

Karyomorphology of five taxa of *Tanacetum* sect. *Xanthoglossa* from Iran

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In Flora Iranica area, *Tanacetum* comprised 18 sections and 54 species. Considering new findings, *Tanacetum* species in Iran has been increased to 37. No detailed information on the karyotype analysis of the studied species was found in the literature. Therefore, in the present study, the detailed karyomorphology of six populations belonging to five taxa of *Tanacetum* sect. *Xanthoglossa* were studied. The basic chromosome number of the *Tanacetum* has been reported to be $x=9$. Mitotic chromosome number of studied taxa were found to be diploid ($2n=2x=18$) for *T. argyrophyllum*, *T. oligocephalum* (Khoy), *T. uniflorum* (no previous karyological records are known for this species) and *T. pinnatum*. According to the symmetry classes of Stebbins, class 2A was found predominant karyotype class among the studied *Tanacetum* except for tetraploid ($2n=4x=36$) *T. polycephalum* subsp. *Heterophyllum* which was placed in 2B. Karyotype analysis of each taxon has been presented and discussed.

Keywords: Asteraceae, diploid, Flora Iranica, karyology, tetraploid**کاربومورفولوژی پنج آرایه از *Tanacetum* بخش *Xanthoglossa* در ایران**

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در فلورا ایرانیکا، جنس *Tanacetum* (کاسنیان) شامل ۱۸ بخش و ۵۴ گونه است. طبق یافته‌های جدید، تعداد گونه‌های این جنس در ایران به ۳۷ عدد افزایش یافته است. هیچ اطلاعات دقیقی در مورد تجزیه و تحلیل کاربوتیپ گونه‌های مورد مطالعه در نوشتجات وجود ندارد. بنابراین، در مقاله حاضر، کاربومورفولوژی دقیق شش جمعیت متعلق به پنج گونه از بخش *Xanthoglossa* مورد مطالعه قرار گرفت. عدد پایه کروموزومی این جنس $x=9$ گزارش شده است. عدد کروموزومی میتوزی آرایه‌های *T. argyrophullum*، *T. oligocephalum* (خوی)، *T. uniflorum* (تاکنون هیچ اطلاعات کاربوتیپی در مورد این گونه یافت نشده است) و *T. pinnatum* دیپلوئید ($2n=2x=18$) بود. با توجه به کلاس‌های تقارن Stebbins، کلاس 2A، کلاس غالب در بین آرایه‌های مورد مطالعه بود به جز *T. polycephalum* subsp. *heterophyllum* که تتراپلوئید و دارای کلاس 2B بود. در بررسی حاضر، تجزیه و تحلیل کاربوتیپ هر آرایه مورد بحث قرار گرفته است.

واژه‌های کلیدی: تتراپلوئید، دیپلوئید، فلورا ایرانیکا، کاربوتیپی، کاسنیان

Introduction

Tanacetum L. (*Compositae-Anthemideae*), with approximately 160 species, after *Artemisia* L. (ca. 522 species) and *Anthemis* L. (175 species), is the third largest genus in *Anthemideae*. Members of this genus are found mainly in Asia, Europe, North America, and northern Africa (Oberprieler *et al.* 2006, 2007, 2009). In Flora Iranica area, *Tanacetum* is comprised of 18 sections and 54 species. Considering new findings, *Tanacetum* species in Iran has been increased to 37 (Podlech 1986, Mozaffarian 2005, 2008, Djavadi 2008, Majdi *et al.* 2010, Sonboli *et al.* 2010a,b, 2011, Kazemi *et al.* 2014a,b, Olanj & Sonboli 2021).

The basic chromosome number of *Tanacetum* has been reported to be $x=9$. Most of the karyological studies in *Tanacetum* have determined the chromosome number of $2n=18, 27, 36, 54, 72$, and 90. (Chehregani & Mehanfar 2008, Chehregani & Hajisadeghian 2009, Majdi *et al.* 2010, Chehregani *et al.* 2011, Inceer *et al.* 2012, Olanj *et al.* 2013, 2015, Ghasemkhani *et al.* 2013, Javadi 2017, Hatami *et al.* 2019, Sadeghian *et al.* 2019, Kurşat 2020).

