CARYOLOGIA Vol. 64, no. 3: 302-308, 2011

Chromosome number variation in *Tanacetum polycephalum* Schultz Bip. (L.) (Asteraceae) in west of Iran

Chehregani Abdolkarim [1,*], Morteza Atri[1], Jalal Sarmadi[1] and Mahtab Asgari[2]

[1]Lab. of Plant Cell Biology, Dept. of Biology, Bu-Ali Sina Univ., Hamedan, I.R. Iran.
[2]Department of Biology, Payame-Nour University, Hamedan, I.R. Iran.

Abstract — Mitotic chromosome numbers are reported for fourteen populations of *Tanactum polycephalum* Schultz Bip. (L.) collected from west of Iran. In *T. polycephalum,* chromosome numbers were varied from 2n= 2x=18 to 2n=6x= 54. One diploid population (2n=2x=18) and two populations with both diploid and tetraploid chromosome numbers (2n=2x=18 and 36) were identified. Five populations with chromosome number (2n=4x=36) and a population with two different chromosome numbers (2n=4x=36 and 54) were found for the first time. In this species, four populations with hexaploidy (2n=6x=54) were detected for the first time. Results indicate that polyploidy is confirmed as the most significant evolutionary trend in chromosome number within this species. This species is also a problemic and polymorph taxnon and our results indicate that its polyploidy can be regarded as the reason.

Key words: biodiversity, chromosome number, polyploidy, *Tanacetum polycephalum*.

INTRODUCTION

The Asteraceae is the largest plant family. The family comprises more than 1600 genera and 23000 species (Kadereit and Jeffrey 2007; Funk *et al*. 2009). Its many genera and species, its worldwide distribution and the fact that it comprises many useful plants have made it the subject of many karyological studies (Watanabe 2002). Many karyological and cytological studies have been performed in the Asteraceae (Carr *et al.* 1999; Vallès *et al*. 2005; Chehregani and Mehanfar 2008; Chehregani and Hajisadeghian 2009). The most common basic chromosome number in the Anthemideae is x=9, although x=8 and x=10 have also been reported by some researchers (Carr *et al*. 1999; Vallès *et al*. 2005; Chehregani and Hajisadeghian 2009). Nevertheless; it seems that chromosome numbers are currently known for less than 40% of species of the family; so new studies are still necessary to improve the knowledge of these plants (Volkova and Boyko 1986; Vallès *et al*. 2005). The native flora of Iran comprises about 8000 angiosperm species. Chromosome counts on Iranian material have so far been carried out for about 1500 species, but in many cases only a single chromosome count has been studied (Ghaffari and Kelich 2006).

Polyploidy is currently considered a prominent force in plant evolution and represents the most common mode of sympatric speciation in plants (Wendel and Doyle 2005 and references cited therein; Chehregani *et al.* 2010). Polyploids, moreover, may have superior levels of adaptability and higher probabilities of survival than their diploid relatives (Levin 1983; Thompson and Lumaret 1992; Soltis and Soltis 2000). Chromosome data currently available show polyploidy is the most significant evolutionary trend in chromosome number within Asteraceae (Carr *et al.* 1999; Vallès *et al*. 2005; Chehregani *et al.* 2010).

The genus *Tanacetum* (L.), formerly *Pyrethrum* (Zinn.), is a large, poorly defined classification group in the Asteraceae (Compositae)

*Corresponding author: e-mail: chehregani@basu.ac.ir

containing polymorph species, many of which have applications as herbal medicines (Brown *et al.* 1999). *Tanacetum* is one of the largest genera of the family Asteraceae, containing 250-500 taxa depending on the opinions of the authors who have studied the genus (Mc Arthur and Plummer 1978; Mabberley 1990; Ling 1991a; b; 1995a; b; Bremer and Humphries 1993; Kadereit and Jeffrey 2007; Funk *et al*. 2009). It is distributed throughout the Northern Hemisphere, with very few representatives (not more than 10 species) in the Southern Hemisphere. Many species of the genus are very abundant and significant in a range of habitats and are used as sources of medicines, food, forage or other useful products. West and central Asia are two important speciation centers of the genus (Valles *et al.* 2005). *Tanacetum polycephalum* is an unknown and problemic species in the genus. It seems that the genus is a polyphyletic complex and should be revised carefully regarding its species and subgenus (Judd *et al.* 1999; Watson *et al.* 2000; Valles *et al.* 2003).

