

Tanacetum isfahanicum (Compositae-Anthemideae), a new species from Iran and its phylogenetic position based on nrDNA ITS data

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Received: 26.03.2023 • Accepted/Published Online: 05.07.2023 • Final Version: 27.07.2023

Abstract: *Tanacetum isfahanicum* Sonboli and Naderifar (Compositae-Anthemideae) is described as a new species from Isfahan and Fars provinces of Iran. It belongs morphologically to *Tanacetum* sect. *Xanthoglossa* and differs from its the closest relative, *T. polycephalum*, by shape of basal and cauline leaves, shape of capitula, length and width of involucre and color of achene ribs. The phylogenetic position of the new species within the genus *Tanacetum* is established based on nrDNA ITS sequences variation and its close relationship with *T. polycephalum* is corroborated.

Key words: Anthemideae, Asteraceae, morphology, new species, nrDNA ITS, *Tanacetum*

1. Introduction

Tanacetum L. with c. 154 species is the second largest genus in the subtribe Anthemidinae Dumort., tribe Anthemideae of the family Compositae. The members of the genus are distributed mainly in Europe, SW Asia, northern Africa and North America (Oberprieler et al., 2022).

The species of the genus *Tanacetum* are perennials, characterized by epaleate receptacles, solitary or numerous capitula, disciform or discoid, in lax to dense corymbs, oblong achenes with longitudinal ribs and a coroniform or auriculate pappus (Tzvelev, 1995; Oberprieler et al., 2006). Based on Flora Iranica (Podlech, 1986), 25 species of this genus are growing in Iran. After publication of Flora of Iran (Mozaffarian, 2008), in which the number of species has been increased to 31, several new species e.g., *T. joharchii* Sonboli and Kaz. Osaloo (Sonboli et al., 2010a) and *T. tarighii* Sonboli (Kazemi et al., 2014a) and new records such as *T. punctatum* Desr. (Djavadi, 2008), *T. zahlbrucknerii* Nab. (Sonboli et al., 2010b), *T. fisherae* Aitch. and Hemsl. (Sonboli et al., 2011a) and *T. tenuissimum* Trautv. (Olanj and Sonboli, 2021) were reported from Iran.

Tanacetum sect. *Xanthoglossa* is comprising 17 species in Iran and characterized by 2–3-pinnatifid or 2–3 pinnatisect leaves, heterogamous capitula, solitary or numerous, with yellow-rayed, disciform or radiate, oblong achene with coroniform pappus (Podlech, 1986; Tzvelev, 1995). During a comprehensive morphological study of undetermined *Tanacetum* species deposited in the Herbarium of Medicinal

Plants (MPH), Shahid Beheshti University of Tehran, Iran (Thiers, 2008), we concluded that the specimen collected from Isfahan (MPH-1172) and Fars (MPH-1667) provinces could be considered as a new undescribed species of the genus *Tanacetum* from Iran. The evolutionary relationships between the new species and related taxa in a molecular phylogenetic framework is reconstructed and discussed based on the information obtained from nrDNA ITS sequence variation.

2. Material and methods

2.1. Taxon sampling and molecular study

Deposited specimens at the Herbarium of Medicinal Plants and Drugs Research Institute (MPH) of Shahid Beheshti University of Tehran, Iran were identified and studied based on identification keys and descriptions available in literature (Grierson, 1975; Podlech, 1986; Tzvelev, 1995; Mozaffarian, 2008). A minimum of three to five herbarium specimens (MPH) were examined from each population in terms of morphometric features. Five new sequences from *Tanacetum* species were generated and sequences of 13 other *Tanacetum* species were retrieved from GenBank (Guo et al., 2004; Sonboli et al., 2010a, 2011b, 2012; Kazemi et al., 2014a). *Tanacetopsis eriobasis* and *Sclerorhachis platyrachis* were selected as outgroups (Oberprieler et al., 2007; Sonboli et al., 2010a; Hassanpour et al., 2018). Voucher information and accession numbers of all sequences are depicted in Table 1.