As far as author's survey is ascertained, no previous karyological records are known for *T. uniflorum* Sch.Bip., while chromosome number counts have been performed for *T. oligocephalum* (DC.) Sch.Bip., *T. pinnatum* Boiss., *T. polycephalum* Sch.Bip. subsp. *heterophyllum* (Boiss.) Podlech, and *T. argyrophyllum* (K. Koch) Tzvelev (Ghaffari & Kelich 2006, Chehregani & Mehanfar 2008, Chehregani & Hajisadeghian 2009, Chehregani *et al.* 2011, Ghasemkhani *et al.* 2013, Chehregani *et al.* 2014, Olanj *et al.* 2015, Mirzadeh Vaghefi *et al.* 2019, Moradi Behjou *et al.* 2022). In addition, no detailed information on the karyotype analysis of the studied species was found in the literature. Therefore, the present survey is focused on the chromosome number and karyomorphology of six populations of five taxa from Iran including *T. oligocephalum* (Khoy and Salmas in W. Azarbijan

Province, Iran), *T. pinnatum*, *T. argyrophyllum*, *T. polycephalum* subsp. *Heterophyllum*, and *T. uniflorum*.

It is noteworthy to state that, *Tanacetum* is known for its medicinal importance due to the presence of several bioactive compounds such as flavonoids, terpenoids, and sesquiterpene lactones. These compounds have been found to possess a wide range of therapeutic properties including antiinflammatory, antioxidant, antimicrobial, antifungal, and antitumor activities. *Tanacetum* species have been traditionally used in the treatment of various ailments such as fever, headache, digestive disorders, and menstrual disorders. In addition, several studies have reported the potential of *Tanacetum* species in the treatment of chronic diseases such as cancer, diabetes, and cardiovascular diseases. Therefore, the genus *Tanacetum* holds great promise as a source of natural medicines for the prevention and treatment of various diseases (Yousefzadi *et al.* 2009, Esmaeili *et al.* 2010, Devrnja *et al.* 2017).

Materials and Methods

Six populations representing five taxa of *Tanacetum* sect. *Xanthoglossa* was studied in North and Northwestern of Iran. Herbarium samples were preserved at the Medicinal Plants Herbarium (MPH) of Shahid Beheshti University (Tehran, Iran). These species were identified according to Tzvelev (1961), Grierson (1975), Podlech (1986), and Mozaffarian (2008). Table 1 summarizes the locations, altitude, collectors, and herbarium vouchers number of all studied taxa. Rootlets were obtained from germinated achenes on wet filter paper in Petri dishes at 24 °C. Samples were pretreated with 0.002 M 8-hydroxyquinoline for 3–3.5 h at room temperature. The roottip was then fixed in a solution of ethanol and glacial acetic acid (3:1) overnight and stored in ethanol 70% (v/v) at 4 °C. Meristems were hydrolysed in 1N HCl at 60 °C for 10 min, rinsed for 2–3 min in tap water, and then stained in 2% (w/v) aqueous aceto-orcein and squashed on slides.

Table 1. Locality and voucher information of the studied *Tanacetum* species in Iran

Taxon	Locality along with related data	Herbarium voucher number
<i>Tanacetum oligocephalum</i> (DC.) Sch.Bip.	W. Azarbijan Province: Khoy, Chaldoran road (Firuraq), 18 km after Khoy, 1580 m, 2011, Olanj	MPH-1914
<i>T. oligocephalum</i> (DC.) Sch.Bip.	W. Azarbijan Province: Salmas, Salmas-Silvana road, 5 km after Mamaka, 1800 m, 2011, Olanj	MPH-1911
<i>T. pinnatum</i> Boiss.	Tehran Province: Lavasan, Galanduak, Kurdian, 1900–2000 m, 2007, Sonboli <i>et al.</i>	MPH-1118
<i>T. argyrophyllum</i> (K. Koch) Tvzelev	W. Azarbijan Province: Meshkinshahr, Lahrood-Shabil, hot spring, 1680 m, 2011, Olanj	MPH-1884
<i>T. polycephalum</i> Sch.Bip. subsp. <i>heterophyllum</i> (Boiss.) Podlech	Mazandaran Province: Siah-Bishe, Chalus road, Pol-e Zanguleh, Yoush road, Golestanak, 2800 m, 2011, Sonboli & Mehregan	MPH-1797
<i>T. uniflorum</i> Sch.Bip.	W. Azarbijan Province: Khoy, Firouragh road, Pasak to Hesar, towards Ghiziljir spring, 1500–1700 m, 2008, Sonboli <i>et al.</i>	MPH-1327

