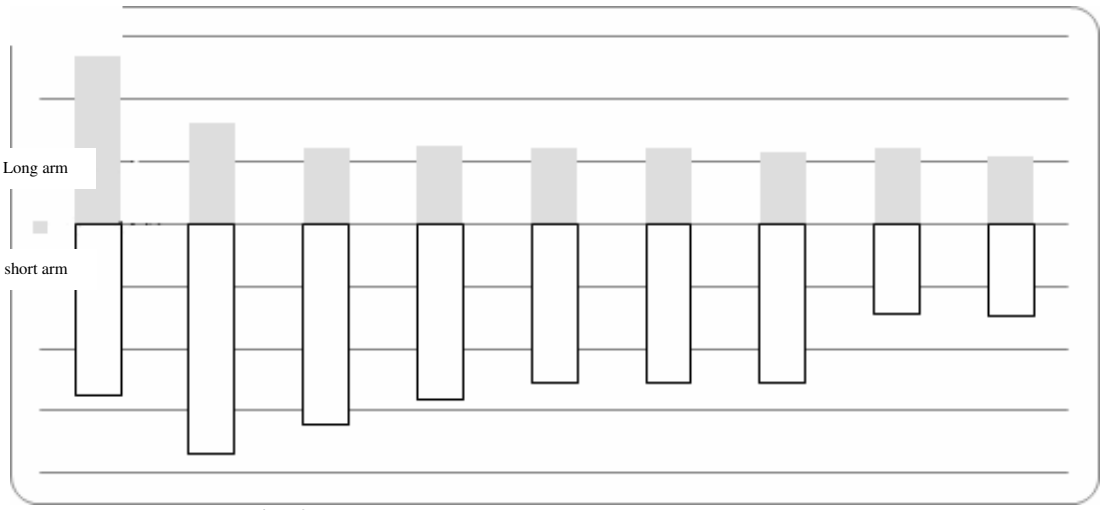


Fig. 3: Ideogram drawn for *C.regia* var. *cynarocephala.*

Table 2: Karyotypic traits including arm length (LA), short arm length (SA), total length of chromosome (TL), centromeric index (CI), arm rate (AR), r-value, percentage of relative length domain difference (%) RL), L / L + S, A_i (mean asymmetry), for *C. regiavarcynarocephala.*

	Long length	**Short length**	**Total length of chromosome**	**Centrumeric index**	**Arm rate**	**r- value**	**%RL**	**L/L+S**	**A_i**
1	1.379	1.338	2.717	0.492	1.03	0.97	15.24	0.5	0.5
2	1.84	0.802	2.642	0.306	2.29	0.435	14.81	0.69	0.69
3	1.605	0.605	2.21	0.273	2.65	0.376	12.39	0.72	0.72
4	1.406	0.617	2.023	0.304	2.27	0.438	11.34	0.69	0.69
5	1.28	0.611	1.891	0.323	2.09	0.477	10.6	0.67	0.67
6	1.281	0.603	1.884	0.32	2.12	0.47	10.56	0.67	0.67
7	1.28	0.582	1.862	0.312	2.19	0.454	10.44	0.68	0.68
8	0.732	0.535	1.332	0.461	1.19	0.857	7.47	0.53	0.53
9	0.717	0.615	1.267	0.422	1.34	0.73	7.1	0.57	0.57

Table 3: Calculating the percentage of total form (%TF), relative length of the shortest chromosome (%S), percentage of long arm relative length (%ASK), intra-chromosomal symmetry index (A1), inter-chromosomal symmetry index (A2), karyotype formula (KF), relative domain difference (%DRL), Stibbins symmetry index (SA), total length of genome (TL), for *C. regia* var. *cynarocephala.*

	%TF	%S	%ASK	A_1	FK	%DRL	A_2	SA	TL
*C.regia*var. *cynarocephala*	**36.33**	**46.63**	**65.48**	**0.45**	3m+6sm	**1.45**	**0.24**	**2**A	36.01

C. kabirkuhensis

It is a diploid array with a chromosome number of 2n = 2x = 18. This is the first report of a chromosome number for this taxon, according to the 2008 chromosomal counting list provided by Mr. Watanabe for the sunflower family. Images of mitotic zone, karyogram and ideogram are shown on page 74. In Table 4 and Table 5, Karyotypic indices were calculated for *C. kabikuhensis*. Karyogram, ideogram and karyotypic morphology were first reported for *C. kabirkuhensis*.

