KARYOLOGICAL STUDY OF SOME ONOPORDUM L. (ASTERACEAE) SPECIES IN IRAN

E. Aghababaeyan, M. Pakravan & A. Tavassoli

Received 2015. 07. 21; accepted for publication 2015. 12. 15

Aghababaeyan, E., Pakravan, M. & Tavassoli, A. 2015. 12. 31: Caryological study of some *Onopordum* L. (Asteraceae) species in Iran.- *Iran. J. Bot. 21 (2): 152-157*. Tehran.

The somatic chromosome numbers and karyotype of five species of *Onopordum* L. *(Asteraceae)* from Iran were investigated. Our results confirm that all of the species are diploid (2n=34). First chromosome reports are presented for *O. leptolepis* DC., *O. heteracanthum C. A. Mey.* and *O. carduchorum Bornm & Beauverd.* Besides, the chromosome numbers of O. *armenum Grossh., O. carduchorum* and *O. acanthium* L. are reported from Iran for the first time, confirm the previous reports. Karyotype formulae for *O. armenum*, *O. carduchorum* and *O. leptolepis* were similar (1Sm+16m), for *O. heteracanthum* and *O. acanthium* were respectively 17m and 1M+16m+2B. Satellites were commonly found on submetacentric pairs, being also variable in morphology and location.

Elham Aghababaeyan, Maneezheh Pakravan (Correspondence <mpf176@yahoo.com>) & Akhtar Tavassoli, Faculty of Biological Science, Alzahra University, Sheikh-Bahaee Sq., Tehran, I. R. Iran.

Keywords: chromosome; Iran, karyotype, *Onopordum*,

بررسی کاریولوژیک برخی از گونه های Onopordum L. (تیره کاسنی) در ایران

الهام آقابابایان، کارشناس ارشد، دانشگاه الزهراء

منیژه پاکروان، دانشیار، دانشگاه الزهراء

اختر توسلی، استادیار دانشگاه الزهراء

تعداد کروموزوم های سوماتیک و کاریوتایپ در پنج گونه از Onopordum از ایران مورد بررسی قرار گرفت. نتایج ما سطح دیپلوییدی (2n=۳۴) را در تمام گونه ها تایید نمود. کاریوتایپ برای گونه های O. leptolepis, O. heteracanthum و O. carduchorum برای اولین بار ارائه می‌شوند. علاوه بر آن اعداد کروموزومی برای گونه های O. armenum, O. carduchorum و O. acanthium برای اولین بار از ایران گزارش شده و تایید کننده گزارشات قبلی می باشد. فرمول کاریوتایپی برای O. armenum, O. carduchorum وO. leptolepis مشابه بوده (1Sm+16m) برای O. heteracanthum و O. acanthium بترتیب 17m و 1M+16m+2B بود. ماهواره ها در بیشتر کروموزوم های متاسنتریک دیده شد ولی در شکل و جایگاه کروموزومی خیلی تنوع داشت.

INTRODUCTION

The genus *Onopordum* L. has about 40 species in the world, belongs to *Cardueae* tribe, native to Europe (mainly the Mediterranean region), N Africa, the Canary Islands, the Caucasus, and west and central Asia (Keleonikos, 2006). There are seven species and one subspecie of *Onopordum* in Iran (Rechinger, 1991), that one is endemic (Mozaffarian, 1996; Aghababaeyan et al. 2014). From a karyological point of view, the genus *Onopordum* has been poorly understood. In particular, relatively little is known about the Iranian species and only the chromosome numbers for two species of the genus is reported from Iran (Ghahremaninejad et al 2013). T o date only the karyotypes of about one to two species has been described (Garcia et al. 2006). The genus has basic chromosome number of X=17 (Lopez-Vinyallonga, et al., 2010). In this paper chromosomal data of five species of *Onopordum* are reported.

The aim of the present work was the evaluation of the chromosome data of some *Onopordum species* in Iran and characterization of the karyotypic details. The

results are discussed in relation to the taxonomic position of the species.