Microphotographs of the best metaphase plates were selected (at least three) by using a BX-51 Olympus microscope and finally recorded with a SSCDC58AP color video camera. The nomenclature adopted for chromosome morphology follows Levan *et al.* (1964). In order to determine the number of chromosomes, 20 samples were counted, while at least three samples were measured to construct ideograms. In each metaphase plate, the following parameters were measured: Chromatin length (X), Intrachromosomal (A1), Interchromosomal (A2) Asymmetry indices were calculated by Romero Zarco (1986) method, Karyotype form percentage (TF%), Relative length (RL), Mean centromeric index (CI), and Arm ratio (AR). Karyotype classification follows, using Stebbins classifications (1971).

Results

The pictures of the somatic metaphase chromosomes, karyotype formula, and parameters of the studied species and populations are depicted in tables 1–3 and figures 1–2. The karyomorphological details of the studied species are given below:

1. *Tanacetum oligocephalum* (DC.) Sch.Bip.

The chromosome number in the populations of Khoy and Salmas were found to be $2n=18$ and $2n=36$,

respectively (Olanj *et al.* 2015). While, in the diploid Khoy population, the karyotype formula was $2n=2x=18=2M+10m+6sm$, with no observable satellite chromosome, the karyotype formula of tetraploid Salmas population was $2n=4x=36=4M+22m+8sm+2st$ with chromosomes number 11 and 18 showing a microsatellite on their short arms. The chromosomes showed a relative length (RL) ranged between 9.42–13.03 μm in Khoy and 4.09–7.88 μm in Salmas population, respectively. Based on karyotype classification of Stebbins (*l.c.*) method, two studied population of *T. oligocephalum* were placed in class 2A (Tabs 2–3 & Figs 1A–1B, 2A–2B).

Distribution: Iran (Northwestern parts), Turkey, and Caucasus.

2. *Tanacetum pinnatum* Boiss.

Chromosomes were counted as $2n=18$. This count confirms the previous ones, (Chehregani & Mehanfar 2008, Chehregani *et al.* 2014, Olanj *et al.* 2015, Mirzadeh Vaghefi *et al.* 2019). Two ploidy levels viz. $2n=2x=18$ and $2n=4x=36$ of this species have been illustrated in different localities in Hamedan Province by Chehregani *et al.* 2014. Mirzadeh *et al.* (2019) have reported chromosome number $2n=18$ from the population of Tehran (Karaj-Chalus), with karyotypic formula $8m+1sm$. In the present survey, for the second time, a different

karyotypic formula viz. $2n=2x=18=6M+10m+2sm$ of other population of Tehran (Lavasan) and for the first time karyomorphological information are presented (no satellites were observed in the karyotype). Based on karyotype classification (Stebbins *l.c.*), the studied population of *T. pinnatum* was placed in class 2A. The relative length of chromosomes were ranged between 9.40–14.25 μm (Tabs 2–3 & Figs 1C–2C).

Distribution: Iran (North, Northwestern & Central parts), Turkey, Iraq, and Caucasus.

3. *Tanacetum argyrophyllum* (K. Koch) Tzvelev

Two ploidy levels ($2n=2x=18$ and $2n=4x=36$) have been reported for this species (Inceer *et al.* 2012, Olanj *et al.* 2015, Kursat 2020, Moradi Behjou *et al.* 2022). The karyotype formula of diploid from Meshkinshar population is reported for the first time viz. $2n=2x=18=4M+12m+2sm$. Just one microsatellite was observed on short arm of the chromosome 5. Total chromatin length (X) was 50.7 μm and the relative length of chromosomes ranged between 8.16–13.41 μm . Based on the karyotype classification (Stebbins *l.c.*), the studied population of *T. argyrophyllum* included in class 2A (Tabs 2–3 & Figs 1D–2D).

Distribution: Iran (Northwestern parts), Turkey (Northeastern & Central parts), Iraq (Northeastern parts), and Caucasus (Eastern and Southern parts).

