

New CHROMOSOME COUNTS OF SIX TAXA OF CARDUEAE (ASTERACEAE) IN IRAN

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In this study, the chromosome numbers of six Iranian taxa belonging to tribe Cardueae were counted. The chromosome numbers in *Jurinea cartilaginea* Mozaff. $2n=30$, *J. carduiiformis* (Jaub. & Spach) Boiss. $2n=34$, *Aegopordon berardioides* Boiss. $2n=34$, *Nikitinia leptoclada* (Bornm. & Sint.) Iljin $2n=30$, *Centaurea amadanensis* Schultz-Bip. var. *gymnoclada* (Jaub. & Spach) Wagenitz $2n=18$ and *Cousinia argentea* Mehregan & Assadi $2n=26$ were determined. Of the chromosome counts reported four are new to the world. Based on this study, three taxonomic changes are confirmed and a new suggestion is presented here.

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شمارش های کروموزومی جدید برای شش آرایه از طایفه *Cardueae* (تیره کاسنی) در ایران

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در این پژوهش عدد کروموزومی شش آرایه از طایفه *Cardueae* در ایران شمارش گردید. اعداد کروموزومی برای *Jurinea cartilaginea* Mozaff. $2n=30$ ؛ *J. carduiiformis* (Jaub. & Spach) Boiss. $2n=34$ ؛ *Aegopordon berardioides* Boiss. $2n=34$ ؛ *Nikitinia leptoclada* (Bornm. & Sint.) Iljin $2n=30$ ؛ *Centaurea amadanensis* Schultz-Bip. var. *gymnoclada* (Jaub. & Spach) Wagenitz $2n=18$ و *Cousinia argentea* Mehregan & Assadi $2n=26$ به دست آمد. چهار مورد از اعداد کروموزومی آرایه شده، گزارش های کروموزومی جدیدی برای دنیا می باشند. بر اساس این مطالعه سه جابه جایی موقعیت تاکسونومی تأیید و یک تغییر موقعیت سیستماتیک پیشنهاد می شود.

INTRODUCTION

The rediscovery of Mendel's Laws in the year 1900 caused the formulation of 'chromosome theory of inheritance' and the foundation of cytogenetic. In the last few decades of the last century, the interest in plant cytogenetic that combined study and use of structural and numerical changes of chromosomes largely declined. However, with reviving the interests in chromosomal data in a new molecular form, plant

cytogenetic had a re-birth (Gupta 2006). Chromosomal data such as number, morphology, staining characteristics and behavior of chromosomes profoundly affected the evaluation of taxonomic investigations (Stace 2000).

The Asteraceae contain the largest number of described species of any plant family, 24,000-30,000, distributed in 1600-1700 genera occurring on all continents except Antarctica. In Asteraceae (Compositae), chromosome

Table 1. The list of studied taxa in present survey (All samples were collected by Parishani and Mirtadzadini). Genera and species are arranged in alphabetical order.

No Taxon	Collection site & altitude (meter)	Coordinates	Herbarium vouchers
1 <i>Aegopordon berardioides</i> Boiss.	Isfahan, Khomeinishahr, Ladour fountain, 1981 m	N 32°42'35" E 51°26'53"	18096 HUI
2 <i>Centaurea amadanensis</i> Schultz-Bip. var. <i>gymnoclada</i> (Jaub. & Spach) Wagenitz	Kordestan, Saqqez to Marivan, after Soote village, 1747 m	N 35°59'42" E 46°25'56"	18097 HUI
3 <i>Cousinia argentea</i> Mehregan & Assadi	Khorasan, Torbat-Heydariyeh to Mashhad, near to Robot-Sefid, 1100 m	N 34°52'51" E 47°23'31"	18098 HUI
4 <i>Jurinea carduiformis</i> (Jaub. & Spach) Boiss.	Isfahan, University of Isfahan, 1743 m	N 32°35'45" E 51°39'32"	18101 HUI
	Kerman, East Kerman, 1220m	N 30°17'30" E 57°01'59"	18102 HUI
5 <i>J. cartilaginea</i> Mozaff.	Khuzestan, Behbahan to Ramhormoz, Maghar strait, 944 m	N 31°00'14" E 50°06'20"	18099 HUI
6 <i>Nikitinia leptoclada</i> (Bornm. & Sint.) Iljin	Khorasan, north west Bojnord, between Pish Ghalae and Tanghe Torkaman, 808 m	N 37°44'51" E 56°56'28"	18103 HUI