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Table 1. List of taxa used for the current molecular phylogenetic analyses. The two GenBank accession numbers for nrDNA ITS of some taxa represent ITS1 and ITS2 regions, respectively. Newly deposited sequences are highlighted in bold.

Taxon	Source (location & voucher)	GenBank accession No.
Outgroups		
<i>Tanacetopsis eriobasis</i> (Rech.f.) Kovalevsk.	Oberprieler et al. 2007	AM774427
<i>Sclerorhachis platyrachis</i> (Boiss.) Podlech	Hassanpour et al. 2018	LC313944
Ingroups		
<i>Tanacetum abrotanifolium</i> (L.) Druce	Sonboli et al. 2012	AB683260
<i>T. aureum</i> (Lam.) Greuter & al.	Sonboli et al. 2012	AB683275
<i>T. bodjnurdense</i> (Rech.f.) Tzvelev	Sonboli et al. 2012	AB683274
<i>T. canescens</i> DC.	Sonboli et al. 2011b	AB608331
<i>T. coccineum</i> (Willd.) Grierson	Sonboli et al. 2011b	AB608333
<i>T. isfahanicum</i> sp.nov.	Iran: Isfahan: Semirom. MPH-1172 Iran: Fars: Abadeh. MPH-1667	QR345424
<i>T. joharchii</i> Sonboli & Kaz. Osaloo	Sonboli et al. 2010a	QR345425
<i>T. kotschyi</i> (Boiss.) Grierson	Sonboli et al. 2010a	AB523748
<i>T. parthenium</i> (L.) Sch.Bip.	Kazemi et al. 2014a	AB998546/ AB998554
<i>T. argyrophyllum</i> (K.Koch) Tzvelev	Iran: W. Azerbaijan: Urmia. MPH-2935	QR345427
<i>T. azerbaijanicum</i> (Podlech) Sonboli & Behjou	Iran: W. Azerbaijan: Khoy. MPH-1326	QR345426
<i>T. polycephalum</i> Sch. Bip. subsp. <i>polycephalum</i>	Iran: Markazi: Arak. MPH-1696	QR345428
<i>T. sonbolii</i> Mozaff.	Sonboli et al. 2012	AB683323
<i>T. tabrisianum</i> (Boiss.) Sosn. & Takht.	Kazemi et al. 2014a	AB998550
<i>T. tarighii</i> Sonboli	Kazemi et al. 2014a	AB998551/ AB998559
<i>T. tenuisectum</i> (Boiss.) Podlech	Sonboli et al. 2010a	AB523747
<i>T. vulgare</i> L.	Guo et al. 2004	AY603264

* GenBank accession numbers of submitted sequences will be included before publication.

2.2. DNA extraction, amplification, and alignment

DNA was extracted from leaves of herbarium specimens by using the modified CTAB DNA extraction protocol of Doyle and Doyle (1987). nrDNA ITS was amplified using the primers ITS AB101 and ITS AB102 designed by Douzery (1999) and PCR amplifications were carried out in a 20 µL volume containing 1.0 µL of template DNA (5 ng/µL), 0.5 µL of each primer (5 pmol/µL), 10 µL of 2X Taq DNA Polymerase Master Mix Red (Amplicon, Cat. No. 180301; 150 mM Tris-HCl (pH = 8.5), 40 mM (NH₄)₂SO₄, 3.0 mM MgCl₂, 0.4 mM dNTPs, 0.05 U/µL Amplicon Taq DNA polymerase, inert red dye, and a stabilizer), and 8.0 µL of distilled water. The PCR procedures for nrDNA ITS

consisted of 4 min at 94 °C for predenaturation followed by 25–35 cycles of 1 min at 94 °C for denaturation, 30–60 s at 52–55 °C for primer annealing, and 2 min at 72 °C for primer extension, finally, followed by 7 min at 72 °C for final primer extension. PCR products were sequenced and all of the sequences were aligned using MAFFT version 7.313 (Kuraku, 2013; Katoh, 2019) as implemented in PhyloSuite version 1.2.2 (Zhang et al., 2020).