4. *Tanacetum polycephalum* Sch.Bip. subsp. *heterophyllum* (Boiss.) Podlech

The chromosome number in the populations of Mazandaran ($2n=36$) and Hamedan ($2n=54$) were reported by Olanj *et al.* (2015). Here, the karyotype formula and karyomorphological parameters for Mazandaran population (endemic taxon to Iran and Iraq), is presented for the first time. The karyotype formula was $2n=4x=36=4M+22m+8sm+2st$. No chromosome with satellite was observed. The studied population of *T. polycephalum* Sch.Bip. subsp. *heterophyllum* from Mazandaran province of Iran included in class 2B on the basis of karyotype classification method (Stebbins *l.c.*). The chromosomes showed a relative length ranged between 3.71–8.11 μm (Tabs 2–3 & Figs 1E–2E).

Distribution: Iran (Western and Southern parts), and Iraq (Northeastern parts).

5. *Tanacetum uniflorum* Sch.Bip.

The chromosome number and karyomorphology of *T. uniflorum* is reported for the first time. The karyotype formula was $2n=2x=18=2M+10m+6sm$. The chromosome pair 7 showed a satellite. Based on the karyotype classification (Stebbins *l.c.*), the studied population of the species placed in class 2A. The relative length of chromosome were ranged between 8.40–14.84 (Tabs 2–3 & Figs 1F–2F).

Distribution: Iran (Northwestern parts), Turkey, and Caucasus.

Table 2. Karyotype analysis of the studied *Tanacetum* species in Iran

Taxon	2n	Ploidy level	Karyotype formula	Chromosome length range (μm)	X (μm)	A1	A2	SC	TF%
<i>Tanacetum oligocephalum</i> (Khoy)	18	2x	2M+10m+6sm	2.92–4.04	31	0.28	0.12	2A	38
<i>T. oligocephalum</i> (Salmas)	36	4x	4M+22m+8sm+2st	3.09–5.96	75.6	0.28	0.19	2A	41.5
<i>T. pinnatum</i>	18	2x	6M+10m+2sm	3.57–5.41	38	0.18	0.13	2A	45
<i>T. argyrophyllum</i>	18	2x	4M+12m+2smt	4.14–6.8	50.7	0.24	0.15	2A	37
<i>T. polycephalum</i> subsp. <i>heterophyllum</i>	36	4x	4M+16m+14sm+2st	3.3–7.2	88.7	0.37	0.21	2B	38
<i>T. uniflorum</i>	18	2x	2M+10m+6sm	3.53–6.24	42	0.31	0.18	2A	41

Table 3. Chromosomal characteristics of the studied *Tanacetum* species in Iran

Pair No.	RL	AR	CI	Type	Pair No.	RL	AR	CI	Type
<i>T. oligocephalum</i> (Khoy)					<i>T. pinnatum</i>				
1	13.03	1.71	36.88	m	1	14.25	1.42	41.40	m
2	12.96	1.23	44.78	m	2	12.38	1.12	47.02	m
3	11.71	2.38	29.48	sm	3	11.27	1.03	49.30	M
4	11.32	1.96	33.62	sm	4	11.09	1.21	45.37	m
5	11.03	1.05	48.83	M	5	10.96	1.06	49.04	m
6	10.74	1.2	45.35	m	6	10.43	1.02	49.49	M
7	10.16	1.61	38.41	m	7	10.27	1.61	38.21	m
8	9.64	2.36	29.77	sm	8	9.96	1.05	48.68	M
9	9.42	1.56	39.04	m	9	9.40	2.01	33.05	sm
<i>T. argyrophyllum</i>					<i>T. uniflorum</i>				
1	13.41	1.22	45.15	m	1	14.84	1.28	43.91	m
2	12.74	1.49	40.25	m	2	12.96	1.84	35.23	m
3	12.29	1.05	48.80	M	3	11.99	1.04	49.01	M
4	11.99	1.02	49.34	M	4	11.80	1.22	44.96	m
5	11.14	2.11	32.21	m	5	11.06	1.17	46.24	m
6	10.91	1.3	43.40	sm	6	9.87	1.11	47.47	m
7	10.08	1.16	46.18	m	7	9.71	2.19	31.37	sm
8	9.29	1.44	40.98	m	8	9.37	1.89	34.52	sm
9	8.16	1.44	40.82	m	9	8.40	2.43	29.18	sm
<i>T. oligocephalum</i> (Salmas)					<i>T. polycephalum</i> subsp. <i>heterophyllum</i>				
1	7.88	1.11	47.48	M	1	8.11	2.03	33.05	sm
2	7.38	1.13	46.95	M	2	6.84	1.59	38.56	m
3	6.77	1.63	38.09	M	3	6.66	1.14	46.63	m
4	6.23	1.94	33.97	sm	4	6.59	1.36	42.38	m
5	6.07	1.32	43.14	M	5	6.20	1.01	49.70	M
6	6.03	1.01	49.78	M	6	6.16	1.04	49.02	M
7	5.75	1.02	49.43	M	7	6.10	1.09	47.74	m
8	5.73	1.06	48.50	M	8	5.96	1.40	41.69	m
9	5.28	1.06	48.62	M	9	5.65	1.62	38.23	m
10	5.26	1.29	43.72	M	10	5.57	2.75	26.66	sm
11	5.17	2.03	32.99	sm	11	5.48	1.18	45.78	m
12	5.12	1.56	39.02	M	12	5.03	1.85	35.03	sm
13	5.01	1.08	48.02	M	13I	4.92	1.28	43.90	m
14	5.00	1.39	41.80	M	14	4.75	2.88	25.79	sm
15	4.96	3.12	24.27	st	15	4.48	2.91	25.59	sm
16	4.17	1.31	43.49	M	16	3.96	2.16	31.65	sm
17	4.13	2.90	25.64	sm	17	3.83	2.15	31.77	sm
18	4.09	2.32	30.10	sm	18	3.71	3.29	23.33	st