number range changes from $n=2$ to high level of polyploidy with $n=114$ (Funk *et al.* 2005). This family includes 43 tribes that Cardueae is one of them. The Cardueae is monophyletic and based on the molecular survey, includes five sub-tribes (*Carlininae*, *Echinopsinae*, *Carduinae*, *Centaureinae*, and *Cardopatiinae*) (Susanna and Garcia-Jacas 2007, Funk *et al.* 2009).

Jurinea Cass. and *Aegopordon* Boiss. from *Jurinea-Saussurea* group of *Carduinae*, *Cousinia* Cass. from the *Arctium-Cousinia* group of *Carduinae* and *Centaurea* L. and *Nikitinia* Iljin form the *Centaureinae* belong to the tribe Cardueae (Häffner 2000, Susanna *et al.* 2003, Funk *et al.* 2009). Although, until render the article of Susanna *et al.* (2002), *Nikitinia* Iljin was known one *Carduinae*, but then it was recognized as one *Centaureinae*.

Based on the latest molecular investigation (Susanna *et al.* 2006), *Outreya carduiformis* Juab. & Spach is transferred to *Jurinea* and also *Jurinea pungens* Boiss. was transferred to *Cousinia* on the basis of the study of Mehregan and Assadi (2009). *Jurinea cartilaginea* was newly added to the Iranian list of this genus (Mozaffarian 1988).

The several reports of cytological studies on Cardueae in Iran have been investigated for different taxa that in some of these cases, only a simple chromosome counts has been studied (Afzal-Rafii 1980, Amirimoqadam *et al.* 2011, Aryavand 1975 and 1977, Bakhshi-Khanaki, 1995a, 1995b and 1996, Djavadi 2005, 2007 and 2012, Djavadi & Attar 2010, Djavadi *et al.* 2007, Djavadi & Ghaffari 1999, Estilai 1977, Ghaffari 1984, 1986, 1987a, b, 1988, 1989, 1999a, 1999b and 2006, Ghaffari

& Chariat-Panahi 1985, Ghaffari & Djavadi 1998, Ghaffari *et al.* 2000, Ghaffari *et al.* 2006, Ghaffari & Kelich 2006, Ghaffari & Shahraki 2001, Ghaffari & Tajik 2007, Garcia-Jacas *et al.* 1998, Garcia-Jacas *et al.* 2009, Garnatje *et al.* 2004, Garnatje *et al.* 2006, Hellwig 1994, Hidalgo *et al.* 2007, Khidir & Knowles 1970, Lopez-Vinyallonga *et al.* 2009, Nouroozi *et al.* 2010, Ranjbar & Negaresh 2013, Romaschenko *et al.* 2004, Schank & Knowles 1964, Sheidai *et al.* 2000, Sheidai *et al.* 2006, Sheidai *et al.* 2009, Sheidai *et al.* 2012a & b, Sheidai *et al.* 2013, Susanna *et al.* 2003b, Vilatersana *et al.* 2000, Yazdani *et al.* 2013).

The purpose of this investigation is to give information concerning the chromosome counts of angiosperm taxa of the Iranian flora and help to complete index of plant chromosome number.

MATERIAL AND METHODS

The chromosome counts were obtained from mitotic cells following Sharma and Sharma (2002). Root meristems from germinating cypselas collected in the wild were used. The germination duration was between 5 days to 2 weeks, depending on the species. Samples were pretreated with 0.002 M 8-hydroxyquinoline at 4°C for 8 h. The material was fixed with Carnoy for 24 h at low temperature. Before staining, the material was hydrolyzed with 1 N HCl for 20 minutes at room temperature. It was stained with 1% aceto-orcin and mounted in 45% acetic acid (Sharma & Sharma 2002, Singh 2002). For all the counts, at least three plates from different individuals were examined. The best metaphase plates were photographed using a BX40 microscope (Olympus, Tokyo, Japan) with digital

camera attachment. The examined plant materials are presented in Table 1 that they were collected from different localities in Iran, in 2010-2013. The herbarium vouchers are preserved in the herbarium of Department of Biology at the University of Isfahan (HUI).