2.3. Phylogenetic analyses

Phylogenetic relationships of 17 species belonging to *Tanacetum* sect. *Xanthoglossa* and other sections were inferred by the maximum parsimony method (MP) and Bayesian inference (BI) analyses. MP analysis was

conducted using PAUP* 4.0 version beta 10 (Swofford, 2002) using the heuristic search with 100 random addition of sequence replicates with the tree bisection reconnection (TBR) branch swapping. Finally, supporting branches were calculated with the same options and 1000 bootstrap replicates (Felsenstein, 1985). The best-fitted substitution models for nrDNA ITS were found by the program of MrModeltest2 (Nylander, 2004) and based on the Akaike Information Criterion (AIC) (Posada and Buckley, 2004). In order to reconstruct the phylogenetic relationships with Bayesian inference (BI), the resulting model, SYM+I, was placed in the software program MrBayes version 3.2.7 (Ronquist et al., 2012). Analysis was done using two parallel searches with 4 Markov chains Monte Carlo

(MCMC) for each and was run for 5,000,000 generations. The initial 25% of trees were discarded as burn-in and a 50% majority rule consensus tree was built using the remaining trees.

3. Results

Tanacetum isfahanicum Sonboli and Naderifar, **sp. nov.** (Figure 1, 2; Table 2).

Holotype: Iran. Isfahan, Semirom, Semirom toward Shahreza, Mehrgerd road, Vardasht toward Fathabad, 31° 34' N, 51° 32' E, 2400 m a.s.l., 18 June 2007 Sonboli, Kanani and Gholipour (MPH-1172). – **Paratype:** Iran. Fars, Abadeh, Abadeh toward Semirom, Hanna dam, 31° 13' N, 51° 46' E, 2360 m a.s.l., 3 June 2010 Kanani (MPH-1667).