Fig. 4: Mitotic zone of *C. kabirkuhensis.*

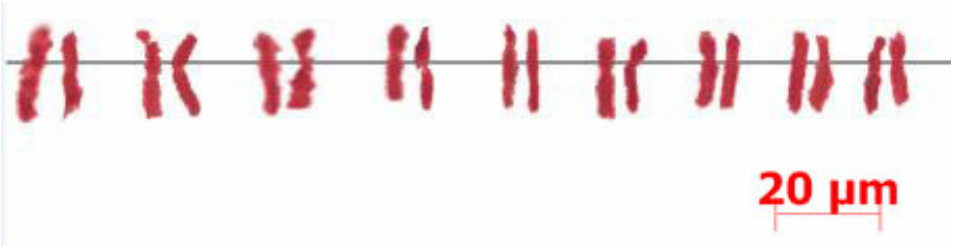


Fig. 5: Karyogram drawn for *C. kabirkuhensis.*

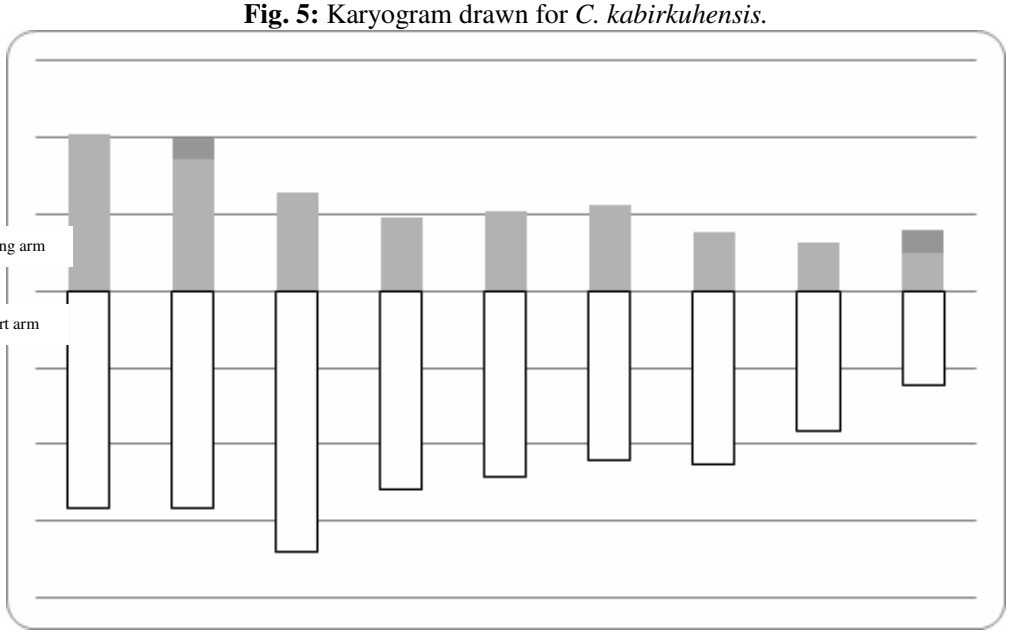


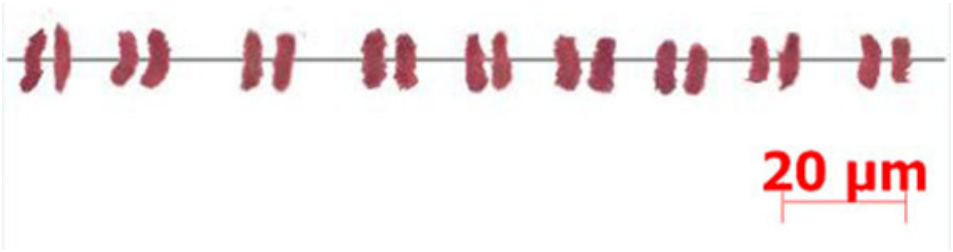
Fig. 6: Ideogram plotted for *C. kabirkuhensis.*

Table 4: Karyotypic traits including arm length (LA), short arm length (SA), total length of chromosome (TL), centromeric index (CI), arm rate (AR), r-value, percentage of relative length domain difference (%) RL), L/L + S, A_i (mean asymmetry), for *C. kabirkuhensis.*

