Tanacetum zahlbruckneri (*Compositae-Anthemideae*), an enigmatic record from Iran and its phylogenetic position

گونه *Tanacetum zahlbruckneri* (*Compositae-Anthemideae*)، یک رکورد معمایی از ایران و موقعیت فیلوژنتیکی آن

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Abstract

Tanacetum zahlbruckneri is reported as a new record from northwestern Iran. A complete description, diagnostic characters and a distribution map of *T. zahlbruckneri* are presented. The phylogenetic position of *T. zahlbruckneri* in a concise molecular framework based on the sequences of ITS nrDNA data are presented. A taxonomic conclusion about the previously reported samples of this enigmatic species is given.

Keywords: *Tanacetum*, *Asteraceae*, new record, ITS, phylogeny

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چکیده

گونه *Tanacetum zahlbruckneri* از شمال غرب ایران (ارومیه، سرو) برای فلور ایران گزارش می‌شود. این گونه معمایی پیش از این دو بار به اشتباه از ماکو و دره قاسملوی ارومیه برای ایران به عنوان گزارش جدید معرفی شده بود. شرح کامل گیاه‌شناسی، صفات متمایز کننده و پراکنش گونه در دنیا به همراه جایگاه فیلوژنتیکی آن براساس داده‌های توالی ITS nrDNA در چارچوب مولکولی در کنار سایر گونه‌های جنس *Tanacetum* ارایه شده است. در نهایت یک نتیجه‌گیری تاکسونومیکی براساس شواهد مورفولوژیکی و مولکولی برای روشن شدن جایگاه تاکسونومیکی این گونه ارایه شده است.

واژه‌های کلیدی: *Tanacetum*، *Asteraceae*، گزارش جدید، ITS، فیلوژنی

Introduction

Asteraceae, as the largest family of angiosperms, comprising over 1600 genera and 23000 species distributed in the worldwide (Jeffrey 2007). *Tanacetum* L. (*Asteraceae-Anthemideae*) is the third largest genus after *Artemisia* L. and *Anthemis* L. within the tribe, comprising around 160 species (Oberprieler *et al*. 2007a). In the Flora Iranica area, the genus is represented by 54 species (Podlech 1986). Considering recent findings, the species number of *Tanacetum* in flora of Iran is increased to 35 (Mozaffarian 2005, Djavadi 2008, Sonboli *et al.* 2010).

Chrysanthemum zahlbruckneri Nab. was originally introduced from Hakkari province, eastern Turkey (Nabelek 1925). Grierson (1975) transferred this species to the genus *Tanacetum* as *T. zahlbruckneri* (Nab.) Grierson, and speculated its relationship with *T. coccineum* (Willd.) Grierson and *T. silaifolium* (Steven) Sch.-Bip. than *T. corymbosum* (L.) Sch.-Bip. with which it was originally compared. Once *T. zahlbruckneri* (Nab.) Grierson, recorded from northwestern of Iran, Maku (Mozaffarian 1991), but this specimen in the recent treatment for flora of Iran (Mozaffarian 2008) was determined as *Tripleurospermum caucasicum*. Once again, Djavadi (2008) misdetermined one specimen of IRAN herbarium (Matin & Daneshpajouh, IRAN-12625) as *T. zahlbruckneri* from Ghasemloo valley, southwest of Urmia (W Azarbaijan province), and published it as a new record for flora of Iran. The main objective of the present study was aimed to characterize the correct taxonomic and phylogenetic position of the enigmatic *T. zahlbruckneri* for the flora of Iran. Therefore, a concise molecular study, based on ITS nrDNA sequences of 16 representatives of *Tanacetum*, was undertaken to infer the phylogenetic position of *T. zahlbruckneri* and not for phylogeny reconstruction of the genus *Tanacetum* as a whole.

Materials and Methods

Herbarium specimens of the genus *Tanacetum* available in several herbaria (G, W, MPH, TARI and IRAN) and herbarium of the research centre of natural resources and agriculture of Urmia were revised and reidentified using relevant literatures (Podlech 1986, Grierson 1975, Nabelek 1925, Mozaffarian 2008). Among them, a specimen deposited in herbarium of the research centre of natural resources and agriculture of Urmia was considered to be an actual representative of *Tanacetum zahlbrukneri* and reported here as a new enigmatic record for the flora of Iran.