MATERIALS AND METHODS

Samples of the *Onopordum* species (*O. leptolepis* DC., *O. heteracanthum* C.A. Mey., *O. carduchorum Bornm &* Beauverd, O. *armenum Grossh.* and *O. acanthium* L.) were collected from different localities of Iran during 2011 to 2013 (table. 1). At least sample s of 14 population were collected. Voucher are preserved at Alzahra University herbarium (ALUH). Mitotic studies were done with young roots obtained by germinating seeds from wild seeds. The root tips were treated with 0.002M 8-hydroxyquinoline for about 4-8 h at 4°C temperature, fixed in Farmer's fixative – ethanol : acetic acid (3 : 1) (Löve & Löve 1975) and stored at 4 ° C in 70% ethanol. Material was hydrolyzed by 1 M hydrochloric acid for 3 minutes and stained with acetic Orceine solution for about 18-24 h and gently heated during 3 minutes. Finally 45% acetic acid was added to stop stain reaction and the tips were squashed on clean slides. Metaphase plates were photographed with a digital camera (DP 12) mounted on an Olympus B51 microscope. Whenever possible, chromosome morphology and idiograms were analyzed in three or four mitotic metaphase plates per population, preferably from different plants.

Table 1: Voucher of *Onopordum* studied specimens. Abbreviations: ALUH: Alzahra University Herbarium.

Species	**Collecting data**
*O. acanthuim*L	Zanjan, Aghababaiyan, 90110 ALUH
	Azerbaijan: 8 km to Kaleibar from Meshkinshahr, Aghakuchaki, 11034 ALUH
O. carduchorum Bornm & Beauv	Gilvan, Aghababaiyan, 90111 ALUH
	Lorestan, Pol-e Dokhtar, Aghababaiyan, 11038 ALUH
	Lorestan, Khoram abad, Aghababaiyan, 11040 ALUH
O. *armenum Grossh.*	Azarbayjan Sharghi, 8km Kalibar, Aghakuchaki, 90112 ALUH
	Mianeh, Aghababaiyan, ۱۱۰۱۴ ALUH
	Ghazvin, 10 Km to Ghazvin, Aghababaiyan, 11015 ALUH
O. heteracanthum C.A. Mey.	Isfahan, Najafabad to Tehran road, Aghababaiyan, 90113 ALUH
	Ghazvin, 10 Km from Ghazvin to Zanjan, Aghababaiyan, 11025 ALUH
	Isfahan, Aliabad, Aghababaiyan, 90115 ALUH
	Kermanshah, Gilane Gharb, Latechegha, Najafian, 90116 ALUH
O. leptolepis DC.	Kohkiluie Buierahmad, Sisakht, Aghababaiyan, 90117 ALUH
	Alborz, Golshahr, Aghababaiyan, 90118 ALUH

All chromosome sizes were measured with computer–aided program Image Tools 3.0 . The parameters measured for each metaphase chromosome spread included Total Chromosome Length of the haploid complement (TL), Total Form percent (TF%: Ratio between the shortest arms of the chromosomes and their total length). The TF% value was considered to be close to 50% in most symmetric karyotypes and less than 50% based on the degree of asymmetry, (Huziwara, 1962), S% (equals to length of the shortest chromosome divided on length of the longest chromosome, Stebbins, 1971) D. R. L (the relative maximum Subtract the relative minimum) (Huziwara, 1962) and Karyotype formulae: according to their arm ratios (long/short) designated by the position of the centromere. The terminology of Levan et al. (1964) has been used for describing the morphology of chromosomes and the pairs established. The terminology of Stebbins (1971) has been used for the apparent size of chromosomes and general karyotype asymmetry.

RESULTS AND DISCUSSION

Chromosome numbers and formulas of the species studied are shown in table 3, Metaphase plates and ideograms are presented in Figs. 2 & 3. According to table 2 studied species have basic chromosome number of x=17.

All of the studied populations were diploid (2n=34). As far as we determined from the literature (table 2) and the chromosome number databases; Index to Plant Chromosome Numbers (Missouri Botanical Garden; http://mobot.mobot.org/W3T/Search/ipcn.html) and Index to Chromosome Numbers in the Asteraceae (WATANABE 2002,http:// www-asteraceae.cla.kobe-u.ac.jp/index.html), our reported number of 2n=34 and karyotype characters are the first report for following species: *O. carduchorum, O. heteracanthum* and *O.*

leptolepis and our report for *O. acanthium* and *O. armenum* agree with the previous reports but are the first report from Iran (table 2). According to our data, the shortest chromosome measured is pair no. 17 of *O. acanthium* (o.85 µm)(table.4), the longest is pair no. 1 of *O. heterocanthum* (3.41 µm) (Tab. 3&8). Two B-chromosomes have been found in O. acanthium. Chromosome pairs No. 1,9 in O. acanthium, No. 11, 15 in O. heterocanthum, No. 3, 4 in O. leptolepis, No. 4 in O. armenum, and No. 2,3,5 in O. cardochorum had satellites on short arms (figs.2& 3). The presence of satellites is variable and their presence or absence cannot be used to characterize species of taxonomic groups (Fritsch & Astanova 1998).

