

Biosystematics and phylogeny of *Tanacetum fisherae*, a new record from Iran

مطالعه بیوسیستماتیکی و فیلوژنتیکی گونه *Tanacetum fisherae*

Received: 21.09.2011 / Accepted: 28.12.2011

دریافت: ۱۳۹۰/۶/۳۰ / پذیرش: ۱۳۹۰/۱۰/۷

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Abstract

The chromosome number ($2n=5x=44+1B$) of *Tanacetum fisherae*, as a new record from high mountains of southern Iran (Kerman province: Hazar mountain) is reported. A new ploidy level (pentaploidy) for the genus is presented for the first time. The studied population was aneuploid, having lost one chromosome out of the 45 expected in an $x=9$ -based pentaploid. The distribution map, description and micromorphological characteristic of the achene are given. A taxonomic conclusion on the basis of molecular data and phylogenetic position of *T. fisherae* is also discussed.

خلاصه

گونه *Tanacetum fisherae* به عنوان رکورد جدیدی برای فلور ایران از استان کرمان گزارش می‌شود. عدد کروموزومی ($2n=5x=44+1B$) و همچنین سطح پلوئیدی پنتاپلوئیدی برای جنس *Tanacetum* برای نخستین بار ارائه می‌گردد. نقشه پراکندگی این گونه در دنیا و ایران، شرح کامل و صفات ریزریخت شناسی میوه ارائه شده است. یک نتیجه‌گیری تاکسونومیک براساس داده‌های مولکولی (nrDNA ITS) و مورفولوژیکی برای روشن شدن جایگاه تاکسونومیک و فیلوژنتیکی این گونه پیشنهاد شده است.

واژه‌های کلیدی: کروموزوم، *Asteraceae* ITS، *Xylanthemum* *Tanacetum*

Keywords: *Asteraceae*, Chromosome, ITS, *Tanacetum*, *Xylanthemum*

Introduction

Tanacetum L., the third largest genus after *Artemisia* L. and *Anthemis* L., belonging to the tribe *Anthemideae* of *Compositae*, consists of ca. 160 species worldwide (Oberprieler *et al.* 2006). In Flora Iranica area, this genus is represented by 18 sections and altogether 54 species (Podlech 1986). During the revision of the genus *Tanacetum* (2004–10), new species and records from different provinces of Iran have been found (Sonboli *et al.* 2010a, b). The species number of *Tanacetum* in the flora of Iran based on the recent findings (Mozaffarian 2005, Djavadi 2008, Sonboli *et al.*, 2010a, b) together with the new species recorded herein is increased to 36.

The most problematic subtribe in Bremer & Humphries' (1993) classification based on a cladistic analysis of mainly morphological data was the so-called 'Tanacetinae' Bremer & Humphries including genera: *Tanacetum*, *Xylanthemum* Tzvelev, *Tanacetopsis* (Tzvelev) Kovalevsk, *Hippolytia* Poljakov, *Heliocauta* Humphries, *Lepidolopha* C. Winkl. and *Opisthopappus* C. Shih. The genus *Xylanthemum* was described to accommodate four species that were removed from *Tanacetum* L. by Tzvelev (2000) on the basis of their shrubby habit, discoid capitula, flat receptacles, auriculate pappus and myxogenic cells in the epidermis of the pericarp. Podlech (1986) relegated the generic rank of *Xylanthemum* to a section of *Tanacetum*, with seven species: *T. pamiricum* (Hoffm.) Bornm., *T. lingulatum* (Boiss.) Bornm., *T. rupestre* Pop. ex Nevsky, *T. gillettii* Podl., *T. macropodum* Hemsl. & Lace, *T. paghmanense* Podl., and *T. fisherae* Aitch. & Hemsl.

Many karyological data concerning chromosome numbers have already been published in the *Tanacetum* from different countries (Baltisberger 1990, Ghaffari & Kelich, 2006, Inceer & Hayirlioglu-Ayaz 2007, Chehregani & Mehanfar 2008, Chehregani & Hajisadeghian 2009, Chehregani *et al.* 2011, Sonboli *et al.* 2011). The most common basic chromosome number in the genus was found to be $x=9$. Chromosome counts of some *Tanacetum* species

originating from Iran has been presented in Table 1. As can be seen several ploidy levels ranging from $2x$ to $10x$ for the different taxa has been reported. To our knowledge, there is no information on the karyology of *T. fisherae*.