There are only three chromosome number reports for *T. polycephalum*. Diploid chromosome number $2n=2x=18$ was reported for an Armenian population of this species (Khandjan 1992). Our prior reports showed also $2n=8x=72$ for a population collected from Hamedan, Iran (Chehregani and Hajisadeghian 2009) and $2n=10x=90$ for a population from Zanjan, Iran (Chehregani and Mehanfar 2008). Nevertheless, *T. Polycephalum*, particularly the Iranian populations, are poorly known in terms of karyology and there are few chromosome counts that were report different chromosome numbers for a species. Our aims in this paper are to study chromosome number and polyploidy in different populations of *Tanacetum polycephalum* in the west of Iran, to provide more information about ploidy level variation in this poorly known species.

MATERIAL AND METHODS

Plant materials - Studied plant samples were collected from the west of Iran, mainly Hamedan province (Fig. 1). Herbarium vouchers of all species studied are deposited in the herbarium of Department of Biology; Faculty of Science; Bu-Ali Sina University; Iran (BHU). The locations, collectors and dates are shown in the Table 1.

Karyological studies - Chromosome counts were made on somatic metaphases using standard squash techniques. Seeds collected in the wild were used in the present study. Root-tip meristems were obtained by germinating seeds on wet filter paper in Petri dishes at approximately 20°C. Samples were pretreated with

Fig. 1 — A map of Iran (left) that shows Hamedan province in the west of Iran and a map of Hamedan province (right) that plant collection areas were indicated (*).

TABLE 1 — The populations of *Tanacetum polycephalum* that were subjected for karyiological studies.

Date of collection	Chromosome number	Collector	Altitude (m)	Locations	Herbarium vouchers	Name of species	
3 December 2008	2n=18	Sarmadi	2313	Kermanshah, Assadabad road km 40, east slope	23062	*Tanacetum polycephalum*	1
2 December 2008	2n=36	Sarmadi	2211	Hamedan, Ganj Nameh, above the waterfall, western slope	23058	*T. polycephalum*	2
2 December 2008	2n=36	Sarmadi	2199	Hamedan, Ganj Nameh, above the waterfall high rooks, western slope	23059	T. polycephalum	3
8 December 2008	2n= 36	Sarmadi	2173	Hamedan, Khangormaz protected area, western slope	23065	*T. polycephalum*	4
10 December 2008	2n=36	Sarmadi	2390	Hamadan, Razan, Bughaty mountain, western slope	23068	*T. polycephalum*	5
6 December 2008	2n=36	Sarmadi	2389	Hamadan, Razan, Bughaty mountain end of road, south slope	23070	*T .polycephalum*	6
2 December 2008	2n=36	Sarmadi	2351	Zanjan, Shirinsoo Road, Aghdaghi mountain, east slope	23071	*T .polycephalum*	7
4 December 2008	2n=18,36	Sarmadi	2206	Hamedan, Ganjnameh, above the waterfall , north slope	23060	*T. polycephalum*	8
6 December 2008	2n=18,36	Sarmadi	2396	Zanjan, Shirinsoo Road, Aghdaghi mountain, western slope	23069	T .polycephalum	9
4 December 2008	2n=54	Sarmadi	2304	Hamedan, Hamedan toAsadabad road km 40, western slope	23061	*T. polycephalum*	10
2 December 2008	2n=54	Sarmadi	2033	Hamedan, Asadabad, Rasulabad Village, Almagholagh mountain, northwest slope	23063	*T. polycephalum*	11
8 December 2008	2n=54	Sarmadi	2277	Hamedan, Kabudrahang, three way of Goltape to Kabudrahang, Soobashy mountain, northwest slope	23066	*T. polycephalum*	12
10 December 2008	2n=54	Sarmadi	2321	Hamedan, Kabudrahang, three way of Goltape to Kabudrahang, Soobashy mountain, north slope	23067	*T. polycephalum*	13
8 December 2008	2n= 36,54	Sarmadi	2072	Hamedan, Asadabad, Rasulabad Village, Almagholagh mountain , western slope	23064	*T. polycephalum*	14