	Long length	Short length	Total length of chromosome	Centrumeric index	Arm rate	r- value	%RL	L/L+S	A_i
1	2.829	2.039	4.868	0.418	1.38	0.72	15.25	0.58	0.16
2	2.829	1.729+0.248	4.806	0.412	1.23	0.698	15.04	0.58	0.17
3	3.407	1.275	4.682	0.272	2.67	0.37	15.01	0.72	0.45
4	2.596	0.954	3.55	0.268	2.72	0.367	11.12	0.73	0.45
5	2.424	1.051	3.475	0.302	2.3	0.433	10.87	0.69	0.39
6	2.202	1.12	3.322	0.337	1.96	0.508	10.41	0.66	0.32
7	2.27	0.765	3.035	0.252	2.96	0.337	9.51	0.74	0.49
8	1.823	0.623	2.446	0.254	2.92	0.341	7.64	0.74	0.49
9	1.22	0.492+0.288	2	0.44	1.38	0.639	6.26	0.61	0.22

Table 5: Calculating the percentage of total form (%TF), relative length of the shortest chromosome (%S), percentage of long arm relative length (%ASK), intra-chromosomal symmetry index (A1), inter-chromosomal symmetry index (A2), karyotype formula (KF), relative domain difference (%DRL), Stibbins symmetry index (SA), total length of genome (TL), for *C. kabirkuhensis.*

	%TF	%S	%ASK	A_1	FK	%DRL	A_2	SA	TL
C.kabirkuhensis	32.22	41.08	67.59	0.53	3m+6sm	2.86	0.38	2A	67.8



C. rahiminejadii

It is a diploid array with a chromosome number of 2n = 2x = 18. According to Negaresh and Rahiminejad (2015), it is a rare monopoly element in western Iran in the Kermanshah area. This is the first report of a chromosome number for this taxon, according to the 2008 chromosomal counting list provided by Mr. Watanabe for the sunflower family. Images of mitotic zone, karyogram and ideogram are shown on page 74. In Table 6 and Table 7, Karyotypic indices were calculated for *C. rahiminejadii*. Karyogram, ideogram and karyotypic morphology were first reported for *C. rahiminejadii*.

Table 6: Karyotypic traits including arm length (LA), short arm length (SA), total length of chromosome (TL), centromeric index (CI), arm rate (AR), r-value, percentage of relative length domain difference (%) RL), L / L + S, A_i (mean asymmetry), for *C. rahiminejadii.*

	Long length	Short length	Total length of chromosome	Centrumeric index	Arm rate	r- value	%RL	L/L+S	A_i
1	2.117	1.14	3.257	0.35	1.85	0.538	13.93	0.64	0.29
2	1.856	0.97	2.826	0.343	1.91	0.522	12.08	0.65	0.31
3	1.888	0.83	2.718	0.3	2.27	0.439	11.62	0.69	0.38
4	1.875	0.767	2.642	0.29	2.44	0.409	11.3	0.7	0.41
5	1.852	0.752	2.604	0.288	2.46	0.406	11.13	0.71	0.42
6	1.682	0.782	2.464	0.317	2.15	0.464	10.53	0.68	0.36
7	1.803	0.607	2.41	0.251	2.97	0.336	10.3	0.74	0.49
8	1.613	0.661	2.274	0.29	2.44	0.409	9.72	0.7	0.41
9	1.361	0.823	2.184	0.37	1.63	0.604	9.34	0.62	0.24

Table 7: Calculating the percentage of total form (%TF), relative length of the shortest chromosome (%S), percentage of long arm relative length (%ASK), intra-chromosomal symmetry index (A1), inter-chromosomal symmetry index (A2), karyotype formula (KF), relative domain difference (%DRL), Stibbins symmetry index (SA), total length of genome (TL), for *C. rahiminejadii.*

	%TF	%S	%ASK	A_1	FK	%DRL	A_2	SA	TL
C.rahiminejadii	31.36	67.05	68.05	0.54	1m+8sm	1.07	0.11	3A	48.33