The genus *Xylanthemum* in other taxonomic treatments and some regional flora has been considered as a separate genus (Bremer & Humphries 1993, Ghafoor 2002, Oberprieler *et al.* 2006, 2009). Oberprieler *et al.* (2007) based on a phylogenetic analysis of the tribe *Anthemideae* inferred from nrDNA ITS data placed *Xylanthemum* in the *Handeliinae* subtribe. Recently, owing the close relationship of the *X. tianschanicum* (Krasch.) Muradyan to the type species of *Richteria* Kar. & Kir. (*Richteria pyrethroides* Kar. & Kir.) which is supported by morphological characteristics, the species is excluded from *Xylanthemum* and the combination *Richteria tianschanica* (Krasch.) Sonboli & Oberpr. has been introduced (Sonboli & Oberprieler 2010). However, the generic circumscription of the genus *Xylanthemum* and taxonomic position of its taxa is still unclear and controversial. In this study the taxonomic status and phylogenetic position of *T. fisherae* based on nrDNA ITS sequences, chromosomal and micromorphological data has been discussed.

Materials and Methods

- Morphology

Herbarium specimens of the genus *Tanacetum* available in several herbaria (IRAN, TARI, MPH, G, W) and herbarium of the research centre of natural resources and agriculture of Kerman were revised using relevant literatures (Podlech 1986, Tzvelev 2000, Ghafoor 2002, Mozaffarian 2008). Comparative morphological characteristics of *T. fisherae* and its close relatives i.e. *T. pamiricum* and *T. rupestre* were studied under stereomicroscope (Blue Light, USA). For scanning electron microscopy mature achenes were directly mounted on stub using double adhesive tape and coated with gold in sputtering chamber and observed under SEM (Philips-XL30).

Table 1. List of Iranian *Tanacetum* taxa and their chromosome counts retrieved from published data

Taxa	2n	n	Reference
<i>T. chiliophyllum</i> (Fisch. & Mey.) Sch. Bip.	18, 27	–	Chehregani & Hajisadeghian (2009)
<i>T. kotschyi</i> (Boiss.) Grierson	18	–	Chehregani & Mehanfar (2008)
<i>T. paradoxum</i> Bornm.	16	–	Sonboli <i>et al.</i> (2011)
<i>T. parthenium</i> (L.) Sch. Bip.	36	–	Chehregani & Mehanfar (2008)
<i>T. pinnatum</i> Boiss.	18	–	Chehregani & Mehanfar (2008)
<i>T. polycephalum</i> Sch. Bip.	72	–	Chehregani & Hajisadeghian (2009)
	90	–	Chehregani & Mehanfar (2008)
	18, 36, 54	–	Chehregani <i>et al.</i> (2011)
<i>T. polycephalum</i> subsp. <i>duderanum</i> (Boiss.) Podl.	–	18	Ghaffari & Kelich (2006)

- Cytological method

Root-tip meristems were obtained from wild-collected achenes germinated on wet filter paper in Petri dishes at room temperature. Seedlings were pretreated with 0.002 M 8-hydroxyquinoline at room temperature for 3 h. Material was fixed in absolute ethanol and glacial acetic acid (3:1) for 24 h at 4° C and stored in alcohol fixative (ethanol 70%) at 4° C. Samples were hydrolysed in 1N HCl for 10 min at 60° C, rinsed in tap water for a minimum of 2–3 min and then stained and squashed on slides with 1% aqueous aceto-carmine. The chromosomal formula was calculated on the basis of at least five metaphase plates. The best metaphase plates were photographed with Olympus BX-51.

- Molecular method

Total genomic DNA was extracted from leaves taken from herbarium specimen with the DNeasy plant mini kit DNA extraction (Qiagen) following the manufacturer's protocol. The detail of PCR program for the amplification of the ITS nrDNA have already been published (Sonboli *et al.* 2010a). A dataset consisting of 28 accessions was analyzed (Table 2). *Ursinia anthemoides* Gaertn. was considered as the outgroup. Phylogenetic analyses of the ITS nrDNA dataset (ITS1+ITS2 only) were reconstructed with maximum parsimony (MP) method using PAUP* (Swofford 2002) and Bayesian inference (BI) using MrBayes (Ronquist & Huelsenbeck 2003), employing the same search strategies as in Sonboli *et al.* (2010a). For the Bayesian analysis, the GTR+G model was selected using the program MrModeltest (Nylander 2004) as implemented

in MrMTgui (Nuin 2005) based on the Akaike information criterion (Posada & Buckley 2004).

