Tanacetum erzincanense (Asteraceae), a new species from Erzincan, Turkey

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Abstract: In this study, Tanacetum erzincanense Korkmaz, Kandemir & Ilhan is described as a new species for science from Erzincan Province. It was compared with close species by using morphological and random amplified polymorphic DNA data. It resembles T. germanicopolitanum (Bornm. & Heimerl) Grierson and T. pinnatum Boiss. It is closer to T. germanicopolitanum in terms of genetic and morphological aspects. Morphologically, it differs from T. germanicopolitanum by having oblong-oblancoalate basal leaves in outline with flabelliform and fewer leaf segments, less dense indumentum, smaller and globose capitula, and shorter apical appendage in inner phyllaries. T. pinnatum shows difference from T. erzincanense with linear-lanceolate and sparsely pinnatifid leaf segments, less dense indumentum, and clearly larger and fewer capitula. Pollen properties and results of the soil analysis are also given.

Keywords: Genetic, ecology, morphology, Tanacetum, Compositae

1. Introduction
Asteraceae includes about 23,000 species in the world. It is also one of the biggest plant families in Turkey. Many species have been described to science from Turkey recently (Aytaç and Duman, 2013; Öztürk and Çetin, 2013; Yıldız et al. 2013). Tanacetum L. is the third largest genus of Asteraceae-Anthemideae (Sonboli et al., 2012). The genus is represented by about 160 species worldwide. The species are scattered in Europe, Asia, North Africa, and North America. It has mostly perennial, rarely annual species. Some species of the genus are widely cultivated. The habits of the species vary from herbs to subshrubs (Oberprieler et al., 2007). Forty-six species are found in Turkey belonging to the genus (Güner, 2012).

2. Materials and methods
The samples belonging to the new species were collected in 2012 and 2013 between Mantarlı and Akyurt villages in Çayırlı-Erzincan by means of a project supported by the Scientific Research Project Unit of Erzincan University. To determine the morphological boundaries of the new species, additional plant specimens were collected 4 times from the field over 2 years. Some of the living material was set in formalin-aceto-alcohol for anatomical studies and the other plant species that grow with new species were recorded in the field work.

Attempts were made to describe the samples using Flora of Turkey (Grierson, 1975), Flora of USSR (Schischkin and Bomrov, 2000), and Flora Iranica (Podlech, 1986). The specimens were also compared with supposedly related species (Tanacetum germanicopolitanum and Tanacetum pinnatum) in the herbaria ANK and GAZI. Besides herbarium specimens, additional materials of related species were collected from their natural habitats in Çankırı (T. germanicopolitanum) and Kars (T. pinnatum) provinces.

The soil analyses were conducted in the soil laboratory of the Erzincan Horticultural Research Institute. The saturation % and constitution, salt %, pH, lime %, organic matter %, and phosphorus (P) and potassium (K) kg/ha in the soil samples were determined according to the methods described by Tüzün (1990) (total salt quantitation), Hindistan and İnceoğlu (1962) [determination of soil reaction (pH)], Çağlar (1949) [lime (CaCO₃) determination], Ulgen and Ateşalp (1972) [phosphorus (P₂O₅) determination], Doll and Lucas (1973) [potassium (K₂O) determination], Ulgen and Ateşalp (1972) (determination of organic matter), and Tüzün (1990) (classification of the soils).

For scanning electron microscopy (SEM) studies, the pollen was treated with 70% alcohol and then dried before mounting on stubs with gold. Photomicrographs were taken with FEI INSPECT S50 electron microscopes. The palynological terminology mainly follows that of Erdtman (1952).