- Molecular procedure

Total genomic DNA was extracted from leaves taken from herbarium specimens 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 followed Sonboli *et al*. (2010). Once the position of *T. zahlbrukneri* in the full dataset of *Anthemideae* (data not shown) was established, a dataset consisting of 18 accessions was reanalyzed (Appendix). *Tanacetopsis eriobasis* and *Richteria pyrethroides*, which already belonged to the *Tanacetum* s.l., was considered as outgroups. 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 Kazemi *et al*. (2009). For the Bayesian analysis, the SYM+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

- Taxonomic note

During revision of the genus *Tanacetum* in different herbaria of Iran, a single specimen of research centre of natural resources and agriculture of Urmia, Iran, at a glance was determined as *T. zahlbruckneri* according to the Flora of Turkey (Grierson 1975). When it was checked with a specimen of *T. zahlbruckneri* from

Turkey which was deposited in TARI together with the image of type specimen (Nabelek 1925), determination was successfully achieved. It was identified as *T. zahlbruckneri*, an endemic species in SE Turkey.

Tanacetum zahlbruckneri (Nab.) Grierson, Notes Roy. Bot. Gard. Edin. 33: 435 (1975)

Syn.: *Chrysanthemum zahlbruckneri* Nab., Spisy Prir. Fak. Masarykovy Univ. No. 52: 20 (1925)

Type: [Turkey C9 Hakkari] mons maidanoke supra pagum Hasitha (Asutka), 1700–1800 m, No. 3598 (photo!).

Diagnosis: Rhizomatous herb. Leaves 2-pinnatisect, with narrow linear and acuminate terminal lobes, radical leaves with long petiole. Capitula radiate, 2–5 (usually 3) per stem; ray florets white. Achene 5-ribbed, coronula, c. 0.2 mm high, marginiform, denticulate.

Perennial, 20–40 cm high. Rhizome woody, dark brown, many-stemmed. Stem herbaceous, lower parts becoming black, erect, moderately whitish-pilose, slightly ribbed, leafy. Radical leaves 6–10 cm long (including petiole); petioles as long as lamina or longer, fragile, whitish-pilose, pale yellow, widened, on the upper side canaliculate; lamina obovate or oblong in outline, 2-pinnatisect; primary segments 4–5 paired, each finely dissected into 3–5 simple or trifid, narrowly linear or lanceolate, acuminate lobes, 10–20 × 0.5–1.0 mm, sparsely pilose. Cauline leaves similar to basal one, lower ones petiolate, upper ones sessile, auriculate. Inflorescence corymbous with sturdy branches, usually 2–5, rarely solitary, nearly erect, whitish-ribbed, sparsely pilose, 5–12 cm long. Capitula large, 3.5–5 cm diameter (including ray flowers), without ray flowers 1.5 cm wide and 1.0 cm high, nearly hemisphericous. Involucral bracts numerous; outer ones ovate-triangular, acute, hirsute, with dark brown scarious margins, lacerate-ciliate, 6 × 3 mm; inner ones longer, 10 × 4 mm, obovate-oblanceolate, above the middle with black or brownish scarious margins, Receptacle convex. Ray flowers pistillate; with white or pale sulphur yellow ligules, elliptic-obovate, apex trilobulate, 15 × 7 mm; tube very short, c. 1 mm. Disc flowers tubular, bisexual, yellow; tube cylinder, c. 2 mm long; limb campanulate or funnel-shaped, c. 2 mm. Achenes brown, obscurely pentagonous, 5 × 2 mm, attenuate at base, subincurved, tuberculate with sessile glands, truncate at apex, with short coronula, c. 0.2 mm high, marginiform, denticulate (Figs 1 & 2).

Fig. 1. *Tanacetum zahlbruckneri* (Nab.) Grierson: Habit and habitat photograph taken from the locality collected and recorded in the present study.

Specimens examined: Iran, W Azarbaijan province, Urmia, Sero road, Golsheykhan village, Koreshams mountain, 1850 m, Alizadeh *et al.* 602 (MPH!); *ibid*, 1990 m, 37° 40′ 23.5″N, 44° 40′ 12.8″E, 2 June 2010, Sonboli *et al.* 1412 (MPH!). Turkey, Bitlis, Van, 10 km S.E. of Pelli, 8 July 1954, Davis & Polunin, D. 22538 (TARI!).