Results and Discussion

- Morphology

During the revision of the genus *Tanacetum* in different herbaria of Iran (2004–2010), some specimens in the herbarium of the Research Centre of Natural Resources and Agriculture of Kerman province (Iran), TARI and TUH were found that have already been identified as *T. pamiricum*. According to Flora Iranica (Podlech 1986) and Flora of USSR (Tzvelev 2000) these specimens were preliminarily identified as *T. cf. fisherae*. When they were checked with specimens of *T. fisherae* from Afghanistan and isotype specimen in the herbarium G, determination was successfully carried out. It was characterized as *T. fisherae*, which is previously known as a species distributed in east and centre of Afghanistan, Pakistan and Tajikistan (Pamir-Alai region). *T. fisherae* was not reported before from Iran in the relevant literatures (Podlech 1986, Mozaffarian 2008). Therefore, it is recorded here for the first time from Iran.

Morphologically, *T. fisherae* is closely related to *T. rupestre* and *T. pamiricum*. The diagnostic morphological characters of *T. fisherae* with allied species are given in Table 3. *T. fisherae* can be easily distinguished from *T. pamiricum* by its pappus structure (entire, unilateral not divided to the base into 3–6 oblong scales), segments of pinnate leaves remote, terete and mucronulate (not approximate and linear), peduncle 3–15 cm long (not 6 cm),

margin color of involucre bracts dark brown (not brownish). *T. fisherae* resembles *T. rupestre*, but differs in having more densely hairy leaves, petiole longer than lamina, peduncle length up to 3–4 cm, diameter of involucre 4–5 mm and achene size 1.8–3.0 mm. A complete description along with the synonyms, type specimen details and specimens examined are presented as follows:

Table 2. List of taxa used for the current molecular study. ^aThe two accession numbers for nrDNA ITS of some taxa represent ITS1 and ITS2 regions, respectively

