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Karyotype Analysis in Five Species of *Carthamus* L. (Asteraceae) from Iran

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ABSTRACT

The genus *Carthamus*, a member of the family Asteraceae, includes about 25 species worldwide and 7 species in Iran. In this study, chromosome numbers and karyotypes of 13 populations of five species of the genus *Carthamus* (Asteraceae family) were investigated. Also pollen sterility percent has calculated in each species. The findings confirmed the triploid chromosome numbers for two species, *C. tinctorius* and *C. dentatus*, respectivly with 24 and 20 chromosomes, aligning with previous reports. *C. lanatus* was identified as a polyploid with 44 chromosomes. Additionally, one population of *C. dentatus* was recognized as a triploid with 30 chromosomes, as first-time report. Among the three populations of *C. turkestanicus* studied, two populations confirmed the previously reported allopolyploid with 64 chromosomes, while one exhibited a new finding of 60 chromosomes. Karyotype analyses revealed that all populations possess asymmetrical karyotypes. The karyotype formulas were as follow: 4m+ 5sm+ 2st+ t for *C. tinctorius* (2n= 2x= 24), M+ 19m+ 2Sm for *C. lanatus*, M+ 7m+ 2sm for *C. dentatus* (2n= 2x= 20), M+7m+2Sm for the population of *C. dentatus* with 2n= 30, and M+ 17m+ 13sm+ T for *C. turkestanicus* (2n= 64). A cluster analysis by Ward method has separated the diploid and polyploids species. These karyological data significantly enhance our understanding of *Carthamus* and will prove to be a valuable resource for future research on this genus.

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Introduction

The genus *Carthamus* L., (Asteraceae), with about 25 species worldwide distributed with 7 species in Iran (Dittrich et al., 1991). *Carthamus tinctorius* L., commonly known as safflower, is the only cultivated species in the genus, with its wild origin remaining unknown. The wild *Carthamus* species are distributed from Spain and North Africa across the Middle East to northern India (Kummar, 1991). Some species, such as C. *creticus* L., C. *lanatus* L., and C. *leucocaulos* Sibth. & Sm., have colonized other regions, including Argentina, Australia, California, and South Africa, where they can be invasive (Ashri and Knowles, 1960; Estilai and Knowles, 1978). Delimitation of *Carthamus* and its close ally, *Carduncellus* Adans, has been challenging due to morphological similarities and convergent evolution of several variable characters used by taxonomists (Vilatersana *et al*., 2000a). Additionally, the sectional classification of *Carthamus* has been a challenge (Knowles, 1959; López González, 1989; Vilatersana *et al*., 2000a), which showed in Table 1. According to López González (1989) classification system, Iranian *Carthamus* grouped under three sections: *Carthamus*, *Atractylis* Rchb. and *Odonthagnathis.* Since hybridization and polyploidy are common in *Carthamus* species, the determination of species boundaries requires the use of various biosystematics methods. The analysis of alcohol dehydrogenase allozymes by Efron *et al*. (1973) and allozymes by McPherson *et al*. (2004) have carried out for this purpose. Karyological studies have been done very little in *Carthamus* species and they have been limited to chromosome counting (Yazdani *et al*. 2013; Ghahremaninejad

et al. 2013). Also, karyological studies have been done in a small number of the species (Uysal *et al*., 2018; Carapetian and Zarei, 2015). Despite of karyological studies of Carapetian and Zarei (2015), no information on the size of the chromosomes is provided for *C. turkestanicus* and only the total size is mentioned, while in the present research the chromosomes are precisely measured and idiogram and karyogramm are provided. Although these researches provide some information of the karyology which are useful to determine the range of species. The aim of this research is to provide information about karyology of this genus to clarify the position of the species.

Table 1. Taxonomic classification for the genus *Carthamus* based on López González (1989).

