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

Maryam NADERIFAR 1,*, Ali SONBOLI 2,*, Shahrokh KAZEMPOUR OSALOO 1

1 Department of Plant Biology, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
2 Department of Biology, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran

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 coryms, 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 epaleate receptacles, 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 platyrrhachis* 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.

* Correspondence: a–sonboli@sbu.ac.ir

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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

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.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Source (location &amp; voucher)</th>
<th>GenBank accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outgroups</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Tanacetopsis eriobasis</em> (Rech.f.) Kovalevsk.</td>
<td>Oberprieler et al. 2007</td>
<td>AM774427</td>
</tr>
<tr>
<td><em>Sclerorhachis platyrachis</em> (Boiss.) Podlech</td>
<td>Hassanpour et al. 2018</td>
<td>LC313944</td>
</tr>
<tr>
<td><strong>Ingroups</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Tanacetum abrotanifolium</em> (L.) Druce</td>
<td>Sonboli et al. 2012</td>
<td>AB683260</td>
</tr>
<tr>
<td><em>T. aureum</em> (Lam.) Greuter &amp; al.</td>
<td>Sonboli et al. 2012</td>
<td>AB683275</td>
</tr>
<tr>
<td><em>T. bodnurdense</em> (Rech.f.) Tzvelev</td>
<td>Sonboli et al. 2012</td>
<td>AB683274</td>
</tr>
<tr>
<td><em>T. canescens</em> DC.</td>
<td>Sonboli et al. 2011b</td>
<td>AB608331</td>
</tr>
<tr>
<td><em>T. coccineum</em> (Willd.) Grievson</td>
<td>Sonboli et al. 2011b</td>
<td>AB608333</td>
</tr>
<tr>
<td><em>T. isfahanicum</em> sp.nov.</td>
<td>Iran: Isfahan: Semirom. MPH-1172</td>
<td>QR345424</td>
</tr>
<tr>
<td><em>T. joharchii</em> Sonboli &amp; Kaz. Osaloo</td>
<td>Sonboli et al. 2010a</td>
<td>QR345425</td>
</tr>
<tr>
<td><em>T. kotschyi</em> (Boiss.) Grierson</td>
<td>Sonboli et al. 2010a</td>
<td>AB523748</td>
</tr>
<tr>
<td><em>T. parthenium</em> (L.) Sch.Bip.</td>
<td>Kazemi et al. 2014a</td>
<td>AB998546/AB998554</td>
</tr>
<tr>
<td><em>T. argyrophyllum</em> (K.Koch) Tzvelev</td>
<td>Iran: W. Azerbajian: Urmia. MPH-2935</td>
<td>QR345427</td>
</tr>
<tr>
<td><em>T. azerbaidjanicum</em> (Podlech) Sonboli &amp; Behjou</td>
<td>Iran: W. Azerbajian: Khoy. MPH-1326</td>
<td>QR345426</td>
</tr>
<tr>
<td><em>T. polycephalum</em> Sch. Bip. subsp. polycephalum</td>
<td>Iran: Markazi: Arak. MPH-1696</td>
<td>QR345428</td>
</tr>
<tr>
<td><em>T. sonboli</em> Mozaff.</td>
<td>Sonboli et al. 2012</td>
<td>AB683232</td>
</tr>
<tr>
<td><em>T. tabrisianum</em> (Boiss.) Sosn. &amp; Takht.</td>
<td>Kazemi et al. 2014a</td>
<td>AB998550</td>
</tr>
<tr>
<td><em>T. tarighii</em> Sonboli</td>
<td>Kazemi et al. 2014a</td>
<td>AB998551/AB998559</td>
</tr>
<tr>
<td><em>T. tenuisectum</em> (Boiss.) Podlech</td>
<td>Sonboli et al. 2010a</td>
<td>AB523747</td>
</tr>
<tr>
<td><em>T. vulgare</em> L.</td>
<td>Guo et al. 2004</td>
<td>AV603264</td>
</tr>
</tbody>
</table>

* GenBank accession numbers of submitted sequences will be included before publication.
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.](image-url)
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

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**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).
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, terminal lobe obovate, mucronate. Shape and structure of the middle similar to basal, primary segments 16–18 paired, 4–7 mm long, terminal lobe obovate, mucronate. Syntflorescence 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, spathulate, 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

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

<table>
<thead>
<tr>
<th>Characters</th>
<th><em>T. isfahanicum</em></th>
<th><em>T. polycephalum</em> subsp. <em>polycephalum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal leaves:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shape</td>
<td>Oblanceolate</td>
<td>Lanceolate</td>
</tr>
<tr>
<td>Cauline leave:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shape of upper</td>
<td>Oblong</td>
<td>Triangular</td>
</tr>
<tr>
<td>Shape of middle</td>
<td>Oblanceolate</td>
<td>Triangular</td>
</tr>
<tr>
<td>Capitula:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shape (often)</td>
<td>Hemiglobular with obconical base</td>
<td>Hemiglobular, ovate with obconical base</td>
</tr>
<tr>
<td>Involucre:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Width (mm)</td>
<td>3–3.8</td>
<td>5</td>
</tr>
<tr>
<td>Outer phyllary color</td>
<td>Light yellow with middle green rib</td>
<td>Straw-yellow with middle brown rib</td>
</tr>
<tr>
<td>Outer phyllary margin</td>
<td>Light yellow membranous</td>
<td>Straw-yellow membranous, often brown in apex</td>
</tr>
<tr>
<td>Ray floret:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Achene ribs color</td>
<td>Light yellow</td>
<td>Straw-yellow</td>
</tr>
</tbody>
</table>
Figure 3. Distribution map of *Tanacetum isfahanicum* Sonboli & Naderifar in Iran.

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.
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. azerbaidjanicum* and *T. argyrophyllum* are not sisters to *T. polyccephalum* 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

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Conflict of interest

The authors declare that they have no conflicts of interest.

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