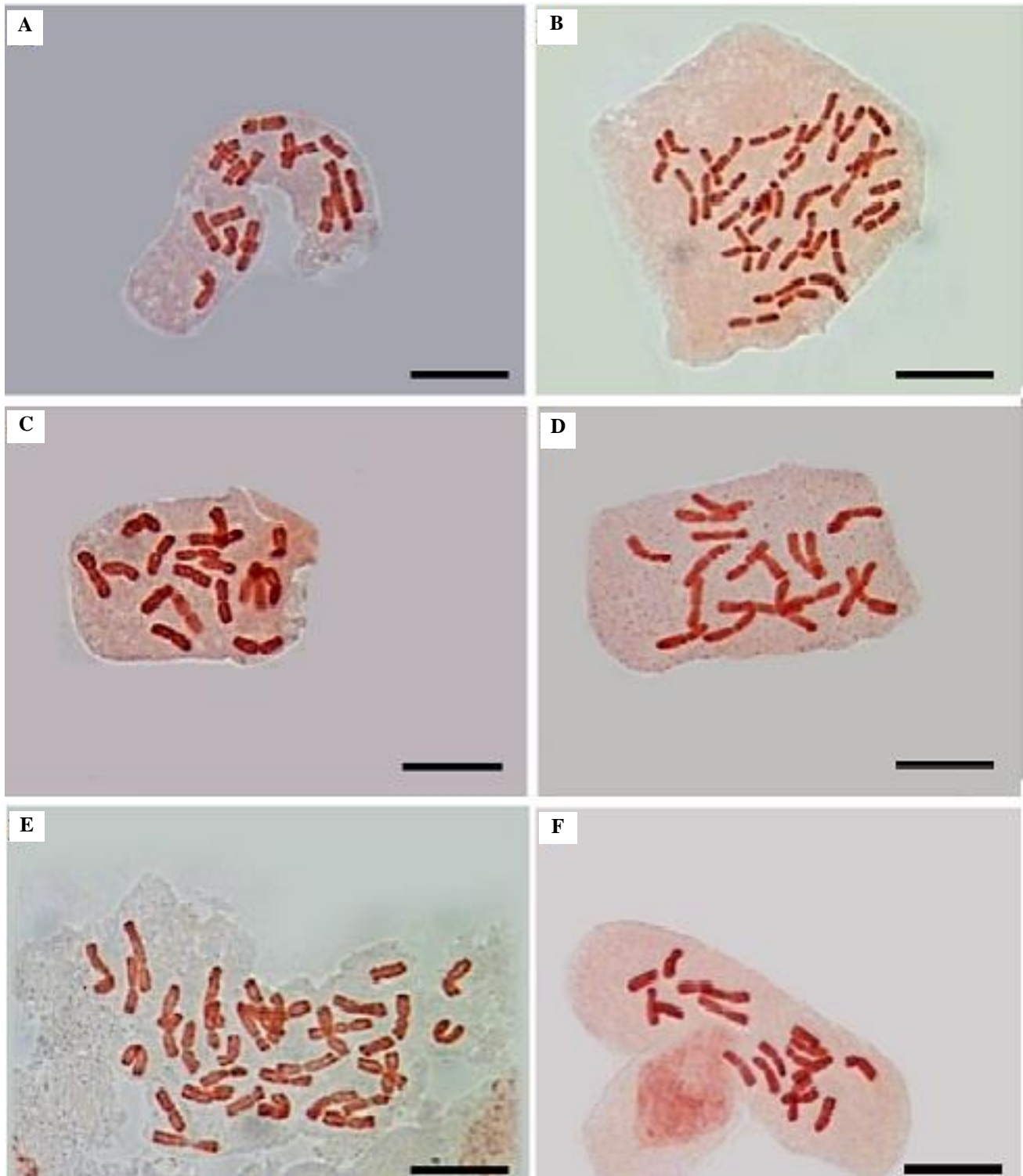


Fig. 1. Mitotic metaphase of *Tanacetum* taxa: A. *T. oligocephalum* (Khoy) ($2n=2x=18$), B. *T. oligocephalum* (Salmas) ($2n=4x=36$), C. *T. pinnatum* ($2n=2x=18$), D. *T. argyrophyllum* ($2n=2x=18$), E. *T. polycephalum* subsp. *heterophyllum* ($2n=4x=36$), F. *T. uniflorum* ($2n=2x=18$) (Bars = 10 μ m).