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Received: 01.11.2013 • Accepted: 06.08.2014 • Published Online: 02.01.2015 • Printed: 30.01.2015
Genomic DNA was extracted from powdered leaf materials using the QIAGEN DNA extraction kit (QIAGEN, Germany) according to the manufacturer’s instructions. The purity and quantity of genomic DNA was determined spectrophotometrically and confirmed using 0.8% agarose gel electrophoresis. Nine primers were used to generate random amplified polymorphic DNA (RAPD) profiles. PCR amplification reactions were carried out in 30 µL (final volume) of reaction mixture containing: 10X buffer at 3.0 µL, dNTPs (10 mM) 1.2 µL, magnesium chloride (25 mM) 1.2 µL, primer (5 µM) 2.0 µL, Taq polymerase (5 units) 0.4 µL, water 19.2 µL, and sample DNA 3.0 µL (100 ng/µL). The thermal cycler (Eppendorf Company, Germany) was programmed for 2 min at 95 °C; 2 cycles of 30 s at 95 °C, 1 min at 37 °C, and 2 min at 72 °C; 2 cycles of 30 s at 95 °C, 1 min at 35 °C, and 2 min at 72 °C; 41 cycles of 30 s at 94 °C, 1 min at 35 °C, and 2 min at 72 °C; and a final 5-min extension at 72 °C, then brought down to 4 °C.

The PCR products (27 µL) were mixed with 6X gel loading buffer (3 µL) and loaded onto agarose (1.5% w/v) gel electrophoresis in 0.5X TBE (Tris-Borate-EDTA) buffer at 70 V for 150 min. The gel was stained in ethidium bromide solution (2 µL EtBr/100 mL 1X TBE buffer) for 40 min and visualized under UV light in the Bio Doc Image Analysis System with the UVIsoft analysis package (UVIsoft, UK).

PCR products were scored as the presence (1) or absence (0) of band for each genotype and analyzed. Data were used to calculate a Jaccard (1908) similarity index, from which a UPGMA dendrogram was constructed. All of the experiments in this study were repeated twice.

3. Results and discussion

**Tanacetum erzincaense** Korkmaz, Kandemir & Ilhan sp. nov. (Figure 1)

- **Type:** B7 Erzincan, Çayırlı, between Mantarlı and Akyurt villages, 39°43′709″N, 40°10′118″E, 1622 m, 02.07.2012, steppe, M. Korkmaz & V. İlhan 3249 (holotype: GAZI, isotypes: NGBB, ANK).

**Diagnosis:** The new species is related to *T. germanicopolitanum* (local endemic to Çankırı) and *T. pinnatum*. Not only were herbarium materials of the related species examined, but additional specimens were also collected from their natural habitats during the study.

**Description:** Perennial with woody rhizomes. Stems erect, rarely ascending, 18–34 cm high, angular to terete with ridges, 2–3.5 mm in diam., grayish, tomentose to subglabrous, corymbosely branched above. Basal leaves 2–10 cm (incl. petiole), oblong to oblanceolate in outline, densely grayish generally in young leaves to sparsely tomentose, grayish to greenish color in old leaves with 2–5 pairs of lateral segments, rarely only 1 terminal leaflet in young leaves of basal leaves; lateral segments 7–13 × 7–9 mm, flabelliform, ovate to obovate or orbicular in outline, cuneate, rarely rounded in young basal leaves, apically 3–7 rounded to acute lobes or pinnatifid, sometimes the lowest segments almost entire in young leaves, ultimate segment larger than lateral one, tripartite, separation more than half of segment, sometimes near to base, trilobed, each lobe (2)–3–5-toothed; caule leaves similar to basal leaves, decreasing in size and pairs of lobes, upper ones sessile, the lowest pair usually entire; the uppermost leaves near capitula with a few segments or reduced to entire bracts. Capitula 12–110 per stem. Involucr 3–4 × 2.5–3.5 mm; phyllaries 3 series, 2.5–3 × 1.5–2 mm long, white tomentose; outer one linear lanceolate with narrow scarious margin and shorter than inner one; inner phyllaries oblong with distinct and ±lacerate scarious whitish to brownish c. 0.5-mm-long appendages, longer than outer one. Ray flowers somewhat longer than disk flowers, 6–8; ligule with 2–3 teeth, 2–2.5 mm, elliptic to rotund; style arms divergent (sometimes turned down), achenes whitish to brownish, ±curved, tuberculate. Disk flowers c. 2 mm, yellow; tube verrucose; anthers exerted, whitish; style bifid, divergent, whitish in young flowers, brownish in old ones; achenes as long as tube, median ones ±straight, outer one slightly curved. *Fl.* June–July in steppe.