Sect. Carthamus (n=12)	Sect. Odonthagnathis (n=10 ,11)	Sect. Atractylis (n=22, 32)	Taxa removed from Carthamus
C. curdicus Hanelt	*C. boissieri* Halacsy	*C. creticus* L.	*Phonus rhiphaeus* G. López
C. gypsicola Ilj.	*C. dentatus* (Forssk.) Vahl	*C. lanatus* L.	*Phonus arborescens* L. G. López
C. oxyacanthus Bieb.	*C. divaricatus* Beguinot & Vacc.	*C. turkestanicus* Popov	
C. palaestinus Eig.	*C. glaucus* Bieb.	**López González (1989) Uncertain placement (n= 12)**	
C. persicus Willd.	*C. leucocaulos* Sibth. Et Sm.	*C. nitidus* Boiss.	
C. tinctorius L.	*C. tenus* (Boiss. & Bl.) Bornm.	*Femeniasia balearica* Susanna	

n= Chromosome number

Materials and methods

Plant materials

A total of thirteen populations from four different species were sampled across various regions of Iran for cytogenetic analysis. The species examined included *Carthamus tinctorius* L., *C. lanatus* L., *C. turkeastanicus* Popov, and *C. dentatus* (Forssk.) Vahl., *C. glaucus* Beid. All specimens were collected by the authors and are stored at the herbarium of the department of plant sciences at Alzahra University (Table 2).

Karyological analysis

Root tips were obtained from seedlings grown from seeds collected in the field. These root tips were stored at 2-3°C in the dark for one week to synchronize cell division. For pretreatment, root tips were exposed to 0.002 M 8-hydroxyquinoline for 4-8 hours at room temperature, then fixed in a mixture of ethanol and glacial acetic acid (3:1 ratio) for 24 hours. After fixation, the samples were washed with distilled water, stored in 70% ethanol, and stained with aceto-orcein (Khayati *et al.*, 2014).
A total of 54 individuals including 13 populations were analyzed. Except of *C. glaucus* (with only one specimen available) at least 3 accessions, at least 4 individuals of each accession and at least 10 plates were examined in other species. In *C. lanatus* and *C. tinctorius* about 30 to 98 cells, in *C. dentatus* 25 cells, in *C. tuekestanicus* 20 cells and 10 cells from *C. glaucus* of each individual were examined. Chromosomal analysis was conducted using an Olympus B51 microscope, and images were captured with an Olympus C-5060 digital camera. Chromosome features such as the short arm length (s), long arm length (L), and total chromosome length (TL) were measured. The arm ratio (r= L/S) was calculated and used for chromosome classification according to the guidelines by Knowles (1964).

Table 2. List of species studies, province and voucher specimens

Species	Province	Collector	ALUH No.*
C. tinctorius L.	Fars: Darab; Hoseyn abad village	Aghakoochaki	38880
C. tinctorius L.	Isfahan: Mahdieh	Aghakoochaki	38803
C. tinctorius L.	Isfahan: Babukan	Aghakoochaki	38804
C. lanatus L.	Azarbayjane: Ahar	Aghakoochaki	38828
C. lanatus L.	Azarbayjane: Kaleibar	Aghakoochaki	38829
C. lanatus L.	Ardebil: Givi	Aghakoochaki	38834
C. turkestanicus Popov	Azarbayjane: Kaleibar	Aghakoochaki	38831
C. turkestanicus Popov	Ardebil: Givi	Aghakoochaki	38835
C. turkestanicus Popov	Azarbayjane: Ahar	Aghakoochaki	38830
C. dentatus (Forssk.) Vahl	Fars: Darab; Hoseyn abad village	Aghakoochaki	38879
C. dentatus (Forssk.) Vahl	Ardebil: Givi	Aghakoochaki	38833
C. dentatus (Forssk.) Vahl	Azarbayjane: Ahar	Aghakoochaki	38827
C. glaucus Beid.	Azarbayjane: Kaleibar	Aghakoochak	38832

*****ALUH No: All the specimens were preserved in Alzahra University Herbarium (ALUH)

Additional chromosomal parameters included the shortest chromosome length as a percentage of the total chromosome length (S%), the range of relative chromosome length difference (D.R.L.), the intrachromosomal asymmetry index (A1), and the total form percentage (TF%). Karyotype asymmetry was evaluated following the categories proposed by Stebbins (1971), providing insights into the chromosomal evolution and diversification among the species studied.

Statistical analysis

Analysis of variance (ANOVA) was utilized to assess the significance of chromosomal variations within and between species. Ideograms were constructed based on average measurements from each species. The results of karyological studies analyzed by multivariate analysis of variance using cluster analysis (CA) by Ward method to study relationships among the taxa by using SPSS statistics software (SPSS 25). Ten quantitative characters were measured and used for statistical studies (Table 3).

Table 3. The number of chromosomes and Karyotypes of the studied populations of *Carthamus*.