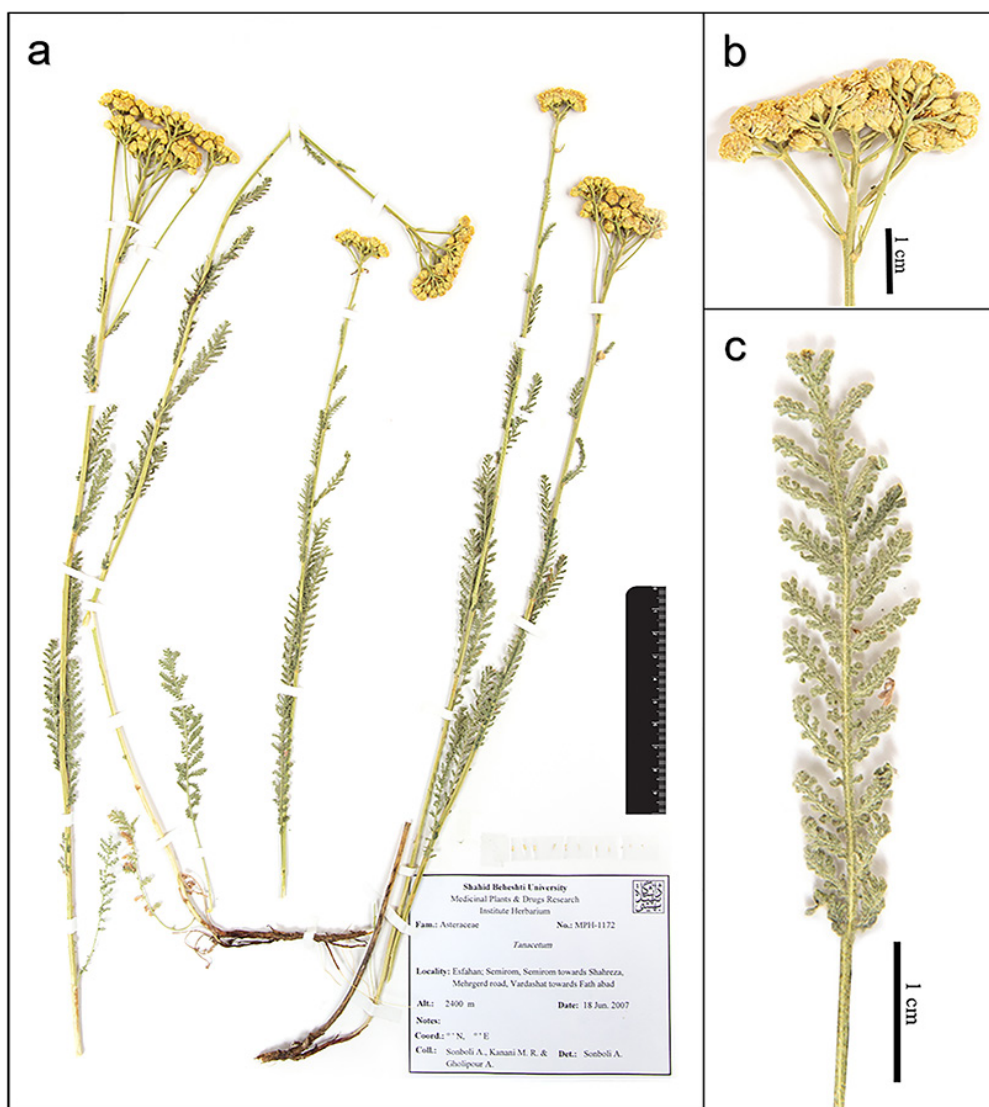


Figure 1. *Tanacetum isfahanicum* Sonboli & Naderifar, a. Photo of the holotype specimen, b. Synflorescence, c. Middle cauline leaf.