Determined pollen properties are indicated as monad. Shape: spheroidal; outline in polar view: circular; ornamentation: echinate-perforate; aperture number: 3; aperture type: colporate (Figure 2).

**Examined specimens.** *Tanacetum germanicopolitanum*: B7 Erzincan, Çayırlı, between Mantarlı and Akyurt villages, 39°43′709″N, 40°10′118″E, 1622 m, 23.06.2013, steppe, M. Korkmaz & V. İlhan 3673 (paratypes: GAZI, NGBB, ANK). *Tanacetum germanicopolitanum*: A4 Çankırı: from Çankırı to Kastamonu, Hıdırlık Tepe, around reservoir, 36T0549600E, 4495439N, 959 m, 05.06.2013, T. Körüklü 1-56-2013 (4 sheets) (ANK); Paphlagonia.: Ad appidum Typem. 2 cycles of 30 s at 95 °C, 1 min at 37 °C, and 2 min at 72 °C; 41 cycles of 30 s at 94 °C, 1 min at 35 °C, and 2 min at 72 °C; and a final 5-min extension at 72 °C, then brought down to 4 °C.

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- **Tanacetum germanicopolitanum:** B7 Erzincan, Çayırlı, between Mantarlı and Akyurt villages, 39°43′709″N, 40°10′118″E, 1622 m, 23.06.2013, steppe, M. Korkmaz & V. İlhan 3673 (paratypes: GAZI, NGBB, ANK). *Tanacetum germanicopolitanum*: A4 Çankırı: from Çankırı to Kastamonu, Hıdırlık Tepe, around reservoir, 36T0549600E, 4495439N, 959 m, 05.06.2013, T. Körüklü 1-56-2013 (4 sheets) (ANK); Paphlagonia.: Ad appidum *Canikiri in vinetis derelictis vallis Cakmalki-dere c. 800 m, 16.06.1929, Bornm. 14254! (E, K); *Canikiri (Papaglonien) Stappenhügel auf Kalk Westlich ob der Stadt. 850 m, 20 Juni 1955, Hub.-Morath 1300! (E); A4 Çankırı. Çankırı’nın bir costas, Devrendi Deresi 850 m, 23.06.1976, N. Çelik 190! (ANK); A4 Ankara-Çankırı Yolu, 81. km, 800–840 m, 05.07.1995, andezit steppe, Z. Aytaç & H. Duman 5777! (GAZI). *Tanacetum germanicopolitanum*: B8: Kars, Kaghizman, Kandemir 10347 (NGBB); between Erzurum and Tamrut (at present known as Şendurak village, Oltu, Erzurum), 18.06.1976, 1300–1500 m, N. Çelik 189! (ANK). The locations of the examined specimens are indicated in Figure 3.
Tanacetum erzincanense can be distinguished from Tanacetum germanicopolitanum by its oblong-oblanceolate basal leaves in outline with 3–5 paired lateral segments (not narrowly linear-oblanceolate with 7–11 paired), leaf segments with flabelliform, ovate to obovate or orbicular in outline, usually equal to its width (not flabelliform or oblong to oblanceolate, usually longer than its width) 3–4 × 2.5–3.5 mm involucre (not 6–8 × 4–5 mm), various appendages c. 0.5 mm long in inner phyllaries (not c. 1 mm long), and 6–8 ray flowers (not 5) (Table 1).