Species*	TL	2n	SD	A_1	D.R.L.	TF%	S%	AT	KF
C. tinctorius L.	22.83	24	1.02	1.04	7.05	32	39	3B	4m+5sm+2st+t
C. lanatus L.	119.25	44	3.25	0.63	3.26	45	46	3B	1M+19m+2Sm
C. turkestanicus Popov	75.11	64	1.71	1.44	3.43	37	35	3B	M+17m+13sm+1T
C. dentatus	23.57	30	0.9	0.67	6.49	38	37	3B	2M+7m+5sm+T
C. dentatus (Forssk.) Vahl	13.98	20	1	0.92	8.15	42	47	3B	M+7m+2Sm

*****Species are listed following the order of Table 1; TL = Total chromosome length; SD = Size of the shortest chromosome (μm); A_1= Intrachromosomal asymmetry index; D.R.L. = Difference of range of relative length; TF = Total form percentage; AT = Stebbins karyotype asymmetry type, KF = Karyotype formula.

Results and Discussion

Karyological studies indicate that *Carthamus tinctorius* and *C. dentatus* are diploid with chromosome numbers 2n= 24 and 2n= 20 respectively, which having basic chromosome numbers of x= 12 and x= 10 (Table1; Figs. 2A1-31; B1-3). Additionally, one population of *C. dentatus* was recognized as a triploid with 30 chromosomes (Fig. 1B1-3), which is reported for the first time from Iran. While, *C. lanatus* and *C. turkestanicus* are polyploids. *C. lanatus* having a chromosome number of 2n= 44 (Fig. 3 A1-3) and *C. turkestanicus* has 2n= 64 (Table 2; Fig.1A1-3), as previously reported by Vilatersana *et al*. (2000b) and Carapetian and Zarei (2015).

C. lanatus is hypothesized to be an allopolyploid, potentially originating from hybridization between species with chromosome numbers 2n=20 and 2n=24, followed by chromosome doubling (Khidir and Knowles, 1970b). Alternatively, Estilai and Knowles (1978) propose that it could be an autopolyploid originating from *C. divaricatus* with a basic chromosome number of x=11. In contrast, Khidir and Knowles (1970a) suggest that *C. turkestanicus* resulted from independent historical hybridization events involving an ancestral form of *C. lanatus* (n=22) and another species with n=10.

Statistical analyses and Ward's dendrogram (Fig. 4) based on karyological data, revealed close relationships between *C. lanatus* and *C. turkestanicus*. These two species exhibit similar leaf shapes and floret colors, which has led to the classification of *C. turkestanicus* as a subspecies of *C. lanatus* in certain taxonomies (Vilatersana *et al*., 2000a; 2005) due to their morphological similarities.

C. turkestanicus is a species reported with high level of self-compatibility (Khidir & Knowles, 1970b). Evidence for the relationship between *C. lanatus* and *C. turkestanicus* comes from the results of hybridization experiments where species with n=10 were crossed with *C. lanatus* (Khidir and Knowles, 1970b). These crosses exhibited several meiotic irregularities, including quadrivalent formations, suggesting substantial genetic differences and potential reciprocal translocations (Khidir and Knowles, 1970a).