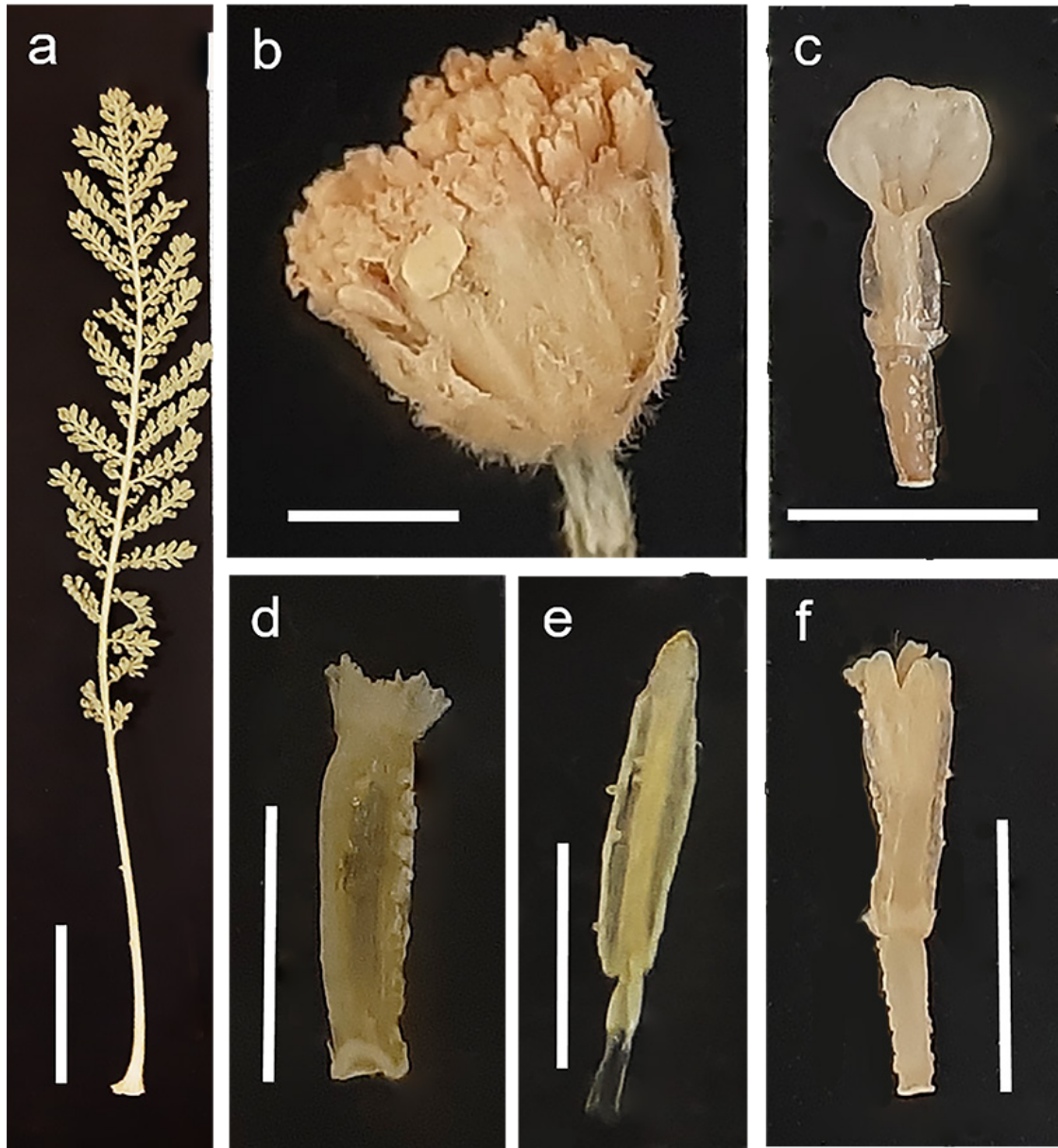


Figure 2. *Tanacetum isfahanicum* Sonboli & Naderifar: a. Basal leaf, b. Capitulum, c. Marginal ray floret, d. Achene of tubular floret, e. Stamen of tubular floret, f. Tubular floret. Scale Bar = 2 mm except for d and e (= 1 mm) and basal leaf a (= 2 cm).

3.1. Diagnosis

Tanacetum isfahanicum is closely related to *T. polycephalum* subsp. *polycephalum* but distinguished from it in having oblanceolate basal leaves (not lanceolate), oblanceolate and oblong middle and upper cauline leaves, respectively (not triangular), 7–9 capitula in every branch versus 10–11, involucre width of 3–3.8 mm versus 5 mm and by light yellow with middle green ribs of outer phyllary (not straw-yellow with middle brown ribs) (Table 2).

3.2. Description

Perennial herb, 42–50 cm high, densely covered by appressed whitish villous bifid and simple nonglandular and sessile glandular hairs, slender and dark brown rhizome, stems numerous, grayish–light green, angled, striate, ligneous at the base, branched only at apex, leafy. Leaves often appressed on a stem. Basal leaves 2-pinnatisect, oblanceolate, petiolate, 12.5 cm long, 1.6 cm wide; primary segments 18 paired, 2–12 mm long, 3–4

Table 2. Diagnostic characters of *Tanacetum isfahanicum* sp. nov. and its relative taxon.

Characters	<i>T. isfahanicum</i>	<i>T. polycephalum</i> subsp. <i>polycephalum</i>
Basal leaves: Shape	Oblanceolate	Lanceolate
Cauline leave: Shape of upper Shape of middle	Oblong Oblanceolate	Triangular Triangular
Capitula: Shape(often)	Hemiglobular with obconical base	Hemiglobular, ovate with obconical base
Involucre: Width(mm)	3–3.8	5
Outer phyllary color Outer phyllary margin	Light yellow with middle green rib Light yellow membranous	Straw-yellow with middle brown rib Straw-yellow membranous, often brown in apex
Ray floret: Achene ribs color	Light yellow	Straw-yellow