Taxa	Source and collection data	GenBank accession No. ^a
<i>Anthemidiinae</i>		
<i>Gonospermum fruticosom</i> (C. Smith ex Link) Less.	Sonboli & Oberprieler (2010)	FN827335/FN827336
<i>Lugoa revoluta</i> (C. Smith ex Link) DC.	Sonboli & Oberprieler (2010)	FN827337/FN827338
<i>Tanacetum canescens</i> DC.	Oberprieler (2004b)	AJ864584/AJ864604
<i>T. chiliophyllum</i> (Fisch. & Mey.) Sch. Bip.	Oberprieler (2004b)	AJ864585/AJ864605
<i>T. corymbosum</i> (L.) Sch. Bip.	Francisco-Ortega <i>et al.</i> 2001	AF155254/AF155291
<i>T. fisherae</i> Aitch. & Hemsl.	Iran: Kerman, Poormirzaii & Ebrahimi 5366 (MPH)	Submitted
<i>T. joharchii</i> Sonboli & Kaz. Osaloo	Sonboli <i>et al.</i> (2010a)	AB523746
<i>T. kotschyi</i> (Boiss.) Grierson	Sonboli <i>et al.</i> (2010a)	AB523748
<i>T. parthenium</i> (L.) Sch. Bip.	Sonboli & Oberprieler (2010)	FN823080
<i>T. pinnatum</i> Boiss.	Oberprieler (2004b)	AJ864588/AJ864608
<i>T. tenuisectum</i> (Boiss.) Podl.	Sonboli <i>et al.</i> (2010a)	AB523747
<i>T. vulgare</i> L.	Francisco-Ortega <i>et al.</i> (2001)	AF155263/AF155299
<i>Handeliinae</i>		
<i>Handelia trichophylla</i> (Schrenk) Heimerl	Oberprieler <i>et al.</i> (2007)	AM774422
<i>Lepidolopsis turkestanica</i> (Regel & Schmalh.) Poljakov	Vallès <i>et al.</i> (2003)	AF504190/AF504163
<i>Microcephala discoidea</i> (Ledeb.) K. Bremer <i>et al.</i>	Watson <i>et al.</i> (2002)	AY127677/AY127678
<i>Pseudohandelia umbellifera</i> (Boiss.) Tzvelev	Oberprieler <i>et al.</i> (2007)	AJ880330/AM774468
<i>Richteria pyrethroides</i> Kar. & Kir.	Oberprieler <i>et al.</i> (2007)	AM774425
<i>R. tianschanica</i> (Krasch.) Sonboli & Oberpr.	Oberprieler <i>et al.</i> (2007)	AM774430
<i>Sclerorhachis platyrhachis</i> (Boiss.) Podlech ex Rech. f.	Oberprieler <i>et al.</i> (2007)	AM774426
<i>Tanacetopsis mucronata</i> (Regel & Schmalh.) Kovalevsk.	Oberprieler <i>et al.</i> (2007)	AM774428
<i>Trichanthemis aulieatensis</i> (B. Fedtsch.) Krasch.	Oberprieler <i>et al.</i> (2007)	AM774429
<i>Xylanthemum fisherae</i> (Aitch. & Hemsl.) Tzvelev	Sonboli & Oberprieler (2010)	FN827333/FN827334
<i>Artemisiinae</i>		
<i>Ajania fastigiata</i>	Vallès <i>et al.</i> (2003)	AF504169/AF504142
<i>Artemisia vulgaris</i> L.	Oberprieler & Vogt (2000)	AJ3296389/AJ3296424
<i>Artemisiella stracheyi</i> (Clarke) Ghafoor	Oberprieler <i>et al.</i> (2007)	AM774421
<i>Filifolium sibiricum</i> (L.) Kitam.	Sanz <i>et al.</i> (2008)	AF504160/AF504187
<i>Hippolytia dolichophylla</i> (Kitam.) K. Bremer & Humphries	Oberprieler (2004a)	AJ748784/AJ748785
Outgroup		
<i>Ursinia anthemoides</i> (L.) Poiret	Himmelreich <i>et al.</i> (2008)	AM774473

Table 3. Diagnostic morphological characters of *Tanacetum fisherae* and allied species

Character	<i>T. fisherae</i>	<i>T. rupestre</i>	<i>T. pamiricum</i>
Leave			
Indumentum	Sparsely hirsute, densely glandulous-punctate	Appressed hairy, dense pubescence	Appressed hairy, glandulous-punctate
Lamina	Pinnate, oblong, with remote, terete, mucronulate segments	Pinnate, ovate or oblong, segments less remote, linear, integer or apically bi- or trifid	Pinnate, broadly oval, segment ternis linear
Petiole length of lower leaves	As long as lamina	Longer than lamina	Longer than lamina
Peduncle length (cm)	Up to 15	Up to 3–4	Up to 6
Involucre			
Diameter (mm)	5–8	4–5	5–8
Margin color of bracts	Dark-brown	Dark-brown	Brownish
Achene size (mm)	3.5–4.0	1.8–3.0	0.4–4.0
Pappus			
Size (mm)	1.5–2	1.2–2.0	1.2–1.8
Structure	Entire, unilateral	Entire, unilateral	Divided to the base into 3–6 oblong scales

Tanacetum fisherae Aitch. & Hemsl. J. Linn. Soc. Bot.

19: 170, 1882 (Figs 1–3).

Syn.: *Pyrethrum fisherae* (Aitch. & Hemsl.) Boiss., Fl.

Or. Suppl. 300 (1888). *Chrysanthemum fisherae* (Aitch. & Hemsl.) Rech. f. In: K. Danske Vid. Selsk., Biol. Skrift., viii. No. 2 (Symb. Afghan. II.) 45 (1955). *Xylanthemum fisherae* (Aitch. & Hemsl.) Tzvel., Fl. USSR, 26: 271–274 (2000).

Type: Afghanistan: Jaji, Hariab district, Sikaram mountain, Seratigah and Sergal, 3000–3800 m, J.E.T. Aitchison. 366 (K; iso G!).