RESULTS

The provided results of each species are given below:

Jurinea cartilaginea Mozaff.

This taxon is endemic to south west of Iran and is found in a limited area of north east of Khuzestan province (Mozaffarian 1988). The chromosome number in this species showed $2n=30$ (Table 1, Fig. 1). According to our data, this is the first chromosome count for this species.

J. carduiiformis (Jaub. & Spach) Boiss.

J. carduiiformis is an endemic taxon in *Flora Iranica* range (Rechinger 1979). We studied two populations of this taxon, one from a population of Kerman province, and another from a population of Isfahan province. It exhibited $2n = 34$ (Table 1, Fig. 2) in both of the mentioned populations. This is the third count for this species. Our results did not agree with the previous reports of $n = 16$ by Ghaffari (1986 and 1988). This is a new different chromosome number for this taxon.

Aegopordon berardioides Boiss.

This taxon is endemic to Iran, Afghanistan and Pakistan (Rechinger 1979). It exhibited $2n=34$ (Table 1, Fig. 3). In here mixoploidy is shown in the same preparations. Where diploid chromosomes ($2n=2x=34$) were counted, tetraploid cells ($2n=4x=68$) were observed also (Fig. 7). With this study, the chromosome number of the taxon is given for the first time.

Cousinia argentea Mehregan & Assadi

It is determined that the somatic chromosome number is $2n = 26$ (Table 1, Fig. 4). This taxon is endemic to north east of Iran (Rechinger 1979, Mehregan & Assadi 2009). According to our data, the chromosome number of the taxon is reported here for the first time.

Centaurea amadanensis Schultz-Bip. var. *gymnoclada* (Jaub. & Spach) Wagenitz.

As far as we reviewed, this is the third count for this species, and also this is the first count for this variety. This variety is endemic to west of Iran according to *Flora Iranica* (Wagenitz 1980). In our new count, chromosome number observed is $2n = 18$ (Table 1, Fig. 5) that is in accordance with the previous reports (Garcia-Jacas *et al.* 1998b, Ghaffari & Shahraki 2001). In this taxon, mixoploidy is observed in the same preparations. Where diploid chromosomes ($2n=2x=18$) were counted, tetraploid cells ($2n=4x=36$) were determined also (Fig. 8).