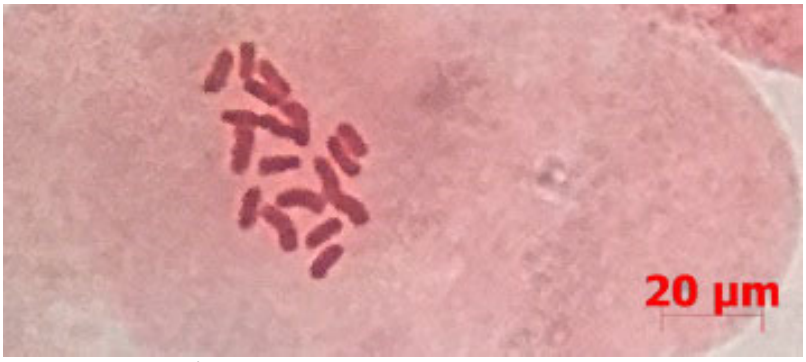


Fig. 7: Mitotic zone of *C. rahiminejadii.*

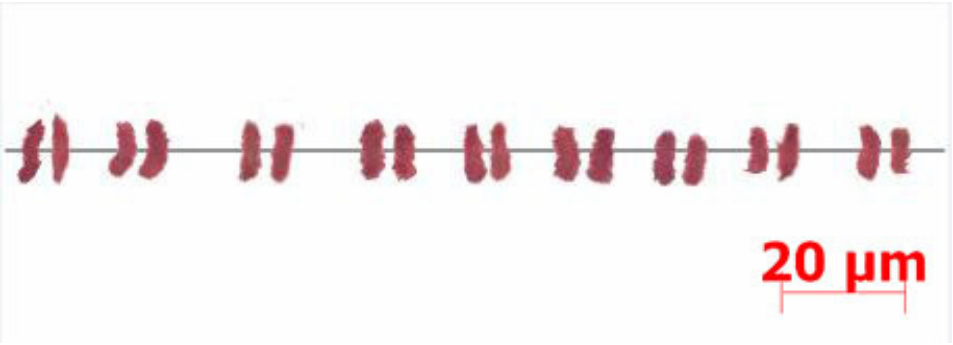


Fig. 8: Karyogramdrawn for species *C. rahiminejadi*i.

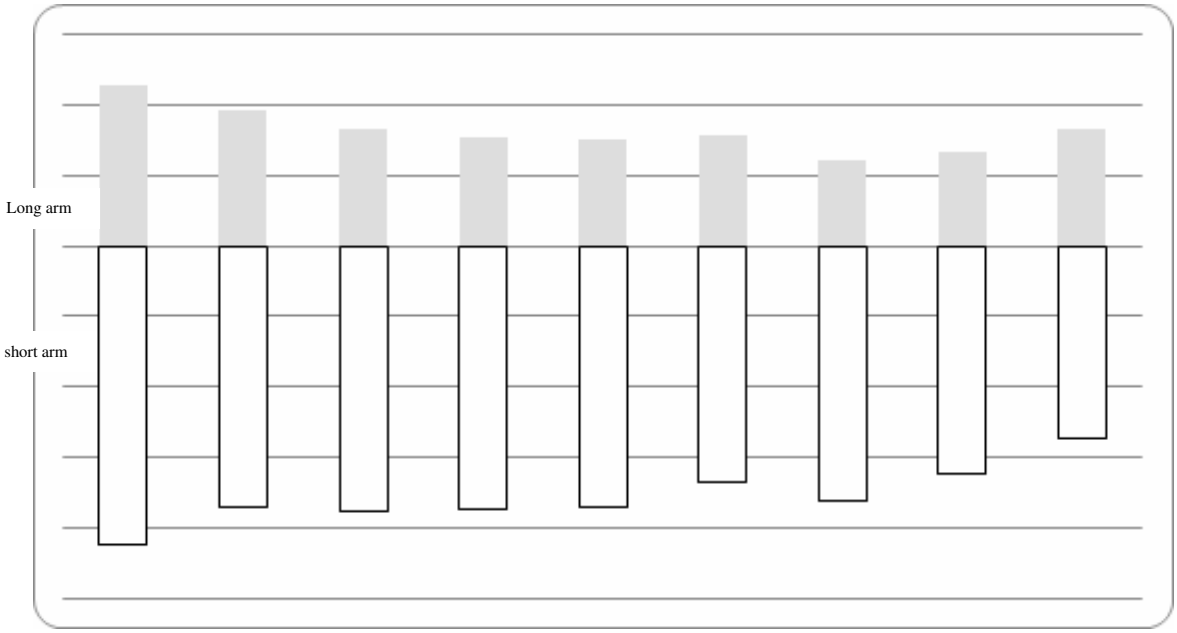


Fig. 9: Ideogram drawn for *C. rahiminejadii.*

C. ravansarensis

It is a diploid array with a chromosome number of 2n = 2x = 18. This is the first report of a chromosome number for this taxon, according to the 2008 chromosomal counting list provided by Mr. Watanabe for the sunflower family. Images of mitotic zone, karyogram and ideogram are shown on page 80. In Table 8 and Table 9, Karyotypic indices were calculated for C. ravansarensis. Karyogram, ideogram and karyotypic morphology were first reported for C. ravansarensis.