Perennial. Small undershrubs, up to 35 cm high, dingy-green with two-branched hairs and with thick woody rootstocks. Stems highly branched near to base, lignifying for a considerable distance, evenly-foliated, upright or ascending. Leaves sparsely hairy, hirsute, densely punctate-glandulous, petiole of lower leaves as long as lamina; lamina oblong, pinnately cut up to axis, with 2–4 remote lateral segments; segments oblong to linear, subobtuse; uppermost leaves reduced, sessile, less divided (to entire). Capitula solitary, numerous on long (up to 15 cm long) upright peduncles. Involucres 5–8 mm in diameter and 5–7 mm high, more or less hairy; involucre bracts with dark-brown membranous margin, external bracts triangular, 3 × 1.5 mm, internal ones oblong, 5 × 2 mm. Corollas of tubular flowers yellow,

3.0–3.5 mm long, 5-toothed at apex; teeth 0.5 mm long. Achenes 3.5–4.0 mm long, c. 1.0 mm wide; longitudinally with 5 distinct ribs, punctate-glandulous, corona-shaped pappus c. 2.0 mm long, entire, one sided, irregularly sinuate-toothed at apex.

Flowering & Fruiting: June–August.

Habitat: Stony and broken-stony slopes, at heights above 2400 m.

Distribution: Afghanistan, Pakistan, Central Asia (Tajikistan: Pamir Alai regions), Iran (Kerman province) (Fig. 4).

Specimens examined: Iran: Kerman province, Kerman, 40 km from Jiroft on the road to Kerman, Kuh-e Sarzeh, 3000–3300 m, 13.6.1977, Assadi & Miller, TARI! (sub *T. pamiricum*); Baft, Gugher, Bondar, *Mirtajeddin*, 31985! (TUH); Kerman, Mehr mountain, north and east slopes, 2400–2935 m, 16.6.2004, Poormirzaii & Ebrahimi 5366!; Afghanistan: Gardez, Safed kuh, in mountains east of Altimur pass, Rech. 31997 W!; Khost: southeast slopes of Sata Kandao pass, inter Gardez and Khost, 2200–2800 m, 8.7.1965, Rech. 32129 W!; Ghazni, inter Sariab and Dasht-e Nawar, 3100 m, 18.7.1967, Rech. 37237 W!; Bamian, inter Bamian and Band-e Amir, 2800–3000 m, 13.7.1962, Rech. 18202 W!.

- Cytology

The constant chromosome number found in all metaphase plates was $2n=44+1B$ (Fig. 5). To our knowledge, this is the first report of chromosome number in *Tanacetum fisherae*, indicating a pentaploid level ($5x$) based on $x=9$. A new ploidy level (pentaploidy) is reported for the first time for the genus. While most chromosomes are metacentric or submetacentric, one subtelocentric chromosome was found. The karyotype formula is given as $2n=5x=25m+15sm+4st$. The studied population is aneuploid, having lost one chromosome out of the 45 expected in a $x=9$ -based pentaploid; this may be arisen from the irregular meiosis phenomenon that occurs in odd-number polyploids. The diploid

chromosome number ($2n=2x=18$) has already been reported in *Tanacetum parthenium* Sch. Bip., *T. albipannosum* Hub.-Mor & Grierson, *T. macrophyllum* Sch. Bip., *T. coccineum* (Willd.) Grierson ssp. *chamaemelifolium* (Sommier & Levier) Grierson, and *T. sorbifolium* (Boiss.) Grierson from Turkey (Inceer & Hayirlioglu-Ayaz 2007). There is also report of $2n=10x=90$ for *T. polycephalum* Sch. Bip. from Iran (Chehregani & Mehanfar 2008). The presence of B-chromosome has already been reported from *Tanacetum corymbosum* (L.) Sch. Bip. ($2n=36+3B$; Baltisberger 1990) and in other *Anthemideae*, such as *Ajania fruticulosa* (Ledeb.) Poljakov ($2n=36+4B$; Garcia *et al.* 2006).



Fig. 1. Type specimen of *Tanacetum fisherae* (iso-G!).



Fig. 2. Scanned image of *Tanacetum fisherae*.