Table 2: A summary of the chromosomal status of *Onopordum* species.

Species	**2n**	**n**	**Previous references**
O. acanthium	34		Ge et al. 1988
	34		Dempsey et al. 1994
	34		Albers et al. 1998
	34		Lövkvist et al. 1999
	34		Morton, 1977, 1981
	34		Van Loon et al. 1980
	34		Kuzmanova et al. 1979
	34		Moore & Ferankton, 1962
	34		Skalinsk 1971
	34		Podlech 1969
O. armenum	34		Gukasian et al. 1990
O. leptolepis	_	17	Ghaffari, 1989
	34		Podlech & B 1974
	34		Podlech & 1969
O. heteracanthum		17	Aryavand, 1975
		17	Ghaffari et al. 1985

Karyotype formula as 1Sm+16m belongs to O.armenum, O. cardochorum and O. leptolepis (table 3). Karyotype formula as 1M+16m+2B belongs to O. acanthium and karyotype formulated as 17m belongs to *O. heterocanthum* (table 3).

Species can thus be distinguished by a combination of karyotype formula and position of satellites in a particular chromosome pair (Table 2). Karyotype length and asymmetry index are also useful to discriminate some taxa.

This genus has relatively asymmetric karyotype, with chromosomes varying in mean total chromosome lengths from 1.3 (in *O. acanthium*) to 2.1µm (in *O. heteracanthum*). As presented in Table 3, the metacentric (M and m) chromosomes dominated the observed karyotypes and the second frequency belongs to the submetacentrics. The highest TL was found in *O. heteracanthum* (35.91µm) and the lowest chromosome length was scored in *O. acanthium* (23.61µm) (Table 3). The degree of karyotype asymmetry as indicated by TF% values ranged from 41.38% (*O. carduchorum*) to 42.61% (*O. acanthium*) (Table 3). As the TF% values were near to 50%, we can conclude that type of chromosomes were metacentric to submetacentric. Also S% (Stebbins 1971) indicating symmetry index was from 34.79% (*O. heteracanthum*) to 41.49% (*O. acanthium*). Following Stebbins' karyotype classification (1971), all species fall into the 3B-category.

The karyological characters may be essential for drawing significant conclusions on the relative closeness and distance of the various taxa. However, they are not enough to draw any definitive taxonomic conclusion in the genus.

Detailed karyotype analyses, including the use of chromosome banding techniques, combined with molecular studies required to establish the sub generic natural classification system of *Onopordum*. Our present data in here will throw some more light on the caryological knowledge of the genus.

Table 3. Karyotype analysis of the *Onopordum* species (TL=Total haploid chromatin length, X=Mean Chromosome Length, TF%=Total Form percent, D.R.L.= difference of range of relative length; TF= total form percentage; AT = Stebbins karyotype asymmetry type; S%=Length of the shortest chromosome divided on length of the longest chromosome, KF= karyotype formulae, n=chromosome number).

Species	**T.L.**	**X**	**TF%**	**D.R.L**	**ST**	**S%**	**KF**	**2n**
O. acanthium	23.61	1.3	42.61	5.12	3B	41.49	1M+16m+2B	**34**
O. carduchorum	25.34	1.4	41.38	6.01	3B	36.14	1Sm+16m	**34**
O. armenum	30.33	1.7	41.84	5.06	3B	39.81	1Sm+16m	**34**
O. leptolepis	31.74	1.8	41.4	6.02	3B	35.27	1Sm+16m	**34**
O. heteracanthum	35.91	2.1	41.97	6.2	3B	34.79	17m	**34**