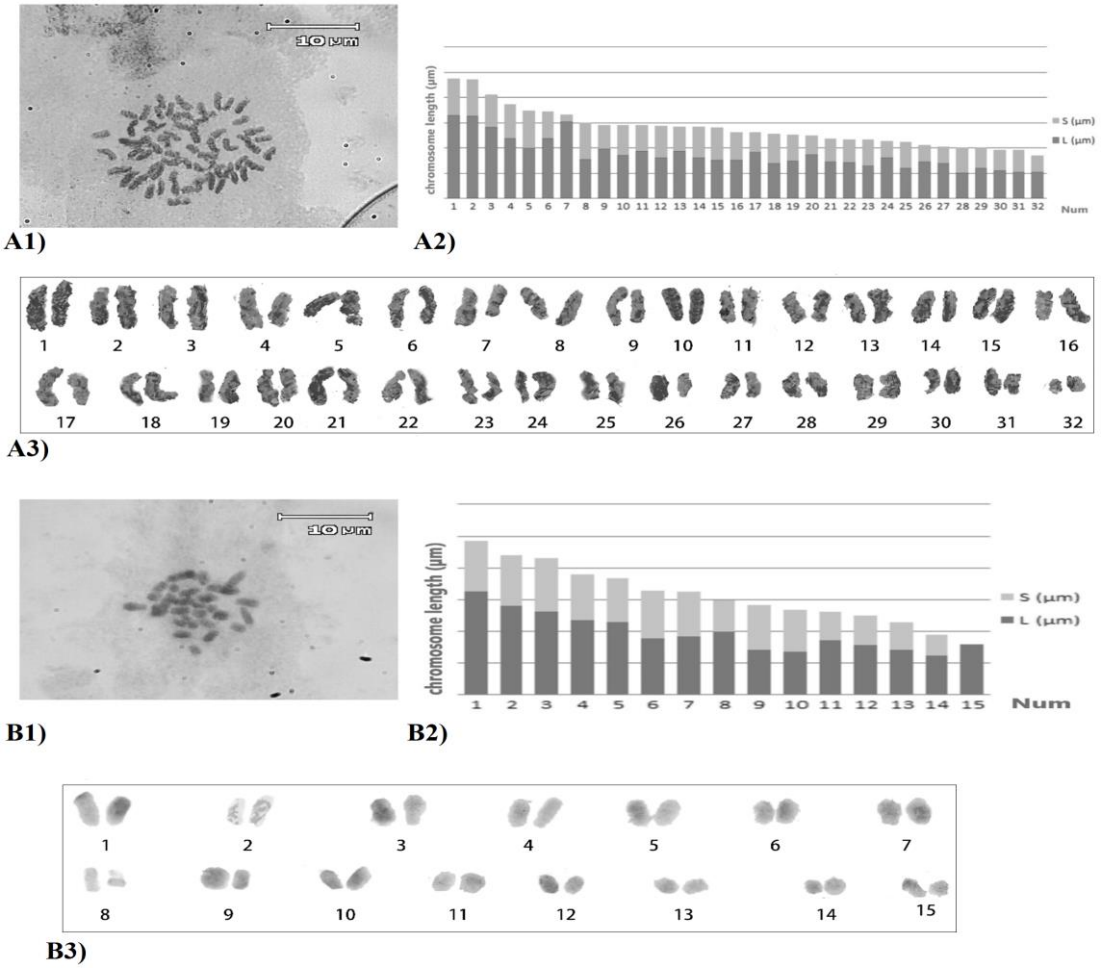

Fig. 1. Chromosomes of *Carthamus* species: A1-3) *C. turkestanicus* (2n=64), A1: Somatic metaphases, A2: Ideogram, A3: Karyogramm; B1-3) *C. dentatus* (2n=30), B1: Somatic metaphases, B2: Ideogram, B3: Karyogramm.

Crosses between *C. lanatus* (n=22) and *C. turkestanicus* (n=32) produced hybrids with 22 bivalent and 10 univalent chromosomes during meiosis I, further supporting the genetic relationship and complexity between these species (Khidir and Knowles, 1970a; Khidir and Knowles, 1970b; Estilai and Knowles, 1978).

The geographical ranges of *C. turkestanicus* and *C. lanatus* are overlapped in an area in the northwestern of Iran. In this region, distinguishing between the two species morphologically is challenging, which may indicate the presence of an introgressive population between these closely related species in an area of sympatry (Grant, 1971).

Analysis of alcohol dehydrogenase allozymes by Efron *et al*. (1973) revealed that *C. lanatus* and *C. turkestanicus* share a unique allele for one subunit of this enzyme, which is not found in other *Carthamus* species. These findings, along with hybridization studies, support the hypothesis that, one of the parents of *C. turkestanicus* could be *C. lanatus* (McPherson *et al*., 2004).

Garnatje *et al*. (2006) proposed that another potential parent of *C. turkestanicus* might be *C. glaucus* subsp. *glaucus* (2n=20). This species, which grows in Iran, shares a common habitat with *C. turkestanicus* and *C. lanatus*. In the Kaleibar area, all three species grow abundantly and coexist sympatrically, suggesting that these two species could be the parents of *C. turkestanicus*. The ten univalents observed by Khidir and Knowles (1970a) in the hybrids of *C. turkestanicus* and *C. lanatus* may originate from *C. glaucus* subsp. *glaucus*, although further investigation is needed. These results are in agreement with Carapetian and Zarei (2015).