0.05% colchicine for 2h 30min at room temperature. The material was fixed in 3:1 v/v absolute ethanol:glacial acetic acid for a minimum of 24h at 4°C. Meristems were hydrolysed in 1M hydrogen chloride (HCl) for 30min at room temperature. They were then stained in 2% acetic orcein for a minimum of 3h at 4°C (CHEHREGANI *et al.* 2010). Squashes were made in 45% acetic acid. Photographs were taken through a Zeiss Axiostra microscope with a Canon G10 digital camera.

To access the existence of published chromosome counts in the studied species, we used the most recent reports of chromosome numbers for this species (CHEHREGANI and MEHANFAR 2008; CHEHREGANI and HAJISADEGHIAN 2009), as well as 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).

RESULTS AND DISCUSSION

Fourteen Iranian populations belonging to species of *Tanacetum polycephalum* Schultz Bip. (L.) were studied in this research work. Based on our results the basic chromosome number in the all studied populations is x=9, but they are different regarding poloidy level. We present the data and chromosome number of different studied populations grouped by increasing chromosome number.

Only a population with chromosome number 2n=2x=18 was detected (Fig. 2A). This population was collected from Kermanshah province in the west of Iran near to Iraq boundary (Table 1). This finding is in accordance with a previously reported data (KHANDJIAN 1992) and is different from our prior reports for two different populations collected from Hamedan and Zanjan provinces (CHEHREGANI and MEHANFAR 2008; CHEHREGANI and HAJISADEGHIAN 2009). It seems that this difference is due to adaptation for different ecological and geographical conditions in each population that is accompanied with polyploidy. Similar data were reported in the some species of *Artemisia* (Chehregani, et al. 2010). In six populations tetraploidy (*2n=4x=36*) was detected (Figs. 2B-G). Five populations were collected from different regions of Hamedan province (Figs. 2B-F and H); and a population was collected from Zanjan province in north of Hamedan (Table 1 and Fig. 2G). In the populations studied in Hamedan, a population was collected from a natural protective area namely Khangormaz (Fig. 1D) and two populations were collected from Razan at the east of Hamedan province (Figs. 2 E-F). Although the previously published literatures showed a high degree of polyploidy for this species (Chehregani and Mehanfar 2008; Chehregani and Hajisadeghian 2009), but this is the first report of tetraploidy for this species.

In two populations both diploid (*2n=2x=18*) and tetraploid (*2n=4x=36*) chromosome numbers were observed. One of them is collected from the capital city of Hamedan (Figs. 2 H, I)