- General distribution

Phytogeographically, *Tanacetum zahlbruckneri* is an Irano-Turanian element (Fig. 3) which is growing in mountainous meadows at 1800–2600 m. This species is mainly distributed in SE Turkey, where is known as its

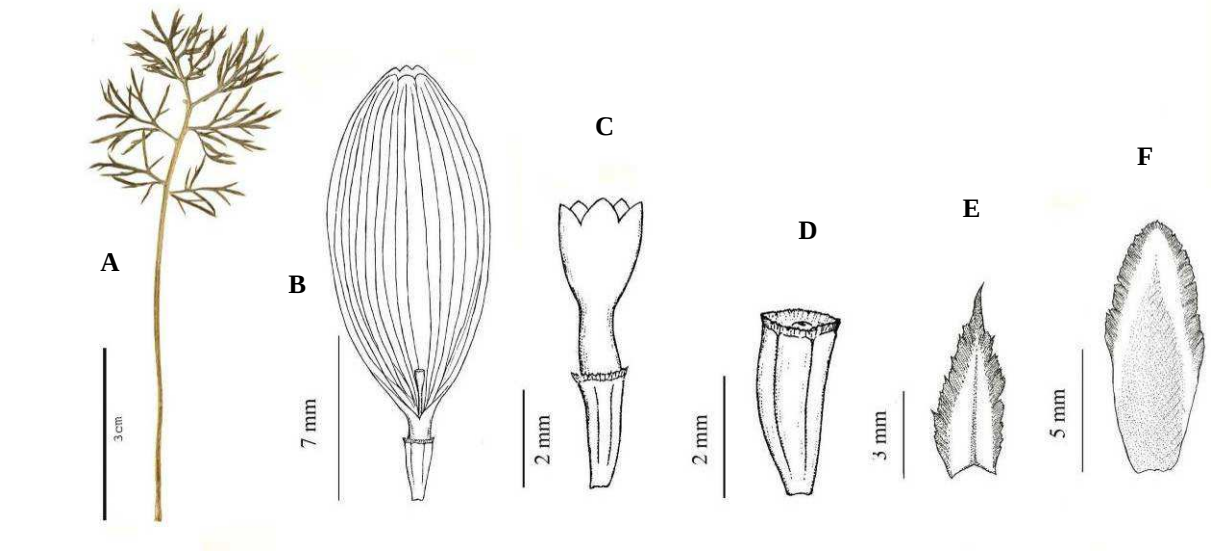


Fig. 2. Drawing of individual parts of capitulum and basal leaf of *Tanacetum zahlbruckneri*: A. Basal leaf, (B) Ray floret, C. Tubular floret, D. Achene, E. Outer involucral bract, F. Inner involucral bract.

type locality, as well as several collection sites, especially regions adjacent to northwest of Iran. The specimen identified here as *T. zahlbrukneri* and its locality, where it has been collected from northwest of Iran (Sero road, a border area close to the type locality) seems to be true representative of this species in Iran.

- Molecular analysis

The length of the ITS nrDNA sequences varied from 454 to 475 bp of which, 55 characters were parsimony informative. The MP analysis resulted in 36 most parsimonious trees of 135 steps with a consistency index (CI) of 0.837 and retention index (RI) of 0.780. On the Bayesian tree (Fig. 4), *T. zahlbrukneri* accessions occupy a position in a well supported clade with 1.0% posterior probability and 72% bootstrap percentage as sister to a clade consisting of *T. kotschyi*, *T. corymbosum* and *T. parthenium*.

