

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

Mustafa KORKMAZ, Ali KANDEMİR*, Veli İLHAN, Nalan YILDIRIM DOĞAN
Department of Biology, Faculty of Arts and Science, Erzincan University, Erzincan, Turkey

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Abstract: In this study, *Tanacetum erzincanense* Korkmaz, Kandemir & İlhan 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-ob lanceolate 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.

Key words: 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 Çayırılı-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 Tüzüner (1990) (total salt quantitation), Hindistan and İnceoğlu (1962) [determination of soil reaction (pH)], Çağlar (1949) [lime (CaCO₃) determination], Ülgen and Ateşalp (1972) [phosphorus (P₂O₅) determination], Doll and Lucas (1973) [potassium (K₂O) determination], Ülgen and Ateşalp (1972) (determination of organic matter), and Tüzüner (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).

* Correspondence: akandemir@erzincan.edu.tr

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 UVIssoft analysis package (UVIssoft, 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 erzincanense Korkmaz, Kandemir & İlhan sp. nov. (Figure 1)

Type: B7 Erzincan, Çayırılı, 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; cauline 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. Involucre 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, Çayırılı, 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 Cankiri in vinetis derelicts vallis Cakmakli-dere c. 800 m, 16.06.1929, Bornm. 14254! (E, K); Cankiri (Paphlagonien) Stappenhügel auf Kalk Westlich ob der Stadt. 850 m, 20 Juni 1955, Hub.-Morath 1300! (E); A4 Çankırı. Çankırı'nın batısı, Devrendi Deresi 850 m, 23.06.1976, *N. Çelik* 190! (ANK); A4 Ankara-Çankırı Yolu, 81. km, 800–840 m, 05.07.1995, andezit step, Z. Ayaç & H. Duman 5777! (GAZI). *Tanacetum germanicopolitanum*: B8: Kars, Kağızman, *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.



Figure 1. General habit of *Tanacetum erzincanense*.

Tanacetum erzincanense can be distinguished from *Tanacetum germanicopolitanum* by its oblong-ob lanceolate basal leaves in outline with 3-5 paired lateral segments (not narrowly linear-ob lanceolate 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 ob lanceolate, 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.

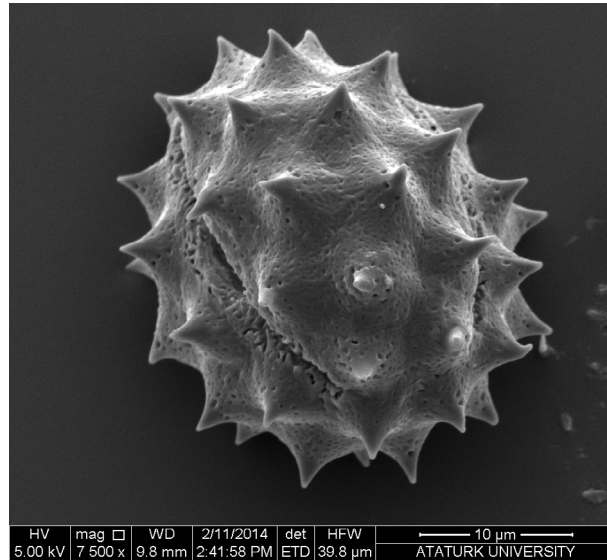


Figure 2. Pollen morphology of *Tanacetum erzincanense*.