T. pinnatum is recorded from Erzurum-Oltu, Kars, Kağızman, and Van-Erciş, and it also grows in Iraq, Iran, and the Caucasus. Some characters of the phyllaries and flowers of T. erzincanense are similar to those of T. pinnatum. However, T. pinnatum is quite different with its leaves with narrow, linear leaf segments and fewer and larger capitula. The number of capitula is at most 25 per stem in T. pinnatum. General habits of T. erzincanense and related species are indicated in Figure 4, and capitula and basal leaves are shown in Figure 5.
According to ITS and trnH-psbA data, *Tanacetum germanicopolitanum* is close to *T. pinnatum* Boiss. (Sonboli et al., 2012). PCR-based RAPD was used to identify genetic variations between plant species since its application does not need any prior information about target sequence on the genome (Khanuja et al., 1998). RAPD markers were successfully used to study genetic diversity in plant species such as those from *Vanilla* Mill. (Besse et al., 2004), *Diospyros* L. (Akbulut et al., 2008), Oleaceae (Zheng et al., 2009), *Thymus* L. (Sunar et al., 2009), and *Allium* L. (Mukherjee et al., 2013). Thus, the new species was compared with *T. germanicopolitanum* and *T. pinnatum* Boiss. by using RAPD markers.

Results of RAPD analysis are summarized in Table 2. Nine of the 34 initial primers produced clear and reproducible polymorphic bands among the 3 *Tanacetum* species. Those 9 random primers generated a total of 62 RAPD bands (Figure 6). The size of the amplicons ranged from 250 to 2700 bp. Primer OPB-03 gave the highest number of RAPD products (10). Primers OPY-7 and OPY-13 gave the lowest number of RAPD products (5) (Table 2). In total, 92.1% of the bands were polymorphic.
The dendrogram realized from the RAPD markers grouped the 3 genotypes into 2 major clusters (Figure 7). Cluster 1 consists of *T. pinnatum*. Cluster 2 consists of *T. erzincanense* and *T. germanicopolitanum*. The greatest similarity was observed between *T. erzincanense* and *T. germanicopolitanum* (0.124), while the greatest dissimilarity was observed between *T. pinnatum* and *T. germanicopolitanum* (0.963).

*Tanacetum erzincanense* grows on the steppe of the Çayırlı district (Erzincan Province) at an altitude of 1622 m. Vegetation of the area is formed by herbaceous plant species including...
Achillea millefolium L. subsp. millefolium L., Bellevalia gracilis Feinbrun, Bupleurum rotundifolium L., Centaurea polypodiifolia Boiss., Dorycnium pentaphyllum Scop. subsp. anatolicum (Boiss.) Gams, Euphorbia virgata Waldst. & Kit., Hedysarum nitidum Willd., Medicago × varia Martyn, Nigella latisecta P.H.Davis, Onobrychis armena Boiss. & Huet, Onobrychis stenostachya Freyn subsp. krausei (Sirj.) Hedge, Ononis spinosa L., Pisum sativum L. subsp. sativum var. arvense (L.) Poiret, Teucrium polium L., and Ziziphora tenuior L.
According to the results of soil analysis for the new species, saturation value was 75.00. Soil pH was 7.59. Electrical conductivity was 0.0007 S and organic matter was 2.32%. Lime (CaCO₃) was 38.80%. Salt concentration was 0.034%, phosphorus was 1.282 kg/ha, and potassium concentration was 18.37 kg/ha. The results of the soil analysis demonstrated that the soil properties of the new species are as follows: rough texture, slightly alkaline, medium amount of organic matter, high amount of lime, saltless, low phosphorus content, and moderate potassium content.

According to the results of soil analysis for *Tanacetum germanicopolitanum*, saturation value was 50.00. Soil pH was 7.59. Electrical conductivity was 0.00043 S; organic matter was 1.74%. Lime (CaCO₃) was 34.10%. Salt concentration was 0.014%, phosphorus was 4.81 kg/ha, and potassium concentration was 35.10 kg/ha. The results of the soil analysis indicate that the soil properties of *T. germanicopolitanum* are rough texture, slightly alkaline, low amount of organic matter, slightly salty, low phosphorus content, and moderate potassium content.

Table 1. Diagnostic characters of *Tanacetum erzincanense* and *Tanacetum germanicopolitanum*.