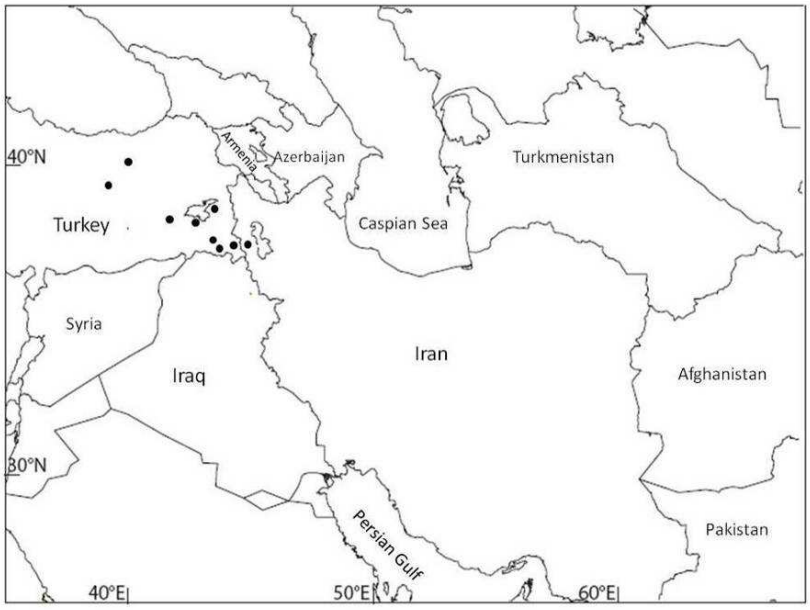
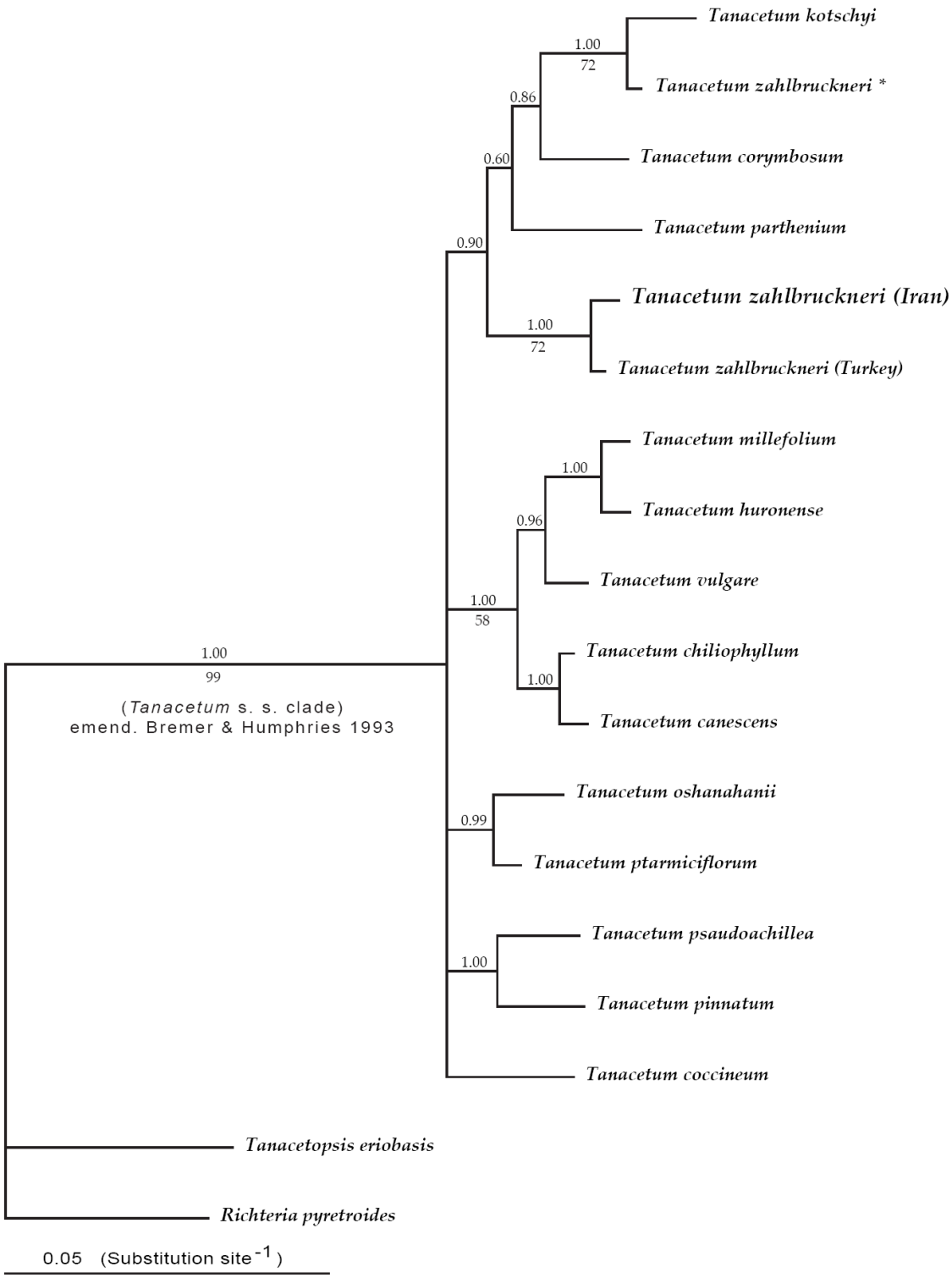