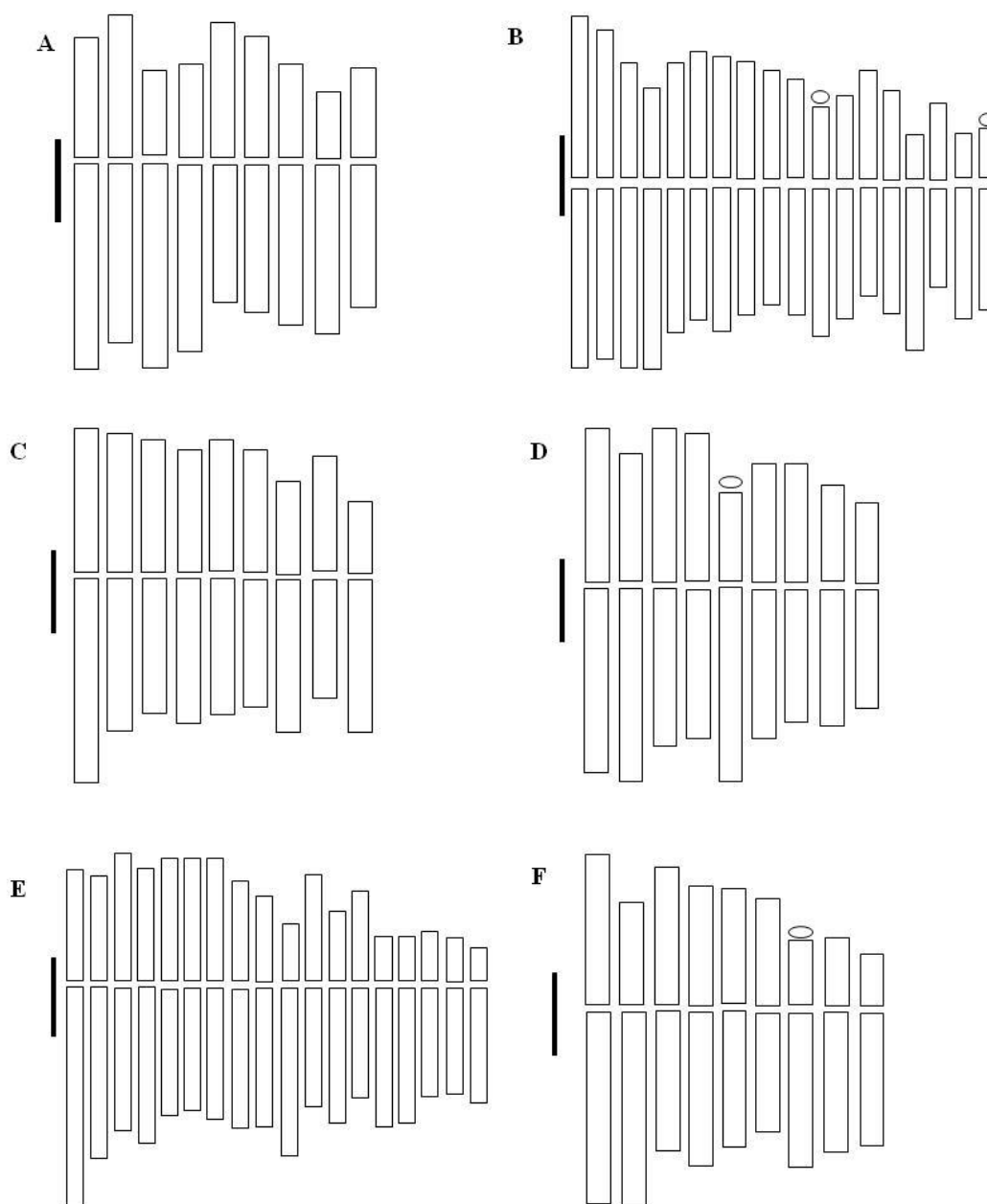


Fig. 2. Ideograms of studied *Tanacetum* taxa: A. *T. oligocephalum* (Khoy) ($2n=2x=18$), B. *T. oligocephalum* (Salmas) ($2n=4x=36$), C. *T. pinnatum* ($2n=2x=18$), D. *T. argyrophyllum* ($2n=x=18$), E. *T. polycephalum* subsp. *heterophyllum* ($2n=4x=36$), F. *T. uniflorum* ($2n=2x=8$) (Bars = 1 μ m).

Discussion

In the present study, karyotype characteristics were presented in five taxa belonging to six populations, of which chromosome number of *T. uniflorum* has been reported for the first time. The predominant basic chromosome number in *Anthemideae* (*Asteraceae*) has been reported to be $x=9$ (Inceer & Beyazoglu 2004, Valles *et al.* 2005, Inceer *et al.* 2012, Olanj *et al.* 2015). Three taxa and one population of *T. oligocephalum* (Khoy) were diploid ($2n=18$), while other population of *T. oligocephalum* (Salmas), and *T. polycephalum* subsp.

heterophyllum were tetraploid. In the studied taxa, chromosome type m was found as the predominant type followed by sm and M.

According to the karyotype classification based on symmetry degree (Stebbins *l.c.*), two classes of 2A and 2B were observed among the studied taxa. *Tanacetum oligocephalum* (Khoy and Salmas), *T. pinnatum*, *T. argyrophyllum*, and *T. uniflorum* were placed in 2A and *T. polycephalum* subsp. *Heterophyllum* included in 2B. This finding revealed the role of polyploidy as an evolutionary mechanism in general (Stebbins *l.c.*). The