mm wide; secondary segments 8 paired, 1–2 mm long, 0.6–2 mm wide, in turn triparted, terminal lobe obovate, mucronate. Cauline leaves sessile except for lowest, upper oblong, 2–pinnatisect; primary segments 11–14 paired, 2.5–3.2 mm long; secondary segments 2–4 paired, 0.5–4.5 mm long, terminal lobe obovate, mucronate. Shape and structure of the middle similar to basal, primary segments 16–18 paired, 4–7 mm long, secondary segments 5–6 paired, 1–7 mm long, terminal lobe obovate, mucronate. Synflorescence compound corymb, capitula often hemiglobular, obconical at the base, (12)40–52(80) on single stem, in every branch 7–9, involucre 4–5 mm long, 3–3.8 mm wide, phyllaries imbricate, 3–4 seriate, outer ovate–triangular, acute, yellow with middle green rib, narrow and membranous border, inner obovate, membranous border, broadened at apex; ray florets pistillate, corolla yellow, spatulate, 1.9 mm long, 2.2 mm wide; disc florets bisexual, often funnelform, funnelform–campanulate rarely, 5–lobed at apex, yellow, 2–2.4 mm, anther theca base obtuse–cordate, apical appendage elliptic, 2–2.2 mm, style 2.1–2.3 mm, bifid at the tip, often exerted. Achenes oblong–cylindrical, 5–6 longitudinal ribs, 1.4 mm long, 0.3–0.4 mm wide, pappus coroniform, serrate, 0.2 mm.

3.3. Distribution and ecology

Tanacetum isfahanicum is a perennial and suffruticose plant and occurs in mountainous places in an area of approximately 50 km of Isfahan and Fars provinces at an altitude of between 2360 and 2400 m a.s.l. It is an Irano – Turanian element and endemic of Iran (Figure 3).

3.4. Phenology

Flowering May–June and fruiting June–July.

3.5. Etymology

The species epithet refers to the Isfahan Province of Iran, where the new species is native.

3.6. Molecular study

The aligned dataset of nrDNA ITS with 641 characters contained 20 parsimony informative, 556 conserved, and 65 variable sites. The best tree obtained from the maximum parsimony analysis from 59 steps showed a consistency index (CI) of 0.881 and a retention index (RI) of 0.932. The strict consensus tree of the MP analysis (tree not shown) is topologically similar to that of the Bayesian analysis. The tree resulting from Bayesian analysis along with both bootstrap (BS) and posterior probability (PP) support values is shown in Figure 4. The monophyly of *Tanacetum* (PP = 1, BS = 100) is corroborated by the Bayesian tree of nrDNA ITS. Also, the close relationship of *Tanacetum isfahanicum* and *Tanacetum polycephalum* subsp. *polycephalum* (PP = 0.79, BS = 60) compared to the other sampled taxa has been shown (Figure 4).

4. Discussion

In the present study, *Tanacetum isfahanicum* is proposed as a new species from Isfahan and Fars provinces of Iran and its close relationship with *T. polycephalum* subsp. *polycephalum* in the molecular phylogenetic framework is corroborated (Figure 4). The review of taxonomic literature clearly revealed the controversial subsp. classification of