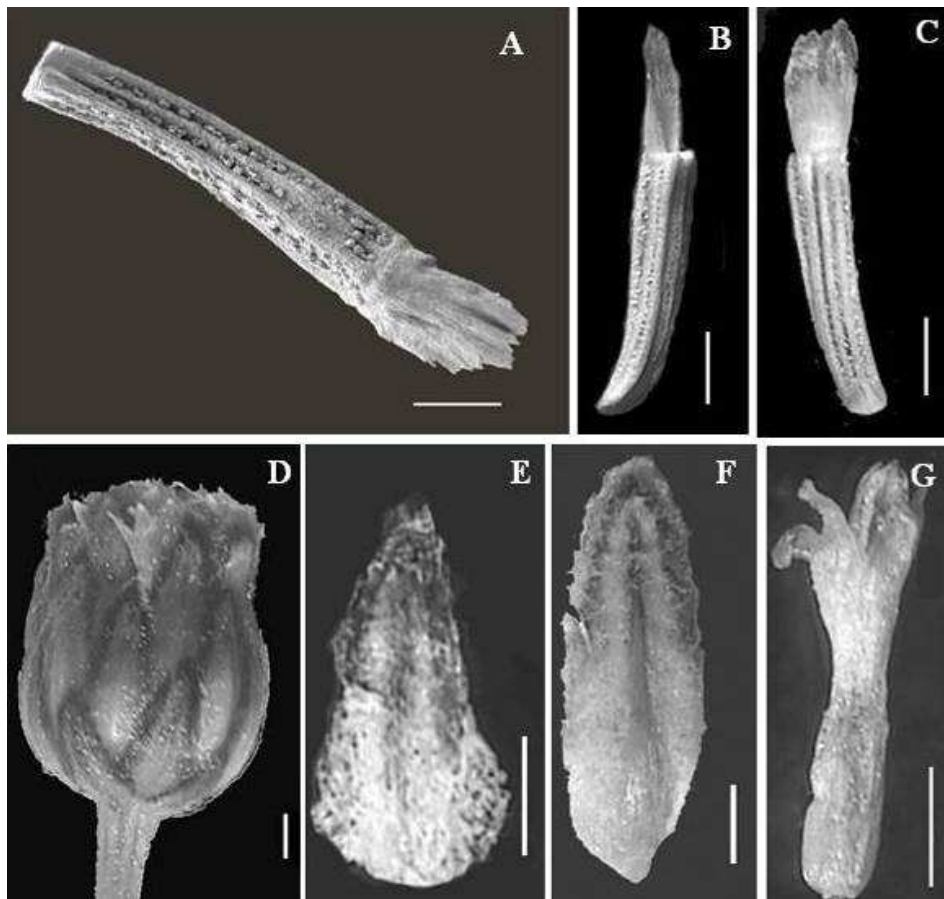


Fig. 3. SEM micrographs of *Tanacetum fisherae*: A–C. Achene, D. Capitulum, E. Outer bract, F. Inner bract, G. Tubular floret.