<table>
<thead>
<tr>
<th>Characters</th>
<th><em>T. erzincanense</em></th>
<th><em>T. germanicopolitanum</em></th>
<th><em>T. pinnatum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower leaves</td>
<td>Oblong-oblancoleate in outline with 3–5 paired lateral segments. Lateral segments flabelliform, ovate to obovate or orbicular in outline and usually equal to its width.</td>
<td>Narrowly linear-oblancoleate in outline with 7–11 paired lateral segments. Segments oblong to oblancoleate and usually longer than its width.</td>
<td>Oblanceolate in outline with 4–6 paired lateral segments. Lateral segments linear lanceolate and clearly longer than its width.</td>
</tr>
<tr>
<td>Capitula</td>
<td>(12–)30–110 per stem</td>
<td>10–40 per stem</td>
<td>4–25 per stem</td>
</tr>
<tr>
<td>Involucre</td>
<td>3–4 × 2.5–3.5 mm</td>
<td>6–8 × 4–5 mm</td>
<td>6–7 × 5–6 mm</td>
</tr>
<tr>
<td>Phyllaries</td>
<td>3 series, appendages c. 0.5 mm in inner one</td>
<td>3–4 series, appendages c. 1 mm in inner one</td>
<td>3 series, appendages c. 1 mm in inner one</td>
</tr>
<tr>
<td>Habitat</td>
<td>Steppe</td>
<td>Chalky hill-steppe</td>
<td>Stony slopes, rock crevices</td>
</tr>
<tr>
<td>Indumentum of leaves</td>
<td>Sparsely and leaf surface visible</td>
<td>Dense and leaf surface invisible</td>
<td>Densely and leaf surface ±visible</td>
</tr>
</tbody>
</table>

Table 2. Total number of amplified fragments and number of polymorphic fragments generated by polymerase chain reaction using selected random decamers.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’–3’)</th>
<th>Length of amplified bands</th>
<th>No. of bands</th>
<th>No. of polymorphic bands</th>
<th>Polymorphism rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-1</td>
<td>AGTCAGCCAC</td>
<td>750–1800</td>
<td>6</td>
<td>5</td>
<td>83.3</td>
</tr>
<tr>
<td>C-10</td>
<td>TGTCTGGGGTC</td>
<td>300–2000</td>
<td>9</td>
<td>8</td>
<td>88.88</td>
</tr>
<tr>
<td>OPA-4</td>
<td>AATCGGGGCTG</td>
<td>500–2300</td>
<td>8</td>
<td>8</td>
<td>100</td>
</tr>
<tr>
<td>OPB-03</td>
<td>CATCCCCCCTG</td>
<td>400–2500</td>
<td>10</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>OPH-16</td>
<td>TCTCAGCTGG</td>
<td>250–2200</td>
<td>8</td>
<td>7</td>
<td>87.5</td>
</tr>
<tr>
<td>OPY-7</td>
<td>AGAGCGGCTCA</td>
<td>750–2000</td>
<td>5</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>OPY-13</td>
<td>GGGTCTCGGT</td>
<td>500–1800</td>
<td>5</td>
<td>4</td>
<td>80</td>
</tr>
<tr>
<td>OPW–8</td>
<td>GACTGCTCTCT</td>
<td>750–2700</td>
<td>7</td>
<td>6</td>
<td>85.7</td>
</tr>
<tr>
<td>OPY-19</td>
<td>TGAGGGTCCCA</td>
<td>300–1600</td>
<td>5</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>250–2700</td>
<td>63</td>
<td>58</td>
<td>92.1</td>
</tr>
</tbody>
</table>
Tanacetum erzincanense is an endemic species and the IUCN category was determined by considering World Conservation Union categories (IUCN Species Survival Commission, 2014). According to the field observations during 3 years, the area of occupancy is estimated to be less than 10 km² and the species is known from only 1 population. The population is very near to the villages and under the threat of excessive animal husbandry. As a result, the IUCN category of the new species was evaluated as “Critically Endangered”, (CR)B2ab (i,ii).

Acknowledgments
The specimens were collected during a project supported by the Scientific Research Project Unit of Erzincan University (Project No.: EUBAP 2011-10.01.05). We would like to thank the Unit for its financial support. Some T. germanopolitanum specimens were collected from Çankırı by Tuğrul Körüklü from ANK. Research Assistant Selim Çöğenli from Atatürk University helped with the SEM studies. We also thank them.

References