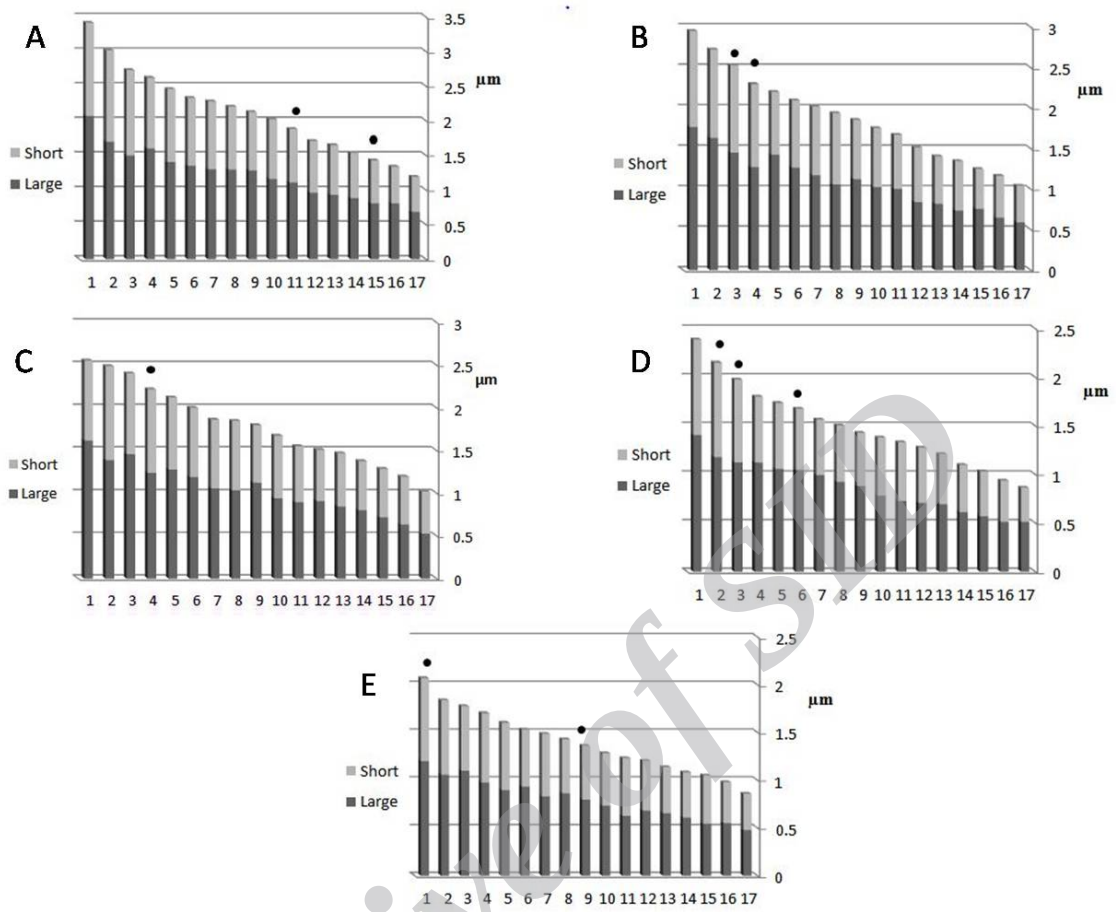

Fig. 1: Ideograms. A, *O. heteracanthum*; B, *O. leptolepis*; C, *O. armenum*; D, *O. carduchorum*; E *O. acanthium*.