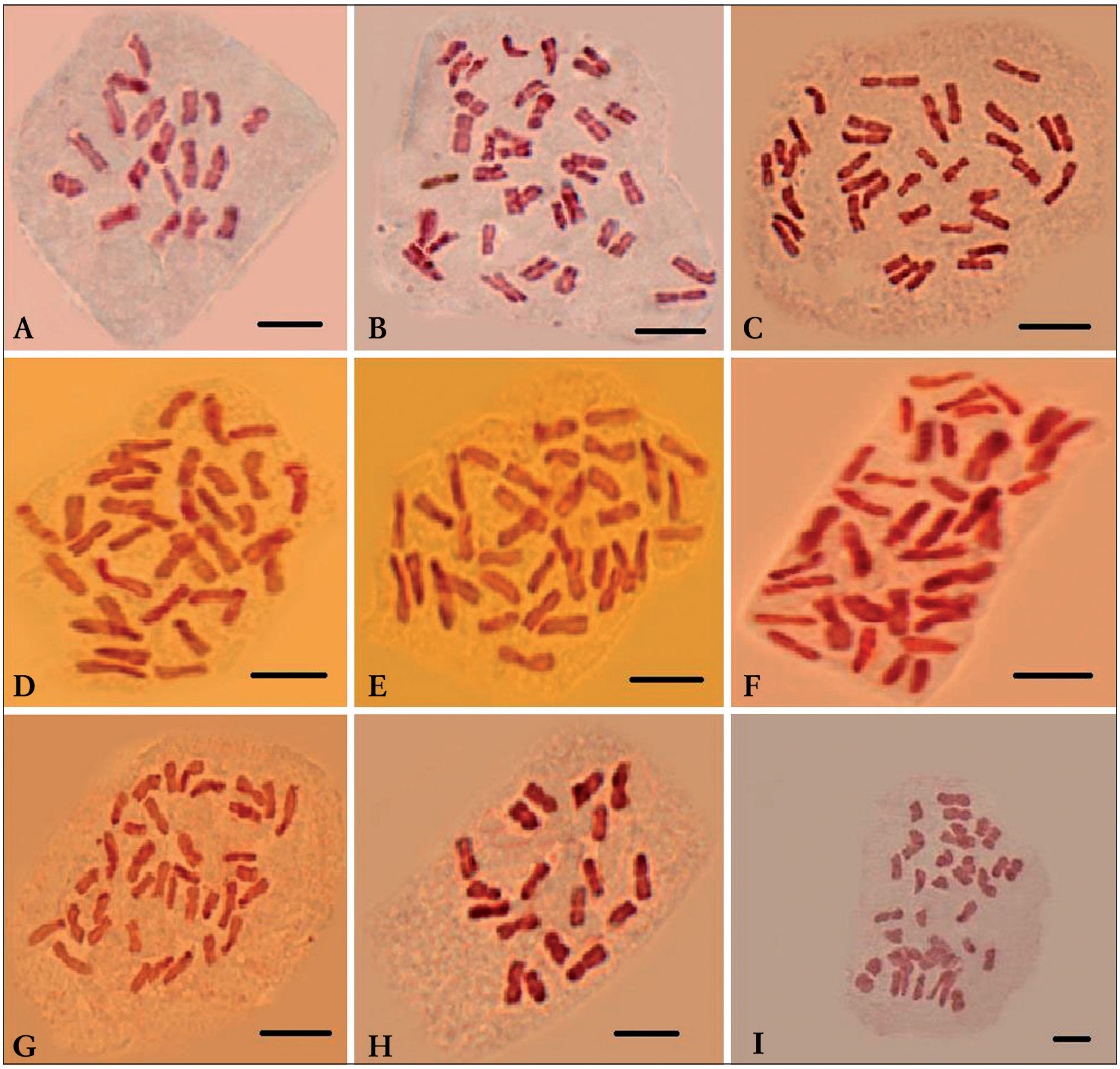

Fig. 2 (A-I) — Light micrographs of mitotic metaphase chromosomes of *T. polycephalum*: (A) *T. polycephalum* somatic count (*2n=18*). (B) *T. polycephalum* (*2n=4x=36*). (C) *T. polycephalum* (*2n=4x=36*). (D) *T. polycephalum* (*2n=4x=36*). (E) *T. polycephalum* (*2n=4x=36*). (F) *T. polycephalum* (*2n=4x=36*). (G) *T. polycephalum* (*2n=4x=36*). (H) *T. polycephalum* (*2n=2x=18*). (I) *T. polycephalum* (*2n=4x=36*). Scale bars = 5µm.

and the other one collected from Zanjan province at the north of Hamedan (Figs. 2 J, K). Presence of two or more different chromosome numbers and ploidy levels in the same population was also reported previously in *Artemisia* species (CHEHREGANI *et al.* 2010).