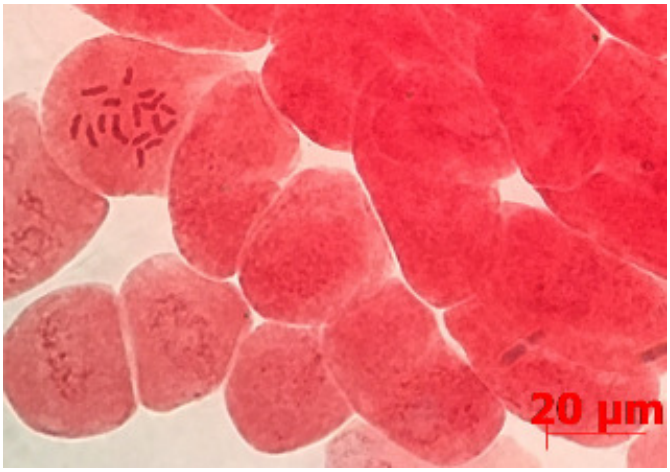


Fig. 10: Mitotic zone of species *C. ravansarensis.*

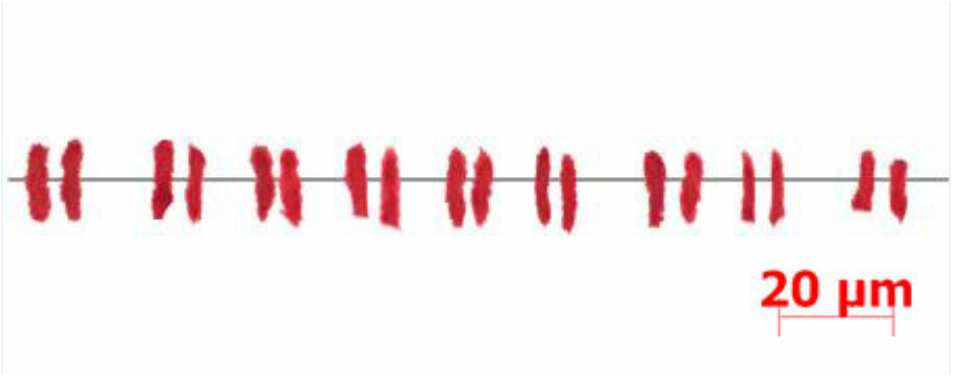


Fig. 11: Karyogramdrawn for species *C. ravansarensis.*

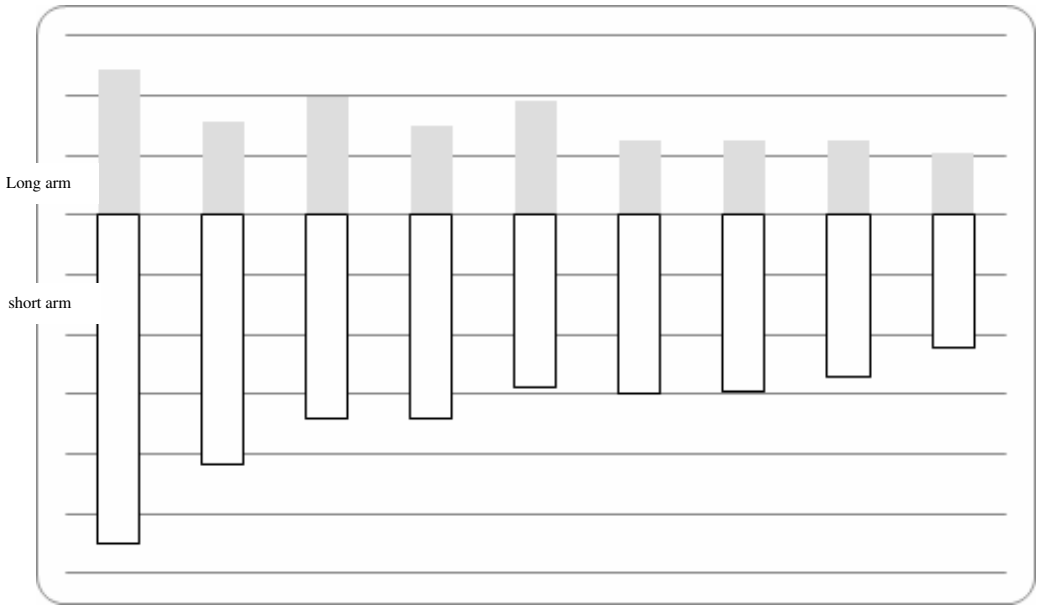


Fig. 12: Ideogram drawn for *C. ravansarensis*.