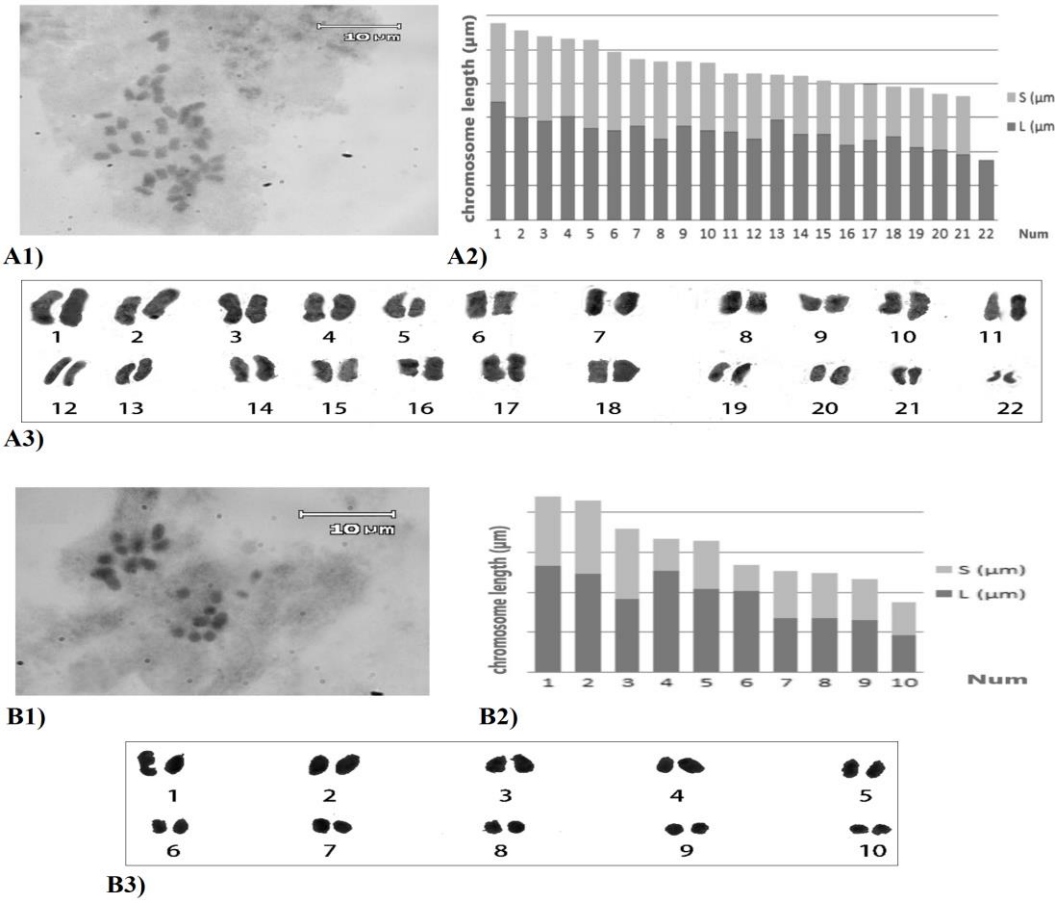

Fig. 2. Chromosomes of *Carthamus* species: A1-3) *C. tinctorius* (2n=24), A1: Somatic metaphases, A2: Ideogram, A3: Karyogramm; B1-3) *C. dentatus* (2n=20), B1: Somatic metaphases, B2: Ideogram, B3: Karyogramm.

Following Stebbins' (1971) karyotype classification, all species fall into the 3B-category (Table 3). The quantitative data of chromosomes (Table 3) indicate the asymmetrical karyotype for these species, but the results of Malik & Srivastava' karyological studies on *C. tinctorius* showed that the chromosomes of this species belong to1B and 1A type and then could be considered symmetrical or slightly asymmetrical karyotype (Malik and Srivastava, 2009). In plant karyotypes, variation arises due to ecological adaptation, phylogenetic differentiation, and changes in chromosome morphology. Genome size diversity spans over 2400-fold across angiosperms. Metaphase chromosome size estimates vary significantly. Plant karyotypes serve as dynamic canvases reflecting adaptation, evolution, and ecological specialization.

The most of content A_1 (1.44) is seen in *C. turkestanicus* Popov and the least of it is in *C. lanatus* (0.63).