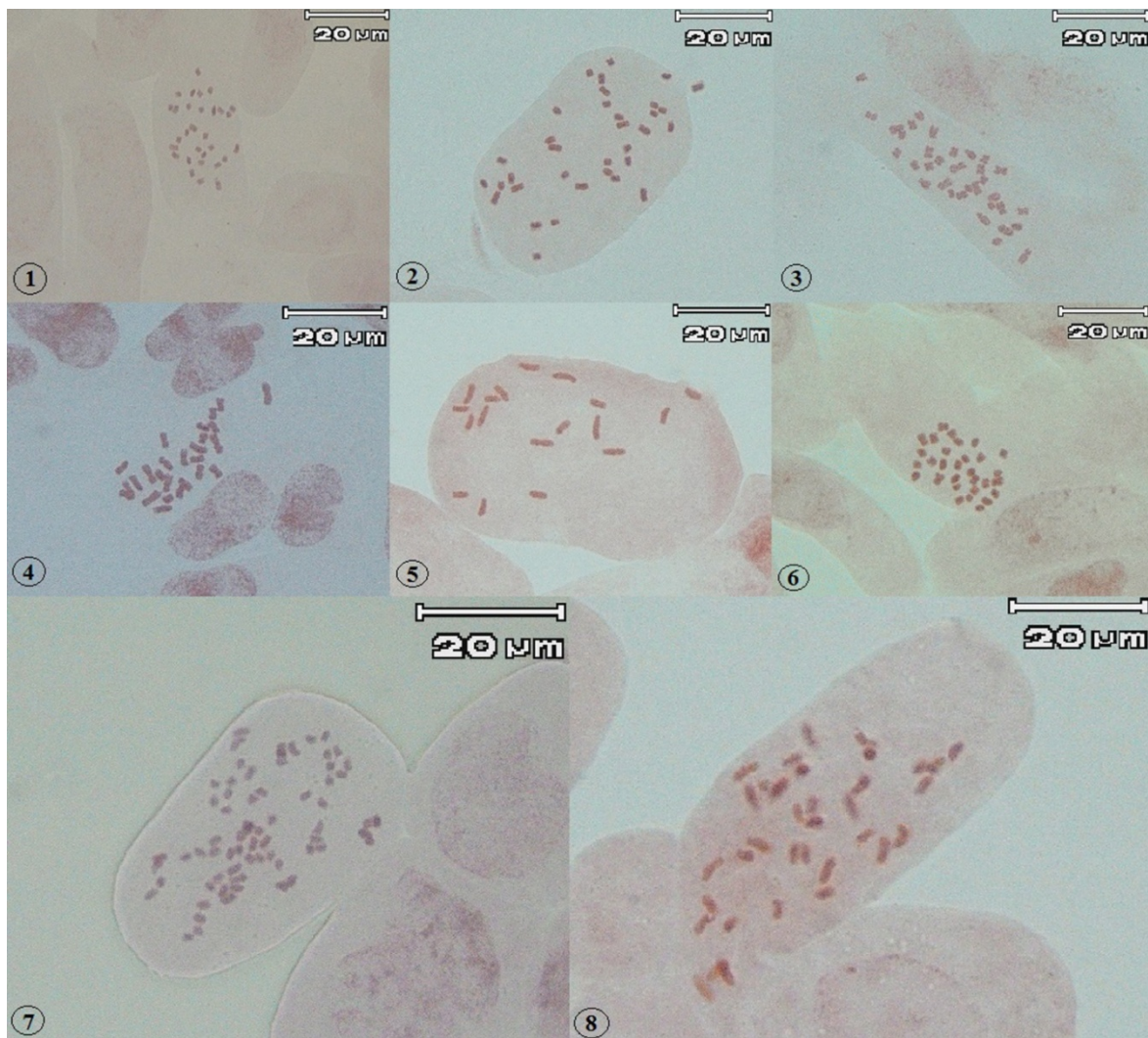
Nikitinia leptoclada (Bornm. & Sint.) Iljin

It exhibited $2n=30$ (Table 1, Fig. 6). The monotypic genus is endemic to the Kopet Dag mountains in south of Turkmenistan and north east of Iran (Rechinger 1979, Häffner 2000). This is the first chromosome count for this species.

DISCUSSION

Variation in chromosome number is probably through hybridization, euploidy, and aneuploidy in Asteraceae (Funk *et al.* 2009, Häffner 2000). It may be suggested based on the data that the tribe Cardueae could be subdivided into two main phyletic groups which one with $x = 9$ and 8 and the second with $x = 6, 5$ and 4 (Missouri Botanical Garden, <http://www.tropicos.org/Project/PCN>; Watanabe 2013, <http://www.lib.kobe-u.ac.jp/products/asteraceae/e-index.html>). Nevertheless, the chromosome numbers differ considerably among different genera of the tribe and the differences are correlated with appreciable morphological variability and these characters will render them useful in construction of general classification. Chromosomes data are capable to provide information to confirm natural boundaries for generic delimitations. Akhtar (2005) argued that chromosomal features are basic to an understanding of relationships in Cardueae and it is more useful at generic level.

Based on the chromosome numbers reported of the genus *Jurinea* (Missouri Botanical Garden, <http://www.tropicos.org/Project/PCN>; Watanabe 2013, <http://www.lib.kobe-u.ac.jp/products/asteraceae/e-index.html>), $2n=30$ in *Jurinea cartilaginea* is not far-fetched. Nonetheless, the suggestion of changing systematic position of this species and the possibility of its separation from *Jurinea* or at least changing its section, can be taken into account on the bases of the basic chromosome number proposed for *Jurinea* ($n=x=17$). The above suggestion can be strengthened based on the fact that *J. cartilaginea* is unique for some of its cypsela features such as the mucilage coat of cypsela during soaking (which does not happen in the other Iranian *Jurinea* species), the inner papus of 5-7 long bristles with a broader base than the base of the other bristles, and pentagonal structure which are unique among the other *Jurinea* species. On the other hand, this reduction in basic chromosome number ($n=x=17$ compare with $n=x=15$) may be related to climatic and ecosystem conditions of the habitat of this taxon. Because this plant lives in a semi-arid environment, very warm (at summer) and partly mountainous; likely this descending dysploidy could be correlated with taxon adaptation to the warm and xeric conditions (the same reports in Selvi & Bigazzi 2002, Watanabe *et al.* 1999).