In the four populations chromosome number was evaluated as hexaploid ($2n=6x=54$). Two populations were located in Assadabad, between the Kermanshah and Hamedan provinces (Figs. 2 L, M), and two others were collected from Kabotarahang at the north of Hamedan (Figs. 2 N, O). This is also the first report as $2n=6x=54$ for *Tanacetum polycephalum*. Finally, in a population that was collected from a mountain in Assadabad region, two different chromosome numbers ($2n=4x=36$ and $2n=6x=54$) were determined (Figs. 2 P, Q).

Our results indicated that the chromosome number and also ploidy variation is common in the species *Thanacetum polycephalum.* It seems that polyploidy is confirmed as the most significant evolutionary trend in chromosome number within the species. Expansion and diversifica-

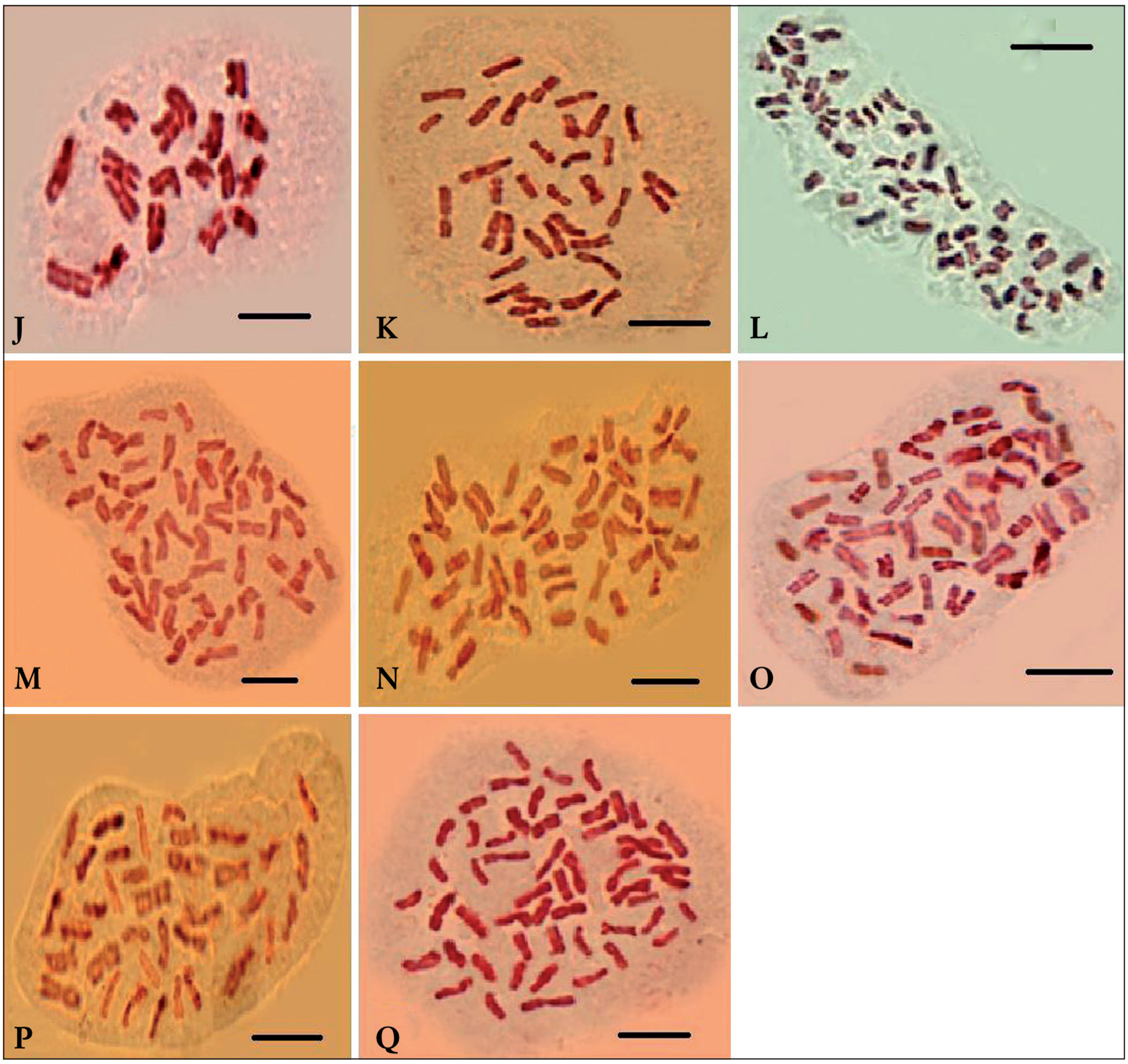

Fig. 2 (J-Q) — Light micrographs of mitotic metaphase chromosomes of *T. polycephalum*: (J) *T. polycephalum* ($2n=2x=18$). (K) *T. polycephalum* ($2n=4x=36$). (L) *T. polycephalum* ($2n=6x=54$). (M) *T. polycephalum* ($2n=6x=54$). (N) *T. polycephalum* ($2n=6x=54$). (O) *T. polycephalum* ($2n=6x=54$). (P) *T. polycephalum* ($2n=4x=36$). (Q) *T. polycephalum* ($2n=6x=54$). Scale bars = 5μm.

tion of the genus have been accompanied by several genome duplications which have led to acquisition of the tetraploid, hexaploid, octaploid and decaploid levels. On the other hand, *Tanacetum polycephalum* is a polyphyletic complex that has very variable characters (JUDD *et al.* 1999; WATSON *et al.* 2000; VALLES *et al.* 2003; CHEHREGANI and MEHANFAR 2007). Watson *et al.* (2002), with molecular data, showed that *Tanacetum* is a problemic genus. We can conclude that the reason of its variation and instability is chromosome number variations that is resulted in problems regarding its taxonomical situation and definition of its species.