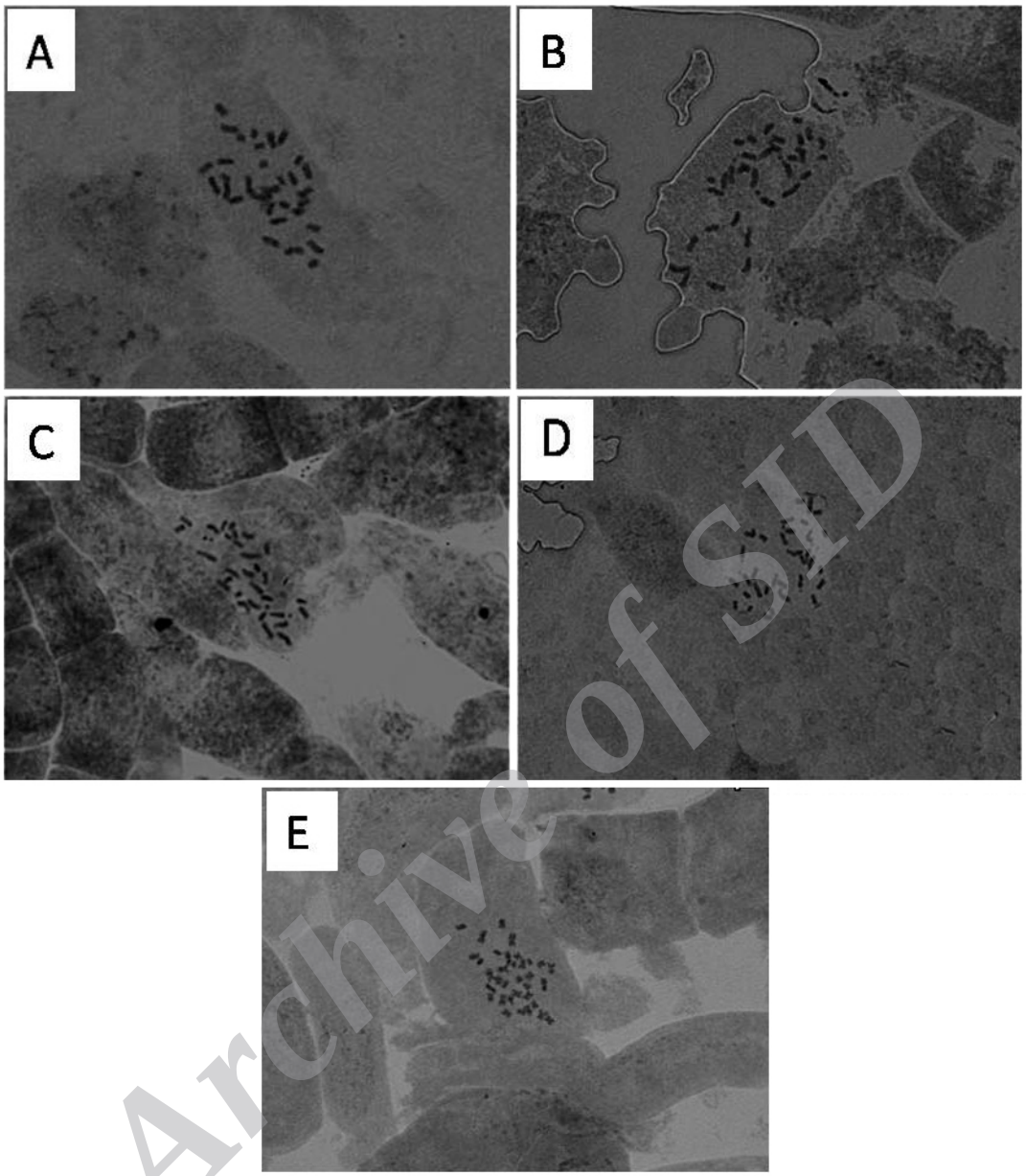

Fig 2: Somatic chromosomes. A,: *O. heteracanthum*; B, *O. leptolepis*; C; *O. armenum*; D, *O. carduchorum*; E, *O. acanthium*.

REFERENCES

Aghababaiyan, E. 2013: Systematic study of the genus *Onopordum* L. (Asteraceae) in Iran. unpublished Msc. Thesise , Alzahra University, Tehran.

Aghababaeyan, M., Pakravan,M. & Ghahremaninejad, F. 2014.: *Anatomical Study and Pollen Micromorphology of Onopordum L. in Iran*. Not. Sci. Biol. 6(1):66-76.

Albers, F. & Pröbsting, W. 1998: In R. Wisskirchen & H. Haeupler, Standardliste der Farn- und Blütenpflanzen Deutschlands. Bundesamt für Naturschutz & Verlag Eugen Ulmer, Stuttgart.

Aryavand, A. 1975: Contribution a l'etude cytotaxonomique de quelques angiospermes de l'Iran. -Bot. Not. 128: 299–311.

Davis, P. 137:. Asteraceae in: Davis, P. (ed.) Flora of Turkey and the east Aegean Islands. vol.5: 356-369, Edinb. Univ. Press.

Dempsey, R. E., Gornall, R. J. & Bailey, J. P. 1994: Contributions to a cytological catalogue of the British and Irish flora, 4. - Watsonia 20: 63–66.

Fritsch, R.M. & Astanova, S.B. 1998: Karyotypes in different sections of Allium L. Subge. Melanocrommyum (Webb et Berth) Rouy from central Asia. - Fedd. Repert. 109: 539-549.

Fritz, E. & Saukel, J. 2011: Secretory structures of subterranean organs of some species of the Cardueae, and their diagnostic value. Acta Biol Cracov Bot., 53/1: 63-73.

Garcia-Jacas, N., Susanna, A. , Hidalgo, O. , Vilatersana, R. & Garnatje, T. 2006: The Cardueae (Compositae) revisited: insights from ITS, trnL-trnF, and matK nuclear and chloroplast DNA analysis. Ann. Missouri. Bot. Gard. 93: 150-171.