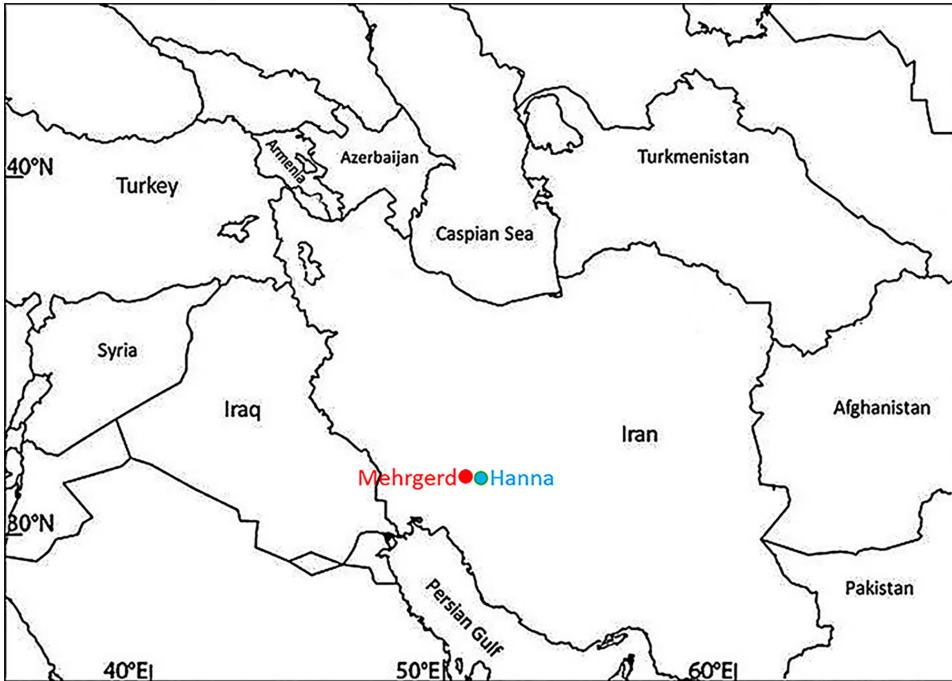
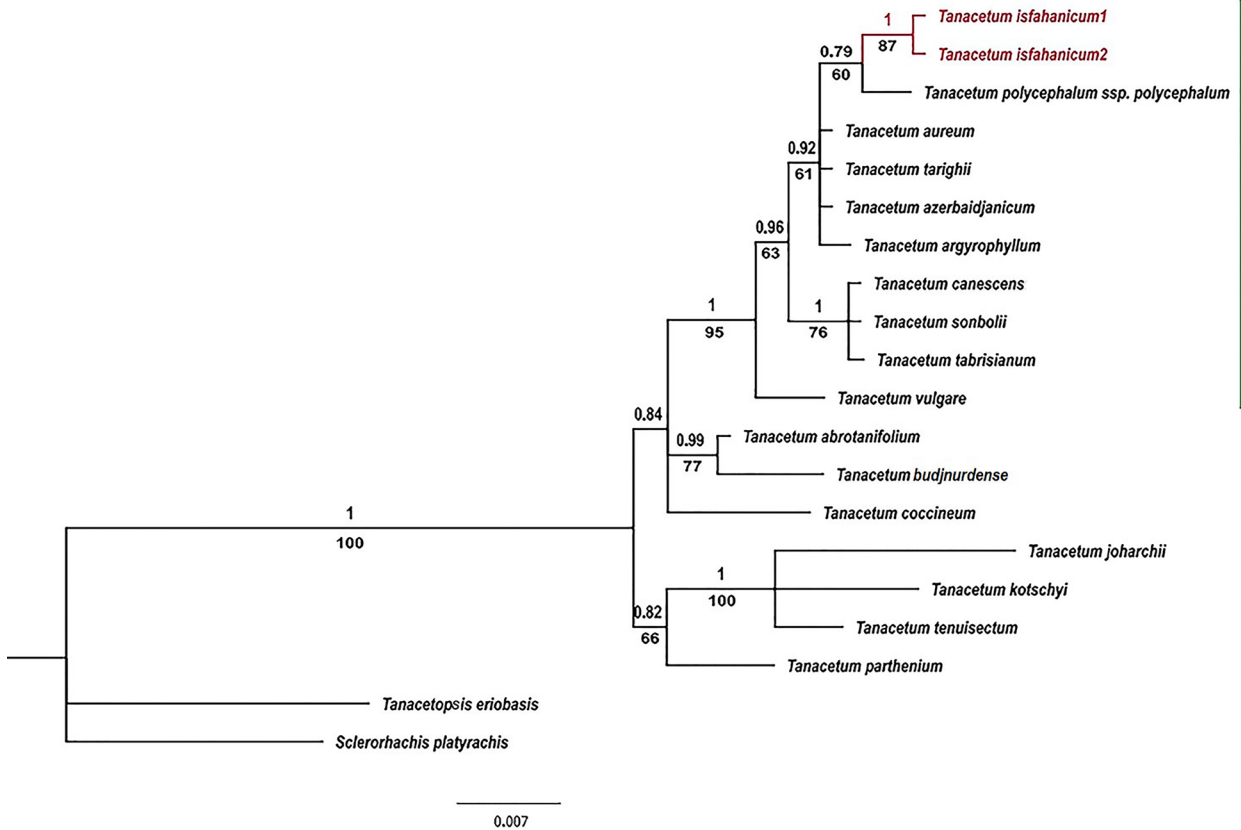