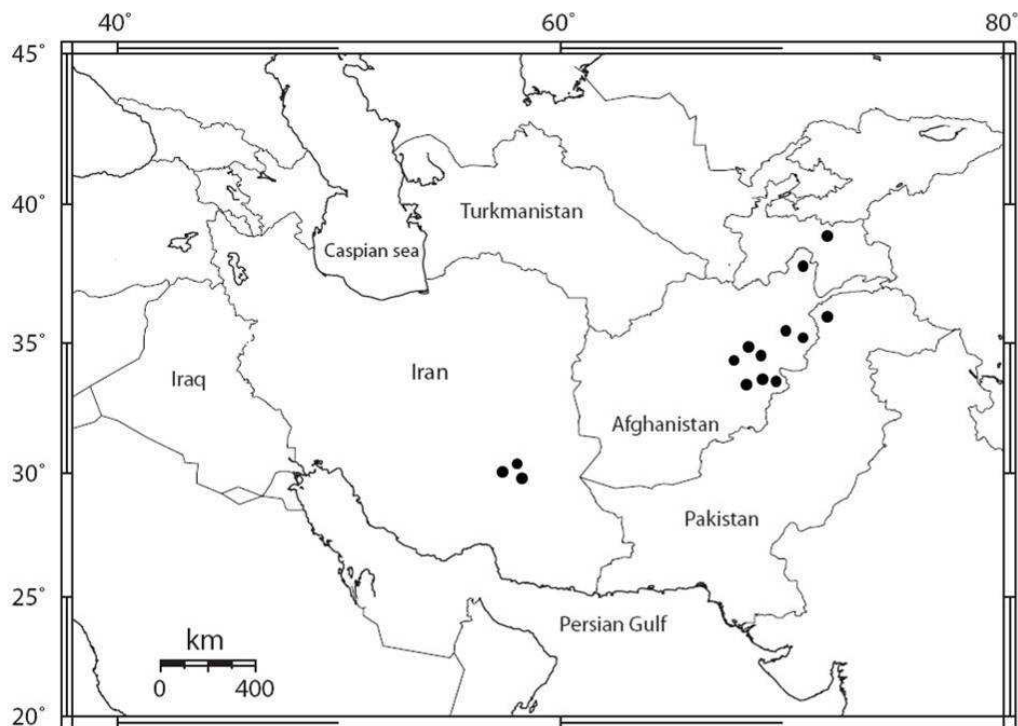


Fig. 4. Distribution map of *Tanacetum fisherae*.

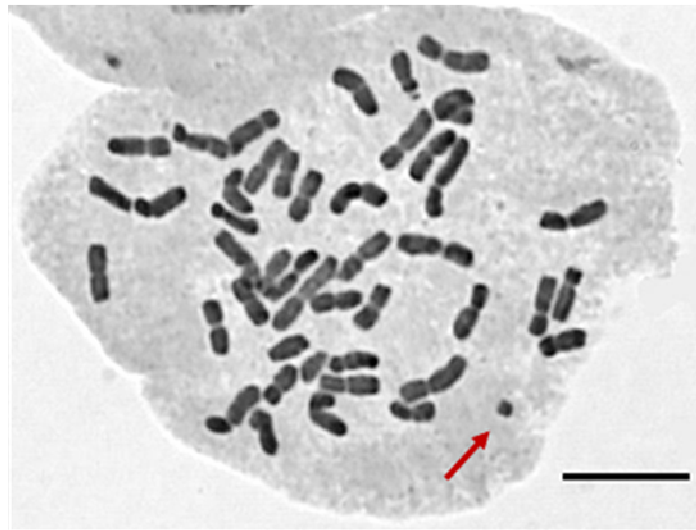


Fig. 5. Mitotic metaphase of *Tanacetum fisherae* ($2n=5x=44+1B$). Arrow shows B-chromosome (Bar = 10 μ m).

- Molecular phylogeny

The aligned dataset of nrDNA ITS consisted of 501 nucleotide sites, of which 115 sites were parsimony informative. Of the remaining nucleotide sites, 298 sites were constant and 88 variable. The MP analysis resulted in 416 most parsimonious trees of 347 steps with a consistency index (CI) of 0.706 and retention index (RI) of 0.806. The strict consensus tree of the MP analysis (tree not shown), is topologically similar to that of the Bayesian analysis (Fig. 6). *T. fisherae* originating from Iran along with a sample identified as *X. fisherae*, the type species of the genus *Xylanthemum*, from Afghanistan formed a well-supported clade nested in the *Tanacetum* group with high posterior probability (pp=1) and bootstrap value of 89%. This clade has been appeared as sister to the representatives of *Tanacetum* sect. *Xylopyrethrum*.

Taxonomic conclusion

The taxonomic position of *Xylanthemum* as a separate genus or a section of the genus *Tanacetum* is still unclear and controversial. While the majority of the authors (Bremer & Humphries 1993, Tzvelev 2000,

Ghafoor 2002, Oberprieler *et al.* 2006, 2007, 2009) accepted *Xylanthemum* as a distinct genus, Podlech (1986) relegated it to a section of *Tanacetum*. Based on a cladistic analysis of morphological characters the genus *Xylanthemum* was found to be closer to the genus *Tanacetum* of *Anthemidiinae* subtribe than the representatives of the subtribe *Handeliinae* (Sonboli 2009). Recently, the phylogenetic position of *X. fisherae* has been shown to be related to the representatives of *Tanacetum* (Sonboli & Oberprieler 2010). As a consequent, based on the results obtained here the retaining of *T. fisherae* in its original taxonomic position under the genus *Tanacetum* is highly supported and the combination of *X. fisherae* should be considered as a synonym.