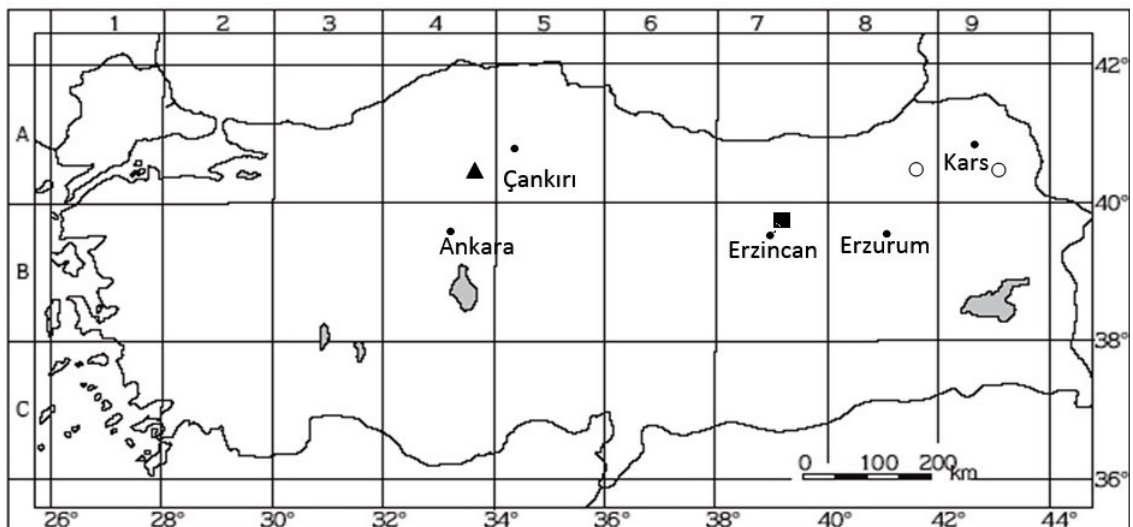


Figure 3. The distribution of *Tanacetum erzincanense* (■), *Tanacetum germanicopolitanum* (▲), and *Tanacetum pinnatum* (○).

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.



Figure 4. General habits of *Tanacetum erzincanense* (A), *T. germanicopolitanum* (B), and *Tanacetum pinnatum* (C).

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 Çayırılı district (Erzincan Province) at an altitude of 1622 m. Vegetation of the area is formed by herbaceous plant species including



Figure 5. Capitula and basal leaves: *Tanacetum erzincanense*: A- capitula, D- basal leaves. *Tanacetum germanicopolitanum*: B- capitula, E- basal leaves. *Tanacetum pinnatum*: C- capitula, F- basal leaves.

Achillea millefolium L. subsp. *millefolium* L., *Bellevia 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.

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

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

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

Primer	Sequence (5'–3')	Length of amplified bands	No. of bands	No. of polymorphic bands	Polymorphism rate (%)
A-1	AGTCAGCCAC	750–1800	6	5	83.3
C-10	TGTCTGGGTC	300–2000	9	8	88.88
OPA- 4	AATCGGGCTG	500–2300	8	8	100
OPB- 03	CATCCCCCTG	400–2500	10	10	100
OPH- 16	TCTCAGCTGG	250–2200	8	7	87.5
OPY- 7	AGAGCCGTCA	750–2000	5	5	100
OPY-13	GGGTCTCGGT	500–1800	5	4	80
OPW- 8	GACTGCCTCT	750–2700	7	6	85.7
OPY- 19	TGAGGGTCCC	300–1600	5	5	100
Total		250–2700	63	58	92.1

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 *T. 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.



Figure 6. Results of gel electrophoresis of PCR products obtained.

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).

References

- Akbulut M, Ercisli S, Yıldırım N, Orhan E, Agar G (2008). The comparison of persimmon genotypes (*Diospyros kaki* Thunb.) by using RAPD and FAME data. Roum Biotechnol Lett 13: 3851–3858.
- Aytaç Z, Duman H (2013). A new species and 2 new records from Turkey. Turk J Bot 37: 1055–1060.
- Besse P, Silva DD, Bory S, Grisoni M, Bellec FE, Duval MF (2004). RAPD genetic diversity in cultivated vanilla: *Vanilla planifolia*, and relationships with *V. tahitensis* and *V. pompona*. Plant Sci 167: 379–385.

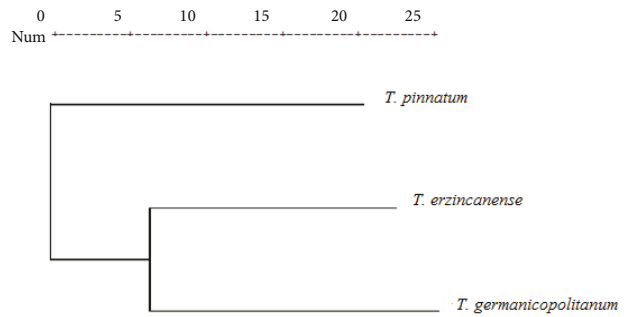


Figure 7. UPGMA dendrogram showing the relationship of 3 *Tanacetum* species.