Figure 3. Distribution map of *Tanacetum isfahanicum* Sonboli & Naderifar in Iran.



Sect. Xanthoglossa

Figure 4. Fifty percent majority rule consensus tree resulting from Bayesian analysis of nrDNA ITS sequences of studied taxa. Values above and below the branches represent Posterior probabilities (PP) and MP bootstrap (BS), respectively.

T. polycephalum. According to the Flora Iranica (Podlech, 1986), *T. polycephalum* is the most morphologically variable species amongst the *T.* sect. *Xanthoglossa* with seven subspecies namely: *T. polycephalum* Sch.Bip. subsp. *polycephalum*, *T. polycephalum* subsp. *argyrophyllum* (K.Koch.) Podlech, *T. polycephalum* subsp. *heterophyllum* (Boiss.) Podlech, *T. polycephalum* subsp. *duderanum* (Boiss.) Podlech, *T. polycephalum* subsp. *farsicum* Podlech, *T. polycephalum* subsp. *junesarensis* (Bornm.) Podlech and *T. polycephalum* subsp. *azerbaidjanicum* Podlech (Podlech, 1986). Mozaffarian (2008) in his treatment for Flora of Iran, decreased the number of subspecies of *T. polycephalum* to three i.e. *T. polycephalum* subsp. *polycephalum*, *T. polycephalum* subsp. *argyrophyllum*, and *T. polycephalum* subsp. *duderanum*. Recently, according to the results of a taxonomic revision of *T. polycephalum* Sch.Bip. species complex (Moradi Behjou et al., 2022), *T. polycephalum* subsp. *azerbaidjanicum* Podlech and *T. polycephalum* subsp. *argyrophyllum* (K.Koch) Podlech are regarded as distinct species, namely *T. azerbaijanicum* (Podlech) Sonboli & Behjou and *T. argyrophyllum* (K.Koch) Tzvelev. The Bayesian tree topology of nrDNA ITS confirmed *Tanacetum* s. str. (Sonboli et al., 2012) as a well-supported, monophyletic genus (PP = 1, BS = 100) in which species belonging to the *T.* sect. *Xanthoglossa*

are accommodated in a well-supported clade with the highest posterior probability (PP = 1) and 95% bootstrap percentage. The molecular phylogenetic study supported that *T. azerbaijanicum* and *T. argyrophyllum* are not sisters to *T. polycephalum* and they could be assigned at the species and not subspecies level in accordance with the morphological study of Moradi Behjou et al. 2022 (Figure 4). Also, the 50% majority rule consensus tree obtained from nrDNA ITS sequences data in the present study revealed the phylogenetic relationship of *T. tarighii* and *T. aureum* and related taxa within the *T.* sect. *Xanthoglossa* (Kazemi et al., 2014a, 2014b).

Acknowledgments

The authors thank the research council of Tarbiat Modares university for financial support and the cooperation of Medicinal Plants and Drugs Research Institute Herbarium of Shahid Beheshti University (MPH) officials. Also, the authors gratefully appreciate the Iran National Science Foundation (INSF) for financial support of this research under project no. 92024588.

Conflict of interest

The authors declare that they have no conflicts of interest.

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