Table 8: Karyotypic traits including arm length (LA), short arm length (SA), total length of chromosome (TL), centromeric index (CI), arm rate (AR), r-value, percentage of relative length domain difference (%) RL), L / L + S, A_i (mean asymmetry), for *C. ravansarensis.*

	Long length	Short length	Total length of chromosome	Centrumeric index	Arm rate	r- value	%RL	L/L+S	A_i
1	2.749	2.749	3.96	0.305	2.27	0.44	17.89	0.69	0.38
2	2.093	2.093	2.88	0.273	2.65	0.45	13.01	0.72	0.45
3	1.708	1.708	2.696	2.696	1.72	0.578	12.18	0.63	0.26
4	1.708	1.708	2.446	2.446	2.31	0.432	11.05	0.69	0.39
5	1.439	1.439	2.339	2.339	1.49	0.667	10.57	0.61	0.2
6	1.495	1.495	2.11	2.11	2.43	0.411	9.53	0.7	0.41
7	1.48	1.48	2.095	2.095	2.4	0.415	9.46	0.7	0.41
8	1.359	1.359	1.974	1.974	2.2	0.452	8.92	0.68	0.37
1	1.106	1.106	1.628	1.628	2.11	0.471	7.35	0.67	0.35

Table 9: Calculating the percentage of total form (%TF), relative length of the shortest chromosome (%S), percentage of long arm relative length (%ASK), intra-chromosomal symmetry index (A1), inter-chromosomal symmetry index (A2), karyotype formula (KF), relative domain difference (%DRL), Stibbins symmetry index (SA), total length of genome (TL), for *C. ravansarensis.*

	%TF	%S	%ASK	A_1	FK	%DRL	A_2	SA	TL
C.ravansarensis	30.24	41.11	66.85	0.55	1m+8sm	2.33	0.19	2A	46.68

C. shahuensis

It is a diploid array with a chromosome number of 2n = 2x = 18. This is the first report of a chromosome number for this taxon, according to the 2008 chromosomal counting list provided by Mr. Watanabe for the sunflower family. Images of mitotic zone, karyogram and ideogram are shown on page 82. In Table 10 and Table 11, Karyotypic indices were calculated for C. shahuensis. Karyogram, ideogram and karyotypic morphology were first reported for C. shahuensis.