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- Çağlar KÖ (1949). Toprak Bilgisi. Ankara, Turkey: Ankara Üniversitesi Ziraat Fakültesi Yayınları (No.: 10) (in Turkish).
- Doll EC, Lucas RE (1973). Testing soils for potassium, calcium and magnesium. In: Westerman RI, editor. Soil Testing and Plant Analysis. Madison, WI, USA: Soil Science Society of America, pp. 181–227.
- Erdtman G (1952). Pollen Morphology and Plant Taxonomy. Uppsala, Sweden: Almqvist & Wiksell.
- Grierson AJC (1975). *Tanacetum* L. In: Davis PH, editor. Flora of Turkey and the East Aegean Islands, Vol. 5. Edinburgh, UK: Edinburgh University Press, pp. 256–292.

- Güner A (2012). Türkiye Bitkileri Listesi. İstanbul, Turkey: Ali Nihat Gökyiğit Vakfı Yayınları (in Turkish).
- Hindistan M, İnceoğlu İ (1962). Toprakta pH tayini. Ankara, Turkey: Tarım Bakanlığı, Topraksu Genel Müdürlüğü, Toprak ve Gübre Araştırma Enstitüsü (in Turkish).
- IUCN Species Survival Commission (2014). Guidelines for Using the IUCN Red List Categories and Criteria. Version 11. Gland, Switzerland: IUCN.
- Jaccard P (1908). Nouvelles recherches sur la distribution florale. *Bul Soc Vaudoise Sci Nat* 44: 223–270 (in French).
- Khanuja SPS, Shasany AK, Daroker MP, Kumar S (1998). DNA fingerprinting of plant genetic resources: the need of time. *J Med Arom Pl Sci* 20: 348–351.
- Mukherjee A, Sikdar B, Ghosh B, Banerjee A, Ghosh E, Bhattacharya M, Roy SC (2013). RAPD and ISSR analysis of some economically important species, varieties, and cultivars of the genus *Allium* (Alliaceae). *Turk J Bot* 37: 605–618.
- Oberprieler CH, Vogt R, Watson LE (2007). Tribe *Anthemideae* Cass. In: Kadereit JW, Jeffrey C, editors. *The Families and Genera of Vascular Plants*, Vol. 8: Flowering Plants, Eudicots, Asterales. Heidelberg, Germany: Springer-Verlag, pp. 342–374.
- Öztürk M, Çetin Ö (2013). *Inula tuzgoluensis* (Asteraceae), a new species from Central Anatolia, Turkey. *Turk J Bot* 37: 825–835.
- Podlech D (1986). *Tanacetum* L. In: Rechinger KH, editor. *Flora des Iranischen Hochlandes und der Umrahmenden Gebirge, Persien, Afghanistan, Teile Von West-Pakistan, Nord-Iraq, Azerbaidan, Turkmenistan*. Graz, Austria: Balogh Scientific Books, pp. 88–148 (in German).
- Schischkin BK, Bobrov EG (2000). *Compositae*. In: *Flora of the USSR*, Vol. XXVI. Washington, DC, USA: Smithsonian Institute Libraries.
- Sonboli A, Stroka K, Osaloo SK, Oberprieler C (2012). Molecular phylogeny and taxonomy of *Tanacetum* L. (Compositae, Anthemideae) inferred from nrDNA ITS and cpDNA trnH-psbA sequence variation. *Plant Syst Evol* 298: 431–444.
- Sunar S, Aksakal O, Yildirim N, Agar G, Gulluce M, Sahin F (2009). Genetic diversity and relationships detected by FAME and RAPD analysis among *Thymus* species growing in eastern Anatolia region of Turkey. *Roum Biotechnol Lett* 14: 4313–4318.
- Tüzüner A (1990). *Toprak ve Su Analiz Lab. El Kitabı*. Ankara, Turkey: Tarım Orman ve Köyişleri Bakanlığı Köy Hizmetleri Genel Müdürlüğü (in Turkish).
- Ülgen N, Ateşalp M (1972). *Toprakta Bitki Tarafından Alınabilir Fosfor Tayini*. Ankara, Turkey: Köy İşleri Bakanlığı, Topraksu Genel Müdürlüğü, Toprak ve Gübre Araştırma Enstitüsü Yayınları 21 (in Turkish).
- Yıldız B, Arabacı T, Dirmenci T (2013). Two new species of *Cirsium* (Asteraceae) and notes on allies from Turkey. *Turk J Bot* 37: 1045–1054.
- Zheng DJ, Liang YF, Liu GM, Yan DH, Linghu CD, Tian YH (2009). RAPD analysis of germplasm resources of *Kudingcha* species in Oleaceae. *Agric Sci China* 8: 784–792.