Fig. 3. Distribution map of *Tanacetum zahlbruckneri* in Iran and Turkey.



* This sample referred to Djavadi (2008) where it has been recorded as *T. zahlbruckneri* from Iran.

Fig. 4. Fifty percent majority rule consensus tree with mean branch length resulting from the Baysian analysis of ITS nrDNA sequences. Posterior probabilities are presented above branches and MP bootstrap values (>50) are below them.

Discussion

After a thorough survey of herbarium specimens (G, W, MPH, TARI and IRAN) and studies of specific descriptions of *Tanacetum* in relevant literatures (Nabelek 1925, Grierson 1975, Podlech 1986), it was concluded that the specimen determined as *T. zahlbruckneri* by Djavadi (2008), could not be considered as an actual representative of *T. zahlbruckneri* for Iran. The position of *T. zahlbrukneri* in the reconstructed molecular phylogeny is congruent with our interpretation of its morphological characters and confirmed its distant affinity to *T. kotschyi*.

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Appendix: List of taxa used for the current molecular study. [a] The two accession numbers for ITS nrDNA of some taxa represent ITS1 and ITS2 regions, respectively; [b] This specimen referred to Djavadi (2008) where it has been reported as a new record for Iran

Taxon	Source & collection data	GenBank
Ingroup		
Tanacetum canescens DC.	Oberprieler 2004	AJ864584/AJ864604
T. chiliophyllum (Fisch. & C.A. Mey.) Sch.-Bip.	Oberprieler 2004	AJ864585/AJ864605
T. coccineum (Willd.) Grierson	Oberprieler 2004	AJ864586/AJ864606
T. corymbosum (L.) Sch.-Bip.	Francisco-Ortega *et al*. 2001	AF155254/AF155291
Tanacetum huronense Nutt.	Francisco-Ortega *et al*. 2001	AF155256/AF155293
T. kotschyi (Boiss.) Grierson	Sonboli *et al*. 2009	AB523749
Tanacetum millefolium (L.) Tzvelev.	Guo *et al*. 2004	AY603263
Tanacetum oshanahanii Marrero Rodr., Febles & Suarez	Francisco-Ortega *et al.* 2001	AF155259/AF155296
T. parthenium (L.) Sch.-Bip.	Oberprieler 2004	AJ864587/AJ864607
T. pinnatum Boiss.	Oberprieler 2004	AJ864588/AJ864608
T. pseudoachillea Winkler.	Francisco-Ortega *et al.* 2001	AF155260/AF155300
Tanacetum ptarmiciflorum (Webb & Berth.) Sch.-Bip	Francisco-Ortega *et al.* 2001	AF155262/AF155298
T. vulgare L.	Francisco-Ortega *et al.* 2001	AF155263/AF155299
T. zahlbruckneri (Nab.) Grierson	Urmia, Sero road, Golsheykhan village, Sonboli *et al*., MPH-1412	-
T. zahlbruckneri (Nab.) Grierson	Turkey, Bitlis, Van, 10 km S.E. of Pelli, Davis & Polunin, D. 22538	-
T. zahlbruckneri (Nab.) Grierson [b]	Urmia, Ghasemloo valley, Matin Daneshpajouh, IRAN-12625	-
Outgroup		
Tanacetopsis eriobasis (Rech. f.) Kovalevsk. = *Tanacetum eriobasis* (Rech. f.) S. Koval.	Oberprieler *et al.* 2007b	AM774427
Richteria pyrethroides Kar. & Kir. = *Tanacetum pyrethroides* (Kar. & Kir.) Sch.-Bip.	Oberprieler *et al.* 2007b	AM774425