lowest intrachromosomal asymmetry index (A1) was obtained for *T. pinnatum* with 0.18 and the highest value, characterized in *T. polycephalum* subsp. *Heterophyllum* with 0.37. While, the studied population of *T. oligocephalum* from Khoy (W. Azerbaijan Province) showed the lowest interchromosomal asymmetry index (A2) value of 0.12, the highest value (0.21) characterized in *T. polycephalum* subsp. *heterophyllum* (Table 2).

The shortest total chromosome length (31 μm) and the shortest mean chromosome length (2.92 μm) were characterized in *T. oligocephalum* (Khoy) while the longest total chromosome length (88.7 μm) and the longest chromosome pair (7.2 μm) were observed in *T. polycephalum* subsp. *heterophyllum*.

Karyotype length and genome size are positively correlated (Garnatje *et al.* 2004, Garcia *et al.* 2005, Olanj *et al.* 2015). According to Olanj *et al.* (2015), in

Tanacetum taxa, *T. polycephalum* subsp. *heterophyllum* and *T. oligocephalum* (Khoy) showed the highest (2C=18.10 pg) and the lowest (2C=7.67 pg) genome size, respectively. Different ploidy levels have already been recognized in *Tanacetum* (Chehregani & Mehanfar 2008, Chehregani & Hajisadeghian 2009, Majdi *et al.* 2010, Chehregani *et al.* 2011, Inceer *et al.* 2012, Olanj *et al.* 2013, 2015, Kurset 2020, Moradi Behjou *et al.* 2022). Polyploidy seems to be common in *Asteraceae* and plays an important role in the speciation (Chehregani & Hajisadeghian 2009, Ghasemkhani *et al.* 2013).

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