C. lanatus, with the highest number of metacentric chromosomes (19) and no submetacentric chromosome, as well as the highest TF percentage and the lowest A1, is more karyotypically symmetric than the others are. Conversely, *C. tinctorius*, with the lowest TF percentage and fewest metacentric chromosomes, has the most asymmetrical karyotype. The *C. dentatus* exhibits differing degrees of karyotype symmetry, with the diploid form (2n=20) being more symmetric compared to the triploid form (2n=30) as shown in Table 3. Jacas & de la Serna (1992) have noted that dysploidy, or changes in the base number of chromosomes, is common in the subtribe Centaurinae and typically does not affect taxonomic classifications.

In this study, we identified two diploid populations (2n= 20) and one triploid population (2n=30) of *C. dentatus* (Table 3; Fig. 1B1-B3). Pollen sterility was observed at 60% in the triploid population, while the diploid populations exhibited only 20% sterility. Despite the high sterility, the triploid *C. dentatus* was fertile, producing numerous seeds, suggesting apomictic reproduction, as Asker & Jerling (1992) indicated that odd-ploidy levels and disrupted microsporogenesis could indicate apomixis, commonly seen in triploids. However, further cytoembryological studies are necessary to confirm this hypothesis.

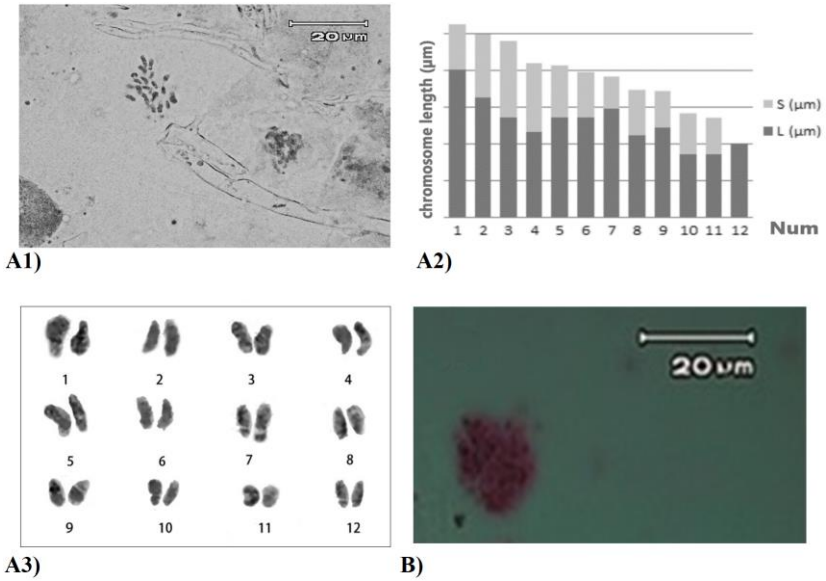

Fig. 3. Chromosomes of *Carthamus* species: A1-3) *C. lanatus* (2n=44), A1: Somatic metaphases, A2: Ideogram, A3: Karyogram; B) *C. glaucus*.

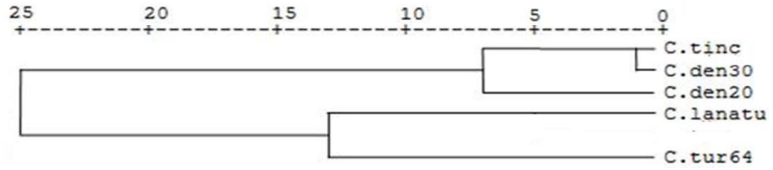

Fig. 4. Dendrogram using Ward method for *Carthamus* species based on karyotype characters: C. tinc= *C. tinctorius*, C. den30= *C. dentatus* (2n= 30), C. den 20= *C. dentatus* (2n= 20), C. lanatu= *C. lanatus*, C. tur64= *C. turkestanicus* (2n= 64).

The dendrogram based on karyological data clearly differentiates the species into two clusters (Fig. 4). The first cluster contains *C. tinctorius* and *C. dentatus*, both diploids, while the second cluster groups together polyploid species and those of hybrid origin. Within each cluster, the different populations are closely related. This study represents a step towards understanding the karyological characteristics at the species level. A combination of additional karyological analysis and molecular studies is necessary to make significant conclusions about the relationships and distinctions among the taxa, as current data are insufficient for resolving taxonomic discrepancies within the genus.

Authors' Contributions:

A.A.: Resources, Software, Validation, Visualization, Investigation,
M.P.: Conceptualization, Methodology, Investigation, Data Curation, Writing - Original Draft, Review & Editing, Supervision, Project administration.

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Disclosure Statement

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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