In conclusion the most common basic number in the Anthemideae is x=9, although x=8 have been also reported (VALLÈS *et al*. 2005; CHEHREGANI and HAJISADEGHIAN 2009). In this research work, in *Tanacetum polycephalum,* chromosome numbers were determined as $2n=2x=18$, $2n=4x=36$ and $2n=6x=54$. We are reporting the first tetraploidy and hexaploidy level for *T. polycephalum.* Prior reports showed diploidy (KHANDIJAN 1992), octaploidy and decaploidy (CHEHREGANI and MEHANFAR 2009; CHEHREGANI and HAJISADDEGHIAN 2009). Our results indicate that the chromosome number variation and also ploidy variation is common in the species *Tanacetum polycephalum.* It seems that polyploidy is the reason of the polymorphism in this species. Expansion and diversification of the species have been accompanied by several genome duplications which have led to acquisition of the tetraploid, hexaploid and decaploid levels.

REFERENCES

BREMER K. and HUMPHRIES C.J., 1993 — *Generic monograph of the Asteraceae-Anthemideae.* Bulletin of Natural History Museum, London (Botany), 23: 71-177.

BROWN A.M.G., EDWARDS C.M., HARTMAN T.V.P., MARSHAL J.A., SMITH R.M., DAVEY M.R., POWER J.B. and LOWE K.C., 1999 — *Sexual hybrids of Tanacetum: biochemical, cytological and pharmacological characterization*. Journal of Experimental Botany, 50: 435-444.

CARR G.D., KING R.M., POWELL A.M. and ROBINSON H., 1999 — *Chromosome number in Compositeae* XVIII. American Journal of Botany, 86: 1003-1013.

CHEHREGANI A. and MEHANFAR N., 2007 — *Achen Micro-Morphology of Anthemidea (Asteraceae) and its allies in Iran with emphasis on systematic.* International Journal of Agriculture and Biology, 9: 486-488.

CHEHREGANI A. and HAJISADEGHIAN S., 2009 — *New chromosome counts in some species of Asteraceae from Iran.* Nordic Journal of Botany, 27: 247-250.

CHEHREGANI A. and MEHANFAR N., 2008 — *New Chromosome Counts in the Tribe Anthemideae (Asteraceae) from Iran*. Cytologia, 73: 189-196.