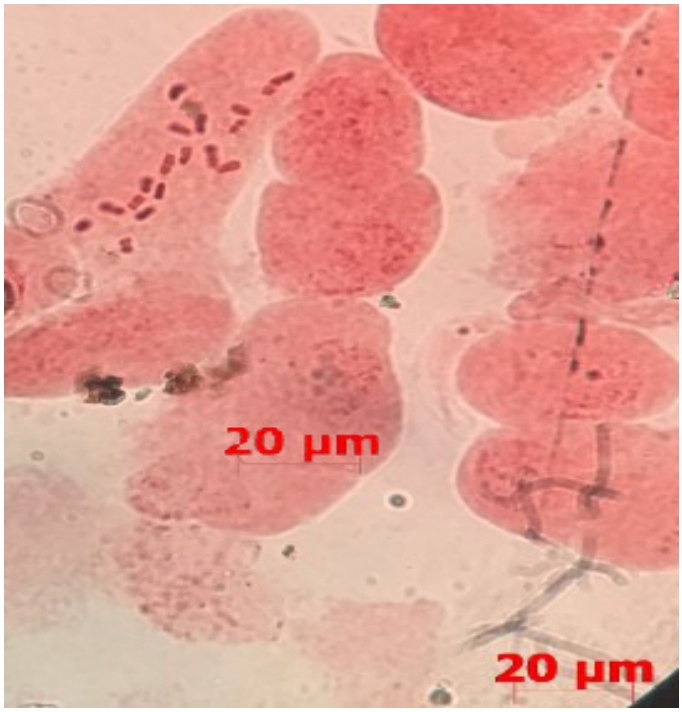


Fig. 13: Mitotic zone of *C. shahuensis.*

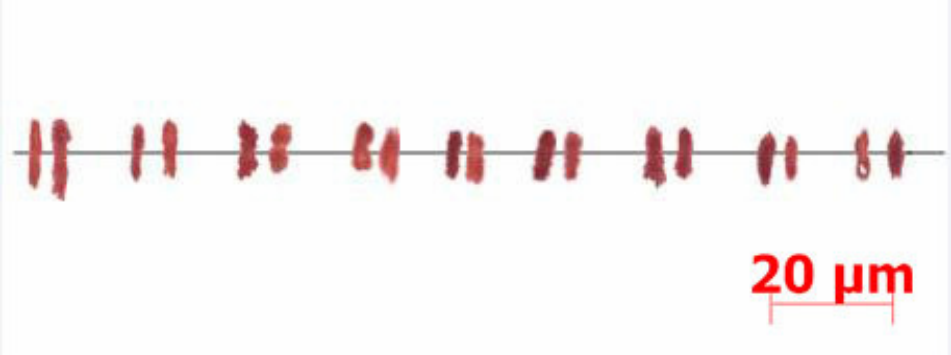


Fig. 14: Karyogram drawn for species *C. shahensis.*

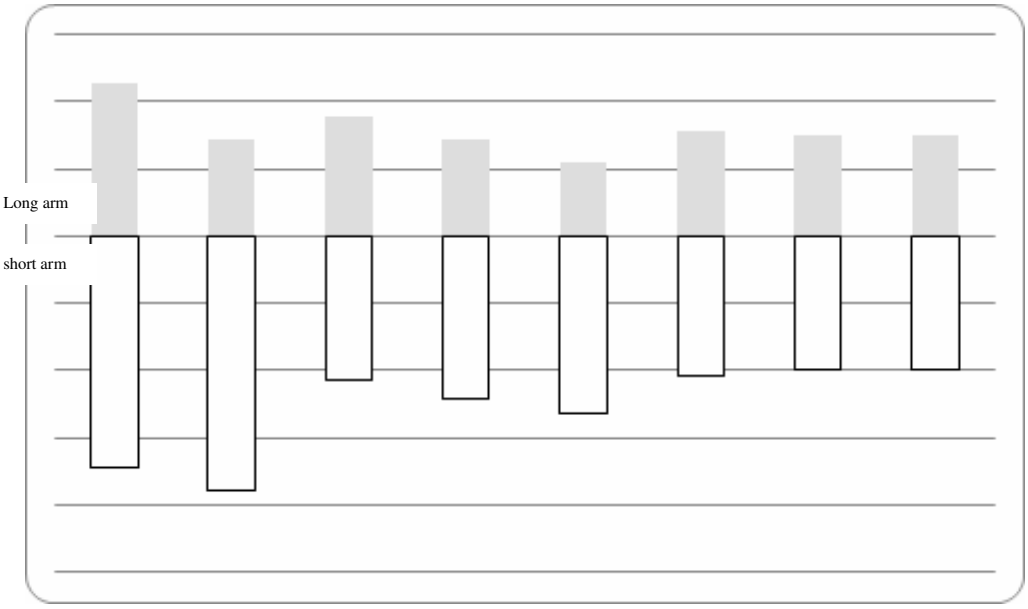


Fig. 15: Ideogram drawn for C. *shahuensis.*

Table 10: Karyotypic traits including arm length (LA), short arm length (SA), total length of chromosome (TL), centromeric index (CI), arm rate (AR), r-value, percentage of relative length domain difference (%) RL), L / L + S, A_i (mean asymmetry), for *C. shahuensis.*

	Long length	Short length	Total length of chromosome	Centrumeric index	Arm rate	r- value	%RL	L/L+S	A_i
1	1.72	1.135	2.855	0.397	1.51	0.65	15.49	0.6	0.2
2	1.897	0.72	2.617	0.275	2.63	0.379	14.09	0.72	0.44
3	1.071	0.884	1.955	0.45	1.36	0.825	10.52	0.54	0.09
4	1.213	0.72	1.933	0.452	1.68	0.593	10.41	0.62	0.25
5	1.326	0.548	1.874	0.292	2.41	0.413	10.09	0.7	0.41
6	1.37	0.46	1.83	0.251	2.97	0.335	9.85	0.74	0.49
7	1.047	0.775	1.822	0.425	1.35	0.74	9.81	0.57	0.14
8	1.207	0.613	1.82	0.336	1.96	0.507	9.8	0.66	0.32
9	0.986	0.746	1.732	0.43	1.32	0.756	9.32	0.56	0.13

Table 11: Calculating the percentage of total form (%TF), relative length of the shortest chromosome (%S), percentage of long arm relative length (%ASK), intra-chromosomal symmetry index (A1), inter-chromosomal symmetry index (A2), karyotype formula (KF), relative domain difference (%DRL), Stibbins symmetry index (SA), total length of genome (TL), for *C. shahuensis.*

	%TF	%S	%ASK	A_1	FK	%DRL	A_2	SA	TL
C.shahuensis	35.49	60.66	64.52	0.45	5m+4sm	1.46	0.18	3A	37.86

Cytotaxonomy is a branch of cytogenetic science that compares karyological features for classification and evolutionary studies. Reduction is done in total length of chromosomes to shorten mitotic cell cycle and produce smaller cells that accelerate the growth of small annual plants at a given time. Another step is to reduce the dysploidy, in which the chromosome number is reduced by moving fragments from one chromosome to another. The association between subtractive dysploidy and increase in total length of chromosome, different length of cell chromosomes, and karyotype asymmetry may be related to such chromosomal alterations (Watanabe *et al.*, 1999).