Acknowledgements

The authors would like to thank Research and Technology Council of Shahid Beheshti University (Tehran, Iran) for financial support. We would like to express our kind appreciation to Prof. Dr. Joan Vallès (University of Barcelona, Spain) for his invaluable comments.

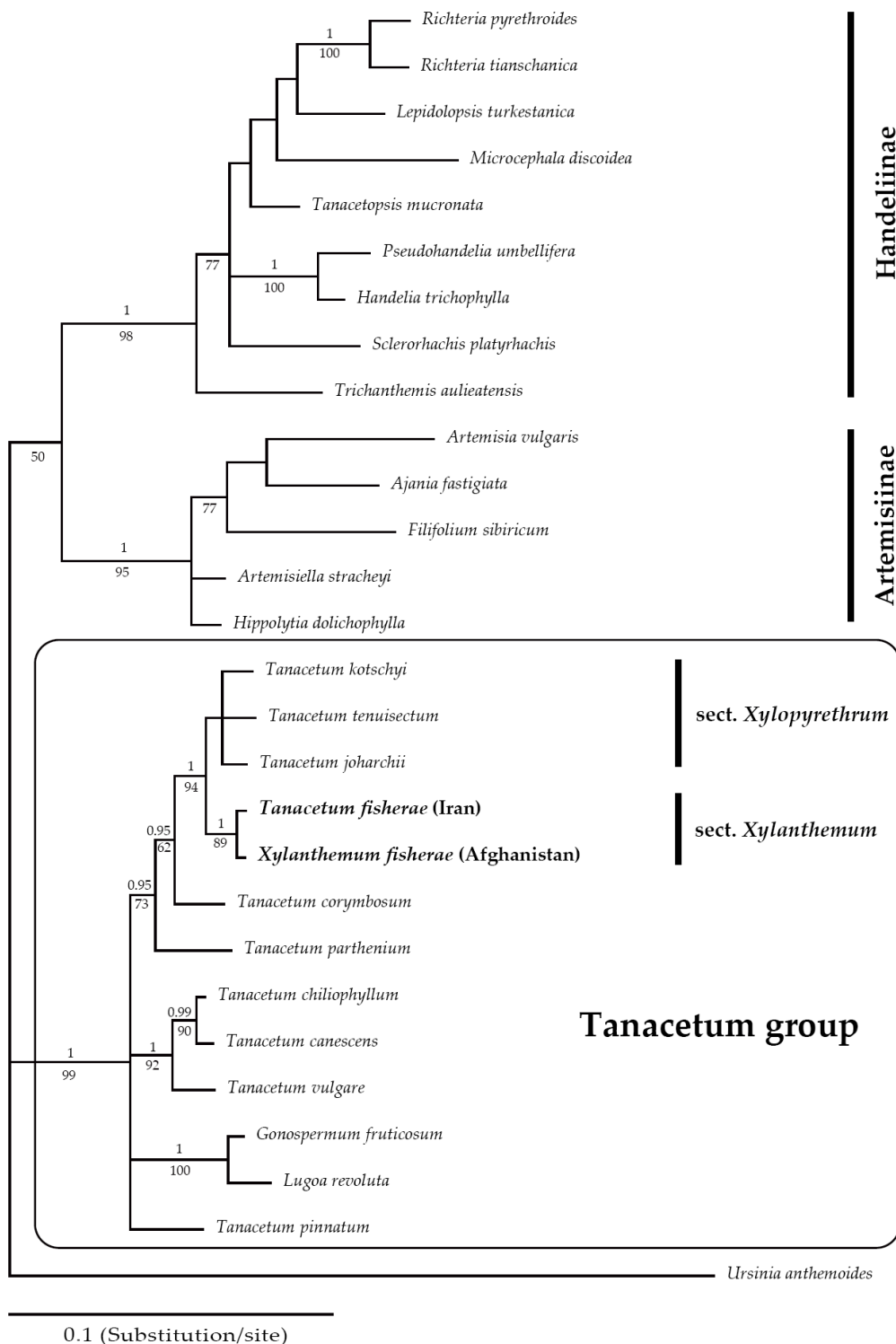


Fig. 6. Fifty percent majority rule consensus tree with mean branch length resulting from the Bayesian analysis of nrDNA ITS sequences. Posterior probabilities are presented above branches and MP bootstrap values (>50) are below them.

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