Figs. 1-6. Somatic metaphases: 1- *Jurinea cartilaginea* $2n=30$; 2- *J. carduiformis* $2n=34$; 3- *Aegopordon berardioides* $2n=34$; 4- *Cousinia argentea* $2n=26$; 5- *Centaurea amadanensis* var. *gymnoclada* $2n=18$; 6- *Nikitinia leptoclada* $2n=30$.

Figs. 7-8. Polyploidy in Somatic metaphases: 7- *Aegopordon berardioides* $2n=4x=68$; 8- *Centaurea amadanensis* var. *gymnoclada* $2n=4x=36$.

Susanna *et al.* (2006) transferred *Outreya carduiformis* to *Jurinea carduiformis* on the bases of molecular similarity. Based on the chromosome numbers reported for *Jurinea* which mostly are $2n=34$ (Missouri Botanical Garden, <http://www.tropicos.org/Project/IPCN>; Watanabe 2013, <http://www.lib.kobe-u.ac.jp/products/asteraceae/e-index.html>), the exhibited chromosome number in our study for this species ($2n=34$) can confirm this changing.

The obtained chromosome number for *Aegopordon berardioides* in the present study ($2n = 34$) shows more similarities between the two genera *Aegopordon* and *Jurinea*, which can be taken as evidence for a systematic

change of *Aegopordon berardioides* to *Jurinea berardioides*, a suggestion provided by Susanna *et al.* (2006) on the basis of a molecular investigation (of course, it was not indexed in IPNI yet).

It was mentioned in the introduction that Mehregan and Assadi (2009) transferred *Jurinea pungens* to their *nomen novum* *Cousinia argentea*. According to the chromosome number given for *Cousinia argentea* in the present study, we can confirm their decision. This is in accordance with $2n=26$ and $n = 13$ for *Cousinia* reported by: Afzal-Rafii, (1980), Djavadi (2005, 2007, 2012), Djavadi & Attar (2010), Djavadi & Ghaffari (1999), Ghaffari (1984, 1986a), Ghaffari & Chariat-

Panahi (1985), Ghaffari & Djavadi (1998), Ghaffari *et al.* (2006), Lopez-Vinyallonga *et al.* (2009a), Sheidai *et al.* (2006), Sheidai *et al.* (2012) and Susanna *et al.* (2003b).

Considering the chromosome numbers reported for the section *Paraphysis* and the other related sections of the genus *Centaurea* (Bakhshi-Khanaki 1995a, 1995b and 1996, Ghaffari 1986, 1988 and 1989, Ghaffari & Shahraki 2001, Garcia-Jacas *et al.* 1998, Ranjbar & Negareh 2013), we concluded that the common basic chromosome number in these sections can be $x=9$. Also, the chromosome number obtained for *Centaurea amadanensis* var. *gymnoclada* in this research supports this issue.

At 1960, Iljin changed *Jurinea leptoclada* to *Nikitinia leptoclada* based on the different morphological characters such as receptacle subcylindrical, warty filaments and multi-seriate pappus. Then Susanna *et al.* (2002) did another systematic change in the position of the subtribe of this taxon. They transferred it from *Carduinae* to *Centaureinae* based on a study of pappus characters. The diploid number ($2n=30$) along with the considerably small size of the chromosomes comparing to that of the *Jurineas*' confirm the changes offered above, surely.

Based on our study we suggest that the dysploidy and polyploidy can be regarded as the main mechanisms of chromosomal evolution in *Cardueae*. Indisputable, detailed karyotype analyses combined with molecular studies can help to overcome the unresolved taxonomic confusions of *Cardueae* plants.

Finally, comprehensive researches including morphological, cytological, and molecular characters should be directed to evaluate the various cytogenetic hypotheses, reappraisal the current classifications, and understand the phylogenetic relationships among the genera in *Compositae*.

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