Ge, C.j.Y.k.L., Wan, P., Li, Y.x. & Jiang, F.H. 1988: Observations on the chromosome numbers of medicinal plants from Shandong Province (V). J. Shandong Coll. Traditional Chin. Med. 12: 55–57.

Ghaffari, S. M. 1989: Chromosome studies in Iranian Compositae. Iran. J. Bot. 4: 189–196.

Ghaffari, S. M. & Chariat-Panahi, M. S. 1985: Chromosome counts of some angiosperms from Iran. Iran. J. Bot. 3: 67–73.

Ghahremaninejad F., NejadFalatoury A., Mahmoodi M., Fereidounfar S., Hoseini E.: *Plant chromosome book of Iran*. First edition 2013; House of Biology, Tehran.

Gukasian, A. G. & Safarian, A. B. 1990: Chromosome numbers of some representatives of Armenian flora. Biol. Zurn. Armen. 43: 259–260.

Huziwara, Y. 1962: Karyotype Analysis in some genera of Compositeae VIII. Further studies of choromosome of *Aster*. -Amer. J. Bot., 49: 116-119.

Kleonikos G. Stavridakis, 2006: Wild edible plants of Crete. 960-631-179-1, Rethymnon Crete, ISBN.

Kuzmanova, B., Georgieva, S. & Nikolova, V. 1979: In IOPB chromosome number reports LXIV. Taxon 28:408.

Levan, A., Fedge, K. & Sandberg, A. 1964: Numenclature for centrometric position on chromosome. Heriditas, 52: 210-220.

Lopez-Vinyallonga, S., Susanna, A. & Garcia-Jacas, N. 2010: Chromosome numbers in the genera *Cousinia*, *Olgaea* and *Syreitschikovia* (Compositae). Folia Geobot., 45: 201-214.

Löve, Á. & Löve, D. 1975: *Plant chromosomes*. J. Cramer, Vaduz.

Lövkvist, B. & Hultgård, U. M. 1999: Chromosome numbers in south Swedish vascular plants. Opera Bot. 137: 1–42.

Moore, R. J. & Frankton. 1962: Cytotaxonomic studies in the tribe *Cynareae*. – Canad. J. Bot. 40: 281-293.

Morton, J. K. 1977: A cytological study of the Compositae (excluding *Hieracium* and *Taraxacum*) of the British Isles. -Watsonia, 11: 211-223.

Morton, J. K. 1981: Chromosome numbers in Compositae from Canada and the U.S.A. Bot. J. Linn. Soc. 82: 357–368.

Mozaffarian, V. 1996: A dictionary of Iranian plant name. Farhang Mo'asser, Tehran (Persian).

Podlech, D. & Dieterle, A. 1969: Chromosomenstudien an afghanischen Pflanzen. - Candollea**.** 24: 185-243.

Rechinger, K.H. , Dittrich, M. , Petrak, F. and Wangenitz, G. 1991: Compositae III._ Cynareae in Rechinger K. H. (ed.), Flora Iranica, CompositeaeIII, 139-a. p.186, Acad. Druck.u.Verlastantal. Graz.

Stebbins, G. L. GR. 1971: Chromosomal evalution in Higher plants. Edward Arnold, London..

Van Loon, J. C. & Kieft, B. 1980: In Chromosome number reports LXVIII. -Taxon 29: 538–542.

Watanabe 2002*: Index to chromosome numbers in Asteraceae*. http://www-asteraceae.cla.kobe-u.ac.jp/index.html

Malik, R. A. & Gupta, R. C. 2013: Meiotic studies in some selected members of Gamopetalae from Kashmir Himalaya. -Plant Syst. Evol.. 299 (8): 1549-1560.

Khoskyhos, D. W. & Raven, P. H. 1974:Chromosome nubmers in Compositae. X. -Amer. J. Bot. 61 (8): 909-913.

Skalinska, M., Czapik, R., Piotrowicz, M., 1959: Further studies in chromosome numbers of Polish angrosperms (Dicotyledons). -Acta Soc. Bot. Polon., Warszawa. 28: 487-529.

Gukasian, A.G. & Safarian, A.B. 1990: Chromosome numbers of some representatives of Armenian flora.- Biol. Zhurn. Armen. 43: 259-260.

Podlech, D. & Bader, O. 1974: Chromosomenstudien an Afghanischen Pflanzen. II. -Mitt. Bot. Staatssamml. Munchen. 11: 457-485.