The present research confirms the studies of the section *Cynaroides*; the chromosomal base number for this section is x = 9. In this study, we performed also the chromosomal counting and determination of karyological traits for the species of *C. kabirkuhensis*, *C. rahiminejadii*, *C.ravansarensis, C. shahuensis*, *C. regia* var. *cynarocephala* for the first time.

In terms of A2, *C.rahiminejadii* is the least symmetric species. Karyotypic Formula for *C. ravansarensis* is 1m + 8sm, 5m + 4sm for *C.shahuensis*, 3m + 6sm for *C.regia* var. cynarcephala, 3m + 6sm for *C.kabikuhensis* and1m + 8sm for *C.rahiminejadii*. In species *C.kabirkuhensis*, minisatellite was found on chromosome 2 and chromosome 9. The two varieties of *C.regia* var. regia and *C.regia* var. cynarocephala are very similar in appearance (by % TF,% ASK, A1); whereas karyotypicallythe variety of *C. regia* var. cynarocephala is more asymmetric than other varieties. Here, *C.shahuensis* (by % TF,% ASK, A1) shows significant similarity with these two varieties, especially *C.regia* var. cynarocephala. The karyotypic formula of all species is a combination of metacentric and sub-metacentric chromosomes. The highest %S was in *C. rahiminejadii* (67.05). The highest total length of genome was obtained in *C. kabirkuhensis* and the lowest in *C. regia* var. cynarocephala. There was no significant difference between species of section Cynaroides in % ASK parameter.

As mentioned, chromosome length and centromere position are two important factors in measuring chromosomes. The species *C. rahiminejadii* shows less difference in length of chromosomes examined by parameter A_2 and has more symmetric karyotype**.**

In terms of total genome content, species in Kermanshah province are more similar than *C. kabirkuhensis* species with different dispersion range (in terms of genome total length and dispersion range).

There are also morphological similarities between the species under study, in terms of the shape of collar, texture, and position of the appendages, and the number of eyelashes. Although *C. rahiminejadii* differs in some important respects, such as the density of felt-like crackers on filarias, the small appendages that cover a small portion of the filarias are pale brown or brown (Negaresh and Rahiminejad, 2016). The small ones that cover a small portion of the filarias and are brown or pale brown (Negaresh and Rahiminejad, 2016).

Two species of *C. ravansarensis* and *C. rahiminejadii* have the same karyotypic formula. In the monograph of Negaresh and Rahiminejad (2018), *C. ravansarensisis* morphologically similar to *C. regia* var. regia and *C. rahiminejadii* similar to C. gigantean subsp. reshingeri. Based on the present study, it is probable that there be more closely relation between *C. ravansarensis* and *C.rahiminejadii*. In the monograph of Negaresh and Rahiminejad (2018), *C. kabirkuhensis* is similar to *C. shahuensis*. Based on Monograph of Negaresh and Rahiminejad (2018), *C. shahuensisis* morphologically similar to the variety of *C. regia* var. regia. The present study confirms this similarity in terms of karyotypic indices (% TF, % ASK, A1). The two most important dispersion areas of section Cynaroides are Turkey and Iran, as Turkey is closer to the Mediterranean region, so it is likely to be of Turkish origin, and because of the high number of species of this section in Iran, it is likely that Iran is home of speciation and variation of this section. This is the section. The morphological studies of Negaresh & Rahiminejad and a

series of observations show some hybrid characteristics between species. In addition, in spite of the great variation in morphological and pollination characteristics, they did not differ in chromosome number. The base chromosome number in the section Cynaroides suggests the possibility of a widespread evolution by homoploidy. This evidence suggests that homoploidy and pollination may play an important role in speciation in this section. Thus, homoploidy has been found in the center of diversity in western Iran (8). While tetraploidy occurred in remote areas (Greece). Finally, homoploidy in the section Cynaroides corresponds to the evolution suggested by morphological studies and nuclear DNA sequences (8). In this study, it was found that the chromosomal bases number in species of the section Cynaroides is x = 9; it confirms previous studies and suggests that homoploidy transformation is occurring among the species of this section.

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