CHEHREGANI A., Atri M., Yousefi S. and Jalali F., 2010 — *Polyploidy variation in some species of the genus Artemisia L. (Asteraceae) in Iran.* Caryologia, 63: 168-175.

FUNK V.A., SUSANNA A., STUESSY T. and BAYER R. (eds.), 2009 — *Systematics, Evolution and Biogeography of the Compositae.* International Association for Plant Taxonomy, Washington D.C.

GHAFFARI S.M. and KELICH K., 2006 — *New or rare chromosome counts of some angiosperm species from Iran.* Iran Journal of Botany, 12: 81-86.

JUDD W.S., CAMPBELL C.S., KELLOGG E.A. and STEVENS P.F., 1999 — *Plant systematic a phylogenetic approach*. Massachusetts, USA.

KADEREIT J.W. and JEFFREY C. (eds.), 2007 — *Flowering plants* vol. VIII. Eudicots. Asterales. Springer-Verlag Press. Berlin Heidelberg.

KHANDJIAN N., 1992 — *The taxonomic significance of the achene's structure in the subtribe Anthemideae (Asteraceae).* Botanicheskii Zhurnal, 77: 89-98.

LEVIN D.A., 1983 — *Polyploidy and novelty in flowering plants.* The American Naturalist, 122: 1-25.

LING Y.R., 1991a — *The old world Seriphidium (Compositae).* Bulletin of Botanical Research. (Harbin), 11:1-40.

LING Y.R., 1991b — *The old world Artemisia (Compositae).* Bulletin of Botanical Research. (Harbin), 12:1-108.

LING Y.R., 1995a — *The new world Artemisia L. In Advances in Compositae Systematics*. Royal Botanic Gardens, Kew, U.K. pp. 255-281.

LING Y.R., 1995b — *The new world Seriphidium (Besser) Fourr.* In Advances in Compositae Systematics. Royal Botanic Gardens, Kew, U.K. pp. 283-291.

MABBERLEY D.J., 1990 — *The plant book*. Cambridge University Press, Cambridge, U.K.

MC ARTHUR E.D. and PLUMMER A.P., 1978 — *Biogeography and management of native western shrubs: a case study, section Tridentatae of Artemisia.* Great Basin Natural Memories, 2: 229-243.

SOLTIS P.S. and SOLTIS D.E., 2000 — *The role of genetic and genomic attributes in the success of polyploids*. Proceedings of the National Academy of Sciences, 97: 7051-7057.

THOMPSON J.D. and LUMARET R., 1992 — *The evolutionary dynamics of polyploid plants: origins, establishment and persistence.* Trends in Ecology and Evolution, 7: 302-307.

VALLÈS J., GARNATJE T., GARCIA S., SANZ M. and KOROBKOV A.A., 2005 — *Chromosome numbers in the tribes Anthemideae and Inuleae (Asteraceae) in Kazakhstan.* Botanical Journal of Linean Society, 148: 77-85.

VALLÈS J., TORRELL M., GARCIA-JACAS T., VILATERSANA N. and SUSANNA A., 2003 — *The genus Artemisia and its allies: phylogeny of subtribe Artemisiinae*

(Asteraceae) based on nucleotide sequence on nuclear ribosomal DNA internal transcribed spacers (ITS). Plant Biology, 5: 274-278.

VOLKOVA S.A. and BOYKO E.V., 1986 — *Chromosome numbers in some species of Asteraceae from the southern part of the Soviet Far East.* Botanicheskii Zhurnal, 71: 16-93.

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

WATSON L.E., EVANS T.M. and OLURAT E.T., 2000 — *Molecular phylogeny and biogeography of tribe Anthemideae (Astraceae) based on chloroplast gene ndhf.* Molecular Phylogenetic and Evolution, 15:59-69.

WENDEL J. and DOYLE J., 2005 — *Polyploidy and evolution in plants.* In: Henry RJ, ed. Plant diversity and evolution: genotypic and phenotypic variation in higher plants. Wallingford: CABI, 97-117.

Received November 1st 2010; accepted April 2nd 2011